University of Cyprus

DEPARTMENT OF BIOLOGICAL SCIENCES

ECOLOGICAL AND BIOLOGICAL RESPONSES TO ENVIRONMENTAL CHANGES, IN THE ENDEMIC SCLERACTINIAN CORAL *Cladocora caespitosa* (LINNAEUS, 1767), IN CYPRUS

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LOUIS HADJIOANNOU

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LOUIS HADJIOANNOU

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Louis Hadjioannou	……..
(Candidate's name in full)	(signature)

Περίληψη

Η παρούσα διατριβή διερευνά τις οικολογικές και βιολογικές αποκρίσεις του μεσογειακού κοραλλιού *Cladocora caespitosa,* σε περιβαλλοντικές αλλαγές. Το σκληρακτήνιο αυτό είδος κοραλλιού, το οποίο χτίζει αποικίες, έχει ανομοιότυπη κατανομή κατά μήκος της Μεσογείου και παρουσιάζει σχετικά μεγάλους πληθυσμούς και στην Κύπρο. Μελετήσαμε πληθυσμούς που βρίσκονται σε αφθονία σε δύο περιοχές στη νοτιοανατολική ακτή της Κύπρου, στην περιοχή ΄Κρυό Νερό', όπου καταγράφονται λιγότερα θρεπτικά συστατικά, και στην περιοχή 'Λιοπέτρι', η οποία είναι πιο πλούσια σε θρεπτικά λόγω ανθρωπογενών δραστηριοτήτων.

Μέσω της συστηματικής παρακολούθησης των αποικιών στους θερμότερους μήνες των 2014 και 2015, καταγράψαμε την εκδήλωση θνησιμότητας που είχε ως αποτέλεσμα την απώλεια του «χρωματισμένου ιστού» και την αύξηση των πρόσφατων «νεκρωτικών» περιοχών σε κοράλλια και στις δύο περιοχές μελέτης. Η θνησιμότητα των κοραλλιών ήταν σημαντικά μεγαλύτερη το 2015, κάτι που συμπίπτει με παρατεταμένες περόδους υψηλών θερμοκρασιών (>29 ^{o}C). Παρατηρήθηκαν σημαντικές διαφορές μεταξύ των περιοχών οι οποίες αποδίδονται κυρίως στις αυξημένες συγκεντρώσεις θρεπτικών ουσιών στη μία από τις περιοχές. Επιπλέον, κατά τη διάρκεια μιας ακραίας χειμερινής ανεμοθύελλας, το 7% των αποικιών στο 'Κρυό Νερό' έχασε κατά μέσο όρο το 50% των «χρωματισμένων ιστών» εξαιτίας των κυμάτων που προκάλεσε η καταιγίδα, τα οποία χτυπώντας στα βράχια ώθησαν μεγάλους ογκόλιθους να καταρρεύσουν, με αποτέλεσμα να πέσουν πάνω στις αποικίες.

Οι ρυθμοί ανάπτυξης (~2,9 mm/έτος) των κοραλλιών στην Κύπρο βρέθηκαν να είναι παρόμοιοι με αυτούς της δυτικής Μεσογείου. Πειράματα 'κοινού περιβάλλοντος' έδειξαν οτι τα κοράλλια που συλλέχθηκαν από την περιοχή με λιγότερα θρεπτικά και μεταμοσχεύθηκαν στην περιοχή με περισσότερα θρεπτικά είχαν, τουλάχιστον βραχυπρόθεσμα, πολύ μεγαλύτερους ρυθμούς αύξησης (~6,2 mm/έτος). Υποθέτουμε ότι αυτό οφείλεται στις συνθήκες αυξημένων θρεπτικών συστατικών.

Αξιολογήσαμε τη σεξουαλική κατάσταση και τον αναπαραγωγικό κύκλο των *C. caespitosa* από την Κύπρο, λαμβάνοντας υπόψη κάποιες περιβαλλοντικές παραμέτρους (θερμοκρασία επιφάνειας θάλασσας και επιφανειακή πρωτογενή παραγωγή) και τις συγκρίναμε με αυτές από τη δυτική Μεσόγειο και την Αδριατική. Προσδιορίσαμε τις αποικίες των *C. caespitosa* ως γονοχωριστικές, με την ωοτοκία να λαμβάνει χώρα στα τέλη του καλοκαιριού, όπως και εκείνες από τη δυτική Μεσόγειο, αλλά σε αντίθεση με εκείνες της Αδριατικής, οι οποίες

έχουν περιγραφεί ως ερμαφρόδιτες, η ωοτοκία των οποίων λαμβάνει χώρα στην αρχή του καλοκαιριού. Διαπιστώσαμε ότι η θερμοκρασία είναι σημαντικός παράγοντας για την ανάπτυξη των γαμετών και της επαγωγής της αναπαραγωγής αλλά δεν εντοπίσαμε σημαντικότητα σε σχέση με την πρωτογενή παραγωγή.

Συλλέξαμε ζωντανά δείγματα κοραλλιών από τις αποικίες και των δύο περιοχών, τα οποία διατηρήσαμε σε ενυδρεία κάτω από συνθήκες θρεπτικών ίδιες με εκείνες στις περιοχές συλλογής. Ακολούθως, εκθέσαμε τα κοράλλια σε σταδιακά αυξημένες θερμοκρασίες προκειμένου να διερευνηθεί ο ρόλος του ιστορικού των θρεπτικών στοιχείων στην επίδραση της απόκρισης των κοραλλιών *C. caespitosa* σε θερμικό στρες. Τα κοράλλια που αναπτύχθηκαν σε συνθήκες φτωχών συνθηκών σε θρεπτικά υπέστησαν λεύκανση και μειώθηκε σημαντικά η περιεκτικότητά τους σε πρωτεΐνες, καθώς επίσης και οι ρυθμοί της φωτοσύνθεσής τους. Αντίθετα, οι αποικίες που αναπτύχθηκαν σε πλούσια θρεπτικά δεν παρουσίασαν σημάδι λεύκανσης ή αλλαγή στον συνολικό μεταβολισμό τους. Τα αποτελέσματά μας δείχνουν ότι το ιστορικό των θρεπτικών ουσιών στο περιβάλλον μπορεί να επηρεάσει την απόκριση των σκληρακτίνιων κοραλλιών υπό θερμικό στρές. Επιπλέον, τα αποτελέσματά μας υποδηλώνουν ότι τα κοράλλια που ευδοκιμούν σε περιβάλλοντα πλούσια σε θρεπτικά είναι πιθανόν εκείνα με υψηλή ετεροτροφική ικανότητα.

Abstract

The present thesis explores the ecological and biological responses to environmental changes in the Mediterranean coral *Cladocora caespitosa* in Cyprus. This colonial scleractinian coral species has a patchy distribution along the Mediterranean Sea and holds relatively large populations in Cyprus. We studied colonies at two areas situated in the southeast of Cyprus that hold an abundance of *C. caespitosa* colonies, a naturally low-nutrient site (Kryo Nero) and an anthropogenically impacted high-nutrient site (Liopetri).

Through systematic monitoring of colonies in the warmest months of 2014 and 2015, we observed a mortality event that resulted in the decline of 'Pigmented tissue' and an increase of 'Recently necrotic' areas in corals at both study sites. Coral deterioration was significantly more in 2015, associated with prolonged periods of high temperatures (>29 ^{o}C). Differences in effect were observed between the sites and attributed mainly to the elevated nutrient concentrations. In addition, during an extreme winter windstorm, 7% of the colonies at Kryo Nero lost an average of 50% of pigmented tissue due to storm-generated waves, which forced boulders to collapse on top of them.

We measured growth rates (~2.9 mm/yr) to be similar as in the Western Mediterranean. Interestingly, common garden experiments showed that corals collected from the low-nutrient site and transplanted in the high-nutrient had, at least in the short term, much larger growth rates (6.2 mm per year) assumingly due to the elevated nutrient conditions.

We assessed the sexual condition and reproductive cycle of *C. caespitosa* from Cyprus considering some environmental parameters (sea surface temperature and surface primary production) and compared them with the ones from Western Mediterranean and Adriatic Sea. We identified colonies of *C. caespitosa* in Cyprus to be gonochoric, with spawning occurring at the end of the summer, much like the ones from Western Mediterranean, but in contrast to the ones from the Adriatic, which have been described as hermaphroditic that spawn at the beginning of the Summer. We found temperature to be an important driving factor for gamete development and spawning, but no association with primary production had been detected.

We collected samples of colonies from both sites (Low-nutrient vs high-nutrient), maintained under the right nutrient conditions in aquaria and exposed to temperature increase in order to investigate the role of the nutrient history in influencing the response of *Cladocora caespitosa* to thermal stress. Colonies grown in nutrient-poor conditions bleached

and significantly decreased their protein content and rates of net photosynthesis. On the contrary, colonies grown under nutrient enrichment presented no sign of bleaching and no change in their overall metabolism. Our results show how nutrient history can influence the response of scleractinian corals to thermal stress. In addition, they suggest that corals with a high success in nutrient rich environments are likely those with a high heterotrophic capacity.

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Chapter 1: Introduction

The present thesis aims to contribute to the understanding of ecological and biological factors that affect the Mediterranean coral *Cladocora caespitosa* in Cyprus. An overview of the most relevant scientific literature on the biology/ecology of *Cladocora* and relevant topics is given here in this introductory chapter.

Scleractinian corals belong to one of the oldest extant taxa (Class: Anthozoa, Phylum: Cnidaria) of animals in the world. They are simple animals that can form very complex and diverse communities, and can live either solitary or in colonies, composed of rigid, semi-rigid, or soft structures, often attached to different hard or soft substrates. Cnidarian fossil remains can be traced back to the earliest fossils from the early Cambrian era (~540 Mya) (Han et al., 2010) whereas scleractinian fossils suddenly appear in the fossil record in the mid-Triassic (~240 Mya) (Stolarski et al., 2011). The large fossilized reefs of *Cladocora caespitosa* that have been found, have been used to characterize the warmer climatic phases of the Pleistocene when this species formed true reefs in both the eastern and western Mediterranean Sea (Peirano et al. 2004). The Mediterranean, occupying 1.1% of the surface of the world's oceans and 0.3% of all salt water, hosts more than 200 species of corals (Aguilar, 2007), with thirty-two being indigenous and two of them (*C. caespitosa* and *Balanophylia europaea*) being endemic (Zibrowius, 1980). The Levantine Sea, despite the fact that is considered to be ultra-oligotrophic, with higher salinity and temperature levels than the rest of the Mediterranean, still hosts at least twelve coral species around the island of Cyprus, including the two aforementioned endemics (Jimenez et al., 2010). In Cyprus and other Mediterranean locations, coral reefs of *Cladocora caespitosa* have been present at least since the Pliocene, as fossilized remains reveal (Bernasconi et al., 1997; Aguirre and Jimenez, 1998; Dornbos and Wilson, 1999). *Cladocora caespitosa* is a colonial scleractinian coral species, with a patchy distribution along the Mediterranean Sea. It hosts endosymbiotic dinoflagellates (zooxanthellae), usually in high densities (depending on a variety of environmental factors), in its polyps (Muller-Parker and D'Elia, 1997). Both the host and the symbiont cells benefit from this relationship by exchange of organic and inorganic molecules. Consequently, environmental and physiological conditions that result in changes in the relationship between the animal host and the symbiotic algae may have profound ecological effects (Muller-Parker and D'Elia, 1997; Berkelmans and van Oppen, 2006; Visram et al., 2006). The genus *Cladocora*, until relatively recently included in the family Faviidae, has been revised and was assigned at first to Caryophilliidae (Romano and Cairns,

2000) and later on, based on molecular data, considered as a member of Oculinidae (Fukami et al., 2008; Kitahara et al., 2010). However, Kitahara et al. (2010) suggested that a further revision was needed with the genus at the moment being classified as *Scleractinia incertae sedis* (Hoeksema and Cairns, 2019). The species has been described as constructional but does not contribute significantly to the framework of reefs (ahermatypic) (Schumacher and Zibrowius, 1985). Colonies of *C. caespitosa* occur in west and central Mediterranean, on a wide range of substrata, from 5 to 40m depth and in differing hydrodynamic environments (Laborel, 1961; Zibrowius, 1980; Schiller, 1993) and communities (Kersting and Linares, 2012; Kersting et al. 2017), though rarely found deeper than 30m (Kružic et al., 2008). The coral has a phaceloid form with separated corallites growing upward (Peirano, 2007) in a parallel fashion, building large, often irregular in shape, colonies of >1m in diameter (Figure 1.1A) (e.g. Kružic et al., 2008). Given the right conditions, colonies can also fuse into banks and produce reef-like structures (Kružic et al., 2008). The corallites are generally closely spaced and may exceed a height of 20cm in old colonies, whereas their diameter typically ranges from 4-5mm and are circular to irregular, depending on the degree of packing (Schiller, 1993). They use their protruding tentacles to prey on a wide range of particles, from bacteria and dissolved organic matter to macrozooplankton (Ferrier-Pagès et al., 2011) (Figure 1.1B). However, apart from the heterotrophic strategy, they also rely on autotrophic feeding, in association with the zooxanthellae, which supply most of its carbon requirements (Schiller, 1993; Hoogenboom et al., 2010).

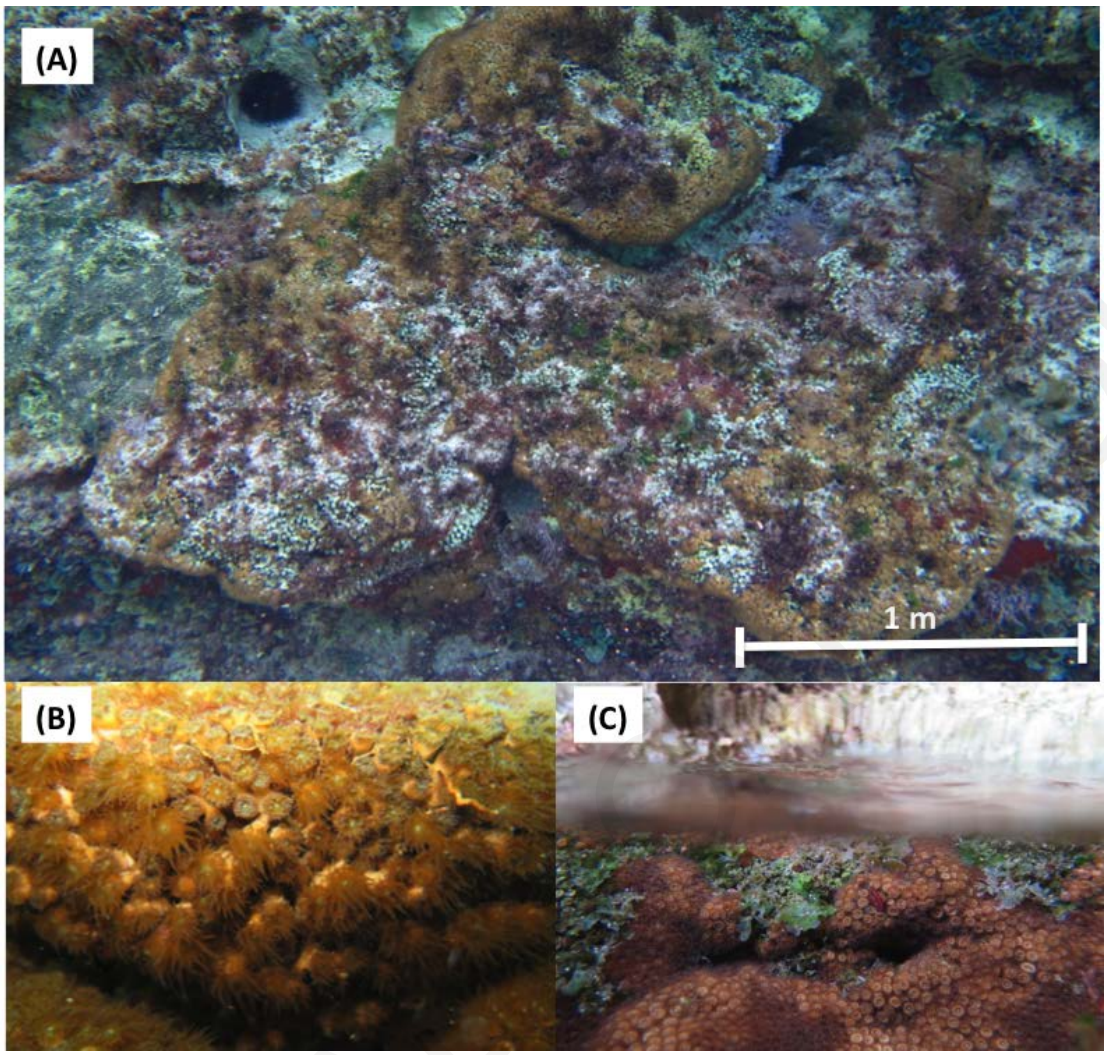

Figure 1.1. (A) Large colony of *C. caespitosa* at Kryo Nero, (B) close up of polyps with protruding tentacles, (C) *C. caespitosa* at intertidal zone.

Even though *C. caespitosa* is a conspicuous species, large bioconstructions of this coral are very rare at the present time, and most of the existing *C. caespitosa* populations are built up of small, disperse colonies. Some *C. caespitosa* bioconstructions have been described in the literature, but the distribution patterns and main population characteristics have been intensively studied only for a few of them (Schiller, 1993; Peirano et al., 2001; Kružić and Pozar-Domac, 2003; Kružić and Benkovic, 2008; Kersting and Linares, 2012; Kersting et al. 2017). *Cladocora caespitosa* is considered to be a long-lived, slow-growing species, with growth rates ranging from 1.3 to 5mm year^{-1} based on research conducted, using different methods, on communities in the Adriatic Sea, the Ligurian Sea, northwest Mediterranean, south Italy and Tunisia (see review in Kersting and Linares, 2012).

The association of *C. caespitosa* with a variety of other invertebrate organisms has been shown to be positively correlated. The architectural structure of *C. caespitosa* is an important factor affecting the associated invertebrate populations. The base and dead parts, as well as

the living parts of *C. caespitosa,* provide suitable sites of attachment for many animal species, with abundance and biomass directly related to the volume of the host colony (Arvanitidis and Koukouras, 1994; Chintiroglou, 1996; Koukouras et al. 1998; Pitacco et al. 2014).

Understanding the reproduction biology and strategies of marine organisms, is essential in comprehending population dynamics, and is consequently a necessity for their management and conservation planning (Fadlallah, 1983). This becomes even more important in the light of evidence for decreasing reproductive efficiency of corals due to increasing temperatures, by reducing individual fecundity, egg quality, lowered fertilization success and reduced recruitment through effects on post- fertilization processes (Baird and Marshall, 2002; Linares et al., 2008; Albright and Mason, 2013; Airi et al., 2014). Two studies in the last decade have produced different results with respect to the reproductive strategies followed by *C. caespitosa*. In the Adriatic, *C. caespitosa* has been described as hermaphroditic, with the release of eggs/sperms (spawning) occurring at the beginning of the summer period (Kružić et al., 2008). A more recent study by Kersting et al. (2013) found each colony to be of distinct sex (gonochoric) in the western Mediterranean, with spawning occurring at the end of the summer period. The recruitment and survival of juvenile corals are also essential for population recovery, especially after major disturbances (Adjeroud et al., 2007; Coles and Brown, 2007). Kersting et al. (2014) found low recruitment rates in *C. caespitosa.* Despite the fact that a low recruitment rate is considered normal in natural conditions for long-lived species such as *C. caespitosa,* under catastrophic impacts these species are highly vulnerable due to their slow growth dynamics (Hughes and Tanner, 2000; Linares et al., 2007; Kersting et al., 2014).

Research on *C. caespitosa* from past decades has shown that it is threatened by increasing disturbances affecting coastal ecosystems, such as extreme storms, elevated seawater temperatures, invasive species, and outbreaks of corallivorous species (Rodolfo- Metalpa et al., 2005, 2006; Kružić et al. 2008, 2013; Kersting et al., 2013, 2014, 2015). Non-native species invading coastal ecosystems is considered to be a major threat to marine biodiversity (Molnar et al. 2008) with the Mediterranean Sea being a marine hot spot for non-indigenous species (Zenetos et al. 2010, 2012). The invasive macrophyte *Caulerpa racemosa* var. *cylindracea* appears to pose a threat to *Cladocora caespitosa* in the Adriatic Sea (Kružić et al. 2008) while it is also found to interact with Cladocora along with *Lophocladia lallemandii* in the NW Mediterranean (Kersting et al. 2014), affecting corals negatively when acting synergistically with warming (Kersting et al. 2015). Sea Surface Temperatures

(SSTs) have been shown to increase globally (by 0.3 ºC to 1.0 ºC) over the last millennium (Salinger, 2005) with increases in the Mediterranean, and especially the Levantine Basin, being particularly intense (Perez et al., 2000). These anomalies, recognized largely to human-induced climate change (Oreskes, 2005), have been largely documented in the Mediterranean (Bethoux et al., 1990; Vargas-Yanez et al., 2008) and have been shown to affect greatly the coralligenous communities in the marine environment (Cerrano et al., 2000; Rodolfo-Metalpa et al., 2005; 2006; Kersting et al., 2013). A rising trend of higher than average anomalies in the Levantine Sea has been reported by Samuel-Rhoads et al. (2010). The trend, which started in the late 1990s, along with evidence of ecological disturbance, should act as an alarming call for the future as it can cause considerable changes on a regional and global scale. Elevated seawater temperatures have been shown in recent years to affect greatly the health of *C. caespitosa* by causing even mass mortality events (Perez, et al. 2000; Rodolfo-Metalpa et al., 2005; Garrabou et al., 2009; Kersting and Linares, 2009, Jimenez et al., 2016). Kersting et al. (2013) used data collected over an 11-year period, in which they recorded mortality of *C. caespitosa* during nine summers, and found significant association between necrosis and temperature increase. In Cyprus, a similar episode was documented in 2012, when SST anomalies were recorded alongside with *C. caespitosa* mortalities (Jimenez et al., 2016). In addition, bleaching (the expulsion of endosymbiotic zooxanthellae) of a substantial amount of *C. caespitosa* polyps, in various locations, was recorded in Cyprus, something that has scarcely been observed in the Mediterranean. Apart from temperature increase, another important biotic stressor that can adversely affect coral fitness, and accelerate rates of coral decline, is the direct consumption by corallivores (Knowlton et al., 1990, Rotjan et al., 2006). However, there have been few studies referring on either obligate or facultative corallivores and their effects in the Mediterranean. Kružić et al. (2013) described tissue mortality in *C. caespitosa* caused by the gastropod *Coralliophila meyendorffi*. In addition, direct observations of predation of *C. caespitosa* have been documented from the gastropods *Babelomurex cariniferus* (Kersting et al., 2013) and *Cerithium* sp., the polychaete *Hermodice carunculata*, the echinoderm *Paracentrotus lividus*, the parrotfish *Sparisoma cretense* and Paguroidea spp. hermit crabs (Jimenez et al., 2016; L. Hadjioannou, pers. obs.). Elevated sea temperature possibly supports the increase of corallivore populations, but this phenomenon deserves further research (Kružić at al., 2013; Jimenez et al., 2016).

Specific objectives of the current thesis:

1) To characterize the populations of *Cladocora caespitosa* colonies at two selected study sites in Cyprus in order to:
   a) identify substrate cover, colony morphology, size-frequency distribution and spatial density cover.
   b) compare between a population at a low-nutrient site and a population at a high-nutrient site.
   c) compare these populations with others from different regions in the Mediterranean.

2) To systematically monitor the selected populations of *C. caespitosa* and record physicochemical parameters. Use the data obtained, in order to:
   a) describe potential mortality events that environmental disturbances might have on the coral populations.
   b) identify the growth rate of *C. caespitosa* in Cyprus and look into the effects of elevated nutrient concentrations.
   c) compare between populations at high-nutrient and low-nutrient sites.

3) To describe the reproductive plasticity employed by *C. caespitosa* in the Mediterranean by:
   a) determining gametogenic cycle in Cyprus.
   b) compare with the reproductive strategies of *C. caespitosa* described for W Mediterranean and Adriatic Sea considering differences in environmental parameters.

4) To investigate the thermal tolerance of the coral *C. caespitosa* acclimated to two different nutrient environments, in order to assess the effect of nutrient supply on the response to thermal stress.

CHAPTER 2:

Overview of *Cladocora caespitosa*, description of its status and study sites in Cyprus

Overview of *Cladocora caespitosa*, description of its status and study sites in Cyprus

Abstract

In this study, we characterize the populations of *C. caespitosa* at two contrasting sites in southeast Cyprus, 'Kryo Nero' a naturally low-nutrient area and 'Liopetri' an anthropogenically high-nutrient area, and compare them with populations from other parts of the Mediterranean. We used visual census techniques to evaluate and compare the distribution, abundance and condition of the colonies at both sites. At Liopetri (N=235) colonies were almost always found on horizontal rocky substrate, whereas at Kryo Nero (N=101) almost half of the colonies were found on vertical rocky substrate. 'Rock covered with macroalgae' was the dominant substrate (70-80%) at both areas. The highest coral cover is found at 3-4 m depth with the highest density being 2 colonies per m^2. The highest density in Cyprus is much less than the highest densities identified at study sites in other parts of the Mediterranean (~6 per m^2). In addition, coral colonies in Cyprus are larger on average and flatter in shape, than the ones from other parts of the Mediterranean, presumably due to different hydrodynamic regimes. The majority of colonies in Cyprus fall into the class of 20-30 cm in diameter, larger than the majority of colonies in W Mediterranean and Adriatic.

However, the very small spatial extend of *C. caespitosa* in the study sites in Cyprus and their proximity to the coast compared to other regions in the Mediterranean, we postulate that the populations at our study sites are potentially more vulnerable to extreme events.

Keywords

Spatial distribution, size distribution, coral density, colony size, sphericity, substrate cover.

Introduction

In the southeast of Cyprus, two sites have been known to hold relatively large populations of *C. caespitosa* colonies. The two study sites selected hold an abundance of *C. caespitosa* colonies and are situated in the southeast of Cyprus, in the Levantine Sea. 'Kryo Nero' is found adjacent to Ayia Napa town, whereas 'Liopetri' is found ~12 km to the west of it (Figure 2.1). The latter is considered to be of high-nutrient composition due to the inflow of nutrients from a nearby fish-farm hatchery and also from agricultural activities. As described by Jimenez et al. (2016), Liopetri site is mainly composed of marine terraces fringing the coastline and exposed to incoming swells, particularly during winter months, with the hard bottom traversed by fractures and channels from ancient streambeds. Kryo Nero is situated at a small cove encircled by cliffs and partially submerged sea caves and is highly turbulent, especially during winter months, even though the site is considered less exposed to the incoming swells (Jimenez et al., 2016). The bottom at Kryo Nero where colonies of *C. caespitosa* are found is very irregular and shallow (<8 m), made up of large boulders and slabs collapsed from the cliffs surrounding the area, as well as from ancient sea caves, whose inner chambers are also evident around the cliffs in the cove (Jimenez et al., 2016). There is higher heterogeneity of the bottom and shoreline at Kryo Nero, compared to Liopetri; hence, benthic communities are more diverse with macroalgae being the dominant benthic group (Jimenez et al., 2016).

In this study, we aim to build on the characterizations of Kryo Nero and Liopetri sites, by identifying the substrate cover and describing the spatial distribution and density of *C. caespitosa*. In addition, we estimate the size frequency and colony morphology and compare with other regions in the Mediterranean.

