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Human impacts on oligotrophic marine ecosystems: case studies from Cyprus, Mediterranean Sea

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HUMAN IMPACTS ON OLIGOTROPHIC MARINE ECOSYSTEMS: CASE STUDIES FROM CYPRUS, MEDITERRANEAN SEA

by

DEMETRIS KLETOU

A thesis submitted to the University of Plymouth in partial fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Highly oligotrophic marine waters cover about a third of the Earth's surface and have diverse communities that are affected by multiple stressors, such as ocean acidification, warming, marine litter, pollution, overfishing. The low nutrient Mediterranean Sea has high biodiversity and endemism but is heavily impacted by these human pressures and a recent major influx of invasive alien species that compete, predate and infect indigenous species. Aquaculture effluents and eutrophication alter water quality and add pressure on coastal ecosystems that are already subject to habitat loss and degradation due to coastal developments. This PhD dissertation reviews human impacts on highly oligotrophic marine ecosystems (**Chapter 2**). It then presents case studies that cover multiple impacts around Cyprus.

A major impact to the Mediterranean Sea is immigration of Indo-Pacific species, which has accelerated in recent years due to climate change and the widening of the Suez Canal. **Chapter 3** compares populations of the seagrass *Halophila stipulacea* both in their alien and native environments. It also presents the detection of the lionfish *Pterois miles* providing the first evidence that a lionfish invasion in the Mediterranean is underway.

Shallow rocky reefs covered by canopy-forming brown seaweed and meadows of *Posidonia oceanica* are declining rapidly throughout the Mediterranean. I studied these important ecosystems across the most industrialised coastline of Cyprus. **Chapter 4** characterises macroalgal communities along an impact gradient and shows that macroalgae are robust indicators to assess the ecological status of coastal waters. Communities dominated by canopy-forming *Cystoseira* at pristine sites, shifted to turf and filamentous opportunistic seaweed on industrialised coastlines. **Chapter 5** characterises sediment chemical variables, delivers the first habitat cartography, describes structural descriptors of *P. oceanica* and assesses the ecological condition across Vasiliko Bay. Despite mounting pressures, ancient *P. oceanica* meadows in Vasiliko Bay have among the highest shoot densities across the whole Mediterranean. However, lower shoot densities and more epiphytes were noted near fish farm sites. In **Chapter 6** monitoring of *P. oceanica* descriptors from fixed plots established at the lower limits of the meadows near fish farms shows that a management decision to relocate farms into deeper waters had prevented further declines in seagrasses.

Chapter 7 presents Integrated Multi-Trophic Aquaculture (IMTA) trials carried out off southern Cyprus. The concept of IMTA is to cultivate organisms (e.g. seaweed, filter feeders, detritivores) around finfish cages, converting waste effluents into product and mitigating environmental impacts. Trials lasted three years and identified opportunities and challenges that impede IMTA adoption by fish farms in warm oligotrophic conditions. Taken as a whole, this thesis demonstrates that ecology of the eastern Mediterranean Sea is changing rapidly due to multiple anthropogenic stressors, but strategic management can help halt, and in some cases reverse, the damage done.

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ACKNOWLEDGEMENTS

This PhD took almost eight years to complete. A lot has happened during this time, loved ones were lost, loved ones were born. Developing as a researcher and climbing up the PhD ladder has taken its toll. The biggest was imposed to my beloved ones, those to whom my presence matters, and my scientific choices have restricted our time together. This academic achievement has only been possible because of the harmony they brought into my life. To my lovely wife who endured and supported me from the first day to this day, my beautiful kids who came into my life and gave me purpose, my parents and grandparents, siblings and best friends who gave me strength and motivation. I walked a long and difficult path and I was never alone, for that I am forever grateful.

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areas: the case of Vasiliko Bay', conducted in 2011-2014 and co-funded by the Research Promotion Foundation of the Republic of Cyprus and the European Regional Development Fund (grant agreement SMEs/Product/0609/74). The research leading to **Chapter 7** has been undertaken as part of the *Increasing Industrial Resource Efficiency in European Mariculture* (IDREEM; www.idreem.eu) project, conducted in 2012-2016 and funded by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 308571.

AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

The study and research on which this application is based was undertaken while the author was employed by Marine & Environmental Research (MER) Lab at Cyprus. The research leading to most PhD Chapters was funded by: (i) the COST (European Cooperation in Science and Technology) Action CA 15121, (ii) the Research Promotion Foundation of the Republic of Cyprus and the European Regional Development Fund (grant agreement SMEs/Product/0609/74), and (iii) the European Union's Seventh Framework Programme (FP7/2007-2013; grant agreement no. 308571).

Relevant scientific workshops, seminars and conferences were regularly attended at which work was often presented. Posters were presented at the European Marine Biology Symposium 2013, European Aquaculture Society 2014 and MarCons 2017. An oral presentation was given at the Aquaculture Europe 2016 (European Aquaculture Society) annual conference. Up to now, four Chapters (2, 3.2, 4 and 6) are published in peer-reviewed book or journals. More publications have resulted from side work with Plymouth University as my affiliation.

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LIST OF PUBLICATIONS

Book Chapters

Kletou D. and Hall-Spencer J. (2012) Threats to Ultraoligotrophic Marine Ecosystems, Marine Ecosystems, Dr. Antonio Cruzado (Ed.), ISBN: 978-953-51-0176-5, InTech, pp. 1-34. https://doi.org/10.5772/34842

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Kletou D., Hall-Spencer J. and Kleitou P. (2016) A lionfish (*Pterois miles*) invasion has begun in the Mediterranean Sea. *Marine Biodiversity Records* 9:46 https://doi.org/10.1186/s41200-016-0065-y

Kletou D., Kleitou P., Savva I., Attrill M., Antoniou C. and Hall-Spencer J. (2018) Seagrass recovery after fish farm relocation in the eastern Mediterranean. *Marine Environmental Research* 140, 221-233. https://doi.org/10.1016/j.marenvres.2018.06.007

Kletou D., Savva I., Tsiamis K. and Hall-Spencer J.M. (2018) Opportunistic seaweeds replace *Cystoseira* forests on an industrialised coast in Cyprus. *Mediterranean Marine Science* 0, 598-610. http://dx.doi.org/10.12681/mms.16891

Presentations

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Kletou D. (2014) Integrated Multi-Trophic Aquaculture Trials in Cyprus: The Challenges of Warm, Oligotrophic Waters. Poster presented at *Aquaculture Europe 2014, Donostia–San Sebastián, Spain.* https://www.was.org/easonline/AbstractDetail.aspx?i=3950

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Kletou D., Savva I., Kleitou P., Antoniou C., Rotini A., Conte C., Luciana M., Chiquillo K., Willette D. and Gidon W. (2017) Differences in morphological, physiological and genetic traits between native and invasive populations of *Halophila stipulacea.* Poster submitted to COST Action CA 15121: *Advancing marine conservation in the European and contiguous seas (MarCons).* http://dx.doi.org/10.13140/RG.2.2.19809.20322

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Rousou M., Ganias K., **Kletou D.**, Loucaides A. and Tsinganis M. (2014) Maturity of the pufferfish *Lagocephalus sceleratus* in the southeastern Mediterranean Sea. *Sexuality and early development in aquatic organisms* 1, 35-44. http://dx.doi.org/10.3354/sedao00005

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Nguyen H. M., Kleitou P., **Kletou D.,** Sapir Y. and Winters G. (2018) Differences in flowering sex ratios between native and invasive populations of the seagrass *Halophila stipulacea*. *Botanica Marina*, 61(4), 337-342. https://doi.org/10.1515/bot-2018-0015

ABBREVIATIONS

AMT	Atlantic Meridional Transect
ANOSIM	ANalysis Of SIMilarity
ANOVA	ANalysis Of VAriance
AP	Alkaline Phosphatase
APA	Alkaline Phosphatase Activity
BI	Blue Island Plc
C	Carbon
C_4Cl_6	Hexaclorobutadiene
C_6Cl_6	Hexachlorobenzene
$CaCO_3$	Calcium carbonate
Chl. *a*	Chlorophyll *a*
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ions
CTD	Conductivity, Temperature and Depth
CWA	Clean Water Act
CYCOFOS	Cyprus Coastal Ocean Forecasting and Observing System
DDT	DichloroDiphenylTrichloroethane
DFMR	Department of Fisheries and Marine Research
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphorous
E/L	Epiphyte : Leaf
EBSA	Ecologically or Biologically Significant Areas
EEI	Ecological Evaluation Index
EIA	Environmental Impact Assessment
EM	EMAT
EQR	Ecological Quality Ratio
ESC	Ecological Status Class
ESG	Ecological Status Group
EU	European Union
FAO	Food and Agriculture Organisation
Fe	Iron
Fe^{2+}	Ferrous iron
FI:FO	Fish in : Fish out
FP7	Framework Programme 7
GDP	Gross Domestic Product
GES	Good Environmental Status
GIS	Geographical Information System
GOBI	Global Ocean Biodiversity Initiative
H^+	Hydrogen ions

H_2CO_3	Carbonic acid
Hg	Mercury
HSD	Honestly Significant Difference
IAS	Invasive Alien Species
ICCAT	International Commission for the Conservation of Atlantic Tunas
IDREEM	Increasing Industrial Resource Efficiency in European Mariculture
IMTA	Integrated Multi-Trophic Aquaculture
IUCN	International Union for Conservation of Nature
LAI	Leaf Area Index
lmg	Relative importance
LNG	Liquefied Natural Gas
LOD	Limit Of Detection
LOI	Loss On Ignition
LPG	Liquefied petroleum gas
MedGIG	Mediterranean Geographical Intercalibration Group
MMY	Meat Mussel Yield
MPA	Marine Protected Area
MSFD	Marine Strategy Framework Directive
N	Nitrogen
N_2	Nitrogen gas
NASA	National Aeronautics and Space Administration
NH_4^+	Ammonium ions
nMDS	non-metric MultiDimensional Scaling
NO_2^-	Nitrite ions
NO_3^-	Nitrate ions
OM	Organic Matter
P	Phosphorous
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	PolyBrominated Diphenyl Ethers
PCBs	PolyChlorinated Biphenyl
PMN	Posidonia Monitoring Network
PO_4^{3-}	Phosphate ions
PREI	*Posidonia oceanica* Rapid Easy Index
RDA	Redundancy (ordination) analysis
REF	Reference
RELIONMED	Preventing a LIONfish invasion in the MEDiterranean through early response and targeted Removal
SE	Standard Error
SeaWiFs	Sea-viewing Wide Field of view sensor
SIMPER	SIMilarity PERcentage procedure
SIMPROF	SIMilarity PROFile Analysis
SSS	Side Scan Sonar
SW	Seawave Fisheries
TPM	Total Particulate Matter
UNCLOS	United Nations Convention on the Law of the Sea
WFD	Water Framework Directive

1. INTRODUCTION

Water bodies are often classified on the basis of surface chlorophyll *a* concentration, the photosynthetic pigment that is present in all photoautotrophs. Oligotrophic waters have chl. *a* concentration between 0.06 - 1 µg/L (Shushkina *et al.*, 1997). Ultraoligotrophic marine areas (chl. *a* <0.06 µg/L) occur within subtropical gyres at mid-latitudes and are quite often referred to as the ocean deserts, however their immense size (16-28% of the surface of the earth) makes their contribution to the global carbon cycle very important (McClain *et al.*, 2004; Signorini *et al.*, 2015). Periods of ultraoligotrophy also occur in the eastern Mediterranean and the northern Red Sea, particularly during summer (Labiosa *et al.*, 2003; Siokou-Frangou *et al.*, 2010). In **Chapter 2**, ultraoligotrophic open-ocean gyres and enclosed Seas are compared and the main threats to these systems are described. Despite the low nutrient background levels, organisms evolved and specialised in nutrient-depleted waters and extreme diversity of picoplankton is recorded. Prokaryotes and viruses are significant contrinutors of carbon export in the nutrient-depleted ocean (Guidi *et al.*, 2016). Picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* dominate the euphotic zone in oligotrophic seawater and while *Synechococcus* has a much broader geographical distribution distribution, *Prochlorococcus* is restricted to warm oligotrophic water (Flombaum *et al.*, 2013). These diverse prokaryotes are the smallest and most abundant photosynthetic organisms on Earth exhibiting a wide range of adaptations to cope with low nutrient conditions and variable light levels (Biller *et al.*, 2015). Photosynthetic picoeukaryotes such as the exceptionally diverse haptophytes are also important producers, thought to contribute 30-50% of the total photosynthetic standing stock across the world ocean with their competitive success in oligotrophic water attributed to their mixed mode of nutrition being able to photosynthesize as well as engulf bacteria (Liu *et al*., 2009; Unrein *et al*., 2014).

In coastal ultraoligotrophic marine ecosystems (i.e., the eastern Mediterranean and the northern Red Sea) benthic primary producers provide food and create structurally complex habitats. The Red Sea is a biodiversity and endemism hotspot, home to over three hundred zooxanthellate scleractinian coral species (Veron *et al*., 2009), that support over a thousand fish species (Golani & Bogorodsky, 2010). In the Mediterranean Sea, the photophilic macroalgal assemblages (e.g. *Cystoseira* forests) covering the rocky shallow reefs and the seagrass *Posidonia oceanica* (Linnaeus) Delile, 1813, that grows at 0 – 50 m in depth (Duarte, 1991) form diverse and complex habitats. The Mediterranean basin covers <0.8% of the world ocean surface and is <0.3% of its volume, but it is home to 4-18% of the world's recorded species, depending on the taxonomic group (from 4.1% of the bony fishes to 18.4% of the marine mammals) (Coll *et al*., 2010).

Subtropical open-ocean ecosystems are far removed from human civilization yet despite this remoteness they are exposed to major threats such as: (i) climate change i.e., warming, acidification and deoxygenation (Gruber, 2011), with impacts that include decreased ocean productivity, altered food web dynamics, reduced abundance of habitat-forming species, shifting species distributions, and a greater incidence of disease (Hoegh-Guldberg & Bruno, 2010; Agusti *et al*., 2019), and (ii) marine litter that cause entanglement of marine fauna, are ingested by consumer species from all trophic levels including the small heterotrophic plankton, aid dispersal of invasive species to non-native waters, and bioaccumulate organic contaminants (Gregory, 2009; Rochman *et al*., 2016). Enclosed marine ecosystems surrounded by humans, are exposed to additional significant pressures including: alien invasions, chemical and noise pollution, overexploitation of resources, eutrophication, aquaculture and habitat loss; all of which are described in **Chapter 2**.

Biological invasions are one of the most important drivers of biodiversity loss and a major pressure to ecosystems resulting in environmental and economic adverse impacts (Early *et al*., 2016). They are considered as the second greatest human-caused agent of species endangerment and extinction (Bellard *et al*., 2016), and responsible for significant impacts on ecosystem processes and functions that are fundamental to human well-being (Katsanevakis *et al*., 2014a). The eastern Mediterranean recently underwent one of the most intense and exceptionally rapid changes in terms of number of arrivals, number of established alien species and related impacts, compared to other Mediterranean regions (Galil *et al.,* 2018a). These profound alterations are fostered by climatic changes (i.e., warming) and the increasing anthropogenic pressures (e.g., pollution, habitat destruction, shipping, enlargement of the Suez Canal), which are rendering the basin a more favourable environment for tropical invaders that increase their abundance and cause declines in native species (Raitsos *et al.*, 2010; Lejeusne *et al.*, 2010; Givan *et al.*, 2017; Arndt *et al.,* 2018). There are now at least 821 alien multicellular species in the Mediterranean Sea, of which more than 600 are well-established (Zenetos *et al.*, 2017). Considering the increasing frequency of Lessepsian immigration events, there is a great need for continuous monitoring and assessment of the invasion processes (Galil *et al.*, 2015). Cyprus in the eastern Mediterranean, being located only approximately 370 km from the Suez Canal is the first European country to be affected by the Lessepsian invasions and offers an ideal site for setting up early response and detection systems, as well as for understanding the invasion dynamic processes and how climate change shifts species abundance and ranges.

Chapter 3 presents two case studies related to the study of Lessepsian immigrants in the Mediterranean. The first demonstrates a monitoring approach that compares seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867, both in their native (northern Red Sea) and alien environments (eastern Mediterranean), and the second demonstrates the early detection of the lionfish *Pterois miles* (Bennett, 1828) invasion in the Mediterranean, that led to the rapid response initiated by the EU LIFE project titled ‘’RELIONMED’’. *Halophila stipulacea* is an Indo-Pacific species that has been present in the Mediterranean for almost a century (Forti, 1927) and although it has remained constricted to the eastern basin for many decades, it has recently displayed a westwards progression (Gambi *et al.*, 2009). Despite its tropical native range, it was found to survive, photosynthesise and grow within a broad range of temperatures (10 to 30°C) (Georgiou *et al.*, 2016). Driven by on-going climate change, it is likely that the species will expand its range throughout the Mediterranean and therefore, it is important to monitor its establishment and invasiveness. In the first part of **Chapter 3**, permanent monitoring stations have been set up in the invaded Mediterranean range of the seagrass species (Limassol, Cyprus) and its native range (Gulf of Aqaba, Israel) at 3 m depth. The monitoring systems set a strong basis for further research and present the first comparisons between the two populations (indigenous and alien). Follow up monitoring can yield useful information about the dynamics of this seagrass in both native and non-native habitats, which is important for both managing the species and anticipating impacts of climate-driven range shifts.

The lionfish (*Pterois miles*) is native to the warm, tropical waters of the South Pacific and Indian Oceans. It has been recognised as one of the most ecologically harmful marine fish invasions to date (Albins & Hixon, 2013), and displayed a remarkable expansion, establishing at the eastern coasts of the USA, Bermuda, the entire Caribbean region and the Gulf of Mexico in less than 30 years (Ferreira *et al.*, 2015). Following an unsuccessful Mediterranean introduction in 1991 (Golani & Sonin, 1992), the lionfish was recorded in 2012 off Lebanon, and has relatively quickly proliferated and spread, reaching the central Mediterranean Sea in just three years (Bariche *et al.*, 2017; Azzurro *et al.*, 2017). Unless controlled quickly, the Mediterranean outbreak in lionfish may result in a cascade of adverse impacts on economically important species and habitats. The second part of **Chapter 3**, used sightings derived from citizen scientists to demonstrate the early detection of the lionfish invasion in the Mediterranean, providing the first robust evidence of the species establishment and expansion in the Mediterranean basin. Early detection and eradication measures are crucial and the most cost-efficient way to prevent the establishment and spread of an invasive species. This study led to the rapid response by the EU with a four-year LIFE project Nature and Biodiversity project, RELIONMED aiming to REmove LIONfish from the MEDiterranean, which initiated on September 2017.

Although the coastal zone covers less than 3% of the Earth’s surface it hosts about two thirds of the world’s population (Hyun *et al.*, 2009). The human ecological footprint is growing worldwide, and the impacts are most intense on coastal marine ecosystems where multiple pressure drivers are acting synergistically (Halpern *et al*., 2008). The Mediterranean coastline hosts a population of about 250 million people and about one third of all global tourism (Randone *et al*., 2017). Inevitable coastal developments are degrading priority habitats and keystone species are being lost at unprecedented rates.

In the Mediterranean Sea, the most productive and biodiverse coastal marine ecosystems are the rocky reefs covered with canopy-forming fucoid algae and seagrass *P. oceanica* meadows. These vulnerable ecosystem-engineers provide three-dimensional biogenic structure, food, shelter and substrata for diversified assemblages of species and enhance coastal primary productivity. Seagrass and algal beds cover less than 0.5% of the global ocean but account for over 5% of the world’s ecosystem services (Costanza *et al.,* 2014). Declines are hard to recover since these perennial species have slow growth rates. While rocky reefs and seagrass ecosystem have been intensively studied there is almost nothing published in the literature from the warm and oligotrophic eastern Mediterranean around Cyprus.

Cyprus the third largest Mediterranean island, is presently undergoing very rapid changes in coastal use (Hadjimitsis *et al.*, 2016) but there are no published studies about the impact of this expansion on marine ecology. Baseline information on marine biota and sensitive ecosystems is lacking. The **next three Chapters** draw from case studies on priority habitats (rocky reefs and seagrass meadows) conducted across steep gradients of impact at a 10 km long coastline, encompassing Vasiliko Bay, off southern Cyprus. Vasiliko Bay is the most industrialised coastline of the island, affected by multiple anthropogenic pressures (Fig. 1-1). The national mariculture industry concentrates on the western side of the bay at approximately 1-3 km from the shore. The coastline in the central eastern side of the bay is modified and heavily industrialised. It hosts the country’s largest power station that uses heavy fuel oil-fired steam and combined cycle gas turbines supplied from a single point mooring receiving buoy about 2 km offshore that has the capacity to accommodate up to 80 kt fuel oil tankers. At the eastern tip of the bay is the country’s largest cement production plant that has been in operation since 1967 and supplied by nearby clay quarries. A new clinker production line was launched in 2011 with the capacity to produce 6000 tons per day. It produced around two megatons of cement and clinker in 2012.

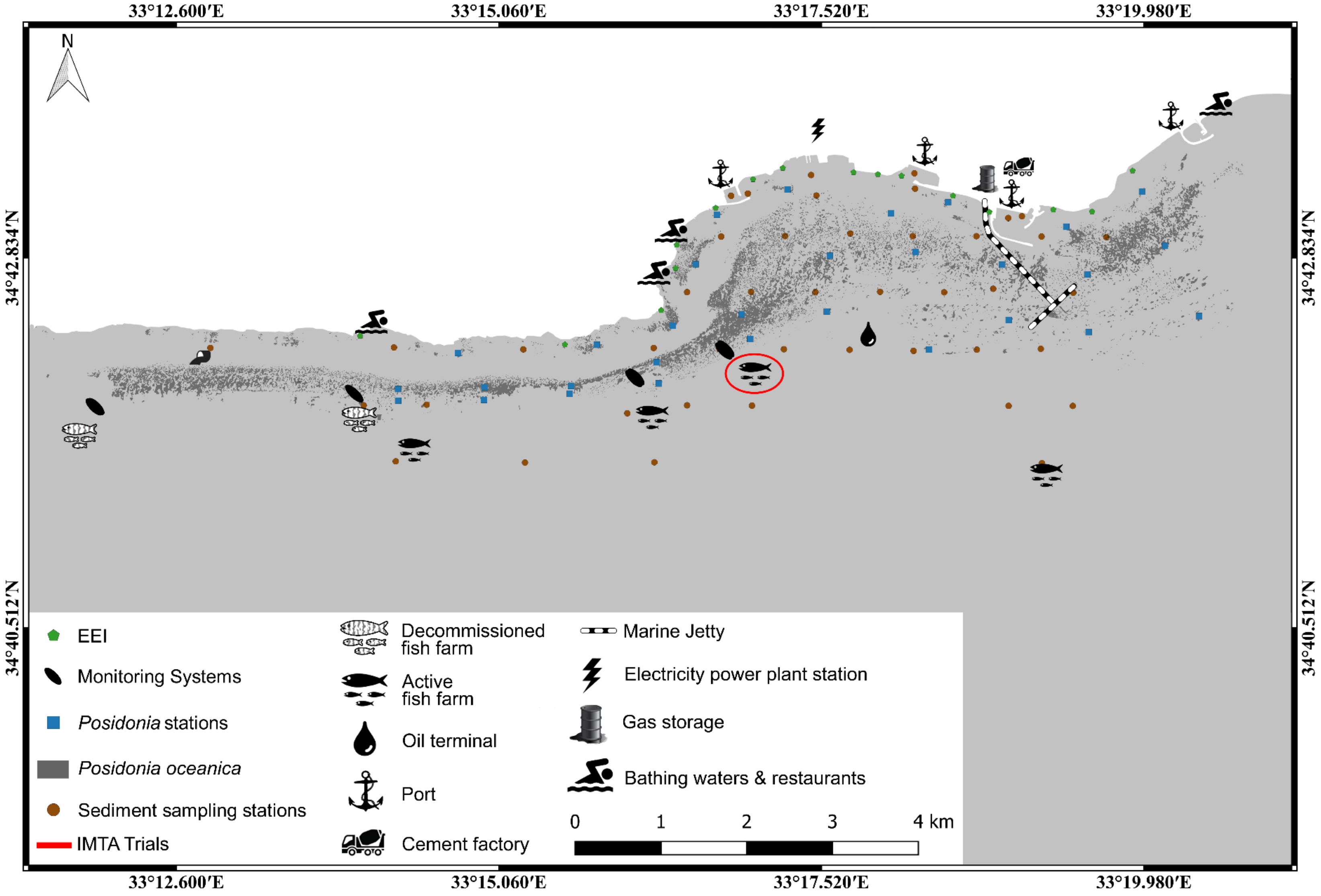

Figure 1-1. Map of Vasiliko Bay and nearby areas, showing main anthropogenic pressures and sampling sites used in the studies described in Chapter 4 (Macroalgae-EEI), Chapter 5 (*Posidonia oceanica* and sediment sampling sites), Chapter 6 (*P. oceanica* Monitoring Systems) and Chapter 7 (IMTA trials).

There are three ports: Vasiliko port a major importer/exporter of dirty cargo mostly used by the cement factory, Archirodon port that includes a dry dock for ship maintenance and repairs and a small shelter for fish farmers and fishing boats, and ‘Evangelos Florakis’ Naval Base. During this PhD programme of research, the human developments in Vasiliko Bay further intensified. The fish farms relocated deeper but expanded production significantly. A desalination plant with the capacity to supply the power station with 60 000 m^3 of desalinated water per day was constructed in 2014. The recent discovery of gas in the eastern Levantine has resulted in the construction of the longest marine jetty (dredging started in 2012) in the region and facilities of various multi-national corporations for the storage and management of petroleum and oil products with a total storage capacity of storage of 858,000 m^3. Governmental plans for further development are underway in the framework of a Master Plan dedicated to expansion of hydrocarbon-based heavy industries in the region. It includes the construction of new marine jetties, pipelines and multiple marine berths. Large areas of land that have been expropriated by the government will be used to construct the nation’s energy centre. Developmental plans are being realised as land reclamation and dredging in the eastern side of the bay have recently started. Despite this extraordinary anthropogenic footprint concentrated on such a small geographic area, there have been no scientific efforts to assess the degree of impact on the local marine ecosystem and its capacity to tolerate these pressures. This PhD programme of research represents the first scientific effort to monitor and assess the condition of the most important habitats “rocky reefs and *P. oceanica* meadows”, across gradients of pressure in Vasiliko Bay.

On the rocky substrata in the upper-sublittoral of the Mediterranean Sea, brown algae of the genus *Cystoseira* form structurally complex canopy with diverse understorey assemblages, facilitate the growth of other species and offer high nursery value for many invertebrates and fish species (Duarte, 2000; Bulleri *et al.*, 2002; Mangialajo *et al.*, 2008;

Cheminée *et al*., 2013; Pitacco *et al*., 2014). Development along European coastlines is taking place at alarming rates and various factors such as water pollution, nutrient enrichment, sediment loading, physical disturbance, invasive species, overfishing and resulting trophic cascades that lead to overgrazing, and climate change have contributed to major die back of *Cystoseira* forests (Sala *et al.*, 2011; Milazzo *et al.*, 2004; Thibaut *et al.*, 2005; Mangialajo *et al.*, 2008; Sales *et al.*, 2011; Tsiamis *et al.*, 2013; Strain *et al.*, 2014; Bianchi *et al.*, 2014; Mineur *et al.*, 2015; Thibaut *et al.*, 2015).

Macroalgal communities respond predictably to anthropogenic disturbances, so they can be used as bioindicator for assessment of the ecological status of coastal waters. In **Chapter 4** the macrophytic communities covering natural and modified hard substrata in the upper sublittoral zone were investigated in 2012-2013, across a gradient of anthropogenic pressures in Vasiliko Bay. To assess whether this coastline is being managed effectively to maintain the good ecological status target, set by European Directives, the macroalgal Ecological Evaluation Index (Orfanidis *et al*., 2011) that has been developed for the Mediterranean waters was applied.

Seagrasses contribute significantly to the economy of coastal countries and human well-being (Barbier *et al.*, 2011; Campagne *et al.*, 2015; Dewsbury *et al.*, 2016). Their meadows are among the most productive ecosystems on Earth but are also among the most threatened, declining at unprecedented rates (Orth *et al.*, 2006; Waycott *et al.*, 2009; Unsworth *et al.*, 2014). The key services they provide include: coastal protection from erosion, water purification, transfer of matter and energy up the trophic levels; carbon sequestration and provision of complex habitat for enhanced biodiversity (Barbier *et al.*, 2011; Campagne *et al.*, 2015).

The endemic Mediterranean seagrass *P. oceanica* forms structurally complex meadows that are the climax stage of many upper subtidal bottoms extending from the surface down to depths of 40-45 m in oligotrophic clear waters, and supporting hundreds of associated species (Piazzi *et al.*, 2016). *Posidonia oceanica* has been identified by the European Union Water Framework Directive (WFD, 2000/60/EC) as a key bioindicator of ecosystem quality and in the Marine Strategy Framework Directive (MSFD, 2008/56/EC) as an indicator of the Good Environmental Status (GES) for marine areas. *Posidonia oceanica* meadows are protected by Conventions ratified by most countries of the Mediterranean such as the Berne and Barcelona Conventions, the European Habitats Directive (92/43/EEC) and the fishing legislation (council regulation 1626/94) and are included in the International Union for Conservation of Nature (IUCN) Red List. Despite the strong protection status, *P. oceanica* declined significantly during the last decades particularly in the western Mediterranean (Marbà *et al*., 2014; Telesca *et al.*, 2015). Efforts to conserve *P. oceanica* relied on the establishment of marine protected areas, which seem to be insufficient to guarantee the protection of *P. oceanica* meadows (Montefalcone *et al.,* 2009). *Posidonia oceanica* seems to be largely affected by salinity fluctuations, turbidity and the increase of sedimentation rate, anchoring and trawling (Pergent *et al.*, 2012). Large regressions of this phanerogam at coastal areas are considered irreversible at human time-scales (Boudouresque *et al.*, 2009). Even if optimal conditions return, the area will still require centuries to be recolonized as *P. oceanica* is among the slowest growing seagrass species in the world (Duarte, 2002; Kendrick *et al.*, 2005).

Despite regressions of *P. oceanica* reported around the Mediterranean, little is known on this seagrass in the warm and salty Levantine basin (eastern Mediterranean). It is absent from the coasts of Syria, Lebanon and Israel, eastern Egypt and eastern Turkey, but Cyprus represents an exception to this trend. Located in the heart of the Levantine basin, it has surface waters in the warm period near the upper tolerance thermal limits of the species (Marbà & Duarte, 2010; Celebi *et al.,* 2006; Telesca *et al.*, 2015), yet an isolated population of *P. oceanica* occupies the upper subtidal (depths ranging from 0 to

>40 m) around the perimeter of the whole island representing the easternmost reported population of the species. But not all is good, while this isolated seagrass population is threatened in many areas around the island, such as in the case of Vasiliko Bay, the most industrialised coastline of Cyprus, earmarked by the government for further development. In Vasiliko Bay, the seagrass *P. oceanica* forms ancient meadows that extend down to a depth of approximately 30-35 m. Fish farms in the western side of the bay have operated for many years above seagrass beds, possibly inducing irreversible declines; the farms gradually expanded to deeper waters. On the other side of the bay, industrial pollution and repeated dredging on different parts of the meadow to position pipelines, construct or enlarge ports, marine jetties and berths pose a real and growing threat to the highly biodiverse *P. oceanica* meadows.

Chapter 5 draws from data collected in 2012-2013 and aims to provide a baseline of physicochemical parameters in the sediments and of the condition of the *P. oceanica* seagrass meadows across Vasiliko Bay. Physicochemical variables, such as pH, organic content, granule size, alkalinity, concentrations of iron, mercury, hexachlorobutadiene and hexachorobenzene were determined. The distribution of seagrass meadows was mapped across a 10 km long coastline using a combination of aerial photographs for the shallows and a Side Scan Sonar (SSS) for the deeper meadows. Structural indicators such as shoot density, leaf morphometrics, and epiphytic and leaf biomass were determined from sites at different depth zones. The ecological status was classified based on WFD (2000/60/EC) biotic indices PREI (*P. oceanica* Rapid Easy Index) (Gobert *et al.*, 2009) and BiPo (Lopez y Royo *et al.*, 2010a).

One of the major threats to *P. oceanica* is the development of aquaculture along Mediterranean coasts (Pergent Martini *et al.*, 2006). Regression was attributed to reduction of light penetration under the cages (Ruiz *et al.*, 2001), increase of nutrients in the water column that lead to increased epiphyte biomass, resulting in reduced light conditions (Delgado *et al.*, 1997), enhanced herbivory (Holmer *et al.*, 2003), sulphide invasion into the roots (Frederiksen *et al.*, 2007), and high input of organic matter and nutrients to the sediment (Cancemi *et al.*, 2003; Apostolaki *et al.*, 2007; Diaz-Almela *et al.*, 2008). Organic matter enrichment has been suggested to be the most important regression driver (Pérez *et al.*, 2008). The mariculture industry of Cyprus has expanded exponentially from 210 tonnes in 1994 to 6625 tonnes in 2016, now exceeding 80% of the total fisheries production in Cyprus. Most fish farms have traditionally been concentrated around the Vasiliko area. Fish farming started in the mid-nineties with small production units (100-300 tonnes per year), using floating cages starting at depth of 22-28 m and located over seagrass meadows. Some of these farms are now each licenced to produce 1000-1800 tonnes per year, but a prerequisite to receiving expansion permits by the national authorities was to relocate cages in deeper water and further away from *P. oceanica* meadows. Now, the shallowest cages in Vasiliko-Moni area are found at a depth of about 37 m, but seagrass meadows still exist within the impact zone of aquaculture effluents (Holmer *et al.*, 2008).

Despite the temporal and spatial scale of this development, no studies have been conducted to monitor the effects of the fish farm units on the adjacent *P. oceanica* meadows. In **Chapter 6**, four *P. oceanica* observatory systems were established to monitor seagrass meadows at their deepest limits near fish farms that have relocated and expanded deeper and at a reference (decommissioned fish farm) site in the Vasiliko-Moni area (Fig. 1-1). The systems were set up according to the 'Protocol for the setting up of Posidonia meadows monitoring systems «MedPosidonia» Programme' (Pergent, 2007). When data were first collected in 2012-2014, the three active farms had a total production *ca* 2.5 kt yr^{-1} and operated shallow cages near the seagrass meadows investigated or had just relocated nearby to deeper water as they expanded. When data collection was repeated three to five years later in 2017, the three farms had a total production >4 kt yr^{-}

[1]. Each monitring system includes 11 numbered markers positioned at 5 m intervals and anchored at the edge of the meadow, allowing photography and collection of sediment and *P. oceanica* data from the same fixed positions. The aims of **Chapter 6** were to: evaluate the monitoring systems' durability in exposed sea conditions, assess progression or regression of *P. oceanica* meadow's edge in half a decade that lapsed between samplings, evaluate changes in *P. oceanica* and sediment descriptors between the two sampling periods for each fish farm and examine whether fish farm or environmental drivers are affecting these descriptors. Across the Mediterranean Sea, seagrass monitoring is extensive, but the adoption of different sampling designs and methods may result in erroneous comparisons (Lopez y Royo *et al.,* 2010b). The first and follow-up data presented in **Chapter 6** enable microscale long-term monitoring of the seagrass condition from the same fixed plots, increasing statistical confidence by removing the confounding variable of intra-meadow patchiness and providing a case study on how fish farm impacts on seagrass beds can be monitored effectively, thus assisting integrated coastal management decisions.

Fish farming has faced an unprecedented growth, production increased from around 0.5% of global fish supplies in 1970 to around 53% by 2015 (FishStatJ, 2017). This increase has been largely due to the rising human population combined with the increased per capita fish consumption from an average of 9.9 kg in the 1960s to over 20 kg in 2015 (FAO, 2016). Fisheries are over-exploited, remaining stagnated at around 90 million tonnes since 2000 and are unable to supply this growing demand (Costello *et al.,* 2016; Jennings *et al.,* 2016). However, the growth of aquaculture has often been linked to a range of adverse environmental and social effects, which has raised public conflict across the world (Osmundsen & Olsen, 2017). Currently, most finfish are produced intensively in monoculture systems stocked with carnivorous species driven by globalizing trade initiatives and economic benefits, with sustainability remaining as a key issue (Bostock *et al.*, 2010). Some of the most important bottlenecks of fish farming include the depletion of wild fish stocks to produce fish meal and fish oil used in the aquaculture feed and the degradation of coastal and aquatic ecosystems from the organic and nutrient enrichments (Naylor *et al.*, 2000; Holmer *et al.*, 2005). Improving the efficiency of resource utilization through better management, integration or technological advancements is crucial for the sustainable development of aquaculture. An appealing concept that is receiving increasing attention for its potential to improve ecological efficiency and promote sustainability in finfish production, is Integrated Multi-Trophic Aquaculture (IMTA) (Chopin, 2012; Lembo *et al.*, 2018). The multi-trophic concept refers to the integration of extractive species from different trophic levels in a finfish system (Chopin, 2006). Extractive species include seaweed, filter-feeder and deposit-feeder invertebrates that can absorb dissolved and particulate fish farm effluents and convert waste into secondary product, diversifying seafood production and mitigating the environmental impacts associated with fish farming. Despite the potential that lies in this "green" technology, most IMTA in Europe is still operating at research or pilot scale and there is little commercialisation. Over the last years, there has been a growing effort to understand the trade-offs between IMTA and finfish monoculture and to identify the bottlenecks that impede adoption of IMTA. In the Mediterranean Sea, and particularly in the eastern basin, the most important barrier to IMTA development is likely the unsuitable environmental background conditions (Kleitou *et al.*, 2018). Seaweeds offer ideal bioremediation in IMTA systems (Troell *et al.*, 2003; Neori *et al.*, 2004; Granada *et al.*, 2016), but the lack of nutrients in oligotrophic water are unsuitable for massive seaweed production. Several studies have shown that bivalves have enormous potential as biofilters of fish farm effluents (Reid *et al.*, 2010; MacDonald *et al.*, 2011; Lander *et al.*, 2013), but most of the research was carried out in in mesotrophic and eutrophic waters, where bivalve cultivation is economically sustainable (Sarà, 2007).

Chapter 7 elaborates on the experiences and lessons learnt from a series of IMTA pilot trials in Cyprus that aimed to explore the application of IMTA to biomitigate fish farm impacts. Experiments initiated in the summer 2013, lasted for *ca* three years and tested different designs and extractive invertebrates. Specifically, there were: (i) three trials (2013-2016) with the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819, using longlines placed at different depths and in empty and stocked fish cages, (ii) two trials (2013-2016) with the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816), the first using 1m^2 benthic enclosures with different stocking densities and the other rearing sea urchins in baskets in the water column and feeding them with seaweed collected from the farm's structures, (iii) one trial (2014-2015) with the European flat oyster *Ostrea edulis* Linnaeus, 1758, placed in inserts within baskets, (iv) one trial (2015-2016) with the green ormer *Haliotis tuberculata* Linnaeus, 1758, reared in baskets and fed with seaweed, (v) one trial (2015) with the blue crab *Callinectes sapidus* Rathbun, 1896, placed in baskets hung on the side of fish cages and fed with dead fish that would otherwise be discarded, and lastly (vi) one trial (2016) with native sponges using the threaded line and mesh methods to cultivate the sponges in the water column. The aim was to identify bottlenecks and opportunities for IMTA in exposed, oligotrophic and warm marine waters by monitoring the performance (growth, survival) of the IMTA co-cultivars and identifying "first hand" practical limitations.

In summary, this PhD programme aimed to review human impacts on ultraoligotrophic marine ecosystems (**Chapter 2**) and then draw from several case studies carried out in the highly oligotrophic coastal waters of Cyprus in the eastern Mediterranean, covering a range of major impacts such as Invasive Alien Species (IAS), coastal modification and industrialisation with the associated chemical pollution, loss of habitat and aquaculture, and demonstrating methods to assess, monitor and mitigate human impacts. **Chapter 3** demonstrates early detection (lionfish *P. miles*) and monitoring (seagrass *H. stipulacea*) of marine IAS. **Chapters 4 and 5** focus on priority marine ecosystems (rocky reefs covered with *Cystoseira* forests and seagrass *P. oceanica* meadows) and demonstrate methods to carry out mapping, ecological characterisation and quality assessments using these biological quality elements. Shifts of macroalgal communities and variation in *P. oceanica* descriptors across gradients of anthropogenic impact are presented. **Chapter 6** demonstrates the first effort to establish fixed plots at the deeper limits of seagrass meadows near fish farms off Cyprus, allowing microscale and standardised monitoring of seagrass meadows from the same positions over long time scales, removing the effects of intra-meadow variance and sampling uncertainties, and providing a robust tool for monitoring the effects of aquaculture on the protected *P. oceanica*. **Chapter 7** presents the case of IMTA as a tool to biomitigate fish farm impacts and presents several bottlenecks and opportunities identified during IMTA experimentation in offshore warm and oligotrophic waters. Finally, **Chapter 8** discusses main conclusions and steps forward.

2. THREATS TO ULTRAOLIGOTROPHIC MARINE ECOSYSTEMS

Remote marine areas with low productivity are amongst the least explored and understood ecosystems of the biosphere

2.1 Introduction

Marine phytoplankton account for ~1% of the world's photosynthetic biomass but for nearly half of the world's primary production (Field *et al*., 1998; Bryant, 2003). Water bodies are often classified on the basis of surface chlorophyll *a* concentrations, the photosynthetic pigment that is present in all primary producers (Table 2-1).

Table 2-1. Classification scheme based on chlorophyll *a* concentrations proposed by Shushkina et al. (1997).

Water body	Chl. *a* (mg m^{-3})
Ultraoligotrophic	<0.06
Oligotrophic	0.06-0.1
Mesotrophic	0.1-0.3
Eutrophic	0.3-1
Hypertrophic	>1

Data from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS, NASA) show that ultraoligotrophic marine areas occur within subtropical gyres at mid-latitudes and cover about 16-28% of the Earth's surface (Fig. 2-1) (McClain *et al*., 2004). Despite their low productivity, subtropical gyres account for 30-50% of global oceanic primary productivity (Karl *et al*., 1996). The subtropical gyres of the North Pacific, North Atlantic, South Pacific, South Atlantic and South Indian Ocean are ultraoligotrophic year-round with the lowest productivity found in the South Pacific gyre near Easter Island (Morel *et al*., 2010). Periods of ultraoligotrophy also occur in the eastern Mediterranean and the north Red Sea, particularly during summer (Labiosa *et al*., 2003; Siokou-Frangou *et al*., 2010). In this **Chapter** ultraoligotrophic areas are compared and the main threats to these systems are described.

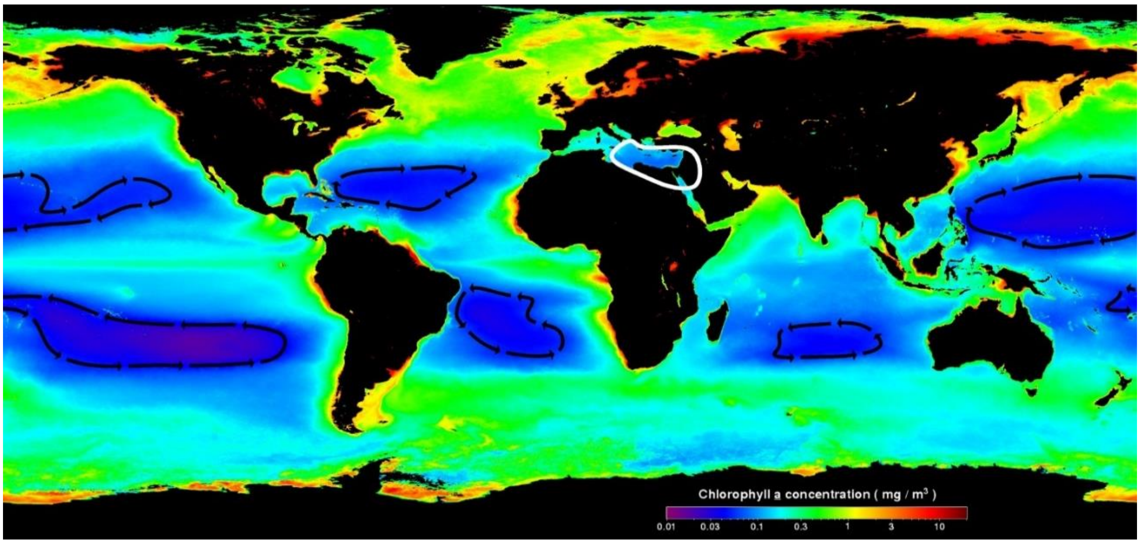

Figure 2-1. World map of surface ocean chlorophyll *a* concentration. Areas within black arrows and the white line indicate ultraoligotrophic (<0.06 mg m^{-3}) open ocean and enclosed systems, respectively. Image from SeaWiFS Project NASA/GSFC and GeoEYE, data 1997-2010.

2.2 Ultraoligotrophic ecosystems

Satellite data underestimate phytoplankton productivity in ultraoligotrophic waters since light penetrates deep into the highly transparent waters, with distinct phytoplankton communities found at different depths and a peak in chlorophyll concentrations as deep as 150 m (Morel *et al*., 2007; Malmstrom *et al*., 2010). Phytoplankton sizes range from picoplankton (0.2–2 μm in diameter), through nanoplankton (2-20 μm in diameter) to microplankton (>20-200 μm). Nutrient rich conditions favour microplankton (e.g. diatoms and dinoflagellates), which are large enough to be eaten by copepods and krill, that in turn are consumed by zooplanktivorous fish. These short, simple food webs have efficient energy transfer to larger consumers (Sommer *et al*., 2002). In ultraoligotrophic waters, picoplankton (Fig. 2-2) seem better able to acquire nutrients than large phytoplankton as they have a higher surface area to volume ratio (Raven, 1998).

Picoplankton are too small to be ingested by copepods and instead are eaten by microplanktonic protists which then feed mesozooplankton (Christaki *et al.*, 2002; Calbet, 2008) or they form aggregates that can then be consumed by crustacean and gelatinous zooplankton (Lomas & Moran, 2011). The complex food webs that occur in ultraoligotrophic waters result in less efficient energy transfer to higher trophic levels. Ephemeral phytoplankton blooms can occur in ultraoligotrophic areas and during these events herbivorous plankton proliferate rapidly given their short generation times (Eden *et al.*, 2009). During blooms myriads of vertically migrating grazers such as copepods, euphausiids and gelatinous zooplankton feed higher trophic groups such as squid, fish and other vertebrates (Seki & Polovina, 2001).

Low phosphorous (P) and nitrogen (N) concentrations normally limit primary production in ultraoligotrophic systems. A spring peak in chl. *a* concentrations usually occurs when longer days allow phytoplankton to thrive due to the greater nutrient availability that follows winter mixing (Morel *et al.*, 2010). Competition for P may have shaped the evolution of marine microbes; the dominance of picocyanobacteria genera such as *Prochlorococcus* and *Synechococcus* in low P environments is thought to be due in part to their ability to form lipid membranes that require less P than most other organisms (Van Mooy *et al.*, 2006; Dyhrman *et al.*, 2009). Picocyanobacteria and picoeukaryotes carry genes encoding for enzymes like alkaline phosphatase (AP) that hydrolyze dissolved organic phosphorous (DOP) and *PstS* genes which are related to the high-affinity uptake of phosphate (Moore *et al.*, 2005; Martiny *et al.*, 2009). Many plankton are able to fix $N_{2,}$ although this ability can be limited by a lack of trace elements such as iron (Tyrrell, 1999; Kustka *et al.*, 2003). The ability to fix N_2 should be ecologically advantageous in ultraoligotrophic environments where the most abundant forms of N are dissolved N_2 gas and dissolved organic nitrogen (DON). In ultraoligotrophic surface waters N_2 fixing bacteria typically have much lower abundances than non-N_2 fixing picocyanobacteria and picoeukaryotes but N_2 fixation increases in importance with depth (Dekas *et al.*, 2009). N_2 fixing cyanobacteria, such as *Trichodesmium* spp., occur in many warm, calm and oligotrophic waters (Capone *et al*., 1997) and are a seasonal and episodic phenomenon in ultraoligotrophic waters. So far, research efforts have focused on colonial *Trichodesmium* spp. but free trichomes, which seem more important in oligotrophic systems, have received little attention (Taboada *et al*., 2010). Primary production in ultraoligotrophic areas is usually dominated by unicellular N_2 fixing bacteria (e.g. *Crocosphaerra* and UCYN clades), non- N_2 fixing picocyanobacteria (e.g. *Prochlorococcus* and *Synechococcus* spp.) and small eukaryotes (e.g. haptophytes) (Malmstrom *et al*., 2010; Moisander *et al*., 2010). Surface-ocean microbial growth is mostly supported by regenerated production, such as DON (e.g. urea) and ammonia oxidation by nitrification which occurs in bacteria and archaea (Zehr & Kudela, 2011).

Even though photosynthetic picoplankton are dominated numerically by *Prochlorococcus* and *Synechococcus*, much of the carbon is fixed by photosynthetic picoeukaryotes such as the exceptionally diverse haptophytes (Grob *et al*., 2011). Picohaptophytes are thought to contribute 30-50% of the total photosynthetic standing stock across the world’s oceans with their competitive success attributed to their mixed mode of nutrition as some are able to photosynthesize as well as engulf bacteria (Liu *et al*., 2009). Recent applications of molecular techniques demonstrate high diversity in the microbial parts of the food web (DeLong, 2009) and a previously unimagined diversity of eukaryotes (Massana & Pedrós-Alió, 2008). Even though the phytoplankton abundance is lowest in oligotrophic waters, the diversity of small-sized phytoplankton seems to peak in these areas (Cermeño & Figueiras, 2008; Kirkham *et al*., 2011). How such a diversity of plankton can coexist on limited resources is intriguing and was dubbed the ‘paradox of plankton’ by Hutchinson (1961). Explanations range from prolonged coexistence and niche segregation to mesoscale turbulence of the ocean (Roy & Chattopadhyay, 2007; Perruche *et al.*, 2010).

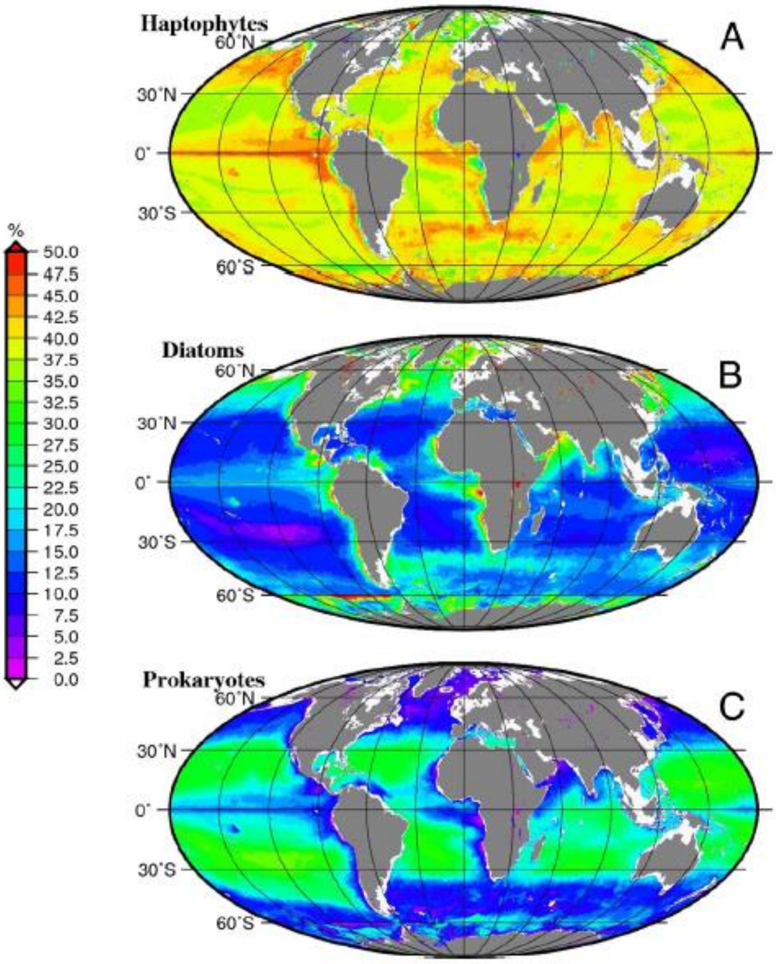

Figure 2-2. Accessory pigments based on relative contribution of (A) haptophytes, (B) diatoms and (C) photosynthetic prokaryotes to total chl. *a* biomass in the euphotic layer for the year 2000. Image from Liu et al. (2009).

2.2.1 Open ocean systems – Subtropical Gyres

Data from monitoring stations off Bermuda and Hawaii are revolutionizing our understanding of mid-latitude gyre dynamics. Once thought of as homogeneous unchanging ocean desserts, we now know that these ultraoligotrophic ecosystems are both physically and biologically dynamic. The gyres circulate clockwise in the northern hemisphere and anticlockwise in the southern hemisphere due to the Coriolis effect.

Ekman pumping (water moving to the right of the wind) and geostrophic flow cause downwelling of relatively warm surface waters at the subtropical convergence near the 20^{o} – 30^{o} latitude (Pedlosky, 1998). The gyres have deep pycnoclines and even deeper nutriclines (e.g. nitrate, phosphate, and silicate) (McClain *et al*., 2004) and expand in area in summer. In most gyres chl. *a* concentrations peak in spring following the winter mixing while in the North Atlantic a secondary peak occurs at the end of September and in the North Pacific chl. *a* concentration is higher during stratified conditions in the summer (Morel *et al*., 2010). Episodic blooms are also detected in all the gyres during the stratified conditions (Wilson & Qiu, 2008). The temporal and spatial complexity that characterizes ultraoligotrophic systems is perhaps best illustrated by describing the primary producers of these systems in turn, since although they have features in common, each gyre is ecologically distinctive.

2.2.1.1 North Atlantic

The Sargasso Sea is probably the most studied open ocean system in the world (Steinberg *et al*., 2001). This subtropical gyre receives iron-rich Saharan dust (Marañón *et al*., 2010) but has extremely low P concentrations, possibly as a result of iron enhanced N_2 fixation (Wu *et al*., 2000). In January-April waves deepen the mixed layer and bring nutrients into the euphotic zone. Subsequent stratification retains nutrients in the surface waters, promoting N_2 fixation, primary production and blooms of phytoplankton such as *Trichodesmium* spp. (Taboada *et al*., 2010). As summer progresses the uptake of P by prokaryotes causes P limitation, although DOP is also utilised and can support ~25-30% of annual primary production (Mather *et al*., 2008; Lomas *et al*., 2010). In summer a distinct shallow-water microbial community develops in the region of lowest nutrients, with a deep chlorophyll maximum community and an upper mesopelagic community (Treusch *et al*., 2009). Bacteria seem to be more concentrated in the surface waters while Archaea (e.g. *Crenarchaeota*) seem better adapted in the mesopelagic layer

(Schattenhofer *et al*., 2009). Picoplankton (*Prochlorococcus* and *Synechococcus* spp. and picoeukaryotes) dominate carbon fixation in the subsurface chlorophyll maximum, while in surface waters the nanoplankton (e.g. some haptophytes, pelagophytes, small diatoms and dinoflagellates) make significant contributions to productivity (Poulton *et al*., 2006). *Prochlorococcus* is twice as abundant in the deep chlorophyll zone than at the surface, but is almost absent below 200 m (Schattenhofer *et al*., 2009; Riemann *et al*., 2011). *Prochlorococcus* clades have a succession of blooms as each responds differently to the seasonal changes in light, temperature and mixing. *Prochlorococcus* peak in abundance during late summer and autumn whilst *Synechococcus* is scarce then but can occasionally become more abundant than *Prochlorococcus* during winter when the Sargasso Sea is more deeply mixed (Malmstrom *et al*., 2010).

Even though photosynthetic picoeukaryotes are less abundant than picocyanobacteria, they cause the observed variations in chl. *a* and peak in abundance during winter/spring (Riemann *et al*., 2011). They are extremely diverse and dominated by haptophytes and chrysophytes, neither of which was traditionally considered to be important in carbon fixation (Kirkham *et al*., 2011). Rates of carbon fixation are comparable to those in the South Atlantic subtropical gyre and peak during the spring blooms (Poulton *et al*., 2006). The North Atlantic gyre appears to be net heterotrophic in autumn and balanced in spring (Gist *et al*., 2009) although it may be net autotrophic annually (Kähler *et al*., 2010). Despite being ultraoligotrophic, the Sargasso Sea is the spawning site of Atlantic eels. We now know that the picoplankton and nanoplankton make significant contributions to carbon export into deeper zones via settling of aggregates and/or consumption of those aggregates by mesozooplankton (Lomas & Moran, 2011). In turn, mesozooplankton (e.g. heterotrophic athecate dinoflagellates and ciliates) feed copepods which may in turn be available to organisms at higher trophic levels, such as the larvae of Atlantic eels (Andersen *et al*., 2011).

2.2.1.2 South Atlantic

Much of our knowledge of the South Atlantic low nutrient gyre comes from the Atlantic Meridional Transect (AMT) programme which has been undertaken semi-annually since 1995 along a 13,500 km transect between 50^{o}N and 52^{o}S (Robinson *et al*., 2009). Nutrient concentrations are lower than in the North Atlantic gyre, yet the southern system appears to be more autotrophic (Gist *et al*., 2009). Nitrate concentrations are below detection limits, and iron concentrations are also very low but soluble reactive P is almost an order of magnitude higher than in the North Atlantic gyre. As P is a more bioavailable nutrient source than DOP; reduced alkaline phosphatase activity (APA) is detected which results in accumulation of DOP (Mather *et al*., 2008). The microbes seem to be adapted to higher organic loading and utilize organic inputs more efficiently than heterotrophic bacteria of the North Atlantic gyre (Martinez-Garcia *et al*., 2010). Unlike the North Atlantic gyre, N_2 fixation is very low and is possibly limited by iron (Moore *et al*., 2009).

