Characterization and Analysis of the Microbiota of *Bangia atropurpurea* and other Freshwater and Marine Algae in North America

by

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Author's Declaration

This thesis consists of materials all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of Contributions

Algal samples were acquired through the sampling efforts of Vanessa Poletto Borges and Dr. Kirsten Müller.

Dr. Trevor Charles, Dr. Michael Lynch and Dr. Jiujun Cheng of Metagenom Bio Inc. (Waterloo, Ontario) sequenced the V4 region of the 16S rRNA gene from all DNA samples.

Abstract

Algae are a diverse polyphyletic group of photosynthetic organisms that contribute to forming rich ecosystems within marine and freshwater habitats. Many factors contribute to algal function and growth, such as the chemistry of their aquatic environment as well as interactions with other organisms through competition, parasitism, and mutualism. An often-overlooked symbiotic relationship is between alga and the microbial community that inhabits its surface or intercellular space. In fact, studies investigating the algal microbiome are limited and comparative analyses are rare. This study examines microbial community compositions of *Bangia atropurpurea* (freshwater Rhodophyta), *Bangia fuscopurpurea* (marine Rhodophyta), and *Cladophora glomerata* (freshwater Chlorophyta) to gain more information on bacterial diversity and composition changes across environment, species, and time. Microbial taxonomy was determined through sequencing of the V4 region of 16S rRNA gene. Sequence data was filtered, sorted, and analysed with Qiime2 and R. Using MEGA to construct maximum likelihood phylogenetic trees, the identification of genera within biologically relevant phyla such as Cyanobacteria, Planctomycetes, Verrucomicrobia, and Bacteroidetes were explored. Comparisons of algal microbiota isolated from different host species or geolocations demonstrated that bacterial communities likely assemble in alga-specific and environment-specific patterns. Sampling the microbiota of *Bangia atropurpurea* 14 years apart showed a significant decrease in bacterial diversity over this time, which may be attributed to loss of certain temporary associations. Future studies can use this data to inform larger metagenomic surveys and further investigate the relationships between bacteria and their algal hosts. This will provide valuable insight into the microbial effect on algal function which may contribute to the mitigation of harmful invasions and blooms that cause damage to the local ecosystem.

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List of Abbreviations

AP	ascorbate peroxidase
ASV	amplicon sequence variant
BAN_MAX	*Bangia maxima* sample
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide Basic Local Alignment Search Tool
BLO_02	*Bangia atropurpurea* (2002) sample
BLO_16	*Bangia atropurpurea* (2016) sample
bp	base pairs
CAT	catalase
CLAD_ON	*Cladophora glomerata* sample
Csp	cold shock protein
DHAR	dehydroascorbate reductase
DOC	dissolved organic carbon
GR	glutathione reductase
LPSN	List of Prokaryotic names with Standing in Nomenclature
MEGAX	Molecular Evolutionary Genetics Analysis 10
METE	cobalamin-independent methionine synthase
METH	cobalamin-dependent methionine synthase
mirlyn	Multiple Iterations of Rarifying for Library Normalization
NCBI	National Center for Biotechnology Information
NF_F	*Bangia fuscopurpurea* sample
nifH	nitrogenase
NPQ	non-photochemical quenching
nSSU	nuclear small subunit
OTU	operational taxonomic unit
POD	peroxidase
PSII	photosystem II
PVC	Planctomycetes, Verrucomicrobia, and Chlamydiae
QIIME2	Quantitative Insights into Microbial Ecology 2
*rbc*L	Rubisco spacer
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
SSU	small subunit
SOD	superoxide dismutase
V4	variable region four

Chapter 1: Introduction

1.1- Bacteria and Microbe-Host Interactions

Bacteria perform fundamental roles in the function and health of many environments in which they exist. In soil, aquatic, and extreme ecosystems, microbes, including Bacteria, Archaea, fungi, and other single-cellular organisms, contribute to metabolic processes that can improve quality and nutrient availability for local species (Alivisatos *et al*. 2015). The term "microbiome", which was initially defined by Whipps *et al*. (1988) encompasses not only the entire community of microorganisms associated with a host or ecosystem, but also any associated metabolic by-products and genetic material. With a growing body of literature and increased research efforts on host-associated microbial communities (Lloyd-Price *et al*. 2016; Mendes *et al*. 2013; Sehnal *et al*. 2021), the need arises for terminology that better describes the precise nature of the microbial community that is being studied. When referring only to the community of associated living cells, the term "microbiota" should be used. The microbiome includes non-living genetic elements such as viruses and phages, along with microbial metabolic products and structural elements that are not native to the host (Berg *et al*. 2020). Most existing literature on host-associated microbial communities refers to the living cells as the microbiome although "microbiota" is now the more accurate and accepted term. On a holistic level, a single host exists as an entire community of Bacteria, Archaea, and viruses that live on or within the host (Whitman *et al*. 1998). This collective community of the host and all associated organisms is referred to as the holobiont, which was first introduced by German theoretical biologist Adolf Meyer-Abich (1943) and popularized by Lynn Margulis (1991). The holobiont is an ecologically and evolutionarily significant unit that comprises symbiotic relationships among all its constituent organisms (Baedke *et al*. 2020). It is important to note that symbiotic interactions are

not one-on-one, rather are intertwined amongst all members of the holobiont. Unfortunately, there are computational and ecological barriers to investigating these relationships due to myriad complexities such as number of interactions, transcriptomic pathways, and external covarying factors (Singh & Reddy 2016). Meta-omics approaches, such as metagenomics, metabolomics, or meta-transcriptomics are also necessary to distinguish between a true bacteria-host symbiotic interaction and the presence of a bacterium that does not have a significant relationship with the host organism. These in-depth approaches are indispensable to achieving a deeper understanding of multi-species interaction and provide a fuller picture of how gene products and different metabolic pathways result in symbiotic relationships between two or more organisms (Patwary *et al*. 2021).

1.1.1 - Types of Bacteria-Host Interactions

Bacteria-host symbioses exists in many capacities and forms (Kalia 2015). Two or more organisms that live together and closely interact, regardless of net positive or negative effects, are said to be part of a symbiotic relationship (Leung & Poulin 2008). The close range between microbe and host allows for exchange of nutrients, production of metabolites, or elimination of toxins (Kalia 2015). These interactions can broadly be mutualistic, commensal, or parasitic which can occur between individual and groups of organisms belonging to different taxa (Hirsch 2004). The specific type of interaction can vary depending on the host organism, the metabolic functions of the microbe, the location of the microbe in relation to the host, and other factors (Singh & Reddy 2016). In this project, these interactions will be considered in the scope of the bacterial community and its algal host.

A mutualistic interaction involves a relationship between the microbial community and its host from which both parties benefit (Leung & Poulin 2008). Bacteria-host mutualism frequently

involves nutrient exchange and material processing. The host often benefits by acquiring new physiological capabilities and adapting to novel environmental niches (Relman 2008; Leung & Poulin 2008). Mutualistic bacteria-host interactions are often indispensable to both host and microbe; hosts can die without an associated microbial community and bacteria may not thrive in the host without the shared nutrients (Gilbert & Neufeld 2014). Often a species of microbe can fill a metabolic niche that the host is not able to accomplish on its own (Kazamia *et al*. 2012). Mutualism and symbiosis are two distinct terms that are often used interchangeably in ecological literature but should be distinguished from one another (Wilkinson 2001). In this report, the term "symbiosis" will be reserved for any interaction between two different species, and "mutualism" will be used specifically for mutually beneficial relationships between two organisms.

Parasitism is another type of symbiotic interaction, where one organism benefits and as a direct result of this, the other party incurs a fitness cost (Leung & Poulin 2008). There are numerous examples of bacterial, viral, nematode, and fungal parasitism, across all ecosystem levels, such as the infection of plants by nematodes and mycorrhizal fungi (Guegan 2005). There are also many instances of parasitism of algae, such as the adelphoparasitism observed between closely related red algal species. The colonization of host alga *Sarcodiotheca gaudichaudii* by *Gardneriella tuberifera,* and *Gracilariopsis lemaneiformis* by *Gracilariophila oryzoides* has been observed to result in fusion between host and parasitic cells, leading to loss of photosynthetic function and spread of infection (Goff & Zuccarello, 1994). Often the parasite can suffer costs due to the host experiencing a loss in fitness as its resources are seeped (Leung & Poulin 2008). The parasitic organism cannot continually benefit by stealing the host's resources as eventually the host will not be able to acquire any more nutrients.

Commensalism is a third type of symbiotic relationship, which refers to the interaction where one party benefits and there no net gain or loss for the other organism (Munguia *et al*. 2009). Microbial commensalism has long been observed in human epidemiology when people carrying certain disease-causing bacteria did not experience any symptoms (Casadevall & Pirofski 2000). Commensalism is a common feature in the root microbiome (rhizosphere) of land plants (Hirsch 2004). There is also evidence that some species of bacteria are reliant on commensal relationships with other bacteria (Ohno *et al*. 1999). This is the case with *Symbiobacterium thermophilum*, a thermophilic bacterium that could be grown in a laboratory culture only when co-cultured with *Bacillus* sp strain S (Ohno *et al*. 1999). Commensalism is wide-spread throughout many ecosystems and organism interactions, and can promote or prevent the survival of other members of a microbiome (Casadevall & Pirofski 2000).

1.2 – Algae

"Algae" is a broad non-taxonomic term that is used to represent the diverse polyphyletic group of chloroplast-containing photosynthetic organisms that cannot be classified as plants. However there are some exceptions, such as the non-photosynthetic green algae of genera *Prototheca* and *Helicosporidum* that underwent evolutionary loss of photosynthetic genes in the plastid and nuclear genomes (Suzuki *et al*. 2018). Adding to this complexity, algae have a long evolutionary history that can be traced back to the formation of living organisms 3.5 years ago (Schopf *et al.* 2007). Fossil records of cyanobacteria in the form of stromatolites have been found to date back 3.3 to 3.5 billion years (Schopf & Packer 1987) and fossilized Rhodophyta dating back about 1.2 billion years has been discovered in the Hunting Formation in arctic Canada (Butterfield *et al*. 1990). Currently, algae exist in a wide array of morphologies, sizes, photosynthetic pigments, forms of reproduction, and habitats (Barsanti *et al*. 2008b) and are

significant ecologically, economically, and culturally on a global scale (Voort *et al*. 2015; Wells *et al*. 2017). As a result, it is vital to understand all aspects of the biological functions of algae, including interactions with its associated microbiota.

1.2.1 – Associated Microbiota of Aquatic Ecosystems and Algae: A Brief Review

Symbiotic relationships have been well researched in terrestrial plants, the human gut and skin, and aquatic organisms (Mendes *et al*. 2013; Schmidt *et al*. 2018; Sehnal *et al*. 2021). Valuable findings in land plant rhizobiomes and aquatic organisms have also demonstrated the relevance and importance of the microbial community to the host organism (Mendes et al., 2013; Sehnal et al., 2021; Uroz et al., 2010; Weinert et al., 2011). Compared to the wealth of literature on terrestrial plant and aquatic vertebrate microbiomes, there is a noticeable deficiency in the research of microbial communities of algae and their aquatic environments. Existing studies have found evidence for the effects of water chemistry on algal microbial community composition, effects of bacterial community on the invasiveness of an algal species, and host- and environment-specific assemblages of bacteria.

Microbes in aquatic habitats are vital to both the organisms living within the body of water and the surrounding ecosystem, and frequently the quality of drinking water is also evaluated by the presence of pathogenic or harmful microorganisms (Alexander *et al*. 2015). Bacteria in the water column may form close associations with various aquatic organisms, thus modulating their functionality and health (Sehnal *et al*. 2021). As a result, if the microbial community composition of the water column is modified by rapid environmental changes caused by natural or anthropogenic effects, the microbiota of aquatic organisms may also be affected (McCormick & Cairns 1994).

Large changes in nutrient availability can result in imbalance of microbes, such as in the case of large blooms of microbial alga cyanobacteria, which produce toxins such as microcystin (Wood *et al*. 2017). Increased dissolved carbon dioxide concentration in oceans, due to climate change, also severely impacts the bacterial community in the water column and in algal growth (Minich *et al*. 2018). For example, a noticeable dysbiosis, or imbalance, of the bacterial community of the oceanic water and microbiota of kelp *Macrocystis pyrifera* occurred due to the increase in CO_2 (Minich *et al*. 2018). In the water column, this change in water chemistry was linked with a large increase in abundance of seven genera within order Rhizobiales and a decrease in abundance of genera within phyla Bacteroidetes and Verrucomicrobia. The kelp microbiota was also affected by change in temperature, which resulted in a decrease in bacteria from Alteromonadales and increase in Rhodobacterales and Rhizobiales microbes (Minich *et al*. 2018). Different environmental stressors result in varied shifts of bacterial community composition, which indicates that the aquatic microbiota is sensitive to natural or anthropogenic changes.

Microbiomes of marine algae are well characterized across different locales in the world, and it has been demonstrated that core algal microbiota are formed depending on species and environment of the alga. The bacterial communities of two invasive marine red algae species belonging to the genus *Asparagopsis* showed host-specific assemblage of bacteria (Aires *et al*. 2016). The two species, *A armata* and *A. taxiformis*, occupy both tropical and temperate regions, and are observed in native and invaded mainland and offshore islands (Aires *et al*. 2016). Using these environmental differences, researchers noted that there are unique bacteria to each species, as well as to each environment. However, mainland habitats are more encumbered by anthropogenic pressures than offshore waters. This study shows that anthropogenic changes in

the environment may affect the species composition of the bacterial community due different nutrient sources and higher stress habitat (Aires *et al*. 2016). The research also provides insight into the assemblage of microbiota based on the environment.

Alga-specific bacterial communities have been observed in some algae, such as the marine brown alga *Saccharina japonica* (Balakirev *et al*. 2012). Each of the three distinct morphological forms of this alga, TYP, LON, and SHA, harbours unique microbes which could indicate an intermediate stage in its evolution brought on by reproductive isolation (Balakirev *et al*. 2012). Although the different types of algae do not occupy the same environment, some of the unique bacterial associations were attributed to the specific algal host rather than the habitat differences. TYP-specific lineages included bacteria such as *Colwelliaceae, Idiomarina,* and *Alcanivorax;* the LON-associated community consisted of *Psychromonas, Vibrio,* and *Cobetia;* SHA-specific lineages included bacteria such as *Gammaproteobacteria*, *Arenicella*, and unclassified *Alteromonadales* (Balakirev *et al*. 2012). Certain bacteria have also been shown to contribute specific protein products for the benefit of the alga, which may further drive species-specific symbioses. For example, in brown alga *Ectocarpales siliculosus,* there are close associations with bacteria from order Rhizobiales that produces proteins vital for algal hormone production such as auxin and cytokinins (Dittami *et al*. 2014). Species-specific associations between bacteria and algal host may be driven by co-evolutionary factors or the metabolism of proteins vital to algal function.

1.2.2 - Algal Response to Abiotic Environmental Stressors

Due to the wide variety and complexity of algal sizes and habitats, these organisms have a broad range of ideal conditions for growth and reproduction (Barsanti *et al*. 2008b). Many abiotic factors, such as temperature, pH, nutrient availability, light access, O_2 and CO_2 levels,

salinity, and turbidity, are significant contributors to proper algal function depending on the species (Butterwick *et al*. 2005; Barsanti *et al*. 2008a). Nutrient availability is universal factor that affects all types of algae, from microscopic cyanobacteria to filamentous macroalgae (Mueller *et al*. 2016; Wood *et al.* 2017). Influx of nutrients has been well described to cause algal blooms and disruptions to the ecosystem (Lyons *et al*. 2014). There is also a general positive correlation between light intensity and algal growth, often observed in algae that inhabit shallow waters or are close to shore, such as *Bangia atropurpurea (*Schlesinger *et al*. 1981). One of the effects of increased algal growth due to light and nutrient exposure is a higher output of dissolved organic carbon (DOC), observed in phytoplankton and seaweeds (Cherrier *et al*. 2015; Mueller *et al*. 2016). Although interactions with biotic factors such as surrounding fauna, flora, and epiphytic macro-organisms are important to consider, extreme changes in certain abiotic factors can result in a variety of algal adaptations manifested as physical and genetic modifications (Rai & Gaur 2012).

As abiotic factors can contribute to improved algal growth, improper quantities of these conditions require innate algal adaptations to evade cellular damage (Fogg 2001). Environmental stressors in the scope of algal function are defined by Borowitzka (2018) as factors that disturb homeostasis. The primary types of stressors that algae experience are physical damage, radiation, extreme temperatures, osmotic stress, desiccation, nutrient deficiency, extreme pH, or toxic substances (Fogg 2001). Algae have evolved many innate protective mechanisms to defend against these stressors, and some of these adaptive responses may contribute to the invasiveness or spread of an alga. In response to radiation and extreme light intensity, some algae employ non-photochemical quenching (NPQ), a regulatory pathway that redistributes excess light and radiation from excited chlorophylls (Kaňa *et al*. 2019; Müller *et al*. 2001). Intertidal marine red

algae, such as genus *Gelidium,* have been observed to downregulate photosystem II (PSII) in response to increased sun exposure (Figueroa & Gómez 2001). Changes in salinity and osmotic stress can also be managed by accumulation of various salts and saccharides, depending on the algal species (Oren 2002). For example, freshwater green alga *Stichococcus bacillaris* produces sorbitol and proline for intracellular osmoregulation (Brown & Hellebust 1978). Red alga *Bostrychia scorpiode* accumulates potassium, sodium, and chlorine ions, as well as sorbitol and dulcitol, which act as organic osmolytes to lower turgor pressure (Karsten & Kirst 1989). Finally, a significant algal adaption is against reactive oxygen species (ROS), such as O_2^- and H_2O_2, by increased production of antioxidants (Cruz de Carvalho 2008). Redox reactions catalyzed by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR) balance the detrimental effects of these reactive molecules (Wang *et al*. 2019). An increase in O_2^- and H_2O_2 concentrations were observed in freshwater *Bangia* exposed to hypersaline conditions and marine *Bangia* in hypo-saline water, and was followed by a linear increase in antioxidant enzyme production (Wang *et al*. 2019). Defense against ROS production is also observed in high-intertidal seaweeds such as *Porphyra* (Rhodophyta) during long periods of drought with frequent low-tide events (Ji & Tanaka 2002). Desiccation stress was observed to trigger a significantly higher production of reactive oxygen species, and these levels subsequently dropped with increased production of antioxidants such as CAT, GR, ascorbate peroxidase (AP), and dehydroascorbate reductase (DHAR), as well as rehydration (Contreras-Porcia *et al*. 2011). Although these innate defenses are extremely effective against environmental stressors and adaptation to new habitats, the microbiome likely contributes to algal acclimation to novel conditions.

1.2.3 – Putative Roles of the Microbiome in Algal Function and Adaptation to Stressors

Although algae have many built-in genetic and transcriptomic pathways that respond to environmental stress, symbiotic relationships with microbes may bolster these responses. In a review by Ghaderiardakani *et al*. (2020) examination of bacteria-alga interactions show how mutually-beneficial relationships result in adaptations and tolerance to various environmental stressors. The bacterial communities of algae have been associated with increased cold tolerance, adaptation to salinity changes and other oxidative stressors, and tolerance to harmful metals (Ghaderiardakani *et al*. 2020). Bacterial symbionts also have been shown to have neutral or commensal associations with algae such as the degradation of algal cell wall polysaccharides (Descamps *et al*. 2006; Cardman *et al*. 2014). Long-term co-evolution between alga and bacteria has strengthened these associations over time and novel interactions are formed under new conditions.

1.2.3.1 - Microbes Associated with Cold Tolerance

Various Antarctic seaweeds have been reported to be associated with bacteria that may contribute to their adaptation to cold environment and harsh radiation during low tides. The proportion of bacterial phyla differs between algae that inhabit cold environments and those in temperate or tropical waters (Gaitan-Espitia & Schmid 2020). Gram-positive bacteria such as Actinobacteria are strongly associated with Antarctic algae. Three co-occuring algae, *Adenocystis utricularis, Monostroma hariotii,* and *Iridaea cordata* were observed to have an abundance of bacteria from phylum Actinobacteria and a low proportion of Firmicutes in their microbiota (Alvarado *et al*. 2018). The carotenoid pigmentation of these microbes may offer protection against oxidative damage, which is particularly important in times of high light radiation during the low tide (Leiva *et al*. 2015). Algae that occupy temporal habitats and

experience temperature shifts have been observed to have seasonal variation in their bacterial communities. This is documented in the microbiota of marine brown alga *Sargassum muticum* where warmer months were associated with a decrease abundance of bacteria from Proteobacteria and Actinobacteria, along with increase in Planctomycetes (Serebryakova *et al*. 2018). Some of these phyla also contain psychrophilic and psychrotolerant bacteria that thrive in extremely cold temperatures normally below 0°C and up to 15°C in some cases (De Maayer *et al*. 2014). Psychrophilic bacteria have evolved differential gene transcription depending on the temperature. Cold shock proteins (Csps) are one class of proteins that are upregulated in cold temperatures, and function by chaperoning RNAs and preventing mRNA breakage during transcription and translation (Lee *et al*. 2013). Such broad shifts in microbial community composition indicates that different metabolic functions are required at different temperatures, and useful enzymes produced by the different bacteria may be conferred to the algal host. Although there is no experimental evidence of increased cold-tolerance in Arctic seaweeds as a direct result of association with these microbes, there are clear differences in the bacterial profiles of algae occupying colder habitats. Additionally, presence of cold-resistant proteins in bacteria associated with algal microbiota has not been confirmed to directly result in cold tolerance of the host. This is an area of study that requires more research, as existing literature indicates that cold resistance could be the basis of some bacteria-alga symbiotic relationships.

1.2.3.2 - Microbes Associated with Salinity Tolerance

Changes in salinity levels are another environmental stressor that algae adapt to with innate cellular pathways, but their microbial communities may also confer some tolerance. As Dittami *et al*. (2016) observe, there are many studies that observe the salinity adaptations within the alga itself, but not many to do with changes to the bacterial community composition. Various

marine strains of brown alga *Ectocarpales* show positive correlations between certain bacterial species and adaptations to lower salinity levels than normal (Dittami *et al*. 2016). There were some differences in the genera present at the normal or lowered salinity levels, such as higher abundance of a bacterium from order Sphingomonadales in the bacterial communities of algae in the diluted water samples. However, the ratio of phyla abundance did not significantly change between the two experimental conditions (Dittami *et al*. 2016). This study concluded that although the bacterial community composition does shift with changing salinity, it may not provide any direct compounds to help the alga adapt to lower salinity (Dittami *et al*. 2016). Salt-tolerant bacteria have been observed to form close relationships with terrestrial plants and coral in conditions of increased salinity (Röthig *et al*. 2016; Ansari *et al*. 2019). In both alfalfa *Medicago sativa* and coral *Fungia granulosa*, salt-tolerant bacterium *Pseudomonas* sp. from family Rhodobacteraceae was strongly associated with host adaptation to the increased salinity stressor (Röthig *et al*. 2016; Ansari *et al*. 2019). A protein produced by *Pseudomonas* that directly contributes to salt tolerance has not been elucidated at this time. However, there is a consistently strong correlation between host salt tolerance and this bacterium in the literature which may also lend to algal adaptation to this stressor.

1.2.3.3 - Microbes Associated with Tolerance to Conductivity Changes

Another less-studied environmental stressor is changes in conductivity. Although this metric is related to salinity, it is also affected by any negatively charged anions such as chloride (Cl^-), nitrate (NO_3^-), sulfate (SO_4^{2-}), and phosphate (PO_4^{3-}), or positively charged cations such as sodium (Na^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) (EPA, 2012). Due to the variety of factors that can contribute to ion levels in a body of water, such as nutrient input from agricultural sources, it is difficult to accurately measure and use conductivity as a metric for stress. Perhaps

because of these difficulties, the effects of changing conductivity on algae or bacteria have not been well studied. In the freshwater green alga *Pseudokirchneriella subcapitata* (now known as *Raphidocelis subcapitata*), increased ion concentrations triggers a toxin response similar to that observed in high saline conditions (Simmons 2012). Bacterial response to conductivity changes is equally as unknown, due to the large number of factors that can affect bacterial community composition in an environment such as a freshwater lake. The monophyletic cluster SOL, a group of filamentous bacteria in the family Saprospiraceae, were studied to assess the differences in bacterial assemblage in lakes with low or high conductivity levels (Schauer *et al*. 2005). Ultimately, conductivity is an important stressor to consider in the case of freshwater algae such as *Bangia atropurpurea*. This alga has recently been observed to invade Lake Superior, which has lower conductivity levels compared to the other Great Lakes (Shea *et al*. 2014). The broad scope of conductivity changes makes it difficult to perform experimental analysis despite the potential role of ion changes in algal adaptation and invasion. Currently there is not enough literature to draw any conclusive inferences on the effect of changes in conductivity on microbiota compositions and functions.

1.2.3.4 – Algal Dependence on Microbial Synthesis of Vitamin B_{12}

The synthesis of vitamin B_{12}, otherwise known as cobalamin, can be accomplished by only a few bacterial and archaeal species, despite the necessity of this molecule as a cofactor for the synthesis of methionine in many organisms (Martens *et al*. 2002). Land plants and some fungi are not dependent on vitamin B_{12} because these organisms do not have cobalamin-dependent enzyme methionine synthase (METH) and they use a B_{12}-independent form of methionine synthase, METE, instead (Eichel *et al*. 1995). If a functional METE gene is present, the organism has no need for vitamin B_{12} and does not synthesize it. Many algae exhibit vitamin

B_{12} auxotrophy, as cobalamin is required for METH function but is not synthesized by the alga itself and must be acquired from external sources (Helliwell *et al*. 2011). A survey of algal genomes found that most algae from phylum Rhodophyta, including *Bangia fuscopurpurea* (Croft *et al*. 2005), and many algae from Chlorophyta, including *Cladophora glomerata* (Hoflmann 1990), require environmental cobalamin uptake for survival. Genomic sequencing of B_{12}-related genes by Helliwell *et al*. (2011) revealed that dependence on vitamin B_{12} could be an evolved characteristic in some algal species. For instance, auxotrophic green algae *Volvox carteri* and *Gonium pectorale* have a non-functional pseudogene for METE while a closely related B_{12}-independent alga, *Chlamydomonas reinhardtii*, has a functional METE gene, indicating that the METE gene could have undergone deleterious mutations throughout the evolution of the auxotrophic algae resulting in B_{12} dependency (Helliwell *et al*. 2011). Mutualism between auxotrophic algae and cobalamin-synthesizing bacteria has been demonstrated in a few species, such as between green alga *Lobomonas rostrata* and three bacteria from order Rhizobiales, *Mesorhizobiom loti, Rhizobium leguminosarum,* and *Sinorhizobium meliloti* (Kazamia *et al.* 2012). In a vitamin B_{12}-free culture, the alga was able to survive only in the presence of the cobalamin synthesizers (Kazamia *et al*. 2012). Although not all algae require a symbiotic relationship with bacteria to obtain vitamin B_{12}, this is a limiting factor for many species and requires mutualism with microorganisms.

1.2.4 - Algae of Interest, their Microbiota, and Habitats

In the present study, the microbiota of several North American algae were examined; *Bangia atropurpurea, Cladophora glomerata, Bangia fuscopurpurea, and Bangia maxima. B. atropurpurea* and *C. glomerata* are both freshwater algae found across the Laurentian Great Lakes (Lowe *et al*. 1982). The marine alga *B. fuscopurpurea* has been isolated from various

marine habitats, while closely related red seaweed *B. maxima* has been found only in a specific niche off the coast of California (Lynch *et al*. 2008). These algae are ecologically important within their ecosystems, but not all algal microbiomes or microbiota are characterized despite important functional symbioses and adaptation to environmental stressors.

1.2.4.1- *Bangia atropurpurea*

Bangia atropurpurea is an invasive species found in the Laurentian Great Lakes in North America (Lin & Blum 1977). This alga belongs to the evolutionarily complex order Bangiales (Rhodophyta), which are represented in the fossil record dating 1.2 to 1.6 billion years ago (Butterfield *et al*. 1990). Phylogenetic analyses based on *rbc*L and 18S rRNA genes showed that *B. atropurpurea* likely originated in European freshwater sources and were possibly transported to the Laurentian Great Lakes in North America via ballast water of ships (Müller *et al*. 1998). This was further corroborated by a karyological analysis that showed a difference in chromosome number and size between the freshwater and marine *Bangia* (Müller *et al*. 2003). The phylogenetic analysis of the *rbc*L and 18S rRNA genes also confirmed that the freshwater *B. atropurpurea* appears to be a distinct species from the marine *Bangia fuscopurpurea*, with an estimated divergence time of 174 to 265 million years (Müller *et al*. 1998).