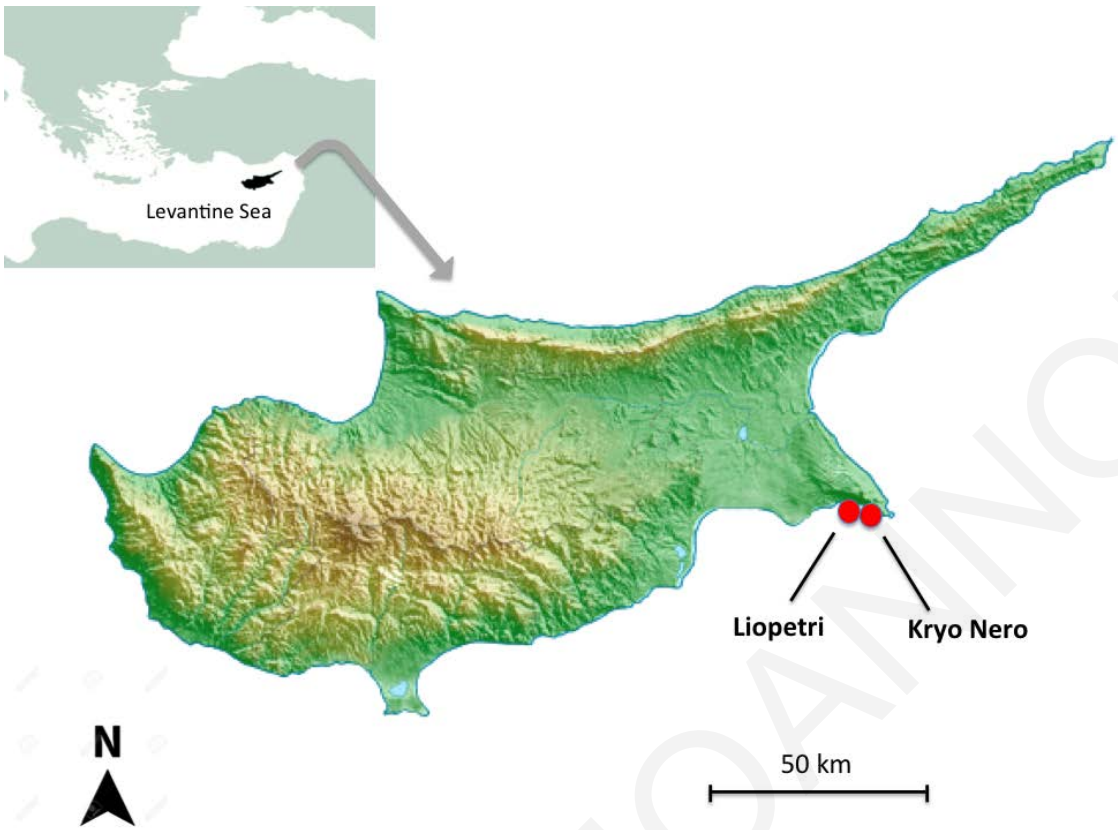

Figure 2.1. Study sites location (red dots) at the southeastern coast of Cyprus.

Materials and methods

Study sites

The study sites were characterized from the point of view of corals; hence, we evaluated and compared the distribution, abundance and condition of *Cladocora* colonies at both study sites.

To map the colonies of *C. caespitosa* at Kryo Nero and Liopetri, we performed visual census by 90 transects of 50m long and 5m wide, with distance of 5m apart, while snorkelling perpendicular to the coast, and recorded the location of each colony observed with the use of a handheld GPS (Garmin Fenix 3). Two heatmaps were created to represent the spatial distribution of the colonies over the sample areas. The analysis method was based on KERNEL density estimation (KDE) algorithm. More specifically, a magnitude per unit area from the sighting points was calculated in order to fit a smoothy tapered surface (raster format) to each point. The search radius within which to calculate density was 5m based on the input dataset. We used the KDE technique since it has been shown to have advantages

over other methods of aggregating point data, such as spatial clustering, choropleth mapping, and quadrat mapping (Yu and Ai, 2014). Finally, the ArcGIS™ software system provided by ESRI was used for the process, analysis, and creation of heatmaps, display, visualization and quality control of spatial data.

Substrate cover was estimated for six different classes, namely: rock covered with macroalgae (e.g. *Cystoseira* spp., bare rock, *C. caespitosa*), encrusting algae (e.g. *Lithophyllum* spp., *Neogoniolithon* spp., *Mesophyllum* spp.), sponge (e.g. *Cliona* sp., *Aplysina* sp.) and other (e.g. Sand/gravel, urchins); with the use of chain transect method (Rogers et al., 1994). Six linear transects of a 10m chain were conducted in May 2015, in parallel to the shore, at two different locations around the two study sites; one location within the core area where corals are situated, and one location 200m to the west of core area. The measurements in the core areas were repeated in November 2015.

Size frequency and colony morphology

We followed the same methodologies as Kersting and Linares (2012) in order to estimate the size frequency and colony morphology of *C. caespitosa* in our study sites. We measured the maximum and minimum diameters (D1, D2) and height (H) of 130 colonies (Liopetri: 70, Kryo Nero: 60) and recorded also their orientation (horizontal or vertical), with the use of SCUBA and measuring tape, to estimate first the relative relationship between measurements and then to compare colony sizes and size frequencies between sites. We also investigated the shape of the colonies by using the Index of Sphericity (Riedel, 1996) with values ranging from 0 (nonspherical) to 1 (perfect sphere). The size/frequency distribution of the populations was analysed in terms of descriptive statistics using skewness (Sokal and Rohlf, 1995). We used Kruskal-Wallis statistical analysis and Mann-Whitney tests for pairwise comparisons, to compare the colony sizes and average degree of sphericity between the sites (Kryo Nero vs Liopetri) and between orientations (horizontal vs vertical). All analyses were computed using PAST statistical package (Hammer and Harper, 2001).

Results

Spatial distribution, density and substrate cover

At Kryo Nero, the colonies recorded (N= 101) were found from <1m (Chapter 1, Figure 1.1C) to a maximum of ~4m depth, with the majority of them found at ~3m depth. At

Liopetri (N= 235), colonies were found in similar depths, except from two colonies situated at 10m depth, within an underwater ancient river channel. Those two deeper colonies are the deepest that have been recorded from all around Cyprus up to date. All colonies at Liopetri were found occurring on horizontal rocky substrate, apart from one single colony occurring on vertical rocky substrate. At Kryo Nero, 57% of the colonies occurred on horizontal rocky substrate and 43% on vertical rocky substrate. The highest coral cover was found between 3-4m depth and the highest coral density was 2 colonies per m^2 occurring only in small clusters at both locations (Chapter 1, Figure 1.1A, B). The highest coral densities were found to be within ancient river channels seemingly associated with flat seafloor morphology, but this requires a more elaborate investigation.

The sites are clearly dominated by one type of substrate, 'Rock covered with macroalgae' (70-80%) and no significant difference between them (Figure 2.2). Liopetri and Liopetri-West had significantly more 'Bare rock' (21.4- 26% on average) than Kryo Nero and Kryo Nero-West (<3% on average), with significant difference between both sites at Liopetri and both sites at Kryo Nero (Mann-Whitney pairwise; $p<0.05$). *Cladocora caespitosa* was detected at similar cover percentages at the two main sites (Figure 2.3; Kryo Nero: 6.4 ± 4.9 %; Liopetri: 4.4 ± 3.1 %). 'Encrusting algae' was higher at Kryo Nero (Figure 2.3; 2.6 ± 1.3 %) and significantly higher than the rest of the sites (Mann-Whitney pairwise; $p<0.05$) whereas 'Sponge' was negligible at Liopetri (<1%) and significantly smaller than the rest (~2.5 %).

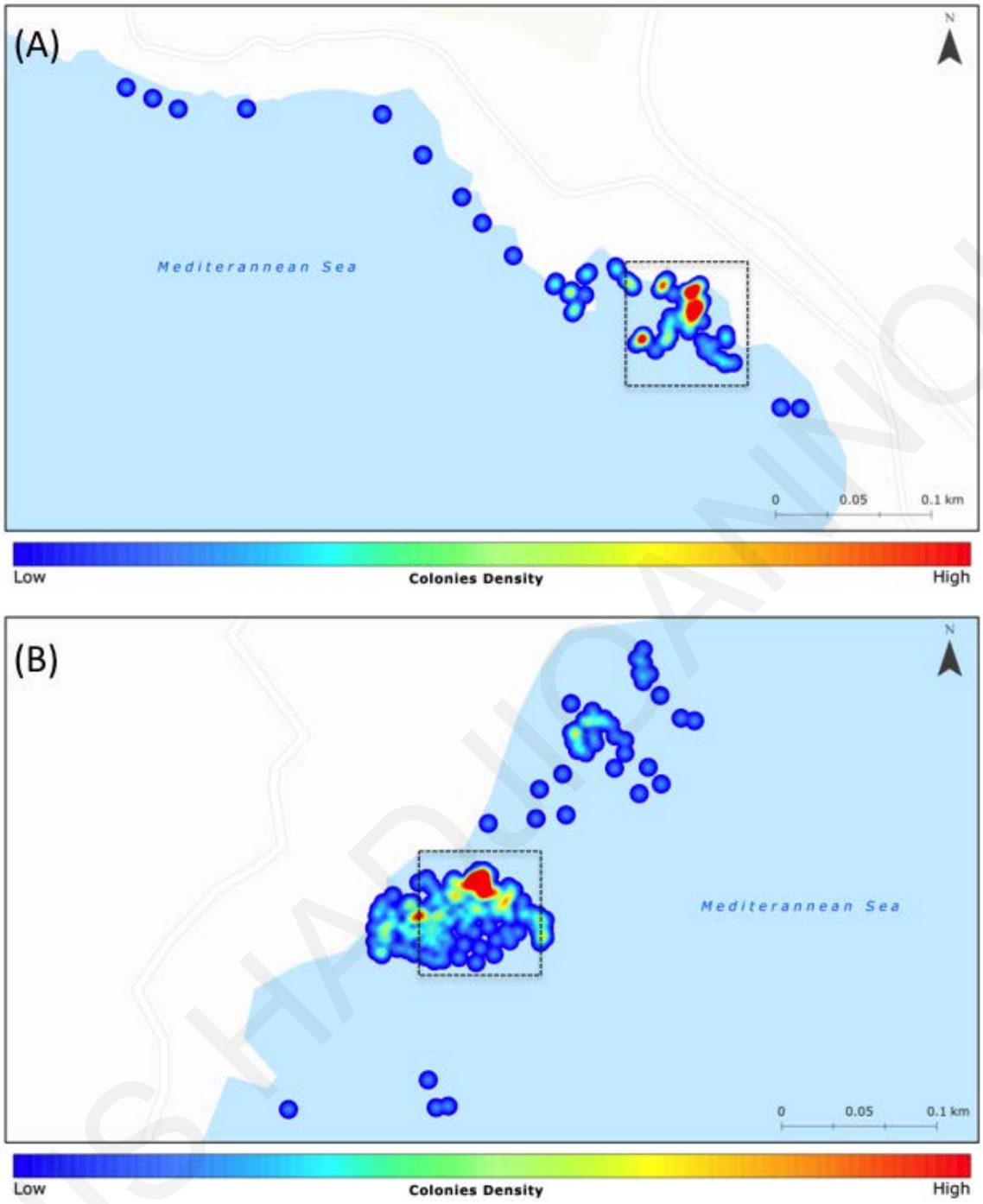

Figure 2.2. Spatial distribution and colony density of *C. caespitosa* at (A) Kryo Nero and (B) Liopetri. In boxes are the core areas where colonies were measured and monitored.

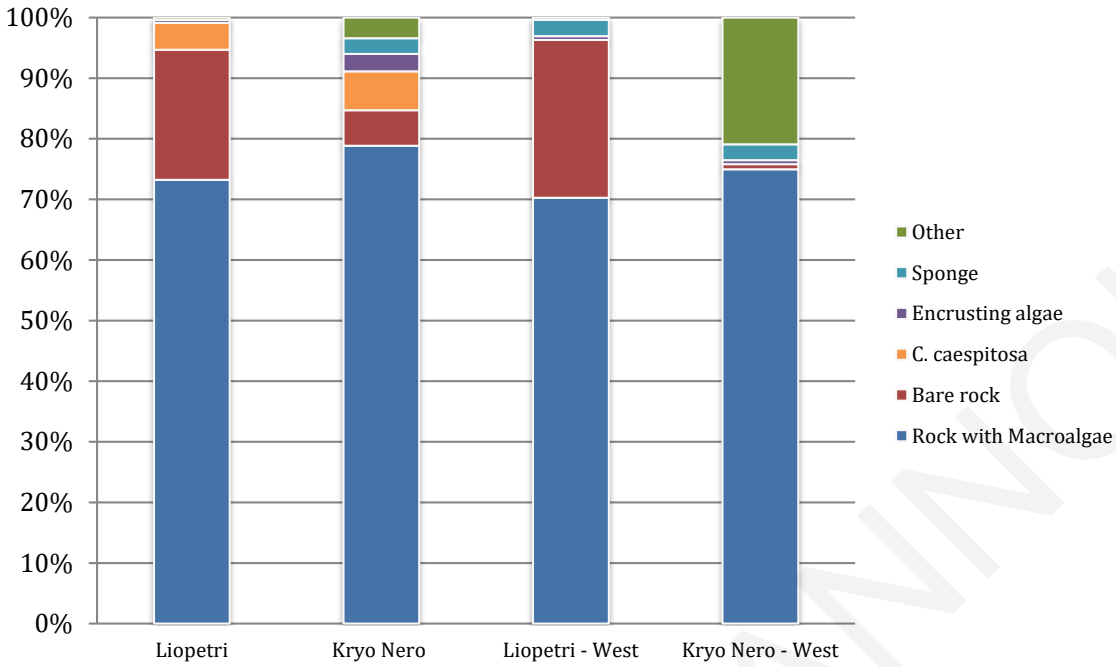

Figure 2.3. Substrate cover percentage at Liopetri, Kryo Nero, Liopetri West and Kryo Nero West.

Size frequency and colony morphology

Following Kersting and Linares (2012) methodology we identified the maximum diameter (D1) as a single size descriptor, since the correlation between both D1-D2 (r^2: 0.89; $p<0.001$) and D1-H (r^2: 0.75; $p<0.001$) showed a positive and significant relationship. Using D1 as the main descriptor, we classified the colonies into 17 classes (0-10, 10-20, 20-30...160-170 cm) and through the skeweness of the distributions we describe them as unimodal with prevalence of smaller colonies in the populations of both Liopetri (g1= 2.7) and Kryo Nero (g1= 2.07). The majority of the colonies at both Liopetri (32%) and Kryo Nero (23%) fall into the class of 20-30 cm (D1). The largest colony was found at Kryo Nero (D1: 164 cm) whereas at Liopetri the largest one was much smaller (D1= 106 cm) (Figure 2.4). It is important to note that some of the larger colonies measured were probably a built-up of several originally independent colonies that fused over time. In addition, the average size of colonies at Kryo Nero (42.5 ± 29.8 cm) was significantly higher than Liopetri (30.25 ± 13.6 cm) (Mann-Whitney pairwise; $p<0.01$). At Kryo Nero, the average size of colonies on a vertical orientation (40.2 ± 26.3 cm) was higher than the ones with horizontal orientation (32.4 ± 28 cm) but not significantly, even though horizontal colonies reached larger sizes (Figure 2.5). At Liopetri, vertical colonies are almost non-existent; therefore none were included in our measurements. However, the colonies with vertical orientation were not as unimodal (g1: 0.49) as the horizontal ones (g1= 2.7) (Figure 2.5).

The average index of sphericity showed no significant difference between Kryo Nero (0.46 ± 0.15) and Liopetri (0.44 ± 0.16) and neither between vertical (0.47 ± 0.19) and horizontal (0.45 ± 0.1).

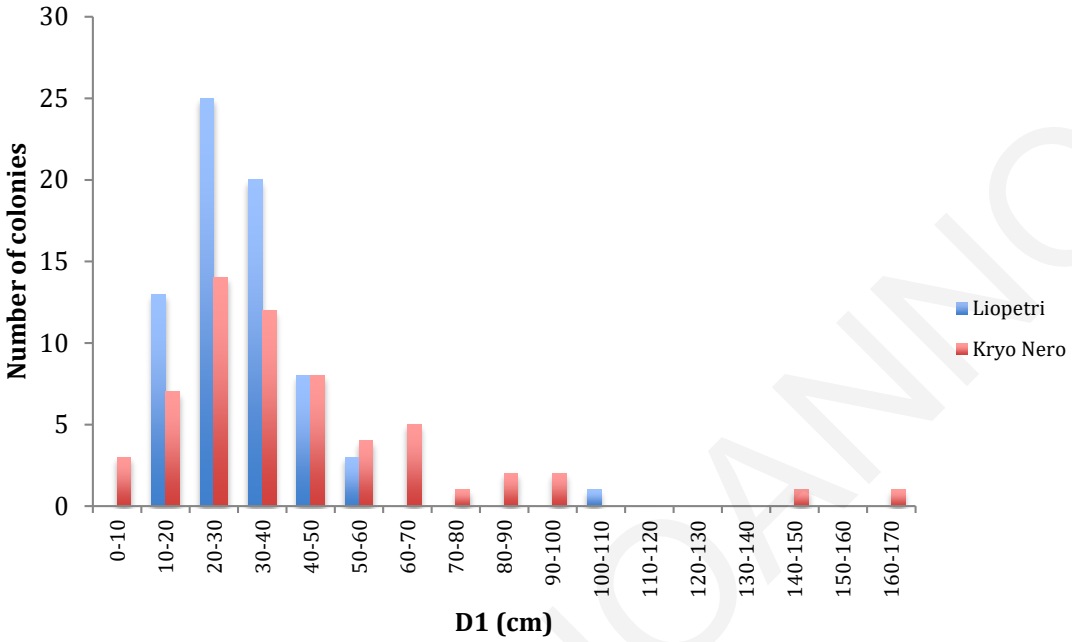

Figure 2.4. Size distribution (D1) of colonies at Liopetri and Kryo Nero.

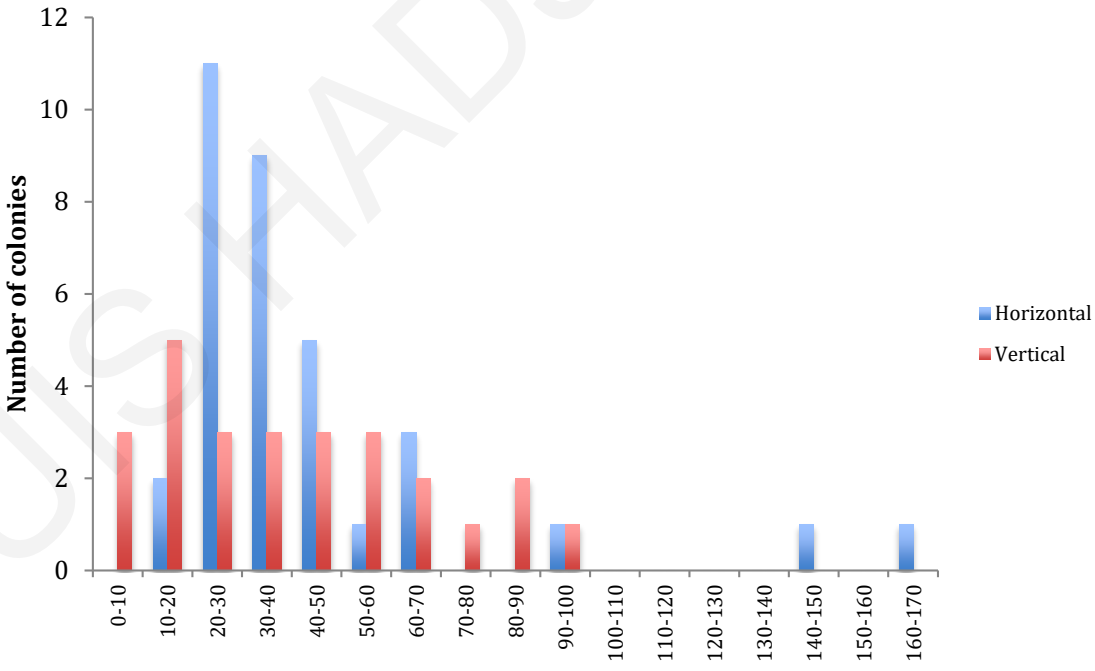

Figure 2.5. Size distribution (D1) of Horizontal vs Vertical colonies at Kryo Nero.

Discussion

The results of this general characterization of the study sites show that the *C. caespitosa* populations at the studied locations are very localized, with high densities present only in very confined and small areas.

Even though colonies at Kryo Nero where significantly larger in diameter than Liopetri, agreeing with the study by Jimenez et al. (2016), there was evidently more abundance at Liopetri, disagreeing with the finds of the same study. However, the colonies at Kryo Nero occur in more abundance on shallower vertical and subvertical surfaces and dim areas below ledges and algal rims whereas colonies at Liopetri are mostly found on horizontal rocky substrates. The maximum colony density recorded (2 per m^2) found only in small patches in both study sites; the rest of the colonies found in a density of 1 per m^2, are much lower than the average density recorded at the Columbretes (Kersting and Linares, 2012; 5.5 per m^2) and in the Gulf of Trieste (Lipej et al., 2016: 6.52 per m^2). The number of colonies observed in our study sites was also much lower than the ones recorded in other studies in the West Mediterranean (Kersting and Linares, 2012) and Adriatic (Kružić et al., 2012).

Despite this, average coral cover at our study sites (Kryo Nero: 6.4 ± 4.9 %; Liopetri: 4.4 ± 3.1 %) was higher than the average cover in the Columbretes (1.9%) though Kersting and Linares (2012) found that in some areas coral cover reached values up to 80%; but also much lower than the average cover in the Gulf of Trieste (Zunino et al., 2018; 21 ± 19 %) though they also had found a much higher mean colony coverage in one of their sites (56%). The clustered distribution appears to be similar to the pattern observed in other regions, and is probably related to a combination of different parameters, such as the particular reproductive strategies followed by *C. caespitosa*, sea-bottom morphology and hydrodynamism (Kersting and Linares, 2012) as well as the availability of suitable rocky substrate at our study sites. The philopatric dispersion strategy followed by *C. caespitosa* (Kružić et al., 2007) could explain the occurrence of the colonies in clumped distributions, since the mechanisms that force the eggs to stay near the parental colonies will restrict the new colonies to develop near the parental ones (Kersting and Linares, 2012).

Size frequency and colony morphology

Colonies at our study sites were found to be most frequently between 20-30 cm, bigger than the most frequent size classes found in the Gulf of Trieste (Zunino et al., 2018: 5-10 cm) and

the Columbretes (Kersting and Linares, 2012: 10-20 cm). Therefore, it is not surprising that the mean diameter of our study sites (35.9 ± 23.3 cm, Max: 164 cm, Min: 6 cm), with the mean at just Kryo Nero being significantly even higher (42.5 ± 29.8 cm), was also larger than the mean diameter found in the Ligurian Sea (Peirano et al., 1999: 13 ± 4.6 cm; Rodolfo-Metalpa et al., 2005; 12.78 ± 6.42), Mljet (Kružić et al., 2012: 32 ± 3.01 cm), the Gulf of Trieste (Zunino et al., 2018: 11.6 ± 7.5 cm, Max: 68 cm, Min: 2 cm) and the Columbretes (Kersting and Linares, 2012: 31.48 ± 21.02 cm, Max: 150 cm, Min: 2 cm).

Regarding colony morphology, the average index of sphericity at our sites (0.45 ± 0.16) showed that they are flatter in shape than the ones from Columbretes (Kersting and Linares, 2012: 0.55 ± 21), the Gulf of Trieste (Zunino et al., 2018; range: 0.43 ± 0.19 to 0.74 ± 0.2) and Croatian Adriatic (Kružić and Benkovic, 2008; range: 0.53 ± 0.03 to 0.83 ± 0.06). Colony morphology has been proved crucial for the ability of corals to cope with sedimentation. The sphericity of colonies is considered important in the early phases of sand shedding and enhances run-off, determining the ability of a colony to clear sand quickly (Riegl, 1995). In addition, morphological variability has been related to differences in the current regime, with flatter colonies being linked to high hydrodynamism (Riedl, 1966; Kružić and Benkovic, 2008, Kersting and Linares, 2012; Zunino et al. 2018). This implies that the study sites in Cyprus are less sheltered than the other regions studied and subjected to stronger currents. However, in agreement with Zunino et al. (2018) we hypothesize that other factors, such as competition with macroalgae, may also influence the growth dynamics of the colonies. In addition, mass mortality events, diseases (Riegl and Purkis, 2015) and corallivory from large-bodied corallivores such as parrotfish of the genera *Sparisoma* and *Scarus* (Miller and Hay, 1998; Roff et al., 2011), some of which occur in abundance in our study sites (Hadjioannou, pers. obs.) can also have an effect on the survival and growth of coral colonies. With no available data to strongly support either theory, we conclude that this matter requires further investigation. Furthermore, the size of the colonies could be related to strong hydrodynamism coupled with the absence of shelter, preventing the growth of larger colonies (Kersting and Linares, 2012; Kersting et al. 2017; Zunino et al. 2018). It could be said that this comes in contrast to our previous assumption, that stronger currents in Cyprus could be the cause of the flatter shape of the colonies in Cyprus, since the mean diameter of colonies in Cyprus is larger than the other regions. However, it is important to take into account also that colonies might break partially and regrow from the remaining bottom parts. Further investigations into the hydrodynamism and other important physical (e.g. sedimentation, nutrient loading and thermal stress) and biological (e.g. diseases,

competition and corallivory) parameters are required to explain the characteristics of *C. caespitosa* populations in our study sites.

Conclusion

Considering the very small spatial extend of *C. caespitosa* in the study sites in Cyprus and their proximity to the coast compared to other regions in the Mediterranean, we postulate that the populations at our study sites are potentially more vulnerable to extreme events from warming and/or storms.

Cladocora caespitosa has already been added to the endangered category (Endangered A4a) of the IUCN Red List (Casado-Amezua et al. 2015). With environmental changes being so eminent, and global warming seemingly being an important threat for this temperate coral, research on the scale of the threat is essential. The endemic scleractinian coral *C. caespitosa* is the only one with bank-building capabilities in the Mediterranean. It has been the focus of recent research, in the light of changes in the climate, with most evident the increase in temperature (e.g. Rodolfo- Metalpa et al., 2006; Kersting et al., 2013). Large fossilized reefs found, have been used to characterize the warmer climatic phases of the Pleistocene when *Cladocora* formed true reefs in both the eastern and western Mediterranean Sea (Peirano et al., 2004). In Cyprus, Cladocora reefs are known from Late Quaternary periods when impressive frameworks were constructed in several locations such as Karpas (Agios Therissos; Galili et al., 2015; Abu Alhaija, pers. comm.), Akamas (Frebourg et al., 2014), and from earlier times, post-Messinian, at Meniko (Dornbos et al., 1999). Compared to the fossil distribution, extant colonies have decreased extensively (Laborel, 1987) with the causes of this historic reduction not fully clear but almost undoubtedly associated with environmental changes. Today, such decreases seem to be continuing (Morri et al., 2001), with the decline being reinforced by recurrent mass mortality events recorded for *C. caespitosa* during the past decades (Perez et al., 2000; Rodolfo-Metalpa et al., 2005; Garrabou et al., 2009; Kersting and Linares, 2009, Jimenez et al., 2013a,b, 2016) and which are possibly caused by climate warming (Lejeusne, 2010). The Levantine Sea, the easternmost and the least nutrient-rich part of the Mediterranean, is a marine laboratory where ecological processes develop in demanding environmental conditions due to damaging human activities and climate change. Despite the fact that in Cyprus *C. caespitosa* colonies are not as extensive as in other parts of the Mediterranean, being in the eastern boundaries of the Levantine basin, it presents an ideal station for studying the response of highly sensitive organisms, such as *C. caespitosa*, to changes in the environment. With

recent warming events, recorded in Cyprus, found to affect *C. caespitosa* populations around the island, in an unprecedented manner (Jimenez et al., 2013a,b; 2016), monitoring the communities, population dynamics, and their ecological connectivity with other species (e.g. corallivores) will provide important data on a species that can serve as a proxy for predicting future changes. Thus, *C. caespitosa* can potentially be used as a reference species for future work on the changing Mediterranean climate, allowing the study of climate cycles at short (annual), medium (decadal) and long (millennial) terms (Peirano et al., 2003). Results will provide indications that will signify the extent that climatic changes can have on the marine environment as a whole.