Prochlorococcus is more abundant than in the North Atlantic gyre although its contribution in the mesopelagic zone is minimal (Schattenhofer *et al*., 2009). SAR11 heterotrophs occur at lower abundances than in the North Atlantic gyre, but still make up about 25% of all picoplankton cells (Mary *et al*., 2006). Larger picoprokaryotes are found in the South Atlantic gyre compared to the North Atlantic (Schattenhofer *et al*., 2009). Small photosynthetic picoeukaryotes of a size <3 µm seem to play a crucial role in oceanic primary production. Phylogenetic analyses using both plastid and nuclear rRNA genes reveal a high diversity especially in the members of haptophytes and chrysophytes, with the latter dominating the South Atlantic gyre and associated with higher light intensities (Kirkham *et al*., 2011). Chrysophytes were previously only known from freshwater systems and their significance in marine oligotrophic systems is poorly understood.

2.2.1.3 North Pacific

Stratification of surface waters usually inhibits marine primary productivity as nutrients become depleted in the euphotic zone. However, at the ALOHA monitoring station stratification and productivity are not strongly correlated (Dave & Lozier, 2010). Presumably allochthonous nutrients maintain new production during stratified periods but it is not well understood how these nutrients are supplied. Unicellular diazotrophs frequently dominate N_2 fixation in late winter and early spring, while filamentous diazotrophs (heterocyst-forming cyanobacteria and *Trichodesmium* spp.) fluctuate episodically during the summer (Church *et al*., 2009). The picocyanobacteria seem well adapted to P starvation by exhibiting significant increases in APA (Moore *et al*., 2005). In the past, a shift from eukaryotic to prokaryotic dominance transformed the North Pacific gyre from a N-limited to a P-limited system (Karl *et al*., 2001). There is now molecular evidence for an increase in N-limited strains of *Prochlorococcus* suggesting that the gyre may be returning to a N-limited phase (Van Mooy & Devol, 2008).

Picophytoplankton are dominant contributors (averaging 91%) to euphotic zone chl. *a* concentrations (Li *et al*., 2011). Picocyanobacteria such as *Prochlorococcus* spp. and heterotrophic bacteria, though incapable of N_2 fixation, represent the vast majority of the total cell abundance throughout the euphotic layer (Duhamel *et al*., 2011). *Proclorococcus* spp. are consistently dominant numerically year-round. Here plankton communities can be distinguished as epipelagic, mesopelagic and bathypelagic (Eiler *et al*., 2011) with distinct *Prochlorococcus* clades at different depths (Malmstrom *et al*., 2010).

In summer chl. *a* concentrations peak and the phytoplankton is supported by N_2 fixation and dominated by a few genera of large diatoms and the cyanobacterium *Trichodesmium* (Dore *et al*., 2008). Filamentous organisms, specifically heterocyst-forming cyanobacteria and *Trichodesmium* spp. fluctuate episodically during the summer resulting in highly variable fixation rates, possibly triggered by mesoscale physical processes (e.g. eddies, and wind-generated waves) that input nutrient rich waters in the euphotic zone and can cause blooms in the microbial communities (Fong *et al*., 2008).

There is uncertainty as to whether the system is a C sink or source. Net community production is calculated to be closely balanced or slightly negative (net heterotrophic) due to tightly coupled respiration and gross community production (Viviani *et al*., 2011), but high oxygen concentrations below the mixed layer may be consistent with an ecosystem that is a net producer of fixed C (net autotrophic) throughout the year (Riser & Johnson, 2008).

2.2.1.4 South Pacific

The South Pacific gyre is the largest oceanic gyre and has the clearest waters ever described with a chlorophyll maximum as deep as 180 m (Ras *et al*., 2008). Far from continental sources it receives the lowest atmospheric iron flux in the world (Wagener *et al*., 2008). Both phytoplankton and heterotrophic bacteria are limited by N within the centre gyre, but not by iron which only limits primary production at the border of the gyre (Bonnet *et al*., 2008). In the surface waters (<180 m), nitrate ions are undetected and only trace quantities of regenerated N are found. Despite N limitation, no evidence of N_2 fixation exists and *nifH* gene abundances are extremely low compared to North Pacific gyre (Bonnet *et al*., 2008). This suggests that the autotrophic communities are adapted to living at low iron levels, and that the common photoautotrophic N_2 fixing organisms are not favoured due to their elevated iron quotas. In spite of strong N depletion leading to low chlorophyll biomass, the South Pacific gyre with its characteristic reduced vertical mixing can accumulate organic matter (Raimbault *et al*., 2008) that can sustain active regeneration processes during stratification (Raimbault & Garcia, 2007).

In the clear waters of the gyre centre autotrophic eukaryotes shift to smaller cells (<2 μm) compared to more eutrophic conditions (Masquelier & Vaulot, 2008). Flow cytometry sorting carried out in the most oligotrophic areas of the gyre allowed the recovery of several novel lineages of photosynthetic picoeukaryotes such as a clade of prasinophytes that were only found in the central region of the gyre (Shi *et al*., 2011). Pelagophytes, chrysophytes and haptophytes are the dominant picophytoplankton (Shi *et al*., 2011). Coccolithophores an important group of unicellular calcifying haptophytes, even though at low abundances they grow down to 300 m deep with maximum cell concentrations recorded between the depths of 150–200 m (Beaufort *et al*., 2008). In addition, high taxonomic diversity is also detected in the microzooplankton tintinnids that is inversely related to chlorophyll concentration and positively to the depth of the maximum chlorophyll layer (Dolan *et al*., 2007). Furthermore, larger microplankton (e.g. diatoms) can adapt to the ultraoligotrophic conditions of this region by forming symbiotic relationships with other species (Gómez, 2007).

There is now growing evidence that this oceanic expanse, once thought to be net heterotrophic may be net autotrophic. The deep layers, below the euphotic zones, may be significant contributors to C fixation fuelling heterotrophic processes in the upper layer (Claustre *et al*., 2008). However, this remains a debate; as some studies show that net community production is closely balanced or slightly net heterotrophic (Viviani *et al*., 2011) while data from oxygen sensors deployed on profiling floats suggest that the system is net autotrophic throughout the year (Riser & Johnson, 2008).

2.2.1.5 South Indian

The Indian subtropical gyre is probably the least studied gyre. Research has so far focused in the Arabian Sea (north-western Indian Ocean) and extensive regions of the oceanic gyre remain unknown. In late winter (austral summer) warm and salty subtropical water is separated from deeper water (Tsubouchi *et al*., 2009). This pronounced vertical stratification prevents nutrient transport into the euphotic zone leading to low seasonal nitrate and orthosilicic acid concentrations that limit primary production by microplankton so that nanoplankton and picoplankton dominate productivity. The turnover rate of nanoplankton and picoplankton seems to be closely coupled to microzooplankton grazing and low nutrient concentrations (Thomalla *et al*., 2010).

About 90% of chl. *a* observed at the surface and at the deep chlorophyll maximum (up to 120 m depth) is attributed to the picophytoplankton fraction, while picoeukaryotes account for up to 50% of the chl. *a* measured (Not *et al*., 2008). Prokaryotic *Prochlorococcus* and eukaryotic prochlorophytes, haptophytes and pelagophytes seem to dominate the oligotrophic waters of the Indian Ocean, though a large fraction of the eukaryotic genomes sampled and a significant flagellate (small phototrophic protist) remain unidentified (Not *et al*., 2008; Schlüter *et al*., 2011). Greater variation in the picoeukaryotic assemblages has been observed vertically in the upper 200 m of the water column than horizontally across the entire southern Indian oceanic expanse.

2.2.2 Enclosed systems

The low primary production observed in open-ocean subtropical gyres relates to their isolation from freshwater and airborne nutrient sources. Few coastal regions are ultraoligotrophic, although the eastern Mediterranean and the northern Red Sea become ultraoligotrophic during the warmer parts of the year (Labiosa *et al*., 2003; Siokou-Frangou *et al*., 2010).

2.2.2.1 Eastern Mediterranean

The Mediterranean connects through the Strait of Gibraltar to the Atlantic Ocean in the west, the Bosporus Strait to the smaller enclosed Black Sea in the northeast, and the Suez Canal to the Red Sea and Indian Ocean in the southeast. Evaporation exceeds precipitation and river run off (the main rivers are the Ebro, Rhone, Po, Danube and Nile) with surface waters increasing in salinity from west to east. Atlantic surface water enters through the Strait of Gibraltar and moves eastwards, sinking to 200-500 m depth in the eastern Mediterranean before circulating back west and exiting through the Strait after about 80-100 years and with nearly 10% more salt content (Bas, 2009).

Nutrients mainly enter the system through the Straits of Gibraltar and Bosporus, from wind-driven Saharan dust deposits and from river discharges mainly in the north. The eastern Mediterranean has the lowest nutrient content. Here dams have resulted in drastic reductions in freshwater flow; the Aswan dam on the Nile, for example, restricts the amount of silica entering the Mediterranean (Turley, 1999). In the eastern Mediterranean aeolian inputs can account for 60-100% of the bioavailable N and 30-50% of the bioavailable P (Krom *et al.*, 2010). The unusually high ratio of N to P (~28:1) observed in the eastern Mediterranean (it can sometimes reach 105:1) is due to high N inputs from rivers and atmospheric deposition (Krom *et al.*, 2010; Markaki *et al.*, 2010).

In the western Mediterranean, winter mixing of surface waters with nutrient-rich deeper waters causes a winter-spring phytoplankton bloom composed of mostly diatoms, some flagellates and coccolithophorids (Goffart *et al.*, 2002). The bloom is less-pronounced in the eastern Mediterranean (D'Ortenzio & Ribera d'Alcalà, 2009), chl. *a* concentration is <0.1 mg m^{-3} on average, with the maxima occurring in late winter - early spring and minima in late summer (Siokou-Frangou *et al.*, 2010). In summer a sharp thermocline at 10-20 m results in nutrient depletion in the surface mixed layer. During this stratified period, primary production in the eastern Mediterranean is both N and P limited, and during the winter mixing it becomes P limited (Thingstad *et al.*, 2005; Tanaka *et al.*, 2011). As in other ultraoligotrophic systems, the microbial loop is in a dynamic equilibrium in which grazing pressure, competition and nutrient concentrations can shift the limiting nutrient.

The importance of N_2 fixation in the eastern Mediterranean is under investigation. There are low concentrations of diazotrophic cyanobacteria, possibly due to P and iron limitation. The N_2 fixation rates decrease from west-east but may sustain up to 35% of the primary production in the eastern basin and can be stimulated occasionally by Saharan dust events (Bonnet *et al*., 2011; Ridame *et al*., 2011) The diazotrophic community is dominated by unicellular picocyanobacteria, although N_2 fixation has also been detected within picoeukaryotes (Le Moal *et al*., 2011).

Picoplankton dominate the most nutrient limited areas of the Mediterranean (Tanaka *et al*., 2007). Larger diatoms and dinoflagellates become abundant after intermittent nutrient pulses associated with upwelling, fronts and gyres (Siokou-Frangou *et al*., 2010). Over 85% of chl. *a* in the eastern basin is found in ultraplankton (<10 μm), that comprises cyanobacteria (*Synechococcus* spp. are dominant), chlorophytes, prasinophytes and haptophytes (Denis *et al*., 2009). Coccolithophores are more abundant and diverse in the eastern basin (Ignatiades *et al*., 2009). In summer, dinoflagellates dominate the larger plankton fraction in offshore areas of the eastern Mediterranean whereas diatoms are more prevalent during winter mixing and in inshore waters where anthropogenic eutrophication is evident (Aktan, 2011).

Most studies describing phytoplankton biomass dynamics in the Mediterranean Sea stress that low nutrients cause low primary production (bottom-up control). However, the planktonic food webs are very efficient at minimizing C export to deeper waters, benefiting predators that control the plankton biomass (top-down control) (Siokou-Frangou *et al*., 2010). A P addition experiment in the eastern Mediterranean during the stratified period (Krom *et al*., 2005), had an unexpected outcome. A decrease in chl. *a* concentration was observed while egg-carrying copepods increased, revealing a tightly controlled microbial planktonic web (Thingstad *et al*., 2005). Efficient top-down control can help explain a ‘Mediterranean paradox’, the fact that Mediterranean fisheries are richer than anticipated on the basis of chl. *a* and nutrient concentrations.

Highly productive, benthic photoautotrophs in the sublittoral zone are significant contributors to C fixation, transfer energy up the food webs and can partly explain why

Mediterranean fisheries are richer than anticipated. Benthic primary producers, such as the seagrass *Posidonia oceanica* (Linnaeus) Delile, 1813, which grow at 0 – 50 m in depth (Duarte, 1991) the coralline algal habitats (e.g. maerl) which grow in low light conditions (Ballesteros, 2006) and macroalgal assemblages (e.g. *Cystoseira* forests) in the shallows form diverse and complex habitats. The Mediterranean basin ranks contains about 7% of the world's marine biodiversity (Bianchi & Morri, 2000). Even though it covers <0.8% of the world ocean surface and is <0.3% of its volume, it is home to 4-18% of the world's recorded species, depending on the phylum considered. Approximately 17 000 marine species occur in the Mediterranean Sea and this inventory is expanding rapidly, especially for microbes and deep-sea species (Coll *et al.*, 2010). An unusually high level of endemism is observed and the region hosts a number of species of conservation interest, such as 71 species of sharks, rays and chimaeras (Cavanagh & Gibson, 2007), sea turtles *Dermochelys coriacea* (Vandelli, 1761), *Chelonia mydas* (Linnaeus, 1758), *Caretta caretta* (Linnaeus, 1758), 9 permanent resident species of cetaceans (Reeves & Notarbartolo, 2006) and the critically endangered Mediterranean monk seal *Monachus monachus* (Hermann, 1779). The southeastern Mediterranean Sea has, on paper, the lowest species richness but this is influenced by the fact that there have been relatively sparse research efforts in this part of the Mediterranean.

2.2.2.2 North Red Sea

The Red Sea is thought to owe its name to intense phytoplankton blooms, but they are very rare in this oligotrophic system. It connects to the Mediterranean Sea through the narrow and shallow (~8 m) Suez Canal in the north and exchanges water with the Indian Ocean through the Bab el Mandeb strait (130 m deep) and the Gulf of Aden in the south. There are no permanent rivers and scant rainfall so seawater entering through the Bab el Mandeb strait gets saltier as it progresses northwards. Like the Mediterranean Sea, the north Red Sea is heavily influenced by seasonal changes in physical and chemical characteristics of the water column. Oligotrophic to ultraoligotrophic conditions prevail in the northern region during the summer and autumn stratified period, while in the winter, conditions become eutrophic (Lindell & Post, 1995; Labiosa *et al*., 2003). The Gulf of Aqaba, at the northeast tip of the Red Sea is about 165 km long, very deep (~1800 m) but very narrow (maximum width <25 km), bounded by desert and separated from the Red Sea by the shallow (240 m) Strait of Tiran. Here, phytoplankton populations have a large spring bloom (with chl. *a* peak at around 3 mg m^{-3}) and smaller autumn bloom but in the summer levels average ~0.2 mg m^{-3} (Labiosa *et al*., 2003).

Although N:P ratios are lower in the summer many phytoplankton species appear to be P limited and even though P is below detection limits, APA is consistently low in the picophytoplankton fraction indicating the absence of P limitation, while larger phytoplankton express increased APA especially during the stratified period indicating P limitation (Mackey *et al*., 2007). N_2 fixation rates are consistently low and are higher during the deep mixing season. Diazotrophic populations are dominated by the smaller N_2 fixing organisms (Foster *et al*., 2009). Small unicellular cyanobacteria (e.g. *Cyanothece* spp.), are the most abundant N_2 fixing organisms, while larger filamentous *Trichodesmium* spp., occur in surface waters especially in the winter when soluble reactive P is more abundant (Mackey *et al*., 2007). Inputs of aerosol N to surface waters represents an important source of ‘new’ N in this region (Aberle *et al*., 2010).

The planktonic communities are characterized by low abundances and the dominance (95%) of ultraplankton (0.2-8 μm) (Berninger & Wickham, 2005; Al-Najjar *et al*., 2007). During the summer and autumn, stratified surface waters become nutrient depleted and picophytoplankton dominate. In winter, nutrient concentrations increase and larger phytoplankton become more abundant. This pronounced seasonal succession of major taxonomic groups is observed with *Prochlorococcus* dominating during the stratified summer period but being almost absent during the winter and chlorophytes with cryptophytes dominating during the winter mixing but being almost absent during the summer (Al-Najjar *et al*., 2007). Larger cells (>8 µm) are dominated by dinoflagellates and ciliates (Berninger & Wickham, 2005). The ciliates prey on the dominant picoautotrophs so that this primary production then becomes available to metazoan grazers (Claessens *et al*., 2008). Stable isotope analyses have revealed a complex and diverse planktonic community that include herbivores and a large variety of omnivores (e.g. non-calanoid copepods) (Aberle *et al*., 2010). It appears top-down and bottom-up controls operate simultaneously in the North Red Sea with small cells being controlled by grazing while larger cells (e.g. diatoms) are limited by nutrient availability (Berninger & Wickham, 2005).

Despite periods of ultraoligotrophic conditions in the water column, the seafloor of the Red Sea is a biodiversity and endemism hotspot (Roberts *et al*., 2002). Coral reefs thrive in the warm and nutrient-poor environment. The proliferation of corals in oligotrophic conditions is attributed to their mixed mode of nutrition achieved by an ancient mutualistic symbiosis between the coral polyp and zooxanthellae microalgae. The Red Sea is home to over three hundred zooxanthellate scleractinian coral species (Veron *et al*., 2009), that support a myriad of species, including over a thousand fish species (Golani & Bogorodsky, 2010). The nutrient-poor waters of the Gulf of Aqaba harbour among the highest levels of endemism for marine organisms, especially molluscs and echinoderms and there are exceptionally diverse fringing reefs, steeply sloping to depths of up to 150 m (Fricke & Schuhmacher, 1983).

2.3 Threats

The human population now exceeds 7 billion compared to around 800 million in the year 1750 and an estimated 9.4 billion by 2050 (Raleigh, 1999). This rapid population increase has been matched with environmental degradation and global biodiversity loss.

Marine litter is now ubiquitous, and resources are being exhausted at alarming rates. The major stressors of anthropogenic climate change on the world's marine ecosystems are warming, acidification and deoxygenation (Gruber, 2011) with impacts that range from decreased ocean productivity, altered food web dynamics, reduced abundance of habitat-forming species, shifting species distributions, and a greater incidence of disease (Hoegh-Guldberg & Bruno, 2010). This section considers how ultraoligotrophic marine ecosystems are being altered by Man, and to what extent these systems may be vulnerable to the multiple stressors that are present.

2.3.1 Open ocean systems

Subtropical open ocean ecosystems are far removed from human civilization yet despite this remoteness rapid changes are underway, such as ocean acidification and the accumulation of marine litter.

2.3.1.1 Climate change

Remotely-sensed ocean colour data show that ultraoligotrophic marine regions have expanded by about 15% in the past decade (Polovina *et al.*, 2008) and that the growth of these provinces may be accelerating as they get larger (Irwin & Oliver, 2009). Significant decreases in chl. *a* concentration have also been recorded in most subtropical gyres (Signorini & McClain, 2011). Polovina et al. (2011) predict that ocean warming will expand the area of the subtropical biome by ~30% by 2100 due to increased water stratification and restricted supplies of nutrients to the upper water column. In such areas, large and efficient C fixing eukaryotic species are outcompeted by smaller eukaryotic and prokaryotic plankton and productivity falls.

During the past 100 years, rising atmospheric greenhouse gas concentrations have increased global surface ocean temperatures by ~0.7 °C (Trenberth *et al.*, 2007). The deep ocean remains relatively cool, so a density gradient is developed which increases upper ocean stratification which can lower the oxygen and nutrient contents of the water. Ocean warming and increased stratification of the upper ocean may lead to 1-7% declines in dissolved oxygen in the ocean interior with implications for ocean productivity and nutrient cycling (Keeling *et al*., 2010). Large expansions of the oxygen minimum zones have occurred horizontally and vertically in all tropical and subtropical oceans and it is estimated that since 1960 deoxygenated areas have increased by 4.5 million km^2 (Stramma *et al*., 2010). The implications of ocean warming and deoxygenation on the functioning of ultraoligotrophic systems are poorly known yet alterations in food webs can be expected since warming will favour some microbes and plankton over others (Marinov *et al*., 2010; Sarmento *et al*., 2010).

Ocean acidification results from the uptake of anthropogenic carbon dioxide (CO_2) of which around one third is absorbed by the oceans (Sabine *et al*., 2004) where it reacts with water to form carbonic acid (H_2CO_3) which further dissociates into hydrogen ions (H^+) and carbonate ions (CO_3^{2-}). Increased H^+ ions lower the pH of the water. Surface waters of the oceans have been acidified by an average of 0.1 pH units compared with pre-industrial levels (Doney, 2010). Model simulations predict that ocean pH will decrease by 0.2 to 0.3 pH units by the end of the twenty first century (Orr *et al.*, 2005). The ecological effects of ocean acidification remain uncertain yet there are widespread concerns over the effects on calcified organisms since uptake of atmospheric CO_2 leads to a decrease in carbonate concentrations and increases calcium carbonate ($CaCO_3$) dissolution (Riebesell *et al*., 2009; Rodolfo-Metalpa *et al*., 2011). The calcifying plankton that occur in ultraoligotrophic systems (e.g. coccolithophores, foraminiferans, and pteropods) may have a reduced ability to construct their $CaCO_3$ shells. Beaufort et al. (2011) for example found a significant decrease in coccolith mass at sites all over the world as pCO_2 concentrations increase, although there were exceptions with heavily calcified coccolith morphotypes found in some low pH areas. Biogeochemical disruptions are also possible due to ocean acidification, although the ecological effects of these remain unknown. For example, experimental decreases in pH lower microbial nitrification (oxidation of ammonia into nitrite) rates (Beman *et al*., 2011). When stimulated by pCO_2, N_2 fixation rates appear to increase in filamentous non-heterocystous *Trichodesmium* spp. (Barcelos e Ramos *et al*., 2007) and the unicellular *Cyanobium waterburyi* Komárek, 1999 (Fu *et al*., 2008), but decrease in heterocystous diazotrophs (Czerny *et al*., 2009). Changes in nitrification and N_2 fixation rates have the potential to cause fundamental alterations to the marine environment. Elevated pCO_2 in cultured organisms and in a few mesocosms reveal contradicting results with some prokaryotic species and communities exhibiting increased production when others are adversely impacted (Liu *et al*., 2010). It is clear that our understanding of the potential impacts of acidification on the overall biogeochemistry of marine waters is limited by the lack of *in situ* experiments (except in some coastal areas with CO_2 vents) and the inconsistency or lack of data for several taxa. In addition, it is unreasonable to try to predict changes to organisms and ecosystem functioning because of decreasing pH/increasing CO_2 alone when they are also being subjected to changes in other stressors (i.e. deoxygenation and warming) (Denman *et al*., 2011).

2.3.1.2 Marine Litter

During the last 60 years, the global production of plastic has increased from 1.5 million tonnes to 265 million tonnes (PlasticsEurope, 2011). The light plastic particles (e.g. polyethylene and polypropylene) that enter water bodies then float and drift with the currents and can be transported over large distances. The massive, swirling vortexes of water in the mid-latitude subtropical gyres trap floating debris in the central slower moving water masses. Accumulating plastic was discovered in 1972 in the Sargasso Sea, with increasing amounts recorded with time, such as in the North Pacific gyre where up to 334,271 pieces per km^2 and a startling 6:1 biomass ratio of zooplankton to plastic were recorded (Moore *et al*., 2001). Similar observations have been made in the North Atlantic gyre (Law *et al*., 2010). Models and observations show that all five subtropical gyres are litter aggregation hotspots (Maximenko *et al*., 2011).

This is a problem since plastic can degrade to microscopic pieces (Thompson *et al*., 2004) that adsorb persistent organic pollutants such as PolyChlorinated Biphenyl (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), DichloroDiphenylTrichloroethane (DDTs), PolyBrominated Diphenyl Ethers (PBDEs), alkylphenols, and bisphenol A (Rios *et al*., 2010). Planktonic plastic loaded in organic pollutants can easily be mistaken for prey and upon ingestion the pollutants bioaccumulate (Harwani *et al*., 2011), while the plastic remains undigested and can sometimes clog the digestive tract of the organism likely leading to starvation and subsequent death. Top predators have been consistently reported victims of this plastic menace. 34% of 408 dissected leatherback turtles (Mrosovsky *et al*., 2009), 28% of 106 dolphins accidentally captured in artisanal fisheries (Denuncio *et al*., 2011) and 9.2% of 141 mesopelagic fishes from 27 species in the North Pacific subtropical gyre (Davison & Asch, 2011) had plastic in their stomachs. Every albatross chick egested bolus examined from the North Pacific colonies contained plastic (Young *et al*., 2009). 134 different types of nets causing stomach rupturing and emaciation were found inside two stranded male sperm whales in Argentina (Jacobsen *et al*., 2010), and the list goes on. It is now recognized that the environmental impacts of plastic debris are wide-ranging and include among others entanglement of marine fauna, ingestion by consumers from all trophic levels including the small heterotrophic plankton, dispersal of invasive species to non-native waters, and bioaccumulation of organic contaminants (Gregory, 2009).

How the biocommunities inhabiting the deoxygenated, acidified, warm waters of the ultraoligotrophic subtropical gyres will respond to changes brought about by the ‘Marine Debris Era’ remains to be seen.

2.3.2 Enclosed systems

Due to the proximity of humans, enclosed ultraoligotrophic systems are exposed to multiple anthropogenic stressors. The benefits supplied by marine biodiversity to human health are enormous and include: i) seafood (high-quality protein, minerals and vitamin D and omega-3 fatty acids) with antioxidant properties and cardio and cancer protective effects, ii) marine organisms such as sharks, algae and sponges supply a large variety of bioactive metabolites some of which are used to treat human diseases and, iii) maritime leisure activities provide physical and psychological effects to users such as recreational fisheries, diving, snorkelling, and whale watching (Lloret, 2010). To sustain such benefits improvements are required in the ways that we manage ultraoligotrophic seas.

The North Red Sea is a biodiversity hotspot with high levels of endemism and stunning fringing reefs that can extend to depths of 150 m. Protecting the threatened coral reefs of the enclosed North Red Sea is a real challenge as there are multiple stressors already in effect. Ocean warming slows coral growth and increases bleaching events (Cantin *et al*., 2010). Future acidification is a significant threat that is expected to increase bioerosion and decrease the net calcification rates (aragonite formation) of stony corals (Silverman *et al*., 2009; Rodolfo-Metalpa *et al*., 2011). Furthermore, the coral reefs of the North Red Sea attract thousands of visitors that can contribute to impacts on coral reefs (Hasler & Ott, 2008). Submerged marine litter in coral reefs of the North Red Sea with an overall mean density of 2.8 items/m^2 and overall mean weight of 0.31 kg/m^2 is another major concern (Abu-Hilal & Al-Najjar, 2009). Bioaccumulation of toxic contaminants among coral tissue of the North Red Sea is high (Ali *et al*., 2010). Moreover, coastal development has resulted in increasing demand for freshwater. Seawater desalination plants are being constructed that discharge high salinity water often contaminated with other chemicals (Hoepner & Lattemann, 2003).

The Mediterranean coasts support a high density of inhabitants, distributed in 21 countries with a population of about 450 million (cf. 246 million in 1960), of which 132 million live on the coast (26,000 km in length). In addition, 200 million tourists per year visit Mediterranean coastal countries. During the past one hundred years, the eastern Mediterranean has been subjected to the effects of two important events, the opening of the Suez Canal in 1869 (discussed below) and the construction of the Aswan High Dam in 1964. Before the construction of the Aswan Dam, nutrient enrichment extended along the Egyptian coast and was detected off the Israeli coast and sometimes off southern Turkey. It provided for dense blooms of phytoplankton off the Nile Delta (Nile bloom) which in turn provided nourishment to sardines, other pelagic fishes and crustaceans. Huge declines have been observed in these fisheries in the years following the Aswan Dam construction. Since the late 1980s the recovery of total fish landings in the region reveal that the pelagic ecosystem is adjusting but the mismatch between extremely low primary productivity and relatively high levels of fish production remains a puzzle - ‘the Levantine Basin Paradox’ (Dasgupta & Chattopadhyay, 2004). Whether this recent increase in fisheries is due to increased fishing efforts, recovery of fish stocks or nutrient enrichments by anthropogenic activities is not yet clear.

Human activities have been reducing biodiversity of the Mediterranean Sea at all levels. The major stressors in the eastern Mediterranean appear to be: climate change, alien species invasions, pollution, fishing impacts, eutrophication and aquaculture, and habitat loss (Claudet & Fraschetti, 2010; Coll *et al*., 2010; Durrieu de Madron *et al*., 2011). Often these stressors act synergistically and have cumulative negative impacts on a great number of taxonomic groups. The Mediterranean Sea is perhaps the most investigated marine environment in the world, however research efforts have been concentrated in the northwestern Mediterranean so much less is known about human-environmental interaction in the ultraoligotrophic waters of southeastern Mediterranean.

2.3.2.1 Climate change

The effects of global climate change are likely to affect chemical and physical properties of the water and act synergistically with other anthropogenic stressors (Gambaiani *et al*., 2009). Climate change impacts in the Mediterranean may provide useful insights for potential impacts elsewhere as the region is well monitored. As in many other regions; sea temperatures are rising, acidification is underway, extreme climatic events and related disease outbreaks are becoming more frequent, native species are being displaced and invasive species are spreading (Lejeusne *et al*., 2010).

Increased warming across the Mediterranean increases stratification of the water column further restricting nutrient availability in ultraoligotrophic zones and is related to increased mortality of the endemic seagrass *P. oceanica* (Diaz-Almela *et al*., 2009). Higher temperatures may disrupt juvenile life histories stages of numerous organisms (Hawkes *et al*., 2007; Byrne, 2011) and cause mass mortalities of adults (Garrabou *et al*., 2009). In addition, increasing temperatures may also contribute to higher frequencies of disease outbreaks as tropical microbial pathogens are expected to spread (Danovaro *et al*., 2009). Rising water temperatures are altering biogeographic boundaries and leading to a progressive homogenization of Mediterranean marine biota. Changes include: an increase in abundance of eurythermal species and a decrease in cold stenothermal species as well as northward species shifts and mass mortalities during unusually hot summers (Coll *et al*., 2010). Warm-water fish like *Thalassoma pavo* (Linnaeus, 1758), *Sphyraena* spp., *Epinephelus* spp., *Sparisoma cretense* (Linnaeus, 1758) and *Sardinella aurita* Valenciennes, 1847, have spread northwestwards (Sara *et al*., 2005). Certain cold-water species have been replaced, for example the distribution of the cave-dwelling crustacean *Hemimysis speluncola* Ledoyer, 1963, has contracted and been replaced by *H. margalefi* Alcaraz, Riera & Gili, 1986, a warm water species that was previously unknown in the region (Chevaldonné & Lejeusne, 2003). Non-indigenous warm water species of algae, invertebrates and fish are enlarging their geographical ranges (Bianchi, 2007). Invasive tropical fauna and flora are most evident in the southern Mediterranean where they now form a significant portion of the biota and some outcompete native species (Lasram & Mouillot, 2009). Predicted levels of warming for the end of this century lie beyond the thermotolerance levels of the developmental stages of many metazoa (Byrne, 2011).

Ocean acidification may also alter the ecology of the Mediterranean, although the evidence to date is sparse. Israel and Hophy (2002), found that acidifying seawater to pH 7.8 with CO_2 did not adversely affect growth and photosynthesis in a wide range of Mediterranean chlorophyte, rhodophyte and phaeophyte algae whereas Invers et al. (1997) found that this level of acidification enhanced photosynthesis in the Mediterranean seagrasses *P. oceanica* and *Cymodocea nodosa* (Ucria) Ascherson, 1870. However, the ability of seagrasses to benefit from the increased CO_2 in the water column is questionable, since it is associated with loss of phenolic substances in seagrass leaves that likely result in increased predation pressure (Arnold *et al*., 2012). Martin and Gattuso (2009) found that the Mediterranean encrusting coralline alga *Lithophyllum cabiochae* decreased calcification when elevated pCO_2 conditions were combined with high temperatures (pH 7.8; seasonal temperature +3 °C). Investigations into the effects of acidification at a natural volcanic CO_2 vent off Ischia in Italy show that seagrasses and certain seaweeds were able to benefit from the elevated CO_2 levels (Martin *et al*., 2008; Porzio *et al*., 2011) but that around 30% of the coastal biodiversity was lost at mean pH levels predicted for 2100 (Hall-Spencer *et al*., 2008). This is partly because ocean acidification disrupts recruitment of organisms from the plankton (Cigliano *et al*., 2010), and partly because peak summer temperatures increase the susceptibility of some organisms to shell and skeleton dissolution (Rodolfo-Metalpa *et al*., 2011). Calcareous systems such as vermetid reefs and mussel beds, as well as deep and shallow coral communities, appear to be especially vulnerable in ultraoligotrophic regions where organisms lack food and are therefore less able to allocate resources for coping with multiple stressors. In contrast, carbon limited organisms, like seagrasses, may make use of the extra pCO_2 and if their habitats are protected they may thrive due to higher photosynthetic rates.

2.3.2.2 Alien species

Warm-water species are found in the Mediterranean due to Atlantic influx, Lessepsian migration, introductions by humans and present-day sea warming (Bianchi, 2007). Most of the 955 alien species so far recorded occur in the oligotrophic eastern Mediterranean (Zenetos *et al*., 2010). About 20% of Mediterranean alien species were accidentally introduced from biofouling on ship hulls or in ballast tanks (Galil, 2009). However most (about 67%) Mediterranean alien species came from the Red Sea since the Suez Canal was opened in 1869. More than 600 tropical Indo-Pacific species have been reported entering the Mediterranean where they have established reproducing populations in the Levantine basin and beyond (Coll *et al*., 2010; Costello *et al*., 2010). The rate of invasion of species from the Red Sea into the low nutrient waters of the eastern Mediterranean is increasing due to warming. Now nearly half of the trawl catches along the Levantine coast consist of Erythrean fish, but whilst some are now targeted commercially, others are detrimental to fisheries. In Cyprus, for example, the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) is out-competing native fishes and predating invertebrates such as the *Octopus vulgaris* Lamarck, 1798, and squid; in this region several other invasive species have caused substantial shifts in coastal ecosystems (Katsanevakis *et al*., 2009).

2.3.2.3 Pollution

Like all coastal systems the Mediterranean Sea is affected by numerous anthropogenic contaminants, but due to its enclosed and oligotrophic nature their impacts can be exacerbated. Marine litter is a major problem in the region, causing obstruction of digestive tracts and contaminant bioaccumulation in many marine animals. Persistent organic pollutants tend to bioaccumulate and come from maritime sources, aerosol deposits, urban/industrial activity, river discharges and tend to accumulate in harbour sediments (Gómez-Gutiérrez *et al*., 2007; Thébault *et al*., 2008).

Riverine inputs and air masses from northern and central Europe carry persistent organic pollutants that can reach the eastern Mediterranean basin (Mandalakis & Stephanou, 2002). Large commercial harbours are situated mostly in the northwest Mediterranean and maritime traffic causes noise pollution that adversely affects cetaceans (Dolman *et al*., 2011). Submarine drilling for oil and gas takes place in the south with exploration now underway in the eastern Mediterranean. About 300 000 tonnes of crude oil are released into the Mediterranean every year (Danovaro & Pusceddu, 2007) and can cause environmental damage, especially when chemical dispersants are used in clean-up procedures. An oil spill in Valencia in 1990 was followed by hundreds of dead dolphins being washed up along the Spanish, French, Italian and North African shores and a year later on the beaches of southern Italy and Greece, thought to be due to disease triggered by immunosuppressants in the oil spill (Zenetos *et al*., 2002).

2.3.2.4 Overexploitation of resources

Industrialized fishing has severe impacts on species, habitats and ecosystems (Tudela, 2004). Several fish resources are highly exploited or overexploited (Palomera *et al*., 2007; MacKenzie *et al*., 2009). Several other organisms are also affected by exploitation and include unwanted by-catch (accidental capture in fishing gear). Bottom-trawling is a non-selective fishing method and causes a large mortality of discarded benthic invertebrates which can induce severe biodiversity and biogeochemical changes (Pusceddu *et al*., 2005). Severe population declines have occurred for all top predators during the last 50 years with the Mediterranean Sea described as the most dangerous sea in the world for cartilaginous fishes (Cavanagh & Gibson, 2007). See turtles face entangling, pollution and loss of habitat. Population declines have also been recorded among marine mammals (such as sperm whales, short-beaked common dolphins, common bottlenose dolphins, striped dolphins and monk seals) that face prey depletion, direct killing and fishery by-catch (Reeves & Notarbartolo, 2006). The Mediterranean monk seal is the most endangered seal in the world with less than 600 individuals currently surviving. Remnant populations are fragmented and declining. The species faces a number of threats (i.e. accidental entanglement, exploitation, persecution and tourism) that caused severe declines in abundance (Karamanlidis *et al*., 2008).

There are clearly multiple threats acting synergistically on species of the Mediterranean Sea. For example, in December 2009, a pod of seven male sperm whales stranded along the coasts of Southern Italy. It appears the cause of death was prolonged starvation not from plastic obstruction (even though plastic was found in all dissected individuals) but due to a lack of prey. High concentrations of pollutants in the tissues of the stranded animals led researchers to conclude that prolonged starvation stimulated the mobilization of highly concentrated lipophilic contaminants from their adipose tissue which entered the blood circulation and may have impaired immune and nervous functions (Mazzariol *et al*., 2011).

2.3.2.5 Eutrophication and Aquaculture

Eutrophication in the ultraoligotrophic eastern Mediterranean is disrupting habitats and causing community shifts. Eutrophic conditions favour opportunistic species that may increase productivity and fishery catches but may out compete the highly diverse communities of ultraoligotrophic systems. Eutrophication sources from agriculture, urbanization, river run-offs, and aquaculture. Considering the exponential human population growth and the fact that fisheries are in global decline, aquaculture efforts are predicted to increase to meet growing demand (Duarte *et al*., 2009).

Fin-fish farming can have a number of environmental effects on the surrounding and downstream ecosystems (Holmer *et al*., 2008). Dissolved wastes increase the nutrient loading of the area and particulate wastes increase sediment deposition. In the benthos sedimentation and organic loading can cause biochemical changes affecting the composition and function of benthic communities (Karakassis *et al*., 2000), stimulating the growth of undesirable species that produce toxic metabolic waste that can kill species of conservation significance. Large-scale *P. oceanica* losses adjacent to fish farm cages have been reported across the Mediterranean (Pergent-Martini *et al*., 2006) including the eastern Mediterranean (Holmer *et al*., 2008; Apostolaki *et al*., 2009).

Improved fish farm management may increase their sustainability although culturing carnivorous fish is still likely to come at environmental costs. Integrated multi-trophic aquaculture (culturing organisms from different trophic levels, mimicking natural ecosystem interactions and producing less waste than monoculture systems) may be key to environmental sustainability of aquaculture practices in ultraoligotrophic waters (Chopin, 2006; Angel & Freeman, 2009).

2.3.2.6 Habitat loss

Coastal habitats such as seagrass meadows, mollusc (oyster, vermetid and mussel) reefs, coralligenous maerl formations, and macroalgal assemblages on shallow reefs are examples of complex and highly productive ecosystems. They supply food resources, nurseries and shelter for a large array of species that are protected by international conventions, directives and action plans. A meta-analysis of 158 experiments in the Mediterranean revealed that human activity caused adverse impacts on all habitat types. Fisheries, species invasion, aquaculture, sedimentation increase, water degradation, and urbanization can all have negative impacts on Mediterranean habitats and associated species assemblages (Claudet & Fraschetti, 2010).

Habitat destruction is considered one of the most pervasive threats to the diversity, structure and functioning of marine coastal ecosystems. The loss of habitat structure generally leads to lower abundances and species richness that usually allows opportunistic species to prosper (Airoldi *et al*., 2008). Habitat destruction can also impair the integrity, connectivity and functioning of large-scale processes decreasing population stability and isolating communities (Thrush *et al*., 2006). Continued losses of habitats to coastal development has triggered several international protective measures such as the development of Marine Protected Areas (MPAs), but their efficacy is much questioned (García-Charton *et al*., 2008; Montefalcone *et al*., 2009) as habitat loss continues apace.

Oligotrophic coastal habitats are dominated by slow growing species and intricate food webs. Habitat losses can be considered irreversible, as it would take centuries following the cessation of disturbances for ecosystems to return to their climax state.

2.4 Conclusions

Ultraoligotrophic marine ecosystems cover almost a third of the earth's surface and contribute significantly to global productivity and biogeochemistry. They are, however, amongst the least understood systems on this planet. Once considered to be monotonous oceanic desserts, they are now known to have highly dynamic physical and biological properties with extremely diverse and vertically-distinct planktonic communities.

There is increasing evidence that these systems may be net autotrophic. The water column is dominated by the smallest eukaryotic and prokaryotic picoplankton, which seem well adapted for surviving in oligotrophic conditions. Adaptations range from niche segregation through prolonged coexistence, symbiotic associations, mixed modes of nutrition, lower cellular nutrient requirements, genes encoding for enzymes that regenerate nutrients from allochthonous sources, genes involved in high affinity uptake of nutrients and efficient nutrient uptake due to large surface: volume ratios. Unicellular cyanobacteria and extremely diverse picoeukaryotes dominate primary production in the deep euphotic zones of ultraoligotrophic waters. This production is channelled through the microbial food web (e.g. small ciliates and nanoflagellates) to vertically-migrating gelatinous and crustacean zooplankton and then to higher trophic levels. Phytoplankton blooms mainly occur after winter mixing events, but sporadic blooms can occur during the stratified periods. Such blooms can favour larger planktonic species that in turn may sustain large predators (e.g. leatherback turtles, elasmobranchs, cetaceans, tunas and billfishes).

Environmental metagenomics has revealed the high biodiversity observed in ultraoligotrophic marine systems, although the causes for this high biodiversity remain puzzling (Roy & Chattopadhyay, 2007). In the eastern Mediterranean and north Red Sea biogenic engineers such as corals, seagrasses, and macroalgae form habitats that are biodiversity hotspots of international commercial significance. Exponential growth in the human population has resulted in multiple stressors that act synergistically in the marine environment reducing biodiversity. It is possible that in ultraoligotrophic environments, where resources are scarce, organisms are particularly vulnerable to multiple stressors. Climate change is underway and its impacts may continue for many millennia after cessation of anthropogenic CO_2 emissions (Tyrrell, 2011). Warming increases stratification that keeps nutrients below the thermocline. Deoxygenated regions are expanding and acidification may impair ecological functioning (Byrne, 2011). Predictions for 2100 include substantial changes in biogeochemical processes and the extinction of many tropical coral reefs (Silverman *et al*., 2009). In addition to climate change, marine litter continues to accumulate in ultraoligotrophic subtropical gyres where it is physically degraded to microscopic pieces adsorbing persistent organic contaminants from the surrounding water. Plastic has been found in many consumer species ranging from copepods to large mammals. It may cause starvation, contaminant bioaccumulation, alien species transportation and entanglement. Enclosed ultraoligotrophic systems face additional threats due to their close proximity to Man. Toxic pollutants bioaccumulate and impair the normal physiological functions of organisms causing for example, cetacean strandings. Invasive alien species are spreading and are competing, predating and infecting indigenous species and altering ancient food webs. Marine fish stocks are overexploited with most top predators in decline. Eutrophication decreases water quality which can add pressure on coastal systems subjected to habitat loss and degradation. It is clear that past methods have failed to ensure environmental sustainability yet there are several reasons to be optimistic.

It is now realized that marine ecosystem degradation is a global concern. International efforts to reduce rates of biodiversity loss have led to numerous agreements, conventions or other legal instruments that are coming into force. Such international agreements form the basis of long-term collaboration that is necessary for improved environmental management. For example, the Kyoto Protocol came into force on 2005 and commits the 191 signatories to tackle the issue of global warming by reducing greenhouse gas emissions. Annex 1 countries pledged to reduce their emissions by 5.2% from 1990 levels by the end of 2012. The United Nations Convention on the Law of the Sea (UNCLOS) signed by 161 countries helps control pollution and set guidelines for the protection of the environment and the management of marine natural resources in the world’s oceans. Inter-governmental organizations, like the International Commission for the Conservation of Atlantic Tunas (ICCAT), are charged with the conservation of stocks of highly migratory species. In Europe, the MSFD aims to achieve healthy waters by 2020 with an unprecedented level of cooperation between countries in developing a network of MPAs. Monitoring of environmental quality, biodiversity and long-term changes in community structure through an international coordinated network of MPAs is an approaching reality. Cautious use of Integrated Coastal Zone Management and

Environmental Impact Assessments can help slow the rate of coastal environmental degradation. International partnerships like the Global Ocean Biodiversity Initiative (GOBI) are promising and the identification of Ecologically or Biologically Significant Areas (EBSAs) in the open oceans and deep seas is well underway. It is clear that these international efforts are required to slow the rates of marine environmental degradation.

There are now ample examples where interventions have had positive environmental outcomes. A primary goal among nations should be raise awareness of effective marine environmental protection. For example, the most viable option to reduce litter is to reduce its production in the first place and then to improve reuse and recycling through enhanced environmental awareness (Thiel *et al*., 2011). There is now scientific clarity that ocean warming, acidification and deoxygenation are underway due to CO_2 emissions, so the primary mitigation strategy is to reduce these emissions (Gruber, 2011). There are reasons to be optimistic about improved management of ultraoligotrophic systems as a growing awareness of their value is being accompanied by shifts towards more sustainable ways of obtaining resources (e.g. marine renewables) and dealing with wastes (e.g. carbon capture and storage).

Change is underway…

3. INVASIVE ALIEN SPECIES

3.1 MONITORING THE ALIEN SEAGRASS *HALOPHILA STIPULACEA* IN THE NATIVE AND INVASIVE RANGES

3.1.1 Introduction

Halophila stipulacea (Forsskål) Ascherson, 1867, is a tropical species, native to the Red Sea, Persian Gulf, and Indian Ocean. Following the opening of the Suez Canal, this species soon became a Lessepsian immigrant and established in many parts of the Mediterranean Sea (Gambi *et al*., 2009; Sghaier *et al*., 2011). In 2002, the seagrass was reported in the Caribbean Sea (Ruiz & Ballantine, 2004), and in just over ten years, it spread to most eastern Caribbean island nations and recently to the south American continent (Willette *et al*., 2014; Vera *et al*., 2014). Studies from the Caribbean have shown that *H. stipulacea* is displacing local seagrass species (Willette *et al*., 2014). It has been suggested that the invasiveness of *H. stipulacea* could be attributed to it being able to tolerate a wide range of conditions (reviewed by Gambi *et al*., 2009).

There is growing concern that climate change and ocean acidification are threatening marine ecosystems in both the northern Gulf of Aqaba and the Mediterranean (Jorda *et al*., 2012; Fine *et al*., 2013; Hall-Spencer & Allen, 2015). *Halophila stipulacea* has so far been limited in the eastern basin of the Mediterranean but the on-going warming and acidification of the Mediterranean Sea, together with the declines of native *Posidonia oceanica* (Linnaeus) Delile, 1813 (Marbá & Duarte, 2010; Jorda *et al*., 2012), will likely favour the expansion of *H. stipulacea* (Hall-Spencer & Allen, 2015). Currently, *H. stipulacea* is expanding westwards and it is estimated that it will colonise the whole Mediterranean over the next 100 years (Gambi *et al*., 2009; Sghaie *et al*., 2011; Georgiou *et al*., 2016).

In this study, identical monitoring stations were set up in the Mediterranean (Limassol, Cyprus) and the Gulf of Aqaba (Israel). Data were collected seasonally for one year and the main objective was to identify whether there are any structural differences between the two seagrass populations. The monitoring stations allow morphometric comparisons that will increase our understanding of the dynamics of this seagrass in both its native and non-native habitats, which is important for both managing the species and anticipating the impacts of range shifts upon climate change.

3.1.2 Materials and Methods

3.1.2.1 Setting up permanent transects

In March-April 2017, two permanent 50 m long transects were set up at norther tip of the Gulf of Aqaba (Israel) and at Limassol (Cyprus) (Fig. 3.1-1). Both sites are within protected swimming areas near the shore (less than 100 m), at the same depth (3 m) and have similar seabed topography. Seawater temperature varied less at the Red Sea site than the Mediterranean site, which can reach 28 °C during late summer and fall to <16 °C in the winter (Fig. 3.1-1).

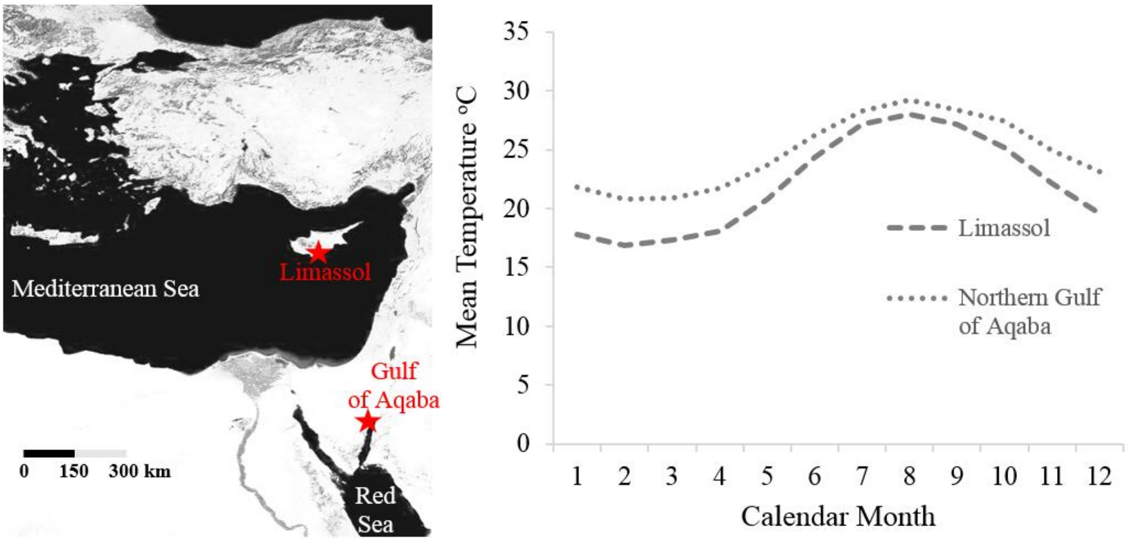

Figure 3.1-1. Study sites in the Mediterranean Sea (Limassol, Cyprus) and Gulf of Aqaba (Eilat, Israel) (*left*). Although similar temperatures are reported in the warm period at both sites, lower sea surface temperatures are reported in the eastern Mediterranean compared to the north Red Sea in the cold period (*right*). Long-term monthly sea surface

temperatures for Gulf of Aqaba and Limassol; downloaded from https://www.seatemperature.org (accessed 15/07/2018).

Each transect was marked at 5 m intervals with a pole hammered into the sediment. At each marker, a photo-quadrat (50 cm x 50 cm) was taken (n=11 per transect), to later estimate the % cover of the seagrass and epiphytes and to allow future comparisons from the same fixed positions (Fig. 3.1-2).

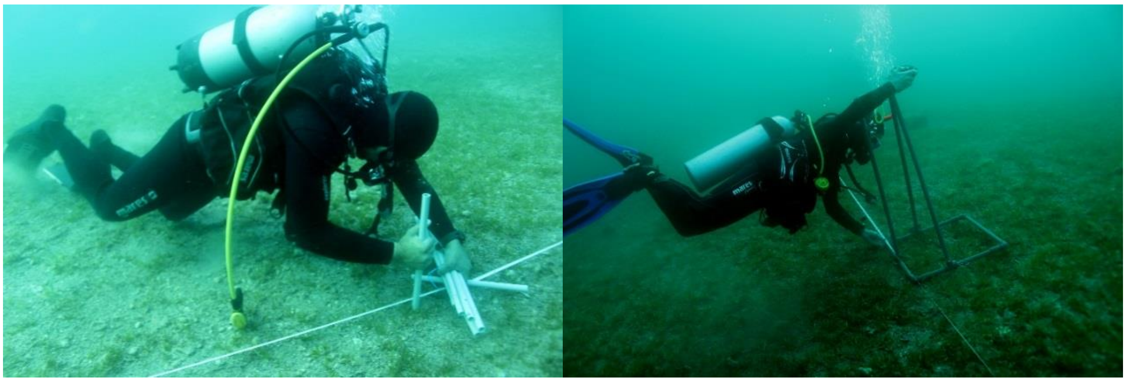

Figure 3.1-2. Setting up *Halophila stipulacea* permanent observatory sites. Two 50 m long transects were set up at Eilat and Cyprus at 3 m depth; each marked with poles placed 5 m apart.

3.1.2.2 Morphological comparisons

At the beginning, middle, and end of each transect, the plants within a quadrat (25 cm x 25 cm) were collected (n=3 per transect), placed in ziploc bags and transferred to the laboratory (Fig. 3.1-3). Measurements were made of the size of leaves, number of leaves per shoot, number of shoots and roots per rhizome, % of apical shoots, rhizome length and internodal distance. The material collected from each quadrat was separated into above ground (leaves and shoots), below ground (rhizomes) and detritus and the dry biomass/weight for each component was later determined. Differences between the two sites for each of the above parameters in the first sampling season (spring, 2017) were tested with a two-sample t-test, using R software. The assumptions for normality and homogeneity of variances were verified with a Shapiro-Wilk test and F-test, respectively.

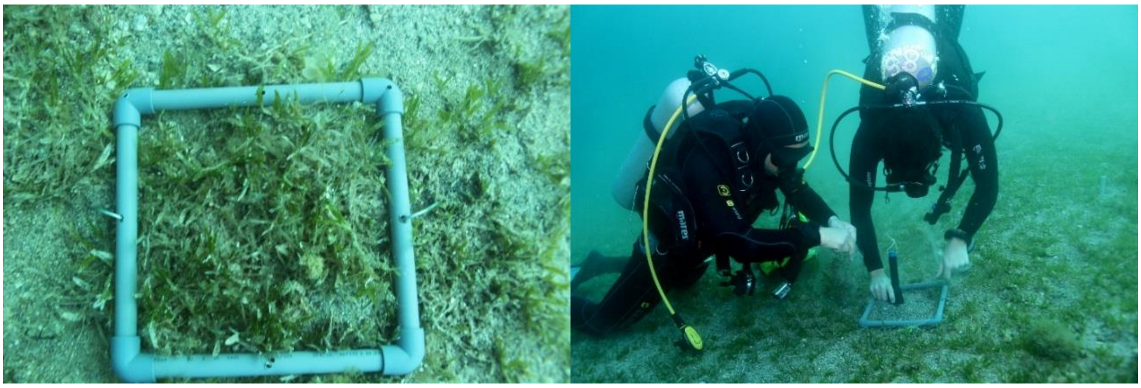

Figure 3.1-3. Plants within three haphazardly placed quadrats (25 x 25 cm) on each transect were collected.

3.1.3 Results

In spring 2017, the number of leaves, roots and shoots per m^2 were not significantly different ($p > 0.05$) between the native and alien *H. stipulacea* meadows. The mean number of roots in the eastern Mediterranean site was 1368 roots per m^2 (range 815 - 2078 per m^2) and in the Gulf of Aqaba site 992 per m^2 (range 496 - 1456 per m^2). The mean number of leaves at the Gulf of Aqaba site was 3099 leaves per m^2 (range 1904 - 4720 per m^2) and in the eastern Mediterranean site 2463 per m^2 (range 1026 - 3393 per m^2). The number of shoots were very similar at the two sites (mean 1280 and 1245 shoots per m^2, in the Gulf of Aqaba site and the eastern Mediterranean site, respectively).

At the same time, in the northern Gulf of Aqaba a significantly higher (two-sample t-test, t value = -6.94, $p < 0.05$) leaf surface area was measured (mean 346 mm^2 leaf^{-1}, range 310 – 411 mm^2 leaf^{-1}) than the eastern Mediterranean site which had smaller leaves (mean 118 mm^2 leaf^{-1}, range 108 – 130 mm^2 leaf^{-1}). The % apical shoots (two-sample t-test, t value = 2.85, $p < 0.05$) and the internodal distance (two-sample t-test, t value = -4.24, $p < 0.05$) were significantly higher in the eastern Mediterranean site (mean 32 % apical shoots, range 30 – 35 % and mean internodal length 1.61 cm, range 1.44 – 1.87 cm) than the northern Gulf of Aqaba site (mean 23 % apical shoots, range 17 – 28 % and mean internodal length 1.04 cm, range 0.99 – 1.12 cm) (Fig. 3.1-4).

Seasonal differences for most seagrass descriptors of the native and the alien *H. stipulacea* were more pronounced during the cold winter months (Fig. 3.1-5). Throughout the year, *H. stipulacea* had higher percent coverage and leaf surface in the native meadow but more apical roots and larger internode distances in the alien meadow (Nguyen *et al.*, in prep.).

