Utilizing the Agarophyte (Gracilaria tikvahiae) as a Biofilter for the Bioremediation of Cobia (Rachycentron canadum) Nitrogenous Effluents in Flow-through Aquaculture Systems

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UTILIZING THE AGAROPHYTE (*GRACILARIA TIKVAHIAE*) AS A BIOFILTER FOR THE BIOREMEDIATION OF COBIA (*RACHYCENTRON CANADUM*) NITROGENOUS EFFLUENTS IN FLOW-THROUGH AQUACULTURE SYSTEMS

By

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The biological and technological feasibility of using the agarophyte *Gracilaria tikkahiae* as a biofilter for the bioremediation of nitrogenous effluents resulting from juvenile cobia culture was examined. Using a thorough literature review of the most relevant published research, optimal parameters are identified and discussed within the context of juvenile cobia culture, while factors maximizing biofiltration and growth of *Gracilaria* within a biofilter system are outlined. The study attempts to match cobia nitrogen excretion rates with rates of algal nutrient uptake to size the initial biomass of algae needed in a filter for the culture of 10,000 juvenile (<30g) cobia. The culture of 10,000 juvenile cobia, 28-70 days post hatch (DPH), produces a maximum of roughly 300g of nitrogen daily which in turn can support roughly 12-27kg of algae (wet weight) per day, depending on uptake rate. The supportable algae biomass is then used to size the initial stocking density of the algae filter. Optimal system components are identified and management considerations are discussed with respect to optimizing growth as a means to maximize biofiltration and nutrient extraction capacity over time.
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CHAPTER 1. INTRODUCTION.

The future of the world’s seafood supply relies on the aquaculture industry developing in a sustainable and responsible manner. Global consumption of seafood has been rising steadily since the start of the 20th century, fueling global market demand and increasing fishing pressures on wild stocks. Fish and products derived from fisheries provide an important source of food and income for much of the world, especially in developing countries. Fish provided roughly 3.0 billion people with almost 20% of their intake of animal protein, highlighting the importance of fisheries in global development and poverty reduction (FAO, 2012). Global catch landings from marine capture fisheries have stagnated at 90 million tonnes, as the rate at which we are extracting seafood resources has exceeded the capacity for the environment to produce them, resulting in the ecological collapse of many large fisheries (FAO, 2012). Technological advances such as radar and remote sensing, improved fishing vessels and the expansion of fishing grounds were responsible for the increase in the annual fishing capacity of global fisheries, which was once believed to be an inexhaustible supply of seafood. The resulting collapse of many fisheries, such as the Peruvian Anchoveta in the 1970’s and Cod stocks in New England circa 1990, has proven that only through improved fisheries management and aquaculture will global fish stocks survive and prosper for future generations (Pauly et. al., 2002).

The modern aquaculture industry has been growing at a rate of 9% and now accounts for nearly half of the worlds’ seafood production (Marine Aquaculture Task
Force, 2007; Bostock et al., 2010). With the human population expected to reach 10.1 billion by 2100, many view the aquaculture industry as the only feasible way to meet the growing demand for seafood (UN, 2011). As such, aquaculture is the fastest growing food producing sector, yielding an estimated 57.2 million tons of seafood in 2010 (FAO 2011b). The growth of the industry has expanded 12 times within the last three decades, owing a large proportion of its expansion to aquaculture development in China, which accounted for over 60% of the world’s aquaculture production by volume (FAO, 2012). In 2010, the production of farmed food fish (including mollusks and all other marine animals) was 59.9 million tons, of which roughly 2/3rd being grown using supplement feeds (FAO, 2012). The trend has shown an increase in the production of fed specimens, particularly finfish which exhibit fast growth and good market suitability. The associated environmental issues with aquaculture are a barrier to progress for the industry as the global consciousness towards stewardship of the environment is increasing.

1.1. INDUSTRY ISSUES AND SUSTAINABILITY

While the industry has shown great promise during its development in recent years, it has faced harsh criticism and debate, particularly with respect to its monospecific production of carnivorous marine finfish as it may lead to eutrophication and pollution of coastal environments. Nitrogen and phosphorous are the primary source of pollutants from aquaculture facilities which can lead to eutrophication (Lazzari & Baldisseroto, 2008). Symptoms of eutrophication include oxygen depletion, increased frequency of harmful algal blooms, acidification, turbidity and natural reduction of animal populations, which can affect the wellbeing of both humans and animal communities alike (Folke & Kautsky, 1992; Chopin et al., 2001). In response to these concerns, academics and
industry officials have been researching ways to reduce the environmental impacts of marine farming by developing more sustainable practices and standards. The long term sustainability of monoculture crops is threatened by rising costs of energy and feed, disease, environmental regulation compliance and public opposition (Neori, 2008). As the demand for fish continues to grow to meet human consumption and as a component of protein in aquaculture feeds, efficient utilization of aquaculture feed resources is paramount to the sustainable development of the industry.

Typical finfish often retain 30% of nitrogen contained in feed, releasing the rest into the culture environment through uneaten feed & excretion (Schuenhoff et. al., 2003). While a single, well situated aquaculture facility may not have detectable environmental effects off-site, geographical concentration and rapid intensification of farm and cage operations have a cumulative impact on the environment as exemplified by the Chilean salmon industry (Benetti et. al., 2008b; Buschmann et. al., 1996). Due to the potential impacts of wide-spread aquaculture activities, many nations are beginning to create regulations for aquaculture effluents, which include compliance with local water quality standards containing numerical data and mandated usage of environmentally sustainable practices (Boyd, 2003). These regulations, along with a growing consumer consciousness for more eco-friendly products, will ultimately drive the industry to change its current practices.

One proposed solution is to move aquaculture operations to land-based systems, thereby allowing for treatment of waste effluents via biological or chemical means. Land based systems permit more effective control over culture conditions and full containment
of waste products, which can allow for a variety of treatment options in either recirculating or flow-through systems (Cahill et. al., 2010). One form of biological treatment involves utilizing fish effluents to supply nutrients for seaweed culture, a concept known as integrated multi-trophic aquaculture (IMTA), a key development for sustainable aquaculture.

An integrated multi-trophic aquaculture system is defined as: An output from one subsystem, which otherwise may have been wasted, becomes an input for another subsystem resulting in greater efficiency of output of desired products from the land/water area under a farmer’s control’ (FAO, 2009). The concept promotes nutrient recycling within the system through bioremediation, which is considered the most relevant benefit from integrated farming but is often the least valued in global industry terms (FAO, 2012). In essence, wastes produced from the cultivation of fish are used in the cultivation of seaweeds, providing nutrients required for growth. With solar energy, the plants turn the excess nutrients such as nitrogen, carbon & phosphorous into valuable, marketable biomass (Neori et. al., 2004). This promotes greater efficiency in the system by utilizing otherwise wasted nutrients, which is significant as feed is a core operation cost of all finfish aquaculture facilities (FAO, 2009).