CHAPTER 3*:*

Cladocora caespitosa in a rapidly changing environment

***Cladocora caespitosa* in a rapidly changing environment**

Abstract

The effect of warming events, extreme windstorms and anthropogenically-induced increase in nutrients at two populations of the scleractinian coral *Cladocora caespitosa* in the Southeast of Cyprus were studied. One of the sites, Kryo Nero, is occurring in a naturally low-nutrient state, while the other, Liopetri, has been subjected to elevated nutrient concentrations as a result of a fishfarm hatchery and agricultural activities. Through systematic monitoring of colonies of both areas in the warmest months of 2014 and 2015, we observed a mortality event which resulted in the decline of 'Pigmented tissue' and an increase of 'Recently necrotic' areas in corals of both study sites. Coral deterioration was significantly more in 2015, associated with prolonged periods of high temperatures (>29 ^{o}C). Necrosis was widespread in the high-nutrient area of Liopetri, (95% of the colonies with 17% of necrotic surface area on average). The difference in effect between the sites is attributed mainly to the elevated nutrient concentrations. At Kryo Nero, after an extreme windstorm that took place in January 2015, 7% of the colonies lost an average of 50% of pigmented tissue. The storm-generated waves that hit the cliffs above the colonies, forcing boulders to collapse on top of them. The perspective for physical/structural recovery of both Cladocora populations is optimistic. Based on the growth rates (~2.9 mm/yr) measured in situ through the alizarin staining technique, recovery could be similar to other Cladocora populations with similar growth rates in the western Mediterranean. Interestingly, common garden experiments showed that corals collected from the low-nutrient site and transplanted in the high-nutrient had at least in the short term, much larger growth rates (6.2 mm per year) assumingly due to the elevated nutrient conditions. It is hypothesized that growth of transplanted colonies will eventually decrease due to other forcing factors operating at Liopetri (e.g. overgrowing algae and disease). Systematic monitoring of the sites is required to further understand the extent of the destruction from a rapidly changing environment, how they recover in relation to the frequency of disturbances (e.g. storms) and which conservations measures need to be enforced.

Keywords

Extreme event, SST warming, windstorm, coral growth, mortality, high-nutrient, common garden experiments, Cyprus

Introduction

Coralligenous organisms are particularly vulnerable to extreme events that are more often than not linked to anthropogenically-induced climate change (Hoegh-Guldeberg, 2011). These events include extreme tides and high solar radiation (Anthony and Kerswell, 2007), coastal and tropical storms (Gleene and Leyte Morales, 1998; Lirman et al. 2001; Teixido et al. 2013), ocean acidification (Madin et al. 2012), extreme temperature increases (Cerrano et al. 2000; Rodolfo-Metalpa et al. 2005; Kersting et al. 2013; Jimenez et al. 2016; Goulet et al. 2017), often linked to the El Niño effect (Jimenez and Cortez, 2003; Elvan Ampou et al. 2017) as well as inter-related phenomena such as disease (Precht et al. 2016) and corallivory outbreaks (Kružić et al. 2013; Jimenez et al. 2016).

Increasing sea-surface temperatures (SSTs) globally have been estimated to range between 0.3-1.0 ^{o}C in the last millennium (Salinger, 2005). In the Levantine Sea, increases over the past two decades have been exceptionally intense (Samuel-Rhoads et al. 2010), with some projections predicting a rise during the 21st century by amounts ranging from 0.44 to 2.53 ^{o}C, the highest expected in all the Mediterranean (Shaltout and Omstedt, 2014). Global warming has been found to affect corals by causing bleaching (the expulsion of symbiotic zooxanthellae) and subsequently mass mortality events on a global scale on a number of occasions recorded since at least the 1980s (Hughes et al., 2017). Evidently, SST anomalies have also affected corals and coralligenous communities in the Mediterranean (Cerrano et al., 2000; Rodolfo-Metalpa et al., 2005,2006; Garrabou et al., 2009; Kružić and Popijac, 2015; Jimenez et al., 2016), including *Cladocora caespitosa* for which mortalities, associated to abnormally warm SST events, have been described on a number of localities in the past 20 years (Kersting et al. 2013; Kružić and Popijac, 2015; Rodolfo-Metalpa et al., 2005; Jimenez et al., 2016; Kružić et al., 2016). *Cladocora caespitosa* is one of the two endemic scleractinian coral species in the Mediterranean but the only one that has the ability to form large bioconstructions at present (Morri et al. 1994; Kružić and Pozar-Domac, 2003; Kersting and Linares, 2012). Even though it has been found to form extended reefs in the past, from the upper Pliocene up until the Holocene (Bernasconi et al., 1997; Aguirre and Jimenez, 1998; Peirano et al., 1998), it is now restricted mostly to a patchy distribution throughout the Mediterranean. The largest bioconstructions at present, which have been well studied, are found in the Adriatic Sea, at Mljet Island (Kružić and Pozar-Domac, 2003) and in Western Mediterranean around the Columbretes Islands (Kersting and Linares, 2012). In Cyprus, *C. caespitosa* are almost always found in the first 3 m of depth (this study), whereas in the rest of the Mediterranean they are more commonly found from 5 to 40 m depth

(Casado-Amezua et al., 2015). Colonies of *C. caespitosa* are found scattered around the island of Cyprus, always within a few meters from the coast and many times under rocky cliffs, exposing them to possible destruction from extreme weather events, such as windstorm-generated swells. These extreme events, though rare in occurrence, can cause abrupt and dramatic ecological change within short periods of time (Turner et al., 1998; Easterling et al., 2000; Jentsch et al., 2007). The effect of these events on coral reefs has been widely recorded in tropical regions (Woodley et al., 1981; Harmelin-Vivien, 1994; Gardner et al., 2005; Walker et al., 2008) but in temperate regions such as the Mediterranean it has rarely been recorded (Teixido et al., 2013) despite of evidence of storm-forcing on population structure (Zunino et al., 2018). With global trends showing an increase in wind speed and wave height over the past 2 decades (Young et al. 2011), high impact weather systems will remain an important risk also in the Mediterranean basin (Nissen et al. 2014).

Besides the risk of destruction on corals from the effects of climatic changes, anthropogenic alterations in ambient nutrient concentrations can also have adverse effects on coral health. Elevated nutrient concentrations under both laboratory and field experimental exposures have been shown to significantly suppress calcification rates, skeletal density of corals, reproduction as well as increasing their susceptibility to bleaching (Stambler et al., 1991; Ferrier-Pagès et al., 2000; Loya, et al., 2004; Dunn, et al. 2012; Wiedenmann et al. 2013). However, there have been cases where corals have responded positively to the addition of nutrients, such as having increased growth (Bongiorni et al., 2003) and reduced susceptibility in bleaching during seasonal loss of coral's zooxanthellae (McClanahan et al., 2003).

In this study, we describe the effect of prolonged periods of higher than average SSTs during two summer seasons (2014-2015) on two populations of *C. caespitosa* at sites with differing nutrient regimes and the partial destruction of one of the populations, situated under cliffs, from the effects of an extreme winter windstorm. We also describe and compare the growth rates of *C. caespitosa* at the two sites, through common garden experiments, and compare with the growth rates from other regions in the Mediterranean.

Materials and methods

Study sites

The two study sites selected hold an abundance of *C. caespitosa* colonies and are situated in the southeast of Cyprus, in the Levantine Sea. 'Kryo Nero' is found adjacent to Ayia Napa

town, whereas 'Liopetri' is found ~12 km to the west of it (Chapter 2, Figure 2.1). The latter is considered to be of high-nutrient density due to the inflow of nutrients from a nearby fish-farm hatchery and also from agricultural activities (Hadjioannou et al. in press).

Environmental parameters

Nutrient concentration levels in the study sites were measured on a monthly basis from both locations (35 times between 2012-2015 from Liopetri; 12 times between 2014-2015 from Kryo Nero) and analyzed using standard spectrophotometric methods (Strickland and Pearson, 1968). We also employed two Star-Oddi starmon mini temperature loggers, one at each study site (~4m depth), to record seawater temperature (SWT) fluctuations with a 30-min interval for the period between June 2014 to June 2016 at Kryo Nero and July 2015 to June 2016 at Liopetri. Anomalies in SWT during the study period were calculated based on SST records (2003-2015) derived from the MODerate-resolution Imaging Spectroradiometer (MODIS) instrument on board both Aqua and Terra sun synchronous satellites (Feldman and McClain; http://oceancolor.gsfc.nasa.gov/). Satellite-derived SSTs match closely shallow (5-6m depth) SWTs from Cyprus (Georgiou et al. 2019) allowing thus its use with confidence to compare and analyze both data sets.

The wind and wave conditions during the storms of January and February 2015 were characterized by observations and forecasts obtained from the Cyprus Department of Meteorology. The University of Athens SKIRON regional atmospheric system was used also to predict wind behaviour at the study site. The Oceanography Center, University of Cyprus WAM4 wave model was used to provide an estimate of wave magnitude and direction for the Levantine basin, including the study site. It is driven by the SKIRON 3-hourly wind input (10 m wind speed and direction), both with 5 km horizontal resolution (Zodiatis et al., 2014). Both systems are described in Zodiatis et al. (2014), in which a 10-year reanalysis of wave conditions is presented. A regression analysis was run to examine the correlation between wind speed and wave height.

To estimate the mass of rubble that dropped in the study area, we measured the dimensions of 10 random boulders and calculated their weights based on their dimensions and the known density of the calcarenite rock formation of the study area. Boulders were classified into two classes based on their shape (ellipsoid or triangular prism) and the following equations were used to calculate the volume.

Equation for calculating the volume of boulders with ellipsoid shape:

$$V = \frac{4}{3}\pi a b c$$

Equation for calculating the volume of boulders with triangular prism shape:

$$V = \frac{1}{4}h\sqrt{-a^4+2(ab)^2+2(ac)^2-b^4+2(bc)^2-c^4}$$

The volumes were then multiplied by the known density of calcarenite limestone (~2 g cm^{-3}) from Cyprus (Modestou et al., 2015) and their weight was calculated. Images of the damaged colonies, before and after the storm, were analyzed and area percentage cover was estimated using the aforementioned method.

Mortality

With the use of SCUBA, we tagged individually 70 colonies at Kryo Nero and 60 colonies at Liopetri, which we visited regularly to monitor their condition with the use of photographic equipment (Canon G16). To assess the impact from temperature increases, we monitored all colonies at both sites on a monthly basis between the summer periods of June – October 2014 and 2015. To assess the impact from winter windstorms, permanently tagged colonies at Kryo Nero were visited and photographed during the winter months of December 2014, January and March 2015. The images obtained were analyzed with the use of photoQuad - photo quadrat analysis software (Trygonis and Sini, 2012) in order to calculate the percentage area cover falling under each of the following categories (Jimenez et al., 2016): 'Pigmented' (pigmented), 'Old necrosis', 'Recent necrosis, 'Affected' (bleached or decoloured) and 'Covered' (either live or dead, covered predominantly by macroalgae, such as Cystoseira spp. or other encrusting organisms such as sponges and bryozoans) (Figure 3.1). 'Recent necrosis' was easily recognizable from the clean shiny-white coloration of the bare skeleton, whereas 'Old necrosis' had a paler-white coloration, often covered by a layer of algae depending on how recent the mortality happened. Images of the colonies were analyzed and area percentage cover was estimated using the aforementioned method (Kersting et al. 2013).

We used Kruskal-Wallis statistical analysis and Mann-Whitney tests for pairwise comparisons, to compare the tissue condition between different sampling periods, according to the categories mentioned above. All analyses were computed using PAST statistical package (Hammer and Harper, 2001).

Common garden growth experiments

In order to estimate the growth rates and test the effect of high-nutrient density in the seawater, in *C. caespitosa* in Cyprus, we used the alizarin red staining technique (Lamberts, 1978). At least 20 live polyps from 10 different colonies from each site were collected and stained for 12 hours in plastic tanks (100 l) aerated with an air pump. Colonies were cemented back to the study sites using Standard Yellow-Grey Milliput epoxy. Ten polyps from each of the 10 colonies were cemented back to the same location from where they were collected at each site (control) and also transplanted 10 polyps out of each colony from Kryo Nero to Liopetri and from Liopetri to Kryo Nero for the common gardens experiment. All corallites were collected 12 months later, cleared of organic material and tissue by submersing them in H_2O_2 (30%) for 24 h and then polished with an electric mini-borer until the alizarin mark limit was clearly noticeable following Kersting and Linares (2012). Measurements were then performed using a caliper to the nearest 0.01 mm from the edge of the calyx to the upper limit of the staining (Schiller, 1993; Rodolfo-Metalpa et al., 1999; Kersting and Linares, 2012). We used one-way analysis of variance (ANOVA) to compare growth rates from each experimental location.

All statistical analyses were computed using PAST statistical package (Hammer and Harper, 2001). Comparisons with $p<0.05$ were considered significant.

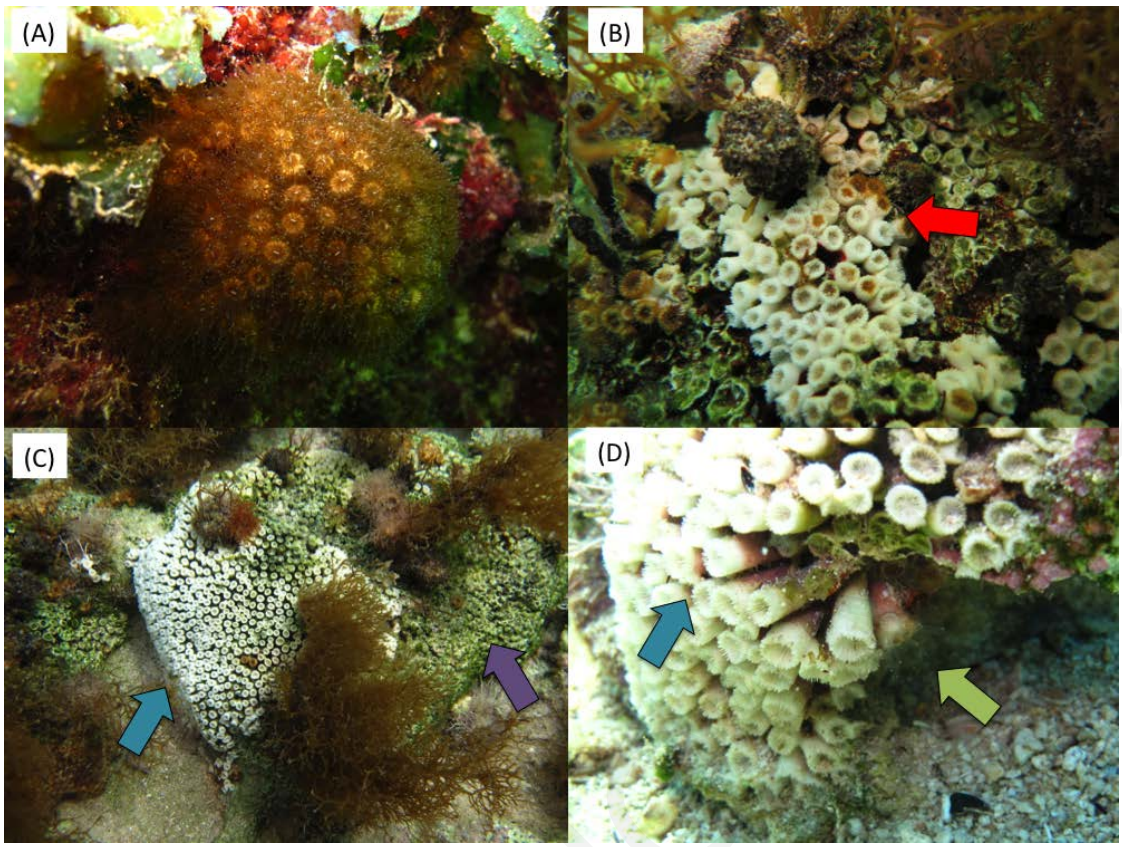

Figure 3.1. (A) Fully pigmented *Cladocora caespitosa* colony. (B) Colony with affected polyps (red arrow). (C) Recently necrotic (blue arrow) and old mortality (purple arrow) areas. (D) Bleached polyp (green arrow) and recently necrotic area (blue arrow**).**

Results

Environmental parameters

Nutrients

The results of nutrient analyses showed considerably higher concentrations at Liopetri than Kryo Nero (Figure 3.2). Mean nutrient concentrations at Liopetri were 1232 ± 826 µg L^{-1} for nitrate (NO_3^-), 92 ± 14 µg L^{-1} for ammonium (NH_4^+), 24 ± 6 µg L^{-1} for phosphate (PO_4^{3-}) and 5 ± 0,2 µg L^{-1} for nitrite (NO_2^-). At Kryo Nero, mean concentrations equaled 74 ± 84 µg L^{-1} NO_3^-, 13 ± 16 µg L^{-1} NH_4^+, 12 ± 8 µg L^{-1} PO_4^{3-}, 8 ± 6 µg L^{-1} NO_2^-.

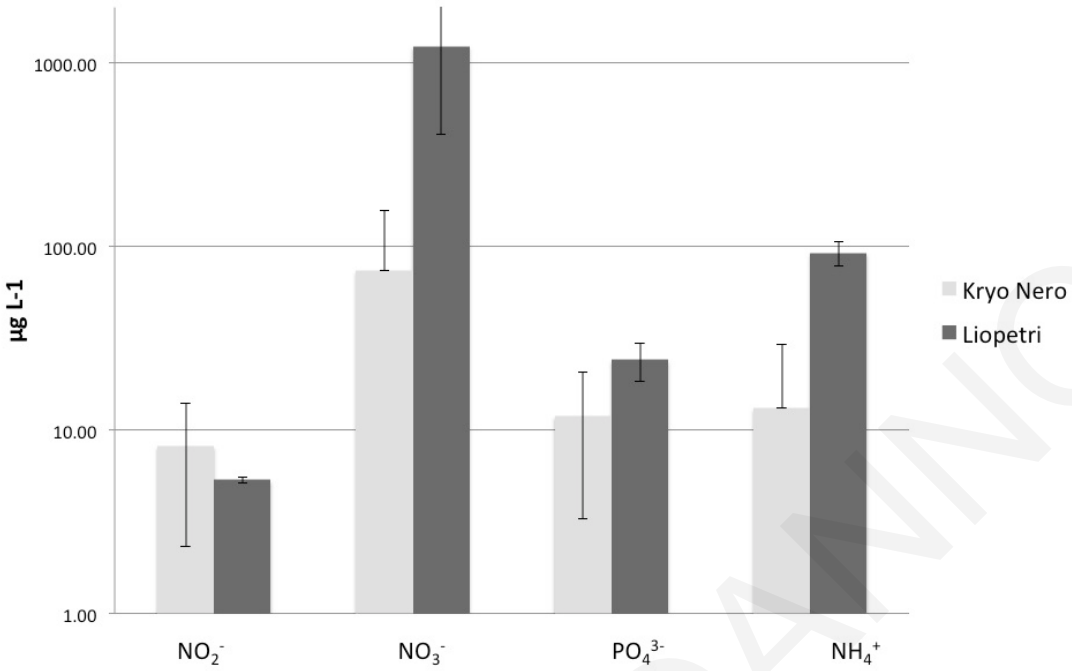

Figure 3.2. Mean (± SD) nutrient concentration levels at Kryo Nero and Liopetri from water samples obtained on a monthly basis (35 times between 2012-2015 from Liopetri; 12 times between 2014-2015 from Kryo Nero).

SST and SWT

Sea water temperatures recorded at the two sites showed the summer months of July-September to be warmer and prolonged in 2015 compared to 2014, with similar trends and minor differences occurring between the two sites (Figure 3.3). No SST anomalies were recorded in summer of 2014, whereas in 2015 we had the second warmest since 2003 (Figure 3.4). Monhtly mean SSTs recorded since 2003 show a clear increasing trend in anomalies (Figure 3.5).

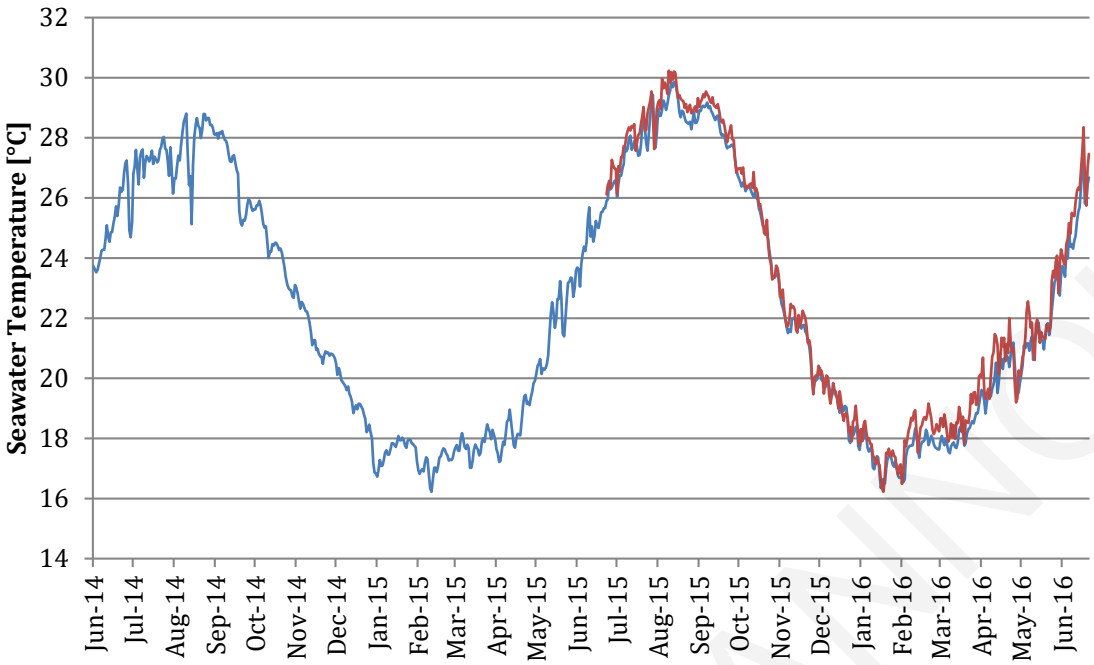

Figure 3.3. Mean daily seawater temperature (4m depth) at the study sites (Blue line: Kryo Nero; Red line: Liopetri).

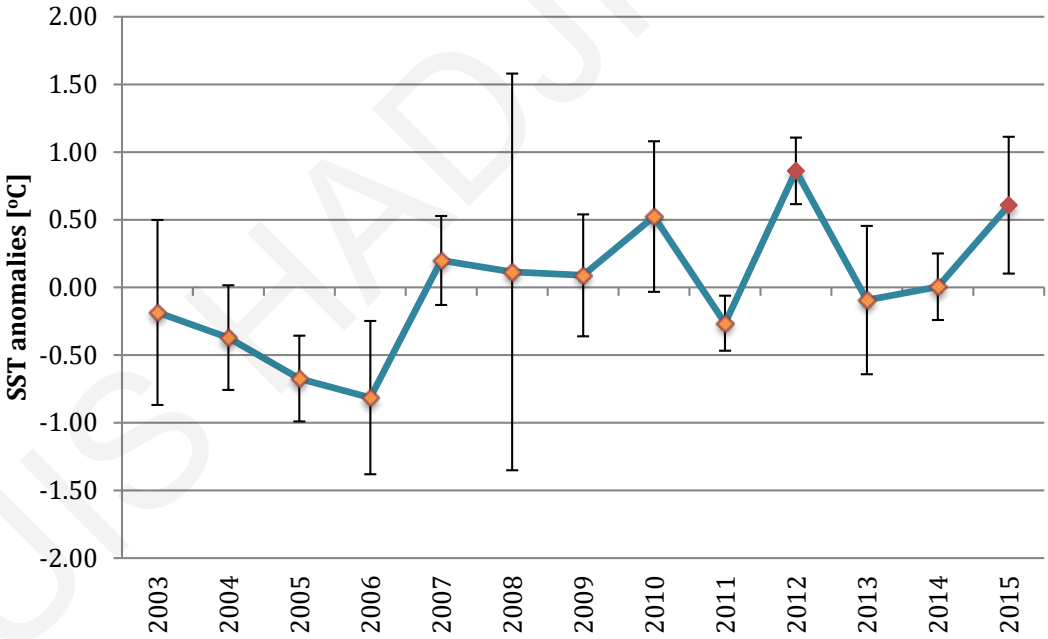

Figure 3.4. Summer (July, August, September, October) mean (± SD) SST anomalies recorded off Ayia Napa between 2003-2015 (2012 and 2015 in red marker) (Source of data: http://oceancolor.gsfc.nasa.gov/).

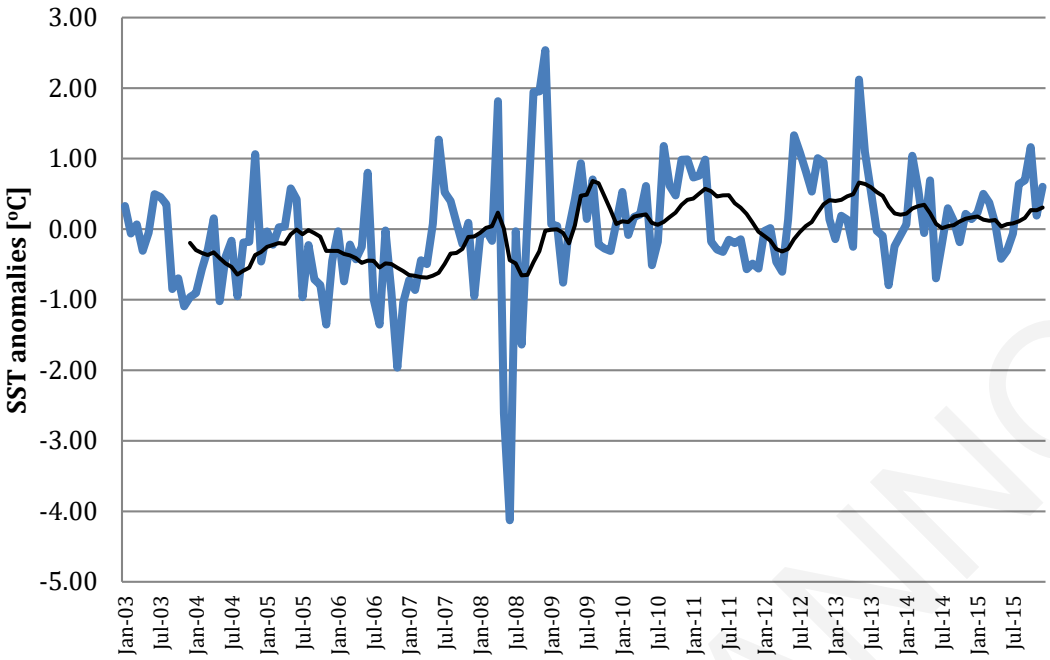

Figure 3.5. Monthly mean SST anomalies recorded off Ayia Napa between 2003-2015 (blue line: SST anomalies, black line: 12-point average).

Wind speed, direction and wave height

For observed winds, meteorological stations nearby (Paralimni and Xylophagou) showed wind speed increasing between 5th-7th of January 2015, from 3 to 22 m s^{-1}, over a period of 24 hours, then falling over the next 20 hours to 5 m s^{-1} and briefly up to 10 m s^{-1} again before calming down, a total of 72 hours of strong winds (Figure 3.6A). Wind direction was steadily from W to SW during the storm (Figure 3.6B). Note the orientation of the coast at Kryo Nero coral site is generally WNW to ESE. The University of Athens SKIRON regional atmospheric system predicted similar wind behavior at Kryo Nero location, but with slightly lower intensity peaks and valleys. The operational forecasts during the storm indicated a rise in significant wave height (average height of the highest 1/3 of the waves, not the maximum) from less than 1 to 4,4 m over the first 24 hours, followed by a decrease to 2,8 m, increase to 4 m before falling to 1 m almost three days later, clearly driven by local winds. During the storm, the wave direction was the same as the wind: from the W-SW. Comparison of the model forecast with a real current meter near the south-central coast showed excellent agreement. An analysis of the 10-year hindcast of 3-hourly wave conditions at the coral site showed waves with significant wave height greater than 4 m have only occurred 5 times from 2001-2010. Note that a similar storm occurred also between the 10th-13th of February 2015 (Figure 3.6C, D).

