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Thermal and Microbial Effects on Brown Macroalgae: Heat Acclimation and the Biodiversity of the Microbiome

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THERMAL AND MICROBIAL EFFECTS ON BROWN MACROALGAE: HEAT ACCLIMATION AND THE BIODIVERSITY OF THE MICROBIOME

By

Charlotte Terry Carrigan Quigley

B.A. Colby College, 2009

A DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctorate of Philosophy

(in Marine Biology)

The Graduate School

The University of Maine

December 2018

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Dissertation Advisor: Dr. Susan H. Brawley

An Abstract of the Dissertation Presented
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This dissertation examines effects of stress on brown algal biology from a macroscopic scale by examining whole aquaculture crops, to a microscopic level by examining the macroalgal microbiome across the vertical stress gradient of the intertidal zone and across their latitudinal biogeographic ranges. Thermal stress negatively affected seedstock gametophytes of the kelp *Alaria esculenta* isolated from northern and southern locations in Maine. However, prior thermal stress had a positive effect on growth of the next-generation sporophytes. *Alaria esculenta* has potential as a kelp crop in Maine's sea vegetable aquaculture sector and implementing this protocol may allow the sea vegetable industry to increase crop yields. Studies found that stress gradients that influence distributions of brown macroalgae, specifically *Fucus* spp., can affect the microbial composition of the macroalgal microbiome. Various methods of describing macroalgal microbiomes were examined with a common garden approach using a lab-cultured strain of *Porphyra umbilicalis*. Methods examined included different preservation techniques, differences between algal tissue types, variability across the algal thallus, and type of analytical pipeline (e.g. mothur versus MED) used. Results were applied to *in situ* studies of the natural microbiome of *Fucus* spp.: each host species of the high, mid-, and low intertidal zones had a different microbiome. Manipulative transplants of mid-*zone F. vesiculosus* into the high zone assessed algal-associated bacterial tolerance to stress. Trans-Atlantic surveys of microbial diversity of *F. vesiculosus* found a biogeographic break in microbial community structure that correlated with sea surface temperatures and environmental stress across latitudes. These studies expand current knowledge of the direct and indirect effects of stress on phaeophytes across multiple scales.

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CHAPTER 1

INTRODUCTION

Phaeophyceae: Brown Macroalgae

The Phaeophyceae (Ochrophyta) contains a diverse group of taxa that vary from small filamentous species (e.g., Ectocarpales) to macroalgae such as kelps (e.g., Laminariales) with a heteromorphic life history comprised of microscopic, filamentous gametophytes but large (≥ 30 m), complex sporophytes (Graham et al. 2016). While still debated, the number of orders described ranges from 19 to 20 (Silberfeld et al. 2014, Guiry and Guiry 2015). These orders are split into four subclasses: Discosporangiophycidae, Ishigeophycidae, Dictyotophycidae, and Fucophycidae (Silberfeld et al. 2014). Most brown algal orders are thought to have diverged 130 to 100 million years ago (Mya) in the lower Cretaceous ("brown algal crown radiation," Silberfeld et al. 2010), but the oldest fossils of brown algae date to the Miocene Epoch (23 – 5.3 Mya; Parker and Dawson, 1965).

The order Fucales (Bory, 1827) includes some of the most common littoral macroalgae worldwide; adults are diploid and produce gametes by meiosis (Graham et al. 2016). The congeners *Fucus spiralis*, *F. vesiculosus*, and *F. distichus* subsp. *edentatus* occupy the high, mid- and low levels of the rocky intertidal zone in the North Atlantic. The order Laminariales (Migula, 1909) consists of a monophyletic lineage of three families commonly referred to as kelps, which have a pronounced heteromorphic life history alternating between microscopic filamentous gametophytes and large sporophytes that often form beds or forests (Hurd et al. 2014). Of a total of ~12 *Alaria* species that inhabit the North Pacific, *Alaria esculenta* is the only species that migrated successfully from the North Pacific to the North Atlantic about ~3.5-5.4 Mya (early Pliocene; Adey et al. 2008, Bolton 2010, Vermeij 2012).

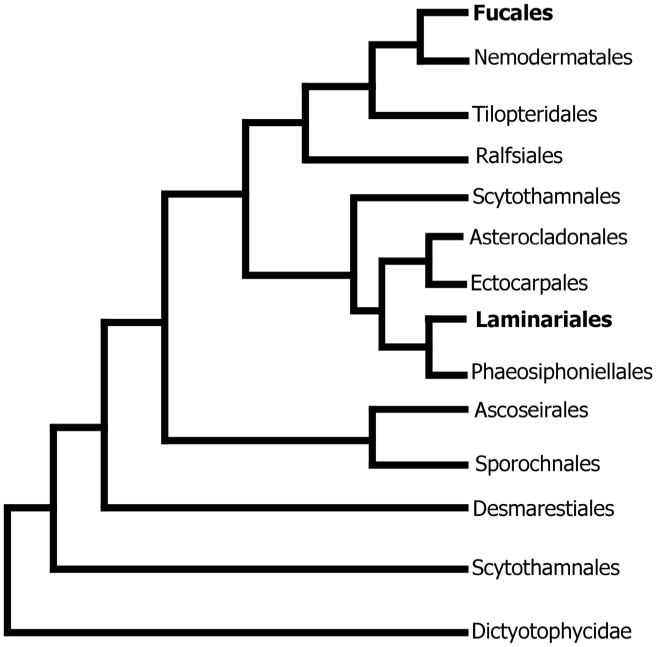


Figure 1.1 Phylogenetic tree of the families in the subclass Fucophycidae (adapted from Silberfeld et al. 2014). Families of interest in this dissertation are bolded.

Marine brown algae are ecosystem engineers (Jones et al. 1997), acting as biological elements that directly affect the biota around them. Therefore they are key to the diversity of organisms in a community or ecosystem. These can be in the form of intertidal fucoid beds or subtidal kelp forests. Brown algae are important structural elements and primary producers of up to 50% of the total carbon fixed in global intertidal zones (Gattuso et al. 2006, Hurd et al 2014) and phaeophytes affect many other biotic and abiotic factors associated with these habitats (Jones et al. 1997). Intertidal communities are a complex web of interacting organisms, and foundational species that directly affect the lower trophic levels have a strong effect on the system's overall diversity (Baiser at al. 2013).

Local and Global Environmental Stressors

Abiotic factors that affect intertidal macroalgae include irradiance, salinity, wave exposure, inorganic nutrient supply, and length of emersion which affect levels of stress from temperature and desiccation. Lower intertidal species are restricted from higher zone by their inability to handle the more severe levels of stress (e.g. Baker 1910, Schonbeck & Norton 1978, Harley & Helmuth, 2003, Williams & Dethier 2005). Warmer temperatures and associated desiccation subject intertidal organisms to stress (e.g. Brawley and Johnson 1991, 1993, Jueterbock et al. 2013). Biotic stressors such as competition and predation also affect intertidal species distributions. Grazers also contribute to variations in abundance and zonation of taxa in the intertidal zone with strong variations due to biogeographic history across the North Atlantic related to whether grazers such as the limpet *Patella vulgata* are present (Hawkins et al. 1992). Stress-tolerant organisms are often excluded from an optimal lower intertidal zone by a stress-intolerant, but competitively superior organism; intertidal species that occupy higher areas of the intertidal zone grow as well or better in the lower intertidal or subtidal zone when competition is reduced (Connell 1961, Schonbeck and Norton 1980, Lubchenco 1980, Serrão et al. 1999). Biotic factors such as predation and competition can vary with different exposure levels (abiotic), and be further complicated by physical disturbance by organisms (e.g. macrophyte whiplash and herbivore movement; Menge 1976). The relative effect of abiotic versus biotic factors influencing intertidal structure changes between zones (Menge 1976).

Abioic factors often control upper boundaries of vertical distribution of intertidal organisms, whereas lower boundaries are controlled by biotic factors. These factors often interact and have additive effects (Jenkins et al. 2008, Williams et al. 2013). The resulting biological changes associated with increased stress include shifts in species' ranges, phenological changes (e.g. times of reproduction, molting, etc.), species invasions, and overall reduction of biological diversity (Lima and Wethey 2012).

At a global level, sea temperatures are the most extensive and severe impacts of climate change on coastal ecosystems across the globe (Halpern et al. 2008), but changing air temperatures are especially important to determining shifts in the intertidal biota. Near-shore sea temperatures (1981- 2012) indicate that 71% of coastlines worldwide are warming significantly; however, the rates of warming, changes in temperature extremes, and changes in seasonal patterns differ spatially (Lima and Wethey 2012). The Gulf of Maine Coastal Current contains two principal branches that cause subtle coastal differences in sea surface temperature (SST, Pettigrew et al. 2005), providing the need to explore effects of differing water temperatures on coastal environments at the local scale. The Gulf of Maine is also experiencing some of the fastest rates of coastal warming in the world (Lima and Wethey 2012, Pershing et al. 2015, Thomas et al. 2017). Stress factors are complex and act at various scales, and are important to investigate in light of climate change.

Brown Algae in Aquaculture

Consumption of macroalgae by humans dates back thousands of years. The earliest verified date is from 14,000 years ago in the mountains of Chile, where archaeologists found the remains of 9 species of macroalgae, including brown algae, indicating that these people used seaweeds harvested from distant coastlines in their diets (Dillehay et al. 2008). Subsequent accounts of macroalgal consumption come from around the world. In Japan in 701 AD the Law of Taiho established that certain edible seaweeds were of such high value that they could be used to pay taxes to the Emperor's Court (Nisizawa et al. 1987). The First Peoples of the Northwest Coast of North America have been utilizing brown macroalgae for millennia (Turner 2001).

Macroalgae are nutritional foods, with different species offering healthy levels of vitamins, minerals, proteins, and/or fiber (MacArtain et al. 2007, Tibbets et al. 2016, Wells, et al. 2017). Seaweed farming is practiced in over 50 countries world-wide, expanding by 8 % in the last decade (FAO 2016). As of 2014, 27 % of aquacultural production by volume was seaweeds (FAO 2016). The global industry is estimated to be worth more than USD$ 6 billion per annum, 85 % of which comprises food products; as of 2015, total seaweed production was 30.4 million tonnes (Ferdouse et al. 2018). The third most important farmed macroalgal species world-wide (2.3 million tonnes) is the brown alga *Undaria pinnatifida* (Japanese wakame; Ferdouse et al. 2018), a Pacific relative to the Pacific/Atlantic species *Alaria esculenta*. While kelps (Laminariales) are the major brown macrophytic contributors to aquaculture (e.g. *Alaria esculenta, Laminaria digitata, Laminaria hyperborea, Saccharina latissima, Saccharina japonica, Sargassum fusiforme*), *Fucus* species are also consumed as whole food products in Europe (pers. obs.) and in British Colombia by members of the First Nations (Turner 2017). Sea vegetable farming is highly sustainable, requiring no fertilizers or irrigation, thus can be important to future food production.

Goals and Specific Objectives: From Macro to Micro

This dissertation consists of two main lines of enquiry that examine the effects of stress on different aspects of brown algal biology from a macroscopic or whole organismal scale, to a microscopic level by examining microbial composition of selected intertidal macroalgae across the vertical stress gradient of the intertidal zone to comparisons over their latitudinal biogeographic ranges. The first examined the effects of thermal stress on the kelp *Alaria esculenta* to determine its potential as an aquaculture crop in Maine's sea vegetable industry (Chapter 2). The specific objective was to predict the response to future warming temperatures in the Gulf of Maine by imposing stressful temperatures on gametophytes isolated from northern and southern locations on the Maine coast. Subsequent assessment of the next-generation sporophytes provided a protocol that may allow the sea vegetable industry to increase crop yields, and this work showed that the gametophyte stage is resilient to predicted climate change.

Many brown macroalgae occupy intertidal habitats, which are defined by various stressors experienced at different levels based on intertidal position, including temperature. The second line of enquiry in this dissertation addressed how those stress gradients that influence distributions of brown macroalgae, specifically *Fucus* spp., affect the microbial composition of the macroalgal microbiome. The first objective was to determine the best methods to describe macroalgal microbiomes (Chapter 3) by utilizing a common garden approach with a lab-cultured red macrophyte, *Porphyra umbilicalis*. The effects on the microbiome of three stabilization techniques, differences between tissue types, and variability across the algal thallus were assessed by two analytical approaches, mothur and MED. These results were applied to *in situ* analyses of *Fucus* spp. across vertical and latitudinal scales (Chapters 4 and 5). Surveys of the natural microbiome of *Fucus* congeners occupying the high, mid-, and low intertidal zones, and manipulative transplants of mid-*zone F. vesiculosus* into the high zone determined whether stress tolerant (upper intertidal zone) or stress intolerant (lower intertidal zone) bacterial taxa exist (Chapter 4). Trans-Atlantic surveys at 11 sites over 2 summers examined correlations between microbial diversity of *F. vesiculosus* across latitudes and degree of environmental stress across latitudes (Chapter 5). This work extends current understanding of direct and indirect effects of stress on phaeophytes across multiple scales, and offers many new avenues for continued research.

CHAPTER 2

TEMPERATURE TOLERANCE OF MAINE STRAINS OF THE KELP *ALARIA ESCULENTA* AND ITS SUITABILITY FOR AQUACULTURE IN THE GULF OF MAINE

Introduction

The harvest and aquaculture of seaweeds is more than a $6 billion industry worldwide, and the United States imports an average of $63 million worth of edible seaweeds annually (Ferdouse et al. 2018). Market demand and interest in integrated aquaculture offer increased opportunities for sea vegetable crops that can fill the growing domestic market. The pristine coastline with existing waterfront industries and communities along the Gulf of Maine (GOM) offer an ideal location for expansion of aquaculture. The future of sea vegetable aquaculture, however, will depend on having diverse crops that are tolerant to coastal warming attributable to climate change.

Seaweed production in the northwestern Atlantic and many other places provides a variety of services including wave attenuation and erosion prevention (Gaylord et al. 2007, Zhu and Zou 2017), carbon sequestration and the reduction of ocean acidification (OA; Chung et al. 2013, Kim et al. 2015), anthropogenic bioremediation (Kim et al.

2015) and aquaculture biomitigation (i.e., integrated multitrophic aquaculture; Chopin et al. 2012, Reid et al. 2013, He et al. 2014), biofuels production (Wargacki et al. 2012), and whole food production (Kim et al. 2015, Kim et al. 2017, Royer et al. 2018). In New England, these areas of study almost exclusively examine one species: the sugar kelp *Saccharina latissima*. Increasing the diversity of macroalgae grown on sea farms may offer new insights into ecosystem services. The subarctic kelp *Alaria esculenta* can sequester almost twice the nutrients per wet weight as *S. latissima* (Reid et al. 2013), indicating its potentially greater value for bioremediation, and as a food. *Alaria esculenta* can produce blades under high sedimentation rates, whereas *S. latissima* cannot (Zacher et al. 2016). In Ireland, farmed *Alaria esculenta* was reported to grow an average of 5 cm per day at the peak of the season (Birkett et al. 1998). Faster-growing blades increase food production, as well as all other services. While kelps, in general, are high in nutrients, *A. esculenta* exceeds *S. latissima* in protein, vitamins A, B_{12}, C and E, while it is lower in iodine (Schiener et al. 2015, Wells et al. 2017). Iodine content remains constant in *A. esculenta*, whereas levels in *S. latissima* can vary across locations (Roleda et al. 2018), leading to difficulty in determining healthy daily consumption of foods. Due to its low iodine content relative to other kelps, its high nutrient content, and mild flavor (Tibbets et al. 2016, Wells et al. 2017), *A. esculenta* has good potential in US markets and is an excellent candidate for diversifying Maine sea vegetable aquaculture. Furthermore, it is related to *Undaria pinnatifida* ("wakame"), a traditional Northwest Pacific food kelp that is commercially valuable and used in many value-added foods (FAO 2016, Wells et al. 2017, Ferdouse et al. 2018).

The aquaculture candidate *Alaria esculenta* L. Greville (Laminariales, Phaeophyceae) is the dominant subtidal kelp in the northwestern Atlantic and inhabits rocky shores that have seawater temperatures from -2 to 17 °C (Adey and Hayek, 2011). The light-brown, ruffled blade has a prominent midrib and can reach 3 – 4 m in length. While originally found in Long Island Sound and abundant in Massachusetts (Taylor 1957), there is strong, but weakly documented evidence of range retraction since the mid-1960s to Cape Ann (northern Massachusetts), where it is patchy at best (Adey and Hayek, 2011, Mathieson and Dawes 2017, pers. obs.). It is found on rocky points with high wave energy throughout Maine and contributes to a significant proportion of biomass from subtidal surveys of the GOM (Adey and Hayak 2011).

Aquaculture crops must demonstrate their capacity to handle variable environments. Average monthly sea surface temperatures are highest in August in the GOM, ranging from roughly 12 °C in the north to 18 °C in the south (Fig. 2.1). Long-term coastal sea surface temperatures (SST) are warming in the GOM (1 ± 0.3 °C /100 y; Shearman and Lentz 2010, Lima and Wethey 2012). In fact, warming rates over the past 33 years alone are at 0.4 °C/decade (~0.03 °C /year; Pershing et al. 2015, Thomas et al. 2017), and summer seasonal duration is increasing by ~2 days/year (Thomas et al. 2017). This rate has increased by a factor of 7 within the past decade (2004-2013; Pershing et al. 2015). Not only are GOM SSTs rising at a faster rate than 99 % of the rest of the global oceans (Pershing et al. 2015), they are predicted to continue to rise even faster with another 3 – 4 °C increase possible over the next century (Fernandez et al. 2015). Thus, it is necessary to test whether the subarctic kelp *Alaria esculenta* (Adey & Hayak 2011) can tolerate temperature changes predicted for the GOM.

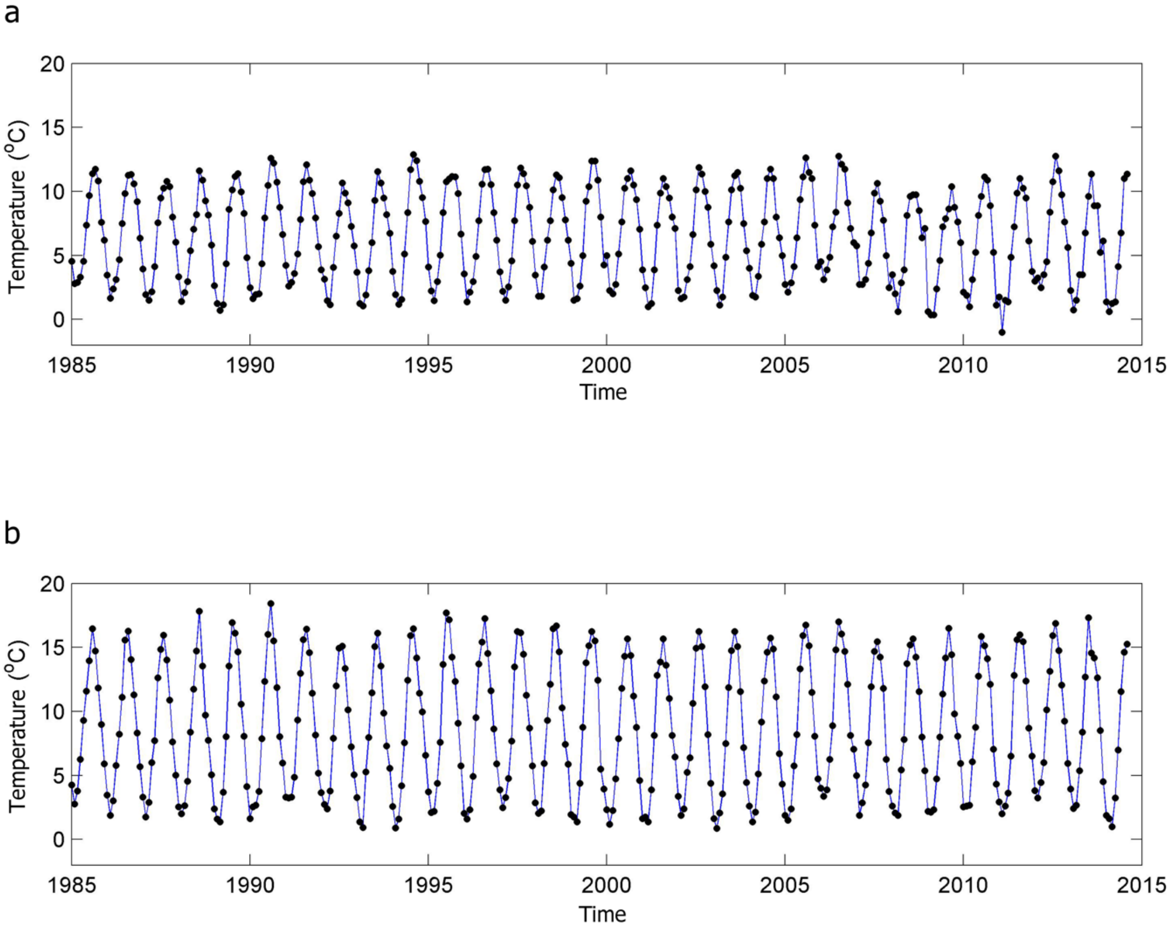


Figure 2.1. Median monthly sea surface temperature of satellite data of a roughly 30 km^2 coastal area at Lubec (a) and Two Lights (b) from 1984 to 2014; Courtesy of R. Weatherbee from the Oceanography Data Lab, University of Maine.

Kelps inhabit the coastlines of every continent except Antarctica, but an overall global trend or trajectory in global kelp abundance is lacking over the past 50 years.

Declines in kelp biomass in 38 % of ecoregions, increases in 27 %, and no change in 35 %, while accounting for all members of the Laminariales, reflect regional differences and drivers of change in kelp abundances (Krumhansl et al. 2016). Localized anthropogenic development poses risks for otherwise stable kelp forests (Pfiester et al. 2018). Human-induced loss of predatory megafauna such as finfish or sea otters can greatly influence their associated kelp ecosystems, including abrupt phase shifts to alternate stable states through trophic cascades; loss of apex predators increases herbivory on kelps by sea urchins, resulting in complete loss of kelp beds (Steneck et al. 2013, Estes et al. 2016). Climate-induced effects on kelps may not be limited to direct effects of thermal stress: "Tropicalization" occurs when temperate habitats gain tropical species that establish and then affect temperate ecosystem. For instance, a tropical herbivorous fish caused a 70 % decrease in abundance of Australian populations of the temperate kelp *Ecklonia radiata* (Zarco-Perello et al. 2017). While all of these direct and indirect factors might influence kelp abundances, kelp studies from the GOM have found a constantly negative rate of change of kelp abundance throughout the past 50 years (Krumhansl et al. 2016), correlating with increases in SST (Pershing et al. 2015, Krumhansl et al. 2016, Thomas et al. 2017). Rising SST might be the strongest influence on kelp abundance in coastal New England and may affect future distributions of *A. esculenta*.

Genetic diversity is important to the ability of a species to adapt to environmental change. In the context of this study, Kraan and Guiry (2000a) found little genetic difference among European and Canadian populations of *A. esculenta* populations using

RUBISCO spacers. Various kelps, including populations of *Alaria esculenta* from New England and Nova Scotia also had low degrees of genetic polymorphism based on allozyme variation for 20 isozymes (Neefus et al. 1993). Although such findings are of great importance, the particular markers used have relatively low resolution and further studies of *A. esculenta* population genetics in the GOM are needed with better DNA-based markers such as microsatellites. Fast-growing *Alaria esculenta* strains were produced by crossing bogeographically distinct Irish populations, but no genetic variation was found with internal transcribed spacers; morphological differences were considered unlikely to be due to genetic adaptation, but instead to be based on ecotypic variation caused by temperature tolerance (Kraan et al. 2000b). Examining temperature ecotypes of *Alaria esculenta* in the GOM should be of value to the aquaculture industry.

Visible and UV radiation, salinity, sedimentation, and herbivory are known to affect *Alaria esculenta*, but temperature has the largest effect on macroalgal growth, reproduction, and survival (Lüning 1990, Fredersdorf et al. 2009, Hurd et al. 2014, Zacher et al. 2016, Park et al. 2017). Kelps have a complex life history, alternating between microscopic gametophytes and macroscopic sporophytes (Graham et al. 2016). Determining how temperature affects both stages in the life history of *A. esculenta* is necessary, because gametophytes act as seedstock for the sporophytic stage, which is the commercial crop. Macroalgal species with heteromorphic life stages often have different optimal temperatures for spore development, gametophyte growth, gametogenesis, and sporophyte growth, all of which can influence the biogeographic distribution of a species

(Wiencke and Dieck 1989, tom Dieck 1993, Izquierdo et al. 2002, Lind and Konar 2017, Hargrave et al. 2017). In Arctic populations of *Alaria esculenta* from Spitsbergen, Norway, spore germination had low optimal temperatures (2 – 12 °C; Müller et al. 2008, Fredersdorf et al. 2009), whereas gametophytic upper survival temperatures (19 – 21 °C; tom Dieck 1993), optimal gametophyte growth (15 °C; Park et al. 2017), and optimal temperatures for photosynthesis in sporophytes (13 – 17 °C; Fredersdorf et al. 2009) were higher, demonstrating a difference in optimal temperatures between life stages. Overall, kelps have high tolerance to increased temperatures, although levels of tolerance can vary within a species (i.e., thermal ecotypes; Kraan et al. 2000b, Müller et al. 2008). While there are species-specific temperature effects on kelp microscopic stages, gametophytes are hardier than spores and sporophytes for various members of the Laminariales (van den Hoek 1982, tom Dieck 1993, Wiencke et al. 2007, Müller et al. 2008, 2012, Zacher et al. 2016, Park et al. 2017).

This study investigates the temperature tolerance of the edible kelp *Alaria esculenta* with the aim of evaluating its potential as a sea vegetable crop in the warming GOM. I surveyed the reproductive phenology of *Alaria esculenta* across most of the coast of Maine in order to understand the natural availability of reproductive material for aquaculture (seedstock sourcing). I then cultured zoospores from a northern ("Downeast") and a southern population to assess gametophyte (seedstock) response to gradual thermal acclimation and determine how tolerant gametophytes are to higher temperatures. Cultures isolated from both populations were exposed to elevated temperatures (or maintained under control conditions). These acclimated strains (and control cultures) were then crossed to produce sporophytes that were grown up in a common garden to assess the effects of strain location and previous thermal acclimation on the growth of next-generation sporophytes (crop yield).

Methods

Study Organism

Kelp life history stages alternate between microscopic gametophytes and macroscopic sporophytes. Mature plants bear sporophylls, which are specialized pairs of bladelets on the stipe below the blade. When sporophylls are ripe, they release zoospores that settle on rock (or other substrate) to develop into either male or female gametophytes. Mature gametophytes produce either sperm or eggs. Mature eggs produce a pheromone that causes release of sperm from antheridia on adjacent male gametophytes, and the sperm swim up the pheromone gradient to fertilize the egg. The zygote is retained on the female gametophyte and the juvenile sporophyte germinates from the zygote (Lüning and Müller 1978, Marner et al. 1984). Haploid gametophytes act as seedstock for the commercial crop, the diploid sporophytes; both life history stages in *Alaria esculenta* are important to the aquaculture industry.

Study Sites

Site locations for reproductive phenological surveys spanned most of coastal Maine. Each location had two subsites 1 – 7 km apart. Lubec sites representing northern Maine were at Quoddy Head State Park (44.813306, -66.952102; permit #2014-28) and Carrying Place Cove Road (44.803451, -66.981868). Schoodic sites were on the Schoodic Peninsula in Acadia National Park (permit # ACAD-2016-SCI-0010) at Schoodic Point (44.333744, -68.058047) and Blueberry Hill (44.338621, -68.044180). Pemaquid sites representing lower, mid-coastal Maine were at Pemaquid Point Lighthouse Park (43.836364, -69.505763) and Chamberlain (43.884844, -69.473678; Fig. 2.2).

Figure 2.2. Map of study sites: triangles indicate reproductive phenology survey sites, circles indicate locations where sorus material was collected for gametophyte thermal acclimation experiments and subsequent sporophyte grow-out, and the square indicates the Springtide Seaweed, LLC. farm where sporophytes were grown and harvested.

Sites where material was collected for thermal acclimation experiments span Maine's range of coastal sea surface temperatures. Using monthly average SST satellite data from the last 30 years, I determined that Lubec waters were representative of colder water profiles in the Gulf of Maine (30-year SST summer average of 10.9°C), where macroalgal aquaculture is currently underway, and that Cape Elizabeth experienced warmer water temperatures that matched those of the retreating boundary of *Alaria* *esculenta* in northern Massachusetts (30-year SST summer average of 15.4°C; courtesy of the Satellite Oceanography Data Lab, University of Maine, Fig. 2.1). Sporophylls to produce gametophyte seedstocks were collected from Lubec (Quoddy Head State Park, 44.813306, -66.952102) and from Cape Elizabeth at Dyer Point (43.564946, -70.196510), just outside of Two Lights State Park. The location of the open water sea farm used for grow-out of the sporophytes produced from the thermal acclimation experiments was in Frenchman's Bay near Sorrento, ME (Springtide Seaweed, LLC.; 44.459287, -68.176394), a site located between Lubec and Two Lights (albeit closer to Lubec).

Reproductive Phenology

I sampled each of the six subsites every two months for two years (2014 – 2016). I used random numbers to identify 30 plants on a 60 m transect line placed parallel to the shore through the *A. esculenta* bed exposed at low spring tides. Plants were classified in the field as reproductively mature when having at least one ripe sporophyll with a deep brown area indicative of mature zoospores. I determined the reproductive proportion of plants for each of six transects every 2 mo with the exception of one January collection from Lubec, because winter ice scour had decimated the population. Representative samples are archived in the University of Maine Herbarium (MAINE-A-4551through 4602, MAINE-A-5177 through 5275). Proportions were transformed using a logit transformation. I used a univariate analysis of variance to examine the effects of location (Lubec, Schoodic, and Pemaquid), month, and year, blocking by subsite.

Gametophyte Thermal Acclimation

A convenience sample of 4 reproductively mature sporophytes were collected from Two Lights (TL) in the north and 4 additional sporophytes from Lubec (Lu) in the south. Six ripe sporophylls were selected from each individual. Sporophylls were washed with sterile seawater, treated for two min in 1 L of 0.01% betadine solution to eliminate ciliates and other contaminants, rinsed again in sterile seawater, wiped dry, wrapped in moist paper towels, held at 4 °C in the dark overnight (20 – 24 h), and placed into circulating 12 °C sterile seawater (4 μmol photons m^2/s, 12:12 L:D photoperiod) to obtain zoospores (South 1970, Gordon and Brawley 2004, Redmond et al. 2014). Zoospore strains (i.e., TL1, TL2, TL3, TL4, Lu5, Lu6, Lu7, Lu8) are defined as mixed sister genotypes from sorus tissue from one adult individual. Zoospores were plated into replicate Petri dishes and placed on orbital shakers (130 rpm; VWR Orbital Shaker, 980001, Radnor, PA) to provide water motion in 12 °C environmental chambers (Percival Scientific, Perry, IA). Gametophyte cultures from each seedstock were maintained in constant light [4 μmol photons m^2/s to promote vegetative growth (Gordon and Brawley 2004) while inhibiting gametogenesis; i.e., production of eggs and sperm, Lüning & Neushal 1978] with weekly sterile seawater changes with a modified nutrient supplement (1/4-strength West-McBride modification of ES [Anderson, 2005] and using a 1-fold reduced Fe-EDTA solution) until gametophytes reached 10+ cells in length (~2 mo).

Replicate cultures (8 replicate Petri dishes per strain, 4 strains per site) were either maintained at 12 °C as controls (n = 32) or underwent a gradual thermal acclimation from 12 to 22 °C, with an increase of 1 °C/12 h (n = 32) and were then maintained at the final temperature of 22 °C for 3 days to determine prolonged effects of temperature. Replicate cultures were assessed for gametophyte health. The same gametophyte filaments in each Petri dish were tracked throughout the experiment. Three character states were used in monitoring the condition of gametophytes during the experiment: healthy, unhealthy, and dead. Filaments classified as healthy maintained constant color and normal cell size (Fig. 2.3a). Any sign of plasmolysis, organelle damage, loss of color or mottling, etc. indicated stress injury (Zhang et al. 2013), and led to the gametophyte being categorized as unhealthy (Fig. 2.3b). A gametophyte with complete loss of cytoplasmic contents was categorized as dead. Gametophytes (2428 individuals) were monitored and categorized daily at an inverted light microscope (253 – 519 gametophytes per replicate Petri dish). After logit-transforming the healthy proportion of gametophytes, I performed a repeated measures multivariate analysis of variance (MANOVA), applying a Greenhouse-Geisser correction for departure from sphericity to examine the effect of source location (Lubec versus Two Lights) and treatment (thermal acclimation versus control).

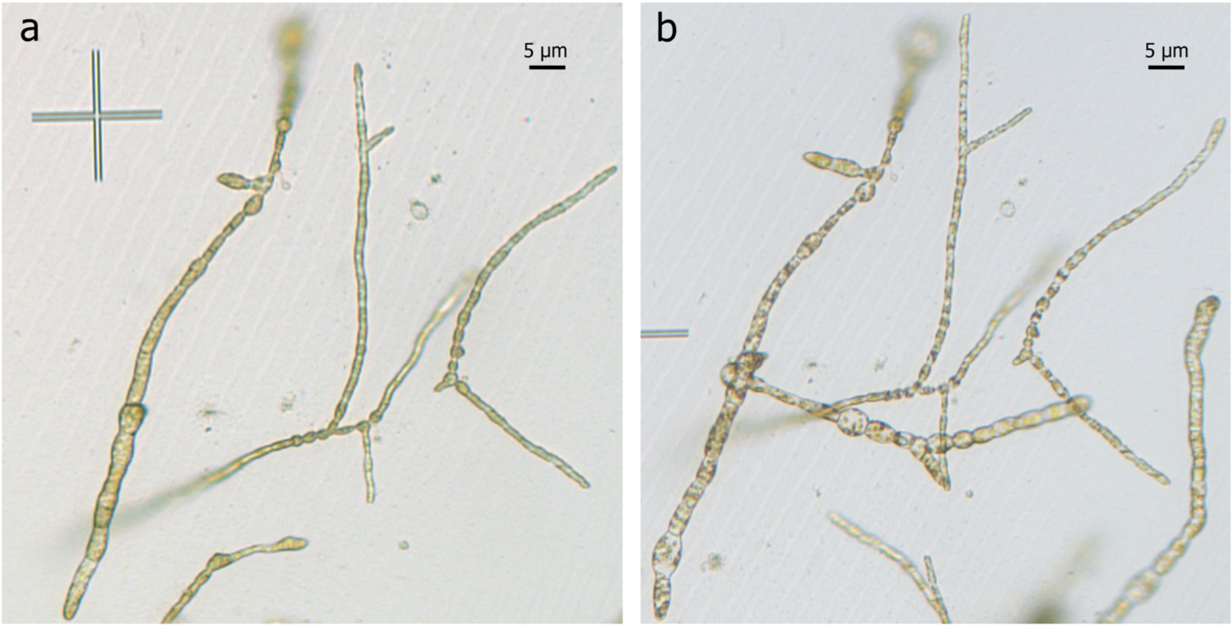


Figure 2.3. Example of a (a) healthy character state from Two Lights, Day 1 of thermal acclimation, and (b) an unhealthy character state where plasmolysis is evident in the same representative gametophytes on Day 10 of thermal acclimation.

Experimentally acclimated cultures were gradually returned to the control temperature of 12 °C (1 °C/12 h). Culture conditions were set for vegetative growth (see above) for 3 mo with monthly media changes. Control cultures from the same seedstocks were maintained under the same conditions. I mixed TL3 with TL4, both previously acclimated, into one culture (TL3/4_Accl.) to reduce potential inbreeding. I also mixed control cultures of TL3 and TL4 that had consistently been kept at 12 °C to produce culture TL3/4_Ctrl. I mixed Lu7 with Lu8 in the same manner to make Lu7/8_Accl. and Lu7/8_Ctrl. Strains TL1, TL2, Lu5, and Lu6 contained filamentous brown algal contaminants that could affect interpretation in further experiments and were not used.

Each mixed culture was seeded onto 2 kurlon lines (~10 m) using a spray-seeding technique (n = 8 lines). First, gametophytes were blended for 1 minute at medium speed with an immersion blender, checking the mixture with a microscope to produce fragments with 5 – 10 cells. Then, I gravity-fed a stream of blended gametophytes in seawater into a stream of filtered, compressed air to create a fine spray that was evenly applied to two spools of kurlon line dampened with seawater. Spools settled for ~8 min and then were carefully placed in a 10 gal (37.85 L) aquarium, with no bubbling or agitation for the first 24 h. The process was repeated for each mixed culture; seeded lines were maintained in separate 10 gal (37.85 L) aquaria for one week to ensure gametophyte attachment. Aquaria were then placed under conditions that promote gametogenesis (10 °C, 40 µmol m^2/s, 14:10 L:D photoperiod; Munda and Lüning 1977, Lüning 1990, Gordon and Brawley 2004).

Sporophyte Grow-Out

To compare next-generation sporophyte grow-out as a function of previous thermal acclimation, seeded lines were placed into a 1.25 m-high transparent Kallwall tank. Duplicate seeded spools were stacked in racks suspended in the tank using randomized numbers, and grown out for 3 mo at the Center for Cooperative Aquaculture Research (Franklin, ME), with biweekly UV-treated and filtered seawater changes with full-strength modified West-McBride enrichment under conditions that support sporophyte growth (10 °C, 40 µmol m^2/s, 14:10 L:D photoperiod; Munda and Lüning 1977, Lüning 1990, Gordon and Brawley 2004). Juvenile (sporophyte) blades were selected at random (3 sections per line, ~2.5 cm in length) to measure blade surface area (MAINE Accession #005569-005576) using ImageJ imaging software (nih.gov). Seeded lines were transplanted to a sea farm in Frenchman's Bay near Sorrento, ME, and randomly placed on long lines at the beginning of the sea vegetable season (12 Oct 2017). After 4 mo of grow-out (9 Feb 2017), adult (sporophyte) blades were sampled at three locations per line using randomized numbers (~5 cm section of line); I measured blade surface area using ImageJ software (MAINE Accession #005610-005649). A final collection was made on 10 Apr 2017 at the beginning of the harvest season after an additional 2 mo of grow-out time: Mature blades were again selected at random (3 sections per line, each of ~5 cm length), imaged, and preserved as herbarium specimens (MAINE Accession #005650-005695). I used a nonparametric permutational multivariate analyses of variance (PERMANOVA) to examine the effects of site (Lubec versus Two Lights) and treatment (previous thermal acclimation versus control).

Results

Reproductive Phenology

I surveyed three locations along Maine's coast every two months for two years to determine when *A. esculenta* was reproductive within each population. All populations across locations had a dip in reproductive maturity in summer (see spline interpolation line on Fig. 2.4). The average proportion of populations that were reproductive in May and July was 0.26, but with a lot of variability across transects, as well as inter-annual variability. A greater proportion (0.71) of blades had sporophylls in colder months (i.e., November and January). Sample time (month) was the only significant factor that affected reproductive phenology (ANOVA, Month: F(5, 41) = 12.68, $p < 0.001$). In contrast, site had no significant effect on the proportion of individuals that were reproductive (Site: F(2, 2) = 16.32, $p = 0.058$), although average reproductive proportions do decline from north to south: 0.69, 0.52, 0.40 at Lubec, Schoodic, and Pemaquid, respectively. There was also no difference between the two years of the survey (Year: F(1, 41) = 1.83, $p = 0.18$). These survey data signify seasonal differences in reproductive populations, but reproductive phenology does not differ statistically across the sites surveyed.

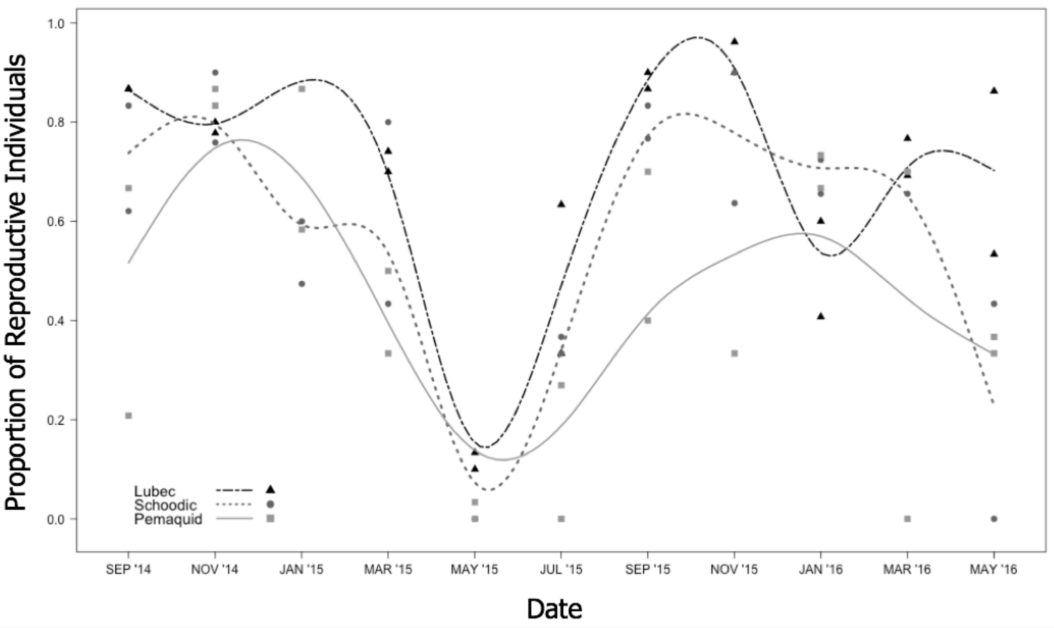


Figure 2.4. Proportion of individuals of *Alaria esculenta* that were reproductive across time for much of the three sites spanning the Gulf of Maine: Lubec, Schoodic, and Pemaquid (2 subsites each). Cubic spline interpolation was applied to distinguish patterns.