Although there has not yet been any characterization of microbial communities of *B. atropurpurea*, its cellular structure may allow for unique communities of bacteria to form. The algal filaments consist of a thin layer of cells that are up to 50 microns in length and 6 to 10 microns in width (Belcher 1959). The filaments are very small and as of now, there is no literature indicating the presence of endogenous bacteria within *Bangia atropurpurea* or characterizing the exogenous microbial communities on its cell surface. However, it is possible that there are endophytic bacteria present within the cell wall, as this is demonstrated in

filamentous marine green algae in genus *Byropsis* (Hollants *et al*. 2011). The cell wall chemistry and composition of *Bangia atropurpurea* has been investigated in depth to determine if it this contributes to its salinity adaptations (Youngs *et al*. 1998). This is also a good indicator of what bacteria could prosper on the cell surface. In red algae, cell surface polysaccharides are mostly agars or carrageenans (Usov 1998). Carrageenans are highly sulfated sugars, which have been hypothesized to contribute to salinity adaptation (Aquino *et al*. 2004). In *B. atropurpurea,* the agars are mostly water soluble 3- and 4- linked galactosyl and 4-linked 3,6-anhydrogalactosyl. There are also 6-O-methyl galactosyl, and insoluble galactosyl and mannosyl residues (Youngs *et al*. 1998). A scanning electron microscopy study noted that this cell wall is unsuitable to the growth of eukaryotic epiphytes due to its mucilaginous surface but is hospitable for many bacterial species (Lowe *et al*. 1982). Apart from this, there have been no studies on the bacterial communities of *Bangia atropurpurea* and there is a considerable gap in molecular classification of the bacteria associated with the alga.

1.2.4.2 - *Cladophora glomerata*

Cladophora glomerata (Chlorophyta) is a freshwater green alga that occupies a similar habitat as *Bangia atropurpurea*. In the Laurentian Great Lakes, *C. glomerata* becomes a nuisance during the warmer months as it starts blooming uncontrollably (Smith *et al*. 2019). Despite growing in the same lakes, *B. atropurpurea* and *C. glomerata* blooms rarely co-occur as the former grows more in the winter months and closer to the shore, while the latter thrives during the summer in deeper waters about 1 meter below the surface (Lowe *et al*. 1982). In terms of morphological characteristics, *C. glomerata* is also a filamentous alga that is made up of cylindrical cells 100-400 microns long and 40-100 microns in diameter (Johnson *et al*. 1996). This alga is not only a host to bacterial epibionts, but it also provides ideal breeding surface for other algal and eukaryotic

epiphytes (Lowe *et al*. 1982; Higgins *et al*. 2008). During its growth stage, *Cladophora* is a massive nutrient sink for carbon, phosphorus, and nitrogen, but in its decomposition stage of life it releases these molecules back into the water (Higgins *et al*. 2008). The cell wall of *Cladophora glomerata* has a few unique features which fosters distinct epiphytic species. Its cell wall consists of two layers; the inner wall is cellulose and the outer wall is mostly pectin and chitin. Interspersed throughout the cell wall are sulfated polysaccharides called ulvans, a feature that is shared among algae of class Ulvophyceae (Pankiewicz *et al*. 2016). Cell surface macromolecules on both green algae (ulvans) and red algae (agar, carrageenans) are known to have a wide range of biological activities such as anticoagulation, anticancer, and antiviral (Duarte *et al*. 2004; Surayot *et al*. 2016). In a microbiome study on closely related marine green alga *Ulva spp.,* 235 associated bacterial species had enzymatic activity for cellulose and ulvan degradation (Odaneth 2017). These bacteria spanned 10 known genera and did not seem to have any other enzymatic role, which may indicate that ulvan breakdown is the specialized function of these bacterial taxa (Odaneth 2017). Although this test was not conducted for *C. glomerata,* it is very likely that specialized polysaccharide-degrading bacteria are present on the cell surface of this alga.

Of all freshwater algal microbiome and microbiota studies, many have focused on *Cladophora glomerata*. One study noted that the epiphytes are vital for processing many substrates produced by *C. glomerata* (Zulkifly *et al*. 2012). The sequences observed spanned 9 phyla, mostly within Bacteriodetes and Proteobacteria but also included taxa within Cyanobacteria, and some other unknown bacteria (Zulkifly *et al*. 2012). The bacterial functions include cellulose degradation, methane oxidation and nitrogen fixing, which are maintained by a vast variety of bacterial species. The epiphytic community on *C. glomerata* contained several genera of bacteria that have nitrogen-fixing capabilities, including *Dechloromonas, Blastobacter,*

and *Devosia* (Zulkifly *et al*. 2012). Similarly, mutual nutrient transfer was inferred due to the presence of the bacterial genus *Cetobacterium* which is known to produce vitamin B_{12}, a molecule that is required for the growth of certain algae (Croft *et al*. 2005). Although 16S rRNA gene classification of the epibiome provided a wealth of information, whole-genome shotgun sequencing was not performed to confirm presence of the relevant enzymes that play a role in mutualism as hypothesized in this study.

A later study by the same authors delved deeper into the total epiphytic community by performing a metagenomic analysis of 16S, 18S and 28S rRNA sequences (Graham *et al*. 2015a). The 18S and 28S sequencing revealed a much larger group of potentially symbiotic eukaryotic organisms ranging from diatoms, protists, and fungi (Zulkifly *et al*. 2012; Graham *et al*. 2015a). Again, from the taxonomic classification the presence of certain interactions and hence postulated metabolic genes were inferred using protein databases. This analysis yielded evidence of nitrogen-fixing and cellulose processing genes in the bacterial species present on *Cladophora* (Graham *et al*. 2015a). The two studies on the microbial community of *C. glomerata* provided evidence for a vastly diverse epiphytic community of both prokaryotic and eukaryotic symbionts, as well as functionality and purpose of these epiphytes (Graham *et al.* 2015a). Although determining functional genes by using protein databases presented more information, the findings were limited by inference and lack of whole-shotgun sequencing. Ideally, whole-shotgun sequencing would provide evidence of specific functional genes present in the organism. This would remove the need for inference and allow for conclusive findings. However, determining the microbial community composition using 16S rRNA gene sequencing and inferring microbial function is the first step in finding microbes of interest and possible interactions with their host.

1.2.4.3 - The Laurentian Great Lakes

Bangia atropurpurea and *Cladophora glomerata* occupy similar niches in the Laurentian Great Lakes, a collection of five lakes in North America that covers a surface area of 244,000 km^2 (Paver *et al*. 2020). Changes in abiotic factors, such as ion concentrations and nutrient input, within these lakes can result in algal adaptations and restructuring of microbial communities in the water column and host microbiota (Paver *et al*. 2020; Mahdiyan *et al*. 2021). Introduction of invasive species into the Great Lakes since the 1800's has changed the natural food webs and ecosystem, and has far reaching effects felt to this day (Mills *et al*. 1993; Ricciardi & MacIsaac 2000). These factors also have significant impacts on the microbial compositions of the Laurentian Great Lakes (Paver *et al*. 2020).

Long term trends of ion concentrations in the Great Lakes show significant increases of Na^{1+}, $Ca2^{+}$, Mg^{2+}, Cl^{-}, and SO_4^{2-} in Lake Ontario between 1965 and 2009 (Chapra *et al.* 2012). This translates into a significant upwards trend in conductivity in Lake Ontario, a trend also noticeable in Lakes Eerie and Michigan. Lake Superior has experienced the least changes in ion concentrations within this time frame due to fewer anthropogenic impacts in this region (Chapra *et al.* 2012). However, a survey of *Bangia atropurpurea* distributions across the Great Lakes between 1995 and 2002 revealed an increase in Lake Superior conductance, from 60 to 110µS/cm in 1995 to a range of 110 to 130µS/cm in 2002 (Shea *et al.* 2014). Even though the conductivity levels of Lake Superior are still relatively low (Poghosyan & Sturchio, 2015), the positive trend suggests that Lake Superior may become an ideal environment for *Bangia atropurpurea* to invade. This further invasion into more ionized water can be bolstered by the bacterial communities of this alga. In both freshwater and marine algae, bacteria play an

important role in algal health and survival, so it is feasible that the microbiota of *Bangia atropurpurea* could contribute to its success in occupying new environments.

The water quality of the Laurentian Great Lakes has also been affected by nutrient input, namely phosphorus, nitrogen, and dissolved organic carbon (DOC) (Mahdiyan *et al.* 2021). The temporal study of water quality in the Great Lakes from 1976 to 2011 showed some important trends. Primarily, DOC concentrations increased in Lakes Ontario, Eerie, Huron, and Michigan, and is significantly correlated with a steady rise in temperature over the years (Mahdiyan *et al.* 2021). Concentrations of phosphorus and nitrogen are steadily declining, due to mandated changes in human practices such as the 1972 US-Canada Water Quality Agreement (Dove & Chapra 2015). The decline in nutrient concentration is not equal for phosphorus and nitrogen. Lake Ontario is experiencing an overall large rate of phosphorus decrease, but there is still a slight total nitrogen increase in some areas of this lake (Mahdiyan *et al.* 2021). Overall, maintaining water quality of the Great Lakes has important ecological implications for algal growth.

Distinct microbial communities have been observed within the water columns of different Great Lakes (Paver *et al.* 2020). Certain bacterial taxa were significantly enriched depending on the lake, likely due to the wide variation in abiotic environmental factors (Paver *et al.* 2020). For example, the lower lakes (Ontario and Eerie) had a higher abundance of bacteria from Actinobacteria, while the upper lakes (Huron, Michigan, and Superior) were enriched in certain Betaproteobacteria and Verrucomicrobia oligotypes (Paver *et al.* 2020). There was also a higher abundance of a certain actinobacterial oligotype in the warmer, well-lit, and higher nutrient waters of Lakes Eerie and Ontario, while not being detected in any of the upper lakes (Paver *et al.* 2020). These findings indicate that depth, light availability, temperature, and nutrient

availability highly correlate with the presence of certain bacterial taxa. Since these factors may vary seasonally and daily, the microbial composition of the water column is subject to changing throughout the day and year. This is an important aspect to consider in future algal microbiome and microbiota research as seasonal and daily samples may provide greater insight into the resident microbial communities.

1.2.4.4 - Marine Red Algae *Bangia fuscopurpurea* and *Bangia maxima*

Bangia fuscopurpurea and *Bangia maxima* are marine red algae belonging to phylum Rhodophyta. While *B. fuscopurpurea* has been observed to occupy many marine habitats from temperate to subtropical regions, *B. maxima* has only been confirmed to grow on littoral boulders in Bolinas Bay, California (Gardner, 1927; Lynch et al., 2008). The samples collected by Lynch *et al*. (2008) showed that within this bay in California, *B. fuscopurpurea* is growing a few meters away from the *B. maxima* site. Despite phylogenetic and geographical similarities, *B. maxima* has some distinct morphological features such as large multiseriate filaments that form hollow tubes, which differ from the smaller filaments of *B. fuscopurpurea* (Lynch *et al.* 2008). Phylogenetic analysis supported by *rbc*L gene and nSSU rRNA gene sequencing revealed that *Bangia maxima* and *Bangia fuscopurpurea* collected from the same sample site are extremely closely related and fall within one clade (Lynch *et al.* 2008). Due to phylogenetic closeness of these two algae based on *rbc*L sequences and their geographic closeness, it is postulated that *B. maxima* evolved from an eastern Pacific population of *B. fuscopurpurea* (Lynch *et al.* 2008). In addition, the geographical dispersal of *B. fuscopurpurea* across four distinct clades indicates that there is likely multiple cryptic species within this taxa and could be distinguished with further phylogenetic analysis (Müller *et al.* 2003; Lynch *et al.* 2008).

The microbiota of *Bangia fuscopurpurea* and *Bangia maxima* have not yet been examined. However, closely related marine alga *Porphyra umbilicalis* (Rhodophyta) has had its bacterial community characterized which may provide some insight into the microbiota of marine *Bangia*. Originally, *Bangia* and *Porphyra* were paraphyletic genera, but with the advent of molecular techniques, it was possible to clarify this phylogeny (Sutherland *et al.* 2011). Using the nSSU rRNA gene and plastid *rbc*L gene, the systematics of the Bangiales order was refined to resolve the non-monophyly of the *Bangia* and *Porphyra* genera, but with a particular emphasis on the foliose genera (Sutherland *et al.* 2011). Marine *Bangia* and *Porphyra* both inhabit the intertidal zone and experience high levels of oxidative, radiation, and desiccation during low tide, which may result in similar cellular adaptations to these stressors (Brawley *et al.* 2017). The surface of *B. fuscopurpurea* mainly contains galactans consisting of DL-galactose, 3,6-anhydro-L-galactose and 6-O-methyl-D-galactose (Wu & Ho 1959). The polysaccharide most common in *Porphyra* is porphyran, which is sulfated and mainly consists of 3-linked α-L-galactosyl and 4-linked β-D-galactosyl residues (Morrice *et al.* 1984). Agar and carrageenan polysaccharides on *Bangia atropurpurea* and *Bangia fuscopurpurea* are very similar in structure to porphyran (Jiang *et al.* 2019). Although such research has not been conducted on *B. fuscopurpurea*, similarities in polysaccharide structure could yield formation of some similar commensal relationships on the surfaces of *B. fuscopurpurea* and *P. umbilicalis*.

The bacterial community associated with *Porphyra umbilicalis* is well described in the literature (Miranda *et al.* 2013; Kim *et al.* 2016). In one study, seven phyla were represented in the microbiota; Bacteroidetes, Proteobacteria, Actinobacteria, Chloroflexi, Plantomycetes, Firmicutes, and Deinococcus-Thermus (Miranda *et al.* 2013). The most abundant bacteria associated with this alga from phyla Bacteroidetes, Proteobacteria, and Planctomycetes are

known for their abilities to digest the polysaccharides of the cell wall. Another study further examined the diverse range of bacteria from phylum Planctomycetes that are associated with *P. umbilicalis* (Kim *et al.* 2016). Despite being in smaller proportions within the bacterial community, Planctomycetes were able to survive when *P. umbilicalis* blades were treated with antibiotics in the lab which indicates potential antibiotic-resistance genes and possibly a robust association with the alga (Kim *et al.* 2016). Initial 16S rRNA gene sequencing resulted in the differentiation of three unique Operational Taxonomic Units (OTUs) on the genus level: *Rhodopirellula* and two unknown genera. A further whole-genome sequencing allowed for metagenomic analysis of these taxa which revealed specific gene products. Overall, several sequences were found within these bacteria that contain sulfatase domains or polysaccharide-degrading properties (Kim *et al.* 2016). Additionally, the authors searched for selenoprotein sequences, which have been observed to arise during stressful conditions. Two of the Planctomycetes OTUs contained all of the genes required to create selenoproteins, which may indicate an adaptive benefit when in a highly oxidative environment (Kim *et al.* 2016). The presence of such planctomycetal species with these functional genes in *Porphyra umbilicalis* provides some indication that there is a potential symbiotic relationship between alga and bacteria.

1.3 –Phylogenetic Analyses of Bacterial Communities Using the 16S rRNA Gene

Since the discovery of the universally conserved 16S rRNA gene that codes for a portion of the small subunit ribosomal RNA (SSU rRNA), it has been used as a marker for taxonomic classification of all Bacteria and Archaea (Woese & Fox 1977). Prior to the advent of nucleotide sequencing, morphological characteristics were used to classify bacterial species. Sequencing of

the 16S rRNA gene simplifies taxonomic classification and provides insight into bacterial evolution (Woese 1987).

1.3.1 –Classification of Bacteria with 16S rRNA Hyper-variable Regions

The 16S rRNA gene sequence encodes a portion of the SSU rRNA and contains hyper-variable and highly conserved regions, which makes it possible to develop primers for PCR amplification and use these fragments to generate phylogenetic comparisons (Yang *et al.* 2016). Amplifying a large portion of the 16S rRNA gene was initially thought to be the most accurate way to get an accurate alignment, but through an experimental process it was determined that targeting a shorter piece of the 16S rRNA gene sequence yields better matches (Jenkins *et al.* 2012). These shorter variable regions are numbered from V1 to V9, and each region has varying degrees of accuracy in representing diversity of a bacterial community when amplified and used in sequence alignments (Kim *et al.* 2011; Yang *et al.* 2016). One or more of these variable regions can be amplified, but experimentally it was found that using three consecutive variable regions, V1-V4 or V4-V6, provided the best phylogenetic resolution of a bacterial community (Kim *et al.* 2011; Yang *et al.* 2016). Using either of these variable sub-regions provides unique advantages to conducting a phylogenetic analysis of a microbial community. V1-V4 regions are more divergent, as a result it is more sensitive to a highly diverse level of bacterial species that are present in smaller quantities (Kim *et al.* 2011). Regions V4-V6 were found to be ideal for identification of bacteria at the phylum level and discovery of novel phyla (Yang *et al.* 2016). Algal metagenome studies vary in terms of variable regions of the 16S rRNA gene that are amplified; some use V1-V3 regions (Zulkifly *et al.* 2012), others use V5-V7 and V8-V9 (Miranda *et al.* 2013). For the purposes of this project, V4 will be used in the current study.

Although using this region will not produce the most in-depth resolution of bacterial taxonomy, it is satisfactory for this preliminary characterization of the bacterial communities of algae.

1.3.2 – Construction of Phylogenetic Trees using Amplicon Sequence Variants

The amplified sequence of 16S rRNA gene is used to conduct taxonomic profiling and construction phylogenetic tree. In the past, this was done by grouping similar sequences that had a certain threshold of dissimilarity into Operational Taxonomic Units (OTUs), which represent a taxonomic level (i.e species). There are many glaring issues with this system, like the reliance on a thorough reference database to construct the OTUs (Callahan *et al.* 2017). OTUs are clusters of sequence reads that are grouped based on the percent of nucleotides that the sequences have in common, where the similarity threshold for an OTU is chosen somewhat arbitrarily. Amplicon sequence variants (ASVs), on the other hand, rely on single nucleotide differences which will show individual un-grouped sequences that are not yet in the reference database thus displaying the entire diversity of a microbial community (Callahan *et al.* 2017). ASVs are also more easily reproducible and data sets of the same genetic locus can be combined, and result in accurate phylogenetic trees (Callahan *et al.* 2017). In an analysis of a microbial community, it is important to give equal representation of each isolate regardless of abundance. This will prevent rare species from being lost in the community data. Using ASVs in the construction of phylogenetic trees will show the full breadth of the bacterial community within each sample.

1.4 – Objectives

The primary focus of this study is to characterize the bacterial communities of *Bangia atropurpurea* to gain more information about the factors affecting algal function. Algal microbiota are poorly represented, especially red freshwater algae such as *B. atropurpurea.* This research will help supplement future studies on the effects of microorganisms on algal function.

The first goal is to use 16S rRNA gene analyses to classify the bacteria in the microbiota associated with various samples of freshwater and marine algae, including *Cladophora glomerata, Bangia fuscopurpurea* and *Bangia maxima*. Phylogenetic trees were created to closely examine the taxonomies of bacterial ASVs from certain phyla. These analyses will allow for the characterization of potential core microbiota of each algal sample. In the scope of this study, a core microbiota or bacterial community refers to the bacteria that are likely consistently associated with the algal host. This is the first time that the microbiota of *B. atropurpurea* and *B. fuscopurpurea* have been characterized to any extent.

The second objective is to compare algal microbiota across time, species, and environment. *Bangia atropurpurea* is the central focus of these comparisons to define its microbial communities in the scope of other algae based on similarities in taxonomy or habitat locale. The comparisons of the algal microbiota were done by finding overlap in bacteria and unique genera between pairs of algal samples. Temporal differences in the bacterial microbiota of *B. atropurpurea* were examined by comparing two microbial communities from this alga collected 14 years apart. Alga-specific associations of the microbiota were investigated using data from *B. atropurpurea* and *C. glomerata*. A bacterial species that is unique to an algal taxon could indicate a co-evolutionary relationship or symbiotic association that potentially affects the function of the alga. Finally, bacterial assemblages specific to the environment of the alga were explored by comparing *B. atropurpurea* and *B. fuscopurpurea*. Examining the differences and similarities in broad microbial community composition and specific bacterial taxa will shed more light on how bacteria assemble based on species and location, as well as what significant changes to algal microbiota may occur over many years.

Chapter 2: Materials and Methods

2.1 – Algal Samples

Fresh algal filaments were not collected at a field site due to COVID-19 restrictions. Frozen samples were selected from the -80°C long-term storage freezer of the Müller lab in University of Waterloo, Ontario. The five samples were chosen to cover a range of taxa, sample time and locality, as shown in table 1.

Table 1 Summary of algal samples chosen from the Müller lab archival freezer. Factors for sample choice were taxonomy, seasonality, and locality. Samples range from freshwater to marine environments and cover red and green algae. Most samples were sourced in summer months, except for the marine *Bangia fuscopurpurea*.

Taxa	**Sample Code**	**Location of Sampling**	**Date of Sampling**
Bangia maxima	BAN-MAX	Marin Country, California (Pacific Ocean)	June 6th, 2004
Bangia atropurpurea	BLO-02	Burlington, Ontario (Lake Ontario)	August 29th, 2002
Bangia atropurpurea	BLO-16	Burlington, Ontario (Lake Ontario)	August 8th, 2016
Cladophora glomerata	CLAD-ON	Burlington, Ontario (Lake Ontario)	June 2nd, 2005
Bangia fuscopurpurea	NF-F	Ferryland, Newfoundland (Atlantic Ocean)	November 22nd, 1998

All samples were in the form of algal filaments, except for *Bangia maxima* which was a frozen DNA extraction from this alga. Each sample contained several filaments collected from one site at the location indicated in Table 1. Algal sampling followed protocols outlined in previous publications (Lynch *et al*, 2008; Müller *et al*, 1998). This included the manual cleaning of filaments under a microscope to remove other macroalgal species. The samples were rinsed with sterile lake water, and then stored in the long-term freezer until DNA extraction.

2.2 - DNA Extraction and Sequencing

DNA was extracted using the Qiagen DNeasy Plant Mini Kit, from which the protocol was followed with no modifications. Filaments from each algal sample were further frozen with liquid nitrogen to allow for pulverization into a fine powder with a mortar and pestle. Samples were placed in respective test tubes and further lysed with TissueRuptor and TissueLyser from the DNeasy kit. DNA was progressively extracted through a series of centrifugations at room temperature and short incubations at 65°C. The only exception to these extraction steps was *Bangia maxima,* for which the extraction process followed a phenol-chloroform protocol (Lynch *et al.* 2008). This method of DNA extraction does not seem to result in significantly different quantities or qualities of bacterial genomic material compared to other commercially available kits (Janabi et al., 2016). Extracted DNA was tested for quality and quantity using a Nanodrop. Entire sample DNA was extracted, including the genetic material of the alga itself and any microbial species present on the outside or within the filament. Of each DNA extraction, a 20µL sample was sent to Metagenom Bio Inc. (Waterloo, Ontario, Canada) for isolation of the V4 region of the 16S rRNA gene using PCR. Amplicon sequences were generated using the Illumina MiSeq technology with primers 515F (5' GTGYCAGCMGCCGCGGTAA 3)' (Parada *et al.* 2016) and 806R (5' GGACTACNVGGGTWTCTAAT 3') (Apprill *et al.* 2015). In the present study, using only the V4 region was more cost-effective based on primer availability and was sufficient for the objectives of this experiment.

2.3 - Data Analysis

Paired-end amplicon sequences were provided by Metagenom Bio Inc. in raw fastq files, as well as the table summaries of taxonomy and amplicon sequence variants. To ensure quality of data, the raw sequences were denoised, truncated, and filtered manually in Quantitative

Insights into Microbial Ecology (QIIME2) (Bolyen *et al.* 2019). Once 16S rRNA data was processed, it was uploaded into R (R Core 2020) for further analysis. After this analysis, sequences from compelling phyla or classes were isolated to incorporate in a taxonomic tree in Molecular Evolutionary Genetics Analysis across computing platforms, version 10 (MEGAX) (Kumar *et al.* 2018).

2.3.1 - QIIME2 Workflow

Paired-end amplicon sequences were uploaded into QIIME2. The sequences were first demultiplexed and then primers were trimmed using the DADA2 plugin. With the same plugin, sequences were further filtered, denoised, merged, and chimeras were removed. The remaining sequences were sorted into individual ASVs with unique identifiers. Taxonomic classification of the ASVs was completed using a pre-existing model that was trained on the Silva 138 99% OTUs from the V4 region of the 16S rRNA gene (Bokulich *et al.* 2018). The full resulting taxonomies, from phylum to species, were transferred and further analysed in Microsoft Excel to ensure that all ASVs were properly classified. Within the genera and family taxonomies, those that were labelled as "uncultured" or had no corresponding taxonomy based on the SILVA database were individually classified with the NCBI BLAST database, specifically using the BLASTn algorithm (Altschul *et al.* 1997). Any other errors or misclassification were fixed at this time as well.

2.3.2 - R Workflow

After finalizing sequence classification in Microsoft Excel, the database containing the taxa and taxon counts for each sample was transferred to R. There were three analyses completed in R. The chloroplast and mitochondria sequence counts were removed from the database for all analyses.

First, Venn diagrams were generated to display similarities and differences of unique genera across the five samples (Hanbo 2018). The samples were divided into a few categories. First the distribution of bacterial genera were compared between marine red algae (*Bangia fuscopurpurea* and *Bangia maxima*), freshwater red algae (*Bangia atropurpurea* 2002 and 2016), and freshwater green algae (*Cladophora glomerata*). Bacterial members found in both microbiota of the *Bangia atropurpurea* samples from 2002 and 2016 were examined to test for change in diversity or community composition over time. These bacteria were also characterized in an effort to elucidate a core microbiota of this alga. The *Bangia atropurpurea* sample from 2002 was also compared to the *Bangia fuscopurpurea* sample from 1998 to examine differences in bacterial compositions across aquatic habitats. Finally, the 2002 *Bangia atropurpurea* microbiota was examined in relation to the 2005 *Cladophora glomerata* microbial community to compare microbes in different algal species inhabiting the same location. The 2002 *Bangia atropurpurea* sample was used for many of these analyses as it was collected at a nearer time to the algal specimens it was compared to (see table 1 for exact date of sampling). For each of these groups, taxon counts from the database were sorted to determine which bacteria were present in both or just one of the samples in question. These values were used to construct Venn diagrams with the aid of the VennDiagram package in R and colourblind-friendly colours were chosen with the RColorBrewer package (Hanbo 2018). The corresponding bacterial taxa for each pairwise comparison and for the three-category comparison were exported into Microsoft Excel to create supplementary table 4 (Microsoft 2021).

Next, abundance plots were generated to display the relative abundances of the genera and the phyla using ggplot2 (Hadley 2016). Relative abundance of each ASV was determined manually and data was reorganized with functions from the Tidyr package and plotted using the

ggplot2 package (Hadley 2016). Several abundance plots were created: relative abundances of all phyla, relative abundances of all genera, and relative abundances of genera from phyla Proteobacteria, Bacteroidetes, Cyanobacteria, and Plancyomycetes.

Lastly data were rarefied and alpha diversity was determined, in order to visualize the diversity within and among the samples (Cameron *et al.* 2020). The rarefaction process was accomplished with the phyloseq and *mirlyn* packages (McMurdie & Holmes 2013; Cameron *et al.* 2020). The *B. maxima* sample was removed from this data due to extremely low quantities of unique bacterial taxa which would have skewed the rarefaction and alpha diversity results. Data was rarefied with a replication of 1000 and no replacement of subsampling. Alpha diversity values were generated from this rarefied database using the Shannon diversity index.

2.3.3 - MEGAX Workflow

ASVs from phylum Cyanobacteria, PVC superphylum, and phylum Bacteroidetes were further examined in a phylogenetic tree using MEGAX (Kumar *et al.* 2018). The closest matches of 16S rRNA sequences from the NCBI BLAST database were added to the alignment for each ASV. The sequences for each tree were aligned with ClustalW. Evolutionary history in the phylogenetic trees was inferred using the maximum likelihood method with a bootstrap value of 1000 and the 16S rRNA V4 region sequence from *Escherichia coli* was the outgroup for each tree. The best model for each tree was selected based on the lowest Bayesian Information Criterion (BIC) score. The best model for determining evolutionary history of the Cyanobacteria and the PVC trees was the Kimura 2-parameter model, with a discrete Gamma distribution to model evolutionary rate differences. The best model for determining evolutionary history of the Bacteroidetes tree was the General Time Reversible model, with a discrete Gamma distribution to model evolutionary rate differences (Kimura 1980; Kumar *et al.* 2018).