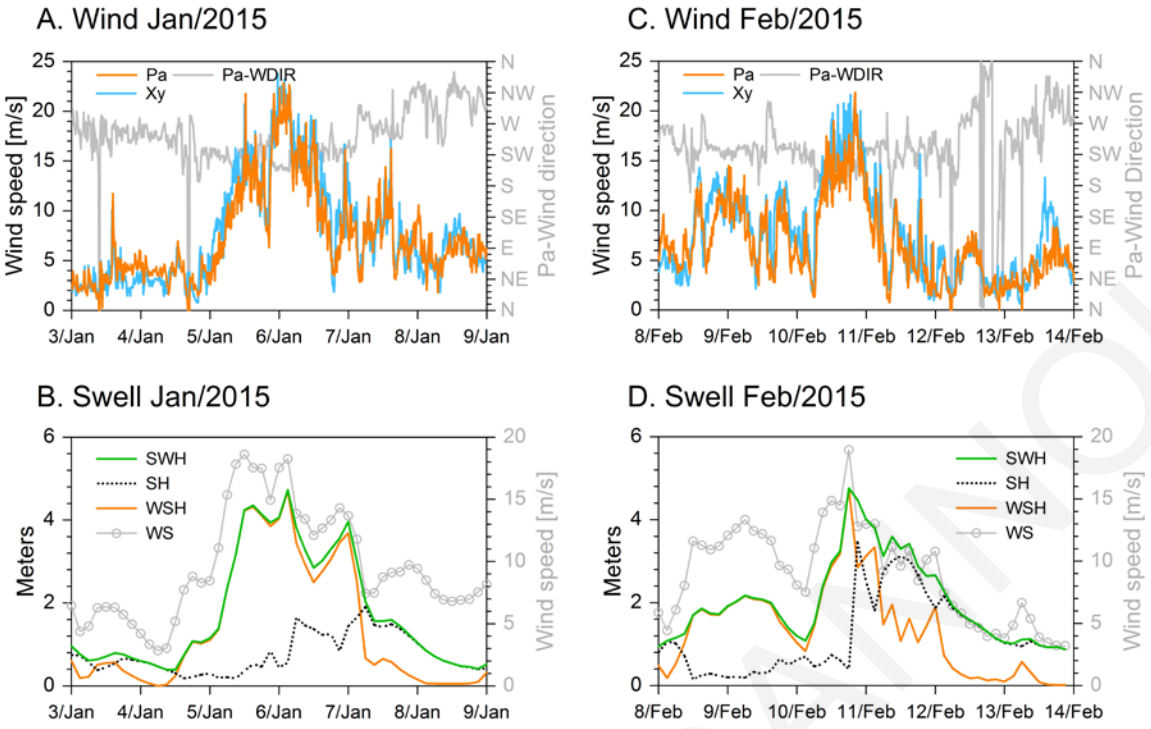

Figure 3.6. Wind speed (m/s), wind direction and swell height (SH: m) records from meteorological stations from January (A, B) and February (C, D). (Pa: Paralimni station, Xy: Xylophagou station, WDIR: Wind direction, SWH: Significant wave height, SH: Swell height, WSH: Wind sea height, WS: Wind speed) (Source of data: The Oceanography Center, University of Cyprus WAM4 wave model).

Cladocora Mortality

Temperature induced

Between the months of June 2014 - October 2014 and June 2015 - October 2015 there were significant changes in percentage cover of *C. caespitosa* colonies both within and between Kryo Nero and Liopetri sites (Figure 3.7).

At Kryo Nero, 'Pigmented' tissue was at its highest percentage in June 2014 (Figure 3.7; 47.1 ± 27.6%) and declined gradually, though not significantly, throughout August and October 2014. 'Pigmented' tissue showed a rebound in June 2015 (Figure 3.7; 46.3 ± 27.6%), possibly and partly due to a decline in 'covered' percentage, but declined again gradually, not significantly, throughout August and September, reaching its lowest percentage in October 2015 (Figure 3.7; 36.1 ± 29.5%). Comparatively, Kryo Nero pigmented tissue had significantly higher percentages of 'Pigmented' tissue in all months of both years (Mann-Whitney pairwise tests; $p<0.001$). At Liopetri, the second highest percentage of 'Pigmented' tissue was recorded in June 2014 (Figure 3.8; 14.6 ± 7.8%), which also declined gradually throughout August and October 2014. 'Pigmented' tissue was at its

highest in June 2015 (Figure 3.8; 16.3 ± 12.5%) but declined again gradually throughout August and September, reaching its lowest percentage in October 2015 (Figure 3.8; 9 ± 5.8%). The differences in 'Pigmented' percentage between June 2014 - October 2014 and June 2015 - October 2015 were statistically significant at both locations (Mann-Whitney pairwise tests; $p<0.01$). At the start of the study (June 2014), all colonies monitored at both sites had at least partly 'Pigmented' tissue, by the end of it however (October 2015) 2% of the colonies at Kryo Nero and 4% at Liopetri had lost completely all 'Pigmented' tissue (Table 3.1).

'Old necrosis' was observed in more colonies at Liopetri than Kryo Nero in both years (Table 3.1). However, percentage cover for 'Old necrosis' was higher at Kryo Nero. An increasing trend through the months was observed at both locations, with the highest percentage cover at both Kryo Nero (Figure 3.7; 29.5 ± 26.7%) and Liopetri (Figure 3.8; 25.9 ± 20.9%) found in October 2015 being significantly higher than June 2014 and 2015.

During periods of temperatures not showing anomalies, 'Recent necrosis' was at its lowest in June, for 2014, at both Kryo Nero (Figure 3.7; 0.2 ± 1.5%) and Liopetri (none recorded) and highest for 2014 in October at both Kryo Nero (Figure 3.7; 4.59 ± 9.39) and Liopetri (Figure 3.8; 4.47 ± 5.52). The same pattern was observed in 2015, with the lowest percentages recorded in June at both Kryo Nero (Figure 3.7; 0.09 ± 0.45) and Liopetri (Figure 3.8; 0.15 ± 0.56), gradually increasing in August and September before reaching peak in October (Figures 3.7, 3.8; Kryo Nero: 11.43 ± 24.2; Liopetri: 17.03 ± 20.42). Mann-Whitney pairwise tests showed 'Recent necrosis' to be significantly higher ($p<0.001$) at Liopetri compared to Kryo Nero in August and October 2014 and August to October 2015. Substantially more colonies were affected by 'Recent necrosis' in 2015 (Liopetri: 100% vs Kryo Nero: 45%) than 2014 (Liopetri: 81% vs Kryo Nero: 32%) (Table 3.1). In 2014, at Kryo Nero only 32% of colonies showed 'Recent necrosis', whereas at Liopetri 68%. Comparatively, in 2015, a much higher percentage of colonies affected both sites (Kryo Nero: 45%, Liopetri: 100%) with the larger amounts of 'Recent necrosis' percentage cover observed coinciding with higher SST anomalies (Figure 3.4).

'Affected' cover was at almost negligible percentages during the months of 2014 at both sites (Figures 3.7, 3.8; <1%), though a larger number of colonies were affected in October at Liopetri (Table 3.1; 21%) than Kryo Nero (Table 3.1; 5%). A much higher number of colonies were also affected in September 2015 at Liopetri (Table 3.1; 33%) compared to Kryo Nero (Table 3.1; 18%) as well as October 2015 (Table 3.1; Liopetri: 26%; Kryo Nero: 12%). Average percentage cover of 'Affected' at Liopetri reached 1.77 ± 11.83 (Table 3.1)

in August 2015 but stayed <1% in all other months. At Kryo Nero, the highest percentages were observed in September 2015 (Table 3.1; 3.47 ± 15.63) and October 2015 (Table 3.1; 3.34 ± 15.65) but stayed <1% in all other months.

The cover percentage of 'Covered' was significantly higher at Liopetri than Kryo Nero in all months of both years (Mann-Whitney pairwise tests; p<0,01). In addition, 'Covered' was higher in 2014 than 2015, in all months, at both locations (Mann-Whitney pairwise tests; p<0.05). The highest percentage at Kryo Nero was recorded in August 2014 (Figure 3.7; 40.2 ± 24.1%) whilst the highest percentage at Liopetri was recorded in October 2014 (Figure 3.8; 75.9 ± 10.7%). All colonies at both sites, apart from 3 (4%) in September and October 2015 at Kryo Nero (Table 3.1), had some amount of their areas covered by macroalgae and/or other organisms.

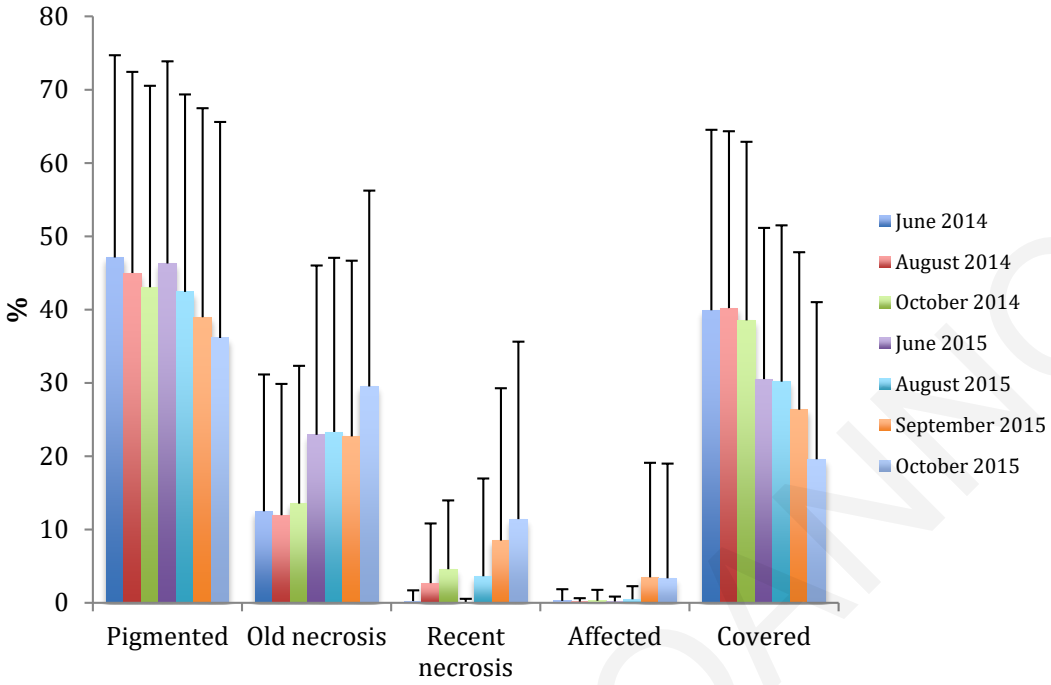

Figure 3.7. Percentage cover of different categories on *C. caespitosa* through summer months of 2014 and 2015 at Kryo Nero (mean ± SD).

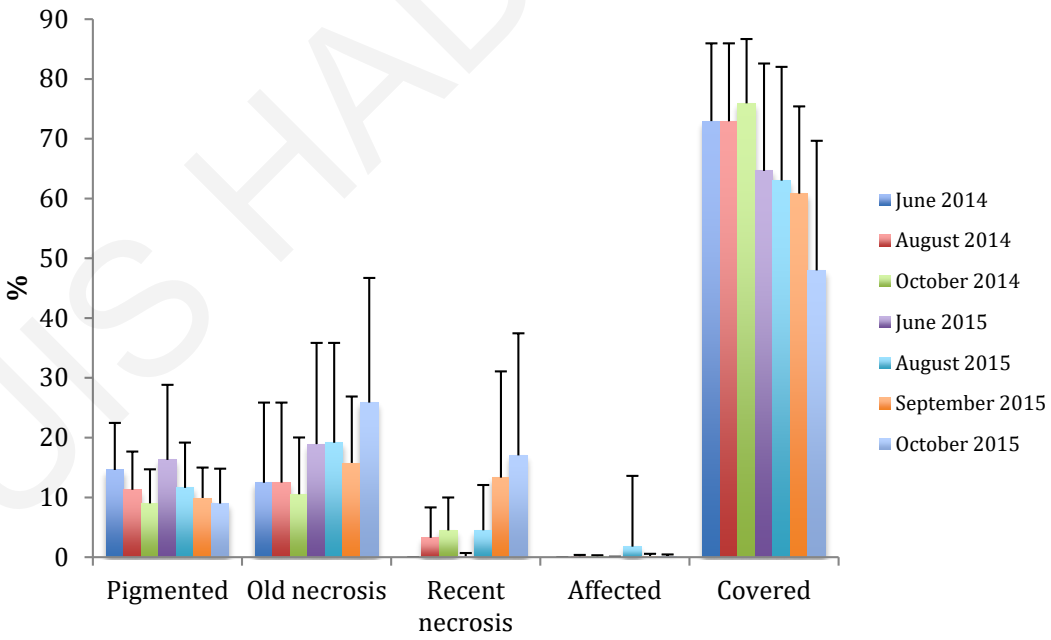

Figure 3.8. Percentage cover of different categories on *C. caespitosa* through summer months of 2014 and 2015 at Liopetri (mean ± SD).

Table 3.1. Percentage of *C. caespitosa* colonies exhibiting each of the categories per site and period.

Kryo Nero

	Pigment	Old necrosis	Recent necrosis (accumulating necrosis)	Affected	Covered
June 2014	100	58	3	3	100
August 2014	100	58	18 (3)	11	100
October 2014	100	63	32 (5)	5	100
June 2015	100	73	4	10	100
August 2015	100	73	24 (2)	14	100
September 2015	98	61	43 (18)	18	96
October 2015	98	80	45 (22)	12	96

Liopetri

	Pigment	Old necrosis	Recent necrosis (accumulating necrosis)	Affected	Covered
June 2014	100	85	0	0	100
August 2014	100	88	69 (0)	13	100
October 2014	100	88	81 (46)	21	100
June 2015	100	88	8	2	100
August 2015	100	88	73 (6)	21	100
September 2015	96	95	98 (70)	33	100
October 2015	96	91	100 (76)	26	100

Wind-storm generated

Out of seventy colonies monitored at Kryo Nero during the period December 2014 to March 2015, about 7% (N=5) showed obvious signs of mechanical damage from the storm (Figure 3.9). Impacted colonies suffered immediate reductions in average 'Healthy' tissue of up to 49.6 ± 15.9% (Figure 3.10), whereas within 2 months after the storm the percentage changed slightly to 49.8 ± 14.9% (Figure 3.10). Before the storm, the condition of the impacted colonies (N=5) consisted of a much higher percentage of 'Healthy' tissue (Table 3.2, Figure 3.10; 61.5 ± 26.7%) than 'Broken' (Table 3.2; Figure 3.10; 13.4 ± 15.3%) or covered (Table 3.2; Figure 3.10; 25.1 ± 14.1%). After the storm, the healthy tissue was dramatically reduced (Table 3.2; Figure 3.10; 11.9 ± 10.8%) while 'Broken' (Table 3.2; Figure 3.10; 46 ± 32.9%) and 'Covered' (Table 3.2; Figure 3.10; 42.1 ± 38.9%) increased. Two months after the storm, the 'healthy' tissue remained at similar levels (Table 3.2; Figure 3.10; 11.7 ± 11.8%) while 'Broken' increased (Table 3.2; Figure 3.10; 55.9 ± 37.2%) due to a decrease in 'Covered' coverage (Table 3.2; Figure 3.10; 32.4 ± 39.4%) by the disappearance of epibionts.

Table 3.2. Area percentage condition through time of five *C. caespitosa* colonies affected by the windstorm of winter 2015 at Kryo Nero (N/M: not measured)

		Colony no.	Healthy	Broken	Covered
Before Storm	**Dec-14**	**1**	75,0	10,6	14,4
		2	79,6	0,0	20,4
		3	87,4	0,0	12,6
		4	33,2	20,3	46,5
		5	32,2	36,1	31,7
	Average		**61,5**	**13,4**	**25,1**
	SD		**26,7**	**15,3**	**14,1**
After Storm	**Jan-15**	**1**	6,8	75,4	17,8
		2	15,9	62,6	21,5
		3	24,8	46,0	29,2
		4	0,0	0,0	100,0
		5	N/M	N/M	N/M
	Average		**11,9**	**46,0**	**42,1**
	SD		**10,8**	**32,9**	**38,9**
2 months after the Storm	**Mar-15**	**1**	0,0	100,0	0,0
		2	11,6	72,0	16,3
		3	24,8	44,1	31,0
		4	0,0	0,0	100,0
		5	22,2	63,4	14,5
	Average		**11,7**	**55,9**	**32,4**
	SD		**11,8**	**37,2**	**39,4**

After the storms, a large amount of boulders of various volumes were observed scattered around the locality, with large boulders found lying next to the damaged colonies (Figure 3.11). Empty sockets in the cliff formations directly above the coral communities indicated their detachment from the vertical walls, triggered by the force of the wind-generated waves from the storm. Boulders (N=10) measured in the locality were estimated to vary in volume (MIN: 0.007 m^3, MAX: 0.942 m^3) and weight (MIN: 1 kg, MAX: 1900 kg) with the smaller and lightest easily moved again ("erratics") by the surge following the main storms.

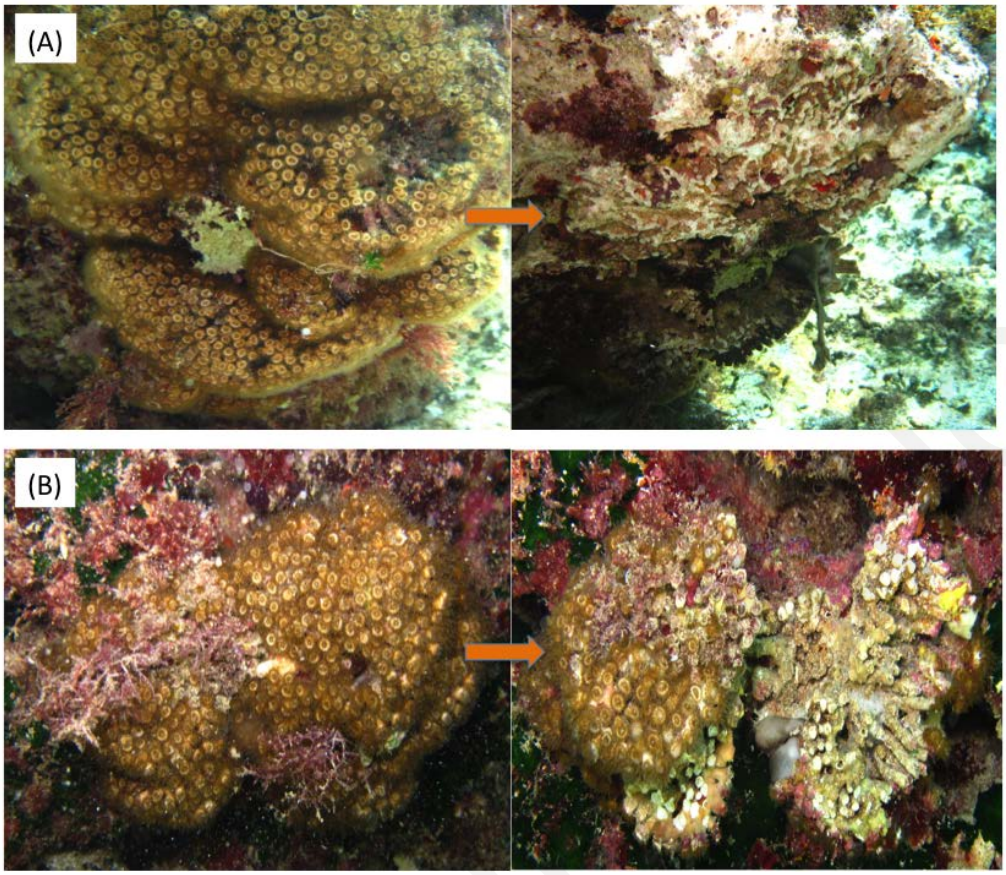

Figure 3.9. Photos of colonies (A) and (B) before and after the January 2015 storm showing clear signs of mechanical damage.

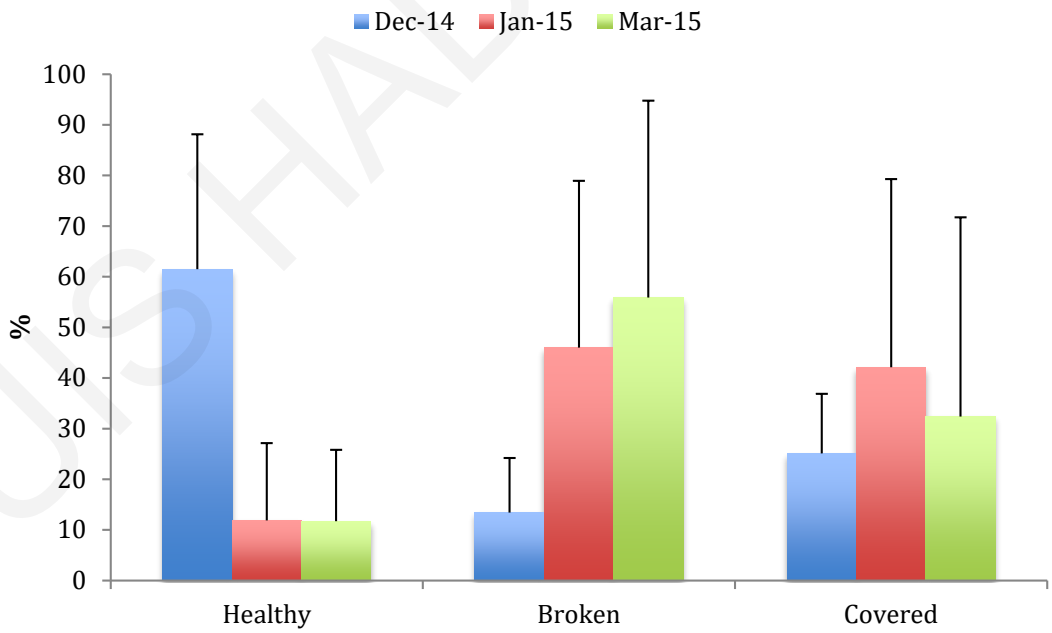

Figure 3.10. Area percentage before the storm (December 2014), 10 days after the storm (January 2015) and 2 months after the storm (March 2015) of 5 tagged colonies (out of 70) of *C. caespitosa* according to general condition (mean ± SD).

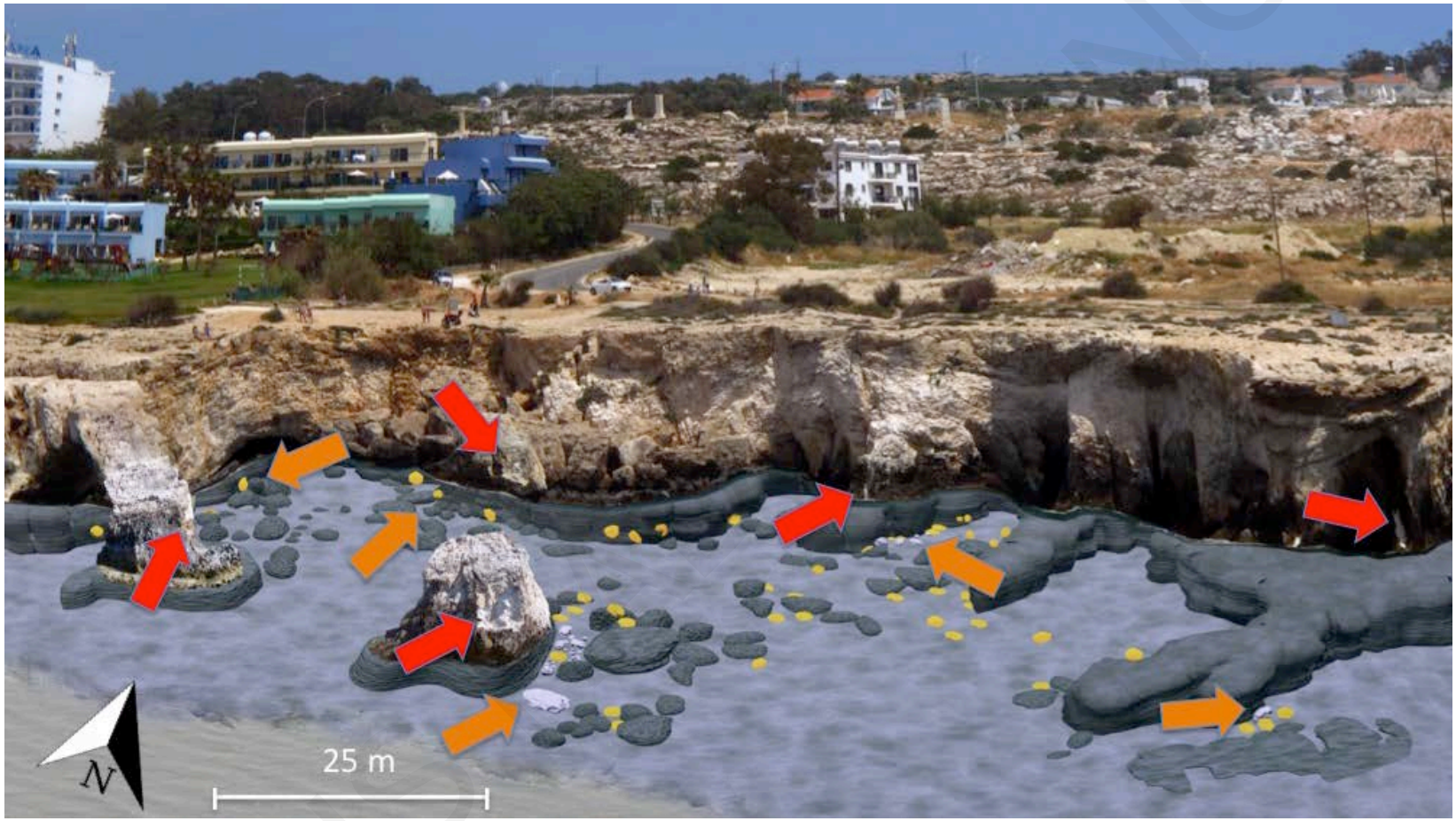

Figure 3.11. Kryo Nero study site, photograph of terrestrial part merged with an underwater image created using Blender open source 3D computer graphics software (https://builder.blender.org/download), showcasing the positions of *C. caespitosa* monitored colonies (yellow structures), sockets on the cliffs (indicated by red arrows) from fallen boulders (indicated by orange arrows) due to the windstorm of January 2015.

Growth rates from common garden experiments

Average annual growth measured using the alizarin staining technique revealed no significant difference in growth rates between "control"corallites from Kryo Nero (Figure 3.12; N: 67, 2.94 ± 1.05 mm) and Liopetri (Figure 3.12; N: 31, 3.39 ± 1.54 mm) and from Liopetri to Kryo Nero (Figure 3.12; N: 15, 3.04 ± 0.93 mm). However, significant differences (ANOVA; p<0,001) were observed between the growth rates of corallites collected from Kryo Nero and transplanted at Liopetri (Figure 3.12; N: 20, 6.29 ± 1.13 mm) and all the rest.

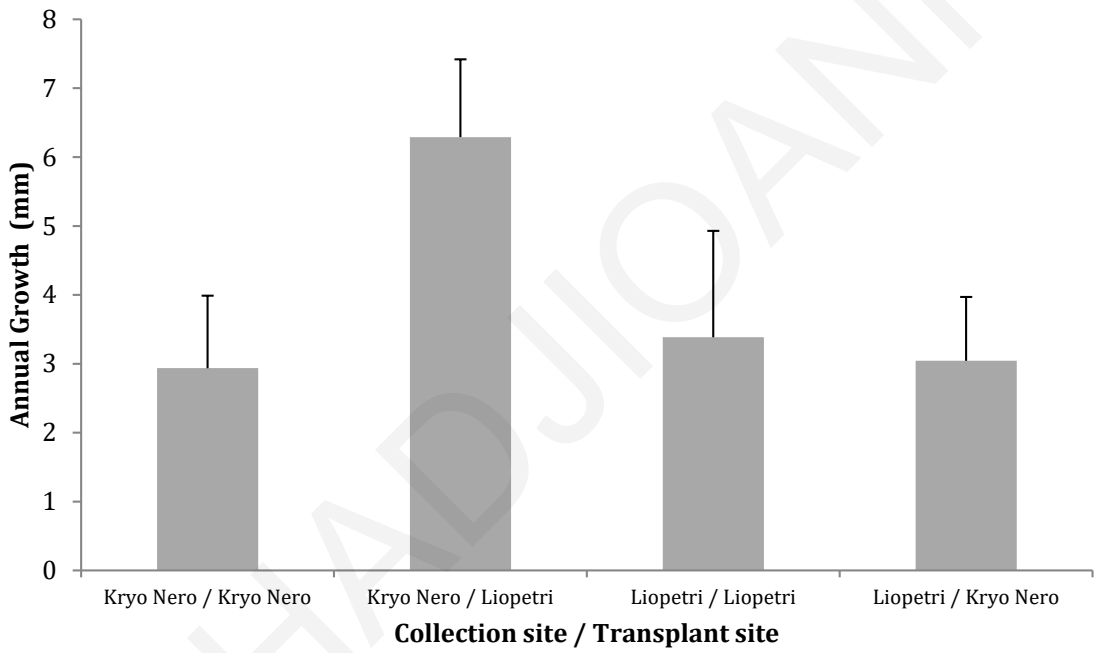

Figure 3.12. Average annual growth of *C. caespitosa* at different collection/transplant sites from common garden experiments, obtained using the alizarin staining technique (mean ± SD).

