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Bioactive Compounds of Important Macroalgae in New England

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BIOACTIVE COMPOUNDS OF IMPORTANT MACROALGAE IN NEW ENGLAND

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Introduction

When most people hear the word seaweed, they think about the stuff that gets washed up on beaches or the kelp forest featured in films like *Finding Dory*. Some may have even heard about seaweed through a collection of odd laws throughout the United States, where in New Hampshire it is illegal to collect and remove seaweed from beaches throughout the night (*Section 207:48 In Night.*, n.d.).

Seaweed is also known as marine macroalgae and can be found in the ocean as well as washed up on beaches. Macroalgae are large, visible organisms that are multicellular, while microalgae are unicellular or simple multicellular organisms and typically require a microscope to be viewed as individual organisms. Marine macroalgae can be grouped into three different phyla. There are the green (*Chlorophyta*), the brown (*Phaeophyta*), and the red (*Rhodophyta*) phyla that macroalgae are classified into. *Chlorophyta* and *Rhodophyta* are considered to belong to the plant kingdom under the supergroup Archaeplastida while *Phaeophyta* belong to the Chromista kingdom under the supergroup of Stramenopila as seen in **Figure 1** (Burki et al., 2020; *Systematics of the Chromista*, n.d.). The difference underlying how these macroalgae are classified is in their pigments, how they collect and store energy, and their evolutionary relationships (*Non-Native Seaweed in Massachusetts*, 2013).

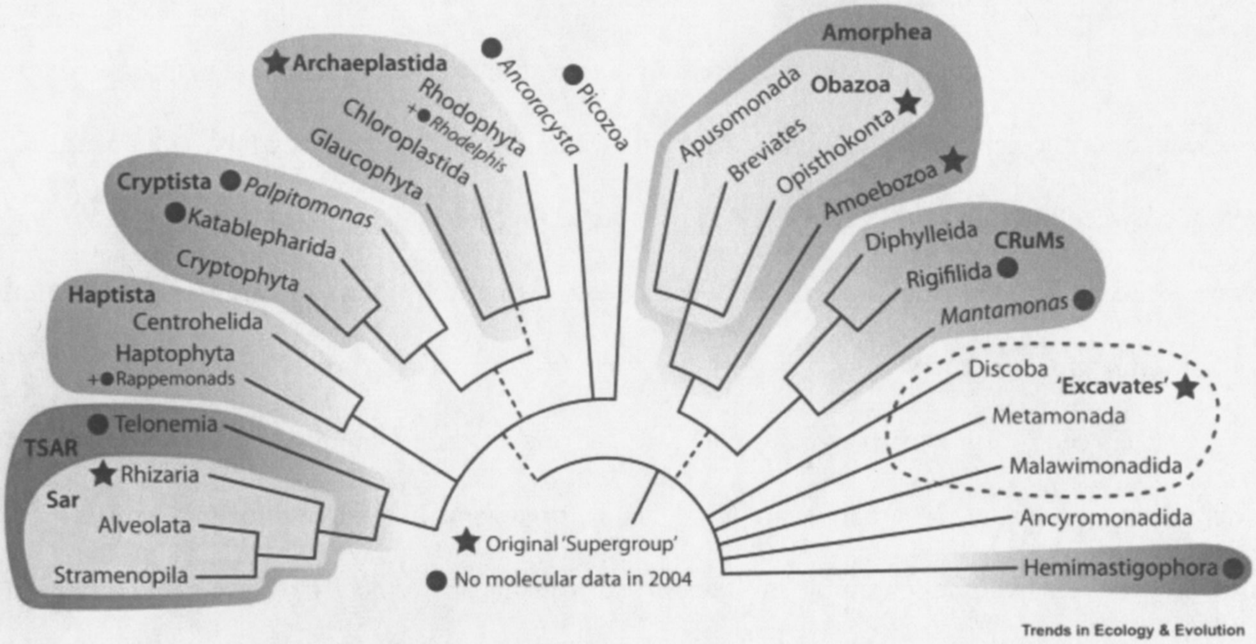


Figure 1 The phylogenetic tree featuring the branching of the green algae (*Chloroplastida*), red algae (*Rhodophyta*), and brown algae (*Stramenopila*). This phylogenetic tree demonstrates only one representative species per phyla. A phylogenetic tree is a visual representation of these evolutionary relationships from a common ancestor. While a tree is suggestive of the evolutionary relationships, it is not a definitive evolutionary history. (Burki et al., 2020)

Macroalgae can be found all over the world in different marine environments. The ecosystems that macroalgae are a part of are in estuaries, rocky coastlines, sandy beaches, and coral reefs among others (Baweja et al., 2016). Macroalgae grow in the environments that support their physical, chemical, and biological needs like light availability, substrates to grow on, and the availability of nitrogen and phosphorus to perform biological functions. In these environments, macroalgae serve as the base of marine food webs (Baweja et al., 2016). The most hospitable environments for macroalgae can be found along the coastlines of the world, but some also occur as free-floating mats further out in the ocean, like the floating mat of *Sargassum* seaweeds known as the *Sargassum* belt in the Atlantic Ocean that stretches from Western Africa to the Caribbean (Wang et al., 2019) **Figure 2** displays the global distribution of seaweed growth.

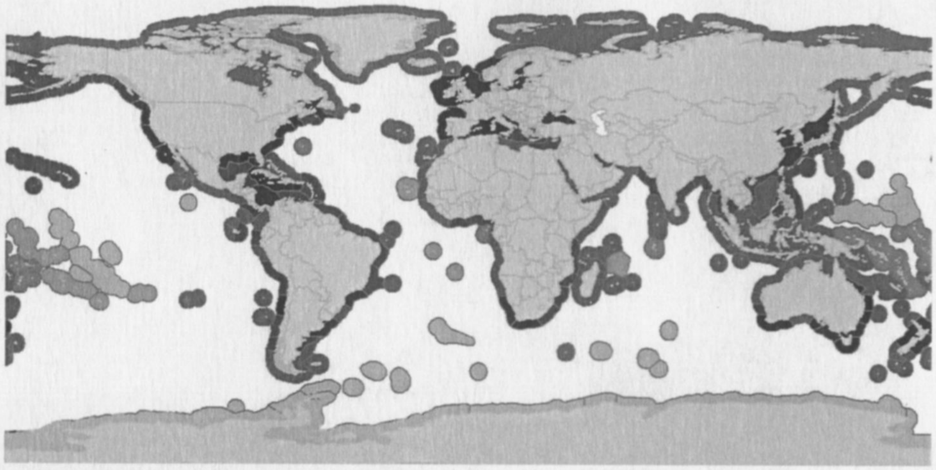


Figure 2 The areas highlighted in blue on the ocean indicate where macroalgal growth occurs.

While macroalgae are common along the coastlines of the world and all three types can be found, some species of macroalgae appear in larger abundances than others based on their location in the intertidal and sub-tidal zones in which they reside, as depicted in **Figure 3**. Intertidal regions are the areas between high-tide lines and low-tide lines, and subtidal regions are below the low-tide lines and are constantly underwater (*Story Map Series*, n.d.).

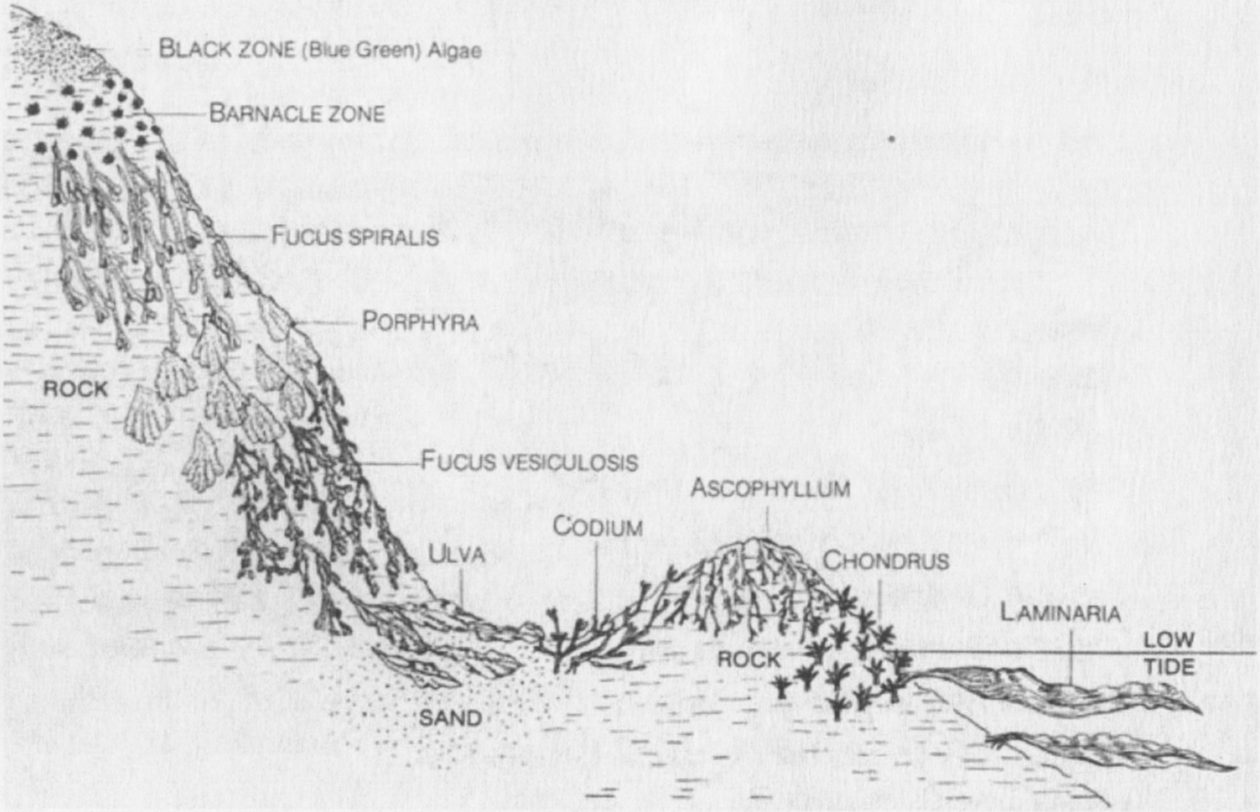


Figure 3. A pictorial representation of the intertidal and subtidal regions. The low tide line on the right represents the beginning of the subtidal region, each species of seaweed above this line are considered to be in the intertidal region (Taylor & Villalard, 1985).

Within these tidal regions, invasive species can be found alongside native species. An invasive species is considered to be organisms that are not native or originate in an ecosystem and cause harm to native species by outcompeting for natural resources (*Invasive Species*, n.d.). Invasive species are a widespread environmental problem threatening ecosystems worldwide on land and sea, through direct threats of preying on native species, outcompeting them for food and resources, introducing new diseases and affecting the reproduction of native species. Indirect threats consist of changing food webs through replacing the native food sources or providing little food value to other wildlife (*Invasive Species*, n.d.). Examples of invasive species that have invaded and found a home in the United States are the Burmese python, which inhabits the everglades in Florida, and milfoil, a freshwater plant that has invaded at least ninety different bodies of water in Maine and New Hampshire (Society, 2011; "The invasive species that nobody is talking about," n.d.). There are some species of macroalgae that are invasive to different regions and have been growing more rapidly than the native species, depleting native numbers through competition for resources and lack of a natural predators. Invasive species of macroalgae have been introduced to New England waters through shipping and aquaculture, cultivation of aquatic plants and animals for food (*Non-Native Seaweed in Massachusetts*, 2013).