Gametophyte Thermal Acclimation

Replicate gametophyte seedstock aliquots from northern and southern populations (Lubec, ME and Two Lights, ME, respectively) were either maintained at a control temperature of 12 °C or gradually acclimated to 22 °C (current summer SST in parts of the GOM) to assess gametophyte health in response to high temperatures. Over 90% of the gametophytes in control treatments were categorized as healthy throughout the experiment (Fig. 2.5a, c). Whether or not the gametophytes were maintained as controls or acclimated to 22 °C was the only factor that significantly affected gametophyte health throughout the experiment (Repeated Measures MANOVA with Greenhouse-Geisser correction ($\varepsilon = 0.39$), Treatment:Day $F(144, 24) = 6.31$, $p = 0.002$). Heat-acclimation of both Lubec and Two Lights seedstocks caused a decrease in the proportion of healthy gametophytes throughout the experiment; at the end of the experiment, almost 75% of acclimated cultures from Lubec were healthy (Fig. 2.5b), whereas only about 25% of Two Lights' acclimated cultures were healthy (Fig. 2.5d). Interestingly, whether the seedstock was isolated from sporophytes collected from the northern or southern Gulf of Maine did not contribute significantly to gametophyte health over time (Repeated Measures MANOVA with Greenhouse-Geisser corrections for departure from Sphericity ($\varepsilon = 0.39$), Treatment:Day $F(144, 24) = 0.91$, $p = 0.423$). However, an analysis of variance of the proportion of gametophytes that were healthy on the final day of the experiment found some effect of location (ANOVA, $F(1, 6) = 4.13$, $p = 0.08$; i.e. marginal differences between Lubec and Two Lights). Because the proportions of gametophytes that were healthy between the different locations differed greatly (25 % versus 75 %), I consider the effect of location to have marginally biological significance.

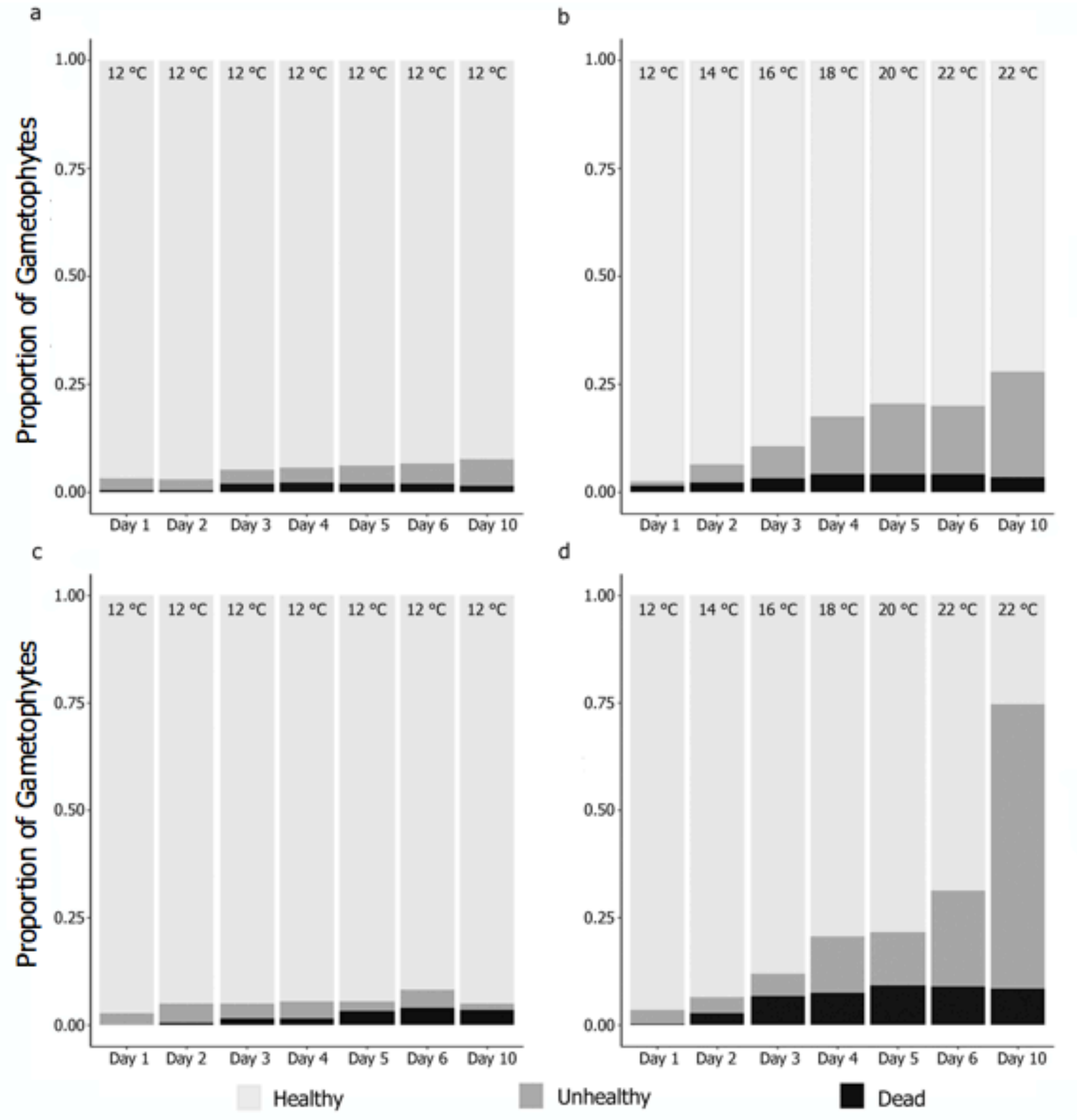


Figure 2.5. Proportions of gametophytes produced from zoospores at (a) Lubec (northern location) at control temperature and (b) gradually acclimated, or (c) Two Lights (southern location) at control temperature and (d) gradually acclimated that are healthy, unhealthy, or dead over a 10-day gradual thermal acclimation (and corresponding controls).

In order to understand the lethal limit for *Alaria esculenta* gametophytes, I exposed aliquots of both heat-acclimated and control cultures from both source locations to an unnatural, extreme temperature of 34 °C (taken from 12 °C to 34 °C, 2 °C/day, while assessing character health state of individual gametophytes daily). Loss of some cultures due to contaminants, limits on culture chamber capacity (stopped at 34 °C to protect chamber), and extreme variability within cultures limited my ability to make quantitative comparisons or determine the LT_{50} for these northern and southern strains. One conclusion, however, is that the unnaturally high temperature of 34 °C does not kill all cultures.

Sporophyte Grow-Out

I promoted gametogenesis by changing culture conditions (see Methods) and crossed seedstock gametophyte cultures to generate sporophyte blades on spools of kurlon line and stacked all spools in one large transparent Kallwall tank, to measure how juvenile growth (i.e., blade surface area) is affected by thermal acclimation at the haploid stage. Both previous acclimation and source location affected the surface area of juvenile blades (PERMANOVA; Treatment: pseudo-$F(1, 243) = 135.7$, $p < 0.001$, Location: pseudo-$F(1, 243) = 49.1$, $p = 0.010$). There was a significant interaction between the main factors of treatment and location (pseudo-$F(1, 243) = 49.6$, $p < 0.001$). Juvenile sporophytes produced from previously acclimated gametophytes had larger blade surface areas than corresponding controls (Fig. 2.6a). Thermally-acclimated seedstocks from Lubec produced the largest juvenile blades with a mean surface area of 2.88 cm^2 (± 3.55 cm^2 SD) compared to Lubec controls averaging 0.50 cm^2(± 0.54 cm^2), Two Lights acclimated blades averaging 1.09 cm^2(± 1.47 cm^2), and Two Lights controls averaging 0.51 cm^2(± 0.63 cm^2); there was a high level of variability (Fig. 2.6). These data indicate that previous thermal acclimation of gametophytes increases juvenile sporophyte growth, but the seedstock source location affects how effective previous acclimation increases growth: strains from northern populations produced larger juvenile blades compared to those from southern populations.

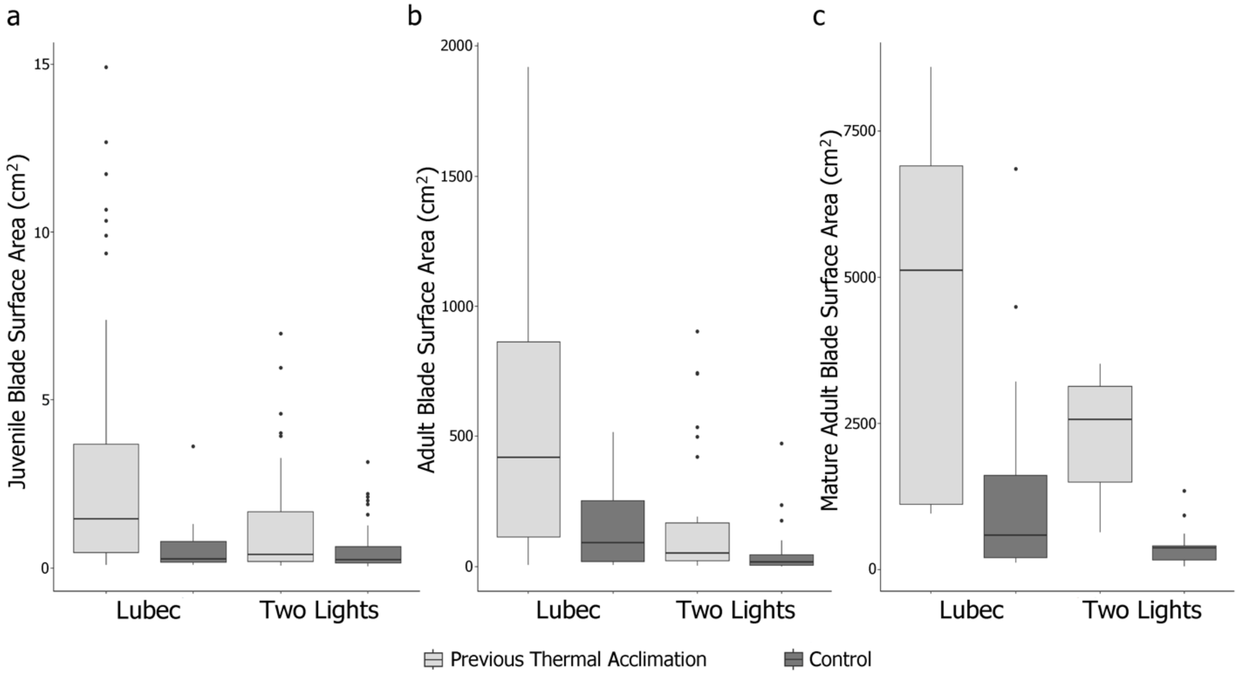


Figure 2.6. Blade surface areas of (a) juvenile, (b) adult, and (c) mature sporophytes produced from gametophytes from two sources, Lubec and Two Lights, that were either thermally acclimated or maintained at control temperatures.

I transferred the spools of juvenile blades from the nursery-based common garden experiment to a sea farm in Frenchman's Bay (Sorrento, ME) for grow-out on long lines, and measured the surface area of the blades at a midpoint during the growing season. Adult blades held to juvenile growth patterns: previous acclimation and source location affected blade surface area (Treatment: pseudo-F(1, 109) = 2055446, $p < 0.001$; Location: pseudo-F(1, 109) = 1692615, $p < 0.001$; Treatment:Location: pseudo-F(1, 109) = 570250, $p = 0.023$). As was true of juveniles, thermally acclimated seedstocks from Lubec had an average blade size of 570.1 cm^2 (± 564.42 cm^2 SD), roughly three times more than thermally acclimated blades produced from Two Lights seedstocks (182.4 cm^2 ± 266.18 cm^2 SD), and far more than either control seedstock (mean of 157.4 cm^2 ± 166.58 cm^2 SD from Lubec and 54.4 cm^2 ± 102.03 cm^2 SD from Two Lights); again, there is a high degree of variability. Thermal acclimation can increase *Alaria esculenta* growth on a sea farm during the growing season, and seedstock source influences how effective this yield increase is at a midpoint in the growing season.

I collected from long lines for a final analysis at the time of the commercial spring harvest. Mature, harvest-ready blades were still affected by previous acclimation (pseudo-F(1, 42) = 67352766, $p = 0.003$), and by source location (pseudo-F(1, 42) = 32033555, $p < 0.001$; no interaction between factors). A lot of growth occurred during the spring months of the growing season. Thermally acclimated blade surfaces areas from Lubec averaged 4601.38 cm^2 (± 3232.10 cm^2) and 2233.01 cm^2 (± 1063.43 cm^2) from Two Lights, whereas control blade surface areas from Lubec averaged 1464.39 cm^2 (±

2026.47 cm^2) and 416.83 cm^2 (± 370.07 cm^2) from Two Lights. At the time of harvest, thermal acclimation of gametophytes influenced the crop yield of sporophytes, as did the site (Two Lights versus Lubec) from where gametophytes were collected.

Discussion

Reproductive phenology surveys showed that seedstock availability did not differ among the three sites surveyed along the Maine coast. Although not significant, annual average reproductive proportions do decline from north to south. The proportion of reproductive individuals varied throughout the year, as would be expected; however, on average, over 25 % of all subpopulations over the two year survey were still reproductive even during summer. Thermal acclimation significantly decreased in the proportion of healthy gametophytes over the course of the experiment. Gametophytes from the southern location had reduced health at the final exposure to 22 °C compared to gametophytes sourced from the northern location. Across three time points throughout the *Alaria esculenta* farming season, sporophytes produced from gametophytes that were previously thermally acclimated were larger than controls, and those produced from gametophytes sourced from Lubec, the northern location, grew to be significantly larger than those sourced from the south.

Reproductive Phenology

To my knowledge, this study represents the first survey of the reproductive seasonality of *Alaria esculenta* in the Northwest Atlantic. While it is limited to the Gulf of Maine, it offers a comparative dataset to similar surveys from across the North Atlantic. Seasonal fertility of *A. esculenta* was explored in Arctic populations in Svalbard, Norway, and all sori were fully developed in June (same qualitative assessment used in this study), but the reproductive proportion fell to 20% by the end of July with almost all zoospores spent by late September. The drastic decline of sporophyll fertility at the beginning of September, along with zoospore release and germination rates monitored in the laboratory, showed that the reproductive season of *A. esculenta* in the Arctic ends in September, with peak fertility in June and July (Olischlager and Wiencke 2013). The reproductive season is reported to occur from November to March in *A. esculenta* populations in the United Kingdom; sporophylls are produced in late fall and early winter and persist on plants after sporulation as vegetative structures during the summer for an unknown duration (Birkett et al. 1998). Reproductive sorus tissue is present year-round in the GOM, even though it is lower in summer. In this study, differences in reproduction between summer and winter seasons (different photoperiod),without significant differences across locations that differ in SST (Fig. 2.1), support the hypothesis that photoperiod is key to zoospore production in Maine. As daylegth shortens in winter, photosynthesis will decline, lowering ATP levels and fixed carbon available for activities such as zoospore production.

Olischlager and Wiencke (2013) did not follow reproduction throughout the year; however, the polar day lasts from April to August, which corresponded to the reported reproductive season. The United Kingdom experiences intermediate daylengths, and also has an extended reproductive season. Maine, at lower latitudes, does not experience polar night and has relatively long days, even in winter (9:13 L:D photoperiod on shortest day), and I report year-round reproductive populations. Lüning (1990) reported that kelps in the Arctic stop growing during the summer and store photosynthates to use under the ice during the winter when there is limited light available; vegetative growth occurred, and zoospore production began in winter. My finding that zoospores are produced by *A. esculenta* in the GOM year-round is consistent with more photosynthetic capability due to longer winter days in Maine compared to more northern latitudes.

Zoospore viability was not measured in this study, and sorus presence does not necessarily correlate with zoospore release or germination (Olischlager and Wiencke 2013). However, sporophylls that I classified as reproductive often released zoospores (as judged by brown secretions) during low tide in the field and also onto herbarium paper during pressing. Sorus tissue collected in summer for use in subsequent acclimation studies supplied zoospores that had high germination rates (pers. obs., data not shown).

Winter storm damage and ice scour will occasionally clear-cut a bed of *A. esculenta*, leaving only the stipes behind. While ice scour is no longer a common occurrence in lower intertidal zones in Maine, it is more common in northern locations (e.g. Lubec sites in this study, January 2015) than southern ones. Beds removed via ice scour can return within one year, sometimes within the same season (Keats et al. 1985). Vegetative gametophytes may be established already in *A. esculenta* understory in protected turfs and crevices, acting as a seed bank (Müller et al. 2012, Bringloe et al. 2017); removal of the sporophyte canopy by ice scour may allow new sporophytes to develop, grow, and replace the previous *A. esculenta* bed (Keats et al. 1985).

Gametophyte Thermal Acclimation

Gametophytes were negatively affected by thermal exposures reaching 22 °C, and gametophytes from the southern population were affected to a greater extent. The majority were able to maintain a healthy state until the prolonged 3-day exposure at 22 °C, after which damage to cellular structure was visible at higher proportions in the filaments. Physiological stress such as from high temperature is often tied to oxidative stress, where inhibition of photosynthesis restricts electron flow through the electron transport chain causing reactive oxygen species (ROS) to form. Lipid, protein, and DNA damage can result (reviewed by Hurd et al. 2014). Higher temperatures may cause DNA damage but can also increase DNA repair (Müller et al. 2008). DNA repair rates increase with rising temperature in algae (Pakker et al. 2000), up to a point. It may be that 22 °C is a critical temperature that beings to promote stress for *A. esculenta* in the GOM.

Previous studies in the Arctic put the upper survival temperatures of *A. esculenta* gametophytes at 19 – 21 °C (tom Dieck 1993), far warmer than the waters they inhabit; thus, such gametophytes may not be threatened by climate change. Origins of the Laminariales are believed to be in the Northwest Pacific during the Miocene Epoch (23 – 5.3 Mya), a time of warmer global climates than preceding or following epochs (Bolton 2010, Vermeij 2012), when kelp-dominated communities first appear in the fossil record (Parker and Dawson 1965). Thus, kelps evolved in relatively warm waters. It is hypothesized that kelps colonized the Arctic and North Atlantic ~3.5-5.4 Mya when the Bering Strait opened (early Pliocene; Adey et al. 2008, Bolton 2010, Vermeij 2012). Very few species from very few genera survived the trip through the Bering Strait despite continuous availability of coastline (Lüning 1990). It is notable that *Alaria esculenta* is the only species of *Alaria* to inhabit the North Atlantic, whereas ~12 species inhabit the North Pacific (excluding lectotypes of *Alaria esculenta* and debated infraspecific species, based on morphological and/or genetic differences; Guiry and Guiry 2015, Lane et al. 2006). Species that survived come from genera with high levels of morphological diversity (i.e., there are many diverse species in the *Alaria* genus, increasing the pool of genetic diversity), which may explain their ability to colonize colder waters that other kelps could not survive (Bolton 2010). The higher-than-ambient temperature survival of *A. esculenta* may be due to the relatively recent range expansion and their ability to survive broad ranges of ancient thermal exposure; rising sea surface temperatures are hypothesized not to affect Arctic populations (tom Dieck 1993, Park et al. 2017, reviewed by Hurd et al. 2014). Kelps expanding their range towards higher latitudes increased their cold tolerance by decreasing their survival, growth, and reproductive temperature from previous, warmer optimal temperatures (Wiencke et al. 1994). Kelps that have expanded ranges from the temperate North Pacific through the Arctic and down to the temperate North Atlantic demonstrate a robust ability to handle a broad range of temperatures; however, the southern edge of temperate expansions are now experiencing extreme warming in SST (Pershing et al. 2015, Thomas et al. 2017), possibly surpassing their ancient optimal temperatures, especially in the GOM.

While only marginally significant, gametophytes from the southern population (Two Lights) had a more negative response to elevated temperature during the acclimation experiment than those from the northern population (Lubec). Temperate populations that are closer to the biogeographical boundary may not have as much ability to respond to stress. Physiological acclimation can be limited to the temperatures found in the biogeographical range where each species occurs (Dalhoff and Somero 1993). Sea surface temperatures of 22 °C are rare even at the southern limit of *Alaria esculenta* in New England (e.g. anomaly in 2013, spike in SST 3 °C above SST trends for GOM; Pershing et al. 2015).

Organisms on the edge of a distribution are often small and fragmented, and are more susceptible to genetic drift, which may reduce adaptive potential; two opposing instances occur at edge populations: local adaptation or maladaptation (Pearson et al. 2009, Nicastro et al. 2013, Araujo et al. 2014, Jueterbock et al. 2014, Saada et al. 2016). Local adaptation occurs when populations are genetically diverse and have higher potential to adapt to environmental changes. Most species in which certain populations have had historical exposure to warmer waters would be expected to have local adaptations to handle these thermal stresses. Central and edge populations of *Fucus vesiculosus* contain two distinct genetic lineages (Nicastro et al. 2013, Assis et al. 2014). The southern lineage was more tolerant to experimental high temperatures than the northern lineage, demonstrating local adaptation (Saada et al. 2016); however, rapid range retraction caused extinction of most of the populations comprising the southern lineage (Nicastro et al. 2013) and populations are already adaptively limited. The consequences of chronic heat stress are evident: *F. vesiculosus* from the southern edge of the population has lost the ability to recover from heat shock (Mota et al. 2015). The other response seen in edge populations is maladaptation, where a species may be at its limits for stress response, and there is little adaptive potential left in regions of low genetic diversity. Jueterbock et al. (2014) found population ecotypes of *Fucus serratus* that differed in thermal stress resistance. While populations at the southern distributional edge still maintained good levels of photosynthetic performance in response to heat stress, heat shock protein (HSP) patterns show limited responsiveness to further heat stress (Jueterbock et al. 2014). Edge populations of *F. serratus* had higher expression of HSPs than central populations when exposed to the same control temperature, and the edge population was maladapted to desiccation and heat stress (Pearson et al. 2009). *F. serratus* has isolated, fragmented edge populations with small population sizes, low genetic diversity, and poor dispersal capacity (Pearson et al. 2009). This is limiting adaptation (genetic change) due to declining genetic diversity at the southern edge of the biogeographic range due to thermal die-offs. Jueterbock et al. (2018) expect southern populations to disappear by 2100 if genetic rescue from other populations does not occur. Southern Maine strains of *Alaria esculenta* do not handle thermal stress well, which might be evidence of maladaptation.

Kelps have low dispersal capacity (Merzouk and Johnson 2001), which supports a more geographically structured genetic diversity, but evidence of maladaptation in edge communities has been demonstrated in kelps. For instance, in Australian kelp forests, populations of *Ecklonia radiata* from warmer latitudes had less genetic variation, less physiological response to loss of canopy, and less ability to recover from a heat wave, suggesting a lack of capacity to adapt to thermal stress (Wernberg et al. 2018). While speciation of *A. esculenta* is established (Neefus et al. 1993, Kraan and Guiry 2000a, Kraan et al. 2000b), the genetic diversity at population levels within the GOM is still unknown, and is hypothesized to be low, because it is low in other kelps (Wernberg et al. 2018). Low genetic diversity may explain why, in the GOM, strains from the southern edge populations (Two Lights) show maladaptation to thermal exposure, compared to strains from the northern populations' (Lubec) responses, which might be more typical of *A. esculenta* at higher latitudes. Preliminary results from gene expression analyses of certain HSPs from gametophytes in my thermal acclimation experiment (not shown) show similar patterns to maladapted populations of *F. serratus*: while strains both populations respond to thermal exposure, the southern strains were less responsive to increasing temperatures, but maintained higher levels at control temperatures than those from the northern population (Quigley et al. in prep). Species' resistance to temperature can often change in response to constant stress; this is especially true at the edges of a species distribution, where populations are already close to their temperature limits (reviewed in Hurd et al. 2014). While there was no statistical effect of location on gametophyte health over time in this study, there was a more drastic decrease in health in the southern strains toward the end of the heat stress acclimation experiment. This may be reflective of loss of stress responsiveness of populations at the southern boundary due to the constant level of stress that the population may be experiencing, and possibly due to limited genetic diversity of *Alaria esculenta*, limiting adaptive potential to select for temperature tolerance. If true, without genetic rescue from diverse populations (Jueterbock et al. 2018) or mutations that favor advantageous strategies such as asexual reproduction (Coleman and Wernberg 2018), fragmented edge populations of *A. esculenta* such as Two Lights might become at risk of extinction.

Sporophyte Grow-Out

Sporophytes that were thermally acclimated had larger blade surface areas, and those from Lubec had blades up to double the size of their southern counterparts from Two Lights. These patterns held throughout the growing season. There is a high degree of variability in blade surface area (although, less in fully mature blades), but differences between treatments and locational effects are statistically supported. Some effect of thermal exposure transferred from the gametophytic life history stage to the sporophytic life history stage. Acclimation occurs when an individual shows phenotypic adjustments to a change in the environment, whereas adaptation is when certain individuals in a population survive a change due to genetic differences from other individuals, causing a genetic change at the population level (Davison and Pearson 1996). Almost all gametophytes regained health after a recovery period, and only ~10 % of all gametophytes died (data not shown), not lending much possibility for natural selection in one generation. I propose a hypothesis to explain this phenomenon: epigenetic heat stress memory affecting the timing of gametogenesis.

Differences in sporophyte size may be accounted for by delays in gametogenesis. While not quantitatively measured, sporophytes were initially found on kurlon lines (both under the microscope and by eye) over the course of a few days after conditions were switched to promote gametogenesis (pers. obs.; thermally acclimated sporophytes from

Lubec were seen first). Mean surface areas of the smallest sporophytes in the final collection point (TL_Ctrl mean = 417 cm^2) were comparable to mean surface areas of the largest sporophytes in the previous collection 2 mo prior (Lu_Accl mean = 570.1 cm^2). In *Laminaria digitata*, sporophyte development was retarded at lower temperatures, leading to the hypothesis that lower temperatures could cause delays in gametogenesis (Zacher et al. 2016). Heat stress exposure might advance the timing of gametogenesis. This study aimed at determining crop yield in the traditional growing season for kelps in Maine (September – April); crop yield in this study is directly applicable to common practices in sea vegetable aquaculture. Continued collections of sporophytes from the sea farm into the summer months might have confirmed whether all cultures would eventually produce sporophytes of the same size. However, blades often disintegrate or break due to epiphyte load that increases with warming waters. Summer collections might not reflect true growth and would not have been applicable to the sea farming industry. While there is evidence that heat stress might speed up gametogenesis, in my study I promoted gametogenesis after recovery time at 12 °C. An explanation for these results may be epigenetic heat stress memory.

Epigenetic responses to stress are not yet well studied in macroalgal systems. To my knowledge there is only one study that examined changes in methylation of macroalgal genomes under environmental stress (Yu et al. 2018), but such responses are well studied in higher plants. Transposable elements, that are normally silenced by small interfering RNAs, have been indicated in heat stress memory. For example, *ONSEN* is a transposable element that is reactivated in *A. thaliana* by prolonged heat stress when small interfering RNAs no longer silence them. New *ONSEN* copies arise through retrotransposition into mutants with deficient production of siRNAs and can be transmitted into the next generation (Ito et al. 2011). Retrotransposition increases genetic variation, which natural or artificial selection can act upon. These retrotranspositions mean that heat-inducibility can be conferred onto neighboring genes; *ONSEN* can "hijack" heat stress response, and the population may now adapt to stressful conditions over time (Stief et al. 2014b). Perhaps previous thermal exposure of *A. esculenta* gametophytes promoted heat stress memory via siRNAs; this might initiate a faster transition to gametogenesis when I altered light regimes, compared to gametophytes that did not have exposure to induce such a memory. If thermal exposure of *Alaria esculenta* gametophytes to 22 °C included transposition of heat stress memory to various genes and to the next generation, my thermal exposure protocol could be important for selective breeding in kelp aquaculture in Maine by increasing temperature tolerance in cultivated strains.

There have been reports of transgenerational effects of stress exposure at both morphological and epigenetic levels, specifically, memory triggered by heat stress exposure in *Arabidopsis thaliana* (Suter and Widmer 2013, Migicovsky et al. 2014). Heat stress in previous generations expedited flowering (i.e., reproduction) in *A. thaliana*, but the effect was reversible after multiple generations of no heat stress (Suter and Widmer 2013). Transgenerational epigenetic inheritance was offered as explanation (Suter and

Widmer 2013). Stress effect is heritable and may undergo adaptation. Migicovsky et al. (2014) found that heat exposure caused changes in genome methylation, and histone modification in differentially expressed genes that led to differences in progeny phenotype (including the tendency to bolt earlier, i.e., transitioning from vegetative to reproductive).

There is evidence of epigenetic heat stress memory, even transgenerationally, in marine macrophytes. Shallow-water populations of the seagrass *Posidonia oceanica* have optimized phenotypic variation to deal with higher levels of light and heat compared to deeper populations through the exclusive upregulation of heat-responsive genes (chaperone and antioxidant genes) and epigenetic responses (e.g. methylation of DNA or histones; Marin-Guirao et al. 2017). In most cases, demethylation in response to stress usually coincides with expression of previously inactivated genes to respond to the stress. Global genomic methylation decreased with exposure to high temperature in the red macroalga *Pyropia haitanensis*; 29 methyl sites mapped to gene coding regions that were involved in various physiological and regulatory pathways, most notably photosynthesis and abiotic stress response (Yu et al. 2018). Transposons are also activated in response to high temperature stress, creating true genetic change, and increasing the potential for *P. haitanensis* to adapt to abiotic stresses associated with increased temperature (Yu et al. 2018). Embryos of *Fucus vesiculosus* that had higher survival rates, and greater lengths when exposed to heat stress were cultured from parental material that had been previously exposed to higher temperatures (Li and Brawley 2004), also a potential example of transgenerational heat stress memory in macroalgae.

A more stressful environment may speed up stress adaptive processes via epigenetic mechanisms. This would mean southern populations, which were historically exposed to higher temperatures, would have a higher response to heat memory. Compared to Two Lights, Lubec might be considered a more typical population of the *A. esculenta* metapopulation of the North Atlantic, but it is still exposed to temperate waters at the end of the range in the Northwest Atlantic, thus adaptation via epigenetic mechanisms may be possible. As an edge population, *A. esculenta* from Two Lights may be genetically limited in its ability to respond to heat stress, even a heat stress memory. Population genetic and epigenetic studies of *Alaria esculenta* in the Gulf of Maine will be of great value to the sea vegetable aquaculture industry.

Conclusions for the Sea Vegetable Aquaculture Industry

This study provides a better understanding of the thermal tolerance of *Alaria esculenta* in the Gulf of Maine and offers protocols that may be implemented in kelp aquaculture, in general. Reproductive seedstock of *Alaria esculenta* may be available to sea farmers practically year-round, at least in low proportions, across source locations in

Maine. Bearing in mind winter storms and ice scour, I suggest collecting seedstocks in late fall rather than winter. While responsive to thermal stress, a large proportion of seedstock gametophytes remain healthy at temperatures not expected to be common in the Gulf of Maine for decades, although, northern seedstocks fare better and might be more useful for the industry. Aquaculturists should bear in mind differences in seedstock strains and how sourcing locations might affect production, especially if aquaculture endeavors coincide with range boundaries of crop species. An important component of sea vegetable aquaculture is crop yield. Sporophytes that were thermally acclimated had higher blade surface areas, and those seedstocks from the northern population had blades up to double the size of their southern counterparts. Predictions of warming for the Gulf of Maine, along with most coastal waters worldwide, include longer summers, which might shorten growing seasons in the future; having temperature-tolerant fast-growing crops will become more important. While I caution that other effects must be explored further (e.g. disease resistance, expression of stress traits) and only a few strains representing two populations with unknown genetic structure were examined, applying my thermal acclimation protocol in sea vegetable nurseries may be able to increase crop yields and speed up harvest time for farmers, and further development of protocols may aid kelp aquaculture in other locations around the globe. *Alaria esculenta* is an excellent candidate for expansion in Maine’s sea vegetable aquaculture sector, now and in the future.

CHAPTER 3

A COMMON GARDEN EXPERIMENT WITH *PORPHYRA UMBILICALIS* (RHODOPHYTA) EVALUATES METHODS TO STUDY SPATIAL DIFFERENCES IN THE MACROALGAL MICROBIOME

Introduction

Next generation sequencing has expanded understanding of host-specific and seasonal variation in macroalgal microbiomes (e.g., Brodie et al. 2016, Braus et al. 2017; references therein), while coupled biodiversity and metagenomic studies are testing assembly rules or functional aspects of host-microbe associations (e.g., Burke et al. 2011a, b, Kim et al. 2016, Zozaya-Valdés et al. 2017). Understanding the co-evolutionary and ecological relationships between host macroalgae and their bacterial communities has become a feasible goal. Recent characterizations of macroalgal microbiomes via various hypervariable regions of the 16S rDNA have revealed many more operational taxonomic units (OTUs; bacterial "species") than previously known from particular macroalgae (see reviews by Goecke et al. 2010, Egan et al. 2014). The extent to which OTU specialization occurs in different regions of a macroalga, however, is poorly known. Such specializations could be functionally important based upon numerous studies in humans (e.g., Human Microbiome Project 2012).

Unfortunately, studies of microbial variability across an algal thallus are complicated when epiphytic eukaryotes with their own complicating microbiota are collected in the field or when thalli are recently grazed, opening the possibility of deposition of grazer-specific microbes onto the thallus. Here, I used a common garden approach in laboratory experiments to address the spatial variability of the microbiome in clonal material of the strain of *Porphyra umbilicalis* Kützing (Rhodophyta) that was used for the *Porphyra umbilicalis* genome project (strain Pum1, Brawley et al. 2017). I grew individuals in a common garden from neutral spores before subsampling blades to examine potential regional specialization of the microbiome with amplicons of the V6 region of the 16S rDNA. Additionally, I tested whether several different stabilization and preparation techniques for the macroalgal microbiome are equally suitable. Techniques that phycologists have used in the past to prepare algal thalli for DNA extraction include freeze-drying followed by surfactant enzymatic washes (e.g., Burke et al. 2009), flash-freezing (Miranda et al. 2013), and freezing at -20 °C (Bondoso et al. 2017). However, phycologists often need to collect samples in locations that are not near a laboratory, in which case silica gel desiccation would be ideal if it does not distort recovery of the microbial community. Finally, I compared microbial community structure as determined by the mothur pipeline for Illumina sequences (Kozich et al. 2013) to that of the minimum entropy decomposition pipeline (MED, Eren et al. 2015). MED uses Shannon entropy to group sequences into amplicon sequence variants (ASVs) that can indicate ecologically distinct associations of host bacteria not revealed by percent identity-based classification of OTUs (Eren et al. 2015). The three null hypotheses addressed are: (1) there is no significant difference between the microbiomes of non-adjacent samples of blade margin, (2) there is no significant difference in recovery of the microbiome among several preparative techniques, and (3) there is no significant difference in microbial community structure between blade margins and holdfasts.

Methods

Common Garden Culture of Pum1

I tested different techniques for stabilization and recovery of the microbiome of *Porphyra umbilicalis* (Rhodophyta) with a common garden design. The isolate used (Pum1) was obtained from a plant growing at Schoodic Point, Maine (44°20’1.68” N; 68°3’29.14” W) on April 3, 2008, and clonal progeny belonging to the 15th generation were used for this common garden experiment. Pum1 had been treated with antibiotics (penicillin, streptomycin) prior to the 7th generation used by Miranda et al. (2013) for comparative studies of the microbiome of clonal lab plants and wild plants in a 454 pyrosequencing study. The isolation and purification techniques used to prepare strain Pum1 for the recent *P. umbilicalis* genome project eliminated virtually all eukaryotic contaminants (Brawley et al. 2017), and the first antibiotic treatments with penicillin/streptomycin reduced the bacterial richness and relative abundance on Pum1 compared to wild blades (e.g. a strong shift in the Bacteroidetes from Sphingobacteriia to

Flavobacteriia, Miranda et al. 2013). The reduced microbiome of Pum1 blades at the time of the present study still included several hundred OTUs, and made this technical comparative study more tractable.

Individuals were grown by I. Mendonça by standard techniques (Royer et al. 2018) from neutral spores to 5 cm in length in the same 5 L glass carboy (common garden design) with vigorous aeration at 12 °C, in 40 µmol m^2 s^{-1} [T8 fluorescent tubes], at 12:12 (L:D), in 3.5 L of West-McBride enriched (Andersen, 2005), sterile seawater that was changed weekly. Plants were randomly assigned between two transparent cylinders (cylinder I, n=3 blades; cylinder II, n=3 blades), with each cylinder placed in a different culture chamber (I36LLVLC8, Percival, Perry, IA). Individuals were grown out while maintaining their normal polarity by anchoring one of the small (1 cm^2) subtending blades that develop at the base of the holdfast and ~ 1 mm of the main holdfast edge with sterilized plastic aquarium clips (Seaweed Clips, Ocean Nutrition Americas, Newark, NJ). These anchored blades moved naturally in the culture medium from their tethered positions by creating water motion with bubbling from a filtered air supply, which simulated natural conditions (Royer et al. 2018).

After 3 weeks of additional growth, the large blades were rinsed with sterile seawater, spread on a sterile surface, and adjacent pieces (1.5 cm^2) of blade margin and subtending vegetative region were cut and processed using sterile techniques to produce 6 replicates of each preparative treatment (Fig. 3.1): sections ‘A’ and ‘C’ -- flash-frozen in liquid N_2, stored at -80 °C, and ground in liquid N_2 to a fine powder with a mortar and pestle; sections ‘B’ -- flash-frozen, lyophilized, and powdered (= Geno/Grinder 2000, SPEX SamplePrep, Metuchen, NJ; 2 min, 600 strokes/min, with zirconia beads); and sections ‘D’ -- dried with silica gel and powdered via the Geno/Grinder procedure. Additionally, the holdfast microbiome was assessed on 2 of the 3 plants/cylinder (sections ‘E’, n=4) by the same preparative treatment as sections ‘A.’ The remaining two holdfast samples were not included in the analysis due to low sequence coverage and their preparation by a different technique. Culture, excision of tissue, preservation, and DNA isolation were performed by I. Mendonça and S. Brawley.

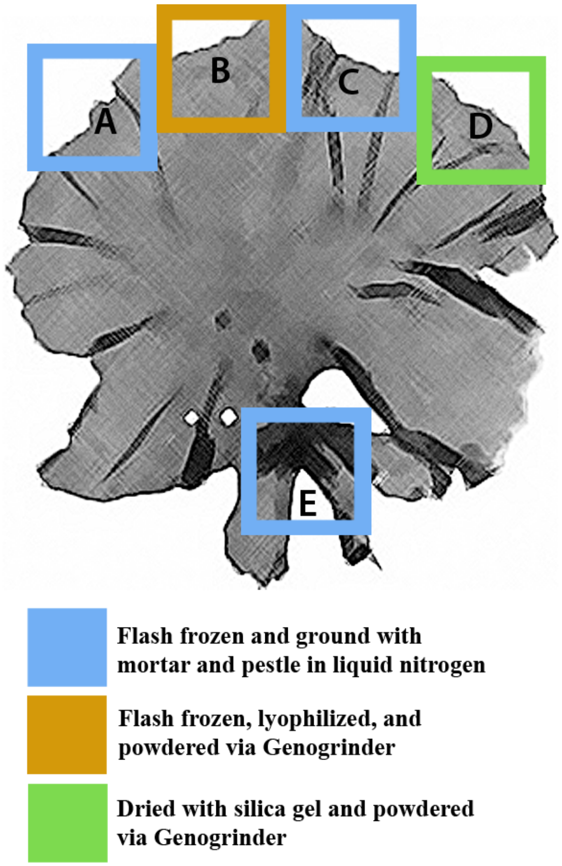


Figure 3.1. Experimental preservation techniques applied to replicates (n=6, Pum1 blades) at positions ‘A’ – ‘E’: sections ‘A’ and ‘C’ – flash-frozen in liquid N_2, stored at -80 °C, and ground with a mortar and pestle; sections ‘B’ – flash-frozen, lyophilized and powdered via Geno/Grinder; and sections ‘D’ – dried with silica gel and powdered via Geno/Grinder; ‘E’ holdfasts – flash frozen as for ‘A’ and ‘C’ (n=4).