Discussion

The results from monitoring the two *C. caespitosa* populations in 2014-2015 suggest that SST anomalies and extreme windstorms are strongly associated with mortality events. In addition, it appears that elevated nutrient concentrations may alter their response to warming events as well as have an effect on their growth rates.

Temperature-induced mortality

During the summer/autumn months of 2014 and 2015 a number of *C. caespitosa* colonies were observed to undergo partial or total necrosis at the two locations, evident also by simultaneous reduction in 'Pigmented' healthy tissue. However, there was a clear difference in response, both between years (2014 vs 2015) as well as between sites in 2015 (Kryo Nero vs Liopetri). From the on-site data loggers, the maximum temperature recorded at Kryo Nero in 2014 (August: 28.8 ^{o}C), was lower than the maximum temperature recorded in 2015 (August: 29.85 ^{o}C). More importantly, temperatures were above 28 ^{o}C from mid-August to mid-September, whereas in 2015 temperatures over 28 ^{o}C where prolonged and lasted from the end of July to the end of September. In many cases, same colonies were observed being affected through the months of August to October with gradual increased necrotic coverage, being the highest in August and September. The SST difference between the two years is mainly evident from the average monthly SST anomalies in August 2014 (0.3) and August 2015 (0.64) (Figure 3.13), as well in as the average summer SST anomalies (2014: 0.01; 2015: 0.61), clearly showing that 2015 was a warmer year. In parallel, the significant reduction in 'Pigmented', healthy tissue, follow a similar decreasing trend. In addition, there is evidently an increased accumulation of 'Old mortality' in both sites as cover percentages peak in October 2015 assumingly as a result of previous year necrosis and reduction in cover by macroalgae and/or other organisms, exposing old mortalities. In October 2012, Jimenez et al. (2016) found that 93% of monitored colonies at Liopetri had gradually undergone recent necrosis, suffering 15% of average recent necrosis cover, slightly lower to the one recorded in October 2015 (17%) at the same site (this study). Average summer SST anomaly in 2012 was the highest in the last 15 years (Figure 3.4; 0.86); hence, Jimenez et al. (2016) associated that mortality event with the temperature anomalies. However, even though the average summer temperature of 2012 was warmer in 2012 than 2015 (Figure 3.4; anomalies 0.61C), the maximum temperature recorded was similar and prolonged in the months of August and September. Similarly, Kersting et al. (2013) found variable average necrotic percentages, of up to 25%, in an 11-year study (2002-2012) of *C. caespitosa* at the Columbretes Islands (W. Mediterranean), also associated with high SST anomalies but more closely related to the interannual temperature context and delayed thermal stress after extreme summers.

Given the significantly lower recent necrosis recorded in 2014, when summer SST were average, and the similarity in response in the years 2012 and 2015, when summer period was warmer (high SST anomaly), we also conclude to an association of mortality events with temperature anomalies.

The results do not come as a surprise, since elevated temperatures are known to cause mortality events in scleractinian corals globally (Glynn, 1984; Hoegh-Guldberg, 1999; Hughes et al. 2017) including *C. caespitosa* and other Mediterranean species (Garrabou et al. 2001; Rodolfo-Metalpa et al. 2005; 2006a; 2006b; Kersting et al. 2013; Kružić and Popijač, 2015; Jimenez et al. 2016, Kružić et al. 2016).

However, the fact that recent necrosis was observed also in 2012, despite average conditions, suggests that other factors also come into play, such as interannual temperature context and delayed thermal stress after extreme summers (Kersting et al. 2013), disease (Precht et al., 2016) and corallivory outbreaks (Kružić et al., 2013; Jimenez et al. 2016). It is also important to note that, in 2015, very low average cover percentage (maximum 3.4% in September at Kryo Nero; 1.76% in August at Liopetri) was 'Affected' by bleaching or decolouring, in contrast to the 2012 event, which had a much higher percentage of 'Affected' area (~10%) (Jimenez et al. 2016). It is important to note however that most of the 'Affected' area consisted of mostly decolouring of polyps. Bleaching was observed only in very few polyps (<5). It is possible that the 'Affected' area in 2015 was larger, but was concealed by the large average percentage cover of macroalgae.

Lastly, we need to consider the fact that *C. caespitosa* at Liopetri underwent significantly greater percentage of 'Recent necrosis' than Kryo Nero. Nutrient enrichment, along with increased seawater temperature, high coastal population and overfishing has been described as one of the most impactful stressors to coral reefs (Halpern et al. 2008) and there is evidence suggesting that nutrients may increase corals' susceptibility to bleaching when sea surface temperatures rise (Wooldridge, 2009; Cunning and Baker, 2012; Wiedenmann et al. 2012; Vega Thurber et al. 2014). Considering the fact that corals at both sites were subjected to similar environmental conditions, apart from nutrient concentrations (nitrate, ammonium and phosphate), we postulate that elevated nutrient concentration pose additional stress on the already thermally stressed *C. caespitosa* at Liopetri, making them more susceptible to necrosis. Further research through long-term monitoring is needed to confirm this assumption.

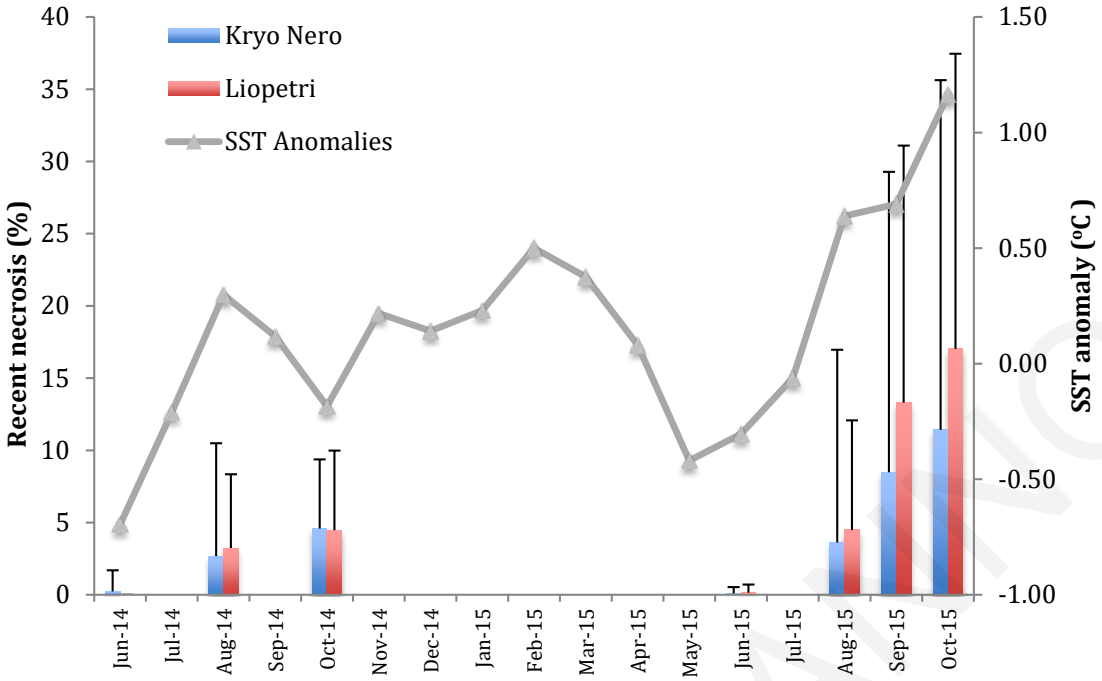

Figure 3.13. Average 'Recent recrosis' percentage cover at both sites and montly mean SST anomalies recorded off Ayia Napa during the study period (mean ± SD).

Wind-storm induced mortality

The extreme event of January 2015, which hit the southeast coast of Cyprus, coincided with damage of an estimated 7% of the colony population which resulted in an average reduction of ~50% of the pigmented healthy tissue on the affected colonies, observed two months after. The sockets in the bedrock directly above the colonies affected, in conjunction with the boulders observed next to the colonies leaves no room for doubt that the damage observed was mechanical, due to the falling of the boulders, which varied in size, with some of them being particularly large and heavy (up to 1900 kg).

The storm indisputably also left the colonies vulnerable to various potential after-effects, such as succession by macroalgae, exposure to bacterial infections and corallivory. Storm-induced conditions, such as turbulence, can be long-lasting and control corals and other benthic communities' structure (Voorhies, 2018; Shedrawi et al., 2017, Zunino et al., 2018). *Cladocora* populations with skewed structure to small size have been explained by storm high frequency (Guresen et al., 2015; Zunino et al., 2018) In addition, there are anecdotal reports of uprooted sponges brought onshore in the area of Pafos, in the southwest of Cyprus, at about the same period (Kontoghiorges, unpublished), indicating that destruction from extreme events was not so localized.

The general lack of long-term environmental and biological data in the Levantine Sea necessitates the need to study the ecological effects of extreme climatic events, which are largely expected to increase in severity, on a global scale. Considering that *C. caespitosa* in Cyprus are mostly found in the first five meters of depth, many times under cliffs and rocks susceptible to erosion events, it is reasonable to believe that with more extreme events being predicted such as the one recorded in the winter of 2015, further destruction is expected. The vulnerability of *C. caespitosa* at this shallow depths, and so close to the shore, was also noted by Schiller (1993) who inferred that the formation of large banks in shallow water in the bay of Piran, was probably prevented by physical disturbance. In addition, evidence that colony sizes of *C. caespitosa* can be limited by storm disturbances has been reported (Schiller, 1993; Kersting et al. 2017; Zunino et al. 2018). Given that there is a growing risk of an intensified storm climate, in terms of either storm duration (Gönnert, 2004), intensity (Weisse et al. 2005), or frequency (McInnes et al. 2003) in many parts of the world, it is critical that we improve our understanding of coastal dynamics. Such data are required to help us predict the behavior of sensitive organisms living in coastal areas, under changing storm frequencies and intensities.

Growth

The annual growth rate, measured for *C. caespitosa* found in the naturally low-nutrient site at Kryo Nero, are similar to the ones identified by previous studies in the Mediterranean (see review in Kersting et al. 2012). Perhaps it is surprising that the growth rate of corals situated in the low-nutrient waters of Cyprus (2.94 ± 1.1 mm) are found to be slightly higher than the ones from more nutrient-rich regions such as Illa-Grossa (West Mediterranean) (2.5 ± 0.8 mm) (Kersting et al. 2012), measured following the same alizarin technique. In general, though, the growth rates from Kryo Nero were within the spectrum of growth found in other studies, the lowest recorded in Tunisia (2.3 ± 0.2 mm; Peirano et al. 2009) and the highest in La Spezia, Italy (4.8 ± 1.7 mm; Rodolfo-Metalpa et al. 1999). Growth rates from the high-nutrient area of Liopetri (3.38 ± 1.54 mm) were slightly higher than the ones from Kryo Nero but the difference was not significant. However, the corals transplanted from Kryo Nero to Liopetri showed a staggering, significant difference by doubling their growth rates (6.29± 1.13 mm). Research on the growth rates of corals from the tropics have reported high coral extension rates when adjacent to sewage outfalls with high nutrient concentrations (Van Woesik, 1992; Risk et al. 1993; Dollar, 1994; reviewed in Lough and Barnes, 1997), while whole colonies of *Montastrea annularis* at eutrophic areas exhibited increased

extension rates at first but then decreased (Tomascik and Sander, 1985). These patterns suggest that coral extension rates may respond positively to moderate increases in productivity (Tomascik and Sander, 1985; Lough and Barnes, 1992; Edinger et al. 2000) and for short periods of time. However, increase in extension rates has been found to be inversely related to skeletal density (Dodge and Brass, 1984; Bosscher, 1992; Lough and Barnes, 1992; Edinger et al. 2000). Edinger et al. (2000) suggested that low density, high extension skeletal growth may be a sclerochronological signal of eutrophication effects on corals. In consequence, normal to high extension rates and low-density skeletal growth may be a typical result of coral growth under eutrophic conditions (Atkinson et al. 1995; Steven and Broadbent, 1997; Dunn et al. 2012). The above are often associated with high coral bioerosion, typified by eutrophied reefs, a trend that has been documented separately in many parts of the world (Rose and Risk, 1985; Risk and Sammarco, 1991; Lough and Barnes, 1997; Edinger et al. 2000; Prouty et al. 2017). Bioerosion is more readily observed at Liopetri coral site, with coral rubble scattered on the seabed among colonies in abundance (Hadjioannou, pers. observation).

Considering that corals collected and transplanted at Kryo Nero did not have the same growth rates, we conclude that the factor behind the increase in growth rate at Liopetri, at least in the short term, is the elevated nutrient concentration. We postulate that the corals collected and transplanted from/to Liopetri did not show the same growth rate due to acclimation of their metabolism to the increased nutrient concentrations. Further research is required to measure skeletal density (through the use of micro CT scanning) at the two sites, in order to be able to attribute the bioerosion observed at Liopetri to the decrease of density.

Conclusion

Interactions between the physical environment and living organisms, forming the basis of the science of ecology, can be complex and difficult to monitor. However, they can also be quite simplistic and straightforward in concept. Either way, complex or simple, they can go unnoticed if no systematic monitoring is followed. Naturally, for living organisms to be able to survive and potentially form viable populations, they must come to terms with a dynamic environment eventually adapting to the physical changes that take place in their surroundings (Bates et al. 2018). There are cases however when no-matter how well adapted the species are to their environment, extreme events can severely damage populations. Even though there is growing evidence showing that catastrophic weather events such as floods, droughts, storms and heat-waves has risen by almost 10-fold in the past 50 years (Easterling et al.,

2000; Beniston and Stephenson, 2004), still the true extend of environmental destruction during these events has not been well documented, hence not well known (Wingfield et al., 2011). There have also been conflicting results from different studies investigating the effects of nutrient increases on coral biology (Tomascik and Sander, 1985; Stambler et al. 1991; Ferrier-Pagès et al. 2001; Szmant, 2002; McClanahan, et al. 2003; Wiedenmann et al. 2013), making it difficult to generalize trends and becoming the focus of debates (Szmant, 2002).

Continuous and systematic monitoring should be conducted, particularly on highly sensitive organisms found in affected regions, in order to identify the long-term effects of extreme climatic events and anthropogenic intrusions such as elevating nutrient concentrations. The results will be detrimental to the identification of solutions and direction towards conservation.

CHAPTER 4:

Divergent response of high-nutrient and low-nutrient acclimated populations of the temperate coral *Cladocora caespitosa* to warming.

Divergent response of high-nutrient and low-nutrient acclimated populations of the temperate coral *Cladocora caespitosa* to warming.

Abstract

Anthropogenic nutrient enrichment and increased seawater temperatures are responsible for coral reef decline. In particular, they disrupt the relationship between corals and their dinoflagellate symbionts (bleaching). However, such symbiosis may acclimate or adapt to the environment in which it lives, and may change the bleaching threshold. This study focused on the role of the nutrient history in influencing the response of the Mediterranean scleractinian coral *Cladocora caespitosa* to thermal stress. Colonies living naturally in two nutrient-poor (<0.5µM nitrogen, <0.2µM phosphorus) and nutrient-rich (ca. 10-20µM nitrogen, 0.4µM phosphorus) locations were sampled, maintained under the right nutrient conditions, and exposed to temperature increase. Colonies grown in nutrient-poor conditions bleached and significantly decreased their protein content and rates of net photosynthesis. On the contrary, colonies grown under nutrient enrichment presented no sign of bleaching and no change in their overall metabolism. These results are important in that they show that nutrient history can influence the response of scleractinian corals to thermal stress. In addition, they suggest that corals with a high success in nutrient rich environments are likely those with a high heterotrophic capacity. Further investigations of under-studied coral groups are thus required in the future to understand the processes leading to coral resistance to environmental perturbations.

Keywords: coral physiology, high-nutrients, Cyprus, photosynthesis, calcification, Symbionidaceae, temperate corals.

Introduction

The health of scleractinian reef-building corals is rapidly declining with ever increasing global threats, such as long-term ocean warming, which induces the loss of coral symbionts and/or photopigments, known as bleaching (Hoegh-Guldberg et al. 2017). In recent years, shallow-water tropical reefs have already undergone massive bleaching events, followed by coral mortality (Precht et al. 2016; Heron et al. 2016; Hughes et al. 2017, 2018). Alongside deterioration in reef environment from global threats, local disturbances such as overfishing, nutrient runoff and pollution are likely to lower the resilience of corals to environmental change (Burke et al. 2011; Kennedy et al. 2013, Duprey et al. 2016, Zaneveld et al. 2016). In particular, anthropogenic seawater nutrient enrichment, due to the use of chemical fertilizers or to discharges of human and animal wastes can cause shifts in trophic dynamics of coral reef ecosystems (Szmant, 2002), loss of coral cover and diversity (Fabricius, 2005), increased coral diseases (Thurber et al. 2017) and susceptibility to bleaching (Wiedenmann et al. 2013). It has also been associated to coral reef decline by disturbing the fine balance between the host and its symbiotic algae (D'Angelo and Wiedenmann, 2014). Seawater enrichment with nitrate seems to be more detrimental for corals than ammonium enrichment (reviewed by Shantz and Burkepile 2014), especially under imbalanced nitrogen to phosphorus ratio (Wiedenmann et al. 2013).

Despite the overall detrimental effect of nutrient enrichment on corals, it has been shown that some corals can acclimate or adapt to relatively eutrophicated and nutrient enriched environments (Peirano et al. 1999; Bongiorni et al. 2003; Sawall et al. 2011; 2014). For many Brazilian reefs, for example, there are no reports of diseases, and bleaching events have high recovery rates of corals (Migotto, 1997; Castro & Pires, 1999), despite the fact that they are both affected by high sedimentation levels (Addad & Martins-Neto, 2000) and nutrient enrichment (Costa et al., 2000). In some other cases, corals respond positively to nutrient addition, by increasing growth and metabolism (Meyer & Schultz, 1985), especially under elevated pCO_2 (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011), or thermal stress (McClanahan et al. 2003; Beraud et al. 2013). A reduced susceptibility to bleaching was also noticed (McClanahan et al. 2003), in particular in regions with small-scale upwelling (Riegl & Piller, 2003). Overall, these antagonistic observations suggest that more research has to be done to better understand the adaptation or acclimation of corals to nutrient enrichment. Mediterranean corals, such as the scleractinian symbiotic coral *Cladocora caespitosa*, are among the few examples of corals that can be found both in areas with low-nutrient (Levantine basin, Cyprus, Krom 1995) and

high-nutrient concentrations (Schiller, 1993a; Peirano et al. 1999; Kružić and Benkovic, 2008; Kersting and Linares, 2012). They are thus the perfect model to study their responses and adaptations to the nutrient levels of their living environment. In addition, they are threatened by global warming, showing several episodes of mortalities (Rodolfo-Metalpa et al, 2005, 2006; Kružić et al. 2008, 2014; Kersting et al. 2013, 2014; 2015; Jimenez et al. 2016), due to the significant increase in sea surface temperatures (SSTs) of the Mediterranean and the Levantine Sea over the past years (Perez et al. 2000; Samuel-Rhoads et al. 2013). Corals, as well as other sessile organisms such as gorgonians, are key species of the Mediterranean Sea, and their mortality can have significant consequences for the ecosystem functioning and the overall biodiversity of this Sea. It is therefore urgent to understand how temperature, but also nutrient conditions, can affect their physiology and their chance to survive both global and local changes.

In this study, we have investigated the thermal tolerance of the coral *C. caespitosa* acclimated to two different nutrient environments, in order to assess the effect of nutrient supply on the response of such coral species to thermal stress. For this purpose, colonies of *C. caespitosa* were sampled in two contrasting but close environments of Cyprus island: a low-nutrient environment, since the waters around Cyprus, being centrally located in the Levantine basin, are considered to be one of the most oligotrophic ocean bodies of the globe (Krom, 1995); a high-nutrient enriched location, situated in front of a fish-farm hatchery where a large population of *C. caespitosa* thrives. Nutrification of the area, reflected in the surrounding marine biota, has been documented through appropriate and environmental impact assessments of the effects of the fish-farm hatchery to the surrounding area. We hypothesize that the main physiological traits of the coral colonies will be different between nutrient-enriched and poor conditions and that the colonies will also present a different response to thermal stress.

Materials and methods

Study sites and sample collection

Coral colonies originated from two areas in Cyprus, both holding >100 colonies of *C. caespitosa* at very shallow depths (<4m). ‘Kryo Nero’ site (i.e. low-nutrient site), with clear waters, is found on the coast of Ayia Napa village in the South-east of Cyprus (34°58.949' N, 34°1.014'E). ‘Liopetri’ site (high-nutrient site) lies approximately 10 km west of ‘Kryo Nero’ right in front of a fish-farm hatchery and very close to an agricultural area (34°57.537'N, 33°53.755'E) (Chapter 2, Figure 2.1).

Prior to the experiments, water samples were collected from both locations (35 times between 2012-2015 from Liopetri; 12 times between 2014-2015 from Kryo Nero) and analyzed at a certified analytical laboratory to determine nutrient concentrations using standard spectrophotometric methods (Strickland and Parsons, 1968). Nutrient analyses showed significantly higher concentrations at Liopetri than in Kryo Nero. Mean nutrient concentrations at Liopetri were 1232 µg L^{-1} or 19.87 µM for nitrate (NO_3^-), 92 µg L^{-1} or 5 µM for ammonium (NH_4^+) and 24 µg L^{-1} or 0.24 µM for phosphate (PO_4^{3-}). At Kryo Nero, mean concentrations equaled 74 µg L^{-1} or 1.2 µM NO_3^-, 13 µg L^{-1} or 0.72 µM NH_4^+, 12 µg L^{-1} or 0.12 µM PO_4^{3-} (Chapter 3, Figure 3.2).

Coral fragments (of 6-8 polyps) were collected from 36 large colonies at Liopetri and Kryo Nero, end of November 2015. They were identified, kept in separated bags containing the original seawater and rapidly transported to the aquarium system of the Centre Scientifique de Monaco (CITES nº CY/exp/005/2015). Here, each fragment was divided in two smaller fragments of 3-4 polyps, making a total of 72 fragments, which were distributed into 12 tanks, so that each tank contained 6 different original colonies. All tanks were maintained at the seawater temperature at the time of collection (17°C).

Experimental setup

Six tanks were maintained under low nutrient condition (ca. 0.5 µM NO_3^-, 0.1 µM NH_4^+ and 0.2 µM PO_4^{3-}) whereas the other six received high nitrogen levels (6-7 µM NO_3^- and 5-6 µM NH_4^+). These concentrations were lower than the mean in situ concentrations of either site, but where applied continuously to the corals for the 6 weeks experiment. Nutrient enrichment was thus performed using a peristaltic pump, which continuously supplied the experimental tanks with a solution of NO_3^- and NH_4^+ at a rate of 15 ml h^{-1}, together with a 12 L h^{-1} seawater flow-through. Nutrient concentrations were monitored twice a week with an auto-analyzer (Alliance Instrument, France), according to Tréguer and Le Corre (1975). Light (100 ± 10 µmol photons m^{-2} s^{-1}, with a 12:12h photoperiod) was provided by HQI lamps and set up to the mean daily irradiance received by the corals at the time of collection (daily photon flux density of 4 mol m^{-2}). It was measured using a spherical quantum sensor (LiCor LI-193, Lincoln, NE, USA). Colonies were fed twice a week with nauplii of *Artemia salina*.

Corals were kept three weeks under the two nutrient conditions and at 17°C (control). Two aquaria per nutrient condition were kept as control while seawater temperature was slowly

increased (0.5°C per day) in two other aquaria to 24°C and the last two aquaria to 29°C. Once the last two aquaria reached 29°C, corals were all maintained for 10 days before the physiological measurements described below were performed. While 17°C corresponds to the temperature at the time of collection, 24°C and 29°C represent respectively the mean annual temperature in Cyprus and the mean maximal temperature recorded in summer times using a Star-Oddi starmon mini temperature logger.

Measurements

Calcification and release of organic carbon and nitrogen

Calcification rates were assessed using the Total Alkalinity (TA) method. Six nubbins from each condition (3 per tank) were placed in separate sealed containers with 350 mL of 0.22 µm-filtered seawater (FSW). An extra container with only FSW was also incubated to serve as control. All containers were placed in a water bath at the right temperature (17°C, 24°C, 29°C) and light and incubated for 6 hours. Stirring was applied by magnetic stir bars. At the beginning and end of the incubation period, three seawater samples (50 mL) were collected from each container. The TA was measured in duplicate by automatic titration using a Metrohm Titrando 888 following Dickson et al. (2007).

The same coral nubbins were used to estimate the total organic carbon (TOC) and nitrogen fluxes (TN) with the use of Shimadzu TOC-L analyser**,** according to the established beaker incubation technique (e.g. Naumann et al. 2010). Briefly, corals were transferred without aerial exposure into acid-washed and seawater-rinsed 250 ml glass beakers filled with 0.2 µm filtered seawater. Three control beakers containing only seawater were also prepared. All beakers were placed in a water bath and incubated for 6 h as described above. After 6 h, corals were removed from the incubation beakers and kept for surface determination. Before and after incubations, seawater subsamples were drawn by sterile syringe from the thoroughly homogenised incubation media to quantify TOC and TON concentrations. Subsamples were transferred into pre-combusted (450°C, 5 h) glass vials, acidified with phosphoric acid (20%, 250 µl) to pH <2 and kept frozen (−20°C) until analysis.

Photosynthesis/respiration

Rates of net photosynthesis (P_n) and respiration (R) were measured using six nubbins per condition (three per tank). Each nubbin was placed in a temperature-controlled airtight chamber filled with ~50 ml of 0.45 µm-FSW, equipped with optodes (OCY-4 micro,

PreSens, Germany), and continuously stirred using magnetic stirrers. The optodes were calibrated before each treatment using nitrogen gas (N_2) and air saturated water for 0% and 100% oxygen saturation values respectively. Measurements were performed during 15 minutes initially at 150 µmol photons m^{-2} s^{-1}, 20 minutes in total darkness and 15 minutes at 300 µmol photon m^{-2} s^{-1}. Rates of gross photosynthesis (P_g) were calculated by adding R to P_n. Photosynthetic efficiencies (P_g/zoox) were calculated by normalizing P_g to symbiont density. Each rate was expressed per polyp surface area (µmol O_2 h^{-1} cm^{-2}) or per symbiont cell (µmol O_2 h^{-1} symbiont $cell^{-1}$) according to Rodolfo-Metalpa et al. (2006). Samples were frozen for later determination of tissue parameters (symbiont, chlorophyll, and protein concentrations).

Tissue parameters were determined according to Hoogenboom et al. (2010). Coral tissue was removed from the skeleton with an airbrush, using 0.45 µm filtered seawater and homogenized with a potter tissue grinder. A 1 mL sub-sample was used to determine symbiont density with a Beckman coulter counter (France). Protein content was assessed in another 1 mL sample according to Smith et al. (1985) by the use of a BCAssay Protein Quantification Kit (Uptima, Interchim) and a Xenius® spectrofluorometer (SAFAS, Monaco). In order to measure Chlorophyll-a concentration, the remaining 5 mL sub-sample was centrifuged at 8000 g for 10 min at 4°C. After removing the supernatant, symbionts were resuspended into 5 mL acetone and placed at 4°C overnight. Chlorophyll a and c_2 concentrations were determined following the method of Jeffrey and Humphrey (1975) by the use of a spectrophotometer (Safas, Monaco). Data were normalized to the nubbin surface area (cm^2). The main Symbiodiniacae genotype hosted by *C. caespitosa* in each location was checked according to the protocol of Santos et al. (2002). Symbionts from both sampling sites belong to clade B.

Statistical analyses

Two-way analysis of variance (ANOVA) was used to compare TA, TOC, TN, P_n, P_g, P_g/zoox, symbiont density, chlorophyll-a/chlorophyll-c2 and protein concentrations between nutrient conditions and temperatures. When significant interaction effects were detected, Tukey's HSD multiple comparison tests were conducted to examine the differences. All data were checked prior to analyses for normal distribution and were log-transformed when required. All analyses were computed using PAST statistical package (Hammer et al. 2001). Comparisons with $p<0.05$ were considered significant.

Results

Effect of thermal stress under different nutrient conditions

Overall, two different patterns in the response of *C. caespitosa* to temperature could be observed, depending on the nutrient conditions. Indeed, many physiological parameters were affected by temperature and nutrient enrichment, with a significant interaction between the two (two-way ANOVA, Table 4.1).