Since the earliest record of macroalgal use, civilizations across the globe have been harvesting macroalgae as a food source, medicines, iodine supplements, and hydrocolloids (Baweja et al., 2016). Some of the earliest records of macroalgal utilization by humans date back as far as at least 35,000 BCE when Tasmanians used kelp to craft water carriers (Baweja et al., 2016). Other accounts dating back 1500 years have seaweed first being consumed by the Japanese (*Seaweed.Ie :: Information on Marine Algae*, n.d.). In 600 BCE, Sze Teu had written in China that "Some algae are a delicacy fit for the most honored guests, even for the king himself" (*Seaweed.Ie :: Seaweed as Human Food*, n.d.). In the Greek and Roman Empires, seaweed from the Mediterranean have been used as medicines and as early as 100 BCE, the ancient Greeks had been using a red algae species to treat parasitic worm infections (ethnoherbalist, n.d.).

Later during the Asuka Era (600-700 CE) and the Nara Era (700-800 CE), it was said that only the Japanese aristocracy consumed seaweed because only wild seaweed was available, limiting the food source (*Seaweed.Ie :: Information on Marine Algae*, n.d.). The species *Palmaria palmata*, commonly known as dulse and in Icelandic as sol, had first been recorded as being used in Iceland in 961 CE, where in the Icelandic Sagas it was recorded as a highly valued food source (ethnoherbalist, n.d.).

There are records of *Porphyra* being eaten in Wales as early as 1600 CE (*Seaweed.Ie :: Seaweed as Human Food*, n.d.). It was not until the Middle Ages in Japan that seaweed was domesticated during the Tokugawa Era (1600-1800 CE). The shogun, Ieyasu Tokugawa had ordered fishermen in the town of Shinagawa (presently a part of the Tokyo metropolitan area) to bring him fresh fish daily. In order to fulfill the shogun's orders, the fishermen built an offshore fence to start a fish farm, where they found that seaweed preferred to grow on the fence, beginning the cultivation of seaweed (*Seaweed.Ie :: Information on Marine Algae*, n.d.).

In more recent years, macroalgae have still been utilized as a nutrition source and as part of drug and bioactive compounds discovery (Baweja et al., 2016). When French chemist, Bernard Courtois, was working in 1811 to extract saltpeter (Potassium nitrate), a strong oxidizer, from seaweeds as a gunpowder for Napoleon, he noticed the seaweed ashes released a violet-colored gas and crystals, iodine (PubChem, n.d.; *The Science of Seaweeds*, 2017). In the beginning of the 19^{th} century, *Chondrus crispus* had been recommended as a health remedy in Ireland (*Seaweed.Ie :: Seaweed as Human Food*, n.d.).

Closer to home in New England, farmers have used macroalgae as fertilizer and animal feed supplements, and as additives in pharmaceuticals and food. The benefit of using macroalgae in this way is because when they break down, macroalgae add their organic matter back to the environment (White & Keleshian, 1994).

More recently, researchers have been looking into the potential antimicrobial properties that macroalgae contain. Since macroalgae are renewable, eco-friendly, and an easily obtainable source of bioactive compounds (Parsaeimehr & Lutzu, 2016), the use of macroalgae to study new origins to combat antimicrobial resistance stems from microbes becoming resistant to antimicrobials in circulation, like penicillin. Bacteria, like *Streptococcus pneumoniae, Neisseria gonorrhoeae, Listeria*, and *Clostridium* have grown and adapted to become resistant to penicillin by the utilization of a penicillinase enzyme that cleaves a chemical bond in the structure of penicillin, rendering it useless against the bacteria (CDC, 2020). The bioactive compounds of interest in macroalgae have been shown to exhibit antibacterial properties, which has resulted in the ability to stunt or completely stop the growth of microorganisms (Pérez, Falqué, & Domínguez, 2016).

As there are no previous studies that compile antimicrobial properties of invasive species of macroalgae in New England, I believe that it would be beneficial to the region to determine the usefulness of the bioactive compounds found in the invasive species that can then lead to a method for the management of the invasive species. By compiling known information about the antimicrobial properties of abundant native and invasive macroalgae for identification of those with antimicrobial compounds will be used to as a means of determining which species would be suitable for future potential as antimicrobial products. A further understanding of the uses of the antimicrobial activity of the invasive macroalgae will not only provide a better picture on how to manage the invasive species, but also a renewable and eco-friendly source of bioactive compounds that are able to fight microbes.

Biology of Seaweeds

The basic body structure of the seaweed is composed of a thallus (a plant with no real roots, stems, or leaves - these anatomical terms only apply to vascular plants) which could be branched filaments, hollow tubes/bladders, spongy or solid, or a simple or compound blade (Taylor & Villalard, 1985). The holdfast is a section of the seaweed that attaches the thallus to the substrate it lives in or on. In some species, a stipe is present, connecting the holdfast to the blade, like in the *Laminaria* (Taylor & Villalard, 1985). It is also possible for some species of seaweed to grow on another plant, usually other algae, these are known as epiphytes (Taylor & Villalard, 1985).

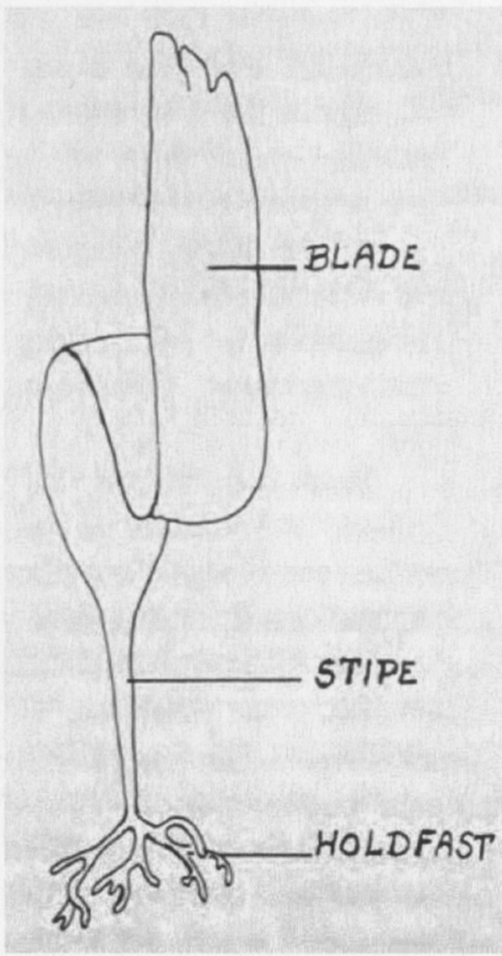


Figure 7. Simple body structure of a *Laminaria* (Taylor & Villalard, 1985).

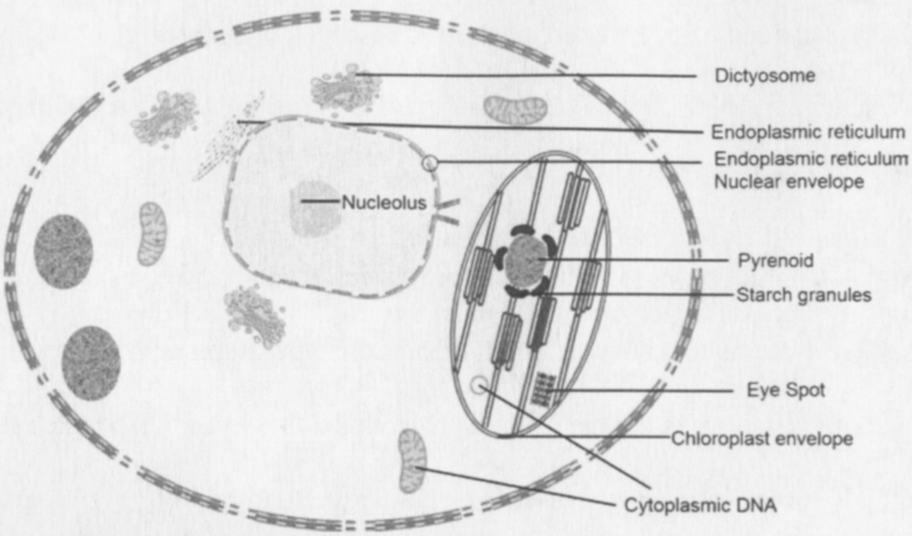
Seaweed thalli can be classified into different categories: filamentous, cylindrical, siphonous and siphonocladous, flattened or foliaceous, complex, and others like spheres, fans, cups, and balls. Filamentous are formed from vegetative divisions on the transverse plane, resulting in branched and heterotrichous seaweeds (Baweja et al., 2016). Cylindrical are formed from vegetative division of the traverse plane in circular orientations, resulting in mostly branched seaweeds (Baweja et al., 2016). Siphonous and siphonocladous are formed by enlarged and elaborated thalli that do not follow cytokinesis in nuclear divisions, with siphonous organized as saccate, uniaxial, and multiaxial, and siphonocladous are special to green seaweeds and are organized as filaments and multinucleate (Baweja et al., 2016). The flattened or foliaceous thalli form as primary filament cells that divide in all directions, losing the filamentous structure (Baweja et al., 2016). Complex thalli are flat foliose or tubular that form from the division of cells in more than two planes (Baweja et al., 2016).

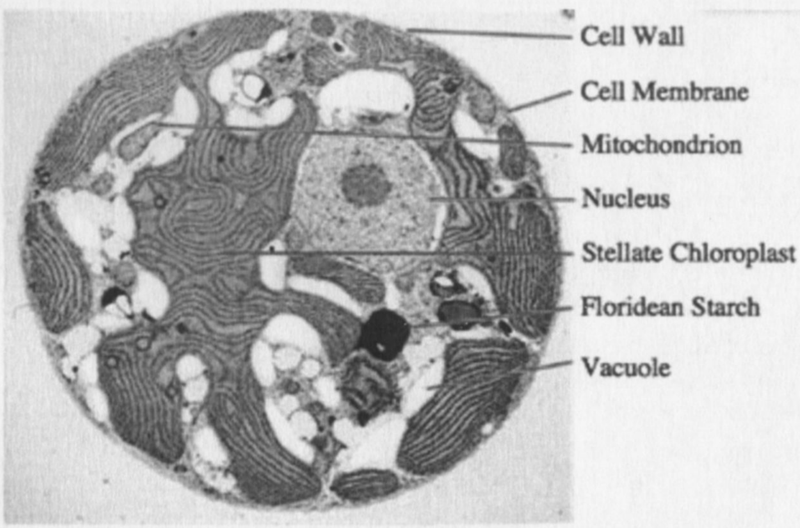
While the holdfast may look like roots, the only purpose is to attach the seaweed to ocean floor or other solid structures. Unlike land plants, seaweed do not absorb any nutrients or water from their roots, rather, their nutrients are absorbed through the blades via the water column (*The Botanical Ocean*, n.d.). Similar to land plants, seaweeds perform photosynthesis as a means of obtaining food (*The Botanical Ocean*, n.d.). Through photosynthesis, carbon dioxide and water are absorbed through the blades and light from the sun is captured in the chloroplast and converted to useable energy to create sugars for the seaweed to use. A byproduct of photosynthesis is oxygen, and it is estimated that 30-50% of the net global oxygen available to humans is produced by all photosynthetic algae (*The Botanical Ocean*, n.d.). While seaweeds are not as complex as land plants, they are still primary producers. Being at the base of the food chain, they are relied upon by most marine life, and it is estimated that between 2-10% of global primary production results from seaweed (*The Botanical Ocean*, n.d.).

Of all green algae present on Earth, only about 10% is found in the oceans and are globally distributed but green seaweeds tend to reside in shallow coastal waters (Baweja et al., 2016). Green algae are multicellular, oxygenic, and photosynthetic eukaryotes, a simple representation of a cell can be seen in **Figure 8**. Within the phyla of Chlorophyceae, green algae have chloroplasts that process chlorophyll a & b, carotenes, and xanthophylls (Baweja et al., 2016).

