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# Polyculture bioremediation: An analysis of potential nitrogen assimilation and removal by *Mya Arenaria*, *Gracilaria Tikvahiae* and *Ulva Lactuca* harvests in the Corsica River, MD

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POLYCULTURE BIOREMEDIATION: AN ANALYSIS OF POTENTIAL NITROGEN  
ASSIMILATION AND REMOVAL BY *MYA ARENARIA*, *GRACILARIA TIKVAHIAE*  
AND *ULVA LACTUCA* HARVESTS IN THE CORSICA RIVER, MD

Kathleen Hemeon

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## ABSTRACT

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**Kathleen Hemeon**

**Polyculture bioremediation: an analysis of nitrogen assimilation and removal by *Mya arenaria* and *Gracilaria tikvahiae* harvests in the Corsica River, MD**

An analysis of nitrogen bioremediation in a tidal tributary of the Chesapeake Bay by the use of a hypothetical bivalve and macroalgae polyculture covering 1%, 3% and 5% of the Corsica River bottom area. This study was performed to illustrate the role ecosystem services play in managing diffuse watershed pollution, particularly nitrogen, resulting in water quality and living resource degradation. Excess concentrations of nitrogen in the Corsica River estuary lead to seasonal eutrophication and subsequent hypoxic events. *Mya arenaria* L. and *Gracilaria tikvahiae* (McLachlan, 1979) were chosen for this theoretical study due to their high assimilative capacities for nitrogen and established commercial value, whereas *Ulva lactuca* L. was analyzed as a biofouling harvest to increase the harvest nitrogen sink. *M. arenaria* nitrogen assimilation was calculated from literature values of nitrogen content in tissue, whereas *G. tikvahiae* was simulated from an existing macroalgae submodel. *M. arenaria* nitrogen removal ranged from 1000 kg N to 7000 kg of nitrogen per year and did not reflect *M. arenaria* mortality or nitrogen remineralization from biodeposits. Simulation of the model indicates that *G. tikvahiae* can remove between 51-255 kg of nitrogen per year and *U. lactuca* only removes 35-103 kg of nitrogen per year. Results indicate that the polyculture of *M. arenaria* and *G. tikvahiae* in the Corsica River can adequately reduce net nitrogen levels and demonstrate the use of bioremediation as a possible nutrient management tool for estuary restoration.

Keywords: Bioremediation, nitrogen assimilation, nutrient management, *Mya arenaria*, *Gracilaria tikvahiae*, Corsica River, eutrophication

## 1 INTRODUCTION

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### 1.1 BACKGROUND AND CONTEXT

The Chesapeake Bay currently experiences seasonal eutrophication that not only reduces water clarity, but also results in the proliferation of harmful algal species, oxygen depletion at depth, the wide spread loss of seagrass and benthic algae and the decline of commercial and recreational species that are affected by habitat loss (Kemp et al., 2005). Current strategies to control eutrophication primarily focus on reducing nutrient discharge into the estuarine environment from identifiable sources with little management once the nutrients enter the waterways (Kemp, Testa, Conley, Gilbert & Hagy, 2009).

The purpose of this study is to explore the introduction of bivalve and macroalgae polycultures in a tidal tributary of the Chesapeake Bay as a nitrogen management strategy. The native and marketable species of soft shell clam *Mya arenaria* L. and agar producing red-algae *Gracilaria tikvahiae* (McLachlan, 1979) were examined as culture species, while the nitrogen assimilation of the green algae *Ulva lactuca* L. was included as a biofouling harvest that can be collected off of the *M. arenaria* planting structures. By quantitatively assessing the potential nitrogen removal by nitrogen assimilation into the tissue of polyculture species, the implementation of bioremediation methods can be considered as a watershed restoration action strategy.

#### **Chesapeake Bay**

The Chesapeake Bay holds 68 trillion liters of water and nearly 193 billion liters of fresh water enter the bay each day from tidal tributaries (Chesapeake Bay Program, 2012). Over 2,700 species of plants and animals reside in the Chesapeake Bay and nearly 173 of those species are

shellfish and the Bay's fisheries produce over 220 million kilograms of seafood each year (2012). Chesapeake Bay is the largest estuary in the United with a watershed sprawling over 167,000 km<sup>2</sup> and seven jurisdictions (Fig 1) (Boesch, Brinsfield & Magnien, 2001).