Table 4.1 Results of the two-way ANOVAs (p value) testing the effect of temperature and nutrient condition on the physiological parameters of *C. caespitosa*. Net photosynthesis (Pn) and gross photosynthesis (Pg) at 100 and 300 µmoles photons m^{-2} s^{-1} normalized to surface area (cm^{-2}) or symbiont cell (symbiont), chlorophyll a (Chl a) or c2 (Chl c2) concentration, total organic carbon (TOC) and nitrogen (NTN) fluxed. NS: non significant.

	Temperature	Nutrient	Interaction
Calcification	<0.001	0.05316	<0.001
P_n (100) (cm^{-2})	<0.001	NS	<0.05
P_n (300) (cm^{-2})	<0.001	<0.001	NS
P_g (100) (cm^{-2})	<0.001	NS	<0.001
P_g (300) (cm^{-2})	<0.001	<0.05	NS
P_g (100) (symbiont)	<0.001	NS	<0.05
P_g (300) (symbiont)	<0.001	NS	<0.05
Respiration	<0.001	<0.05	NS
Symbiont density	<0.01	<0.05	<0.05
Chl a (µg cm^{-2})	<0.001	NS	NS
Chl c2 (µg cm^{-2})	<0.001	NS	NS
Protein (µg cm^{-2})	NS	<0.05	<0.001
TOC	<0.001	NS	<0.05
TN	NS	<0.001	<0.01

In the low nutrient condition, we observed a partial loss of symbionts (bleaching) at temperatures higher than 17°C ($p<0.05$; Table 4.2, Figure 4.1). As expected, chl-a and chl-c_2 concentrations followed the same pattern as the symbiont density, and were significantly affected by temperature conditions (two-way ANOVA, $p<0.001$; Table 4.1, Figure 4.1). Tukey post-hoc tests showed a loss of chl-a ($p<0.05$; Table 4.2, Figure 4.2) and chl-c2 ($p<0.01$; Table 4.2, Figure 4.1) from 17°C to 24°C and 29°C. Protein concentration also

significantly decreased from 17°C to 29°C ($p<0.01$; Table 4.2, Figure 4.1). As a consequence of bleaching, net photosynthesis, P_n (cm^{-2}) measured at 100 µmole photons m^{-2} s^{-1} was significantly lower at 24° C ($p<0.05$; Table 4.2, Figure 4.3) and 29° C ($p<0.01$; Table 4.2, Figure 4.3) compared to 17°C. Pn measured at 300 µmole photons m^{-2} s^{-1} however increased with temperature ($p<0.05$; Table 4.2, Figure 4.3). Since the dark respiration was significantly higher at 29°C when compared to 24°C and 17°C ($p<0.05$; Table 4.2, Figure 4.3), gross photosynthesis, measured at both 100 and 300 µmole photons m^{-2} s^{-1} was also significantly higher at 27°C and 29°C compared to 17°C ($p<0.001$; Table 4.2, Figure 4.3). The photosynthetic efficiency of the symbionts (Pg/symbiont) tended to be higher at 29°C compared to both 17°C and 24°C ($p<0.01$; Table 4.2, Figure 4.3). Finally, calcification rates, measured through total alkalinity, significantly increased from 17 to 24°C ($p<0.05$; Table 4.2, Figure 4.2), before decreasing again to the initial value at 29°C ($p<0.001$; Table 4.2, Figure 4.2). Concerning the organic carbon and nitrogen fluxes, TOC was significantly lower at 29°C when compared to both 17°C ($p<0.001$; Table 4.2, Figure 4.2) and 24° C ($p<0.001$; Table 4.2, Figure 4.2). There were no significant differences in the TN fluxes with temperature conditions.

In the high nutrient condition, there was no significant change in symbiont density with increased seawater temperature (Table 4.2, Figure 4.1). Despite this, chl-a and chl-c_2 were significantly lower at 24°C compared to 17°C ($p<0.05$; Table 4.2, Figure 4.1). Protein concentration was however higher at 29°C compared to 17°C ($p<0.05$; Table 4.2, Figure 4.1), and was the highest between nutrient treatments ($p<0.001$; Table 4.3, Figure 4.1). While P_n at 100 µmole photons m^{-2} s^{-1} was significantly lower at 29°C compared to lower temperatures ($p<0.05$; Table 4.2, Figure 4.3), P_n measured at 300 µmole photons m^{-2} s^{-1} was significantly higher ($p<0.05$; Table 4.2, Figure 4.3). As respiration rates increased at 29°C, ($p<0.001$; Table 4.2, Figure 4.3), P_g (at both 100 and 300 µmole photons m^{-2} s^{-1}) was also higher ($p<0.001$; Table 4.2, Figure 4.3). Overall, the photosynthetic efficiency of the symbionts increased with temperature ($p<0.05$; Table 4.2, Figure 4.3). Finally, calcification rates significantly increased with temperature ($p<0.001$; Table 4.2, Figure 4.2). Concerning the organic carbon and nitrogen fluxes, TOC and TN fluxes were significantly higher at 24°C when compared to 17°C ($p<0.001$; Table 4.2, Figure 4.4) and 29°C ($p<0.001$; Table 4.2, Figure 4.4).

Table 4.2. Results of the Tukey post-hoc test on the effect of temperature and nutrient condition on the physiological parameters of C. caespitosa. Net (Pn) and gross photosynthesis (Pg) at 100 and 300 µmoles photons m-2 s-1 normalized to surface area (cm-2) or symbiont cell (symbiont), chlorophyll a (Chl a) or c2 (Chl c2) concentration, total organic carbon (TOC) and nitrogen (NTN) fluxes. HN: High nutrient, LN: Low nutrient, NS: non significant.

	Nutrient	17° C - 24° C	24° C - 29° C	17° C - 29° C
Calcification	HN	<0.05	<0.001	<0.001
	LN	<0.05	<0.001	NS
P_n (100) (cm^{-2})	HN	NS	<0.05	0.0576
	LN	<0.05	NS	<0.01
P_n (300) (cm^{-2})	HN	NS	<0.001	<0.001
	LN	NS	<0.001	<0.05
P_g (100) (cm^{-2})	HN	NS	NS	<0.001
	LN	NS	<0.001	NS
P_g (300) (cm^{-2})	HN	NS	<0.001	<0.001
	LN	NS	<0.001	<0.001
P_g (100) (symbiont)	HN	<0.05	NS	<0.05
	LN	NS	<0.01	<0.01
P_g (300) (symbiont)	HN	NS	NS	<0.01
	LN	NS	<0.001	<0.001
Respiration	HN	NS	<0.001	<0.001
	LN	NS	<0.001	<0.001
Symbiont density	HN	NS	NS	NS
	LN	<0.05	NS	<0.01
Chl a (µg cm^{-2})	HN	<0.05	NS	NS
	LN	<0.05	NS	<0.05
Chl c2 (µg cm^{-2})	HN	<0.05	NS	NS
	LN	<0.01	NS	<0.01
Protein (µg cm^{-2})	HN	NS	NS	<0.05
	LN	NS	NS	<0.01
TOC	HN	<0.001	<0.001	NS
	LN	NS	<0.001	<0.001
TN	HN	<0.05	0.0592	NS
	LN	NS	NS	NS

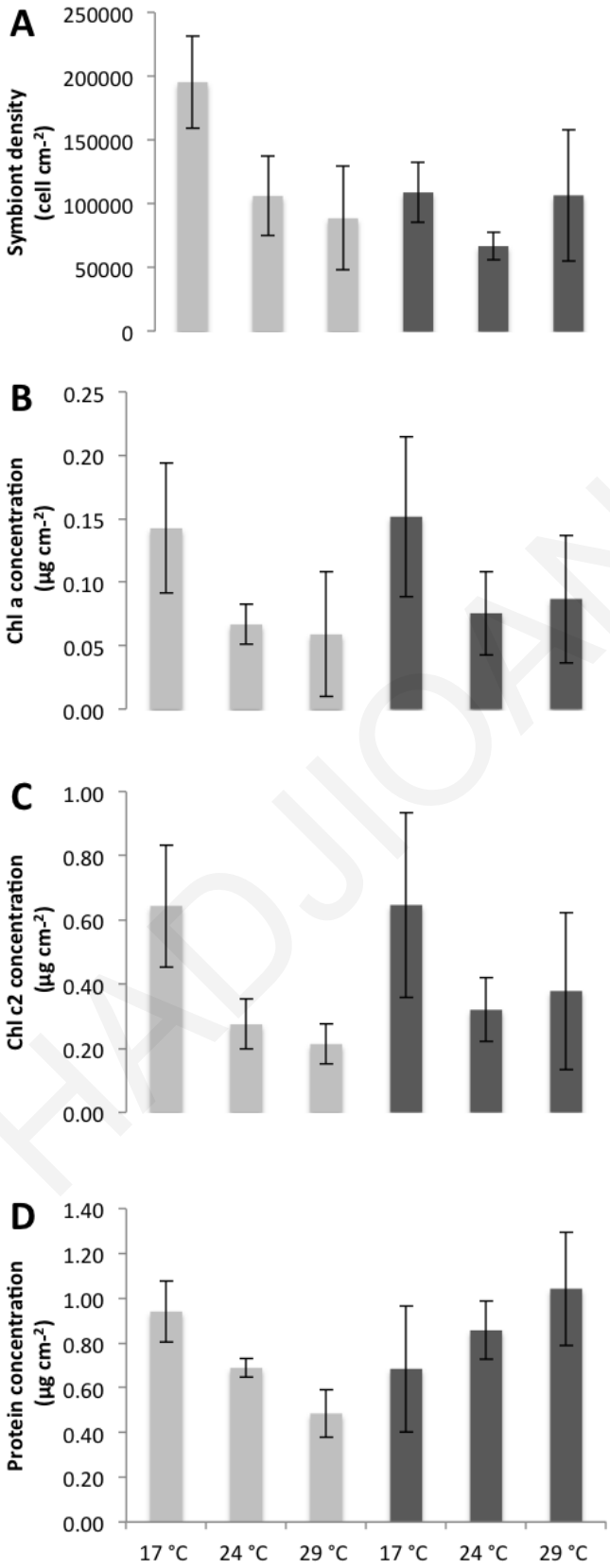

Figure 4.1. Symbiont density (A), concentrations in Chlorophyll-a (B), Chlorophyll-c2 (C) and protein (D) in nubbins maintained under low nutrient (LN, light grey) and high nutrient (HN, dark grey) conditions at different temperatures.

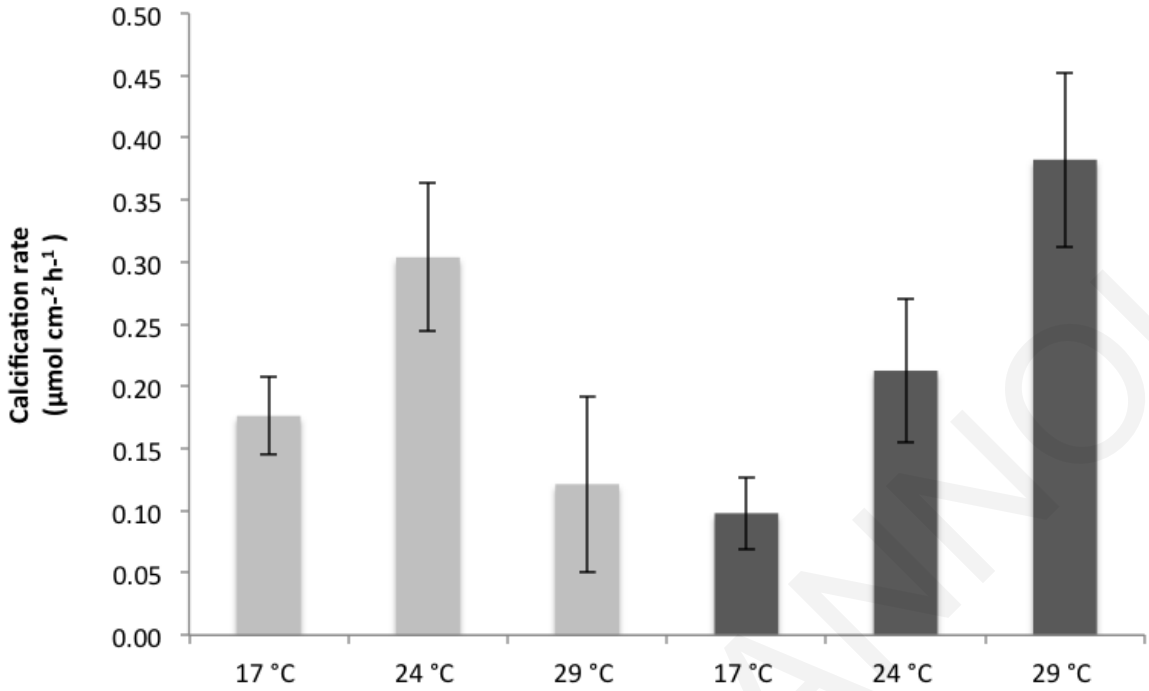

Figure 4.2. Calcification rate of *C. caespitosa* under low nutrient (light grey) and high nutrient (dark grey) levels at different seawater temperatures.

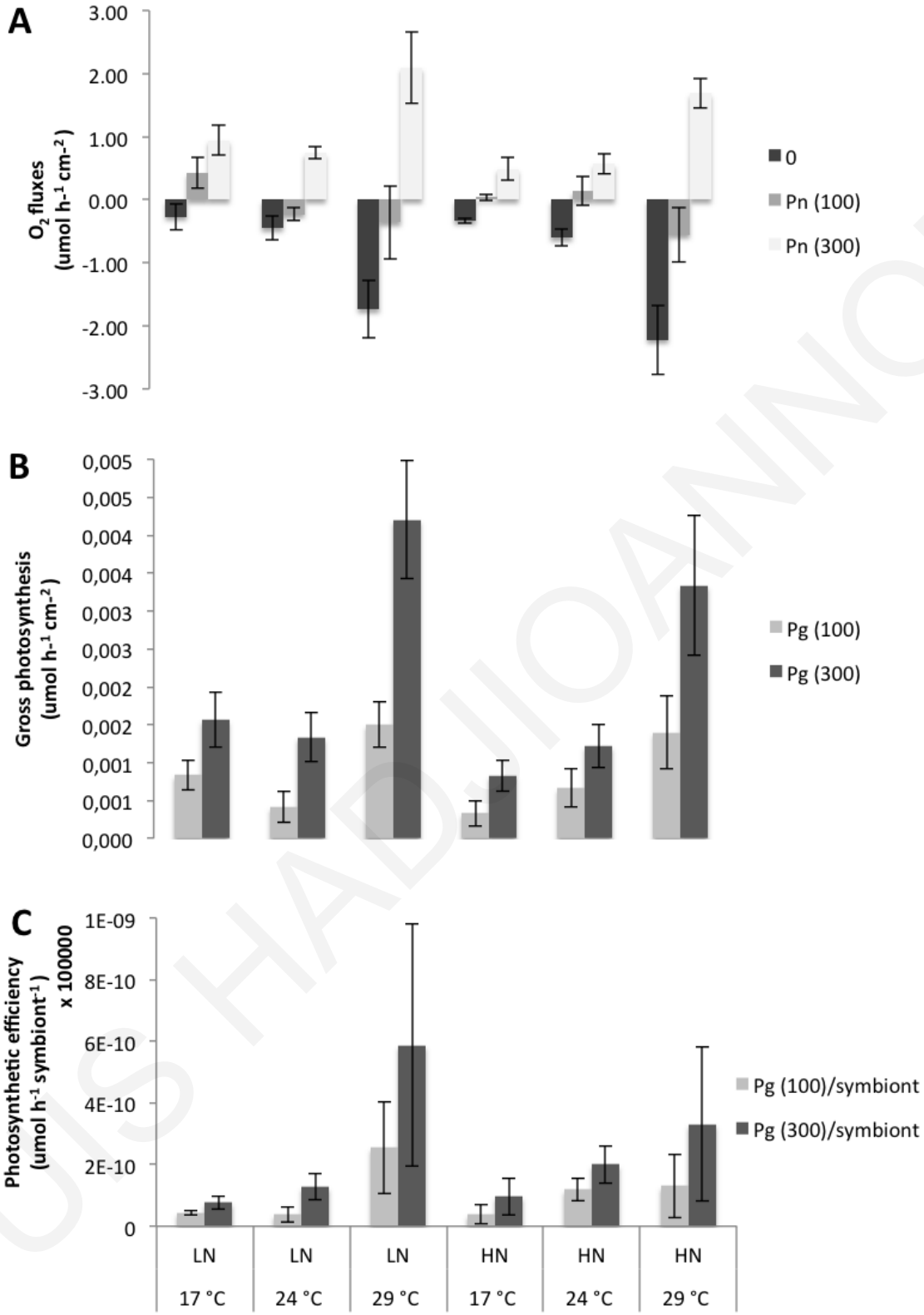

Figure 4.3. Average net photosynthesis (Pn) (A), respiration rates, gross photosynthesis (Pg) (B) and photosynthesis efficiency (Pg/symbiont) (C) of *C. caespitosa* under different temperatures, light intensities (0, 100 and 300 µmol photon $m^{-2} \cdot s^{-1}$) and nutrient levels (high (HN) and low (LN) nutrient).

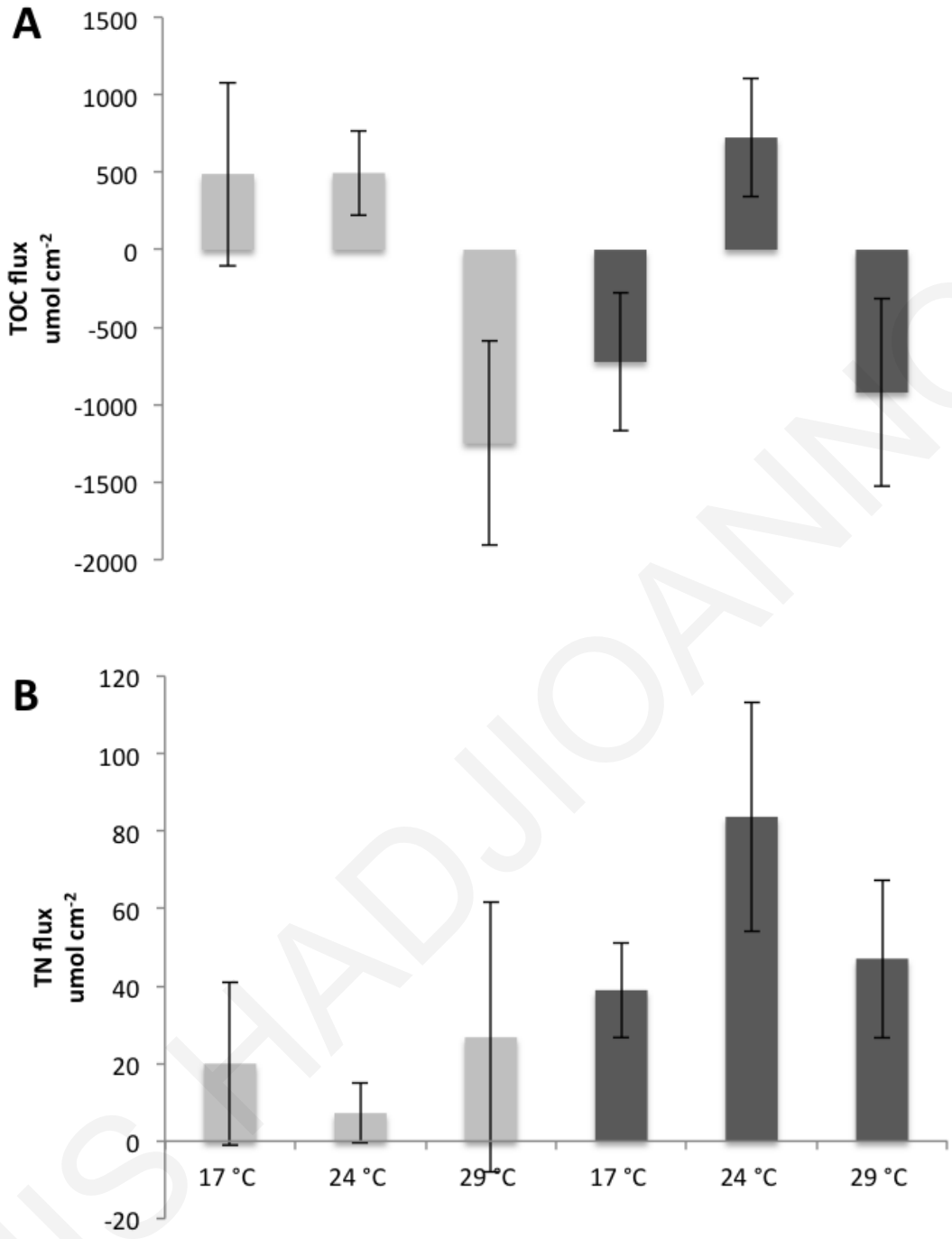

Figure 4.4. A) Total organic carbon (TOC) and B) total nitrogen (TN) fluxes under low nutrient (light grey) and high nutrient (dark grey).

Low nutrient vs high nutrient

Nutrient enrichment did not change the symbiont density nor the chlorophyll content within the tissue of *C. caespitosa* at all temperatures. Protein density also did not change with nutrient enrichment at 17°C and 24°C. The only changes were observed at 17°C for Pn at 300 µmole photons m^{-2} s^{-1} and Pg at 100 µmole photons m^{-2} s^{-1} which were both higher under low nutrient conditions (Table 4.3) and calcification at 29°C, which was higher under

high nutrient level (Table 4.3). TOC and TN fluxes were also slightly changed depending on the nutrient conditions, with however no clear trend.

Table 4.3. Results of the Tukey's post-hoc test on the effect of nutrient conditions on the physiological parameters of *C. caespitosa*. Net (Pn) and gross photosynthesis (Pg) at 100 and 300 µmoles photons m-2 s-1 normalized to surface area (cm-2) or symbiont cell (symbiont), chlorophyll a (Chl a) or c2 (Chl c2) concentration, total organic carbon (TOC) and nitrogen (NTN) fluxes. NS: non significant.

	17° C	24° C	29° C
Calcification	NS	NS	<0.001
P_n (100) (cm^{-2})	NS	NS	NS
P_n (300) (cm^{-2})	<0.01	NS	NS
P_g (100) (cm^{-2})	<0.01	NS	NS
P_g (300) (cm^{-2})	NS	NS	NS
P_g (100) (symbiont)	NS	0.051	NS
P_g (300) (symbiont)	NS	NS	NS
Respiration	NS	NS	NS
Symbiont density	NS	NS	NS
Chl a (µg cm^{-2})	NS	NS	NS
Chl c2 (µg cm^{-2})	NS	NS	NS
Protein (µg cm^{-2})	NS	NS	<0.001
TOC	<0.01	NS	NS
TN	NS	<0.001	NS

Discussion

Although many coral species are vulnerable to increased sea surface temperature and eutrophication (Hoegh-Guldberg, 1999; Fabricius et al. 2013, Hall et al. 2018), some may acclimate or even adapt to these stressors, at both the physiological and molecular levels (i.e. Middelbrook et al. 2008). For example, thermal history led to acclimation in several coral species (Brown et al., 2002; Ulstrup et al., 2006; Howells et al. 2012; Oliver and Palumbi 2011; Kruegger et al. 2017; see Hughes et al. 2018 for an alternative view) and some corals are able to grow in nutrified or eutrophic environments (Sawall et al. 2011; Moura et al., 2016). Although the above studies have highlighted the importance of understanding the flexibility of coral responses to environmental stressors, most of them have focused on the acclimation to high temperature conditions rather than eutrophication or nutrification. It is

however important to understand the ability of different coral species to acclimate to high nutrient conditions, as this is going to affect many reefs in the future, due to the increasing urbanization of many coastal areas (e.g. Duprey et al., 2016). The present study is thus one of the few that has focused on experimentally testing the effect of the long-term nutrient history on the bleaching susceptibility of a scleractinian coral species (Vega Thurber et al., 2013; Hall et al., 2018). Mediterranean corals such as *C. caespitosa* are good examples of coral species able to thrive both in low-nutrient and nutrient-enriched environments. In addition, they experience large temperature variations between summer and winter conditions (Shaltout and Omstedt, 2014). The results of this study are important because they show that the nutrient history can influence the response of some scleractinian corals to thermal stress and therefore have implications for the understanding of the bleaching process and coral resilience (Pawlik et al. 2016, Mumby & Steneck 2018). We indeed observed that colonies acclimated to very high levels of nitrogen did not bleach at high seawater temperatures, on the contrary to those grown under low-nutrient conditions. Our results provide novel insights into the particular resilience of Mediterranen corals to nutrification, as also observed in some particular occasions with tropical corals (Sawall et al. 2011; Moura et al. 2016). Although pollution has been correlated with the decline of *C. caespitosa* in one particular region in the Mediterranean (El Kateb et al. 2016) our results suggest that further investigation of under-studied coral groups are needed in the future to understand the processes leading to such coral resilience to environmental perturbations.

Colonies of *C. caespitosa* maintained under low nutrient concentrations presented a response to thermal stress similar to many tropical and temperate coral species (Hoegh-Guldbergh, 1999; 2011; Rodolfo-metalpa et al. 2006; Kružić and Popijač, 2015). Temperature increase induced a significant bleaching of *C. caespitosa* (decrease in both symbiont density and areal chlorophyll content), followed by a decrease in the net photosynthesis measured at the in situ irradiance of 100 µmole photons m^{-2} s^{-1}. Higher irradiance (300 µmole photons m^{-2} s^{-1}), applied over a short-term incubation, however allowed maintaining and even increasing photosynthesis at high temperature. Such high irradiance is recorded in surface water in summer-time for short daily periods in the North-West Mediterranean (Ferrier-Pagès et al. 2015) and certainly for longer periods in Cyprus. However, since high irradiance also tends to induce oxidative stress (Lesser and Farrell 2004), it is not likely to reduce bleaching and/or mortality under thermal stress. Mortality and bleaching of *C. caespitosa* has thereby been recorded in 2012 in Cyprus, concurring with temperature anomalies (Jimenez et al. 2016), and overall in the Mediterranean Sea (Rodolfo-Metalpa et al. 2000; Kružić et al. 2012; 2014; Kersting et al. 2013;). A similar effect of high temperature on *C. caespitosa* was observed

in laboratory thermal stress experiments (Rodolfo-Metalpa et al. 2006; Kersting et al. 2015). Despite significant bleaching, calcification rates were boosted under high temperature conditions. This is similar to in situ observations in the North-West Mediterranean Sea showing higher growth rates of *C. caespitosa* in summer, compared to almost no growth in winter, at temperatures of 12°C (Peirano et al. 2005). The growth of *C. caespitosa* in a previous thermal stress experiment (Rodolfo-Metalpa et al. 2006) was also significantly enhanced during the first 3 weeks of temperature increase, contrary to another Mediterranean coral, *Oculina patagonica*, whose growth was rapidly impacted by thermal stress (Rodolfo-Metalpa et al. 2006). In tropical corals, the thermal optimum for calcification generally occurs between 26°C and 28°C, after which there is an inverse temperature dependency (Reynaud-Vaganay et al. 1999; Edmunds 2005). Calcification of *C. caespitosa* may follow the same trend, at least until the energetic reserves in coral tissue are able to sustain such high growth rate. Since *C. caespitosa* bleached under high temperatures, it is expected that the subsequent energy shortage (Rodriguez and Grottoli, 2007) would have again reduced calcification if the thermal stress was applied over a longer-term period.

One of the major observations of this study is the particular acclimation of *C. caespitosa* to long-term nutrification, since the colonies were close to a fish farm and within a cultivated area. The same can be observed in other parts of the Mediterranean Sea, such as close to the city of La Spezia (North West Mediterranean Sea), where many colonies also thrive next to a fish farm and a river mouth in a very turbid environment (Peirano et al. 2005). High nitrogen supply did not disrupt the symbiotic association between the host and the symbionts, since symbiont density was comparable between high-nutrient and low-nutrient sites. This is in contrast with tropical corals, which usually experience a significant increase in symbiont density with nitrogen enrichment (Tanaka et al. 2014; Ezzat, et al. 2016), that may lead to a disruption of the symbiosis, and a decrease in rates of photosynthesis and calcification (Marubini and Davis, 1996; Marubini and Atkinson, 1999; Wooldridge, 2016). It has to be noticed that *C. caespitosa*, under natural conditions, can afford relatively high symbiont densities, particularly in the North-West Mediterranean Sea, where it can host more than 2 and up to 6 $x10^{-6}$ zooxanthellae cm^{-2} (Rodolfo-Metalpa et al. 2006; Hoogenboom et al. 2010). The lack of nitrogen enhancement of symbiont growth can be partly due to the fact that symbionts are already nutrient-repleted, due to the particular heterotrophic nature of *C. caespitosa*, which mainly feed on planktonic prey throughout the year (Ferrier-Pagès et al. 2011).

