Text: Thesis Proposal Draft 4

Audience: Thesis Committee

Genre: Proposal

Comments: Form on github to upload comments, or verbally in class that I can add to github file

Preferred Comments: Overall structure/flow, grammar, is each section explained well enough for non-seaweed people to understand

# **Project Description**

## ***Summary***

Marine algae encompass the large photosynthetic group that are responsible for producing half to three-quarters of the earth’s oxygen supply, which includes phytoplankton and macroalgae. Macroalgae is the term used to describe the types of red, brown, and green seaweed found in the water. These macroalgae are responsible for providing food and shelter for other marine organisms, as well as a multi-billion-dollar industry for human uses (*Macroalgae: The Facts*).

The aquaculture of macroalgae has been occurring in countries in Asia for centuries, with seaweed being a staple in the diet of many Asian cultures (Kim, J.K. et al. 2019; Moreira et al. 2021). This once solely Asian industry has spread through Europe (Araújo et al. 2021) and was established in the US in the early 1980s and 90s. Despite the development of kelp farms on both the east and west coast of the US (Grebe et al. 2019), overall effort in the macroalgae in the US has been small. Little progress has been made to expand this multibillion-dollar industry in the US.

One aspect of macroalgae aquaculture that has been neglected in the US is the diversification of cultured species. Currently, the macroalgae aquaculture effort in the US has been focused on kelp. However, efforts to cultivate new macroalgal species would notably expand the US industry and open the doors to a largely untapped market that could produce millions of metric tons in yield per year (Kim et al. 2019). The work proposed here will help diversify aquaculture in the US by detailing the culturing conditions for *Wildemania amplissima*, a species of nori. This work has the potential to significantly alter the macroalgae aquaculture industry in the US and contribute to enhanced understanding of the optimal growth conditions for this species.

## ***Background***

 Macroalgae are extremely important to marine environments, they provide food and sheltered habitats for various invertebrates and organisms, and act as nursey ground for juveniles (Macreadie et al. 2017). Some macroalgae species efficiently sequester inorganic waste in the environment, including phosphorous and nitrogen, which can lead to harmful algal blooms (Redmond et al. 2014, Mooney-McAuley et al. 2016). Macroalgae are highly productive and fast-growing organisms, with kelp forests being ‘four times more productive per square metre than any intensively farmed crop on land’ making them an incredible resource to sustain in the ocean. The aquaculture of macroalgae is also a large industry worldwide, valued around 11 billion dollars annually (*Macroalgae: The Facts*).

To compete with the global industry, US macroalgae aquaculture will need to expand. Currently in the US, kelp is primarily farmed because cultivation methods are well known, it requires little human involvement (Mooney-McAuley et al. 2016).

Kelp is ecologically important because they provide vertical habitats for many invertebrates and other organisms, and act as shelter for juvenile fish by decreasing ocean currents and giving them places to hide; because of this kelp forests are highly biodiverse (Steneck et al. 2002). Species of kelp are known for their uses as food sources, pharmaceuticals, bioremediation, and more recently as forms of biofuel (Flavin et al. 2013).

Kelp aquaculture is worldwide, with growing interest and action beginning in the Gulf of Maine. With various methods used for structuring longline grow out, including submerged parallel lines or buoy suspension of lines, methods are dependent on locality conditions. Nurseries are where the kelp spores are housed prior to placement onto longlines. The objective of a nursery is to provide a mimicked version of the location’s environment conditions for the sporophytic kelp, allowing for optimal growth and higher survival. Nylon twine wrapped PVC pipes provide the substrate necessary for kelp spore attachment on growth, usually a four-to-six-week process. These spools are then transferred to grow out locations, where the spools are unwrapped onto the longlines and monitored for growth rate and fouling until it is time to harvest (Flavin et al. 2013).

Another species that has the potential to be highly beneficial for both humans and marine ecosystems is nori. Nori is the common name given to the foliose species within the order Bangiales that are known to grow into edible leaf-like sheets. The species occur in eight foliose genera include *Boreophyllum, Clymene, Fuscifolium, Lysithea, Miuraea, Porphyra, Pyropia,* and *Wildemania* (Sutherland et al. 2011). In general nori is high in vitamins, minerals, antioxidants, proteins, and can be eaten as a substitute for finfish (Knoop et al. 2020). Nori is commonly known as the wrapper to sushi, but is also used in pharmaceuticals, cosmetics, and biofertilizers (Kılınç et al. 2013).