Chapter 3: Results

3.1 - DNA Extraction and Sequence Reads Quality

Quality and concentration of DNA was tested using a nanodrop spectrophotometer, for which the values are shown in table 2. *Bangia maxima* (BAN-MAX) and *Bangia fuscopurpurea* (NF-F), both of which are marine samples, had the highest concentration of genetic material. The three freshwater samples, *Bangia atropurpurea* (BLO-16 and BLO-02) and *Cladophora glomerata* (CLAD-ON), had the lowest amounts of DNA. The A260/280 ratios indicate purity of the sample, with a ratio of around 1.8 suggests a pure DNA sample and deviations from this ratio could be caused by changes in the pH of the solution (Thermo Scientific 2012). Although a discrete cut-off has not been established, generally a ratio of 1.8±0.2 at this absorbance indicates an acceptable level of DNA purity (Lucena-Aguilar *et al*. 2016). The BLO-02, BLO-16, and CLAD-ON samples contained less pure DNA extractions based on their A260/280 readings.

Table 2 Nanodrop values and DNA concentration for the extracted DNA of each sample. Concentration of DNA indicates quantity of DNA present which was taken from absorbance readings at 260/280 nm.

Alga	**Sample**	**Concentration of DNA (ng/µL)**	**A260/280**
Bangia maxima	BAN-MAX	51.6	1.78
Bangia atropurpurea	BLO-02	8.8	2.13
Bangia atropurpurea	BLO-16	8.5	2.08
Cladophora glomerata	CLAD-ON	13.5	1.53
Bangia fuscopurpurea	NF-F	32.3	1.82

Initial read counts, shown in table 3, ranged from 4829 to 23,914 forward sequences with a mean of 12,730 and a total of 63,651 forward reads. There were an equal number of reverse reads for each sample. Denoising and trimming primers with DADA2 resulted in a mean sequence length of 253 base pairs (bp). Less than 2% of sequences had a length of 252 bp or 254 bp. DADA2 statistics also show percent of reads that passed through filtration, denoising,

merging and removal of chimeras (Mohsen *et al.* 2019). *Bangia atropurpurea* from Lake Ontario in 2002 and 2016 (BLO-02 and BLO-16) had the highest percent of reads after filtration (95.18% and 96.85%, respectively). *Cladophora glomerata* (CLAD-ON) contained slightly more chimeras and other repeated sequences that were subsequently filtered out or merged.

Demultiplexed and filtered sequence reads produced a total 294 amplicon sequence variants and after further narrowing down to the genus level, 152 unique ASVs remained. Demultiplexing reads allows categorization and assignment of raw sequence data to their respective samples of origin. Additionally, sequence reads are filtered to remove low-quality sequences. Despite the *Bangia maxima* (BAN-MAX) sample having a large number of reads, these corresponded to only three unique ASVs in two bacterial phyla (table 3), whereas the other algal samples had a larger diversity of bacterial taxa. The *Bangia atropurpurea* samples had a similar number of bacterial phyla, but *B. atropurpurea* 2016 (BLO-16) had a markedly lower genus diversity compared to the 2002 sample (table 3). Of the 152 ASVs that were classified within QIIME2 using a classifier trained with the SILVA database, 22 ASVs did not have a close taxonomic match within this classifier (Bokulich *et al.* 2018). These missing ASVs were classified manually using the NCBI database, specifically the MegaBLAST algorithm within the BLASTn database (Altschul *et al.* 1990; Morgulis *et al.* 2008). This successfully established a sufficiently close taxonomic match at the genus level with an E-value of $<1e^{-50}$, indicating a high-quality match but does not confirm exact taxonomy (Altschul *et al.* 1990). Some taxonomic classifications that were completed with the SILVA database were incorrect and were corrected using the NCBI database. For example, 15 sequences of order Burkholderiales were incorrectly classified as class Gammaproteobacteria rather than Betaproteobacteria. The correct taxonomic

names were confirmed with the List of Prokaryotic names with Standing in Nomenclature (LPSN) database (Parte *et al.* 2020).

Table 3 Sequence counts for forward and backward reads for each sample after demultiplexing in QIIME2. Filtration through DADA2 includes denoising, merging and removing chimeric reads. Percent of initial reads indicates the number of reads left after all filtration is complete.

Sample	Forward & Reverse Read Count Before Filtering	Forward & Reverse Read Counts After all Filtration	Percent of Initial Reads	Number of Unique Phyla	Number of Unique Genera
BAN-MAX	23914	22274	93.14	2	3
BLO-02	16925	16109	95.18	8	62
BLO-16	6327	6128	96.85	7	26
CLAD-ON	4829	4317	89.4	11	68
NF-F	11656	10794	92.6	8	46

3.2 - Distribution of Bacterial Taxa Across Algal Microbiota

This study aims to examine the microbiota of *Bangia atropurpurea* in several perspectives, and to characterize the bacterial community of this alga for the first time. This involves various analyses to compare the microbiota of other freshwater and marine algae, including *Bangia maxima, Bangia fuscopurpurea,* and *Cladophora glomerata*. First, a sample-wide comparison of the microbiota was conducted between the three broad categories of samples: freshwater red algae, marine red algae, and the freshwater green alga. Next, comparisons were made between pairs of algal samples, with *B. atropurpurea* as the focus of each analysis. All pairwise analyses involves the 2002 sample of *B. atropurpurea* due to the higher diversity and similar sample collection dates. The temporal analysis aimed to examine the differences in the *B. atropurpurea* microbiota from 2002 to 2016. The alga-specificity analysis was done with the 2002 *B. atropurpurea* sample and the *C. glomerata* sample. Finally, environment-specificity of the microbiota was analysed with *B. atropurpurea* from 2002 and *B.*

fuscopurpurea. Based on an alpha diversity analysis (Figure 1), microbiota from *Cladophora glomerata* and *Bangia fuscopurpurea* had the highest diversity. The bacterial communities of both *B. atropurpurea* samples had lower diversities. *Bangia maxima* was omitted from the pairwise analyses due to low number of ASVs but was included in the 3-category analysis (Figure 2). These investigations into the bacterial communities of algae aim to provide a fuller understanding of the *Bangia atropurpurea* microbiota in three different scopes.

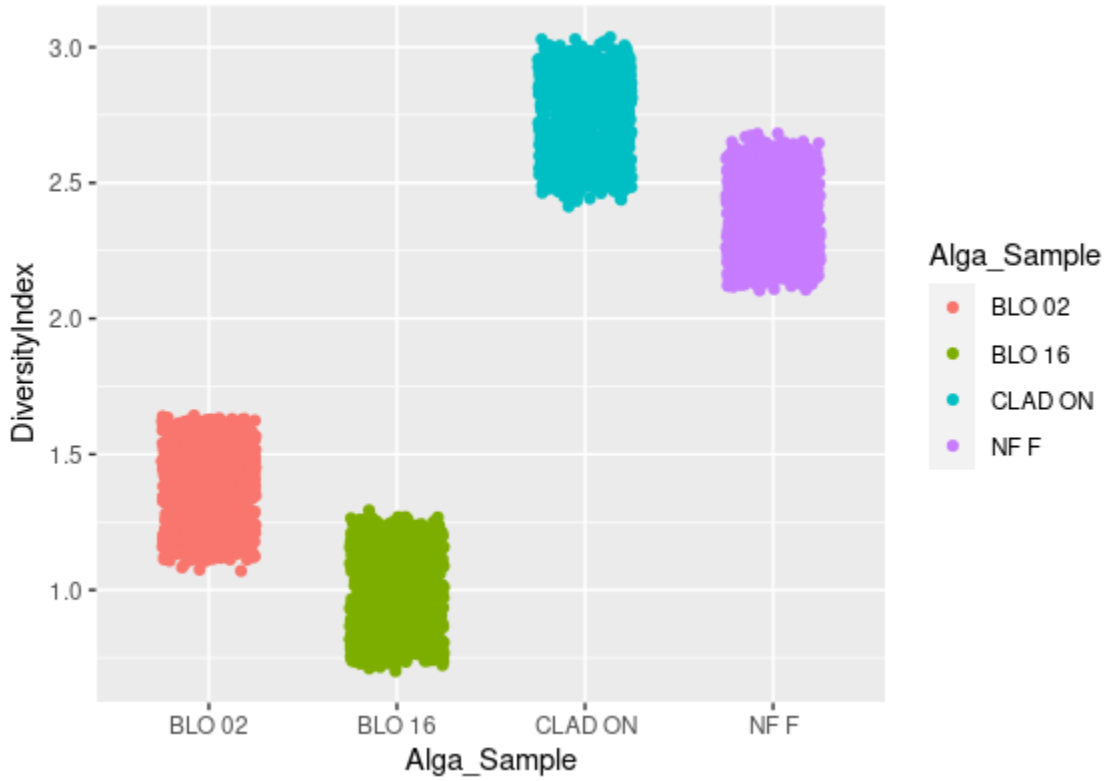


Figure 1 Alpha diversity of microbial genera from 2002 and 2016 samples of *Bangia atropurpurea*, *Cladophora glomerata*, and *Bangia fuscopurpurea.* Diversity analysis was constructed with the Shannon index using the *mirlyn* package (Cameron *et al.* 2020) in R. BLO 02: *Bangia atropurpurea* (2002), BLO 16: *Bangia atropurpurea* (2016), CLAD ON: *Cladophora glomerata*, NF F: *Bangia fuscopurpurea.*

3.2.1 - Bacterial Phyla in Freshwater Red, Freshwater Green, and Marine Red Algae

The unique bacterial genera as well as the overlap in genera of across three categories of algae are shown in figure 2A. The categories of this Venn diagram are freshwater Rhodophyta

(*Bangia atropurpurea* 2002 and 2016), marine Rhodophyta (*Bangia fuscopurpurea* and *Bangia maxima*), and freshwater Chlorophyta (*Cladophora glomerata*). Figure 2B illustrates the distribution of bacterial genera across phyla and groups of algae. Only two unique bacterial genera were observed across all groups of algae; *Lewinella* and *Gemmobacter* of phyla Bacteroidetes and Proteobacteria, respectively. These two phyla were also the most abundant and well distributed amongst all samples, except for *B. maxima*. Genera from phyla Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Verrucomicrobia were observed in all three groups of algae. The groups of algae that do not occupy the same environments have very little overlap in bacterial community members. The marine and freshwater red algae were observed to have 2 bacterial genera in common, both from Bacteroidetes. *B. fuscopurpurea* and *C. glomerata* had an overlap of 6 genera, from Proteobacteria and Bacteroidetes. Cyanobacteria were primarily found in the freshwater red algae, with one cyanobacterial isolate in *B. fuscopurpurea* and none in *C. glomerata*. Bacteria from phylum Planctomycetes were observed in *C. glomerata* and *B. fuscopurpurea*, but not in the freshwater *Bangia* samples. Phyla Acidobacteria and Deinococcus-Thermus were only observed in the freshwater samples. These broad trends in bacterial community compositions indicate the compositions of the putative core microbiota, or overlap in bacterial species, within each group of samples, and bacteria that are unique to each category. However, there also appears to be some overlap in bacterial genera between samples and across all three groups of algal samples.

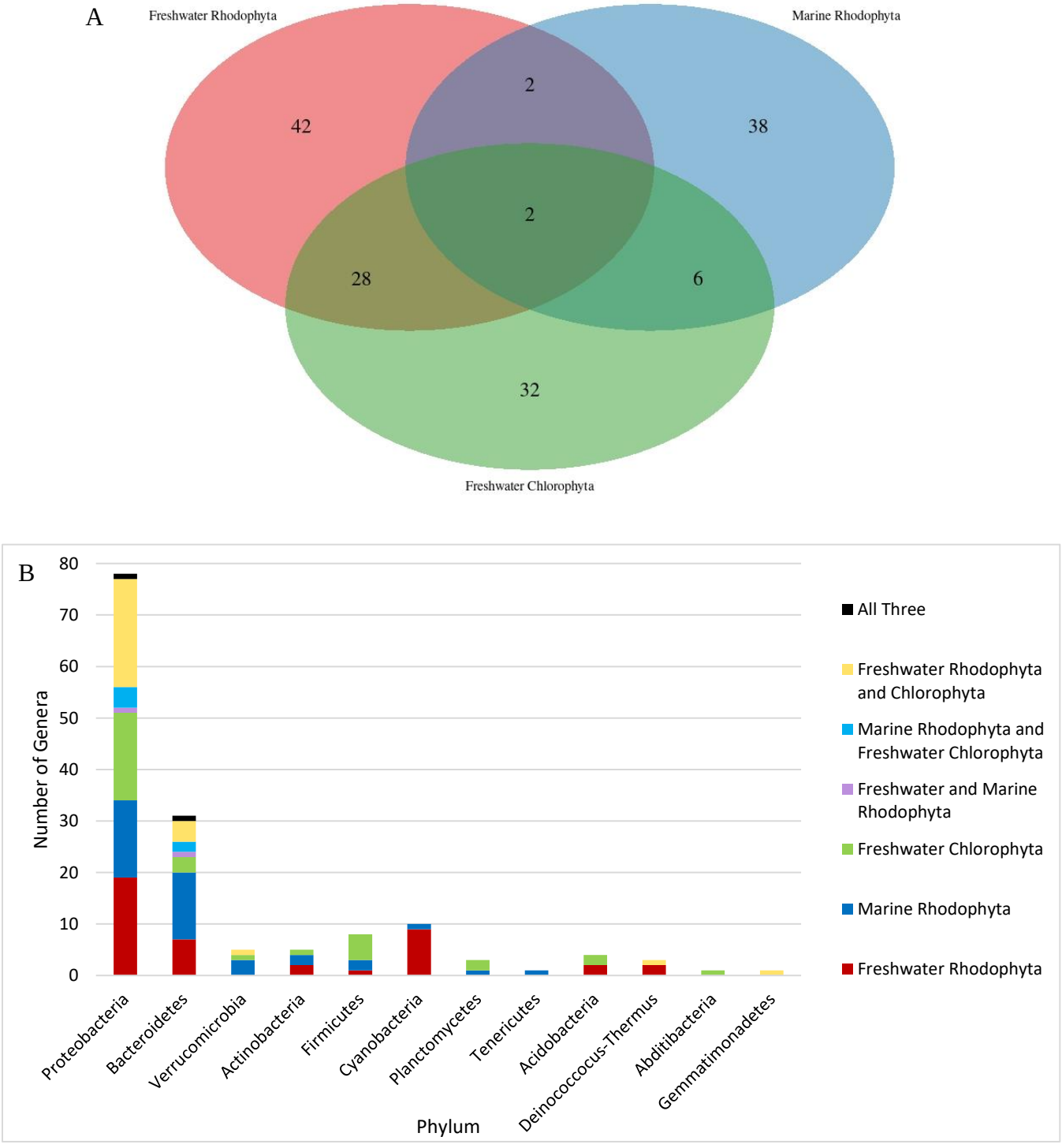


Figure 2 Distribution of bacterial phyla across three categories of algae. A) Three category Venn diagram illustrating the number of genera that are unique to or overlap between marine red algae, freshwater red algae, and the freshwater green alga. B) Bar plot of the number of genera distributed across each phylum, divided by each category of algae and overlap between the categories.

3.2.2 - Bacterial Phyla Represented in the Microbiota of *Bangia atropurpurea* from 2002 and 2016

Comparing the microbiota of *Bangia atropurpurea* from 2002 and 2016 appears to indicate a loss of bacterial diversity across the two samples. For example, there is an overlap of fourteen bacterial genera (figure 3A) between the two samples but overall, the 2016 *B. atropurpurea* sample has a much smaller group of unique bacterial genera. In fact, the sample from 2016 has 12 unique genera compared to 48 unique genera from the 2002 sample, which is also depicted in the alpha diversity analysis (figure 1). In comparing the composition of phyla and abundances of genera from each phylum between the two *B. atropurpurea* samples (figure 3B), there are some key similarities and differences. Bacterial genera from phyla Proteobacteria, Bacteroidetes, Cyanobacteria, Deinococcocus-Thermus, and Verrucomicrobiota, are present in both samples. However, the 2002 sample has more unique genera from Proteobacteria, Bacteroidetes, and Cyanobacteria than the 2016 sample. Interestingly, the composition of phyla within the microbiota also appears to have changed between the fourteen years of sampling. For example, the sample from 2002 contains genera from three phyla that are not present in 2016: Actinobacteria, Acidobacteria, and Firmicutes. Additionally, phylum Gemmatimonadota is represented only in the 2016 *B. atropurpurea* microbiota. Although there are some broad similarities in the bacterial microbiota of the two *Bangia atropurpurea* samples, there are also many substantial differences which could be attributed to the fourteen-year sampling difference.

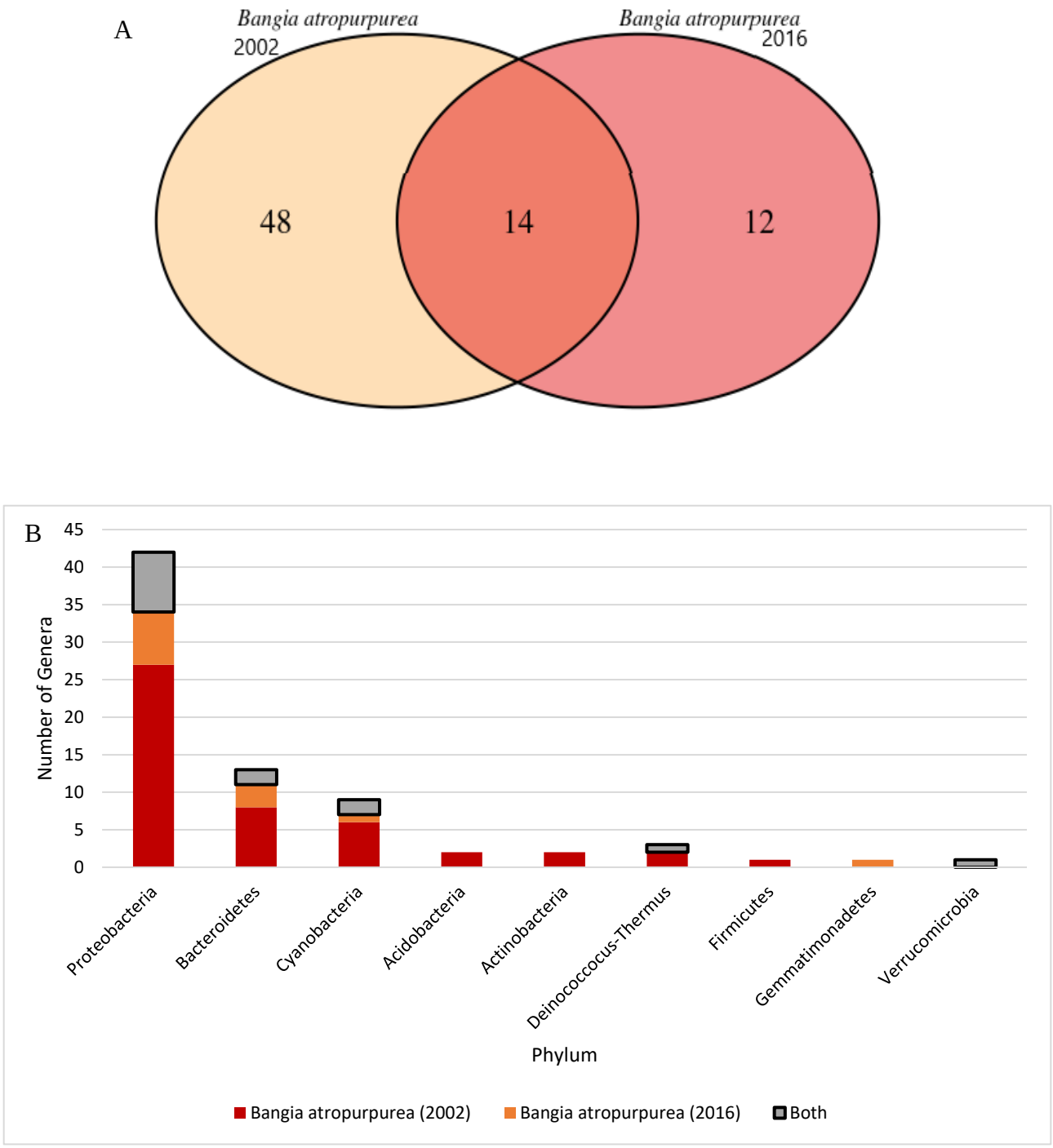


Figure 3 Distribution of bacterial genera between *Bangia atropurpurea* 2002 and *Bangia atropurpurea* 2016. A) Pairwise Venn diagram illustrating the number of unique bacterial genera for each alga sample and the number of bacterial genera that are found in both samples. B) Bar plot illustrating the distribution of bacterial genera per phylum across both samples. This includes genera that are found in only one sample and those that were isolated from both samples.

3.2.3 - Bacterial Phyla Represented in the Microbiota of *Bangia atropurpurea* and *Cladophora glomerata*

To investigate potential species-specific bacterial community members, the microbiota of *Bangia atropurpurea* from 2002 and *Cladophora glomerata* were compared. As observed in the other algal samples, each of these freshwater algae have large distinct core microbiota. The overlap in bacteria between these two *Bangia* and *Cladophora* is 25 bacterial genera (figure 4A), which is the largest overlap in bacterial community composition in relation to the other microbiota comparisons. Despite being distantly related algal species, *C. glomerata* and *B. atropurpurea* inhabit similar environments in the Laurentian Great Lakes, attached to rocks or hard surfaces in the littoral zone (Lin & Blum 1977; Higgins *et al.* 2008). Most of this similarity in bacterial community is attributed to overlap of bacteria from phylum Proteobacteria, from which there are 20 identical genera. There is also an overlap in bacterial genera from Bacteroidetes, Deinococcocus-Thermus, and Verrucomicrobiota. The microbiota of *C. glomerata* has bacteria from three phyla that *B. atropurpurea* does not: Planctomycetes, Abditibacteria, and Gemmatimonadetes. Cyanobacterial sequences were isolated from *B. atropurpurea* but not from *C. glomerata*. There are many similarities in bacterial community composition at the phylum level between *B. atropurpurea* and *C. glomerata* which could be potentially attributed in part to the occupancy of similar habitats. There are also many genera that seem to occupy the microbiota of one alga or the other, indicating a potential species-specific assemblage of bacteria.

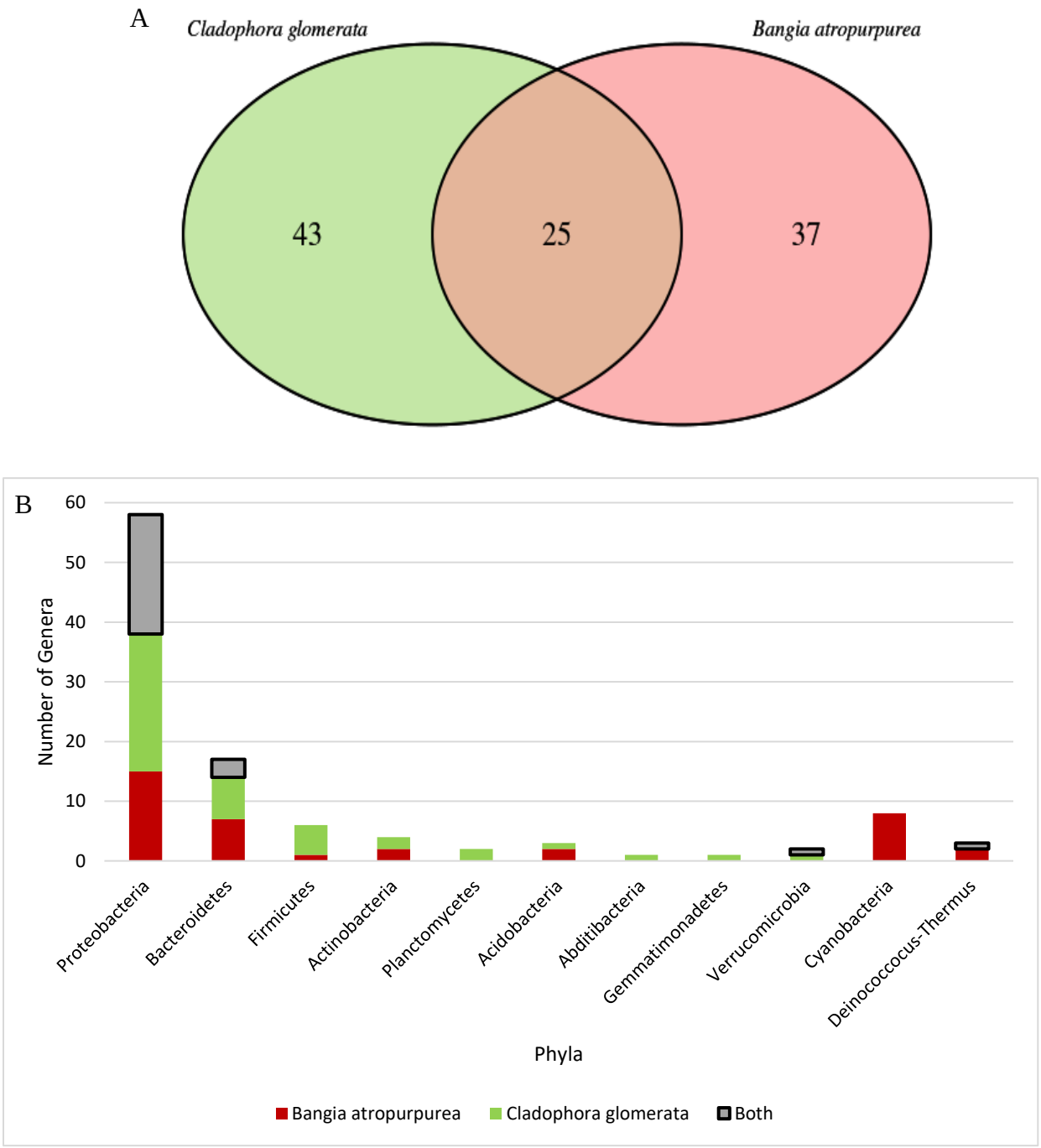


Figure 4 Distribution of bacterial genera between *Bangia atropurpurea* 2002 and *Cladophora glomerata*. A) Venn diagram illustrating the number of unique bacterial genera for each alga sample and the number of bacterial genera that are found in both samples. B) Bar plot illustrating the distribution of bacterial genera per phylum across both samples. This includes genera that are found in only one sample and those that were isolated from both samples.

3.2.4 - Bacterial Phyla Represented in the Microbiota of *Bangia atropurpurea* and *Bangia fuscopurpurea*

To investigate the effect of geolocation and potential environmental factors on composition of microbial communities, the microbiota of *Bangia atropurpurea* and *Bangia fuscopurpurea* were compared. Freshwater red alga *B. atropurpurea* and marine red alga *B. fuscopurpurea* are evolutionarily closely related but occupy differing aquatic habitats. The microbial community compositions of *B. atropurpurea* and *B. fuscopurpurea* appear to have the least in common compared to the other pairwise analyses, with only 3 identical bacterial genera, illustrated in figure 5A. Figure 5B shows that these common genera span Proteobacteria and Bacteriodetes, the most abundant phyla. While the observed overlap in microbial composition between these two algae is very small, their unique bacterial communities are large. Both algae have bacteria from phyla Cyanobacteria, Actinobacteria, Firmicutes. Verrucomicrobiota. Deinococcus-Thermus and Acidobacteria are unique to *B. atropurpurea*, while Planctomycetes and Tenericutes are phyla observed only in the microbiota of *B. fuscopurpurea*, albeit in very low abundances. These vast differences in the bacterial communities at the phylum level between *B. atropurpurea* and *B. fuscopurpurea* indicate that microbiota could be influenced by the environment.