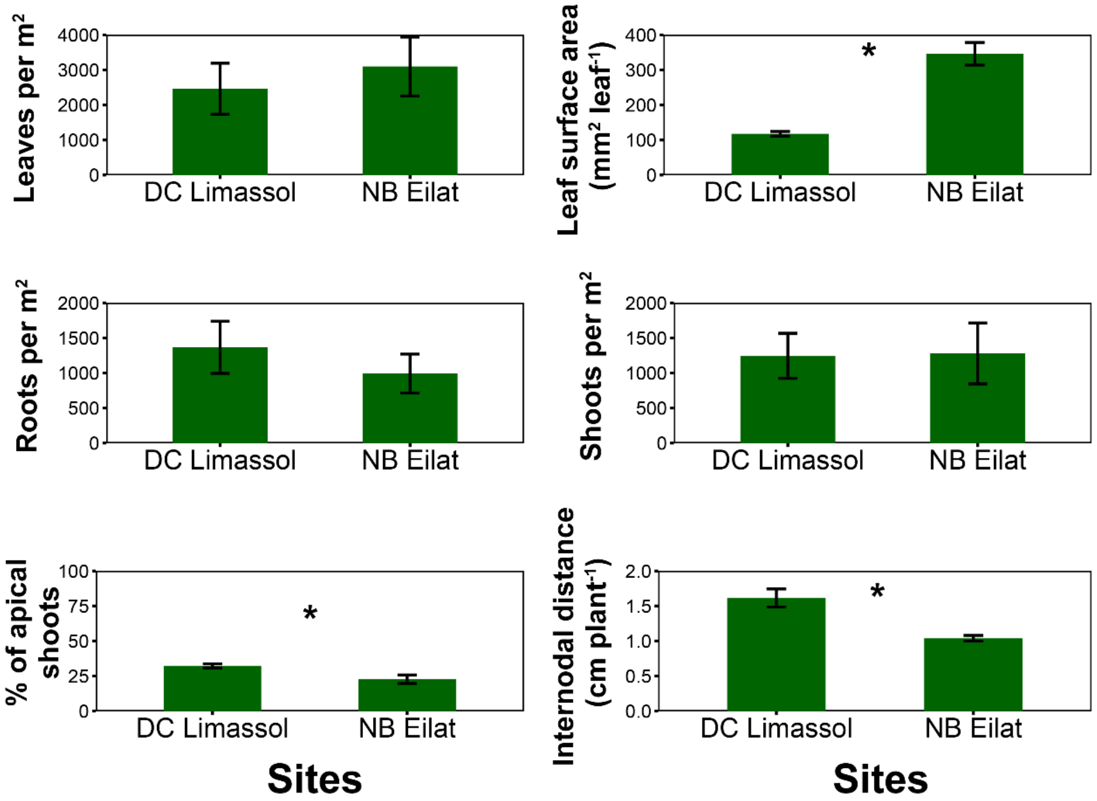

Figure 3.1-4. Comparisons of plant morphology in spring 2017, between a study site at Cyprus in the eastern Mediterranean (DC Limassol) and at the northern tip in the Gulf of Aqaba (NB Eilat). N=3 quadrats sampled from each site, +/- SE. * in the middle of the panel denotes significant differences (two-sample t-test, $p < 0.05$) between the two sites.

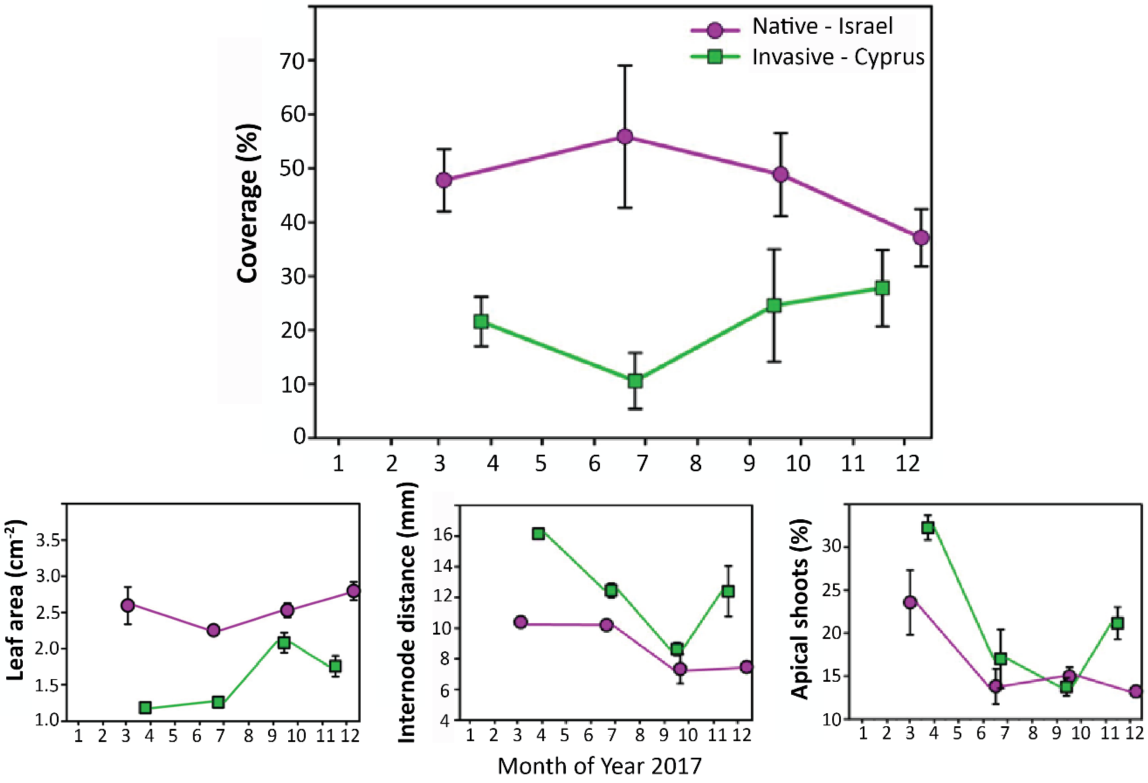

Figure 3.1-5. Seasonal comparisons (2017) in plant morphology in Cyprus and the Gulf of Aqaba. N=11 for cover estimates and n=3 quadrats per season for the rest of the seagrass descriptors, +/- SE. Significant differences (two-way ANOVA, $p < 0.01$) were detected in all seagrass descriptors presented Nguyen *et al.*, (in prep).

Only three quadrats were taken on each sampling occasion and this amount of replication was too low to detect significant difference between above ground, below ground and detritus biomass between the two sites. In spring 2017, higher total biomass was recorded on the native seagrass meadows in the northern Gulf of Aqaba (mean 135.60 g m^{-2}) compared to the site off Cyprus (mean 87.83 g m^{-2}). Above ground biomass was much higher at the northern Gulf of Aqaba site (mean 54.32 g m^{-2}) than at the Mediterranean site (mean 15.95 g m^{-2}). Below ground biomass was also higher at the northern Gulf of Aqaba site (mean 72.24 g m^{-2}) compared to the Mediterranean basin (mean 53.40 g m^{-2}) while the biomass of detritus was higher in the Mediterranean site (mean 18.47 g m^{-2}) compared to the northern Gulf of Aqaba site (mean 9.04 g m^{-2}) (Fig. 3.1-6).

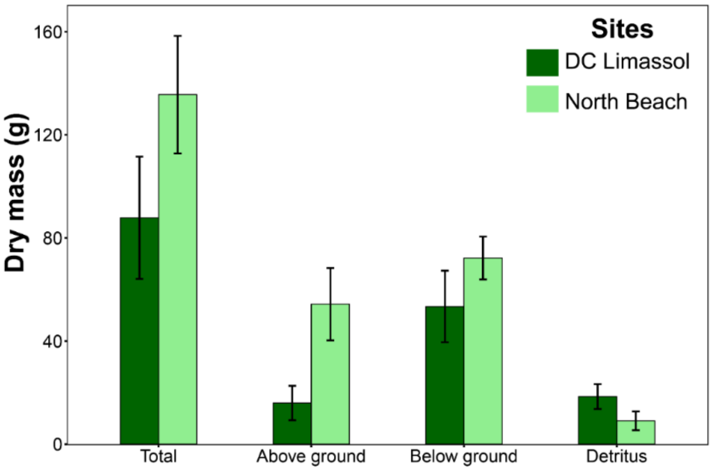

Figure 3.1-6. Dry biomass of *Halophila stipulacea* at 3 m depth in spring 2017, off Cyprus (DC Limassol) and off north beach in the Gulf of Aqaba (North Beach, Eilat). N=3 quadrats sampled from each site, +/-SE. No significant differences (two-sample t-test, $p > 0.05$) were detected between the two sites in any parameter.

3.1.4 Conclusions

The monitoring stations provide baseline data that allow, for the first time, comparisons between alien and native populations of *H. stipulacea*. Continued monitoring of the two transects set up in the native (Red Sea) and non-native (Mediterranean Sea) range will enhance understanding of the dynamics of the species in the two different environments. Photo-quadrat analysis will help indicate seasonal changes in % epiphytic and seagrass coverage in the Mediterranean and the Red Sea. Plant collection allows comparisons of morphological parameters such as leaf number and surface area, shoot number and % of apical shoots, root number, internodal length, and biomass (above ground, below ground and detritus).

This study used the same methodology to compare alien species with native conspecifics, demostrating a new approach to monitoring IAS. However, seagrass meadows are dynamic and display high intra-meadow variability, thus higher spatial coverage is needed for this monitoring to be efficient and comparisons to be valid. Further replication across many sites in both regions is necessary to ensure that the differences observed between the native and alien populations in this study are not site specific. In this study, morphological differences were detected in the plant populations from the native and non-native sites. Coverage and leaf area were significantly higher in the native population, while the alien seagrass had higher number of apical shoots and internodal distance. These differences may be due to differences in water temperature, since they were more pronounced during the winter months. The colder waters result in shrinkage of *H. stipulacea* leaf area and hence cover and productivity are lowered, which may be limiting the spread of this seagrass. In the Mediterranean, the introduction of *H. stipulacea* has not negatively impacted native seagrass meadows (Duarte, 2002), however warming and acidification may increase the spread and allow it to outcompete native pioneer photosynthetic species, like it has in the western Atlantic (Willette & Ambrose, 2012; Hall-Spencer & Allen, 2015; Steiner & Willette 2015).

This study has encouraged further research, to better understand the two populations. Living *H. stipulacea* collected from the Mediterranean and the Red Sea have been transported in a controlled temperature, light, and salinity mesocosm to the Dead Sea-Arava Science Center in Israel. Physiological responses to thermal stress will be assessed, contributing to a predictive understanding of responses and adaptive potential of *H. stipulacea* seagrass in the Gulf of Aqaba and the Mediterranean Sea. Previous studies have demonstrated that genotypic diversity contributes to thermal tolerance (Ehlers *et al*., 2008). DNA samples obtained from *H. stipulacea* specimens collected in the sites at the northern Gulf of Aqaba and Cyprus in the eastern Mediterranean were compared with the 2bRAD genotyping. Genetic comparisons targeting the associated epiphytic microbiome were also carried out, benefiting from the growing knowledge of the associated bacterial communities on *H. stipulacea* (Mejia *et al*., 2016; Rotini *et al*., 2017). The microbial work will help to further increase our understanding of the functionality of seagrasses and their associated microbiome and will allow comparisons among microbiomes associated with native and invasive *H. stipulacea* and with different seagrass species.

3.2 A LIONFISH (*PTEROIS MILES*) INVASION HAS BEGUN IN THE MEDITERRANEAN SEA

3.2.1 Abstract

Few sightings of the alien lionfish *Pterois miles* have been reported in the Mediterranean and it was questionable whether the species could invade this region like it has in the western Atlantic. In the present work, evidence from divers and fishermen shows that lionfish have recently (2014-2015) increased in abundance and within a year have colonised almost the entire south eastern coast of Cyprus, likely due to sea surface warming. At least 23 individual fish are reported of which 6 were removed. Groups of lionfish exhibiting mating behaviour have been noted for the first time in the Mediterranean. Managers need this information and should alert stakeholders to the potential ecological and socio-economic impacts that may arise from a lionfish invasion. Actions could involve incentives to engage divers and fishermen in lionfish removal programmes, as these have worked well at shallow depths in the Caribbean. Given that the Suez Canal has recently been widened and deepened, measures will need to be put in place to help prevent further invasion.

Keywords: lionfish, *Pterois miles*, biological invasion, non-indigenous, alien species, Mediterranean Sea

3.2.2 Introduction

The Mediterranean Sea represents around 0.8 % area and 0.3 % volume of the world ocean, but it contains about 6.3% of the world's described macrophytes and metazoans and is a biodiversity hotspot with estimated 17,000 species (Coll *et al.*, 2010). Mediterranean ecosystems face multiple anthropogenic threats such as climate change, over fishing and alien invasions (**Chapter 2**; Kletou & Hall-Spencer, 2012). Mediterranean seawater temperatures are steadily increasing, and alien species are spreading, causing community shifts and tropicalization (Lejeusne *et al*., 2010; Montefalcone *et al.*, 2015). There are now >1000 alien species in the Mediterranean and the majority are thermophilic species that have entered the eastern basin through the Suez Canal (Katsanevakis *et al*., 2014b). Recent enlargement of this canal coupled with sea surface warming is raising concerns that this problem will get worse (Galil *et al*., 2015).

Lessepsian fish invasion rates are increasing rapidly; *ca* 40 % of the 130 alien fish species now living in the Mediterranean have been reported since 2001 and these have expanded their geographical range during this time (Zenetos *et al*., 2012). The bluespotted cornetfish *Fistularia commersonii* (Rüppell, 1838) colonised almost the entire Mediterranean in just 7 years (Azzuro *et al*., 2012) where it now poses a threat to local ecosystems since it feeds on a large variety of prey (41 taxa) and targets juveniles of the commercial fish *Boops boops* (Linnaeus, 1758) and *Spicara smaris* (Linnaeus, 1758) (Bariche *et al*., 2009). The pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) has now reached the central Mediterranean (Azzuro *et al*., 2014) and is expanding west (Suissi *et al*., 2014). It eats economically important cephalopods *Sepia officinalis* (Linnaeus, 1758) and *Octopus vulgaris* (Lamarck, 1798) and is classed as a fisheries pest (Kalogirou, 2013). Some countries have initiated invasive fish eradication programmes, for example the Cypriot authorities have been compensating fishermen for their pufferfish catches during the reproductive season, resulting in massive removals of pufferfish biomass from the eastern Mediterranean (Rousou *et al*., 2012).

Few worldwide fish invasions of similar magnitude are documented; the introduction of the red lionfish *Pterois volitans* (Linnaeus, 1758) and the devil firefish *P. miles* (Bennett, 1828) in the western Atlantic is one of the fastest and most ecologically harmful marine fish introductions to date (Albins & Hixon, 2013). In their native range, *P. miles* occur in the Indian Ocean from South Africa to the Red Sea and Persian Gulf, and east to Sumatra while *P. volitans* are observed in both the western and central Pacific and western Australia. *Pterois volitans* looks similar to *P. miles* but has an additional dorsal fin ray and an extra anal fin ray (Schultz, 1986). A decade after either intentional or accidental aquarium release in Florida, *P. volitans* became fully established throughout the southeast United States and the Caribbean Sea, extending to South America and much of the Gulf of Mexico (Schofield *et al*., 2015). The alien lionfish population has low genetic diversity and is thought to originate from a small founder population in the southeast United States (Betancur-R *et al*., 2011). The lionfish are generalist carnivores and can feed on a large variety of fish and crustaceans although large individuals prey almost exclusively on fish (Côté *et al*., 2013b). Lionfish spawn every four days year-round, producing around two million buoyant gelatinous eggs per year (Morris *et al.*, 2009). Their eggs are planktonic and can ride the ocean currents and cover large distances for about a month before they settle (Ahrenholz & Morris, 2010). In the western Atlantic, lionfish are more abundant than in their native region and have become the dominant predator on coral reefs with a great impact on native reef fishes, decreasing the abundance of >40 prey species by 65 % on average, in just two years (Green *et al*., 2012). Their dramatic invasive success results from a combination of factors such as early maturation and reproduction, anti-predatory venomous defences and ecological versatility of the lionfish coupled with naïve prey and the overfishing of native predators (Côté *et al*., 2013a).

In the Mediterranean Sea, one *P. miles* was recorded in 1991 off Israel (Golani & Sonin, 1992). After two decades of no records, two specimens were captured off the coast of Lebanon in 2012 (Bariche *et al*., 2013). Soon after, two more were reported in a newspaper article, captured off Cyprus and in 2014, another specimen was captured in Turkey. Then in 2015 two more from Cyprus and another from Rhodes in Greece (Turan *et al*., 2014; Oray *et al*., 2015; Crocetta *et al*., 2015; Iglésias & Frotté, 2015). Recently, several records were reported from south Turkey indicating a westerly migration of the species towards the Aegean Sea (Turan & Öztürk, 2015). Hitherto, lionfish were reported only occasionally in the eastern Mediterranean Sea and their successful invasion in the Mediterranean Sea is questionable due to unfavourable oceanographic conditions that limit the wide dispersion of lionfish larvae (Johnston & Purkis, 2014).

This **Chapter** presents evidence that the threat of lionfish invasion in the eastern Mediterranean Sea is real and probably underway. Sightings of the lionfish *P. miles* from coastal waters of south Cyprus are becoming more frequent in contrast to recent model predictions (Poursanidis, 2015). Furthermore, groups of lionfish have been sighted together for the first time in the Mediterranean.

3.2.3 Materials and Methods

In 2015 reports of lionfish sightings on electronic social media/networks and within the local community of divers and fishermen became more frequent. To map the distribution of lionfish around Cyprus, information was collated from local divers and spearfishermen who reported lionfish encounters in the coastal waters. In-person and telephone interviews were conducted. Interviewees with proof of lionfish sightings willingly provided the evidence (photographs and/or videos) for the purposes of this study. Date of sighting, locality (area and depth) and other information were recorded for validated lionfish sightings. In addition, governmental officers of the Department of Fisheries and Marine Research (DFMR) of the Ministry of Agriculture, Rural Development and Environment shared information and specimens captured in trammel nets by local coastal fishermen.

3.2.4 Results

The lionfish *P. miles* has spread rapidly and colonised almost the entire south eastern coast of Cyprus, from Limassol (south) to Protaras (south east) in just one year (Fig. 3.2-1). Divers and government officers that were questioned in this study mentioned >10 additional lionfish sightings and captures that were unfortunately not photographed so are not included in this database.

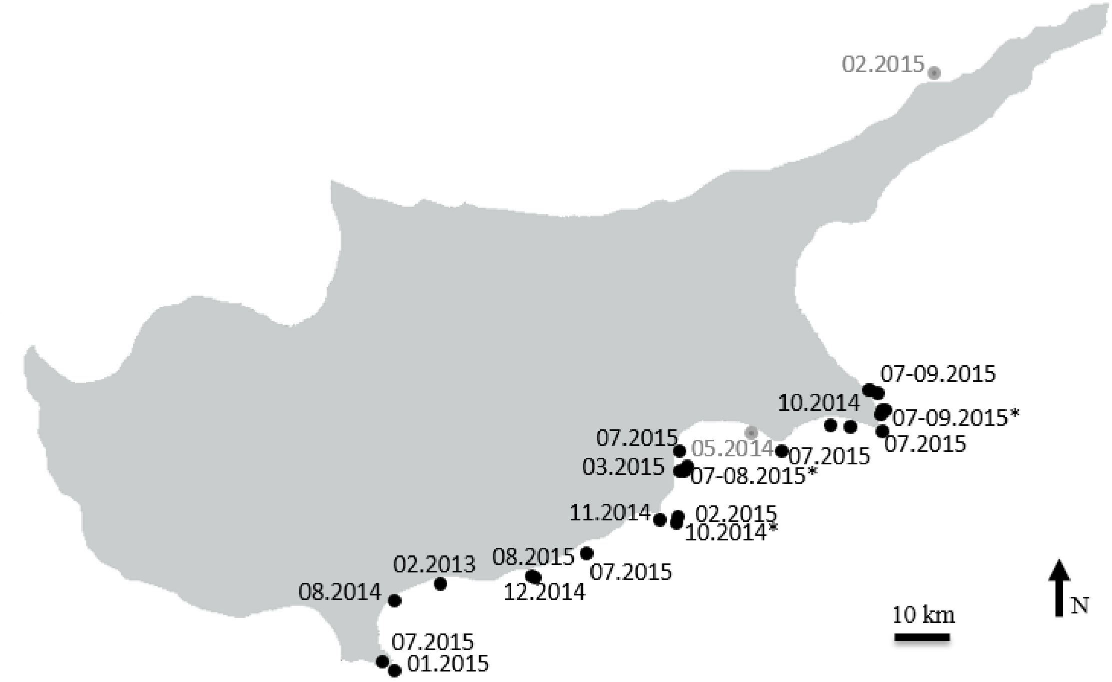

Figure 3.2-1. Updated lionfish reports from the island of Cyprus. Oray et al. (2015) and Iglésias & Frotté (2015) sightings are presented in grey. Locality points on the map are roughly estimated based on the information provided by the person who reported the lionfish sighting. * indicates the presence of a lionfish pair.

Officers from DFMR provided four specimens for the purposes of this study. One is now alive in an aquarium at DFMR offices and another is among the first two undocumented reports made from Cyprus back in February 2013 (Bariche *et al.*, 2013). More details about these specimens are presented in Fig. 3.2-2. Most validated reports of lionfish come from scuba divers and freedivers across south Cyprus. Here 24 new sightings of at least 19 different fish are listed (Fig. 3.2-3, Table 3.2-1).

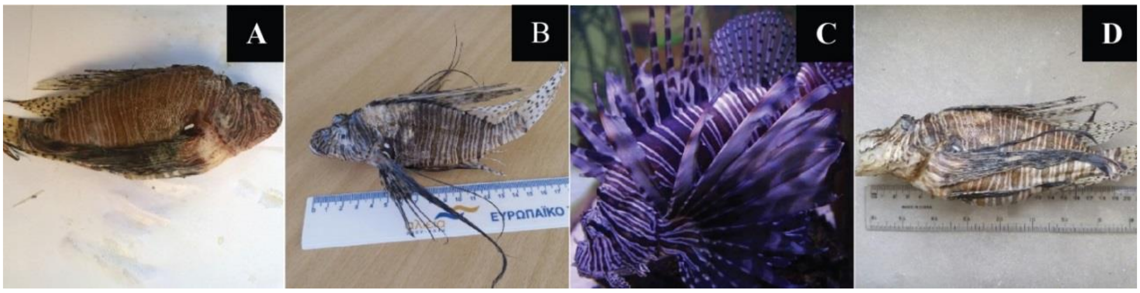

Figure 3.2-2. Photographs of lionfish from the south Cyprus captured by professional fishermen using trammel nets. (A) was captured in Amathounta, Limassol in February 2013 and it is one of the first two lionfish that were reported in Cyprus (Bariche *et al.*, 2013); (B) was captured at Cape Gata, Akrotiri, Limassol in January 2015 at the depth of 15 m; (C) is a living specimen captured at Cape Kiti, Larnaca in May 2015 at the depth of 40 m; (D) was captured at Ayios Theodoros near Zygi in July 2015 at the depth of 15 m.

Three pairs of lionfish have been photographed, one on the Zenobia wreck at 20 m depth, another near Cape Kiti at 42-43 m (Photo D, Fig. 3.2-3) and one more at Cyclops caves near Cape Greco at 12-15 m depth. The Cyclops pair has been photographed many times by different recreational scuba divers both as singletons or together (Photos C, I, J, O, Q, W and X, Fig. 3.2-3). The distinctive stripe pattern of these two individuals allows their identification through photos and tracking of their movement. They were documented together at the same rock formation for a period of two months between July and September 2015 (Photos O, Q, W and X, Fig. 3.2-3). It is noteworthy that one individual from the pair named β with a unique stripe pattern on its left side, may have been reported on the other side of Cape Greco, around 10 km from Cyclops caves, several months earlier in October 2014 (Photo C, Fig. 3.2-3). The other individual, named α, has very distinctive stripes on its right side and was first sighted at Cyclops caves on 7 of July 2015 and the next day it was reported towards Green bay, about 2 km to the north east (Photos I and J, Fig. 3.2-3). Two weeks later it was found back at Cyclops caves with β where they remained together for at least two months. About a week before the pairing was sighted at Cyclops caves, a third lionfish was reported at the site but it was not sighted again thereafter (Photo L, Fig. 3.2-3). Recently, a different lionfish has joined the pair (Photo X, Fig. 3.2-3).

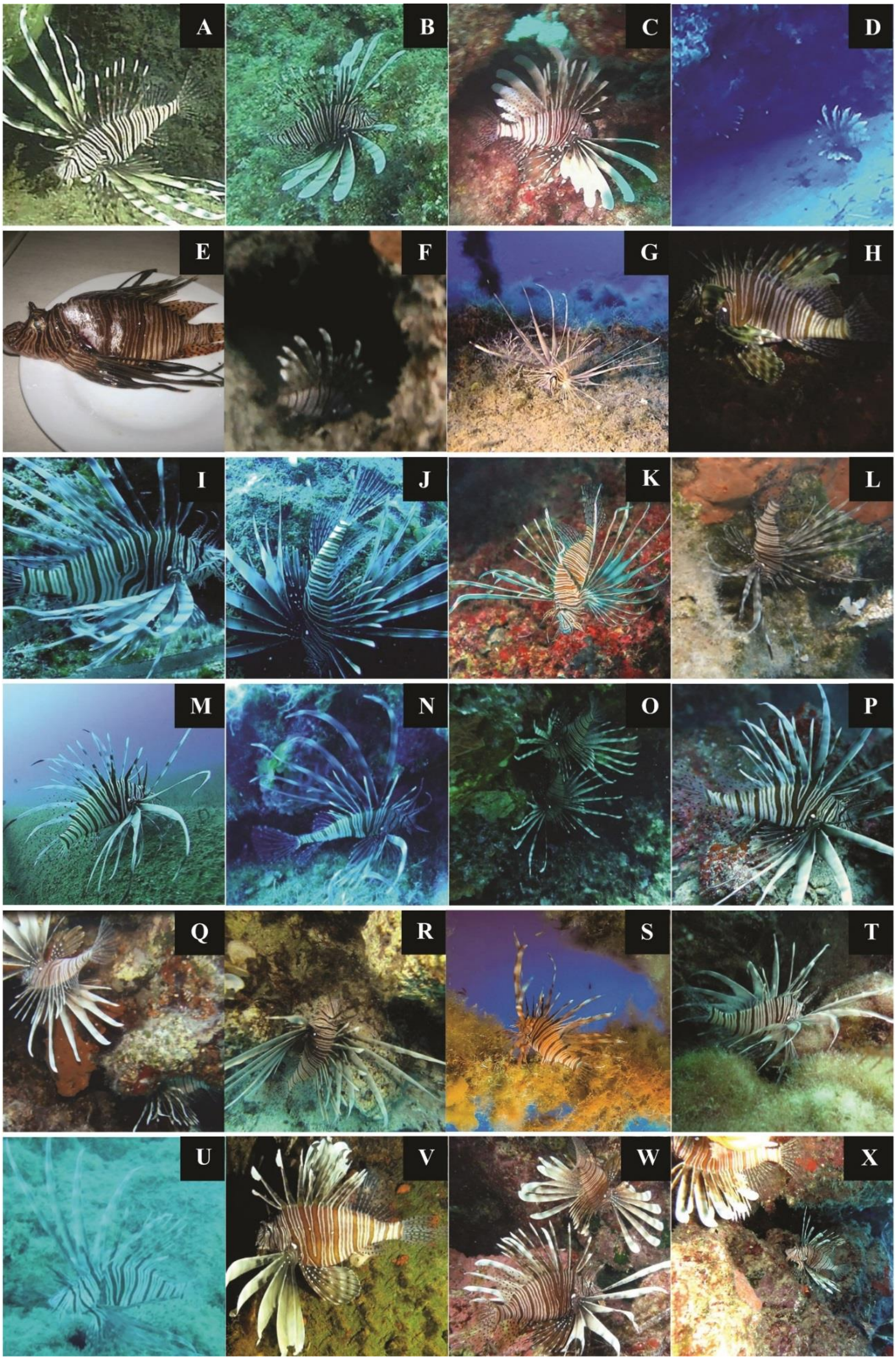

Figure 3.2-3. Photographs of lionfish from the south coast of Cyprus captured by recreational scuba divers, free-divers or spear-fishermen. Further details are given in Table 3.2-1.

Table 3.2-1. Lionfish sightings details from divers who took the photographs in Figure 3.2-3. Coordinates are best available estimates.

Photo	Date	Recorded by	Method of detection	Site	Depth (m)	Type of Substrate	Latitude (N)	Longitude (E)
A	28.08.2014	Stathis Georgiou	Freediving / Spearfishing	Limassol Marina (external side of wave breakers)	15	Artificial hard	34º39.9'	33º2.4'
B	08.10.2014	Tasos Charalambous	Freediving / Spearfishing	Near Ayia Napa port, Famagusta	09-10	Natural hard	34°58.8'	34°0.3'
C	15.10.2014	Tasos Charalambous	Freediving / Spearfishing	2 km from Ayia Napa port towards Cape Greco, Famagusta	10-11	Natural hard	34°58.4'	34°1.8'
D	25.10.2014	George Vasiliou	Freediving / Spearfishing	Cape Kiti, Larnaca	42-43	Natural hard	34°49.6'	33°38.4'
E	23.11.2014	Constantinos Afxentiou	Freediving / Spearfishing	Cape Kiti, Larnaca	10	Natural hard	34°48.7'	33°36.3'
F	20.12.2014	Giannis Ioannou	Freediving / Spearfishing	Vasiliko Port (external side of wave breakers), Larnaca	5	Artificial hard	34°42.9'	33°19.0'
G	28.03.2015	Andreas Makris	Recreational scuba diving	Alexandria Wreck, Larnaca	30	Wreck	34°53.6'	33°39.2'
H	05.07.2015	George Pavlides	Freediving / Spearfishing	Akrotiri, Limassol	13	Natural hard	34°33.0'	33°1.0'
I	07.07.2015	Vagelis Gavalas	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	13	Natural hard	34°59.1'	34°4.6'
J	08.07.2015	Wolf Werner	Recreational scuba diving	Green Bay, Protaras, Famagusta	12	Natural hard	35°0.1'	34°4.1'
K	10.07.2015	Maria Papanikola	Recreational scuba diving	Cape Greco, Famagusta	10	Natural hard	34°57.7'	34°4.4'
L	16.07.2015	Wolf Werner	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	9	Natural hard	34°59.1'	34°4.6'
M	18.07.2015	Elia Sotiris	Freediving / Spearfishing	Oil pipeline, near Larnaca port	16	Pipeline	34°56.2'	33°39.0'
N	19.07.2015	Sotiroula Tsirponouri	Recreational scuba diving	Zenobia Wreck, Larnaca	20	Wreck	34°53.8'	33°39.5'
O	21.07.2015	Vagelis Gavalas	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	12-14	Natural hard	34°59.1'	34°4.6'
P	25.07.2015	Maria Papanikola	Recreational scuba diving	Xylofagou, Larnaca	14	Natural hard	34°56.7'	33°50.2'
Q	05.08.2015	Wolf Werner	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	14	Natural hard	34°59.1'	34°4.6'
R	07.08.2015	Wolf Werner	Recreational scuba diving	Konnos Bay, Protaras, Famagusta	12	Natural hard	34°59.3'	34°4.7'
S	09.08.2015	Loizos Loizou	Recreational scuba diving	Zenobia Wreck, Larnaca	20	Wreck	34°53.8'	33°39.5'
T	17.08.2015	Vagelis Gavalas	Recreational scuba diving	Green Bay, Protaras, Famagusta	03-04	Natural hard	35°0.1'	34°4.1'
U	20.08.2015	Andreas Gkinos	Freediving / Spearfishing	Vasiliko Port (external side of wave breakers), Larnaca	14	Artificial hard	34°42.9'	33°19.0'
V	29.08.2015	Wolf Werner	Recreational scuba diving	Zenovia wreck, Larnaca	20-22	Wreck	34°53.8'	33°39.5'
W	08.09.2015	Vagelis Gavalas	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	14	Natural hard	34°59.1'	34°4.6'
X	21.09.2015	Royce Hatch	Recreational scuba diving	Cyclops Caves, Protaras, Famagusta	15	Narural hard	34°59.1'	34°4.6'

3.2.5 Discussion

The possibility of a lionfish invasion in the Mediterranean Sea and the potential ecological and socio-economic impacts that may follow have been largely neglected by the regional scientific community, managers and other stakeholders. Few validated reports of lionfish sightings exist from the region, which creates muted concern and uncertainty about the ability of the species to colonise the Mediterranean. Furthermore, model simulations have suggested that this would be unlikely to happen (Johnston & Purkis, 2014) although warming and acidification of Mediterranean waters due to CO_2 emissions may require new modelling approaches (Hall-Spencer & Allen, 2015). The lionfish first appeared in the eastern Mediterranean at about the same time that they were first reported off Florida. There they spread rapidly and colonised almost all warm parts of the east coast of United States, the Gulf of Mexico and the entire Caribbean Sea (Schofield *et al.*, 2015) whereas more than two decades passed for a second sighting to occur in the Mediterranean Sea.

Here, >20 lionfish sightings (mostly by divers) are reported from the coastal waters of south Cyprus during over the past year, providing evidence that the lionfish invasion in the Mediterranean Sea is becoming a clear threat that requires the immediate attention of officials and the engagement of stakeholders. It appears that the lionfish have found an empty niche, formed reproductive populations and are now established. Lionfish are normally solitary animals and only gather in groups to mate (Fishelson, 1975). While some lionfish stay put, like one that remained on the Zenobia wreck for at least 6 weeks, some travel up to 2 km a day (α from the Cyclops pair).

In the western Atlantic, lionfish have been found in a wide variety of habitats including hard bottom and patchy reefs, seagrass beds and wrecks (Claydon *et al.*, 2012). The lionfish reported in this study were found at depths ranging from 3–43 m and the majority were found on vertical rock walls or at the entrance of small caves. Three were found on breakwater defences, three were on wrecks and one was spearfished near an oil pipeline. So far, no lionfish have been spotted in seagrass *Posidonia oceanica* (L. Delile, 1813) meadows. Genetic research is needed to assess dispersion pathways of the lionfish and to find out whether they have come from Lessepsian migration and/or through aquarium release.

Lionfish have very few documented natural predators due to their venomous spines. Native groupers in the western Atlantic have learned to eat this non-indigenous species (Maljković *et al*., 2008) and in at least three cases reported herein groupers have been seen close to lionfish. It remains to be seen whether native groupers such as *Epinephelus marginatus* (Lowe, 1834) will learn to prey on lionfish and control their invasion. Restrictions on fishing seem sensible to help threatened IUCN Red List species such as the Mediterranean dusky grouper, as these may in turn help control the spread of invasive fish (Mumby *et al*., 2011).

Judging from the recent increase in *P. miles* in the eastern Mediterranean Sea, its few natural predators, the dispersal capabilities of their planktonic larvae and its ability to adapt to a range of habitats, it is suspected that a rapid expansion throughout the Mediterranean Sea may soon follow with significant impacts on local ecosystems and fisheries. Furthermore, CO_2 emissions which are leading to warming of the Mediterranean Sea and the construction of a deeper and wider Suez Canal are expected to increase invasion rates (Galil *et al*., 2015; Hall-Spencer & Allen, 2015).

Lionfish are slow moving and easy to collect by divers and we know, from the western Atlantic experience, that early detection and a rapid response is the first line of defence to mitigate impacts of the lionfish invasion (Morris *et al*., 2009). Targeted removal by divers has reduced lionfish abundance at shallow depths in the western Atlantic (Frazer *et al*., 2012; Albins & Hixon, 2013). Mediterranean stakeholders (especially divers and fishermen) should be engaged with education and outreach, training and the provision of incentives for removal efforts. The fact that only about a tenth of the lionfish reported by divers in this study were removed from the ecosystem is testimony to the lack of knowledge about the potential impacts of a lionfish invasion. It is believed that many will be motivated to aid removal efforts. When the venomous dorsal, pelvic fin and anal fin spines are removed, the lionfish is safe to consume and this can be promoted. Removal programs should be combined with efforts to restore populations of potential predators of lionfish, such as the dusky grouper.

4. OPPORTUNISTIC SEAWEEDS REPLACE *CYSTOSEIRA* FORESTS ON AN INDUSTRIALISED COAST IN CYPRUS

4.1 Abstract

Seaweeds are affected by humans worldwide, although no studies have assessed this in Cyprus. The Water Framework Directive requires ecological assessments of European coastal waters using biological indicators. Macroalgal community metrics in the upper subtidal across *ca* 10 km of shoreline were investigated, encompassing undeveloped areas with limited human access as well as the most industrialised and impacted coast of Cyprus (Vasiliko Bay). Quadrats (20 x 20cm) were used to survey the algal communities in summer 2012 and spring 2013. Of the 51 recorded taxa, *Cladophora nigrescens* and *Laurencia caduciramulosa* (a non-native species) are new records for Cyprus. Brown algae of the genus *Cystoseira*, e.g., *Cystoseira barbatula*, formed dense forests covering rocky substrata on shorelines with limited human access. *Cystoseira* decreased in abundance around bathing waters and was very rare in heavily industrialised parts of the bay. In impacted areas, fleshy and filamentous opportunistic species such as opportunistic *Ulva* and *Chaetomorpha* species with lower biomass than perennial species, proliferated in spring. The Ecological Evaluation Index (EEI-c) used was a robust biotic index reflecting anthropogenic stress. Without improved management, the Marine Strategy Framework Directive targets may not be met on some coastlines of Cyprus since seaweed forests are in decline and are further threatened by imminent development.

Keywords: ocean sprawl, eastern Mediterranean, macroalgae, biological indicators, *Ulva*, *Cystoseira*, ecological assessment, Marine Strategy Framework Directive.

4.2 Introduction

The human ecological footprint is growing worldwide, and this is especially obvious on rocky shores (Halpern *et al.*, 2008). Although the coastal zone is less than 3% of the Earth's surface, it is home to about 60% of the world's population, and this is expected to rise to 80% by 2050 (Hyun *et al.*, 2009). The policy responses to this reality in Europe are the MSFD (2008/56/EC), which is an attempt to achieve or maintain 'good environmental status' by 2020, and the WFD (2000/60/EC) that aims to achieve 'good ecological status' in coastal waters. A range of biological indicators have been developed to assess environmental and ecological status based on biological quality elements.

Studies worldwide have shown that seaweeds integrate the effects of water quality; in degraded conditions long-lived species tend to be replaced by short-lived, opportunistic species that form less complex habitats (Murray & Littler, 1978). Their responsiveness to anthropogenic disturbances makes macroalgae a key element used to classify the coastal waters in different ecological status classes (ESC). There are no studies of seaweed communities across gradients of human impacts in Cyprus, and this setting is interesting since it is highly oligotrophic (**Chapter 2**; Kletou & Hall-Spencer, 2012). Numerous macroalgal indicators have been designed to assess ecological quality, each tailored to different biogeographic provinces (Neto *et al.*, 2014). The Ecological Evaluation Index continuous formula (EEI-c) has been adopted in the central and eastern Mediterranean to assess the ESC's of coastal waters using benthic macrophytes (Orfanidis *et al.*, 2001; 2011). Here, this index was applied in coastal surveys off Cyprus.

Although all marine ecosystems have been impacted by humans, rocky reefs are amongst the most impacted as they have multiple pressure stressors acting synergistically (Firth *et al.*, 2016). Undeveloped shores of the Mediterranean often have a continuous belt of *Cystoseira* spp. 'forests' that support a diverse range of associated species (Bulleri *et al.*, 2002; Cheminée *et al.*, 2013; Pitacco *et al.*, 2014). *Cystoseira* forests can host richer and more abundant juvenile fish assemblages compared to turf algae or barren reefs (Thiriet *et al*., 2016; Cheminée *et al*., 2017). There are *ca.* 40 species of *Cystoseira* described so far and all these perennial brown fucoids, except *C. compressa*, are included in the Barcelona Convention as they are of high marine conservation importance. There has been a major global loss of canopy-forming algae and of *Cystoseira* forests throughout the Mediterranean; urbanisation, nutrient enrichment, sediment loading, physical disturbance, invasive species, overfishing and marine heat waves have all contributed to these losses (Strain *et al*., 2014; Mineur *et al*., 2015).

Cyprus is presently undergoing very rapid changes in coastal use (Hadjimitsis *et al*., 2016) but there are no published studies about the impact of this expansion in resource exploitation on marine ecology. Baseline information on marine biota and sensitive ecosystems is lacking. A few macroalgal investigations were carried out at pristine locations of Cyprus for WFD and MSFD, which resulted in high ecological assessments (Stavrou & Orfanidis, 2012). In the present study, surveys were conducted along a 10 km stretch of coast to assess whether ocean sprawl is being managed effectively to maintain this good ecological status. Algal assemblages on natural and modified hard substrata in the upper sublittoral zone were analysed across a gradient of anthropogenic pressures. The surveys covered shores with limited human access, bathing waters and the most industrialised parts of Cyprus – the aim was to describe seaweed communities on shores with low to high levels of human influence, to identify patterns of change in macrophytic coverage and biomass that might be relevant to coastal pressures, substratum type and seasonality, and to assess the ESC's using the biotic index EEI-c that has been developed for Mediterranean waters.

4.3 Materials and Methods

4.3.1 Study area

Some areas to the west of Vasiliko Bay have not been developed and access is limited to recreation. At the western side of the bay there are restaurants and bathing waters, and fish farms offshore. By stark contrast, the east of the bay has a completely developed foreshore; there is a naval base, a crude oil import terminal, the main power station in the region, a desalination plant and a large cement plant. The recent discovery of major gas reserves in the eastern Levantine (Ruble, 2017) has triggered further developments in eastern Vasiliko Bay; infrastructure has been built including a 1.2 km long offshore jetty and fuel storage facilities on land. Further coastal disruption is underway, such as land reclamation west of Vasiliko port and construction of a liquefied petroleum gas and bitumen storage area east of the port, where heavy dredging is anticipated to create an approach canal to the berth.

Sixteen rocky coastal sampling sites were selected along *ca* 10 km of coastline extending from Agios Georgios westwards to Zygi eastwards and encompassing Vasiliko Bay (Fig. 4-1). Conglomerate is the dominant substratum at sites 14-16. All other sites were limestone bedrock, the dominant intertidal and shallow sublittoral substratum. Sites 11 and 13 were exceptions, as they were breakwaters made of quarried limestone boulders that have been in place for several decades. Coastal defence boulders were also present at sites 9, 10 but sampling was made on natural submerged hard substrata.

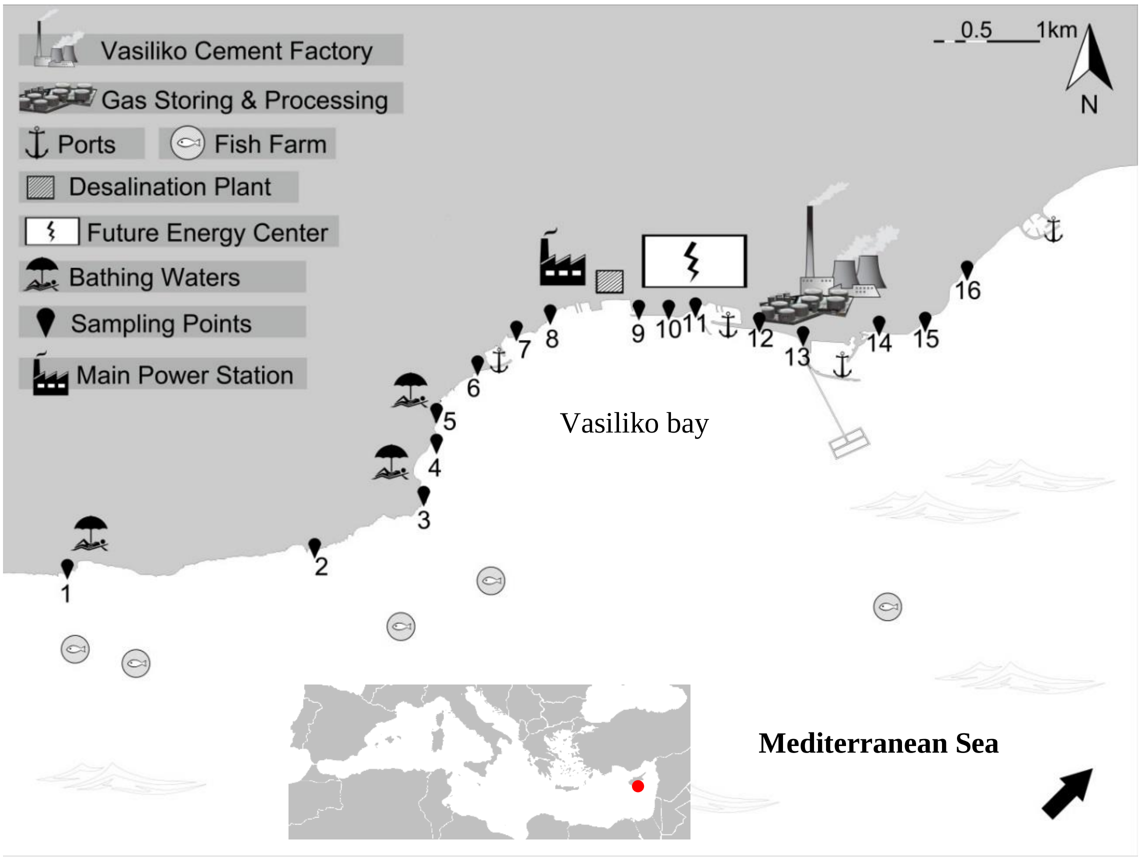

Figure 4-1. Coastal developments in Vasiliko Bay, south Cyprus in the eastern Mediterranean Sea (red dot). There are bathing waters in the west with small natural beaches surrounded by limestone bedrock. The coastline in central-eastern parts of the bay is heavily industrialised, whereas west of the bay there is little coastal modification. The arrow below represents the dominant surface current direction.

4.3.2 Field and laboratory work

At each sampling site, four to six replicate macroalgal samples were taken from smooth horizontal surfaces in the upper subtidal (0.3-1.5 m below the water level), in the summer (June-July of 2012) and spring (March-April of 2013). Each sampling unit was a 0.04 m^2 photoquadrat (20 cm x 20 cm) placed haphazardly over vegetated hard substrata. Macroalgae within the quadrat were then scraped off with a chisel and transported to the laboratory. Vertical photographs were also taken of the scraped area that allowed an estimation of coverage of small and encrusting species (e.g., coralline algae). To minimise the adverse impacts of scraping on *Cystoseira* forests, parts of the holdfast attached to the substratum were left behind to allow regeneration. In the laboratory macroalgae were sorted to the lowest possible taxonomic level, and the abundance of each taxon was estimated as percent coverage of the sampling surface. The surface covered by each sorted taxon in vertical projection was quantified within a transparent cuboidal container filled with seawater and having at its bottom a square 20 x 20 cm matrix divided in 100 squares, where each square represented 1% of sampling surface. *In situ* photographs of quadrats were processed to estimate percent coverage of obvious species and where appropriate modify the estimations made in the laboratory. Sorted macroalgae were blotted on filter paper and weighed (wet weight) and then dried and reweighed (dry weight). Photomicrographs to aid identification of macroalgae species were taken using Olympus CX41 microscope and Olympus SZ stereoscope fitted with a Q. Imaging Micropublisher 5.0 RTV camera. For nomenclature the AlgaeBase taxonomic database was used (Guiry & Guiry, 2018). To assess ecological quality, the abundance of the two Ecological Status Groups (ESG I and ESG II), the Ecological Evaluation Index continuous formula (EEI-c) and the Ecological Quality Ratio (EQR) were calculated for each site following Orfanidis et al. (2011).

4.3.3 Statistical analysis

Preliminary analysis indicated that where macroalgae formed dense canopies, calculations of percent coverage based on *in situ* vertical photographs underestimated short, encrusting and sciophilic macroalgae species that develop under the dense canopy of taller photophilic species. Thus, data obtained using the scraping method and quantified in the lab were combined with those from *in situ* photo-quadrats of scraped substrata to add coverage of encrusting algae. Average seaweed coverage (%) and dry biomass (g m^{-2}) was calculated for all late-successional ESG I and opportunistic ESG II species. To identify the effects of sites, time and their interaction (sites x time) on coverage (%) and dry mass (g m^{-2}) for both ESG I and ESG II, a two-way analysis of variance (ANOVA) was computed. The fixed factor sites comprised 16 site levels and the fixed factor time comprised the two sampling seasons: summer 2012 and spring 2013.

The seasonal macroalgal abundance data % coverage were square-root transformed and analysed using PRIMER v7.0.13. A non-metric multidimensional scaling (nMDS) analysis based on Bray Curtis dissimilarity was undertaken (number of restarts: 100) and a Similarity Profile Analysis (SIMPROF) was used to distinguish statistical differences in macroalgal communities among sites. In addition to this, a one-way and a two-way analysis of similarity (ANOSIM) were performed as complementary analysis based on sites, time and the crossing of the two.

The level of anthropogenic stress at each sampling site was calculated using the MALUSI index (Papathanasiou & Orfanidis, 2018). The MALUSI stress index considers different intensities of indirect and direct pressures (such as agriculture, urbanisation, industrialisation, sewage outfall, aquaculture, fresh water and sediment run off) around a 3 km radius of the study site. Sampling sites were then grouped into three categories based on the MALUSI index scores (2-4 = low stress; >4-8 = medium stress, and >8 = high stress). Sampling sites were also grouped based on substratum type (natural limestone, natural conglomerate and ‘modified’). The ‘modified’ sampling sites were those on the external side of port breakwaters or where there was coastal hardening. Comparisons of the macroalgal community structure were conducted using one-way ANOSIM. Similarity percentage procedure (SIMPER) analysis was used to identify the species that contributed most to the dissimilarities between different levels of each category and the top three species that contributed to the similarity within each level of category across the two seasons (Clarke *et al*., 2014).

To assess differences in ecological quality between grouped sites, EQR scores were analysed using a Kruskal-Wallis test followed by a Dunn’s pairwise comparison with a Bonferroni correction for the substratum type and stress category (Dinno, 2016). A Mann-Whitney test was used for seasonal comparisons. Main and interaction effects between sites and time were identified using a two-way ANOVA and to see how EEI-c score matched with the MALUSI index scores, a Pearson's Correlation was computed.

For all two-way ANOVA analyses, the normality of errors and homogeneity of variances were visually inspected and tested via a Shapiro-Wilk test and Levene's test, respectively. To achieve the normal distribution of errors and homogeneity of variances, the data were square-root transformed and the analysis was repeated, without requiring proceeding to a non-parametric test. For the Pearson's Correlation analysis, a power transformation was conducted, and normality of data and equal variances were verified with a Shapiro-Wilk test and F-test, respectively. For all the statistical analyses the significance level α was set at 0.05. Graphical material was generated with R-studio v3.4.2 package: ggplot2 (Wickham, 2016).

4.4 Results

4.4.1 Macroalgal abundance and biomass

A diverse range of macroalgal taxa was sampled from the upper subtidal, including 21 Ochrophyta, 15 Rhodophyta and 11 Chlorophyta (Table 4-1, Table 4-6 in Appendix 1). *Cladophora nigrescens* Zanardini ex Frauenfeld, 1855, *Chondrophycus* cf. *glandulifera* (Kützing) Lipkin & P.C.Silva, 2002 and the alien *Laurencia caduciramulosa* Masuda & Kawaguchi, 1997 are new records for Cyprus. Two more non-native species were sampled (*Caulerpa cylindracea* Sonder, 1845 and *Stypopodium schimperi* (Kützing) M.Verlaque & Boudouresque, 1991), though in small proportions. Two seagrasses (*Posidonia oceanica* (Linnaeus) Delile, 1813, *Cymodocea nodosa* (Ucria) Ascherson, 1870), a Chrysophyte and Cyanobacteria were also found in samples from hard substrata.

Table 4-1. Taxa recorded, and % coverage in 134 quadrats (0.04 m^2) sampled on hard substrata at 0.3 - 1.5 m depth across Vasiliko Bay in late summer 2012 and early spring 2013. Late-successional (Ecological Status Group I) and opportunistic species (Ecological Status Group II) are separated in five categories based on their sensitivity to pressures (Orfanidis *et al.*, 2011). Taxa with an asterisk correspond to non-native introductions. New records for Cyprus appear in bold.

Taxa	ESG	Summer Coverage (%)	Spring Coverage (%)
CYANOBACTERIA			
Cyanobacteria	*IIB*	0.21	0.02
CHLOROPHYTA			
Acetabularia acetabulum (Linnaeus) P.C.Silva	*IC*	0.19	0
Anadyomene stellata (Wulfen) C.Agardh	*IC*	0.97	0.39
**Caulerpa cylindracea* Sonder	*IIA*	0.21	0.75
Chaetomorpha aerea (Dillwyn) Kützing	*IIB*	0.08	3.71
Chaetomorpha linum (O.F.Müller) Kützing	*IIB*		
Cladophora laetevirens (Dillwyn) Kützing	*IIB*	5.91	2.8
***Cladophora nigrescens* Zanardini ex Frauenfeld**	*IIB*		
Dasycladus vermicularis (Scopoli) Krasser	*IIA*	4.1	4.28
Flabellia petiolata (Turra) Nizamuddin	*IC*	0.01	0
Ulva intestinalis Linnaeus	*IIB*	4.59	7.16
Ulva linza Linnaeus	*IIB*		
CHRYSOPHYTA			
Chrysophyte sp.	*IIB*	0.14	0
OCHROPHYTA			
Cladostephus spongiosum (Hudson) C. Agardh	*IIA*	6.6	3.02
Cystoseira barbata (Stackhouse) C. Agardh	*IB*	0.86	2.38
Cystoseira barbatula Kützing	*IA*	26.04	29.44
Cystoseira cf. *elegans* Sauvageau	*IA*	0	0.08
Cystoseira compressa (Esper) Gerloff & Nizamuddin	*IB*	1.57	1.14
Cystoseira crinitophylla Ercegovic	*IA*	2.09	0
Cystoseira foeniculacea (Linnaeus) Greville f. *foeniculacea*	*IA*	8.88	0.36
Dictyopteris polypodioides (A.P. De Candolle) J.V. Lamouroux	*IIA*	1.41	1.88
Dictyota dichotoma (Hudson) Lamouroux	*IIA*	1.06	4.96
Dictyota implexa (Desfontaines) J.V. Lamouroux	*IIA*	0	0.73
Dictyota mediterranea (Schiffner) G. Furnari	*IIA*	5.41	17.55
Feldmannia irregularis (Kützing) Hamel	*IIB*	0	0.97
Feldmannia simplex (P. Crouan & H. Crouan) Hamel	*IIB*	0.12	0
Halopteris scoparia (Linnaeus) Sauvageau	*IIA*	15.1	13.55
Hydroclathrus clathratus (C. Agardh) M. Howe	*IIA*	0.03	0.04
Padina pavonica (Linnaeus) Thivy	*IB*	5.95	11.17
Sargassum vulgare C. Agardh	*IB*	0.04	1.66
Scytosiphon lomentaria (Lyngbye) Link	*IIB*	0	0.07
Sphacelaria cirrosa (Roth) C. Agardh	*IIA*	4.7	3.71
**Stypopodium schimperi* (Kützing) M. Verlaque & Boudouresque	*IIA*	0.31	0.09
Taonia atomaria (Woodward) J. Agardh	*IB*	0	0.1

Taxa	ESG	Summer Coverage (%)	Spring Coverage (%)
RHODOPHYTA			
Botryocladia botryoides (Wulfen) Feldmann	*IIA*	0.01	0
Chondria dasyphylla (Woodward) C. Agardh	*IIA*	0	0.1
***Chondrophycus* cf. *glandulifera* (Kützing) Lipkin & P.C Silva**	*IIA*	0.01	0.13
Dasya corymbifera J. Agardh	*IIB*	1.81	1.25
Herposiphonia secunda (C. Agardh) Ambronn	*IIB*	0.04	0
Jania rubens (Linnaeus) J.V. Lamouroux	*IC*	17.46	6.67
Jania virgata (Zanardini) Montagne	*IC*		
***Laurencia caduciramulosa* Masuda & Kawaguchi**	*IIA*	0	0.16
Laurencia obtusa (Hudson) Lamouroux	*IIA*	0.17	0.02
Corallinaceae	*IC*	0.91	0.8
Peyssonnelia sp.	*IC*	0.18	0
Polysiphonia sp.	*IIB*	0.1	0
Rytiphlaea tinctoria (Clemente) C. Agardh	*IB*	0.49	0.85
Spermothamnion flabellatum Bornet	*IIB*	0.03	0
Wrangelia penicillata (C. Agardh) C. Agardh	*IIB*	0.39	0.43
TRACHEOPHYTA			
Cymodocea nodosa (Ucria) Ascherson	*IB*	0.54	0.14
Posidonia oceanica (Linnaeus) Delile	*IA*	0.41	0.31

There were 4-10 macroalgal taxa per sampling site with the lowest diversity recorded at site 10, a heavily industrialized area (MALUSI stress index score = 10). At all sites, there was >100% algal coverage due to multiple layers of vegetation, except at site 7, near the naval port, and on conglomerate substrata (sites 14 – 16, Fig. 4-2). Canopy-forming *Cystoseira* and other ESG I species dominated on undeveloped shores, but their abundance was low in industrialised areas. For example, total coverage of ESG I species at site 2 was 121 ± 15%, while at site 11 coverage of the same group dropped to 15 ± 4%. The total coverage of opportunistic ESG II species on industrialised coasts matched the coverage of *Cystoseira*-dominated sites (e.g., ESG II coverage was 111 ± 9% at site 13) but their biomass was lower (ESG I biomass at site 2 was 1.11 ± 0.27 kg m^{-2}, while ESG II biomass at site 13 was 0.37 ± 0.07 kg m^{-2}) (Fig. 4-2). The highest total biomass was found at site 2, which also had the highest coverage of *Cystoseira*. Abundance and biomass were significantly correlated (R = 0.943, $p = 0.001$), as expected.