However, IMTA offers more than just greater efficiency. IMTA has the potential to diversify products, leading to reduced risk, greater profits, increased socio-economic benefits and decreased environmental effects (FAO, 2009; Barrington et. al., 2009). Both industry and public members are realizing the potential of IMTA, however no large-scale commercial operation has yet to shift from its traditional monoculture practices to that of
IMTA due to potential loss of revenue and increased complexity of operations. Pilot studies are showing that IMTA can be both environmentally and economically beneficial. As environmental regulations in developed countries are getting more demanding, the implementation of IMTA may become more widespread. (Chopin et. al., 2004; Nobre et. al., 2010; Neori et. al., 2004).

The integration of algae culture as a tool for bioremediation has been proven through various studies with various different species of macroalgae (Chopin et. al., 2001; Neori, 2008; Neori et. al., 1996; Schuenhoff et. al., 2003). Many common genera of macroalgae used in these studies, such as Ulva, Gracilaria, Porphyra, Enteromorpha, Laminaria & Chondracanthus will possess varying nutrient uptake and bioremediation efficiency rates in different environmental conditions, making selection of a suitable species a key factor when designing an integrated system (Chopin et. al., 2001; FAO, 2009). The type of species chosen should be geographically endemic, the cultivation should be feasible with available technologies and should it be tailored to the desired outcome of the crop. If the outcome is a marketable product, then factors such as market price, agar production or even methane production potential should be considered. However, if bioremediation is the goal, nutrient uptake rates, growth rates and biofiltration efficiency are more important.

Traditional methods of filtering harmful nutrients from recirculating systems involve the use of ‘biofilm’ bacterial filters. These methods work by utilizing nitrifying bacteria to oxidize the highly toxic ammonia into nitrate via a two-step process. The nitrate will continue to build up in the system and eventually becomes toxic to fish,
prompting the need for a water exchange. Respiration from the bacteria competes with
the cultured organisms for oxygen, lowers the dissolved oxygen levels, and consequently
leads to acidification of the water through metabolic processes. In contrast, seaweed
biofiltration has the ability to absorb the full range of nitrogenous wastes (ammonium,
nitrite and nitrate), and can even outperform traditional bacterial biofilters resulting in
less ammonium and undetectable nitrates in the system (Cahill et. al., 2010).

Red algae from the genus Gracilaria has been proven to be great candidates for
biofiltration because they possess a high photosynthetic efficiency, high productivity and
the ability to produce a commercially valuable byproduct (Edding et. al., 1987; Capo et.
al., 1999). Gracilaria can either be cultured attached to rafts or long-lines, or free-floating
in suspension in tanks or raceways. The latter is more suitable to land based systems,
allowing for control over certain culture parameters and accessibility for routine
maintenance (Santelices & Doty, 1989).
Chapter 2. STUDY OBJECTIVES.

The goal of the project is to examine the biological and technological feasibility of using the red alga *Gracilaria tikvahiae* in a tumbling algae filter as a tool for bioremediation of cobia (*Rachycentron canadum*) fingerling effluents resulting from commercial aquaculture practices. The project will involve utilizing the underlying biological and physiological principles of these organisms to facilitate their culture, maximize the efficiency of the biofilter, and calculate the initial biomass of algae to be used in a flow-through aquaculture system.

The objective is to explore the parameters of an aquaculture system which uses *G. tikvahiae* to remove the ammonium-based nitrogen from aquaculture wastes for a sample system of 10,000 juvenile cobia, aged 28-70 days post hatch. By quantifying nutrient loads, the supportable biomass of algae needed to achieve maximal bioremediation is used to size the biofilter, while constraints and limitations in the context of a flow-through aquaculture system will be examined. A small sample size of 10,000 fish was chosen to be a modular number which can then be scaled up linearly based on the number of fish cultured.

2.1. SCOPE OF INVESTIGATION

The process for the aquaculture of cobia is complex and involves many different levels of operation (see figure 1). The hatchery phase (Phase 1) involves the spawning of cobia broodstock to produce viable eggs that can be hatched into larvae. Larvae are grown to roughly 28 ‘Days Post Hatch’ when they become fingerlings and are ready to
begin grow-out for market suitability (Benetti et. al. 2008, 2008b).

**Figure 1.** The four phases of cobia (*Rachycentron canadum*) production include a hatchery phase, nursery phase, nearshore growout and offshore growout phase.

This study will focus on the nursery phase (Phase 2) and the bioremediation of effluents that are produced during this phase of production. Currently there are few studies that look at the nursery phase, with the majority of studies focused on intensive hatchery production or grow-out of adults.

During the nursery phase, cobia at 28 DPH (~1.0g) can be grown in flow-through tanks or raceways until 70 DPH (~30g). 30 grams is considered the minimum size to stock cobia juveniles in ocean cages, as smaller cobia are insufficiently resistant to ocean currents and bacterial infections, and occasional size grading is often undertaken during this time (Liao et. al., 2004). Once 30g is reached, the cobia can be transferred to nearshore or ocean cages for the adult portion of their grow-out to market size (Phase 3 & 4), which takes roughly 1.5-2 years (Liao et. al., 2004).
Chapter 3. **CULTURED SPECIES: BIOLOGY AND OPTIMAL CULTURE CONDITIONS.**

3.1. **COBIA (RACHYCENTRON CANADUM):**

3.1.1. **BACKGROUND**

Cobia (*Rachycentron canadum*), the sole member of the family Rachycentridae, is a widely distributed migratory fish found in temperate and subtropical waters worldwide (Briggs, 1960; Schaffer & Nakamura, 1989). Cobia larviculture has been developing in the United States since the 1970’s while commercial culture of cobia has been underway since the 1990’s in Southeast Asia, mostly driven by its excellent aquaculture performance and comparatively low production costs (Liao et. al., 2004; Benetti et. al., 2008). Certain characteristics that make cobia excellent for aquaculture production include rapid growth rates, high fecundity, ease of natural and induced spawning, high disease resistance and high survival rates (Benetti et. al., 2008b; Holt et. al., 2007; Liao et. al., 2004). The extremely rapid growth rate of cobia allows the fish to reach sexual maturity within 1.5-2 years when the animal reaches roughly 8-10kg (Liao et. al., 2004). While cobia culture in Taiwan accounts for roughly 80% of 5cage culture, the industry in the Americas is still incipient, with projects developing in the United States, Mexico, Brazil, Belize, Panama and Puerto Rico (Benetti et. al. 2007, Liao et. al., 2004).