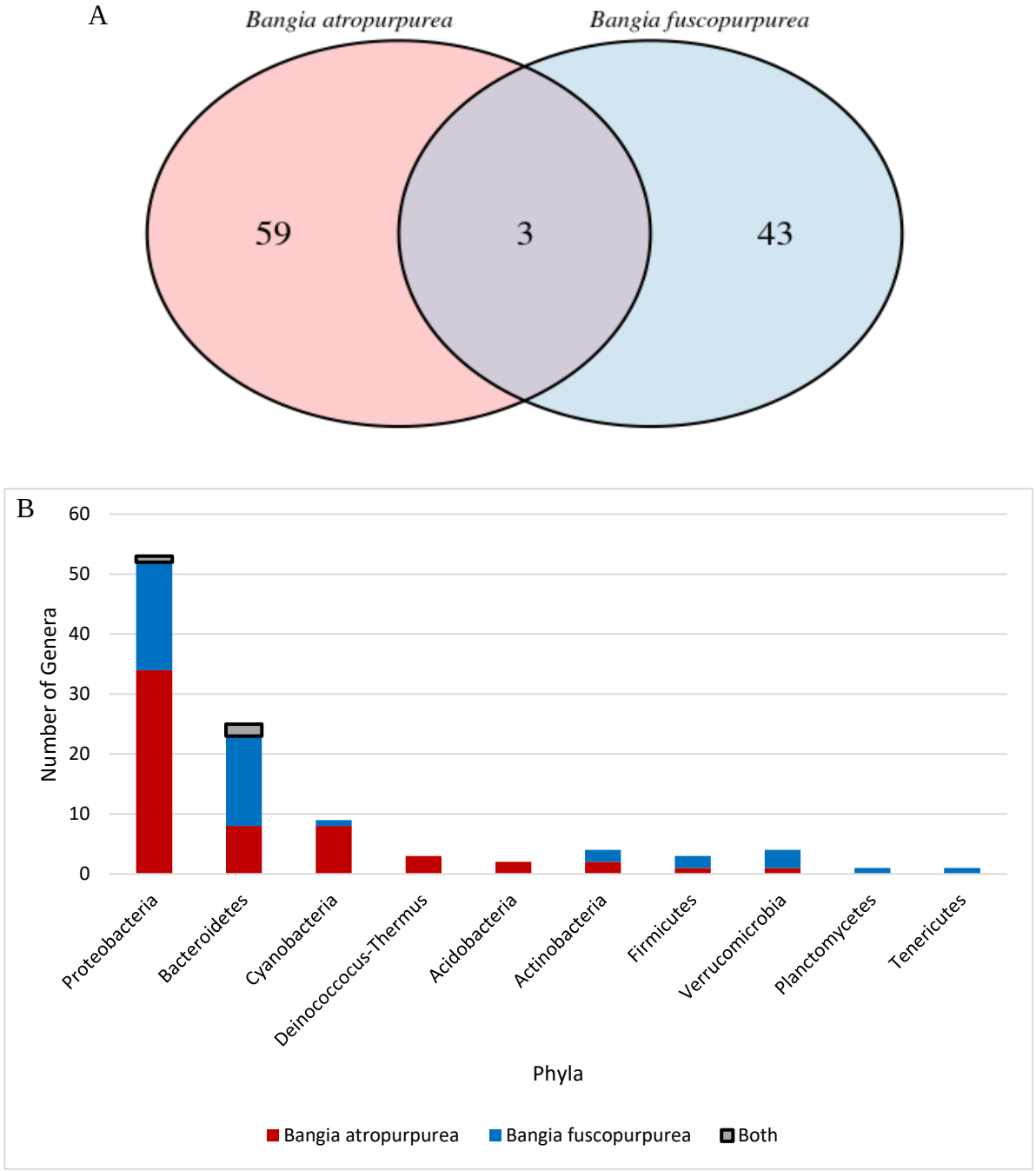


Figure 5 Distribution of bacterial genera between *Bangia atropurpurea* 2002 and *Bangia fuscopurpurea*. A) Venn diagram illustrating the number of unique bacterial genera for each alga sample and the number of bacterial genera that are found in both samples. B) Bar plot illustrating the distribution of bacterial genera per phylum across both samples. This includes the genera that are found in only one sample, and those that were isolated from both samples.

3.3 - Relative Abundances and Distributions of Bacterial Genera Within Phyla

Across the algal samples, there were certain genera that were more abundant in relation to others. This distribution and composition are illustrated in the relative abundance bar plots. The relative abundances of all phyla across all 5 algal samples are illustrated in figure 6A. Figures 6B and C show the relative abundance of the genera from the two most abundant and prevalent phyla, Proteobacteria and Bacteroidetes. There are several genera that are more abundant within certain algae, which may be indicative of larger trends. Microbes within other phyla may also be biologically important despite their considerably lower abundance in these algal samples. See table 4 in Supplementary Information for a more detailed breakdown of relative abundances of genera across each phylum.

3.3.1 - Genera within Phylum Proteobacteria

Proteobacteria was the most abundant phylum based on number of sequence reads, and the most diverse with 68 ASVs corresponding to unique bacterial genera across all algal samples (figure 6B). The overabundance of proteobacterial sequence reads may be skewed due to a few prevalent bacterial genera in the algal microbiota. In *Bangia fuscopurpurea*, genus *Granulosicoccus* (Gammaproteobacteria) is most abundant with 4735 reads assigned to this taxonomy. *Duganella* (Betaproteobacteria) stands out as most common in *C. glomerata* with 2075 reads. Figure 6B provides a thorough visualization of the relative abundances of proteobacterial genera across all algal samples.

There were interesting patterns in the distributions of bacteria across three proteobacterial classes; Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Within class Alphaproteobacteria, the 2016 sample of *Bangia atropurpurea* had one unique bacterial taxon, genus *Aquidulcibacter*, and seven taxa that were also sequenced in the 2002 sample.

Gammaproteobacteria is the least represented class in the 2016 *B. atropurpurea* sample, with only one ASV from genus *Aeromonas*. The 2 proteobacterial ASVs from *Bangia maxima* both belonged to class Gammaproteobacteria. An ASV classified as genus *Psychrobacter* was also sequenced in the *C. glomerata* sample, and the ASV assigned to genus *Cobetia* was unique to *B. maxima.* In the 2002 *B. atropurpurea* sample there are a few microbes that were at an abundance of greater than 100 reads, such as 318 reads assigned to genus *Pseudomonas* (Gammaproteobacteria) in the 2002 sample of *B. atropurpurea*. There were also 242 reads assigned to *Pseudonomas* in the microbial community of *C. glomerata,* but no *Pseudomonas* detected in the 2016 *B. atropurpurea.* Genera *Sphingorhabdus, Porphyrobacter, Paucibacter,* and *Gemmobacter* were detected in all algae from Lake Ontario (*B. atropurpurea* 2002 & 2016, and *C. glomerata*). Within class Betaproteobacteria, the 2016 *B. atropurpurea* sample had more bacterial taxa and a greater diversity than the 2002 sample. *B. fuscopurpurea* also had a large abundance and diversity of reads from class Alphaproteobacteria, and presence of 2 genera from class Oligoflexia that were not present in any other algal samples. Phylum Proteobacteria contains a diverse group of bacteria with many metabolic functions in aquatic habitats (Nold & Zwart 1998). The distribution of bacterial classes and genera from this phylum may be indicative of species-specific symbioses and interactions.

3.3.2 - Genera within Phylum Bacteroidetes

Phylum Bacteroidetes was the second most abundant group, with 28 unique bacterial genera shown in figure 6C. Sequence reads of certain bacterial genera were overabundant in this phylum, most notably an ASV classified as genus *Jiulongibacter* with 3340 reads in the 2002 sample of *B. atropurpurea* and an ASV with 1039 reads classified as genus *Lewinella* in *B. fuscopurpurea.* Both genera were also present in all the other algal microbiota at significantly

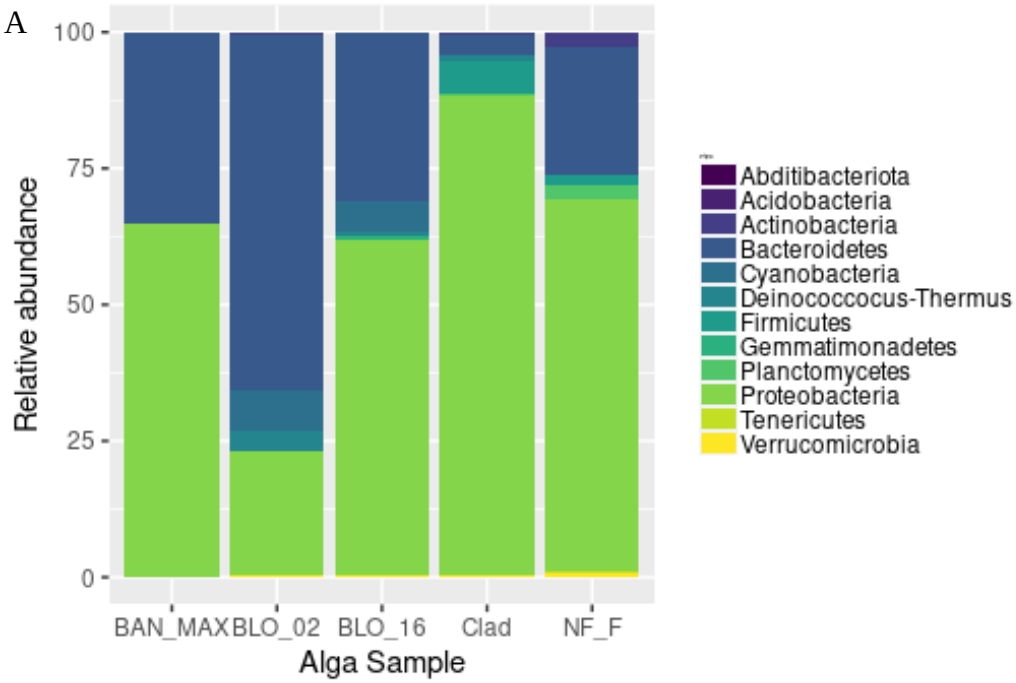
lower quantities and were absent from *B. maxima*. *B. fuscopurpurea* had the most unique genera from Bacteroidetes, as shown in figure 6C. There were 6 orders from this phylum represented across all samples: Flavobacteriales, Marinilabiales, Sphingobacteriales, Cytophagales, Chitinophagales, and Saprospirales (Figure 9). A more detailed summary of the distribution of bacterial genera within these orders can be found in section 3.4.3.

3.3.4 - Genera within other Phyla

There were many phyla that were at lower abundances with less reads and bacterial genera, which can be found in table 4 in Supplemental Information. Bacteria from Cyanobacteria were primarily found in the two *B. atropurpurea* samples, and one cyanobacterial isolate was sequenced in *B. fuscopurpurea*. The 2002 sample of *B. atropurpurea* had 8 unique genera from this phylum, and the 2016 sample had 3 genera which are described in detail in section 3.4.1. Planctomycetes reads were isolated only from the microbiota of *C. glomerata* and *B. fuscopurpurea*, of which 2 genera associated with the green alga and 1 genus in the marine red alga. Phylum Verrucomicrobiota had 5 representative genera, 3 of which were only in *B. fuscopurpurea*. Genus *Luteolibacter* from this phylum was only in the algal samples from Lake Ontario, although another ASV sequenced from *Bangia fuscopurpurea* was clustered closely with this genus in the phylogenetic tree (figure 8). Actinobacteria were present in low abundances within the microbiota of the Lake Ontario algae, but higher quantities of reads from this phylum were in *B. fuscopurpurea,* such as ASV assigned to genera *Illumatobacter* and *Acidimicrobium*. From phylum Firmicutes, 5 genera were unique to *C. glomerata*, 2 phyla were unique to *B. fuscopurpurea,* and one genus from this phylum was in the 2002 sample of *B. atropurpurea*. Genus *Deinococcus* from phylum Deinococcus-Thermus was also distributed among all Lake Ontario algae. The phyla at the lowest abundances were Abditibacteria,

Acidobacteria, Tenericutes, and Gemmatimonadota, with 1 to 3 genera at a read count of less than 10 in each phylum. There was a high diversity of phyla from most of the algal samples. The consistent presence of bacteria at lower abundances may indicate their importance for a healthy algal microbiota.

A



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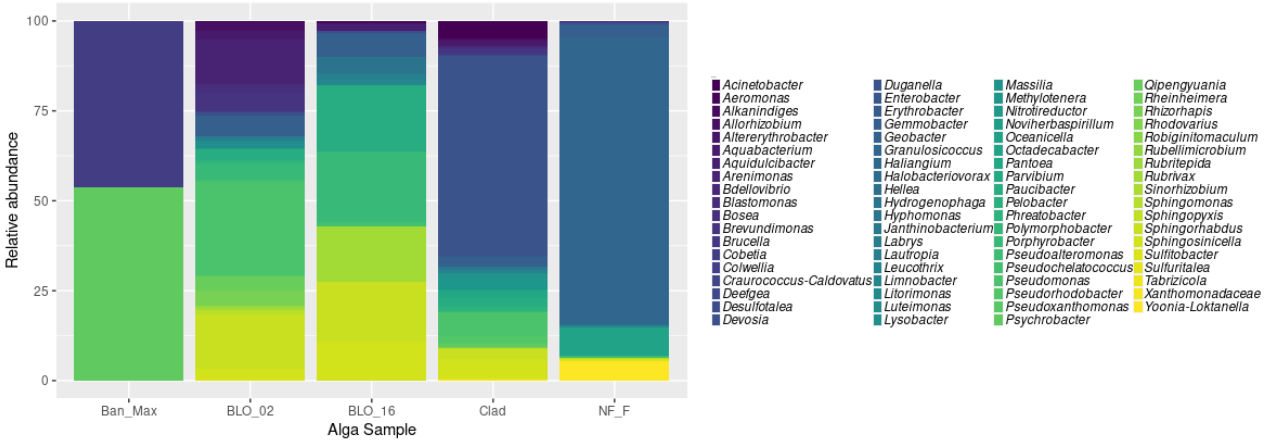

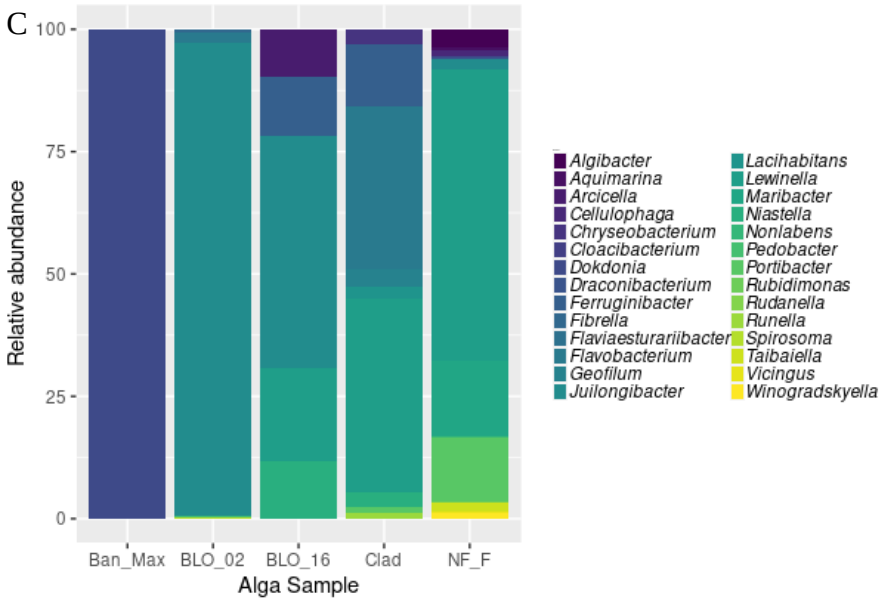

Figure 6 Relative abundance barplots of bacterial taxa present in each sample. Relative abundances of bacteria are shown from A) phyla, B) genera within phylum Proteobacteria, C) genera within phylum Bacteroidetes. Ban_Max: *Bangia maxima*, BLO_02: *Bangia atropurpurea* (2002), BLO_16: *Bangia atropurpurea* (2016), Clad: *Cladophora glomerata*, and NF_F: *Bangia fuscopurpurea*.

3.4 - Phylogenetic Tree Analysis

The trees were constructed with all unique ASVs at the species level. As a result, there may be more ASVs within the trees than what is shown in the Venn diagrams and relative abundance plots (figures 2-6). For example, there are 60 unique ASVs in the Bacteroidetes phylogenetic tree at the species level, but 28 sequences assigned to genera within Bacteroidetes. Any duplicate sequences were removed from the tree analysis to avoid redundancy.

3.4.1 - Cyanobacteria

There were 16 ASVs assigned to phylum Cyanobacteria, which are shown in the phylogenetic tree (figure 7). The only cyanobacterial sequence found in *Bangia fuscopurpurea* was ASV16 and it did not closely align with any sequences from the NCBI database, but was distantly clustered with *Acaryochloris marina* and *Synechococcus* genera. One ASV (ASV9) was

found only in the 2016 sample of *Bangia atropurpurea* was closely clustered with *Tapinothrix clintonii*. Two ASVs were observed in both *B. atropurpurea* samples; ASV11 was clustered with *Trichocoleus desertorum*, and the ASV6 had no close match. There were several ASVs with very close taxonomic matches, at bootstrap values greater than 90. For example, ASV13 aligned with the V4 16S rRNA sequence for *Tychonema bourrellyi* with a bootstrap value of 91. ASV8 clustered with *Chamaesiphon minutis* in 99% of the replicates. These close matches with classified Cyanobacteria will allow a better understanding of which cyanobacterial species are interacting with the algae.

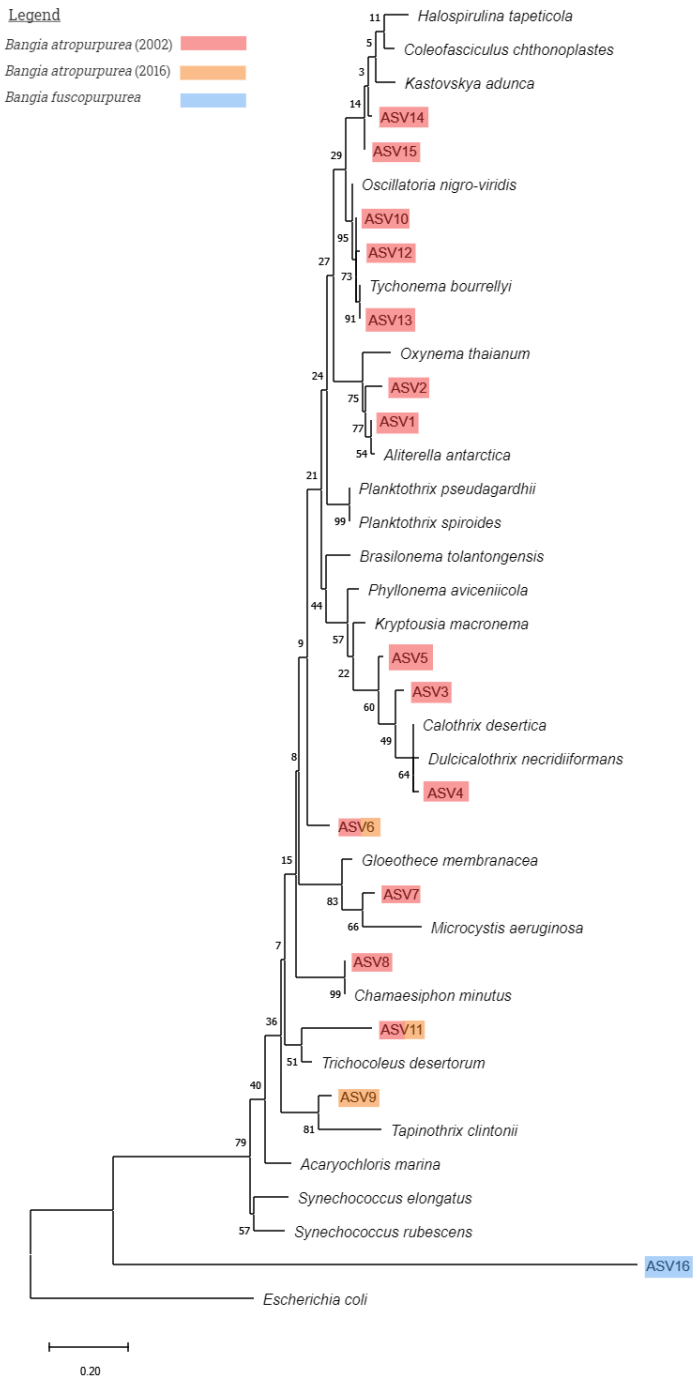

Figure 7 Phylogeny of sequences classified as phylum Cyanobacteria. Tree was constructed using maximum likelihood with bootstrap values, percent out of 1000 replicates, at the node of each branch. ASVs are colour coded to show which algal sample the sequence originated from.

3.4.2 – PVC Superphylum

The PVC superphylum is a monophyletic group consisting of Verrucomicrobia, Planctomycetes, and several other phyla (Wagner & Horn 2006). Sequences from Verrucomicrobia and Planctomycetes were present in some of the algal microbiota examined in this study. Phylogenetic analysis of these sequences will provide a better understanding of the verrucomicrobial and planctomycetal bacteria that are associated with freshwater and marine algae (figure 8).

3.4.2.1 - Verrucomicrobia

Verrucomicrobial sequences were observed to be lower in abundance but prevalent throughout nearly all algal microbiota sampled in this study, as shown in figure 2B. Overall, there were 7 unique ASVs at the species level with one ASV (ASV3) present in both *B. atropurpurea* samples and *C. glomerata* (figure 8). This sequence clustered closely with *Luteilibacter yoenseiesis* and *Luteilibacter luojiensis* in the phylogenetic tree. The other ASVs were primarily isolated from the marine *B. fuscopurpurea* or freshwater *C. glomerata* and broadly grouped with different genera in the phylogenetic tree. For instance, ASV5 grouped with genus *Prosthetibacter* and ASV1 had close alignments to sequences from genus *Rubritalea*. Although bacteria from phylum Verrucomicrobia are present in lower abundance, they are persistently present in some algal samples.

3.4.2.2 - Planctomycetes

Planctomycetal sequences were also present in lower abundances, with 3 ASVs isolated from *Cladophora glomerata* (ASV9 & ASV10) and *Bangia fuscopurpurea* (ASV8). ASV8 clustered closely with *Phycisphaera mikurensis* at a bootstrap value of 100 and ASV9 was closely clustered with *Pirellula stayeli*. The sequence of ASV10 closely grouped with

Fimbriiglobus ruben at a bootstrap value of 90 and was also clustered with *Zavarzinella formosa*. Despite the narrow distribution amongst the algal samples, planctomycetal sequences were abundant in *B. fuscopurpurea* with 231 reads, and more than one planctomycetal ASV was present in *C. glomerata*.

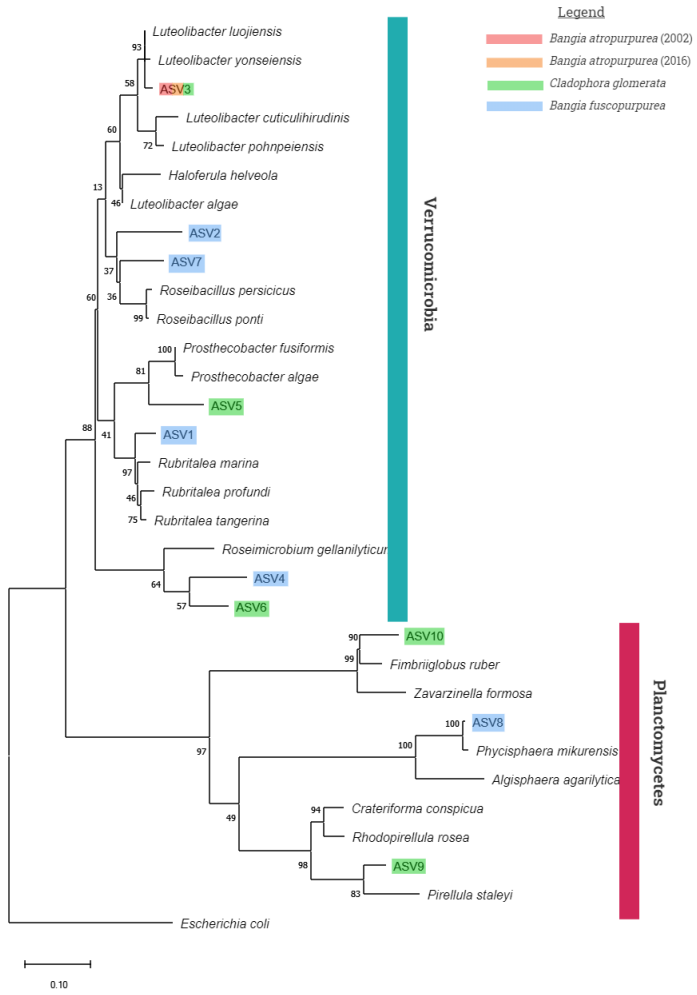

Figure 8 Phylogeny of sequences classified as phyla Verrucomicrobia and Planctomycetes. Tree was constructed using maximum likelihood analysis and bootstrap values, in percent out of 1000 replicates, are indicated at the node of each branch. ASVs are colour coded to show which algal sample the sequence originated from.

3.4.3 - Bacteroidetes

The phylogenetic tree of ASVs assigned to phylum Bacteroidetes is shown in Figure 9. This tree has a total of 60 sequences at the species level, which were distributed across all algal samples. Some ASVs were not assigned to an algal sample due to inconsistencies between the SILVA database and the NCBI database. Out of all Bacteroidetes ASVs, 46 were assigned to an algal sample; 26 were sequenced from *Bangia fuscopurpurea*, 12 from *Bangia atropurpurea* (2002), 6 from *Bangia atropurpurea* (2016), 14 from *Cladophora glomerata*, and 1 from *Bangia maxima.* Bacterial sequences spanned 6 orders: Flavobacteriales, Marinilabiales, Sphingobacteriales, Cytophagales, Chitinophagales, and Saprospirales. Freshwater algae *B. atropurpurea* and *C. glomerata* had lower abundance of bacteria from order Saprospirales, and higher bacterial abundance and diversity from Chitinophagales and Cytophagales. ASVs isolated from the marine alga *B. fuscopurpurea* dominated orders Flavobacteriales and Saprospirales, but 2 ASVs in Saprospirales (ASV39 and ASV42) were isolated from freshwater algae as well as marine. Some genera were more abundant, such as four ASVs (31, 55, 33, and 5) assigned to genus *Flavobacterium* in the freshwater *Bangia* and *Cladophora*. There were also multiple sequences classified as genus *Lewinella*, namely ASVs 10, 53, 47, 29, and 43, that were isolated from *B. fuscopurpurea* and *C. glomerata*. The only Bacteroidetes sequence found in *B. maxima* was also isolated from *B. fuscopurpurea* (ASV 23), which clustered with genus *Dokdonia*. ASV39 was isolated from all algal samples except for *Bangia maxima* but did not have a close grouping in the tree. The presence of ASV38 in both samples of *Bangia atropurpurea* and in *Bangia fuscopurpurea* is of interest, but clustered with bacteria from three different genera: *Jiulongibacter sediminis, Arcticibacterium luteifluviistationis,* and *Taeseokella kangwonensis*. There is a high level of diversity and abundance of bacteria from Bacteroidetes in the algal microbiota, which are likely involved in many different symbioses with the algal host.