DNA Extraction, Amplification, and PNA Clamps

Microbial sequences of the V6 hypervariable region of the 16S rDNA were amplified following physical disruption of samples by grinding them in liquid nitrogen to powder with a mortar and pestle or by bead-beating dry sample (de Bruin & Birnboim 2016). Following this physical disruption step, samples were extracted using the Qiagen DNeasy Plant MiniKit protocol (Germantown, MD). Two blanks (Qiagen columns and kit reagents) were processed through sequencing as controls. Based on the findings of Lundberg et al. (2013), I employed genus-specific peptide nucleic acid (PNA) clamps that were developed and tested by Dr. Hilary Morrison of the Marine Biological Laboratory where the sequencing for this work was conducted (Quigley et al. 2018). The PNA clamps avoided the complication of amplifying eukaryotic host plastid and mitochondrial 16S rDNA to recover only host-associated bacteria are the focus of my study. H. Morrison downloaded *Porphyra* spp. plastid and mitochondrial 16S genes from NCBI's GenBank reference database and aligned the 16S V6 primers to them. The EMBOSS command *splitter* (Rice et al. 2000) generated all possible 16-mer antisense sequences for the region between the primers. H. Morrison evaluated these sequences using the guidelines provided by PNA Bio (PNA Bio, Newbury Park, CT). Criteria for rejection were a purine stretch of 6 nt or longer, > 50 % purine content, > 35 % G content, and self-complementarity. PNA sequences were *Porphyra* mitochondrion, 5' CACTAAATGACATACA and *Porphyra* chloroplast, 5’ GTTCGCATTCCCTAAG (Quigley et al. 2018).

Sequencing and Demultiplexing

Bacterial V6 amplicon libraries were generated at Marine Biological Labs (Woods Hole, MA) by H. Morrison in a two-step protocol as described in Eren et al. (2013), with the addition of the PNA clamps described above. The PNA clamps against both plastid and mitochondrial 16S rDNA were combined, preheated at 65˚C for 5 min, and included in the PCR reaction at 2 µM with 0.2 µM V6-specific primers. The reaction mix included 200 µM each dNTPs, 2 mM $MgSO_4$, 0.66 units Platinum Hi-Fidelity Taq polymerase, and 1X Platinum Hi-Fidelity buffer (Quigley et al. 2018). The reactions were initially activated at 94˚C for 3 min followed by 25 cycles of 94˚C for 30 s, 78˚C for 10 s (PNA annealing step), 60˚C for 45 s, and 72˚C for 1 min, and given a final extension cycle at 72˚C for 1 min. Reactions were prepared in triplicate, plus a single no-template control. Triplicate reactions were pooled after cycling, the products visualized on the Caliper LabChip High Sensitivity assay (Perkin Elmer, Waltham MA), and purified with the Qiagen MinElute kit (Qiagen). Cleaned product (10-15 µL) was used in fusion primer PCR with the following components: 200 µM dNTPs, 2 mM $MgSO_4$, 1.3 units Hi-Fi polymerase, 1X Hi-Fi buffer, and 0.25 µM of each fusion primer, containing the Illumina sequences necessary to bind the products to the sequencing flow cell and to bind the sequencing primers (Quigley et al. 2018). These second-round amplifications were activated at 94˚C for 3 min followed by 10 cycles of 94˚C for 30 s, 60˚C for 45 s, and 72˚C for 1 min with a final extension at 72˚C for 2 min. The fusion products were visualized on a Bioanalyzer High Sensitivity DNA assay chip, purified with 1.8 volumes of Agencourt AMPure XP beads (Beckman Coulter, Brea, CA), and quantified with a Picogreen assay (Invitrogen, Carlsbad, CA). Products were pooled products in equimolar concentration, quantified (KAPA Biosystems, Wilmington, MA), and sequenced using a paired-end 2 X 101 nt protocol on an Illumina HiSeq 1000 run at the Marine Biological Laboratory (Woods Hole, MA; Quigley et al. 2018).

Individual amplicon libraries contained a unique combination of barcode (sequenced in read 1) and index (sequenced in a short indexing read). Datasets were demultiplexed by index using Illumina's CASAVA program v. 1.8 (Hosseini et al. 2010), and by barcode using custom python scripts. These curated reads served as input to microbial community structure analyses.

Bioinformatic Processing: mothur and MED

The preprocessed V6 16S rDNA sequences were analyzed using two software packages: mothur (Kozich et al. 2013) and Minimum Entropy Decomposition (MED, Eren et al. 2015). The former is a reference-based alignment and clustering process to analyze community sequence data, while the latter does not require preliminary classification and clustering.

For the analysis by mothur, I used a series of commands that closely followed the standard operating procedure (SOP; https://www.mothur.org/). Paired-end reads were merged to make contigs (command *make.contigs*); contigs longer than 175 bp or with any ambiguous bases were removed (*screen.seqs*), and replicate sequences were removed (*unique.seqs*). The remaining unique sequences were aligned (*align.seqs*) to a SILVA alignment database v.123 (Pruesse et al. 2007; https://www.arb-silva.de) that was previously trimmed to the 16S V6 hypervariable region (alignment positions 31188 to 33284). Sequences were removed (*screen.seqs*) if they did not align to the SILVA database, contained homopolymers longer than 8 bases, or were outside the V6 alignment. All alignment gaps were removed (*filter.seqs*). The following applications of *unique.seqs* and *pre.cluster* commands grouped sequences with any redundancy created by trimming sequence ends, or that had only one base difference between sequences. The UCHIME algorithm (Edgar et al. 2011) (*uchime.chimera*) split the data by sample to find and remove chimeric sequences (*remove.seqs*). Sequences were classified (*classify.seqs*) using the SILVA reference taxonomy v.128 and the Wang method (Wang et al. 2007) at a bootstrap cutoff of 80. Any sequences classified as chloroplast, mitochondria, Eukaryota, or Archaea, as well as any unclassified sequences (unknown) were removed (*remove.lineage*). Sequences were clustered using the average neighbor clustering technique (*cluster.split*) which bins the sequences by taxonomic level, in this case to genus, and then assigns an operational taxonomic unit (OTU) for each cluster; a cutoff level of 0.03 clustered sequences at a 97% similarity level, and *make.shared* generated a counts table of the number of sequences of each OTU present in each sample. One blade sample from plant 3 in chamber II was eliminated from further analysis, because only 512 sequences remained due to low initial sequence depth. The two Qiagen controls were also eliminated from further analysis due to uneven sequencing coverage (Control-1 = 712 remaining sequences; Control -2= 13,724 sequences) and small but detectable algal contamination in Control-2. Importantly, the OTUs determined in Control-1 were in very low abundance and were not found in any equal amount in the algal samples (i.e., contaminants were so rare that they failed to amplify and sequence at a detectable level in algal samples). The 2,396,770 sequences in the remaining 29 samples were used in further analysis.

For the MED analysis, paired-end reads were pre-processed, merged, and trimmed of primer/adapter sequences using Illumina-utilities (Eren et al. 2013; https://github.com/meren/illumina-utils). Merged reads were retained only if there were no mismatches between the two reads, resulting in very high-quality datasets. The Minimum Entropy Decomposition algorithm generates operational taxonomic unit (OTU) equivalents termed "amplicon sequence variants" (ASVs; Callahan et al. 2017) using Shannon entropy to identify information-rich positions that serve to partition a dataset. MED's ASV identification (decomposition) process resembles a bifurcating tree, rather than sequence clustering. The method is based on oligotyping (Eren et al. 2013) and is described by Eren et al. (2015).

The 29 samples analyzed by MED were identical to those I analyzed by mothur. Reads do not go through an alignment step. Rather, terminal gaps are added so that all reads have equal length (79 characters). Alignment is unnecessary, because Illumina reads are of a consistent length and post-trimming length variation is assumed to be biologically significant rather than a sequencing artifact. Sequence data were concatenated into a single fasta file. The second input file contained sample metadata.

The MED pipeline version 2.1 command "decompose" was run with default parameters except that the minimum substantive abundance (M) was set to 10. This is a filtering option that requires the frequency of the most abundant unique sequence in any ASV to be greater or equal to M (no such filtering is done by mothur). MED output includes an abundance matrix comparable to that generated by mothur.

Statistical Analysis

All downstream analyses for both the mothur and MED outputs were produced in R statistical software version 3.3.3 (The R Foundation for Statistical Computing, 2017). Distance matrices were produced with the *vegdist* function (*vegan* version 2.4-4, Oksanen et al. 2017) for both the Jaccard and Morisita-Horn indices. The Jaccard index is based on the number of “taxa” present in each sample, where all species are weighted and counted equally. The Morisita-Horn index is based on the relative abundance of “taxa”; thus, it is less influenced by sample size and species richness. Both indices are invaluable in determining how different regions of the thallus and sample preservation techniques affect recovery of a microbial “taxon” or its relative abundance in the microbial community. Statistical comparisons were performed with the *adonis* function, a nonparametric permutational multivariate analysis of variance. I blocked samples (*strata* function) to account for potential differences between the two environmental chambers. The samples were analyzed in four MANOVAS: mothur output applied to the Jaccard index, and to the Morisita-Horn index, and MED output applied to the same two ecological community indices. A Holm *p*-value correction minimized false discovery rates to 5% (Holm, 1979) for all multiple comparisons (*pairwise.adonis* function; Arbizu 2017). Mann-Whitney U tests (R v. 3.3.3) compared sequence percentages of specific taxa of interest between replicate samples of holdfast (‘E’) and blade margins (‘A’).

Core microbial communities were created using custom R scripts. Any OTU/ASV ≥ 0.1 % of the total sequences found in a group of treatment replicates (i.e., for ‘A’, ‘B’, ‘C’, ‘D’, and ‘E’; Appendix 3.1) was considered a core member of the mothur or MED community. Sequence comparison between ASVs and representative OTU sequences revealed matches within one bp. Only in one case did this difference of one base result in different taxonomic assignments (i.e., ASV0053 was classified as a Planctomycete, but the corresponding OTU0005 sequence, which differed by 1 bp, was classified as

"Unknown"). In some cases, despite exact sequence matches, the taxonomic assignments still differed between mothur's OTU assignment using Silva v.128 and the VSEARCH (Rognes et al. 2016) MED ASV assignment using Silva v.128. Because these mothur analyses pertained to clustered sequences that could differ by 3%, I used the taxonomy assigned to ASVs for these analyses, because each ASV was based on a single sequence. Sequences will be permanently archived in the public archive VAMPS (MBL, Woods Hole) and submitted to the SRA at NCBI.

Results

PNA Clamp Efficiency

Collaborator Hilary Morrison evaluated the efficiency of the PNA clamps for *Porphyra* spp. prior to this study by comparing the mitochondrial and plastid sequences in amplicon libraries produced from *Porphrya* spp. field samples (including epiphytes) generated with and without the PNA clamps (Quigley et al. 2018). No host mitochondrial amplification occurred, which is consistent with the imperfect match of the primers to V6 regions of the mitochondrial 16S rDNA. Without PNA clamps, however, up to 97 % of the tag sequences were identified as "Chloroplast" of which up to 95 % of the sequences were an exact match to the reference *P. umbilicalis* plastid 16S gene (Quigley et al.

2018). When PNA clamps were included, exact matches dropped to a maximum of 7 % (Table 3.1).

Table 3.1. Efficacy of peptide nucleic acid clamp design for the genus *Porphyra* based on amplicons generated from *Porphyra* spp. field samples. Amplicons were generated with and without PNA in the reaction mix (courtesy of Dr. Hilary Morrison).

Dataset	Total Tag Sequences	Exact Match to Plastid 16S	Identified as 'chloroplast'	Percent Host Plastid	Percent Any Plastid
P. linearis	558599	528770	534294	95%	96%
P. linearis +PNA	482817	33803	55011	7%	11%
P. umbilicalis	774292	353793	725683	46%	94%
P. umbilicalis + PNA	948857	6162	409103	1%	43%

The mothur analysis identified 662 OTUs that had ≥ 10 assigned reads over all 29 samples in the common garden experiment; the MED analysis identified 988 ASVs using the same data and threshold. The number of OTUs identified per pooled, replicate treatment sections are: 'A' – 342, 'B' – 178, 'C' – 261, 'D' – 215, and 'E – 272. The number of ASVs in 'A-E' are 608, 374, 556, 453, and 550, respectively.

Core Community Comparisons

The core-defined taxa represented a small number of total OTUs or ASVs, but accounted for most of the sequences recovered across sample types, typically 30-39 taxa. The core OTUs represented 96.90% to 98.72% of the sequences by sample type (i.e., A-E) in the final mothur analysis, and core ASVs represented 95.33% to 97.50% of the sequences per group in the final MED analysis (Appendix 3.1). The cores were dominated by Proteobacteria, especially by Alphaproteobacteria (e.g., *Sulfitobacter* sp. and other Rhodobacteraceae) and Gammaproteobacteria, but single representatives of Deltaproteobacteria and Betaproteobacteria were present (Fig. 3.2, Table 3.2). Several Bacteroidetes (e.g., *Fabibacter* sp. and *Dokdonia* sp.) were so abundant that this phylum accounted for almost one third of all sequences in any sample of the core (Table 3.2, Appendix 3.1). Actinobacteria and Planctomycetes were also present at lower levels in the core. All core members were present in each sample (e.g., a 'C' core member was present in every 'C' sample); differences between samples in the same group (i.e., 'A' – 'E') lie outside of that group's core community composition (< 0.1 % e.g., Fig. 3.3).

Table 3.2 Comparison of microbiomes of blade margin and holdfast regions of *Porphyra umbilicalis* (composite ASV analysis of core taxa, see Appendix 3.1).

Comparison of Blade and Holdfast Regions

Taxonomy	Number of ASVs		% Sequences		Classified Genera of Interest for Group
	Blade ‘A’	Holdfast ‘E’	Blade ‘A’	Holdfast ‘E’	
Actinobacteria	**3**	**1**	**4.2**	**2.5**	
Bacteroidetes	**4**	**4**	**33.1**	**32.3**	
Cytophagia	1	1	22.8	14.3	*Fabibacter*
Flavobacteriia	1	1	8.9	12.8	*Dokdonia*
Sphingobacteriia	1	1	0.6	3.9	(Saprospiraceae ASV0028)
Planctomycetes	**2**	**3**	**0.9**	**3.2**	
Planctomycetacia	1	1	0.3	1.2	*Blastopirellula*
Phycisphaerae	0	1	0	0.4	
OM190	1	1	0.6	1.6	
Proteobacteria	**22**	**20**	**56.7**	**57.9**	
Alphaproteobacteria	10	11	29.4	43	*Sulfitobacter, Sphingorhabdus, Hyphomonas*
Betaproteobacteria	1	1	8.7	2	*Methylotenera*
Deltaproteobacteria	1	1	7.6	5.1	
Gammaproteobacteria	10	7	10.9	7.8	*Pseudohongiella, Haliea*
Unknown	**1**	**2**	**0.4**	**1.6**	
Total (phylum level)	**32**	**30**	**95.3%**	**97.5%**	

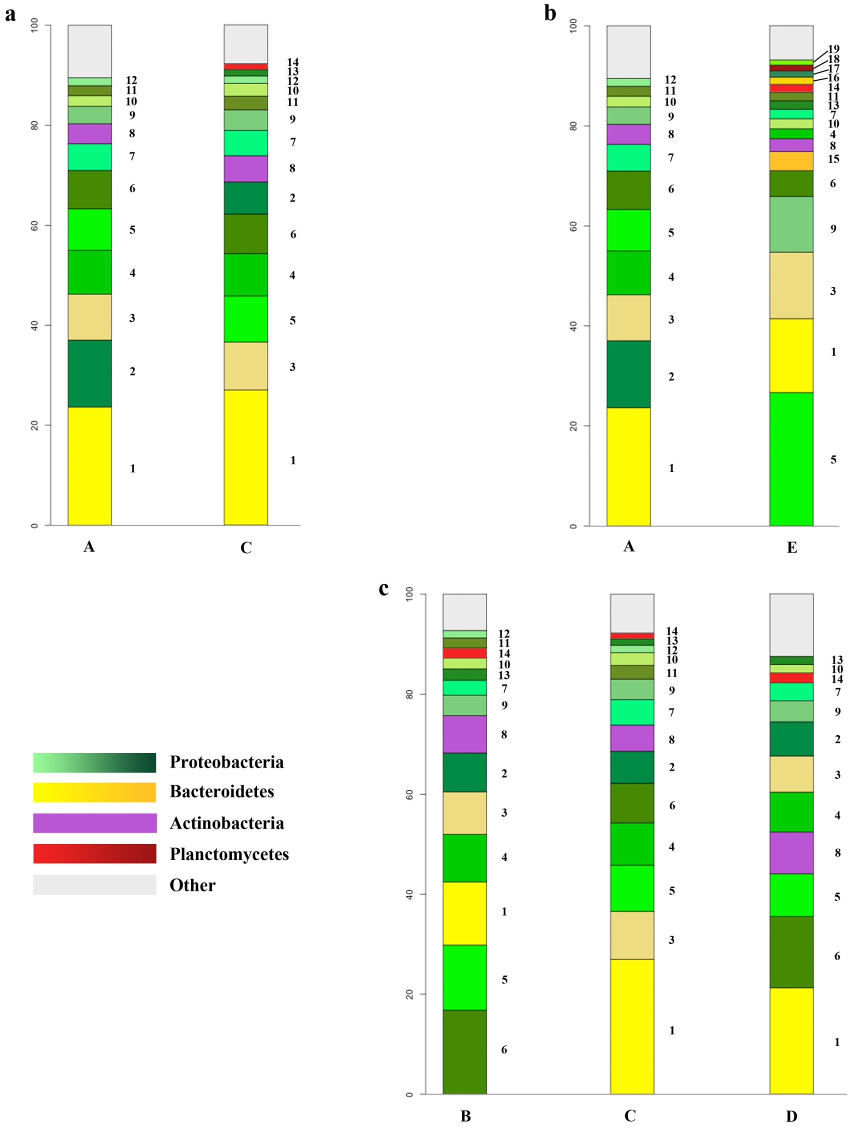


Figure 3.2. Relative abundance of taxa accounting for > 1% of sequences: (a) ‘A’ and ‘C’ samples to examine positional effect of blade margin; (b) ‘A’ and ‘E’ samples to examine regional effects of blade margin versus holdfast; and (c) ‘B,’ ‘C,’ and ‘D’ samples to examine preservation effects (n = 6 for all groups). The same pattern and order of taxa were found between relative abundance measures of MED and mothur analyses (< 1% difference), therefore only the mothur analysis is shown. Sequences differed by up to one bp, but most sequences matched exactly; thus, the taxonomy assigned by VSEARCH to

the MED output sequences using the Silva reference database (v128), was used: (1) ASV0040/OTU0879: Bacteroidetes; Cytophagia; Cytophagales; Flammeovirgaceae; *Fabibacter*, (2) ASV0012/OTU0508: Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae, (3) ASV0037/OTU0648: Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; *Dokdonia*, (4) ASV0353/OTU0435: Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae; *Methylotenera*, (5) ASV0023/OTU0683: Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; *Sulfitobacter,* (6) ASV0007/OTU0689: Proteobacteria; Deltaproteobacteria; Myxococcales; Nannocystaceae, (7) ASV0491/OTU0981: Proteobacteria; Gammaproteobacteria; Oceanospirillales; Oceanospirillaceae; *Pseudohongiella*, (8) ASV0019/OTU0254: Actinobacteria; Acidimicrobiia; Acidimicrobiales; MarineGroupSva0996, (9) ASV0016/OTU0509: Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae, (10) ASV0024/OTI0978: Proteobacteria; Gammaproteobacteria, (11) ASV0010/OTU0777: Proteobacteria; Gammaproteobacteria, (12) ASV0206/OTU0324: Proteobacteria; Alphaproteobacteria, (13) ASV0356/OTU0326: Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae, (14) ASV0053/OTU0005: Planctomycetes; OM190, (15) ASV0028/OTU0193: Bacteroidetes; Sphingobacteriia; Sphingobacteriales; Saprospiraceae, (16) ASV0492/OTU0119: Bacteroidetes; Bacteroidetes; Incertae Sedis; OrderIII; Unknown Family; *Balneola*, (17) ASV0203/OTU0217: Proteobacteria; Alphaproteobacteria, (18) ASV0003/OTU0137: Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; *Blastopirellula*, (19) ASV0771/OTU0980: Proteobacteria; Gammaproteobacteria; Clade KI89A.

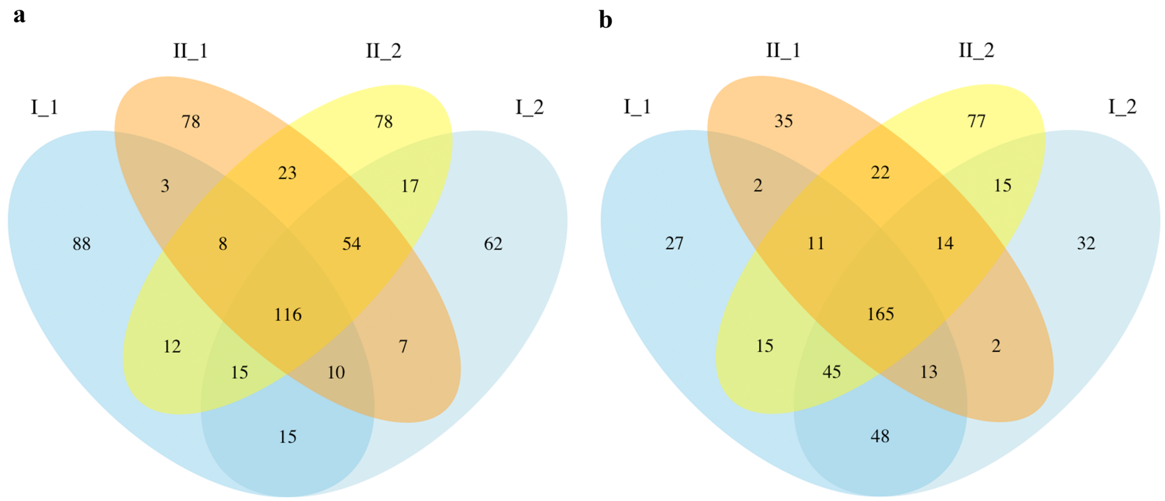


Figure 3.3. Venn diagrams of the number of shared ASVs of Individuals 1 and 2 in Chambers I and II (n=4) in (a) ‘A’ treatments on blade margins and in (b) ‘E’ treatments on holdfasts. All ‘A’ core members reside in the 116 central ASVs; All ‘E’ core members reside in the 165 central ASVs.

Microbial Communities between Blade Positions: A vs. C

I investigated how uniform the microbiome was along the blade margin by comparisons of ‘A’ and ‘C,’ which were located 1.5 cm apart on the blade margin and otherwise prepared identically. Regardless of analytical method or distance measure, all permutational analyses determined that there was no significant difference (mothur, Jaccard: F(1,10) = 0.94, p = 0.357; MED, Jaccard: (F(1,10) = 0.94, p = 0.343, mothur, Morisita-Horn: (F(1,10)=1.72, p = 0.182; MED, Morisita-Horn: (F(1,10)=1.76, p = 0.171, see Table 3.3) between samples excised from the different locations along the blade margins, leading to failure to reject the null hypothesis that they would be similar. The dominant taxon was classified as *Fabibacter* sp. (Cytophagales, Bacteroidetes; ASV0040/OTU0879), accounting for 22.84 % and 26.06 % of ASVs, and 23.65 % and 26.99 % of the OTUs, for ‘A’ and ‘C,’ respectively (Fig. 3.2a); *Dokdonia* sp. (Flavobacteriales, Bacteroidetes; ASV0037/OTU0648) represented 8.87 % and 9.26 % of ASVs, and 9.18 % and 9.59 % of OTUs assigned to ‘A’ and ‘C’ sequences, respectively. The ‘A’ position samples accounted for twice the abundance of an unclassified Rhodobacteraceae (Rhodobacterales, Alphaproteobacteria; 13.35 % (ASV) and 13.40 % (OTUs); ASV0012/OTU0508) compared to the ‘C’ position (only 6.39 % ASVs and 6.40 % OTUs, only 1.5 cm away); however, a planctomycete (ASV0053/OTU0005) was twice as abundant in ‘C’ compared to ‘A’ (1.13 % versus 0.61% in both ASVs and OTUs). Overall, 2-3 additional taxa were assigned to the ‘A’ core with a cut-off of 0.1% sequence abundance (see Appendix 3.1). These minor differences in taxa and similar relative abundances support my acceptance of the null hypothesis that position on the blade margin did not affect microbial composition within the sample size used (1.5 cm^2).

Table 3.3 Results of nonparametric permutational multivariate analyses of variance.

COMPARISON	ANALYSIS METHOD	DISTANCE MATRIX	F STATISTIC	P-VALUE	ADJUSTED P-VALUE
‘A’ and ‘C’	mothur	Jaccard	F(1,10)=0.94	0.357	-
(Position)	MED	Jaccard	(F(1,10)=0.94	0.343	-
	mothur	Morisita-Horn	(F(1,10)=1.72	0.182	-
	MED	Morisita-Horn	(F(1,10)=1.76	0.171	-
‘B’, ‘C’, and ‘D’	mothur	Jaccard	F(2,17)=2.89	0.012**	-
(Preservation)			Pairwise: B vs. C	-	0.012**
			Pairwise: C vs. D	-	0.012**
			Pairwise: B vs. D	-	0.196
	MED	Jaccard	F(2,17)=2.87	0.015**	-
			Pairwise: B vs. C	-	0.018**
			Pairwise: C vs. D	-	0.018**
			Pairwise: B vs. D	-	0.172
	mothur	Morisita-Horn	F(2,17)=5.94	0.001**	-
			Pairwise: B vs. C	-	0.006**
			Pairwise: C vs. D	-	0.044**
			Pairwise: B vs. D	-	0.325
	MED	Morisita-Horn	F(2,17)=5.62	0.002**	-
			Pairwise: B vs. C	-	0.003**
			Pairwise: C vs. D	-	0.034**
			Pairwise: B vs. D	-	0.331
‘A’ and “E’	mothur	Jaccard	F(1,7)=8.08	0.056*	-
(Region)	MED	Jaccard	F(1,7)=8.38	0.056*	-
	mothur	Morisita-Horn	F(1,7)=17.15	0.056*	-
	MED	Morisita-Horn	F(1,7)=17.59	0.056*	-

Microbial Communities among Stabilization Techniques: B vs. C vs. D

To understand the consequences of different preservation and processing techniques, I analyzed the effect of three stabilization techniques on recovery of the microbiome. The same statistical conclusions pertaining to type of preservation treatment were found with mothur and MED. The Jaccard distance measure detected significant differences among samples from the three preservation techniques (mothur: F(2,17)=2.89, p = 0.012; MED: F(2,17)=2.87, p = 0.015, Table 3.3), and Holm-corrected pairwise comparisons found that this stemmed from differences between hand-grinding flash-frozen samples under liquid nitrogen (= ‘C’ samples) compared to the other two methods (i.e., lyophilization and powdering with a Geno/Grinder after initial flash-freezing = ‘B’ samples; silica gel desiccation followed by powdering with a Geno/Grinder = 'D’ samples). There was no significant difference between ‘B’ and D’ treatments (Table 3.3), but because hand-grinding in liquid nitrogen (‘C’) recovered a significantly different microbial community (i.e., had fewer ASVs/OTUs) compared to ‘B’ and ‘D,’ the null hypothesis is rejected. The same statistical differences were found with the Morisita-Horn distance matrix (mothur: F(2,17)=5.94, p = 0.001; MED: F(2,17)=5.62, p = 0.002; see Table 3.3).

Despite ‘C’ communities being statistically different from ‘B’ and ‘D’ communities, no core members were uniquely recovered in 'C' (Fig. 3.2c). Many differences in core assemblies were due to the relative abundance of individual taxa differentially recovered. The same two prominent Bacteroidetes taxa (*Fabibacter* sp.; ASV0040/OTU0879 and *Dokdonia* sp.; ASV0037/OTU0648) accounted for 35.32 % (ASVs) and 36.59 % (OTUs) of the core sequences from samples that were hand-ground in liquid nitrogen (‘C’), compared to 20.53 % ASVs and 21.14 % OTUs from ‘B’ samples and 27.58 % ASVs and 28.45 % OTUs from 'D' samples (Appendix 3.1). Differences in overall taxon membership were evident in comparison of regions ‘B’, 'C', and ‘D’ among individuals (Fig. 3.4), despite the similarity of the core taxa, which constitute ≥ 96.31 % of ASVs and 97.60 % of OTUs. Taxa unique to ‘B’ cores included *Propionibacterium* sp. (Propionibacterales, Actinobacteria; ASV0054/OTU0387) and *Haemophilus* sp. (Pasteurellales, Proteobacteria; ASV1132/OTU0948). Nine taxa were unique to ‘D’ cores, versus ‘B’ and ‘C,’ many of which are Gammaproteobacteria (Enterobacteriales, Oceanospirillales, Alteromonadales, Pseudomonadales). Differences in relative abundance, and the fact that both ‘B’ and ‘D’ cores had unique taxa, while ‘C’ did not, supports my rejection of the null hypothesis that preservation techniques recover the same microbiome.

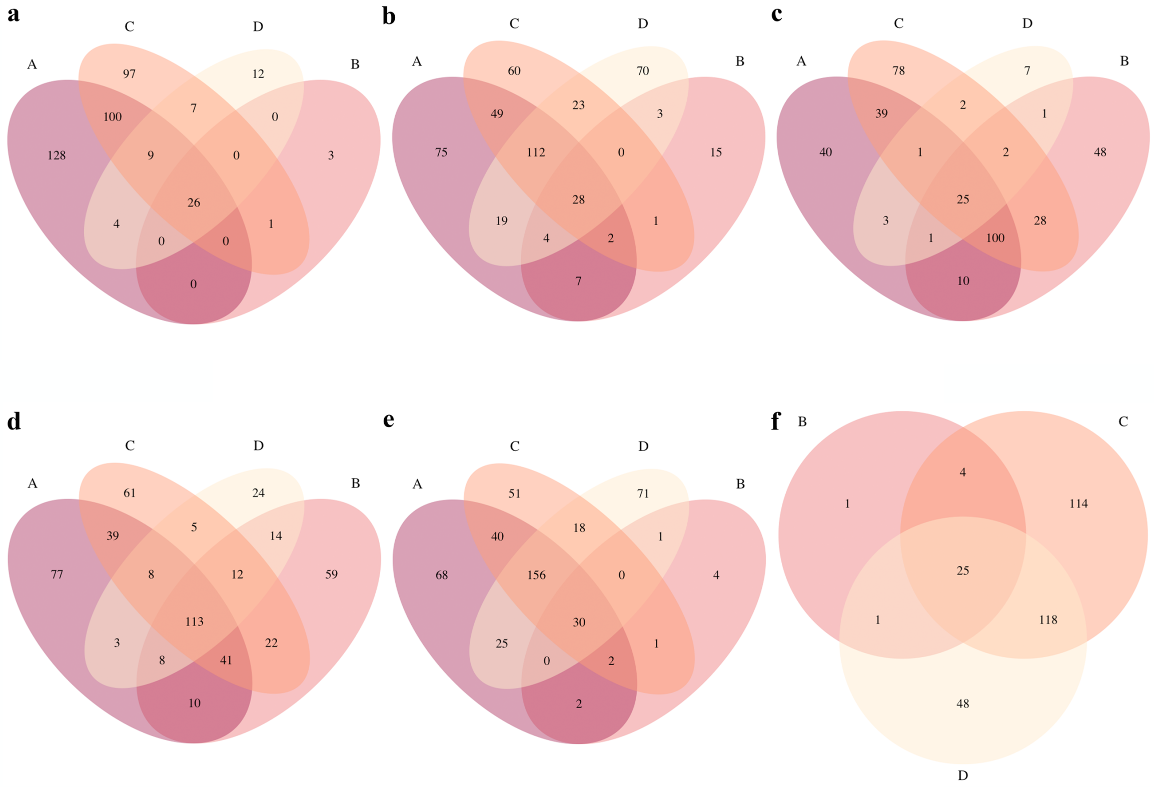


Figure 3.4. Venn diagrams of the number of shared ASVs of each treatment sample (‘A’ – ‘D’) on each individual: a) Individual 1 from Chamber I, b) Individual 2 from Chamber I, c) Individual 3 from Chamber I, d) Individual 1 from Chamber II, e) Individual 2 from Chamber II, and f) Individual 3 from Chamber II (excluding ‘A’ because it was not included in statistical analyses).

Microbial Communities between Regions: A vs. E

Because the *Porphyra umbilicalis* thallus contains functionally different regions, I analyzed the microbial communities associated with the blade margin versus the holdfast. All permutational analyses found a marginal statistical difference in the microbial community between algal blade margin ('A') and holdfast ('E') samples, irrespective of analysis pipeline or distance measure (mothur, Jaccard: F(1,7)=8.08, p = 0.056; MED, Jaccard: F(1,7)=8.38, p = 0.056; mothur, Morisita-Horn: F(1,7)=17.15, p = 0.056; MED, Morisita-Horn: F(1,7)=17.59, p = 0.056; see Table 3.3) This analysis used 575 permutations (n=4 replicates for each of 'A' and 'E'), as opposed to the default 999, because I restricted comparisons between 'A' and 'E' to the same preservation/stabilization technique (lower sample number). Some of the most abundant taxa in both 'A' and 'E' cores were still the Bacteroidetes *Fabibacter* (ASV0040/OTU0879) and *Dokdonia* spp. (ASV0037/OTU0648); *Fabibacter* sp. had about one third more total reads in blade margins than holdfasts, but replicates varied, thus the two regions did not have significantly different abundances of *Fabibacter* sp. (ASVs: Mann–Whitney U = 11, n1 = n2 = 4, p = 0.486, two-tailed). The alphaproteobacterium *Sulfitobacter* sp. (Rhodobacteraceae; ASV0023/OTU0683) was three times more abundant in holdfasts than blade margins (Fig. 3.2b; ASVs: Mann–Whitney U = 0, n1 = n2 = 4, p = 0.029, two-tailed), making it the most abundant taxon in the holdfast. As a group, Rhodobacteraceae were more abundant in holdfasts (40.01 % ASVs and 40.16 % OTUs) compared to blade margins (25.99 % ASVs and 26.07 %

OTUs; Appendix 3.1). Planctomycetes had greater taxonomic diversity and abundance in holdfasts compared to blade margins (ASVs: Mann–Whitney U = 18, n1 = n2 = 12, p = 0.002, two-tailed), while Gammaproteobacteria and Actinobacteria were more abundant and diverse in blades than holdfasts (Table 3.2). Lastly, the Alphaproteobacteria (Hyphomonadaceae) *Hyphomonas* sp. (ASV0237/OTU0092) and *Algimonas* sp. (ASV0359/OTU432) were present in blade margin core communities (> 0.1 % in 'A'), but were present in far lower relative abundances in holdfast samples (<< 0.1 % in 'E'; ASVs: Mann–Whitney U = 64, n1 = n2 = 8, p = 0.001, two-tailed). The overall relative abundance and number of ASVs/OTUs classified as either Bacteroidetes or Proteobacteria were nearly the same in holdfast versus blade margin, but at a subphylum level, there were important differences in distribution. These taxon comparisons and the marginal significant difference between overall 'A' versus 'E' microbial communities (Table 3.3), led us to reject the null hypothesis that microbiomes of blade margins and holdfasts are not different.

Discussion

Here, I and my collaborators (Quigley et al. 2018) developed and evaluated techniques for preservation and analysis of the *Porphyra umbilicalis* microbiome, aided by use of a single genotype (Pum1) to minimize host effects and to eliminate extraneous microbial variability that would complicate such studies if wild thalli were used.

Enrichment of some members of the microbiome was observed in samples of holdfast versus blade margin, and below I consider how this might affect *P. umbilicalis*.

Importance of PNA Clamps

The PNA clamps that were developed to block amplification of the host's plastid V6 16S rDNA were highly effective and can be applied to a variety of other microbial studies of *Porphyra* spp. *sensu lato.* Additionally, my techniques can be used to develop similar PNA clamps for other macroalgae. Surface treatments (enzymatic, mechanical) can remove the microbiome without disrupting the host thallus and avoid the organellar 16S rDNA problem, but this may miss important members of the microbiome that lie within macroalgae. The bacterial V6 recovery would have been too low to permit statistically valid comparisons of the effect of different preparative and analytical techniques on the microbiome without these PNA clamps (Quigley et al. 2018).

ASVs versus OTUs

ASVs and OTUs recovered similar microbiomes both in terms of percent of total sequences, and order of relative abundance within each core community. MED identified more distinct microbial taxa (988 ASVs versus 662 OTUs), but both analyses led to the same statistical conclusions for all three tested hypotheses, which is a robust result considering the differences in computational approaches. Mothur aligns sequences to a reference database (e.g., SILVA, Pruesse et al. 2007, used here), then pre-clusters all sequences that are within one nucleotide of each other, and clusters sequences that differ by less than a fixed sequence dissimilarity threshold (3% for this analysis and most others; Schloss et al. 2009, Westcott and Schloss, 2015). In contrast, MED does not align or pre-cluster sequences, but divides the sequences into groups of amplicon sequence variants based on the frequency of alternative bases at variable nucleotide positions, and continues to partition the ASVs further until all meaningful nucleotide variability is resolved (Eren et al. 2013, 2015). Taxonomy assigned through VSEARCH is more accurate, because it is based on a single MED sequence. Most comparisons between OTU and ASV construction in previous studies found that MED provides finer biological resolution. Often one OTU can be resolved into multiple ASVs, which better assesses ecological dynamics at a sequence variant level (Eren et al. 2013, Needham et al. 2017). In my analysis, core community members are equivalent, with sequences matching within one base pair. The average sequence length was 60 bp, meaning that sequences that differ by a single base would fall into the same mothur OTU, but not the same ASV.

Macroalgal Microbiome Distribution and Preservation

In evaluation of core microbiomes (≥ 0.1% of sequences within a sample group), I found that the core taxa were distributed evenly at the blade margin using subsamples of 1.5 cm^2. The type of powdering technique used during preparation of the samples for DNA analysis led to quantitative differences in taxon recovery, but silica gel desiccation was as effective as flash-freezing and lyophilization for microbiome recovery.

Examination of lab-grown *Porphyra umbilicalis* with scanning electron microscopy (Royer et al. 2018) showed that blade surfaces at a fine scale can be visually diverse (bacterial filaments, cocci, rods) and uneven. A certain level of patchiness is likely for any macroalgal surface, and it is important to evaluate what size of sample section recovers the community accurately in a cost-effective manner (e.g., Penton et al. 2016). Here, I determined that a single 1.5 cm^2 section adequately captured Pum1 microbial biodiversity (equivalent core taxa) for the blade margin, because 'A' and 'C' replicates prepared identically, but excised 1.5 cm apart, did not have significantly different bacterial communities.

This common garden study determined that the algal microbiome was recovered interchangeably by either flash-freezing/lyophilization/powdering ('B') or silica gel desiccation/powdering (‘D’). The third technique, flash freezing in liquid nitrogen/hand-grinding with a mortar and pestle (‘C’), recovers core taxa present in either 'B' or 'D' or in both 'B' and 'D' cores, and no taxa in the Qiagen column control were found in ‘C’ samples. Therefore, I rule out possible contamination for distinguishing ‘C,” yet I do not understand the basis for the difference in the two different powdering techniques. Previous studies have established that variation introduced by different preservation techniques, while detectable, usually does not outweigh differences found in the microbial communities of different species, or even individual samples (Hammer et al. 2015, Lauber et al. 2010, Song et al. 2016). This also applies to differences in DNA extraction techniques (Rubin et al. 2014), or sequencing platform (Tremblay et al. 2015), whereas use of primers that recover different hypervariable regions has greater effects (e.g., Tremblay et al. 2015, Clooney et al. 2016). Nonetheless, as microbiologists move towards identification of minor changes in host-microbial interactions, use of artificial mock communities that simulate the taxa on the host of interest may be the only safeguard for fine-scale understanding of the consequences of different preservation techniques. Without determining how to consistently and effectively capture those minor differences, studies will need larger sample sizes and possibly deeper sequencing to account for preservation discrepancies, wasting valuable time and money. Here, however, I found that most core OTUs or ASVs from Pum1 were universally recovered, whichever stabilization and preparation technique was used. It is particularly valuable to know that silica gel was as effective as flash-freezing/lyophilization, because phycologists often work in remote locations without access to lyophilizers, dry ice, or liquid nitrogen. Silica gel and flash-freezing/lyophilization techniques offer diverse ways to recover large quantities of the macroalgal microbiome in large-scale studies, with standardized and time-efficient processing techniques (i.e., mechanized bead-beating of dry material versus hand-grinding material under liquid N_2 using mortars and pestles). Researchers may now decide which recovery method is best for their studies of macroalgal microbiomes.

