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Research Article

Maltese Microalgae and Global Climate Variability

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Abstract. The biodiversity of cyanobacteria and microalgae growing in terrestrial and marine habitats around the Maltese islands is currently being investigated, as limited knowledge exists about the phototrophic microorganisms inhabiting this geographical area. New strains of the genera *Oculatella*, *Albertania*, *Nodosilinea*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Calothrix* and *Jenufa* have been recorded recently. Since the phototrophic microbial biodiversity is currently largely unknown, there is an imminent risk of undescribed microorganisms being lost as a result of changes in microbial community structures due to global climate variability (GCV). We describe a six-month experiment to assess the effects of GCV on two Maltese microorganisms, the filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402. Each strain was cultured under environmental conditions associated with GCV; a temperature (T) of 26 °C, enhanced ultraviolet radiation (UVR) and an increased CO_2 concentration. Elevated T stimulated growth and biomass accumulation of *Nostoc* AD0303, whereas growth of *Jenufa* AD0402 was partially inhibited. Increased UVR had the most prominent effect on cellular morphology. *Nostoc* AD0303 presented as aggregated filaments, whereas *Jenufa* AD0402 exhibited thicker cell walls. These UV-protecting mechanisms allowed both strains to accumulate biomass at a significantly higher rate than the control. An increase in CO_2 concentration resulted in inhibition of growth in *Jenufa* AD0402 and bleaching of filaments in *Nostoc* AD0303, both leading to culture death. A lower CO_2 concentration and re-introduction of air subsequently allowed *Jenufa* AD0402 to grow. So far, this demonstrates that the effects imposed by climate variability are strain-specific, making changes at an ecosystem level difficult to predict.

Keywords: cyanobacteria, microalgae, climate change, *Nostoc*, *Jenufa*

1 Introduction

Global climate variability (GCV) has affected organisms throughout evolutionary history. Currently, however, advocates in the scientific community argue that there is a rapid pace of change (Bradshaw, 2006). This rapid change in climatic parameters on a global scale could be accounted for by anthropogenic greenhouse gas emissions. An increase in temperature (T) has resulted, particularly, but not exclusively, due to CO_2 emitted from fossil fuel combustion (Montzka, Dlugokencky & Butler, 2011). Global levels of CO_2 reached an average concentration of 406.00 ppm in October 2018 (NOAA, 2018). Foster and colleagues suggest that the CO_2 level will soon reach its highest in at least 50 million years; at around 600 ppm by 2050 (Foster, Royer & Lunt, 2017). Different climatic models have predicted that this value will continue to rise and may even double by the year 2100. Ozone layer depletion, through the past use of chlorofluorocarbons, is another significant consequence, and this is expected to expose the Earth's surface to higher levels of ultraviolet radiation (UVR) (Sivasakthivel & Reddy, 2011).

The latest report from the Intergovernmental Panel on Climate Change discusses the consequences of climate change that could be avoided by limiting global warming to 1.5 °C, rather than 2 °C or more (IPCC, 2018). The aim is to reduce challenging impacts on ecosystems, human health and global well-being.

To date, most research studies focusing on the effects of GCV on ecosystems and biodiversity have been concerned with higher animals and plants (Parmesan,

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2006). Research studies conducted on the effects of GCV on microorganisms have been few in comparison, and most have focused on bloom-forming cyanobacteria and their toxicity to humans and other animals (Paerl & Paul, 2012; Hense, Meier & Sonntag, 2013; Moe, Haande & Couture, 2016). On the other hand, research into the effects of GCV on non-pathogenic strains of eukaryotic microalgae and cyanobacteria is generally lacking. Since autotrophic microorganisms are important primary producers in both marine and freshwater ecosystems, a change in their populations may cause a cascading effect throughout the biocoenosis (Gragnani, Scheffer & Rinaldi, 1999).

Most of the research into the effects of elevated temperature, CO_2 and UVR has been concerned with marine and freshwater cyanobacteria and microalgae (Karlberg & Wulff, 2012; Lürling, Mello, van Oosterhout, de Senerpont Domis & Marinho, 2018; Visser et al., 2016). Studies about the ecophysiology of terrestrial cyanobacteria and microalgae such as the *Nostoc* and *Jenufa* strains applied in this research study have been mostly overlooked. The effect of changes in environmental parameters on terrestrial organisms may be different to that observed in aquatic organisms. Exposure may be higher in terrestrial microalgae and these may be susceptible to desiccation, which alters their growth and photosynthetic rates (Häubner, Schumann & Karsten, 2006).

The main objectives of this research were to investigate the biodiversity of cyanobacteria and microalgae in the Maltese islands, which is severely understudied at present, and to monitor the effects of GCV on two strains isolated from microalgal biofilms. These included a filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402. These were subjected to future environmental conditions related to climate variability within a laboratory setting, i.e. an increase in T, UVR and CO_2 concentration.