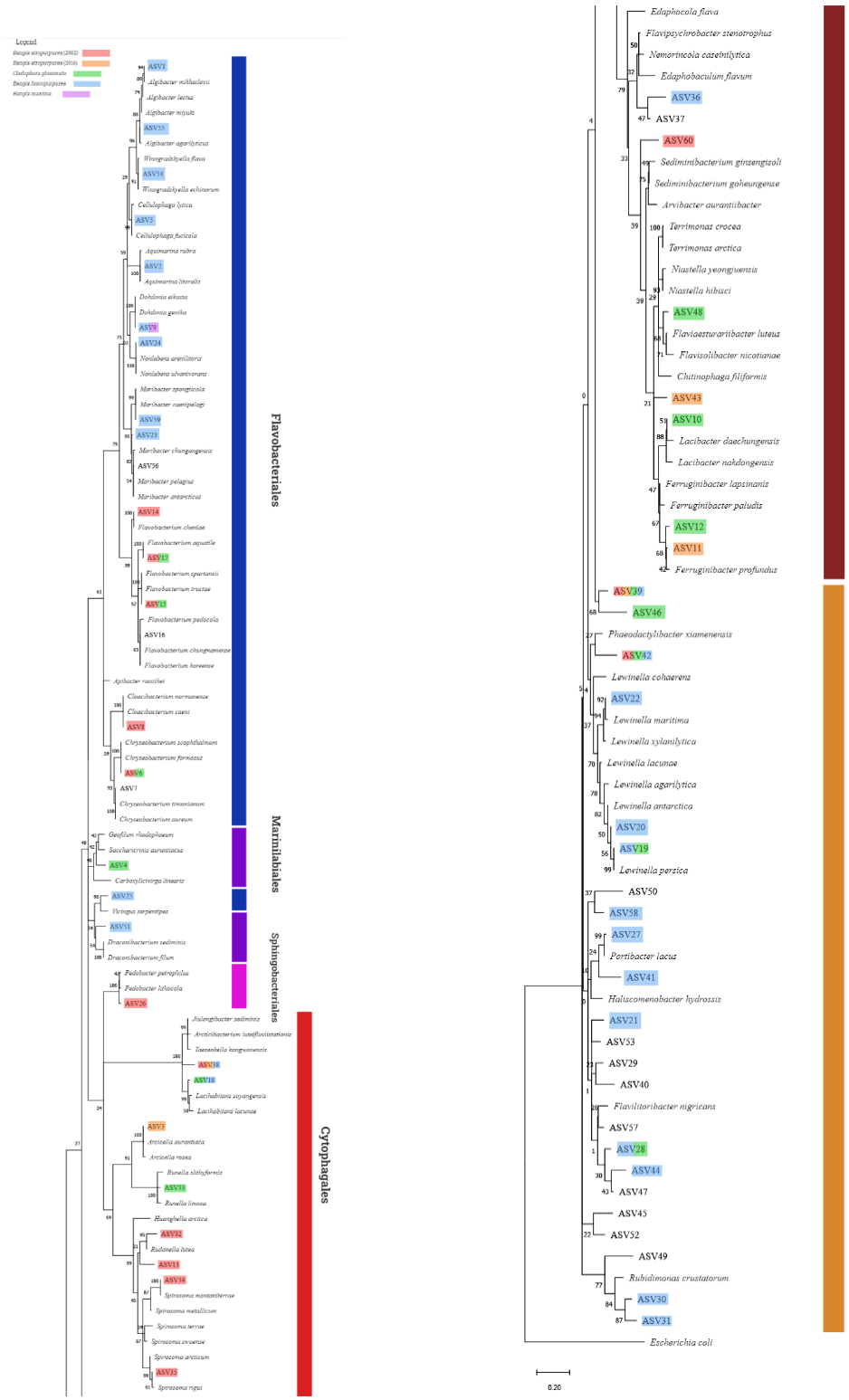

Figure 9 Phylogeny of sequences classified as phylum Bacteroidetes. Tree was constructed using a maximum likelihood analysis and bootstrap values, in percent out of 1000 replicates, are indicated at the node of each branch. Sequences are grouped into orders, indicated by the coloured bars on the right of the tree. ASVs are colour coded to show which algal sample the sequence originated from.

3.5 - Taxonomic Nomenclature Discrepancies in the SILVA Database

There were some errors in the bacterial classifications provided by the SILVA database which was used in the QIIME2 workflow. This resulted in discrepancies during the characterization of bacterial communities and creation of phylogenetic trees. These errors were corrected with the NCBI database which uses various scientific publications to confirm nomenclature of organisms (Schoch *et al.* 2020). There were several sequences throughout the samples that were assigned to phyla that are not validly published, as purported by the International Code of Nomenclature of Prokaryotes (Parker *et al.* 2019). In *Bangia fuscopurpurea*, some sequences were classified as belonging to phyla Pastecibacteria, Bdellovibrionota, and Myxococcota. These groups were proposed as separate phyla but are not yet validly published as such and currently remain as classes within phylum Proteobacteria (Parte *et al.* 2020). Sequences categorized as invalidly published phylum Chloroflexi were corrected to phylum Firmicutes (Schoch *et al.* 2020). Several sequences classified as Burkholdariales (Betaproteobacteria) were incorrectly categorized to be within class Gammaproteobacteria. There were also a few samples classified as phylum Desulfobacteriota, which is also not validly accepted and is officially order Desulfuromonadales within class Deltaproteobacteria (Proteobacteria). Finally, SILVA used the name Deinococcota for the phylum which is actually called Deinococcocus-Thermus (Schoch *et al.* 2020). The correct nomenclature based on International Code of Nomenclature of Prokaryotes (Parker *et al*. 2019) will be applied to all bacterial and algal taxonomies in this thesis.

Chapter 4: Discussion

Currently there is a lack of literature on the characterization of freshwater red algal microbiomes and microbiota, particularly from the Laurentian Great Lakes. In this study, the microbiota of freshwater red alga *Bangia atropurpurea* was examined in the scope of a marine red alga, *Bangia fuscopurpurea*, and a freshwater green alga, *Cladophora glomerata*. These comparisons were made to get a sense of any environment-specific and species-specific bacterial assemblages that may occur amongst these algal taxa. To date there have been some studies investigating patterns in the assemblage of algal microbiota in relation to environment and species (Dittami *et al.* 2016; Eigemann *et al.* 2013; Morrissey *et al.* 2019), but none on freshwater red algal microbiota. A temporal study of *B. atropurpurea* bacterial communities was also conducted with two samples of this alga collected in 2002 and 2016. Research on temporal changes in algal microbiomes are also limited to green or brown marine algae and are primarily short-term studies of a few months to a few years (Braus *et al.* 2017; Lachnit *et al.* 2011; Paix *et al.* 2019). In this present study, the bacterial communities of *Bangia atropurpurea*, *Bangia fuscopurpurea* and *Bangia maxima* are characterized for the first time. The microbiota of *B. maxima* was extremely small, with only two unique genera, and thus will not be discussed in this study. *Bangia maxima* is observed to grow on boulders and covered by a large amount of sand, which may be the reason for such low diversity of bacteria associated with this algal taxon (Gardner 1927). The mechanism of DNA extraction for the *B. maxima* samples likely did not have an effect on the lower microbial diversity, as literature suggests that the phenol-chloroform method of DNA extraction does not alter the quantity of bacterial sequence reads (Janabi *et al.* 2016). Comparisons of algal microbiota provide more insight on how these communities have changed over time, how they compare with the microbiota of other ecologically relevant algae,

and how the microbiota may impact algal function. However, it is important to note that the sample size and sequencing depth of the microbial communities in this study are extremely limited. Thus, any suggestions or inferences of bacterial role in the algal microbiome are putative and cannot be confirmed without further metagenomic sequencing and more thorough investigation.

4.1 – Temporal Differences in the Microbiota of *Bangia atropurpurea* Between 2002 and 2016

There were several differences in the microbiota of the two *Bangia atropurpurea* samples from 2002 and 2016. The differences in bacterial community compositions can be observed at the phylum level and at lower taxonomies such as family or genus level. The most striking difference was in the lower diversity of the 2016 sample, which only had twelve unique bacterial genera in comparison to forty-eight unique genera in the 2002 sample. Although this could be an artefact of low sample size and replicates, DNA extraction errors, or limitations of sequencing only the V4 region of the 16S rRNA gene, changes in the water quality of Lake Ontario could potentially contribute to some of the observed differences in bacterial diversity. Studies have shown that there is a slight positive trend in the acidification and salinification of the Great Lakes over the past several years, particularly in Lake Ontario, which may have affected the microbial composition of the water column in myriad ways (Mahdiyan *et al.* 2021; Phillips *et al.* 2015). However, changes in microbial communities of the water column might not be reflected in the microbiota of an alga. For example, there was a lack of bacterial sequences from phyla Acidobacteria and Actinobacteria in the 2016 *Bangia* sample, despite having been reported in the water column in 2008 (Winters *et al.* 2014) and 2014 (Paver *et al.* 2020). In the 2002 sample, the Acidobacteria isolates were characterized as family Blastocatellaceae which have an optimal

growth at pH 4.5-7.9 and prefer freshwater conditions (Pascual *et al.* 2015). Within phylum Actinobacteria, one bacterial isolate in the 2002 *Bangia atropurpurea* sample was classified to be within genus *Geodermatophilis*, which is known to have high resistance to oxidative stress (Hezbri *et al.* 2016; Montero-Calasanz 2020). The lack of certain phyla, especially those that are normally found in lower quantities, could be attributed to poor resolution of the sequencing or weak and temporary associations with the alga. Moreover, changes in the microbiota of *Bangia atropurpurea* could occur independently of the microbial community composition of the water column.

Bacteria from other phyla, including Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, and Proteobacteria, were present in both samples but at a much lower diversity in the 2016 *Bangia atropurpurea* sample. The three bacterial genera from Bacteroidetes unique to the 2016 microbiota were classified as *Arcicella* (closely clustered with *Arcicella aurantiaca*), *Ferruginibacter* (closely clustered with *Ferruginibacter profundus*), and *Niastella* (no close grouping) (figure 9). These are bacterial genera commonly found within freshwater environments, generally with optimal pH of 6-8 and ability to grow in salinities less than 1% NaCl (Sheu *et al.* 2010; Jin *et al.* 2014). Several ASVs isolated from the 2002 sample that were classified within order Flavobacteriales (genera *Flavobacterium* and *Chryseobacterium*) are found in saltwater, freshwater, soils, and plants in warm and cold environments (Akter *et al.* 2015; Bernardet & Bowman 2006). Additionally, three ASVs associated with this algal sample from order Cytophagales and family Spirosomaceae were clustered closely with *Rudanella lutea*, *Spirosoma montaniterrae*, and *Spirosoma rigui* in the maximum likelihood tree. These strains, and other members from order Cytophagales, are able to acclimate and survive in a wide range of salinities, pH, and temperatures (Finster *et al.* 2009; Weon *et al.* 2008). This preliminary

analysis and literature review shows that the bacteria of phylum Bacteroidetes associated with the 2002 *B. atropurpurea* sample seem to be more resistant to harsh environments, whereas the Bacteroidetes isolates from the 2016 sample are potentially more acclimated to the freshwater habitat and could be less adaptable to changes in pH or salinity. Although this correspondence between bacteria and alga cannot be confirmed in this study, future work on the relationship between *Bangia atropurpurea* and Bacteroidetes may elucidate additional findings. Further changes in the pH and salinity of Lake Ontario may cause more drastic differences in the bacterial profile of these algal microbiota.

The difference in the microbiota of the 2002 and 2016 *Bangia atropurpurea* samples is also evident in the diversity and the composition of bacteria within Proteobacteria. Most strikingly, perhaps, is the lack of bacteria from class Gammaproteobacteria in the 2016 sample, except for one ASV classified as *Aeromonas.* In the NCBI database, this ASV aligned at 100% identity and E-value of $8e^{-132}$ with *Aeromonas sobria*, which has been associated with toxic effects in humans and mammals (Daily *et al.* 1981). The 2002 sample had 7 ASVs within Gammaproteobacteria, including one ASV with 314 sequence reads classified as *Pseudomonas*, a genus that contains strains known to be toxic to other organisms, but did not have a conclusive species alignment in the database (Palleroni 2015). *Pseudomonas* has also been implicated in salt-resistance (Ansari *et al.* 2019) which could provide some benefits to the algal host. However, without further metagenomic sequencing it is difficult to confirm the exact functions of this *Pseudomonas* strain. The 2002 sample of *Bangia atropurpurea* also contained many ASVs classified as family Rhodobacteraceae, which have been observed to confer salt resistance in coral in the long term (Röthig *et al.* 2016). Sequences classified as Rhodobacteraceae were also associated with *Cladophora glomerata*, but not the 2016 *B. atropurpurea* sample. Although

a direct symbiotic relationship in the scope of algal salt-resistance and presence of Rhodobacteraceae has not been established, the coral research indicates that members of this bacterial order can potentially help the host become more resistant to salt stress. This change in abundance of bacteria with potential salt-tolerant abilities could be indicative of temporary associations and may show that this symbiotic relationship is not vital *B. atropurpurea*. The 2016 sample had 2 ASVs classified as family Burkholderiaceae (Betaproteobacteria), a group that was entirely absent from the 2002 sample. The genera *Limnobacter* and *Lautropia* of the family Burkholderiaceae are both strictly freshwater microbes that have oxidase and catalase activity (Rosenberg 2013). Both algal microbiota contained a variety of unique proteobacterial genera with a diverse range of putative functions.

Regardless of the many differences in bacterial diversity and composition, there is evidence for a microbial community that is unique to *Bangia atropurpurea*, as several bacterial genera across 5 phyla were present in both *Bangia* samples despite a 14-year sampling difference. In this analysis, the community of bacteria that appear to be closely associated with both samples of *B. atropurpurea* will be referred to as the core microbiota. A temporal study of *Cladophora glomerata* also demonstrated a putative core algal microbiota consisting of bacterial genera that continually colonized the epiphytic community over the span of 3 years (Braus *et al.* 2017). In *B. atropurpurea*, bacteria that persistently occupied the microbiota and were present in both samples belong to phyla Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, Proteobacteria, and Verrucomicrobia. Some potential core bacteria were also present in other algal microbiota, such as genus *Lewinella* (Bacteroidetes) sequenced from *C. glomerata* and *B. fuscopurpurea*, and an ASV distantly grouped with genus *Jiulongibacter* (Bacteroidetes) was also observed in *B. fuscopurpurea*. Bacteria from these genera have been isolated from a wide

variety of environments and are adaptable to a range of salinities, acidities, and temperatures which may contribute to their persistence in core algal microbiota regardless of species or environment (Yoon *et al.* 2008b; Sly & Fegan 2015; Liu *et al.* 2016). Bacteria from phylum Proteobacteria make up a large portion of the potential core microbiota in *Bangia atropurpurea*. Some of these genera may have protective symbiotic relationships with algae, such as *Sphingosinicella, Paucibacter,* and *Sphingorhabdus* which are known to be toxin degraders (Krishnan *et al.* 2020). Bacteria from phylum Deinococcus-Thermus are known to have protective pathways against oxidative stress, which may also result in a symbiotic bacteria-alga relationship (Lee *et al.* 2016). Some epiphytic bacteria with potential symbiotic functions sequenced from *Bangia atropurpurea* are known to be degraders of algal surface polysaccharides, specifically carrageenans and agars found on Rhodophyta (Michel *et al.* 2006). *Pseudomonas, Alteromonas, Sphingorhabdus,* and *Flavobacterium* are just a few of the known agarase-producing genera (Michel *et al.* 2006) that were sequenced in the *Bangia* samples. However, these agar-degrading genera were not persistently present in both algal samples so are not considered to be part of the core microbiota in this case. From this temporal analysis on the microbial communities of *Bangia atropurpurea* it is evident that adaptable bacterial genera are likely to occupy the core algal microbiota, but those with nonessential functions may be more variable in their associations with the alga. Other temporal studies have also demonstrated the presence of both stable and inconsistent members of the algal microbiota over months and years (Braus *et al.* 2017; Paix *et al.* 2019). For example, in marine brown alga *Taonia atomaria* there were seasonal clustering of certain bacterial OTUs and associated metabolites, but the alga retained a consistent core microbial community of some bacterial sequences over the six sampling months (Paix *et al.* 2019). This evidence may indicate that the bacteria within the core

microbiota have symbiotic relationships with *Bangia atropurpurea*. However, it is not yet fully understood how these symbiotic interactions contribute to the formation of the core microbial community or to algal function.

4.2 – Species-Specific Trends in the Microbiota of *Bangia atropurpurea* and *Cladophora glomerata* from the Laurentian Great Lakes

Some species-specific patterns in the microbiota of *Cladophora glomerata* and *Bangia atropurpurea* are evident. Although the microbial community of *B. atropurpurea* has not been previously characterized, the microbiome of *C. glomerata* has been sampled and sequenced on three separate occasions: in 2011 by Zulkifly *et al*. (2012), in 2012 by Graham *et al*. (2015a), and in 2014 by Braus *et al*. (2017). Although these studies sampled algae from Lake Mendota, Wisconsin, USA, any similarities in bacterial community members with the Lake Ontario sample may be attributed to *Cladophora*-specific assemblage. Using the bacterial genera observed in these Lake Mendota studies to support the bacterial sequence data from *Cladophora* in the present Lake Ontario study, a better comparison of *Bangia* and *Cladophora* microbial communities might be made.

There were many similarities in bacterial genera sequenced from the Lake Ontario *Cladophora glomerata* sample in this study and from Lake Mendota in 2011 (Zulkifly *et al.* 2012) and 2012 (Graham *et al.* 2015a). Bacterial genera responsible for methane degradation, such as *Methylotenera* (Proteobacteria), were observed in higher abundances by Zulkifly *et al*. (2012). This genus was also observed in the microbiota of the *C. glomerata* sample in this present study and not in *B. atropurpurea*, indicating a possible specific association between methanotrophic bacteria and *Cladophora glomerata*. Bacterial genera known to reduce Fe(III), *Ferruginibacter* (Bacteroidetes) and *Geobacter* (Proteobacteria) were sequenced in *Cladophora*

from Lake Mendota and Lake Ontario, but not from *B. atropurpurea* (Zulkifly *et al.* 2012; Graham *et al.* 2015a). Even some less abundant genera were also found associated with *Cladophora* regardless of the lake it was sampled from: *Deinococcus* (Deinococcus-Thermus), *Pirellula* (Planctomycetes), *Luteolibacter* (Verrucomicrobia), and *Gemmatimonas* (Gemmatimonadetes) (Zulkifly *et al.* 2012; Graham *et al.* 2015a). Of these rare bacterial taxa, Planctomycetes and Gemmatimonadetes were not present in *B. atropurpurea*. Presence of these bacterial genera in *C. glomerata* across different lakes, indicates potential for symbiotic relationships to persist due to species-specific interactions. Alga-specific symbioses could be present as some bacterial genera are not present in the bacterial community of *B. atropurpurea*. Interestingly, bacteria from phylum Firmicutes were present in high abundance only in the Lake Ontario sample of *C. glomerata*. Phylum Abditibacteria was a rare bacterial taxon present only in *Cladophora* from Lake Ontario. The distribution of Abditibacteria and Firmicutes in this case may be indicative of environment-specific microbes or a temporary association rather than a consistent species-specific interaction between bacteria and alga.

There were also some bacterial genera and phyla that seem to be specific to *Bangia atropurpurea.* For instance, bacterial sequences classified as phylum Cyanobacteria were abundant and persistent in both *B. atropurpurea* microbiota examined in this study. These sequences closely clustered with several different cyanobacterial genera including *Tychonema*, *Calothrix*, and *Microcystis*. The *Cladophora* microbial communities from Lake Mendota contained low abundances of cyanobacterial sequences and were not part of the putative core microbiota (Graham *et al.* 2015a; Braus *et al.* 2017), while the *Cladophora* sample from Lake Ontario had no associations with this bacterial phylum. There may be a species-specific association of Cyanobacteria with *B. atropurpurea* as it seems that bacteria from this phylum do

not abundantly populate the *Cladophora* microbiota. Another potential *Bangia*-specific symbiont is an ASV that grouped with a cluster of taxa within order Cytophagales (Bacteroidetes) consisting of *Jiulongibacter sediminis, Taeseokella kangwonensis,* and *Arcticibacterium luteifluviistationis.* There were a large number of sequence reads assigned to this ASV in both *B. atropurpurea* samples and in *B. fuscopurpurea*, but none in *C. glomerata* from this study nor from the Lake Mendota samples. Although the exact identity of this isolate is unknown, this could indicate a potential *Bangia*-specific bacterial interaction. From this analysis it appears there are less species-specific bacteria associated with *B. atropurpurea* compared to *C. glomerata,* which has been studied in more detail. Further characterization of the bacterial communities associated with *Bangia atropurpurea* is necessary to get a better scope of species-specific symbioses.

Species-specific assemblage of bacterial communities in algal microbiota have also been observed between freshwater green alga *Desmodesmus armatus* and diatom *Stephanodiscus minutulus* in a series of laboratory experiments (Eigemann *et al.* 2013). Over multiple inoculations of xenic algal samples with bacterial cultures, 55-65% of bacterial species remained consistently present in *D. armatus* indicating a close association with the alga (Eigemann *et al.* 2013). Additionally, host-specific microbial community compositions were observed amongst three different morphological forms of brown alga *Saccharina japonica* (Balakirev *et al.* 2012). The bacterial assemblages were also found to be uncorrelated with the depths which the different algal strains inhabit. (Balakirev *et al.* 2012). This provides more evidence for species-specific bacterial community compositions.

4.3 – Environment-Specific Patterns in the Microbiota of *Bangia atropurpurea* and Marine Red Algae

The vast differences in the microbial communities of *Bangia atropurpurea* and *Bangia fuscopurpurea* could be indicative of the effect environment has on algal microbiota composition. Due to low sample quantities and lack of environmental data, direct correlation between environmental conditions and bacterial community composition of the alga cannot be confirmed. Despite genetic and morphological similarities between these two algae (Sutherland *et al.* 2011), there were only 3 ASVs found in both algal microbiota. There were ASVs clustered with *Jiulongibacter* and *Lewinella* (Bacteroidetes), and an ASV closely grouped to *Gemmobacter* (Proteobacateria). As previously mentioned, bacteria from genera *Jiulongibacter* and *Lewinella* are known to be adaptable to a variety of environmental changes and stressors (Liu *et al.* 2016; Sly & Fegan 2015). Research on the *Porphyra umbilicalis* microbiota by Miranda *et al*. (2013), collected in fall 2010 and winter 2011 from Acadia National Park, Maine, was used to supplement the microbial community composition data obtained from *Bangia fuscopurpurea* in the present study. These two algae are closely related and occupy similar habitat niches (Sutherland *et al*. 2011), which makes the comparison worthwhile and provides a better sense of the microbial community members that are associated with marine red algae.

Although the *P. umbilicalis* samples are taken from a location further south on the east coast of North America than the *B. fuscopurpurea* sample, there are many similarities in the microbiota of the two algae due to the marine environment and the close evolutionary relatedness (Sutherland *et al*. 2011). Both *B. fuscopurpurea* and *P. umbilicalis* had a large abundance of sequence reads assigned to genera *Lewinella* (Bacteroidetes) and *Granulosicoccus* (Proteobacteria). Certain less abundant genera were present in both marine samples, such as

Ilumatobacter from phylum Actinobacteria and *Phycisphaera* (Planctomycetes). Bacteria classified as genus *Phycisphaera* have thus far been only isolated and characterized from marine Rhodophyta (Fukunaga & Kurahashi 2009). Although this does not eliminate the possibility that this bacterium is present in other habitats, its consistent extraction from marine sources may indicate a specific association with the marine environment of this algal group. Bacteria from phylum Actinobacteria are known to associate with Antarctic algae due to their resistance against cold temperatures (Leiva *et al.* 2015). The large number of actinobacterial sequence reads assigned to genera *Ilumatobacter* and *Acidimicrobium* isolated from *B. fuscopurpurea* may be indicative of a useful symbiotic association for this alga which was sampled from the Northern Atlantic Ocean (table 1).

Within the most abundant phyla, Bacteroidetes and Proteobacteria, there were similar distributions of bacteria from lower taxonomies. For instance, both *B. fuscopurpurea* and *P. umbilicalis* microbiota had more diversity and abundance of bacterial genera from families Saprospiraceae and Flavobacteriaceae, both of which have been primarily isolated from marine habitats, and other extreme environments in the case of Flavobacteriaceae (Jooste & Hugo 1999; McIlroy & Nielsen 2014). Although bacterial isolates classified as Flavobacteriaceae were observed in the freshwater algae, they were not as numerous or diverse as those isolated from the marine alga. Bacteria from Saprospiraceae are able to degrade complex proteins and carbon sources from surfaces to which they attach (McIlroy & Nielsen 2014). The complex surface polysaccharides on algal cells may be one of the many nutrient sources these bacteria consume. Additionally, members of the Saprospiraceae have been implicated in resistance to changes in conductivity (Schauer *et al.* 2005). This association between bacteria and alga is more prevalent in marine *Bangia* rather than the freshwater species, as the *B. atropurpurea* samples contained

few sequences from this bacterial family. However, there has been evidence of a further invasion of *B. atropurpurea* into Lake Superior, which is generally less conductive than the lower Great Lakes (Shea *et al.* 2014). A microbiome study of *Bangia atropurpurea* from Lake Superior may reveal stronger associations with conductivity-resistant bacteria due to this major difference in water chemistry. Finally, the freshwater *B. atropurpurea* had more bacteria from order Cytophagales, which are primarily found in freshwater environments and cannot easily adapt to seawater (Reichenbach 1992). Cytophagales bacteria isolated from the Laurentian Great Lakes are known to be cellulose degraders (Reichenbach 1992), which could be the basis for a symbiotic association between this bacterial taxon and algae.

There was also much similarity in the bacterial community composition within phylum Proteobacteria. Within class Alphaproteobacteria, bacterial genera from families Rhodobacteraceae and Sphingomonadaceae were present in both marine and freshwater algae but more prevalent in the marine algal microbiota. Rhodobacterceae are a diverse group of bacteria that are able to adapt to non-marine environments due to genome plasticity and loss of certain genetic pathways that are not needed in freshwater (Simon *et al.* 2017). Sphingomonodaceae are an opportunistic group of bacteria that have been isolated from a variety of drinking water sources and can degrade pollutants such as polycyclic aromatic hydrocarbons and chloroaromatic compounds (Stolz 2009; Vaz-Moreira *et al.* 2011). Bacteria classified as family Sphingomonodales were more prevalent in freshwater *B. atropurpurea*, which is an observation that is in line with literature findings that show a higher abundance of this bacterial group in algae exposed to less saline water samples (Dittami *et al.* 2016). Order Bdellovibrionales (class Oligoflexia), was present only in the microbial communities of the marine algae *B. fuscopurpurea* and *P. umbilicalis*. Bdellovibrionales is a poorly defined group,

but contains a diverse range of bacteria isolated from freshwater, marine, and soil sources that have exhibited predatory behaviour against other bacteria (Snyder *et al.* 2002). There were many clear delineations between the marine and freshwater bacterial communities of *Bangia atropurpurea, Bangia fuscopurpurea,* and *Porphyra umbilicalis*, likely due to the environment-specific presence of many bacteria.

There are distinct differences in the water chemistry between marine and freshwater environments, such as salinity, nutrient concentration, and pH level that cause these differences in microbial community composition (Cavender-Bares *et al.* 2001). The effect of different environmental conditions on microbial community assemblage was also evident in the microbiota of marine green algae *Caulerpa cylindraeca* and *Caulerpa prolifera* (Morrissey *et al.* 2019). The variations in bacterial community composition have statistically significant correlations with differences in nutrient availability. For example, about 11% of variation in Actinobacteria and 36% of proteobacterial variation in the algal rhizobiome was significantly correlated with nitrite concentration (Morrissey *et al.* 2019). Environmental factors may be one of the biggest contributors to shaping the microbial community composition of algae, and metagenomic sequencing coupled with rigorous water chemistry testing may elucidate these relationships.

4.4 – Potential Bacteria-Alga Symbioses

4.4.1 – Resistance to Oxidative Stress

Oxidation resistance is an important component of algal survival, especially closer to the shoreline in the intertidal or splash zone where solar radiation is more extreme. Multiple sequence reads from the 2002 and 2016 *Bangia atropurpurea* samples and the *Cladophora glomerata* sample were classified as a bacterium from genus *Deinococcus* from phylum

Deinococcus-Thermus. The V4 16S rRNA sequence of this bacterial isolate aligned very closely to *Deinococcus knuensis, Deinococcus seolensis,* and *Deinococcus aquaticus* at a 100% sequence identity and E-value of $8e^{-132}$ for all three alignments. As with many other bacteria from genus *Deinococcus,* all three of these potential matches for the isolates observed on *B. atropurpurea* and *C. glomerata* shows presence of oxidase and catalase genes through experimentation. These genes code for proteins that confer resistance to gamma radiation (Lee *et al.* 2016, 2017). Protection to the algal host may be offered if the bacterial isolates contain these genes, but further metagenomic sequencing is required to confirm these functions and interactions.

4.4.2 –Protection against Toxins

A mutualistic protective relationship may be occurring involving Proteobacterial isolates from genera *Paucibacter, Aeromonas,* and *Sphingophyxis*. These taxa were observed in the microbiota of all the Lake Ontario algae, where microcystin-producing Cyanobacteria are known to occur and bloom in warmer months (Boyer, 2008). Several strains of *Paucibacter* has been shown to degrade microcystin through various genetic pathways (Bourne *et al.* 2001; You *et al.* 2014). Specific gene pathways, such as the *mlr* genes, and expressed proteins have also been elucidated from species within genus *Sphingopyxis* (Maghsoudi *et al.* 2016). Additionally, *Sphingomonas* sp. and *Aeromonas* sp. have shown microcystin-degrading abilities (Bourne *et al.* 2006; Mankiewicz-Boczek *et al.* 2015). Bacteria capable of microcystin degradation outside of the phylum Proteobacteria have also been found. Actinobacterial strains belonging to *Arthrobacter* spp. (family Micrococcaceae), *Brevibacterium* sp. (Brevibacteriaceae), and *Rhodococcus* sp. (Nocardiaceae), have been reported to break down microcystin but do not have the same genetic pathways or enzyme products as Proteobacteria with this capability (Manage *et*

al. 2009). Although these Actinobacterial strains have not been isolated from any algal microbiota in this study, it is important to note that microcystin degrading enzymes can be produced from a variety of bacteria through multiple pathways. Often the presence of microcystin degrading enzymes is coupled with presence of enzymes that can break down other harmful cyanobacterial and non-cyanobacterial peptides (Mou *et al.* 2013; Santos *et al.* 2021). Although microcystin is a hepatotoxin that primarily affect the livers of humans and animals, there is some evidence for the antagonistic effects against photoautotrophic organisms such as algae (Babica *et al*. 2006). The presence of bacteria that can break down microcystin and other toxic peptides in the microbiota of algae living amongst toxin-producing Cyanobacteria is a possible indicator of mutualistic symbiosis that may protect the algal host.