3.1.2. **HATCHERY PRODUCTION**

Hatchery technology for cobia larviculture has been developed extensively at the University of Miami Experimental Hatchery (UMEH) and at the University of Texas Marine Science Institute (UTMSI), with survival rates ranging from 7-15% during the
early rearing phase (0-28 DPH) and roughly 25% for the post-larval phase (Benetti et al., 2008; Faulk et al., 2007). Cobia reach an average size of 3-5cm and 1.0g weight during the hatchery phase after which they are ready to commence the nursery phase of the production cycle (Benetti et al., 2008). Adequate survival and growth depends greatly on using the correct culture protocol and best management practices available.

3.1.3. Broodstock and Spawning

Success of the entire operation begins with maintaining healthy broodstock, through use of high quality feeds, acceptable water quality and prophylactic health treatments. Cobia are broadcast spawners and can spawn throughout the year in the wild, with a peak during the spring and autumn months when water temperature averages 23 – 27 °C (Liao et al., 2004). Wild broodstock cobia have been shown to spawn in captivity through temperature manipulation, with spawning occurring at 24-26°C shortly after sunset (Benetti et al., 2008b). Viable eggs are collected in egg collectors, and placed in incubators for the hatching process. Overall hatching rates of 50-90% have been reported (Benetti et al., 2008b; Faulk et al., 2007).

Cobia are euryhaline and eurythermal, and are found in a wide range of environmental conditions (Shaffer & Nakamura, 1989). Optimal culture conditions for larval rearing are from 25°32 ºC with a salinity range of 26-34ppt (Benetti et al., 2008b; Faulk et al., 2007). Chen et al. (2009) investigated the effects of different salinities on juvenile cobia, and found that specific growth rate (SGR) & food conversion efficiency were maximized at 30ppt and then decreased as salinity reached 35ppt. Salinity and temperature during the nursery phase should be comparable to the parameter range during larval rearing. Lower temperature and higher stocking density have been reported to
decrease the growth rate of cobia grown in open ocean cages (Benetti et al., 2010). Dissolved oxygen levels are always maintained above saturation, which through the use of supplemental oxygen can reach 6.5-9.0mg/L (Benetti et al., 2008).

3.1.4. **Cobia diet and energetics**

Cobia are voracious carnivores, which must derive a large amount of energy from feeds to supplement their high growth rates and metabolisms (Shaffer & Nakamura, 1989; Watson & Holt, 2010). Cultured cobia are fed a diet of formulated feed in pellet form, which can vary in their protein and lipid content. Both feed type and ration level are major factors in determining the energy budget of cobia (Sun et al., 2006). High quality feed brands such as Otohime™ typically contain anywhere from 45-52% crude protein and 12-16% lipid content. Chou et al. (2001) found optimal levels of protein and lipid content for maximal growth to be 44.5% and not over 6% respectively. Furthermore, Craig et al. (2006) supported that claim, demonstrating that growth was the same at 40% and 50% crude protein levels, stating that the US industry dietary feed standards of 58% crude protein and 16% lipid content is over formulated with respect to protein. Feeding rate is commonly 5-7% body weight (BW) for larvae, 5% for the nursery stage of 2-35g and is incrementally reduced down to 2-3% for cobia 100g or more.

3.1.5. **Nitrogen excretion**

With respect to dietary protein content, excess protein is catabolized and excreted as nitrogenous wastes such as ammonia and urea (Craig et al., 2006). In open water systems, this can lead to excess eutrophication, while in recirculating aquaculture systems (RAS) it can lead to a buildup of ammonia and consequently an increase in production costs and a decrease in productivity due to mortality and other secondary effects. Feeley
et. al. (2007) found that total ammonia nitrogen (TAN) excretion of juvenile cobia scaled with increasing mass, and were among the highest values in reported literature. Nitrogen excretion, fecal production and specific growth rate was also shown to increase significantly with increasing ration level for cobia being fed 2-8% body weight per day (Sun et. al., 2006). Rates of nitrogen excretion measured in California Halibut (P. californicus) were shown to peak roughly 4-6 hours after feeding, which closely matches the excretion peaks for other teleost fishes which peaked 3-6.5 hours after being fed (Merino et. al., 2007).

Figure 2. Image showing a tank reared cobia at the Smithsonian Marine Ecosystem Exhibit. Credit: the author.
3.2. **The Red Alga, *Gracilaria tikvahiae***

3.2.1. **Background**

*Gracilaria tikvahiae* (formerly *Gracilaria foliiefera var. angustissima*) is a marine macroalgae in the division Rhodophyta, which is endemic to warm-water coastal regions along the entire United States east coast, throughout the Caribbean and down the northern coast of South America (McLachlan, 1979). Experiments at Harbor Branch Oceanographic Institute in Florida looked at the culture viability of 42 separate species of seaweed, and found *G. tikvahiae* to be a prime phycoculture candidate based on a variety of factors, such as year round viability, persistence in the vegetative (non-sexual) reproductive state, high growth rates, yield and nutrient uptake rate (Lapointe & Ryther, 1978). The total organic yield of *G. tikvahiae* grown under ideal conditions was shown to reach 36.5 t/ha/year, a rate of production that exceeds most known agricultural crops (Lapointe & Ryther, 1978). The genus Gracilariales is one of the most important commercially, with applications in agar extraction, bioconversion to methane, fertilizer and wastewater treatment (Hanisak, 1990). Furthermore, *G. tikvahiae* is one of the most thoroughly investigated and best characterized species in the Northwest Atlantic (McLachlan, 1979). Whilst Rhodophytes are typically red, their colors can range from green, black or yellow which is the result of ordinary genetic controls and occasionally their nutritional status (Santelices & Doty, 1989).
Gracilaria can either be cultured while attached to surfaces such as rafts or rope lines, or it can be grown floating in suspension, the latter being perfectly suitable for intensive land-based culture systems. Nutrients in open-water systems are difficult to monitor & control due to dilution and currents, and hence can make management and integration challenging and often inefficient (Chopin et al., 2001). Fish effluents from land-based tank culture can be channeled through pipes into a separate bio-filtration culture module, thereby allowing for efficient treatment by biological means. Seaweed culture modules can be engineered for efficiency, and culture parameters such as temperature, pH and solar irradiation, can be controlled for optimal culture of each seaweed species. Furthermore, since no nutrients are lost to the environment, rates of nutrient uptake and discharge can be calculated, allowing for a mutually beneficial and balanced ecosystem to be engineered within the culture system (Neori et al., 2004).
Increasing algae biomass production and nutrient removal is of the highest priority for optimization of the algae filter. In general, biomass production and nutrient removal were negatively correlated with algae filter density, and positively correlated to the biological and physical limits of light intensity, temperature and water flow.