In terms of macroalgal aquaculture, nori has been harvested for centuries, but industrial expansion was hindered by the lack of knowledge of nori’s life history and reproduction. A turning point for the industry came in the early 1950s when a scientist, Mary Drew-Baker, discovered the complexity of the life history of *Porphyra umbilicalis*. This transformation in the understanding of the reproductive life history of this nori species allowed for the ‘domestication’ of seaweed through artificial seeding of nori and the birth of the industry that is well known today (Harris et al. 2013). Nori is also a candidate for integrated multi-trophic aquaculture systems (IMTAs) (Redmond et al. 2014), as studies have shown that nori can take up a significant amount of inorganic nutrients, specifically nitrogen, which is very common waste product in finfish fisheries (Kim et al. 2007). However, widespread nori cultivation in the US has been hampered by lack of understanding of optimal growth requirements and reproductive cycle of native species.

Nori has a heteromorphic life cycle, alternating between a gametophyte blade and microscopic filamentous sporophytes. Reproduction occurs when male gametes are released from the male thalli section of the blade and fertilize the egg in the female carpogonia. Once fertilized, mitosis occurs resulting in mature zygotospores which are then released into the environment. Once the zygotospores settle onto a substrate, typically shells, they bore into the surface and enter the conchocelis stage. The conchocelis grows in its vegetative form as ‘red fuzz’ on the surfaces it attaches to. Conchosporangial filaments form once the conchocelis are triggered, indicating the occurrence of meiosis in mature conchosporangium and formation of four identical haploid spores. Conchospores are released and settled onto suitable substrate, typically shells, rocks, or other macroalgae, and grow into haploid gametophyte blades (Redmond et al. 2014).

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Figure 1) The life cycle of nori from the New England Handbook: Nursey Systems, Chapter 6: Nori (Redmond et al. 2014)

Each of these growth stages have unique optimal culturing conditions, therefore describing the stage-specific growth conditions will be necessary for successful nori aquaculture cultivation.

In addition, distribution of nori species varies regionally. Species found in Asia are not native to US coasts (Yarish et al. 1999). Nori distribution also varies with elevation, with species ranging from the high intertidal region to shallow subtidal. Some species, like *Wildemania amplissima*, and *Wildemania miniata*, are subtidal, where they are less likely to encounter desiccation events from low tides relative to mid- or high intertidal species such as *Porphyra umbilicalis* or *Pyropia leucosticta* (Krishnamurthy 1972). Given the high diversity in environmental conditions that are optimal for the different nori genera, cultivation methodology will not be universal for all.

In New England, *W. amplissima* is a native species of nori, growing along the coast north of Cape Cod. This species grows in the low intertidal to subtidal range, in areas with constant water flow and motion. *W. amplissima* can appear in various colors depending on life stages and age. The colors range on a scale of pale pink to dark red, and olive yellow to brown (Sutherland et al. 2011). The blades generally have ruffled edges and grow in an oblong shape. While most species of nori are monostromatic, *W. amplissima* is one of several distromatic species. Blades are monoecious (Sutherland et al. 2011) with male thalli typically located along the edges of the blade and female thalli in the middle (or represented by the reddest portion of the blade). The species is commonly found attached to shells, rocks, or epiphytically on other macroalgae. *W. amplissima* has been found to grow to great lengths, with samples being found up to six feet long. This species can be found on both coasts of the US, with the climate in New England providing a suitable habitat for *W. amplissima* to grow natively in large abundances (Yarish et al. 1999).

This project seeks to use the geographic preference of W. amplissima to develop cultivation methods specific to this species. While attempting to introduce this new macroalgae, this project will also focus on creating similarities in the methods used by kelp farmers to allow for an easier incorporation into this already established industry. The goal of this project is to develop optimal methodology for seeding and growing out *W. amplissima,* while maintaining a similarity in methodology between kelp and nori cultivation. To complete this goal, the methodology developed will need to determine the best conditions for triggering and growth of each life stage, as well as the ideal techniques and technology needed for seeding longlines.

## ***Specific Research Questions***

Incorporation of *Wildemania amplissima* into the existing New England macroalgae aquaculture industry will require development of reliable cultivation methods. The goal of this project is to develop optimal methodology establishing nori culture, for seeding nori lines, and growing out *W. amplissima,* while maintaining a similarity in methodology between kelp and nori cultivation. To complete this goal, methodology must be developed for the optimal conditions for triggering and growth of each life stage, as well as the ideal techniques and technology needed for seeding longlines. The methodology that will be developed will answer these questions:

Question 1)

What are the optimal conditions to successfully establish and grow the various filamentous stages of *W. amplissima*?

1. What laboratory conditions will result in reliable zygotospore release from fertile blades of *W. amplissima*?

Hypothesis: Zygotospore release can be triggered from fertile *W. amplissima* blades through temperature shocking for extended periods in colder chambers, drying and rehydrating, or maceration of fertile blades.