The cyanobacterial strain selected for this study is morphologically classified within the genus *Nostoc* Vaucher ex Bornet & Flahault, 1886 (Nostocaceae, Nostocales). This strain of *Nostoc* forms a very thick subaerial biofilm in the entrance chambers of hypogea (Zammit, Billi, Shubert, Kaštovský & Albertano, 2011; Zammit, Sánchez-Moral & Albertano, 2011). Morphologically, colonies of *Nostoc* consist of trichomes surrounded by an extracellular sheath or a gelatinous matrix. Heterocytes may be present within the trichome, however this is dependent on the amount of nitrogen available (M. D. Guiry & Guiry, 2018).

The microalga chosen for this study belongs to the genus *Jenufa* Němcová, M. Eliáš, Škaloud et Neustupa and was described in 2011 as part of the Chlorophyceae (incertae sedis). *Jenufa* strains form subaerial biofilms on stone surfaces in humid environments (Zammit, Billi, Shubert et al., 2011; Zammit, Sánchez-Moral & Albertano, 2011). Morphologically, this is a coccal microalga with roughly spherical or ovoid cells and a lobed chloroplast.

Morphological features were noted at the beginning of the study since changes in morphology brought about by T, CO_2 and UVR have been observed in other species of cyanobacteria and microalgae (Giordanino, Strauch, Villafañe & Helblung, 2011).

2 Materials and Methods

2.1 Sampling of Microbial Communities

Phototrophic biofilms and microalgal biomats were sampled from different calcareous substrates by non-invasive sampling methods which employed different techniques to remove the biofilm but not the underlying substrate (Zammit, De Leo, Albertano & Urzì, 2008). Biofilm communities were sampled from various microhabitats in hypogea and along rocky shores, such as smooth or rough rock surfaces and crevices. In littoral zones, biofilms in rock pools were either submerged in seawater or located in the splash zone. The diversity of microorganisms within these biofilms and biomats was observed by light microscopy as described below.

2.2 Microscopy

The morphological features of the microorganisms present in the microbial communities and those of isolated strains were examined by light microscopy. Temporary slides were prepared for biofilm and biomat samples and these were observed via a Nikon Eclipse Ti-S inverted microscope at a 200x and 400x magnification. Light micrographs were taken using a Nikon camera via NIS-Elements microscope imaging software. Cultured strains were observed using an Olympus BX-51 microscope equipped with DIC and a DP73 camera at a 1000x magnification. Identification was based on reference books for microalgae, cyanobacteria and diatoms (John, Whitton & Brook, 2002; Komárek & Anagnostidis, 1998, 2005; Komárek, 2013; Ettl & Gärtner, 2014; Škaloud, Rindi, Boedeker & Leliaert, 2018).

2.3 Culture Conditions

Cyanobacterial biofilms were grown on BG-11 medium (Rippka, Deruelles, Waterbury, Herdman & Stanier, 1979) and microalgal mats on Bold's Basal Medium (BBM) (Nichols & Bold, 1965), f/2 (Guillard, 1975) and SN (Waterbury, Watson, Valois & Franks, 1986) media. Individual strains were isolated by subsequent transfer onto fresh media. All isolated microorganisms were added to the Maltese Microalgal Culture Collection (MMCC) (Zammit, 2016).

The two strains chosen for the initial experiment related to climate variability were the cyanobacterium *Nostoc* AD0303 and the microalga *Jenufa* AD0402, strains of which have been found to be ubiquitous in terrestrial habitats. An isolated colony of *Jenufa* AD0402 from solid nutrient medium was inoculated into 100 mL of liquid BBM and an isolated colony of *Nostoc* AD0303 was inoculated into liquid BG-11 medium. These strains were cultured under environmental conditions that were similar to those of the natural environment from where they were sampled; 18 °C T, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. Strains were allowed to accumulate biomass until they reached an optical density of 0.6 during exponential growth phase, measured as described in Section 2.5.

2.4 Experimental Design

Three experimental set-ups and two controls were included in the six-month study. These were prepared as follows. For each experiment, 25 mL of the liquid culture was made up to 1 L with autoclaved medium in a glass culture flask. Experiments were carried out in duplicate.

In the first experiment, cultures were grown at an elevated temperature of 26 °C, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. In the second experiment, a UV-chamber was designed in which light was provided by a UV-lamp (PHILIPS Blacklight Blue F8 T5 TL 8W/ 08) to emit UV-A (340 nm–400 nm) over a 10-hour photoperiod. The cultures were maintained at 18 °C, 75% RH and at a light intensity of 40 µE, also for a 10-hour photoperiod.

For the CO_2 experiment, the culture flasks were fitted with a Duran GL-45 dual port cap to allow an inflow of filtered CO_2 from one port, and a filtration system using a Duran pressure compensation attachment, fitted with a 0.2 µm filter, on the second port. The flow rate of CO_2 was set at 0.2 L/ min. Five different CO_2 experiments were carried out varying the amount of CO_2, as follows: 10 hours of CO_2 daily for 2 weeks, 2 hours of CO_2 daily for 2 weeks, 30 minutes of CO_2 daily for 1 week, 15 minutes of CO_2 daily for 2 weeks and finally, 15 minutes of CO_2 and 15 minutes of air weekly for 8 weeks.