The Chesapeake Bay is a partially mixed estuary located on the mid-Atlantic coast of the United States and is bordered by Virginia to the south and Maryland to the north (Sowers & Brush, 2014). This region was historically formed by the retreat of a continental ice sheet that carved out the Susquehanna River valley, which was subsequently flooded during the Pleistocene glacier melts and sea level rise during 7400 and 8200 years before present (Bratton, Colman, Thieler & Seal II, 2003; Pritchard, 1967 as cited in Bricker, Rice & Bricker,

2014). The present day estuary extends north to south for over 300 km and is on average 20 km wide with a deep narrow central channel remnant of the historic river valley and central depths reaching up to 54 meters and is lined by wide, shallow sills typically only 8 meters deep (Kemp et al., 2005; Bratton et al., 2003). Although the bay itself is bordered by just two states, its extensive watershed extends over seven jurisdictions and has a long history of anthropogenic intervention (Fig 1).

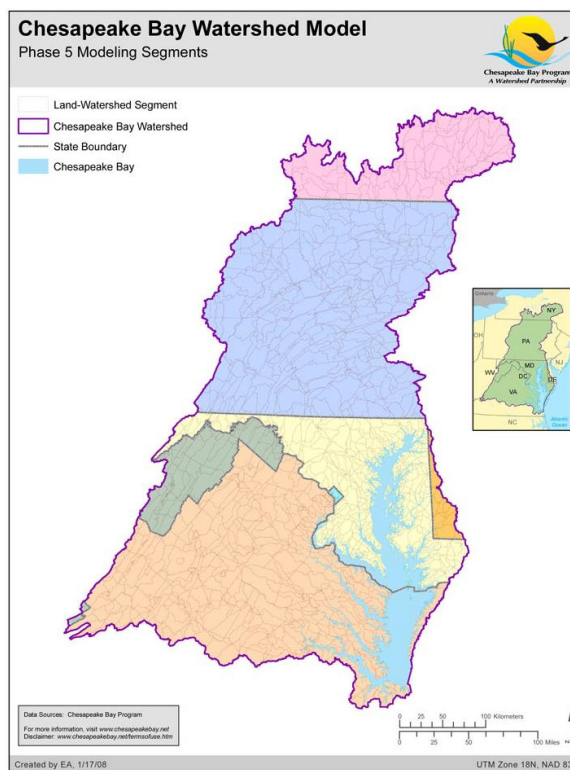


Figure 1 Map of Chesapeake Bay watershed. Reprinted from the Chesapeake Bay Program (<http://www.chesapeakebay.net/maps>)

Chesapeake Bay has been affected by human activity for centuries for its comprehensive provisioning of natural resources. Sowers and Brush (2014) identify five periods of major land use change in the Chesapeake Bay watershed:

1. Late precolonial (~1400s): Native American populations cleared less than 1% of the land
2. Early colonial (1665-1720): Tobacco agriculture took root, but employed less than 20% of the land
3. Developing agriculture (1720-1800): Wheat became the dominant crop and 60% of the land was devoted to agriculture
4. Intensive agriculture (1800-early 1900s): Identified with peak deforestation where 80-90% of the watershed was used for cultivation. Nearly half of the watershed was deforested during this time
5. Urbanization and afforestation (early/mid 1900s-present): Some land is reclaimed by forest as human populations centralize and land use shifts from intense agriculture to developed land (Fig 2)

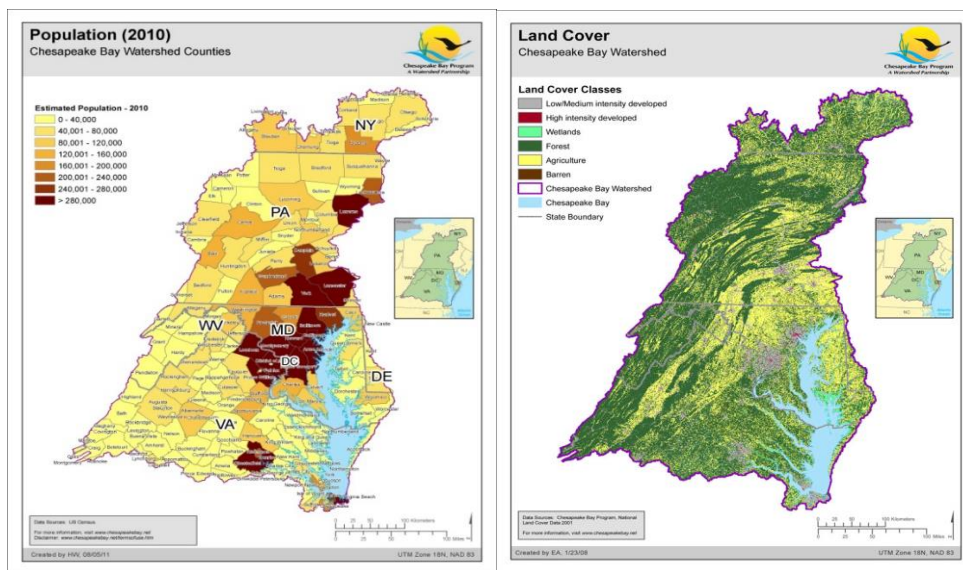


Figure 2 Watershed population size by county and land use patterns. Reprinted from the Chesapeake Bay Program (<http://www.chesapeakebay.net/maps>)

With the advent of modern fertilizers, greater crop yields can be produced on smaller and less fertile land, resulting in intensive agricultural practices in regions not used for urban development (Fig 2) (Kemp et al., 2005).