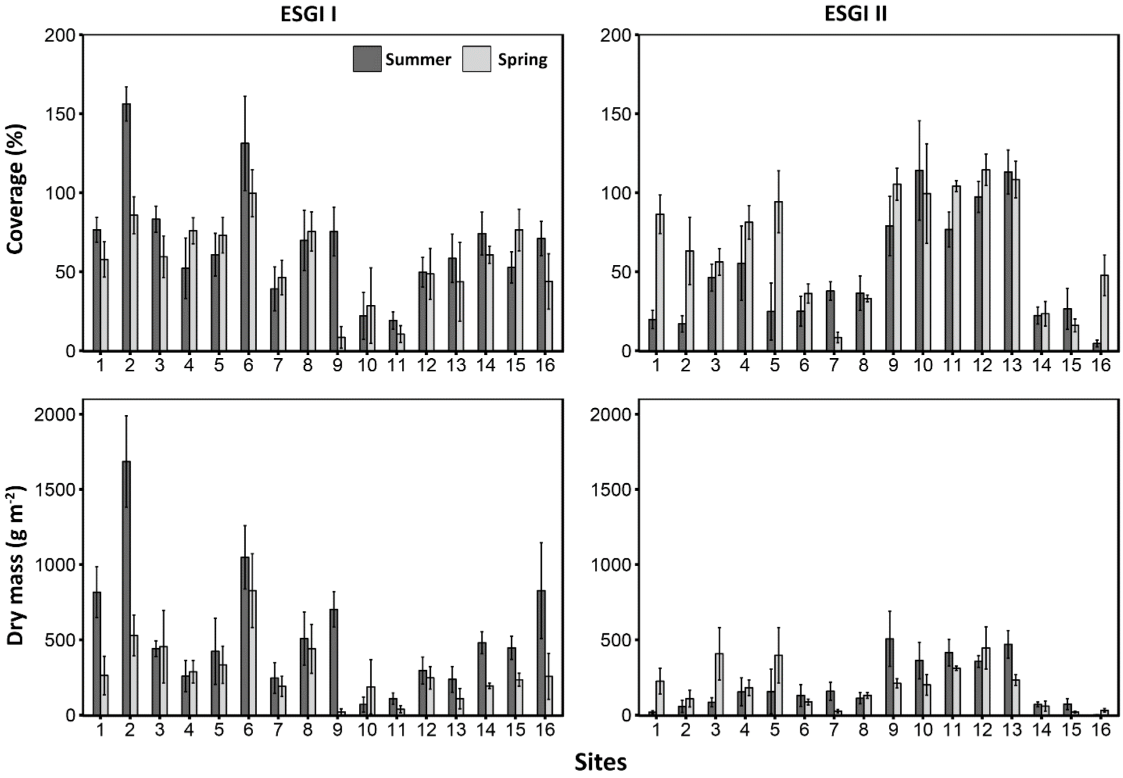

Figure 4-2. Contribution to average total coverage (top panel) and average dry mass (bottom panel) of macroalgae separated into Ecological Status Group I (left) and ESG II (right), and season of sampling (error bars = SE, n = 4-6), for sampling sites across Vasiliko Bay, Cyprus in 2012-2013.

There was a significant interaction between the effects of the sites and time on the ESG I coverage and biomass. The effect of time was observed in most sites, and although ESG I coverage and biomass were reduced at some sites (i.e., 2, 6, and 9) between samplings, it increased in other sites (Table 4-2). The interactive effects between sites and time were also significant on the ESG II coverage, whereby all sites showed a change in coverage from one sampling period to the other. Significant interaction between the effects of sites and time were notable in biomass of ESG II species as well. Although the biomass of opportunistic macroalgae was different between the sites, it only changed at a few sites between the two sampling periods and overall time did not affect the biomass of ESG II species (Table 4-2).

Table 4-2. Two-way ANOVA of Coverage (%) and Dry mass (g m^{-2}) for two ecological macroalgal groups: ESG I and ESG II, based on sites, season and the interaction of the two.

Variable	Effects	df	Sum of squares	Mean square	F value	*p*-value
ESG I						
Coverage (%)	Site	15	91511	6101	7.016	< 0.001
	Season	1	4797	4797	5.517	< 0.050
	Site x Season	15	23281	1552	1.785	< 0.050
Dry mass (g m^{-2})	Site	15	5416	361	6.508	< 0.001
	Season	1	1189	1189	21.427	< 0.001
	Site x Season	15	1792	120	2.153	< 0.050
ESG II						
Coverage (%)	Site	15	661	44	11.636	< 0.001
	Season	1	49	49	12.870	< 0.001
	Site x Season	15	183	12	3.229	< 0.001
Dry mass (g m^{-2})	Site	15	3300	220	7.309	< 0.001
	Season	1	5	5	0.182	> 0.050
	Site x Season	15	1042	69	2.307	< 0.010

4.4.2 Community structure

Shifts in macroalgal community structure were observed across a gradient of stress (Fig. 4-3; Table 4-3). Late-successional species, particularly *Cystoseira barbatula* Kützing, 1860 and to a lesser extent *Cystoseira foeniculacea* (Linnaeus) Greville f. *foeniculacea*, formed dense aggregations at the sites with limited human influence. Their canopy was often partly covered by epiphytes (e.g., the *Jania* spp., *Dictyota mediterranea* (Schiffner) G.Furnari, 1997, *Sphacelaria cirrosa* (Roth) C.Agardh, 1824 and *Wrangelia penicillata* (C.Agardh) C.Agardh, 1828) and there was a diverse understorey of Corallinaceae and fleshy seaweeds (e.g., *Padina pavonica* (Linnaeus) Thivy, 1960, *Dasycladus vermicularis* (Scopoli) Krasser, 1898, *Anadyomene stellata* (Wulfen) C.Agardh, 1823, *Rytiphlaea tinctoria* (Clemente) C.Agardh, 1824, *Cladophora* spp.). On modified coasts *Cystoseira* forests were almost absent, here opportunistic seaweed (*Halopteris scoparia* (Linnaeus) Sauvageau, 1904, *Cladostephus spongiosus* (Hudson)

C. Agardh, 1817, *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux, 1809, *Dictyota dichotoma* (Hudson) J.V.Lamouroux, 1809 and nitrophilous green algae *Ulva* spp. and *Cladophora* spp.) dominated.

Figure 4-3. Macroalgal community shifts across Vasiliko Bay, southern Cyprus in 2013. A climax community with *Cystoseira* spp. and *Posidonia oceanica* with several layers of vegetation covered limestone rocky shores with limited human access (left picture). Perennial species co-existed with bushy opportunists, at sites with moderate anthropogenic impact (middle picture). On heavily industrialised coasts opportunistic species dominated (right picture).

Macroalgal community structure differed across sites (one-way ANOSIM, R = 0.6, $p < 0.05$) depending largely on levels of stress and substratum type and to a lesser extent on the time of sampling (Fig. 4-4; Table 4-3). The macroalgal community at highly stressed sites was different compared to medium and low stress sites. The macroalgal communities were also affected by substratum type (Table 4-3), for example *P. pavonica* was more abundant on conglomerate than on limestone substrata and *C. barbatula* was the most abundant species on natural substrata but was absent from modified substrata where it was replaced by *H. scoparia* turf. The macroalgal assemblages within Vasiliko Bay changed between the two sampling periods, though the effect of time was not strong (Table 4-3), mainly because it was only prominent in some sites (two-way ANOSIM, R = 0.4, $p < 0.05$; Fig. 4-4). Spring blooms of green algae were recorded at some sites; for example, *Ulva* spp. increased from 0% to 54% coverage at the industrial site 9 and *Chaetomorpha* spp. increased from 0-2% to 11-52% coverage on conglomerate substrata (Table 4-4).

Table 4-3. Pairwise differences in macroalgal community composition across Vasiliko Bay, southern Cyprus, calculated using ANOSIM (R statistic and Significance level). The average dissimilarity and main taxa responsible for these differences calculated by SIMPER analysis are given as well as their average percent coverage.

Pairwise groups of sites	**R statistic**	**Sign. level**	**Average Dissimilarity**	**Main taxa responsible for dissimilarity**	**Av. Coverage %**
			Seasons		
Summer, Spring	0.069	0.001	74.96	*Jania* spp.	17.5, 6.7
			Stress Category		
Low, Medium stressed	-0.063	0.899	63.71	*D. mediterranea*	33.5, 8.5
Low, High stressed	0.557	0.001	83.85	*C. barbatula*	58.8, 6.2
Medium, High stressed	0.494	0.001	84.11	*H. scoparia*	0.4, 42.7
			Rocky Substratum		
Limestone, Modified	0.714	0.001	85.76	*H. scoparia*	3.2, 49
Limestone, Conglomerate	0.421	0.001	74.51	*P. pavonica*	4.9, 21.3
Modified, Conglomerate	0.649	0.001	87.92	*H. scoparia*	49, 0.6

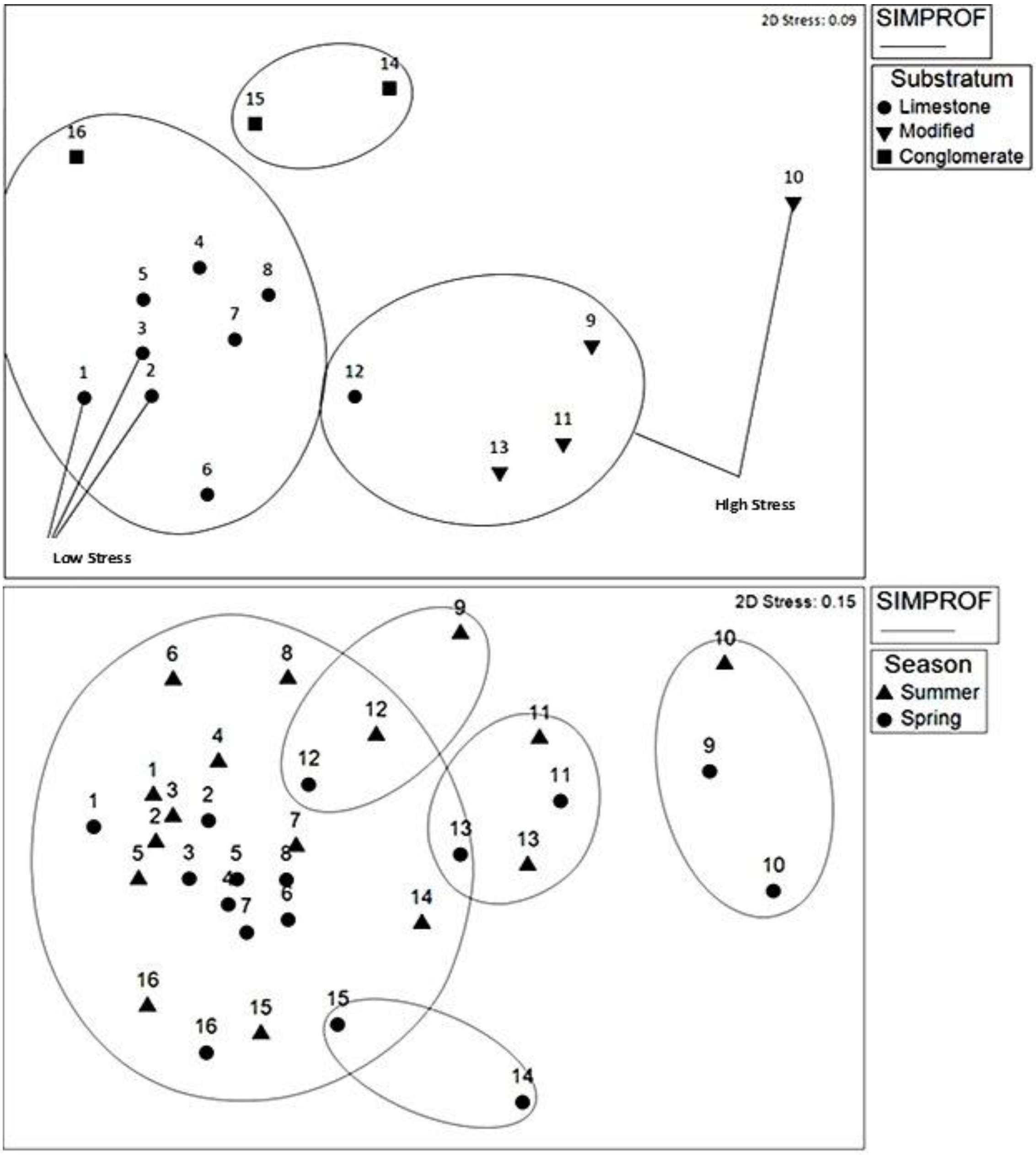

Figure 4-4. Macroalgal community similarities tested with a SIMPROF test (significant different groups are assigned with a SIMPROF line) and displayed as a non-metric multidimensional scaling (nMDS) plot based on Bray-Curtis similarities. The top panel was run with the average macroalgal % coverage at each site, separated by substratum type and stress level (high stressed and low stressed sampling sites are noted; all others were classified as medium stressed based on MALUSI index scores). The bottom panel was run with the average seasonal macroalgal % coverage at each site, separated by season of sampling.

Table 4-4. The three species with maximum contribution (%) to site similarity, their contribution % to the similarity, and the site with their highest abundance within each category for summer and spring, generated via SIMPER analysis of similarity.

Category	**Summer**			**Spring**		
MALUSI stress categories	Top 3 species with max. contribution (%) for site similarity	Contr. (%)	Site with the highest contr.	Top 3 species with max. contribution (%) for site similarity	Contr. (%)	Site with the highest contr.
Low stressed sites	*C. barbatula*	47.1	2	*C. barbatula*	40.6	2
	Jania spp.	24.6	2	*D. mediterranea*	34.9	1
	D. mediterranea	18.9	3	*D. vermicularis*	5.9	3
Medium stressed sites	*C. barbatula*	37.2	16	*C. barbatula*	35.3	8
	Cladophora spp.	22.1	15	*P. pavonica*	21.3	15
	Jania spp.	8.2	6	*Jania* spp.	12.2	6
High stressed sites	*H. scoparia*	44.6	13	*H. scoparia*	53.0	11
	Jania spp.	17.7	9	*Ulva* spp.	12.3	9
	Cladophora spp.	4.5	11	*Jania* spp.	7.2	12
Substratum						
Limestone	*C. barbatula*	37.1	2	*C. barbatula*	43.7	2
	Jania spp.	18.1	6	*D. mediterranea*	20.2	1
	D. mediterranea	12.9	3	*Jania* spp.	11.2	6
Modified	*H. scoparia*	51.1	13	*H. scoparia*	54.7	11
	Jania spp.	17.5	9	*Ulva* spp.	18.6	9
	Cladophora spp.	5.0	11	*D. dichotoma*	9.4	10
Conglomerate	*C. barbatula*	38.2	16	*P. pavonica*	34.2	15
	Cladophora spp.	25.0	15	*Chaetomorpha* spp.	27.0	16
	P. pavonica	19.9	14	*C. barbata*	16.6	15

4.4.3 Ecological status class

Shifts in macroalgal communities across the study area were well reflected by the EEI-c biotic index and further supported by the MALUSI stress index (Fig. 4-5, MALUSI data Table 4-7 in Appendix 1). The two indices had a significant negative correlation on averaged seasonal values (Pearson's correlation, $\rho = -0.647$, $p < 0.01$) and in the summer (Pearson's correlation, $\rho = -0.729$, $p < 0.01$), but not in the spring (Pearson's correlation, $\rho = -0.487$, $p = 0.056$). Overall, there was significant inter-site variability in EQR reflected on both sampling periods (two-way ANOVA, df = 15, F = 8.808, $p < 0.05$). Low ESC was recorded at sites 10 – 13 in both seasons. Good-High ESC was assessed at the other sites but in most cases, spring ecological assessments produced lower EQR values due to the increase in abundance of opportunistic species (Fig. 4-5). The highest ESC scores were assessed at sites 2 and 6, which also had the highest macroalgal biomass whereas the lowest was assessed at sites 10 and 11, which had among the lowest species diversity and biomass. The overall EQR of Vasiliko Bay was similar in spring and summer (Man-Whitney test, W = 5106, $p = 0.09$), although the effect of time on EQR was prominent on some site levels, showing significant differences in sites 1, 2, 5, 7, 9 and 16 (two-way ANOVA, df = 1, F = 8.035, $p < 0.05$). No interaction effect was observed between sites and time (two-way ANOVA, df = 15, F = 1.559, $p > 0.05$). Significant differences of the EQR score were also observed between the different levels of coastal stress as well as between modified and natural substrata (Table 4-5). No differences in the EQR scores were detected between natural substrata limestone and conglomerate and between low and medium stressed sampling sites.

Table 4-5. The pairwise comparisons based on the EQR score calculated with the EEI-c index (Orfanidis *et al*., 2011), and statistical differences between different seasons, substrata and stress category in Vasiliko Bay, Cyprus.

Groups	Average EQR	Statistical test	df	test statistic	*p*-value
Season (Summer, Spring)	0.59, 0.48	Mann-Whitney	-	W = 5106.0	>0.050
Substratum		Kruskal-Wallis	2	$\chi^2 = 42.3$	<0.001
Limestone, Modified	0.63, 0.17		-	z = 5.7	<0.001
Limestone, Conglomerate	0.63, 0.75	Dunn's test	-	z = 1.5	>0.050
Conglomerate, Modified	0.75, 0.17		-	z = 5.7	<0.001
Stress category		Kruskal-Wallis	2	$\chi^2 = 53.7$	<0.001
High, Medium	0.19, 0.70		-	z = -6.9	<0.001
High, Low	0.19, 0.71	Dunn's test	-	z = -5.3	<0.001
Low, Medium	0.71, 0.70		-	z = 0.4	>0.050

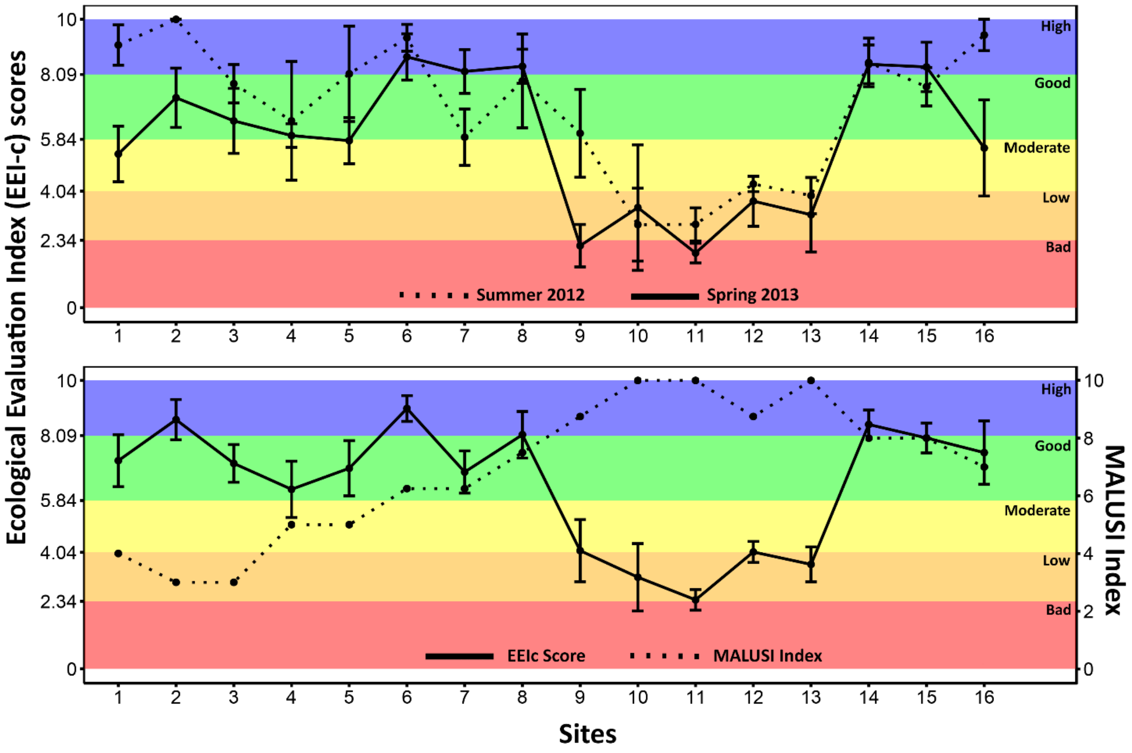

Figure 4-5. Top panel: Mean values (error bars = SE, n = 4-6) of Ecological Evaluation Index (EEI-c) across Vasiliko Bay, Cyprus, in summer 2012 and spring 2013. Lower panel: Mean values (error bars = SE, n = 8-10) of EEI-c and MALUSI index across Vasiliko Bay, Cyprus, for the period summer 2012 to spring 2013. Ecological status classes colour categorisation corresponds to EEI-c scores.

4.5 Discussion

The surveys on the southern coast of Cyprus identified 51 taxa of macrophytes. Three species are reported for the first time from Cypriot waters, expanding the existing checklist of seaweed species (Tsiamis *et al*., 2014). One of these, *L. caduciramulosa*, is native to SE Asia and was described for the first time from the Mediterranean Sea by Furnari et al. (2001).

The results are consistent with global observations that human impacts combine to cause loss of perennial canopy-forming brown seaweeds and a proliferation of opportunistic macroalgae (Scherner *et al*., 2013; Strain *et al*., 2014). In the surveys, canopy-forming *Cystoseira* dominated shallow subtidal hard substrata showing good environmental quality of waters, to which human access was limited to recreation. Algal biomass was considerably higher than at impacted sites as there were more perennial species present, an indication of a healthy shallow rocky reef ecosystem (Panayotidis *et al*., 2004; Sala *et al*., 2012). The canopy of *C. barbatula* diminished near industrialised areas and got replaced by simpler communities, dominated by stress-resistant and ephemeral species such as *H. scoparia* and *Ulva* spp. Similar community shifts from canopy-forming fucoids to bushy, turf or fleshy opportunistic species have been widely reported across gradients of impact around the Mediterranean Sea (Benedetti-Cecchi *et al*., 2001; Thibaut *et al*., 2005, 2015; Arévalo *et al*., 2007; Mangialajo *et al*., 2008; Orlando-Bonaca *et al*., 2008; Perkol-Finkel & Airoldi, 2010; Pinedo *et al*., 2013; Tsiamis *et al*., 2013; Iveša *et al.*, 2016; Badreddine *et al*., 2018; Orlando-Bonaca & Rotter, 2018), but this is the first time it is reported from the oligotrophic waters of Cyprus.

Opportunistic algae dominated in spring at some impacted sites, but they did not approach the high levels of biomass found in unimpacted *Cystoseira* forests. Blooms of green algae (*Ulva* and *Chaetomorpha* spp.) occurred on highly stressed shores during spring, which may be due to eutrophication, whereas a bloom of *D. mediterranea* was recorded in spring on the western side of the study area reflecting the typical annual cycle of Dictyotales (Tronholm *et al*., 2008).

The most significant factors that affected shallow subtidal communities were human stress level, calculated with the MALUSI index, and the type of substratum available for seaweed growth. On breakwaters and coastline defences *Cystoseira* spp. were almost absent, even though these were constructed using natural limestone boulders several decades ago. This emphasises the fact that man-made structures do not function as surrogates of natural rocky reefs (Bulleri & Chapman, 2010), as they are composed of different assemblages of species and can have significantly lower abundances of large perennial algae (Ferrario *et al*., 2016). Despite differences in macroalgal community structure, the two natural substrata studied (limestone and conglomerate), had similar

ESC, as assessed with the EEI-c, mainly because macroalgal community structure was dominated by species of the same ESG. The averaged and the summer ESC scores were negatively correlated with the MALUSI stress index, which demonstrates that the EEI-c is a robust way of assessing the environmental quality of coastal waters as it is unaffected by natural variability of communities due to different type of substratum and in summer, macroalgal community shifts from perennial species to opportunistic species reflect an increasing gradient of anthropogenic stress. The macroalgal biotic index (EEI-c) differed at some sites between the two survey periods as opportunistic seaweed increased abundance in spring, even at low stressed sites. This confirms the need to sample in summer to accurately assess the ESC of coastal waters using macroalgal-based indicators (Orfanidis *et al*., 2011). Spring assessments can produce the 'worst' ESC scores, but unlike summer assessments, they may not always be representative of anthropogenic stress.

As in many places around the world, a single human generation has transformed the coastline of Cyprus creating a heavily industrialised foreshore in Vasiliko Bay. Despite major alterations to the area, there had been no assessments on the marine ecosystem impacts of these developments. High ESC was assessed in other coastlines of Cyprus monitored for WFD and MSFD (Stavrou & Orfanidis, 2012). In this study, low ESC was assessed along industrialised coastlines where artificial breakwaters and coastal hardening had modified the shores. There was likely a combination of several impacts such as contamination from ports, cement dust deposition, litter, warm water from a power station, brine from a desalination unit and possibly waste effluents from fish farms operations. Major industrial developments are still underway in Vasiliko Bay, in 2017 land reclamation decimated the last remnant of *Cystoseira* habitat in the eastern side of the bay. It is recommended that *Cystoseira* forests receive more attention when coastal developments are evaluated in Cyprus. The baseline data on macroalgal communities in

Cyprus will allow future comparisons and ecological assessments in the region. The bad ESC scored along the modified, industrial coastline should alert those responsible for managing the use of coastal marine resources in Cyprus as attempts may be needed to meet the obligations of the European MSFD.

In summary, it is not too late to conserve *Cystoseira* forests by raising public awareness and mitigating human impacts on coastal ecosystems (Gianni *et al*., 2013). The disappearance of these fucoid forests leads to systems with lower biodiversity and reduced ecosystem services to humanity (Chapin *et al*., 2000; Cardinale *et al*., 2012). Shallow reefs around parts of Cyprus are still covered with luxuriant *Cystoseira* forests, but this habitat is threatened by coastal developments. At the industrialised and modified forefront of Vasiliko Bay, the *Cystoseira* canopy was diminished and was replaced by opportunistic algae that had lower biomass per unit area. Ecological assessments with a macroalgal WFD index EEI-c, showed that the coastal waters in this side of the bay, may not meet the quality targets of European Directives. Despite the ecological degradation, industrialisation is expanding, and construction has begun for a booming hydrocarbon industry, now that large gas reserves have been located. Furthermore, there are approved government plans to construct a new port to serve fish farmers, near the dense *Cystoseira* forests west of Vasiliko Bay. As pressures continue to mount, it remains to be seen whether the MSFD will be applied to ensure that marine resources are managed sustainably in Cyprus.

5. BASELINE CHARACTERISATION OF VASILIKO BAY SEDIMENTS AND CONDITION OF THE SEAGRASS *POSIDONIA OCEANICA*

5.1 Abstract

Vasiliko Bay in southern Cyprus, is affected by multiple and mounting anthropogenic pressures. Most of the national mariculture production concentrates in the western side of the bay. The coastline on the eastern side of the bay has been modified and is heavily industrialised with ports, a power station, a desalination unit, cement factory and a major new oil terminal and gas storage facilities, while further development plans are underway as the area has been earmarked by the government to create the largest hydrocarbon processing, storing and transport centre in the eastern Mediterranean. This study aimed to carry out the first scientific investigation to characterise the sediments and *Posidonia oceanica* seagrass. The meadows were mapped in high resolution and seagrass structural descriptors such as shoot densities from a total of 500 quadrats (0.16 m^2) and leaf morphometric analyses from a total 700 shoots were conducted in 2012 and 2013 at 30 sites spread across the bay. An extensive ancient seagrass meadow was revealed, covering about 200 ha across 10 km of coastline over soft substratum at *ca* 10-30 m depth and over hard substratum at *ca* 0-6 m depth. Across all sites, depth was the most important driver of shoot density and foliar surface area. Across the shallow sampling sites, these descriptors of *P. oceanica* were lower within the bay near the industry. Across the intermediate depth sampling sites, heavier epiphytic biomass, lower leaf number, mass and foliar surface area were measured in the western side, which may indicate eutrophication and light attenuation even at large distances from fish farming operations. In the eastern side of the bay part of the meadow was dredged to construct a marine jetty. Despite the heavy anthropogenic pressures, good ecological status was assessed with both

BiPo and PREI and the *P. oceanica* across heavily impacted Vasiliko Bay had among the highest shoot densities reported across the Mediterranean Sea. This raises implications for managers as coastal developments need to respect the protection afforded by European legislation and International Conventions. The findings from this understudied region increase our knowledge regarding the status of *P. oceanica* meadows across gradients of impact in its upper thermal and easternmost limits of its existence and demonstrate that even impacted seagrass meadows of Cyprus are perhaps among the most pristine of the entire Mediterranean.

5.2 Introduction

Seagrass meadows provide valuable ecosystem services but are facing a worldwide crisis as they are being lost at an unprecedented rate, with water quality and other localised stressors placing seagrass meadows among the most threatened ecosystems on earth (Orth *et al.*, 2006; Waycott *et al.*, 2009; Unsworth *et al.*, 2014). It is estimated that seagrasses are declining at a rate 7% year^{-1} globally (Waycott *et al.*, 2009). Seagrass and algal beds cover less than 0.5% of the global ocean but account for over 5% of the world's ecosystem services with an estimated value equivalent to estuaries and over five times larger than that estimated for tropical forests and cropland (Costanza *et al.*, 2014). Seagrasses are important candidates to partially mitigate anthropogenic CO_2 leakage, since they act as significant carbon sinks, forming natural hotspots for carbon sequestration (Kennedy *et al.*, 2010; Fourqurean *et al.*, 2012; Russell *et al.*, 2013; Mazarrasa *et al.*, 2015).

The endemic Mediterranean seagrass *Posidonia oceanica* (Linnaeus) Delile 1813, supports the highest carbon burial rates and forms the largest organic carbon stocks compared to ten other seagrass species (Lavery *et al.*, 2013). *Posidonia oceanica* sequestration rate can reach 1.09 TgCyr^{-1}, estimated to range from 71 to 273 kgCm^{-2}yr^{-1}, representing 11 to 42% of the CO_2 emissions produced by the Mediterranean countries since the beginning of the Industrial Revolution (Pergent *et al*, 2014). It forms structurally complex meadows which extend in oligotrophic clear waters to depths of 45 m; they cover a known area of 12,247 km^2 while almost half of the Mediterranean coastline, mostly from the southern and eastern basins, still remains uncharted (Telesca *et al.,* 2015). *Posidonia oceanica* plants, and the biogenic matte that their roots develop, stabilise the sediment, reduce wave power protecting the coastline from erosion, sequester nutrients and contaminants, oxygenate the water with up to 14 $Lm^{-2}d^{-1}$, transfer nutrients to food webs, and provide an essential habitat for many protected and commercial species contributing to fisheries and maintaining cultural values (Boudouresque *et al.,* 2012; Campagne *et al.,* 2015; Jackson *et al.,* 2015). The seagrass is an ecosystem engineer associated with distinct marine Mediterranean biocommunities. Its canopy and rhizomes are highly biodiverse with 660 species of epibionts reported in the literature (reviewed by Piazzi *et al.,* 2016). Hundreds more mobile species are associated with the habitat of *P. oceanica,* such as 122 fish taxa, 120 species of crustaceans, many echinoderms including 16 species of sea cucumbers, 171 mollusc species reported in the western Mediterranean alone and extremely diverse polychaete communities with a total (epifauna and infauna) of 394 polychaete species (annelids) reported from just the eastern Mediterranean (Díaz-Almela & Duarte, 2008; Çinar, 2013; Urra *et al.,* 2013; Zubak *et al.,* 2015). Also recorded in the meadows are many iconic and protected species such as the large bivalve *Pinna nobilis* Linnaeus, 1758, the giant gastropod *Tonna galea* (Linnaeus, 1758), the seahorse *Hippocampus hippocampus* (Linnaeus, 1758) and the turtle *Chelonia mydas* (Linnaeus, 1758).

Seagrasses have been recognized in the European Union WFD (2000/60/EC) as key coastal ecosystem engineers and identified as appropriate bioindicators of ecosystem quality (Marbà *et al.,* 2013). Recently, within the MSFD (2008/56/EC), *P. oceanica* has been selected as an indicator of GES for marine areas. Even though *P. oceanica* meadows are protected by Conventions ratified by most countries of the Mediterranean, such as the Berne and Barcelona Conventions, the European Habitats Directive (92/43/EEC) and the fishing legislation (council regulation 1626/94), the species is included in the IUCN Red List with large declines estimated at 34% in the last 50 years (Marbà *et al.*, 2014; Telesca *et al.*, 2015). Significant losses of *P. oceanica* have also been recorded around fish farms where seagrass meadows shift from carbon sinks to carbon sources (Pergent-Martini *et al.*, 2006; Apostolaki *et al.*, 2009). Regardless of the general decline, the meadows can remain relatively stable over time in some localities (Bonacorsi *et al.*, 2013; Guillén *et al.*, 2013). *Posidonia oceanica* seems to be more resilient to seawater temperature variations, most contaminants and the invasions of alien species, but it is sensitive to salinity fluctuations, turbidity and increase of sedimentation rate, anchoring and trawling (Pergent *et al.*, 2012). Large regression of this phanerogam in coastal areas is considered irreversible within human time-scales (Boudouresque *et al.*, 2009). Even if optimal conditions return, the area will still require centuries to be recolonized as *P. oceanica* is the slowest growing seagrass species in the world (Duarte, 2002; Kendrick *et al.*, 2005).

Despite the regression of *P. oceanica* reported around the Mediterranean, little is known regarding the presence of *P. oceanica* in the warm, salty and oligotrophic Levantine basin (eastern Mediterranean), somewhat limited by the uncertainty on the current distribution of the species in the eastern basin (Telesca *et al.*, 2015). It has not been reported from the coasts of Syria, Lebanon and Israel, eastern Egypt and eastern Turkey and it has been suggested that the high temperature and salinity, or increase in turbidity due to heavy anthropogenic disturbance on the coast and river discharges, are limiting its distribution in the Levantine basin (Celebi *et al.*, 2006). Cyprus forms a phenomenal exception to this pattern. Despite the fact that it is located in the heart of the Levantine basin, with surface waters in the warm period near the maximum temperature tolerance limit reported in the literature for this species (Celebi *et al.,* 2006; Marbà & Duarte, 2010; Telesca *et al.,* 2015), *P. oceanica* occupies the upper subtidal (depths ranging from 0 to >40 m) around the perimeter of the whole island representing the easternmost reported population of the species. It is estimated that *P. oceanica* beds cover an area of 9,040 ha in about 30% of the Cyprus coastline surveyed (Telesca *et al.,* 2015). The explanation for this paradox may lie in the gene pool of the *P. oceanica* meadows, which seems to be isolated from those in the western and central Mediterranean by having less affinity and a higher number of unique alleles (Arnaud-Haond *et al.,* 2007; 2010; Serra *et al.,* 2010; Chefaoui *et al.,* 2017). Ancient and genetically isolated meadows surrounding Cyprus may be more tolerant to warming than the remaining populations around the Mediterranean. Other factors contributing to the enigmatic distribution of *P. oceanica* around Cyprus may be the occasional small upwelling summer events that cool surface waters of south Cyprus, the lack of permanent rivers and the presence of reservoirs that minimise the inflow of murky freshwater in coastal water bodies, and the relatively low anthropogenic and industrial footprint at the majority of the island's coastline.

However, ancient seagrass meadows in many areas around the island are threatened by changes in water quality induced by human pressures such as urbanisation and coastal modification, fish farming, dredging and others. In only a few years, several km of the coastline around the island of Cyprus have been transformed. Vasiliko Bay is the most industrialised coastline of Cyprus, affected by multiple anthropogenic pressures, and with further developments underway. Due to the high socioeconomic benefits associated with this heavy industrialisation and the multi-industry services that the bay offers, there is a general tendency to downplay the impacts of this development on priority and protected marine ecosystems, which receive less weight in managers' decisions. In Vasiliko Bay, the seagrass *P. oceanica* forms ancient meadows that extend down to a depth of approximately 30 m. Fish farms in the western side of the bay have operated for many years above seagrass beds, possibly inducing local irreversible regressions; the farms gradually expanded in deeper waters. On the other side of the bay, industrial pollution and repeated dredging of different parts of the meadow to position pipelines, construct or enlarge ports, marine jetties and berths to accommodate large vessels pose a real and growing threat to the highly biodiverse *P. oceanica* meadows.

It is important that the distribution and ecological status of this priority habitat is considered and integrated into Vasiliko's industrial planning and management. This study carried out in 2012-2013 aimed to describe physicochemical parameters in the sediments across the bay, to present the first scientific survey to map the geographic distribution of *P. oceanica* in high resolution and to define basic descriptors of *P. oceanica* meadows such as shoot density, leaf biometry and epiphytic biomass. The forcing drivers that correlate with the variability of *P. oceanica* descriptors were identified and the ecological status of Vasiliko Bay assessed using *P. oceanica* as a biological quality element and WFD biotic indices. Lastly, due to the oligotrophic nature of Cyprus coastal waters, which also define the upper thermal limits of *P. oceanica* it was reviewed whether *P. oceanica* descriptors are different compared to other sites around the Mediterranean.

5.3 Materials and Methods

5.3.1 Study area

Vasiliko Bay is a small semi-enclosed bay, with a central location in southern Cyprus (eastern Mediterranean) approximately 25 km east of the town of Limassol. The national mariculture industry is concentrated on the eastern side of the bay (seven out of nine licenced units) at approximately 1-3 km from the shore, with most of the active units expanding and contributing significantly to organic loading. The total annual licenced production of only the four farms operating near the Vasiliko area during 2013 was about 4 kT/yr, representing about two thirds of the total aquaculture production of Cyprus (Fig. 5-1).

The coastline in the central and eastern sides of the bay has been entirely modified and is heavily industrialised. In the central regions of the bay, the largest power plant of Cyprus operates using heavy fuel oil-fired steam and combined cycle gas turbine units supplied offshore, by a single point mooring at about 2 km from the coastline and two submarine pipes of 0.5 m in diameter. The largest cement manufacturing plant on the island operates in the eastern part of the bay, producing around 6 kT of clinker per day and has a clinker silo with a total capacity of 100 kT. There are three ports in the bay (Fig. 5-1). Vasiliko port on the eastern tip of the bay is a major importer/exporter of different cargoes including wheat, coal, perlite, cement, gravel, scrap iron and others. Archirodon is a small port nearby, which includes a dry dock for ship maintenance and repairs and a small shelter for fish farmers and fishing boats. Furthermore, there are active clay quarries, a recently constructed marine jetty, and facilities of various multi-national corporations for the storage and management of petroleum and oil products with a total storage capacity of storage of 858,000 m^3. Governmental plans for further development are underway in the framework of a Master Plan dedicated to the expansion of hydrocarbon-based heavy industries in the region. Large areas of land have been expropriated by the government for constructing a Liquefied Natural Gas (LNG) plant and new marine jetties and multiple marine berths capable of handling vessels up to Suez-max size that will transfer Liquefied petroleum gas (LPG), bitumen and other oil-based products are planned. More dredging is anticipated, over or near seagrass meadows to construct jetties and lay pipes, expand Vasiliko port and construct a new port west of Vasiliko Bay to accommodate the fish farmers of the area. Plans implemented at this moment include land reclamation west of Vasiliko port to construct fuel storage facilities. These huge projects have the potential to jeopardise the integrity of *P. oceanica* meadows, hence it is now more important than ever to increase awareness about the condition of this priority habitat and inform coastal zone management decisions.

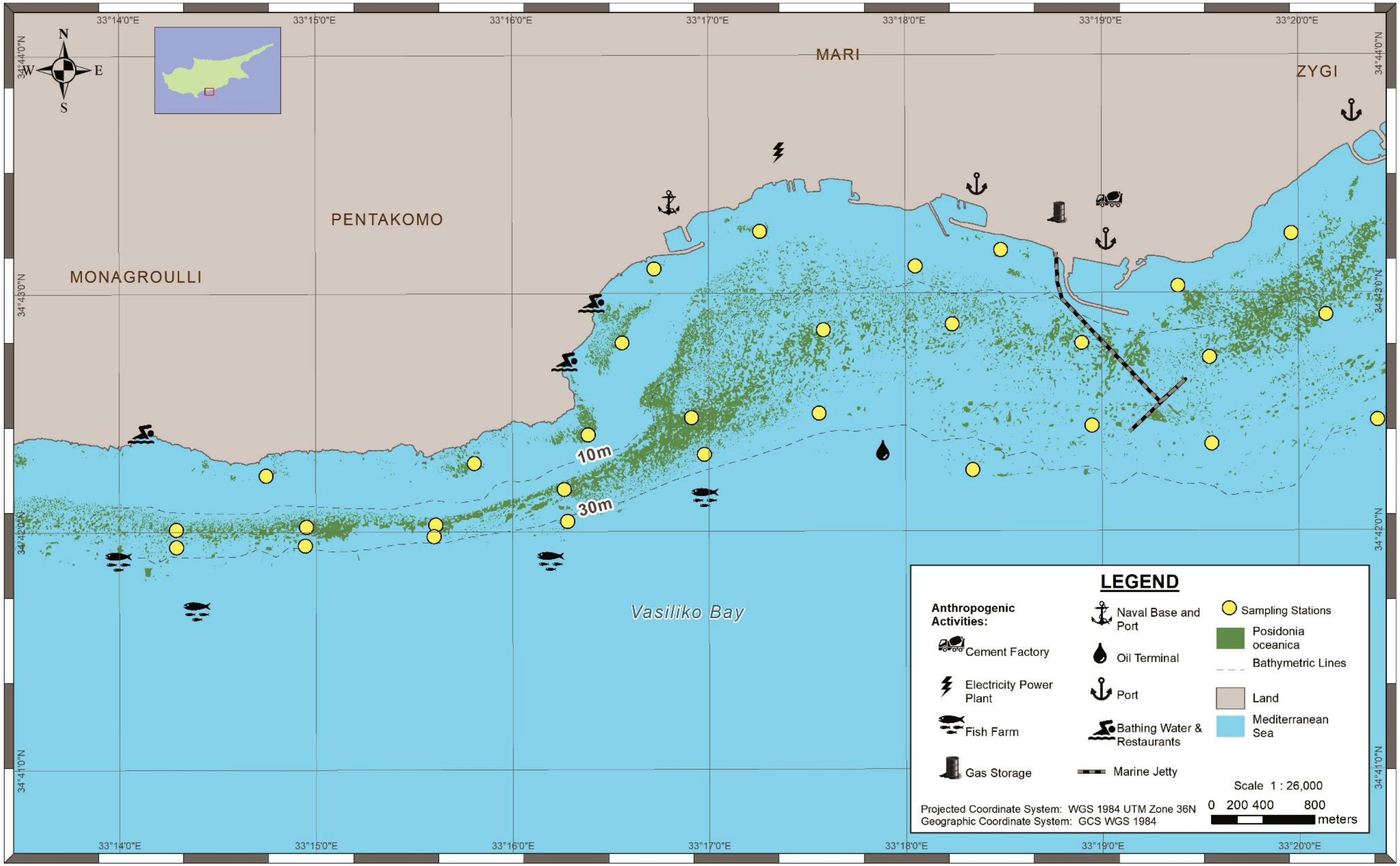

Figure 5-1. *Posidonia oceanica* spatial distribution, sampling sites and anthropogenic pressures across the study area.

5.3.2 Sediment characterisation

To describe important sediment parameters across the Vasiliko Bay, samples were collected in the summer of 2013, from 41 sites, in most cases spaced at 750 m intervals, forming equilateral triangular grids, except in the shallows, where the sampling sites were positioned around the anthropogenic activities on the coastline. The samples were obtained with a 0.1 m^2 Van-Veen grab and the pH was measured from the surface sediment (~ 5 cm), which was collected and kept frozen until analysis.

Afterwards samples were dried at 100 ^oC until constant weight and granulometry was conducted using an Endecotts Octagon sieve shaker for two replicates per site. The data were then processed with a particle size analysis software (GRADISTAT). Fine sediment passing through the 212 μm sieve was homogenised, five replicates of 1.5 g from each site were combusted at 550 ^oC and the organic content was determined as % weight loss following ignition. The particle size distribution and organic content were also determined from additional samples collected during the same period downstream of four fish farm units of the area (0, 50, 200 and 500 m from the cages).

Other chemical variables of the sediment that were determined include: (i) alkalinity expressed as % dry mass of calcium carbonate determined by titration with sulphuric acid (0.01N) (Limit Of Detection/LOD 0.1%); (ii) reactive ferrous iron (Fe^{2+}) determined by extraction with 1,10-phenanthroline/sodium acetate reagent deaerated with nitrogen and measured with a spectrophotometer at 510 nm (LOD 1 mg/kg); (iii) total iron determined by digestion in a microwave with concentrated acids and later analysed by inductively coupled plasma – optical emission sprectrometry (LOD 1 mg/kg); and three priority hazardous substances, namely: (iv) mercury determined by digestion in a microwave with concentrated acids and later analysed by inductively coupled plasma – optical emission sprectrometry (LOD 1 mg/kg); (v) hexachlorobutadiene and (vi) hexachorobenzene determined by extraction with n-Hexane in an ultrasonic bath and analysed by gas chromatography – mass spectrometry (LOD 0.01 mg/kg). The values obtained from the chemical analyses were processed with ArcGIS (v.10.2.2) software and interpolated using the algorithm of the 'Nearest Neighbour' to display heat maps across the whole area studied.

5.3.3 Seagrass mapping

Mapping was carried out to assess the distribution of the seagrass meadows from Zygi port to Ayios Georgios Alamanou in Limassol, covering an area about 10 km of coastline. Among the different methods used to map seagrasses, aerial photography and satellite imaging have been demonstrated to be efficient methods for mapping shallow meadows, while the use of a SSS appears to be a more powerful tool, particularly suitable for mapping deeper meadows (Belsher *et al.*, 1988; Pasqualini *et al.,* 2000; Piazzi *et al.,* 2000; Leriche *et al.,* 2006). These methods associated with Geographical Information System (GIS) analysis constitute an efficient tool for seagrass management (Douven *et al.*, 2003).

In Cyprus, the waters are oligotrophic with very low turbidity values. For mapping the upper subtidal from 0-15 m depth, IKONOS (1 m resolution, 2011) and GeoEye (0.5 m resolution, 2010) satellite images and higher resolution aerial images (2009-2010) from the Department of Lands and Surveys were georeferenced in UTM zone 36N, compared, and the aerial images that had the largest resolution were selected to digitise the shallow *P. oceanica* meadows with the ArcGIS software. For mapping the depth zone over 15 m, a SSS C-MAX system was used, composed of the CM2 digital towfish equipped with a wing depressor, sonar transceiver unit, depth sensor, tow cable, electric winch and connected computer. During 2012-2013, the fish was towed at slow and steady 5 knot speed in transect lines (at 25 or 50 m intervals) parallel to the coastline with complete overlapping of coverage in each transect. Some small areas in the vicinity of the fish farms with obstacles in the water column (e.g. ropes and chains) were not mapped. Data obtained from the digital towfish were processed in SonarWiz 5 software which included bottom tracking, setting the LBK algorithm to account for the sheave offset, georeferencing in UTM zone 36N, and producing a mosaic of the mapped area, which allowed the digitisation of *P. oceanica* meadows with ArcGIS. Several features (sand, matte, living *P. oceanica* and rocky reefs) were selected and confirmed with point dives.

5.3.4 Assessment of *P. oceanica* meadows

To assess the general *P. oceanica* condition, the seagrass was monitored at 30 sites spread across the area of study at different depths (Figure 5-1). Ten sites were set up at each depth zone: (i) shallow 5 m depth, (ii) intermediate depth 15 m and (iii) at the lowest limits of the meadows, which ranged between 22 – 33 m. The sites at intermediate 15 m depth and lower limits were set at about 1 km intervals. At the shallows, the 5 m sites were set across different patches spread out at uneven distances. The 5 m and 15 m sites were sampled twice, while the lower limits were sampled one time (Table 5-1).

5.3.4.1 Shoot Densities

Shoot densities were determined within 0.16 m^2 quadrats, that were consistently placed at about 1.5 m from the edge of the meadow investigated. In total, 500 quadrat measurements were recorded from all the 30 sampling sites; 20 replicate measurements from each of the sites of 5 m and 15 m depth and 10 from the lower limit sites were transformed to shoot density m^{-2}. At the lowest limits, the proportion of horizontal (plagiotropic) shoots was determined to enable the assessment of the type of lower limit (Montefalcone, 2009).

Table 5-1. *Posidonia oceanica* sampling sites, geographical location, time of sampling and replicate numbers.

Depth (m)	Number of Site	Coordinates		Time of Sampling	Total number of quadrat (0.16 m^2) measurements	Total number of shoots analysed
		Latitude	Longitude			
5 ± 1	1	34° 42.237'N	33° 14.747'E	Autumn 2012, Summer 2013	20	20
	2	34° 42.288'N	33° 15.806'E		20	20
	3	34° 42.408'N	33° 16.385'E		20	20
	4	34° 42.793'N	33° 16.560'E		20	20
	5	34° 43.104'N	33° 16.722'E		20	20
	6	34° 43.261'N	33° 17.261'E		20	20
	7	34° 43.113'N	33° 18.050'E		20	20
	8	34° 43.181'N	33° 18.486'E		20	20
	9	34° 43.029'N	33° 19.387'E		20	20
	10	34° 43.249'N	33° 19.964'E		20	20
15 ± 1	1	34° 42.010'N	33° 14.290'E	Autumn 2012, Spring 2013	20	40
	2	34° 42.022'N	33° 14.951'E		20	40
	3	34° 42.030'N	33° 15.610'E		20	40
	4	34° 42.178'N	33° 16.263'E		20	40
	5	34° 42.479'N	33° 16.912'E		20	40
	6	34° 42.846'N	33° 17.583'E		20	40
	7	34° 42.870'N	33° 18.239'E		20	40
	8	34° 42.790'N	33° 18.898'E		20	40
	9	34° 42.730'N	33° 19.547'E		20	40
	10	34° 42.908'N	33° 20.140'E		20	40
25.2	1	34° 41.938'N	33° 14.291'E	Autumn 2012, Autumn 2013	10	10
27.2	2	34° 41.942'N	33° 14.945'E		10	10
27.8	3	34° 41.977'N	33° 15.607'E		10	10
32.4	4	34° 42.045'N	33° 16.279'E		10	10
26.1	5	34° 42.329'N	33° 16.970'E		10	10
27.2	6	34° 42.497'N	33° 17.560'E		10	10
32.5	7	34° 42.258'N	33° 18.341'E		10	10
22.3	8	34° 42.443'N	33° 18.948'E		10	10
23.5	9	34° 42.367'N	33° 19.558'E		10	10
30.3	10	34° 42.467'N	33° 20.401'E		10	10

5.3.4.2 Leaf Morphometrics and Epiphytes

Seagrass morphometric analysis was conducted using the technique of Giraud (1977), on a total of 700 shoots collected using a non-destructive approach (Gobert *et al.*, 2012). Observations such as presence of epiphytes presence, herbivory marks, necrosis were noted for 20 (10 x 2 samplings), 40 (20 x 2 samplings) and 10 orthotropic shoots from each sampling site at the 5 m, 15 m and lower limits, respectively. The foliar surface and the Leaf Area Index (LAI) was calculated for all sites. The LAI expresses the total surface area (m^2) of *P. oceanica* canopy in 1 m^2 of benthic surface (LAI = mean foliar surface area shoot^{-1} x mean shoot density m^{-2}). The LAI values were interpolated using the algorithm 'Nearest Neighbour' ArcGIS (v.10.2.2) software and a heat map was generated.

The ratio of epiphyte to leaf dry mass was estimated for a total of 400 *P. oceanica* shoots collected from the 15 m depth sites. The epiphytes were gently scraped off from the leaves with a scalpel and placed on dry pre-weighted Whatman GF/F. Leaves and epiphytes from each shoot were placed in a drying oven at 60 ^{o}C until constant weight.

5.3.4.3 Ecological Status Class

The ESC was assessed with WFD (2000/60/EC) seagrass indices PREI (Gobert *et al.*, 2009) and BiPo (Lopez y Royo *et al.*, 2010a), applied in both seasons (autumn 2012 and spring 2013) at each of the ten pairs (15 m depth and lower limit sites), set at about 1 km intervals. The metrics that were used in the calculations included: (i) depth at the lower limits of the meadow, (ii) characterisation of the meadow type at the lower limit, (iii) shoot density at 15 m depth (mean of both seasons), (iv) foliar surface shoot^{-1} at 15 m depth (seasonal mean) and (v) dry weight Epiphyte/Leaf (E/L) at 15 m depth for PREI only (seasonal mean). The reference conditions used in the calculation of the ESC scores were: (i) those suggested by Lopez y Royo et al. (2010a) for BiPo and applied in the central and western Mediterranean, (ii) the reference values set by the national authority of Cyprus for PREI following the intercalibration MedGIG exercise and (iii) the reference conditions adapted for the area of study and the time of sampling, set for the shoot density and foliar surface shoot^{-1} as the average of the three highest values (after the highest) measured during the survey (Gerakaris *et al.*, 2017). For E/L dry weight the reference was set at zero considering that healthy seagrass leaves have minimal epiphytic biomass (Gobert *et al.*, 2009), and for the lower limit, the reference depth was set at 33 m determined from the mapping with SSS and confirmation dives (for reference condition values used, see Table 5-3 in Appendix 2).

5.3.5 Statistical analyses

The spatial differences in the *P. oceanica* descriptors across sampling sites were identified by comparing sites of the same depth (5 m and 15 m) using a 1-way ANOVA, followed by a Tukey's honestly significant difference (HSD) test. The importance of season and the location of the sites on the development of epiphytes on *P. oceanica* meadows was examined via a two-way ANOVA using the E/L dry weight descriptor. To determine whether the two biotic indices (BiPo and PREI) follow the same pattern across sites, averaged EQR seasonal results were tested using a Pearson's Correlation analysis. For all the parametric tests, the assumptions for normality and equal variances were verified using a Shapiro-Wilk test and Bartlett test, respectively. When assumptions were violated, $\log_{10}$ or square root transformations were conducted, depending on the skewness of the residuals. In the case of 1-way ANOVA analyses, when assumptions were not met, the analysis proceeded with the non-parametric Kruskal-Wallis rank sum test, followed with a post-hoc Dunn's test with Bonferroni correction, to locate the pairwise differences. For all the statistical analyses the significance level α was set at 0.05, and their computation was carried out by R (v 1.0.153). For the Dunn's test, the "Dunn.test" package was used (Dinno, 2017) and graphical output was generated via the package ggplot2 (Wickham, 2016).

Redundancy (ordination) analysis (RDA) was further carried out to identify trends and general patterns that would explain the variation of the *P. oceanica* descriptors (shoot density, number of leaves, foliar surface, E/L biomass, herbivory and coefficient A) observed across the sites against proxies of anthropogenic pressures, depth and time. The analysis consisted of three runs, where the first included the *P. oceanica* descriptors collected from all the sampling sites (shallow, intermediate and lower limits). This analysis did not include time, since lower limits were sampled only once. The second run included only the shallow sites (5 m), and the last run only the sites from the intermediate depth (15 m). The proxies of anthropogenic pressures comprised: 1) number of anthropogenic pressures within 1 km radius from each site, 2) distance to the nearest anthropogenic pressure, 3) direction from the nearest anthropogenic pressure (excluded from the first part of the analysis) and 4) geographical position within the bay (Bay area) defined by regions of anthropogenic pressure gradient. In the case of shallow sites, the pressure gradient was defined as outer bay to inner bay (low to high pressure), where the latter has the highest cumulative land-based anthropogenic pressures; intermediate depth as western to eastern bay (high to low pressure), where western side hosts almost all of the fish farm units; the whole study area as western, central and eastern bay. The RDA was conducted using the "vegan" package (Oksanen *et al.,* 2018) in R-studio (version 3.5.0). Prior to computation, all *P. oceanica* descriptors were log (1+x) transformed. When a right angle between a *P. oceanica* descriptor and predictor (arrow) was formed on the RDA plot, a zero correlation was assumed, and when it was lower than 20°, a strong correlation was implied. If a descriptor was found at ~180° from the predictor, it was then interpreted as strong negative correlation. RDA was further complemented with two permutation ANOVA tests including: 1) a test to identify the significance of all the predictors together, 2) type III test (permutation = 500) to analyse the marginal effects when each predictor was eliminated from the model while the others remained. This allowed to identify which of the predictors had a significant role in the variance of the model.

5.4 Results

5.4.1 Sediment characterisation

The range and mean ± standard error of sediment metrics across 41 sites are summarised below and extrapolated as heat-maps (Fig. 5-2). The pH in the surface sediment ranged between 7.01 - 8.12 and averaged at 7.45 ± 0.03. The organic content of surface sediment ranged from 1.86% to 11.12% and averaged at 5.44 ± 0.40%. Most organic enriched sediments were detected near the fish farms and ports. Based on the average particle size of the sediment grains (Folk and Ward method), the sites are characterized by very coarse silt, fine sand and very fine sand. Mean particle geometric diameter ranged between 52 to 483 μm, fine sand was more prominent in the deeper sites, particularly near the fish farms in the western side of the bay and in the shallow sites near the ports. Values for carbonates ranged between 0.1 and 33.1% and averaged at 12 ± 1.05%. The highest alkalinity values were determined near the fish farms. Ferrous iron ranged between 16845 – 84411 mg/kg and averaged at 52376 ± 2222 mg/kg. Total iron was slightly higher, and the highest concentrations were measured at sites located near the coastline at the central-eastern side of the bay. Mercury was often below the detection limits of 1 mg/kg. Concentrations above 1 mg/kg were detected at five sites in the central-eastern side of the bay, with the highest near the outflow of the power station. Hexachlorobutadiene ranged between 0.01 – 0.46 mg/kg and averaged at 0.09 ± 0.02 mg/kg. The highest concentration was measured in the centre of the bay. Hexachlorobenzene ranged between 0.01 – 0.28 mg/kg and averaged at 0.03 ± 0.01 mg/kg. The highest concentration was measured near the naval base, being over four times higher compared to the rest of the samples.