The optimization of an algae filter will require a thorough understanding of its physiological parameters (nutrient uptake kinetics, storage, nutrient type preference etc.), biological parameters (growth rates, life history stages, nutrient pre-history) and physical and chemical parameters (light, temperature, water flow etc.) (Chopin et. al., 2001).

The following will attempt to address in detail some of these parameters with respect to Gracilaria tikvahiae in order to establish optimal culture conditions needed to maximize biofiltration.

3.2.2. GROWTH RATE

The growth rate of Gracilaria tikvahiae and its relationship with nutrient concentration reflects the unique evolutionary history of this alga. Multiple laboratory experiments confirm that G. tikvahiae can utilize and thrive on extremely low nitrogen concentrations (1-2 µM) in the surrounding medium (DeBoer et. al., 1978; LaPointe & Ryther, 1978; Peckol et. al., 1978). Specific growth rate increases with increasing nitrogen concentration up to 1.5 µM, beyond which increasing the concentration of nitrogen has no substantial effect (DeBoer et. al., 1978). This adaptation has resulted from the evolution of this alga in environments where nutrient availability is sparse and sporadic, which allows them to rapidly absorb the smallest traces of nutrients present in the water. Specific growth rates in laboratory settings with supplemental nutrients ranged from 5-20%/day (DeBoer et. al, 1978; Parker, 1982), while in situ measurements of
growth rates ranged from 3-8%/day (Peckol et. al., 1978; Pennimen et. al., 1986). These ranges will be used to estimate the growth and yield of *Gracilaria tikvahiae* in the biofilter.

3.2.3. **Light Intensity**

Lapointe & Ryther (1978) found a close correlation between solar irradiation and yield of *G. tikvahiae*, with higher yields obtained during the summer time where incident solar radiation was highest, which makes sense in the context of a photosynthetic organism. Cultures of *G. tikvahiae* are grown best under full sunlight with no shade cloth (D Hanisak, *pers. comm.*, 2 April 2014).

3.2.4. **Temperature**

Optimal temperatures ranged between 12°C up to 34°C throughout the year. There was a positive correlation between yield and temperature, with higher yields obtained during the summer months (Lapointe & Ryther, 1978). While this effect was found mainly to be due to solar radiation, temperature was found to have a second order effect. Pennimen *et. al.* (1986) corroborated these finding by studying the *in-situ* growth of *Gracilaria tikvahiae*, and found that maximum growth coincided with season temperature and/or irradiation maxima.

3.2.5. **Aeration**

Aeration of algae in suspension is necessary to achieve optimal growth and survival. The aeration of seaweed increases photosynthetic efficiency by tumbling the seaweed inside the trough, which allows maximum light absorption while minimizing self-shading. Furthermore, aeration aids in nutrient absorption by decreasing the diffusion boundary layer, while simultaneously increasing the availability of metabolic gases such
as carbon dioxide (Hanisak & Ryther, 1984). *G. tikvahiae* requires relatively large amounts of aeration for growth, a factor that could hamper large scale production due to the costs associated with providing aeration. Ryther *et. al.* (1983) demonstrated that periodic aeration of 15min/hour, for a total of 6hour/day was as effective as continuous aeration, a finding that has important implications for the energy costs associated with culture.

### 3.2.6. Stocking Density

The density of macroalgae plays an important role in the growth rate in tumbling land-based systems. As above, self-shading becomes a problem in suspension systems and decreases productivity by limiting light. At very high densities of culture the macroalgae is no longer able to tumble and becomes stagnant. Lapointe & Ryther (1978) examined initial stocking densities ranging from 0.4- 4.8 kg/m$^2$, and found that maximum yields were obtained in all trials when density was between 2.0 – 3.0 kg/m$^2$. As such, this will be the target density in our system.

### 3.2.7. Nutrient Uptake and Kinetics

Nitrogen, found in the forms of ammonium (NH$_4$), nitrite (NO$_2$) and nitrate (NO$_3$), is the nutrient most commonly cited to limit growth in natural ecosystems (Hanisak, 1990). For *G. tikvahiae*, growth rates were identical when either ammonium or nitrate was supplied; however the uptake rate was much faster for ammonia rather than nitrate, showing a preference for the former compound (Lapointe & Ryther, 1978; Ryther *et. al.*, 1981). Ammonium is often the preferred source of nitrogen for plants because the incorporation of nitrate requires additional metabolic energy and enzymatic activity (Goldman and Horne, 1983). Most species of algae in nature live most of their lives in
nutrient-limited environments, and as such have the ability to rapidly uptake nutrients and store them in excess, also known as luxury consumption. When nutrients are supplied in culture at high ambient levels (>100 μmoles N/ℓ), *G. tikvahiae* was able to absorb the nitrogen to undetectable levels within 8 hours (Ryther, 1981). Furthermore, the algae was able to grow for an additional two weeks without any supplemental nutrient addition, showing the capacity for growth on luxury consumption. The algae cannot continue such high rates of uptake indefinitely when supplied a high concentration of nutrients, and eventually reaches a saturation point (Hanisak, 1990). Saturation levels are overcome as the organism grows and needs more nutrients to supplement its growth. Therefore, having optimal growth parameters within the culture unit will allow maximal uptake. At low ambient levels of nutrient concentration (1-20 μmoles N/ℓ), the saturation level is rarely reached and nutrient metabolic requirements are greater than uptake rates (DeBoer *et al.*, 1978). Interestingly, increasing the nitrogen concentration past 1.5 μmoles N/ℓ of the culture had almost no effect on the growth, showing the ability of this red alga to utilize very low Nitrogen concentrations for growth (DeBoer *et al.*, 1978). These findings are sensible in the evolutionary context of this species, however it is important to acknowledge that the nitrogen uptake is dependent on environmental conditions such as light, temperature and water motion.

### 3.2.8. NUTRIENT UPTAKE, DAY VS. NIGHT

Nutrient uptake rates were compared under full sunlight and at night in a controlled experiment by Ryther *et al.* (1981). The results show that nitrogen uptake in the dark was significantly lower, resulting in a 50% lower uptake rate during the night
time. These results have important findings for algae biofilters as their nutrient uptake capabilities will be reduced during the night time.