Pum1 Microbiome Composition and Region-Specific Functional Predictions

The relative rank positions in abundances of Bacteroidetes and Proteobacteria on Pum1 appear to have changed over 4 additional years in culture and passage through 8 additional generations of the life history. Bacteroidetes dominated the recovered microbiome (~80% V8 sequence abundance, 42% OTU diversity) compared to Proteobacteria (14 % V8 sequence abundance, 32 % OTU diversity) when Pum1 was harvested for pyrosequencing in 2011 (see Miranda et al. 2013, Table S1), but Proteobacteria were more abundant than Bacteroidetes in the present study (see Figure 3.2 and Table 3.2). Various technical and biological factors may affect these estimates. Technically, the present study used V6 tags and represents a deeper sequencing of the microbiome with Illumina HiSeq compared to the earlier pyrosequencing study that used V8 tags for quantitative comparisons. Biologically, as each new generation is established from parent neutral spores, some bacterial OTUs may be lost, causing rarer OTUs to become more prominent and detectable. Importantly, however, the Pum1 blade still grows normally to maturity. This provides opportunity to consider what important roles some taxa may play.

Many species in the Bacteroidetes (especially Flavobacteriia), Alphaproteobacteria, Gammaproteobacteria and Planctomycetes are able to derive carbon from the breakdown of macroalgal cell wall polysaccharides (e.g., Goecke et al. 2010, Barbeyron et al. 2016, Thomas et al. 2012, Kim et al. 2016). *Dokdonia* (Flavobacteriia) and *Blastopirellula* (Planctomycetes), found across all sample cores of Pum1, use macroalgal carbon (Thomas et al. 2012, Kim et al. 2016), and *Fabibacter* (1st, 2nd, or 3rd most abundant ASV/OTU in each of the 5 cores) might have such capabilities, because it is also found in coral mucus (Pereira de Castro et al. 2010). *Methylotenera* (Betaproteobacteria) is a methylotroph (Wang et al. 2014, Taubert et al. 2015) and consumes small organic molecules, while *Pseudohongiella* likely reduces nitrate (Xu et al. 2016); both are present across all cores.

Sulfitobacter (Rhodobacteraceae, Alphaproteobacteria) may be one of the most significant members of the microbiome of Pum1, and is in high relative abundance across cores (≥ 8.26 %), including > 26 % relative abundance in the holdfast core. Some *Sulfitobacter* synthesize vitamin B_{12} (Dogs et al. 2017), which might be used by *P. umbilicalis* in its natural habitat (Brawley et al. 2017). Four strains of *Sulfitobacter* increase the cell division rate of the diatom *Pseudo-nitzchia multiseries* PC9, and

Sulfitobacter SA11 was demonstrated to synthesize the plant growth regulator IAA (Amin et al. 2015). *Sulfitobacter* sp. MS3 is one of the Alphaproteobacteria (i.e., in addition to *Roseovarius* sp. MS2 and *Paracoccus* sp. UL2, E34) that stimulate cell division in multiple species of the green macroalga *Ulva* (Spoerner et al. 2012, Grueneberg et al. 2016, Ghaderiardakani et al. 2017). It is possible that in addition to a *Sulfitobacter*, other Alphaproteobacteria such as *Hyphomonas* (found in 'A,' 'B,' and 'C' cores), might supply Pum1 with important morphogens. Fukui et al. (2014) reported that *Hyphomonas* supports normal development from protoplasts of a relative of *Porphyra*, *Pyropia yezoensis* (nori). In the *Ulva* system, bacteria such as *Sulfitobacter*, which stimulate cell division, act in combination with many strains of *Maribacter* (Bacteroidetes; e.g., *Maribacter* sp. MS6) or *Microbacterium* sp. EC19 (Actinobacteria) to support normal morphogenesis in multiple species of *Ulva* (Ghaderiardakani et al. 2017, Weiss et al. 2017). Whether the few Bacteroidetes and Actinobacteria present in the Pum1 microbiome have symbiotic roles will be of interest. A lottery effect where different bacterial taxa can provide the same stimulatory or morphogenetic services to algae has been invoked to understand seasonal changes in the microbiome of wild populations that still maintain normal algal morphology (see Burke et al. 2011a, Ghaderiardakani et al. 2017). However, there is equally strong evidence that the associations between a bacterium and a macroalgal host can be acutely specific (see Amin et al. 2015, Weiss and Wichard 2017). Thus, potentially important morphogenetic bacteria in *Porphyra umbilicalis* (e.g., Pum1) must be isolated and tested.

Because morphogenetic bacteria cluster around the *Ulva* rhizoid (Spoerner et al. 2012, Ghaderiardakani et al. 2017), the microbial differences I observed in Pum1 between blade margin ('A') and holdfast ('E') are particularly relevant. *Sulfitobacter* sp. (ASV0023) was the most abundant bacterium recovered from the holdfast, where it was three times more abundant than at the blade margin. The association with the blade margin might lead to seeding of neutral spores with *Sulfitobacter* as they are released from parent blades. The relative abundance of Alphaproteobacteria taxa is about 50% higher in the holdfast cores compared to the blade margin cores; overall, Rhodobacteraceae were more abundant in the holdfast. Two Bacteroidetes (Sphingobacteriia, Saprospiraceae ASV0028/OTU0193; Flavobacteriia *Dokdonia* sp. ASV0037/OTU0648) are more abundant in the holdfast. The largest "drift" in particular taxa on Pum1 may be among the Planctomycetes, because *Blastopirellula* (ASV0003/OTU0137) was not recovered by Miranda et al. (2013), and *Rhodopirellula* and three planctomycetes that were newly recognized (see Kim et al. 2016 for their assembled genomes) were not recovered from Pum1 in the present study. I show here that Planctomycetes, even at very low relative abundances, are significantly more common in the holdfast compared to blade margins. The holdfast is a thick mass of extracellular polysaccharide that Planctomycetes can feed on (Kim et al. 2016). Knowing that the Planctomycetes have particular affinity for the holdfast may make it possible to isolate new taxa for the first time, and is an excellent example of how information on regional specificity of the microbiome may help to find needles in the haystack.

Conclusions

My colleagues and I (Quigley et al. 2018) discovered that microbial composition is evenly distributed across the examined portions of the blade margin at a scale of 1.5 cm^2, and that certain sample preservation techniques are interchangeable, which is of value to phycologists who work in remote field sites. This study is one of the first to examine community differences among thallus regions, specifically between blade margin and holdfast, finding that potentially important symbionts have higher affinities for specific regions. Finally, this research is a robust demonstration of the strengths of Minimum Entropy Decomposition (MED) to assess microbial community assembly.

CHAPTER 4

MICROBIAL CHARACTERIZATION OF INTERTIDAL MACROALGAL COMMUNITIES ACROSS A STRESS GRADIENT

Introduction

The steep environmental gradients of the intertidal zone have long attracted attention of biologists interested in the effects of stress on species distributions (e.g. Baker 1909, 1910, Stephenson & Stephenson 1949, Connell 1961, Schonbeck & Norton 1978, Little & Kitching, 1996, Valdivia et al. 2011). Invertebrates and macroalgae are well-studied; lower intertidal species are restricted from higher zones by their inability to handle stressful levels of temperature, desiccation, and light, especially during long aerial exposures at spring tides each month (e.g. Baker 1910, Schonbeck & Norton 1978, Harley & Helmuth, 2003, Williams & Dethier 2005). In New England, the stress gradient plays a major role in defining rockweed vertical distributions with *Fucus spiralis*, *F. vesiculosus*, and *F. distichus* inhabiting the upper, mid-, and lower zones, respectively. Stress often involves interactions among environmental factors; for example, on a sunny low tide, the rocky substratum and benthic organisms will heat and desiccate faster than on a cloudy day. Despite the extensive attention given for over a century to analysis of stress in macroscopic organisms in the intertidal zone, to my knowledge, whether marine intertidal bacteria follow the ecological paradigm developed to understand stress-related zonation of marine algae and invertebrates is unknown. The major objective of this research is to determine whether intertidal bacteria are different in the high, mid-, and low zones of the rocky intertidal zone. Here I examine this question in the context of macroalgal-associated bacteria to create a tractable subset of intertidal bacteria for my research by descriptive and experimental analysis of the microbiome of the three *Fucus* congeners *F. spiralis*, *F. vesiculosus*, and *F. distichus* subsp. *edentatus*.

Associated bacteria of intertidal algae, while poorly known, may be essential to their macroalgal hosts that provide important ecosystem services. Pioneering studies found that bacteria are required for normal development and morphogenesis (e.g. Fries 1970, Provasoli and Pintner 1980, Tatewaki et al. 1983). Green (*Ulva* spp.), brown (*Fucus* spp.), and red (*Pyropia* spp.) algae were unable to maintain their multicellular morphologies when made axenic, but, when re-inoculated with seawater containing marine bacteria, some recovery occurred (Fries 1975, Fries 1977, Provasoli & Pintner 1980, Yamazaki et al. 1998). Matsuo et al. (2005) discovered and described the structure of a signaling molecule from a member of the Bacteroidetes called “thallusin.” Thallusin appears to be solely responsible for the restoration of the natural foliaceous morphology of the green alga *Monostroma oxyspermum*, which becomes single-celled under axenic conditions. Many algal-associated bacteria have the metabolic pathways needed to degrade algal polysaccharides such as alginate, fucoidan, and cellulose (Armstrong et al. 2001, Kazamia et al. 2012, Labourel et al. 2014, Kim et al. 2016; reviewed by Goecke et al. 2010) and the carbon supply and niche supplied to bacteria may have led to mutualistic associations that led to the evolution of multicellularity (Miranda et al. 2013). Overall, algal survival and morphogenesis require a symbiosis between macroalgae and certain bacteria (Ghaderiardakani et al. 2017, Weiss et al. 2017), and these associations vary due to natural fluctuations such as salinity (Lachnit et al. 2009, Dogs et al. 2017) or seasonality (Michelou et al. 2013, Miranda et al. 2013). Given that some bacteria are required for morphological integrity and, therefore, the ecological function of the macroalgae, it is essential to understand how sensitive these associations may be to increased stress, and the intertidal zone offers an ideal location to examine this question.

Here, I used analyses of natural host microbiomes of differently zoned *Fucus* congeners and a transplant experiment to investigate the zonal distribution of bacterial ASVs (amplicon sequence variants). Restricting my analyses to these host microbiomes is a tractable approach to understand how tolerant or intolerant bacterial taxa are. Previous studies showed that closer-related macroalgal species inhabiting different geographic locations share more similar bacterial communities when compared to more distantly-related sympatric species (Lachnit et al. 2009, Barrot et al. 2011). Thus, the biochemical and cell wall compositions of an alga appear to affect the composition of its microbiome. The three *Fucus* species examined here can demonstrate how environmental stresses affect the composition of the associating microbial community across vertical scales, because differences in stress tolerance among intertidal *Fucus* species are well established (e.g. Baker 1909, 1910, Schonbeck and Norton 1978, 1980, Collén and

Davison 1999a,b), but the cell wall composition of all three congeners is alginate, fucans, and a small amount of cellulose (Kloareg and Quantrano 1988).

Bacterial communities might be expected to differ on a vertical scale, from high to low zones, because of the intrinsic degree of stress resistance of different taxa, and because relationships between the host and its microbiome may differ due to differential exposure to environmental stress. My goals were to survey the microbiome of three *Fucus* congeners to see if intertidal position affects bacterial community structure, as it does for macroalgae and invertebrates, and to test differences with a manipulative experiment where *F. vesiculosus* was transplanted from its native mid-zone to the high zone, under different treatments. These studies aimed to answer the following questions:

> (1) Do macroalgae have distinct microbiomes, that is, are bacterial communities of macroalgae different from those of the surrounding water column? (2) Do bacteria follow established stress-determined upper boundaries of distribution of macroalgae and invertebrates? (3) When exposed to increased stress, such as when *Fucus vesiculosus* is transplanted from mid- to high zone, do host-associated bacteria change? and (4) Do microbiomes of transplanted *F. vesiculosus* become more similar to microbiomes of the high zone fucoid *F. spiralis*, and (5) which algal-associated bacteria respond to increased stress?

Methods

Natural Survey and Manipulative Experiment

I explored the effect of intertidal position on the microbiome of three *Fucus* congeners (Phaeophyceae) using both surveys and manipulative experiments at Acadia National Park (permit #ACAD-2017-SCI-0006), Schoodic Point, Maine, during the summer of 2016. Using random numbers to select sampling locations, I sampled three individuals each from two 20 m transects (A and B; located 60 m apart) of *F. spiralis* in the high zone, *F. vesiculosus* in the mid-zone, and *F. distichus* subsp. *edentatus* in the low zone. Two rounds of collections were taken two weeks apart (7/7/16 and 7/20/16). The holdfast, a reproductive receptacle, and a vegetative blade tip were harvested from each individual. I collected a water sample directly above each transect before the tide receded from that area of the intertidal zone (n = 6, one per transect in each zone) during each collection to determine if macroalgal and water column microbiomes differ in composition.

I conducted an experimental transplant of *Fucus vesiculosus* individuals simultaneously with my studies of the natural community. This allowed us to consider potential shifts in the microbiome in response to intertidal position within a vertical stress gradient. For transect A, I selected at random 48 *F. vesiculosus* from the mid-zone, and randomly assigned them to one of three treatments. I back-transplanted 16 individuals within the native mid-zone in 4 clumps (n = 4 clumps/transect, control treatment; Fig. 4.1). Each individual in the clump was attached with a zip-tie to a small stainless-steel eyebolt anchor set into a plastic socket in a hole drilled into the rock substratum. Holdfasts of transplants rested on the substratum. The remaining 32 individuals were transplanted into the high zone. Individuals were clustered in a clump to create a canopy effect as a central individual surrounded by three other transplanted *F. vesiculosus*, near patches of high zone *F. spiralis*, without allowing the transplants to come into direct contact with any *F. spiralis*. Half of the interspersed transplanted clumps (transplants) were undisturbed following transplant for the two-week period (n = 4 clusters/transect, dry treatment) to expose them to high intertidal stress, while the other interspersed half of the clumps were watered with seawater during daytime low tides when native mid-zone *Fucus vesiculosus* individuals were underwater (n = 4 clumps/transect, sea-watered treatment). The exact same design was employed with 48 additional randomly collected *F. vesiculosus* on Transect B. Several blade tips were collected at the start (7/7/16), a midpoint (7/11/16), and end (7/20/16) of this manipulative experiment (collected from a different individual per cluster at each time point to prevent wounding responses/stress); the holdfast and several receptacles were also harvested at the end point from the central individual from the cluster. Every algal sample was harvested, washed with sterile seawater, placed in a sterile Falcon tube, placed on ice for transport, flash-frozen in liquid nitrogen, and stored at -80 °C until DNA extraction. Water samples were collected in 1 L sterile polypropylene bottles, transported on ice, and immediately pre-filtered through a

5.0 µm sterile filter, followed by a 0.2 µm sterile filter that retained the bacterial community of the water column. Filters were also flash-frozen, and stored in a -80 °C freezer until DNA extraction.

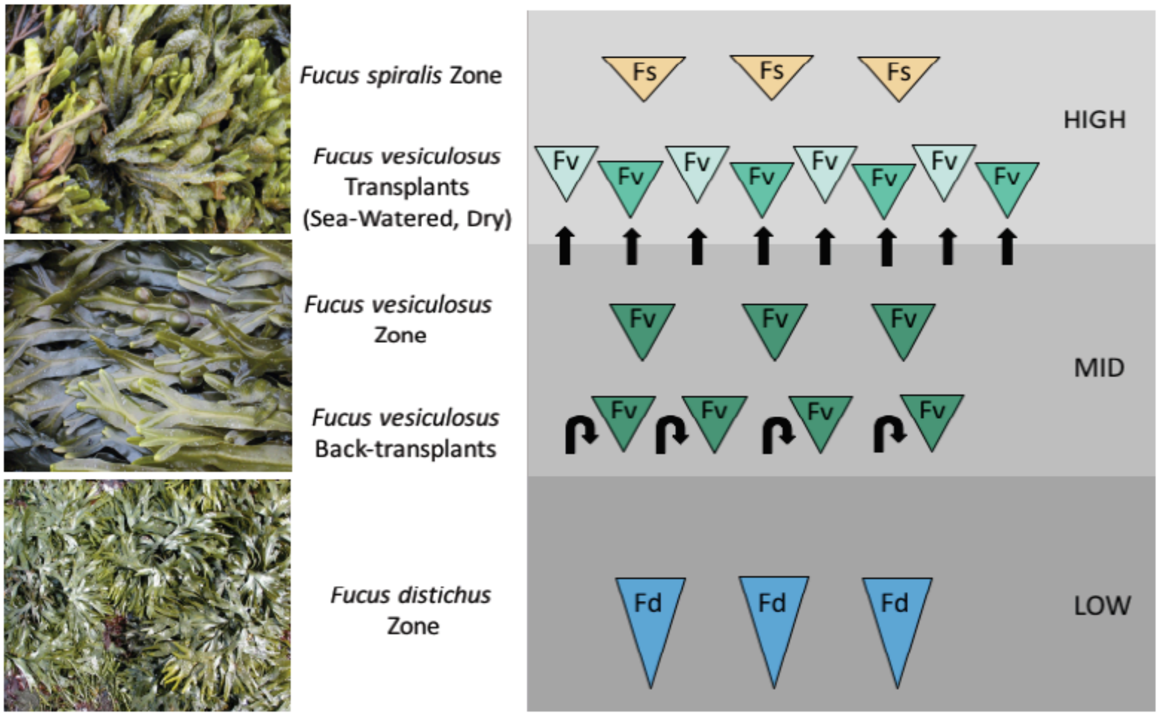


Figure 4.1. Photographs of the three *Fucus* spp. a) inhabiting the three intertidal zones; b) schematic of experimental design of one representative transect (n =2), Fs = *Fucus spiralis*, Fv = *F. vesiculosus*, Fd = *F. distichus*, and shading of green denotes treatment of *F. vesiculosus* (dark = back-transplant control, medium = sea-watered treatment of transplant to high zone, light = dry treatment of transplants to high zone). Diagram drawn for clarity of treatment; no pseudoreplication in positioning was present.

Environmental Data Collection

Thermal loggers (iButton, DS1921G-F5#, Maxim, San Jose, CA) were deployed from 6/24/16 - 7/15/16 with a sample rate of 15 min. Each iButton was wrapped in parafilm and encapsulated in a thin layer of Z-Spar (A-788 Splash Zone Epoxy, West Marine, Watsonville, CA) that attached each iButton to the rock substratum. In total, 8 sensors were deployed in pairs to capture the span of microhabitat temperatures within a zone: one covered by algal canopy (covered; C) and one uncovered (exposed; E), in the mid-zone (n = 2 pairs/transect) and high zone (n = 2 pairs/transect). My team also measured irradiance during low tide from 7/10/16 to 7/18/16 using a LI-COR spherical quantum sensor (SPQA3718, Lincoln, NE) read by a LI-COR Light Meter (LI-250, Lincoln, NE).

Analysis was carried out by Mr. Kyle Capistrant-Fossa (M.S. student, Brawley lab) in R-Studio (R 3.5.1) in the following ways: (1) a hierarchical cluster analysis was performed by computing a Euclidean dissimilarity matrix (pairwise difference) of Z-Score normalized iButton records, and then clustering records based on Ward's Sum of Square Errors method (package *pvclust*, Suzuki and Shimodaria 2006; function *ward.D2*, Murtagh & Legendre 2014); (2) time series were filtered to only contain temperatures of daytime exposures and descriptive statistics were computed using custom scripts; and (3) patterns of exposure were determined by linking iButton records relative to the NOAA buoy (#8413320, Bar Harbor, ME) tidal height using custom scripts.

DNA Extraction, Amplification, and Sequencing

Each sample was lyophilized, and pulverized using a Geno/Grinder (SPEXSamplePrep, Metuchen, NJ; 2 min, 600 strokes/min, with 2.4 mm zirconium beads). I extracted DNA using the Qiagen DNeasy Plant MiniKit protocol (Germantown, MD). The V4 hypervariable region of the 16S rDNA was amplified at the Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA by collaborator Dr. Hilary Morrison using genus-specific peptide nucleic acids (PNA) designed to block amplification of the host 18S and plastid 16S genes (Table 4.1). The final 100 μl reaction mix contained 1X Platinum HiFi Taq polymerase, 2 units of HiFi Taq (Life Technologies, Carlsbad, CA), 2 mM $MgSO_4$, 0.32 μM amplification primers, 0.2 mM dNTPs, and 1μM PNA mix. The amplification primers are Illumina fusion primers designed to bind directly to the MiSeq flow cell and universally amplify the bacterial V4 region of the small subunit rRNA gene: 515F (5' GTGYCAGCMGCCGCGGTAA 3') and 806RB (5' GGACTACNVGGGTWTCTAAT 3'). The PNA mixture was denatured for 5 min at 65 ^{o}C before it was added to the PCR master mix. Amplification cycling began with a 3 min initial denaturation at 94 ^{o}C, 30 cycles of denaturation at 94 ^{o}C (30 s), PNA annealing at 78 °C (10 s), fusion primer annealing at 50 °C (1 min), and elongation at 72 °C (1.5 min), ending with a final 10 min extension step.

Table 4.1. PNAs used to block amplification of host rRNA (designed by Dr. Hilary Morrison).

PNA ID	5' - 3' sequence
FucusCP1R	CTACAAACGCTTTACGCC
FucusCP1F	TACTGGGCTATTACTGAC
FucusCP2F	AGCTCAACTTCAAACATG
FucusCP2R	CGGTGGTCCTTCCAATCT
Fucus18sF	ATTCTTGGATTTATGGAA
Fucus18sR	GCCACAAATCCAACTACG

The amplification products were cleaned, quantified, and pooled as previously described prior to sequencing (Quigley et al., 2018). Each pool, containing up to 96 amplicon libraries, was sequenced on an Illumina MiSeq using the version 3 sequencing kit and protocol.

Bioinformatic Processing and Statistical Analysis

Paired-end reads were demultiplexed by index using on-instrument software and by barcode using a custom pipeline (Quigley et al., 2018). Paired-end reads were merged, trimmed of primer sequences, and quality filtered (Eren et al., 2013). The final datasets served as input to Minimum Entropy Decomposition analysis (Eren et al., 2015).The average number of high quality, merged V4 reads was 80,582; the smallest dataset had 2,849 reads and the largest > 700,000. The MED pipeline version 2.1 command "decompose" was run with default parameters except that the minimum substantive abundance (M) was set to 10 as described in Quigley et al. (2018). Dr. Morrison created a reference 16S V4 database from the SILVA reference taxonomy v.128 (Pruesse et al. 2007; https://www.arb-silva.de). The MED analysis identified 8,221 ASVs (amplicon sequence variants) that had ≥ 10 assigned reads across the 231 samples. Taxonomy was assigned to these ASVs using VSEARCH (Rognes et al. 2016) and my and collaborators' custom V4 database. Sequences will be deposited to the SRA at NCBI (GenBank).

All statistical analyses were performed in R statistical software version 3.3.3 (The R Foundation for Statistical Computing, 2017). The Morisita-Horn distance index was used to examine species diversity using measures of "taxon" relative abundance (*vegdist* function, *vegan* version 2.4-4, Oksanen et al. 2017; additional analyses exploring species richness, using the Jaccard distance index, Table 4.2). Nonparametric permutational multivariate analyses of variance (*adonis* function, *vegan*) were used to compare water column versus algal samples, species across intertidal zones (*F. spiralis*, *F, vesiculosus*, and *F. distichus*), tissue types (blades, receptacles, and holdfasts), and treatment effects in my transplant experiment (back-transplant control, sea-watered, or dry). I blocked samples to account for possible differences between transects A and B (*strata* function). I corrected for multiple comparisons (*pairwise.adonis* function, Arbizu 2017) using a Holm *p*-value correction, to minimize false discovery rates (Holm 1979). All figures were produced in *ggplot2* (Wickham, 2010). To assess differential abundance of ASVs between two given factors, I applied a Wald significance test (alpha = 0.05) to data matrices using the *DESeq* function (*DESeq2*, Love et al. 2018); an ASV had to account for at least 0.1% of the total reads in an individual sample to be included in analyses. These studies compared a total of 24 groups (grouped by species, treatment, time point, and/or tissue) in which ASVs were analyzed. Core abundance communities (CACs) for these groups were created using custom R scripts and are defined as all ASVs that account for 0.1 % of the total sequences found in any single group of treatment replicates.

Table 4.2. Statistical analyses using Jaccard distance matrix to determine differences in presence/absence among groups. Nonparametric permutational multivariate analyses of variance, blocking for transects.

COMPARISON	DISTANCE MATRIX	FACTOR	F STATISTIC	P-VALUE	ADJUSTED P-VALUE
Water Column vs. *Fucus* spp.	Jaccard	sample type	F(1,104)=9.8224	0.001*	-
3 *Fucus* spp. x 3 Tissues	Jaccard	day	F(1,88)=1.3420	0.118	-
		species	F(2,88)=9.9142	0.001*	
			Pairwise: Fs vs. Fv	-	0.003*
			Pairwise: Fv vs. Fd	-	0.003*
			Pairwise: Fs vs. Fd	-	0.003*
		tissue	F(2,88)=13.4596	0.001*	
			Pairwise: H vs. R	-	0.003*
			Pairwise: H vs. B	-	0.003*
			Pairwise: R vs. B	-	0.102
		species:tissue	F(4,88)=4.1310	0.001*	
Transplanted vegetative *F. vesiculosus* over time	Jaccard	day	F(2,1)=26.6384	0.001*	-
		treatment	F(2,1)=3.8693	0.038*	-
		trt effect: start	F(2,23)=1.3773	0.078	-
		trt effect: end	F(2,23)=1.7131	0.027*	-
			Pairwise: C vs. D	-	0.033*
			Pairwise: C vs. W	-	0.110
			Pairwise: W vs. D	-	0.659
Up-planted *F. vesiculosus* (dry) vs. *F. spiralis*	Jaccard	species	F(1,33)=3.9736	0.001*	-
		tissue	F(2,33)=3.9779	0.001*	-
			Pairwise: H vs. R	-	0.003*
			Pairwise: H vs. B	-	0.003*
			Pairwise: R vs. B	-	0.321
		species:tissue	F(2,33)=1.4710	0.040*	-

Reported p-values for all statistical comparisons: *significant, alpha value of 0.05.

Results

Environmental Intertidal Comparisons

Exposed iButtons reached temperatures that were up to 10 ºC higher than canopy-covered iButtons within both the mid-zone and the high zone (Table 4.3). Mid-zone maximum temperatures were 4 – 6 ºC lower than ibutton maximum temperatures in the high zone (i.e., comparing exposed to exposed and covered to covered). The length of exposure of iButton sites to air varied between the two high zone transects, because of topographic differences between transects A and B; however, mean daily exposure at low tide was 2 – 4 h longer in the high zone compared to the mid-zone (Table 4.3). Mean temperatures and ranges in temperature at iButton sites between the high and mid-zones offered less insight than total exposure periods and temperature maxima to explain any stress-related differences in microbiomes found associated with *Fucus* spp. The length of time that transplants were exposed to the air during daytime (sunrise to sunset) low tides was 9.3 ± 1.6 h at the beginning of the experiment (near the highest spring tide of the tidal cycle), but decreased to 6.7 ± 3.3 by the end of the temperature records. The timing of high tide shifted to cover the intertidal zone for a greater portion of each day, decreasing exposure time and, I hypothesize, stress towards the end of the 2 week experiment.

Table 4.3. Descriptive statistics of temperatures recorded by iButtons during daytime exposure to the air from 6/25/16 - 7/15/16 (courtesy of Kyle Capistrant-Fossa).

iButton	Daily Mean Temp (°C) (SD)	Daily Mean Range (°C) (SD)	Daily Mean Exposure (h) (SD)	Min (°C)	Max (°C)
A.High.C	18.51 (3.04)	10.18 (5.03)	12.03 (0.90)	11	36.5
B.High.C	15.09 (2.01)	5.20 (2.52)	8.84 (1.18)	10.5	32.5
B.High.E	23.68 (7.21)	19.03 (9.12)	8.84 (1.18)	10	42.5
A.Mid.C	17.66 (3.81)	8.55 (4.31)	6.34 (1.32)	11	30
A.Mid.E	20.91 (6.76)	13.95 (7.59)	5.75 (1.31)	10	40
B.Mid.C	14.82 (2.13)	4.10 (2.02)	6.33 (1.32)	10.5	28.5
B.Mid.E	22.32 (6.84)	13.24 (6.92)	6.33 (1.32)	10.5	37.5

Hierarchical clustering analyses included all time points, at low tide and high tide, day and night. Records clustered by position in the intertidal zone, with 100 % approximately unbiased (AU) p-values and 100 % bootstrap probabilities (Fig. 4.2), showing that zone was the main factor accounting for differences in temperature records. Additionally, records clustered between exposed and covered iButtons (highly supported; ≥ 98%, Fig. 4.2).

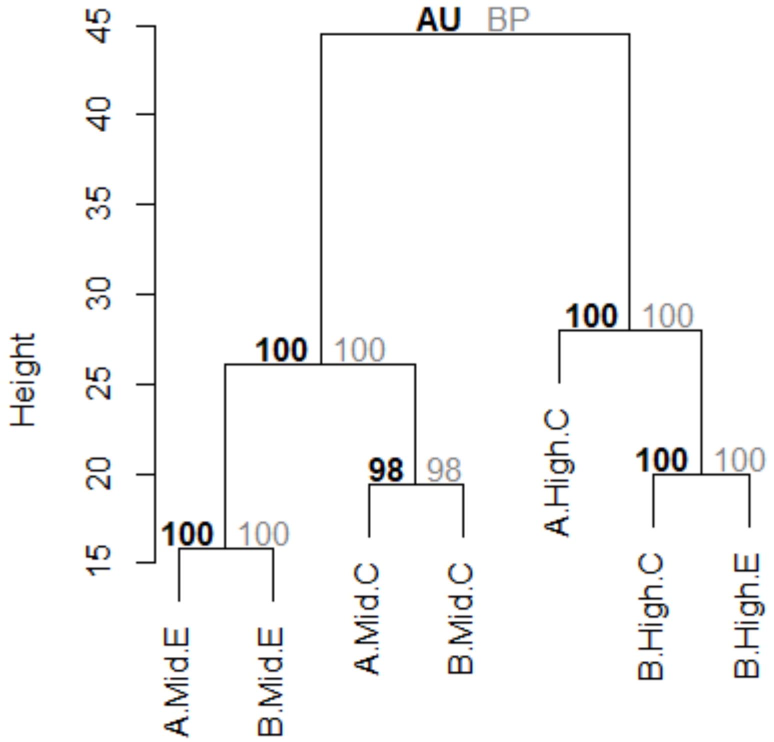


Figure 4.2. Neighbor-joining tree generated from a Euclidean dissimilarity matrix using iButton records, with each branch representing a single record and giving approximately unbiased (AU) p-values (bold) and bootstrap probabilities (BP, in grey; courtesy of Kyle Capistrant-Fossa).

Mean morning Li-Cor irradiance measurements were 1918 ± 811 µmol photos/m^2/s (n = 34); midday means measured 2350 ± 585 µmol photos/m^2/s (n = 32); and afternoon means were 1674 ± 678 µmol photos/m^2/s (n = 30). As expected, irradiance was greatest during midday, and most days throughout the experiment were sunny. Algae and associated microbiomes experienced daytime irradiances between 142 to 2815 µmol photos/m^2/s.

Water Column versus Host Microbiomes

To examine whether fucoid microbiomes were composed of bacteria that are unique from that of the surrounding water column, I compared all water column samples to that of all *Fucus* species collected. Permutational analyses found a significant difference between microbiomes of the water column versus those associated with the three *Fucus* congeners, regardless of intertidal level (sample type: $p = 0.001$, Table 4.4). Seawater microbiomes were composed predominantly of Proteobacteria, and Alphaproteobacteria and Gammaproteobacteria were most abundant. Significant numbers of Epsilonproteobacteria and Betaproteobacteria were also recovered (Fig. 4.3a). The microbiome of *Fucus* species samples was also mainly composed of Proteobacteria, but class-level microbial composition on host algae differed: Gammaproteobacteria dominated most tissues with far fewer Alphaproteobacteria, and a low abundance of Betaproteobacteria and Deltaproteobacteria. The classes Acidimicrobiia and Flavobacteriia (Bacteroidetes) were more abundant in the water column, whereas Planctomycetacia (Planctomycetes) was more abundant in fucoid communities (Fig. 4.3a). In response to my first question, macroalgae do have distinct microbiomes that differ from those of the surrounding water columns (Table 4.4).

Table 4.4. Nonparametric permutational multivariate analyses of variance using the Morisita-Horn distance index to assess microbial community diversity, blocking for transects.

COMPARISON	FACTOR	F STATISTIC	P-VALUE	ADJUSTED P-VALUE
Water Column vs. *Fucus* spp.	sample type	F(1,104)=17.2814	0.001*	
3 *Fucus* spp. x 3 Tissues	day	F(1,88)=0.559	0.712	
	species	F(2,88)=27.830	0.001*	
		Pairwise: Fs vs. Fv	-	0.012*
		Pairwise: Fv vs. Fd	-	0.003*
		Pairwise: Fs vs. Fd	-	0.003*
	tissue	F(2,88)=62.476	0.001*	
		Pairwise: H vs. R	-	0.003*
		Pairwise: H vs. B	-	0.003*
		Pairwise: R vs. B	-	0.121
	species:tissue	F(4,88)=12.152	0.001*	-
Transplanted blade *F. vesiculosus* over time	day	F(2,1)=1149.05	0.016*	-
	treatment	F(2,1)=537.52	0.029*	-
	exp. start: trt	F(2,23)=0.64569	0.6	-
	exp. end: trt	F(2,23)=2.3162	0.069**	-
	end	Pairwise: C vs. D	-	0.039*
		Pairwise: C vs. W	-	0.054**
		Pairwise: W vs. D	-	0.788
Up-planted *F. vesiculosus* (dry) vs. *F. spiralis*	species	F(1,33)=8.1328	0.001*	-
	tissue	F(2,33)=10.9750	0.001*	-
		Pairwise: H vs. R	-	0.003*
		Pairwise: H vs. B	-	0.003*
		Pairwise: R vs. B	-	0.255

Reported p-values for all statistical comparisons: *significant, alpha value of 0.05, ** marginally significant value of 0.10.

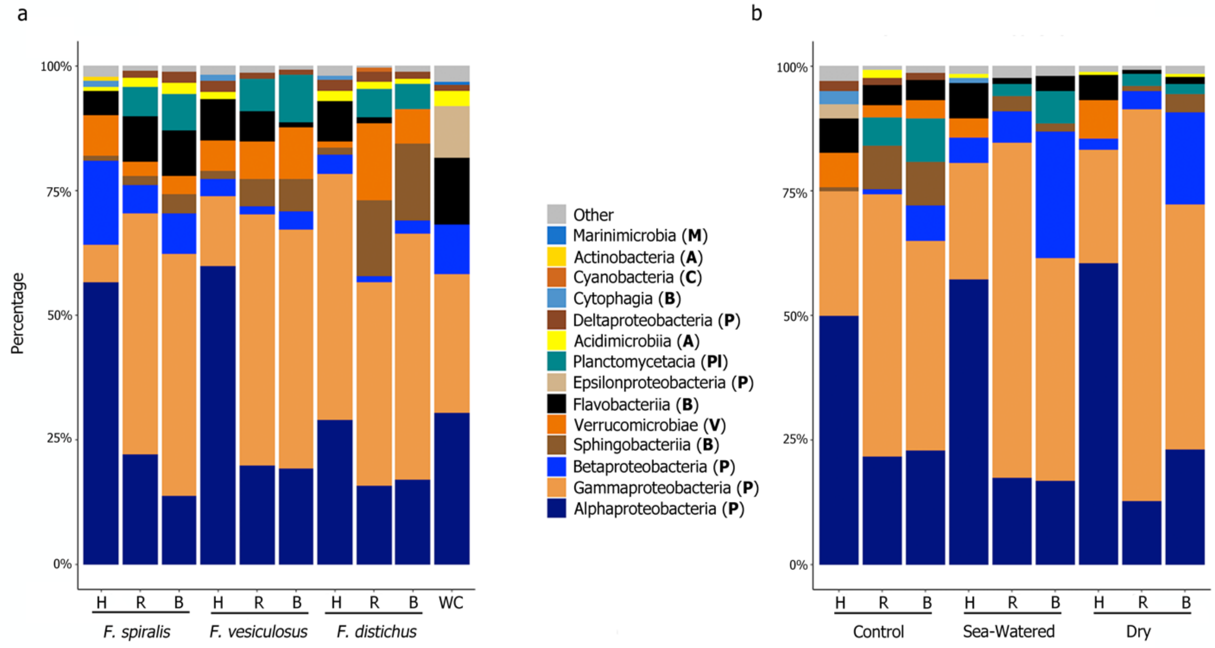


Figure 4.3. Class-level composition of (a) natural fucoid microbiomes plus water column samples, and (b) transplanted *F. vesiculosus* bacterial communities at the end of the 2 week experiment (H = holdfast, R = receptacle, B = blade tip). Phyla notations for each class: A = Actinobacteria, B = Bacteroidetes, C = Cyanobacteria, M = Marinimocrobia, P = Proteobacteria, Pl = Planctomycetes, V = Verrucomicrobia.

Natural Survey of Host Microbiomes of Fucus Congeners

The microbiomes of the three *Fucus* congeners were characterized to examine whether they were distinctive. Using permutational analyses, I determined that each *Fucus* species had a statistically distinct microbial community ($p \leq 0.012$, Table 4.4, Fig. 4.4a). Microbiomes also were significantly different between holdfast communities and other tissue types (Table 4.4, Fig. 4.4a), suggesting functionally specific algal-bacterial relationships. A significant interaction was found between species and tissue, meaning that how the holdfast communities differed from those of other tissue types varied among fucoid species (p = 0.001, Table 4.4). While all three tissues types of all three *Fucus* species (9 groups, Fig. 4.3a) were dominated by Proteobacteria, the class composition within Proteobacteria varied. Gammaproteobacteria dominated receptacle and blade communities of all host species. Alphaproteobacteria were the most common component of holdfast communities of *Fucus spiralis* and *F. vesiculosus*, whereas the low-zone *F. distichus* holdfast was composed mostly of Gammaproteobacteria. Betaproteobacteria were more abundant in *F. spiralis* communities, whereas Verrucomicrobiae and Sphingobacteriia were more abundant in mid- and low zone fucoids (Fig. 4.3a).

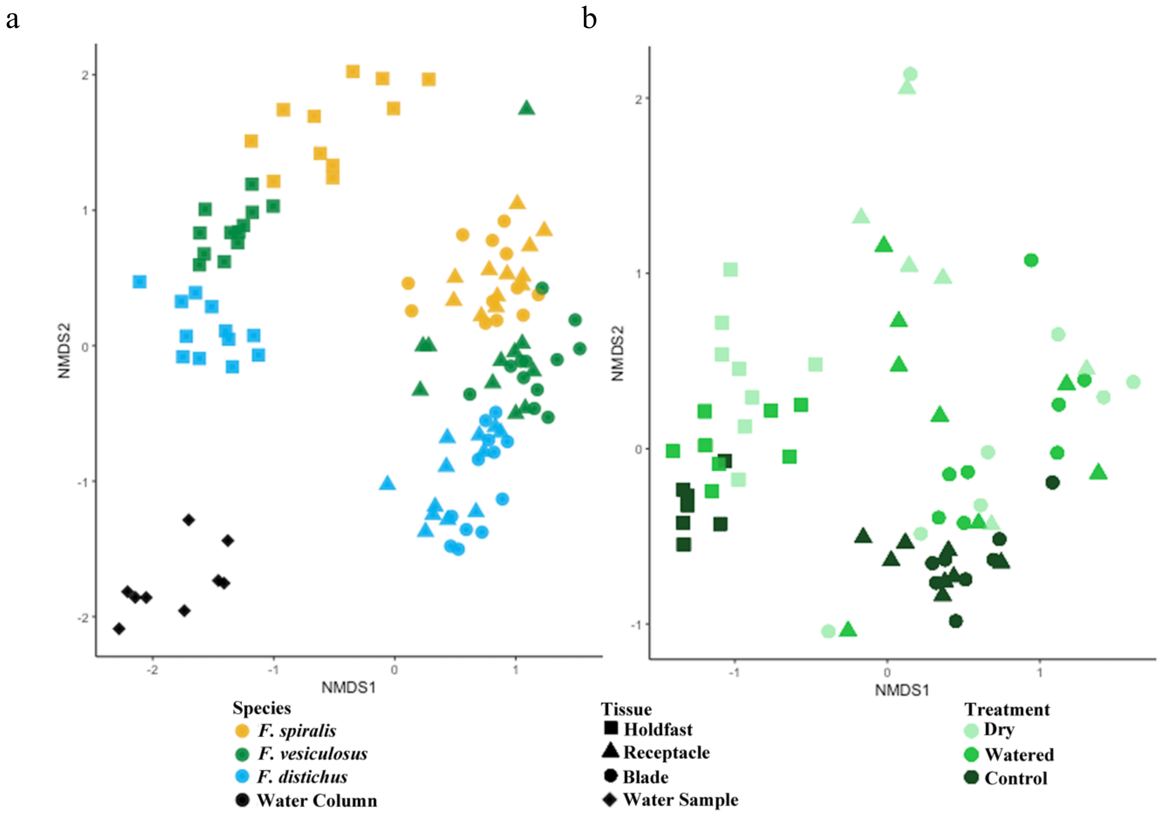


Figure 4.4. NMDS ordination plots of (a) natural *Fucus* species communities plus water column samples and (b) transplanted *F. vesiculosus* at the end of the 2 week experiment. Analyses include three tissue types (holdfast, receptacles, and blades).