In the tropics, chronic nutrient enrichment has direct but also indirect effects on corals (reviewed in D'Angelo and Wiedenmann 2014). It enhances the prevalence and severity of coral disease (Vega Thurber et al. 2013, 2017), leads to imbalance N:P ratios within the coral tissue (Wiedenmann et al. 2013, Rosset et al. 2017), and mostly increase the density and productivity of macroalgae, which in particular overgrow and replace corals (Mc Cook et al. 2001), alter the coral microbial communities and interfere with recruitment of planulae by allelopathic interactions (Morrow et al. 2016; Beatty et al. 2018). However, *C. caespitosa* banks can be observed in Spain in the middle of a high algal coverage of *Dictyopteris polypodioides*, *Halimeda tuna*, *Cystoseira sauvageauana* and *Cystoseira compressa* (Kersting and Linares, 2012), though the algae were never observed overgrowing coral colonies. In addition, *C. caespitosa* were found to exhibit toxic activity, which might explain the low overgrowth of living colony parts by *Caulerpa racemosa*, an invasive algal species found overlapping with coral colonies in NW Mediterranean (Kersting et al. 2013). Finally, the high particle content of the water can also represent an important food source for this heterotrophic coral species and explain its presence in eutrophic environments (Ferrier-Pagès et al. 2011). The same observation was made in tropical areas, where the increased productivity of nutrient enriched waters has benefited corals with a high heterotrophic capacity (Fabricius, 2005; Hoogenboom et al. 2012; Anthony, 1999). In the light of these observations, we suggest that corals with a high success in nutrified environments are likely those with a high heterotrophic capacity.

Another major observation of this study is that high levels of seawater dissolved inorganic nitrogen avoided bleaching of *C. caespitosa* under high temperatures and maintained the physiological functions. Although moderate inorganic nitrogen supply (ca. 1-3 µM) has been shown to promote coral growth and metabolism (Tanaka et al. 2007; Sawall et al. 2011), in particular under elevated pCO_2 (Langdon and Atkinson 2005; Holcomb et al. 2010; Chauvin et al. 2011) or thermal stress (Beraud et al. 2013), other studies on tropical corals have also suggested that elevated inorganic nitrogen levels may impact corals by decreasing their thermal thresholds for bleaching. Nitrogen addition indeed tends to enhance symbiont growth inside the coral host tissue and increase oxidative stress (Nordemar et al. 2003; Wooldridge, 2009; Wooldridge and Done 2009; Wiedenmann et al. 2013; Vega Thurber et al. 2013). To reconcile these two opposite observations, Wiedenmann et al. (2013), as well as some other studies (Ezzat et al. 2016; Rosset et al. 2017) demonstrated that an imbalance N:P ratio was the key factor explaining coral bleaching. A condition where phosphorus is in limited amount while nitrate is fully available indeed promotes coral bleaching (Wiedenmann et al. 2013; Ezzat et al. 2016; Rosset et al. 2017). In this study, while the

seawater N:P ratio was very high and should have induced bleaching in *C. caespitosa*, the contrary was observed. A plausible explanation is that the internal N:P ratio of the coral tissue was not imbalance, due to the high degree of heterotrophy of this coral species (Ferrier-Pagès et al. 2011), which may have provided large amounts of organic phosphorus to the coral. Heterotrophy may have also avoided carbon limitation of the symbionts under high nutrient supply (Wiedenmann et al. 2013; Tremblay et al. 2016). Such carbon limitation has often been reported in coral-dinoflagellate symbiosis (Marubini et al. 2008; Tansik et al. 2017), enhancing bleaching under thermal stress (Tremblay et al. 2016; Krueger et al. 2018; Slavov et al. 2016). Since the physiological traits of the coral host are partly shaped by the dominant symbiont type present within its tissues (Little et al. 2004), we also suggest that the symbionts of *C. caespitosa* have particular adaptation to nutrification and can provide ecological advantages to *C. caespitosa* in high-nutrient conditions. Symbionts in *C. caespitosa* belong predominantly to formerly clade B (now *Breviolum* sp.), which is common in the Mediterranean temperate and subtropical regions (Finney et al. 2010; Rodriguez-Lanetty et al 2001; LaJeunesse et al. 2012) though symbionts belonging to clade A have also been found in *C. caespitosa* from the W Mediterranean (Casado- Amezúa et al. 2014).

Cladocora caespitosa is an emblematic coral of the Mediterranean Sea, and its conservation is an important concern now that its bioconstructions are endangered by the climate change effects (Kersting & Linares, 2012). A better knowledge of its response to environmental stressors is thus needed to further understand how this species can be preserved. This study conclusively demonstrates that the long time scale acclimation to high nutrient levels can reduce the bleaching susceptibility of *C. caespitosa* and has not necessarily adverse effects on its growth. This is maybe due to the high heterotrophic capacities of the coral host, which can maintain a balanced C:N:P ratio within the tissues and counterbalance the nutrient-enhancement of symbiont growth. However, this coral model need more in depth studies to fully understand the different acclimation or adaptation ways to nutrification.

CHAPTER 5:

Insights into the reproductive plasticity of the temperate coral *Cladocora caespitosa*

Insights into the reproductive plasticity of the temperate coral *Cladocora caespitosa*

Abstract

Reproductive strategies, highly important for maintaining viable populations, are greatly affected by environmental parameters that can have a strong influence on successful establishment of organisms. In this study, we assess the sexual condition and reproductive cycle of the Mediterranean endemic scleractinian coral *C. caespitosa* from Cyprus (East Mediterranean) considering some environmental parameters (sea surface temperature and surface primary production) and compare them with the ones from western Mediterranean and Adriatic Sea. We identified colonies of *C. caespitosa* in Cyprus to be gonochoric, with spawning occurring at the end of the summer, much like the ones from western Mediterranean, but in contrast to the ones from the Adriatic, which have been described as hermaphroditic that spawn at the beginning of the summer. We found temperature to be an important driving factor for gamete development and spawning, but no association with primary production has been detected. Further comparative studies from varying latitudinal gradients are needed to explain the plasticity observed, including the continuous monitoring of seawater temperature and light irradiance at the colony depth.

Keywords: Mediterranean, Levantine, scleractinia, gametes, subtropical/temperate corals, SST, primary productivity, anomalies.

Introduction

Variable reproductive traits are used to promote the evolution of corals, such as sexuality (gonochorism vs hermaphrodism) and reproductive modes (brooding vs spawning) as well as the common occurrence of asexual reproduction. Hermaphroditic broadcast spawning is the most common sexual pattern recorded in scleractinian corals, followed by moderately common gonochoric broadcast spawning, while hermaphroditic and gonochoric brooding are relatively uncommon (Harrison, 2011). However, there are examples where some species exhibit changes in sexual condition among populations, such as in the scleractinian coral *Porites asteroides*, classified as hermaphroditic in Puerto Rico (Szmant, 1986) but in Panama recorded to have mixed separate gonochoric and hermaphroditic colonies (Soong, 1991).

The most important environmental parameters that appear to strongly influence the sexual reproductive cycles of corals are water temperature and photoperiod (Glynn et al. 2000; Penland et al. 2004). In addition, sexual reproductive success in corals can be negatively affected by a wide range of natural and anthropogenic disturbances, such as thermal stress (Randall and Szmant, 2009; Yakovleva et al. 2009), ultraviolet radiation (Wellington and Fitt, 2003; Torres et al., 2008), lowered salinity (Vermeij et al., 2006; Humphrey et al., 2008) and increased nutrients (Tomascik and Sanders, 1987; Harrison and Ward, 2001), among others (reviewed in Harrison, 2011).

Seawater temperature change, an important parameter known to control gametogenetic cycles and planulae release in shallow water scleractinian corals (Richmond and Hunter, 1990; Fadlallah, 1996; Baird et al., 2009; Harrison, 2011), has been an extensively studied subject. Sea surface temperatures (SSTs) have been shown to increase globally (by 0.3 ^{o}C to 1.0 ^{o}C) over the last millennium (Salinger, 2005) with increases in the Mediterranean and the Levantine Sea being particularly intense (Perez et al., 2000, Samuel-Rhoads et al., 2013). These anomalies, recognized largely to anthropogenically induced climate change and global warming (Oreskes, 2005), have been documented in the Mediterranean (Bethoux et al., 1990; Vargas-Yanez et al., 2008) and have been shown to affect greatly the coralligenous communities in the marine environment (Cerrano et al., 2000; Rodolfo-Metalpa et al., 2005; 2006; Kersting et al., 2013). This becomes even more important in the light of evidence that increasing temperature decreases the reproductive efficiency of corals by reducing individual fecundity, egg quality, lowered fertilization success and reduced recruitment, through effects on post-fertilization processes (Baird and Marshall, 2002; Linares et al., 2008; Albright and Mason, 2013; Airi et al., 2014).

The colonial zooxanthellate scleractinian coral *C. caespitosa* has a patchy distribution along the basin. Colonies of *C. caespitosa* occur on a wide range of substratum, from 5 to 40m depth in W Mediterranean in differing hydrodynamic environments (Laborel, 1961; Zibrowius, 1980; Schiller, 1993) but, in general, it is rarely found below 30m (Kružic et al., 2008), though in Cyprus they are predominantly found at depths between 1-5m (Hadjioannou, in prep.). In Cyprus, colonies of *C. caespitosa* can be found in a fragmented pattern around the island with very few recorded localities holding relatively large communities. C*ladocora caespitosa* colonies have been described as hermaphroditic in the Adriatic with the release of eggs/sperms (spawning) occurring at the beginning of the summer period (Kružić et al., 2008), whereas in the western Mediterranean Kersting et al. (2013) found colonies (and polyps) to be of distinct sex (gonochoric) with the spawning occurring at the end of the summer period.

Nothing on the reproductive strategy of this species is known from the E Mediterranean, hence, the aim of this study was to describe the reproductive strategy and gametogenic cycle of *C. caespitosa* in the Levantine Sea, and compare with the results previously reported from W Mediterranean and the Adriatic Sea in order to explore potential differences among areas considering the differing environmental parameters.

Materials and Methods

Study site

Kryo Nero, situated on the Southeast corner of Cyprus is a site which hosts >100 colonies of variable sizes (5-170 cm diameter) within an area of ~3500 m^2, at depths of <1-5m and at distances of 1-30m away from the shoreline. In order to identify the reproductive strategy followed by *C. caespitosa* in Cyprus, we first identified, through preliminary histological analyses, the sex of four colonies (2 male, 2 female; 30-100 cm in diameter), showing no signs of recent mortality, from Kryo Nero (Figure 5.1c). The colonies were individually marked for easy identification and sampled by SCUBA on a monthly basis between September 2014 and October 2015. Temperatures were recorded continuously in half-hour intervals for the duration of the study period using a data logger (Starmon mini Star-Oddi-Iceland).

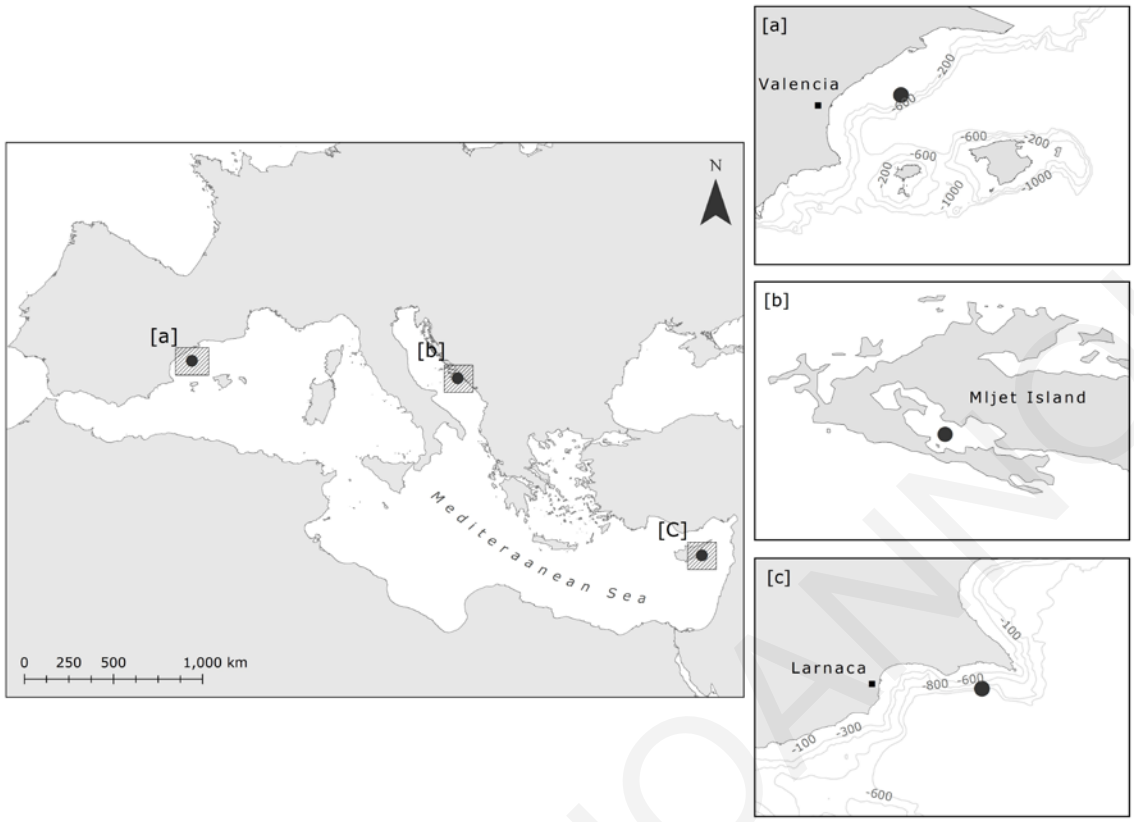

Figure 5.1. Sites where *C. caespitosa* have been studied in the Mediterranean (a) Columbretes Island Marine Reserce, Spain (b) Mljet Island, Croatia and (c) Kryo Nero, Cyprus. Black dots indicate the sampling location for satellite data.

Environmental parameters

One of the main problems encountered when trying to compare environmental parameters between regions is the lack of monitoring stations and buoys that result in both spatial and temporal gaps in important parameters such as sea surface temperature (SST) and chlorophyll (Chl-α) records. The aforementioned monitoring difficulties can be effectively solved by the use of remote sensing, which allows obtaining spatially detailed, regularly and continuously repeated datasets. Satellite infrared (IR) radiometers provide a wide spatial coverage record of SST in near-real time, uniquely resolving existing difficulties in monitoring with traditional field observation methods. The continuous acquisition of satellite data improves the quality of the information extracted from a remotely sensed data set (Kozlov et al. 2014; Casey and Cornillon, 1999; Topouzellis et al. 2015).

Annual and seasonal mean variation of Sea Surface Temperature (SST) and surface primary production (Chl-a) datasets (with accuracy of ± 0.4^{o}C) for Columbretes Islands, Mljet Island and Kryo Nero (Figures 5.5, 5.6) were derived from MODerate-resolution Imaging Spectroradiometer (MODIS) instrument on board both Aqua and Terra sun synchronous

satellites (Feldman and McClain; http://oceancolor.gsfc.nasa.gov/). Anomalies were also calculated from the same dataset for the periods during which polyp collection was obtained. The MODIS/Terra and MODIS/Aqua SST Thermal monthly L3 Global (spatial resolution of 4630m), which configured on a 0.05º latitude/longitude climate modeling grid (CMG) were used. A total of 312 images covering a time frame of a 13-year period (2003 - 2015) were used and all data where grouped to examine the annual mean variation and seasonal variation respectively for both SST and Chl-a, following Georgiou and Akcit, (2016). For the processing, displaying, analysis and quality control of the MODIS data, GIS open source software was used in addition with Python programming language.

Satellite-derived data have been validated with in situ measurements in order to confirm their suitability as a proxy for ambient surface temperature in the Kryo Nero area (Georgiou et al. 2019).

Coral reproduction

Four polyps were collected from each of the colonies sampled each month. By the end of the experiment, we had collected 56 polyps from each of the four colonies. The polyps extracted per month were immersed immediately in Helly's fixative for 12-16 hours (Peters et al. 2005), decalcified in HCl (37%), dehydrated through a graded alcohol series and transversally dissected before embedded in paraffin. The height and width of each polyp was measured with the use of a stereoscope equipped with a micrometer before dissection. Polyps were then cross-sectioned to 5 μm thick, and cross-sections (with 20 μm distance between) from each polyp were then stained with haematoxylin-eosin before examined under a light microscope for identification of gametes.

In order to estimate potential fecundity, we counted the number of all oocytes and spermaries identified from 31 male and 24 female polyps in 5 cross-sections (5 μm width) each separated by 20 μm. We measured also the dimensions (minimum and maximum diameter) of oocytes, in a maximum of 20 oocytes per polyp, considering only the oocytes where the nucleoulus was visible in order to avoid repeat counting. Spermary dimensions were not recorded due to the impossibility of measuring it accurately.

We used Pearson's product moment correlation coefficient to measure the strength of linear association between oocyte size (diameter) and seawater temperature, as well as between polyp size (diameter) and number of gametes at the study site, using PAST statistical

package (Hammer et al. 2001). We also used one-way ANOVA to compare between male and female polyp height (H) and width (W).

Results

Sexual condition and reproduction cycle

All polyps examined in each colony were found to be of the same sex (Figure 5.2); therefore, we conclude that colonies of *C. caespitosa* from E Mediterranean are gonochoric.

We found oocyte development to begin during springtime, as early as April in some female colonies, whereas spermary development appears to begin later, at the beginning of the summer period (July). From all polyps analyzed during the reproductive months, 25% (9/36) of female polyps and 40% (10/25) of male polyps did not contain any gonads. In male polyps, the highest mean number of spermaries per polyp detected in the mesenteries, within 105 μm of width, was observed in September 2014 (Figure 5.3A; n=5 slides/6 polyps; 359 ± 12 SD). A much smaller number was observed in August 2015, though only from one polyp, (Figure 5.3A; n=5 slides/1 polyp; 108) and in September 2015 (Figure 5.3A; n=5 slides/7 polyps; 170 ± 55 SD). A very small amount of spermaries was also observed in one polyp from October 2015 (Figure 5.3A; n=5 slides/1 polyp; 7). As mentioned above, male gametes were observed also in polyps collected before August 2015, but gonads were not counted due to low image quality. In female polyps, the lowest numbers detected in the mesenteries were from June 2015 (Figure 5.3B; n=5 slides/4 polyps; 13 ± 0.5) and followed an increasing pattern through the months to reach the highest in September 2015 (Figure 5.3B; n=5 slides/7 polyps; 33 ± 18). Similar counts were also observed in September 2014 (Figure 5.3B; n=5 slides/6 polyps; 25 ± 2).

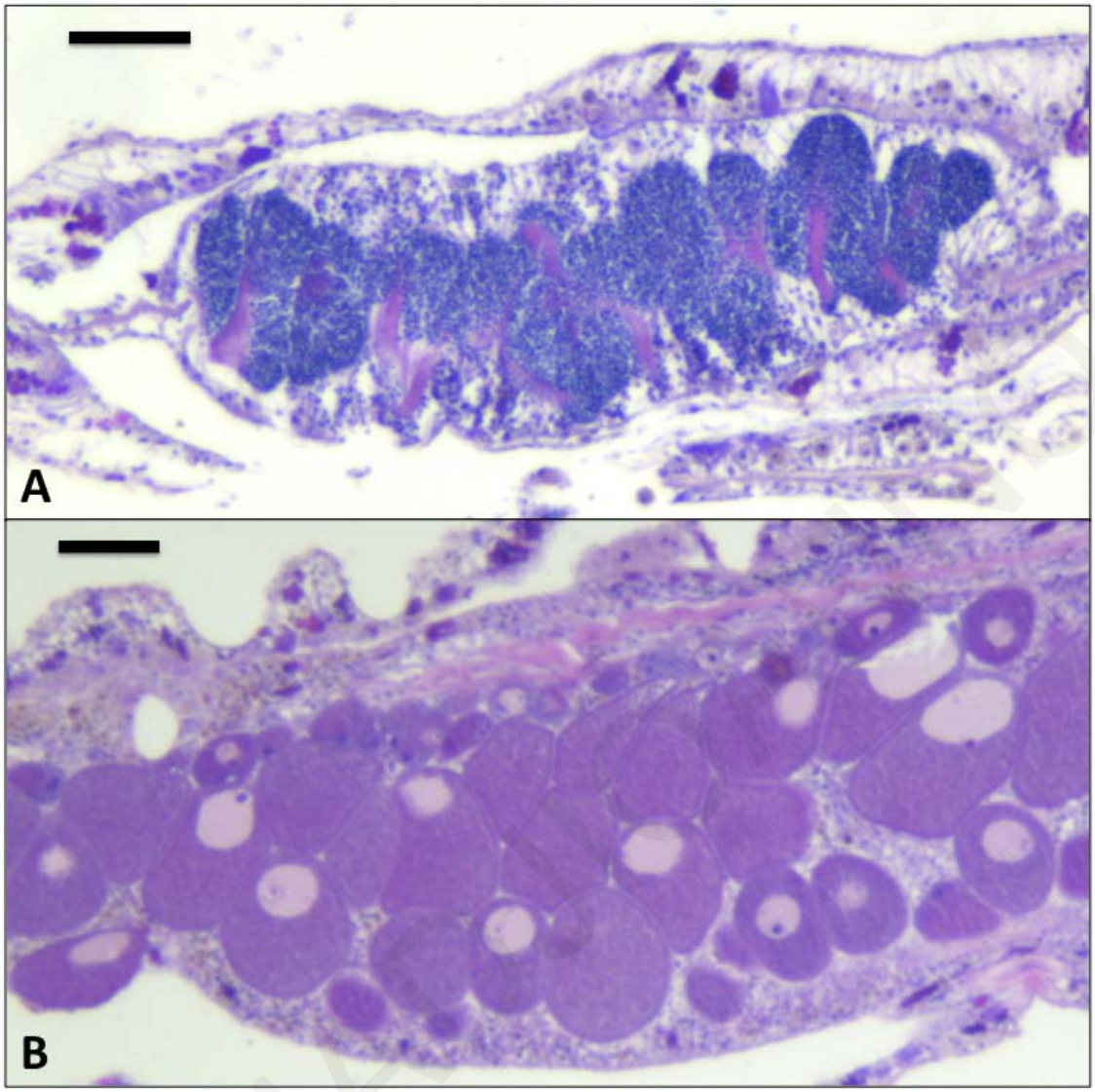

Figure 5.2. Gonads of *C. caespitosa* from Kryo Nero (Cyprus). (A) Male mesentery packed with spermaries filled with spermatozoa, the tails of the spermatozoa are well visible (pink) indicating an advanced maturity stage (September 2014). (B) Female mesentery packed with oocytes containing an oval nucleus and a spherical nucleolus (August 2015). Scale bar 50 μm.

Female gonads were observed in polyps before June 2015 but were not counted due to low image quality, hence they were also not included in the graph. Based on the observed pattern of gradual increase in gonad numbers, through the months, and subsequent lack of them, we conclude that spawning in *C. caespitosa* in Cyprus occurs at the end of the summer, beginning of autumn (September-October). A similar increasing pattern was observed in the mean oocyte sizes (diameter), found to be smallest in April 2015 (Figure 5.3C; 10 ± 4 μm), and gradually increased through the summer months to reach maximum size in September 2015 (Figure 5.3C; 103 ± 15 μm). Similar sizes were observed in September 2014 (Figure 5.3C; 109 ± 18 μm).

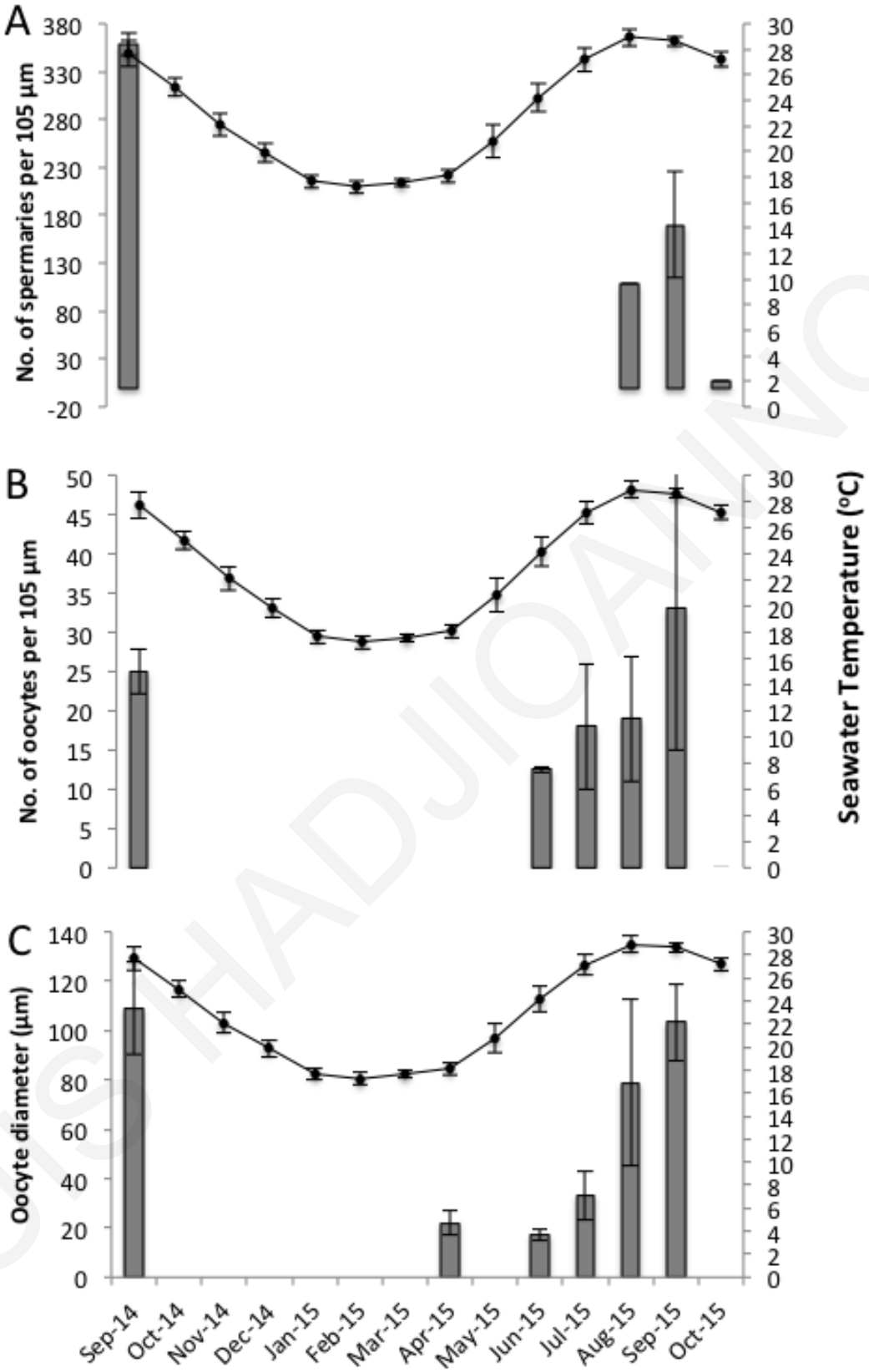

Figure 5.3. *Cladocora caespitosa* from Cyprus. (A) Number of spermaries per 105 µm, (B) number of oocytes per 105 µm, (C) oocyte diameter and seawater temperature in Kryo Nero. Oocyte and spermaries are shown in bars (monthly average ± SD), and SWT is shown as smoothed line (monthly average ± SD).

Environmental parameters

With regards to the monthly average temperatures recorded in situ, the lowest temperature recorded in Kryo Nero was in February 2015 (Figure 5.3; 17.2 ± 0.5 ^{o}C), gradually increasing to reach the highest average temperatures in August 2015 (Figure 5.3; 28.9 ± 0.7 ^{o}C) and September 2015 (Figure 5.3; 28.6 ± 0.4 ^{o}C).