Two controls were set up. The first was used as a control for the increased T and increased UVR experiments. Here the strains were cultured at 18 °C, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. In the second control, cultures were grown under the same conditions described above, but in addition, air was provided via a pump, in order to replicate the agitation created by the inflow of CO_2.

2.5 Analysis

2.5.1 Growth Curves and pH

2 mL aliquots of each culture were used to measure *in vivo* absorbance for chlorophyll *a* at a wavelength of 680 nm and the optical density at 790 nm respectively using a Shimadzu UV-2501PC spectrophotometer. The pH of each aliquot was also measured. Both tests were carried out bi-weekly.

2.5.2 Morphology

Wet-mount slides of each culture were prepared bi-monthly and viewed microscopically as described in Section 2.2. For each of the strains, different morphological characters were noted and the dimensions of individual cells were taken.

2.5.3 Pigment Assays

Estimation of chlorophyll *a* and *b*, carotenoids and phycobiliproteins were conducted at the end of the study. Cell lysis was carried out as follows. 10 mL of culture were aliquoted into 15 mL centrifuge tubes and centrifuged at 6000 rpm until a cell pellet formed. The supernatant was removed, the pellet transferred to a 2 mL microcentrifuge tube and washed with 2 mL of distilled water. The samples were frozen at −20 °C overnight, thawed and placed in a sonicator for 15 minutes at 250 Hz. Additionally, for the strain *Jenufa* AD0402, a 4-hour drying period was carried out at 60 °C.

Analyses for chlorophyll *a*, *b* and carotenoids were conducted on both strains. 0.25 mL of glass beads and 1.5 mL of pure methanol were added to the cell pellet, the tubes were vortex shaken for 15 minutes and incubated in darkness overnight at 4 °C. The concentration (µg/ml) of chlorophyll *a* (C_a), chlorophyll *b* (C_b), and total carotenoids (C_{x+c}) were calculated using the equations proposed by Lichtenthaler (1987).

A phycobiliprotein assay was also carried out for cultures of *Nostoc* AD0303. Following cell lysis, 0.25 mL of glass beads and 1.5 mL of phosphate buffer (0.01 M NaH_2PO_4, 0.15 M NaCl, pH 7) were added to the pellets and the tubes were vortex shaken for 15 minutes. The samples were then incubated overnight in darkness at 4 °C. The concentration of phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) were calculated using the equations proposed by Bennett and Bogorad (1973).

2.5.4 UV-Absorbing Compounds

Cultures were tested for the presence of scytonemin and mycosporine-like amino acids (MAAs) at the end of the six-month study, using a method adapted from Rastogi and Incharoensakdi (2013). The solvent was evaporated using a Savant SpeedVac SVC 100H Centrifugal Evaporator. The extracts were scanned for typical peaks of

both MAAs and scytonemin between 200 nm and 800 nm using a Shimadzu UV-2501PC spectrophotometer.

2.5.5 Biomass Yield

The biomass yield for each culture was calculated by freeze-drying at the end of each experiment. 50 mL of culture was centrifuged at 4000 rpm until a cell pellet was formed. The pellet was frozen at −20 °C and lyophilised to a powder. The tube was weighed beforehand, and the amount of biomass was calculated by subtracting the mass of the empty tube from the mass of the tube containing the powder.

2.5.6 Data Analysis

Means were calculated from measurements taken in duplicate. Differences between the means ($P < 0.05$) for treatment and experimental controls were determined using the independent t-test on IBM SPSS v. 25.

3 Results

3.1 Microbial Communities

In both terrestrial hypogean environments and the marine littoral zone, the biofilms and biomats consisted of highly diverse microbial communities made up of both phototrophic and heterotrophic microorganisms. In each habitat investigated so far, filamentous cyanobacteria were found to be the dominant microorganisms making up the microbial biofilm structure. Upon initial transfer to culture conditions, the biofilms and their constituent microorganisms grew at a very slow rate. Many of the microorganisms exhibited intricate relationships and proved to be difficult to isolate in culture.

Cyanobacteria possessing fine filaments and having a simple morphology, were found in all biofilm and biomat samples. They belonged to the genus *Leptolyngbya* (Fig. 1a) and the recently described genera *Nodosilinea* (Perkerson et al., 2011), *Oculatella* (Zammit, Billi & Albertano, 2012) and *Albertania* (Zammit, 2018).

For biofilms isolated from terrestrial environments, filaments of the heterocytous genera *Nostoc* and *Fischerella*, as well as coccal cyanobacteria belonging to *Chroococcidiopsis*, *Gloeocapsa* and *Asterocapsa spp.* were isolated in culture. Microalgal strains belonged to the genera *Jenufa*, *Pseudopleurococcus* and *Trentepohlia*. All these strains are presently being characterised by a more comprehensive multiphasic approach. The characteristics of the Maltese *Jenufa* strains are described in a separate study (Zammit & Agius, 2019).