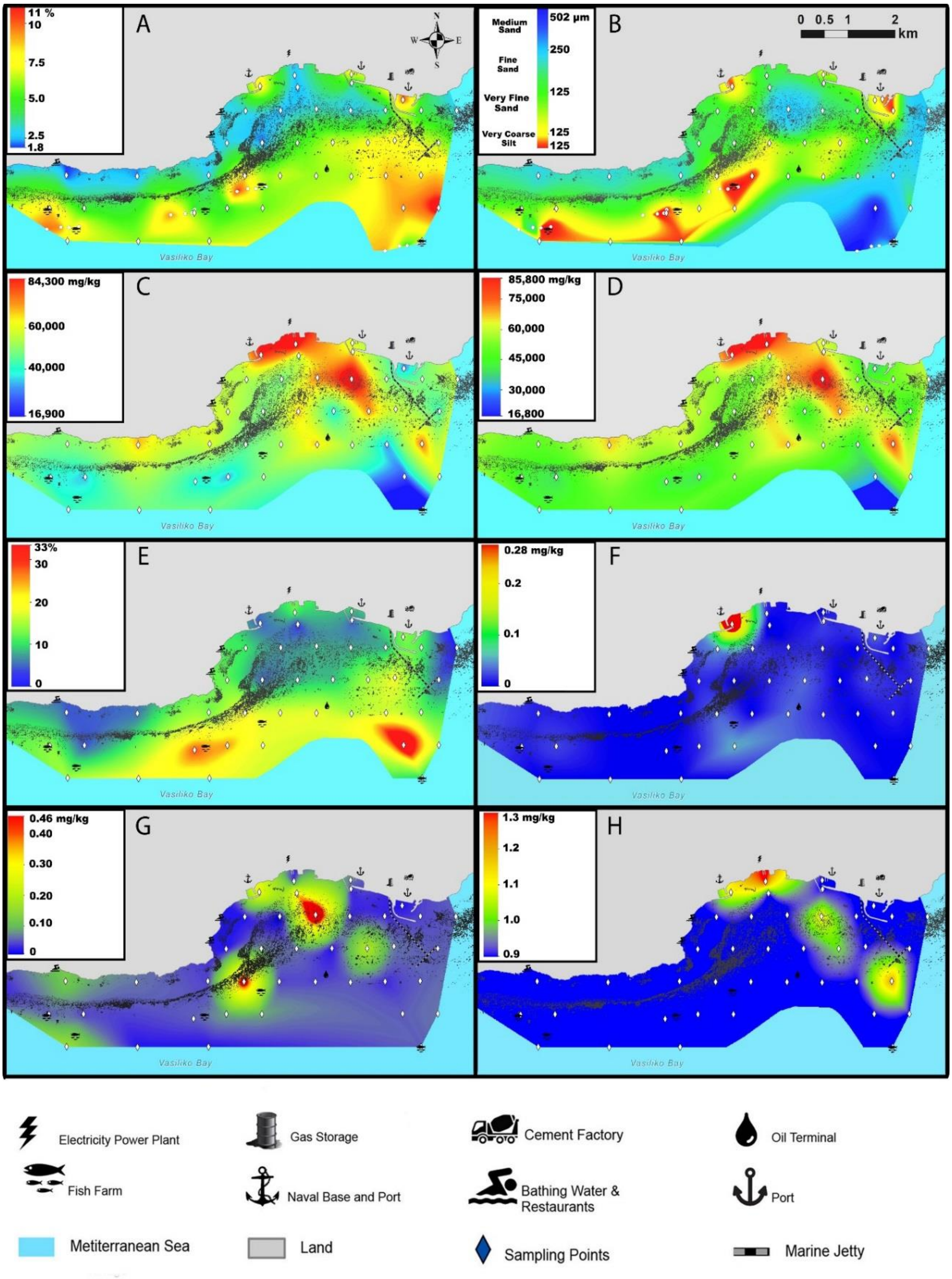

Figure 5-2. Concentration heat maps of sediment chemical variables. A: mean organic matter OM (%), B: mean geometric granule size (µm), C: ferrous iron Fe^{2+} (mg/kg), D: total iron Fe (mg/kg), E: alkalinity $CaCO_3$ (%), F: hexachlorobenzene C_6Cl_6 (mg/kg), G: hexachlorobutadiene C_4Cl_6 (mg/kg), H: mercury Hg (mg/kg). Maps produced in ArcGIS using interpolations of the algorithm ‘Nearest Neighbour’.

5.4.2 Mapping and visual observations

The mapping revealed an extensive seagrass meadow covering about 192.2 ha, along the 10 km of coastline that was scanned (Fig. 5-1). About three quarters of this seagrass canopy existed at the depth zone 10-30 m. The depth of the lower limits of the seagrass meadows across the ten deepest sampling sites ranged from 22 to 33 m. The most shallow lower limits were detected across Vasiliko port and near the marine jetty that was recently constructed. In the shallows, the seagrass occupied only some sections of the hard limestone, which is the dominant substrata in the intertidal and upper subtidal and extends offshore up to 250 m from the coastline and 6 m maximum depth.

In the shallow and some of the intermediate depth sites, the phanerogam had a dense and healthy canopy with a few calcareous leaf epibionts. A well-developed diverse coralligenous community dominated by rhodophytes and bryozoans was more pronounced under the dense canopy of some sites (e.g. shallow site 2), but absent from the sites near the industry. The seagrass had dense shoots and long leaves with few epiphytes at the 5 m sites. At the western sites of intermediate depth and lower limits, near the fish farms, the meadows appeared influenced with significantly higher epiphytic vegetation and fine particulates covering the canopy (Fig. 5-3). At the intermediate 15 m depth sites, the seagrass forms continuous meadows and the matte is often >3 m vertical height. The dead matte of the seagrass meadows was more common at some intermediate and all deeper sites around the perimeter of fish farms and the Vasiliko port and was usually covered by the chlorophytes *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809 and *Caulerpa cylindracea* Sonder, 1845 and turf algae.

Dredging works for constructing the marine jetty in the eastern parts of Vasiliko Bay coincided between the two annual sampling periods and irreversible impacts were observed on the underlying meadows of sampling sites 8 and 9 at 15 m depth (Fig. 5-4).

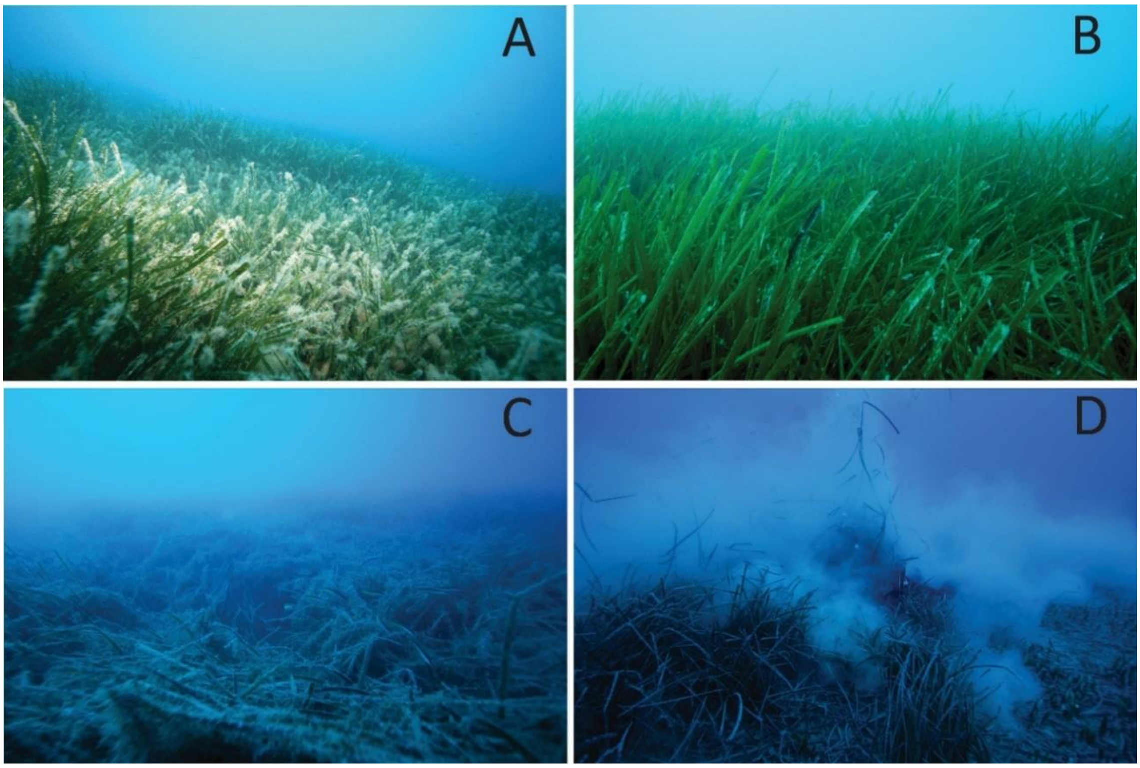

Figure 5-3. A: Site 4 at 15 m across a fish farm, *Posidonia oceanica* covered with epiphytes. B: Site 8 at 15 m, *P. oceanica* leaves had only a few calcareous epibionts (photograph taken on same day as A). C-D: Large biomass of epiphytic vegetation on the leaves of *P. oceanica* and presence of fine particulate matter at the deeper sites near the fish farms.

Figure 5-4. A: Site 8 at 15 m, detached shoots due to abrasion by chains and anchors of the platform used to construct the marine jetty. B: Site 9 at 15 m, what remained of a healthy *Posidonia oceanica* meadow following dredging. C-D: Site 9 at 15 m, large pieces of living matte ripped off and laying sideways or upside down.

5.4.3 *Posidonia oceanica* structural descriptors

The mean values ± SE of all *P. oceanica* structural descriptors measured at the 30 sampling sites are presented in Table 5-4 in Appendix 2.

Large variation in shoot densities was measured among the 5 m depth sites (Kruskal-Wallis rank sum test, $\chi^2 = 61.84$, df = 9, $p < 0.05$). The highest mean densities exceeding 900 shoots m^{-2} were reported at the sampling sites outside the bay area (Fig. 5-5). At the 15 m depth sites, the mean shoot density ranged between 395 and 478 shoots m^{-2} and varied across the sites (1-way ANOVA, F = 2.88, df = 9, $p < 0.05$). Measuring the shoot density at site 9 in spring 2013 was not possible because the *P. oceanica* meadow studied in the autumn of 2012 was dredged for the construction of the marine jetty. The mean shoot density at the lower limit sites ranged between 116 – 345 shoots m^{-2} (Table 5-4 in Appendix 2). A low ratio of plagiotropic rhizomes (<30%) was measured at the sampling sites in the deeper sites. No comparisons among the lower limit sites were performed because the depth and light variables were not constant.

The mean number of leaves $shoot^{-1}$ at 5 m depths differed significantly across sites (Kruskal-Wallis, $\chi^2 = 31.83$, df = 9, $p < 0.05$). Differences across the sites were also observed in the mean number of leaves $shoot^{-1}$ at 15 m (Kruskal-Wallis, $\chi^2 = 54.9$, df = 9, $p < 0.05$), which ranged from 5.4 to 7.3 (Fig. 5-5). The overall mean of all shoots collected was 6 leaves $shoot^{-1}$.

Both 5 m and 15 m depth sites showed inter-variation in foliar surface per shoot (1-way ANOVA, $F_{5m} = 3.74$, $F_{15m} = 8.11$, df = 9, $p < 0.05$). At the shallow sampling sites (5 m), the mean foliar surface per shoot ranged from 213 - 313 cm^2, whereas at the sampling sites of intermediate depth (15 m) it ranged from 147 - 225 cm^2 (Fig. 5-5). At the lower limits, mean foliar surface area values ranged from 102 to 162 cm^2 (Table 5-4 in Appendix 2).

About ten times higher LAI values were recorded at shallow sampling sites compared to the deeper boundaries (Fig. 5-6). The highest values (>21-27 m^{2} canopy m^{-2}) were reported at the 5 m depth sites outside the Vasiliko Bay area. At the 15 m depth sites mean LAI ranged from 5.86 - 10.01 m^{2} canopy m^{-2}. At this depth the lowest values were measured at sites 4 and 5, which are nearest to fish farms. The mean LAI values at the lower limit sites ranged from 1.46 to 4.90 m^{2} canopy m^{-2} (Table 5-4 in Appendix 2).

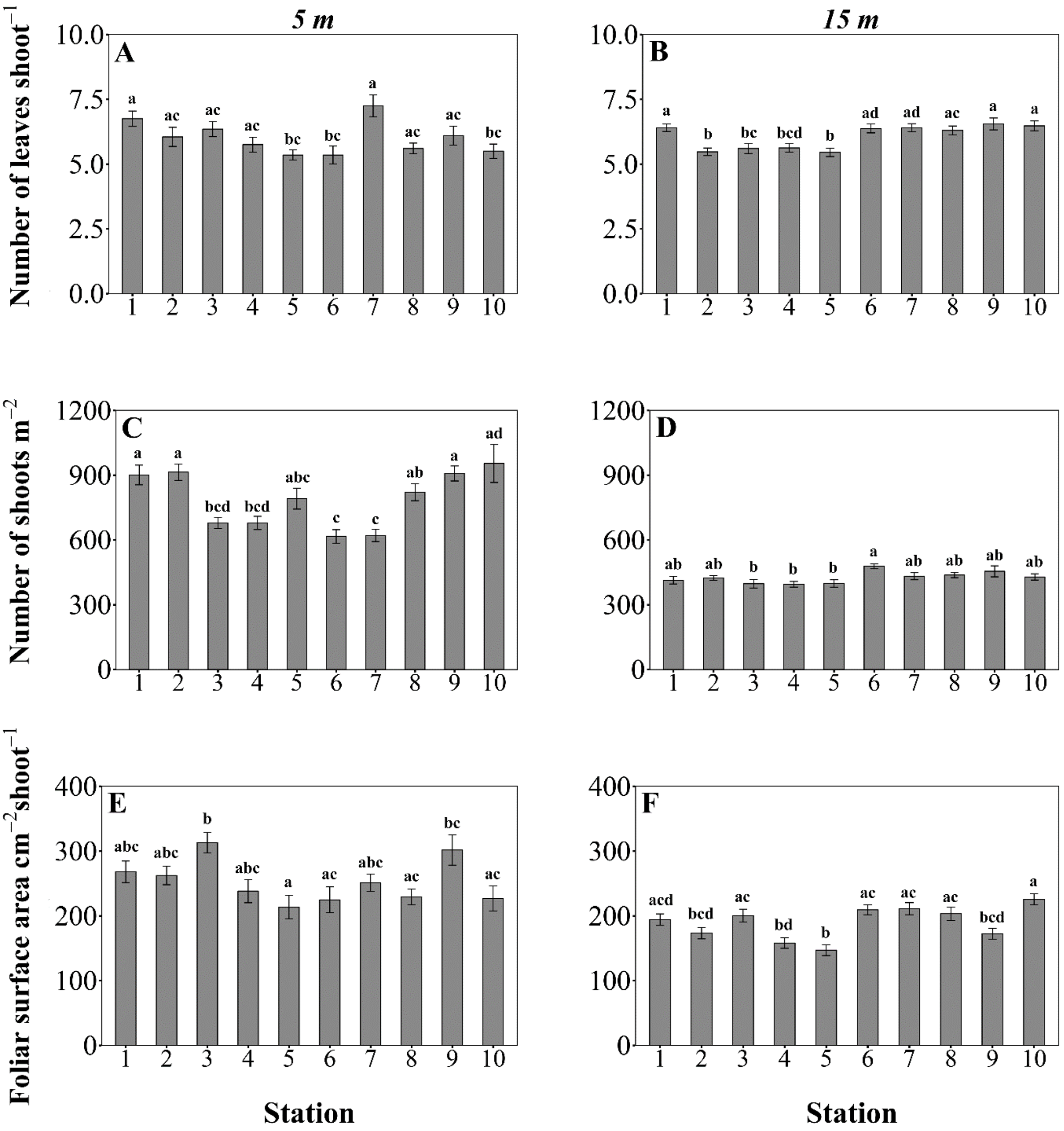

Figure 5-5. *Posidonia oceanica* descriptors at 5 m and 15 m depth sampling sites. The mean number of leaves and foliar surface were calculated from 20 and 40 shoots for the 5 m and 15 m depth sites, respectively. The mean shoot density was calculated from 20 replicate measurements in 0.16 m^{2} quadrat. The error bars represent the SE. Sites within each panel that do not share a letter are significant different at $p < 0.05$. Pairwise comparisons in panels B, E and F were generated by Tukey's (HSD) post-hoc test and for panels A, C and D by Dunn's post-hoc test with Bonferroni correction.

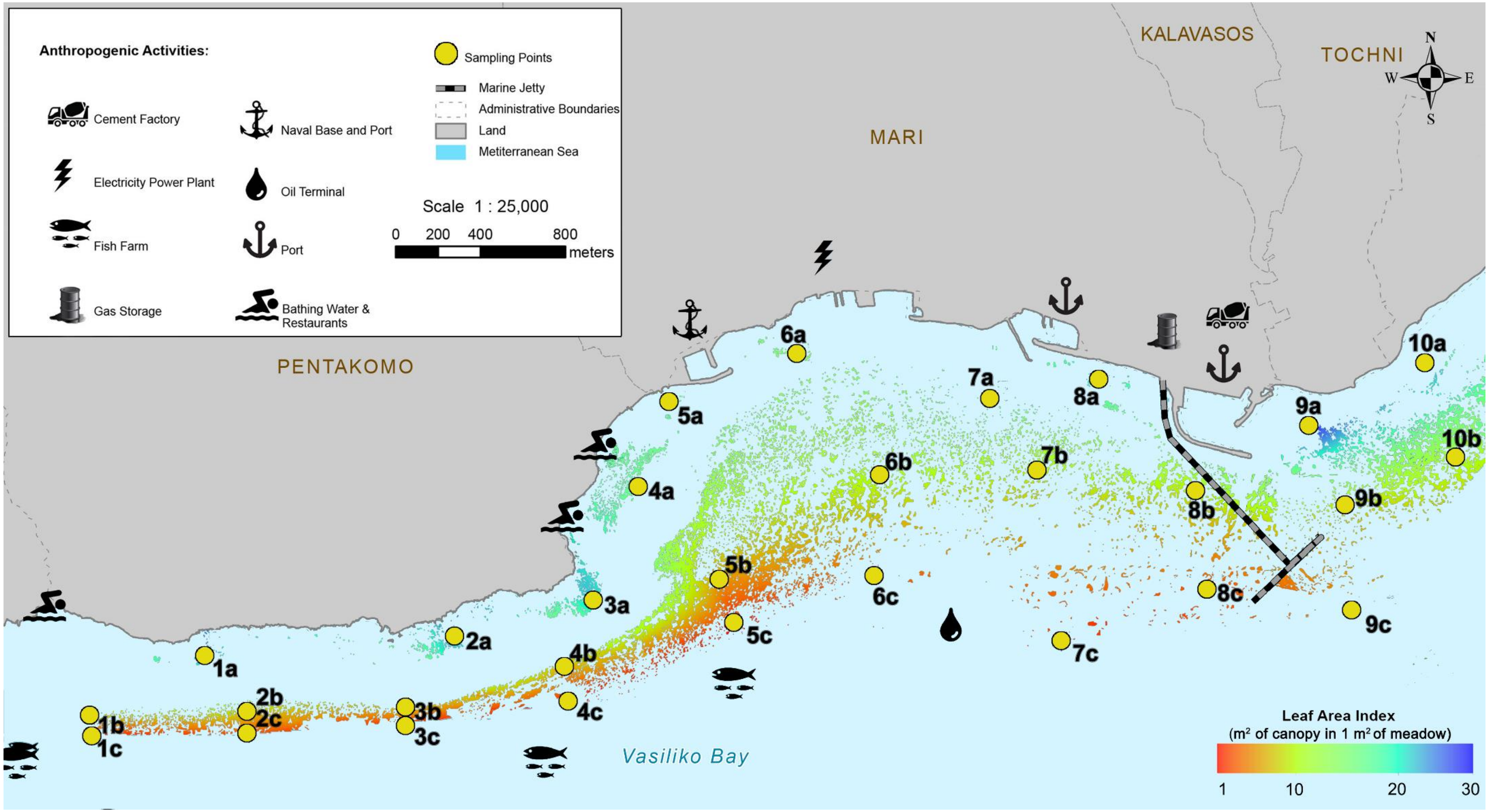

Figure 5-6. *Posidonia oceanica* Leaf Area Index/LAI (m^2 of seagrass canopy in 1 m^2 of seagrass meadow) heat map interpolated using the algorithm of the 'Nearest Neighbour' using the mean LAI values of all sampling sites. High LAI scores are reflected in blue colour. The shallow sites at 5 m depth, intermediate at 15 m depth and deep sampling sites at the lower limits of the seagrass meadows are represented by a, b and c, in respective order.

The dry mass of epiphytes and leaves of *P. oceanica* was estimated only for the sampling sites of intermediate 15 m depth (Table 5-5 in Appendix 2). Confirming the visual observations, E/L varied across the Vasiliko Bay sites, both in spring (Kruskal-Wallis, $\chi^2 = 125.67$, df = 9, $p < 0.001$) and summer (Kruskal-Wallis, $\chi^2 = 139.40$, df = 9, $p < 0.001$) (Fig. 5-7). There was a significant interaction between the main effects (station location and season) (two-way ANOVA, F = 11.87, df = 1, $p < 0.05$). Several sites had higher E/L being in spring (overall mean epiphytic biomass 0.124 g shoot^{-1}) than in the autumn (overall mean epiphytic biomass 0.060 g shoot^{-1}) (Fig. 5-7).

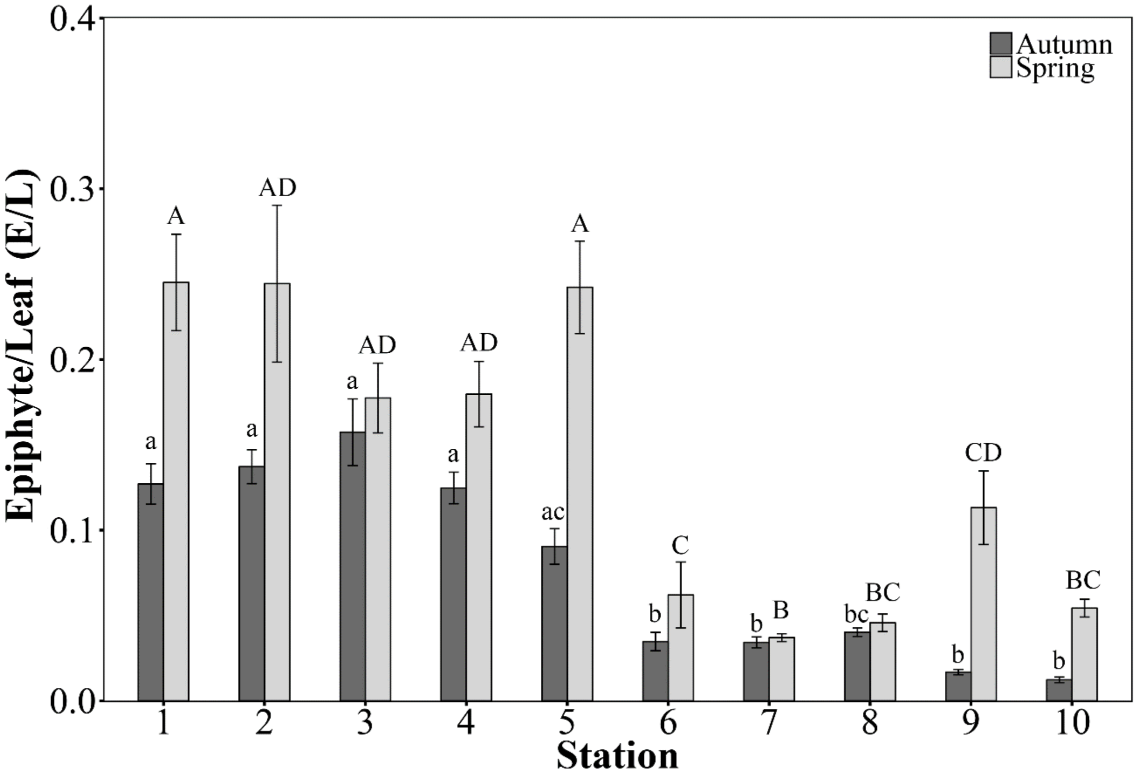

Figure 5-7. Seasonal mean Epiphyte to Leaf dry weight ratio (E/L) calculated for 20 shoots collected from each site at 15 m depth. The error bars represent the SE. Sites from each season that do not share a letter are significant different at $p < 0.05$. Pairwise comparisons are generated by Dunn's post-hoc test with Bonferroni correction.

5.4.4 Main drivers affecting *P. oceanica* descriptors

Vasiliko Bay shows heterogeneous responses of *P. oceanica* descriptors attributed to both abiotic variation and anthropogenic pressure. The major driver within the whole area of study was depth, as it explained most of the variation in the dataset (F=96.7, $p <$

0.01) and showing a strong negative correlation with shoot density and foliar area (Fig. 5-8). No clear patterns were detected with number of leaves, adult leaves that had herbivory marks or that had broken apex (coefficient A), although they might be influenced by a combination of factors including distance to the nearest pressure, depth and the pressure gradient (bay area) (Fig. 5-8).

At shallow sites (5 m), the most controlling factors were the direction of station from the anthropogenic pressure (F=8.54, $p < 0.01$), time (F=16.87, $p < 0.01$) and bay area (F=22.36, $p < 0.001$). The first factors affected foliar surface area, which increased from autumn 2012 to summer of 2013, but was lower at sites located southwestern from the industrialised forefront (Fig. 5-8). Moreover, the bay area seemed to influence the shoot density substantially. All sites located within the bay were characterised by lower shoot densities than the outer bay sites that have little anthropogenic influence (Fig. 5-5 and 5-8).

At sampling sites of intermediate depth (15 m) the variation of *P. oceanica* descriptors was driven by the cumulative effect of four out of five predictors tested: time (F=4.26, $p < 0.05$), bay area (F=16.06, $p < 0.01$), direction (F=42.71, $p < 0.01$) and number of pressures (F=3.6, $p < 0.05$). The bay area and time (autumn 2012 and spring 2013) showed to affect the E/L, whereby higher E/L values were reported in the western side of the bay near the mariculture facilities and during the spring of 2013 (Fig. 5-7 and 5-8). At 15 m depth sites the number of leaves per shoot was affected mostly by the anthropogenic pressure gradient and to a lesser extent by the number of pressures, showing lower values near fish farms (Fig. 5-5 and 5-8). Shoot densities were not strongly affected by any predictor (Fig. 5.5 and 5.8). Foliar area strongly correlated with direction from the nearest pressure source and had lower values in sites located northwards from an anthropogenic pressure.

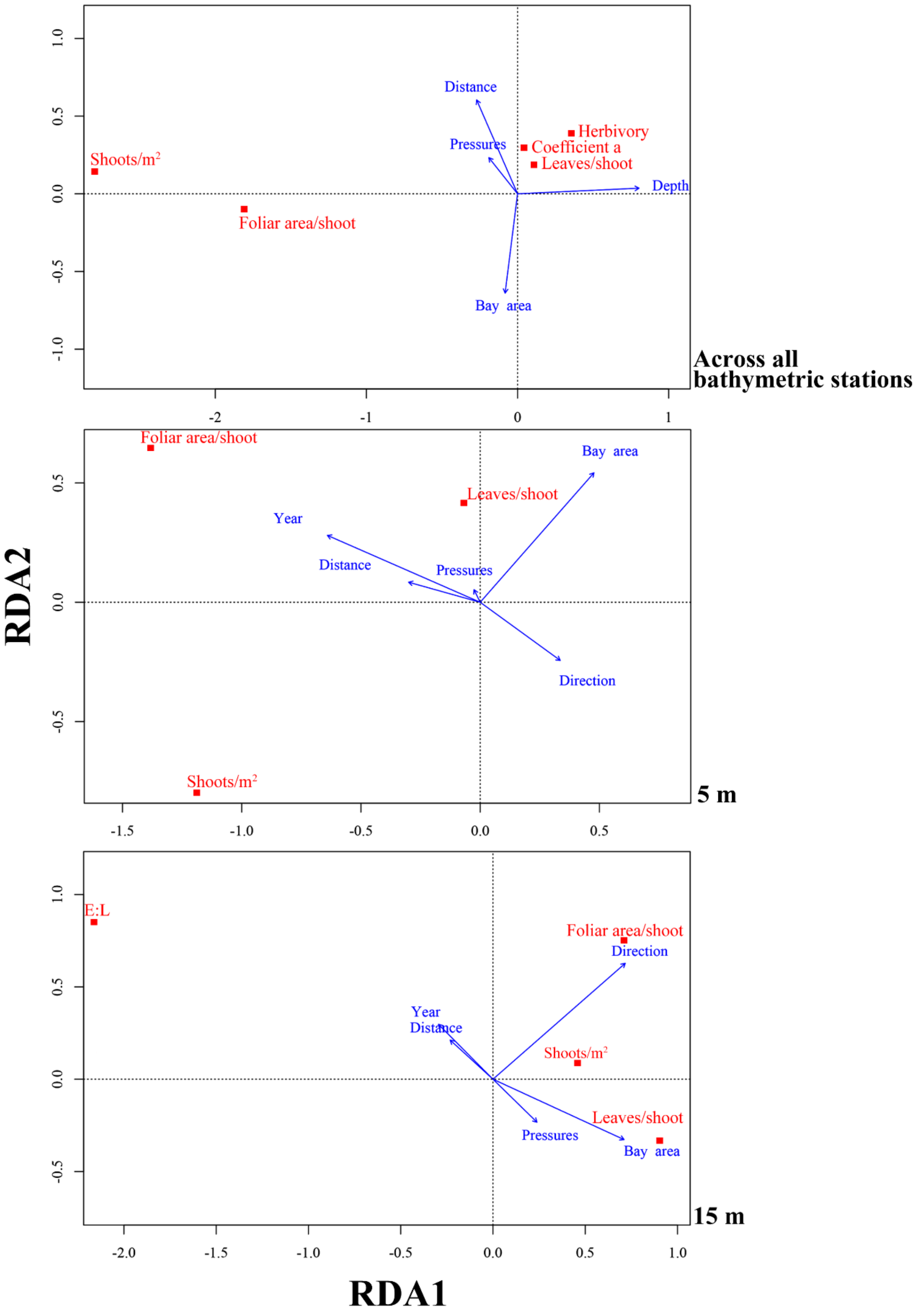

Figure 5-8. RDA between the *Posidonia oceanica* descriptors and proxies of anthropogenic pressures, depth and time across the whole Vasiliko Bay (top panel), shallow 5 m depth (bottom left) and intermediate 15 m depth (bottom right). The model with all the terms together was significant for across all bathymetric sites (df = 4, Variance = 1.12, F = 35.25, $p < 0.001$), 5 m (df = 5, Variance = 0.60, F = 9.73, $p < 0.001$) and 15 m (df = 5, Variance = 0.60, F = 18.90, $p < 0.001$).

5.4.5 Ecological status class

Using the metrics determined in this study (Table 5-6 in Appendix 2) and the site-adapted reference values (Table 5-3 in Appendix 2), the ESC of the Vasiliko basin was estimated to be mostly ‘Good’ with both PREI and BiPo WFD indices. Exceptions were the sampling sites 5 (near a fish farm) and 9 (near the marine jetty) in the spring of 2013, which were estimated to have ‘Medium’ ESC with the BiPo index. The ESC assessed at the sampling sites 7 (in the central area of the bay) and 10 (east of Vasiliko Bay, near Zygi) was assessed marginally ‘High’ with the PREI (Fig. 5-9).

In both seasons, the ESC assessed with the PREI had slightly higher (0.064 ± 0.029) EQR values than the BiPo index, while the seasonal fluctuations in the EQR values were small (0.028 ± 0.024) for both indices (Fig. 5-9). The EQR values between the two biotic indices were highly positively correlated (Pearson’s Correlation, $\rho = 0.93$, $p < 0.05$). The EQR scores using the site-adapted reference values was similar but consistently slightly lower (0.036 ± 0.026) than those assessed against the intercalibrated reference values for PREI and slightly higher (0.029 ± 0.006) than those assessed against the reference values of BiPo, but in either way the evaluation of the ESC was the same.

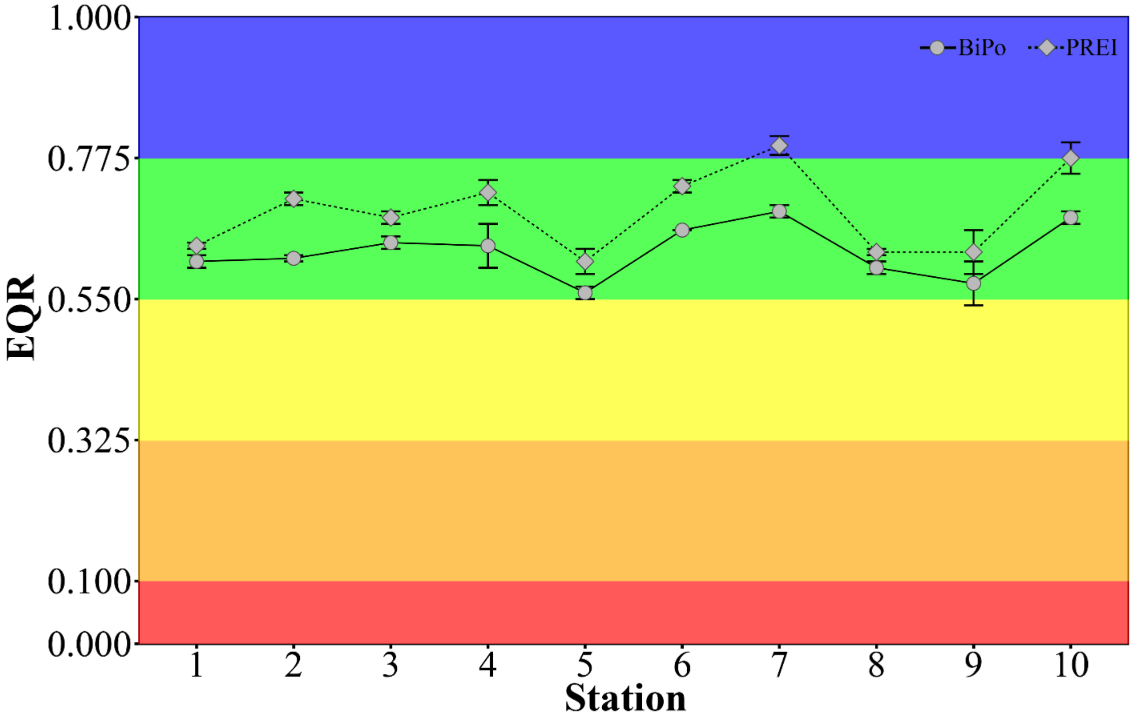

Figure 5-9. Mean (of two seasons) Ecological Quality Ratio estimated with the PREI and BiPo WFD indices, using the adapted reference conditions, at 10 sites set at about 1 km apart. The error bars represent the SE.

5.5 Discussion

This study is the first to develop a high-resolution map and assess the condition of the protected seagrass *P. oceanica* meadows in Vasiliko Bay, Cyprus. The results can guide responsible management planning and add weight to the protected seagrass habitat and the key ecosystem services it provides.

Benchmarks and reference conditions have not yet been set up regarding the concentration of contaminants within the sediments. Higher concentrations of iron, mercury, hexaclorobenzene and hexaclorobutadience were measured in the central region of the bay where the industry concentrates. It is worth exploring whether this coastal chemical contamination is bioaccumulating and the effects of this on the biota. Trace element contamination of Mediterranean coastal waters can be assessed accurately by measuring trace element concentrations in the leaves of the bioindicator *P. oceanica* (Richir *et al.*, 2015).

The resulting map revealed a belt of *P. oceanica* meadow covering most of the soft substratum in the depth zone 10-30, having a total coverage close to 200 ha in *ca* 10 km of coastline. In the shallows, *P. oceanica,* forms patches only over the hard substratum (0-6 m), perhaps limited by the intensified hydrodynamics as it was shown that *P. oceanica* demonstrates preferential colonisation of rocky substrata instead of sandy in high-energy conditions (Montefalcone *et al.,* 2016; Vacchi *et al.,* 2017). At some sampling sites of intermediate depth, the matte exceeded 3 m in height and considering about 1 m/kyr vertical accretion of the matte based on radioactive dating (Lo Iacono *et al.,* 2008; Serrano *et al.,* 2011), and that clones of *P. oceanica* can survive hundreds to thousands of years (Arnaud-Haond *et al.,* 2012), we can assume that the same meadows have occupied the Vasiliko Bay for at least 3 kyr. At the lower limit sites, large expanses of dead matte detected and the low percentage of plagiotropic shoots measured, indicates low vertical expansion of the meadow and are a sign of regressive meadows (Fernández-Torquemada *et al.,* 2008). The shallowest deeper limits of the meadow were recorded across the Vasiliko port and cement plant.

The *P. oceanica* meadows in the central-western part of the bay and near the fish farms, appeared impacted as their canopy was covered in epiphytes and fine matter, probably due to nutrient and particulate enrichments from the fish farms. Here, the highest OM content, alkalinity values and finer granule size (coarse silt) measured in the sediments reflected *in situ* observations. Sedimentation is considered the main driver of the negative environmental changes in *P. oceanica* meadows and deposition rates above 1.5 g organic matter $m^{-2}d^{-1}$ can cause accelerated mortality and declined recruitment (Díaz Almela *et al.,* 2008). The sedimentation rate was not assessed in this study, but a noteworthy *in situ* observation is that the fine matter covering the seafloor at the sites around fish farms was less pronounced in winter and early spring, which may be mainly due to its resuspension and flashing during strong winter storms that preceded. This adds complexity to any efforts trying to assess mariculture sedimentation impacts to marine ecosystems. Intense hydrodynamics during winter storms may be flushing and clearing *P. oceanica* meadows from the fine particles that accumulated over the warm season, buffering to some extent the mariculture impacts to *P. oceanica* meadows of the Vasiliko Bay.

Depth was the most important factor explaining the variation observed in shoot density and foliar area, but did not correlate to leaf number, herbivory and coefficient A. The highest densities, longer leaves and up to ten-fold higher LAI values in the shallows, is a natural consequence of the increased productivity due to higher illumination (Pirc, 1986; Romero *et al.,* 1998; Via *et al.,* 1998; Olesen *et al.,* 2002). To identify spatial differences in *P. oceanica* descriptors across the study area and limit the variability that might be attributed to depth, sites of the same depth (i.e., 5 and 15 m) were compared. At the shallow sites, lower shoot densities measured within the bay may be attributed to physical factors, such as different exposure and hydrodynamics, but also to the multiple pressures exerted by the increased industrialisation on the adjacent coastline. At the western sites of intermediate depth, lower number and mass of leaves per shoot and heavier mass of epiphytes were reported. In seagrasses the number of leaves per shoot is a robust bioindicator that decreases with light attenuation (McMahon *et al.,* 2013), and perhaps the lower number and mass of leaves can be attributed to epiphytic shading that can cause oxidative stress and cell damage in *P. oceanica* (Costa *et al.,* 2015). Increased epiphytic vegetation on *P. oceanica* leaves is indicative of eutrophication and anthropogenic pressures (Balata *et al.,* 2008; Brahim *et al*., 2014), which in this case may be attributed to the increased mariculture activity in the western parts of the bay and/or the steeper slope of the seabed in that section of the bay, which creates upwelling effects. A recent study that established seagrass monitoring systems using fixed plots near the fish farms of the study area, found that *P. oceanica* didn't regress and most structural indicators didn't change over a period of five years (**Chapter 6**; Kletou *et al.,* 2018). A global meta-analysis of indicators in relation to the most common and important drivers of seagrass decline, shows that although structural and demographic descriptors of *P. oceanica* are responsive to degradation, they respond slowly to pressures, while biochemical and physiological indicators present more stressor-specific responses and can detect degradation responses much faster (Roca *et al.,* 2015; 2016). Although no differences in shoot densities across sampling sites of intermediate depth were found, and that these didn't change near fish farms during the last years (**Chapter 6**; Kletou *et al.,* 2018), the epiphytes reflected both temporal and spatial changes. On the western side of the bay across fish farms, opportunistic erect algae covered the seagrass canopy and increased in spring when background nutrients were higher. The epiphytic community can be an indicator of the ecological quality of coastal waters, as it responds faster and is more sensitive to environmental alterations than the host plant (Delgado *et al.,* 1999; Giovannetti *et al.,* 2010; Piazzi *et al.,* 2016).

Evidence from the European MedVeg project that investigated the effects of mariculture on *P. oceanica* meadows around Mediterranean fish farms, including Cyprus, showed that impacts can be felt up to 400 m from the edge of the cages of farms (Marbà *et al.,* 2006; Holmer *et al.,* 2008). In this study, epiphytic overload was detected over larger distances from fish farms. For example, site 2 at 15 m depth had significantly higher epiphytic mass and lower leaf number and mass than all the eastern sampling sites (6-10) of the same depth. At the time of samplings, site 2 was surrounded by three mariculture units. It was located at about 850 m northeast from a newly constructed mooring which rapidly increased to 15 finfish cages by the spring of 2013; 1.4 km east of a mooring that was reduced to 6 finfish cages; and 1.7 km west of the largest mooring in the area that increased to 60 finfish cages. In the absence of any other obvious anthropogenic organic enrichments near the sampling site, the synergistic impacts of mariculture may be responsible for the lower performance of these descriptors compared to the eastern sampling sites. Impacts of fish farming are site-specific and the synergistic effects need to be considered holistically. In the western side of Vasiliko Bay, fish farms operated for years over *P. oceanica* meadows but have gradually relocated in deeper waters. Production has increased significantly over the last decade, yet seagrass meadows persist near several fish farm units (**Chapter 6**; Kletou *et al.,* 2018). The Department of Fisheries and Marine Research is taking steps aiming to designate the western waters of Vasiliko Bay as an aquaculture zone and a large investment is planned to construct a port west of Vasiliko to accommodate the fish farmers of the area. It is important to implement integrated approaches to assess the impacts of mariculture and of the other industries to *P. oceanica* meadows of Vasiliko Bay prior to any long-term planning. The cumulative effect of different stressors reduces the resilience of seagrasses (i.e. the capacity to resist and recover from stress) (Unsworth *et al.,* 2015). In the oligotrophic waters of Cyprus, the synergistic effects of fish farms may not affect most *P. oceanica* structural descriptors at intermediate depths, but are detected in descriptors such as increased epiphytic biomass at distances at least two-fold higher than the 400 m reported. Dispersion of farm wastes can have a large area of influence, spanning km and affecting *P. oceanica* meadows in remote environments, demonstrated by elevated isotopic content in epiphytes and seagrass leaf tissue (Ruiz *et al.,* 2010).

The diversity of European seagrass indicators is particularly striking and includes biochemical, physiological, morphological, structural, demographic, and community measures (Marbà *et al.,* 2013; Romero *et al.,* 2015). The two WFD indices applied in this study (PREI and BiPo), present significant high correlations with human pressures and are highly comparable (Gerakaris *et al.,* 2017). In this study PREI gave consistently slightly higher EQR values. Overall, the ESC of the Vasiliko basin using *P. oceanica* is assessed to be ‘good’, with little differences among sampling sites and season studied.

These indices have been designed to reflect the water quality across basin scales and not to detect localised pressures/degradation. For example, at the lower limits of the seagrass near the mariculture units, there were signs of heavy anthropogenic footprint such as extensive dead matte and fine particulate matter covering the leaves of *P. oceanica* over large distances, which was not reflected in the assessments. Many coastal pressures may not be felt at 15 m depth and the lower limits, sometimes >1 km away from the shoreline.

Maintaining biodiversity and the functional balance of the fauna within a seagrass meadow food web is critical to prevent detrimental trophic cascades (Unsworth *et al.*, 2015). A highly diverse coralligenous algal community was present within the *P. oceanica* shoots at some of the remote sampling stations e.g., site 2 at 5 m depth. This basic compartment of the ecosystem was entirely absent from the other shallow sites near the industrialised coastline of Vasiliko Bay. The key lies in understanding that the ecosystem services of seagrass beds can vary greatly, even over small spatial scales. Two seagrass beds with similar shoot densities and phenological dimensions may be strikingly different in terms of the associated communities they sustain and their ecological services and value. The good quality of a water body and the apparent health of a species, whether emblematic or not, such as the seagrass *P. oceanica*, is not always indicative of the good structure and functioning of the whole ecosystem (Personnic *et al.*, 2014). Thus, an ecosystem-based approach that considers the overall functioning of the *P. oceanica* ecosystem, from primary producers and the litter to top predators, complies with the MSFD and is more informative to managers about the actual status of the ecosystem functioning than single-species WFD descriptors (Boudouresque *et al.*, 2015).

Shoot density is the most basic descriptor to describe *P. oceanica* ecosystem integrity, a reliable and efficient indicator for monitoring the vitality and dynamics of *P. oceanica* meadows as well as revealing stress on this marine ecosystem (Pergent-Martini *et al.*, 2005; Mayot *et al.*, 2006). It is remarkable that despite the intense anthropogenic pressures in Vasiliko Bay and that the water temperatures are near the upper tolerance limits of *P. oceanica*, shoot densities recorded at both 5 m depth and 15 m depth meadows are amongst the highest reported in the literature from across the Mediterranean Sea at the same depths (Table 5-2). This may be due to the extremely low nutrient levels and clarity of the waters in this side of the Mediterranean, allowing more light to reach the benthos and buffering to some extent the effect of suspended wastes from anthropogenic sources. Nevertheless, this finding deserves attention and indicates the high value and condition of these *P. oceanica* meadows compared to the rest of the Mediterranean Sea.

Implementing monitoring programs is an essential step in assessing the ecological status of the seagrass meadows, identifying the causes and potential synergistic effects of stressors to thereby improve the integrated coastal management and conservation of keystone species. Considering the mounting pressures and coastal modification in the central and eastern portions of the bay and the aquaculture expansion westwards, it is questionable whether the marine ecosystems of Vasiliko Bay will retain their good water quality status and maintain the ecosystem services offered not so long ago. During this study, dredging operations to construct the fuel marine jetty at the eastern part of the bay increased turbidity and sedimentation in the area and caused localised irreversible loss of the protected *P. oceanica* meadow habitat in the dredged area. This demonstrates the urgent need to upgrade and improve the quality of marine Environmental Impact Assessment studies but also the evaluation and follow-up monitoring of large projects. In the current economic climate, net economic gain and the contribution of a development to a country's Gross Domestic Product (GDP) should not monopolise the decision making (Hadjimichael *et al.*, 2014). Environmental Impact Assessment (EIA) studies, which largely underpin the decisions for coastal developments, are plagued by inconsistent methods and lack of independent evaluation, leading to perceptions of inadequate scientific rigour (Sheaves *et al.*, 2016). They are often designated insufficient time to determine geographical extent, local drivers and temporal variability of seagrass and its associated environment, leading to biased decisions made based on highly limited data and is thus no surprise that refining EIA processes is identified as one of the most significant strategies for helping to reverse the decline of seagrass meadows and bolstering their resilience (Cullen-Unsworth & Unsworth, 2016).

The findings presented in this study are important to increase awareness of managers and stakeholders, especially now that further plans are underway in Vasiliko Bay, such as the construction of marine jetties over healthy seagrass meadows, dredging in the eastern parts of the bay to lay pipes, a port in pristine shores west of the bay, and more. The *P. oceanica* meadows of Vasiliko Bay are among the densest that remain in the world. Will these ancient meadows continue to withstand the complex pressures that are being enforced to them? Will the slowest growing seagrass in the world continue to dominate and characterise the coastal marine ecosystems of Vasiliko Bay and support iconic wildlife such as sea turtles, monk seals and dolphins observed in the area during samplings? It depends largely on the weight placed on this biological element in management decisions. If, awareness among citizens about the importance/value and threats/impacts on *P. oceanica* is enhanced; informed stakeholders get engaged in the decision process; scientists and consultants are approved, qualified and use scientifically robust methods in their studies; managers consider impacts holistically, evaluate not so lightly, request environmental compensation and take responsible decisions with respect to our natural heritage; and, if mitigation practices, environmental monitoring of approved projects and restoration of marine ecosystems start being enforced, then, the *P. oceanica* meadows of Vasiliko Bay and beyond, will be safeguarded for the next generations.

Table 5-2. *Posidonia oceanica* shoot density at 5±1 m and 15±1 m depths at Vasiliko Bay (mean range of ten different sampling sites at each bathymetric zone) and different Mediterranean sites reported in the literature. Sorted by highest mean reported, in the case of range the lowest extreme was not taken into consideration during sorting.

Depth (m)	Country	Location	Mean shoot densities (shoot m^{-2})	Source
5 ± 1	Malta and Spain	Comino, Majorca	1185 - 1243	Vàzquez-Luis *et al.*, 2015
	Spain	Majorca	384 - 1013	Hendriks *et al.*, 2013
	Cyprus	**Vasiliko Bay**	**617 - 954**	**Present study**
	Tunisia	Mahdia	807	Mabrouk *et al.*, 2011
	Spain	Catalonia	481 - 789	Romero *et al.*, 1999
	Malta	Northern Malta	787	Borg & Schembri, 2000
	Greece	Saronikos Gulf	500 - 750	Panayotidis & Simboura, 1989
	Tunisia	Mahdia	413 - 748	Mabrouk *et al.*, 2017
	Italy	Tuscany	593 - 697	Mauro *et al.*, 2013
	Spain	Catalonia	111 - 627	Alcoverro *et al.*, 1995
	Greece	Southern Aegean Sea	610	Gerakaris & Panayotidis, 2009
	Italy	Sardinia	525	Pergent-Martini *et al.*, 1994
	Italy	Sardinia	69 - 525	Scardi *et al.*, 2006
	Tunisia	Eastern Tunisia	362 - 496	Sghaier *et al.*, 2006; 2011
	Italy	Gulf of Taranto	494	Costantino *et al.*, 2010a
	Spain	Balearic Islands	367 - 484	Marbà *et al.*, 2005
	Italy	Gulf of Naples	473	Pergent-Martini *et al.*, 1994
	Tunisia	Mahdia	456	Mabrouk *et al.*, 2009
	Turkey	Central Aegean Sea	450	Pergent-Martini *et al.*, 1994
	Italy	Gulf of Naples	431	Costantino *et al.*, 2006
	Italy	Monterosso	349	Peirano *et al.*, 2011
	Italy	Gulf of Naples	341	Buia *et al.*, 1992
	Spain	Campomanes	292	Marbà *et al.*, 2005
15 ± 1	Cyprus	Cape Kiti	509 - 568	Kletou *et al.*, 2016
	Greece	Southern Aegean	518	Gerakaris & Panayotidis, 2009
	Malta	Northern Malta	486	Borg & Schembri, 2000
	Cyprus	**Vasiliko Bay**	**395 - 478**	**Present study**
	France	Corsica	176 - 472	Lopez y Royo *et al.*, 2010a
	France	Corsica	211 - 448	Pergent *et al.*, 2015
	Spain	Balearic Islands	433	Marbà *et al.*, 2005
	Italy	Gulf of Naples	244 - 413	Vasapollo, 2009
	Greece	Saronikos Gulf	231 - 406	Gerakaris *et al.*, 2017
	Spain	Catalonia	398	Marbà *et al.*, 2005
	Italy	Sardinia	395	Pergent-Martini *et al.*, 1994
	Italy	Gulf of Naples	220 - 388	Lopez y Royo *et al.*, 2011
	Italy	Gulf of Naples	301-345	Costantino *et al.*, 2006
	Italy	Gulf of Naples	137 - 343	Zupo *et al.*, 2006
	Spain	Catalonia	173 - 340	Romero *et al.*, 1999
	France	Corsica	285 - 334	Lopez y Royo *et al.*, 2011
	Croatia	Northern Adriatic Sea	141 - 324	Guala *et al.*, 2015
	Spain	Catalonia	159 - 288	Lopez y Royo *et al.*, 2011
	Italy	Ligurian Sea	260	Rotini *et al.*, 2013
	Italy	North-Eastern Ionian Sea	225 - 234	Costantino *et al.*, 2010a
	Italy	Southern Adriatic	189	Costantino *et al.*, 2010b
	Italy	Southern Adriatic	135 - 163	Costantino *et al.*, 2010a

6. SEAGRASS RECOVERY AFTER FISH FARM RELOCATION IN THE EASTERN MEDITERRANEAN

6.1 Abstract

Finfish aquaculture has damaged seagrass meadows worldwide as wastes from the farms can kill these habitat-forming plants. In Cyprus, the Mediterranean endemic *Posidonia oceanica*, is at its upper thermal limits yet forms extensive meadows all around the island. Understanding this under-studied isolated population may be important for the long-term survival of the species given that the region is warming rapidly. When fish farming began around Cyprus in the 90s, cages were moored above seagrass beds, but as production expanded, they were moved into deeper water further away from the meadows. Here, the deepest edge of meadows near fish farms that had been moved into deeper waters, as well as at a decommissioned farm site were monitored. Four *P. oceanica* monitoring systems were set up using methods developed by the Posidonia Monitoring Network. Seagrass % coverage, shoot density, % of plagiotropic rhizomes, shoot exposure, leaf morphometry, and sediment organic matter content and grain size were monitored at 11 fixed plots within each system, in 2012-2014 and in 2017. Expansion at the lower depth limit of seagrass meadows was recorded at all monitoring sites. Most other *P. oceanica* descriptors either did not change significantly or declined. Declines were most pronounced at a site that was far from mariculture activities but close to other anthropogenic pressures. The most important predictor affecting *P. oceanica* was depth, hence the availability of light. Monitoring using fixed plots allowed direct comparisons of descriptors over time, removes patchiness and intra-meadow variability, increasing our understanding of seagrass dynamics and ecosystem integrity. It seems that moving fish farms away from *P. oceanica* has helped ensure meadow recovery at the deepest margins of their distribution, an important success story given that these meadows are at the upper thermal limits of the species.

Keywords: aquaculture; bioindicators; Cyprus; ecological monitoring; ecosystem change; eastern Mediterranean; seagrass.

6.2 Introduction

Seagrasses are major contributors to human well-being and the economies of coastal countries (Barbier *et al*., 2011; Campagne *et al*., 2015; Dewsbury *et al*., 2016). Their meadows are among the most productive ecosystems on Earth but are declining at unprecedented rates (Waycott *et al*., 2009; Costanza *et al*., 2014). They provide: coastal protection from erosion, by attenuating waves and stabilising sediments; water purification by assimilating nutrients and pollutants; transfer of matter and energy up trophic levels and sustaining fisheries; carbon sequestration to help mitigate climate change; and provision of structurally complex habitat for enhanced biodiversity, which boosts tourism, recreation, education and research (Barbier *et al*., 2011; Campagne *et al*., 2015). However, there are multiple mounting pressures, including: sediment and nutrient runoff, physical disturbance, invasive species, disease, commercial fishing practices, aquaculture, overgrazing, algal blooms and global warming, all of which have caused major declines in seagrasses, raising awareness of the need to protect, monitor, manage and restore these habitats (Orth *et al*., 2006; Barbier *et al*., 2011).

The endemic seagrass *Posidonia oceanica* (Linnaeus) Delile, 1813, forms one of the most important coastal ecosystems in the Mediterranean Sea. Its rhizomes propagate vertically as well as horizontally, producing reefs called “matte” that can extend many meters down into the sediment and persist for millennia, resulting in the largest documented stores of organic carbon among seagrasses (Buia *et al*., 2004; Lo Iacono *et* *al*., 2008; Fourqurean *et al*., 2012; Lavery *et al*., 2013). The seagrass forms meadows that can extend up to 15 km wide and clone for thousands, possibly tens of thousands of years (Arnaud-Haond *et al*., 2012). The structurally complex meadows are the climax stage of many upper subtidal bottoms extending from the surface down to depths of 40-45 m in oligotrophic clear water, supporting hundreds of associated species (Piazzi *et al*., 2016). An estimated 34% of *P. oceanica* meadows died in the last half century, classifying the *P. oceanica* habitat as an 'endangered' ecosystem (Telesca *et al*., 2015).

The Mediterranean coast is home to about 250 million people and supports about one third of all global tourism, which is anticipated to reach 0.5 billion arrivals per year by 2030 (Randone *et al*., 2017). Residents and tourists place a high demand on seafood. In the last two decades, there has been a dramatic growth in the Mediterranean aquaculture production expanding approximately 5% annually (Massa *et al*., 2017). The development of fish aquaculture along the Mediterranean coasts has caused localised losses of *P. oceanica* (Delgado *et al*., 1997; Pergent *et al*., 1999; Ruiz *et al*., 2001; Cancemi *et al*., 2003; Pergent Martini *et al*., 2006; Diaz-Almela *et al*., 2008; Holmer *et al*., 2008; Pérez *et al*., 2008; Apostolaki *et al*., 2009). Several factors cause this damage including reduction of light under the cages (Ruiz *et al*., 2001), an increase in particulate matter and nutrient concentrations in the water, which can cause an increase in epiphyte biomass (Delgado *et al*. 1997), enhanced herbivory (Holmer *et al*., 2003), expansion of competitive opportunistic seaweed (Holmer *et al*., 2009), sulphide invasion into the roots (Frederiksen *et al*., 2007), and high input of organic matter into the sediments (Cancemi *et al*., 2003; Apostolaki *et al*., 2007; Diaz-Almela *et al*., 2008). Organic enrichment may be the most important factor as this can lead to anoxic and toxic benthic conditions causing high *P. oceanica* mortality (Pérez *et al*., 2008). Seagrass loss can continue even after several years of fish farming cessation as the matte itself begins to rot (Delgado *et al*., 1999; Apostolaki *et al*., 2010). *Posidonia oceanica* losses are considered irreversible over human time-scales, because it grows slowly (only 3-4 cm per year) and has extremely low natural colonization rates (Boudouresque *et al*., 2012). Although sexual reproduction rates are speeded up by warming, seedlings usually settle at the shallow boundaries of seagrass meadows (Balestri *et al*., 2017). Hence, the deep and exposed seagrass meadows near fish farms have the lowest recruitment rates limited to vegetative propagation and horizontal growth under low light conditions.

Today, fish farm cages are moored 1-3 km off Cyprus in water depths of 22-75 m, cultivating mainly gilthead seabream (*Sparus aurata* Linnaeus, 1758) and European seabass (*Dicentrarchus labrax* Linnaeus, 1758). National mariculture production expanded from 210 tonnes in 1994 to 6625 tonnes in 2016, now exceeding 80% of the total fisheries production (data from Department of Fisheries and Marine Research, Cyprus). The number of licenced units has remained the same for many years and the production increase is due to existing units that have expanded production, especially during the last decade. Most fish farms are within the Vasiliko-Moni area in south Cyprus. Fish farming in the area started in the mid-1990s with small production units (100-300 tonnes per year), using floating cages starting at 22-28 m depth and over seagrass meadows. One of these units ceased operations soon after, the rest of the farms expanded and are now each licenced to produce 1000-1800 tonnes per year. A prerequisite to receiving expansion permits by the national authorities was to relocate cages in deeper water and further away from the *P. oceanica* meadows. Currently, the shallowest cages in Vasiliko-Moni area are found at the depth of about 37 m, but seagrass meadows still exist within the impact zone (<400 m) of aquaculture effluents (Holmer *et al*., 2008).