3.2.9 WATER FLOW

Flow rate and turnover rate determine the availability of limiting factors such as nutrients & carbon dioxide, and also the buildup up growth-suppressing secondary metabolites (Hanisak & Ryther, 1984). Maximal yield was proportional to turnover rate, which reached up to 30 culture volumes/day (Lapointe & Ryther, 1978). The data seems to indicate that a greater supply of seawater enhances growth by providing a greater carbon dioxide supply while keeping pH at more stable levels. This has important implications for the culture management as it favors flow-through systems that discharge a great volume of water daily with overall lower nutrient concentration.
Chapter 4. **APPROACH AND METHODOLOGY.**

4.1. **CONCEPTUAL DESIGN**

An algae biofilter will utilize a series of tumbling algae filters in order to filter out inorganic nitrogen from Total Ammonium Nitrogen (TAN) resulting from aquaculture wastes. The design intended to achieve this will consist of separate culture module(s) that will be installed on the effluent outflow line before it reaches the discharge location, in order to biologically treat the effluents before it gets discharged into a local body of water.

Nutrient rich effluents leave the culture tank through a central standpipe, and get pumped up to an elevated sedimentation tank or through mechanical drum filters to remove solid particulates. The water will then be gravity fed via PVC pipes into a series of tumbling algae culture troughs, where the algae will absorb nitrogenous waste. Water exits the algae filter through a standpipe or a bulkhead at the end of the trough, and get discharged back into the local body of water (**Figure 4**).

![Diagram showing the basic layout of an effluent biofiltration unit. The tumbling algae filters will be located after mechanical/sediment filters and before the discharge location.](image)

**Figure 4.** Diagram showing the basic layout of an effluent biofiltration unit. The tumbling algae filters will be located after mechanical/sediment filters and before the discharge location.
4.2. APPROACH

The goal of this project is to examine the biological and technological feasibility of utilizing *Gracilaria tikvahiae* in a tumbling algae filter (TAF) as a tool for bioremediation of cobia effluents resulting from aquaculture practices. More specifically, rates of nitrogen excretion by cobia during the production cycle will be quantified, while rates of nutrient uptake from *G. tikvahiae* will be determined to size the initial biomass of the algae filter. Finally, specific system design components will be identified to form a model system with the goal of maximizing biofiltration.

Due to the wide scope of this project, a comprehensive literature review was undertaken on this issue. Data sets will be derived from the most relevant published research.

The project will focus on the post-hatchery phase of production that involves rearing of juvenile cobia from roughly 30 to 70 days post hatch. During this phase, the cobia juveniles are reared in land-based systems until they are large enough (roughly 30g) to be stocked in ocean cages (Liao et. al., 2004).

4.3. METHODOLOGY AND ASSUMPTIONS

4.3.1. DETERMINING COBIA GROWTH RATE

The growth rate of cobia is not static, but depends on a variety of factors including water temperature, density of culture, water quality, feed rate and quality/protein content of feed. As such, it can be difficult to generate estimates on growth as the results can vary greatly. To compensate this, data was taken from an
experimental procedure that closely matched the desired culture conditions. The 
publication by Faulk et. al. (2007): Growth and survival of juvenile cobia *Rachycentron canadum* in a recirculating raceway system, closely followed the growth of cobia up through 70 days post-hatch with culture parameters that closely matches the target values for this model system.

The growth of cobia from 28-51 DPH is determined by the equation:

\[ Y = 2 \times 10^{-8} x^{4.961} \]

The growth of cobia from 52-70 DPH is determined by the equation:

\[ Y= 9 \times 10^{-9} X^{5.174} \]

4.3.2 DETERMINING NITROGEN EXCRETION

Nitrogen excretion as a product of TAN production is the metric used to derive the total amount of nitrogen excreted by juvenile cobia on a daily basis. Predicting rates of nitrogen excretion can be tricky, as the result depends on variables such as the chosen species, the feed rate and protein content, and the age/size of the fishes. To explore the possibilities within the data, 3 separate data sets (a-c) were generated.

a. **P-TAN generic** is derived from a generic aquaculture reference formula that attempts to quantify the total nitrogen amount resulting from ammonium excretion (Timmons et. al., 2009). The formula doesn’t relate specifically to cobia, but rather to a generic finfish. Hence, this dataset can be used more as a comparison of potential results rather than the absolute TAN production values for cobia. The formula used is:

\[ P\text{-TAN} = BM \times \%BW \times PC \times 0.092 / t \]
Where:

\[
P\text{-TAN} = \text{total ammonia as nitrogen production} \\
BM = \text{fish biomass in the system (mg)} \\
%BM = \text{feeding rate expressed as percentage of fish body weight} \\
PC = \text{Protein content in feed} \\
T = \text{time period (usually 1 day)}
\]

This formula relies on the assumptions that all TAN is excreted during time (t), and that nitrogen not assimilated in feces is quickly washed from the system and does not contribute additional ammonium.

b. Sun et. al. (2006) data set is derived from the publication: Growth, fecal production, nitrogenous excretion and energy budget of juvenile cobia (Rachycentron canadum) relative to feed type and ration level. In this experiment, the nitrogen excretion is examined over a variety of ration levels for juvenile cobia:
Figure 5. Graph showing the nitrogen excretion rates (mgN/g/d) for juvenile cobia over various different ration levels. Source: Sun et. al. (2006)

At the desired ration level of 5% BW/day, the calculated nitrogen excretion is:

\[ U = 0.117RL_d + 0.408 \]

Where:

- \( U \) = Nitrogen Excretion
- \( RL_d \) = Ration level per day (5%BW/d = 5)

Hence:

\[ U = 0.117(5) + 0.408 \]
\[ U = 0.993 \text{ mgN/g/d} \]

c. Feeley et. al. (2007) data set was derived from the publication: Elevated oxygen uptake and high rates of nitrogen excretion in early life stages of cobia *Rachycentron canadum* (L.), a fast-growing subtropical fish.

The ammonia excretion rate for juvenile cobia is predicted by the equation:

\[ U = 3.8906x^{-0.3632} \]
Where:

\[ U = \text{Ammonium (NH}_3 + \text{NH}_4 \text{) excretion rates (mgN/g/h)} \]

\[ X = \text{Wet weight (g) of juvenile cobia} \]

4.3.3 **DETERMINING AMMONIUM UPTAKE RATE BY *Gracilaria tikvahiae* **

The ammonium uptakes rates are not constant but depend on variables highly reliant on culture parameters. As described above in section 3.2, the rate of nutrient uptake is dependent primarily on the ambient nutrient load, the water flow/turbulence and the nutritional history of the algae.

Table 1. Shows the range of ammonium uptake capabilities of *Gracilaria* from published data. Note: some results have had their units converted to achieve uniformity in the data.