Red algae are considered one of the oldest eukaryotic algae groups and are closely related to green algae and plants (Baweja et al., 2016). Red algae have chloroplasts with chlorophyll a, β-carotene, and xanthophylls, as seen in **Figure 8** (*Seaweed.Ie :: Rhodophyta (Red Algae)*, n.d.).

Brown seaweeds are a part of the supergroup Stramenopiles and Phylum Phaeophyta, putting them in a different kingdom than the plant kingdom with red and green seaweeds. The brown coloration comes from fucoxanthin, and some species contain Phaeophyceae tannins, chlorophyll a, c1 & c2, β-carotene, diatoxanthin, violaxanthin (Baweja et al., 2016). Like the green and red seaweeds, brown seaweeds also have chloroplasts, as seen in **Figure 8**.


Dictyosome
Endoplasmic reticulum
Endoplasmic reticulum
Nuclear envelope
Nucleolus
Pyrenoid
Starch granules
Eye Spot
Chloroplast envelope
Cytoplasmic DNA


Cell Wall
Cell Membrane
Mitochondrion
Nucleus
Stellate Chloroplast
Floridean Starch
Vacuole

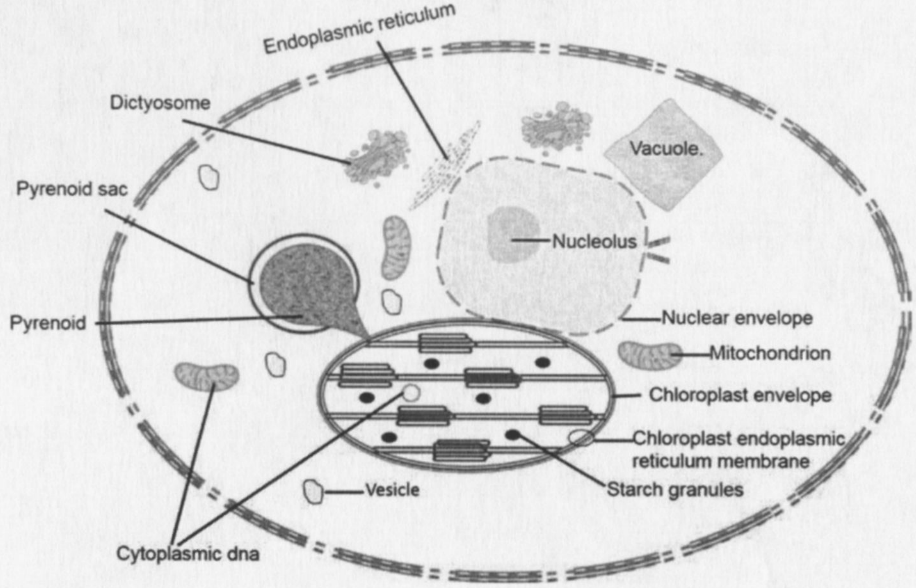

Endoplasmic reticulum
Dictyosome
Vacuole.
Pyrenoid sac
Nucleolus
Pyrenoid
Nuclear envelope
Mitochondrion
Chloroplast envelope
Chloroplast endoplasmic
reticulum membrane
Vesicle
Starch granules
Cytoplasmic dna

Figure 8. A green seaweed cell (top), a red seaweed cell (center), and a brown seaweed cell (bottom). (Baweja et al., 2016; *RED ALGA (RHODOPHYTES) - Ppt Video Online Download*, n.d.)

Macroalgae tend to grow close to their thermal limits and survive through upregulating their stress response systems to survive in long, frequent temperature exposures well below lethal limits (Baweja et al., 2016). It is the heat shock proteins that seaweed has adapted that allow them to alter their cell membrane properties while also developing other physiological and biochemical adaptions as functions of thermal environmental selection pressures (Baweja et al., 2016).

The green seaweeds have the ability to reproduce asexually and sexually (isogamous, anisogamous, or oogamous), yet zoospore formation is a common mode of vegetative reproduction (Baweja et al., 2016).

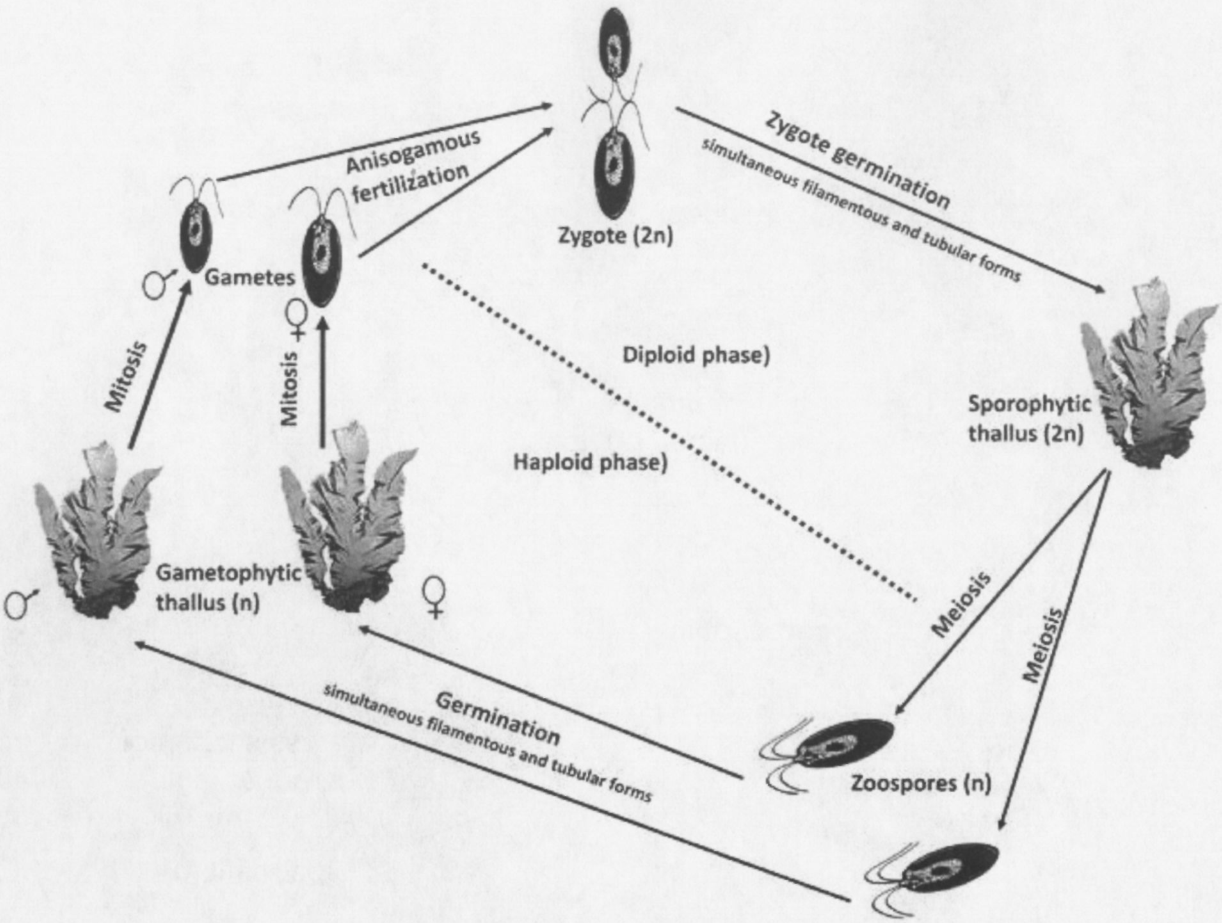


Figure 9 The life cycle of Ulva seaweeds. This model lifecycle is representative of other green seaweeds. (Baweja et al., 2016)

There is diversity and variation in the lifecycle of the red seaweeds, which varies much more than the green and brown seaweeds **Figure 10** (Baweja et al., 2016). They can have up to five free-living morphological phases in their lifecycle (Baweja et al., 2016). Reproduce asexually and sexually, where asexual reproduction can occur with monospores, carpospores, chonchospores, tetraspores, aplanospores, apogamy, and apomeiosis (Baweja et al., 2016).

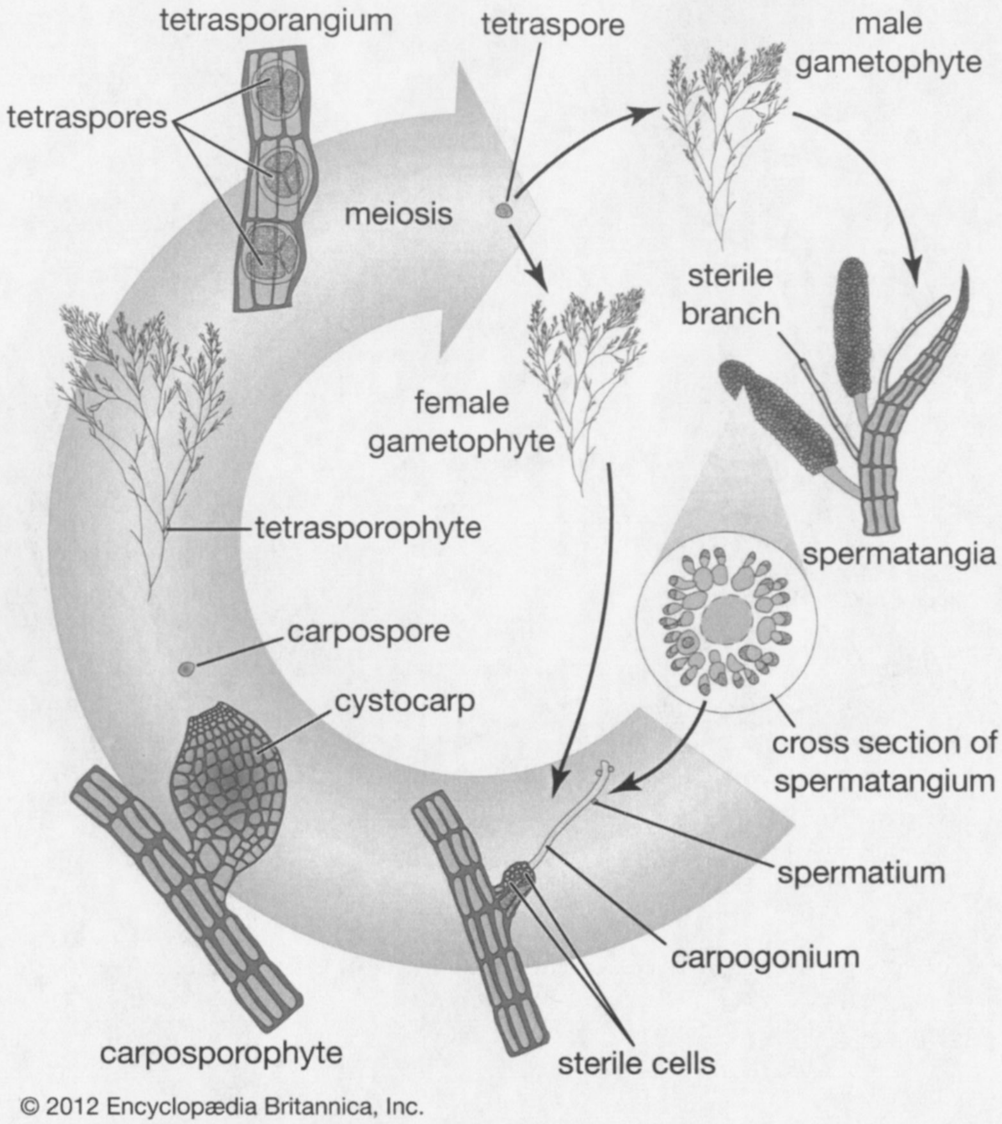


Figure 10 Representative lifecycle of Polysiphonia for red seaweeds. (*Red Algae* | *Protist*, n.d.)