Despite the temporal and spatial scale of this development, no local studies have been conducted to evaluate the effects of the fish farm units to the adjacent *P. oceanica* meadows. In this study, four *P. oceanica* observatory systems were established near major fish farms that have just relocated and expanded deeper and at a reference (decommissioned fish farm) site in the Vasiliko-Moni area. The aims were: to assess progression or regression of *P. oceanica* meadow's edge, evaluate the changes in *P. oceanica* and sediment descriptors between the two sampling periods for each monitoring system and examine whether fish farm or environmental drivers are affecting these descriptors. This study shows how fish farm impacts to seagrass beds can be monitored effectively, assisting integrated coastal management decisions.

6.3 Materials and Methods

6.3.1 Study area

Four *P. oceanica* monitoring systems were set up using standardised methods developed by the Posidonia Monitoring Network (PMN) (Boudouresque *et al*., 2000; Pergent, 2007) at the warmest and easternmost geographic limits of *P. oceanica*. Three systems were deployed at the regressive lower limits of seagrass meadows near active fish farm sites Seawave Fisheries (SW), Blue Island Plc (BI) and EMAT (EM) that recently relocated and expanded production to deeper nearby waters (Fig. 6-1, Table 6-1). During the first data collection, the three farms had a total production *ca* 2.5 kt yr^{-1} and operated shallow cages near the seagrass meadows investigated (EM) or had just relocated to deeper water as they expanded (SW and BI). At the second data collection three to five years later, the three farms had a total production larger than 4 kt yr^{-1} (Fig. 6-1, Table 6-1). The fourth monitoring system (REF) was set up *ca* 300 m far from a licensed small (*ca* 100 t yr^{-1}) production unit, which operated for a few years in the nineties and ceased operations about 15 years ago. This monitoring system was set at stable lower limits of the seagrass meadow, over 3.5 km from the nearest fish farms and resembles the natural deeper boundaries of *P. oceanica* meadows in the coastal area studied (Table 6-1).

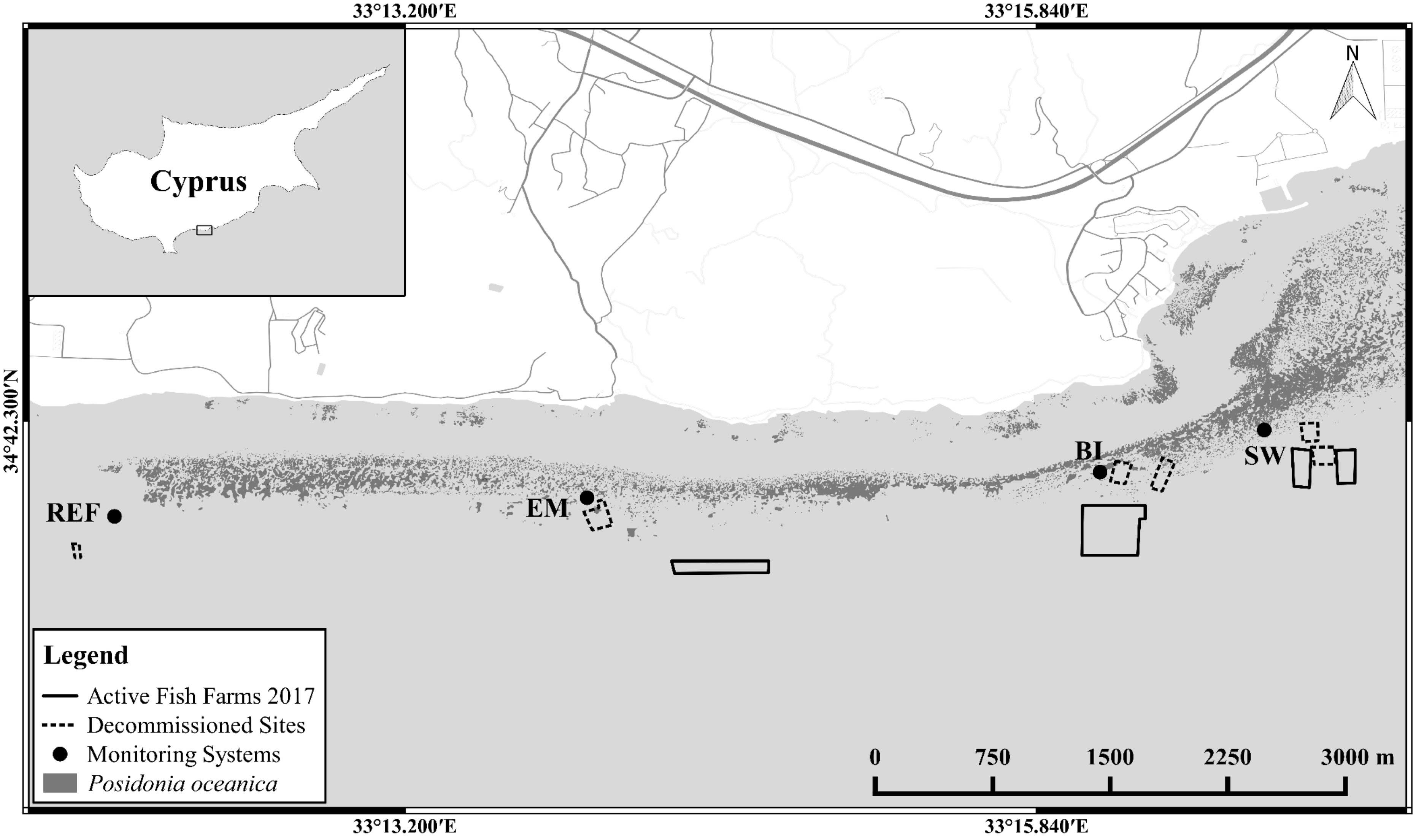

Figure 6-1. Locations of *Posidonia oceanica* monitoring systems and coastal areas occupied by decommissioned and active fish farms. REF = reference station, EM, BI and SW = active fish farms. The surrounding meadows of *P. oceanica* are also shown.

Table 6-1. Details of *Posidonia oceanica* monitoring systems set up near farms operating in south Cyprus.

***Posidonia oceanica* Monitoring System – Fish farm information**	**Distance from the fish farm, depth and time of data collection.**	**Added Value**
SW Established in 1995 with licenced annual production 300 t yr^{-1}. Licenced production increased to 450 t yr^{-1} in 2008, 750 t yr^{-1} in 2010, 1000 t yr^{-1} in 2013, 1250 t yr^{-1} in 2014 and to 1500 t yr^{-1} after 2016. Shallow cages relocated in deeper water in 2011. # of finfish cages in 2017: 36	About 275 m northwest of the existing finfish cages and 240 m west of the previous position of cages, which were relocated deeper in 2011. The monitoring system was set up at regressive lower limits, at 25-26 m depth, in the summer of 2012. First set of data were collected the same period. Second set of data were collected in early autumn 2017.	Future monitoring and comparison with the data presented in this study, will be able to detect whether the relocation but expansion of the fish farm in deeper waters had any impacts on the adjacent *P. oceanica* meadows.
BI Established in 1993 with licenced annual production 300 t yr^{-1}. Licenced production increased to 500 t yr^{-1} in 2004, 900 t yr^{-1} in 2007, 1300 t yr^{-1} in 2009, 1500 t yr^{-1} in 2014 and to 1800 t yr^{-1} in 2017. Shallow cages relocated in deeper water in 2011. # of finfish cages in 2017: 66	About 250 m north of the existing finfish cages and less than 100 m west of the previous position of the cages, which were relocated deeper in 2011. The monitoring system was established at regressive lower limits, at 22-23 m depth, in the autumn of 2012. First set of data were collected two years later in autumn of 2014. Second set of data were collected in autumn 2017.	Future monitoring and comparison with the data presented in this study, will be able to detect whether the relocation but expansion of the fish farm in deeper waters had any impacts on the adjacent *P. oceanica* meadows.
EM Established in 1993 with a licence to produce 100 t yr^{-1}. In 2011 it received permit to produce 1000 t yr^{-1} at a new, deeper (50 m depth) site over 600 m further offshore. The old shallow mooring system was gradually decommissioned and went from 10 cages in 2012, to 6 in 2013 to 2 in 2014 to 1 in 2015. # of finfish cages in 2017: 0 in old and 22 in new mooring.	About 100 m north/northwest of the shallow mooring, which ceased operations gradually. Operations moved and expanded deeper about 750 m southeast from the monitoring system. The monitoring system was established at regressive lower limits, at 21-23 m depth, in the summer / autumn of 2012. First set of data were collected the same period. Second set of data were collected in autumn 2017.	Future monitoring and comparison with the data presented in this study will provide vital information about the recovery rates of the *P. oceanica* meadow following the cessation of mariculture operations in the near vicinity.
REF Farming started in mid-nineties and lasted about a decade (production *ca* 100 t yr^{-1}). It has remained inactive for *ca* fifteen years.	About 300 m northeast from a small production unit, which terminated operations a long time ago. The monitoring system was established at stable lower limits, at 28-29 m depth, in the summer of 2013. First set of data were collected the same period. Second set of data were collected in early autumn 2017.	Future monitoring and comparison with the data presented in this study will provide a point of reference for other monitoring systems and if fish farming initiates near this system it will provide baseline data and vital information about the direct effects of the fish farm on the adjacent *P. oceanica* meadows.

6.3.2 Dispersal of fish farm effluents

To predict the dispersal of fish farm effluents and sedimentation, the dispersion MERAMOD model developed for gilthead sea bream *S. aurata* and European sea bass *D. labrax* farming was simulated (Cromey *et al*., 2012). The simulation was made in 2012, just after the relocation of BI and SW, and before the relocation of EM fish farm. Historical daily sea current data (2005-2010) of the surface waters and the 10 m depth zone in the study area were extrapolated using 3-D interpolations of the Cyprus Coastal Ocean Forecasting and Observing System (CYCOFOS); a validated hydrodynamic flow model covering the Levantine region (Zodiatis et. al., 2003; 2008). Two scenarios were applied that included the coldest-water period (February) and the warmest-water period (August). The latter accounts for the worst-case scenario since maximum biomass/feed input was used. Data incorporated into the MERAMOD model included: daily average current speed and direction for the months of August and February obtained from the CYCOFOS, bathymetric data at each site and a range of husbandry data collated from the managers of the three fish farms (Table 6-2).

Table 6-2. Husbandry data (year 2012) used in MERAMOD for each of the two scenarios.

Data	Scenario 1 (Winter)			Scenario 2 (Summer)		
	SW	BI	EM	SW	BI	EM
Feed input (kg d^{-1})	3665	7500	2200	7500	11000	1200
Feed input (kg $cage^{-1}$ d^{-1})	136	221	183	278	324	200
Max biomass (t)	580	1194	300	600	861	150
Cage diameter (m)	19	22	22	19	22	22
Cage surface area (m^2)	286	390	390	286	390	390
Cage volume (m^3)	4011	5459	5459	4011	5459	5459
No of cages	27	34	12	27	34	6
Feed input per day per unit cage surface area (kg d^{-1} m^{-2})	0.47	0.57	0.47	0.97	0.83	0.51

Waste feed and faeces were assigned a random starting position in the cage volume. An average settling velocity of feed pellets representing 1 to 5 mm pellets and settling velocity of faecal particles for bream and bass (Magill *et al.*, 2006) were assigned to cages according to the percentage of bream and bass being farmed at each site. For particles between sea surface and 5 m depth, surface current speed and direction were used for advection, whereas 10 m current speed and direction were used for particles from 5 m to the sea bed. Predicted flux was scaled to standard units of g m^{-2} yr^{-1} of total dry solids. Numerous model default data were used consistently across sites (Table 6-3), so that differences among sites were primarily driven by differences between the sites in terms of depth, hydrography, feed input and husbandry data in general.

Table 6-3. MERAMOD default data applied across all scenarios.

Model default parameter	Value
Feed wasted, digestibility, water content	5%, 85%, 9%
Wild fish consumption of waste pellets	50 % of wasted pellets are consumed by wild fish and do not contribute to flux
Horizontal dispersion coefficients: k_x, k_y (m^2 s^{-1})	0.4, 0.1
Vertical dispersion coefficient: k_z (m^2 s^{-1})	0.001
Particle trajectory time step (seconds)	60
Feed settling velocity (cm s^{-1})	Mean = 8.4, standard deviation = 4.3
Faecal settling velocity: Sea Bream (cm s^{-1})	0.4 cm s^{-1} (24%), 1.5 (45%), 2.5 (18%), 3.0 (13%)
Faecal settling velocity: Sea Bass (cm s^{-1})	0.4 cm s^{-1} (6%), 1.4 (9%), 2.5 (20%), 3.6 (38%), 4.6 (27%)

6.3.3 Monitoring systems and data collection

The four monitoring systems were set up in 2012-13 according to the ‘Protocol for setting up of *Posidonia* meadows monitoring systems «MedPosidonia» Programme’ (Pergent, 2007). In each monitoring system, 11 numbered cement markers were positioned at 5 m intervals and anchored with 12 mm diameter iron stakes, at the edge of the meadow (total 50 m length). Additionally, 16 mm diameter iron “photostakes”, from where photographs were taken, were hammered 50 cm into the sediment and sticking out 1 m, across each marker and the meadow’s edge.

At every marker, the following variables were recorded by scuba divers: depth and angle to other markers, % seagrass cover in a 0.36 m^2 quadrat, shoot density and % of plagiotropic (horizontally oriented) rhizomes in three fixed quadrats (0.04 m^2), and shoot exposure or burial of orthotropic (vertically oriented) shoots (three replicates taken at both the edge and another three at the inner side of the meadow). Surface sediment samples were collected from each marker by a diver and granulometry was conducted using an Endecotts Octagon sieve shaker after first drying the samples at 100 ^oC until constant weight. The granulometry data were processed with the GRADISTAT particle size analysis software. Fine sediment passing through the 212 μm sieve was homogenised, three replicates of 1.5 g from each marker were combusted at 550 ^oC and the organic carbon was determined as % weight loss following ignition. In addition, about 20 randomly selected orthotropic shoots from each monitoring system were removed and leaf morphometric analyses were carried out using the technique of Giraud (1977), including estimating the foliar surface per shoot, which was later used to estimate the LAI. The past annual *P. oceanica* leaf production rate was calculated following a standardised procedure, known as lepidochronology, which uses the thickness of the scales (previous leaf petioles that remain attached on the rhizome) to determine annual cycles (Pergent, 1990; Pergent & Pergent-Martini, 1991). The lepidochronological analysis involves carefully removing the scales from the rhizomes and ordering them from the older (near the rhizome base) to the more recent (near the living leaves). A cross section was made 10-12 mm above the base of each scale, viewed and photographed under an Olympus CX41 microscope attached to a camera. The thickness (μm) of the central/wider portion of the scale was measured with Image Pro Plus software.

About half a decade following deployment, the monitoring systems were revisited (Table 6-1). Initially an inspection was carried out to record any missing cement markers, labels, or photostakes. Progression or regression of the edge was measured using a measuring tape from the marker's inner side to the rhizome that was furthest from the marker in progression or closest in regression. Data collection from each monitoring system was repeated (except for lepidochronology) using the same methods. At each of the 44 markers, the measurements of shoot density and % of plagiotropic rhizomes were repeated from the same fixed quadrats.

6.3.4 Statistical analysis

To evaluate changes between the two sampling periods for each fish farm, a paired t-test was computed for variables taken from fixed quadrats (i.e. shoot density, plagiotropic rhizomes % and coverage %), following Elzinga et al. (1998). Variables derived from randomly selected shoots (i.e. number of leaves, foliar surface, shoot exposure) within the meadow were compared with a 2-sample t-test (Elzinga *et al.*, 1998). The assumptions for normality and homogeneity of variances were verified using a Shapiro-Wilk (accompanied by inspecting visually the QQ plots) and F test, respectively and if assumptions were violated, $\log_{10}$ or square root transformations were conducted. To calculate the rate of annual leaf production of *P. oceanica* acquired from the lepidochronological analysis and examine the patterns of change over the years (increase, decrease or none), a simple linear regression was performed. When assumptions were not met, the analysis proceeded with the non-parametric Wilcoxon signed rank test for data collected from the fixed quadrats, non-parametric Mann-Whitney U test for data collected from random shoots and non-parametric regression Kendall–Theil Sen Siegel for the lepidochronological data. Shoot progression was compared between the different monitoring sites using the non-parametric Kruskal-Wallis test.

In order to identify the predictors that affect the *P. oceanica* and sediment descriptors, a multiple regression analysis was applied, where all the descriptors were categorised into two environmental predictors (depth and time) and three fish farm associated predictors (distance of a monitoring system to the nearest fish farm, direction of a monitoring system to the nearest fish farm, and the size of production of the nearest fish farm in tonnes). The assumptions for normality and heteroscedasticity were verified via Shapiro-Wilk test and Breusch-Pagan test on either untransformed or cox box transformed data. The multiple regression analysis was further complemented with the relative importance analysis, which aims to identify the factor with the highest controlling effect on the descriptor (Tonidandel & LeBreton, 2011). This was based on the calculation of lmg, the relative contribution of each predictor to the R^2, averaged over the orderings among predictors (Grömping, 2006).

For all the statistical analyses the significance level α was adjusted to 0.05, computation was carried out by R-studio (v 1.0.153) and all the graphic material was generated via the package ggplot2 (Wickham, 2016). The relative importance analysis was conducted via the package relaimpo (Grömping, 2006).

6.4 Results

6.4.1 Fish farm effluents

The scalar and vector averages for the entire period considered indicated that there is alternation of surface currents towards the east and west respectively, but the prevailing average direction of the currents at 10 m depth is west – southwest (Fig. 6-2). The predominant direction of the currents during the two scenarios simulated in the MERAMOD, February and August, were towards east and southwest respectively. For all farms, there were virtually no areas predicted to have deposition greater than 5000 g m^{-2} yr^{-1}; areas of 2500 to 5000 g m^{-2} yr^{-1} were evident for farms BI and SW but not for the small EM farm (before relocation) (Fig. 6-2). The extent of the deposition footprints was high as a result of the reasonably high current and depth. According to the model and driven by the currents direction, the main dispersal of the effluents was not in the direction of the *P. oceanica* monitoring systems.

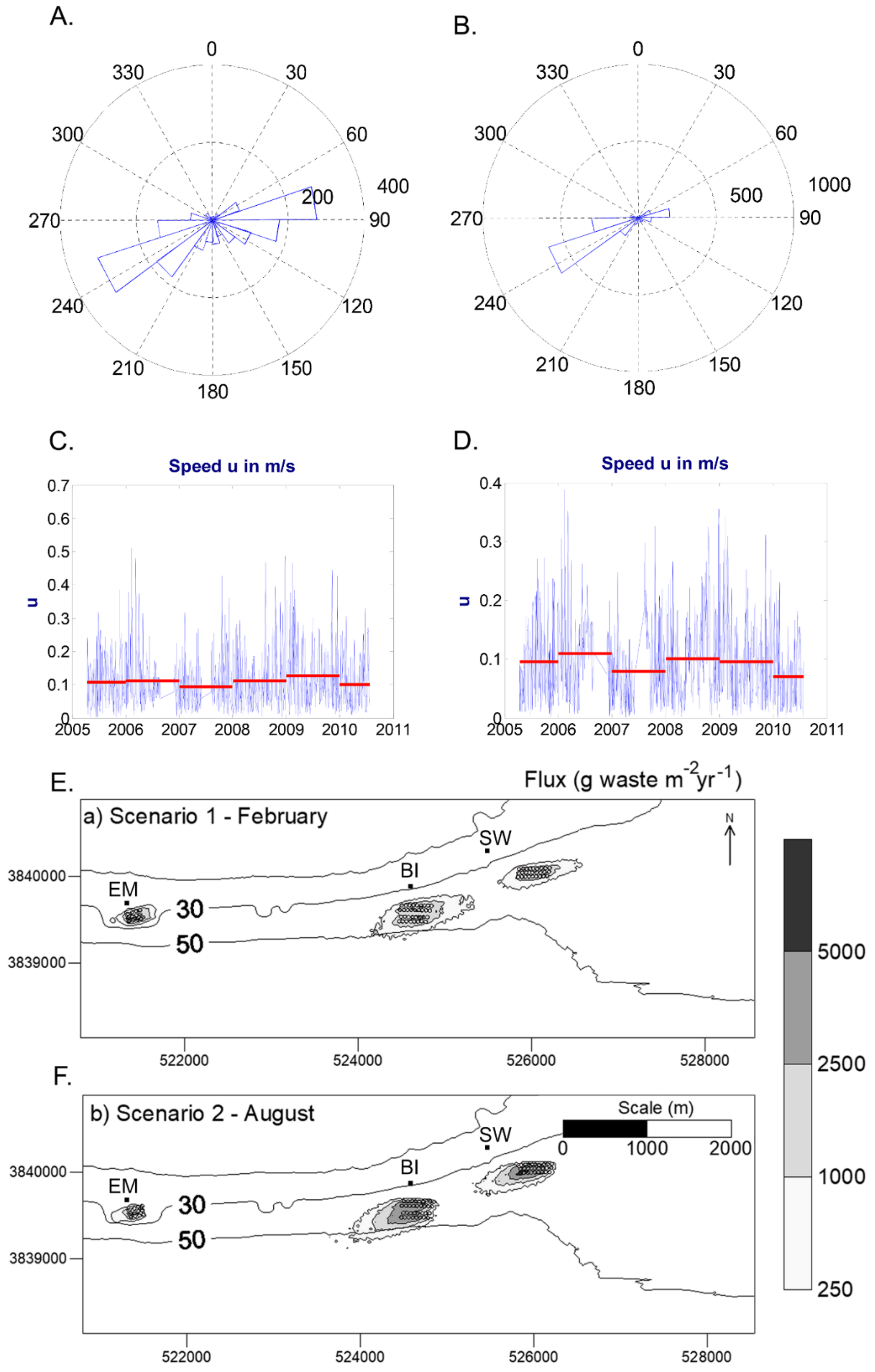

Figure 6-2. *Top panel*: Angle histogram in degrees (rose diagram) showing the frequency of current direction for the whole period 2005-2010 at surface (A) and at 10 m depth (B), estimated with CYCOFOS; *Middle panel*: Sea surface current speed data and annual averages at surface (C) and at 10 m depth (D), estimated with CYCOFOS; *Bottom panel:* MERAMOD predicted waste flux (g m^{-2} yr^{-1}) under winter (E) and summer (F) scenarios.

6.4.2 Field observations and sediments

When the monitoring systems were set up in 2012-13, epiphytes and fine particulate matter were covering the leaves of *P. oceanica* (Fig. 6-3). The lower limits investigated were sharp at the edge with high *P. oceanica* cover, surrounded by dead matte covered predominantly by *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, 1809. Four to five years following the initial deployment, all 44 markers and 132 iron stakes were still in place. Only one photostake and two labels from the initial 44 were missing. Visually, the ecological condition seemed to be improved in 2017 compared to the first surveys. The fine particulates and the epiphytes covering the seagrass leaves were less pronounced, some calcareous organisms (bryozoans and rhodophytes) were found within the rhizomes under the canopy and *C. prolifera* had almost disappeared from the surface of the dead matte (Fig. 6-4). Improvement of the ecological condition was also reflected in sediment variables. Overall, the organic matter in the sediment was reduced by about 15% and the mean grain size enlarged overall by almost 90%, from very fine sand to sand (Folk and Ward method) (Table 6-4).

Figure 6-3. Seagrass meadows near fish farms (SW left, BI right), covered in fine particles and epiphytes during first data collection.

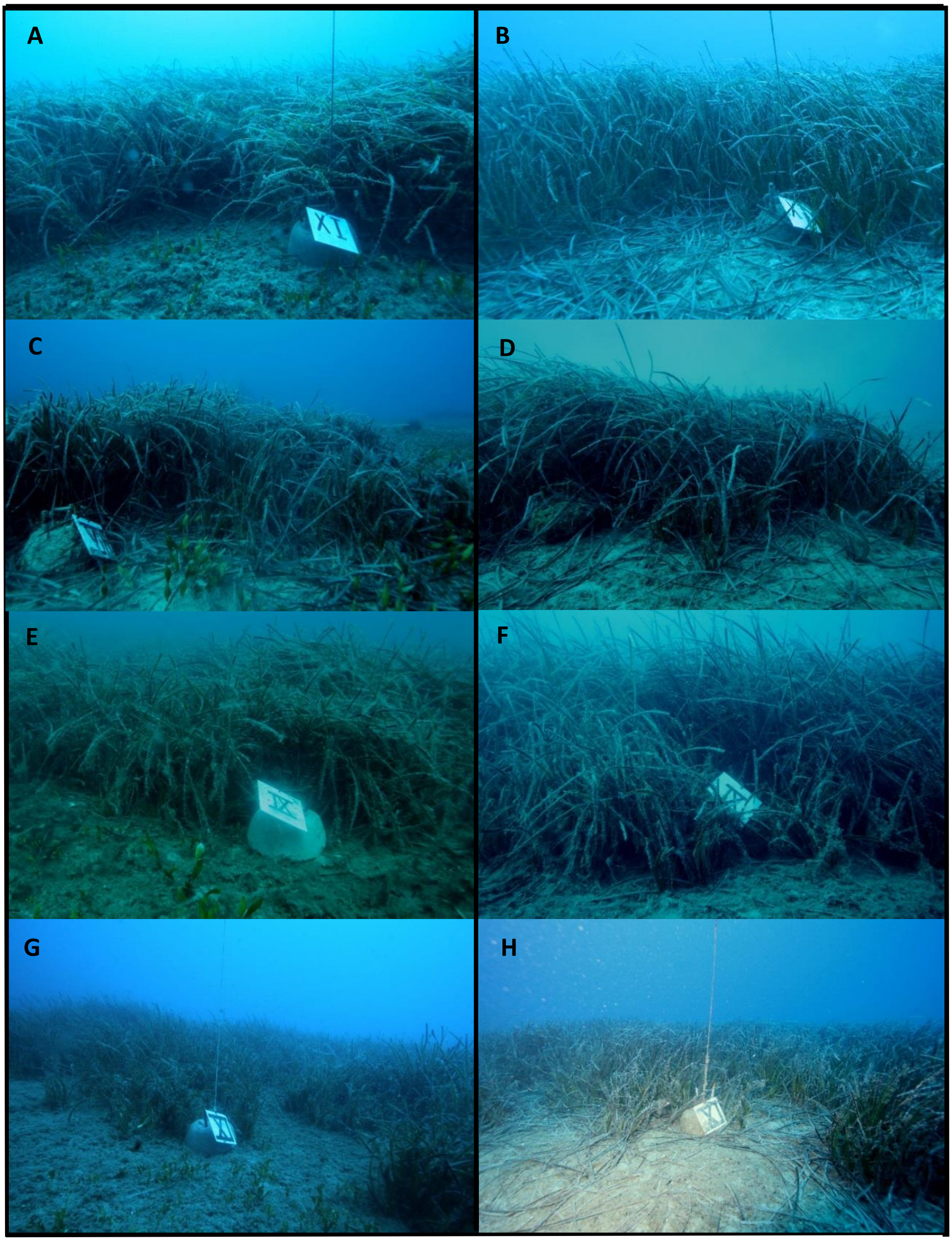

Figure 6-4. Photographs taken from fixed positions during the first data collection (left) and the follow-up monitoring (right) from: i) SW monitoring system - Marker 11 in 2012 (A) and 2017 (B), ii) BI monitoring system - Marker 8 in 2014 (C) and 2017 (D), iii) EM monitoring system - Marker 9 in 2012 (E) and 2017 (F) and iv) REF monitoring system - Marker 11 in 2013 (G) and 2017 (H).

Table 6-4. Coordinates, depth, relative abundance of sand, silt and clay, and the % organic carbon for each of the four monitoring systems at both times of sampling.

Monitoring System	Latitude Longitude	Mean depth (m)	Year of sampling	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)
SW	34°42.262'N	25.9	2012	71.54	23.72	4.74	9.41 ± 0.47
	33°16.791'E		2017	76.97	19.19	3.84	8.23 ± 0.17
BI	34°42.086'N	22.7	2014	76.01	19.99	4.00	8.73 ± 0.29
	33°16.105'E		2017	78.85	17.63	3.53	7.87 ± 0.17
EM	34°41.979'N	22.2	2012	68.09	26.59	5.32	8.35 ± 0.19
	33°13.961'E		2017	80.64	16.13	3.23	7.26 ± 0.22
REF	34°41.901'N	28.8	2013	66.49	27.92	5.58	10.43 ± 0.22
	33°11.986'E		2017	80.28	16.43	3.29	7.74 ± 0.19

6.4.3 *Posidonia oceanica* metrics

The seagrass limit had not regressed between sample dates; on the contrary, it had progressed at all markers (range 1.2 - 9 cm per year). The slowest progression was recorded at the REF monitoring system, which was the deepest and with no farm in its vicinity (mean progression 14.9 cm in 4 years; Fig. 6-5). Progression was higher at the other monitoring systems, but despite varying distances from the cages and different depths among the stations, the shoot progression was not statistically different (Kruskal-Wallis, $\chi 2 = 4.43$, df = 3, $p > 0.05$).

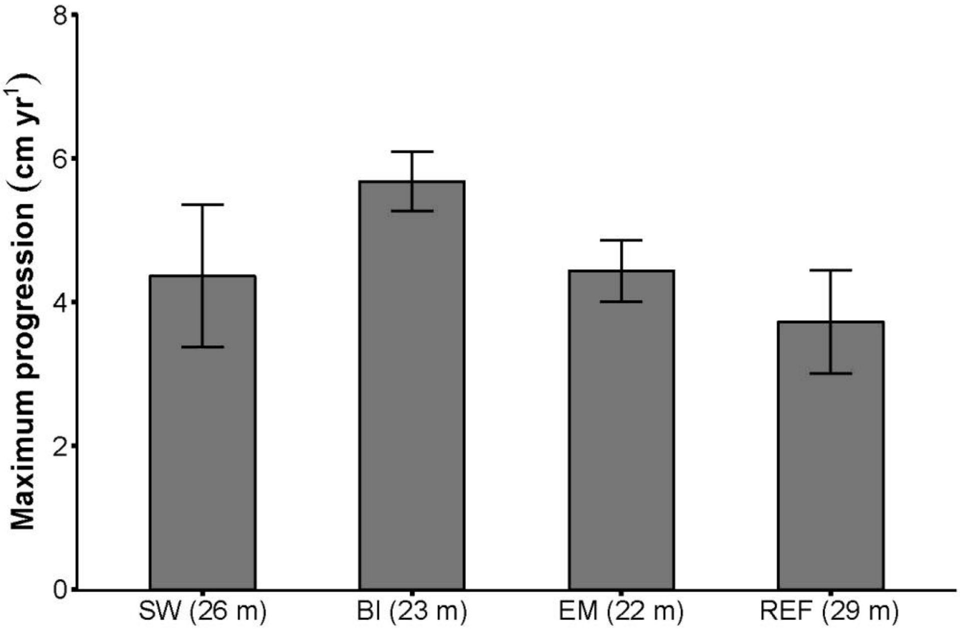

Figure 6-5. Mean maximum progression (± standard error) of *Posidonia oceanica* measured from all markers at each monitoring system.

The monitoring system REF, which had no fish farm in the near vicinity but was closer to the city of Limassol, appeared the most impacted, exhibiting significant decline in almost all the descriptors measured, including seagrass % coverage, % of plagiotropic rhizomes, number of leaves per shoot, foliar surface area and shoot exposure. Shoot density from fixed quadrats was not significantly different between the two samplings (Fig. 6-6, Table 6-5).

The EM monitoring system, which was approximately 100 m north from a small fish farm that gradually relocated and expanded about 750 m southeast from the monitoring system, had no significant change in seagrass coverage, foliar surface area and number of leaves per shoot. However, a significant increase in shoot exposure and a significant decline in shoot density was apparent, as well as in % of plagiotropic rhizomes, which was around half in 2017 compared to the first values in 2013 (Fig. 6-6, Table 6-5).

The SW monitoring system, that was at a distance between 240-275 m northeast from a major fish farm in both sampling periods, appeared to be unaffected over time, with no significant change in any descriptor being detected. Similar results were found at the BI monitoring system, which is located 250 m north from the largest fish farm (previous distance was 80 m west from the decommissioned mooring). In this case, however, while there was no significant change in seagrass coverage, shoot density, % plagiotropic shoots and foliar surface area, the shoot exposure increased and the number of leaves per shoot decreased (Fig. 6-6, Table 6-5).

Table 6-5. Changes of *Posidonia oceanica* and sediment descriptors at each monitoring system between first data collection (2012 for SW and EM, 2013 for REF and 2014 for BI) and follow-up monitoring from the same fixed points (2017). Arrows indicate significant change, – indicates no significant change.

Descriptors	**Monitoring Systems**			
	SW	**BI**	**EM**	**REF**
Coverage (%)	– Wilcoxon signed rank test, V = 14, $p > 0.05$	– Wilcoxon signed rank test, V = 6, $p > 0.05$	– Wilcoxon signed rank test, V = 4, $p > 0.05$	⬇ Paired t-test, df = 10, t =-10.09, $p < 0.05$
Shoot Density (m^{-2})	– Paired t-test, df = 10, t = -1.77, $p > 0.05$	– Wilcoxon signed rank test, V = 40, $p > 0.05$	⬇ Paired t-test, df = 10, t = 4.25, $p < 0.05$	– Paired t-test, df = 10, t = 0.79, $p > 0.05$
Plagiotropic rhizomes (%)	– Paired t-test, df =10, t = 0.87, $p > 0.05$	– Paired t-test, df = 10, t = 0.95, $p > 0.05$	⬇ Paired t-test, df = 10, t = 6.46, $p < 0.05$	⬇ Paired t-test, df = 10, t = 6.81, $p < 0.05$
Leaf Number (shoot^{-1})	– 2-sample t-test, df = 46, t = 0.51, $p > 0.05$	⬇ Mann-Whitney U test, W = 353, $p < 0.05$	– Mann-Whitney U test, W = 246, $p > 0.05$	⬇ Mann-Whitney U test, W = 339, $p < 0.05$
Foliar Surface (cm^{2}shoot^{-1})	– 2-sample t-test, df = 46, t = 1.40, $p > 0.05$	– 2-sample t-test, df = 42, t = 1.96, $p > 0.05$	– Mann-Whitney U test, W = 242, $p > 0.05$	⬇ 2-sample t-test, df = 38, t = 5.86, $p < 0.05$
Shoot Exposure (cm)	– 2-sample t-test, df = 20, t = -1.97, $p > 0.05$	⬆ 2-sample t-test, df = 19, t = -2.83, $p < 0.05$	⬆ 2-sample t-test, df = 20, t = -5.44, $p < 0.05$	⬆ 2-sample t-test, df = 20, t = -2.92, $p < 0.05$
Organic Matter (%)	– Paired t-test, df =10, t = 2.17, $p > 0.05$	⬇ Paired t-test, df =10, t = 4.38, $p < 0.05$	⬇ Paired t-test, df =10, t = 3.38, $p < 0.05$	⬇ Paired t-test, df =10, t = 11.07, $p < 0.05$
Grain size (μm)	– Wilcoxon rank sum test, V = 11, $p > 0.05$	⬆ Paired t-test, df =10, t = -2.74, $p < 0.05$	⬆ Wilcoxon rank sum test, V = 0, $p < 0.05$	⬆ Wilcoxon rank sum test, V = 0, $p < 0.05$

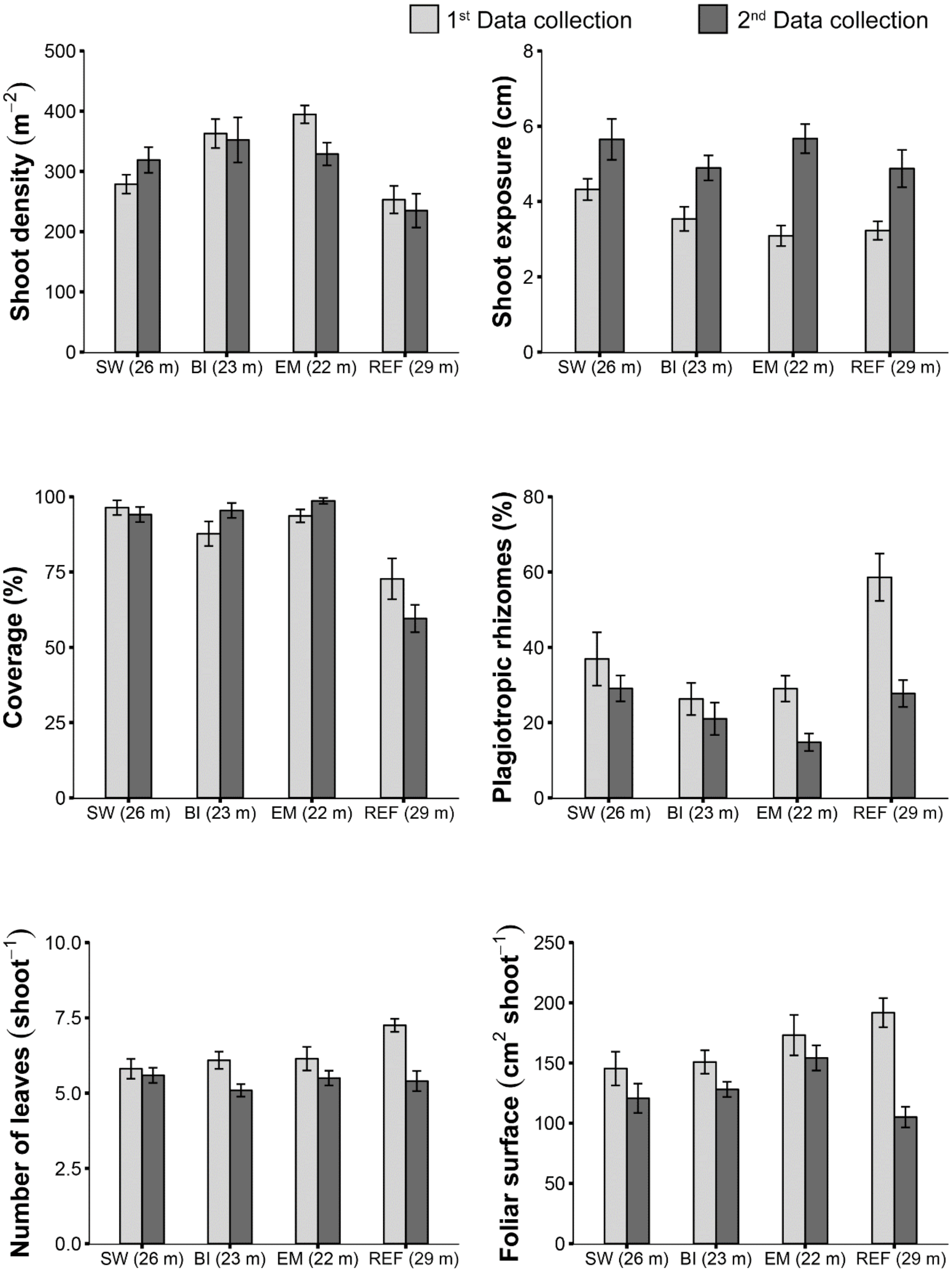

Figure 6-6. *Posidonia oceanica* descriptors determined from fixed positions in the summer-autumn period, firstly in 2012 for SW and EM, 2013 for REF and 2014 for BI (in light grey), and follow-up monitoring in 2017 at all the monitoring systems (in dark grey). Mean ± standard error.

The lepidochronological analysis carried out during the first data collection at each of the monitoring systems showed no significant change in leaf production over the years that preceded sampling and when farms operated shallow moorings nearer to *P. oceanica* meadows (Table 6-6).

The LAI decreased between the two sampling periods across all monitoring systems. The smallest decrease was recorded at the SW monitoring system (from 4.06 to 3.85 m^2 of canopy per m^2), while the largest decrease was recorded at the deepest REF monitoring system (from 4.85 to 2.47 m^2 of canopy per m^2). At both times of data collection, the EM monitoring system, the shallowest site, had the highest LAI values (6.84 m^2 of canopy per m^2 in 2012 and 5.07 in 2017) compared to all other systems.

Table 6-6. Mean number of leaves per shoot ± SE, during lepidochronological years determined from 15-20 vertical shoots collected in 2012-14 from each monitoring system.

Lepidochronological year	**Monitoring System**			
	SW	**BI**	**EM**	**REF**
2012	5.6 ± 0.3	6.9 ± 0.2	7.6 ± 0.4	6.9 ± 0.4
2011	5.7 ± 0.4	6.9 ± 0.4	6.2 ± 0.4	7.2 ± 0.4
2010	5.8 ± 0.4	6.6 ± 0.5	6.6 ± 0.5	7.1 ± 0.4
2009	4.7 ± 0.5	6.4 ± 1.0	6.1 ± 0.3	7.0 ± 0.4
2008	-	-	6.3 ± 0.3	6.5 ± 0.6
2007	-	-	6.1 ± 0.4	6.3 ± 0.5
Rate of change ± SE	0.22 ± 0.17	0.24 ± 0.14	0.06 ± 0.06	0.03 ± 0.08
R^2	0.03	0.04	0.006	0.001
df	50	57	155	84
p	> 0.05	> 0.05	> 0.05	> 0.05
Statistical analysis	Simple linear regression	Simple linear regression	Kendall–Theil Sen Siegel	Kendall–Theil Sen Siegel

6.4.4 Underlying predictors

Out of the five predictors that were investigated, time and depth had the greatest effect on the descriptors studied, but all the predictors considered seemed to play a key role in explaining most of the descriptors and had similar weight in their contribution (Table 6-7). The shoot density was the only descriptor that was not affected over time. Depth, distance and direction from the cages were significant predictors of shoot density and had the largest contribution in relative importance (Table 6-7).

Table 6-7. The source of variation and the relative importance of two environmental and three fish farm related predictors on seven measured descriptors, acquired from the multiple regression analysis. Note: The source of variation in the model takes in account all the predictors, whereas % lmg takes in account the average relative importance of each predictor without and with all the possible combinations with the rest of the predictors.

Variable	**Source of variation**					**Relative Importance**
	Predictor	Df	Sum of squares	*F*	Prob. > *F*	% lmg
Shoot density	Depth	1	2321	6.83	< 0.05	35.9
	Distance	1	1380	4.06	< 0.05	21.7
	Tonnes	1	192	0.56	ns	16.2
	Direction	2	9184	13.52	< 0.001	22.9
	Time	1	248	0.73	ns	3.2
Plagiotropic rhizomes	Depth	1	2.10	6.80	< 0.05	21.3
	Distance	1	1.56	9.65	< 0.05	16.9
	Tonnes	1	0.46	1.48	ns	11.7
	Direction	2	5.96	5.04	< 0.001	24.8
	Time	1	5.70	18.46	< 0.001	25.3
Number of Leaves	Depth	1	0.00	0.17	ns	3.2
	Distance	1	0.00	0.11	ns	16.2
	Tonnes	1	0.00	3.94	< 0.05	20.1
	Direction	2	0.00	0.91	ns	15.4
	Time	1	0.00	6.55	< 0.05	45.1
Foliar Surface	Depth	1	0.23	7.75	< 0.01	21.5
	Distance	1	0.00	0.29	ns	9.7
	Tonnes	1	0.04	1.52	ns	8.9
	Direction	2	0.00	0.02	ns	11.5
	Time	1	0.50	16.80	< 0.001	48.4
Shoot exposure	Depth	1	15.05	15.04	< 0.01	14.7
	Distance	1	3.11	1.48	ns	16
	Tonnes	1	9.10	9.09	< 0.05	9.5
	Direction	2	14.64	3.48	< 0.05	32.1
	Time	1	19.54	9.29	< 0.01	27.8
Grain size	Depth	1	0.00	0.80	ns	3.7
	Distance	1	0.00	12.66	< 0.001	18.9
	Tonnes	1	0.00	100.73	< 0.001	8.7
	Direction	2	0.00	29.17	< 0.001	19.6
	Time	1	0.00	10.04	< 0.05	49.0
Organic matter	Depth	1	0.00	21.67	< 0.001	14.5
	Distance	1	0.01	52.62	< 0.001	15.7
	Tonnes	1	0.00	5.60	< 0.05	9.2
	Direction	2	0.00	2.06	ns	16.6
	Time	1	0.00	12.06	< 0.001	44.0

6.5 Discussion

In Cyprus, mariculture activities are concentrated in an area between the cities of Limassol and Larnaca. Small scale production started here in the mid-nineties in shallow water (< 30 m) over *P. oceanica* meadows and this may have contributed to degradation and regression of the lower limits of the meadows. Thereafter, managers followed a precautionary approach (Pergent-Martini *et al*., 2006) and only allowed new production units to be placed in deeper water while asking for the relocation of the shallow cages when existing farms requested expansion of their production. This study has shown that this management intervention may have been effective in preventing further declines in the lower limits of the meadows studied. It proves the point that local impacts on *P. oceanica* can be managed at the local level (Guillén *et al*., 2013).

These permanent *P. oceanica* monitoring systems are the first in Cyprus and the easternmost seagrass PMN systems in the Mediterranean. Setting up monitoring systems using permanent cement markers is a durable and effective method to monitor the edge of seagrass meadows from fixed positions over medium to long timeframes (Pergent *et al*., 2015) and is substantially more robust than random plots for monitoring seagrasses (Schultz *et al*., 2015). About five years following deployment, all cement markers were still in place, despite some major storms. The results indicate that *P. oceanica* has not regressed during this time and although an overall lower performance was recorded in some *P. oceanica* structural descriptors, this was not detected near fish farms.

Contrary to expectations, *P. oceanica* meadows had progressed at all monitoring stations and although differences among the monitoring systems were not statistically significant, the largest observed progression was recorded near the largest fish farm. There is hope, therefore, that despite major losses of *P. oceanica* from fixed PMN markers at deep meadows in the north-western Mediterranean (Boudouresque *et al*., 2000, 2012; Pergent *et al*., 2015), a decline has not been detected in impacted deep meadow limits in the eastern Mediterranean. This is despite the fact that water temperatures are close to the reported upper limit of the species (Celebi *et al*., 2006). The seagrass horizontal growth rates reported in this study may be overestimated as there was a selection bias for the furthest rhizome from the marker; however, the values obtained are consistent with previous estimations but lower than recolonization rates measured along labelled fixed pegs at shallower healthy patches in the western Mediterranean (Gobert *et al*., 2016). In other studies, *P. oceanica* meadows could survive close to fish farm cages, even though effects on *P. oceanica* descriptors were detected at large distances from the fish cages (Borg *et al*., 2006; Marbà *et al*., 2006; Holmer *et al*., 2008; Rountos *et al*., 2012). In this study, *P. oceanica* descriptors did not clearly detect impacts of the fish farm operations.

The impacts of mariculture on *P. oceanica* meadows are site-specific and dependant on variables, such as the size of the farm and the intensity of feeding, depth and hydrodynamics. In Cyprus, it seems that the decision to relocate the fish farms deeper (southern), in an area dominated by west and east currents, has been successful in mitigating impacts to the *P. oceanica* meadows that stretch northwards. The model simulations presented in this study, showed that the main dispersal of particulate matter is not in the direction of the seagrass meadows investigated. However, only two months were considered and resuspension, which would tend to increase dispersion of waste particles, was not considered in the model. Furthermore, with the estimated velocity of currents, farm effluents can disperse over a distance covering several kilometres (Sarà *et al*., 2006) and affect *P. oceanica* meadows even *ca* 3 km away, in ways that are not always reflected by alterations in structural descriptors (Ruiz *et al*., 2010).

At the sites monitored, improvement was also recorded in sediment variables: the organic matter content decreased, and the mean grain size increased in all monitoring systems, except at SW where the changes were not significant. This, together with the increase in *P. oceanica* shoot exposure measured during the follow-up monitoring, may indicate less sedimentation of suspended fine particulates or resuspension of silty sediments during storms that preceded the second sampling. It is also noteworthy that *C. prolifera,* a highly nitrophilous green seaweed, was very abundant during the first data collection but rare during follow up monitoring, which is another indication of improved water quality condition (Holmer *et al*., 2009).

Across the four monitoring systems assessed, the lowest rates of progression and the highest reduction in the performance of *P. oceanica* descriptors were recorded at the REF monitoring system, which lies far from any aquaculture operations. At both times of sampling, lower seagrass coverage and shoot densities were measured at this monitoring system compared to its shallower counterparts, although the values of shoot densities measured still indicate high ecological condition of the meadows (Pergent *et al*., 1995) and progression of the meadow was still recorded despite a strong dynamic regression at other PMN lower limit reference sites in the Mediterranean (Pergent *et al*., 2015). The deeper water, and consequently the reduced light availability and water circulation, may be the most important limiting factor of the *P. oceanica* descriptors (Martínez-Crego *et al*., 2008). Furthermore, this site was closer to the anthropogenic footprint of Limassol city, which may be affecting the *P. oceanica* meadow. For example, about 1.4 km to the northeast there is a sewage outlet releasing processed effluents generated from Limassol. This monitoring system can provide valuable baseline data if fish farming begins nearby.

The variation in descriptors considered in this study was explained by the cumulative effects of environmental and farm predictors. The variables having the most cumulative effect on *P. oceanica* descriptors, were: water depth followed by direction, and then distance to the nearest fish farm or production tonnage. The multiple regression analysis to identify main predictors was purely suggestive. The creation of more PMN systems at different directions from the fish farms can enable better discrimination of the factors contributing to the changes in structural and demographic *P. oceanica* descriptors.

The PMN protocol allows microscale detection of regression/progression of seagrass lower limits using structural and morphological *P. oceanica* descriptors that are widely applied in generic ecosystem monitoring. Most of the structural indicators considered exhibit marked seasonality and/or strong bathymetric dependence (Marbà *et al.*, 2013). This bottleneck of inherent patchiness and differences of these indicators across the meadow was removed by sampling around the same time of year (summer - autumn period) and from the same fixed plots (same depth). However, data should be interpreted based on the validity of the structural indicators used to reflect stress. The diversity of *P. oceanica* indicators is striking; structural descriptors of *P. oceanica* used in this study such as coverage and shoot density are widely used in monitoring programmes (e.g. EU Water Framework Directive) as they are linked directly to ecosystem integrity and can detect generalized degradation responses (Martínez-Crego *et al.*, 2008; Marbà *et al.*, 2013). A recent global review of seagrass indicators identified structural indicators such as density, coverage and depth limit among the best suited indicators for generic ecosystem monitoring, stress screening and ecological assessment (Roca *et al.*, 2016).

Lower shoot size, shoot density and coverage are commonly reported responses for *P. oceanica* meadows exposed to fish farm effluents (Pergent-Martini *et al.*, 2006), though this was not detected in this study. The number of leaves per shoot are responding consistently to light stress in seagrasses making them a robust bioindicator of degraded water quality (McMahon *et al.*, 2013). Lepidochronological analysis showed that before this study and relocation of farms, the number of leaves per shoot at each monitoring station was stable and not very different across stations. Between the two sampling periods, the leaf number decreased at two stations but declines of this descriptor near aquaculture are not always consistent (Pergent-Martini *et al.*, 2006). The percentage of plagiotropic rhizomes is correlated with water quality (Gobert *et al.*, 2009). On the other hand, the plagiotropic rhizomes remain plagiotropic when surrounding substrate space is sufficient to allow lateral expansion and revert to orthotropic in dense meadows where space is inadequate for colonization (Molenaar *et al*., 2000). This makes any comparisons using this descriptor difficult. Plagiotropic rhizomes are the most common rhizome on edges of meadows, while orthotropics are predominant in continuous meadow (Lapeyra, 2016). Hence the lower plagiotropic rhizome measured from the fixed positions of decommissioned sites (REF and EM) in the follow-up monitoring may be partly explained by the fact that the edge has progressed a little and what used to be the edge of the meadow is now a little further inside.

Structural descriptors are responsive to degradation but are not effective in reflecting early improvements and recoveries because they respond slowly and detect impacts much too late for effective management action to be taken (Roca *et al*., 2015, 2016). Environmental change is first reflected in plant physiology, which modifies seagrass growth and morphology, that induce changes in meadow structure (Collier *et al*., 2012). Thus, physiological indicators present more stressor-specific responses and can detect degradation responses much faster (Roca *et al*., 2015, 2016). At the highest level of cellular response, altered gene expression of stress-related genes are the faster predictors of an imminent seagrass collapse (Ceccherelli *et al*., 2018). One drawback of the PMN protocol is that it only includes structural variables and once a decline in these parameters is sufficiently large to be detected in *P. oceanica* meadows, there is a considerable risk that the seagrass meadow has already degraded irreversibly. The PMN protocol applied in this study can benefit from incorporating early-warning indicators together with the structural *P. oceanica* descriptors considered. The response of the physiological indicators is highly stress-specific, so the choice depends on the objectives of the management strategy (Roca *et al*., 2016).

Despite national and international protection, large declines of *P. oceanica* meadows have been documented since the second half of the 20th century, especially near urban areas of the western Mediterranean (Marbà *et al*., 2014; Telesca *et al*., 2015). The loss of *P. oceanica* meadows may result in the erosion and rapid remineralisation of the carbon-rich matte, accelerating climate change (Pergent *et al*., 2014). Efforts to conserve *P. oceanica* lie mostly in the establishment of marine protected areas, which seem to be insufficient to guarantee the protection of *P. oceanica* meadows (Montefalcone *et al*., 2009). Across the Mediterranean Sea, seagrass monitoring is extensive, but the adoption of different sampling designs and methods may result in erroneous comparisons (Lopez y Royo *et al*., 2010b). Recently, the PMN has refined a standardised methodology for setting up *P. oceanica* monitoring systems, which has been applied in the Euro-Mediterranean region. Comparable temporal monitoring along the edge of the meadow is possible through photography and measurements of vitality parameters from fixed positions. Slow growing seagrasses such as *P. oceanica* are especially suited to fixed-plot monitoring (Schultz *et al*., 2015) as small-spatial scale progression or regression of the meadow can be monitored effectively.

The *P. oceanica* monitoring systems set up and monitored in this study are valuable tools for researchers, managers and decision makers and their application should be promoted. Set up at the deepest boundaries of the meadows, near the compensation depth where the plants are most sensitive to changes in water quality, they form an important indicator of ecological integrity and allow for detection of small losses, which is critical for slow growing *P. oceanica* (Holmer *et al*., 2003; Buia *et al*., 2004). Future comparisons will guide responsible management and increase our understanding regarding the mariculture impacts on *P. oceanica* in the eastern Mediterranean. They can also be compared with other PMN systems set up in other places across the Mediterranean Sea to assess the *P. oceanica* population dynamics in different regions. The use of fixed plot methods using cement markers, like the PMN method applied in the Mediterranean or using quadrats placed over transects like the SeagrassNet method applied globally (www.SeagrassNet.org), allow reliable and effective microscale monitoring of seagrass descriptors from the same positions using standardised methodologies, have high statistical power, and their use should be encouraged and widely adopted in generic ecosystem monitoring (Schultz *et al*., 2015). The disadvantage with the used *P. oceanica* descriptors is that they respond late to generalised pressures. If the management strategy aims to achieve effective early detection of stress imposed on *P. oceanica* by specific anthropogenic activities (e.g. burial, metal pollution, eutrophication, organic enrichment, shading), it is recommended to incorporate stress-specific biochemical and genetic indicators in the monitoring program.

7. CHALLENGES OF INTEGRATED MULTI-TROPHIC AQUACULTURE IN THE EASTERN MEDITERRANEAN

7.1 Abstract

Fish farming is expanding globally to meet an increasing demand for seafood by a growing human population. Integrated Multi-Trophic Aquaculture (IMTA) is widely used in Asia to increase efficiency in finfish production and mitigate environmental impacts by turning waste effluents into valuable products yet it is hardly used at all in the western world. Over a three-year period, IMTA experiments were carried out at a European seabass (*Dicentrarchus labrax*) and Gilt-head seabream (*Sparus aurata*) fish farm in Cyprus to inform developments in Europe. Mussels (*Mytilus galloprovincialis*), abalone (*Haliotis tuberculata*), oysters (*Ostrea edulis*), sea urchins (*Paracentrotus lividus*), crabs (*Callinectes sapidus*) and sponges (*Sarcotragus foetidus* and *Calyx nicaeensis*) were cultivated. Most co-cultivars had little or stunted growth and high mortality. Mussels almost reached commercial size, but their meat yield decreased significantly when water temperatures were highest. Longlines proved to be an unsuitable technique for growing mussels in exposed waters; survival and growth were better when the mussels were reared in net enclosures. Oysters displayed high mortalities and stunted growth, but there was a spike in growth when biofouled baskets were replaced. Herbivores such as abalone and sea urchins survived well in baskets and sea urchins developed gonads when fed with seaweed, which was not readily abundant. Crabs utilised discarded fish tissue, but they became carnivorous in small spaces. Sea urchins reduced the organic matter in the sediment, but mortalities were high at high stocking densities. Benthic IMTA in deep waters is not feasible with the current technology. Sponges seem to be a promising IMTA crop in Cyprus and their commercial niches should be explored further. The trials demonstrate the logistical difficulties of IMTA and that further research is needed before

it becomes a viable commercial option for fish farms in warm, oligotrophic, exposed localities.

7.2 Introduction

Annual per capita fish consumption has risen from an average of 9.9 kg in the 1960s to over 20 kg in 2015 (FAO, 2016). With over a third of the global fish stocks being over-exploited, and more than half fully-fished, supplies from wild caught fisheries have remained at around 90 million tonnes since 2000 (Costello *et al.*, 2016; Jennings *et al.*, 2016). To meet growing demand of an increasing human population the aquaculture industry increased from around 3.5 million tonnes and 0.5% of global fish supplies in 1970 to more than 106 million tonnes and around 53% of global fish supplies by 2015 (FishStatJ, 2017).

The aquaculture industry has expanded into new areas and undergone substantial technological innovations, yet it can have adverse environmental and socioeconomic effects (Osmundsen & Olsen, 2017). These include issues such as privatization of common resources, salinization of land and aquifers, interactions of escaped fish with their surrounding wild conspecifics and coastal ecosystems, introduction of alien species into ecosystems, depletion of wild fish stocks for the production of fish meal and fish oil used in aquaculture feeds, and degradation of coastal and aquatic ecosystems from the chemical pollution and organic enrichment (Naylor *et al.*, 2000; Hall, 2004; Hall-Spencer *et al.*, 2006: Holmer *et al.*, 2005; Primavera, 2006; Martinez, 2009; Savini *et al.*, 2010; Arechavala-Lopez *et al.*, 2017).