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>NH(_4) Uptake rate (µM/g/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parker 1982</td>
<td>Gracilaria tikvahiae</td>
<td>0.7-2µM</td>
</tr>
<tr>
<td>Ryther et. al. 1981</td>
<td>Gracilaria tikvahiae</td>
<td>0.23 – 2.73 µM</td>
</tr>
<tr>
<td>Peckol et. al. 1994</td>
<td>Gracilaria tikvahiae</td>
<td>1-4µM</td>
</tr>
<tr>
<td>Hernandez et. al. 2003</td>
<td>Gracilaria Graciales</td>
<td>1-3µM</td>
</tr>
</tbody>
</table>

The ammonium uptake estimates are derived from independent scientific experiments. Using these ranges, two estimates (lower and upper) will be chosen as theoretical values for nitrogen uptake rates to be used in this study. The lower estimate represents a more conservative value, while the upper estimate represents a median value for reported uptake rates.
The two estimates of ammonium uptake rates chosen for this study are:

**Lower estimate:** 0.5 µM/g/h  
**Upper estimate:** 1.5 µM/g/h

1 µM of NH₄ contains 0.014g of nitrogen. These uptake rates are converted into a more functional form of kilograms of nitrogen absorbed per kilogram of algae per day (kgN/kg/d). Furthermore, the estimates must be adjusted downwards since the nutrient uptake rates are reduced with the absence of direct sunlight. Assuming an average day of 14 hours daylight and 10 hours darkness, with an average of 50% reduction in nutrient absorption during darkness, this would result in a net decrease in uptake rate of 21% from a 24-hour estimate.

The adjusted uptake rates are:

**Lower estimate:** 0.0132 kgN/Kg/d  
**Upper estimate:** 0.0395 kgN/Kg/d

These estimates rely on the assumption that the nutrient uptake rate of algae is a constant, which it never is. Nutrient uptake rates can be much higher under ideal conditions, and vice versa holds true too. These values were chosen as a conservative average estimate of nutrient uptake capabilities when a culture system provides the ideal parameters for growth and absorption, and reproduction.
Chapter 5. **Data.**

5.1. **Cobia Growth Curve**

The growth curve of cobia during this phase of production (28-70DPH) is calculated based on growth data from cobia being fed at 5%BW/day with a feed protein content of 45%, which is the optimal feeding rate (Chou *et. al.*, 2001; Faulk *et. al.*, 2007).

![Cobia Growth Curve](image)

**Figure 6.** Graph showing projected weight gain of juvenile cobia from 28-70DPH being fed at 5% BW/day.

Cobia at roughly 28 days post hatch weigh on average 1.0g, but can range between 1.0 – 3.0g depending on the amount of feed given and the general health of the specimen during its initial stages of life (Benetti *et. al.*, 2008). Their growth rate is gradual until 52 DPH, after which their growth accelerates following a more exponential function.
5.2. **Total feed amount required**

The total amount of pelletized feed required during the duration of the nursery cycle is shown in figure 2 for a sample size of 10,000 fish in culture. The fish are fed at a constant 5% bodyweight, as the gradual decrease in feed rate is only recommended after 100g wet weight is reached.

![Graph showing the increasing amount of feed needed during the nursery trial of juvenile cobia. The feed amount is directly related to the biomass of cobia in the system, which in turn directly effects the nitrogen production in the system.](image)

**Figure 7.** Graph showing the increasing amount of feed needed during the nursery trial of juvenile cobia. The feed amount is directly related to the biomass of cobia in the system, which in turn directly effects the nitrogen production in the system.
5.3. **TOTAL AMMONIA NITROGEN (TAN) PRODUCTION**

Three estimates of the total daily nitrogen production resulting from metabolic ammonium release is summarized in **figure 8**. ‘P-TAN generic’ indicates theoretical production of total ammonia nitrogen (P-TAN) as a result of a generic equation that incorporates the feed rate and protein content in the feed. The remaining two lines show P-TAN resulting from 2 separate ammonia excretion experiments using juvenile cobia. The discrepancy between the generic and cobia specific data sets is large (2 fold).

![Figure 8](image)

**Figure 8.** Graph showing three projections for Total Ammonium Nitrogen excretion from the rearing of juvenile cobia, being fed at 5% BW/d.

While all three estimates produce roughly the same amount of total ammonia nitrogen initially (between 7-15g Nitrogen/day), the generic equation gives an estimate that begins
to surpass the other two estimates starting from 37 DPH, and ends with almost double the amount of Nitrogen production.

The P-TAN production from Sun et. al. (2006) & Feeley et. al. (2007) are the result of similar experimental trials that resulted in comparable results. As such, the data set seems more plausible than a generic formula that doesn’t relate to the specific species in this case. The maximum estimates for these two trials produce an average of 300gN/day for a sample size of 10,000 fish.

5.4. SUPPORTABLE BIOMASS OF SEAWEED

The biomass of seaweed that can be supported is directly related to the total amount of nitrogen produced by the system. The maximum estimates required for the duration of the 40 day cycle will be used to size the intial biomass of the biofilter.

5.4.1. LOWER ESTIMATE: UPTAKE RATE OF 0.5 µM/G/H NH₄

![Graph showing the supportable biomass of Gracilaria tikvahiae when a conservative nutrient uptake rate of 0.5 µM/g/h NH₄ is chosen.](image)

**Figure 9.** Graph showing the supportable biomass of *Gracilaria tikvahiae* when a conservative nutrient uptake rate of 0.5 µM/g/h NH₄ is chosen.
Under the most conservative nutrient uptake rate, the adjusted nutrient uptake rate is 0.0132 KgN/Kg/d. Taking an average of the maximum algal biomass for the Sun/Feeley data sets yields 27kg wet weight, which is used to size the initial biomass of the algae filter. For systems with adult fish, or specimens that have a higher nutrient output, the upper estimate of 50kg of algae (wet weight) may be used for the starting density within the filter.

5.4.2. **Upper estimate: uptake rate of 1.5 µM/g/h NH₄**

![Graph showing the supportable biomass of *Gracilaria tikvahiae*](image)

**Figure 10.** Graph showing the supportable biomass of *Gracilaria tikvahiae* for when the upper nutrient uptake rate of 1.5 µM/g/h NH₄ is chosen.

With an upper nutrient uptake estimate of 1.5 µM/g/h, or an adjusted uptake rate of 0.0395 kgN/Kg/d, supportable biomass of *G. tikvahiae* for the Sun/Feeley data sets reached 12kg. A more conservative estimate which can be used in a wider variety of systems would be 22kg under a high uptake rate.
5.4.3. **Extrapolated biomass estimates for large scale operations**

The initial biomass results derived above from the different data sets are used to extrapolate the supportable initial biomass of algae in the biofilter for cultures of fish up to 150,000 fish. Results are obtained by scaling algae biomass linearly with number of fish in culture.