Brown seaweeds are able to reproduce vegetatively, asexually, and sexually. Asexual reproduction occurs in all seaweeds except for Tilopetridales, Dictyotales, and Fucales (Baweja et al., 2016).

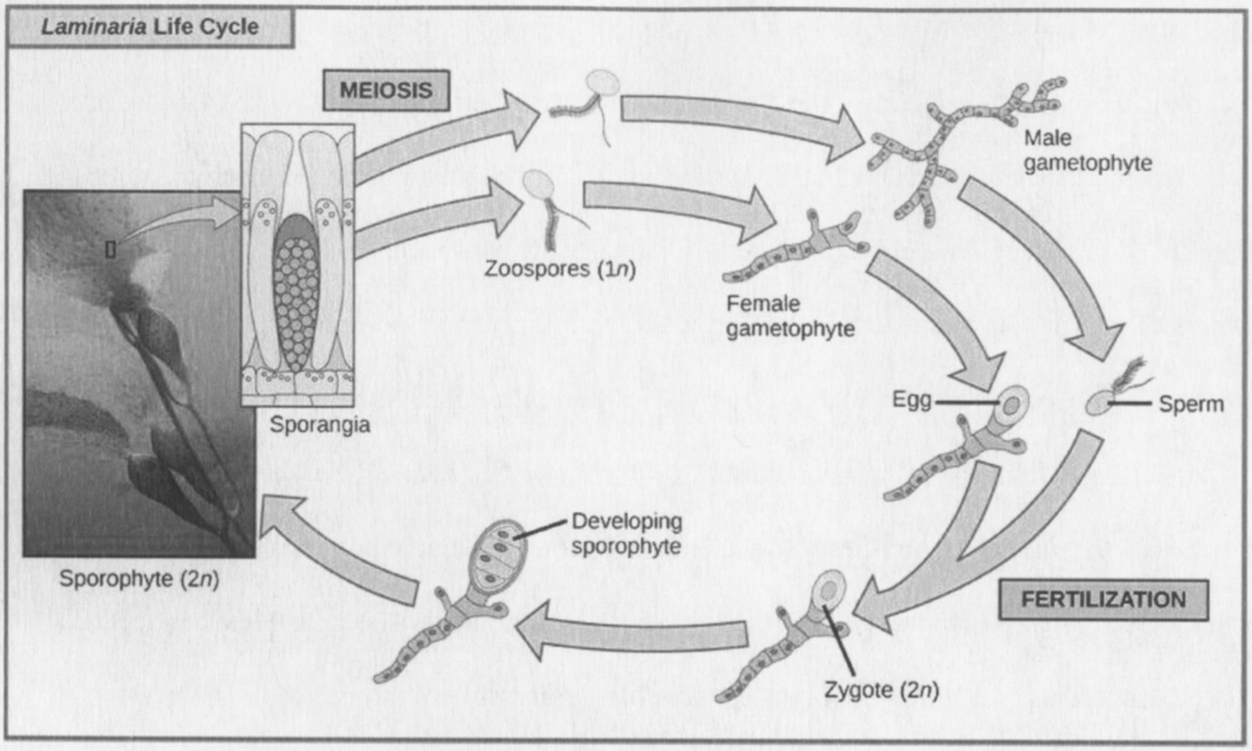


Figure 10. The lifecycle of Laminaria seaweeds. This model lifecycle is representative of most brown seaweeds. (*5.3.2*, 2020)

As *Chlorophyta*, *Phaeophyta*, and *Rhodophyta* each vary in genetic make-up and compounds, a comparison of a species from each classification would give a better idea of where to search for antimicrobial compounds. In a study performed by Salvador et al. (2007), over 80 different species of macroalgae from each classification were tested for antimicrobial activity. The study found that *Phaeophyta* had 84% of the species show antimicrobial activity (Salvador et al., 2007). Considering this impressive statistic, this is a sure-fire direction to head in to begin analyzing the antimicrobial properties.

In order to narrow the field of study, a closer look at macroalgae species in New England both native and invasive, which could be harvested without imposing adverse effects on the ecosystem the data already present on these species will lead to the hopefully intended results. To understand the nature of these invasive species, a geographic introduction is necessary. As of

2013, the Coastal Zone Management of Massachusetts has documented 17 species of invasive macroalgae that have been introduced to the Bay State and surrounding states (*Non-Native Seaweed in Massachusetts*, 2013). According to this report, the majority of macroalgae introduced to the region are *Rhodophyta* at 13 species. Most species have been introduced to the waters of the Gulf of Maine and Cape Cod via hitch-hiking on vessels and organisms. One *Chlorophyta*, *Codium fragile*, is believed to have been introduced to New England waters by oysters brought from the Long Island Sound (*Non-Native Seaweed in Massachusetts*, 2013). Prior to *C. fragile* being introduced to the Gulf of Maine, the macroalgae has been found in the Pacific Ocean near Japan and the North American coastline from Alaska to Mexico (Sept, 2016).

According to *A Field Guide to Economically Important Seaweeds of Norther New England*, there are over 250 species of macroalgae in the Gulf of Maine and the Bay of Fundy (White & Keleshian, 1994). The invasive macroalgae in question are a small portion of the total yet are growing each year. Evidence of these macroalgae being found in multiple regions is indicative of the preferred climate for growth as well as introduction due to ocean travel.

Based on Salvador et. al.'s study, despite the focus of macroalgae on the Iberian coast, one species of interest, a *Phaeophyta*, *Fucus serratus*, which is found in the New England waters is thought to originate in Europe. This species is an invasive species in Europe as well, as provided by the Salvador study (Johnson et al., 2012; Salvador et al., 2007; Sept, 2016).

When actively collecting macroalgae samples, it is possible to identify the species based on physical appearance, however, for species of macroalgae very similar in appearance, they can only really be identified by examining the cellular and reproductive structures of the organism (*Non-Native Seaweed in Massachusetts*, 2013). The need for a genomic analysis for the identification stems from the physical similarities between species of the same phyla. An example of this can be seen in Irish Moss and False Irish Moss if one does not know what differences to look for. Both macroalgae belong to *Rhodophyta*, yet Irish Moss is formally known as *Chondrus crispus* and False Irish Moss is known as *Mastocarpus stellatus*. Due to the similarities in physical appearance as seen in **Figure 4**, it is common to harvest both at the same time. Not only, are these macroalgae similar in physical appearance and habitat, but also in their biochemistry - both are rich in carrageenan, protein, vitamin A, and iodine (White & Keleshian, 1994).

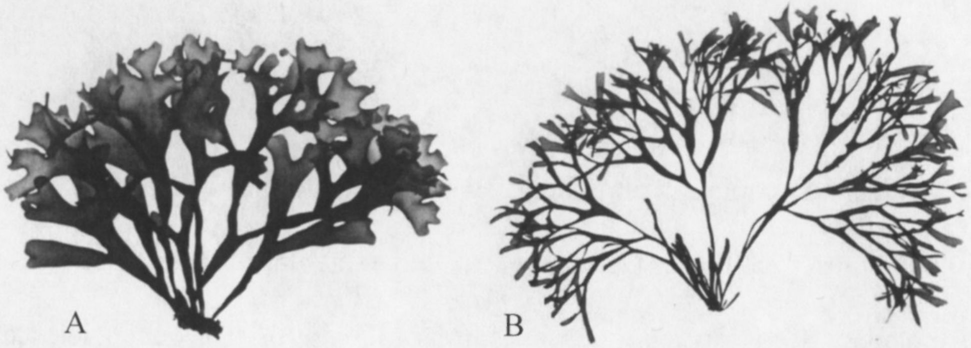


Figure 4. *C. crispus*, the fronds are large and fan-like (A). *M. stellatus*, the fronds tend to grow longer than *C. crispus*, are flattened and the tips tend to curl (B). (*Chondrus Crispus Stackhouse*, n.d.; *Mastocarpus Stellatus (Stackhouse) Guiry*, n.d.)

These *Rhodophyta* are not the only macroalgae commonly mis-identified, the *Phaeophyta* of the *Fucus* genus are often confused. *Fucus vesiculous, Fucus spiralis,* and *Fucus distichus* are very similar in appearance and are known as rockweeds. The differences in physicality can be observed in **Figure 5**. Through a DNA analysis of *F. distichus*, it has been found that this east coast dweller is actually the same species as *Fucus gardneri* which is found on the west coast of the United States and other regions of the world (Sept, 2016).

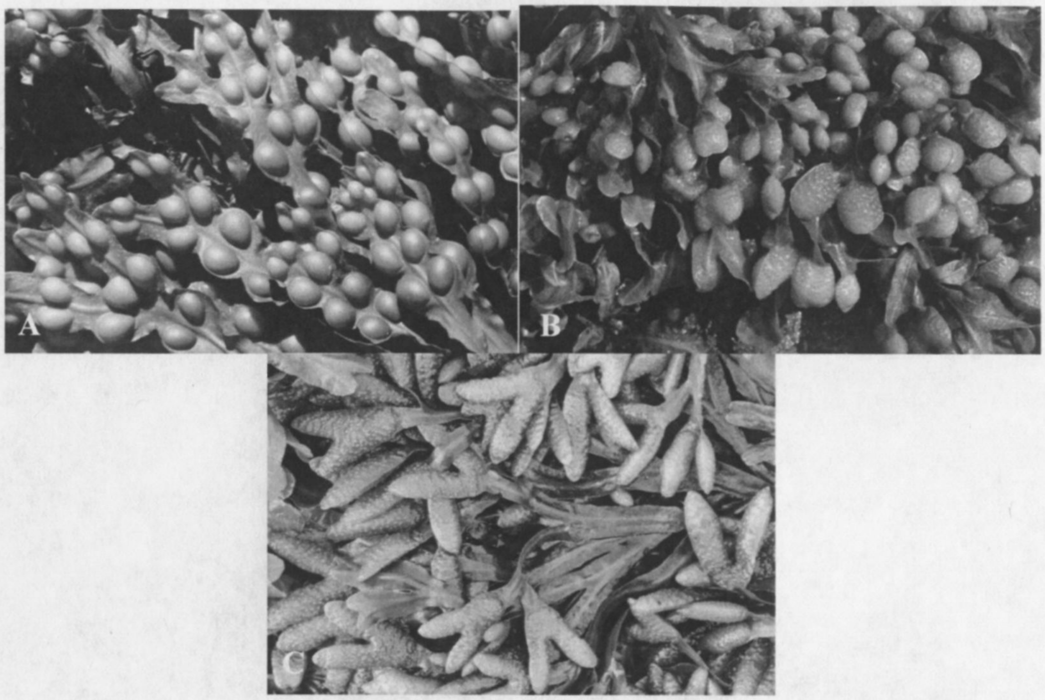


Figure 5. *F. vesiculosus* contains "air bubbles" within the blade, as well as reproductive receptacles on the tips of the blades (A). *F. spiralis* have flat blades and singular receptacles on the tips of the blades (B). *F. distichus* appears similar to *F. spiralis*, the receptacles are paired and more pointed (C). (*Rockweed (Fucus Distichus) at Low Tide in Resurrection Bay in Southcentral Alaska. Spring. Morning.*, n.d.; *Seaweed.Ie :: Information on Marine Algae*, n.d.-a; *Seaweed.Ie :: Information on Marine Algae*, n.d.-b)

The species included in **Figure 4** and **Figure 5** are actually native to the New England region and of the species of macroalgae in New England, have the closest physical similarities. The Invasive species on the other hand, vary much more in physical characteristics. One *Chlorophyceae*, *C. fragile*, is easily identifiable due to its tubular branches (**Figure 6A**). Another species, a *Phaeophyceae, Colopomenia peregrina* (**Figure 6B**), is distinguishable by the collapsed balloon-like shape in adulthood ("Colpomenia peregrina Sauvageau," n.d.). A *Rhodophyceae, Bonnemaisonia hamiferi* (**Figure 6C**) is characterized by hook-like branches and multi-branched, cotton-wool-like tufts ("Seaweed.ie: Bonnemaisonia asparagoides," n.d.).