Sustainable development of aquaculture is a prerequisite for the industry to maintain its increasing stature and importance in meeting future demands for seafood (Subasinghe *et al.*, 2009). Currently, there is a growing trend towards enhanced intensive systems and the production of carnivorous species (Bostock *et al.*, 2010). A significant portion of the fish feed is still wasted in open sea carnivorous finfish systems, with economic and environmental implications (Ballester-Moltó *et al.,* 2017). Improving the efficiency of resource utilization through better management and technological advances is a major research area.

IMTA is receiving increasing attention for its potential to improve efficiency and promote sustainability (Lembo *et al.,* 2018). It has been widely promoted by NGOs, industry actors and scholars as ecologically efficient, environmentally acceptable, product-diversified, profitable and beneficial to society (OECD, 2010; Espinós *et al.,* 2011; Chopin, 2012). Integrated cultivation of fish with shellfish and seaweeds is traditional and common in lagoons and bays in Asia (Neori *et al.,* 2004). The factors that underpin the success of IMTA in these regions remain elusive as information is rarely published (Neori *et al.,* 2004). Outside Asia the IMTA concept is relatively new and despite the fact that it has the potential to alter public perceptions of the aquaculture sector, promoting more sustainable practices (Alexander *et al.,* 2016), there is little commercial uptake, with most of the IMTA systems operating at a research or pilot scale. In contrast, peer-reviewed articles indicate strong evidence of successful IMTA applications and significant growth of IMTA species, a figure which according to Kerrigan & Suckling (2016), overestimates take-up by the industry. Over the last years, there has been a growing interest in understanding the trade-offs between IMTA and monoculture and identify the bottlenecks that are slowing IMTA adoption.

By comparing the trade-offs for an individual farmer or company to diversify from fin-fish production into an IMTA system, Hughes & Black (2016) concluded that in contrast to the situation in Asia, the balance in Europe is not yet sufficiently positive to motivate the large-scale uptake of IMTA. Several impediments of IMTA are discussed in the literature, including: legislation bottlenecks (i.e. bureaucracy and lack of IMTA-specific licences) (Alexander *et al.,* 2016), and lack of evidence that IMTA can be economically profitable (Kinney, 2017; Lance *et al.*, 2017) and environmentally beneficial (Robinson & Reid, 2014; Cubillo *et al.*, 2016). In addition, there are technological and infrastructure complexities (Holdt & Edwards, 2014; Hughes & Black, 2016), unavailability of hatcheries to provide co-cultivar species (Alexander & Hughes, 2017), and inadequate knowledge to enable selection of species and stocking densities (Chopin, 2012) to sustain year-round production (Handå *et al.*, 2013; Skjermo *et al.*, 2014).

To date, the IMTA development has largely been system/site-specific without effective knowledge exchange about logistical difficulties. Effective transfer of knowledge and skills across organisations and countries is often advocated to accelerate long-term industry development (Kumaraswamy & Shrestha, 2002). IMTA needs innovation and its commercialisation depends on collaboration between science and industry and sharing of expertise and lessons learnt. Identifying the correct system configuration takes a lot of trial and error and learning by doing and therefore knowledge-sharing is crucial (Alexander & Hughes, 2017).

In the Mediterranean Sea, and particularly in the eastern basin, the most important barrier to IMTA development is likely the unsuitable environmental conditions (Kleitou *et al.*, 2018). Seaweeds can work well in aquaculture bioremediation (Troell *et al.*, 2003; Neori *et al.*, 2004; Granada *et al.*, 2016), but a lack of nutrients throughout most of the eastern Mediterranean (**Chapter 2**; Kletou & Hall-Spencer, 2012) makes it poorly suited to seaweed farming. Several studies have shown that bivalves have enormous potential as biofilters of fish farm effluents (Reid *et al.*, 2010; MacDonald *et al.*, 2011; Lander *et al.*, 2013) but most were carried out in nutrient rich waters, where bivalve cultivation is economically sustainable (Sarà, 2007). Oligotrophic waters are usually unsuitable for farming suspension feeders, although high current velocities can compensate for the lower quantity of suspended matter and can sustain bivalves (Sarà & Mazzola, 2004).

Bivalves can feed on detritus, so their cultivation in oligotrophic waters may be possible near fish farms. This study elaborates on the experiences and lessons learnt from a series of IMTA trials in Cyprus (eastern Mediterranean) under the framework of IDREEM (*Increasing Industrial Resource Efficiency in European Mariculture*), a European FP7 project. The aim of the experiments was to grow an array of available commercial species using different designs, and to identify bottlenecks and opportunities for IMTA in the oligotrophic eastern Mediterranean by monitoring the performance of the organisms cultured.

7.3 Materials and Methods

7.3.1 Study site

Across Europe, Cyprus in the eastern Mediterranean, has perhaps the most challenging environmental conditions to grow extractive species (Fig. 7-1). The warm and oligotrophic waters are well suited to culture sea bass and bream, but are far from optimum for co-cultivar filter feeders, which may face malnutrition close to their thermal tolerance levels. Background nutrient levels are too low to sustain commercial macroalgal production.

The IMTA experiments were conducted at SW fish farm in Vasiliko Bay, south-east Cyprus. Vasiliko Bay has most of the finfish production units of the island. SeaWave floating cages were moored over soft-bottom benthos at depths 37-42 m about 1.2 km offshore. During the experiments, SW was licenced to produce 1000 tonnes of gilthead sea bream (*Sparus aurata* Linnaeus, 1758) and European sea bass (*Dicentrarchus labrax* (Linnaeus, 1758)) in 2013, 1200 tonnes in 2014-15 and 1500 tonnes from 2016 onwards. The cultivation of fin-fish was conducted in three parallel rows of ten cages.

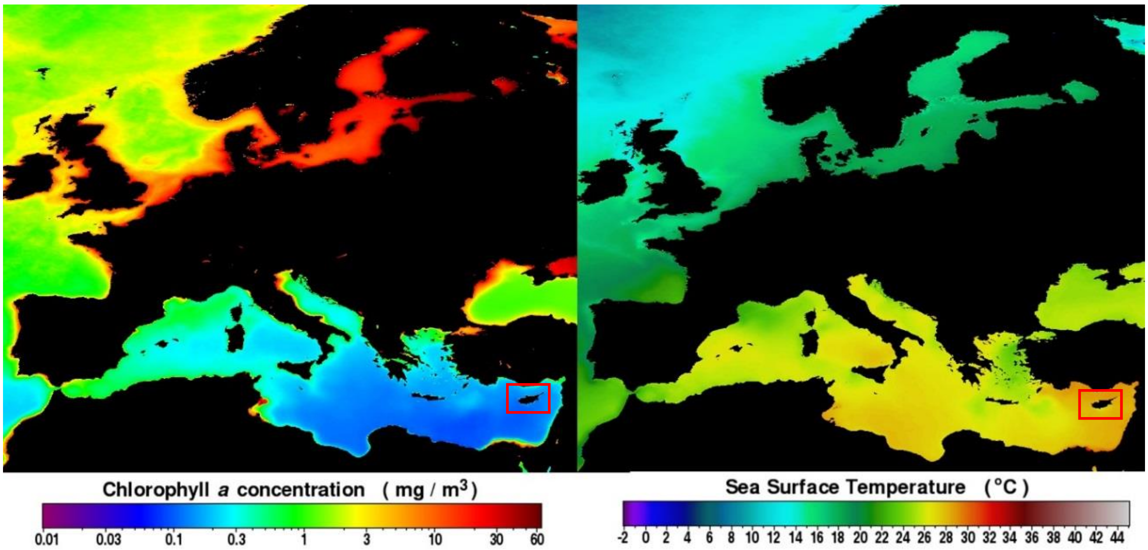

Figure 7-1. Cyprus surface waters (in red box) are the warmest and most oligotrophic in Europe. MODIS-Aqua 2002-2018 mean chl. *a* concentration (left) and 2002-2016 mean summer surface temperature (right) (NASA, 2018).

7.3.2 Environmental conditions

Physico-chemical water column variables were collected monthly for a year in 2012-2013. The water column was profiled with an Idronaut Ocean Seven 316 Plus CTD to determine the dissolved oxygen, temperature, conductivity and pH, and a Cyclops C6 (Turner Designs) to determine chlorophyll *a in vivo* and turbidity. Three replicates of water samples were collected with a Van Dorn sampler from three depths (surface, 15 m and approximately 1 m above the bottom at 36 m) to determine Total Particulate Matter (TPM) and nutrient concentrations. Chlorophyll *a* and pheophytin concentrations were determined on a seasonal basis (autumn was missed). Approximately 2 L of seawater were filtered separately to determine TPM and chl. *a*. For the former, pre-combusted, pre-weighted glass fiber filters were used. The residue and filter were first dried at 100 ^{o}C in a drying oven until constant weight and mass was recorded with a 5 d.p. analytical balance to determine TPM after extracting the weight of the filter. Nutrients and chl. *a* were determined using the Trilogy laboratory fluorometer (Turner Designs). The guidelines of Holmes et al. (1999) and recommended modifications of Taylor et al. (2007) were followed to determine the ammonium (NH_4^+), procedures adapted from Strickland &

Parsons (1972) were followed to determine nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions, and the acidification Method EPA 445.0 (Arar & Colins, 1997) for chl. *a* and pheophytin.

Sediment was collected seasonally for a year using a 0.1 m^2 Van Veen grab (KC Denmark) to carry out granulometric analysis and determine the organic matter content using five replicates of the surface sediment (top 5 cm) below the cages and at increasing distances (50 m, 100 m and 200 m), north from the farm towards the shore. Samples were dried in a drying oven and weighted, then sieved with Endecotts Octagon sieve shaker with mesh sizes ranging from 0.075 mm to 2 mm. Weight in each sieve was measured with a 3 d.p. analytical balance. The OM was calculated based on the Loss On Ignition (LOI). Specifically, 5 g of sediment that passed through the 212 μm sieve were combusted in a muffle furnace at 550 ^oC for 3 hours and the OM was determined as the difference between the dry and LOI weight measured with a 5 d.p. analytical balance.

7.3.3 IMTA trials

IMTA pilot trials started in the summer of 2013 and lasted for *ca* three years. Several experiments with different species were conducted throughout this period, both in the water column and benthos (Fig. 7-2, Table 7-1). The selection was narrowed to native (except for the established blue crab) to the Mediterranean Sea filter feeders, detritivores and herbivores that could be supplied in large numbers and/or were provided by hatcheries/suppliers across Europe.

Table 7-1. Species cultured, methods trialled and duration of the different IMTA experiments carried out in Cyprus.

Extractive Species	Culturing method	Start of experiment	End of experiment
Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819	– Socks hanged on longlines at 3, 15 and 30 m – Socks hanged on longlines at 15 m depth – Socks hanged in juvenile fish and empty cage	Jul. 2013 Nov. 2014 Jul. 2015	Oct. 2014 Sep. 2016 Sep. 2016
Purple sea urchin *Paracentrotus lividus* (Lamarck, 1816)	– Three different concentrations (25, 50, 100) in 1 m^2 benthic enclosures – In Ortac baskets hanged on the side of fish cages at 5-10 m depth, fed with seaweed	Oct. 2013 Sep. 2015	Jul. 2014 Oct. 2016
European flat oyster *Ostrea edulis* Linnaeus, 1758	– In inserts inside of Ortac baskets at 15 m depth	Sep. 2014	Oct. 2015
Green ormer *Haliotis tuberculata* Linnaeus, 1758	– In Ortac baskets hanged on the side of fish cages at 5-10 m depth, fed with seaweed	Jun. 2015	Sep. 2016
Blue crab *Callinectes sapidus* Rathbun, 1896	– In SEAPA baskets hanged on the side of fish cages at 5-10 m depth, fed with dead fish	Jul. 2015	Oct. 2015
Native sponges *Sarcotragus foetidus* Schmidt, 1862 *Calyx nicaeensis* (Risso, 1826)	– In mesh panel, hanged adjacent to fish cages at 10 m depth – Using threaded line in panel method, hanged adjacent to fish cages at 10 m depth – Using wrapped threaded line around the fish farm ropes at 5 m depth	Jun. 2016	Oct. 2016

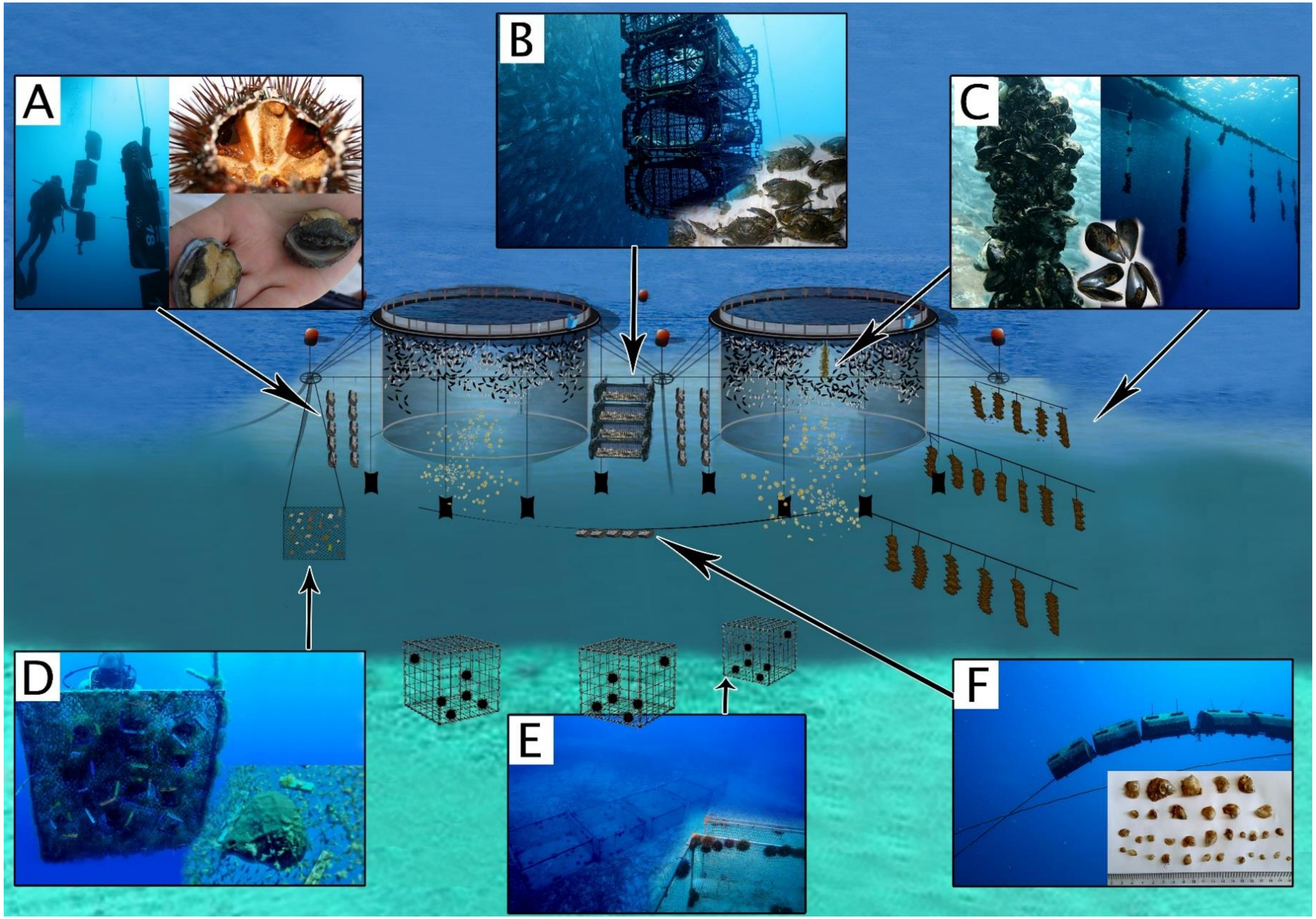

Figure 7-2. IMTA trials in Cyprus. A: Sea urchins and abalone in Ortac baskets; B: Blue crabs in Seapa baskets; C: Mussels hanged in longlines at different depths, in empty and sea bass cage; D: Sponges in mesh panel; E: Sea urchins in benthic enclosures; and F: Oysters in Ortac baskets.

7.3.3.1 Mediterranean mussel

Three batches of *Mytilus galloprovincialis* Lamarck, 1819, were deployed in different years (Table 7-1). A small number of socks containing juvenile mussels were shipped from Greece; transit lasted 3-5 days. In the first trial, the mussel socks were distributed at three depths (shallow at 5 m, intermediate at 15 m and deep at 30 m). About fifty mussels were collected from each depth every two to three months for over a year, to determine shell length and width, wet weight and Meat Mussel Yield (MMY), calculated using the formula: *meat weight after in boiling water for 5 min. * 100 / empty shell weight + boiled meat weight*. The aim was to detect any differences in mussel viability and growth above and below the thermocline. The second batch of mussels arrived late due to embargos at the site of origin and were deployed at the intermediate depth. Soon after, in January 2015, a phenomenal storm with strong bursts of wind reaching 10 beaufort claimed almost the entire batch. This storm also caused unprecedented fish losses from most fish farms of the island. About 100 individuals from the remaining mussels were monitored as above for almost two years (Table 7-1). The last batch was deployed inside fin-fish cages to protect them from storms and predation, some were placed in cages containing juvenile sea bream, others in cages with juvenile sea bass and the rest in an empty cage. The sea bream quickly predated on the mussels. About 100 mussels from each of the empty cage and the sea bass cages were monitored for a year as above. Furthermore, a few mussels from this last batch were placed in Zygi marina (near the study site) to assess the growth and survival of the mussels in warmer and more eutrophic water.

7.3.3.2 Purple sea urchin

Two pilot experiments were conducted with native sea urchins *Paracentrotus lividus* (Lamarck, 1816), one on the benthos and the other in the water column. In the former, ten benthic enclosures (surface area 1 m^2) were deployed at 34 m depth *ca* 100 m downstream from the farm. Young sea urchins were collected from a highly dense local population at a shoreline of south Cyprus. Three replicates of three stocking densities (25, 50, and 100 sea urchins m^{-2}) were set-up and one benthic enclosure was left empty to act as control. Sea urchins could graze and scavenge on the sea floor but were cultivated extensively and not provided with supplementary diet. Surface sediment and five sea urchins were collected from each benthic unit at four intervals in a period of eight months. The shell diameter and height, wet mass and gonadal weight of the sea urchins was measured to calculate the gonadosomatic index (% of gonad weight to total weight) and the sediment was analysed to determine the organic matter content. For the second trial, a small number of Ortac baskets each containing ten juvenile sea urchins, were hanged on the side of the cages at about 5-10 m depth. The sea urchins were fed approximately every two to three weeks with seaweed collected around the farm structures, but the seaweed was not always abundant. Metrics for this IMTA crop were collected at five intervals within a period of a year.

7.3.3.3 European flat oyster

One small batch of tiny native oysters *Ostrea edulis* Linnaeus, 1758, received from a Scottish hatchery after about three days in flight transit, were placed within inserts of Ortac baskets and deployed at 15 m depth near the finfish cages. Three inserts were removed eight times within a year. Observations such as associated fouling taxa, shell dimensions and wet weight of surviving oysters were recorded. The Ortacs were changed after about 10 months in the experiment, following heavy biofouling and stagnated oyster growth. The oysters were monitored two more times before this pilot ended.

7.3.3.4 Green ormer

One batch of native abalone *Haliotis tuberculata* Linnaeus, 1758, was transported from a French hatchery. High mortalities were observed during the first week, most likely due to transit stress and slow response between delivery and deployment. Survivors of two size classes (small and large) were deployed in Ortac baskets arranged vertically in tetrads and deployed on the side of finfish cages at 5-10 m depth. Unfortunately, the tetrad with all the small sized abalone was lost early in the experiment following bad weather and efforts to recover it were unsuccessful. The experiment proceeded with the large abalone, which were fed on seaweed collected from around the farm every two to three weeks, but the seaweed was not always abundant, and the abalone were frequently undernourished. Survival, shell size were monitored eight times for a period of one year. Weight of green ormers was measured less frequently.

7.3.3.5 Blue crab

The blue crab *Callinectes sapidus* Rathbun, 1896, is not native to the Mediterranean but has established and is locally abundant. Only a small number of crabs was used in this pilot. The aim was to investigate whether these scavengers could grow on a diet of fresh fish that die from stress following harvesting events. These fish represent a considerable amount of energy that is wasted or combusted. Ten blue crabs were placed in each Seapa basket arranged in bundles of four and hung on the external side of the fish cages at 5 m depth. They were fed with fish every other week. Their feeding behaviour and survival rates were monitored. This experiment was cut short and surviving blue crabs were removed only about three months later.

7.3.3.6 Native sponges

Following sponge surveys carried out at south Cyprus coastal areas with the assistance of IDREEM partners, it proved difficult to find sufficient quantities of bathing sponges to stock an IMTA unit, thus locally abundant sponge species *Sarcotragus foetidus* Schmidt, 1862 and *Calyx nicaeensis* (Risso, 1826) were used to test the potential of sponges in IMTA application. About 30 explants of sponge were collected from an area near the farm, leaving behind the bulk of the donors to recover. The sponge explants were cultivated using three different methods, namely wrapped threaded line, threaded line in panel, and mesh panel. Specifically, (i) *S. foetidus* explants were threaded onto 3 mm dyneema lines, which was wrapped around the ropes of the cages at approximately 5 m depth, (ii) *S. foetidus* explants were threaded onto 3 mm dyneema lines mounted in parallel on a stainless-steel panel, and (iii) *C. nicaeensis* explants were placed into a polyester mesh (4 mm mesh size) within a stainless-steel panel. In the latter technique, pegs around the explants were used to keep them stationary and attached to the mesh. Both techniques with the panels (i.e. threaded line and mesh) were hanged below the buoys of the fish farms at around 10 m depth.

All the sponges dislodged themselves from the threaded lines a few days following deployment, thus only the *C. nicaeensis* in the mesh panels were visually monitored for about five months. Following the end of the project, the explants were released in the area they were collected.

7.3.3.7 Data analysis

Performance data collected regarding the mussels, sea urchins and oysters were averaged by sampling expedition and treatment and the standard error (SE) was calculated. To characterise the pattern of the crop performance data over time, a trend analysis was conducted using linear and polynomial models (quadratic and cubic). The model was chosen based on two key criteria including the pattern of data and the highest R^2 given by the fitted models.

7.4 Results

7.4.1 Environmental conditions

The entire water column was oligotrophic in summer (chl. *a* < 0.1 μg L^{-1}), becoming mesotrophic in winter and spring (chl. *a* 0.1-0.3 μg L^{-1}) (Table 7-2). At most times, chlorophyll *a in vivo* displayed different vertical distribution in the water column being higher near the bottom (Table 7-3). Temperature was *ca* 17 °C in winter and ranged from 20-28 °C in the summer and early autumn with a pronounced thermocline (Table 7-3). This coincided with a halocline and the salinity gradient across the water column ranged between 38.9 ppt to 39.5 ppt (Table 7-3). During the warm season, the water column turned more acidic and, in some cases, hypoxic with the lowest values of dissolved oxygen 2-3 ppm measured in September (Table 7-3). Ammonia was elevated at the surface water, but most other nutrients were close to minimum detection limits and phosphate was always below (<1 μg L^{-1}) (Table 7-4). At the farm site, the average TPM was 1 mg L^{-1} and ranged between 0.3 – 3.3 mg L^{-1} (Table 7-4).

The surface sediment was composed of coarse silt and fine sand and there were no major differences among sites and seasons (Table 7-5). The finest sediment was collected at station 50 m from the cages at all seasons. The OM averaged at 7.5% across all stations and increased as the distance from the cages increased (Fig. 7-3).

Table 7-2. Chlorophyll *a* and phaeophytin concentrations at different depths and seasons, measured with a laboratory fluorometer.

	Spring	Summer	Winter
	Chlorophyll a (μg L^{-1})		
Surface	0.14 ± 0.01	0.05 ± 0.01	0.12 ± 0.00
15 m depth	0.15 ± 0.01	0.04 ± 0.00	0.12 ± 0.00
Near bottom	0.21 ± 0.01	0.05 ± 0.01	0.10 ± 0.00
	Phaeophytin (μg L^{-1})		
Surface	0.05 ± 0.00	0.01 ± 0.00	0.08 ± 0.01
15 m depth	0.05 ± 0.00	0.01 ± 0.00	0.07 ± 0.00
Near bottom	0.08 ± 0.01	0.02 ± 0.00	0.05 ± 0.00

Table 7-3. Monthly values (2012-2013) of water variables measured with sensors in single vertical deployments of sensors at continuous mode and presented as the average of values measured at the surface (above 2 m depth), at 15 ± 1 m depth, and near the bottom (2 m from the bottom, depth *ca* 36 m).

Calendar Month	1	2	3	4	5	6	7	8	9	10	11	12
						In vivo Chlorophyll *a* (arb. unit)						
Surface	24.9	7	16.7	16.2	6.3	29.9	21.2	6.2	8.0	15.5	20.0	32.4
15 m depth	41.9	13.6	27.3	22.9	9.8	22.6	19.1	12.4	19.2	17.7	29.7	42.7
Near bottom	60.4	18.6	34.4	66.4	21.4	24.1	22.1	19.2	46.0	35.8	27.9	39.3
						Temperature (^{o}C)						
Surface	17.9	17.2	17.0	17.8	20.3	25.1	25.2	27.9	26.0	23.8	20.9	19.0
15 m depth	17.9	17.1	17.0	17.6	19.0	20.8	21.3	24.3	25.9	23.8	20.9	19.0
Near bottom	17.7	17.1	16.9	17.5	18.8	19.9	20.5	20.9	20.9	21.7	20.7	18.7
						Conductivity (mS/cm)						
Surface	50.6	45.9	49.6	50.4	53.1	58.7	58.8	62.2	60.3	57.7	54.3	52.0
15 m depth	50.6	49.8	49.5	50.2	51.6	53.7	54.2	57.5	60.1	57.6	54.3	52.0
Near bottom	50.4	49.8	49.5	50.1	51.4	52.7	53.3	53.6	53.6	54.9	54.1	51.8
						Salinity (ppt)						
Surface	39.17	36.06	39.08	39.10	39.02	39.16	39.12	39.37	39.49	39.46	39.46	39.32
15 m depth	39.17	39.15	39.08	39.11	38.98	39.11	38.98	38.94	39.50	39.46	39.46	39.32
Near bottom	39.13	39.15	39.09	39.11	38.99	39.09	38.96	38.90	38.88	39.18	39.46	39.34
						Dissolved Oxygen (ppm)						
Surface	5.815	6.376	6.932	7.357	7.626	5.438	5.177	4.673	1.987	4.585	4.012	4.496
15 m depth	5.952	6.414	7.191	7.676	8.036	6.794	6.315	5.786	2.264	5.176	3.858	4.447
Near bottom	6.230	6.628	7.407	7.738	8.110	7.196	6.786	6.560	2.767	6.062	4.008	4.499
						pH						
Surface	8.28	8.27	8.26	8.26	8.23	8.13	8.16	8.15	8.18	8.20	8.43	8.48
15 m depth	8.29	8.27	8.27	8.28	8.24	8.19	8.19	8.18	8.17	8.22	8.42	8.47
Near bottom	8.28	8.27	8.28	8.29	8.24	8.19	8.20	8.20	8.21	8.23	8.43	8.49

Table 7-4. Monthly values (2012-2013) measured for total particulate matter and dissolved nitrogen from discrete water samples collected from three different depths.

Calendar Month	1	2	3	4	5	6	7	8	9	10	11	12
Total Particulate Matter (mg L^{-1})												
Surface	0.67 ± 0.36	1.33 ± 0.35	1.52 ± 0.28	1.46 ± 0.47	1.05 ± 0.24	0.85 ± 0.20	0.80 ± 0.06	0.44 ± 0.12	0.41 ± 0.09	0.63 ± 0.05	1.04 ± 0.47	1.52 ± 0.49
15 m depth	0.79 ± 0.17	1.29 ± 0.37	3.34 ± 1.09	1.34 ± 0.38	0.72 ± 0.26	0.66 ± 0.18	0.77 ± 0.14	1.24 ± 0.23	1.12 ± 0.42	0.85 ± 0.51	1.52 ± 0.44	1.75 ± 0.45
Near bottom	0.73 ± 0.06	1.02 ± 0.17	0.72 ± 0.10	0.28 ± 0.08	0.74 ± 0.16	0.35 ± 0.11	0.63 ± 0.09	0.76 ± 0.26	1.12 ± 0.43	0.31 ± 0.03	1.24 ± 0.18	1.38 ± 0.06
Ammonium (NH_4^+) (µg L^{-1})												
Surface	20.6 ± 4.8	28.4 ± 0.9	24.3 ± 0.1	52.0 ± 23.2	36.2 ± 11.7	48.9 ± 10.8	38.0 ± 10.6	40.9 ± 5.6	18.9 ± 3.4	27.2 ± 4.4	46.2 ± 6.3	23.4 ± 1.0
15 m depth	13.8 ± 1.0	3.6 ± 0.9	17.3 ± 0.6	3.2 ± 0.6	15.9 ± 5.8	3.5 ± 0.5	2.2 ± 0.3	6.4 ± 0.1	28.2 ± 1.8	15.1 ± 1.5	23.9 ± 3.8	13.5 ± 4.6
Near bottom	6.5 ± 1.3	3.3 ± 1.4	6.4 ± 1.7	3.0 ± 1.8	4.8 ± 2.3	3.0 ± 1.0	2.5 ± 0.3	3.2 ± 0.6	6.7 ± 1.2	0.6 ± 0.1	2.5 ± 0.7	3.7 ± 1.1
Nitrate (NO_3^-) (µg L^{-1})												
Surface	1.1 ± 0.9	<1	2.5 ± 1.2	1.3 ± 0.9	5.8 ± 3.5	1.5 ± 0.1	4.4 ± 0.1	3.7 ± 0.1	1.6 ± 1.0	<1	2.9 ± 1.3	<1
15 m depth	2.8 ± 0.3	<1	1.1 ± 0.9	< 1	2.4 ± 1.5	<1	5.8 ± 2.5	3.0 ± 1.2	<1	<1	<1	1.2 ± 1.1
Near bottom	0.8 ± 0.2	<1	< 1	< 1	1.5 ± 0.8	<1	3.7 ± 0.3	2.0 ± 0.5	<1	<1	<1	<1
Nitrite (NO_2^-) (µg L^{-1})												
Surface	4.0 ± 1.3	6.4 ± 1.0	< 1	< 1	< 1	< 1	< 1	1.3 ± 0.1	2.0 ± 0.0	1.3 ± 0.0	<1	<1
15 m depth	3.0 ± 0.3	13.0 ± 0.3	< 1	< 1	< 1	< 1	< 1	< 1	2.0 ± 0.0	1.3 ± 0.0	<1	<1
Near bottom	4.3 ± 0.3	10.4 ± 1.0	< 1	1.7 ± 0.3	< 1	< 1	< 1	< 2	2.0 ± 0.0	1.3 ± 0.0	<1	1.7 ± 0.3

Table 7-5. Seasonal sediment type based on granule size at increasing distances from the cages.

Season	Winter			Spring			Summer			Autumn		
%	Silt	Sand	Gravel	Silt	Sand	Gravel	Silt	Sand	Gravel	Silt	Sand	Gravel
Cages	50.8	48.5	0.7	50.6	49.0	0.4	45.7	53.8	0.5	50.4	48.7	0.9
50 m	52.7	47.1	0.2	55.0	44.9	0.1	51.4	48.4	0.2	52.1	47.6	0.3
100 m	49.1	50.4	0.4	44.0	55.2	0.8	49.2	50.2	0.5	49.0	50.7	0.4
200 m	46.5	52.6	0.8	41.4	58.0	0.6	29.2	70.2	0.5	42.9	56.2	0.9

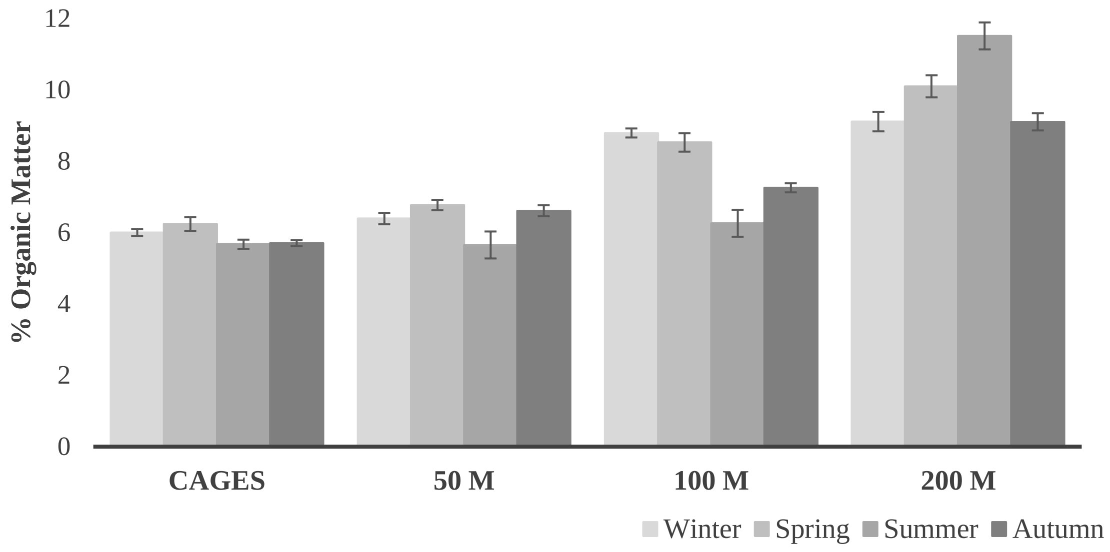

Figure 7-3. Seasonal organic matter content (%) of surface sediment at increasing distances from the fish farm northwards.

7.4.2 IMTA

7.4.2.1 Mediterranean mussel

In the first trial, mussels grew from 2.1 ± 0.1 g to 9.5 ± 0.3 g (mean wet weight ± SE) and 33.9 ± 0.3 mm to 47.5 ± 0.4 mm (mean shell length ± SE), in *ca* one year. Mean mussel meat yield increased very little and peaked in late spring, with the highest mean 22 ± 0.5% recorded at the deepest station (maximum value 32.5%). The mean mussel yield fell to *ca* 16% at all stations during the summer and autumn oligotrophic season. No major differences were detected in mean mussel growth and yield among the three depths investigated and although the deep mussels seemed to perform better, by the end of this trial when the thermocline deepened, and the water column became warm and oligotrophic, mussels from all depths had similar metrics (Fig. 7-4). As the experiment progressed, the socks became less dense, particularly in the shallows, indicating mortalities and/or losses. Turtles were frequently observed by divers eating mussels from the lines.

The second batch of mussels arrived much later than anticipated and their size was close to that of the first batch, despite being one year younger. A catastrophic storm soon after deployment left only a few that doubled in mass from *ca* 8 g in December to *ca* 16 g in July. The mussels lost all the weight they gained in the warm, oligotrophic period that followed, but their mass increased again to *ca* 19 g by next July. Shell length increased little in two years from 43.6 ± 0.9 mm to 51.9 ± 1.3 mm. The mussel meat yield decreased the first year; the lowest mean values 10.7 ± 0.2% were recorded at the end of the warm period and then increased again (Fig. 7-4).

In the third trial, mussels kept inside the empty cage had high survival rates. When dislodged from the socks they were found growing on the net below. Some mortalities were observed in the sea bass cage. There was no significant difference in the mean mussel weight and shell length between the mussels of the empty cage and the sea bass cage (Fig. 7-4). Their mean wet weight increased ten-fold from 0.6 ± 0.1 g to >6 g and their mean shell length almost doubled from 23.3 ± 0.9 mm to 38.6 ± 0.4 mm, in less than one year. The mean mussel meat yield decreased following deployment in cages but increased in late spring and early summer to 18.7 ± 0.3% (maximum value 36%), decreasing thereafter, during the oligotrophic and warm period (Fig. 7-4). The mussels placed within the Zygi port grew faster, reaching mean wet weight 10.4 ± 0.35 g during the same period, but overall the mean mussel meat yield was not any different than those kept in the cages. The mussel meat yields decreased in all trials particularly during the warm and oligotrophic period. This period coincided with a boom of biofouling epibionts, especially seaweeds, which covered the mussels entirely.

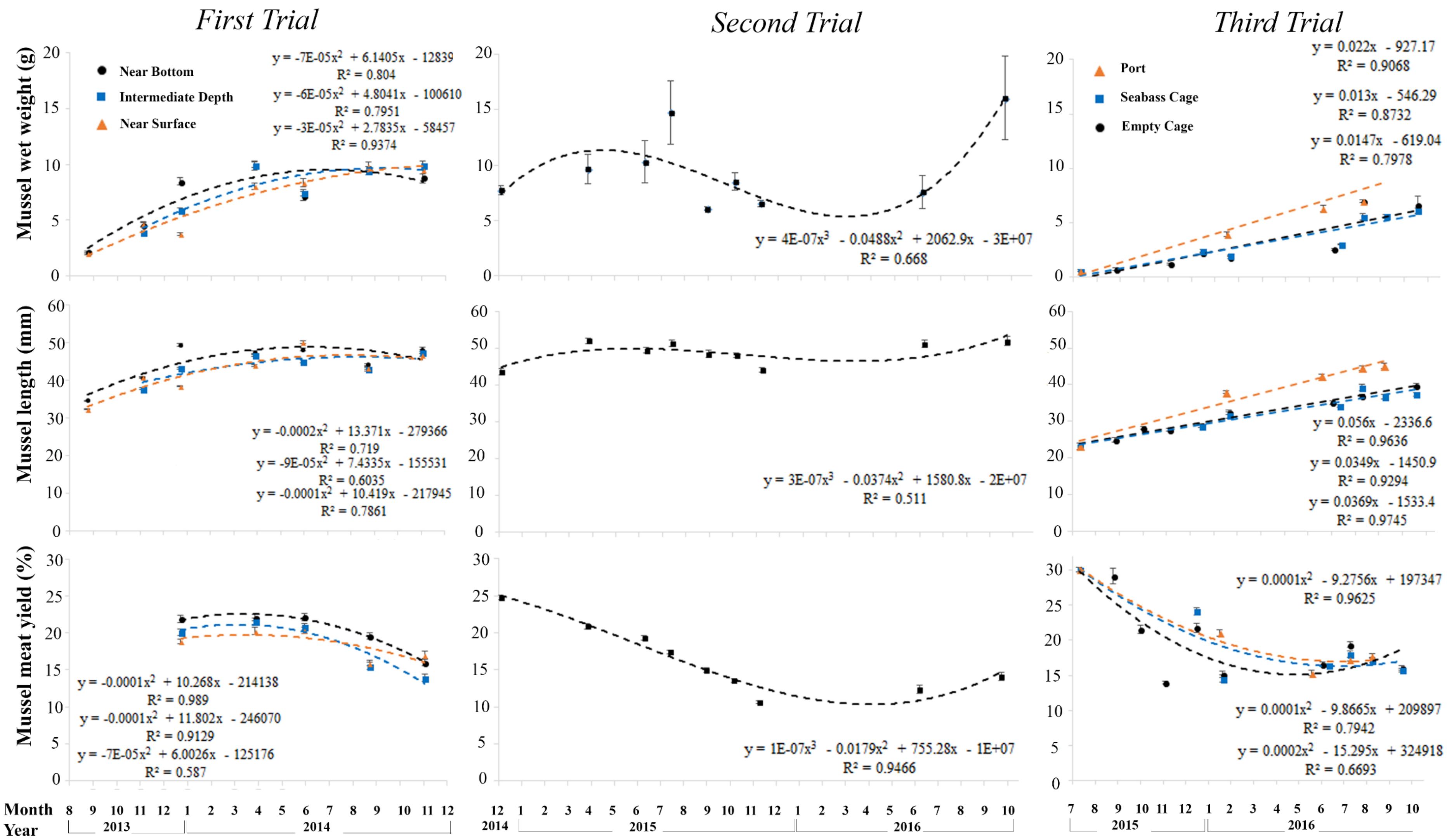

Figure 7-4. Mean ± SE of mussel wet weight (g), length (mm) and meat yield (%) in all trials. For plots of the first trial (*left*), dotted trend lines of all the three depths represent a fit for a second-order polynomial function, for plots of the second trial (*middle*) a fit for third-order polynomial function, and for plots of the third trial (*right*) a linear fit for mussel weight and length and second-order polynomial function for meat yield of mussels reared in empty and sea bass cages, and in the small Zygi port. Equations and R^2 values derived from these fits are presented in symbol sequence.

7.4.2.2 Purple sea urchin

In the first benthic trial, high mortalities in the enclosures were correlated with the sea urchin density. By eight months, about 88%, 75% and 47% of sea urchins died in the enclosures with 100, 50 and 25 individuals per m^2, respectively. Surviving sea urchins did not gain biomass and didn’t develop gonads. Mean gonadal weight did not change much, it was about 0.8 ± 0.1 g in October 2013 and 0.6 ± 0.1 g in June 2014 when this experiment started and ended, respectively. In the end, the sea urchins in the enclosures with the lowest density had slightly higher gonadal weight than the rest but mean gonadosomatic index was always below 2% (Fig. 7-5). During this experiment the sea urchins cleared the seafloor from the green seaweed *Caulerpa* spp, which remained in the control area. The enclosures with sea urchins had lower OM in the surface sediment than the control enclosure (Fig. 7-6).

In the second trial, all sea urchins that were suspended in the water column and fed with macroalgae survived. These sea urchins developed gonads in early autumn as their mean gonadal weight and gonadosomatic index increased in respective order from 1.3 ± 0.2 g and 3.1 ± 0.5% in September 2015 to 3.1 ± 0.2 g and 12.5 ± 0.8% in September 2016 (Fig. 7-5).

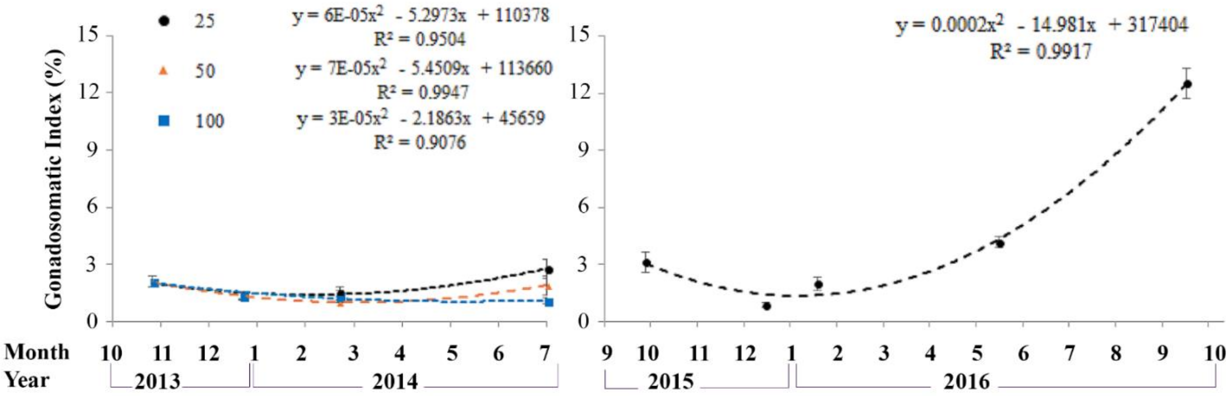

Figure 7-5. Mean ± SE of sea urchin gonadosomatic index (% of gonadal weight to total wet weight), from the first benthic experiment (*left*) that included three different stocking densities, and from the second water column experiment (*right*), where sea urchins were reared in baskets and fed with seaweed. For both plots, dotted trend lines represent a fit for a second-order polynomial function. Equations and R^2 values derived from these fits are presented in symbol sequence.

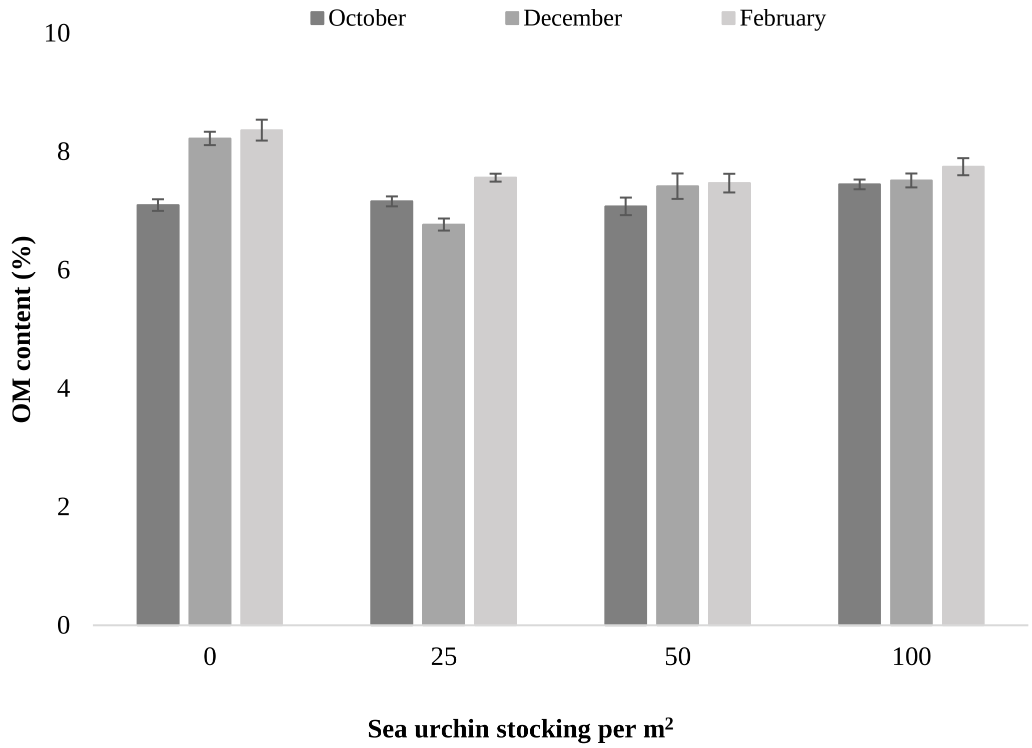

Figure 7-6. Mean ± SE organic matter content (%) of surface sediment in 1 m^2 benthic enclosures with different concentrations of sea urchins. The first measurement in October represents the OM on the first day sea urchins were deployed.

7.4.2.3 European flat oyster

High oyster mortalities were observed. Several taxa (sponges, bryozoans, macroalgae, ascidians, hydrozoans and other) covered the plastic surface of the Ortac baskets and the inserts that contained the juvenile oysters, both externally and internally. Biofouling peaked during the warm season. Crabs and other bivalve species (fan mussel *Pinna nobilis* Linnaeus, 1758, the alien rayed pearl oyster *Pinctada imbricata radiata* (Leach, 1814) and several scallops) were commonly found within the inserts. Survivor oysters gained little mass during the first ten months, mean weight per oyster increased from 0.04 ± 0.00 g to 0.23 ± 0.06 g (Fig. 7-7). However, when the Ortac baskets were replaced, some oysters grew exponentially raising the mean weight to 1.16 ± 0.40 g during the warm and oligotrophic season.

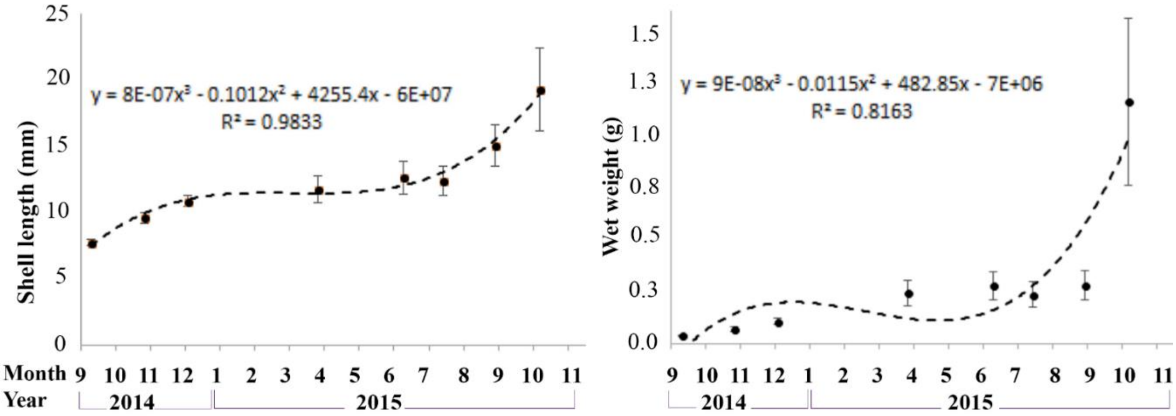

Figure 7-7. Mean ± SE of oyster shell length (mm) and wet weight (g). Oyster baskets were changed in Month 7 following heavy biofouling. For both plots, dotted trend lines represent a fit for a third-order polynomial function. Equations and R^2 values derived from these fits are also presented.

7.4.2.4 Green ormer

The abalone in Ortacs displayed very high survival rates. Only a few shells (dead animals) were found following periods of starvation during the winter when macroalgae were not available. No biofouling developed in the inner side of the Ortac baskets, which were exposed to the abalone. Mature abalone displayed no noticeable growth during the duration of this trial. The shell length remained relatively unchanged and averaged around 45 cm while mean mass fluctuated and ranged between 9.3 to 11.4 g. The largest abalone recorded reached 55.8 cm long and weighted 18.9 g.

7.4.2.5 Blue crab

Several crabs died in the beginning, likely due to transit stress. It was observed that blue crabs were feasting on fresh fish that were provided to them. However, survivor numbers decreased by 70% as cannibalistic behaviour occurred in the Seapa baskets. The trial was cut short as it was decided that it was not worth to continue feeding the few remaining crabs.

7.4.2.6 Native sponges

A drawback in the method applied is that the sponge IMTA units were attached to the farm structure and as a result the wave energy at the surface was transferred to the IMTA structures stocked with sponges. Repeated wave motion quickly resulted in the tear and loss of the sponges that were threaded, thus both techniques (i.e. in panel and wrapped around the ropes of the farm) were unsuccessful. All the sponges in the mesh survived the five months of this pilot. New pinacocyte cells formed at the injured cut ends and the sponges grew over the net, despite heavy biofouling that peaked in summer and cleared after.

7.5 Discussion

The use of IMTA to process the waste effluents of aquaculture production units offers significant environmental and socio-economic benefits (Barrington *et al.*, 2009; 2010). IMTA technology can diversify production, improve efficiency and increase the sustainability of the aquaculture industry. Although it is becoming a commercial reality in many areas of the world, there is little uptake in the western world with several bottlenecks being identified (Hughes & Black, 2016; Kleitou *et al.*, 2018). Knowledge transfer of the experiences and lessons learnt from IMTA experiments open the path for those wishing to develop commercial scale IMTA in Europe (Alexander & Hughes, 2017). There are only a few examples where IMTA has been successful in offshore waters and this was only possible in eutrophic environmental background conditions, suitable for the cultivation of filter feeders and macroalgae (Buck *et al.*, 2017; Kleitou *et al.*, 2018).

IMTA at sea relies on nutrient-rich waters and currents to move the nutrients from fed to extractive species. Off the southern coast of Cyprus, the water column becomes ultra-oligotrophic in summer and total suspended matter is lower than 1 mg L^{-1}. These are among the lowest values reported across the Mediterranean (Siokou-Frangou *et al.,* 2010). During this time, temperature reaches 28 ^{o}C in stratified surface waters, in some cases with low dissolved oxygen concentrations. These environmental conditions prove to be suitable for finfish culture, providing quick returns as fish metabolism and growth is high and losses due to pathogenic infections are minimal. However, these conditions are a major barrier to IMTA development as there are inadequate dissolved nutrients to sustain massive productions of seaweeds and suspended material to grow most filter feeders. Cultivating invertebrates in an open environment is challenged by exposed weather conditions/storms and predation (Holdt & Edwards, 2014; Wilding *et al.,* 2018) and these challenges were also faced in this study.

Mytilus galloprovincialis has been found to display high adaptability to heat stress compared to other *Mytilus* species (Tomanek & Zuzow, 2010) and it is one of the most commercially successful aquaculture species in the Mediterranean basin. The successful commercial cultivation of the species for many years coupled with the existing expertise, availability of seeds and the fact that *M. galloprovincialis* is a local native species makes it a great candidate for the IMTA development of the Mediterranean aquaculture. Mussels reared close to fish farms may ingest at least 20% of their diet from the fin-fish wastes (Lander *et al.,* 2012; Reid *et al.,* 2013). However, studies in open-water or coastal farms have led to contradictory results with several studies providing evidence of direct organic uptake and assimilation (Deudero *et al.,* 2011; Gvozdenovic *et al.,* 2017) and others showing no significant effect (Navarrete-Mier *et al.,* 2010; Irisarri *et al.,* 2013; Sanz-Lazaro *et al.,* 2017). The difficulty and differences in detecting changes in suspended detrital POM and shellfish yield suggest that the nutrient flow dynamics of IMTA configurations are location specific (Ferreira & Bricker, 2016).

In this study, the mussels were challenged by the warm, oligotrophic conditions but little growth was observed particularly during the mesotrophic winter and spring. Mussels almost reached a marketable size in two years, but the mean meat yield was always below marketability preferences, mostly decreasing during the warm oligotrophic season. Several studies have shown that growth, condition and meat yield of the bivalves are associated with the reproduction and the environmental conditions (Sarà & Mazzola, 1997; Okumuş & Stirling, 1998; Çelik *et al.,* 2012). Besides the low food availability, it is possible that a spawning period during the summer might have further depleted growth and body reserves (Rouane-Hacene *et al.,* 2015). In addition, it is possible that the low mussel meat yield was due to the biofouling that peaked when the waters warmed. Studies have shown that mussels might continue growing on socks with heavy biofouling infestations, but their condition index can be reduced significantly (LeBlanc *et al.*, 2007). Biofouling can have manifold effects on the IMTA species' performance, as it can reduce their condition and/or final product marketability by direct competition or predation, and/or restriction of the water flow and ultimately the flux of nutrient particles that pass through the culture enclosures, and/or by interfering with vital functions of the species (e.g., opening and closing of the shell valves) (LeBlanc *et al.,* 2002; Willemsen, 2005). In this study, depth seemed to correlate with mussel performance, but during the oligotrophic period there was no difference among mussels reared at different depths. The rarefaction of the mussels in the socks, was more pronounced in the shallows and that could be due to the combined result of the stronger hydrodynamic energy, high temperature, predation by loggerhead turtles, and/or biofouling that led to mortalities or dislodgment.

It appears that the classical production method using longlines may not be a suitable option for IMTA in exposed conditions, especially for small enterprises, since extractive species are not the core business and their production should be achieved with minimal labour costs and interference. Mechanised systems such as the 'Smart Farm System' (Norwegian innovation) require extensive initial and operating costs but they are more likely to minimize the costs of cleaning, stocking, and harvesting, thus increasing the cost-efficiency and ability of an IMTA system to deal with the complexity of adding production layers (Holdt & Edwards, 2014). The third mussel trial showed that culturing mussels in empty cages or nets could be a promising alternative farming technique in exposed waters as mussels are predator-free and the ones that dislodge form byssal threads at the net below and are thus, not a "lost crop". The co-rearing with seabass has not been found to affect the size and weight of mussels although some mortalities were observed. Mussels (*M. edulis*) have been found capable of inactivating infectious salmon anemia virus and could thus act as a possible biofilter for disease reduction or prevention if placed appropriately around fish cages (Skår & Mortensen, 2007; Molloy *et al.,* 2014). Other studies found that mussels can reduce bacterial communities related to phytoplankton blooms (Voudanta *et al.,* 2016) and that filter feeders can ingest larval stages of invertebrates including sea lice and can thus form a biological control of sea lice in the salmon industry (Bartsch *et al.,* 2013; Webb *et al.,* 2013).

The second bivalve *O. edulis* trialled displayed very high mortality rates and stunted growth. This can be partly explained by thermal stress and predatory crabs (mainly *Pilumnus* sp.), which grew and got trapped in the inserts that contained the oysters. The mortality of the flat oyster increases during the hot summer months when high metabolic and reproductive activities and food unavailability result in negative energy budgets (Montes *et al.,* 2003; Zrnčić *et al.,* 2007). Their slow growth can also be attributed to competing biofouling, since a spike in oyster weight was recorded during the oligotrophic period, following a change of heavily biofouled baskets. Although the *O. edulis* performance can be enhanced with improved care such as prompt replacement of baskets and physically removing any potential predators from the baskets, it is doubtful that this method can form a profitable IMTA configuration given the slow growth and the high labour work that would be required to clean or replace the baskets.

Both *M. galloprovinciallis* and *O. edulis* are native in the Mediterranean but not very common in the oligotrophic eastern basin. Future IMTA efforts in this region could be focused on other locally abundant edible bivalve species like scallops and the endemic fan mussel *P. nobilis*. Seeds of these entered the baskets and were commonly found growing alongside oysters but had faster growth rates and were able to coincide with biofouling and local conditions. The fan mussel is an endangered and protected species and culturing it can assist restoration efforts. The eastern Mediterranean lacks any filter-feeder hatcheries and given the inherent challenging conditions for seaweed production and the high reliance of IMTA in filter-feeders co-product, it is crucial that local promising species are first produced in hatcheries before IMTA develops.

The experiments with the locally abundant deposit feeders and herbivores such as the sea urchin *P. lividus* and abalone *H. tuberculata* displayed remarkable survival rates in the baskets but seaweed diet was required to sustain them and for *P. lividus* to develop gonads. There are several reasons that will deter a fish farmer from cultivating these IMTA crops in exposed, oligotrophic waters using the techniques trialled. The seaweed is not readily abundant around the farm thus it is impossible to self-sustain mass production of herbivores. Furthermore, collection of seaweed to feed organisms in the Ortac baskets is a tedious and time-consuming process. Artificial feed containing fish meal can favour a storage of reserves in reared *P. lividus* (Fernandez, 1997), but the purchase of artificial feed is prohibitively expensive and beyond the 'circular economy' scope of IMTA. In addition, several baskets were lost due to bad weather conditions and technical failures.