Pearson's correlation coefficient showed a strong correlation between oocyte development (in terms of diameter) and seawater temperature (Figure 5.3; $r = 0.83$, $p<0.01$), but showed no correlation between gamete development (in terms of numbers counted) and polyp dimensions (height and width). Also, polyp biometric measurements showed no significant differences (one-way ANOVA; $p>0.05$) between average size dimensions of male polyps (H: 8.60 ± 0.91 mm, W: 7.48 ± 1.03 mm) and female polyps (H: 8.65 ± 0.97 mm, W: 7.23 ± 0.44 mm).

Annual and seasonal mean variation of sea surface temperature (SST) during the year of sampling *Cladocora caespitosa* shows roughly a similar seasonal pattern between the three regions (Figure 5.4). However, the warmest and coldest periods of the year - summer and winter seasons - are similar but not identical between the three regions. For Columbretes and Kryo Nero, summer months were defined as July, August and September, while the winter months as January, February and March. For Mljet Island, summer months were defined as June, July and August, whereas winter months as December, January and February. Summer months at all regions are characterized by high or higher than average temperatures (Figure 5.5) whereas conversely they showed low average Chl-a (Figure 5.6).

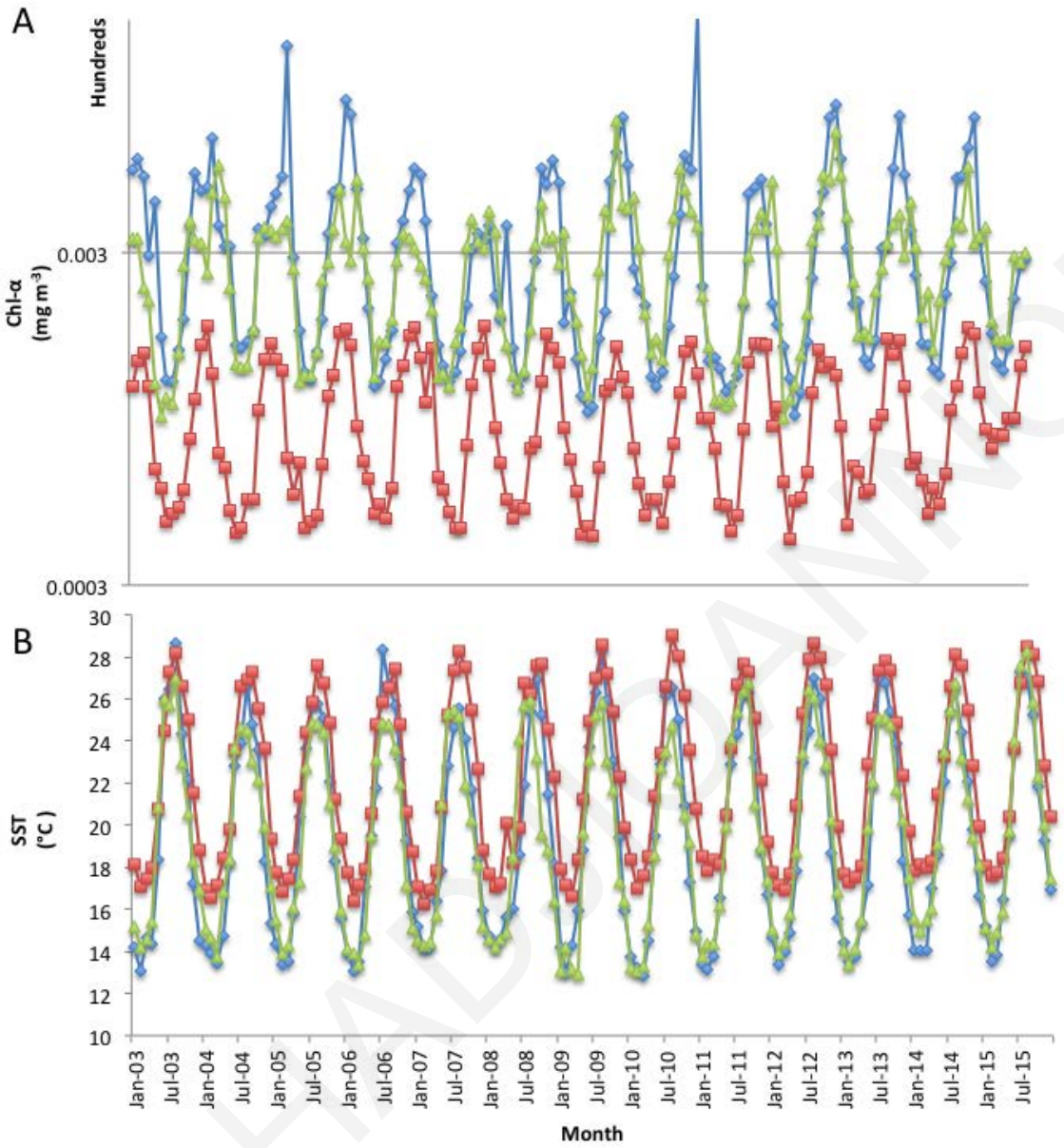

Figure 5.4. Mean monthly surface (A) primary production (Chl-α) and (B) sea surface temperature (SST) at Kryo Nero (red dots), Columbretes Islands Marine Reserve (blue dots) and Mljet Island (green dots), between January 2003 and July 2015.

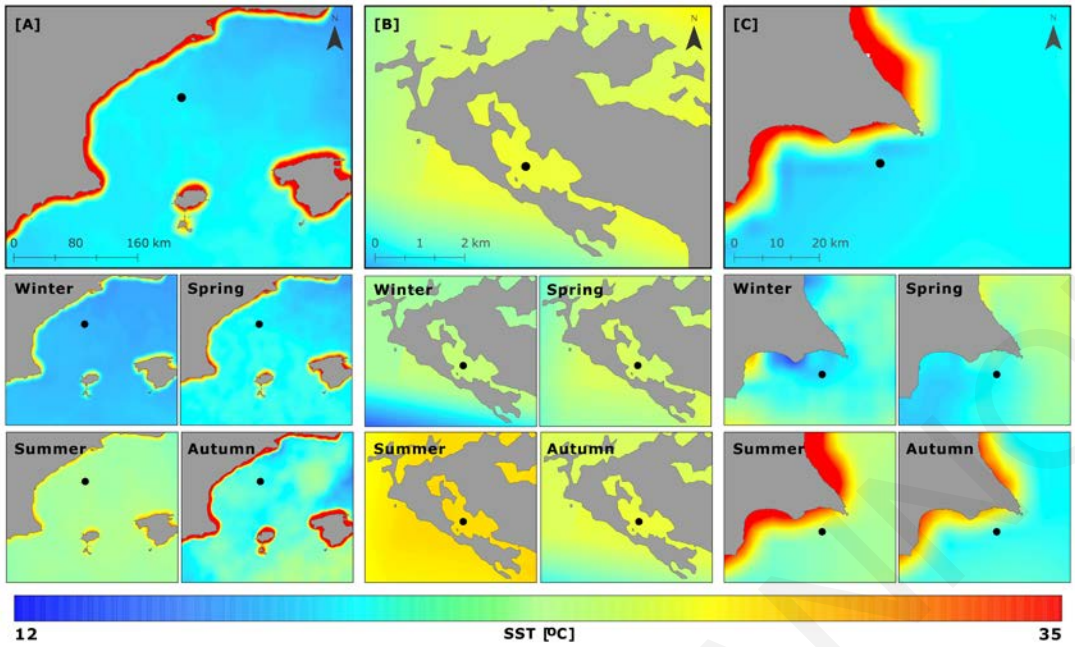

Figure 5.5. Annual mean and seasonal variation of sea surface temperature (SST) during the year of sampling in the region around (A) Columbretes Island Marine Reserve (April 2008 - July 2009), (B) Mljet Island (January 2005 - January 2006) and (C) Kryo Nero (September 2014-October 2015) (MODIS-Aqua sensor data from the NASA Goddard Space Flight Center, Ocean Biology Processing Group, Feldman and McClain; https://oceancolor.gsfc.nasa.gov/cms/). Black dots indicate the sampling location for satellite data.

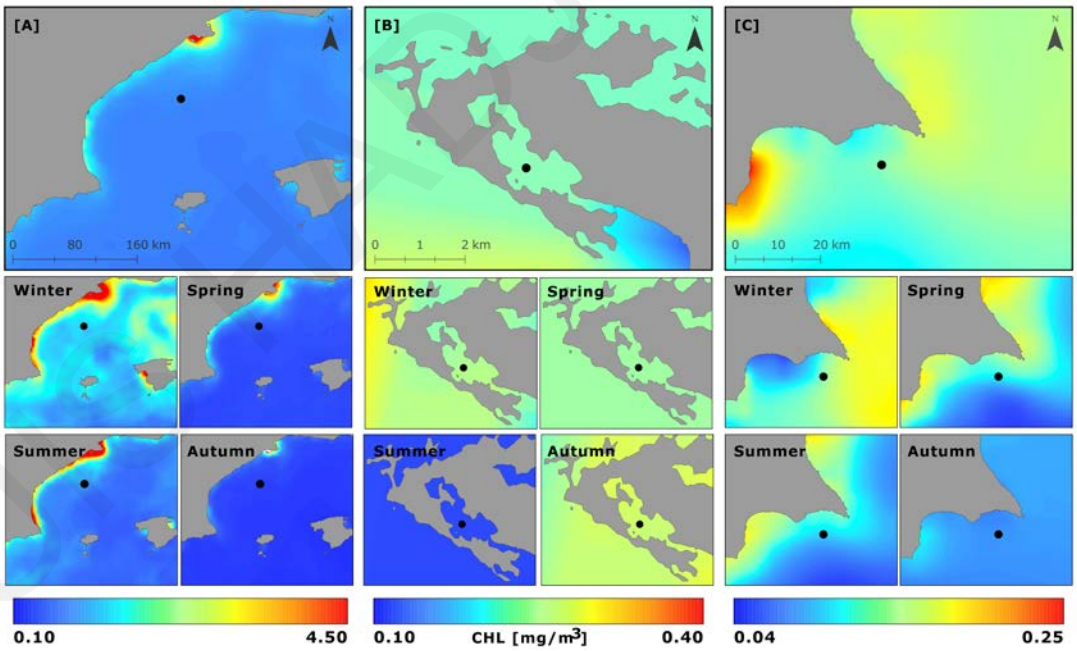

Figure 5.6. Annual mean and seasonal variation of surface primary production (Chl-α) during the year of sampling in the region around (A) Columbretes Island Marine Reserve (April 2008-July 2009), (B) Mljet Island (January 2005-January 2006) and (C) Kryo Nero (September 2014-October 2015) (MODIS-Aqua sensor data from the NASA Goddard Space Flight Center, Ocean Biology Processing Group, Feldman and McClain; https://oceancolor.gsfc.nasa.gov/cms/). Black dots indicate the sampling location for satellite data.

The data obtained from MODIS were in agreement with our in-situ data and can be used for discussing temporal patters and interannual variability (Georgiou et al. in prep.). The highest annual mean was found at Kryo Nero (Figure 5.5C; 22.99 ± 4.22 ^{o}C) and the lowest at Mljet Island (Figure 5.5B; 18.77 ± 4.28 ^{o}C). At Columbretes Islands, the annual mean was found to be 19.72 ± 4.73 ^{o}C (Figure 5.5A). The mean temperature at Columbretes Islands during summer was 24.74 ± 2.55 ^{o}C (Figure 5.5A) and in winter was 13.81 ± 0.74 ^{o}C (Figure 5.5A), the lowest of all regions. The mean temperature at Mljet Island in summer was 24.70 ± 0.24 ^{o}C (Figure 5.5B) and in winter was 14.55 ± 0.84 ^{o}C (Figure 5.5B). At Kryo Nero, the highest mean temperature from all regions in summer was 27.78 ± 0.92 ^{o}C (Figure 5.5C) and in winter was 17.80 ± 0.21 ^{o}C (Figure 5.5C), much higher than the lowest found in other regions.

The seasonal context of SST variability is better represented by computing temperature anomalies for each studied areas, making it easier to highlight and recognize the magnitude of the highs and lows. At Columbretes, the colder than average period was from April to August 2008 while the warmer than average was between September and December 2008 (Figure 5.7A). The year 2009 did not deviate much from the average. At Mljet, the colder than average period was from May to August 2005 and the warmer in January 2005 with minor to no deviations recorded in the rest of the months (Figure 5.7C). At Kryo Nero, the colder than average period was from May to June 2015 and the warmest from August to October 2015 (Figure 5.7E).

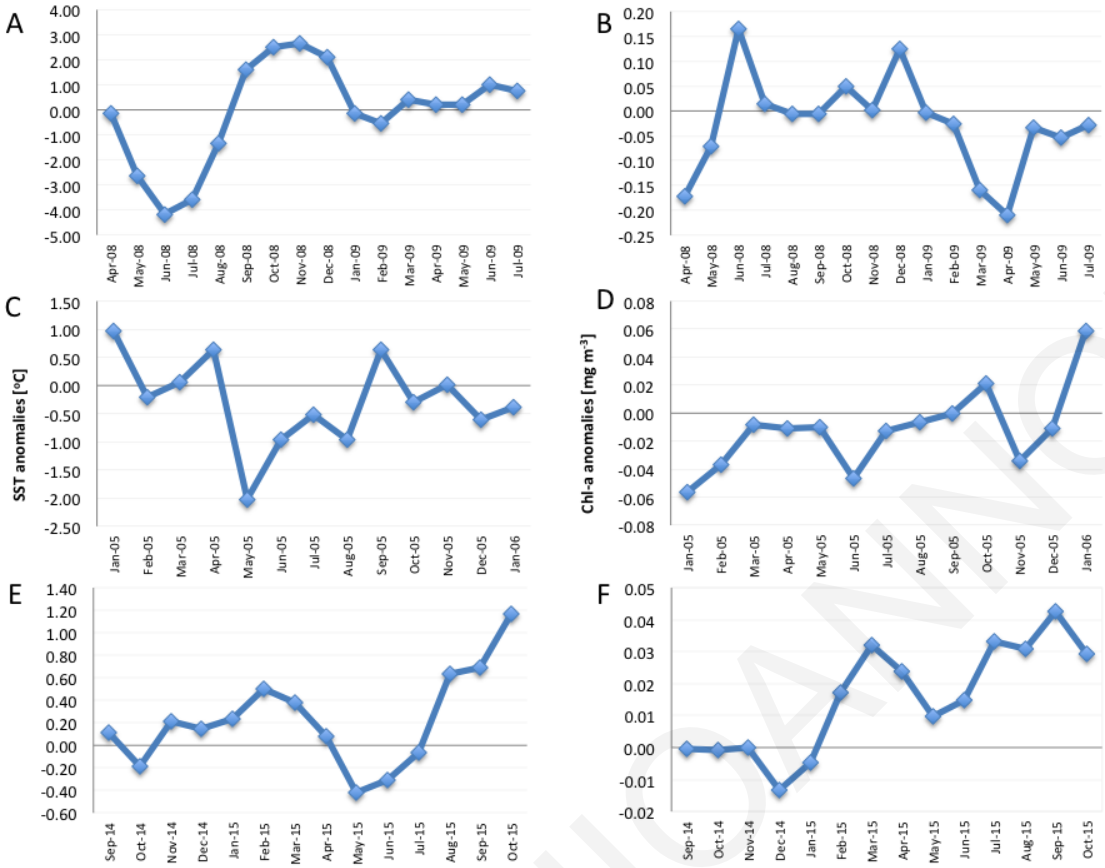

Figure 5.7. Monthly mean sea surface temperature (SST) and Chl-α anomalies off (A, B) Columbretes Island Marine Reserve (April 2008 – July 2009), (C, D) Mljet Island (January 2005 – January 2006) and (E, F) Kryo Nero (September 2014-October 2015).

Annual and seasonal mean variation of surface primary production (Chl-α) during the year of sampling *Cladocora caespitosa* also varied between the three regions. The highest Chl α concentrations at Columbretes Islands were found during winter (Figure 5.6A; 0.51 ± 0.05 mg/m^3); the highest from all regions, and the lowest in summer (Figure 5.6A; 0.13 ±0.02 mg/m^3). The highest Chl α concentrations at Mljet Island were found similarly during winter (Figure 5.6B; 0.35 ± 0.01 mg/m^3) and the lowest during summer (Figure 5.6B; 0.14 ± 0.01 mg/m^3). At Kryo Nero, the highest Chl α concentrations were found also in winter (Figure 5.6C; 0.17 ± 0.01 mg/m^3) and the lowest in summer (Figure 5.6C; 0.089 ± 0.01 mg/m^3), both much lower than the other regions.

At Columbretes, the lowest than average periods of Chl-a concentrations were in April 2008 and March-April 2009, while the highest was in June 2008 (Figure 5.7B). At Mljet, the lowest period was in January 2005 and the highest in January 2006 (Figure 5.7D). At Kryo Nero, the lowest period was December 2014 and the highest between July to October 2015 (Figure 5.7F).

Discussion

Our results show that the sexual reproductive strategy followed by *C. caespitosa* in Cyprus (gonochoric) is similar to the one found in *C. caespitosa* in the W Mediterranean (Kersting et al. 2013) but different to the one followed by *C. caespitosa* in the Adriatic where they were identified to be hermaphroditic with polyps within a colony having both male and female gonads, found in separate mesenteries (Kružić et al. 2008). Spawning in Cyprus was not observed directly during the course of our study, however, based on the fact that both maturation and decrease of spermatic cysts and oocytes inside the mesenteries was more or less synchronized, we conclude that it occurs at the end of the summer period beginning of autumn (September/October) when in-situ temperatures peaked and started to slightly decline (Figure 5.3). This also comes into agreement with the results from *C. caespitosa* in W Mediterranean, which were also found to spawn at the end of the summer period, though with one month difference, coinciding with temperatures in W Mediterranean that reached highest in August and started declining in September (Kersting et al. 2013) (Figure 5.4). On the contrary, a different pattern was observed to take place in the Adriatic, with spawning occurring at the beginning of summer, in June, one month prior to the peak of the sea temperature (Schiller, 1993; Kružić et al. 2008). Several studies have shown that reproductive traits can vary with latitude and geographic location (Rinkevich & Loya, 1979; Kojis, 1986; Baird et al. 2009) especially considering the fact that seasonal changes in sea-water temperatures are an important environmental factor in controlling gametogenetic cycles and planulae release in scleractinian corals (Richmond and Hunter, 1990; Fadlallah, 1996; Harrison, 2011). Crowder et al. (2014) argued that this could demonstrate that some corals display a reproductive plasticity in the timing of gamete release, an important adaptive mechanism for larval survival and fitness with rising ocean temperatures associated with climate change. The differences in the timing of spawning of *C. caespitosa* from different regions in the Mediterranean come in agreement with this argument. However, warmer than average conditions (positive SST anomalies) were recorded during the assumed spawning period in the W Mediterranean and E Mediterranean, whereas colder than average conditions (negative anomalies) were recorded in the Adriatic during the observed spawning, which might have had an effect on the precise timing of spawning. With regards to primary production (Chl-α), spawning coincided with low concentrations in all three regions, though contracting conditions (positive anomalies) were recorded in both W and E Mediterranean, more eutrophic and less oligotrophic, respectively, while less eutrophic (negative anomalies) were recorded in the Adriatic. It is important thus to characterize the sampling periods in

relation to the long-term environmental conditions.

Sexuality of scleractinians tends to be phylogenetically correlated and constant within genera and families (Harrison, 1985; Shlesinger et al. 1998; Fautin 2002; see Airi et al. 2016 for a review), nevertheless, there are plenty of examples where intraspecific diversity in reproductive strategies (mixed or contrasting sexual patterns) has been recorded in different regions (see examples reviewed in Harrison, 2011). In the Mediterranean, this has been observed in the azooxanthellate colonial *Astroides calycularis*, described as hermaphroditic in Algeria (Lacaze-Duthiers, 1873) and in Naples (Cirino et al. 1993) but conversely found to be gonochoric in the Southern Tyrrhenian Sea (Goffredo et al. 2010) and the Northern Alboran Sea (Casado-Amezua et al. 2013). The different sexuality expressed in *A. calycularis* has been attributed to the fact that gonochorism would be advantageous by ensuring cross-fertilization in high population density areas by maintaining the genetic variability of the population (Goffredo et al. 2010). This explanation however can only be rejected in the case of *C. caespitosa,* which follows a philopatric dispersion strategy (Kružić et al. 2007), since population density in the sample site in Cyprus (and possibly the Columbretes also) is much lower than the one found in Mljet Island.

The monthly maximal mean oocyte size found in this study (109 ± 18 μm) was slightly higher than the one recorded from W Mediterranean (88 ± 23 μm) by Kersting et al. (2013), but much smaller than the spawned egg diameter recorded at Mljet (416 ±73 μm) by Kružić et al. (2008). Previous studies from Indopacific corals, such as *Pocillopora verrucosa* (Sier and Olive, 1994) and *Echinopora lamellosa* (Fan and Dai, 1999) found larger egg sizes at higher latitudes compared to tropical populations, a behavior that suggests increased energy investment towards larval survivorship as a response to the unfavorable environmental conditions at higher latitudes (Santacruz-Castro, 2019). This comes in disagreement with our results where *C. caespitosa* eggs from lower, subtropical latitudes (Cyprus) were found larger than the ones from higher, temperate latitudes (Columbretes), though probably not significantly larger. However, Santacruz-Castro (2019) found larger eggs in the reef-building coral *Acropora hyacinthus* in lower latitudes compared to the ones from higher latitudes. Octocorals are also known to present smaller oocyte sizes at higher latitudes compared to tropical locations, attributed to differences in the maternal effects at different latitudes (Tsounis et al. 2006; Huang and Song, 2012). The differences between oocyte sizes in *C. caespitosa* could be explained by a trade-off between gonad size and egg number, as has been reported in other aquatic animals (e.g. fish; Fleming and Gross, 1990), however, we cannot know if this is the case for *C. caespitosa* since the number of oocytes in this study

was not counted for the whole polyp, hence a comparison with the results from Kersting et al (2013) is not possible. Furthermore, the mean diameter of spawned oocytes of *C. caespitosa* from the Adriatic Sea described by Kružić et al. (2008) was 416 ±74 μm, a much higher value than the one reported from W and E Mediterranean. Small changes in oocyte size have been reported in other scleractinian corals (Shlesinger et al. 1998), however, the astonishing difference in size between Mljet and the other sites is something that to our knowledge has not been reported before and should be examined further. Further comparative studies in the larval development mode of *C. caespitosa* from varying latitudinal gradients is needed to explain the reproductive plasticity observed, including the monitoring of sea water temperature and light irradiance at the colony depth.

Research on *C. caespitosa* from the past decade has shown that they are threatened by increasing disturbances affecting coastal ecosystems, such as elevated seawater temperatures, invasive species, and outbreaks of corallivorous species (Rodolfo- Metalpa et al, 2005, 2006; Kružić et al. 2008, 2013; Kersting et al. 2013, 2014; Jimenez et al. 2016; Rilov 2016). Even though *C. caespitosa* is a conspicuous species, large bioconstructions of this coral are very rare at the present time, and most of the existing *C. caespitosa* populations are built up of small, disperse colonies. A rising trend of SSTs higher than average anomalies in the Levantine Sea has been reported by Samuel-Rhoads et al. (2013). The trend, which started in the late 1990s, along with evidence of ecological disturbance (Jimenez et al. 2016; Rilov 2016), should act as an alarming call for the future as it can cause considerable changes on a regional and global scale, affecting the reproductive traits of organisms influenced directly by this parameter. Furthermore, environmental parameters such as temperature, salinity, solar radiation and primary productivity also differ substantially between the Levantine Sea and other regions in the Mediterranean. Hence, variation in these parameters should be taken into consideration when comparing same species populations along different geographical gradients in the region.

Based on our findings and the results from research done in other parts of the Mediterranean (Kružić et al, 2008; Kersting et al. 2013) we postulate that temperature is an important driving factor for gametal development and spawning whereas primary productivity is probably not. Further research is required to reach solid conclusions on the matter. In addition, taking into consideration the interannual variability evidenced by SST anomalies during the study periods, in all three regions, further research is also required to verify the differences in spawning periods. Furthermore, by experiencing successful reproduction, corals can allow the addition of new individuals to existing populations while increasing

dispersal potential (Kinlan et al. 2005), which in turn can result in colonization of new areas (Fiorillo et al. 2013). This information is extremely important and has been proven to be highly significant for the recovery of populations of endangered species, damaged by natural or human disturbances, like in the case of *Cladocora caespitosa* in the W Mediterranean (Kersting et al. 2014). Comprehending the strategies followed will enable us to make vital decisions for management and conservation planning.

Chapter 6: Conclusions

This thesis covered aspects of the biology and ecology of *C. caespitosa* in Cyprus, a Mediterranean endemic scleractinian coral exposed to multiple threats. These include: (i) the description and characterization of coral populations at two differing sites, (ii) the effects of thermal stress from warming events, as a result of increasing trends evident in the past decades, (iii) the effects of an extreme windstorm and (iv) the effects of elevated nutrients resulting from human activities. The general conclusions are listed below:

1) Colonies of *C. caespitosa* in Cyprus are found attached to rocky substrates predominantly at depths of 1-4 m, contrary to the W Mediterranean and the Adriatic Sea where colonies are found deeper (5-40 m).
2) The highest coral cover is found at 3-4 m depth with the highest density being 2 colonies per m^2. The highest density in Cyprus is much less than the highest densities identified at study sites in other parts of the Mediterranean (~6 per m^2). In addition, coral colonies in Cyprus are larger on average and flatter in shape, than the ones from other parts of the Mediterranean, presumably due to different hydrodynamic regimes. The majority of colonies in Cyprus fall into the class of 20-30 cm in diameter, larger than the majority of colonies in W Mediterranean and Adriatic.
3) A gradual mortality event was recorded during a period of prolonged SST anomalies in the summer of 2015. The event affected 100% of monitored colonies at Liopetri (~17% loss of pigmented tissue) and 45% at Kryo Nero (~11% loss of pigmented tissue). Necrosis of pigmented tissue on coral colonies was also recorded in the summer of 2014, but much less than 2015, due to lower SST anomalies. The higher mortality percentage recorded at Liopetri was attributed mainly to the elevated nutrient concentrations.
4) An extreme windstorm, which took place in the winter months of 2015, generated waves that hit the cliffs above the colonies, forcing boulders to collapse on top of them, causing a loss of 50% of pigmented tissue in 7% of the colonies at Kryo Nero.
5) The growth rate of *C. caespitosa* in Cyprus, measured through the alizarin staining technique, was found to be ~2.9 mm/yr, similar to the growth rate in the W Mediterranean. However, common garden experiments showed that corals collected from the low-nutrient site and transplanted in the high-nutrient had, at least in the short term, much larger growth rates (6.2 mm per year) assumingly as a result of elevated nutrient conditions.

6) Through experiments in aquaria we found that *C. caespitosa* colonies exposed to temperature increases and grown under nutrient-poor conditions bleach and significantly decrease their protein content and rates of net photosynthesis. On the contrary, colonies grown under nutrient enrichment present no sign of bleaching and no change in their overall metabolism, showing that nutrient history can influence the response of scleractinian corals to thermal stress. The results from these experiments come into disagreement with our postulations from Chapter 3, where we attributed the higher mortality percentage recorded at Liopetri to the elevated nutrient concentrations. However, during the summer period of 2015, the maximum temperature recorded was higher (29.85 ^{o}C) than the maximum temperature of the experiments in chapter 4 (29 ^{o}C). This temperature difference, in addition to the prolonged high temperatures (>28 ^{o}C for 2 months) that coincided with the higher mortality in Liopetri, could potentially have had a different effect on corals. Moreover, additional factors such as diseases, competition and outbreaks of corallivore populations could work synergistically with temperature increases in situ.
7) We identified colonies of *C. caespitosa* in Cyprus to be gonochoric, with spawning occurring at the end of the summer, much like the ones from W Mediterranean, but in contrast to the ones from the Adriatic, which have been described as hermaphroditic that spawn at the beginning of the Summer. We found temperature to be an important driving factor for gamete development and spawning, but no association with primary production was detected.

We propose that continuous and systematic monitoring should be conducted on *C. caespitosa* colonies covering all regions in the Mediterranean, in order to identify the long-term effects of extreme climatic events and anthropogenic intrusions such as elevating nutrient concentrations. The results will be integral to the identification of solutions and directions towards conservation.

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