4.4.3 – Vitamin B_{12} Synthesis

The mutualistic relationship between auxotrophic algae and microbial synthesizers of vitamin B_{12} has been demonstrated in a variety of studies (Croft *et al.* 2005; Kazamia *et al.* 2012). Although not all algae require an external supply of cobalamin, two algae studied in this present thesis, *Bangia fuscopurpurea* and *Cladophora glomerata*, do need vitamin B_{12} for proper function and growth (Hoflmann 1990; Croft *et al.* 2005). The auxotrophic status of *Bangia atropurpurea* is not confirmed but can be assumed due to its close evolutionary relationship with *B. fuscopurpurea*. There are several bacteria implicated in the de novo production of vitamin B_{12}, including *Pseudomonas denitrificans, Propionibacterium shermanii*, and *Sinorhizobium meliloti*, which are all used in industrial production of this compound (Martens *et al.* 2002). Other bacteria from order Rhizobiales (Proteobacteria), such as *Mesorhizobium loti* and *Rhizobium leguminosarum*, have demonstrated close mutualistic interactions with algae on the basis of vitamin B_{12} synthesis (Kazamia *et al.* 2012). Some cyanobacterial taxa such as genus

Synechococcus have demonstrated production of pseudocobalamin, but this form of vitamin B_{12} may not be used by algae (Helliwell *et al.* 2016). In the present study, sequences classified as genera *Pseudomonas* and *Sinorhizobium* were identified in *B. atropurpurea* and *C. glomerata.* Vitamin B_{12}-synthesizing bacteria from order Rhizobiales were not identified in *B. fuscopurpurea* but it is likely that mutualistic interactions involving microbial synthesis of cobalamin occur in this alga as well. Although exact functions of these bacteria cannot be confirmed, this taxonomic classification indicates potential vitamin B_{12} mutualism between the bacteria and algal host.

4.5 – Phyla of Interest

4.5.1 - Cyanobacteria

The Cyanobacteria were a prevalent group of bacteria in both *Bangia atropurpurea* samples but were not observed in any other algal samples within this project. Cyanobacteria are well represented in all Laurentian Great Lakes and have been observed in the microbiota of *Cladophora glomerata* in several studies, albeit in low abundance (Ibsen *et al.* 2014; Graham *et al.* 2015a; Paver *et al.* 2020). The lack of cyanobacterial isolates in other algal samples in this study could be due to weak associations with the alga or insufficient sampling, and in future studies can be remedied with more sampling from a variety of sites. The sequences classified as Cyanobacteria spanned several orders, including Synechococcales, Cyanobacteriales, Oscillatoriales, and Leptolyngbyales. A bacterial isolate from the 2002 *B. atropurpurea* was closely clustered with *Calothrix desertica,* a nitrogen-fixing cyanobacteria (Stancheva *et al.* 2013). One ASV sequenced from both *B. atropurpurea* samples distantly grouped with genus *Planktothrix*, which is known to contain genes that code for microcystins, a family of hepatotoxins (Christiansen *et al.* 2003). Several ASVs, all of which were observed only in the

2002 *B. atropurpurea* sample, grouped closely with cyanotoxin-producing species *Tychonema bourrellyi* (Buratti *et al.* 2017). Both *Planktothrix* and *Tychonema* have been known to specifically produce toxins microcystin and anatoxin. The presence of toxin-producing cyanobacteria within the algal microbial community may be due to the blooming of these genera in the Great Lakes especially during the summer months (Boyer 2008), although there seems to be a closer relationship between Cyanobacteria and *Bangia* than with *Cladophora* despite occupying similar habitats. Microcystins have also been implicated in allelopathy against algae as they are potent phosphatase inhibitors (Babica *et al*. 2006). If this is the case, the underlying cause for the association between toxin-producing Cyanobacteria and *Bangia atropurpurea* could be an important research focus in the future.

The ability to fix nitrogen is another known feature of some Cyanobacteria. In the 2002 sample of *Bangia atropurpurea*, presence of heterocyst-forming bacteria *Calothrix desertica* was observed (Bauersachs *et al.* 2009). While most macroalgae are capable of assimilating organic nitrogenous compounds such as NO_3^-, NH_4^+, or NO_2^-, reliance on bacterial symbionts for nitrogen fixation, or converting N_2 into these molecules, may be necessary if they are not readily available in the water (Giordano & Raven 2014). Association between nitrogen-fixing Cyanobacteria *Calothrix* sp and *Anabaena* sp and green marine macroalga *Codium decorticatum* has been observed to improve algal nitrogen assimilation (Rosenberg & Paerl 1981). Although studies have shown proof of many Cyanobacteria-driven assimilation of nitrogen in terrestrial plants (Issa et al., 2014), this relationship is poorly described in the scope of algae. Additionally, due to lack of existing research on the *Bangia atropurpurea* microbiota, it is unclear if nitrogen-fixing species are symbiotically significant to this alga. The concentration of reduced nitrogenous compounds are steadily increasing in Lake Ontario and the other Great Lakes,

making them readily available for macroalgal assimilation (Dove & Chapra 2015). This could potentially limit the need for algal association with nitrogen fixing bacterial symbionts.

4.5.2 – The PVC Superphylum

Planctomycetes and Verrucomicrobia are bacterial phyla within the PVC superphylum (Wagner & Horn 2006) that have been observed in many algal microbiota, but exact symbiotic associations are not well understood. A meta-analysis of algal microbiota research by Goecke *et al.* (2013) revealed that bacterial genera from Verrucomicrobia and Planctomycetes are present in varied abundance across several freshwater and marine algae, including Rhodophyta, Chlorophyta, and Ochrophyta (Lachnit *et al.* 2011; Miranda *et al.* 2013; Morrissey *et al.* 2019). The verrucomicrobial and planctomycetal sequences obtained in the present study were primarily isolated from *Cladophora glomerata* and *Bangia fuscopurpurea* and had similar classifications as described in other algal microbiota research. The primary function of Planctomycetes and Verrucomicrobia bacteria seem to be polysaccharide degradation (Cardman *et al.* 2014), but more comprehensive genomic studies have uncovered other functional genes that may be implicated in algal function (Kim *et al.* 2016; Cabello-Yeves *et al.* 2017; Vollmers *et al.* 2017).

4.5.2.2 - Verrucomicrobia

Verrucomicrobial bacteria have been isolated from a diverse range of environments including soil (Kant *et al.* 2011), human and animal guts (Derrien *et al.* 2004), and associated with coral and algae in both marine and freshwater habitats (Yoon *et al.* 2008a). This phylum is divided into 3 classes, Opitutae, Terrimicrobia, and Verrucomicrobiae (*Phylum "Verrucomicrobia"* n.d.), but all ASVs identified in this study were classified as Verrucomicrobiae. A metagenomic analysis of Verrucomicrobia isolated from freshwater reservoirs in Spain revealed several functional genes. This included genes involved in

metabolism of simple and complex carbohydrates, phosphate transporters and enzymes for catalyzing phosphate degradation, and nitrogen fixing (Cabello-Yeves *et al.* 2017). Although these bacteria were not classified to the genus level, the presence of genes coding for polysaccharide-degrading enzymes in freshwater Verrucomicrobia may be indicative of potential symbiotic associations with algae. The presence of the *nif* operon, which is involved in the nitrogen fixation pathway, is rare in freshwater bacteria and may be another symbiotic association with the algal host (Rosenberg & Paerl 1981; Cabello-Yeves *et al.* 2017). This may be relevant to three verrucomicrobial ASVs that were isolated from *Cladophora glomerata* and *Bangia atropurpurea* in the present study. One of these ASVs found in all samples from Lake Ontario (*B. atropurpurea* 2002 & 2016, and *C. glomerata*) was closely clustered with *Luteolibacter yonseiensis* and *Luteolibacter luojiensis*. Both of these strains were found to have extremely low tolerance for salinity but could survive temperatures ranging from 4°C to 30°C (Jiang *et al.* 2012; Park *et al.* 2013). Out of 4 ASVs isolated from *Bangia fuscopurpurea*, two were distantly grouped with genus *Roseibacillus*, one with strains from genus *Rubritalea*, and one distantly grouped with genus *Roseimicrobium*. Representative bacteria from genera *Roseibacillus* and *Rubritalea* have been isolated from marine environments and can tolerate salinity up to 7% NaCl (Yoon *et al.* 2008a; Song *et al.* 2018). Bacteria from genus *Roseimicrobium* have been isolated from soil and has tolerance for lower salinity concentrations (Otsuka *et al.* 2013). Overall, verrucomicrobial samples isolated from the marine alga *Bangia fuscopurpurea* seem to be more adapted to higher salinity and harsher conditions than those isolated from the freshwater algae, which is consistent with bacterial community members from other phyla. At this point there are no detailed functional genomic analyses of these bacterial strains so more direct associations with the algal host cannot be elucidated.

4.5.2.1 - Planctomycetes

There were three ASVs classified as phylum Planctomycetes in this study which clustered closely with *Phycisphaera mikurensis, Pirellula staleyi,* and *Fimbriiglobus ruber* in the maximum likelihood phylogenetic tree (figure 8). *Phycisphaera mikurensis*, which was sequenced in the *Bangia fuscopurpurea* sample in this study, was first isolated and characterized from marine red alga *Porphyra* sp. (Fukunaga & Kurahashi 2009). Although this bacterial isolate was only able to grow in saline environments in culturing experiments (Fukunaga & Kurahashi 2009), order Phycisphaerales, to which this species belongs, is also found in hypersaline, freshwater, wastewater, soil, and animal microbiomes (Spring *et al.* 2018). In a genomic analysis of three Planctomycetes strains isolated from *Porphyra umbilicalis*, several functionally important gene families were identified (Kim *et al.* 2016). These strains fell within a clade that includes genera *Blastopirellula, Pirellula,* and *Rhodopirellula*. The ASV classified as *Pirellula staleyi* isolated from *Cladophora glomerata* in the present study is closely related to the planctomycetes strains examined by Kim *et al*. (2016). The genomic study of these Planctomycetes strains revealed a large group of sulfatase genes, which catalyze the degradation, hydrolysis, or synthesis of compounds that contain esterified sulfate (Kim *et al.* 2016). These planctomycetal strains also contained several genes dedicated to polysaccharide degradation, including agarases, carageenases, and xylanases (Kim *et al.* 2016). *Pirellula staleyi* and *Phycisphaera mikurensis* have several enzymes belonging to these gene families (Kim *et al.* 2016). A large portion of the cell surface polysaccharides on *Cladophora glomerata* are sulfated, which is a likely explanation for the symbiotic relationship between sulfate-degrading Planctomycetes and this alga (Surayot *et al.* 2016). Two of the planctomycetal strains described by Kim *et al*. (2016) also had genes that encode for selenoproteins, which have been shown to offer resistance to oxidative stress by inserting selenocysteine into proteins (Hatfield &

Gladyshev 2002). The last planctomycetal ASV, clustered with *Fimbriiglobus ruber* (order Planctomycetales, family *Gemmataceae*), was first isolated from peat and has been associated with Crustceans, but not algae (Kulichevskaya *et al.* 2017; Ravin *et al.* 2018). This species has been observed to degrade chitin and can be grown on other polysaccharides such as xylan (Ravin *et al.* 2018). Genetic sequencing of *F. ruber* revealed gene pathways coding for chitinase that were acquired through lateral gene transfer, and the ability to obtain nitrogen through chitin degradation was also observed (Ravin *et al.* 2018). The association of this species with *Cladophora glomerata* is not surprising as the alga has a chitinous outer layer of the cell wall (Pankiewicz *et al.* 2016). Despite the diverse array of functions that planctomycetal bacteria demonstrate, the symbiotic relationships with algal hosts seem to have mutualistic or commensal explanations.

4.6 – Conclusions and Future Work

4.6.1 – Characterization of the Bacterial Community of *Bangia atropurpurea*

The bacterial communities of marine and freshwater algae have become of greater interest in recent years, but there is still a significant gap in the research of freshwater red algal microbiomes and microbiota. *Bangia atropurpurea* is an invader of the Laurentian Great Lakes, and understanding the entire biological system of this alga, including the microbiome and its symbiotic associations, is vital to obtaining the full scope of algal function (Graham *et al.* 2015b). Characterization of the microbiota and further metagenomic sequencing may provide more information on how to mitigate the invasion of *B. atropurpurea* as it adapts to novel environmental conditions. Currently there is no published research that investigates the bacterial community composition of *B. atropurpurea* and this project is the first to do so.

The microbiota of two marine red algae, *Bangia fuscopurpurea* and *Bangia maxima,* were also characterized for the first time. The bacterial community of *Bangia maxima* was observed to be of extremely low diversity and abundance, and thus was not discussed at great length. Reasons for this poor associated microbial community are unknown but may be a result of DNA extraction or sequencing errors, or perhaps the unique habitat of this alga (Gardner 1927) is not conducive to formation of a microbiome. Unfortunately, there is no research on microbiomes or microbiota of algae that grow on unsubmerged boulders that can confirm this finding.

Analysis of the bacterial composition of *Bangia atropurpurea* was conducted through several comparisons to other algal microbiota. In the temporal analysis of the *B. atropurpurea* samples from 2002 and 2016, the diversity and abundance of the bacterial community was observed to be much lower in the 2016 sample compared to the 2002 sample. This may be an artefact of having only one experimental replicate for each algal sample and this result might not accurately reflect the true bacterial associations with the algal host. The bacteria phyla that were isolated only from the 2002 sample were those that are generally found in lower abundances in the water column and algal microbiota, such as Acidobacteria and Actinobacteria. The lack of these phyla in the 2016 sample could be a result of low sequencing resolution or reflect temporary bacteria-alga associations that are not always captured with V4 region 16S rRNA gene sequencing. Changes in the bacterial community composition could also be reflective of fluctuations in the water quality and chemistry of the Great Lakes (Paver *et al.* 2020). Bacterial genera from the most abundant phyla, Proteobacteria and Bacteroidetes, had a diverse range of presumed functions in both samples but the bacterial taxa from these phyla in the 2016 *B. atropurpurea* sample were typically more acclimated to average freshwater conditions and not

adaptable to environmental stressors. With this analysis of the *B. atropurpurea* bacterial communities, a putative core microbial community was established. This community consisted of genera with functions that may drive strong associations with the alga, such as degradation of cell surface polysaccharides agar and carrageenan, or protection against oxidative stress.

The microbiota of *Bangia atropurpurea* and *Cladophora glomerata* have some similarities, but there are likely some species-specific bacterial associations for each alga. This analysis between *B. atropurpurea* and *C. glomerata* revealed many broad differences in their microbial communities. Bacterial sequences classified as phyla Planctomycetes or Gemmatimonadetes were found only in *C. glomerata*, while phylum Cyanobacteria was observed solely in *B. atropurpurea.* The bacteria associated with *Cladophora* were substantiated by several other studies (Zulkifly *et al.* 2012; Graham *et al.* 2015a; Braus *et al.* 2017), but more research is needed on the microbiota of *Bangia atropurpurea* to confirm these species-specific associations.

Assemblage of bacteria in algal microbiota is also dependent on environmental factors, such as nutrient input or aquatic conditions (Morrissey *et al.* 2019). The relationship of bacterial community composition to the environment and geolocation was investigated with a pairwise analysis of *Bangia atropurpurea* and *Bangia fuscopurpurea*, which are closely related algae but are found in differing habitats. The microbiota of these two algae had the most divergence in bacterial community composition illustrating environment-specific associations, which is supported by the literature (Balakirev *et al.* 2012; Morrissey *et al.* 2019). Bacteria native to their habitats, whether it be freshwater or saline, are generally acclimated to those conditions and are specific to that locale. As a result, bacteria rarely transition between freshwater and marine environments, but that has been shown to occur (Herlemann *et al.* 2011). In the present study, only two bacterial genera were found in both freshwater and marine algae. The bacteria unique to

the marine *B. fuscopurpurea* were generally more adaptable to environmental stressors which may support algal survival in harsh conditions. Overall, a significant difference in bacterial communities of algae was likely observed due to environmental differences.

4.6.2 – Potential Bacterial Symbionts and Phyla of Interest

Several genera were observed with putative functions that may indicate a close symbiotic relationship with the algal host. An ASV classified as genus *Deinococcus* from Deinococcus-Thermus, isolated from both *Bangia atropurpurea* samples and *Cladophora glomerata,* has been experimentally shown to produce proteins that are resistant against oxidative stress caused by gamma radiation, although presence of these gene products cannot be confirmed in this study (Lee *et al*, 2016, 2017). These algal samples from Lake Ontario also had associations with various proteobacterial genera that have been observed to produce enzymes that degrade microcystin and other toxic peptides. Presence of microcystin-producing Cyanobacteria in Lake Ontario and other Great Lakes could result in these potential protective bacterial associations that may be vital to algal survival and health. Additionally, bacteria from order Rhizobiales that synthesize vitamin B_{12} had associations with auxotrophic algae *Bangia atropurpurea* and *Cladophora glomerata*. Although these symbionts were not observed to associate with *Bangia fuscopurpurea*, there is likely a different bacterial association that provides vitamin B_{12} to this alga. Oxidative stress protection, toxin degradation, and vitamin B_{12} production are only a few of the vital symbiotic interactions that might occur between bacteria and algal host and were observed in this study of algal microbiota.

Other phyla isolated from algal samples have non-vital putative functions that foster the close association between bacteria and host. Association between *Bangia* and Cyanobacteria could arise due to the nitrogen fixing abilities of bacteria from this phylum. The occurrence of

microcystin-producing cyanobacterial genera may reflect a temporary association with the alga simply due to its presence in the water column (Paver *et al*. 2020), and not as a close mutualistic or commensal relationship. As discussed previously, algae may form associations with bacteria that produce protective microcystin-degrading enzymes to mitigate the presence of harmful Cyanobacteria. Bacteria from phyla Verrucomicrobia and Planctomycetes have been observed to form close associations with many algae, and may have vital symbiotic associations despite low abundances in algal microbial communities (Goecke *et al.* 2013). Genomic analyses of bacterial genera from both of these phyla revealed specialized polysaccharide-degrading functions (Cardman *et al.* 2014; Vollmers *et al.* 2017). Some planctomycetal genera were implicated in more protective functions, such as genes encoding selenoproteins. In-depth metagenomic analyses that uncover specific bacterial functions provide an enormous wealth of knowledge to understanding bacteria-alga interactions.

From this preliminary analysis, bacterial associations with *Bangia atropurpurea* seem to be driven by protective, vital, or neutral functions that may be commensal or mutualistic in nature. There may also be parasitic bacterial symbionts but were not observed in the present study. However, it is important to keep in mind that exact functions of associated bacterial isolates cannot be confirmed with 16S rRNA gene sequence classification. Thorough metagenomic analysis is necessary to elucidate presence of functional genes and establish these symbiotic interactions. Although a putative microbiota of *Bangia atropurpurea* has now been established, more research that characterizes the microbial community of this alga is vital to substantiate these findings. Freshwater algal microbiomes, especially those of red algae, are not well studied and characterization of the microbiota is essential to a comprehensive understanding of algal function within the ecosystem.

References

Aires, T., Serrão, E. A., & Engelen, A. H. (2016). Host and environmental specificity in bacterial communities associated to two highly invasive marine species (genus *Asparagopsis*). *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00559

Akter, S., NGO, H. T. T., Du, J., Won, K., Singh, H., Yin, C. S., Kook, M., & Yi, T.-H. (2015). Chryseobacterium formosus sp. nov., a bacterium isolated from an ancient tree trunk. *Archives of Microbiology*, *197*(8), 1011–1017. https://doi.org/10.1007/s00203-015-1137-9

Alexander, J., Bollmann, A., Seitz, W., & Schwartz, T. (2015). Microbiological characterization of aquatic microbiomes targeting taxonomical marker genes and antibiotic resistance genes of opportunistic bacteria. *Science of The Total Environment*, *512–513*, 316–325. https://doi.org/https://doi.org/10.1016/j.scitotenv.2015.01.046

Alivisatos, A., Blaser, M., Brodie, E., Chun, M., Dangl, J., Donohue, T., Dorrestein, P., Gilbert, J., Green, J., Jansson, J., Knight, R., Maxon, M., McFall-Ngai, M., Miller, J., Pollard, K., Ruby, E., & Taha, S. (2015). A unified initiative to harness Earth's microbiome. *Science*, *350*(6260).

Altschul, S F, Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Altschul, Stephen F, Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/https://doi.org/10.1016/S0022-2836(05)80360-2

Alvarado, P., Huang, Y., Wang, J., Garrido, I., & Leiva, S. (2018). Phylogeny and bioactivity of epiphytic Gram-positive bacteria isolated from three co-occurring antarctic macroalgae. *Antonie van Leeuwenhoek*, *111*(9), 1543–1555. https://doi.org/10.1007/s10482-018-1044-6

Ansari, M., Shekari, F., Mohammadi, M. H., Juhos, K., Végvári, G., & Biró, B. (2019). Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (Medicago sativa L.) cultivars at high salinity. *Acta Physiologiae Plantarum*, *41*(12), 195. https://doi.org/10.1007/s11738-019-2988-5

Apprill, A., Mcnally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

Babica, P., Bláha, L., & Maršálek, B. (2006). Exploring the natural role of microcystins - A review of effects on photoautotrophic organisms. *Journal of Phycology*, *42*(1), 9–20. https://doi.org/10.1111/j.1529-8817.2006.00176.x

Baedke, J., Fábregas-Tejeda, A., & Nieves Delgado, A. (2020). The holobiont concept before

Margulis. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *334*(3), 149–155. https://doi.org/10.1002/jez.b.22931

Balakirev, E. S., Krupnova, T. N., & Ayala, F. J. (2012). Symbiotic associations in the phenotypically-diverse brown alga Saccharina japonica. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0039587

Barsanti, L., Coltelli, P., Evangelista, V., Frassanito, A. M., Passarelli, V., Vesentini, N., & Gualtieri, P. (2008a). Oddities and Curiosities in the Algal World. In *NATO Science for Peace and Security Series A: Chemistry and Biology* (pp. 353–391).

Barsanti, L., Coltelli, P., Evangelista, V., Frassanito, A. M., Passarelli, V., Vesentini, N., & Gualtieri, P. (2008b). The world of algae. In *NATO Science for Peace and Security Series A: Chemistry and Biology* (pp. 1–15).

Bauersachs, T., Compaoré, J., Hopmans, E. C., Stal, L. J., Schouten, S., & Sinninghe Damsté, J. S. (2009). Distribution of heterocyst glycolipids in cyanobacteria. *Phytochemistry*, *70*(17–18), 2034–2039. https://doi.org/10.1016/j.phytochem.2009.08.014

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1), 1–22. https://doi.org/10.1186/s40168-020-00875-0

Bernardet, J.-F., & Bowman, J. P. (2006). The Prokaryotes, The Genus Flavobacterium. In *The Prokaryotes: Vol. 7: Proteobacteria: Delta and Epsilon Subclasses. Deeply Rooting Bacteria*. https://link.springer.com/content/pdf/10.1007/0-387-30747-8_17.pdf

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*(1), 1–17. https://doi.org/10.1186/s40168-018-0470-z

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Borowitzka, M. A. (2018). The 'stress' concept in microalgal biology—homeostasis, acclimation and adaptation. *Journal of Applied Phycology*, *30*(5), 2815–2825. https://doi.org/10.1007/s10811-018-1399-0

Bourne, D. G., Blakeley, R. L., Riddles, P., & Jones, G. J. (2006). Biodegradation of the cyanobacterial toxin microcystin LR in natural water and biologically active slow sand filters. *Water Research*, *40*(6), 1294–1302.

https://doi.org/https://doi.org/10.1016/j.watres.2006.01.022

Bourne, D. G., Riddles, P., Jones, G. J., Smith, W., & Blakeley, R. L. (2001). Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. *Environmental Toxicology*, *16*(6), 523–534. https://doi.org/https://doi.org/10.1002/tox.10013

Boyer, G. L. (2008). *Cyanobacterial Toxins in New York and the Lower Great Lakes Ecosystems BT - Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs* (H. K. Hudnell (Ed.); pp. 153–165). Springer New York. https://doi.org/10.1007/978-0-387-75865-7_7

Braus, M. J., Graham, L. E., & Whitman, T. L. (2017). Spatiotemporal dynamics of the bacterial microbiota on lacustrine Cladophora glomerata (Chlorophyta). *Journal of Phycology*, *53*(6), 1255–1262. https://doi.org/10.1111/jpy.12573

Brawley, S. H., Blouin, N. A., Ficko-Blean, E., Wheeler, G. L., Lohr, M., Goodson, H. V., Jenkins, J. W., Blaby-Haas, C. E., Helliwell, K. E., Chan, C. X., Marriage, T. N., Bhattacharya, D., Klein, A. S., Badis, Y., Brodie, J., Cao, Y., Collén, J., Dittami, S. M., Gachon, C. M. M., … Prochnik, S. E. (2017). Insights into the red algae and eukaryotic evolution from the genome of Porphyra umbilicalis (Bangiophyceae, Rhodophyta). *Proceedings of the National Academy of Sciences*, *114*(31), E6361–E6370. https://doi.org/10.1073/PNAS.1703088114

Brown, L. M., & Hellebust, J. A. (1978). Sorbitol and proline as intracellular osmotic solutes in the green alga Stichococcus bacillaris. *Canadian Journal of Botany*, *56*(6), 676–679. https://doi.org/10.1139/b78-074

Buratti, F. M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., & Funari, E. (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology*, *91*(3), 1049–1130. https://doi.org/10.1007/s00204-016-1913-6

Butterfield, N. J., Knoll, A. H., & Swett, K. (1990). A bangiophyte red alga from the Proterozoic of arctic Canada. *Science*, *250*(4977), 104. https://doi.org/10.1126/science.11538072

Butterwick, C., Heaney, S. I., & Talling, J. F. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshwater Biology*, *50*(2), 291–300. https://doi.org/https://doi.org/10.1111/j.1365-2427.2004.01317.x

Cabello-Yeves, P. J., Ghai, R., Mehrshad, M., Picazo, A., Camacho, A., & Rodriguez-Valera, F. (2017). Reconstruction of Diverse Verrucomicrobial Genomes from Metagenome Datasets of Freshwater Reservoirs. *Frontiers in Microbiology*, *8*, 2131. https://doi.org/10.3389/fmicb.2017.02131

Cameron, E. S., Schmidt, P. J., Tremblay, B. J.-M., Emelko, M. B., & Müller, K. M. (2020). *To rarefy or not to rarefy: Enhancing microbial community analysis through next-generation sequencing*. bioRxiv.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A. D., & Teske, A. (2014). Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Applied and Environmental Microbiology*, *80*(12), 3749–3756. https://doi.org/10.1128/AEM.00899-14

Casadevall, A., & Pirofski, L.-A. (2000). Host-Pathogen Interactions: Basic Concepts of Microbial Commensalism, Colonization, Infection, and Disease. In *INFECTION AND IMMUNITY* (Vol. 68, Issue 12). http://iai.asm.org/

Cavender-Bares, K. K., Karl, D. M., & Chisholm, S. W. (2001). Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. *Deep-Sea Research Part I: Oceanographic Research Papers*, *48*(11), 2373–2395. https://doi.org/10.1016/S0967-0637(01)00027-9

Chapra, S. C., Dove, A., & Warren, G. J. (2012). Long-term trends of Great Lakes major ion chemistry. *Journal of Great Lakes Research*, *38*(3), 550–560. https://doi.org/10.1016/j.jglr.2012.06.010

Cherrier, J., Valentine, S. K., Hamill, B., Jeffrey, W. H., & Marra, J. F. (2015). Light-mediated release of dissolved organic carbon by phytoplankton. *Journal of Marine Systems*, *147*, 45–51. https://doi.org/10.1016/j.jmarsys.2014.02.008

Christiansen, G., Fastner, J., Erhard, M., Börner, T., & Dittmann, E. (2003). Microcystin biosynthesis in Planktothrix: Genes, evolution, and manipulation. *Journal of Bacteriology*, *185*(2), 564–572. https://doi.org/10.1128/JB.185.2.564-572.2003