Table 2. Shows the supportable biomass of *G. tikvahiae* over the two uptake rate estimates up to 150,000 fish per trial run.

<table>
<thead>
<tr>
<th># fish in Culture</th>
<th>Supportable Biomass range (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low uptake</td>
</tr>
<tr>
<td>10,000</td>
<td>27.0</td>
</tr>
<tr>
<td>20,000</td>
<td>54.0</td>
</tr>
<tr>
<td>30,000</td>
<td>81.0</td>
</tr>
<tr>
<td>40,000</td>
<td>108.0</td>
</tr>
<tr>
<td>50,000</td>
<td>135.0</td>
</tr>
<tr>
<td>100,000</td>
<td>270.0</td>
</tr>
<tr>
<td>150,000</td>
<td>405.0</td>
</tr>
</tbody>
</table>

5.5. **Growth and yield of Gracilaria tikvahiae**

5.5.1 **Growth estimates for a 12kg starting biomass**

Under the upper uptake estimate of 1.5 µM/g/h for a 10,000 juvenile cobia system, the starting biomass of *Gracilaria tikvahiae* was calculated to be 12kg. As the algae grows, its ability to absorb nutrients increases, allowing further buffering capacity for nutrient absorption. The following graph shows the projected growth rate up through 70 DPH at 4 different relative growth rate percentages (5, 10, 15 and 20%).
The graph also account for routine harvest down to initial biomass, a management technique needed to keep absorption capacity of the filter high.

![Biomass increase as a function of Relative Growth rate](image)

**Figure 11.** Graph showing the projected increases of the initial biomass of *Gracilaria tikvahiae* over 4 different relative growth rate scenarios (5%, 10%, 15% and 20%).

A 12kg starting biomass has the potential to increase to 15kg – 26kg depending on the relative growth rate. The projected yield (or harvest) would range from 3kg – 14kg per week over the duration of the 42 days cycle.

### 5.5.2 Growth estimates for a 27kg starting biomass

Under the lower uptake estimate of 0.5 μM/g/h for 10,000 juvenile cobia, the starting biomass of *Gracilaria tikvahiae* was calculated to be 27kg. The following graph shows the projected growth rate up through 70 DPH at 4 different relative growth rate
percentages (5, 10, 15 and 20 %), accounting for weekly harvest down to initial biomass.

![Graph showing the projected increases of the initial biomass of Gracilaria tikvahiae over 4 different relative growth rate scenarios (5%, 10%, 15% and 20%).](image)

**Figure 12.** Graph showing the projected increases of the initial biomass of *Gracilaria tikvahiae* over 4 different relative growth rate scenarios (5%, 10%, 15% and 20%).

Under these projections, a 27kg starting biomass has the potential to increase to 35kg – 60kg in a single week. Due to the large increase in biomass, harvesting to decrease the biomass in the filter will be necessary, as will be described below in 6.3 *Algae filter upkeep and management*. The harvest yield in this scenario would be 8kg-33kg every weekly harvest.
Chapter 6. **SYSTEM DESIGN, MANAGEMENT AND DISCUSSION.**

6.1. **PRE-ALGAE FILTER WATER TREATMENT**

Effluents in a flow through system leave the main culture tanks containing juvenile cobia and are funneled through PVC pipes towards the discharge location. Prior to being discharged, the effluents will be subjected to a variety of mechanical and biological (algae) filtration devices, in order to purify water before localized discharge which will help minimize any compounding environmental effects that may occur.

Large amounts of organic particulate matter, including fecal waste and suspended particles can be found in the effluents of aquaculture operations. These can cause turbidity in the effluent water, and ultimately lead to decreased photosynthesis by decreasing the amount of sunlight that can reach the algae in the filtration units. It is in the best interest of any aquaculture operation that intends to use algae filtration to remove these particulate wastes through the use of sedimentation tanks and mechanical filters. Since utilization of these filtration methods can often be prohibitively costly, the inclusion of these systems will depend on budgetary constraints that are unique to each operation.

6.1.1. **SEDIMENTATION TANK**

Sedimentation tanks work by allowing suspended solid wastes which have a higher density than seawater to sink out of suspension to the bottom, thereby allowing for the removal of these solids which results in effluents with a lower total dissolves solids (TDS) measurement. More than 95% of aquaculture systems had suspended solids with
particle sizes of 20 microns or less (Chen et. al., 1993). Typical aquaculture sedimentation tanks use a retention time of 15-60 minutes, which depends on the size of the sedimentation tank and its flow rate. Most solid wastes are only slightly more dense than seawater and require longer settling times, so the use of a sedimentation tank may be impracticable in most scenarios. If used, sedimentation tanks must be engineered accordingly to allow for optimal settling of solids, which could allow for the removal of most solids down to less than 100 microns (Chen et. al., 1993).

6.1.2. MECHANICAL/DRUM FILTRATION
Mechanical filters function by running solid particulate wastes through a mesh screen, thereby filtering out particles that are larger than the chosen mesh size. Static mesh screens often get clogged with particles and become ineffective, requiring greater amounts of manual labor and maintenance. Drum filters, also known as rotating screen filters, are mechanical filters that rotate to prevent screen clogging during filtration. Most drum filters are equipped with auto-backwash features to maintain efficiency. Typical aquaculture operations utilize drum filters with mesh screen sizes ranging from 10 to 250 microns.

6.2. ALGAE FILTER CULTURE TROUGHS
The algae culture troughs are the physical tanks that will hold the unattached algae in suspension and make up the actual biofilter unit. There are various possible designs and configurations for these troughs, which can vary with respect to effectiveness and cost. Some possible, cost-effective methods will be described below.
Figure 13. An algae culture trough built by the author at the Smithsonian Marine Station. It utilizes a PVC airline running down the center and is compartmentalized to allow for separate cultures of *Gracilaria tikvahiae* and *Ulva lactuca*.

6.2.1. **TROUGH DESIGN AND WATER FLOW**

Algae culture troughs are easiest to construct and install in a rectangular shape. The troughs are typically constructed out of fiberglass or cement. The dimensions of the trough can vary depending on the size of the biofilter required, which ultimately relies on the nutrient load estimation. Water flow/aeration within the culture is extremely important for the circulation of the algae within the trough. While good circulation allows
for sufficient sunlight to reach the algae, effectively only the algae at the surface of the trough can receive sunlight. Therefore, a measurement of surface area of the trough is used in the sizing of the culture trough.