Larger cyanobacterial filaments (Fig. 1b–d) were also observed in communities colonising the calcareous rock of the marine littoral, including Oscillatorialean strains belonging to the genera *Lyngbya* (Fig. 1b), *Oscillatoria*, as well as the heterocytous *Calothrix* (Fig. 1d) and *Scytonematopsis*. Large spherical cyanobacterial cells of *Stanieria cf. sublitoralis* produced baeocytes by spontaneous multiple fission to form red colonies on the surface of biofilms from the littoral zone. Other coccal cyanobacteria could be morphologically classified into *Aphanocapsa* and *Gloeocapsa spp.* Diatoms of *Navicula* spp. were ubiquitous in the same biofilms and biomats. Developing macroalgal filaments belonging to species of the green macroalgae *Cladophora* and *Ulva* also frequently contributed to the structure of marine biofilms and biomats. These microbial communities also included cryptic coccal microalgae, bacteria, protozoans and fungi.

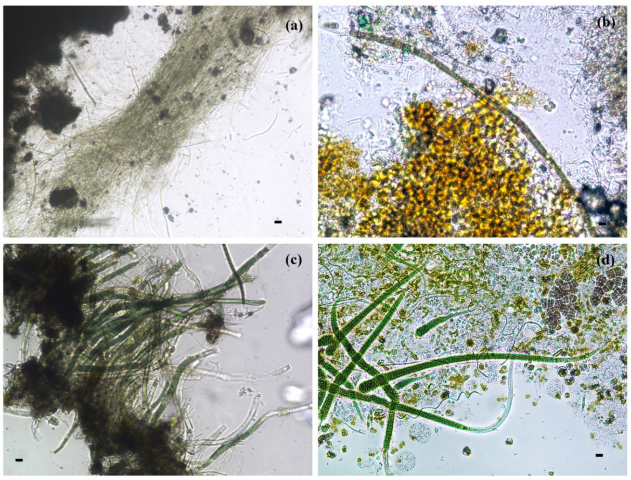

Figure 1: Light micrographs showing biofilm and biomat microorganisms from a rocky shore in Sliema, Malta, (a) simple filaments of the Leptolyngbyaceae, (b) *Lyngbya* filament and coccal microalgae, (c) tufts of cyanobacterial filaments, (d) *Calothrix* filaments with basal heterocyte, surrounded by different types of coccal cyanobacteria and microalgae. Scale bar = 10 µm in (a), (b), (d). Scale bar = 20 µm in (c).

For the climate change experiment, the filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402 were used. Both of these were of common occurrence in biofilms colonising hypogea (Zammit, Billi, Shubert et al., 2011; Zammit, Sánchez-Moral & Albertano, 2011).

3.2 Growth Experiment

Under all treatments, both *Nostoc* and *Jenufa* strains displayed a typical sigmoidal growth curve based on the absorbance of chlorophyll *a* (Fig. 2) and cell density. In this study, two subsequent growth curves were observed, since fresh medium was supplied again midway through the experiment. Under elevated UVR, both strains exhibited growth in culture for the duration of the experiment (Fig. 2).

At elevated temperature, *Nostoc* AD0303 accumulated a higher mean cell density than the control. On the other hand, *Jenufa* AD0402 grown under elevated

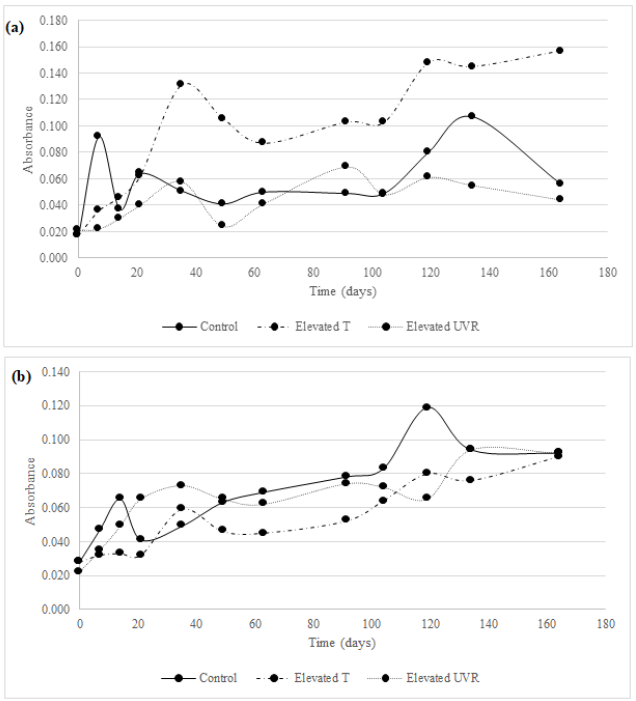

Figure 2: Representative growth curves based on the absorbance of chlorophyll *a* at 680 nm for (a) *Nostoc* AD0303 and (b) *Jenufa* AD0402.

temperature displayed an extended lag phase and a decrease in growth rate but continued to grow steadily over the six- month period.