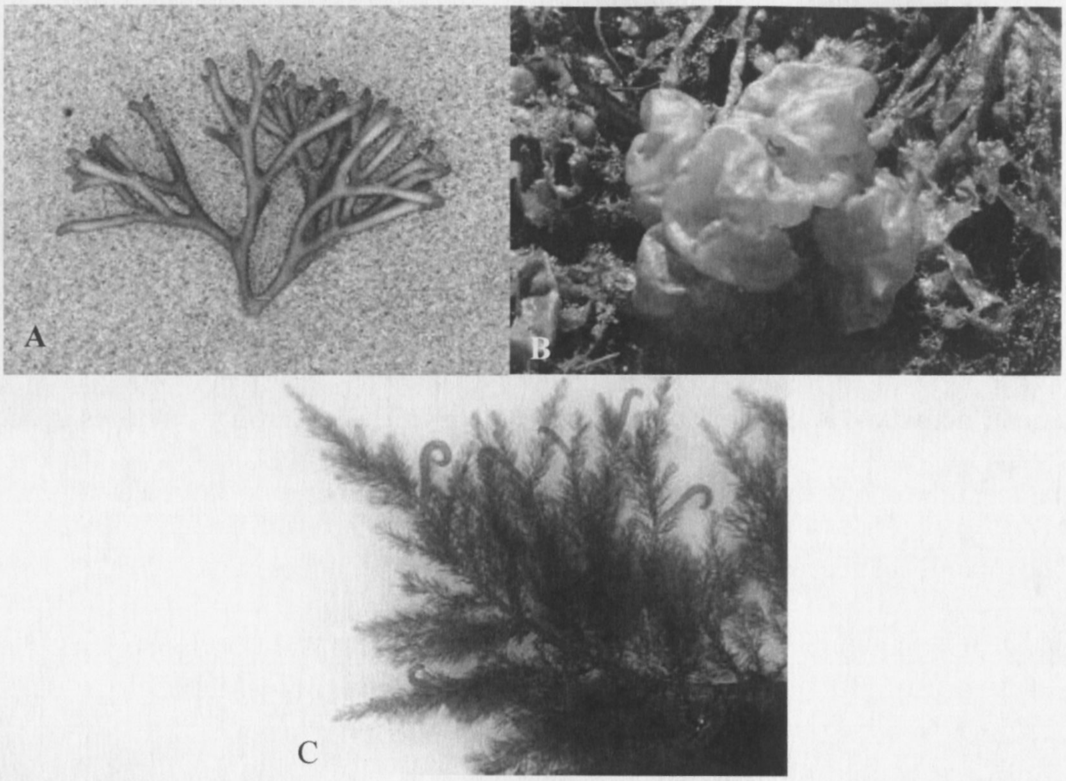


Figure 6. *C. fragile* (A). *C. peregrina* (B). *B. hamiferi* (C). (*Colpomenia Peregrina Sauvageau*, n.d.; *Felty Fingers - Codium Fragile - by Christine Young - JungleDragon*, n.d.; Mosquin, 2015)

It has been found that kelp in the Gulf of Maine and along the New Hampshire seacoast is disappearing and are being replaced with an invasive, shrub-like species, thus changing the base of the marine food chain (Harris et al., 2017). Photographs taken over the course of 30 years of parts of the sea floor demonstrate that the seaweed and the number and types of small creatures had changed significantly (Harris et al., 2017). The invasive red macroalgae replacing the kelp is *Dasysiphonia japonica* and it has 90% coverage in some areas of the Gulf of Maine (Harris et al., 2017). With the disappearance of the kelp forest, researchers are still unsure of the effects this will have on the ecosystem as the *D. japonica* is a shrub-like seaweed which is vastly different from the tall kelps. It is speculated that the change in seaweed is caused by historical fishing practices and warming waters, each having a negative effect on the growth of kelp

(Harris et al., 2017). Previous studies also show that other coasts with kelp forests that experience high levels of human activity have been replaced with mats of introduced seaweeds (Dijkstra et al., 2017).

Seaweeds also face threats from rising ocean temperatures and ocean acidification do effect seaweed growth. The concern that increasing amounts of greenhouse gases from anthropogenic activities, pollution caused by human activities, are causing increases in ocean surface temperature and ocean acidification because of climate change (Baweja et al., 2016). Global temperature and ocean chemistry changes are forcing changes in biological systems to reorganize their communities as new species enter and older species die out (Baweja et al., 2016).

While seaweeds are the primary producers of the oceans and the long-term effects of ecosystem changes are not well known, it is known that changing seaweed communities could result in changes in other communities as well (Baweja et al., 2016).

Based on the physiological and biochemical adaptions of seaweeds, research suggests that seaweeds can thrive in changing sea conditions by migrating or other adaptation strategies (Baweja et al., 2016). Adaptations for temperature changes are beneficial when the initial temperature is cooler, but quickly becomes detrimental at warmer than optimal temperatures, causing physiological changes (Baweja et al., 2016). On the other hand, rising oceanic CO_2 concentrations will likely lead to an increase in the production of oxygen byproduct by many species (Baweja et al., 2016). However, if the increase in CO_2 concentration is in the atmosphere, the pH of the ocean water will become more acidic (Baweja et al., 2016).

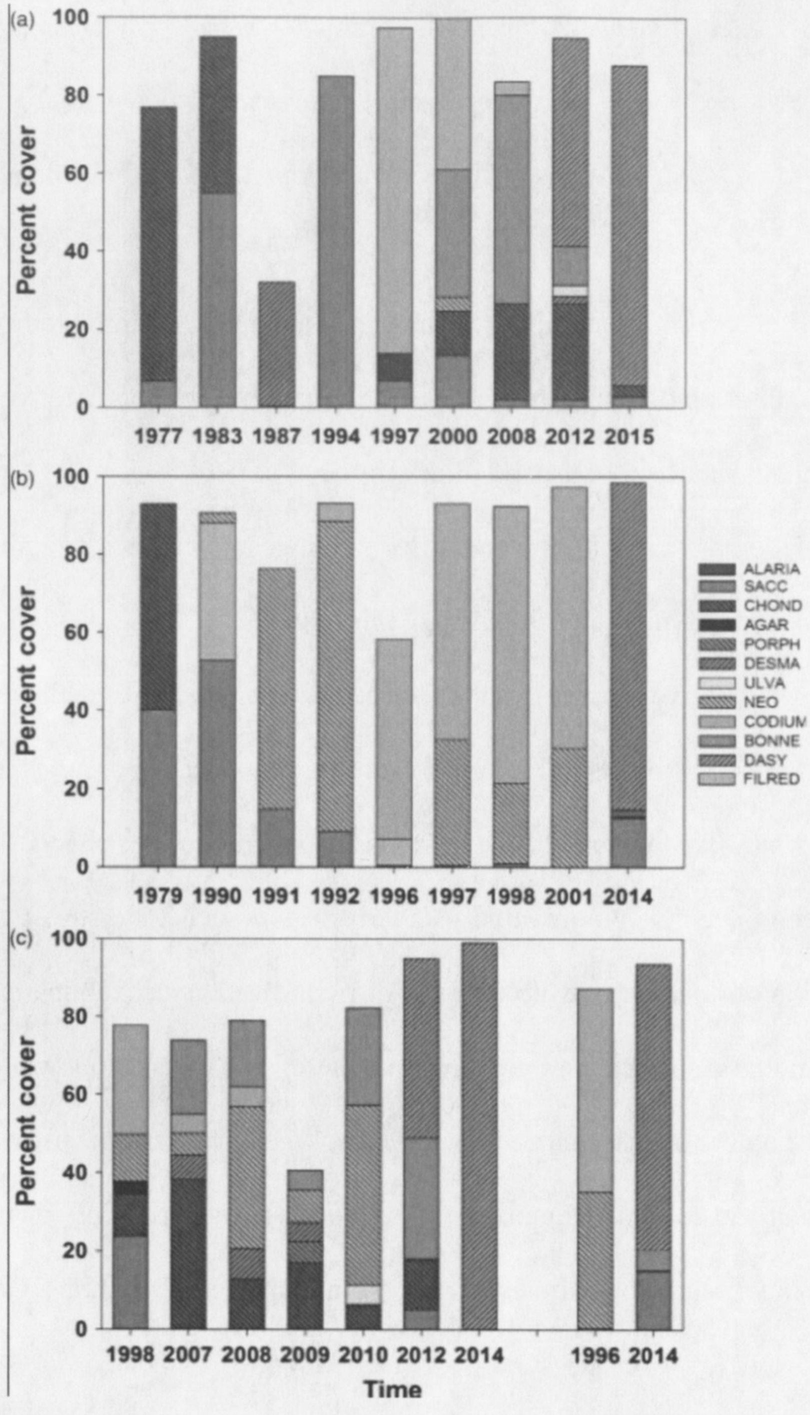


Figure 23. The long-terms trends in changes of seaweed assemblages from four sites at the Isles of Shoals: (a) Star Island, (b) Appledore Island, and (c) White Island on the left and Lunging Island on the right. Blues represent native species and orange/pinks represent invasive species. The abbreviations are as follows: Alaria (*Alaria esculenta*), Sacc (*Saccharina latissima*), Chond

(*Chondrus crispus*), Agar (*Agarum clathratum*), Porph (*Porphyra* spp.), Desma (*Desmarestia aculeata*), Ulva (*Ulva* spp.), Neo (*Neosiphonia* spp.), Codium (*Codium fragile* sp. *fragile*), Bonne (*Bonnemaisonia hamifera*), Dasy (*Dasysiphonia japonica*), Filred (Filamentous red seaweed) (Dijkstra et al., 2017).

Kelp forests are a very productive system. They are a host of high biodiversity and ecological function through offering protection and a source of food for many young fish species, young and old shellfish, seals, and birds (Harris et al., 2017).

Having little to no prior studies on the effects changes in habitat structure have from long-term shifts in foundation species in marine environments, a terrestrial study was used. In this study shifting from native species to an introduced dominant species had increased the canopy heights in terrestrial forests in Hawaii, but the consequences to organisms living in the forest were unknown (Dijkstra et al., 2017). In a marine model, species changes in a reef ecosystem were seen to be much more rapid than terrestrial models (Dijkstra et al., 2017).

Identification of invasive seaweeds can be difficult from visualization alone, like in **Figure 17**, when multiple species are found together there is no physical indication of which seaweed is invasive unless one is aware of what it looks like. The Massachusetts Office of Coastal Management had compiled a guide to which species are considered invasive, as seen in **Table 1**, based on the research of Mathieson et al. from multiple publications, Green et al., Gulbransen et al., Nettleton, and CZM and Low et al (*Non-Native Seaweed in Massachusetts*, 2013).

Figure 17 Native and invasive seaweeds attached to a floating dock. By visualization alone, it can be difficult to distinguish native seaweeds from non-native ones. *Codium fragile* can be seen in the bottom of the figure with the green, tube-like structure (*Non-Native Seaweed in Massachusetts*, 2013).

Table 1 The known invasive macroalgae in Massachusetts waters circa. 2013 (*Non-Native Seaweed in Massachusetts*, 2013).