Core abundance communities (CACs) are defined as all ASVs that account for ≥ 0.1 % of the relative abundance of each group (see Appendix 4.1). In the natural survey, nine groups were analyzed: the three tissue types [holdfast (H), receptacle (R), blade tip (B)] of the three *Fucus* species. For example, the CAC of *F. spiralis* holdfast holds each ASV present in ≥ 0.1 % total abundance. Of the 8,221 ASVs identified in the overall analysis, 351 ASVs composed the nine CACs: in the high zone, *F. spiralis* H, R, and B communities had 61, 77, and 86 ASVs respectively; *F. vesiculosus* in the mid-zone had

97, 89, and 58 ASVs; and in the low zone, *F. distichus* communities had 91, 104, and 76 ASVs. There are more ASVs in common between fucoids from adjacent intertidal zones (low-mid, mid-high) than from intertidal extremes (low-high). Only two ASVs were present in all nine natural CACs: a *Burkholderia-Paraburkholderia* (Betaproteobacteria; ASV02929) and a *Granulosicoccus* (Gammaproteobacteria; ASV03270). The former, ASV02929, was always more abundant in holdfast communities of the respective species, and was also more abundant in *F. spiralis* communities compared to communities lower down in the intertidal zone. The latter, ASV03270, was the most abundant ASV in all three species in receptacle communities (21.75 – 34.73 %), as well as in blade communities (23.32 – 38.15 %). However, it was much less abundant across holdfast communities (0.24 – 1.37 %). *Octadecabacter* ASV13813 (Alphaproteobacteria) was essentially universally present across natural CACs. Although it only accounted for 0.06 % of the relative abundance of *Fucus distichus* vegetative communities, and thus would not be included in that core community (< 0.1%), this was the only ASV to behave in this manner (present in 8 out of 9 cores; in fact, it is present in 23 of the 24 cores analyzed for this study), thus I made an exception to include this ASV. This *Octadecabacter* was more abundant in holdfast communities (2.10 – 14.77 %) but < 1% in receptacle and blade communities. When considering each tissue individually, there were many ASVs that are unique to each species; only 17, 22, and 17 ASV were found in all three *Fucus* species in holdfast, receptacle, and blade tip communities, respectively. Interestingly, there were three representatives of the Rhodospirillaceae, assigned to the AEGEAN-169 marine group (Alphaproteobacteria) following the same patterns of abundance across the thallus in each species (ASV04184 in *F. spiralis*, ASV00914 in *F. vesiculosus*, and ASV00916 in *F. distichus*). Each was in low abundance in holdfast communities, yet is either the second or third most abundant ASV in their respective species' receptacle and blade communities. These results address my second question: Bacteria do have distinctive compositions on closely-related congeners with similar cell wall composition, suggesting that there are zone-specific microbiomes that are responding to differential stress. Further, I found that there are distinct communities across different tissues of the thallus.

Effect of Transplant on Microbial Biodiversity

I transplanted *F. vesiculosus* from its native mid-zone to the high zone to determine whether the microbial community changes in response to new stress levels. A repeated-measure permutational MANOVA assessed treatment effect on the microbial community of blades over three time points. I determined that the treatments (i.e., back-transplanted controls, sea-watered transplants, and dry transplants) significantly affected the blade microbiomes over time (treatment: $p = 0.029$, Table 4.4). The bacterial diversity was the same across treatments on blades at the beginning of the experiment (treatment, $p = 0.60$, Table 4.4), as expected, because no time had elapsed. At the experiment's conclusion, there was a marginally statistical effect of treatment ($p = 0.069$, Table 4.4, Fig. 4.4b). Adjusted pairwise comparisons determined that this effect was due to significant differences in relative abundances between back-transplanted control communities and dry communities ($p = 0.039$, Table 4.4), and control and sea-watered communities (marginally significant p = 0.054, Table 4.4). Of note, back-transplanted control communities were statistically indistinguishable from *Fucus vesiculosus* microbiomes collected in the natural surveys (treatment: F(1,39) = 2.097, p = 0.120), supporting the biological basis for utilizing back-transplants as controls in the comparisons within the manipulative experimental treatments. At a class level, taxonomic composition differed among treatments by the end of the experiment. Alphaproteobacteria and Gammaproteobacteria were more abundant in higher stress treatments (controls < sea-watered < dry). Treatments with increased stress (dry and sea-watered) had reduced taxonomic diversity overall. While Deltaproteobacteria were higher in abundance in controls, they were very low in both the sea-watered and dry treatments across tissue types. This pattern held for Verrucomicrobiae in receptacle and blade communities. There were also noteworthy negative changes in the composition of Flavobacteriia in treatments with increased stress across tissue types (Fig. 4.3b).

CACs, all ASVs that account for ≥ 0.1 % of the relative abundance of a given group, of each *Fucus vesiculosus* tissue type (holdfast, receptacle, and blade) that underwent each treatment (back-transplant control, sea-watered, or dry; e.g. group = sea-watered *F. vesiculosus* receptacle community), contained a range of 40 to 126 ASVs at the conclusion of the experiment (Appendix 4.1). Only four ASVs were present in all nine experimental CACs, three of which were universally present in natural CACs: the *Burkholderia-Paraburkholderia* (ASV02929), the *Granulosicoccus* (ASV03270), and the *Octadecabacter* (ASV13813). The former, ASV02929, was more abundant in blade communities of the higher stress treatments (24.79 % and 18.13 % in the sea-watered and dry treatments, respectively; compared to controls, 6.94 %); it was the second most abundant ASV in blade communities across treatments (albeit at a far lower percentage in the control), whereas it was more abundant in holdfast communities in the natural collections. The *Granulosicoccus* (ASV03270) was the most abundant ASV in blade communities, regardless of treatment; however, in receptacle communities, relative abundance was lower in the dry, high stress treatments (3.50 %) compared to controls (29.91 %) and sea-watered (15.95 %) communities. The *Octadecabacter* (ASV13813), while present across treatments and tissues, was more prominent in holdfast communities. The fourth universal ASV, a member of the Rhodospirillaceae (AEGEAN-169 marine group; ASV00914) was also present in all nine experimental core communities. While always in low abundance in holdfast communities, it was prominent in receptacle and blade communities, with increasing relative abundance with increasing stress: 5.62 – 5.92 % in controls, 8.57 – 9.52 % in sea-watered, and 9.04 – 15.58 % in dry communities. This ASV was unique to *Fucus vesiculosus* in the natural core communities, yet members of the AEGEAN marine group were present in similar patterns in the other fucoids (as discussed above).

Certain ASVs that were unique to holdfast communities regardless of treatment, and that were in high abundance, belong to the genus *Octadecabacter*; both ASV00830 and ASV02241 were in the top three most abundant ASVs in every treatment's holdfast community. There were only three ASVs that were found across all three tissues, but only found in the higher stress communities (i.e., sea-watered and dry transplant treatments, but not back-transplanted controls): *Sulfitobacter* (Alphaproteobacteria; ASV11740), *Alteromonas* (Gammaproteobacteria; ASV08051), and *Psychromonas* (Gammaproteobacteria; ASV07729), which were consistently more abundant in dry versus sea-watered communities. Both *Alteromonas* (ASV08051) and *Psychromonas* (ASV07729), along with a *Pseudoalteromonas* (Gammaproteobacteria; ASV07519) were the only three ASVs that significantly change in abundance in dry blade communities from the beginning to the end of the experiment (Wald Test: log2-fold change ≥ 8.02, adjusted p-value ≤ 0.002). I can confirm that host-associated bacteria do respond to increased stress, because there are significant differences among manipulative transplant treatments.

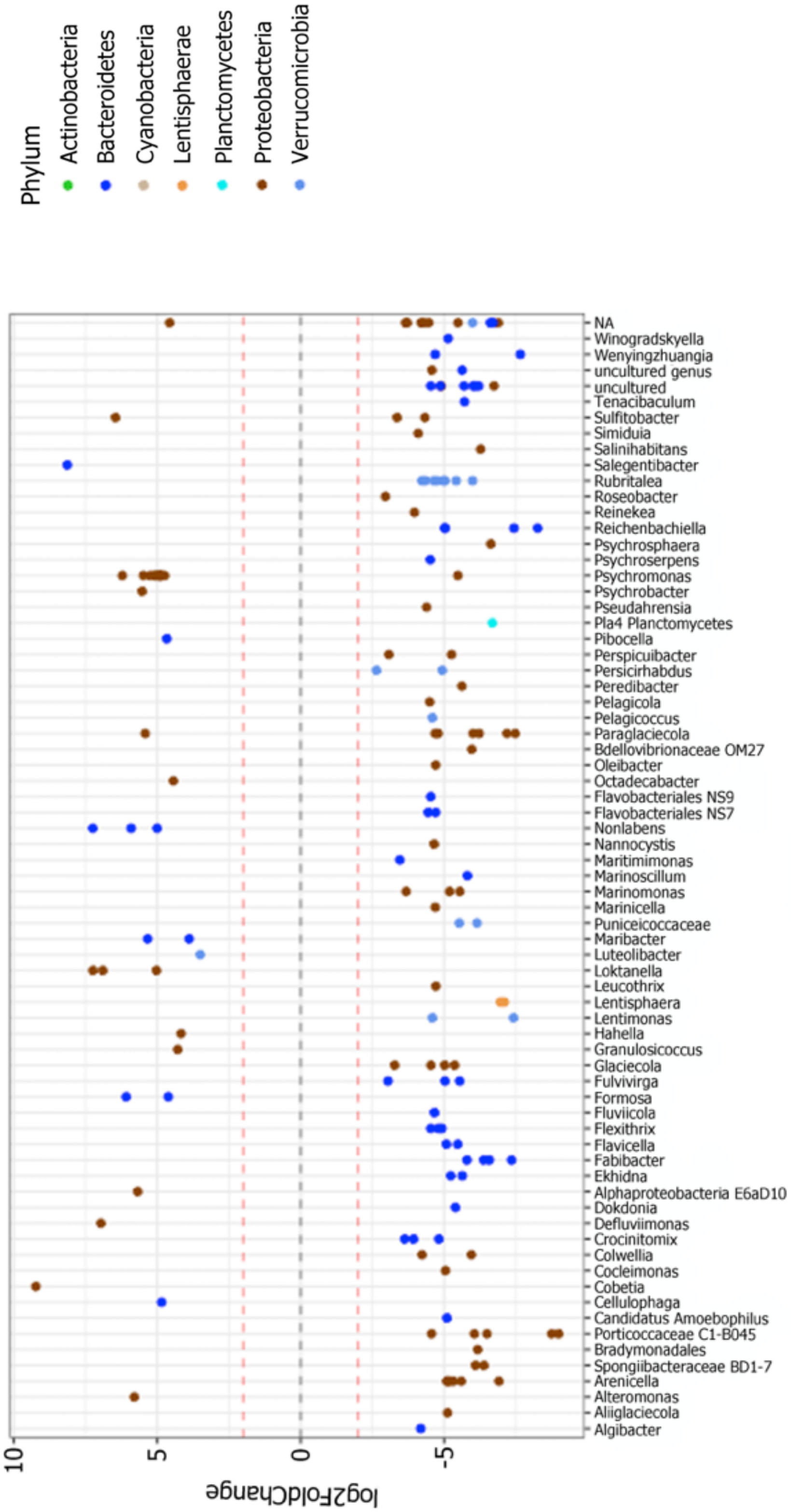
Stress-Responsive Taxa

In order to assess changes in relative abundance of bacteria due to differences in intertidal exposure period and zonal temperature maxima, I compared the microbiomes of natural *Fucus* spp. communities with *Fucus vesiculosus* transplant communities. Firstly, permutational analyses determined that microbial communities of native high zone *Fucus spiralis* remain statistically different microbial communities of *Fucus vesiculosus* transplanted into the high zones (dry treatment: $p = 0.001$; Table 4.5); microbiomes of transplanted *F. vesiculosus* did not change into those of natural *F. spiralis* in the high zone. While the microbiomes of these two species as a whole remained statistically distinct (over the 2 week experiment), there were many ASVs that were statistically significant between *Fucus vesiculosus* controls and dry transplants at the end of two weeks (alpha = 0.05: 157 in holdfasts, 114 in receptacles, and 9 in blades), demonstrating differences in relative abundance that are likely due to differences in the stress levels between high and mid-zones, notably elevated temperature and associated desiccation over a longer period of time in the high zone compared to the mid-zone (Fig. 4.5).

a


Phylum
Actinobacteria
Bacteroidetes
Cyanobacteria
Lentisphaerae
Planctomycetes
Proteobacteria
Verrucomicrobia
NA
Vibrio
uncultured genus
uncultured
Taibaiella
Acidimicrobiales Sva0996
Sulfitobacter
Simiduia
Salegentibacter
Rubritalea
Robiginitomaculum
Ralstonia
Psychromonas
Psychrobacter
Pseudomonas
Pseudoalteromonas
Portibacter
Pleurocapsa
Phyllobacterium
Paraglaciecola
Oligoflexaceae
Marinomonas
Maribius
Loktanella
Litorimonas
Lewinella
Leucothrix
Ilumatobacter
Granulosicoccus
Falsirhodobacter
Dinoroseobacter
Curvibacter
Cobetia
Burkholderia-Paraburkholderia
Myxococcales Blfdi19
Bifidobacterium
Arenicella
Aquimarina
Alteromonas
15
10
5
0
-5
log2FoldChange

b


Phylum
Actinobacteria
Bacteroidetes
Cyanobacteria
Lentisphaerae
Planctomycetes
Proteobacteria
Verrucomicrobia
NA
Winogradskyella
Wenyingzhuangia
uncultured genus
uncultured
Tenacibaculum
Sulfitobacter
Simiduia
Salinihabitans
Salegentibacter
Rubritalea
Roseobacter
Reinekea
Reichenbachiella
Psychrosphaera
Psychroserpens
Psychromonas
Psychrobacter
Pseudahrensia
Pla4 Planctomycetes
Pibocella
Perspicuibacter
Persicirhabdus
Peredibacter
Pelagicola
Pelagicoccus
Paraglaciecola
Bdellovibrionaceae OM27
Oleibacter
Octadecabacter
Flavobacteriales NS9
Flavobacteriales NS7
Nonlabens
Nannocystis
Maritimimonas
Marinoscillum
Marinomonas
Marinicella
Puniceicoccaceae
Maribacter
Luteolibacter
Loktanella
Leucothrix
Lentisphaera
Lentimonas
Hahella
Granulosicoccus
Glaciecola
Fulvivirga
Formosa
Fluviicola
Flexithrix
Flavicella
Fabibacter
Ekhidna
Alphaproteobacteria E6aD10
Dokdonia
Defluviimonas
Crocinitomix
Colwellia
Cocleimonas
Cobetia
Cellulophaga
Candidatus Amoebophilus
Porticoccaceae C1-B045
Bradymonadales
Spongiibacteraceae BD1-7
Arenicella
Alteromonas
Aliiglaciecola
Algibacter
10
5
0
-5
log2FoldChange

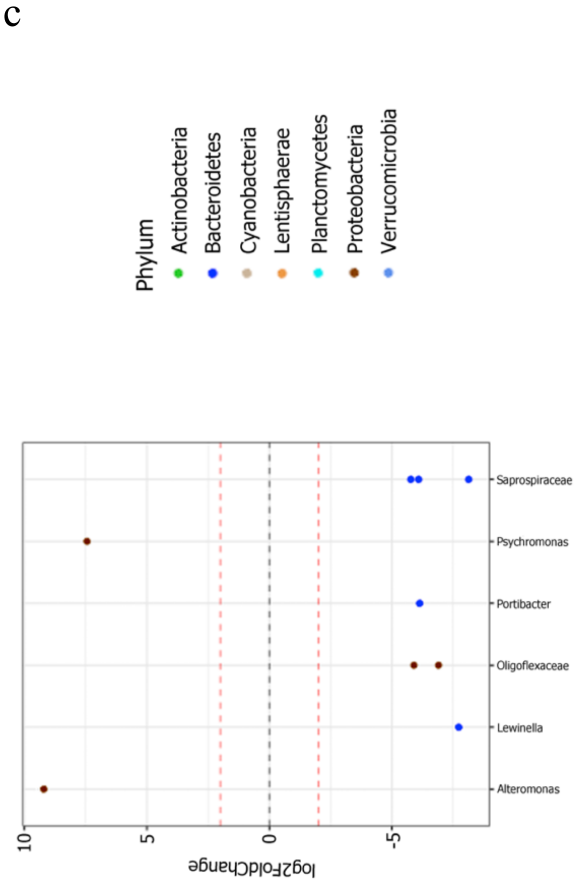


Figure 4.5. Differential abundance of ASVs of dry transplants, (a) receptacle, (b) holdfast, and (c) blade, in comparison the back-transplant controls after 2 weeks. The axis at zero denotes the back-transplant control and any ASV that is more or less abundant is plotted above or below the line, respectively, on a $\log_2$ fold change scale.

Taxa of interest that might be stress responsive are listed in Table 4.5. Many ASVs became more abundant in *Fucus* microbiomes subjected to higher levels of stress, including members of the genera *Cellulophaga*, *Loktanella*, *Maribacter*, *Octadecabacter,* *Cobetia* and *Psychrobacter.* As previously mentioned, *Alteromonas* (ASV08051), *Psychromonas* (ASV07729), and *Pseudoalteromonas* (ASV07519), were the only taxa to increase significantly in abundance over time in blade communities throughout the manipulative transplant experiment. However, they were not the only representatives of their genera to respond to temperature. Numerous additional members of these genera across tissues types were more abundant in dry versus control microbiomes (Table 4.5).

Table 4.5. Stress responsive taxa of interest that differed significantly between back-transplant controls and dry treatments (log2-fold change and adjusted p-value) with additional observations from natural surveys (Fs = *Fucus spiralis*, Fv = *F. vesiculosus*, Fd = *F. distichus*) and transplant experiment (Sea-W = sea-watered treatment, Ctrl = back-transplant control treatment), as well as noted functions of similar taxa found in macroalgal microbiome literature.

Genus	**Class**	**ASV**	**Tissue**	**Natural Survey Observations**	**Transplant Observations**	**Log 2-fold change**	**Adjusted p-value**	**Antibiotic/ Antimicrobial**	**Disease/ Pathogen**	**Algal Polysaccharide Digestion**	**Morphogenesis and Growth**	**Nutrient Provider**
STRESS-TOLERANT												
Cellulophaga sp.	Flavobacteriia	ASV07690	holdfast	in Fs only		= 4.85	= 0.030	**X**	**X**	**X**	**X**	
Loktanella spp.	Alphaproteobacteria	various, n = 3	holdfast		Dry > Sea-W > Ctrl	≥ 5.03	≤ 0.019			**X**		**X**
		ASV11673	receptacle		Dry > Sea-W > Ctrl	= 10.0	< 0.001					
Maribacter sp.	Flavobacteriia	ASV06794	holdfast		Dry > Sea-W > Ctrl	= 3.88	= 0.024			**X**	**X**	
Octadecabacter sp.	Alphaproteobacteria	ASV07405	holdfast	in Fs only		= 4.44	= 0.007			**X**		**X**
Alteromonas spp.	Gammaproteobacteria	ASV08051	blade		> over time in dry	= 9.21	< 0.001	**X**		**X**		
		various, n = 6	receptacle			≥ 5.86	≤ 0.015					
Psychromonas spp.	Gammaproteobacteria	ASV07729	blade		> over time in dry	= 7.44	= 0.003					
		various, n = 10	holdfast			≥ 4.74	≤ 0.011					
		various, n = 19	receptacle			≥ 5.39	≤ 0.010					
Pseudoalteromonas spp.	Gammaproteobacteria	ASV07519	blade		> over time in dry	-	-	**X**	**X**	**X**	**X**	
		various, n = 12	receptacle			≥ 5.67	≤ 0.046					
Cobetia sp.	Gammaproteobacteria	ASV04532	holdfast		Dry > Sea-W > Ctrl	= 9.23	< 0.001			**X**		
			receptacle		Dry > Sea-W > Ctrl	= 11.14	< 0.001					
Psychrobacter sp.	Gammaproteobacteria	ASV03985	holdfast			= 5.52	= 0.024				**X**	
			receptacle			= 10.38	< 0.001					
VARIABLE STRESS-RESPONSE												
Marinomonas spp.	Gammaproteobacteria	various, n = 3	holdfast			≤ -3.67	≤ 0.029			**X**	**X**	
		various, n = 3	receptacle			≥ 5.98	≤ 0.025					
Sulfitobacter spp.	Alphaproteobacteria	ASV07058, ASV07185	holdfast			≤ -3.36	≤ 0.023	**X**		**X**	**X**	**X**
		ASV11665	holdfast			= 6.45	= 0.002					
			receptacle			= 5.96	= 0.034					
STRESS-INTOLERANT												
Roseobacter sp.	Alphaproteobacteria	ASV11717	holdfast	Fd > Fv > Fs		= -2.95	= 0.008	**X**	**X**	**X**		
Lewinella sp.	Sphingobacteriia	various, n = 3	receptacle	Fd > Fv > Fs		≤ -4.91	≤ 0.019		**X**			
		ASV08338	blade	Fd > Fv > Fs		= -7.73	= 0.001					
Glaciecola spp.	Gammaproteobacteria	various, n = 4	holdfast			≤ -3.28	≤ 0.022	**X**		**X**		

Certain genera had multiple ASVs that appeared to exhibit opposite responses to stress. An ASV assigned to *Marinomonas* declined in *F. vesiculosus* holdfasts that were moved as transplants to the high zone, yet increased in receptacles, showing a possible shift in bacterial taxa across the thallus in response to increased exposure. Another group of interest that appeared to vary in response to stress, even within a tissue type, was the genus *Sulfitobacter*. Certain ASVs were significantly less abundant in dry holdfast communities compared to controls, yet another *Sulfitobacter* was more abundant in holdfasts, and was also more abundant in receptacle communities compared to controls (Table 4.5). Examples of a negative response to exposure can be found in ASVs assigned to the following genera: *Roseobacter*, *Lewinella*, and *Glaciecola* (Table 4.5). These numerous examples based on relative abundance differences across high, mid-, and low zones and experimental treatments reveal algal-associated taxa that may be stress-tolerant or stress-intolerant to increases in absolute levels of stress or longer periods of exposure.

Discussion

Three *Fucus* congeners that occupy distinct zones in the northeastern Atlantic's rocky intertidal zone were found to have distinctive microbiomes. This suggests that intertidal marine bacteria may have different stress tolerances, as do their hosts. In further support of the possibility of zone-specific bacteria, microbial composition changed when mid-zone *Fucus vesiculosus* was transplanted to the more physiologically stressful high-zone while microbiomes of back-transplanted controls were not significantly different from microbiomes of unmanipulated *F. vesiculosus*. Using V4 16S rDNA sequences, I described fucoid microbiomes to the level of each individual amplicon sequence variant (ASV). By comparing the relative abundance of certain ASVs under different stress levels, whether across unmanipulated fucoid congeners or in transplant experiments, I found some taxa that are potentially stress responsive. Such responses by these bacteria may have consequences for the algal host. Strong regional differences in microbial composition across the fucoid thallus were found, with holdfast communities being particularly divergent from those of blades and receptacles.

Natural Surveys

Bacterioplankton water-column communities taken from directly above the *Fucus* beds had a different community composition than that of the *Fucus*-associated bacteria; this differentiation in macroalgal microbiomes from the bacteria in the surrounding seawater is consistent with earlier studies (e.g. Lachnit et al. 2009, Michelou et al. 2013, Grueneberg et al. 2016). Each *Fucus* species had a significantly different bacterial community, despite the similar cell wall composition of these congeneric taxa, which supports my hypothesis that macroalgal microbiomes might fit the paradigm that applies to distinctive abundance across the vertical stress gradient of most eukaryotic intertidal species. The upper boundary for most intertidal organisms is driven by abiotic factors (e.g. high and low temperatures, high irradiance, nutrient availability as a function of exposure periods), while lower limits are driven by biotic factors (e.g. herbivory, predation, competition; e.g. Connell 1961, Schonbeck and Norton, 1978, 1980, Lubchenco 1980, Jenkins et al. 2008). These factors can often interact and have additive effects (Williams et al. 2013, Jenkins et al. 2008). While factors that cause stress in the intertidal zone are numerous, sub-optimal temperature, desiccation, and exposure time are found to have strong effects on macroalgal health and growth (Madsen and Maberly 1990, Williams and Dethier 2005, Migné et al. 2015). In *Fucus gardneri*, net photosynthesis increased with seawater temperature to a critical point (Colvard et al. 2014); however, photosynthetic rates decreased in *F. gardneri* during prolonged exposure to the air, as desiccation increased (Williams and Dethier 2005). Upper shore species have the capability to have high rates of photosynthesis in air, using CO_2 as an inorganic carbon source, during the short time before desiccation becomes photosynthetically limiting (Madsen and Maberly, 1990Surif and Raven 1990). However, carbon flux of an individual alga suggests that photosynthetic performance was always higher underwater (utilizing bicarbonate) for macroalgal species inhabiting all levels of the intertidal zone (Migné et al. 2015). Thus, major differences in net photosynthesis, growth, and survival are not attributed to temperature or thallus desiccation, so much as length of time the alga is exposed to air during the day (Wright et al. 2004, Williams and Dethier 2005, Dethier and Williams 2009). Intertidal zones in different locations differ in the length of exposure that intertidal organisms experience (Helmuth et al. 2002, Dethier and Williams 2009), including in this study, by as much as 2 – 4 h longer in the high zone compared to the mid-zone during the daytime low tide.

Bacterial homeostasis and membrane integrity are determined by solute concentration and the osmotic pressure of the surrounding environment (White 2000). It is possible that different bacteria have different osmotic tolerances, stratifying their distribution in an intertidal zone based on exposure time. Sodium translocation is used by some marine bacteria, sodium ion efflux can increase electrochemical gradients in certain bacteria (Dimroth 1990); this has been found in marine Flavobacteriia (Inoue et al. 2013). Additionally, chloride ion pumps, once thought to be exclusively found in haloarchaea, have recently been identified in marine bacteria; Nakajima et al. (2018) suggested that these bacteria acquired the necessary genes through horizontal gene transfer from haloarchaea. More immersion in lower areas of the intertidal zone may be important to certain ASVs.

Various physiological stresses are often caused by oxidative stress, leading to the build-up of reactive oxygen species (ROS; Davison and Pearson 1996), which can result in damage to both the host and its microbiome. ROS formation follows the vertical scale of the intertidal zone, where lower intertidal *Fucus* species had the lowest ROS scavenging activity, and upper intertidal species had higher levels (Collén and Davison 1999a), with each species in each zone having their own ROS-scavenging enzymes and protective mechanisms (Collén and Davison 1999b). It is possible that algae receive further protection from ROS damage through mutualisms with certain bacteria. Morris et al. (2012) found the marine blue-green alga *Prochlorococcus* required symbiotic heterotrophic bacteria to detoxify hydrogen peroxide produced under high light. While macroalgae can use “oxidative bursts” of ROS to defend against bacterial pathogens, resident host-associated bacteria can express oxidases to degrade ROS, preventing or limiting damage to themselves, and possibly aiding their host (reviewed in Egan et al. 2012).

Tissue Effect

Most macroalgal microbiome studies sample from one unspecified tissue type or a subsample of the entire powdered individual. While such investigations are useful for determining broad differences in microbiomes, my study demonstrated strong regionality of the microbiome on the algal thallus. Across all three *Fucus* species, holdfast communities differed from both receptacle and blade communities. Tissues perform specific functions; therefore, associated bacterial communities likely consist of different functional partners. Whereas *Fucus* receptacles and vegetative blades differ in function, both contain an extracellular matrix rich in fucoidan and alginic acid (McCully 1966, 1968; Kloareg and Quantrano 1988); histology suggests that holdfasts have a different polysaccharide composition from vegetative tissues (McCully 1966). Yet it is surprising that communities did not differ more, because large amounts of fucoidan are secreted from conceptacles as gametangia are expelled from receptacles (Speransky et al. 2001), providing a prolific carbon source for bacteria. However, fucoidan is only present after a certain point of maturation, and large releases from receptacles occur only at maturity

(McCully 1968). Vegetative thalli also continuously secrete alginic acid and fucoidan to the thallus surface to replenish the outer layer of polysaccharides that peel off (an anti-epibiont response by hosts); these hydrophilic polysaccharides can prevent desiccation, and may also provide a buffer against sudden changes in osmotic stress by modifying the transport of ions (osmoregulation; McCully 1966). These microenvironments provide ample carbon sources and protection from desiccation and osmotic stress for any associated bacteria. Polyphenolics are present in all *Fucus* tissues, and are thought to have antifouling effects; distinct types of tannin-like polyphenols were identified in thallus and holdfast tissue (McCully 1966). Different tissues may have different defensive compounds that may contribute to differences in bacterial community structure.

Bacterial densities are known to change across macroalgal thalli (Tujula et al. 2006), notably increasing from the tips to the holdfast in some species (Royer et al. 2018). Very few studies, however, have identified specific compositional differences in macroalgal microbiomes across tissue types within a thallus (but see: Staufenberger et al. 2007, Quigley et al. 2018). Quigley et al. (2018) found richness and diversity differences between holdfast and blade margin in a cultured isolate of the red alga *Porphyra umbilicalis*.

Rhizoids are the uniseriate filaments that initially attach an alga to a substrate and develop into the holdfast structure. Certain taxonomic groups of symbiotic bacteria are essential to rhizoid development by supplying required morphogens (Spoerner et al. 2012, Grueneberg et al. 2016, Weiss et al. 2017). A working model for *Ulva* spp. proposed by Spoerner et al. (2012) hypothesized that rhizoid cells excrete some diffusible substance to attract the necessary bacteria, starting a cascade of bacterial activity that ensures cell division, rhizoid attachment, and normal morphological development; this may define holdfast communities as different from others.

How holdfast communities differ from receptacle and blade communities varies among the three *Fucus* species (i.e. there is an interaction between species and tissue). Thallus surface areas increase from high to low zones, and increase in proportion to the holdfasts; these spatial differences might cause differences in bacterial competition and colonization on receptacle and blade surfaces. Holdfast communities also vary in canopy coverage: holdfasts of *F. spiralis* are often exposed to environmental stresses at low tide due to the low stature of thalli; whereas, *F. vesiculosus* holdfasts often are covered by their own fronds, and *F. distichus* holdfasts often are surrounded by other filamentous and foliaceous macroalgae. In this study, covered environments under canopy averaged 3 – 8 °C cooler than exposed microenvironments, which could influence microbial community composition.

While I cannot completely rule out the possibility that sampled holdfasts contain bacterial contaminants from the surrounding substrate or microorganismal epiphytes, care was taken to remove non-fucoid material with aid of a microscope when necessary after collection. Holdfasts of *F. spiralis* were collected from otherwise bare/thin crust-covered substratum and lacked epiphytes or other visible biota, yet distinctions of microbiomes between holdfasts and other tissues (blades, receptacles) remained, lending support that regional differences in tissue that I report are real differences.

Transplant Experiment

Fucus vesiculosus transplants in the high zone clearly experienced more stress than back-transplanted controls, because their emersion time at low tide was 2 – 4 h longer. Their associated bacterial communities became different. This likely reflects a major role of elevated environmental stress. Although direct and indirect biotic effects cannot be discounted without further experimental laboratory work, and I anticipate some will be found. Higher stress experienced by *F. vesiculosus* transplanted to the high zone might have changed their physiology in some fashion that leads to selective effects on the transplant microbiome. Both dry and sea-watered microbial communities differed from back-transplanted controls, dry ones more than sea-watered ones. Sea-watered communities received partial relief from thermal and desiccation stress, and, although they still experienced additional irradiance on sunny days, they have a statistically intermediate composition of bacteria between the two treatment extremes: dry and back-transplanted control. While increased exposure during diel low tides increased stress

(Williams and Dethier 2005), summer low tides in Maine occur in early morning or evening, lessening the potential of exposure stress. Irradiance measures during midday were much higher than either morning or afternoon measurements at my study sites. If longer exposure during low tides had occurred during these increases in irradiance, stress levels would have been higher. Additionally, mean daily exposure time decreased throughout this two week experiment.

Due to the significant effect of tissue type, the treatment effect on taxa was explored on a tissue level. There are far fewer significant changes in relative abundance among treatments for blade samples, compared to other tissue types, making it most practical to compare significant changes over time; three ASVs changed significantly from the beginning to the end of the experiment. All are members of the Alteromonadales, and each had a minimum of an 8-fold increase in relative abundance: *Psychromonas*, *Alteromonas,* and *Pseudoalteromonas*. While less is known of the role of *Psychromonas* in macroalgal communities, *Alteromonas* and *Pseudoalteromonas* produce antibiotics (Rao et al. 2005, Wiese et al. 2009, Goecke et al. 2013), and can cause disease (Vairappan et al. 2001, Wiese et al. 2009, Grueneberg et al. 2016). Although other potential effects are known for these genera, it is likely that these ASVs are increasing in response to stress and may be negatively affecting the *Fucus* host. The meristem of kelps is found at the base of the blades. Kelp blade tips have epiphytized, old tissue at the end of the blade that experience higher drag and perhaps nutrient limitation and disease. Unsurprisingly, Staufenberger et al. (2007) found differences between the bacteria on surfaces of the meristem and the blade tips. Host stress may cause mutualistic bacteria to change their relationships to negatively impact the host (pathogenic relationship), or allow for the invasion of new pathogenic bacteria, as may be the case in *F. vesiculosus* blade tissue, because two of the three significantly-increasing ASVs were not detected, even at low levels, in back-transplant control communities, and were not detected in native *F. spiralis* CAC microbiomes. In prolonged periods of transplant of *F. vesiculosus* to the upper intertidal zone, Schonbeck and Norton (1978) found deterioration of thalli. My experiments were kept to two weeks to avoid such effects, even though a longer period might allow more convergence to a *F. spiralis* microbiome.

Universal Taxa

All universally present taxa either maintained their abundance or positively responded to increase stress. *Burkholderia-Paraburkholderia* (ASV02929), *Granulosicoccus* (ASV03270), and *Octadecabacter* (ASV13813) were found universally throughout the three tissue types and three *Fucus* species. *Paraburkholderia* is the result of a recent split of *Burkholderia* (Sawana et al. 2014) and contains environmental species from very diverse ecological niches including metal-polluted soils, legume nodules, and arsenic-rich marine sediments (Dobritsa and Samadpour, 2016). This may be the first time a member of this genus has been identified in macroalgae or in intertidal environments. *Granulosicoccus* is a highly abundant ASV in receptacle and blade microbiomes across *Fucus* species, and was found in *Fucus* spp. microbiomes previously by Lachnit et al. (2009) and Dogs et al. (2017). A strain closely related to *Granulosicoccus antarcticus* was isolated from *Laminaria hyperborea* and found to digest algal-derived mannitol; it was only detected in natural kelp populations during the growing season and was hypothesized to be an early colonizer of new tissue (Bengtsson et al. 2011). So, a commensal, if not mutual, relationship is already established between brown macroalgae and certain *Grannulosicoccus*. Most *Octadecabacter* ASVs, including ASV13813 are either in higher abundance in, or are exclusive to, holdfast communities, across *Fucus* species and transplant treatments. An unclassified Rhodobacteraceae closely related to *Octadecabacter*, while not inducing complete morphogenesis, produced a new *Ulva* morphotype with enlarged cells and vacuoles (Gruenenberg et al. 2016). Bacteria that produce auxin-like morphogens cause host cell growth and division through vacuole expansion, producing a rhizoid that attaches the alga to the substratum (Spoerner et al. 2012, Gruenenberg et al. 2016). Perhaps *Octadecabacter* produces a morphogen capable of partially stimulating macroalgal production of rhizoids, which might explain why it is associated with holdfast communities. Dogs et al. (2017) isolated an *Octadecabacter* strain from *Fucus spiralis* that can digest glucose, mannitol, and fucose, and also produced and released large amounts of vitamin B_{12}, a vitamin that no eukaryote can produce (Kazamia et al. 2012, Helliwell et al. 2015). Another universally present group of interest is the AEGEAN-169 marine group in the Rhodospirillaceae: one ASV unique to each *Fucus* species is low in abundance in the holdfasts, yet high in receptacles and blades (ASV04184 in *F. spiralis*, ASV00914 in *F. vesiculosus*, and ASV00916 in *F. distichus*). ASVs can identify environmental dynamics, which would otherwise fail to be detected with OTUs (Eren et al. 2013, Needham et al. 2017). In *F. vesiculosus* transplants, the ASV00914 maintained a pattern of low abundance in holdfasts, and high abundance in other tissues, which were slightly more abundant in higher stress treatments, but not to a great extent. Rhodospirillaceae OTUs were found to be more abundant in bleached *Delisea pulchra*, when directly compared to healthy tissue (Zozaya-Valdes et al. 2015) and Rhodospirillaceae have only been found to act as a pathogen (Florez et al. 2015). But the patterns seen in this study do not follow that of a pathogen; perhaps they play an unrecognized role in fucoid microbiomes. All ASVs that are universally present in this study either increase in abundance in response to increased stress, or are unaffected and maintain their abundances across stress levels. These taxa may be important for maintaining the holobiont, and may continue this function in times of stress. It will be helpful to isolate these bacteria and perform reconstitution experiments, applying bacterial strains to axenic algal hosts, to determine their specific role(s).

Stress-Responsive Taxa

I explored ASVs of interest that respond to stress across natural stress gradients across the low, mid-, and high intertidal zones or across stress treatments: back-transplant control, sea-watered, and dry. Representative strains of almost all of these taxa (Table 4.5) have demonstrated the ability to digest algal polysaccharides. An isolate sister to

Loktanella and another sister to *Roseobacter* isolated from the kelp *Laminaria hyperborea* digest alginate, fucoidan, and mannitol (Bengtsson et al. 2011). Over 25 % of isolates from the fucoid *Ascophyllum nodosum* were able to digest alginate and other polysaccharides including isolates of *Cobetia*, *Maribacter*, *Marinomonas*, *Cellulophaga* and *Pseudoalteromonas* (Martin et al. 2015). Isolates of *Glaciecola* and *Alteromonas* also digest algal polysaccharides (reviewed in Goecke et al. 2010). *Loktanella*, *Octadecabacter*, and *Sulfitobacter* strains isolated from *Fucus spiralis* grew to varying degrees on glucose, mannitol, sucrose, and one strain of *Sulfitobacter* grew on fucoidan (Dogs et al. 2017). *Pseudoalteromonas* can degrade various large polysaccharides into mono- and disaccharides that other bacteria can then utilize (Ivanova et al. 2002). This could cause an additive, if not, synergistically negative effect, leading to algal decomposition. A hypothesis (Miranda et al. 2013). for the propensity of algal-associated bacteria to digest cell wall material is that macroalgal-bacterial coevolution led to multicellular algae as a consequence of selection on algal-associated bacteria to produce morphogens that increased the surface area of the bacterial niche and supplied carbon from larger amounts of cell wall polysaccharides. Polysaccharide consumption must be kept in balance for the success of the holobiont, but that may or may not be possible while a host is under stressful conditions.

Antibacterial and antimicrobial compounds produced by algal microbiomes may be responsible for, not only preventing fouling and pathogens, but possibly for maintaining a balance of mutual relationships among *Fucus* and its various bacteria. These relationships may get out of balance when symbionts are stressed. Bacteria with antimicrobial activity are more prominent on macroalgae rather than on phytoplankton (Wiese et al. 2009), suggesting bacterial interspecific competition is necessary on sessile macroalgal surfaces. Certain bacteria will benefit from increases in stress levels, while others will be negatively affected. Both potential stress-tolerant and intolerant ASVs are assigned to taxa that are known for antimicrobial activity. Changes in competitive bacteria will affect algal hosts depending on the roles that these bacteria may play. ASVs from genera with isolates that have previously shown antimicrobial activity that demonstrated positive responses to stress include *Cellulophaga*, *Alteromonas*, and *Pseudoalteromonas. Cellulophaga* isolated from the kelp *Saccharina latissima* (formerly *Laminaria saccharina*) can inhibit yeast (Wiese et al. 2009) and *Alteromonas* isolates from *Ulva lactuca* showed low levels of inhibition against a variety of bacteria, including strong competitors like *Pseudoalteromonas* and *Roseobacter* isolates (Rao et al. 2005). These symbionts may increase their antibiotic abilities in times of stress, outcompeting other bacteria. The genus *Pseudoalteromonas* is of interest to many due to its connection with macroalgal diseases and algicidal abilities (Wang et al. 2008, Wiese et al. 2009, Grueneberg et al. 2016). *Pseudoalteromonas* strains can inhibit members of diverse genera, but also members of its own genus (Holmstrom et al. 2002, Rao et al. 2005, Wiese et al. 2009). Perhaps certain strains of *Pseudoalteromonas* are usually symbiotic or commensal, but become parasitic under stress and outcompete other symbionts. Future experiments can test this.