**Figure 14** shows the 2 configurations that work best for algae culture troughs (Brian Lapointe, *pers. comm.*, 2014). The first relies on a PVC air-line running along the bottom corner of the trough, which moves algae and water in a circular movement. The second requires the use of a malleable plastic sheet folded along the bottom of the trough to create a curved bottom, with a PVC air line in the center that pushes algae up the middle.

![Figure 14. Image showing a side view of the two best configurations for algae culture troughs (B. Lapointe, *pers. Comm.* 2014)](image)

The air line is powered by an air compressor, which must be sized accordingly to generate enough air flow to circulate the algae. Air gets pumped into a 2-inch diameter PVC pipe with 2-3mm holes drilled at roughly 15cm intervals to allow for optimal tumbling within the filter (Neori *et. al.*, 1996).
A longitudinal view of the same culture tank shows the placement of the influent and the effluent standpipe, as well as the air-line which runs down the length of the trough (Figure 15). The inflow of the fish culture effluent is on the opposite side of the standpipe, to force the water to flow down the length of the trough before being discharged.

**Figure 15.** Image showing a longitudinal view of the algae culture tank.

The algae filter troughs, if multiple ones are used, should be arranged in sequence with one another, with water flowing from one through to the next until the discharge point. This is to allow for maximum retention time which leads to greater nutrient absorption. Aeration should be sufficient that the algae is constantly tumbling, and that no dead-spots form within the trough. Sunlight is the critical factor in the growth and optimization of this filter.

### 6.2.2. Algae Filter Upkeep and Harvest Management

As a living filter, the optimal utilization requires maintenance and upkeep to maximize biofiltration. As the algae grows, the density within the filter increases while the growth rate and yield decreases due to a variety of factors such as less sunlight, higher
pH and more secondary metabolites (Hanisak & Ryther, 1984). While the trough design is suggested above in section 6.2, number of troughs and trough size are dependant on the size of the aquaculture operation. Within all systems, the optimal density of the culture troughs should be between 2.0-3.0 kg/m$^2$. It is the responsibility of the on-site manager to create a harvest schedule that maintains the algae within these densities to allow for maximum growth and efficiency. It is also important to note that the density is per meter squared (m$^2$), and hence related to the surface area of the trough as sun light is the driving parameter in growth. For instance, a 20kg biomass algae filter should have a trough surface area of 6.5 – 10 m$^2$ to achieve an optimal density of 2.0 – 3.0 kg/m$^2$.

6.2.3. CULTURE TROUGH MAINTENANCE

It is important to clean and sterilize the trough units in between each trial. All algae can be removed and stored in holding tanks or baskets while the algae filters are serviced. Tanks can be sterilized with 10% hypochlorite solution or 10% HCl, followed by a thorough rinse with freshwater before restocking for the following trial.

6.2.4. EPiphyte Management

An ephiphyte is a plant that grows on another plant, but is not parasitic (at least directly). For algae, an ephiphyte is a non-target species of alga that grows on or with the target species. Although technically this does not leech nutrient directly from the target species, an ephiphyte will compete for both nutrient availability and light. Uncontrolled ephiphyte growth can often smother a culture of algae, slowing its growth and productivity and eventually killing it (Lapointe & Ryther, 1978). In addition to this, ephiphytes can contaminate monoculture algae cultures, rendering them often useless for sale for purposes such as food, agar or phycocolloid production. The two most common ephiphytes for the
culture of *Gracilaria tikvahiae* in Florida was Enteromorpha spp. in the summer and *Giffordia michellae* in the winter (Lapointe & Ryther, 1978; Capo et. al., 1999).

Epiphyte management typically requires the manual removal of epiphytes from the culture system. Time and effort should be spent daily in removing any visible epiphytes in the algae trough, thereby stemming the proliferation of the epiphyte before it can cause deleterious effects on the target crop. Having a pure starting culture also lessens risks of epiphyte contamination.
Chapter 7. **CONCLUSION.**

Sustainable aquaculture practices must be a reality if the industry is to expand and provide sustenance for the world's burgeoning population. Algal biofilters have the potential to be an effective tool in reducing the nitrogenous wastes resulting from land-based aquaculture facilities. This study concluded that the red algae *Gracilaria tikvahiae* which is found extensively throughout the southeastern United States is a viable candidate for phycoculture and use in algal biofilters. Published research shows that 10,000 juvenile (<30g) cobia can produce a maximum of 300g of nitrogen daily. Possible nitrogen uptake rates of *Gracilaria tikvahiae* range from 1-3 μM/g/h. The data correlating nitrogen excretion and uptake suggests that a starting algae filter biomass of 12-27kg of *G. tikvahiae* has the potential for up to 33kg of harvest per week for a sample trial of 10,000 juvenile cobia. These results are then scaled up depending on the number of fish in culture.

*G. tikvahiae*, apart from being one of the most well-studied species in the region, possesses excellent aquaculture qualities that make it suitable for bioremediation. The species has an extremely high growth rate and nutrient uptake capabilities, with the interplay of those two parameters resulting in a greater potential for increased nutrient uptake over time. The reproductive biology of the alga allows it to reproduce almost purely vegetatively throughout the year, making it more effective in a biofilter and its management simpler. Furthermore, its preference for ammonium (NH₄) as a nutrient
source favors aquaculture systems, especially flow through ones that have no bacterial biofiltration and hence low levels of nitrate (NO$_3$) and nitrites (NO$_2$).

Optimal biofilter performance relies on providing ideal parameters for the growth of the algae, as continuous growth will require a continuous nutrient supply when grown in non-saturated conditions. The algae must be provided with sufficient light, water flow, and a low density (2-3 kg/m$^2$), all of which have been shown to maximize growth rate in pilot scale systems.

The design of the algae filter is also paramount to its success. Rectangular algae filter troughs are one of the most typical and effective designs used for biofilters, as surface area is a key factor in efficiency for a filter that relies on sunlight to function. Perforated PVC airlines running down the side or the center of the trough must produce sufficient turbulence to keep the algae tumbling within the filter without forming “dead-zones”. Algae filter troughs connected in series will provide a greater retention time, and hence increase the nutrient absorption efficiency.

The concept of bioremediation, nutrient removal and algae biofilters is more than a novel technology designed to protect the environment. Utilizing this technology provides a variety of economic incentives to a farm operator, such as product diversity, offsetting the external costs of pollution and cleanup, and the production of a valuable by-product. Gracilaria is a key genus for the production of agar which can be used in everything from food to biomedical research.
Ultimately, it is the decision of aquaculture venture owners to endorse and utilize this technology, as the fate of not only the industry but possibly the entire planet relies on the adoption of environmentally, socially and economically responsible practices.
REFERENCES