Phylum/Class	Species
Chlorophyta	*Codium fragile* ssp. *fragile*
Phaeophyceae	*Melanosiphon intestinalis* *Ulonema rhizophorum* *Colpomenia peregrina*
Rhodophyta	*Antithamnion pectinatum* *Bonnemaisonia hamigera* *Dumontiia contorta* *Gracilaria vermiculophylla* *Grateloupia turuturu* *Heterosiphonia japonica* *Lomenaria orcadensis* *Lomentaria clavellosa* *Neosiphonia harveyi* *Porphyra katadae* *Porphyra yezoensis f. narawaensis* *Porphyra yezonensis f. yezoensis* *Rhodymenia delicatula*

Chemistry of Seaweeds

Metabolism is defined as the process of converting compounds known as substrates assisted by enzymes to different compounds known as products. Generally, molecules and compounds can be destroyed to obtain energy that can then be utilized to construct different molecules (Fani, 2012). **Figure 20** shows the breakdown of large molecules is known as a catabolic process and the buildup of new large molecules is known as an anabolic process. Enzymes are protein structures specified to a substrate that help "speed up" a metabolic reaction by lowering the amount of energy that is needed to form the product.

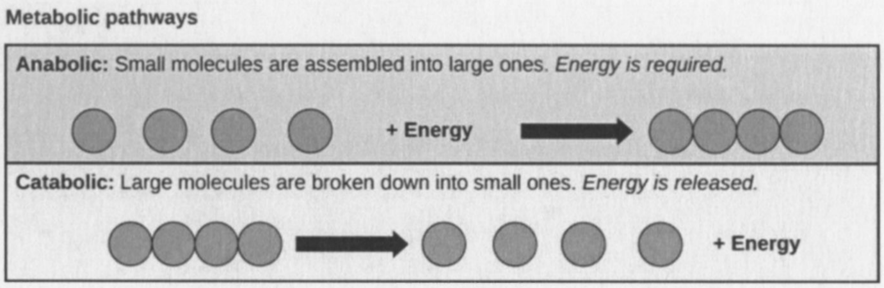


Figure 20 The basic metabolic pathway defined by the breakdown of large molecules (bottom) and the buildup of large molecules (top). (*6.1C*, 2018)

Natural factors enacted on macroalgae, like environmental conditions such as light exposure, temperature, salinity, life stage, reproductive state, age, geographic location, and seasonality factor into the antimicrobial activity of a single or multiple metabolic compounds (Pérez et al., 2016). Metabolites that express antimicrobial activity include polysaccharides, polyunsaturated fatty acids, phlorotannins and other phenolic compounds, carotenoids, and other compounds (Pérez et al., 2016).

Polysaccharides:

Polysaccharides serve as storage or structural components in *Chlorophyta, Rhodophyta,* and *Phaeophyta*. The polysaccharides in the cells of macroalgae are found within the cell wall and include: alginic acid, alginates, carrageenans, agar, laminarans, fucoidans, and ulvans as seen in **Figure 21**. The antimicrobial activity of these polysaccharides is dependent on the size, charge, density, distribution, sulphate content, structural and conformational components (Pérez et al., 2016).

Alginates:

Alginate and alginic acid are the salt and acid forms of the compound. Alginates form with the cations Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}, and play a large role in the cell walls and intracellular matrix of *Phaeophyta* (Pérez et al., 2016). Molecular weight (MW) varies from 500-1000 kDa. A popular component in the textile industry, alginates have been used as bandages for large wounds and burns as well as inclusion in the fire-proofing of a fireman's uniform (*Seaweed.Ie :: Alginates*, n.d.).

Carrageenans:

Major component of *Rhodophyta* cell walls. They have sulphate half-esters attached to the sugar units and depending on the level of sulphation can have the form of kappa (one), lambda (two), and iota (three) (Pérez et al., 2016). Carrageenans are largely used in food processing for their stabilization and thickening properties in addition to displaying antimicrobial properties and acting as a carrier for anti-retroviral drugs in HIV prevention and treatment (*Seaweed.Ie :: Carrageenans*, n.d.).

Agar:

Usually, a mixture of the polysaccharides agarose and agaropectin, and have similar structural and functional properties of carrageenans in *Rhodophyta*. Agarose makes up the majority of agar (Pérez et al., 2016). Agar is often found within labs as a part of the substrate for microbial growth plates, it is also found as an emulsifier in food products (*Seaweed.Ie :: Agars*, n.d.).

Galactans:

Sulphated galactans are the main extracellular polysaccharide in *Rhodophyta* but can also be found in *Phaeophyta* and *Chlorophyta* (Pérez et al., 2016). Galactans have shown antimicrobial activity against *Escherichia coli* in the absence of metal ions (Liu et al., 2020).

Laminarans:

The main storage polysaccharide in *Phaeophyta*, like Laminarias or Saccharinas, can range from 32-35% of the mass, the molecular weight depends on the degree of polymerization (Pérez et al., 2016). Laminarans can help prevent blood clots as an anticoagulant and is a hypolipidemic agent that lowers the levels of certain lipids in the blood (PubChem, n.d.).

Fucoidans:

Along with laminarans, fucoidans are the main water-soluble polysaccharides in *Phaeophyta*. They are a complex heterogenous group that contribute to intercellular mucilage. They can be sulphated polysaccharides bonded to multiple other minor molecules. According to IUPAC, fucoidans is reserved for polysaccharides of algal origins and fucan sulphates to the similar polymers of marine invertebrates. Composition varies widely based on species and geographic origins (Pérez et al., 2016). Some lab studies have suggested that fucoidans have anticancer, anticoagulant, antiviral, and neuroprotective properties but more studies in humans need to be performed to confirm the anticancer properties (*Fucoidan | Memorial Sloan Kettering Cancer Center*, n.d.).

Ulvans:

Ulvans are water-soluble sulphated polysaccharides that are extracted from the intercellular space and in the fibrillar wall of *Chlorophyta*, and account for 18-29% of the dry mass of the macroalgae. Mainly composed of glucuronic acids and iduronic acids units with rhamnose and xylose sulphates (Pérez et al., 2016). Ulvans have been shown to demonstrate antiviral, antioxidant, antihyperlipidemic, anticancer, and immunomodulating properties (Kidgell et al., 2019).

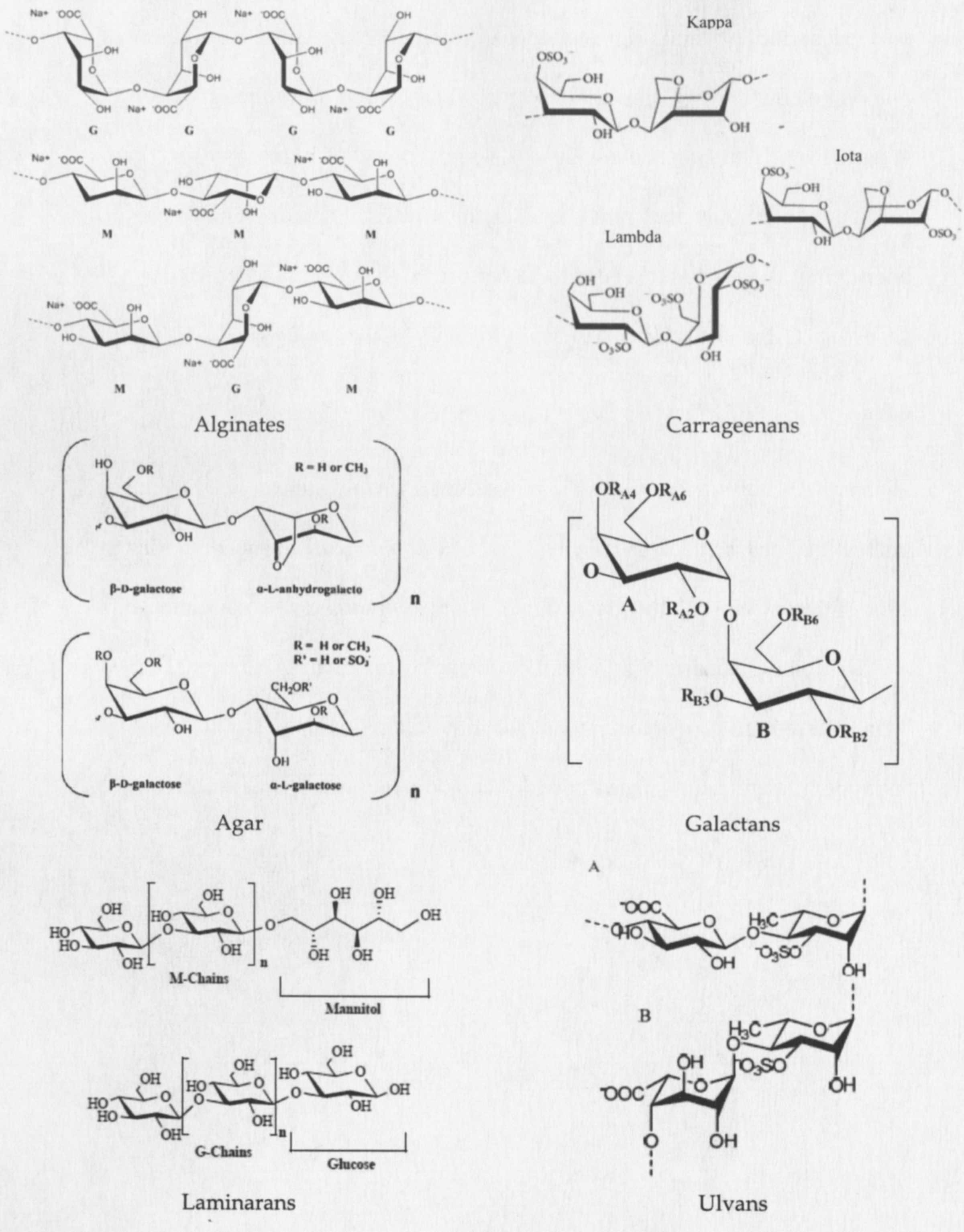


Figure 21 Chemical structures of mostly polysaccharides in macroalgae, including alginates, carrageenan, agar, galactans, laminarans, fucoidans, and ulvans. (Pérez et al., 2016)

Lipids, Fatty Acids, Sterols:

Algal lipids compose 0.12-6.73% of the dry mass and contain mostly phospholipids, glycolipids, and non-polar glycerolipids and can be seen in **Figure 22** (Pérez et al., 2016).

Phospholipids:

Phospholipids are found in the extra-chloroplast, mitochondria, and cell membranes and are 10-20% of total lipid content in macroalgae. They are characterized by higher contents of n-6 fatty acids and the major fatty acids present are oleic, palmitic, stearic, arachidonic, and eicosapentanoic acids. The most abundant phospholipids are phosphatidylglycerol in *Chlorophyta*, phosphatidylcholine in *Rhodophyta*, and phosphatidylcholine and phosphatidylethanolamine in *Phaeophyta* (Pérez et al., 2016). While phospholipids themselves do not contain any antimicrobial properties, they are able to be manipulated to express antimicrobial activity and act as a carrier of other medically active substances (Cohen et al., 2000).

Glycolipids:

Located in the photosynthetic membranes and are more than half of the lipids in the main macroalgal groups. They are characterized by high n-3 polyunsaturated fatty acids with the three major types of glycolipids being: monogalactosyldiacylglycerides, digalactosyldiacylglycerides, and sulfoquinovosyldiacyl glycerides (Pérez et al., 2016). Glycolipids have been used to combat many microbial agents, acting as antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory agents by disrupting the formation of biofilms (Shu et al., 2021).

Triacylglycerol:

Also known as triglycerides, they are most prevalent as glycerolipids, their content ranges from 1-97% and function as storage and energy reservoirs (Pérez et al., 2016). While not presently used as an antimicrobial agent, triacylglycerols are metabolized differently than conventional fats and oils, promoting the reduction of body fat and leading to its use in obesity management (Lee et al., 2012).