Certain ASVs from genera with isolates that have previously shown antimicrobial activity that demonstrated mixed and negative responses to stress include *Glaciecola*, *Roseobacter*, and *Sulfitobacter*. *Glaciecola* and *Sulfitobacter* isolated from the kelp *Saccharina latissima* can inhibit yeast (Wiese et al. 2009). Members of the genus *Roseobacter* are considered to be some of the best competitors in inhibition experiments (Rao et al. 2005, Dogs et al. 2017). Some *Roseobacter* are pathogenic (Case et al. 2011); *Roseobacter*s and various members of the family Rhodobacteraceae can produce an antibiotic called tropodithietic acid (Brinkhoff et al. 2004) that inhibits many marine bacteria. Multiple *Sulfitobacter* strains showed inhibitory effects against a panel of marine bacteria (Dogs et al. 2017). Bacteria with weaker antimicrobial acitivities (e.g. *Glaciecola*) may be out-competed, and the algal host could lose symbionts in times of stress. Stronger competitors such as *Roseobacter*s*,* which may be important in maintaining the proper balance of associated antimicrobial bacteria to fend off pathogens or fouling organisms (Holmstrom et al. 2002), may lose dominant positions, greatly affecting the community structure in times of increased stress.

Effects on macroalgal morphogenesis and growth, as well as nutrient supply may be more prominent in potential stress-tolerant taxa. Bacterial control over growth and morphogenesis was explored in great detail in green macroalgae (Provasoli & Pintner 1980, Matsuo et al. 2003, 2005, Marshall et al. 2006, Singh et al. 2011, Spoerner et al. 2012, Grueneberg et al. 2016, Weiss et al. 2017, Ghaderiardakani et al. 2017). Despite overall differences in bacterial community diversity among the green, red, and brown macroalgal lineages (Lachnit et al. 2009, Barott et al. 2011), bacteria and bacterial morphogens isolated from one algal species are known to effect growth and development of other species in the same lineage (Matsuo et al. 2005, Marshall et al. 2006), and sometimes across lineages (Singh et al. 2011), but specificity is also observed. The microbiome of *Porphyra umbilicalis* contained *Hyphomonas,* which had been found to support normal development in a closely related red alga *Pyropia yezoensis*, and also contained abundant *Sulfitobacter* (Quigley et al. 2018), which stimulate cell division in diatoms and green macroalgae. It is, therefore, possible that bacterial groups known to induce green algal morphogenesis may be relevant to this study of fucoid brown algae; however, they are unlikely to be the same ASVs (strains; Weiss et al. 2017). Bacteria isolated from *Ulva* spp. from the genera *Psychrobacter*, *Cellulophaga*, *Pseudoalteromonas*, *Cobetia*, positively influenced growth and morphology of axenic *Ulva linza* (Marshall et al. 2006), and a strain of *Marinomonas* isolated from *Ulva fasciata* induced algal morphogenesis and growth (Singh et al. 2011). Studies by Spoerner et al. (2012) and many others that build from this work discovered a tripartite system between *Ulva* spp. and two required symbiotic groups of bacteria. The first group consists of bacteria that can produce an MS2 morphogen, which induces cell division and growth, similar to the plant hormone cytokinin. Strains assigned to *Sulfitobacter* (Spoerner et al. 2012), *Pseudoalteromonas* (Grueneberg et al. 2016), and *Cellulophaga* (Ghaderiardakani et al. 2017) have been found in this study of fucoid algae and increase in abundance in response to increased stress. The second group of bacteria important to algal morphogenesis produce MS6, which is required for normal cell wall formation and produce the algal rhizoid, similar to effects of the phytohormone auxin. While two bacteria were previously identified to have this ability (Spoerner et al. 2012, Grueneberg et al. 2016, Weiss et al. 2017), only *Maribacter* is found in my study. Here, *Maribacter* ASV06794 and ASV06944 increased in abundance with increased stress. While morphogens may be functional at low concentrations, their activity can be lost over time without replenishment (Matsuo et al. 2005; i.e. the macroalga takes up the morphogen to maintain normal morphology). Under high-stress conditions, it may be that macroalgae require more morphogen to maintain homeostasis, and it appears that certain taxa known for producing such morphogens increase in abundance with stress and therefore may increase morphogen availability to their fucoid host when needed.

Algal-associated bacteria can provide required nutrients to their host, and examples of potentially stress-tolerant taxa that can produce such nutrients were found in this study. Dogs et al. (2017) found that certain *Loktanella*, *Octadecabacter*, and *Sulfitobacter* strains produced very high concentrations of vitamin B_{12} (cobalamin) in their culture media, which would make B_{12} available to their *F. spiralis* host. Because many algae require an exogenous source of vitamin B_{12} (Croft et al. 2005, Helliwell et al. 2015), and B_{12} is required for fucoid growth (Fries et al. 1993), these genera may be important in maintaining algal growth in times of stress.

Conclusions

In summary, bacterial communities were found to be distinct across zones on algal hosts that are closely related (congeners) and biochemically similar, following well-established patterns of distribution of macroalgal and invertebrate species across the vertical gradient of stress. Further, I demonstrated the importance of examining eukaryote-associated microbiomes on a tissue-specific level, because holdfast microbial communities are different from those of blade and receptacles. While mid-zone bacterial communities on *Fucus vesiculosus* did not become a microbial community identical to the microbiome of high zone *Fucus spiralis* in the 2 week transplant experiment, differences in transplanted communities correlated with increases in environmental stress. Specific ASVs were identified as potentially stress-responsive due to shifts in abundance across vertical zonation in natural surveys of *Fucus* species, as well as changes in abundance due to stress treatments in transplant experiments. Algal polysaccharide digestion and antimicrobial abilities are known from taxa assigned to ASVs with variable stress response; however, taxa known for their involvement in algal morphogenesis and nutrient provision were assigned to ASVs that became more abundant in the host in environments with increased stress, and may be stress tolerant intertidal bacteria. This study provides a rich series of hypotheses about the roles and responses of bacterial taxa to intertidal stress, which can now be tested experimentally. Overall, my results suggest that *Fucus* species which are ecosystem engineers, may be able to maintain symbiotic relationships with some bacteria in times of stress that are important to their role as structural ecosystem engineers.

CHAPTER 5

LATITUDINAL EFFECTS ON TRANS-ATLANTIC MACROALGAL MICROBIOMES OF *FUCUS* AND *PORPHYRA* SPP.

Introduction

Bacteria have important associations with various eukaryotes in ecosystems across the globe, and this holds true within marine algal communities as well. Some bacteria associated with macroalgae are essential to them. Bacteria are required for normal development and morphogenesis (Fries 1970, 1975, 1977, Provasoli and Pintner 1980, Matsuo et al. 2003, 2005, Marshall et al. 2006, Spoerner et al. 2012, Grueneberg et al. 2016, Ghaderiardakani et al. 2017). Many algal-associating bacteria make enzymes that degrade algal polysaccharides (Armstrong et al. 2001, Kazamia et al. 2012, Labourel et al. 2014, reviewed by Goecke et al. 2010, Bengtsson et al. 2011, Martin et al. 2015, Dogs et al. 2017). Anti-microbial and antibiotic activities of algal-associated bacteria ward off foreign colonizing microbia (Rao et al. 2005, Wiese et al. 2008, Goecke et al. 2013, reviewed by Wichard 2015, Dogs et al. 2017, Kim et al. 2017). Overall algal survival likely requires a symbiosis between macroalgae and certain bacteria, yet little is known of whether these association change across environments.

Few studies have explored microbiome-environmental interactions. Examples include changes in microbiomes of algae between regions with different salinities (Lachnit et al. 2009. Dogs et al. 2017), changes in microbial richness and diversity between seasons (Miranda et al. 2013, Goecke et al. 2013), and changes in bacterial composition between reproductive and vegetative states (Michelou et al. 2013, Quigley et al. in prep). Environmental drivers of these changes may have serious effects on macroalgal life cycles, growth, and survival. Intertidal macroalgae act as food sources, nurseries, and habitat refugia (at low tide). Given that some bacteria are required for morphological integrity and, therefore, the ecological function of the macroalgae, it is essential to understand how environmental factors affect these relationships, and how sensitive these associations may be to a warming climate.

Environmental factors vary across latitudinal scales. Spatial patterns of abiotic stress and range retractions typically focus on large-scale trends such as latitudinal temperature gradients (LTG; Deutsch et al. 2008, Chen et al. 2011) or photoperiod. Overall, sea surface temperatures (SST) follow LTGs (Rind et al. 1998) from the tropics to the poles, however, temperatures are more variable at finer scales and do not strictly adhere to latitudinal gradients, whereas photoperiod does adhere to latitudinal gradients. Lüning (1990) concluded that SST tolerance of many seaweed species is at least partially responsible for geographic distributions of macroalgae, regardless of photoperiod. This is demonstrated by the influence of ocean currents on algal distributions, instead of light and daylength, in which a stricter latitudinal distribution would be expected (Lüning 1990). Further still, biotic mitigation of stress can dampen broad-scale thermal trends (i.e. cm-thick mussel and macroalgal beds eliminated temperature differences across sites at different latitudes; Jurgens and Gaylord 2018) and patterns of thermal stress based on organismal temperatures during daily exposure to the air do not follow latitudinal patterns, or those of SST, but rather the timing of low tides (Helmuth et al. 2002). By comparing microbiomes of macroalgae across the North Atlantic, I can assess forcing variables affecting microbiome composition, and can detect possible breaks in community structure and patterns of stress across latitudinal scales. Latitudinal replicates across the Atlantic allow photoperiod to be examined with spatial replication.

I characterized microbial communities of sympatric species *Fucus vesiculosus* (Phaeophyceae) and *Porphyra umbilicalis* (Rhodophyta) that occupy the mid-intertidal zone across the North Atlantic. By working with both red and brown macroalgae, I can assess whether microbial diversity is affected by differences in cell wall composition (Kloareg and Quantrano 1988) across their biogeographical ranges. Eleven sites were selected for trans-Atlantic comparisons, not based solely on latitudes, but on comparable environmental factors such as SST and air temperature. Fucoids are perennials that make up a major structural component of the North Atlantic intertidal zone, acting as ecosystem engineers. The biogeographical distribution of the model species *F. vesiculosus* ranges from North Carolina to Greenland (Muhlin and Brawley 2009) and from the Faroe Islands to Spain, including the Canary Islands off the Moroccan coast

(Lüning 1990, Gallardo et al. 2016). While slightly more constrained, the distribution of *P. umbilicalis* extends to southern New England in the western North Atlantic and to Portugal in the eastern North Atlantic (Brodie et al. 2008, Guiry and Guiry 2015, pers. obs. S. H. Brawley).

The questions this research aims to answer are: (1) Do microbiomes of algal samples differ from those of the surrounding water column and substratum? (2) Are there differences between microbial communities of sympatric hosts associated with differences in cell wall polysaccharides (i.e. *Fucus vesiculosus* versus *Porphyra umbilicalis*)? (3) Does stress level in the intertidal zone affect microbial composition on an identical host (*P. umbilicalis* from high and mid-zones)? (4) Are there correlations between microbial diversity of *F. vesiculosus* across latitudes and degree of environmental stress across latitudes? (5) Are mid-zone microbiomes of *Fucus vesiculosus* from northern sites similar to low-zone microbiomes of a site from an intermediate latitude (i.e. *F. distichus* from Schoodic, ME); are mid-zone microbiomes of *Fucus vesiculosus* from southern sites similar to high-zone microbiomes of a site from an intermediate latitude (i.e. *F. spiralis* from Schoodic, ME)?

Methods

I explored latitudinal effects on the microbiomes of two sympatric mid-intertidal macroalgae with diverse evolutionary history: *Fucus vesiculosus* (Phaeophyceae) and *Porphyra umbilicalis* (Rhodophyta) were collected from 11 trans-Atlantic locations (Fig. 5.1, Table 5.1) during the summers of 2015 and 2016. Using randomized numbers, I sampled three individuals from two 20 m transects (located ~40 – 100 m apart) at two collections per summer. Collections were made at least two days apart. The holdfast, a reproductive receptacle, and a vegetative blade tip were harvested from each individual of *F. vesiculosus*; the holdfast and a portion of the blade margin were collected from *P. umbilicalis*. In most cases, both species were collected from the same transect. However, certain sites did not have both *F. vesiculosus* and *P. umbilicalis* in the same local area (e.g. Portugal sites); in such cases transects sites were as close as possible (< 10 km apart). Two sites were moved between 2015 and 2016 due to availability of a collector at remote sites (i.e. Greenland) or to loss of a population due to unknown factors (i.e. Sidmouth moved to Minehead, England). The biogeographical range of *F. vesiculosus* extends further south than that of *P. umbilicalis*; thus, the latter was not collected at the southern-most collection sites. In some instances, other members of the genus *Porphyra* were collected for comparison [Table 1: (P)] when *P. umbilicalis* was not available; the similar cell wall structure and composition across *Porphyra* species still permits examination of differences in microbial composition between sympatric red algal and brown algal hosts. I also collected a water sample directly above each transect before the tide receded during each collection, as well as a surface scraping of the substratum surrounding the algae on each transect. At Schoodic, ME, I also collected samples of *Fucus* congeners from high, mid-, and low intertidal zones (*F. spiralis, F. vesiculosus,* and *F. distichus* subsp. *edantatus*, respectively; Quigley et al. in prep).

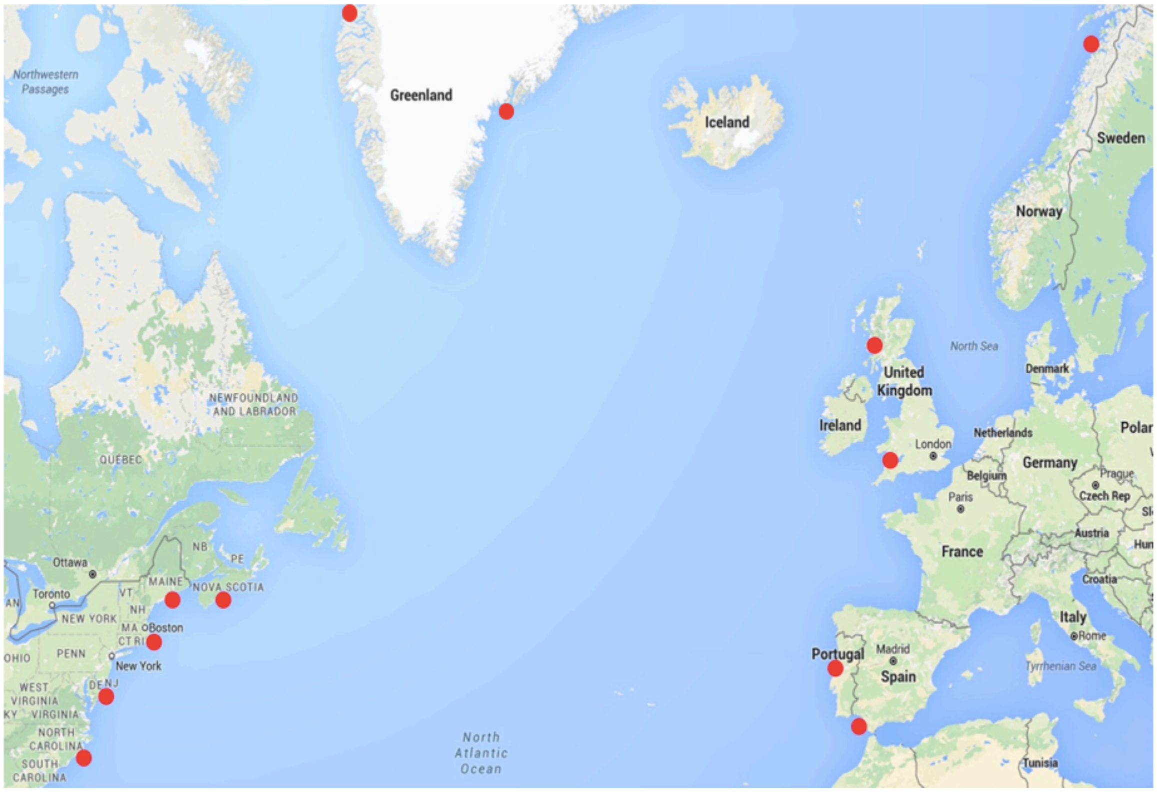


Figure 5.1. Map of collection sites.

Table 5.1. Collection sites and coordinates; F = *Fucus vesiculosus*, P = *Porphyra umbilicalis*, and (P) = *Porphyra* spp.

Site	**Species**	**Latitude, Longitude**	**Year**
Northwestern Atlantic			
Uummannaq, Greenland	F	70.6737 ° N, -52.1202° W	2016
Tasiilaq, Greenland	F	65.6075° N, -37.5667° W	2015
Halifax, NS, Canada	F,(P)	44.6478° N, -63.5714° W	2015, 2016
Schoodic, ME, USA	F,P	44.3340° N, -68.0577° W	2015, 2016
Woods Hole, MA, USA	F	41.5248° N, -70.6742° W	2015
Newport, RI, USA	F,(P)	41.4513° N, -71.3572° W	2016
Lewes, DE, USA	F,(P)	38.7880° N, -75.1603° W	2015, 2016
Beaufort, NC, USA	F	34.7203° N, -76.6745° W	2015, 2016
Northeastern Atlantic			
Bodø, Norway	F,P	67.394898° N, 14.6328° E	2015, 2016
Oban, Scotland, UK	F,P	56.2962° N, -5.6539° W	2015, 2016
Sidmouth, England, UK	F,P	50.675123° N, -3.2462° W	2015
Minehead, England, UK	F,P	51.1832° N, -3.3854° W	2016
Viana do Castelo, Portugal	F	41.6958° N, -8.8512° W	2015, 2016
Amorosa, Portugal	P	41.6426° N, -8.8238° W	2015, 2016
Cádiz, Spain	F	36.4680° N, -6.2521° W	2015, 2016

Each algal sample was harvested with a sterile razor, washed with autoclaved, sterile-filtered seawater, placed in a sterile Falcon tube, and placed on ice for transport. Samples were wrapped in sterile foil sheets at the laboratory, and flash-frozen with liquid nitrogen. Water samples were collected in 1 L sterile polypropylene bottles, transported on ice, and immediately pre-filtered through a 1.0, 5.0 or 7.0 μm sterile filter, followed by a 0.2 μm sterile filter that retained the bacterial community of the water column. Filters and scrapings from the substratum on each transect were also flash-frozen. Samples were either shipped to UMaine on dry ice, and stored at -80 °C, or lyophilized and shipped to UMaine with silica desiccant.

Each sample was lyophilized, and pulverized using a Geno/Grinder (SPEXSamplePrep, Metuchen, NJ; 2 min, 600 strokes/min, with 2.4 mm zirconium beads). DNA was extracted using the Qiagen DNeasy Plant MiniKit protocol (Germantown, MD). I followed the protocol for amplifying the V4 hypervariable region of the 16S rDNA in Quigley et al. (in prep), using genus-specific peptide nucleic acid (PNA) clamps for *Porphyra* (Quigley et al. 2018) and *Fucus* (Quigley et al. in prep). PCR products were cleaned, quantified, and pooled (96 amplicon libraries per pool) as described in Quigley et al. (2018). Pools were sequenced on an Illumina MiSeq using the Illumina sequencing kit (v.3) and protocol. Paired-end reads were demultiplexed (Quigley et al. 2018), merged, trimmed of primer sequence, and quality-filtered (Eren et al. 2013). Datasets were analyzed using Minimum Entropy Decomposition (Eren et al. 2015).

The average number of high quality, merged V4 reads was 85,662; the smallest dataset had 3,045 reads and the largest had 701,180 reads. The MED pipeline version 2.1 command "decompose" was run with default parameters except that the minimum substantive abundance (M) was set to 100 as described in Quigley et al. (2018). The dataset was divided into 7 separate runs (5 runs of 200 samples, 1 run with 100, and 1 run with 123 samples) and then recombined to reflect the presence and abundance of each sequence. The MED analysis identified 14,791 ASVs (amplicon sequence variants) that had ≥ 100 assigned reads across the 1,223 samples. Sequences will be deposited to the SRA at NCBI (GenBank).

Statistical analyses were performed in R statistical software version 3.3.3 (The R Foundation for Statistical Computing, 2017). Microbial diversity was examined with the Morisita-Horn distance index for differences in relative abundance of ASVs (*vegdist* function, *vegan* version 2.4-4, Oksanen et al. 2017). Nonparametric permutational multivariate analyses of variance (*adonis* function, *vegan*) were used to compare water column and substrate scrapings versus algal samples, species (*F, vesiculosus*, and *P. umbilicalis*), tissue types (blades, receptacles, and holdfasts), collection dates, and locations. I blocked samples to account for possible differences between transects (*strata* function). Multiple comparisons were corrected for (*pairwise.adonis* function; Arbizu 2017) using a Holm *p*-value correction (Holm 1979). All figures were produced with *ggplot2* (Wickham, 2010).

Results

To examine whether macroalgal microbiomes were composed of bacteria that are unique from that of the surrounding environment, I compared water column, substratum scraping, and algal samples. I only used sites where both types of environmental samples were taken (n = 8 sites in 2015 (Table 1). Permutational analyses found macroalgae, the water column and the substratum have distinct microbiomes (sample type: F(3, 572) = 11.75, p = 0.001, Fig. 5.2). Water column communities are distinct from those of macroalgae (sample type: F(1, 257) = 10.36, p = 0.001); Substratum scrapings also have a significantly different community than macroalgal communities (sample type: F(1, 254) = 5.16, p = 0.001). In response to my first question, these two macroalgae do have distinct microbiomes that differ from those of the surrounding environment.

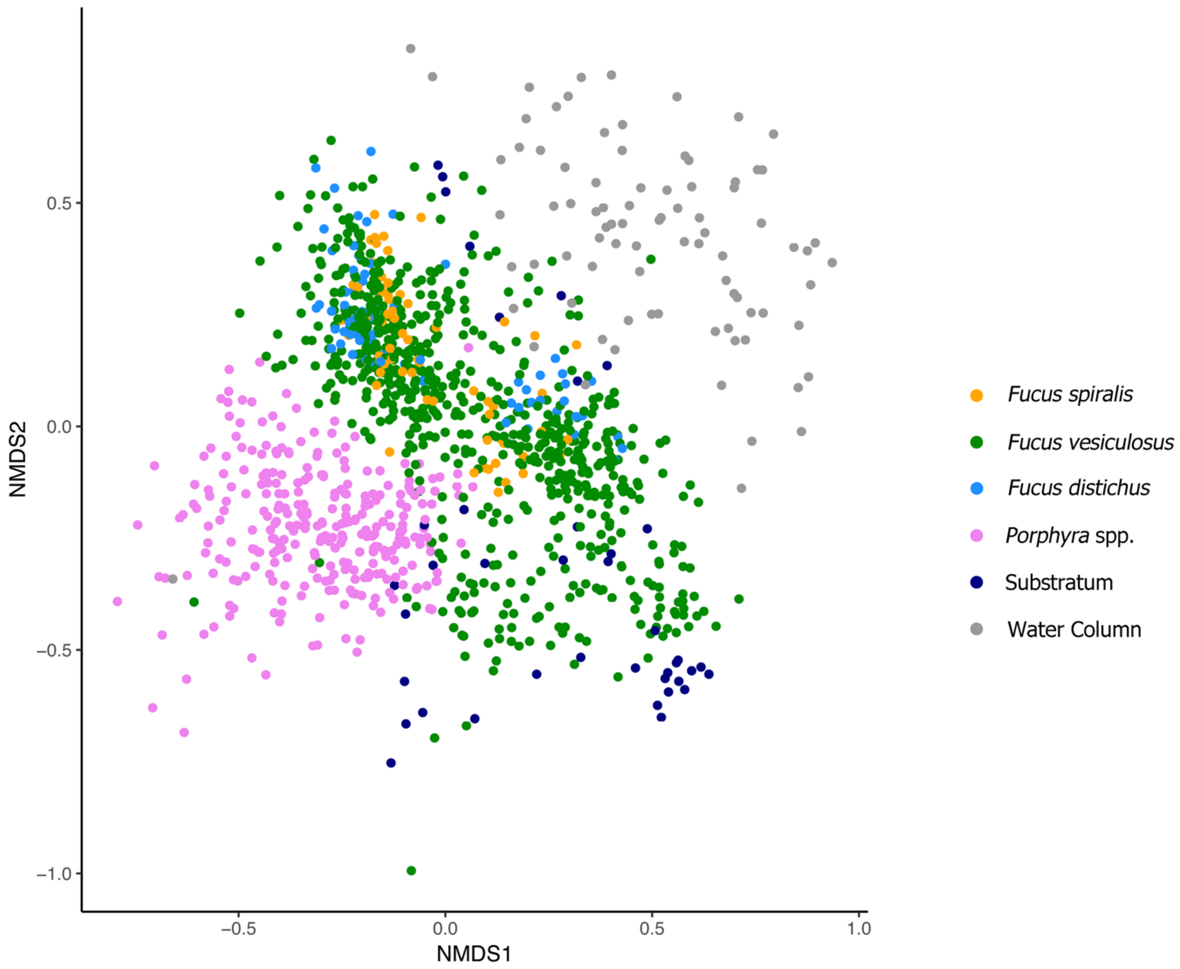


Figure 5.2. Ordination of microbial communities of all macroalgal, water column, and substratum samples across sites over two summer collections (2015 and 2016; n = 1223; NMDS, bray distance index).

Because of differing abilities of certain bacteria to digest various macroalgal polysaccharides, I examined microbial communities of two sympatric hosts with different cell wall polysaccharide compositions and from eukaryotic supergroups (the brown alga *Fucus vesiculosus*, Phaeophyceae, Stramenopila, and the red alga *Porphyra* spp., Rhodophyta, Archaeplastida). I compared vegetative samples from sites where both species were collected (i.e. excluding Greenland sites, Cádiz, and Beaufort) using a permutational analysis. There are significant differences between microbial communities of vegetative *F. vesiculosus* and *Porphyra* spp. (species: F(1, 447) = 110.53, p = 0.001 Fig. 5.3). There is also a significant effect of tissue type (F(3,447) = 44.83, p = 0.001, Fig. 5.3). I can conclude that there are differences between microbial diversity of sympatric hosts with different compositions of polysaccharides in their cell walls.

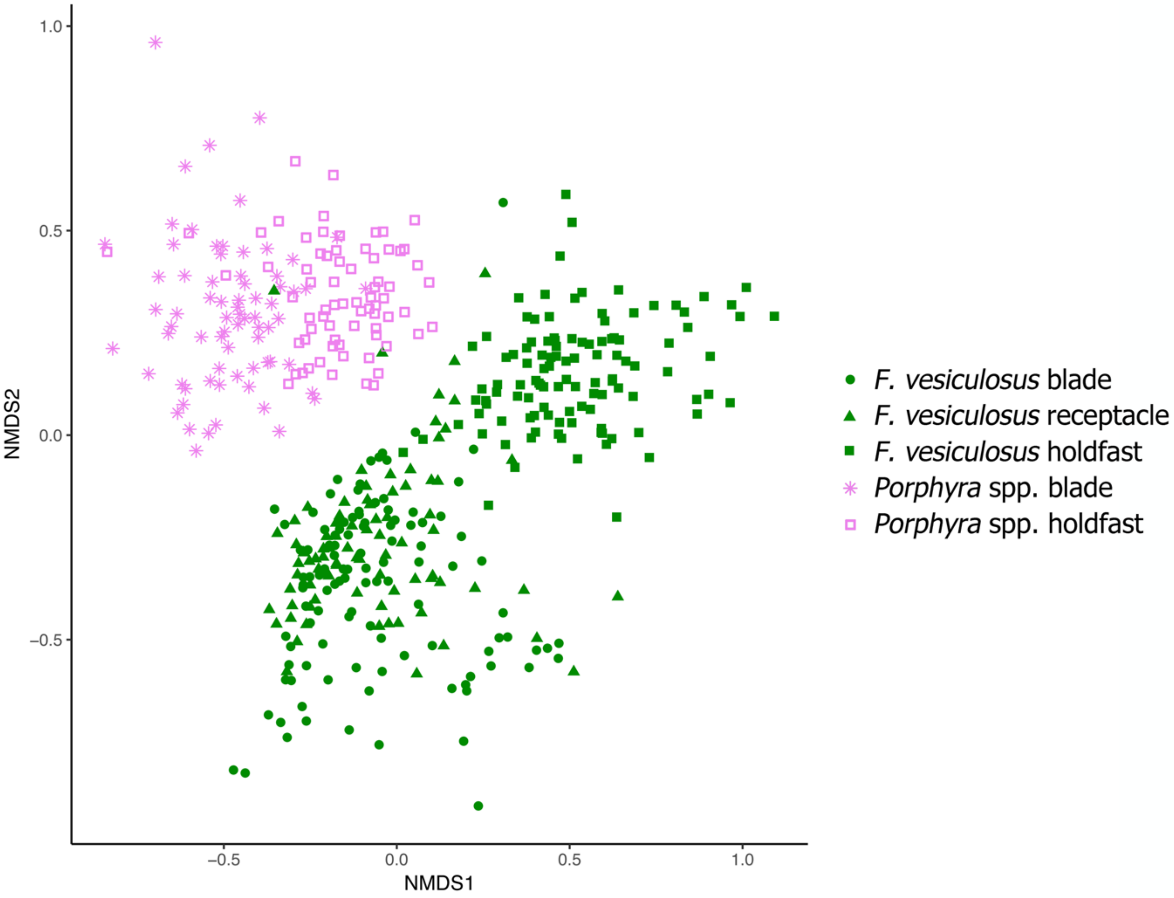


Figure 5.3. Ordination of microbial communities of all tissues types of *Porphyra* spp. and *Fucus vesiculosus* from all sites except Greenland, Cádiz, and Beaufort, where *Porphyra* spp. are not found (NMDS, bray distance index).

In order to validate that the intertidal stress gradient affects microbial composition, not minor differences in congener species, I characterized the microbiome of *P. umbilicalis* from both the high and mid-zones at one site in Schoodic, ME.

Permutational analyses found significant differences between high and mid-zone communities (F(1, 84) = 3.42, p = 0.004), as well as differences in microbial diversity between blade margins and holdfasts (tissue: F(1, 84) = 7.69, p = 0.001). However, there is a lot of variability across community composition collected on different days, years, and even transect. Zonation rather than host species defines microbial composition, but microbial communities of *P. umbilicalis* are diverse.

To examine latitudinal effects on microbial diversity, I compared vegetative microbiomes of *F. vesiculosus* from 11 sites across the North Atlantic. There is significant effect of site on the microbial composition (F(10, 230) = 22.76, p = 0.001); pairwise comparisons found that each site had a significantly different bacterial community, except for Bodø, Norway, Greenland (both sites), England (both sites), and Schoodic, ME (pairwise adjusted p-values ranged from 0.06 to 0.27). There are trends of differences in sites from northern to southern latitudes (Fig. 5.4).

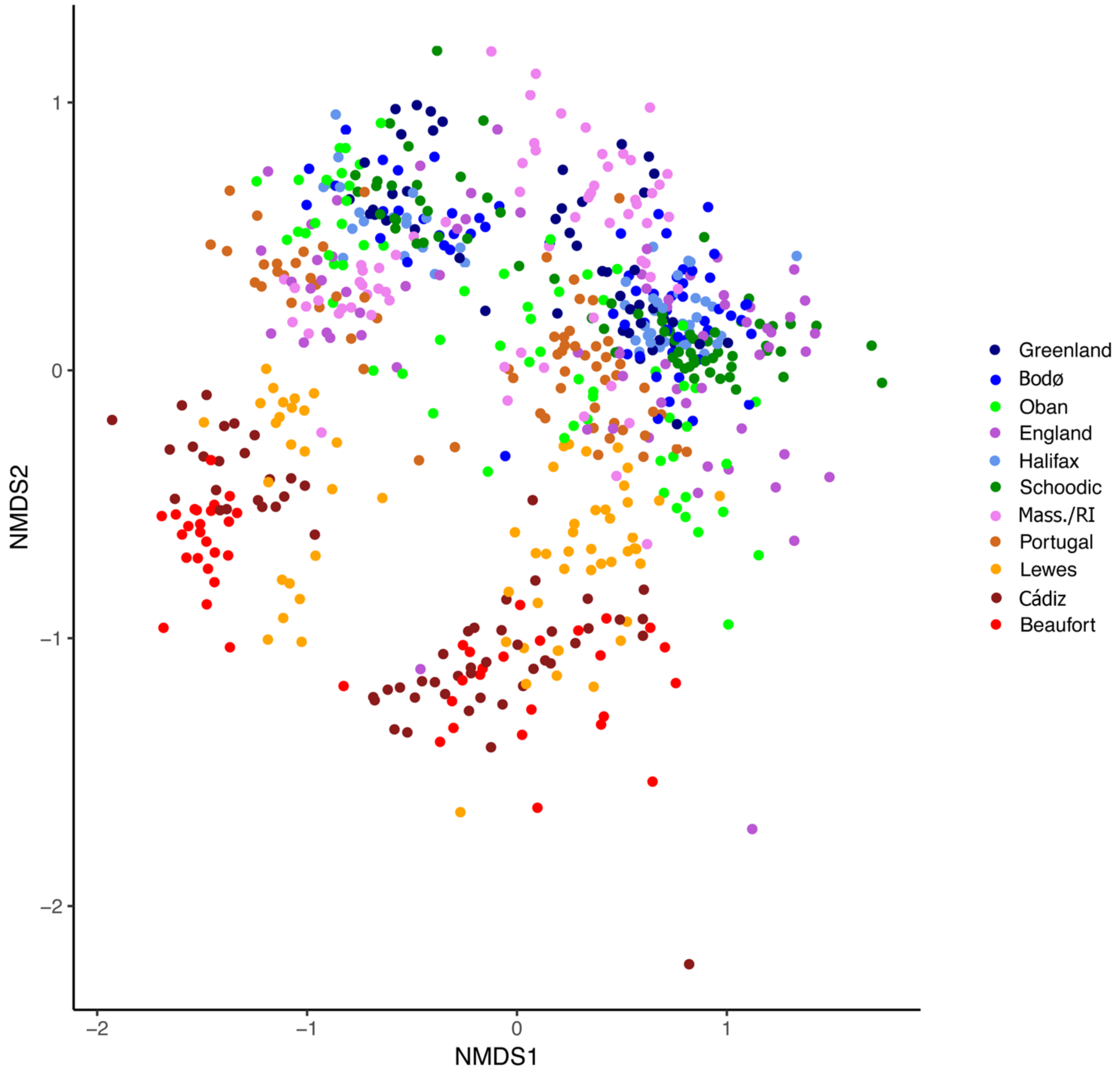


Figure 5.4. Ordination of microbial communities of vegetative blade samples of *Fucus vesiculosus* (NMDS, bray distance index) collected from all sites.

To examine whether stress levels are comparable from vertical to latitudinal scales, I compared microbial compositions of *F. vesiculosus* from sites at southern and northern latitudinal limits of the biogeographic range to *Fucus* congeners from an intermediate latitude (Schoodic ME). For each analysis, the extreme site was “group 1” and was analyzed with *F. vesiculosus* = “group 2”, *F. distichus* = “group 3”, and *F. spiralis* = “group 4” from Schoodic, ME; I examined Bodø, Greenland, Cádiz, and Beaufort. There was a significant effect of group in each analysis (group: p = 0.001for all; Bodø F(3, 95) = 10.11, Greenland F(3, 89) = 10.87, Cádiz F(3, 94) = 20.03, Beaufort F(3, 95) = 28.77). Pairwise comparisons showed that the *F. vesiculosus* microbiome from Bodø, Norway significantly differed from that of *F. spiralis* from Schoodic, ME (p-adj. = 0.006), but not from lower intertidal species (*F. vesiculosus*: p-adj. = 0.090, *F. distichus*: p-adj. = 0.090; Fig. 5.5b). Although the same pattern holds true of populations from Greenland when compared to congener fucoids from Schoodic, ME (Fig. 5.5a), all groups are statistically distinct (alpha level of 0.05, $p \leq 0.030$; of note, an alpha of 0.01 distinguishes *F. spiralis* communities from those of *F. vesiculosus* from Greenland). Microbial composition of *F. vesiculosus* from Beaufort, NC significantly differs from that of all *Fucus* congeners from Schoodic, ME (p-adj = 0.006, Fig. 5.5c). Again, the same pattern held for populations from Cádiz, Spain (p-adj = 0.006, Fig. 5.5d), where *F. vesiculosus* microbial communities from southern latitudes are different from those of all *Fucus* species from intermediate latitudes. Mid-zone microbiomes of *Fucus vesiculosus* from northern sites are similar to mid- and low-zone microbiomes of a site from an intermediate latitude, however, mid-zone microbiomes of *F. vesiculosus* from southern sites are distinct from all *Fucus* congener microbiomes of a site from an intermediate latitude.

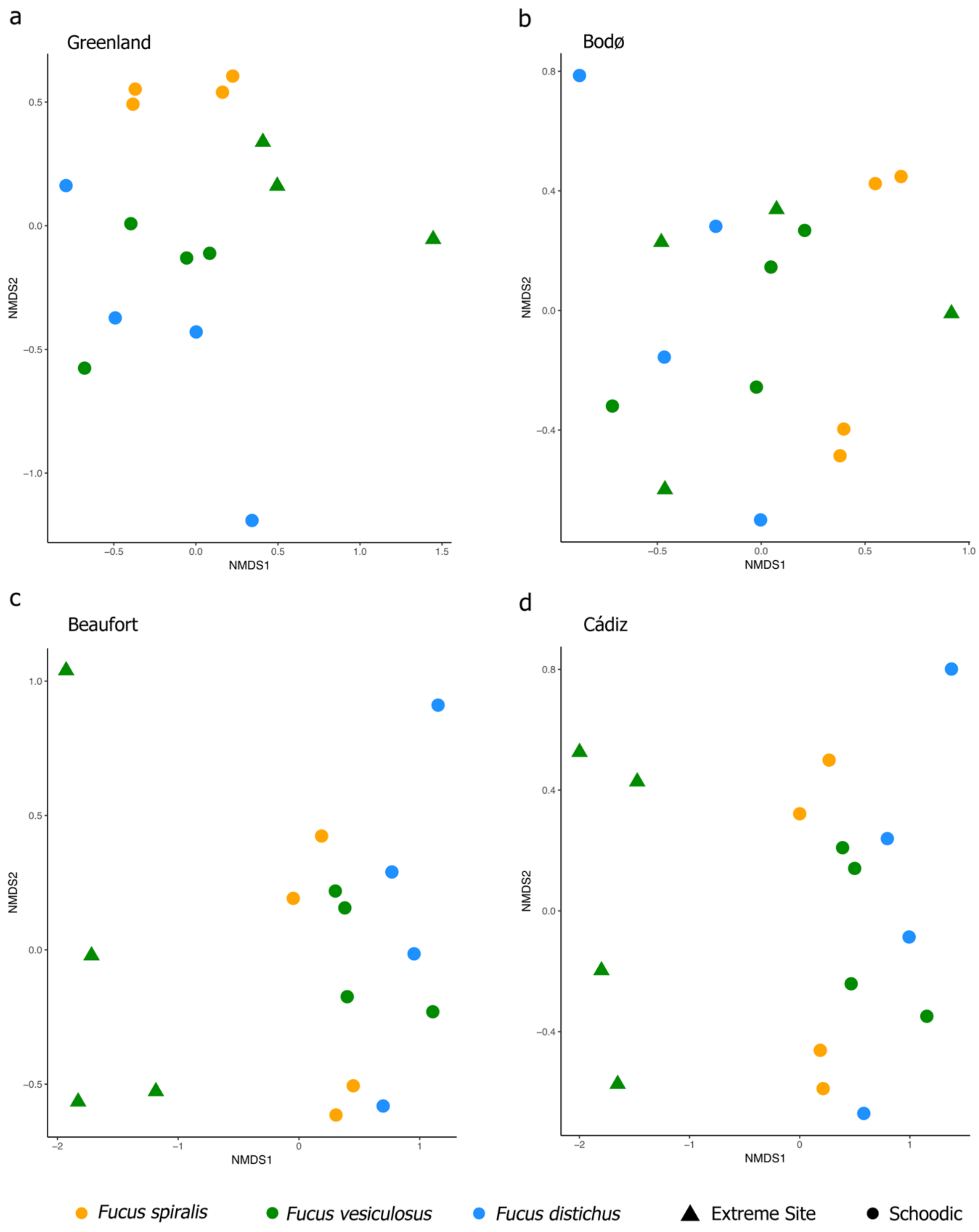


Figure 5.5 Ordination of vegetative microbiomes of *Fucus spiralis* (high zone), *Fucus vesiculosus* (mid-zone), and *Fucus distichus* (low zone) from an intermediate site (Schoodic, ME) compared to the microbiome of vegetative *Fucus vesiculosus* from extreme site: a) Greenland and b) Bodø at extreme northern latitudes, and c) Beaufort and d) Cádiz from extreme southern latitudes, representing the species boundary of *Fucus vesiculosus*.