Contreras-Porcia, L., Thomas, D., Flores, V., & Correa, J. A. (2011). Tolerance to oxidative stress induced by desiccation in Porphyra columbina (Bangiales, Rhodophyta). *Journal of Experimental Botany*, *62*(6), 1815–1829. https://doi.org/10.1093/jxb/erq364

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., & Smith, A. G. (2005). Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature*, *438*(7064), 90–93. https://doi.org/10.1038/nature04056

Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species. *Plant Signaling & Behavior*, *3*(3), 156–165. https://doi.org/10.4161/psb.3.3.5536

Daily, O. P., Joseph, S. W., Coolbaugh, J. C., Walker, R. I., Merrell, B. R., Rollins, D. M., Seidler, R. J., Colwell, R. R., & Lissner, C. R. (1981). Association of Aeromonas sobria with human infection. *Journal of Clinical Microbiology*, *13*(4), 769–777. https://doi.org/10.1128/jcm.13.4.769-777.1981

De Maayer, P., Anderson, D., Cary, C., & Cowan, D. A. (2014). Some like it cold: Understanding the survival strategies of psychrophiles. *EMBO Reports*, *15*(5), 508–517. https://doi.org/10.1002/embr.201338170

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of*

Systematic and Evolutionary Microbiology, *54*(5), 1469–1476. https://doi.org/https://doi.org/10.1099/ijs.0.02873-0

Descamps, V., Colin, S., Lahaye, M., Jam, M., Richard, C., Potin, P., Barbeyron, T., Yvin, J.-C., & Kloareg, B. (2006). Isolation and Culture of a Marine Bacterium Degrading the Sulfated Fucans from Marine Brown Algae. *Marine Biotechnology*, *8*(1), 27–39. https://doi.org/10.1007/s10126-005-5107-0

Dittami, S. M., Barbeyron, T., Boyen, C., Cambefort, J., Collet, G., Delage, L., Gobet, A., Groisillier, A., Leblanc, C., Michel, G., Scornet, D., Siegel, A., Tapia, J. E., & Tonon, T. (2014). Genome and metabolic network of "Candidatus Phaeomarinobacter ectocarpi" Ec32, a new candidate genus of Alphaproteobacteria frequently associated with brown algae. *Frontiers in Genetics*, *5*, 241. https://doi.org/10.3389/fgene.2014.00241

Dittami, S. M., Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., & Tonon, T. (2016). Host-microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *ISME Journal*, *10*(1), 51–63. https://doi.org/10.1038/ismej.2015.104

Dove, A., & Chapra, S. C. (2015). Long-term trends of nutrients and trophic response variables for the Great Lakes. *Limnology and Oceanography*, *60*(2), 696–721. https://doi.org/https://doi.org/10.1002/lno.10055

Eichel, J., González, J. C., Hotze, M., Matthews, R. G., & Schröder, J. (1995). Vitamin-B12-Independent Methionine Synthase from a Higher Plant (Catharanthus Roseus). *European Journal of Biochemistry*, *230*(3), 1053–1058. https://doi.org/https://doi.org/10.1111/j.1432-1033.1995.1053g.x

Eigemann, F., Hilt, S., Salka, I., & Grossart, H. P. (2013). Bacterial community composition associated with freshwater algae: Species specificity vs. dependency on environmental conditions and source community. *FEMS Microbiology Ecology*, *83*(3), 650–663. https://doi.org/10.1111/1574-6941.12022

EPA. (2012). *Conductivity*. United States Environmental Protection Agency. https://archive.epa.gov/water/archive/web/html/vms59.html

Figueroa, F. L., & Gómez, I. (2001). Photosynthetic acclimation to solar UV radiation of marine red algae from the warm-temperate coast of southern Spain: A review. *Journal of Applied Phycology*, *13*(3), 235–248. https://doi.org/10.1023/A:1011126007656

Finster, K. W., Herbert, R. A., & Lomstein, B. A. (2009). Spirosoma spitsbergense sp. nov. and Spirosoma luteum sp. nov., isolated from a high Arctic permafrost soil, and emended description of the genus Spirosoma. *International Journal of Systematic and Evolutionary Microbiology*, *59*(4), 839–844. https://doi.org/https://doi.org/10.1099/ijs.0.002725-0

Fogg, G. E. (2001). Algal Adaptation to Stress — Some General Remarks. *Algal Adaptation to Environmental Stresses*, 1–19. https://doi.org/10.1007/978-3-642-59491-5_1

Fukunaga, Y., & Kurahashi, M. (2009). Phycisphaera mikurensis gen. nov., sp. nov., isolated

from a marine alga, and proposal of Phycisphaeraceae fam. nov., Phycisphaerales ord. nov. and Phycisphaerae. *The Journal of General …*, *275*, 267–275. http://japanlinkcenter.org/JST.JSTAGE/jgam/55.267?from=Google

Gaitan-Espitia, J. D., & Schmid, M. (2020). *Diversity and Functioning of Antarctic Seaweed Microbiomes BT - Antarctic Seaweeds: Diversity, Adaptation and Ecosystem Services* (I. Gómez & P. Huovinen (Eds.); pp. 279–291). Springer International Publishing. https://doi.org/10.1007/978-3-030-39448-6_14

Gardner, N. L. (1927). New Rhodophyceae From the Pacific Coast of North America. II. *University of California Publications in Botany*, *13*(13), 235–272.

Ghaderiardakani, F., Quartino, M. L., & Wichard, T. (2020). Microbiome-Dependent Adaptation of Seaweeds Under Environmental Stresses: A Perspective. *Frontiers in Marine Science*, *7*(December), 1–9. https://doi.org/10.3389/fmars.2020.575228

Giordano, M., & Raven, J. A. (2014). Nitrogen and sulfur assimilation in plants and algae. *Aquatic Botany*, *118*, 45–61. https://doi.org/10.1016/j.aquabot.2014.06.012

Goecke, F., Thiel, V., Wiese, J., Labes, A., & Imhoff, J. F. (2013). Algae as an important environment for bacteria – phylogenetic relationships among new bacterial species isolated from algae. *Phycologia*, *52*(1), 14–24. https://doi.org/10.2216/12-24.1

Goff, L. J., & Zuccarello, G. (1994). The Evolution of Parasitism in Red Algae: Cellular Interactions of Adelphoparasites and Their Hosts. *Journal of Phycology*, *30*(4), 695–720. https://doi.org/https://doi.org/10.1111/j.0022-3646.1994.00695.x

Graham, L. E., Knack, J. J., Graham, M. E., Graham, J. M., & Zulkifly, S. (2015). A metagenome for lacustrine Cladophora (Cladophorales) reveals remarkable diversity of eukaryotic epibionts and genes relevant to materials cycling. *Journal of Phycology*, *51*(3), 408–418. https://doi.org/10.1111/jpy.12296

Graham, L. E., Wilcox, L. W., & Knack, J. J. (2015). Why we need more algal metagenomes. *Journal of Phycology*, *51*(6), 1029–1036. https://doi.org/10.1111/jpy.12344

Hadley, W. (2016). *ggplot2: Elegant Graphics for Data Analysis.* Springer-Verlag. https://ggplot2.tidyverse.org

Hanbo, C. (2018). *VennDiagram: Generate High-Resolution Venn and Euler Plots*. https://cran.r-project.org/package=VennDiagram

Hatfield, D. L., & Gladyshev, V. N. (2002). How Selenium Has Altered Our Understanding of the Genetic Code. *Molecular and Cellular Biology*, *22*(11), 3565–3576. https://doi.org/10.1128/mcb.22.11.3565-3576.2002

Helliwell, Katherine E., Wheeler, G. L., Leptos, K. C., Goldstein, R. E., & Smith, A. G. (2011). Insights into the evolution of vitamin B 12 auxotrophy from sequenced algal genomes. *Molecular Biology and Evolution*, *28*(10), 2921–2933.

https://doi.org/10.1093/molbev/msr124

Helliwell, Katherine Emma, Lawrence, A. D., Holzer, A., Kudahl, U. J., Sasso, S., Kräutler, B., Scanlan, D. J., Warren, M. J., & Smith, A. G. (2016). Cyanobacteria and Eukaryotic Algae Use Different Chemical Variants of Vitamin B12. *Current Biology : CB*, *26*(8), 999–1008. https://doi.org/10.1016/j.cub.2016.02.041

Herlemann, D. P. R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal*, *5*(10), 1571–1579. https://doi.org/10.1038/ismej.2011.41

Hezbri, K., Ghodhbane-Gtari, F., Montero-Calasanz, M. del C., Nouioui, I., Rohde, M., Spröer, C., Schumann, P., Klenk, H.-P., & Gtari, M. (2016). Geodermatophilus pulveris sp. nov., a gamma-radiation-resistant actinobacterium isolated from the Sahara desert. *International Journal of Systematic and Evolutionary Microbiology*, *66*(10), 3828–3834. https://doi.org/https://doi.org/10.1099/ijsem.0.001272

Higgins, S. N., Malkin, S. Y., Todd Howell, E., Guildford, S. J., Campbell, L., Hiriart-Baer, V., & Hecky, R. E. (2008). An ecological review of Cladophora glomerata (Chlorophyta) in the Laurentian Great Lakes. In *Journal of Phycology* (Vol. 44, Issue 4, pp. 839–854). https://doi.org/10.1111/j.1529-8817.2008.00538.x

Hirsch, A. M. (2004). Plant-Microbe Symbioses: A Continuum from Commensalism to Parasitism. In *Symbiosis* (Vol. 37).

Hoflmann, J. P. (1990). Dependence of photosynthesis and vitamin B12 uptake on cellular vitamin B12 concentration in the multicellular alga Cladophora glomerata (Chlorophyta). *Limnology and Oceanography*, *35*(1), 100–108. https://doi.org/10.4319/lo.1990.35.1.0100

Ibsen, M., Kumar, A., Neufeld, J., & Kirkwood, A. (2014). *The role of Cladophora glomerata as a habitat for bacterial communities and a reservoir of antibiotic resistance*. University of Ontario Institute of Technology.

Issa, A. A., Abd-Alla, M. H., & Ohyama, T. (2014). Nitrogen Fixing Cyanobacteria: Future Prospect. In *Advances in Biology and Ecology of Nitrogen Fixation* (pp. 23–48).

Janabi, A. H. D., Kerkhof, L. J., McGuinness, L. R., Biddle, A. S., & McKeever, K. H. (2016). Comparison of a modified phenol/chloroform and commercial-kit methods for extracting DNA from horse fecal material. *Journal of Microbiological Methods*, *129*, 14–19. https://doi.org/https://doi.org/10.1016/j.mimet.2016.07.019

Ji, Y., & Tanaka, J. (2002). Effect of desiccation on the photosynthesis of seaweeds from the intertidal zone in Honshu, Japan. *Phycological Research*, *50*(2), 145–153. https://doi.org/https://doi.org/10.1046/j.1440-1835.2002.00268.x

Jiang, F., Li, W., Xiao, M., Dai, J., Kan, W., Chen, L., Li, W., Fang, C., & Peng, F. (2012). Luteolibacter luojiensis sp. nov., isolated from Arctic tundra soil, and emended description of the genus Luteolibacter. *International Journal of Systematic and Evolutionary*

Microbiology, *62*(Pt_9), 2259–2263. https://doi.org/https://doi.org/10.1099/ijs.0.037309-0

Jiang, Z., Yu, G., Liang, Y., Song, T., Zhu, Y., Ni, H., Yamaguchi, K., & Oda, T. (2019). Inhibitory effects of a sulfated polysaccharide isolated from edible red alga Bangia fusco-purpurea on α-amylase and α-glucosidase. *Bioscience, Biotechnology and Biochemistry*, *83*(11), 2065–2074. https://doi.org/10.1080/09168451.2019.1634515

Jin, L., Lee, H. G., La, H. J., Ko, S. R., Ahn, C. Y., & Oh, H. M. (2014). Ferruginibacter profundus sp. nov., a novel member of the family Chitinophagaceae, isolated from freshwater sediment of a reservoir. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *106*(2), 319–323. https://doi.org/10.1007/s10482-014-0202-8

Jooste, P. J., & Hugo, C. J. (1999). The taxonomy, ecology and cultivation of bacterial genera belonging to the family Flavobacteriaceae. *International Journal of Food Microbiology*, *53*(2–3), 81–94. https://doi.org/10.1016/S0168-1605(99)00162-2

Kalia, Vipin C. (2015). Microbes: The Most Friendly Beings? In Vipin Chandra Kalia (Ed.), *Quorum Sensing vs Quorum Quenching: A Battle with No End in Sight* (pp. 1–5). Springer India. https://doi.org/10.1007/978-81-322-1982-8_1

Kaňa, R., Kotabová, E., Šedivá, B., & Kuthanová Trsková, E. (2019). Photoprotective strategies in the motile cryptophyte alga Rhodomonas salina—role of non-photochemical quenching, ions, photoinhibition, and cell motility. *Folia Microbiologica*, *64*(5), 691–703. https://doi.org/10.1007/s12223-019-00742-y

Kant, R., van Passe, M. W. J., Palva, A., Lucas, S., Lapidus, A., del Rio, T. G., Dalin, E., Tice, H., Bruce, D., Goodwin, L., Pitluck, S., Larimer, F. W., Land, M. L., Hauser, L., Sangwan, P., de Vos, W. M., Janssen, P. H., & Smidt, H. (2011). Genome sequence of Chthoniobacter flavus Ellin428, an aerobic heterotrophic soil bacterium. *Journal of Bacteriology*, *193*(11), 2902–2903. https://doi.org/10.1128/JB.00295-11

Karsten, U., & Kirst, G. O. (1989). Incomplete turgor pressure regulation in the “terrestial” red alga, Bostrychia scorpioides (Huds.) Mont. *Plant Science*, *61*(1), 29–36. https://doi.org/https://doi.org/10.1016/0168-9452(89)90115-5

Kazamia, E., Czesnick, H., Nguyen, T. T. Van, Croft, M. T., Sherwood, E., Sasso, S., Hodson, S. J., Warren, M. J., & Smith, A. G. (2012). Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. *Environmental Microbiology*, *14*(6), 1466–1476. https://doi.org/10.1111/j.1462-2920.2012.02733.x

Kim, J. W., Brawley, S. H., Prochnik, S., Chovatia, M., Grimwood, J., Jenkins, J., LaButti, K., Mavromatis, K., Nolan, M., Zane, M., Schmutz, J., Stiller, J. W., & Grossman, A. R. (2016). Genome Analysis of Planctomycetes Inhabiting Blades of the Red Alga Porphyra umbilicalis. *PLOS ONE*, *11*(3), e0151883-. https://doi.org/10.1371/journal.pone.0151883

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*,

111–120.

Krishnan, A., Zhang, Y., Balaban, M., Seo, Y., & Mou, X. (2020). Taxonomic and Genotypical Heterogeneity of Microcystin degrading Bacterioplankton in Western Lake Erie. *Harmful Algae*, *98*, 101895. https://doi.org/https://doi.org/10.1016/j.hal.2020.101895

Kulichevskaya, I. S., Ivanova, A. A., Baulina, O. I., Rijpstra, W. I. C., Sinninghe Damsté, J. S., & Dedysh, S. N. (2017). Fimbriiglobus ruber gen. nov., sp. nov., a Gemmata-like planctomycete from Sphagnum peat bog and the proposal of Gemmataceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, *67*(2), 218–224. https://doi.org/https://doi.org/10.1099/ijsem.0.001598

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Lachnit, T., Meske, D., Wahl, M., Harder, T., & Schmitz, R. (2011). Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environmental Microbiology*, *13*(3), 655–665. https://doi.org/10.1111/j.1462-2920.2010.02371.x

Lee, J. J., Lee, Y. H., Park, S. J., Lee, S. Y., Park, S., Lee, D. S., Kang, I. K., Ten, L. N., & Jung, H. Y. (2017). Deinococcus knuensis sp. nov., a bacterium isolated from river water. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *110*(3), 407–414. https://doi.org/10.1007/s10482-016-0813-3

Lee, J. J., Lee, Y. H., Park, S. J., Lim, S., Jeong, S. W., Lee, S. Y., Cho, Y. J., Kim, M. K., & Jung, H. Y. (2016). Deinococcus seoulensis sp. nov., a bacterium isolated from sediment at Han River in Seoul, Republic of Korea. *Journal of Microbiology*, *54*(8), 537–542. https://doi.org/10.1007/s12275-016-6253-y

Lee, J., Jeong, K. W., Jin, B., Ryu, K. S., Kim, E. H., Ahn, J. H., & Kim, Y. (2013). Structural and Dynamic Features of Cold-Shock Proteins of Listeria monocytogenes, a Psychrophilic Bacterium. *Biochemistry*, *52*(14), 2492–2504. https://doi.org/10.1021/bi301641b

Leiva, S., Alvarado, P., Huang, Y., Wang, J., & Garrido, I. (2015). Diversity of pigmented Gram-positive bacteria associated with marine macroalgae from Antarctica. *FEMS Microbiology Letters*, *362*(24). https://doi.org/10.1093/femsle/fnv206

Lin, C. K., & Blum, J. L. (1977). Recent Invasion of a Red Alga (Bangia atropurpurea) in Lake Michigan. *Journal of the Fisheries Research Board of Canada*, *34*(12), 2413–2416. https://doi.org/10.1139/f77-326

Liu, Y., Du, J., Lai, Q., Dong, C., Xie, Y., & Shao, Z. (2016). Jiulongibacter sediminis gen. nov., sp. nov., a new member of the family Cytophagaceae, isolated from the surface sediment of the Jiulong River in China. *International Journal of Systematic and Evolutionary Microbiology*, *66*(6), 2347–2353. https://doi.org/https://doi.org/10.1099/ijsem.0.001038

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. In

Genome Medicine (Vol. 8, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13073-016-0307-y

Lowe, R. L., Rosen, B. H., & Kingston, J. C. (1982). A Comparison of Epiphytes On Bangia Atropurpurea (Rhodophyta) and Cladophora Glomerata (Chlorophyta) from Northern Lake Michigan. *Journal of Great Lakes Research*, *8*(1), 164–168. https://doi.org/10.1016/S0380-1330(82)71954-9

Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Ávila, J. A., López-Guerrero, J. A., & Aguilar-Quesada, R. (2016). DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. *Biopreservation and Biobanking*, *14*(4), 264–270. https://doi.org/10.1089/bio.2015.0064

Lynch, M. D. J., Sheath, R. G., & Müller, K. M. (2008). Phylogenetic position and ISSR-estimated intraspecific genetic variation of Bangia maxima (Bangiales, Rhodophyta). *Phycologia*, *47*(6), 599–613. https://doi.org/10.2216/07-47.1

Lyons, D. A., Arvanitidis, C., Blight, A. J., Chatzinikolaou, E., Guy-Haim, T., Kotta, J., Orav-Kotta, H., Queirós, A. M., Rilov, G., Somerfield, P. J., & Crowe, T. P. (2014). Macroalgal blooms alter community structure and primary productivity in marine ecosystems. *Global Change Biology*, *20*(9), 2712–2724. https://doi.org/10.1111/gcb.12644

Maghsoudi, E., Fortin, N., Greer, C., Maynard, C., Pagé, A., Duy, S. V., Sauvé, S., Prévost, M., & Dorner, S. (2016). Cyanotoxin degradation activity and mlr gene expression profiles of a Sphingopyxis sp. isolated from Lake Champlain, Canada. *Environmental Science. Processes & Impacts*, *18*(11), 1417–1426. https://doi.org/10.1039/c6em00001k

Mahdiyan, O., Filazzola, A., Molot, L. A., Gray, D., & Sharma, S. (2021). Drivers of water quality changes within the Laurentian Great Lakes region over the past 40 years. *Limnology and Oceanography*, *66*(1), 237–254. https://doi.org/https://doi.org/10.1002/lno.11600

Manage, P. M., Edwards, C., Singh, B. K., & Lawton, L. A. (2009). Isolation and identification of novel microcystin-degrading bacteria. *Applied and Environmental Microbiology*, *75*(21), 6924–6928. https://doi.org/10.1128/AEM.01928-09

Mankiewicz-Boczek, J., Gągała, I., Jurczak, T., Jaskulska, A., Pawełczyk, J., & Dziadek, J. (2015). Bacteria homologus to Aeromonas capable of microcystin degradation. *Open Life Sciences*, *10*(1). https://doi.org/doi:10.1515/biol-2015-0012

Martens, J. H., Barg, H., Warren, M., & Jahn, D. (2002). Microbial production of vitamin B12. *Applied Microbiology and Biotechnology*, *58*(3), 275–285. https://doi.org/10.1007/s00253-001-0902-7

McCormick, P. V., & Cairns, J. (1994). Algae as indicators of environmental change. *Journal of Applied Phycology*, *6*(5–6), 509–526. https://doi.org/10.1007/BF02182405

McIlroy, S. J., & Nielsen, P. H. (2014). *The Family Saprospiraceae BT - The Prokaryotes: Other Major Lineages of Bacteria and The Archaea* (E. Rosenberg, E. F. DeLong, S. Lory,

E. Stackebrandt, & F. Thompson (Eds.); pp. 863–889). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-38954-2_138

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. In *PLoS ONE* (Vol. 8, Issue 4, p. e61217). PLoS ONE. https://doi.org/10.1371/journal.pone.0061217

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. In *FEMS Microbiology Reviews* (Vol. 37, Issue 5, pp. 634–663). https://doi.org/10.1111/1574-6976.12028

Meyer-Abich, A. (1943). I. Das Typologische Grundgesetz und Seine Folgerungen Für Phylogenie und Entwicklungsphysiologie. *Acta Biotheoretica*, *7*(1), 1–80. https://doi.org/10.1007/BF01603792

Michel, G., Nyval-Collen, P., Barbeyron, T., Czjzek, M., & Helbert, W. (2006). Bioconversion of red seaweed galactans: A focus on bacterial agarases and carrageenases. *Applied Microbiology and Biotechnology*, *71*(1), 23–33. https://doi.org/10.1007/s00253-006-0377-7

Microsoft. (2021). *Microsoft Excel* (Microsoft Office 365).

Mills, E. L., Leach, J. H., Carlton, J. T., & Secor, C. L. (1993). Exotic Species in the Great Lakes: A History of Biotic Crises and Anthropogenic Introductions. *Journal of Great Lakes Research*, *19*(1), 1–54. https://doi.org/https://doi.org/10.1016/S0380-1330(93)71197-1

Minich, J. J., Morris, M. M., Brown, M., Doane, M., Edwards, M. S., Michael, T. P., & Dinsdale, E. A. (2018). Elevated temperature drives kelp microbiome dysbiosis, while elevated carbon dioxide induces water microbiome disruption. *PLoS ONE*, *13*(2), 1–23. https://doi.org/10.1371/journal.pone.0192772

Miranda, L. N., Hutchison, K., Grossman, A. R., & Brawley, S. H. (2013). Diversity and Abundance of the Bacterial Community of the Red Macroalga Porphyra umbilicalis: Did Bacterial Farmers Produce Macroalgae? *PLOS ONE*, *8*(3), e58269-. https://doi.org/10.1371/journal.pone.0058269

Mohsen, A., Park, J., Chen, Y. A., Kawashima, H., & Mizuguchi, K. (2019). Impact of quality trimming on the efficiency of reads joining and diversity analysis of Illumina paired-end reads in the context of QIIME1 and QIIME2 microbiome analysis frameworks. *BMC Bioinformatics*, *20*(1), 1–10. https://doi.org/10.1186/s12859-019-3187-5

Montero-Calasanz, M. del C. (2020). Geodermatophilus. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–28). https://doi.org/https://doi.org/10.1002/9781118960608.gbm00044.pub2

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schäffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics (Oxford, England)*, *24*(16), 1757–1764. https://doi.org/10.1093/bioinformatics/btn322

Morrice, L. M., McLean, M. W., Long, W. F., & Williamson, F. B. (1984). *Porphyran primary structure BT - Eleventh International Seaweed Symposium* (C. J. Bird & M. A. Ragan (Eds.); pp. 572–575). Springer Netherlands.

Morrissey, K. L., Çavas, L., Willems, A., & De Clerck, O. (2019). Disentangling the influence of environment, host specificity and thallus differentiation on bacterial communities in siphonous green seaweeds. *Frontiers in Microbiology*, *10*(APR). https://doi.org/10.3389/fmicb.2019.00717

Mou, X., Lu, X., Jacob, J., Sun, S., & Heath, R. (2013). Metagenomic Identification of Bacterioplankton Taxa and Pathways Involved in Microcystin Degradation in Lake Erie. *PLOS ONE*, *8*(4), e61890. https://doi.org/10.1371/journal.pone.0061890

Mueller, B., Den Haan, J., Visser, P. M., Vermeij, M. J. A., & Van Duyl, F. C. (2016). Effect of light and nutrient availability on the release of dissolved organic carbon (DOC) by Caribbean turf algae. *Scientific Reports*, *6*(March), 1–9. https://doi.org/10.1038/srep23248

Müller, K. M., Cole, K. M., & Sheath, R. G. (2003). Systematics of Bangia (Bangiales, Rhodophyta) in North America. II. Biogeographical trends in karyology: Chromosome numbers and linkage with gene sequence phylogenetic trees. *Phycologia*, *42*(3), 209–219. https://doi.org/10.2216/i0031-8884-42-3-209.1

Müller, K. M., Sheath, R. G., Vis, M. L., Crease, T. J., & Cole, K. M. (1998). Biogeography and systematics of Bangia (Bangiales, Rhodophyta) based on the Rubisco spacer, rbcL gene and 18S rRNA gene sequences and morphometric analyses. 1. North America. *Phycologia*, *37*(3), 195–207. https://doi.org/10.2216/i0031-8884-37-3-195.1

Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology*, *125*(4), 1558–1566. https://doi.org/10.1104/pp.125.4.1558

Nold, S. C., & Zwart, G. (1998). Patterns and governing forces in aquatic microbial communities. *Aquatic Ecology*, *32*(1), 17–35. https://doi.org/10.1023/A:1009991918036

Oren, A. (2002). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology and Biotechnology*, *28*(1), 56–63. https://doi.org/10.1038/sj/jim/7000176

Otsuka, S., Ueda, H., Suenaga, T., Uchino, Y., Hamada, M., Yokota, A., & Senoo, K. (2013). Roseimicrobium gellanilyticum gen. nov., sp. nov., a new member of the class Verrucomicrobiae. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt_6), 1982–1986. https://doi.org/https://doi.org/10.1099/ijs.0.041848-0

Paix, B., Othmani, A., Debroas, D., Culioli, G., & Briand, J. F. (2019). Temporal covariation of epibacterial community and surface metabolome in the Mediterranean seaweed holobiont Taonia atomaria. *Environmental Microbiology*, *21*(9), 3346–3363. https://doi.org/10.1111/1462-2920.14617

Palleroni, N. J. (2015). Pseudomonas. In *Bergey's Manual of Systematics of Archaea and Bacteria* (p. 1). https://doi.org/https://doi.org/10.1002/9781118960608.gbm01210

Pankiewicz, R., Łęska, B., Messyasz, B., Fabrowska, J., Sołoducha, M., & Pikosz, M. (2016). First isolation of polysaccharidic ulvans from the cell walls of freshwater algae. *Algal Research*, *19*, 348–354. https://doi.org/10.1016/j.algal.2016.02.025

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/https://doi.org/10.1111/1462-2920.13023

Park, J., Baek, G. S., Woo, S.-G., Lee, J., Yang, J., & Lee, J. (2013). Luteolibacter yonseiensis sp. nov., isolated from activated sludge using algal metabolites. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt_5), 1891–1895. https://doi.org/https://doi.org/10.1099/ijs.0.046664-0

Parker, C. T., Tindall, B. J., & Garrity, G. M. (2019). International code of nomenclature of Prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, *69*(1), S1. https://doi.org/10.1099/ijsem.0.000778

Parte, A. C., Carbasse, J. S., Meier-Kolthoff, J. P., Reimer, L. C., & Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, *70*(11), 5607–5612. https://doi.org/10.1099/ijsem.0.004332

Pascual, J., Wüst, P. K., Geppert, A., Foesel, B. U., Huber, K. J., & Overmann, J. (2015). Novel isolates double the number of chemotrophic species and allow the first description of higher taxa in Acidobacteria subdivision 4. *Systematic and Applied Microbiology*, *38*(8), 534–544. https://doi.org/10.1016/j.syapm.2015.08.001

Paver, S. F., Newton, R. J., & Coleman, M. L. (2020). Microbial communities of the Laurentian Great Lakes reflect connectivity and local biogeochemistry. *Environmental Microbiology*, *22*(1), 433–446. https://doi.org/10.1111/1462-2920.14862

Phillips, J. C., McKinley, G. A., Bennington, V., Bootsma, H. A., Pilcher, D. J., Sterner, R. W., & Urban, N. R. (2015). The Potential for CO_2-Induced Acidification in Freshwater. *Oceanography*, *28*(2), 136–145. http://www.jstor.org/stable/24861876

Phylum "Verrucomicrobia." (n.d.). LPSN. https://lpsn.dsmz.de/phylum/verrucomicrobia-1

R Core. (2020). *R: A Language and Environment for Statistical Computing* (3.6.3). R Foundation for Statistical Computing. https://www.r-project.org/

Rai, L. C., & Gaur, J. P. (2012). Algal Adaptation to Stress: Some General Remarks. In *Algal Adaptation to Environmental Stresses: Physiological, Biochemical and Molecular Mechanisms* (pp. 2–14).