Nostoc AD0303 exhibited filament bleaching leading to culture death in all CO_2 treatments. Culture death was also observed in *Jenufa* AD0402 growing under increased CO_2 concentration. However, the subsequent introduction of air in the final experiment, allowed cultures of *Jenufa* AD0402 to recover. In the same experiment, filaments of *Nostoc* AD0303 were bleached throughout, and only started to recover 8 weeks after the experiment was terminated.

3.3 Morphology

Cultures of *Nostoc* AD0303 grown at elevated T were characterized by short, rapidly dividing filaments (Fig. 3). A significant reduction ($p < 0.05$) of 0.66 µm was observed in the widths of cells grown at elevated T when compared to the control. Cells of *Jenufa* AD0402 also showed a significant reduction in diameter ($p < 0.05$) of 0.45 µm when compared to that of the control (Fig. 3e).

Cultures of *Nostoc* AD0303 grown under elevated UVR showed the greatest variation in morphology. Filaments became densely aggregated and individual filaments could not be easily discerned under the microscope (Fig. 3c). Cultures of *Jenufa* AD0402 grown under elevated UVR exhibited thicker cell walls (Fig. 3f). Heterocytes were observed in cultures of *Nostoc* AD0303

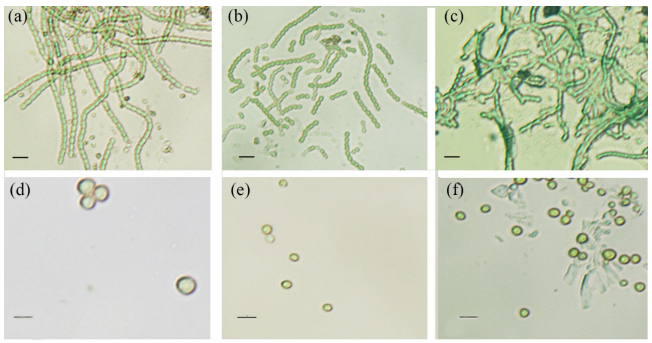

Figure 3: Light micrographs of *Nostoc* and *Jenufa* cultures taken at the end of the six-month study. *Nostoc* AD0303 cultivated (a) under control conditions (b) at elevated T (c) at elevated UVR. *Jenufa* AD0402 grown (d) under control conditions (e) at elevated T (f) under elevated UVR. Scale bars = 10 µm.

grown under increased CO_2 concentration, prior to culture death. Heterocytes were not observed in any of the other experimental cultures. Cultures of *Jenufa* AD0402 grown under elevated CO_2 exhibited a significant reduction in cell diameter ($p > 0.05$) of 1.38 µm.

3.4 Pigment Analysis

Cultures of *Nostoc* AD0303 grown under elevated T and UVR had significantly lower concentrations ($p > 0.05$) of allophycocyanin (93.5%) (Fig. 4a). When grown under elevated UVR, *Nostoc* AD0303 cells contained a significantly lower ($p > 0.05$) concentration of phycocyanin (reduced by 89.7%) and carotenoids (90% lower) (Fig. 4b). Cultures of *Jenufa* AD0402 showed no significant changes in pigment concentration in all experimental treatments (Fig. 5a, b).

3.5 Photoprotective Pigments

No typical absorption maxima for scytonemin and mycosporine-like amino acids were detected.

3.6 Biomass

Nostoc AD0303 produced a significantly greater biomass when cultured at an elevated T, whereas *Jenufa* AD0402 produced a significantly greater biomass when cultured at elevated UVR (Fig. 6). For the CO_2 parameter, the data shown in Fig. 6 is for the final experiment, in which cultures were incubated for 8 weeks.

4 Discussion

The biofilms and biomats studied so far are diverse in morphology and are composed of heterogenous communities of microorganisms. A high diversity of morphologically distinct cyanobacteria and microalgae were found to make up these microbial communities. The species composition varied between different types of biomat communities, while the biofilm samples had