The cultivation of the sea urchins on the benthos appears more natural and biologically sensible. A benthic component of deposit feeders to utilize the vertical fluxes of organic matter to the seabed is also necessary to significantly reduce the organic loading underneath the fin-fish and shellfish aquaculture operations (Cubillo *et al.*, 2016).

In the benthic trials of this study, sea urchins decreased the organic content of the sediment and grazed all the available seaweed, competing to death. The highest survival was recorded at the lowest concentration of sea urchins, which suggests that intraspecific competition occurred. Gonads didn't develop but this experiment didn't last long enough. Sea urchins would probably do better in IMTA systems over rocky substrata. In soft sediments, other deposit-feeders such as sea cucumbers and polychaetes hold some potential (Nelson *et al.,* 2012; Cubillo *et al.,* 2016; Marques *et al.,* 2018). Despite the potential that lies on the benthic IMTA, particularly to minimize the environmental effects of aquaculture effluents, the development of IMTA infrastructure that would allow utilization of three-dimensional space, and increased mobility and food availability should be developed (Robinson *et al.,* 2011). In addition, many fish farms worldwide (including Cyprus) operate at large depths and tend to move deeper (Bostock *et al.,* 2010). To this end, the development of infrastructure that will allow remote control and access from the surface is crucial towards daily practical crop monitoring, feeding if needed, and harvesting.

The trials with detritivore crabs aimed to demonstrate a method to utilise the wasted energy in discarded fresh fish. There is considerable potential to increase the sustainability of the industry by supporting the full utilisation of by-products and mortalities are in fact, a major by-product which is included in the Fish In:Fish Out (FI:FO) calculations (Stevens *et al.,* 2018). For instance, mortalities for the Scottish aquaculture are estimated to be around 6.7% of total annual productions and the disposal of mortalities contributes a significant additional expense to the industry of over £2 million per annum (Stevens *et al.,* 2018). The short experiment with blue crabs *C. sapidus* demonstrated that the crabs become cannibalistic if grown together in small spaces. Feeding them was a time-consuming and dirty process, thus cultivating them together in baskets does not appear to be a viable option. Cannibalism among crustaceans is one of the limiting factors to their culture productivity and profitability in aquaculture (Romano & Zeng, 2017). Mitigation strategies for cannibalism such as the adoption of appropriate stocking densities, supplementary dietary improvements and manipulations with various additive compounds responsible for decreasing aggression are effective to a certain extent (Romano & Zeng, 2017). Small production lines of fish-fed crustaceans can be potentially profitable on land with individual culture, which can eliminate cannibalism altogether or infrastructures specially adapted to the species cultured. A land-system would be more convenient in terms of labour requirements and cost-effectiveness.

The culture of sponges at sea is a relatively cheap alternative solution which capitalizes on their ability to reproduce asexually and regenerate whole specimens from small pieces. Their extraordinary ability to filter large volumes of water with high retention rates is promising for their use as filter-feeders in IMTA systems (Duckworth, 2009). For instance, *Spongia (Spongia) officinalis* Linnaeus, 1759 was found to unselectively filter organic particles of 0.1 – 50 mm in size which includes eukaryotes and bacteria, phytoplankton and detritus (Stabili *et al.*, 2006). Several studies have been conducted on the viability of Mediterranean sponge cultivation of several species including *Axinella verrucosa* (Esper, 1794), *Dysidea avara* (Schmidt, 1862), *Chondrilla nucula* Schmidt, 1862, *Chondrosia reniformis* Nardo, 1847, *Ircinia variabilis* (Schmidt, 1862) and *S. officinalis* (van Treeck *et al.*, 2003; De Caralt 2010; Osinga *et al.*, 2010). Despite promising results and the fact that some companies generate medicines from sponge cultures (e.g., KliniPharm GmbH), there is little published information on their incorporation in IMTA configurations.

Initial conclusions indicate that sponges can be a potential IMTA crop in the oligotrophic waters of the Mediterranean. In this study two different methods of sponge cultivation were tried, and it was quickly realised that the threaded-line, which is the most widely technique to culture marine sponges worldwide, is not suitable in exposed conditions since sponge (*S. foetidus*) explants were dislodged, after forming the "doughnut shape" described by Duckworth et al. (2007). The sponges in the mesh (*C. nicaeensis*) got heavily biofouled during the warm season. Biofouling can reduce water flow and consequently food availability and growth (Duckworth & Battershill, 2003). The biofouling cleared, all sponges healed their pinacoderm, displaying observable growth in the few months of the trial; albeit monitored only with a camera and *in situ* observation.

The fact that *C. nicaeensis* were found to tolerate the biofouling and grow during the warmest and most oligotrophic season is very promising and encouraging. The population of this species has been found to regress in other areas of the Mediterranean (Morri & Bianchi, 2001) and any efforts to cultivate the species can assist restoration. Furthermore, *C. nicaeensis* might have a commercial potential as acetylenes were isolated from its tissue (Steiner *et al.,* 1977). Naturally occurring aquatic acetylenes are of interest since many of them have antitumor, antibacterial, antimicrobial, antifungal, phototoxic, HIV inhibitory, and immunosuppressive properties (Siddiq & Dembitsky, 2008). The enormous potential of sponges in biotechnology, pharmaceuticals, and cosmetics (Sipkema *et al.,* 2005; Wijffels, 2008; Koopmans *et al.,* 2009; Webster & Taylor, 2012) and the fact that they naturally thrive in warm, oligotrophic and exposed waters, and can be cloned asexually makes them ideal IMTA candidates to potentially generate sufficient raw material for exploration and develop substances with unique bioactive profiles without harvesting and disturbing the marine ecosystem. Further experiments are needed to evaluate the synthesis of bioactive metabolites, identify other promising IMTA sponge species, optimise production protocols and ensure successful cloning of initial explants.

The IMTA trials with different extractive taxa in exposed, oligotrophic and warm waters prove that although IMTA offers the opportunity to diversify and increase overall aquaculture production, its development is more challenging at some sites, and faces environmental and technical bottlenecks. Failure of many crops trialled could be due to multiple reasons. Some of the most important are: the environmental unsuitability including oligotrophy, thermal stress and exposed conditions (storms), lack of specialised durable infrastructure adapted to the species cultured and unavailability of hatcheries to produce promising IMTA species that are locally abundant. Despite knowledge gained, several obstacles remain that we need to overcome before IMTA is adopted by fish farms around the Mediterranean. Research efforts should focus in finding successful extractive species and creating market niches, producing large quantities in captivity to stock IMTA units and developing appropriate culturing protocols and specialised infrastructure. The selection of species is highly site-specific. Rearing invertebrates is a complex task and uses different technology that should be channelled to fish farmers. It is also important that IMTA efforts are supported by the stakeholders, such as managers and policy makers, who can provide a range of incentives to fish farmers to stimulate innovations and promote the development of sustainable aquaculture practices.

8. CONCLUSIONS

Since this PhD programme of research started in 2011, the human population rose by over 0.6 billion, equivalent to almost the entire population of Europe today. The anthropogenic footprint is growing worldwide and is more pronounced where land and sea meet and where human activities have been historically concentrated. Although the coastal zone covers less than 3% of the Earth's surface, it is home to about 60% of the world's population, and this is expected to rise to 80% by 2050 (Hyun *et al.*, 2009). Anthropogenic stressors act synergistically and have cumulative impacts on the marine ecosystems. Habitat loss and degradation, fishing impacts, pollution, climate change, aquaculture and eutrophication, alien species invasions, sedimentation increase, water degradation and urbanization are reducing biodiversity at all levels (Coll *et al.*, 2010; Claudet & Fraschetti, 2010; **Chapter 2**).

Ultraoligotrophic marine ecosystems cover almost a third of the earth's surface but are amongst the least understood ecosystems on this planet. The water column of oceanic gyres has extremely diverse and vertically-distinct planktonic communities. The causes for this high biodiversity in these nutrient-limited ecosystems remain puzzling (Roy & Chattopadhyay, 2007). Closed marine ecosystems are not usually limited by nutrient availability as they are enriched by land-based inputs. However, the eastern Mediterranean and north Red Sea form exceptions and while they are mainly oligotrophic, they become ultraoligotrophic during the prolonged warm and dry season. Both coastal regions support biogenic and endemic engineer marine species such as corals, seagrasses, and macroalgae, which form benthic habitats of significant ecological importance. **Chapter 2** reviewed the main threats to ultraoligotrophic open-sea and coastal marine ecosystems. Climate change is real and will continue for many millennia after cessation of anthropogenic CO_2 emissions (Tyrrell, 2011). Warming increases stratification restricting nutrient supply to the upper water column (Polovina *et al.*, 2011). Deoxygenated regions are expanding, and acidification may impair ecological functioning (Byrne, 2011). In addition to climate change, marine litter continues to accumulate in ultraoligotrophic subtropical gyres where it is physically degraded to microscopic pieces adsorbing persistent organic contaminants from the surrounding water (Thompson *et al.*, 2004; Rios *et al.*, 2010). Plastic remnants have been found in many consumer species ranging from copepods to large mammals. Litter may cause ingestion and starvation, contaminant bioaccumulation, alien species transportation and entanglement (Gregory, 2009). Enclosed ultraoligotrophic marine ecosystems must cope with additional threats due to their proximity to human civilisations. Natural coastlines are modified by urbanisation and industrialisation and resulting water pollution can take many forms, e.g. biological, chemical, thermal, saline, light, noise. Invasive alien species are spreading causing community shifts with socio-economic and ecological consequences (Katsanevakis *et al.*, 2009). Marine fish stocks are overexploited with most top predators in severe decline (MacKenzie *et al.*, 2009), yet the culture of carnivorous fish is far from sustainable. Eutrophication decreases water quality; habitats are lost and degraded (Claudet & Fraschetti, 2010). Past methods have failed to ensure environmental sustainability, yet there are several reasons to be optimistic.

International efforts to reduce rates of biodiversity loss have led to numerous agreements, conventions or other legal instruments that are currently in force. The Habitat Directive aims to ensure the conservation of rare, threatened or endemic species and an expanding network of Natura 2000 sites have been established, safeguarding priority habitats against potentially damaging developments. Water policies such as the US Clean Water Act (CWA) and the European WFD (2000/60/EC) aim to set water quality standards and monitor discharge to minimise the impacts of anthropogenic pressures on surface water quality (Hoornbeek, 2004). In Europe, the aim to achieve ‘good ecological status' in all transitional and coastal waters by 2015 was not met during the first WFD cycle, where only about half of the European coastline met this target. Extensions were required to achieve WFD environmental objectives by the end of the second and third cycles in 2021 and 2027, respectively (European Commission, 2012). The effectiveness of the WFD to achieve its objectives is questionable in the literature, while there are problems and delays in its implementation (Voulvoulis *et al*., 2017). The European MSFD (2008/56/EC) aims to achieve GES in marine waters, following an integrated ecosystem-based approach, focused on 11 descriptors related to ecosystem features, human drivers and pressures. However, as the 2020 is approaching it is becoming evident that more effort and time extensions will be required before the GES target is realised and marine use is compatible with the conservation of ecosystems and good status of waters, habitats and resources.

An important prerequisite to effectively conserve ***marine ecosystems*** *lies in our ability to* ***assess*** *and* ***monitor*** *them and to* ***mitigate anthropogenic impacts*** *where we can.*

The core of this PhD focuses on these three aspects and draws from case studies executed in the coastal waters of Cyprus, located in the eastern Mediterranean. The Mediterranean basin covers <0.8% of the world ocean surface but is home to approximately 6.3% of the world's marine macrophytes and metazoans (Coll *et al*., 2010). Despite that it is among the most investigated marine environments in the world, research efforts are regionally biased. The Levantine basin in the Mediterranean has the lowest species richness, which is likely due to the unfavourable environmental conditions prevailing in the area (high salinity and ultraoligotrophy), as well as a consequence of the low intensive sampling effort in this region (Coll *et al*., 2010). There is very little scientific data published from the south and eastern Mediterranean. Cyprus lies in the heart of the Levantine and forms no exception to this pattern. This is reflected by the fact that, despite its proximity to the Suez Canal and that it is probably among the first areas to be colonised by marine Lessepsian immigrant species, only six records of non-indigenous species were first reported in the Mediterranean from Cyprus, compared for example to 200 reported from Israel (Galil *et al*., 2018b). The recorded number of IAS in Cyprus is substantially lower compared to its neighbouring countries and this is partially attributed to the low research effort and citizen science participation (Crocetta *et al*., 2015; Chartosia & Michailidis, 2016).

Biological pollution ranks as the second greatest threat to marine biodiversity and ecological integrity (Streftaris & Zenetou, 2006; Worm *et al*., 2006; Bellard *et al*., 2016), affecting ecosystem services that are fundamental to human well-being (Mooney, 2005; Kettunen *et al.,* 2008; Katsanevakis *et al*., 2014a). The European Regulation 1143/2014 on IAS highlights the importance of prevention, early detection and rapid response, as basic eradication principles. The eastern Mediterranean basin exhibits higher seawater warming (Marbà *et al*., 2015), which is expected to enhance the successful invasion and the establishment of many thermophilic alien species (Bianchi, 2007; Pancucci-Papadopoulou *et al*., 2011; Zenetos *et al*., 2012). The invasion of Indo-Pacific species through the Suez Canal, combined with other human stressors and climate change, have altered the species diversity, food webs, ecosystem processes and functions in the Levantine basin (Goren *et al*., 2016; Galil *et al*., 2018a). **Chapter 3** aimed to bridge the gap and elevate the awareness regarding the significance of Cyprus, largely due to its sentinel location, in acting as an ideal monitoring site to understand IAS dynamics, assess impacts to the Mediterranean coastal ecosystems, and act as an early warning system, as it is at the frontline and likely the first European Union member to be reached by an Indo-Pacific potential invasive species. The first section of **Chapter 3** demonstrated an innovative and effective approach to monitor the seagrass *Halophila stipulacea* (Forsskål)

Ascherson, 1867, in both alien (eastern Mediterranean) and native (northern Red Sea) by establishing fixed sampling plots and comparing seasonal demographic and morphometric variables.

Coverage and leaf area were significantly lower in the alien population. Leaf shrinkage was observable when the surface seawater temperature was below the native range. Colder waters are likely an environmental barrier, for now limiting the expansion of this thermophilic seagrass in the Mediterranean. This alien species has not yet impacted native seagrass meadows (Duarte, 2002), however warming may allow it to spread and outcompete native pioneer species, as has been reported in the Western Atlantic (Willette & Ambrose, 2012; Steiner & Willette, 2015). This work stimulated further research that aims to further enhance knowledge on the *H. stipulacea* dynamics in the Mediterranean compared to its native range in the Red Sea. Parallel studies were implemented (e.g. Nguyen *et al*., 2018) and more are underway involving molecular and physiological traits. Setting up and expanding monitoring networks in both alien and native environments and using standardised protocols to assess the dynamics in each environment, can guide risk assessments and management more appropriately. Fixed plots are ideal for long-term monitoring of sessile benthic marine IAS and offer a strong statistical framework. They are easy to set up, can detect phenological changes in the benthic community, are reliable and their use in monitoring should be further encouraged.

The second section of **Chapter 3** demonstrated the use of citizen science to provide an early warning to a potentially dangerous marine IAS. The lionfish *Pterois miles* (Bennett, 1828) was not thought to pose a threat in the Mediterranean until this PhD **Chapter** was published and provided the first evidence of the lionfish establishment in the Mediterranean waters. The word spread quickly following an unprecedented media coverage (the journal provided a list of >500 posts in news pages in just the first three days). In the western Atlantic, the lionfish demonstrated one of the fastest and most ecologically harmful marine fish introductions to date (Albins & Hixon, 2013). The lionfish has traits that enable its rapid and successful spread across large expanses of marine ecosystems (Morris & Whitfield, 2009). These include: long pelagic larval phase, quick sexual maturity, continuous reproduction and high fecundity, high survival of eggs and larvae, wide ecological niche and ability to cross environmental barriers (e.g. thermal, salinity and deep-water barriers), and several mechanisms such as competitive ability (Côté *et al*., 2013). Furthermore, it is a generalist and aggressive mesopredator, feeding mainly on a wide variety of herbivorous fish and having the ability to destabilize the community through a series of cascading effects (Albins & Hixon, 2008; Morris & Akins, 2009; Albins & Hixon, 2011). Many of the expected impacts include: community shifts (Lesser & Slattery, 2011), biotic homogenisation (Layman & Allgeier, 2012), and degradation of habitat-forming species (Lesser & Slattery, 2011). This section of **Chapter 3** provided an early warning about the growing problem in the Mediterranean, using recorded sightings by divers and fishermen from around the island of Cyprus, and led to a successful application to the *LIFE Nature and Biodiversity* financial instrument of the European Union. The RELIONMED project is a four-year project that aims to build social capital and infrastructure to combat the lionfish invasion in the Mediterranean waters; create a surveillance system that will be established on MedMIS portal to monitor the lionfish distribution and abundance; develop 'Removal Action Teams' and demonstrate the effectiveness of coordinated removals in priority areas such as near/within the Natura 2000 sites and Marine Protected Areas; explore potential small local market niches that would make future removals economically sustainable; assess and analyse risk, develop tools and guides for managers; and transfer cost-effective practices in neighbouring countries to tackle the cross-border issue of lionfish. Like many bioinvasions, the lionfish invasion could severely heighten the impacts on key species and ecosystems, by acting synergistically with other anthropogenic stressors (Lejeusne *et al*., 2010; Mineur *et al*., 2015).

Chapters 4-6 of this PhD programme demonstrated different methods to assess and monitor the two most important coastal marine ecosystems (shallow rocky reefs and meadows of the seagrass *Posidonia oceanica* (Linnaeus) Delile 1813), across gradients of anthropogenic impact in warm and oligotrophic waters of southern Cyprus. The site chosen was Vasiliko Bay, encompassing the most industrialised foreshore around the island with an offshore jetty and fuel storage facilities, power and desalination plant, cement production factory, ports, and more. Recent findings of natural gas in the Levantine have encouraged the government to proceed with the Vasilikos Master Plan and further development such as land reclamation, construction of jetties and berths, and pipeline deployments are underway, aiming to establish the region as the major hydrocarbon processing, storing and transport centre in the eastern Mediterranean. On the other side of the bay, at about 1-3 km from the coastline, most of the island's fish farms have operated for over two decades, expanding and moving deeper over time. Despite the mounting pressures, Vasiliko Bay still holds pristine rocky shorelines covered by lush *Cystoseira* canopy and extensive seagrass meadows that sustain high biodiversity and large iconic predators including turtles, bluefin tunas, cetaceans and monk seals.

Chapter 4 attempted to provide the first robust baseline on the abundance and community structure of macrophytic assemblages on shallow rocky reefs across gradients of impact and assessed the associated ecological status, using a WFD biotic index applied in the eastern Mediterranean. Canopy-forming *Cystoseira* with high biomass dominated hard substrata in the upper subtidal, where human access was limited to recreation. *Cystoseira* canopy decreased near bathing waters and co-existed with other bushy opportunistic macroalgae, but diminished near industrialised and modified coastlines and got replaced by opportunistic communities, composed of stress-resistant and short-lived algal species. Similar macroalgal community shifts have been widely reported across gradients of impact around the Mediterranean Sea (Benedetti-Cecchi *et al*., 2001; Thibaut *et al*., 2005; 2015; Arévalo *et al*., 2007; Mangialajo *et al*., 2008; Tsiamis *et al*., 2013; Orlando-Bonaca & Rotter, 2018).

The most significant factors that affected shallow subtidal microbenthic communities were human stress levels (coastal modification and industrialisation) and the type of substratum (limestone, conglomerate, modified). Temporal and spatial shifts of the macrophytic community were reflected by the Ecological Evaluation Index (EEI-c; Orfanidis *et al*., 2011), which assessed low ecological status near the industry and high at pristine rocky shores. The EEI-c proved to be a robust index that remained unaffected by the natural variability of communities, in this case due to different type of substratum, but detected shifts in macroalgal associations due to water quality changes caused by the industrialisation. The index considers the entire macrophytic community and can provide more information to managers about the actual status of the ecosystem functioning compared to single-species WFD descriptors. However, a disadvantage of the monitoring technique applied lies in its destructive nature, since macroalgae in quadrats need to be removed and collected to assess their coverage on land. Non-destructive methodological approaches should be the way forward. Coupling data obtained through different sampling techniques, such as photographic and *in situ* measurements, can provide a non-destructive alternative to assess the ecological quality of marine habitats (Piazzi *et al*., 2014).

It is dubious that the coastal body of water around industrial Vasiliko will reach good ecological status any time soon. The low index scores at the modified, industrial coastline should alert those responsible for managing the co-existence of multi-layered industrial development, intense coastal seafood production and at the same time maintaining good ecological condition of coastal water bodies to meet the obligations of the European WFD and MSFD and maintain nearby blue flag bathing waters. Recently, land reclamation in the eastern side of the bay killed the last remnant of *Cystoseira* habitat on the industrialised side of the bay. **Chapter 4** is a published article and will help raise awareness about the importance of these fucoid forests. There are not many of them left and their disappearance leads to ecosystems with lower biodiversity and reduced services to humanity (Chapin *et al*., 2000; Cardinale *et al*., 2012). Shallow reefs around parts of Cyprus are still covered in luxuriant *Cystoseira* forests, but this habitat is threatened by rapid coastal developments around the island and disappeared from many areas. In order to minimise this threat, the protected *Cystoseira* forests should receive more weight in the decision-making process concerning the permitting of coastal developments.

During this PhD programme of research, dredging to construct a marine jetty at Vasiliko Bay caused irreversible damage to the underlying *P. oceanica* meadows studied in **Chapter 5**. The EIA carried out did not include sufficient information about the presence, distribution and status of the seagrass beds in the area and the evaluators did not point out this weakness. This demonstrates the urgent need to upgrade and improve the quality of marine impact assessment studies but also the evaluation and follow-up monitoring of projects. EIAs which largely underpin the decisions for coastal developments, are plagued by inconsistent methods and lack of independent evaluation, leading to perceptions of inadequate scientific rigour (Sheaves *et al*., 2015). They are often designated insufficient time, leading to biased decisions made based on highly limited data and is thus no surprise that refining the EIA process is identified as one of the most significant strategies for helping to reverse the decline of seagrass meadows and bolstering their resilience (Cullen-Unsworth & Unsworth, 2016).

Chapter 5 comprised the first scientific survey to describe physicochemical variables in the sediments across Vasiliko Bay, to map the geographic distribution of *P. oceanica* in high resolution, to characterise the meadows' condition using structural descriptors and to assess the ecological status using WFD seagrass indices applied in the Mediterranean. Data presented in this **Chapter** are expected to assist management decisions, especially now that large developments are underway in the bay. At the deeper sediment sampling sites, organic matter content was higher and granule size was finer. Higher concentrations of pollutants were measured in the central region of the bay near the industry, however biota toxicity was not evaluated. Mapping revealed a morphologically complex *P. oceanica* meadow, occupying some areas of the limestone substrata in the shallows and dominating soft substrata forming an almost continuous belt from around 10 to 30 m depth. Despite the mounting pressures in Vasiliko Bay and the high temperature profile, the seagrass *P. oceanica* grows over impressive ancient matte and has some of the highest shoot densities reported from the same depths around the whole Mediterranean Sea (**Chapter 5**).

Overall across all the sampling sites, depth was the most important factor determining the performance of the *P. oceanica* descriptors across the bay. The shallow meadows had significantly higher shoot densities and foliar surface, and consequently up to a ten-fold higher Leaf Area Index scores compared to the deeper lower limits. This proves a point, that in terms of ecosystem services such as C absorption and fixation, not all meadows are the same, some are more important than others. Across the shallow 5 m depth sites, lower shoot densities measured within the bay could be due to different exposure and hydrodynamics, but also due to the multiple pressures exerted by the industry on the adjacent coastline. Across the intermediate 15 m depth sites, the western sites had a lower number and mass of leaves per shoot and significantly higher mass of epiphytes during both seasons studied. Perhaps the lower number and mass of leaves can be attributed to heavy epiphytic shading that restricts light, absorbs nutrients and causes *P. oceanica* cellular damage (Costa *et al*., 2015). The increased epiphytic vegetation on *P. oceanica* leaves is indicative of eutrophication (Balata *et al*., 2008), which may be attributed to the concentrated mariculture activity in the western parts of the bay and/or the steeper slope of the seabed in that section of the bay, which creates upwelling effects. While *P. oceanica* shoot densities were not spatially different across the 15 m depth sites, significantly higher epiphytic biomass was measured at all sites located west of the fish cages even at distances >0.8 km, compared to all sites eastwards from fish cages. This indicates that fish farm impacts can be felt at large distances in oligotrophic waters and that the epiphytic community can be a more sensitive indicator of the ecological quality of coastal waters than *P. oceanica* structural descriptors, as it responds faster and is more sensitive to environmental alterations than the host plant (Delgado *et al*., 1999; Giovannetti *et al*., 2010; Piazzi *et al*., 2016).

The ESC of the Vasiliko basin using *P. oceanica* as a ‘biological quality element’ and the two WFD indices applied (PREI and BiPo) was ‘good’. However, there are shortcomings that may arise from using the WFD descriptors (Romero *et al*., 2015). Seagrass structural and demographic parameters used in most seagrass indices are responsive to degradation but respond slowly and are not stressor-specific or effective in reflecting improvements (Roca *et al*., 2015; 2016). Localised ecosystem degradation often remains undetected. At the lower limit sampling sites near the mariculture units, for example, there were signs of a heavy anthropogenic footprint, such as extensive dead matte and fine particulate matter covering the leaves of *P. oceanica* over large distances, which was not accounted for in the ecological assessments. Similarly, the associated community is unaccounted, for example, a highly diverse coralligenous community was well-developed within the dense seagrass rhizomes of some pristine meadows but this basic compartment of the ecosystem was absent from other anthropogenically-influenced sites. The coralligenous community is composed of sensitive species and indicative of high water quality, thus their use in ecological assessments should be considered. The key lies in understanding that the ecosystem services of seagrass beds can vary greatly, even over small spatial scales. Thus, an ecosystem-based approach that considers the overall functioning of the *P. oceanica* ecosystem and complies with the MSFD, provides more information to managers about the actual status of the ecosystem than single-species WFD descriptors (Boudouresque *et al*., 2015).

The development of finfish aquaculture along Mediterranean coasts has been considered one of the major threats to *P. oceanica*, causing localised losses and degradation of adjacent meadows (Pergent-Martini *et al*., 2006; Diaz-Almela *et al*., 2008; Holmer *et al*., 2008; Apostolaki *et al*., 2009). Organic enrichment seems to be the most important factor as it can lead to anoxic and toxic benthos inducing high *P. oceanica* mortality (Pérez *et al*., 2008). In the island of Cyprus, the mariculture industry started in the mid-nineties and now produces over 6.5 t yr^{-1} exceeding 80% of the total national fisheries production. Most fish farms have historically been concentrated at the western side of Vasiliko Bay. Fish farming started with small production units and floating cages moored in shallow water mostly over seagrass meadows. Significant increases in production occurred over the years, but the national authorities followed a precautionary approach and requested relocation and expansion in deeper water, further away from the seagrass meadows. **Chapter 6**, a published article, demonstrated an effective and robust method to monitor seagrass beds, providing vital information on the seagrass dynamics near fish farms and confirming the argument that impacts on *P. oceanica* can be managed at the local level (Guillén *et al*., 2013). Following the PMN protocol, four *P. oceanica* observatory systems were established near major shallow fish farms that relocated deeper and at a reference site, at the deepest edge of seagrass beds, near the compensation depth where the plants are most sensitive to changes in water quality. They form the easternmost PMN systems and the first in this region of the Mediterranean. Monitoring was repeated from the same 44 fixed plots three to five years later, aiming to assess fish farming impacts on adjacent seagrass beds. Despite major regression of *P. oceanica* from fixed

PMN markers at lower depth limits of meadows in the north-western Mediterranean (Boudouresque *et al.*, 2000; 2012; Pergent *et al.*, 2015), a decline has not been detected in impacted deep meadow limits in the eastern Mediterranean. On the contrary, seagrass beds had progressed at all monitoring stations and the largest mean progression was recorded near the largest fish farm. This is despite the fact that water temperatures are close to the reported upper limit of the species (Celebi *et al.*, 2006). It seems that the decision to relocate the fish farms deeper (southern), in an area dominated by west and east currents, has been successful in mitigating impacts on the *P. oceanica* meadows that stretch northwards, supported by the fact that *P. oceanica* descriptors (coverage, shoot density, leaf morphometrics, rhizome type and exposure) did not clearly detect fish farming impacts in the last three to five years that lapsed between monitoring periods. However, farm effluents is known to disperse over large distances and affect *P. oceanica* meadows even *ca* 3 km away, in ways that are not always reflected by alterations in structural descriptors (Ruiz *et al.*, 2010). The PMN protocol uses *P. oceanica* structural and morphological indicators that are described in **Chapter 6**. The main drawback in their monitoring application is that they respond late and are not ‘stress-specific’ (Roca *et al.*, 2016). By the time a decline in these indicators is sufficiently large to be detected, there is a considerable risk that the seagrass meadow has already degraded irreversibly. The *P. oceanica* structural indicators applied, such as density, coverage and depth limit, are among the best suited indicators for generic ecosystem monitoring, but if the management strategy aims to detect specific pressures in almost real-time then it should incorporate early-warning physiological indicators in the monitoring protocols.

Across the Mediterranean Sea, seagrass monitoring is extensive, but the adoption of different sampling designs and methods may result in erroneous comparisons (Lopez y Royo *et al.*, 2010b). The use of fixed plot methods, like the PMN cement markers applied in **Chapter 6**, allows reliable and effective microscale long-term monitoring of seagrass descriptors from the same positions using standardised methodologies, has high statistical power, is durable and their use should be encouraged and widely adopted in generic seagrass ecosystem monitoring. Seagrass intra-meadow variability is eliminated with fixed-plot monitoring, which is especially suited to slow growing and highly variable seagrass meadows (Schultz *et al*., 2015). Creating numerous monitoring systems that apply standardised methodologies and use a fixed-plot approach, seems to be the best way to advance the long-term monitoring of the endemic bio-engineer *P. oceanica* over large scales, detect local and regional differences in population dynamics, and guide responsible management across the Mediterranean basin.

Our success in conserving marine ecosystems lies in our capacity to assess their status and monitor their condition. The human population is growing and economically developing exponentially and pressures on marine ecosystems will only intensify; therefore, it is crucial to take action that moves beyond monitoring. Such actions include mitigation of anthropogenic activities that can affect ecosystem functioning and integrity. During this PhD programme of research, human population increased by 0.6 billion! Annual per capita fish consumption exceeds 20 kg, but with over a third of the global fish stocks over-exploited and more than half fully-fished, supplies from fisheries have stagnated at around 90 million tonnes since the millennium change (Costello *et al*., 2016; Jennings *et al*., 2016). Aquaculture has grown dramatically to meet this growing demand, reaching a milestone in 2014 when the farmed fish consumption surpassed that of wild caught fish for the first time (FAO, 2016). The efficiency in resource utilisation and sustainability of this rapidly-developing industry is questionable as there is a growing trend towards enhanced intensive carnivorous production systems (Bostock *et al*., 2010). IMTA is a promising solution for improving efficiency and promoting sustainability in the industry (Barrington *et al.*, 2009; 2010; Lembo *et al.*, 2018). IMTA is when secondary commercial species from different trophic levels are cultivated adjacent to the fin fish cages, absorbing fish farm effluents, thus mitigating environmental impacts and basically turning waste into useful biomass product (Chopin, 2012).

Chapter 7 demonstrated IMTA trials carried out at one of the fish (sea bream and sea bass) farms operating in Vasiliko Bay. The work was executed during IDREEM (*Increasing Industrial Resource Efficiency in European Mariculture*); a four-year European FP7 project that aimed to promote uptake of IMTA technology by the industry, by developing seven IMTA units across Europe and assessing the environmental, financial and regulatory context. Out of all the IMTA sites demonstrated in IDREEM, Cyprus, offers the most challenging environmental conditions to grow extractive species. The water column becomes ultra-oligotrophic in summer and total suspended matter is lower than 1 mg L^{-1}. During this time, temperature reaches 28 ^{o}C in stratified surface waters and, in some cases, the water column becomes deoxygenated. Cultivating invertebrates in an open environment is also challenged by exposed weather conditions/storms and predation (Holdt & Edwards, 2014; Wilding *et al.*, 2018). These environmental conditions proved a major barrier to classical IMTA development in this region as there are inadequate background suspended material and dissolved nutrients to sustain massive productions of filter feeders and seaweeds. IMTA trials lasted >3 yrs and included mussels (*Mytilus galloprovincialis* Lamarck, 1819), oysters (*Ostrea edulis* Linnaeus, 1758), sea urchins (*Paracentrotus lividus* (Lamarck, 1816)), abalone (*Haliotis tuberculata* Linnaeus, 1758), blue crabs (*Callinectes sapidus* Rathbun, 1896) and local sponges, aiming to gain valuable first-hand experience and to identify the opportunities and bottlenecks of this technology in the Mediterranean. Most co-cultivars displayed stunted growth and high mortality.

In the one benthic trial that was carried out, sea urchins biomitigated fish farm impacts by decreasing the organic content of the sediment, but were not fed and competition soon led to mortality, especially in the high concentration treatment. The major problem encountered with the benthic experiment involved practical hazards and limited time associated with deep water diving to monitor the co-cultivars. Many fish farms worldwide (including Cyprus) operate at large depths and tend to move deeper (Bostock *et al.,* 2010). To this end, the development of infrastructure that will allow remote control and access from the surface is crucial. Benthic IMTA infrastructure is lacking and should first be developed (Robinson *et al.,* 2011).

In the water column, mussels almost reached a marketable size, but the mean meat yield was always below marketability preferences, mostly decreasing during the warm oligotrophic season when biofouling peaked. Heavy biofouling infestations can significantly reduce the mussel condition index (LeBlanc *et al.,* 2007). In the warm period, there was no major difference in mussel performance cultivated at different depths. Above the thermocline, mussel survival could be attributed to their high adaptability to heat stress compared to other *Mytilus* species (Tomanek & Zuzow, 2010). However, rarefaction of mussel socks proved that longlines may not be a suitable technique to grow mussels in exposed waters, in this case the strong hydrodynamics and predation by turtles caused significant losses of crop. Culturing mussels in empty cages or nets is a promising alternative farming technique in exposed waters as mussels are predator-free and the ones that dislodge are not lost.

Oysters displayed high mortalities and stunted growth, but their growth peaked when biofouled baskets were replaced. Predatory crabs growing and becoming trapped in oyster baskets, competitive biofouling and thermal stress may have contributed to oyster mortality and slow growth rates. Although the oyster performance can be enhanced with improved husbandry, it is doubtful that oysters can form a profitable IMTA configuration given their low growth potential in this environment and the high labour work that would be invested. Future IMTA efforts in this region should focus on other locally abundant, edible bivalve species like scallops and the endemic fan mussel *Pinna nobilis* Linnaeus,

1758. Seeds of these entered the oyster baskets and outgrew the IMTA crop being more compatible with biofouling and local conditions. The eastern Mediterranean lacks any filter-feeder hatcheries and given the high reliance of IMTA in filter-feeders co-product, it is crucial that local promising species are first produced in hatcheries.

Herbivores such as abalone and sea urchins displayed remarkable survival rates in the baskets, but a seaweed diet was required to sustain them and for sea urchins to develop gonads. However, the seaweed is not readily abundant around the farm and collecting them is a time-consuming process, thus it is impossible to self-sustain mass production of herbivores in this environment without artificial feed, which is prohibitively expensive and beyond the ‘circular economy’ scope of IMTA.

The trials with detritivore crabs aimed to demonstrate a method to utilise the wasted energy in discarded fresh fish, which represents a significant portion of energy loss (Stevens *et al*., 2018). The short experiment demonstrated that crabs ate the fish but became cannibalistic if grown together in small spaces, which is in fact limiting crustacean productivity and profitability in aquaculture (Romano & Zeng, 2017). Feeding them was a time-consuming and dirty process and their cultivation in baskets did not appear to be a viable option so the trial was cut short. Small production lines of fish-fed crustaceans may be potentially cost-effective on land using infrastructures specially adapted to the species cultured.

The culture of sponges at sea is a promising cheap alternative solution which capitalizes on their ability to reproduce asexually and regenerate. The sponge trial, although short-lived, demonstrated that sponges kept in a mesh healed their pinacoderm and displayed observable growth; an encouraging outcome. The enormous potential of sponges in biotechnology, pharmaceuticals, and cosmetics (Sipkema *et al*., 2005; Wijffels, 2008; Koopmans *et al*., 2009; Webster & Taylor, 2012), and the fact that they naturally thrive in warm, oligotrophic and exposed waters and can be cloned asexually, makes them ideal IMTA candidates for the region. Further experiments are needed to evaluate the synthesis of bioactive metabolites, identify promising IMTA sponge species, optimise production protocols and ensure successful cloning of initial explants.

The IMTA trials presented in **Chapter 7**, with different extractive taxa in exposed, oligotrophic and warm waters, prove that although IMTA offers the opportunity to diversify and increase overall aquaculture production, there are environmental and technical barriers that restrict its development and uptake by the industry in some regions. Failure of many crops trialled could be due to the environmental unsuitability including oligotrophy, thermal stress and exposed conditions, lack of specialised durable infrastructure adapted to the species cultured and lack of expertise. Further research is needed on finding successful extractive species, producing large quantities of crop in captivity to stock IMTA units and developing appropriate culturing protocols, specialised infrastructure and market niches. Fish farmers will also need training on invertebrate cultivation and incentives should be provided to them to stimulate innovations. For now, IMTA does not seem to be a viable commercial option for fish farms in warm, oligotrophic, exposed localities to mitigate environmental impacts and increase sustainability of the industry.

To conclude, we are entering an era where growing anthropogenic impacts on marine ecosystems are being realised and efforts are undertaken to mitigate them. Ultraoligotrophic marine ecosystems are highly biodiverse with high endemism and are among the least understood marine ecosystems. Pressures are complex and act synergistically (**Chapter 2**). Foundation species such as seagrasses and reef-forming species (macroalgae and coralline community) are vital components in coastal communities by structuring complex habitats for associated species and enhancing resilience against external disturbances (Angelini *et al.*, 2011; Mineur *et al.*, 2015). Such habitat engineers harbour high levels of biodiversity and are contributing significantly to fisheries. In Cyprus, dense forests of canopy-forming *Cystoseira* still cover most rocky shorelines, despite their disappearance and long-term declines reported throughout the Mediterranean (Bianchi *et al*., 2014; Thibaut *et al*., 2015). Large declines of the endemic seagrass *P. oceanica* are also widely reported around the Mediterranean (Marbà *et al*., 2014; Telesca *et al*., 2015). The absence of this keystone species from the coasts of Syria, Lebanon, Israel, eastern Egypt and eastern Turkey was attributed to the high temperature and salinity or increase in turbidity due to heavy anthropogenic coastal impacts and river discharges (Celebi *et al*., 2006). Against the odds, Cyprus still holds some of the healthiest *P. oceanica* meadows forming a continuous belt around the entire island. This easternmost isolated population is a unique marine “ecological jewel” and has high conservational value. It is among the last remnants in the warm eastern Mediterranean and sustains an array of iconic species (for example Cyprus hosts one of the last and largest nesting populations of loggerhead and green turtles) and deserves more attention by scientists and managers.

Effective conservation of priority marine ecosystems and keystone species lies in our ability to assess and monitor them, and where possible mitigate anthropogenic impacts. One of the most serious threats is the influx of Indo-Pacific species that have been crossing the Suez Canal, displacing and outcompeting indigenous species inducing community shifts with socio-economic consequences. **Chapter 3** demonstrated an innovative way to monitor and assess alien seagrass dynamics by comparing both native (Red Sea) and alien (Cyprus) populations. This **Chapter** also demonstrated how citizen-science can provide an ‘early warning’ for a highly invasive species, in this case the lionfish. **Chapter 4** presented how the macrophytic community can be monitored effectively across gradients of human impact (coastal modification, industrialisation and pollution) to assess the ecological status of shallow rocky reefs. It demonstrated that canopy-forming fucoid *Cystoseira* flourished at rocky coastlines with limited human access, but almost vanished from modified industrialised shores. **Chapter 5** produced the first cartographic map of *P. oceanica* revealing the ancient and extensive seagrass meadows that span Vasiliko Bay, a heavily impacted coastal water body in southern Cyprus that has been earmarked by the government for further developments. The condition of the seagrass ecosystem was assessed with structural and morphological *P. oceanica* descriptors and showed that despite the manifold and escalating human pressures, at the most industrialised shore of Cyprus and with seawater temperature near the upper limits reported in the literature, *P. oceanica* forms among the densest meadows across the whole of the Mediterranean Sea. However, these meadows are threatened by dredging and anchoring, aquaculture and industrial pollution. For example, dredging removed all the underlying seagrass beds at one sampling site monitored in this Chapter. It is not too late to raise awareness about the seagrass meadows of Vasiliko Bay to assist coastal management and decision-making. **Chapter 6** demonstrated how human impacts (in this case fish farming) on seagrass beds can be monitored effectively, assisting managers. The *P. oceanica* observatory systems set up at the deepest edge of meadows near fish farm or decommissioned sites, use durable fixed markers that allow microscale and standardised long-term monitoring of seagrass structural and morphological descriptors, from the same positions, removing the effects of intra-meadow variance and increasing precision and reliability in evaluations. Follow-up monitoring three to five years later indicated that, despite the odds, the endemic seagrass edge progressed at all monitoring sites and near fish farms and *P. oceanica* descriptors didn’t change much over time: supportive evidence that impacts can be managed at the local level. Finally, **Chapter 7**, explored the potential of IMTA to mitigate the environmental impacts of finfish farming. It elaborated on the experiences and lessons learnt from benthic and water column pilot trials carried out with six extractive invertebrate taxa and described opportunities and logistical difficulties that prevent IMTA from becoming a viable commercial option for fish farms in warm, oligotrophic, exposed localities.

APPENDICES

Appendix 1. Chapter 4 supplementary tables

Table 4-6. Taxa recorded, and % coverage in 8-10 quadrats at each site, sampled on hard substrata at 0.3 - 1.5 m depth across Vasiliko Bay in late summer 2012 and early spring 2013. Late-successional (Ecological Status Group I) and opportunistic species (Ecological Status Group II) are separated in five categories based on their sensitivity to pressures (Orfanidis *et al*., 2011).

Species/Taxa	**Functional Group**	**Sampling Site**															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Cystoseira cf. *elegans* Sauvageau	IA	0.6															
Cystoseira barbatula Kützing	IA	47.1	83.5	45.8	29.4	51.0	42.4	26.3	26.4			1.3	23.8	2.4	2.6	3.1	44.0
Cystoseira crinitophylla Ercegovic	IA									11.9	6.4						
Cystoseira foeniculacea (Linnaeus) Greville f. *foeniculacea*	IA				12.3		7.8		28.0	12.1	1.5	0.7	5.1		9.1	2.8	
Posidonia oceanica (Linnaeus) Delile	IA				5.8				3.4							0.3	
Cymodocea nodosa (Ucria) Ascherson	IB				0.5												5.4
Cystoseira barbata (Stackhouse) C. Agardh	IB										2.9		3.1	0.9	1.0	7.6	1.0
Cystoseira compressa (Esper) Gerloff & Nizamuddin	IB	1.6					8.9			3.1	0.5	2.8					
Padina pavonica (Linnaeus) Thivy	IB		0.7	2.6	1.4	8.8	8.6	4.9	7.2	0.7	1.5	2.5	1.6	21.4	37.5	2.9	5.4
Rytiphlaea tinctoria (Clemente) C. Agardh	IB	9.7	1.3			0.1											
Sargassum vulgare C. Agardh	IB									0.5	12.5	0.4		0.3			
Acetabularia acetabulum (Linnaeus) P.C. Silva	IC																1.6
Anadyomene stellata (Wulfen) C.Agardh	IC	0.3	1.5	3.1	3.4			0.2	0.2						1.9	1.2	0.6
Flabellia petiolata (Turra) Nizamuddin	IC								0.6								
Jania rubens (Linnaeus) J.V.Lamouroux *Jania virgata* (Zanardini) Montagne	IC	6.2	3.3	16.7	1.7	3.6	46.4	1.7	7.4	13.6		7.2	15.6	25.8	6.3	1.8	
Corallinaceae	IC	1.9	3.8	3.3	0.6	3.4	1.5										
Peyssonnelia sp.	IC				0.6				0.1	0.3				1.1			
Taonia atomaria (Woodward) J. Agardh	IC													0.7			
Botryocladia botryoides (Wulfen) Feldmann	IIA			0.1													
Caulerpa cylindracea Sonder	IIA	0.6		0.4	2.7	0.4			1.9			0.2		1.3	0.1		

Species/Taxa	**Functional Group**	**Sampling Site**															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Cladostephus spongiosum (Hudson) C.Agardh	IIA							4.6	1.6	16.4	7.0	0.7	4.5				
Dasycladus vermicularis (Scopoli) Krasser	IIA	1.1	4.1	1.9	13.5	22.1	7.3	3.5	1.4	0.5		0.9	2.9	1.0			
Dictyopteris polypodioides (A.P.De Candolle) J.V.Lamouroux	IIA								0.4	4.6	0.4	2.8	0.8	15.1	0.6		0.6
Dictyota dichotoma (Hudson) Lamouroux	IIA	1.1			1.3	0.8	5.9	5.3	0.1	5.7	19.0			3.8	2.1	1.5	
Dictyota mediterranea (Schiffner) G. Furnari	IIA	44.1	25.2	31.6	26.8	13.6		4.8	15.5			0.3	1.4	3.4	0.1	0.5	8.1
Dictyota implexa (Desfontaines) J.V.Lamouroux	IIA					5.8											
Halopteris scoparia (Linnaeus) Sauvageau	IIA		2.7						1.4	26.8	12.6	74.1	21.2	75.8	1.7		
Hydroclathrus clathratus (C.Agardh) M. Howe	IIA		0.3		0.1									0.4			
Laurencia caduciramulosa Masuda & Kawaguchi	IIA		0.8	0.4													0.1
Laurencia obtusa (Hudson) Lamouroux	IIA	1.0		0.5			0.1										
Sphacelaria cirrosa (Roth) C. Agardh	IIA	2.3	2.4	1.4	9.7	9.6	1.3	2.2	4.6	6.6			24.5			0.6	
Stypopodium schimperi (Kützing) M.Verlaque & Boudouresque	IIA			1.1					0.2	1.3				0.6		0.1	
Cladophora laetevirens (Dillwyn) Kützing *Cladophora nigrescens* Zanardini ex Frauenfeld	IIB	0.1	2.2	4.2	9.6	3.6		3.5	5.5	1.7	0.2	5.1	0.7	0.5	8.6	12.9	1.0
Chaetomorpha aerea (Dillwyn) Kützing *Chaetomorpha linum* (O.F.Müller) Kützing	IIB		0.1	0.3		3.9									5.9	4.3	16.7
Chondria dasyphylla (Woodward) C.Agardh	IIB	0.5					0.3	0.3									
Chondrophycus cf. *glandulifer* (Kützing) Lipkin & P.C Silva	IIB							0.8				0.1					
Chrysophyte sp.	IIB		0.4		0.8		0.3	0.2									
Cyanobacteria	IIB		0.3				0.3					1.3		0.1			
Dasya corymbifera J. Agardh	IIB	2.6	2.0	0.7	1.2	0.8	4.8	1.1	1.0	0.9		1.0	1.0	6.9	0.1	0.3	
Feldmannia irregularis (Kützing) Hamel	IIB				0.1		6.8		0.8		0.6						
Feldmannia simplex (P. Crouan & H.Crouan).Hamel	IIB						0.2						0.7				

Species/Taxa	**Functional Group**	**Sampling Site**															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Herposiphonia secunda (C.Agardh) Ambronn	IIB	0.1					0.3										
Polysiphonia sp.	IIB						0.9										
Scytosiphon lomentaria (Lyngbye) Link	IIB										0.6						
Spermothamnion flabellatum Bornet	IIB												0.2				
Ulva intestinalis Linnaeus	IIB				0.3			0.3		27.9	66.4	3.1					
Ulva linza Linnaeus																	
Wrangelia penicillata (C. Agardh) C. Agardh	IIB				2.6		2.8							1.2			

Table 4-7. MALUSI index score for each sampling site indicating anthropogenic stress assessed using various pressures.

Site	Urban	Commercial & Industrial	Agriculture	Mariculture	Sediment nutrient release	Sewage outfall	Irregular Fresh Water inputs	Harbour	**SUM**	Background trophic status	Stability of water column	Confin ement	**MA-LUSI**
Site 1	0	0	1	1	0	2	0	0	4	1	1	1	4
Site 2	0	0	1	1	0	0	0	1	3	1	1	1	3
Site 3	0	0	1	1	0	0	0	1	3	1	1	1	3
Site 4	0	0	1	1	0	0	0	2	4	1	1	1.25	5
Site 5	0	0	1	1	0	0	0	2	4	1	1	1.25	5
Site 6	0	1	1	1	0	0	0	2	5	1	1	1.25	6.25
Site 7	0	1	1	1	0	0	0	2	5	1	1	1.25	6.25
Site 8	0	1	2	1	0	0	0	2	6	1	1	1.25	7.5
Site 9	0	1	2	1	0	0	0	3	7	1	1	1.25	8.75
Site 10	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 11	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 12	0	1	3	0	0	0	0	3	7	1	1	1.25	8.75
Site 13	0	1	3	1	0	0	0	3	8	1	1	1.25	10
Site 14	0	1	3	1	0	0	0	3	8	1	1	1	8
Site 15	0	1	3	1	0	0	0	3	8	1	1	1	8
Site 16	0	1	3	0	0	0	0	3	7	1	1	1	7

Appendix 2. Chapter 5 supplementary tables

Table 5-3. Reference condition values used for applying the biotic indices BiPo and PREI to assess the ecological water quality status of the coastal area studied. From the seasonal adapted reference conditions, only the leaf surface area was varied between the two seasons.

	BiPo		**PREI**	
	Reference conditions in western Mediterranean	Reference conditions adapted to study area (Autumn 2012)	Reference conditions set by DFMR *Intercalibration exercise*	Reference conditions adapted to study area (Spring 2013)
Shoot density (shoots/m^2)	599	581	731	581
Foliar surface per shoot (cm^2/shoot)	310	325	176	309
Epiphyte / Leaf Dry Mass (E/L) (g/shoot)	Not applied	Not applied	0	0
Lower Limit of Meadow (m)	38	33	42	33
Type of Meadow (λ)	3 (Progressive)		3 (Progressive)	

Table 5-4. *Posidonia oceanica* descriptors determined at the 30 sampling sites. Mean values ± SE are presented.

Depth (m)	Site #	Shoot density (shoots m^{-2})	Leaf number (leaves shoot^{-1})	Foliar surface (cm^{2}shoot^{-1})	% of adult leaves with herbivory marks	Coefficient A (% of adult leaves with broken apex)	Leaf Area Index
5 ± 1	1	901 ± 46	6.8 ± 0.3	268 ± 17	28.7 ± 4.5	65.4 ± 7.0	24.15
	2	914 ± 38	6.1 ± 0.4	262 ± 14	34.7 ± 6.3	43.6 ± 7.2	23.98
	3	678 ± 26	6.4 ± 0.3	313 ± 16	47.9 ± 7.3	59.6 ± 6.9	21.24
	4	679 ± 31	5.8 ± 0.3	238 ± 18	30.4 ± 7.6	32.8 ± 4.4	16.18
	5	791 ± 48	5.4 ± 0.2	213 ± 18	18.3 ± 6.6	30.2 ± 8.8	16.88
	6	617 ± 31	5.4 ± 0.3	225 ± 20	15.8 ± 4.6	56.7 ± 6.6	13.86
	7	621 ± 28	7.3 ± 0.4	251 ± 13	18.3 ± 5.7	35.8 ± 5.9	15.59
	8	821 ± 39	5.6 ± 0.2	229 ± 12	24.2 ± 5.6	33.3 ± 7.6	18.82
	9	908 ± 35	6.1 ± 0.4	302 ± 23	19.7 ± 6.0	39.6 ± 7.4	27.39
	10	954 ± 88	5.5 ± 0.3	227 ± 19	12.7 ± 5.0	24.6 ± 5.8	21.63
15 ± 1	1	414 ± 18	6.4 ± 0.1	194 ± 9	17.7 ± 3.3	22.7 ± 4.0	8.04
	2	424 ± 11	5.5 ± 0.1	173 ± 9	13.7 ± 3.9	6.7 ± 2.5	7.36
	3	398 ± 19	5.6 ± 0.2	200 ± 10	29.8 ± 5.1	17.3 ± 3.6	7.96
	4	395 ± 13	5.6 ± 0.2	158 ± 8	13.1 ± 3.6	18.1 ± 4.1	6.24
	5	398 ± 17	5.5 ± 0.2	147 ± 8	17.7 ± 3.6	12.7 ± 3.6	5.86
	6	478 ± 11	6.4 ± 0.2	209 ± 8	14.5 ± 2.8	12.9 ± 3.1	10.01
	7	432 ± 17	6.4 ± 0.2	211 ± 9	12.8 ± 3.2	13.6 ± 3.2	9.12
	8	438 ± 12	6.3 ± 0.2	203 ± 10	7.5 ± 2.5	13.1 ± 2.3	8.90
	9	455 ± 18	6.6 ± 0.2	172 ± 9	23.0 ± 3.9	16.8 ± 3.3	7.85
	10	429 ± 14	6.5 ± 0.2	225 ± 9	15.9 ± 3.9	8.2 ± 2.7	9.67
25.2	1	250 ± 22	6.3 ± 0.2	106 ± 9	20.8 ± 7.8	47.5 ± 5.6	2.66
27.2	2	284 ± 22	6.4 ± 0.3	134 ± 29	30.0 ± 10.7	39.2 ± 8.1	3.81
27.8	3	244 ± 11	6.4 ± 0.3	121 ± 11	41.7 ± 6.8	55.8 ± 5.7	2.95
32.4	4	177 ± 12	6.6 ± 0.3	131 ± 11	62.5 ± 6.2	48.3 ± 7.9	2.31
26.1	5	138 ± 15	6.6 ± 0.2	162 ± 11	22.5 ± 9.0	48.3 ± 9.4	2.23
27.2	6	304 ± 12	6.5 ± 0.2	133 ± 12	40.8 ± 12.2	54.2 ± 11.3	4.03
32.5	7	197 ± 15	5.6 ± 0.3	130 ± 11	66.7 ± 9.6	50.1 ± 12.4	2.56
22.3	8	345 ± 22	5.5 ± 0.3	102 ± 11	66.7 ± 10.5	63.3 ± 11.1	3.51
23.5	9	333 ± 15	6 ± 0.2	147 ± 13	40.0 ± 13.0	50.0 ± 5.6	4.90
30.3	10	116 ± 15	5.4 ± 0.3	125 ± 12	36.7 ± 10.2	60.0 ± 6.2	1.46

Table 5-5. Dry weight of *P. oceanica* leaves and epiphytes, and E/L. Mean values ± SE are presented. Sites varied between them (Kruskal-Wallis, $\chi 2 = 228.86$, df = 9, $p < 0.05$) and those that do not share a letter are significantly different at $p < 0.05$. Pairwise comparisons are generated by Dunn's post-hoc test with Bonferroni correction.

Site #	**Foliar dry weight (g shoot^{-1})**	**Epiphytic dry weight (g shoot^{-1})**	**Epiphyte : Leaf (E/L)**
1	1.012 ± 0.046	0.187 ± 0.021	0.186 ± 0.018^{a}
2	0.787 ± 0.048	0.148 ± 0.021	0.192 ± 0.025^{a}
3	0.869 ± 0.048	0.146 ± 0.014	0.167 ± 0.014^{a}
4	0.715 ± 0.045	0.116 ± 0.014	0.152 ± 0.011^{a}
5	0.656 ± 0.037	0.105 ± 0.013	0.166 ± 0.019^{a}
6	1.039 ± 0.049	0.049 ± 0.008	0.048 ± 0.007^{b}
7	1.007 ± 0.047	0.036 ± 0.003	0.036 ± 0.002^{b}
8	1.018 ± 0.057	0.041 ± 0.002	0.043 ± 0.003^{b}
9	0.858 ± 0.037	0.067 ± 0.012	0.081 ± 0.017^{b}
10	1.275 ± 0.086	0.042 ± 0.005	0.038 ± 0.005^{b}

Table 5-6. Data collected from the sampling sites and used for the application of the WFD biotic indices BiPo and PREI.

Site Code	**Shoot density m^{-2} Summer 2012 (10 repl. of 0.16 m^{2})**	**Shoot density m^{-2} Spring 2013 (10 repl. of 0.16 m^{2})**	**Mean shoot density m^{-2} (20 repl. of 0.16 m^{2} quadrat)**	**Mean foliar surface (cm^{2}shoot^{-1}) Summer 2012 (20 shoots)**	**Mean foliar surface (cm^{2}shoot^{-1}) Spring 2013 (20 shoots)**	**Dry mass ratio E/L Summer 2012 (20 shoots)**	**Dry mass ratio E/L Spring 2013 (20 shoots)**	**Depth (m) at lower limits**	**Type of Meadow**
1	417	411	414	189	199	0.120	0.245	25.2	Regressive
2	406	443	424	176	171	0.125	0.244	27.2	Stable
3	339	456	398	190	210	0.158	0.177	27.8	Regressive
4	397	393	395	126	190	0.121	0.180	32.4	Regressive
5	404	393	398	160	135	0.079	0.242	26.1	Regressive
6	479	478	478	215	204	0.036	0.062	27.2	Regressive
7	433	432	432	194	228	0.036	0.037	32.5	Regressive
8	439	436	438	197	210	0.038	0.046	22.3	Regressive
9	455	-	455	204	138	0.017	0.113	23.5	Regressive
10	434	424	429	248	203	0.012	0.054	30.3	Regressive

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