Rationale: In sexual reproduction, fertilization of conchosporangia from spermatangia occurs and results in the release of zygotospores. The release of these zygotospores can be accomplished through stressful events or conditions fertile blades endure. The first method involves macerating the fertile blades and removing the remnants through filtration once zygotospores have been released. Another method involves overnight drying and rehydrating fertile blades (Sahoo & Yarish 2005). The final method is temperature shocking the fertile blades, leaving them in cold chambers overnight and placing them into trays with seawater the next day to isolate any zygotospores released.

1. What conditions will yield mass produced conchocelis? And what conditions are optimal for large growth rates?

Hypothesis: Conchocelis can be mass produced on shells or in well plates or flasks once zygotospores settle onto the surrounding substrate.

Rationale: In nature, conchocelis can be found boring into various calcareous shells, such as clams, oysters, and scallops. Once zygotospores are released, they can be added into a tank or container where they will settle onto the substrate thus beginning the conchocelis stage. The conchocelis grow through the summer and winter months until environmental conditions begin changing to spring conditions. This phase can persist through adverse conditions until the environment is deemed suitable for conchosporangial filament growth and conchospore release (Sahoo & Yarish 2005).

1. What conditions are required for reliable conchosporangial filament formation and conchospore release?

Hypothesis: Conchosporangial filament production and conchospore release can be triggered by mimicking the seasonal change from winter to spring they encounter in nature.

Rationale: *W. amplissima’s* growth season is spring to early summer, where the largest fertile blades being found in late spring/early summer. Fertile blades release zygotospores when water temperatures begin to rise in the beginning of summer. Once zygotospores are released, they settle onto a substrate, typically boring into shells, and grow through the summer and winter months. Once temperatures and daylength begins increasing, occurring in early spring (March/April), conchosporangial filaments form and conchospores are released. Blades grow through late spring and into early summer when the cycle repeats.

The cultivation of this family is highly species specific, *W. amplissima* is a cold-water subtidal species that grows blades in the late spring to early summer range, while other species are the opposite. Sahoo & Yarish state that decreasing water temperature and increasing water agitation will trigger conchospore release for their target species, which happens to have the opposite growing season of *W. amplissima*. The conditions needed for conchospore release in *W. amplissima* would oppose Sahoo and Yarish’s statement due to the difference in growing seasons, as well as the highly specific conditions each species requires.

Question 2)

How will conchospores be seeded onto longline string? What technology must be developed for seeding longlines?

Hypothesis: Attachment of concospores to longline string will be similar to the attachment of kelp spores onto longline string.

Rationale: There are two traditional methods for seeding conchospores onto netting depending on location. In outdoor settings, layers of nets would be suspended in nursery waters and the substrate containing the conchocelis would be suspended under the nets in bags. Once the conchospores were released, the buoyant conchospores would float to the surface and collect on the netting. Indoor seeding used a rotary wheel fixed with layers of netting that would rotate over tanks containing the floating conshospores to encourage attachment (Sahoo & Yarish 2005).

The goal of this experiment is to incorporate the cultivation of nori into the existing kelp industry. To allow for an easier introduction, a similarity to kelp cultivation must be made. To create a similarity in protocols, nori grow out will be done using longlines. The method for attachment will also mimic that of kelp cultivation. There are multiple methods for kelp attachment to longlines, but a common one is the addition of longline string into tanks containing sporophytic kelp to allow for the spores to settle onto the string (Thomas et al. 2021). Conchospore attachment to longline string will be encouraged by adding string to tanks containing conchospores.

## ***Approach***

### Overview

This project is projected to run for two years. The first year will be dedicated to finding reliable sites of *W. amplissima* populations and establishing protocol for the release and growth of different life stages of this species.

The second year of this project will continue to work with controlling the life history stages of *W. amplissima*, as well as focus on the deployment of seeded longlines for grow out trials. Nori is a spring to early summer crop meaning longlines have a short window for deployment. Once the season ends for open water trials, the methods for establishment and growth of *W. amplissima* life stages will be honed and perfected to ensure reliable results. Experiments will also be run to test optimal attachment of conchospores onto longlines, and techniques necessary for large-scale production will be worked on. During this time, outreach to various corporations and symposiums to share the project’s findings and establish connections for future grow out trials will be completed.

### Study Area

This project will be conducted in the lab at UNH and along New England’s coastline, chosen for the abundant populations of wild nori, specifically *W. amplissima*, as well as the established kelp farms and interest in macroalgae aquaculture in the area (Yarish et al. 1999).

### Methods: Question 1a

Though methods have been established in Asia, nori cultivation is very specific. We are able to use some established methods as guides for this project, but the main objectives are to create cultivation protocol specifically for *W. amplissima* and develop methods for gametophyte grow-out on longlines rather than the traditional netting.