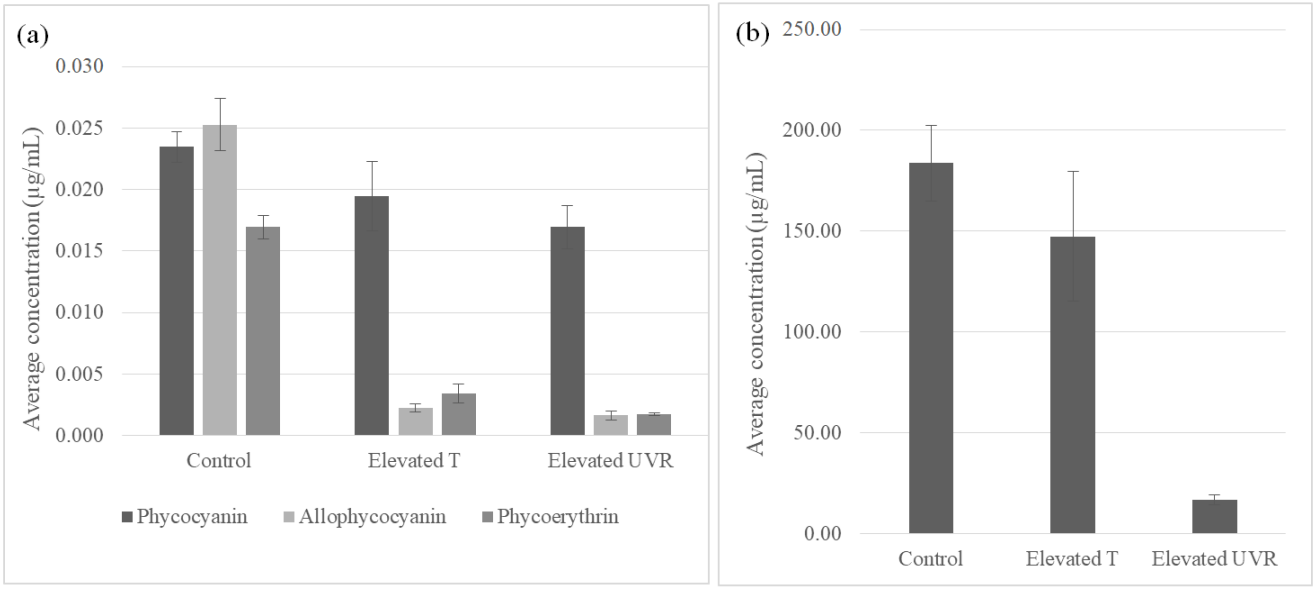

Figure 4: Pigment concentrations of *Nostoc* AD0303, (a) phycobiliproteins, (b) carotenoids. Error bars denote the standard deviation of duplicate samples.

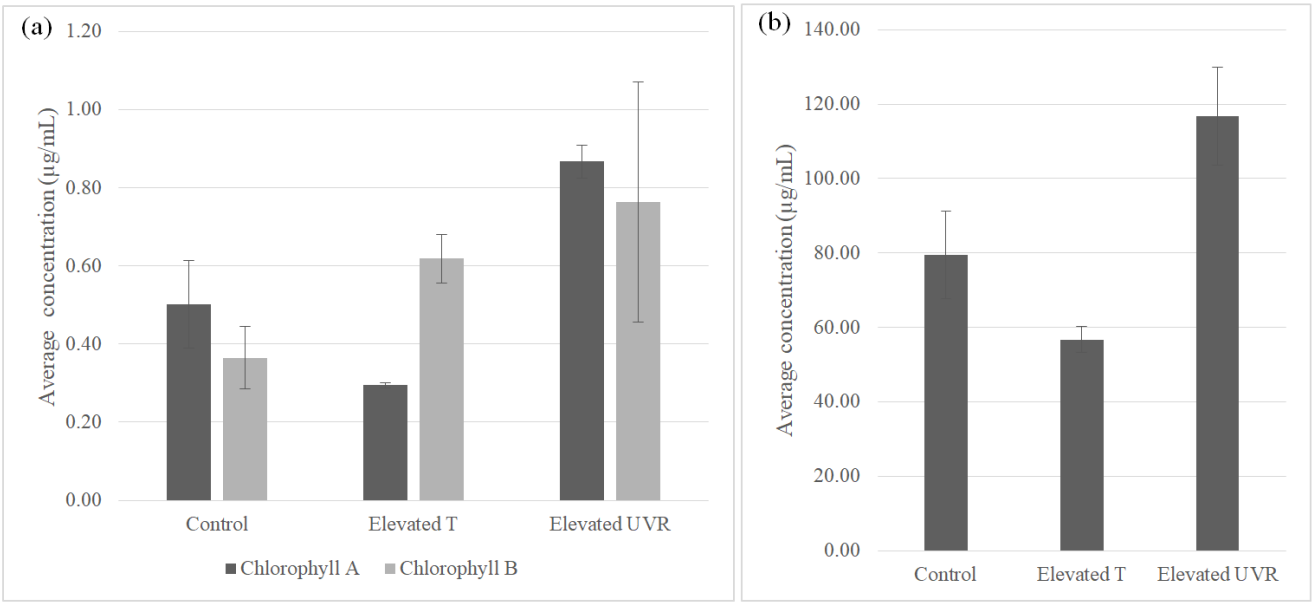

Figure 5: Pigment concentrations of *Jenufa* AD0402 (a) chlorophylls *a* and *b* (b) carotenoids. Error bars denote the standard deviation of duplicate samples.

similar compositions. Thus, the identity and relationships between these microorganisms may be important in shaping the community together with the prevalent biotic and abiotic components. Distinct layers of terrestrial biomats were composed of different types of microalgae and cyanobacteria. On the other hand, aerophytic biofilms in the marine littoral were commonly dominated by cyanobacterial filaments that were arranged in tufts attached to the rock surface (Fig. 1c).