Fatty Acids:

They are carboxylic acids with aliphatic chains and prevalent even carbon numbers, causing the chains to be straight or bent, saturated or unsaturated. With a double bond, fatty acids are unsaturated and can be classified as monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids. PUFAs can be further classified as n-3 or n-6 depending on the position of the first double bond from the methyl end. *Chlorophyta* are rich in C18 PUFAs, mostly α-linoleic, stearidonic, and linoleic acids. *Rhodophyta* are rich in C20 PUFAs, mostly arachidonic and eicosapentanoic acids. *Phaeophyta* are rich in both C18 and C20 PUFAs (Pérez et al., 2016). Fatty acids are used to kill and inhibit bacterial growth by disrupting cell membranes, the electron transport chain, oxidative phosphorylation, enzyme inhibition, disruption of nutrient uptake, generation of peroxidation and auto-oxidation degradation, and lysis of bacterial cells (Desbois & Smith, 2010).

Oxylipins:

Oxygenated products of fatty acids, mainly derived from C16, C18, C20, and C22 PUFAs. They have innate immunity in response to biotic and abiotic stressors, like pathogenic bacteria and herbivores (Pérez et al., 2016). In a study of oxylipin antimicrobial activity, 41 of the 43 derivatives were able to inhibit microbial growth, and 26 of the 41 oxylipins were effective against at least three different microbes (Prost et al., 2005).

Sterols:

They are structural components of cell membranes and regulate membrane fluidity and permeability. Sterols are seen as cholesterol, fucosterol, isofucosterol, and clionasterol in macroalgae (Pérez et al., 2016). Sterols have been shown to have antimicrobial activity (Bhardwaj et al., n.d.)

Phenolic Compounds:

Considered secondary metabolites since they are not directly involved in primary processes like photosynthesis, cell division, algal reproduction. Antimicrobial action is due to alteration of microbial cell permeability and the loss of internal macromolecules or by the interference with the membrane function and loss of cellular integrity and eventual cell death. Range from simple molecules to complex polymers (Pérez et al., 2016). Phenolics are an antioxidant source and have shown antibacterial activity against β-lactamase and biofilm development (Mandal et al., 2017).

Pigments:

Three basic classes of pigments: chlorophylls, carotenoids, phycobiliproteins. Each provide the classification of *Chlorophyceae*, *Phaeophyceae, Rhodophyceae* (Pérez et al., 2016).

Chlorophylls:

Chlorophylls provide green pigmentation from the presence of chlorophylls a and b (Pérez et al., 2016). Chlorophylls have been used to treat wounds as part of a water-soluble solution, clearing the area of suppuration and odor within two to three days of application (Mowbray, 1957). Chlorophylls have also shown reductions in suppuration in secondary infected ulcers, contact dermatitis, and pemphigus in addition to the suggestion of antimicrobial and antibacterial activity against staphylococci, streptococci, and lactobacilli species (Mowbray, 1957).

Carotenoids:

Carotenoids are a class of pigments synthesized by macroalgae and are expressed was yellow, orange, and red in color. Within the typical human diet, fruits and vegetables provide 40-50 carotenoids (*Carotenoids*, 2014). Carotenoids are found in all algae and can be divided into classes of carotenes and xanthophyll/oxycarotenoids (Pérez et al., 2016). The most common carotene is β-carotene while the most common xanthophyll are lutein, fucoxanthin and violaxanthin (Pérez et al., 2016). Mixtures of each of these class members can be found in all three types of seaweeds (Pérez et al., 2016). Carotenoids are antimicrobial agents as well as protectors against oxidative damages, anticancer agents, and treat erythropoietic protoporphyria (a rare genetic metabolic disorder that causes a deficiency in the enzyme ferrochelatase that results in skin hypersensitivity to sunlight and some fluorescent lighting) (*Erythropoietic Protoporphyria (EPP) and X-Linked Protoporphyria (XLP)*, n.d.; Kirti et al., 2014).

Other Compounds:

Lectins:

Bioactive proteins or glycoproteins that bind with glycans, glycolipids, and polysaccharides (Pérez et al., 2016). They have high acidic amino acid content and don't require metal ions for biological activities (Pérez et al., 2016). In marine organisms, lectins can be further classified as C-type lectins, F-type lectins, galactans, intelectins, and rhamnose-binding lectins (Pérez et al., 2016). Lectins produce powerful antimicrobials when they bind with carbohydrates on microbial surfaces (Breitenbach Barroso Coelho et al., 2018). As an antifungal, lectins disrupt chitin-binding of cell walls causing them to disintegrate and act as an antiquorum-sensing therapy, preventing cell-to-cell communication (Breitenbach Barroso Coelho et al., 2018).

Alkaloids:

Biological amines and halogenated cyclic nitrogen-containing compounds are considered alkaloids (Pérez et al., 2016). In seaweeds, alkaloids can be further classified as phenylethylamine alkaloids, indole and halogenated indole alkaloids, and alkaloids like 2,7-naphthyridine derivatives (Pérez et al., 2016). Halogenated compounds are dominant features in Chlorophyta species while most indoles are dominant in Rhodophyta species (Pérez et al., 2016). Alkaloids serve as base structures in antibacterial drugs like metronidazole and quinolones (Cushnie et al., 2014).

Terpenes:

A major class of metabolites in seaweeds, they are derived from the five-carbon precursor isopentenyl pyrophosphate and can be classified in seven categories: hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30) and polyterpenes (>C30) (Pérez et al., 2016). They are found as cyclic and linear C15, C20, and C30 compounds in Chlorophyceae and as halogenated secondary metabolites in Rhodophyceae (Pérez et al., 2016). Most terpenes demonstrate antiviral and antifungal properties (Pérez et al., 2016). In a study with 33 terpene derivatives, 16 showed antimicrobial activity during initial screening, and it was found that higher antimicrobial activity was associated with the presence of hydroxyl groups and lower activity was associated with hydrocarbons (Guimarães et al., 2019).

Halogenated Compounds:

The most common halogenated compounds found in seaweeds are halogenated, like the furanones, bromoditerpenes, bromophenols, and polar compounds (Pérez et al., 2016). A study shows that some halogenated compounds like polybrominated indole demonstrate broad spectrum antibacterial activity against gram-positive bacteria like methicillin-resistance *Staphylococcus aureus*

(MRSA), penicillin-resistance *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (VRE) (Vairappan et al., 2004).

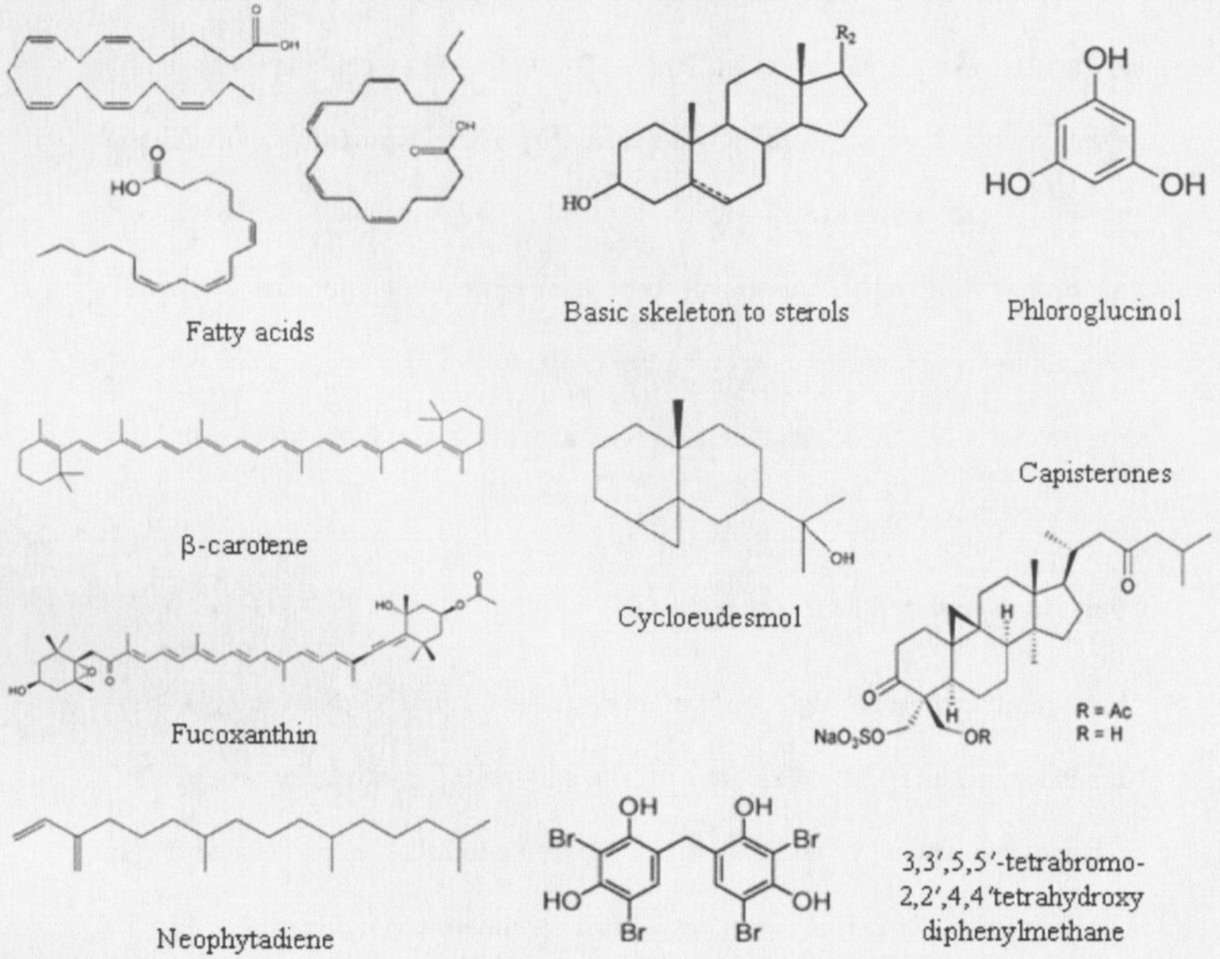


Figure 22 Chemical structures of fatty acids, sterols, phloroglucinol, carotenoids (β-carotene and fucoxanthin, terpenes (neophytadiene and cycloeudesmol) and a brominated compound. (Pérez et al., 2016).

Most, if not all compounds are able to be extracted with organic solvents. Separation and quantification of the compounds can be performed through HPLC or GCMS while identification of these compounds can be performed via different analytical instruments such as, NMR and MS.

Extractions:

Supercritical-Fluid Extraction (SFE)

Used for the extraction of high-value bioactive compounds, like pigments and fatty acids (Sosa-Hernández et al., 2018). This extraction technique is chosen for its selectivity, quick times, and low degradability of the extracted product without non-food grade solvents (Sosa-Hernández et al., 2018). Instead SFE uses supercritical fluids, which are fluids above their critical point and display liquid-like and gas-like characteristics that enhance the transport properties and solvent strength (Sosa-Hernández et al., 2018). Extracts like vitamin E, carotenoids, and PUFAs can be isolated.

Microwave-Assisted Extraction (MAE)

This technique presents high concentration results that are of good quality (Sosa-Hernández et al., 2018). MAE are performed by microwave irradiation, causing polar molecules to move and their dipoles to rotate to heat the solvents (Sosa-Hernández et al., 2018). Advantages of this extraction technique are that minimal solvents are utilized and quick times. Phenolic compounds, phytonutrients, alkaline galactans, carrageenans, and agar can be extracted with MAE (Sosa-Hernández et al., 2018). In *Fucus vesiculosus*, a native species, MAE allowed for the extraction of fucoidan (Sosa-Hernández et al., 2018).