Discussion

Two sympatric macroalgae, *Fucus vesiculosus* and *Porphyra* spp., at 11 trans-Atlantic sites have different microbiomes, most likely due to differences in the polysaccharide composition of cell walls and mucilages between brown and red algae, that may attract different bacteria. Vertical stress regimes that define distinct levels of the intertidal zone also define macroalgal microbial composition; microbiomes of *P. umbilicalis* from both the high and mid zones differed. There are latitudinal effects on (vegetative) blade microbiomes of *Fucus vesiculosus*, and unique assemblages for many sites; one group of northern sites does not differ among sites. Microbiomes of *Fucus vesiculosus* from high northern latitudes are similar to respective *Fucus* congeners that occupy the lower zones of the intertidal vertical stress gradient at the 45th degree in Maine; however, those from southern latitudes at the trailing edge differ from all *Fucus* congeners at an intermediate site, regardless of vertical zonation.

Microbial composition of environmental communities, cell walls, tissues, and intertidal zones

Environmental communities of bacteria from water-column samples taken from directly above the *Fucus* beds and from substratum scraping from each transect had different community compositions than that of the *Fucus*-associated bacteria, although the substratum community appears more similar to those of macroalgae. While earlier work (e.g. Lachnit et al. 2009, Michelou et al. 2013, Grueneberg et al. 2016) found differences between macroalgae and bacterioplankton from water samples, this is the first comparison of the microbial compositions of the substratum surrounding the targeted macroalgae, to my knowledge. The closer clustering of substratum samples to macroalgal samples might indicate that the substratum acts as a recruitment community for establishing macroalgal microbiomes or that juvenile or embryonic stages of the targeted macroalgae lie in the substratal areas around sampled macroalgae. Significant differences between macroalgal and substratum communities rules out the possibility of significant interference of substratum in interpretation of macroalgal microbiomes, within this study. Taxonomic assignment and further experiments are needed to determine if the substratum acts as a recruitment pool for macroalgal microbiomes or whether the microbiomes are shared between the target hosts studied and other Rhodophyta (for P. umbilicalis) or Phaeophyceae (for F. vesiculosus) in the substratum samples.

The microbiomes of two sympatric macroalgae, *Fucus vesiculosus* and *Porphyra* spp., are significantly different from each other. The biochemical composition of macroalgal cell walls is hypothesized to determine the composition of the associated microbiome (reviewed by Goecke et al. 2010). *Fucus vesiculosus* contains alginate, fucans, and a small amount of cellulose; the extracellular matrix is rich in fucoidan and alginic acid (McCully 1966, 1968; Kloareg and Quantrano 1988), which is secreted from conceptacles in large quantities as mature oogonia or antheridia (gametangia) are expelled from receptacles (Speransky et al. 2001), providing an abundant carbon source for bacteria. Cell walls of *Porphyra* spp. contain mannans, and sulfated porphyran (in the agar family; Rees and Conway 1962, Percival 1979, Brawley et al. 2017). Previous studies found differences in bacterial community structure between different algal lineages (Lachnit et al. 2009, Barrot et al 2011, Goecke et al. 2013). Conspecific macroalgae from different locations are more similar in microbial composition than to sympatric macroalgae from different evolutionary lineages (Lachnit et al. 2009). A comparison of the culturable microbiome of *Fucus vesiculosus* to that of another sympatric red macroalga, *Delesseria sanguinea*, found great variation in epibiota between the two, although season affected the level of variation (Goecke et al. 2013).

Microbiomes of both brown and red macroalgae differ among tissue types. Trends shows differences between *P. umbilicalis* blade margin and holdfast communities, as well as between *F. vesiculosus* receptacle and blade microbiomes compared to those of *F. vesiculosus* holdfasts (also see Quigley et al. in prep). Most macroalgal microbiome studies sample from one unspecified tissue type. To my knowledge, only three studies are the exception (Staufenberger et al. 2007, Quigley et al. 2018, Quigley et al. in prep). These studies demonstrate the importance of examining algal microbiomes across the thallus. Bacterial communities associated with different tissues likely consist of different functional partners, because host tissues perform different functions. Within one macroalga, the cell wall composition and defense compounds such as phenolics can differ among tissues types (McCully 1966, 1968, Speransky et al. 2001); these may contribute to differences in bacterial community structure among tissue types.

Porphyra umbilicalis that inhabited high versus mid-zones at the same intertidal site in Schoodic, ME had different microbiomes. Algal-associated bacteria follow the vertical stress regimes that define the distribution of various macroalgae and invertebrates in rocky intertidal zones (Baker 1909, 1910, Stephenson & Stephenson 1949, Connell 1961, Schonbeck & Norton 1978, Little & Kitching, 1996, Valdivia et al. 2011). Compositional differences of intertidal bacteria on three *Fucus* congeners inhabiting distinct levels within the intertidal zone further support these findings (Quigley et al. in prep); but only the comparison of microbial composition of conspecifics between the two zones, performed in this study, fully distinguishes between species-associated and zone-associated microbiome.

Microbiome of *Fucus vesiculosus* across latitudinal gradients

The microbial composition of vegetative blade communities of *Fucus vesiculosus* was analyzed; many sites throughout the North Atlantic had significantly different microbiomes. Ordination analyses show latitudinal trends, where more southern sites are more distant along ordination axes (NMDS; Fig. 5.4). There is less separation among more northern sites. In fact, the microbial diversity of Greenland, Bodø, England, and Schoodic, ME sites do not differ significantly. There appears to be a break in host microbial community structure between sites from northern and northwestern areas of the Atlantic, and all other sites. Both Halifax, NS and Oban, UK sit north of this biogeographic break. It is possible that other factors explain why the microbial diversity of these sites differs from this group of sites. For instance, samples of *F. vesiculosus* in Oban in the summer of 2015 were collected from a more sheltered site with a small amount of freshwater run-off (pers. obs.) and was not reflective of the water motion or exposure of all other sites. The site was moved 6.9 km north the following summer to an exposed, wave-washed site, as true of all other sites in this study. Similarly, there were extreme rainfall events both days before and during collections in the summer of 2015 at Halifax. Previous studies describe the effects of salinity on macroalgal microbiomes (Lachnit et al. 2009, Dogs et al. 2017). While not the predominant factor, Lachnit et al. (2009) found that the region from which a macroalga was collected, which differed in salinity and tidal range, contributed the dissimilarity among epibacterial communities. Microbial communities of *Fucus spiralis* differed between samples collected from a harbor site with freshwater input and an exposed site at full salinity (Dogs et al. 2017). While larger-scale variables (e.g. biogeographic history, see Muhlin and Brawley 2009) may be at play in causing this break among sites, distinct incidences of lower salinities may explain why some sites do not follow this break.

A large-scale forcing variable affecting composition of the microbiome may explain this break in community structure in northern and northwestern sites. Simple latitudinal gradients cannot explain this break, because *F. vesiculosus* blades from sites that differ by over 25° latitude (44.3340° N at Schoodic, ME to 70.6737° N at Uummannaq, Greenland) do not have distinct microbiomes, and other sites that are latitudinally closer to one another do have distinct microbiomes. Thus, photoperiod differs greatly among northern sites, and therefore does not explain microbial diversity of vegetative *Fucus vesiculosus*. Global air temperatures and sea surface temperatures loosely follow latitudinal temperature gradients, in that temperatures cool from equatorial regions to the poles, however, additional factors such as ocean currents and gyres, prevailing winds, and ocean depth influence temperature distributions. Intertidal zones are both marine and terrestrial environments throughout a tidal cycle, so it may be expected that both air and sea temperatures will affect the distribution of intertidal organisms, and their associated microbiomes. However, a transferable distribution model found that the most relevant predictor of the biogeographical range of *Fucus vesiculosus* was extreme SST, although extremes of summer air temperature and humidity were also important (Assis et al. 2014). This coincides with other studies that emphasize the importance of SST in macroalgal distributions (Lüning 1990, Adey and Steneck 2001). In fact, the classic coastal biogeographic regions align well with the biogeographic break in microbiomes identified in this study (Lüning 1990). Biogeographic region 2 is the cold temperate group that is recognized by zoologists (Briggs 1974, Vermeij 1978) and phycologists (van den Hoek 1975, Lüning 1990); it extends from the midcoast of the United States across to England and northward to the Arctic border, the summer 15 °C isotherm occurs within this region from the southern Gulf of Maine across to England, and upwards along the Norwegian coast (Lüning 1990). This isotherm and biogeographic region coincides with the break in microbial diversity found in vegetative blade communities of *Fucus vesiculosus*.

In order to examine the effect of stress on microbial composition, I compared the microbiomes of vegetative *Fucus vesiculosus* from latitudinal extremes to *Fucus* congeners that span vertical extremes within the intertidal zone. Microbiomes of *F. vesiculosus* from Bodø are significantly different from the high zone *Fucus spiralis* microbial community from Schoodic, ME mid-zone bacterial communities. However, this microbiome from a cooler site with less thermal stress is more similar to bacterial communities at slightly warmer sites that inhabit lower levels of thermal stress (mid- and low zone). Trends, although not significant, are similar in Greenland sites on the other side of the North Atlantic. Sites in Greenland differed between years due to collaborator availability; Uummannaq and Tasiilaq are on opposite coasts and may have divergent microbial communities. Further inter-annual investigation is needed. The microbial communities from the southern range boundaries of *Fucus vesiculosus* on both sides of the North Atlantic (Beaufort, NC and Cádiz) differed from all *Fucus* spp. microbiomes across the vertical intertidal gradient from Schoodic, ME. Edge populations of macroalgal distributions are often small and fragmented and can respond to the extreme thermal stress of these latitudes via either local adaptation or maladaptation (Pearson et al. 2009, Nicastro et al. 2013, Araujo et al. 2014, Jueterbock et al. 2014, Saada et al.

2016). The bacterial community may respond in a similar manner: either the community composition differs from those of more northern latitudes in that it contains bacteria that thrive with these high levels of stress and may be advantageous to its algal host, or, like the host, certain bacteria are unable to handle the stress and are eliminated from the microbiome. In the northeastern Atlantic, *Fucus vesiculosus* is undergoing a steady range retraction (Nicastro et al. 2013), and Cádiz, Spain, is a tiny, hold-out populational remnant of this retraction up the Portuguese coast (Mota et al. 2015). The same is occurring in the northwestern Atlantic. In fact, the population at Beaufort, NC was all but locally extinct by the end of this study (pers. obs.). Taxonomic assignment of the ASVs in this study will help to discern why southern extreme edge microbiomes differ so greatly, and further reconstitution experiments might determine if certain taxa are helping trailing edge populations of macroalgae, or whether bacteria are suffering from the same stress effects.

Conclusions

Sympatric macroalgae, *Fucus vesiculosus* and *Porphyra umbilicalis,* which have distinct cell wall composition, have different microbiomes across 11 trans-Atlantic sites; differences in carbon sources of an algal host may determine microbial composition. The vertical stress gradient defines microbial composition, not slight differences in host, because microbiomes of *Porphyra umbilicalis* from mid- and high zones are significantly different. There is a break in microbial composition of vegetative communities of *Fucus vesiculosus* across latitudes that corresponds with sea surface temperatures and summer isotherms across the North Atlantic Ocean. Vegetative microbiomes of *Fucus vesiculosus* from northern latitudes are similar to *Fucus* congeners that occupy the lower zones of the intertidal vertical stress gradient (*Fucus vesiculosus* and *F. distichus*) from an intermediate latitude, however, those from southern latitudes with trailing edge populations differ from all *Fucus* congeners from an intermediate site, regardless of vertical zonation, which may be influenced by range retraction due to extreme thermal stress.

CHAPTER 6

CONCLUDING REMARKS

These studies contribute to our understanding of various stressors on brown macroalgae, both as a crop and as a host of a microbial community. They also provide an array of protocols and tools to continue lines of inquiry relating to the thermal and microbial effects on brown macroalgae. These include thermal acclimation protocols to increase crop yield of the aquaculture candidate *Alaria esculenta*, peptide nucleic acid clamps for two model macroalgae, *Porphyra* spp. and *Fucus* spp., to increase sequencing depth of associated microbiomes, assessment of various algal preservations methods on microbiome recovery for phycologists who often work in remote areas with limited laboratory or equipment access, and analysis of various tissue microbiomes, demonstrating the need to account for microbial community differences across macroalgal structures.

This dissertation consists of two main lines of inquiry, both of which examine effects of stress on brown algae. The first examined the effects of thermal stress on the kelp *Alaria esculenta* to determine its potential as a crop (Chapter 2). Sea vegetable aquaculture is a global industry that is growing rapidly, and its expansion to the northwestern Atlantic includes parts of the United States where one need is to diversify crop species and strains that can withstand warming waters. The kelp *Alaria esculenta* has many favorable traits including high nutritional value and fast growth, but it is a sub-Arctic species and shallow coastal waters are warming in areas such as the Gulf of Maine. This led us to examine the temperature tolerance of *A. esculenta*. I surveyed reproductive phenology of *A. esculenta* for two years at three locations and concluded that seedstock are available to sea farmers practically year-round from across the Maine coast. Gametophytes (seedstock) from a northern and a southern population were gradually acclimated to 22 °C to determine their tolerance to high temperatures. While responsive to thermal stress, a large proportion of seedstock gametophytes remained healthy; differences in northern versus southern responses were marginal, but southern gametophytes suffered more damage when exposed to 22 °C. To determine effects of strain location and previous thermal acclimation on crop yield, acclimated gametophyte strains and, separately, control cultures were then crossed to produce sporophytes that were grown up in a common garden. Sporophytes derived from gametophytes that were thermally acclimated had higher blade surface areas compared to controls, and seedstocks from the northern population produced larger blades their southern counterparts. These results suggest that the application of seedstock thermal acclimation protocols in sea vegetable nurseries might increase crop yields of *Alaria esculenta* in Maine, and possibly elsewhere, but more isolated strains with defined population genetics should be compared in an additional acclimation trial before broad implementation.

The second line of inquiry I addressed was how stress gradients that influence distributions of brown macroalgae, specifically *Fucus* spp., affect the microbial composition of the macroalgal microbiome, across vertical and latitudinal spatial scales. While macroalgal microbiomes are the focus of many recent studies, there is little information about microbial spatial diversity across the thallus. Reliance on field material makes it difficult to discern whether recovered microbiomes belong to the host or its epiphytes, and technical comparisons of macroalgal samples for microbial studies are needed. In Chapter 3, a common garden approach, which avoided the problem of epiphytes and other natural biota (e.g. grazers), was used to examine the microbiome of *Porphyra umbilicalis* (lab culture strain Pum1).My collaborators and I used the V6 hypervariable region of the 16S rDNA with Illumina HiSeq sequencing and developed PNA clamps to block recovery of organelle V6 sequences. The common garden approach allowed us to determine differences in the microbiome at the holdfast versus blade margin. I found a notable increase in the relative abundance of Planctomycetes and Alphaproteobacteria at the holdfast, particularly of the possible symbiont *Sulfitobacter* sp. Non-adjacent 1.5 cm^2 samples of blade margin had microbiomes that were not statistically different. The most abundant phylum in the overall microbiome was Proteobacteria, followed by Bacteroidetes. Because phycologists often work in remote sites, I compared three stabilization and preparation techniques and found silica gel desiccation/bead-beating and flash-freezing/lyophilization/bead-beating to be interchangeable. Core taxa ($\geq 0.1\%$ of sequences) across treatments were similar and accounted for $\geq 95\%$ of all sequences. Finally, statistical conclusions for all comparisons were the same, regardless of which microbial community analysis tool was used: mothur or minimum entropy decomposition (MED).

All results from this laboratory investigation of a model macrophyte were applied to some of the first ever in situ examinations of environmental stresses on macroalgal microbiomes (Chapters 4 and 5). Stress gradients frequently produce distinctive vertical distributions of macroalgae and invertebrates within the intertidal zone. Whether stress tolerant (upper intertidal zone) or stress intolerant (lower intertidal zone) bacterial taxa exist in the intertidal zone is unknown. To examine this question (Chapter 4), I studied host microbiomes of three congeneric brown algae with similar cell wall composition and morphology on a rocky shore in Acadia National Park (Maine). Analysis of both natural microbiomes of *Fucus spiralis* (high zone), *F. vesiculosus* (mid-zone), and *F. distichus* (low zone) and of an experimental transplant were carried out on three different tissue types (holdfast, receptacle, and blade tip) using V4 16S rDNA sequencing. I found that fucoid macroalgae from each zone have significantly different microbiomes, and microbial communities differ between tissue types. Further, transplanting native mid-zone *Fucus vesiculosus* to the high zone found differences in microbial structure among *F. vesiculosus* transplants assigned to various stress-level treatments: microbiomes of dry treatments differed significantly from control (mid-zone) back-transplants, with transplants watered with seawater having an intermediate microbial community. I explored specific ASV (amplicon sequence variants) that were potentially stress-responsive due to changes in abundance across vertical stress gradients in surveys of all three *Fucus* species, as well as changes in abundance due to stress treatments in transplant experiments.

These studies were expanded to understand latitudinal effects on microbial community composition on macroalgal hosts (Chapter 5). Macroalgae maintain important associations with bacteria involved in maintaining normal algal morphogenesis, warding off pathogens and epiphytes with the production of antibiotics, and acquiring nutrients for their macroalgal host. Despite of this symbiosis between macroalgae and certain bacteria, only a few studies have investigated microbiome-environmental interactions. Whether these associations change across environmental scales will be important to study in light of climate change. I characterized microbial communities of two sympatric macroalgae, *Fucus vesiculosus* (Phaeophyceae) and *Porphyra umbilicalis* (Rhodophyta), that occupy the mid-intertidal zone at 11 sites across the North Atlantic. The two algae have different microbiomes most likely due to differences in the polysaccharide composition of cell walls between brown and red algae. By comparing microbiomes of *P. umbilicalis* from the high and mid-zones at one location, I determined that vertical stress regimes that define distinct levels of the intertidal zone also define macroalgal microbial composition. Latitudinal comparisons of blade microbiomes of *F. vesiculosus* found one group of northern sites where there are no statistically significant differences among sites, defining a biogeographical break in microbial community structure that aligns with summer isotherms. Microbiomes of *F. vesiculosus* from cooler northern latitudes are similar to *Fucus* congeners that occupy the lower zones of the intertidal vertical stress gradient at a site at an intermediate latitude; however, those microbiomes of southern latitudes at the trailing edge of their distribution differ from all *Fucus* congeners at the intermediate site, regardless of the vertical stress gradient. There are microbiome-environmental interactions across environmental scales, and more studies should investigate how symbiotic relationships between macroalgae, important members of coastal ecosystems, and bacteria may change in the future.

Overall, these studies have made significant contributions to advancing the understanding of stress effects on brown macroalgae. The information and protocols provided by the studies related to *Alaria esculenta* aquaculture can be applied directly to the sea vegetable aquaculture in the Gulf of Maine and help the industry now and in the future. The studies concerning macroalgal microbiomes provide insight to researchers about microbial composition analysis programs as well as considerations for preservation techniques and tissue sampling. Most importantly, these studies provide some of the first examinations of environmental stress effects on macroalgal microbiomes at both intertidal vertical and latitudinal scales, which will prove important in light of increases in environmental stressors due to climate change.

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APPENDIX 3.1: CORE COMPOSITION OF THE COMMON GARDEN *P. UMBILICALIS* EXPERIMENT

Core composition (≥ 0.1 % relative abundance) by group; defines percentage of sequences assigned to each OTU or ASV per treatment group. Taxa above bold line constitute > 1% of sequences. These are composites across replicates for each comparison (i.e., A, B, C, D, E).

ASV_A	%_A	ASV_A_tax
0040	22.8445697	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0012	13.3545587	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0037	8.8695489	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0353	8.7154939	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0023	8.2640458	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0007	7.6531842	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0491	5.2941334	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0019	3.8958052	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0016	3.4749449	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0024	2.1395783	Proteobacteria;Gammaproteobacteria
0010	1.975253	Proteobacteria;Gammaproteobacteria
0206	1.5771659	Proteobacteria;Alphaproteobacteria
0356	0.9006636	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0423	0.8948586	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0492	0.7859037	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0053	0.6088521	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0028	0.5965724	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0017	0.4829289	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0027	0.4414011	Unknown
0359	0.4268886	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0003	0.3259714	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0771	0.2395667	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0237	0.2324221	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Hyphomonas*
0977	0.1895546	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0205	0.1859823	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0599	0.1804006	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
1179	0.1625392	Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;*Micrococcus*
0774	0.1480268	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0338	0.1210113	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Erwinia*
0203	0.1203415	Proteobacteria;Alphaproteobacteria
0730	0.1201182	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;*Acinetobacter*
0692	0.1064989	Actinobacteria;Actinobacteria;Corynebacteriales;Dietziaceae;*Dietzia*

OTU_A	%_A_OTU	OTU_A_tax
0879	23.6497888	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0508	13.4027821	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0648	9.1780431	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0435	8.7726895	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0683	8.2851939	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0689	7.6800654	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0981	5.3298631	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0254	4.0079999	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_g
0509	3.4838975	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0978	2.1435076	Proteobacteria;Gammaproteobacteria
0777	1.978777	Proteobacteria;Gammaproteobacteria
0324	1.5805662	Proteobacteria;Alphaproteobacteria
0326	0.9008848	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0308	0.8959741	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0119	0.7861537	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0005	0.6082535	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0193	0.5912894	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0979	0.4834778	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0849	0.4406211	Unknown
0432	0.4281212	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0137	0.3272292	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0980	0.2395068	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0092	0.235489	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Hyphomonas*
0681	0.1921858	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0093	0.1866055	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0661	0.1803555	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
0501	0.1709806	Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;*Micrococcus*
0707	0.1479898	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0001	0.1247757	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;*Staphylococcus*
0674	0.1209811	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Erwinia*
0217	0.1203114	Proteobacteria;Alphaproteobacteria
0026	0.1200882	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;*Acinetobacter*
0257	0.1064723	Actinobacteria;Actinobacteria;Corynebacteriales;Dietziaceae;*Dietzia*

ASV_B	%_B	ASV_B_tax
0007	16.7691189	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0023	12.9623145	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0040	12.2775638	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0353	9.4528998	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0037	8.2514883	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0012	7.727285	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0019	7.2688091	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0016	4.1041942	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0491	2.9528864	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0356	2.2638257	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0024	2.1959432	Proteobacteria;Gammaproteobacteria
0053	1.9949896	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0010	1.9939121	Proteobacteria;Gammaproteobacteria
0206	1.484255	Proteobacteria;Alphaproteobacteria
0028	0.7240794	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0492	0.630337	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0423	0.4008297	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0017	0.3830509	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0003	0.3604235	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0237	0.2957735	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Hyphomonas*
0205	0.2866148	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0027	0.2451311	Unknown
0771	0.2133448	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0359	0.2133448	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0054	0.1750936	Actinobacteria;Actinobacteria;Propionibacteriales;Propionibacteriaceae;*Propionibacterium*
0774	0.1643186	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0599	0.1605474	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
1132	0.1422299	Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae;*Haemophilus*
0977	0.1228349	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0236	0.1125987	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
0874	0.1034399	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Marinomonas*

OTU_B	%_B_OTU	OTU_B_tax
0689	16.8275059	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0683	13.0093917	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0879	12.6343695	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0435	9.5102619	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0648	8.5064309	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0508	7.7563864	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0254	7.4648821	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0509	4.1090797	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0981	2.9667707	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0326	2.2711475	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0978	2.1984062	Proteobacteria;Gammaproteobacteria
0005	1.9995797	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0777	1.9963468	Proteobacteria;Gammaproteobacteria
0324	1.4876959	Proteobacteria;Alphaproteobacteria
0193	0.7198703	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0119	0.6304253	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0308	0.4008858	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0979	0.3831046	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0137	0.360474	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0092	0.2958149	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Hyphomonas*
0093	0.2866549	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0849	0.2451654	Unknown
0432	0.2133747	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0980	0.2133747	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0387	0.1751181	Actinobacteria;Actinobacteria;Propionibacteriales;Propionibacteriaceae;*Propionibacterium*
0707	0.1643416	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0661	0.1605699	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
0948	0.1422498	Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae;*Haemophilus*
0997	0.1239298	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Marinomonas*
0681	0.1228521	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0445	0.1153086	Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;*Streptococcus*
0057	0.1126144	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae

ASV_C	%_C	ASV_C_tax
0040	26.0573069	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0037	9.2627049	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Dokdonia
0023	9.1967916	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0353	8.3884108	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0007	7.8910976	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0012	6.385688	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0019	5.103342	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0491	5.0429963	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0016	4.0936292	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0010	2.7308223	Proteobacteria;Gammaproteobacteria
0024	2.5402664	Proteobacteria;Gammaproteobacteria
0206	1.4632035	Proteobacteria;Alphaproteobacteria
0356	1.3333525	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0053	1.1259142	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0492	0.9815155	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0423	0.885609	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0028	0.8604649	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0027	0.496056	Unknown
0017	0.4863576	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0003	0.2868216	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0599	0.2859236	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
0359	0.2853848	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0771	0.27407	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0205	0.2004339	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0774	0.1869639	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0237	0.1858863	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae*;Hyphomonas*
0203	0.1819351	Proteobacteria;Alphaproteobacteria
0977	0.157689	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0944	0.1266182	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;*Staphylococcus*
0002	0.1068622	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*

OTU_C	**%_C_OTU**	**OTU_C_tax**
0879	26.9931049	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0648	9.5940171	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0683	9.230051	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0435	8.4451986	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0689	7.9158587	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0508	6.4021763	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0254	5.2643109	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_g
0981	5.070028	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0509	4.1032823	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0777	2.7370179	Proteobacteria;Gammaproteobacteria
0978	2.5463262	Proteobacteria;Gammaproteobacteria
0324	1.4678949	Proteobacteria;Alphaproteobacteria
0326	1.334303	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0005	1.1278101	Planctomycetes;OM190;OM190_or;OM190_fa;*OM190_ge*
0119	0.9820082	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0308	0.8854054	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingorhabdus
0193	0.8602672	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0849	0.495942	Unknown
0979	0.4862458	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0137	0.2862171	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0432	0.2862171	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0661	0.2858579	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
0980	0.274007	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0093	0.2003878	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0707	0.1869209	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0092	0.1858436	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Hyphomonas*
0217	0.1818933	Proteobacteria;Alphaproteobacteria
0681	0.1576528	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0001	0.1414925	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;*Staphylococcus*
0708	0.1068376	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*

ASV_D	%_D	ASV_D_tax
0040	20.5789391	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0007	14.2359677	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0023	8.5013867	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0019	8.0990818	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0353	7.9262477	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0037	6.997402	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0012	6.8201832	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0016	4.191864	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0491	3.5845699	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0053	1.9512338	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0024	1.6859537	Proteobacteria;Gammaproteobacteria
0356	1.6329707	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0028	0.9946177	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0206	0.9767131	Proteobacteria;Alphaproteobacteria
0492	0.9763477	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0010	0.8236107	Proteobacteria;Gammaproteobacteria
1196	0.7088752	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae
0944	0.6705082	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;*Staphylococcus*
0027	0.5601576	Unknown
0423	0.4914624	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0017	0.4611343	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0559	0.4202095	Firmicutes;Bacilli;Bacillales;Family_XII;E*xiguobacterium*
0003	0.3617456	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
1222	0.3398216	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Pantoea*
0771	0.2276439	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0203	0.2272785	Proteobacteria;Alphaproteobacteria
0774	0.2214321	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0205	0.2049892	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0359	0.2009698	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0977	0.1936618	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0865	0.1775842	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
1188	0.1622374	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Alteromonas*
0758	0.1403134	Proteobacteria;Gammaproteobacteria;Alteromonadales;Colwelliaceae;*Colwellia*
0002	0.1216781	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*
1131	0.1172933	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Neptuniibacter*
0599	0.1107161	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
1197	0.1088891	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;*Pseudomonas*
1285	0.1045043	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Paraglaciecola*

OTU_D	%_D_OTU	OTU_D_tax
0879	21.227657	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0689	14.2787241	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0683	8.542842	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0254	8.3438316	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_g
0435	7.9706414	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0648	7.2184185	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0508	6.83756	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0509	4.2055102	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0981	3.6040971	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0005	1.9554144	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0978	1.6859287	Proteobacteria;Gammaproteobacteria
0326	1.632981	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0193	0.9899399	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0324	0.976064	Proteobacteria;Alphaproteobacteria
0119	0.9727776	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0777	0.8267149	Proteobacteria;Gammaproteobacteria
0679	0.717533	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae
0001	0.7131511	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;*Staphylococcus*
0849	0.5597853	Unknown
0308	0.4911358	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0979	0.4608278	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0575	0.4257728	Firmicutes;Bacilli;Bacillales;Family_XII;*Exiguobacterium*
0137	0.3615052	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0985	0.3428822	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Pantoea*
0217	0.2285881	Proteobacteria;Alphaproteobacteria
0980	0.2274927	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0707	0.221285	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0093	0.2048529	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0432	0.2022968	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;*Algimonas*
0681	0.1960892	Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;*Escherichia-Shigella*
0512	0.1807526	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0494	0.1643205	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Alteromonas*
0028	0.1402202	Proteobacteria;Gammaproteobacteria;Alteromonadales;Colwelliaceae;*Colwellia*
0009	0.1289003	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;*Pseudomonas*
0754	0.12817	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Neptuniibacter*
0708	0.1215972	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*
0010	0.1153895	Proteobacteria;Gammaproteobacteria;Cellvibrionales
0661	0.1106425	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;*Alcanivorax*
0987	0.1044348	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Paraglaciecola*

ASV_E	**%_E**	**ASV_E_tax**
0023	26.5484804	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0040	14.2580269	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0037	12.7740698	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0016	11.1071577	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0007	5.1410162	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0028	3.8710801	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0019	2.4884901	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
0024	1.9956293	Proteobacteria;Gammaproteobacteria
0353	1.9955218	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;*Methylotenera*
0491	1.8785681	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0356	1.6999128	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0010	1.6933557	Proteobacteria;Gammaproteobacteria
0053	1.6029531	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0492	1.4149458	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0203	1.2658514	Proteobacteria;Alphaproteobacteria
0003	1.1590022	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0771	1.0342014	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0022	0.9906663	Unknown
0205	0.7532117	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0027	0.5998175	Unknown
0017	0.5225292	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0002	0.488561	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*
0005	0.4137451	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;SM1A02
0012	0.3981584	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0421	0.3450563	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*
0062	0.2559436	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0206	0.2234804	Proteobacteria;Alphaproteobacteria
0354	0.212731	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;*Anderseniella*
0774	0.2011216	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0423	0.1615637	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*

OTU_E	%_E_OTU	OTU_E_tax
0683	26.6582662	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;*Sulfitobacter*
0879	14.7705922	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;*Fabibacter*
0648	13.290407	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;*Dokdonia*
0509	11.1450569	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0689	5.1570689	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;uncultured
0193	3.8149226	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
0254	2.5682755	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_
0435	2.002692	Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae;Methylotenera
0978	1.9997916	Proteobacteria;Gammaproteobacteria
0981	1.8873194	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;*Pseudohongiella*
0326	1.7070632	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
0777	1.6983619	Proteobacteria;Gammaproteobacteria
0005	1.6063002	Planctomycetes;OM190;OM190_or;OM190_fa;OM190_ge
0119	1.4180946	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_III;Unknown_Family;*Balneola*
0217	1.2594304	Proteobacteria;Alphaproteobacteria
0137	1.1492141	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;*Blastopirellula*
0980	1.0355603	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
0883	0.9682059	Unknown
0093	0.7561524	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;*Rhizobium*
0849	0.5990996	Unknown
0979	0.5225068	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0708	0.490065	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;*Aliiglaciecola*
0762	0.4137944	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;SM1A02
0508	0.3985403	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0709	0.3448287	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;S*phingorhabdus*
0507	0.2557748	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
0324	0.223333	Proteobacteria;Alphaproteobacteria
0717	0.2125907	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;*Anderseniella*
0707	0.2009889	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;*Haliea*
0308	0.1617794	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;*Sphingorhabdus*

APPENDIX 4.1: CORE COMPOSITION OF NATURAL SURVEYS AND TRANSPLANT EXPERIMENTS OF *FUCUS* SPP.

Core composition (≥ 0.1 % relative abundance) by group; defines percentage of sequences assigned to ASV per group. Taxa above bold line constitute > 1% of sequences. These are composites across replicates for natural survey and transplant core communities. Cores are broken up by species, tissue, and/or transplant treatment.

Fs_H_ASV	Fs_H_%	Fs_H_tax
830	16.8369178	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2929	16.0111806	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
9638	12.351496	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Profundibacterium
9841	6.1515185	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
3422	5.3315819	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
7057	4.1108008	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
13813	3.5726759	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2241	1.8876064	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
315	1.5277286	Proteobacteria;Alphaproteobacteria;E6aD10;E6aD10_fa;E6aD10_ge
7405	1.4667016	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3270	1.36785	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4822	0.832142	Actinobacteria;Actinobacteria;Micrococcales;Dermacoccaceae;Kytococcus
4184	0.7674897	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
6923	0.7496045	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
4677	0.7041666	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
13082	0.6926863	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Marivita
5177	0.6802392	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
7334	0.629605	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
4657	0.5689405	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
8596	0.5579436	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
8353	0.4902701	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
11779	0.4818109	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
2934	0.4596962	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
9696	0.4338353	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudoruegeria
3834	0.4332311	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
1638	0.4248927	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Tunicatimonas
8729	0.4103913	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium
3346	0.3908143	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3246	0.3270079	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
13026	0.3134732	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
1639	0.2957089	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Catalinimonas
6251	0.2733525	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
7436	0.2615096	Proteobacteria;Gammaproteobacteria;E01-9C-26_marine_group
12933	0.2276729	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7690	0.220543	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Cellulophaga
4013	0.2200596	Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Marinicella
11821	0.2155883	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Oceanicola

9842	0.2130506	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
9642	0.205679	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
3255	0.1863437	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3276	0.1830809	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
9171	0.1799389	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
9179	0.1798181	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
13128	0.1738967	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
11634	0.1711172	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
5450	0.168942	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
5451	0.1651958	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
5452	0.1512985	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
3340	0.1510569	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
12520	0.1490025	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
2214	0.1400599	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
9505	0.13293	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
9133	0.13293	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4134	0.1302714	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
6622	0.1296672	Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae
11369	0.1285796	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
13750	0.1231415	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
8879	0.1151657	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
7347	0.1104528	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
5751	0.1085192	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Defluviicoccus
9438	0.1006643	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella

Fs_R_ASV	Fs_R_%	Fs_R_tax
3270	34.7280507	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4184	14.6797905	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
2929	5.4251572	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
10966	3.2498505	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
3514	2.888764	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3246	2.452046	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4657	2.1435414	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
5650	2.060995	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
5177	1.5217062	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
11568	1.2301287	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
10057	0.9934381	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
3112	0.9755026	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
3314	0.837349	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8267	0.7898092	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
13813	0.7769158	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
1099	0.7172029	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
3278	0.6276696	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11668	0.5964806	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5078	0.5583047	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
13026	0.5476443	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
3657	0.5472121	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
10075	0.5402972	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
7690	0.5402252	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Cellulophaga
7405	0.52301	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5977	0.4697798	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3848	0.4609201	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Lacinutrix
2962	0.4386628	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
6083	0.4161174	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
4711	0.4032961	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
8353	0.3772212	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
11637	0.3709546	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
8596	0.3516506	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
1803	0.3401258	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
3847	0.3360921	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Lacinutrix
3255	0.3351557	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
546	0.33105	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
4822	0.3295374	Actinobacteria;Actinobacteria;Micrococcales;Dermacoccaceae;Kytococcus

8597	0.3116739	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
9243	0.309441	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
11740	0.2983484	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
8457	0.2724175	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio
717	0.2405803	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2619	0.240004	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
3273	0.2392117	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
242	0.2391397	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
13728	0.2329451	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
3902	0.2325849	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
11677	0.2291275	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
13692	0.2245176	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
659	0.2155859	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
6571	0.2071583	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pibocella
719	0.2010358	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
237	0.194409	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2934	0.1921761	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
8015	0.1902313	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
316	0.189655	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia
2516	0.1794988	Bacteroidetes;Flavobacteriia;Flavobacteriales;Schleiferiaceae;Schleiferia
7541	0.1709993	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
7466	0.1688384	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5453	0.165453	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
542	0.1630039	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
9690	0.1590423	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Aminobacter
549	0.1481658	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
9244	0.1429076	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
8917	0.1408907	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
7278	0.1305184	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
12357	0.1249001	Bacteroidetes;Flavobacteriia;Flavobacteriales;NS9_marine_group;NS9_marine_group_ge
9020	0.122307	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;Peredibacter
9261	0.1219468	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Dokdonia
11720	0.1206503	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
1890	0.1117906	Bacteroidetes;Flavobacteriia;Flavobacteriales;Schleiferiaceae;Schleiferia
7801	0.1068926	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
5253	0.1050198	Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_Incertae_Sedis;Unknown_Family;uncultured
6052	0.1042995	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
5402	0.1028589	Proteobacteria;Deltaproteobacteria;Myxococcales;Sandaracinaceae;uncultured
5297	0.1011302	Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_Incertae_Sedis;Unknown_Family;uncultured
8294	0.1005539	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter

Fs_V_ASV	**Fs_V_%**	**Fs_V_tax**
3270	37.3797917	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2929	7.7074526	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
4184	4.8089619	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_grou
3514	3.9097156	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3246	3.0539116	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4657	2.5493335	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
5650	2.0049109	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
5177	1.7102589	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
3112	1.603407	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
3314	1.4745193	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
1099	1.4176755	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5977	1.0027702	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
11568	0.920113	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
10057	0.8876437	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
6083	0.7079383	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3657	0.7010128	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
2962	0.678617	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
7278	0.6236621	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
10075	0.6172762	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
3255	0.6105305	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3278	0.4863197	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
13813	0.4736378	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
8353	0.4450361	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
3848	0.4248889	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Lacinutrix
717	0.4219208	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
659	0.4094188	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
1803	0.3955676	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
11740	0.3739814	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
8597	0.3630084	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
549	0.3555432	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
719	0.347898	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
242	0.3405227	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5740	0.338544	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Candidatus_Endobugula
3847	0.3373748	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Lacinutrix
8015	0.3264018	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
546	0.3226242	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula

8457	0.3117411	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio
8917	0.2990592	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
2934	0.2823299	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
5078	0.2802612	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
11677	0.2665	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
13026	0.2538181	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
13728	0.2477919	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
9244	0.2461729	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
1170	0.2317821	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Hellea
4822	0.2199996	Actinobacteria;Actinobacteria;Micrococcales;Dermacoccaceae;Kytococcus
5253	0.2169416	Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_Incertae_Sedis;Unknown_Family;uncultur
8929	0.206958	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
5451	0.2051591	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
13692	0.2016513	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
1890	0.2010217	Bacteroidetes;Flavobacteriia;Flavobacteriales;Schleiferiaceae;Schleiferia
9243	0.1988631	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
3422	0.1915778	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
237	0.1914878	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7466	0.1836628	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9020	0.1777266	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;Peredibacter
8258	0.1773668	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
316	0.1761076	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia
12024	0.1756579	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Ahrensia
3363	0.1751183	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3057	0.1738591	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
2516	0.1709809	Bacteroidetes;Flavobacteriia;Flavobacteriales;Schleiferiaceae;Schleiferia
716	0.1689122	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
1315	0.1670234	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_g
5978	0.1650447	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8596	0.1588387	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
9690	0.1586588	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Aminobacter
8294	0.1554208	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
6923	0.1453473	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
4970	0.1414797	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Fretibacter
9261	0.14112	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Dokdonia
9118	0.1359932	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Mesorhizobium
9195	0.1247504	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11720	0.1243906	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
8296	0.1221421	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
667	0.1205231	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured

3902	0.1198036	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
3212	0.1162059	Proteobacteria;Gammaproteobacteria;uncultured;uncultured_fa;uncultured_ge
5546	0.115936	Bacteroidetes;Bacteroidetes_Incertae_Sedis;Order_II;Rhodothermaceae;Rubrivirga
542	0.112878	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
4656	0.1103596	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
10076	0.1087406	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
11888	0.1086507	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
602	0.103434	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
3273	0.1021748	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
747	0.1009156	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera

Fv_H_ASV	Fv_H_%	Fv_H_tax
3422	15.609564	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
13813	14.7744262	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9638	6.5194526	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Profundibacterium
830	6.0203272	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2241	5.7585592	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2929	3.5279626	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
4677	2.9367066	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
9841	2.0043759	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
8596	1.2899416	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
13026	1.1812746	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
12266	0.9933011	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Simiduia
11634	0.9254687	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
9947	0.8899213	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
7109	0.8512242	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11668	0.8445872	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5872	0.8213014	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
4657	0.8173642	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
5177	0.7088098	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
12933	0.6938484	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
914	0.6542513	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
3346	0.5639206	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3834	0.558296	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
11369	0.5305105	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
4014	0.524661	Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Marinicella
8879	0.4497416	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5451	0.430393	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
12517	0.3986703	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
5450	0.3715599	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11480	0.3679601	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Tenacibaculum
11975	0.3641354	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
315	0.3607607	Proteobacteria;Alphaproteobacteria;E6aD10;E6aD10_fa;E6aD10_ge
7436	0.3561485	Proteobacteria;Gammaproteobacteria;E01-9C-26_marine_group;E01-9C-26_marine_group_fa;E01-9C-26_marine_group_ge
8355	0.3495115	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter
464	0.3455743	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
3423	0.3262257	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
4134	0.3192513	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
4485	0.3184638	Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulforhopalus

13621	0.3136267	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Tenacibaculum
14151	0.2929282	Proteobacteria;Alphaproteobacteria;Rhodobacterales
3424	0.2886535	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
13206	0.2859537	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
12520	0.2678426	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
12219	0.2595182	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
11717	0.2556935	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
11912	0.2547935	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
1604	0.2513063	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
10650	0.2472566	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
3270	0.2427569	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
10499	0.2298204	Proteobacteria;Deltaproteobacteria;Myxococcales;BIrii41;BIrii41_ge
5452	0.2284705	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
397	0.2118217	Proteobacteria;Gammaproteobacteria;HOC36;HOC36_fa;HOC36_ge
8354	0.2104718	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter
8729	0.2073221	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium
5250	0.1935981	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
11637	0.1926982	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
4013	0.1866236	Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Marinicella
9706	0.1799866	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
7427	0.1655877	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9521	0.1652502	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
9642	0.1601881	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
1102	0.1586132	Verrucomicrobia;Spartobacteria;Chthoniobacterales;Xiphinematobacteraceae;Candidatus_Xiphinematobacte
3340	0.1581632	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
4575	0.1569258	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;Nannocystis
4294	0.1567008	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
1606	0.1550135	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
8421	0.154676	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
12284	0.154001	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
3673	0.1533261	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
6888	0.1511888	Proteobacteria;Alphaproteobacteria;Rhizobiales;uncultured;uncultured_ge
1638	0.1501763	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Tunicatimonas
2186	0.1468016	Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria;Mitochondria_ge
4926	0.1444393	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Reichenbachiella
11407	0.1434268	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
6622	0.1432018	Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae
3636	0.1339775	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Leptobacterium
11806	0.133865	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Hoeflea
8880	0.133865	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea

9696	0.1311652	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudoruegeria
7468	0.1303778	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
11852	0.1294779	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7958	0.1291404	Bacteroidetes;Flavobacteriia;Flavobacteriales;NS9_marine_group;NS9_marine_group_ge
9372	0.1275655	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
6559	0.1250907	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maritimimonas
6621	0.1227284	Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae
12485	0.1225034	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
6923	0.1222784	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
11740	0.1218284	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
9444	0.1195786	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter
3276	0.1189037	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5749	0.1178912	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
8223	0.1163163	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11831	0.114404	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
11898	0.1121542	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
6251	0.1117042	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
10498	0.1069795	Proteobacteria;Deltaproteobacteria;Myxococcales;BIrii41;BIrii41_ge
11793	0.1059671	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11058	0.1016924	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella

Fv_R_ASV	Fv_R_%	Fv_R_tax
3270	31.1207111	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
914	6.642708	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
718	3.5980434	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3514	3.3269301	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5977	2.075106	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
13859	2.0138116	Proteobacteria;Gammaproteobacteria;Alteromonadales
8015	1.9216538	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4386	1.8691775	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
10966	1.7860107	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
4451	1.7508248	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
7732	1.7450325	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11632	1.6631625	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
2929	1.5754138	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
1099	1.5581234	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4150	1.5308046	Proteobacteria;Gammaproteobacteria
3657	1.3921356	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7278	1.2464641	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
6083	1.2400667	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7690	1.1321746	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Cellulophaga
5650	0.9878864	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
11668	0.8796485	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
8596	0.8774872	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
7075	0.7906895	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
3246	0.769941	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2241	0.7513538	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
13813	0.737608	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
546	0.6804632	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3112	0.6677548	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
5978	0.6624812	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
10057	0.6508967	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
4409	0.6384476	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8597	0.5685944	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
8293	0.5268381	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3314	0.5127465	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8292	0.4111654	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
5923	0.4087448	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
2619	0.4053732	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
8917	0.3860944	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius

549	0.3473639	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
542	0.3233303	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7403	0.3147715	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
4657	0.3074231	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
6926	0.3068179	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
782	0.2546874	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7277	0.2427571	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
4331	0.2322964	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;uncultured;uncultured_ge
3057	0.2272822	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11972	0.2214899	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5177	0.2179453	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
12084	0.2080034	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3306	0.2061879	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3363	0.2035079	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2305	0.2021246	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
12085	0.1998769	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
8918	0.1993582	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
4711	0.1946898	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
10075	0.1859581	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
7735	0.1754974	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3330	0.1723852	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
6732	0.1702239	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
8457	0.1651232	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio
7405	0.1624432	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
8297	0.1606277	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
7155	0.160109	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
719	0.1566509	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
2806	0.1524148	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
11720	0.1522419	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11740	0.1471412	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
1315	0.1456715	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
9402	0.1402251	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
7729	0.1395334	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
747	0.1392741	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
6214	0.138496	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
10967	0.137545	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
2962	0.1365941	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
1716	0.1307153	Proteobacteria;Deltaproteobacteria;Bradymonadales;Bradymonadales_fa;Bradymonadales_ge
9382	0.1279489	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Dokdonia
6923	0.1215514	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina

13692	0.1143759	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
10160	0.1142895	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Robiginitomaculum
6188	0.114203	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
11047	0.1110907	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
717	0.1098804	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8267	0.1078056	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
11718	0.1065952	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
8294	0.1060765	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
8338	0.1047798	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
4970	0.1031372	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Fretibacter
3513	0.1026185	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula

Fv_V_ASV	Fv_V_%	Fv_V_tax
3270	38.1471332	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
914	7.6763271	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
3514	5.453625	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
4451	4.1092141	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
718	4.0554412	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5977	3.5518992	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
2929	3.4732945	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
4386	3.052729	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8015	2.190963	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4150	2.1711151	Proteobacteria;Gammaproteobacteria
6083	2.078171	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3657	1.6979133	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7278	1.3505313	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
8293	1.0100569	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
1099	0.8734823	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5978	0.8657006	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
10057	0.8606293	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
546	0.8462898	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
13813	0.6892554	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3306	0.6425647	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5650	0.6242907	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
3112	0.5716544	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
8292	0.5465604	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
549	0.4639337	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7279	0.4163687	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
542	0.4067507	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3314	0.39416	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
10069	0.3150307	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
782	0.2710506	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8297	0.2644929	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
8918	0.2642306	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
6214	0.236426	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
3057	0.2217369	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8878	0.2178023	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
7277	0.212731	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
719	0.2043372	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
5253	0.1945444	Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_Incertae_Sedis;Unknown_Family;uncultured
3363	0.1863254	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

8338	0.1716362	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
747	0.1591329	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
8917	0.1565973	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
3513	0.1522255	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
8457	0.1519632	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio
3246	0.1486406	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
6732	0.1467171	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
95	0.1376237	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8596	0.137274	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
10075	0.133252	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
4409	0.1330771	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
2934	0.1281807	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
4970	0.125033	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Fretibacter
4331	0.1224974	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;uncultured;uncultured_ge
3278	0.1194371	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7958	0.1117428	Bacteroidetes;Flavobacteriia;Flavobacteriales;NS9_marine_group;NS9_marine_group_ge
8376	0.1085951	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
6188	0.1068464	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
8294	0.1064966	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3273	0.1063218	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

Fd_H_ASV	Fd_H_%	Fd_H_tax
5872	31.7144695	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
3422	3.667216	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
2929	3.6361626	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
12933	2.9777099	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11912	2.8172171	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2241	2.6488855	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
11637	2.4310092	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
13813	2.1036922	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
12517	1.4707656	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
916	1.4556911	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
7039	1.4038349	Proteobacteria;Alphaproteobacteria;Rickettsiales;Mitochondria;Mitochondria_ge
11668	1.175507	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
4134	1.1673668	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
8596	1.1322935	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
11717	1.1154101	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
3346	1.0162201	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3340	0.9963218	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
4677	0.8130163	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
11806	0.7555323	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Hoeflea
11831	0.7412618	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
7109	0.7180472	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5874	0.7034752	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
5177	0.6910136	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
1604	0.6371475	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
10936	0.5904167	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
7958	0.5431833	Bacteroidetes;Flavobacteriia;Flavobacteriales;NS9_marine_group;NS9_marine_group_ge
13432	0.4995679	Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulforhopalus
8353	0.452234	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
11407	0.4277129	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
10498	0.4208791	Proteobacteria;Deltaproteobacteria;Myxococcales;BIrii41;BIrii41_ge
8355	0.4136434	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter
12469	0.3862079	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae
11898	0.3629932	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
6486	0.3577674	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
2962	0.3567624	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
2305	0.3566619	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
13633	0.3498282	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Tenacibaculum
12029	0.3463108	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter

4014	0.3306333	Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Marinicella
3270	0.325106	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12515	0.324503	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae
531	0.3003839	Bacteroidetes;Cytophagia;Cytophagales;Cytophagaceae;uncultured
7529	0.284204	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7377	0.2786767	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
3834	0.2765662	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
13026	0.2721444	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
13951	0.2664161	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
5749	0.2623962	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
1370	0.2597833	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
11740	0.2476233	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
3837	0.2423974	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
11818	0.2390811	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
6888	0.2369706	Proteobacteria;Alphaproteobacteria;Rhizobiales;uncultured;uncultured_ge
7436	0.2270215	Proteobacteria;Gammaproteobacteria;E01-9C-26_marine_group;E01-9C-26_marine_group_fa;E01-9C-26_marine_group_ge
9145	0.2136555	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
6559	0.1826021	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maritimimonas
9191	0.1816976	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5700	0.1778787	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Amphritea
9706	0.1764718	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
12929	0.170643	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8372	0.1684321	Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;uncultured
13449	0.1684321	Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;SEEP-SRB4
5923	0.1645127	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
5899	0.1635077	Deinococcus-Thermus;Deinococci;Deinococcales;Trueperaceae;Truepera
11480	0.1592869	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Tenacibaculum
11759	0.1561715	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
1096	0.1543626	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4760	0.1531566	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
830	0.1518501	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3889	0.1512472	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
9292	0.1483328	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
11339	0.1445139	Verrucomicrobia;Opitutae;Puniceicoccales;Puniceicoccaceae;Lentimonas
11852	0.1437099	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
13728	0.1436094	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
2252	0.142906	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3276	0.141298	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8633	0.1371777	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
10499	0.1360722	Proteobacteria;Deltaproteobacteria;Myxococcales;BIrii41;BIrii41_ge

7466	0.1321528	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5186	0.1278315	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
4713	0.127128	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
13958	0.1262235	Proteobacteria;Gammaproteobacteria;Arenicellales
9511	0.1236106	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
9643	0.1226057	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
6467	0.1202943	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
12481	0.1173799	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
10948	0.112556	Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;Acaryochloris
11801	0.1094406	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
6444	0.1087372	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
13864	0.1060238	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
9375	0.1037123	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina

Fd_R_ASV	Fd_R_%	Fd_R_tax
3270	21.7530649	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
916	6.5340776	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
4150	6.4025647	Proteobacteria;Gammaproteobacteria
4409	5.5187784	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5249	3.2376976	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
3514	3.0651993	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
8015	2.750492	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
783	2.5037047	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5979	1.8982105	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
10093	1.5651254	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
3363	1.5306063	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12316	1.3594693	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
8292	1.2026748	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3657	1.1708783	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
718	1.1515768	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8338	1.145694	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
6214	1.1175439	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
2929	1.0454913	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
3057	1.0050408	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7865	0.9910873	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
10160	0.8644362	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Robiginitomaculum
8083	0.8215547	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
4408	0.8028366	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
6083	0.7885914	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
1605	0.7340415	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
2806	0.7317564	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
5977	0.729131	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
546	0.6266919	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3328	0.6187671	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12430	0.5817685	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
8906	0.5305732	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
11233	0.4993116	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7277	0.4839481	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
8782	0.4808852	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
10058	0.4738841	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
10057	0.4731062	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
8917	0.4317805	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
4331	0.427259	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;uncultured;uncultured_ge

8223	0.3858847	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8779	0.3520463	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7849	0.3431491	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;uncultured;uncultured_ge
5978	0.3214166	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
9857	0.3200067	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;DEV007;DEV007_ge
11047	0.3147073	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3117	0.309262	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
2962	0.305956	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
13813	0.3044488	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
1315	0.3007052	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
11081	0.2890854	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
10043	0.285439	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
4453	0.2851959	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4733	0.2787296	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
12337	0.2755208	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
399	0.2755208	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;DEV007;DEV007_ge
11048	0.2744512	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
11632	0.2707076	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
9511	0.2574347	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
542	0.2452315	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
12517	0.2395918	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
11720	0.2232073	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
9999	0.2230129	Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;FamilyI_ge
8258	0.2181024	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5039	0.2155256	Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;FamilyI_ge
782	0.2144074	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
9155	0.2110527	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4384	0.2005025	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
1498	0.1925291	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;Peredibacter
4149	0.1808607	Proteobacteria;Gammaproteobacteria;uncultured;uncultured_fa;uncultured_ge
8918	0.1774088	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
11352	0.1742486	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
3112	0.173811	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
1236	0.1711856	Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae;Haliangium
3551	0.1706994	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
549	0.1676364	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
10036	0.1619967	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
10017	0.1618995	Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;FamilyI_ge
13585	0.1575724	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
6188	0.1554818	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge

1448	0.1550443	Proteobacteria;Deltaproteobacteria;Myxococcales;Blfdi19;Blfdi19_ge
5038	0.1547039	Cyanobacteria;Cyanobacteria;SubsectionI;FamilyI;FamilyI_ge
11831	**0.1524675**	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
1370	**0.1524675**	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
11417	0.1490642	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
10966	0.1463902	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
2805	0.1423062	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
8293	0.1397294	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
10056	0.1349162	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
8267	0.1337494	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
9127	0.1303947	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
3656	0.1288875	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5650	0.1287903	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
8907	0.1264566	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
10037	0.1212058	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
2795	0.1199904	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
5253	0.1161009	Proteobacteria;Alphaproteobacteria;Alphaproteobacteria_Incertae_Sedis;Unknown_Family;uncultured
10075	0.1120655	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
1541	0.1100236	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
1099	0.109586	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5177	0.1064744	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
6732	0.104238	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
2812	0.1033142	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11232	0.1026822	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11870	0.1016126	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Pseudahrensia
4970	0.1006402	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Fretibacter

Fd_V_ASV	Fd_V_%	Fd_V_tax
3270	23.3185045	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4150	16.052995	Proteobacteria;Gammaproteobacteria
916	8.3558773	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
8015	3.6062434	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
783	3.5630062	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3514	2.686534	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
4409	2.6162996	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5979	2.3969907	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
2929	2.3924796	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
6083	1.8315069	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
718	1.476518	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3057	1.3874758	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
2806	1.1110494	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3363	1.0887021	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
10093	1.018676	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
8292	0.9473312	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
5977	0.9178355	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3657	0.8944472	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
11048	0.8718917	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
6214	0.823727	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8338	0.7703572	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
11233	0.5960899	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8782	0.5915094	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5249	0.553408	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
546	0.538556	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7849	0.5264802	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;uncultured;uncultured_ge
4408	0.5123222	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
9155	0.4558988	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4733	0.4308448	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4149	0.4134944	Proteobacteria;Gammaproteobacteria;uncultured;uncultured_fa;uncultured_ge
12316	0.4115512	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
5978	0.4006552	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
5980	0.3936456	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8376	0.3558217	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
9511	0.3433989	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
11081	0.3276447	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4331	0.3170957	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;uncultured;uncultured_ge
542	0.3075183	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula

1605	0.305922	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
8223	0.2972468	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11047	0.2934298	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8293	0.2853098	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
9857	0.2769816	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;DEV007;DEV007_ge
10057	0.2719153	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
8779	0.2510254	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8906	0.2482493	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
3328	0.225902	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
549	0.215353	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5879	0.2141732	Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae;Haliangium
1315	0.1884946	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
1236	0.1876618	Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae;Haliangium
8258	0.1735733	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7865	0.1724629	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
3356	0.1585825	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
10058	0.1571251	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
3656	0.1548349	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
8878	0.1449104	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
13585	0.1437306	Bacteroidetes;Sphingobacteriia;Sphingobacteriales
8083	0.1358188	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
8918	0.1347084	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
3112	0.1336674	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
7277	0.132904	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
1498	0.1317935	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoracaceae;Peredibacter
3314	0.1315853	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3571	0.1262414	Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio
11232	0.1247146	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
782	0.1204811	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8296	0.1136797	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
4453	0.1107649	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
10043	0.1061844	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
5225	0.1054904	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
10037	0.1040329	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
6188	0.1028531	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
8917	0.1027837	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
8016	0.1023673	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11352	0.1002852	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured

C_H_ASV	C_H_%	C_H_tax
830	12.1395034	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3422	11.1063058	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
2241	10.1459472	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5872	4.9398146	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
13813	2.9636405	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
4677	2.7289827	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
9638	2.0740517	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Profundibacterium
12987	1.7835231	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
7732	1.5548663	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11668	1.4365027	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
9841	1.2734425	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
5923	0.9346993	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
8596	0.8753107	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
9947	0.6468608	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
11065	0.6431361	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Fabibacter
7109	0.6323757	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
13866	0.6015433	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11717	0.5967839	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
8879	0.5926453	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
12284	0.5537426	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
12246	0.5504318	Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
9505	0.5092528	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
10069	0.4904223	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
12266	0.4864906	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Simiduia
1096	0.461866	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
9696	0.4554512	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudoruegeria
12933	0.4440701	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7468	0.4405523	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
4713	0.4372415	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
464	0.4033051	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
12295	0.4024774	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
11634	0.4008219	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
9503	0.3923378	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
5177	0.3821983	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
12517	0.3815775	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
12225	0.3813706	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae
6416	0.3759904	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
6251	0.3679202	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
914	0.3617123	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group

4711	0.3612984	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
13026	0.3552975	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
4575	0.3524005	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;Nannocystis
13864	0.3521936	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
9428	0.3513658	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
12147	0.3486758	Lentisphaerae;Oligosphaeria;P.palmC41;P.palmC41_fa;P.palmC41_ge
10983	0.3213611	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
13908	0.3190849	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Spongiibacteraceae;BD1-7_clade
8624	0.3190849	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
3834	0.3149463	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
3340	0.3103938	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae
11637	0.3085315	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
3636	0.3027375	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Leptobacterium
4451	0.2944603	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
6656	0.2934257	Verrucomicrobia;Opitutae;Puniceicoccales;Puniceicoccaceae;Lentimonas
12980	0.2928049	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
7427	0.2872178	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
10967	0.2801822	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
8880	0.277906	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4926	0.2764575	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Reichenbachiella
13048	0.2733535	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
10278	0.2721119	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Flexithrix
9643	0.2706634	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7334	0.2702496	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
1637	0.2644556	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
8354	0.2636278	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter
3346	0.260317	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3720	0.2586615	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
403	0.2516259	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
10068	0.251419	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
12296	0.2507982	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
11369	0.2350716	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
13206	0.2265875	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
5452	0.2247251	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11831	0.222242	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
10966	0.2212073	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
11912	0.2195519	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
13855	0.2058946	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
4485	0.2044461	Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulforhopalus
4712	0.2027906	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola

8355	0.1998936	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter
4134	0.1938927	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
9402	0.1918234	Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Arcobacter
9521	0.1918234	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
3931	0.1876848	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Riemerella
6888	0.1847878	Proteobacteria;Alphaproteobacteria;Rhizobiales;uncultured;uncultured_ge
2929	0.1837532	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
11338	0.1765106	Lentisphaerae;Lentisphaeria;Lentisphaerales;Lentisphaeraceae;Lentisphaera
2305	0.1734067	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
12170	0.1731998	Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
3625	0.1655434	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;uncultured;uncultured_ge
13021	0.1645087	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
9706	0.1576801	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
6559	0.1545761	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maritimimonas
11113	0.1535415	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
9642	0.1525068	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
10242	0.1508514	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Reichenbachiella
10650	0.1481613	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
11693	0.1477475	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
7057	0.1475405	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
10499	0.1475405	Proteobacteria;Deltaproteobacteria;Myxococcales;BIrii41;BIrii41_ge
8599	0.1444366	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Spongiibacteraceae;BD1-7_clade
9842	0.1421604	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11806	0.1413327	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Hoeflea
1102	0.1407119	Verrucomicrobia;Spartobacteria;Chthoniobacterales;Xiphinematobacteraceae;Candidatus_Xiphinematobacter
11337	0.1382287	Lentisphaerae;Lentisphaeria;Lentisphaerales;Lentisphaeraceae;Lentisphaera
9444	0.1351248	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter
11407	0.1328486	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
3270	0.1299516	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7058	0.1293308	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
1370	0.1289169	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
13869	0.12871	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
5451	0.1272615	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
9845	0.1225021	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11818	0.119812	Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;Nannocystis
1266	0.119812	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7734	0.1185705	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11716	0.1183635	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
7280	0.1175358	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Salinihabitans
13959	0.1165012	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Perspicuibacter

7436	0.1162942	Proteobacteria;Gammaproteobacteria;E01-9C-26_marine_group;E01-9C-26_marine_group_fa;E01-9C-26_marine_group_ge
7491	0.1156734	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Psychrosphaera
5362	0.1150527	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;DEV007;DEV007_ge
13014	0.1121556	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter
9816	0.1109141	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
7185	0.1109141	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
2813	0.1096725	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Simiduia

C_R_ASV	C_R_%	C_R_tax
3270	29.9133804	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
914	5.624463	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169
718	3.8589246	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5977	3.3089559	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3514	3.0207319	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7732	2.3703707	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
8596	1.9914695	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
8015	1.873932	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5872	1.7201999	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
4150	1.703817	Proteobacteria;Gammaproteobacteria
6083	1.3586341	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7735	1.3473946	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11632	1.3331073	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
7278	1.1828041	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
10966	1.1753747	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
8917	1.1111767	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
5978	1.0446928	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
5650	1.0081171	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
13870	0.9681125	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
2929	0.9540156	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
11668	0.913249	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
3657	0.7509444	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
546	0.7042723	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
8918	0.7029388	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
8293	0.7017958	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
2619	0.6576002	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
1315	0.6452178	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
3112	0.6427413	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
13813	0.623882	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
4409	0.5901638	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8730	0.5825439	Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium
2962	0.5808294	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
3363	0.5806389	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8338	0.5223464	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
3314	0.5008201	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
6214	0.4911047	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5177	0.4893902	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
549	0.4333837	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
782	0.4312882	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

10057	0.3922361	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
5923	0.3665188	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
7277	0.3251806	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
3057	0.3137507	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11093	0.3055593	Bacteroidetes;Flavobacteriia;Flavobacteriales;Cryomorphaceae;Crocinitomix
3246	0.2867	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11047	0.2743176	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8292	0.2729841	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
6989	0.2722221	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Aureispira
7279	0.2705076	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
7075	0.2520293	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5225	0.2394564	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
4331	0.2318365	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;uncultured;uncultured_ge
11720	0.2287885	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
10160	0.2272645	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Robiginitomaculum
5087	0.2194541	Proteobacteria;Gammaproteobacteria;Cardiobacteriales;Cardiobacteriaceae;uncultured
7403	0.2124056	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2806	0.2076432	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
1605	0.2011662	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
8297	0.1994517	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
10967	0.1943083	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
542	0.1922128	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3117	0.1887838	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
10075	0.1842119	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
4711	0.1794494	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
11759	0.1784969	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
7406	0.165924	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
3556	0.165924	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
2305	0.1623046	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
8016	0.1588756	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
3328	0.1569706	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5788	0.1563991	Cyanobacteria;Cyanobacteria;SubsectionII;FamilyII;Pleurocapsa
3330	0.1529702	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12337	0.1523987	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
7865	0.1520177	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
6747	0.1514462	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
13585	0.1506842	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11831	0.1468742	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
8258	0.1449692	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8294	0.1388733	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter

8782	0.1386828	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
6188	0.1383018	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
11972	0.1348728	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
8296	0.1329678	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
7541	0.1312533	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
7849	0.1308723	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;uncultured;uncultured_ge
6926	0.1280149	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
8376	0.1251574	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7405	0.1217284	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5793	0.1186805	Cyanobacteria;Cyanobacteria;SubsectionII;FamilyII;Pleurocapsa
4384	0.118109	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8223	0.1179185	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
3513	0.116585	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
6732	0.1163945	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
13692	0.1156325	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
8298	0.114299	Proteobacteria;Gammaproteobacteria;uncultured;uncultured_fa;uncultured_ge
3212	0.114299	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
11002	0.111251	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Simiduia
8083	0.1106795	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
5979	0.1055361	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
600	0.1040121	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
6923	0.1038216	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
3278	0.1036311	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

C_V_ASV	C_V_%	C_V_tax
3270	29.4167696	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2929	6.9355735	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
914	5.9183248	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
3514	4.6073865	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5977	4.1400147	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
718	4.0680572	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7278	3.070966	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
4150	2.4245209	Proteobacteria;Gammaproteobacteria
8015	1.8005771	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
11632	1.5736884	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
6083	1.493293	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8293	1.2361681	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
546	1.1717111	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
13813	1.1435844	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5978	1.134912	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3657	1.1332713	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5650	1.0892061	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
4451	1.0617826	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8596	1.0301401	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
7279	0.8719275	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
3112	0.7598895	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
10057	0.6956668	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
3314	0.6009737	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
549	0.5951139	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
11759	0.5946451	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
4386	0.5819881	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
5087	0.5360479	Proteobacteria;Gammaproteobacteria;Cardiobacteriales;Cardiobacteriaceae;uncultured
6926	0.5318289	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
11047	0.4612777	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7277	0.421666	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
8917	0.3949456	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
8292	0.3853357	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3513	0.3623655	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3255	0.3506461	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
6214	0.3473646	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
8297	0.3412705	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
782	0.3340045	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8338	0.3286135	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella

8918	0.3248633	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
3057	0.3199411	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
3363	0.2939239	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
542	0.2864235	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
8298	0.2711882	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
10075	0.270485	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
8016	0.2657972	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4409	0.2498588	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4970	0.23978	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Fretibacter
600	0.2161068	Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade_fa;KI89A_clade_ge
11888	0.214466	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
6747	0.1905583	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
11720	0.1846986	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11255	0.1790733	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
8781	0.1767294	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
2962	0.175323	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
5788	0.1664163	Cyanobacteria;Cyanobacteria;SubsectionII;FamilyII;Pleurocapsa
2806	0.1647755	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
1170	0.1643068	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Hellea
3306	0.163838	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8294	0.1502434	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
6188	0.14579	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
2210	0.14579	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
719	0.1382896	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
6232	0.1368832	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
7541	0.1338362	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
5793	0.1296172	Cyanobacteria;Cyanobacteria;SubsectionII;FamilyII;Pleurocapsa
7865	0.1261013	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
548	0.1253982	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3848	0.1235231	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Lacinutrix
8083	0.1211792	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
5177	0.1193041	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
6732	0.1176633	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
5225	0.1167258	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
3345	0.115085	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8782	0.1143819	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
1315	0.1141475	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Sva0996_marine_group;Sva0996_marine_group_ge
3246	0.1111004	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

W_H_ASV	W_H_%	W_H_tax
3422	13.08191	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
830	9.661449	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2241	8.820112	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2929	5.005387	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
9638	4.954496	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Profundibacterium
13813	4.918392	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
10984	3.304854	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
10966	3.029849	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
5872	2.812842	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
4677	2.662473	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
11717	1.477764	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
914	1.43225	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_group
11668	1.022048	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
8051	1.013214	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
7057	1.010142	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
9503	0.908936	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
7729	0.864382	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
2305	0.859581	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
10977	0.792558	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
9841	0.761063	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
2606	0.724575	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
4532	0.649679	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Cobetia
9505	0.574398	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
9642	0.555386	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11740	0.53695	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
10069	0.51909	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
4451	0.498734	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
10983	0.478953	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
12933	0.453988	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5177	0.45322	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
9816	0.434976	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
7427	0.409434	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5923	0.397911	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
11673	0.391382	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
11634	0.364112	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
3346	0.355662	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11831	0.344716	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter
11912	0.335498	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
13026	0.315717	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae

3903	0.308036	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Salegentibacter
12284	0.293633	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
9521	0.293056	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
11898	0.27462	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
4711	0.266747	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
4748	0.266747	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
9438	0.265594	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
1638	0.259449	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Tunicatimonas
11407	0.258105	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
12295	0.255608	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
9446	0.25292	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter
3270	0.250615	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7109	0.243317	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
9643	0.2389	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
464	0.233523	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
10967	0.227762	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
8596	0.225073	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
13908	0.219312	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
9704	0.216432	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
6251	0.215087	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
12517	0.212207	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
5451	0.208366	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
7734	0.207406	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
6794	0.207214	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
7468	0.203757	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9706	0.196267	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia
7801	0.194347	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
4713	0.192042	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
11793	0.188201	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
12296	0.187817	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
1604	0.173414	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
4717	0.17111	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
7377	0.167077	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
4134	0.159587	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
10279	0.15517	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Flexithrix
10257	0.152289	Bacteroidetes;Cytophagia;Cytophagales;Flammeovirgaceae;Fulvivirga
11716	0.151521	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
3834	0.14768	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;uncultured
9947	0.14672	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
9444	0.146144	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter

10068	0.145376	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
8879	0.138462	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
6622	0.137118	Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae
13206	0.135582	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
12336	0.135006	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
8353	0.12886	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Cocleimonas
403	0.12694	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
315	0.125788	Proteobacteria;Alphaproteobacteria;E6aD10;E6aD10_fa;E6aD10_ge
2380	0.123099	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Aureispira
12297	0.121179	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
9506	0.120026	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
11720	0.118682	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7732	0.115994	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
7058	0.115801	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
6888	0.114457	Proteobacteria;Alphaproteobacteria;Rhizobiales;uncultured;uncultured_ge
5749	0.112345	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
11113	0.110808	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
658	0.11004	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Ulvibacter
11806	0.107544	Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Hoeflea
5450	0.103127	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11820	0.10159	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
4014	0.100246	Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Marinicella

W_R_ASV	W_R_%	W_R_tax
10966	18.6851794	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
3270	15.9485988	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
914	9.523451	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169
7729	9.0120562	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
2929	5.9653764	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
8051	3.8649473	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
5872	3.241881	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
12003	2.4237927	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas
10984	1.5347221	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
7519	1.361927	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas
3514	1.2916617	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7801	1.2857466	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
3255	1.0991493	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11718	1.0600732	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
718	0.9910627	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5977	0.9001839	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
8267	0.8605701	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
7735	0.7544552	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
13813	0.5752071	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
6232	0.5732354	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
3314	0.5707259	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5923	0.5148005	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
7732	0.4923945	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3657	0.4818189	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
11888	0.4757244	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
11255	0.4569034	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
3112	0.4491957	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
4711	0.4210538	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
6083	0.3629774	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
10968	0.355449	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
11673	0.3507885	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
3345	0.3425431	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
13859	0.3233636	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
11717	0.2645702	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
12138	0.2643909	Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Staphylococcus
7155	0.2640324	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5994	0.2631362	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
546	0.2446736	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
13692	0.2389377	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter

11740	0.2210129	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
5650	0.2029088	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
3057	0.1948427	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5078	0.1928709	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
7278	0.1894652	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
7279	0.1830123	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
10057	0.1790688	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
9145	0.1776349	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3246	0.1731537	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12165	0.1464457	Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
12160	0.1450117	Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
10983	0.1425022	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
2305	0.1356908	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
5177	0.1349738	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
8015	0.1338983	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
542	0.1256529	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
10075	0.1217095	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
11720	0.1204547	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11632	0.1140018	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
7541	0.1140018	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
1500	0.1080866	Proteobacteria;Deltaproteobacteria;Oligoflexales;Oligoflexaceae;Oligoflexaceae_ge
2210	0.1041431	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
11759	0.1019922	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge

W_V_ASV	W_V_%	W_V_tax
3270	36.0211776	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2929	24.7890642	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
914	8.5699626	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169
3514	2.8924717	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5977	1.9675066	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
718	1.8414168	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3657	1.7183182	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
3314	1.5821045	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
546	0.9314076	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
6083	0.8060081	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
4657	0.7530872	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Pricia
3246	0.7344499	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5650	0.6760068	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
13813	0.5996167	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
7278	0.5733863	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
3112	0.5328903	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
7801	0.5312797	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
5078	0.5064299	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
542	0.4120927	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
11637	0.32949	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
8015	0.321667	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
7729	0.3143041	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
8051	0.2915251	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
10057	0.2889941	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
11568	0.2770293	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae
8917	0.2756488	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
7279	0.2669053	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
5978	0.2287103	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7405	0.2015596	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
11720	0.1891347	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
12336	0.182462	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
11759	0.1822319	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
13692	0.1787806	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
7277	0.1665858	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
3255	0.1606034	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
4150	0.1557715	Proteobacteria;Gammaproteobacteria
7690	0.1527803	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Cellulophaga
11740	0.1525502	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
7406	0.1502493	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae

719	0.1458776	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera
549	0.136904	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
4822	0.1283907	Actinobacteria;Actinobacteria;Micrococcales;Dermacoccaceae;Kytococcus
10075	0.1265499	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
11047	0.1201074	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
2934	0.1145852	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
5177	0.109063	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
7541	0.1042311	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tateyamaria
10966	0.104001	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
3306	0.1028506	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus

D_H_ASV	D_H_%	D_H_tax
830	18.9767434	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
2241	8.4126635	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9638	8.0039808	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Profundibacterium
7729	7.7887902	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3422	7.6276012	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
13813	4.2739561	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9841	3.9107913	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
4532	3.8585844	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Cobetia
2606	2.4047835	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
9696	2.3670966	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudoruegeria
2929	2.2295638	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkhold
8051	2.2080285	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
11673	1.8078294	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
4677	1.8058717	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Porticoccaceae;C1-B045
11740	1.1695992	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
5872	1.1457798	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
2305	0.6101689	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
7405	0.5804762	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5451	0.5431156	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
11912	0.5312059	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
5453	0.5060813	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
10977	0.475736	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
3903	0.4750834	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Salegentibacter
5450	0.4617054	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
464	0.426792	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
9503	0.4196135	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
6794	0.4166769	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
9842	0.4147191	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
6888	0.3767059	Proteobacteria;Alphaproteobacteria;Rhizobiales;uncultured;uncultured_ge
5177	0.3641436	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
9816	0.3470132	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
3902	0.2975797	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Nonlabens
13026	0.2811019	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
4451	0.2784915	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
315	0.2735971	Proteobacteria;Alphaproteobacteria;E6aD10;E6aD10_fa;E6aD10_ge
10069	0.2732708	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea
11637	0.2398258	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina
914	0.2339525	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169_marine_g
11634	0.2261214	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Aquimarina

3963	0.214538	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
9505	0.1835401	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Winogradskyella
1604	0.1735882	Actinobacteria;Acidimicrobiia;Acidimicrobiales;uncultured;uncultured_ge
13692	0.1714673	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
403	0.171141	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;uncultured
12933	0.166736	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11898	0.1587418	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9446	0.1553157	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Algibacter
9947	0.1510739	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
13206	0.1484636	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Persicirhabdus
7468	0.1481373	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
9845	0.1476478	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter
8596	0.1442217	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
9642	0.1427534	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
14451	0.1425903	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
11774	0.1407957	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Defluviimonas
11769	0.1360644	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
3270	0.1316594	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
12336	0.1280702	Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix
4134	0.124481	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
12517	0.1243178	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;uncultured
5749	0.1243178	Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella
8729	0.1208918	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Novosphingobium
11793	0.1186077	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
7801	0.1148553	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
11113	0.1119187	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
11717	0.1109398	Proteobacteria;Gammaproteobacteria;Cellvibrionales;Cellvibrionaceae;Simiduia
2813	0.1109398	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter
11668	0.1097978	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
5078	0.1050665	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
5250	0.1027825	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Rubritalea

D_R_ASV	D_R_%	D_R_tax
7729	23.1438612	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3270	16.6404359	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7519	10.4207877	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas
914	9.0358588	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169
7732	6.5384595	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
7735	6.0832844	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
8051	4.8352584	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
2929	3.497491	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholderia
12003	2.4451176	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas
10966	2.0632448	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
2606	1.6635855	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Paraglaciecola
3514	1.2345513	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7279	0.6066306	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
10977	0.570249	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
3112	0.5511149	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
5977	0.5225485	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
718	0.4899398	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5650	0.4322681	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
6083	0.371093	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7278	0.3457606	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
13870	0.3376758	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3314	0.2983297	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8917	0.2972517	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maribius
8015	0.2576361	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
4532	0.2495513	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Cobetia
7733	0.2460479	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3903	0.2441614	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Salegentibacter
546	0.2403885	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
11740	0.2255663	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
3657	0.2129001	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
10075	0.2112832	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
6747	0.2040068	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
549	0.1797524	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
2619	0.1708591	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Reinekea
11673	0.153342	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella
5923	0.1490301	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
10984	0.1452572	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
5978	0.1242367	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
542	0.1169603	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula

13813	0.1121094	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter

D_V_ASV	D_V_%	D_V_tax
3270	38.4100416	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
2929	18.1270654	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Burkholderia-Paraburkholde
914	15.5820365	Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;AEGEAN-169
7729	2.2323909	Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
3255	1.5228833	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
8051	1.1079273	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
718	1.0973338	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
7279	1.0355813	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
6232	0.8268114	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
7278	0.725527	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
5977	0.7151919	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
3514	0.7123497	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
13813	0.636128	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
11255	0.6358697	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Portibacter
11888	0.5495712	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
3345	0.5149485	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3657	0.4787755	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
5994	0.4059127	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;uncultured
5650	0.382917	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
546	0.3803333	Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula
7405	0.3769743	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Octadecabacter
3314	0.3250402	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5177	0.3121213	Actinobacteria;Acidimicrobiia;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
10057	0.2958435	Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus
13692	0.2880921	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
12003	0.2847332	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromo
7519	0.264838	Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromo
3112	0.2596705	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Rubidimonas
11759	0.2495937	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
6083	0.2459764	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
7801	0.243651	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
9145	0.2392586	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
3246	0.1955926	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11740	0.1769893	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
7277	0.1744055	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
4749	0.1676877	Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;Maribacter
4532	0.1555439	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Cobetia
95	0.127639	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
11720	0.1237633	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae

13827	0.123505	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Defluviimonas
8267	0.1232466	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aliiglaciecola
7958	0.1222131	Bacteroidetes;Flavobacteriia;Flavobacteriales;NS9_marine_group;NS9_marine_group_ge
9187	0.1139449	Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus
5978	0.1061936	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
2210	0.1051601	Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularculaceae_ge
12831	0.1051601	Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae;Lewinella
10633	0.1038682	Proteobacteria;Alphaproteobacteria;Caulobacterales;Hyphomonadaceae;Litorimonas
5078	0.1023179	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella
719	0.1012844	Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera

BIOGRAPHY OF THE AUTHOR

Charlotte Terry Carrigan Quigley was born on June 29, 1987 in Boston, Massachusetts. She grew up in Hanover, Massachusetts, and then moved to Dover, Massachusetts where she graduated from Dover-Sherborn Regional High School in 2005. She was accepted at Colby College in Waterville, Maine, where she majored in Biology and minored in Creative Writing. Completing the requirements for her major in the fall of her junior year allowed her the opportunity to study abroad at the University College Cork in Ireland in the Spring of 2008, where she worked on her writing minor. During her senior year, she completed a senior project entitled, "Effect of variation in environmental conditions on exercise physiology in the invasive Green Crab, *Carcinus maenas*." Charlotte earned her Bachelor of Arts degree *cum laude* from Colby College in May, 2009.

She accepted a position abroad as a Resident Naturalist and Administrative Assistant at the University of Georgia Ecolodge in San Luis, Costa Rica, where she led educational courses and experiences for undergraduate students and eco-tourists. In 2010, she moved to the main campus of the University of Georgia in Athens, Georgia, where she was a research technician in the Department of Plant Biology for three years. Her contribution to projects from phylogeography to plant transcriptomics earned her co-authorship on three scholarly publications (published and in review).

Charlotte matriculated at The University of Maine in September 2013 as a member of Dr. Susan H. Brawley's laboratory. She received a $15,000 grant from the USDA SARE program for her projects relating to sea vegetable aquaculture. She received a four-year fellowship from the NSF EPSCoR program as a member of the Sustainable Ecological Aquaculture Network (SEANET); she acted as graduate student liaison for SEANET from 2016 to 2017. She has published one of her dissertation chapters in the *Journal of Phycology* (Quigley et al. 2018). She is a member of the Phycological Society of America, the American Society of Plant Biology, and the Northeast Algal Society. Charlotte T. C. Quigley is a candidate for the Doctorate of Philosophy degree in Marine Biology from the University of Maine in December 2018.