Both *Jenufa* and *Nostoc* strains responded differently to changes in environmental parameters (Fig. 2). The cultures follow a typical sigmoidal growth curve, with a second spurt of log phase growth after three months, when cultures were supplemented with liquid medium. Temperature yielded a positive effect on the growth rate of *Nostoc* AD0303. The final mean cell density recorded for cultures grown at elevated temperature was twice that recorded in the control. The final

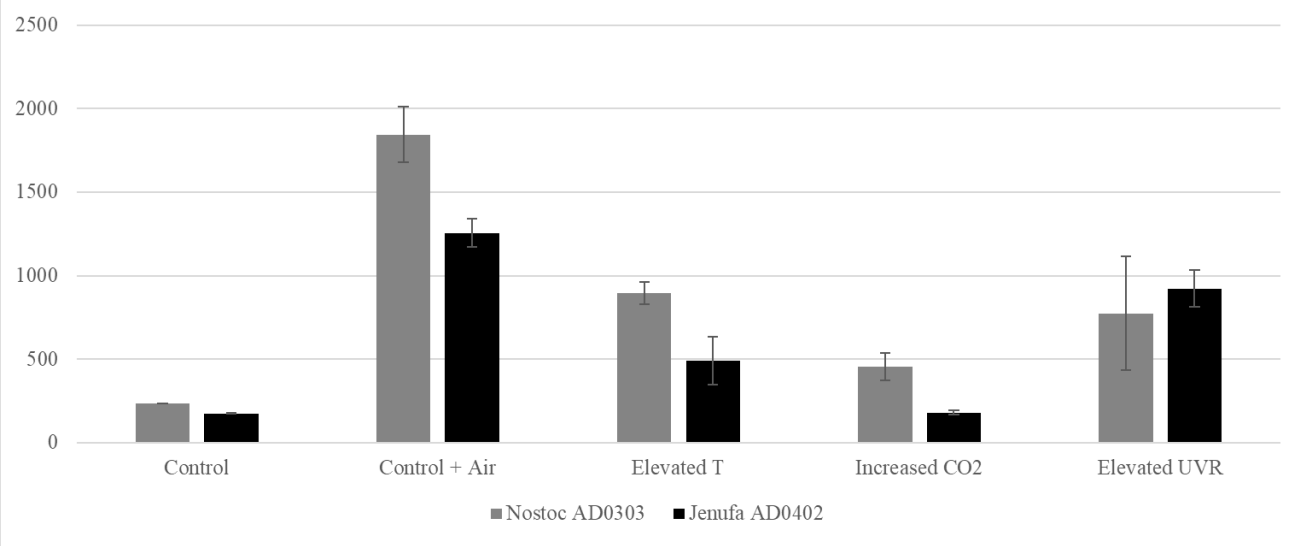

Figure 6: Final biomass yield (µg/L) for both strains. Error bars denote the standard deviation of duplicate samples.

biomass yield at the end of the experiment was four times higher than that of the control. A similar positive effect of temperature on growth has been documented extensively in cyanobacteria, as in the marine cyanobacterium *Synechococcus* and the terrestrial cyanobacterium *Nostoc flagelliforme* (Fu, Warner, Zhang, Feng & Hutchins, 2007; Li et al., 2016). The effects on growth rate are influenced by the photosynthetic rate. A higher photosynthetic rate is determined by carbon fixation rate of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, (RuBisCO), which increases at higher temperatures (Beardall & Raven, 2004). Microscopic observation of *Nostoc* AD0303 showed that cultures consisted of short rapidly dividing filaments throughout the entire experiment (Fig. 3b). Temperature was found to have no effect on the concentrations of chlorophyll *a*, *b* and carotenoids of *Nostoc* AD0303, however significant reductions in allophycocyanin and phycoerythrin were recorded (Fig. 4a, b). This suggests that other pigments may be contributing to the increase in growth rate. This significant reduction in phycobiliproteins contradicts findings published for other cyanobacteria. For instance, the production of phycobiliproteins in *Spirulina platensis* showed the highest concentrations of phycoerythrin and allophycocyanin at temperatures between 30–35 °C (Kumar, Kulshreshtha & Singh, 2011).

Temperature had a different effect on the growth of *Jenufa* AD0402. Unlike cultures of the same strain grown under the different experimental conditions, these cultures showed a lag phase at the start of the study. Moreover, one of the replicates grown under elevated temperature failed to grow and the culture died at day 50. This implies that this specific subaerophytic strain is acclimatised to grow at lower temperatures in its natural habitat and the cells do not easily adapt to changes in temperature. This is corroborated by the significant reduction in cell diameters observed in *Jenufa* AD0402 within the first month. No significant differences in growth rate, cell size or pigment content was noted in experimental cultures compared to the control after six months. This suggests that growth in *Jenufa* cells was not completely inhibited by elevated temperature, but that the cells took longer to acclimatize.