Pressurized-Liquide Extraction (PLE)

Great for the extraction of polar compounds, PLE is used for the extraction of high-value compounds by increasing the temperature and pressure through liquid solvents (Sosa-Hernández et al., 2018). This technique also requires low amounts of solvents and has fast extraction times (Sosa-Hernández et al., 2018). High-value fatty acids, phenolic compounds, and fucoxanthin were extracted (Sosa-Hernández et al., 2018).

Enzyme-Assisted Extraction (EAE)

This technique is highly selective, maximal yield, and quick extraction times (Sosa-Hernández et al., 2018). Enzymes such as ligninolytic, cellulolytic, and proteolytic are used to breakdown the cell walls and membranes (Sosa-Hernández et al., 2018). Alginates and carrageenans, carotenoids, and phlorotannins have been extracted with this technique (Sosa-Hernández et al., 2018).

Solvent Extractions

Most techniques for extraction use methanol or a mixture of methanol and chloroform with the residues from these extractions being used for centrifugation and collection of the lower organic layers (Kumari et al., n.d.).

Analysis and Identification:

Chromatography:

Chromatography is used to separate mixtures into individual components. Thin layer chromatography (TLC) is useful in initial assessments of samples for identifying crude extracts (Misra et al., 2015). TLC is also useful in identifying specific compounds in the crude extracts that have been previously eluted in other chromatographic techniques (Misra et al., 2015). High pressure liquid chromatography (HPLC) is a popular and reliable method of sample analysis. HPLC is usually coupled with a UV-Vis diode array detector for its high sensitivity to changes as the sample flows through the detector (Misra et al., 2015). Ultrahigh-performance liquid chromatography (UPLC) is similar to the HPLC but is able to function well in higher pressures than the HPLC would (Misra et al., 2015). This distinction allows for the separation of much smaller compounds and particles that would not have been noticed in an HPLC, improving the sensitivity, speed, and resolution of the results (Misra et al., 2015). Gas chromatography (GC) utilizes capillary columns and while it is commonly connected to other instrumental forms of analysis, it is a reliable method of separation. This method works best with molecules that have sufficient volatility when heated, like FAMEs (Misra et al., 2015). A common detector paired with the GC is the flame ionization detector (FID) for identification (Misra et al., 2015).

Multi-Analytical Chromatography:

Liquid chromatography (LC) and GC instruments are commonly with other instruments like the mass spectrometer (MS) and NMR. Primarily used for the chemical analysis of polar compounds, but tends to be expensive (Misra et al., 2015). NMR on its own is important for the structure determination of unknown compounds. When paired with LC, unknown complex molecules can be identified (Misra et al., 2015). This instrument analysis technique allows for the identification and quantification of metabolites and other compounds (Misra et al., 2015).

Antimicrobial Testing

Some species produce more bioactive compounds in the winter, like the *Laminaria* type, *Chondrus crispus, Laurencia pinnatifida,* and *Ulva lactuca*. Others like the *Dictyota* type have peak activity in the summer (Parsaeimehr & Lutzu, 2016). Evidence of this can also be observed in the study performed by Salvador et. al. through measuring the diameter of the inhibition halo in millimeters on agar plates with different bacteria. The inhibition halo measures the effectiveness of the potential antibiotic against the bacteria based on how large the resulting diameter is (**Figure 18**).

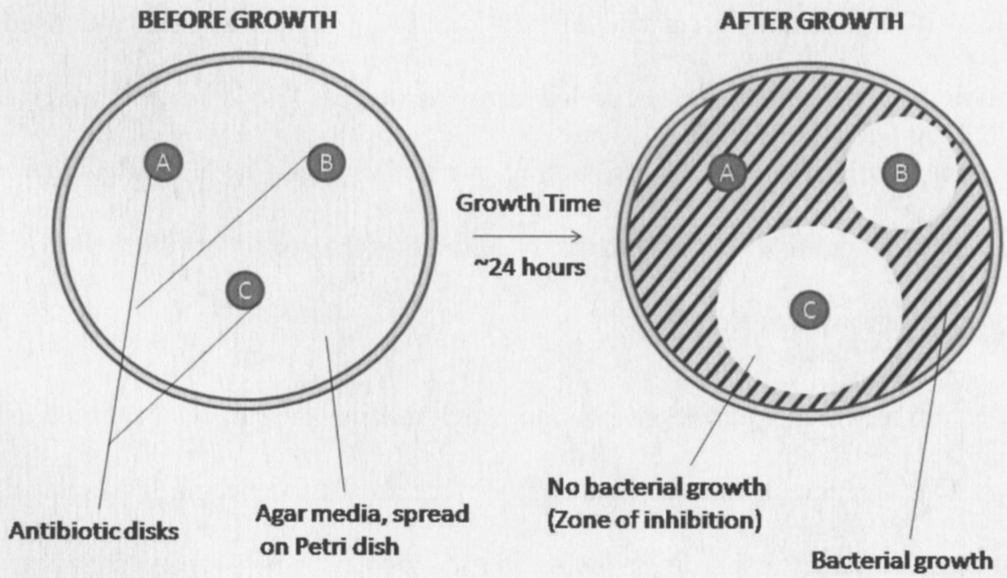


Figure 18. The disc diffusion method showing the resulting inhibition halos (zones of inhibition) after bacterial growth. (*Encyclopedia - Disk Diffusion Test - Microbiology Techniques. The Disk d*, n.d.)

The Salvador et al. study found that there are 16 species of *Phaeophyceae* that produce antimicrobial compounds in the winter, 19 species in the spring, 16 species in the summer, and 12 species in the fall. Some of these species produced antimicrobials throughout each season, while others only produced antimicrobials during one season (Salvador, Garreta, Lavelli, &

Ribera, 2007). On the other hand, Hornsey and Hide had found that in *Ulva lactuca, Chondrus crispus, Codium fragile, Laminaria saccharina,* and *Laminaria digitata*, there is no significant seasonal variation in antibiotic distribution (Hornsey & Hide, 1976.). Instead of solely looking at the antibiotic properties of the seaweeds, Hornsey and Hide studied where in the thallus the antibiotics are located. They found that only *U. lactuca* had equal distribution of antibiotics while *C. crispus* and *C. fragile* demonstrated a concentration of antibiotics in the younger portions of the thalli (Hornsey & Hide, 1976.). *L. saccharina* saw higher antibiotic distribution in the fronds of the thalli and *L. digitata* was more complicated in its antibiotic distribution (Hornsey & Hide, 1976.). In a different study, it was found that *Palmaria palmata* showed a 7.9% inhibition on bacterial growth from a spring sampling against *Vibrio harveyi*, which causes vibriosis in abalone (Nuria et al., 2015). Through further analysis with ^{1}H NMR, to show the effectiveness of the high polarity of the bioactive compounds present and that showed antibacterial properties (Nuria et al., 2015).

Identification of which microbes respond to which macroalgae can be performed through multiple assays. One such methods is the disc diffusion method. Evidence of this technique can be seen in Salvador et al.'s test using *Bonnemaisonia asparagoides* against a few common bacteria (Salvador, Garreta, Lavelli, & Ribera, 2007). The assay works by placing discs of the desired potential antimicrobial on the surface of the bacteria-streaked agar plate and observing the growth patterns of the bacteria. If the selected macroalgae contains antimicrobial compounds, then there will be a ring around the disc in which no bacteria grow, the inhibition halo. To quantify the success of the antimicrobial disc, a comparison of the diameter of the rings are measured in millimeters and are compared. The results of Salvador et al.'s assay can be observed in **Figure 19**.

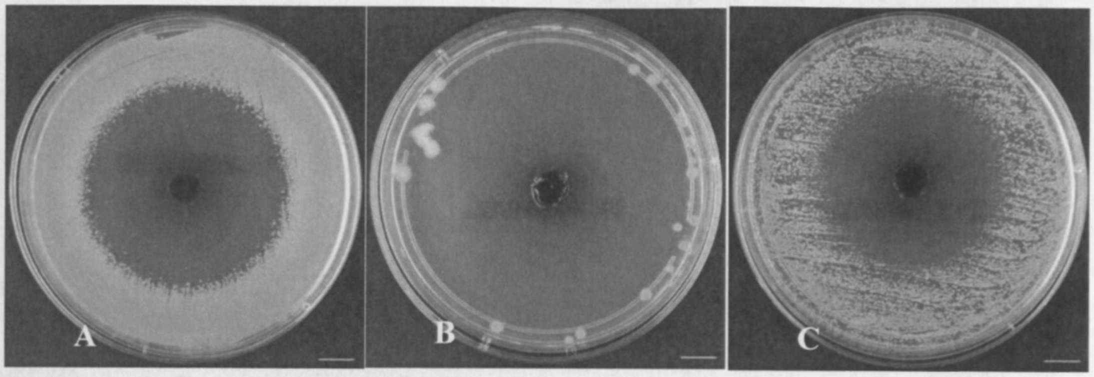


Figure 19. The results of bioassay of solid extracts from *B. asparagoides* from the Salvador et al. study. The inhibition halo for *Bacillus subtilis* shows a solid ring with a clear distinction in the growth (A). The inhibition halo for *Bacillus cereus*, which shows that the microbe did not favor the environment, as very little growth can be seen (B). The inhibition halo for *Candida albicans* shows a clear ring as well as spotted growth surrounding the halo (C). (Salvador et al., 2007)

Another assay can be performed to test for turbidity of the microbe in a liquid culture containing different concentrations of ground-up macroalgae. A spectrophotometry test can be performed to quantify the growth of the microbe in the presence of the macroalgae at the different concentrations. This type of test uses light absorbance to measure the growth rate of the bacteria at across time. When the data is compiled into one graph, the effectiveness of the macroalgae concentration can be determined by the growth curve that takes the longest time and the has the lowest light absorbance.

From analyzing these macroalgae that exhibit antimicrobial properties, their compounds can then be extracted and further analyzed for their individual effectiveness against bacteria. By doing so, it allows for a cataloguing of new antibacterial and antimicrobial compounds that withstand the penicillinase enzyme that most bacteria now have.

Future of Seaweeds

Seaweeds have demonstrated antimicrobial properties across many species that are native and invasive, however, there are concerns when it comes to harvesting them. The goal is to harvest seaweeds at a rate that is not detrimental to the ecosystems they come from. An added benefit would be to study these ecosystems where harvesting is being done to observe if there is a change in the seaweed species populations. A return of native species being the most abundant in the environment would be ideal, but that would be unobtainable solely through harvesting, rather the need for slowing climate change would need to be addressed and that is a larger conversation that requires many parties to agree on.

For the seaweeds in the Gulf of Maine, the kelps have been replaced by the shrubby *D. japonica* due to the changes in the ocean temperature from global warming. In a perfect scenario, the invasive species of seaweeds would be harvested for their antimicrobial properties, but native species also have antimicrobial properties. A more realistic and practical solution would be to arrange offshore sites for the cultivations of all seaweeds that would allow for harvesting without disrupting the ecosystems of the coastlines.

Conclusion

Through extracting and analyzing the compounds that come from seaweeds that grow in ocean off of New England, a catalogue of these properties can be done. A successful cataloguing would list out which compounds come from which species and the results of disc diffusion assays to determine the antimicrobial properties of the seaweeds. Based on the results of the assay and the extractions, seaweeds that are both native and invasive to the region can be identified for their potential use in future antimicrobial products and the feasibility of how harvesting of the seaweeds would affect the ecosystem. While there are still species in New England waters that have to be catalogued for their antimicrobial properties, there are also some species that do have known properties as well. By ethically harvesting seaweeds, the ecosystem is maintained and there is the potential for offshore cultivation of the seaweeds as well.

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