Ravin, N. V., Rakitin, A. L., Ivanova, A. A., Beletsky, A. V., Kulichevskaya, I. S., Mardanov, A. V., & Dedysh, S. N. (2018). Genome analysis of Fimbriiglobus ruber SP5T, a planctomycete with confirmed chitinolytic capability. *Applied and Environmental Microbiology*, *84*(7). https://doi.org/10.1128/AEM.02645-17

Reichenbach, H. (1992). The Order Cytophagales. *The Prokaryotes*, 3631–3675. https://doi.org/10.1007/978-1-4757-2191-1_37

Ricciardi, A., & MacIsaac, H. J. (2000). Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution*, *15*(2), 62–65. https://doi.org/10.1016/S0169-5347(99)01745-0

Rosenberg, E. (2013). The prokaryotes: Alphaproteobacteria and betaproteobacteria. *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*, *1973*, 1–1012. https://doi.org/10.1007/978-3-642-30197-1

Rosenberg, G., & Paerl, H. W. (1981). Nitrogen fixation by blue-green algae associated with the siphonous green seaweed Codium decorticatum: effects on ammonium uptake. *Marine Biology*, *61*(2–3), 151–158. https://doi.org/10.1007/BF00386654

Röthig, T., Ochsenkühn, M. A., Roik, A., van der Merwe, R., & Voolstra, C. R. (2016). Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Molecular Ecology*, *25*(6), 1308–1323. https://doi.org/https://doi.org/10.1111/mec.13567

Santos, A. A., Soldatou, S., de Magalhães, V. F., Azevedo, S. M. F. O., Camacho-Muñoz, D., Lawton, L. A., & Edwards, C. (2021). Degradation of Multiple Peptides by Microcystin-Degrader Paucibacter toxinivorans (2C20). In *Toxins* (Vol. 13, Issue 4). https://doi.org/10.3390/toxins13040265

Schauer, M., Kamenik, C., & Hahn, M. W. (2005). Ecological differentiation within a cosmopolitan group of planktonic freshwater bacteria (SOL cluster, Saprospiraceae, Bacteroidetes). *Applied and Environmental Microbiology*, *71*(10), 5900–5907. https://doi.org/10.1128/AEM.71.10.5900-5907.2005

Schlesinger, D. A., Molot, L. A., & Shuter, B. J. (1981). Specific Growth Rates of Freshwater Algae in Relation to Cell Size and Light Intensity. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(9), 1052–1058. https://doi.org/10.1139/f81-145

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S., & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database : The Journal of Biological Databases and Curation*, *2020*. https://doi.org/10.1093/database/baaa062

Schopf, J W, & Packer, B. M. (1987). Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Science*, *237*(4810), 70 LP – 73. https://doi.org/10.1126/science.11539686

Schopf, J William, Kudryavtsev, A. B., Czaja, A. D., & Tripathi, A. B. (2007). Evidence of Archean life: Stromatolites and microfossils. *Precambrian Research*, *158*(3), 141–155. https://doi.org/https://doi.org/10.1016/j.precamres.2007.04.009

Sehnal, L., Brammer-Robbins, E., Wormington, A. M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C. J., Simonin, M., & Adamovsky, O. (2021). Microbiome Composition and Function in Aquatic Vertebrates: Small Organisms Making Big Impacts on Aquatic Animal Health. *Frontiers in Microbiology*, *12*(March). https://doi.org/10.3389/fmicb.2021.567408

Serebryakova, A., Aires, T., Viard, F., Serrão, E. A., & Engelen, A. H. (2018). Summer shifts of bacterial communities associated with the invasive brown seaweed Sargassum muticum are location and tissue dependent. *PLoS ONE*, *13*(12), 1–18. https://doi.org/10.1371/journal.pone.0206734

Shea, T. B., Sheath, R. G., Chhun, A., Vis, M. L., Chiasson, W. B., & Müller, K. M. (2014). Distribution, seasonality and putative origin of the non-native red alga Bangia atropurpurea (Bangiales, Rhodophyta) in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *40*(1), 27–34. https://doi.org/10.1016/j.jglr.2014.01.004

Sheu, S.-Y., Yang, C.-S., Chen, M.-H., Arun, A. B., Young, C.-C., & Chen, W.-M. (2010). Arcicella aurantiaca sp. nov., isolated from stream water. *International Journal of Systematic and Evolutionary Microbiology*, *60*(12), 2979–2983. https://doi.org/https://doi.org/10.1099/ijs.0.021527-0

Simmons, J. A. (2012). Toxicity of major cations and anions (Na+, K+, Ca2+, Cl−, and SO) to a macrophyte and an alga. *Environmental Toxicology and Chemistry*, *31*(6), 1370–1374. https://doi.org/https://doi.org/10.1002/etc.1819

Simon, M., Scheuner, C., Meier-Kolthoff, J. P., Brinkhoff, T., Wagner-Döbler, I., Ulbrich, M., Klenk, H. P., Schomburg, D., Petersen, J., & Göker, M. (2017). Phylogenomics of Rhodobacteraceae reveals evolutionary adaptation to marine and non-marine habitats. *ISME Journal*, *11*(6), 1483–1499. https://doi.org/10.1038/ismej.2016.198

Singh, R. P., & Reddy, C. R. K. (2016). Unraveling the Functions of the Macroalgal Microbiome. *Frontiers in Microbiology*, *6*, 1488. https://doi.org/10.3389/fmicb.2015.01488

Sly, L. I., & Fegan, M. (2015). Lewinella. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–5). https://doi.org/https://doi.org/10.1002/9781118960608.gbm00358

Snyder, A. R., Williams, H. N., Baer, M. L., Walker, K. E., & Stine, O. C. (2002). 16S rDNA sequence analysis of environmental Bdellovibrio-and-like organisms (BALO) reveals extensive diversity. *International Journal of Systematic and Evolutionary Microbiology*, *52*(6), 2089–2094. https://doi.org/10.1099/ijs.0.02261-0

Song, J., Lim, Y., Joung, Y., Cho, J.-C., & Kogure, K. (2018). Rubritalea profundi sp. nov., isolated from deep-seawater and emended description of the genus Rubritalea in the phylum Verrucomicrobia. *International Journal of Systematic and Evolutionary Microbiology*, *68*(4), 1384–1389. https://doi.org/https://doi.org/10.1099/ijsem.0.002686

Spring, S., Bunk, B., Spröer, C., Rohde, M., & Klenk, H. P. (2018). Genome biology of a novel lineage of planctomycetes widespread in anoxic aquatic environments. *Environmental Microbiology*, *20*(7), 2438–2455. https://doi.org/10.1111/1462-2920.14253

Stancheva, R., Sheath, R. G., Read, B. A., McArthur, K. D., Schroepfer, C., Kociolek, J. P., & Fetscher, A. E. (2013). Nitrogen-fixing cyanobacteria (free-living and diatom endosymbionts): Their use in southern California stream bioassessment. *Hydrobiologia*, *720*(1), 111–127. https://doi.org/10.1007/s10750-013-1630-6

Stolz, A. (2009). Molecular characteristics of xenobiotic-degrading sphingomonads. *Applied Microbiology and Biotechnology*, *81*(5), 793–811. https://doi.org/10.1007/s00253-008-1752-3

Surayot, U., Lee, J. H., Kanongnuch, C., Peerapornpisal, Y., Park, W. J., & You, S. G. (2016). Structural characterization of sulfated arabinans extracted from Cladophora glomerata Kützing and their macrophage activation. *Bioscience, Biotechnology and Biochemistry*, *80*(5), 972–982. https://doi.org/10.1080/09168451.2015.1132149

Sutherland, J. E., Lindstrom, S. C., Nelson, W. A., Brodie, J., Lynch, M. D. J., Hwang, M. S., Choi, H. G., Miyata, M., Kikuchi, N., Oliveira, M. C., Farr, T., Neefus, C., Mols-Mortensen, A., Milstein, D., & Müller, K. M. (2011). A new look at an ancient order: Generic revision of the bangiales (Rhodophyta). *Journal of Phycology*, *47*(5), 1131–1151. https://doi.org/10.1111/j.1529-8817.2011.01052.x

Suzuki, S., Endoh, R., Manabe, R., Ohkuma, M., & Hirakawa, Y. (2018). Multiple losses of photosynthesis and convergent reductive genome evolution in the colourless green algae Prototheca. *Scientific Reports*, *8*(1), 940. https://doi.org/10.1038/s41598-017-18378-8

Thermo Scientific, D. (2012). NanoDrop Lite: Interpretation of Nucleic Acid 260/280 Ratios. *Protocols and Product Manuals*, *T123*, 1.

Uroz, S., Buée, M., Murat, C., Frey-Klett, P., & Martin, F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environmental Microbiology Reports*, *2*(2), 281–288. https://doi.org/10.1111/j.1758-2229.2009.00117.x

Vaz-Moreira, I., Nunes, O. C., & Manaia, C. M. (2011). Diversity and antibiotic resistance patterns of Sphingomonadaceae isolates from drinking water. *Applied and Environmental Microbiology*, *77*(16), 5697–5706. https://doi.org/10.1128/AEM.00579-11

Vollmers, J., Frentrup, M., Rast, P., Jogler, C., & Kaster, A.-K. (2017). Untangling Genomes of Novel Planctomycetal and Verrucomicrobial Species from Monterey Bay Kelp Forest Metagenomes by Refined Binning. *Frontiers in Microbiology*, *8*, 472. https://doi.org/10.3389/fmicb.2017.00472

Voort, M. P. J. van der, Vulsteke, E., & Visser, C. L. M. de. (2015). Macro-economics of algae products. *Marco-Economics of Algae Products Public Output Report WP2A7.02 of the EnAlgae Project, June*, 47.

Wagner, M., & Horn, M. (2006). The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. *Current Opinion in Biotechnology*, *17*(3), 241–249. https://doi.org/https://doi.org/10.1016/j.copbio.2006.05.005

Wang, W. J., Li, X. L., Zhu, J. Y., Liang, Z. R., Liu, F. L., Sun, X. T., Wang, F. J., & Shen, Z. G. (2019). Antioxidant response to salinity stress in freshwater and marine Bangia (Bangiales, Rhodophyta). *Aquatic Botany*, *154*, 35–41. https://doi.org/https://doi.org/10.1016/j.aquabot.2018.12.008

Weinert, N., Piceno, Y., Ding, G. C., Meincke, R., Heuer, H., Berg, G., Schloter, M., Andersen, G., & Smalla, K. (2011). PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: Many common and few cultivar-dependent taxa. *FEMS Microbiology Ecology*, *75*(3), 497–506. https://doi.org/10.1111/j.1574-6941.2010.01025.x

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., & Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, *29*(2), 949–982. https://doi.org/10.1007/s10811-016-0974-5

Weon, H.-Y., Noh, H.-J., Son, J.-A., Jang, H. B., Kim, B.-Y., Kwon, S.-W., & Stackebrandt, E. (2008). Rudanella lutea gen. nov., sp. nov., isolated from an air sample in Korea. *International Journal of Systematic and Evolutionary Microbiology*, *58*(2), 474–478. https://doi.org/https://doi.org/10.1099/ijs.0.65358-0

Whipps, J. M., Karen, L., & Cooke, R. C. (1988). Mycoparasitism and plant disease control. *Fungi in Biological Control Systems*, 161–187. http://www.jphysiolanthropol.com/content/34/1/23

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(12), 6578–6583. https://doi.org/10.1073/pnas.95.12.6578

Winters, A. D., Marsh, T. L., Brenden, T. O., & Faisal, M. (2014). Molecular characterization of bacterial communities associated with sediments in the Laurentian Great Lakes. *Journal of Great Lakes Research*, *40*(3), 640–645. https://doi.org/10.1016/j.jglr.2014.04.008

Wood, S. A., Borges, H., Puddick, J., Biessy, L., Atalah, J., Hawes, I., Dietrich, D. R., & Hamilton, D. P. (2017). Contrasting cyanobacterial communities and microcystin concentrations in summers with extreme weather events: insights into potential effects of climate change. *Hydrobiologia*, *785*(1), 71–89. https://doi.org/10.1007/s10750-016-2904-6

Wu, Y.-C., & Ho, H.-K. (1959). Studies of Water-Soluble Polysaccharides from Red Seaweeds (Pterocladia Tenuis Okam. and Bangia Fusco-Purpurea Lyngb). *Journal of the Chinese Chemical Society*, *6*(1), 84–94. https://doi.org/https://doi.org/10.1002/jccs.195900010

Yoon, J., Matsuo, Y., Adachi, K., Nozawa, M., Matsuda, S., Kasai, H., & Yokota, A. (2008).

Description of Persicirhabdus sediminis gen. nov., sp. nov., Roseibacillus ishigakijimensis gen. nov., sp. nov., Roseibacillus ponti sp. nov., Roseibacillus persicicus sp. nov., Luteolibacter pohnpeiensis gen. nov., sp. nov. and Luteolibacter algae sp. no. *International Journal of Systematic and Evolutionary Microbiology*, *58*(4), 998–1007. https://doi.org/https://doi.org/10.1099/ijs.0.65520-0

Yoon, J., Matsuo, Y., Katsuta, A., Jang, J.-H., Matsuda, S., Adachi, K., Kasai, H., & Yokota, A. (2008). Haloferula rosea gen. nov., sp. nov., Haloferula harenae sp. nov., Haloferula phyci sp. nov., Haloferula helveola sp. nov. and Haloferula sargassicola sp. nov., five marine representatives of the family Verrucomicrobiaceae within the phylum 'Verrucomicrob. *International Journal of Systematic and Evolutionary Microbiology*, *58*(11), 2491–2500. https://doi.org/https://doi.org/10.1099/ijs.0.2008/000711-0

You, D.-J., Chen, X.-G., Xiang, H.-Y., Ouyang, L., & Yang, B. (2014). Isolation, identification and characterization of a microcystin-degrading bacterium Paucibacter sp. strain CH. *Huan jing ke xue= Huanjing kexue*, *35*(1), 313–318. http://europepmc.org/abstract/MED/24720221

Zulkifly, S., Hanshew, A., Young, E. B., Lee, P., Graham, M. E., Piotrowski, M., & Graham, L. E. (2012). The epiphytic microbiota of the globally widespread macroalga Cladophora glomerata (Chlorophyta, Cladophorales). *American Journal of Botany*, *99*(9), 1541–1552. https://doi.org/10.3732/ajb.1200161

Appendix A - Supplementary Figures and Tables

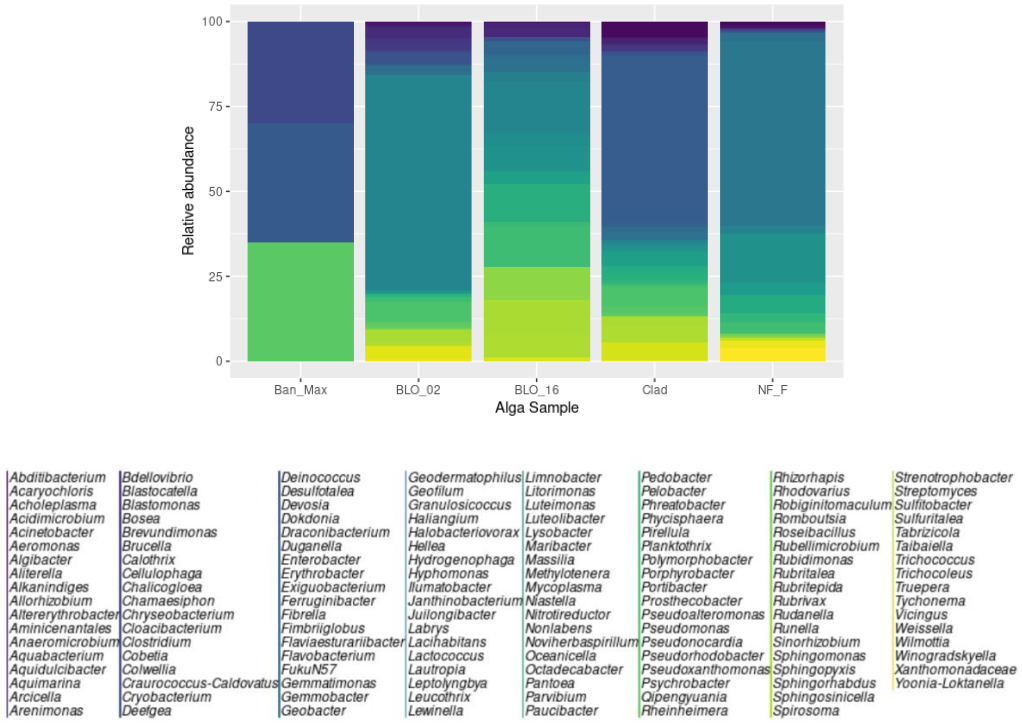

Figure 10 Relative abundance bar plot of all bacterial genera across all algal samples. Ban_Max: *Bangia maxima*, BLO_02: *Bangia atropurpurea* (2002), BLO_16: *Bangia atropurpurea* (2016), Clad: *Cladophora glomerata*, NF_F: *Bangia fuscopurpurea*.

Table 4 Detailed list of microbes and assigned phylum and genus taxonomies. Taxonomies were assigned using the SILVA database in QIIME2 and any nomenclature errors were modified using the NCBI database. Each table shows distribution of bacterial phyla and genera in each pairwise analysis from figures 3 to 5. The pairwise analysis shows unique and overlapped bacteria between: A) *Bangia atropurpurea* 2002 and 2016, B) *Bangia atropurpurea* (2002) and *Bangia fuscopurpurea*, C) *Bangia atropurpurea* (2002) and *Cladophora glomerata.*

A

Microbial genera (and their phyla) unique to *Bangia atropurpurea* (2002)		**Microbial genera (and their phyla) unique to *Bangia atropurpurea* (2016)**		**Microbial genera (and phyla) found in both *B. atropurpurea* samples**	
Acidobacteria	*Blastocatella*	Bacteroidetes	*Arcicella*	Bacteroidetes	*Jiulongibacter*
	Stenotrophobacter		*Ferruginibacter*		*Lewinella*
Actinobacteria	*Geodermatophilus*		*Niastella*	Cyanobacteria	*Trichocoleus*
	Pseudonocardia	Cyanobacteria	*Tapinothrix*		*Planktothrix*
Bacteroidetes	*Chryseobacterium*	Gemmatimonadetes	*Gemmatimonas*	Deinococcocus-Thermus	*Deinococcus*
	Cloacibacterium	Proteobacteria	*Desulfotalea*	Proteobacteria	*Sphingosinicella*
	Fibrella		*Aeromonas*		*Pseudochelatococcus*
	Flaviaesturariibacter		*Hydrogenophaga*		*Gemmobacter*
	Flavobacterium		*Lautropia*		*Labrys*

	Pedobacter		*Limnobacter*		*Paucibacter*
	Rudanella		*Rubrivax*		*Porphyrobacter*
	Spirosoma		*Aquidulcibacter*		*Sphingorhabdus*
Cyanobacteria	*Aliterella*				*Sphingosinicella*
	Calothrix			Verrucomicrobia	*Luteolibacter*
	Chalicogloea				
	Chamaesiphon				
	Tychonema				
	Oscillatoria				
Deinococcocus-Thermus	*Truepera*				
	Deinococcus				
Firmicutes	*Anaeromicrobium*				
Proteobacteria	*Acinetobacter*				
	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium				
	Aquabacterium				
	Arenimonas				
	Blastomonas				
	Bosea				
	Brevundimonas				
	Craurococcus-Caldovatus				
	Devosia				
	Duganella				
	Luteimonas				
	Lysobacter				
	Oceanicella				
	Phreatobacter				
	Polymorphobacter				
	Pseudomonas				
	Pseudoxanthomonas				
	Qipengyuania				
	Rheinheimera				
	Rhizorhapis				
	Rhodovarius				
	Rubellimicrobium				
	Rubritepida				
	Sinorhizobium				
	Sphingomonas				
	Sphingopyxis				
	Sulfuritalea				

B

Microbial genera unique to *Bangia atropurpurea* (2002)		**Microbial genera unique to *Bangia fuscopurpurea***		**Microbial genera found in both algae**	
Acidobacteria	*Blastocatella*	Actinobacteria	*Acidimicrobium*	Bacteroidetes	*Jiulongibacter*
	Stenotrophobacter		*Ilumatobacter*		*Lewinella*
Actinobacteria	*Geodermatophilus*	Bacteroidetes	*Algibacter*	Proteobacteria	*Gemmobacter*
	Pseudonocardia		*Aquimarina*		
Bacteroidetes	*Chryseobacterium*		*Cellulophaga*		
	Cloacibacterium		*Dokdonia*		
	Fibrella		*Lewinella*		
	Flaviaesturariibacter		*Lewinella*		
	Flavobacterium		*Maribacter*		
	Pedobacter		*Maribacter*		
	Rudanella		*Nonlabens*		
	Spirosoma		*Vicingus*		
Cyanobacteria	*Trichocoleus*		*Portibacter*		
	Aliterella		*Rubidimonas*		
	Calothrix		*Taibaiella*		
	Planktothrix		*Draconibacterium Winogradskyella*		
	Chalicogloea	Cyanobacteria	*Acaryochloris*		
	Chamaesiphon	Firmicutes	*Clostridium*		
			Weissella		
	Tychonema	Planctomycetes	*Phycisphaera*		
Deinococcocus-Thermus	*Deinococcus*	Proteobacteria	*Bdellovibrio Halobacteriovorax Desulfotalea*		
	Deinococcus				
	Truepera				
Firmicutes	*Anaeromicrobium*		*Haliangium*		
			Pelobacter		
Proteobacteria	*Acinetobacter*		*Altererythrobacter*		
	Sphingosinicella		*Colwellia*		
	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium		*Erythrobacter*		
	Aquabacterium		*Granulosicoccus*		
	Arenimonas		*Hellea*		
	Blastomonas		*Leucothrix*		
	Bosea		*Litorimonas*		
	Brevundimonas		*Octadecabacter*		
	Craurococcus-Caldovatus		*Pseudoalteromonas*		
	Devosia		*Robiginitomaculum*		
	Duganella		*Sphingomonas*		
	Pseudochelatococcus		*Sulfitobacter*		
	Labrys		*Yoonia-Loktanella*		

	Luteimonas	Tenericutes	*Mycoplasma*
	Lysobacter	Verrucomicrobia	*Luteolibacter*
	Oceanicella		*Roseibacillus*
			Rubritalea
	Paucibacter		
	Phreatobacter		
	Polymorphobacter		
	Porphyrobacter		
	Pseudomonas		
	Pseudoxanthomonas		
	Qipengyuania		
	Rheinheimera		
	Rhizorhapis		
	Rhodovarius		
	Rubellimicrobium		
	Rubritepida		
	Sinorhizobium		
	Sphingomonas		
	Sphingopyxis		
	Sphingorhabdus		
	Sphingosinicella		
	Sulfuritalea		
Verrucomicrobia	*Luteolibacter*		

C

Microbial Genera Unique to *Bangia atropurpurea* (2002)		**Microbial Genera Unique to *Cladophora glomerata***		**Microbial Genera in Both Algae**	
Acidobacteria	*Blastocatella*	Abditibacteriota	*Abditibacterium*	Bacteroidetes	*Chryseobacterium*
	Stenotrophobacter	Acidobacteria	*Aminicenantales*		*Flavobacterium*
Actinobacteria	*Geodermatophilus*	Actinobacteria	*Cryobacterium*		*Lewinella*
	Pseudonocardia		*Streptomyces*	Deinococcocus -Thermus	*Deinococcus*
Bacteroidetes	*Cloacibacterium*	Bacteroidetes	*Geofilum*	Proteobacteria	*Sphingosinicella*
					Acinetobacter
	Fibrella		*Ferruginibacter*		*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*
	Flaviaesturariibacter		*Lacihabitans*		*Aquabacterium*
	Juilongibacter		*Lewinella*		*Arenimonas*
	Pedobacter		*Niastella*		*Blastomonas*
	Rudanella		*Portibacter*		*Bosea*
	Spirosoma		*Runella*		*Brevundimonas*
Cyanobacteria	*Trichocoleus*	Firmicutes	*Acholeplasma*		*Duganella*

	Aliterella		*Exiguobacterium*		*Gemmobacter*
	Calothrix		*Lactococcus*		*Paucibacter*
	Planktothrix		*Romboutsia*		*Phreatobacter*
	Chalicogloea		*Trichococcus*		*Polymorphobacter*
	Chamaesiphon	Gemmatimonadetes	*Gemmatimonas*		*Porphyrobacter*
	Tychonema	Planctomycetes	*Pirellula*		*Pseudomonas* *Pseudoxanthomonas* *Rhizorhapis*
			Fimbriiglobus		
	Wilmottia	Proteobacteria	*Geobacter*		*Sphingopyxis*
Deinococcocus-Thermus	*Truepera*		*Nitrotireductor*		*Sphingorhabdus*
	Deinococcus		*Altererythrobacter*		*Sulfuritalea*
Firmicutes	*Anaeromicrobium*		*Noviherbaspirillum*	Verrucomicrobia	*Luteolibacter*
			Alkanindiges		
Proteobacteria	*Craurococcus-Caldovatus*		*Brucella*		
	Devosia		*Deefgea*		
	Pseudochelatococcus		*Enterobacter*		
	Labrys		*Granulosicoccus*		
	Luteimonas		*Hyphomonas*		
	Lysobacter		*Janthinobacterium*		
	Oceanicella		*Lautropia*		
	Qipengyuania		*Massilia*		
	Rheinheimera		*Methylotenera*		
	Rhodovarius		*Noviherbaspirillum*		
	Rubellimicrobium		*Pantoea*		
	Rubritepida		*Parvibium*		
	Sinorhizobium		*Pseudorhodobacter*		
	Sphingomonas		*Psychrobacter*		
	Sphingosinicella		*Sphingomonas*		
			Tabrizicola		
			Aquidulcibacter		
			Xanthomonadaceae		

Table 5 Relative abundance of each phylum, in percentage and number of ASVs, out of all samples and within each sample. Relative abundance of each phylum is considered based on number of ASVs at the genus level. Total number of ASVs for each algal sample is provided.

Phylum	**Total – 152 ASVs**	***Bangia maxima* – 3 ASVs**	***Bangia atropurpurea* 2002 – 62 ASVs**	***Bangia atropurpurea* 2016 – 26 ASVs**	***Cladophora glomerata* – 68 ASVs**	***Bangia fuscopurpurea* – 46 ASVs**
Proteobacteria	51.97 % (79)	66.66% (2)	56.45% (35)	57.69% (15)	63.23% (43)	41.30% (19)
Bacteroidetes	20.39 % (31)	33.33% (1)	16.13% (10)	19.23% (5)	14.71% (10)	36.96% (17)
Cyanobacteria	7.24 % (11)	0	12.90% (8)	11.54% (3)	0	2.17% (1)
Firmicutes	5.26 % (8)	0	1.61% (1)	0	7.35% (5)	4.35% (2)
Actinobacteria	3.95 % (5)	0	3.22% (2)	0	2.94% (2)	4.35% (2)
Verrucomicrobia	3.29 % (5)	0	1.61% (1)	3.85% (1)	2.94% (2)	6.52% (3)
Planctomycetes	1.97 % (3)	0	0	0	2.94% (2)	2.17% (1)
Deincoccus-Thermus	1.97 % (3)	0	4.84% (3)	3.85% (1)	1.47% (1)	0
Acidobacteria	1.97 % (3)	0	3.22% (2)	0	1.47% (1)	0
Abtidibacteria	0.66 % (1)	0	0	0	1.47% (1)	0
Tenericutes	0.66 % (1)	0	0	0	0	2.17% (1)
Gemmatimonadetes	0.66 % (1)	0	0	3.85% (1)	1.47% (1)	0