Increasing the concentration of CO_2 had an inhibitory effect on the growth rate of both strains. Elevated CO_2 resulted in culture decline and death of one or both strains. In the first experiment, *Jenufa* and *Nostoc* culture death was attributed to the 10-hour duration of CO_2 flow which caused the liquid medium to become acidic (pH = 4.7). The bleaching of cells due to an acidic pH was also documented in *Synechococcus* at a pH lower than 5.0 (Sakomoto, Delgaizo & Bryant, 1998). In our experiment, the flow of CO_2 was subsequently decreased in order to maintain the pH of the media; however, culture growth of both strains was still inhibited. This suggests that the CO_2 was the direct cause of growth inhibition.

In the final CO_2 experiment, a limited weekly supply of CO_2 for 15 minutes was followed by an additional 15 minutes of air. In this experiment cultures of *Jenufa* AD0402 grew steadily till the end of the experiment. During a 20-day period, the mean cell density was the same in the experimental culture and in the aerated control, suggesting that oxygen deprivation had been the cause of culture death in previous experiments. How-

ever, filaments of *Nostoc* AD0303 remained bleached and took over two months to recover after the experiment was terminated.

UVR, specifically UV-A (emission maximum at 320–400 nm) influenced the morphology of both *Jenufa* AD0402 and *Nostoc* AD0303. In general, cyanobacteria may employ a range of mechanisms which allow them to tolerate or avoid exposure to UVR. For instance, motile species may glide away from UVR exposure, however both strains used in this study are non-motile. The formation of a microbial mat consisting of various layers of cells or filaments for protection, is also a possible response (Singh, Häder & Sinha, 2010). In fact, both strains were present as biofilms or biomats in their natural habitat. However, this six-month study was probably too short to result in biomat or biofilm formation. At the end of the UV experiment, cultures were screened for UV-absorbing compounds scytonemin and MAAs. MAAs may occur in both strains but the production of scytonemin is limited to cyanobacteria. *Nostoc flagelliforme*, for instance has been documented to produce both scytonemin and MAAs (Ferroni, Klisch, Pancaldi & Häder, 2010). Scytonemin was also isolated from *Nostoc commune* (Matsui et al., 2012). However, even though the production of UV-absorbing compounds has been recorded in several strains, the *Nostoc* strain AD0303 used in this experiment produced neither of these compounds under the experimental conditions provided here. *Jenufa* AD0402 did not produce any UV-absorbing compounds either. On the other hand, the mechanism that allowed the cultures to thrive in elevated UV-A was a change in cell and filament morphology. In fact, filaments of *Nostoc* AD0303 became aggregated to a point where it was hard to distinguish between individual filaments. This aggregation allowed for the formation of clusters of filaments in which the outer filaments sheltered the inner filaments. The compact structure was the result of thicker sheaths and an extracellular polysaccharide (EPS) matrix around the cells. On the other hand, *Jenufa* AD0402 grown under elevated UVR exhibited thicker cell walls and a significant reduction in cell diameter (Fig. 4f). Similar reactions to UVR exposure have also been documented in the macroalgae *Gelidium floridanum* (Schmidt et al., 2012) and the microalga *Chlamydomonas reinhardtii* (De Lange & Van Donk, 1997). The change in morphology in both *Nostoc* and *Jenufa* strains must have protected the cells adequately to allow for biomass accumulation and growth under elevated UVR.

5 Conclusion

Further knowledge is constantly being gained from the continued study of the biodiversity and ecology of biofilm and biomat forming microorganisms growing in different microhabitats around the Maltese islands. The number of new taxa being discovered routinely suggests that more intensive research in this field is required. The effects of changes in environmental parameters on two of these phototrophic microorganisms, a cyanobacterium and a microalga, were found to be strain specific.

Under increased CO_2, cyanobacterial filaments of *Nostoc* AD0303 became bleached and proved to be less resistant than the microalgal *Jenufa* AD0402 cells. Under elevated UV-A levels, both strains underwent changes in morphology which allowed them to proceed with photosynthesis and growth. This implies that the photosynthetic apparatus was not irreparably damaged. *Nostoc* AD0303 benefitted positively from an elevated temperature, whereas *Jenufa* AD0402 took a longer time to acclimatize, however, at the end of the study there was no significant difference between the experimental culture and the control.

Further studies on different strains of Maltese cyanobacteria and microalgae and their response to GCV should be undertaken, since some of these strains might be used as early indicators of climate change in particular habitats. In view of this, our current studies are investigating the effects of GCV on phototrophs isolated from Maltese rocky shore environments, which are seasonally exposed to stresses such as high UV-radiation, temperature and desiccation.

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