Fertile blades must be collected from sites along New England’s coastline, with the locations being near grow out locations to make sure the area is suitable for *W. amplissima* growth and to prevent the introduction of non-local genotypes. Zygotospore release and extraction can be completed using methods described in the Mariculture of Seaweeds (Sahoo & Yarish 2005) and New England Seaweed Culturing Manual, Chapter 6; Nori (Redmond et al. 2014). Zygotopore release from fertile blades can be accomplished through various methods, which include placing the blades under environmental stressors such as drying out, temperature shocking, or maceration of fertile blades. Once zygotospores have been released they can be filtered out using sieves or isolated using drawn-out glass pipettes.

### Methods: Question 1b

Once zygotospores have been released, they can be transferred into wells or containers housing oyster shells. The zygotospores will settle onto the available substrate and germinate in the containers which marks the beginning of the conchocelis stage. The conchocelis stage will grow until environmental conditions are suitable for conchosporangial filament development and conchospore release (Sahoo & Yarish 2005).

Culture-chamber experiments will be run to find the optimal conditions for the conchocelis growth based on temperature, light levels, and photoperiod. Six chambers will be setup with three different temperatures (10, 14, 18 ͦ C), two different photoperiods (12L:12D, 16L:8D). Within each chamber, two different light levels (30 and 60μM/m²/s) (See Figure 2), the two different light levels will be achieved by placing neutral density strips over specific wells within the culture plates. A total of twelve plates will be made, with two plates in each chamber. The experiment will run for seven weeks, with conchocelis area being calculated weekly using ImageJ. Media will be replaced weekly with autoclaved saltwater treated with Von Stosch enrichment and germanium dioxide. The growth rate will be calculated from linear regression of Area vs Week, Growth Rate (% per week) = Slope/Intercept.

A group of windows with blue glass

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Figure 2) The six-culture chamber setup for determining the optimal conditions for conchocelis growth rates

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Figure 3) Six chamber culture plate example, visualizing the placement of neutral density strips over columns with samples from each location site.

### Methods: Question 1c

Conchosporangial filament production and conchospore release occurs when conchocelis encounter suitable conditions. For *W. amplissima* in New England these conditions are the changing of seasons from winter to spring. To trigger conchospore release in a laboratory setting, conchocelis must be exposed to conditions that mimic this transition between seasons. Conchospores are released in nature around early spring (March/April), this coincides with conditions such as lengthening of days and increasing of sea water temperature, increased water agitation has also been observed to encourage conchospore release (Sahoo & Yarish 2005).

Culture-chamber experiments will be run to determine the necessary conditions to trigger production of conchosporangial filaments and release of conchospores. These experiments will be run similarly to that of the conchocelis culture-chamber experiments, where two chambers will be set at the same temperature (14 ͦ C), with different photoperiods (16L:8D, 8L:16D) and two different light levels (20 and 100μM/m²/s) using neutral density strips. The well plates will be left in the chambers for four weeks, with water changes done weekly using autoclaved saltwater treated with Von Stosch enrichment and germanium dioxide. After four weeks, the temperature in the chamber will be decreased (8 ͦ C), and the individual spore clumps within the wells will be redistributed to new plates for another six to eight weeks (See Figure 3).

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Figure 3) The two-chamber setup for determining the conditions necessary for conchosporangial filament production and conchospore release

### Methods: Question 2

Once conchospores have been released they can be attached to longline string. It is unknown if *W. amplissima* conchospores are buoyant, so the preliminary method for this stage involves the addition of string to a container of conchospores with aeration.

## ***Broader Impacts***

The size of the US macroalgae industry is considerably smaller and newer compared to other countries in this industry. The US currently has a limited scale of macroalgae in production, based solely on kelp, with a need for expansion. The introduction of nori into New England’s mariculture industry could create the large economic impacts seen in European and Asian countries. The global macroalgae market is a multibillion-dollar industry and the expansion into this industry would allow the US to enter untapped revenues and options for more environmentally conscious sources for human and animal food, biofertilizers, and extracts used in pharmaceuticals and cosmetology. Growing the US mariculture industry would open the opportunity for more jobs—farmers, processors, distributors.

An introduction of nori into this industry would not only create jobs, but lengthen the macroalgae industry’s season. Nori would be outplanted in the spring to early summer months, while kelp is grown during winter months. The addition of nori would elongate the season, as well as diversify the macroalgae industry by introducing a new species that holds many economic, environmental, and dietary benefits. Nori is known for its high values of vitamins, minerals, and proteins, making it a staple in diets among many Asian communities. Nori is a candidate for integrated multi-trophic aquaculture systems because of their ability to uptake large amounts of nitrogen from the surrounding waters. The need for expansion within the US macroalgae industry can be aided by the introduction of an economically significant macroalgae such as nori.