### **Nitrogen Loading and Eutrophication**

Changes to the Chesapeake Bay estuary due to land use alteration and anthropogenic inputs have led to the transition from benthic primary producers and consumers to a predominantly pelagic and opportunistic community (Kemp et al., 2005; Sowers & Brush, 2014). Developed land increases the area of impervious surfaces resulting in increased water runoff, carrying with it chemicals and nutrients used by humans. Historic changes in food web dynamics have also been observed in bay sediment cores where the proliferation of planktonic diatom populations has positively correlated with high sedimentation rates and nutrient flows (Sowers & Brush, 2014). As a result, losses have been seen in both the shellfish and finfish industries, in particular oyster, shad, Atlantic croaker and striped bass in direct response to anthropogenic activity in the watershed (Kemp et al., 2005).

Nixon defines eutrophication as the “increase in the rate of supply of organic matter to an ecosystem” (1995, abstract) where the principal source of organic matter is from the overabundance and senescence of phytoplankton. Many events can lead to an increase influx of organic matter, but is primarily a reflection of excess nutrients. As organic matter at the surface expires and settle to the benthos, microbial processes use oxygen to decompose the organic matter and can lead to oxygen deprivation near the sediment (Boesch et al., 2001). In regions such as the Chesapeake Bay with strong stratification, oxygen deplete water at the bottom rarely get replenished with oxygen rich surface water. As turbidity increases due to phytoplankton blooms at the surface, benthic algae and submerged aquatic vegetation can receive inadequate light for primary production, further reducing the concentration of dissolved oxygen at the benthos.

Nutrients which fertilize such excessive phytoplankton blooms can be a result of either point source or nonpoint sources. Section 502 (14) of the Clean Water Act defines point sources as

“any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged. This term does not include agricultural stormwater discharges and return flows from irrigated agriculture.”

Nonpoint sources were then defined as pollution that does not fall into the definition above. Point sources are relatively simple to identify and likewise easier to mitigate through best management practices and technology. Nonpoint source pollution is rapidly becoming the predominant supply of nutrients to waterways and is minimally reduced by watershed nutrient reduction strategies (Rose, Bricker, Tedesco & Wikfors, 2014). This pattern of undermanaged nutrient enrichment from the watershed followed by periods of phytoplankton blooms and hypoxia are common in the Chesapeake Bay, particularly in the tidal tributaries such as the Corsica River.

### **Corsica River**

The Corsica River is one of over 100,000 tidal tributaries located in Chesapeake Bay and is located in Queen Anne County, Maryland (Chesapeake Bay Program, 2012). The watershed sprawls over more than 64 square kilometers and consists of three major sub watersheds (Fig 3) (McCoy, 2003). Over 64% of the Corsica River watershed is devoted to agricultural land and the corresponding deforestation, coupled with intense fertilization, significantly impacts the quality of local surface waters.

The Corsica River was first placed on the federal impaired waters list (303(d)) in 1996 for excessive sediment, nutrient and fecal coliform concentrations and was amended in 2002 to include polychlorinated biphenyl pollution and impacts on biological communities (Town of Centreville, 2004; McCoy, 2003). Actions for managing water quality in the Corsica watershed tackle urban non-point pollution, waste water treatment by improving discharge technology and through the addition of extensive vegetation buffers on the margins of agricultural land (McCoy, 2003). In September 2005, nutrient levels in the watershed reached critical levels resulting in a nutrient induced algal bloom that killed off over 50,000 fish and prompted the development of more intense and broad reaching mitigation policy (Town of Centreville, 2004; Maryland Department of Natural Resources [MD DNR], 2003).

Federal, state and community agencies have collaborated to mitigate nutrient loading and to instill best management practices throughout the watershed and ultimately remove the Corsica River from the impaired water 303(d) listing. Currently, the Corsica River has been designated by the state of Maryland as a high priority watershed for restoration funding and action (Town of Centreville, 2004) and as of late, there has been a strong

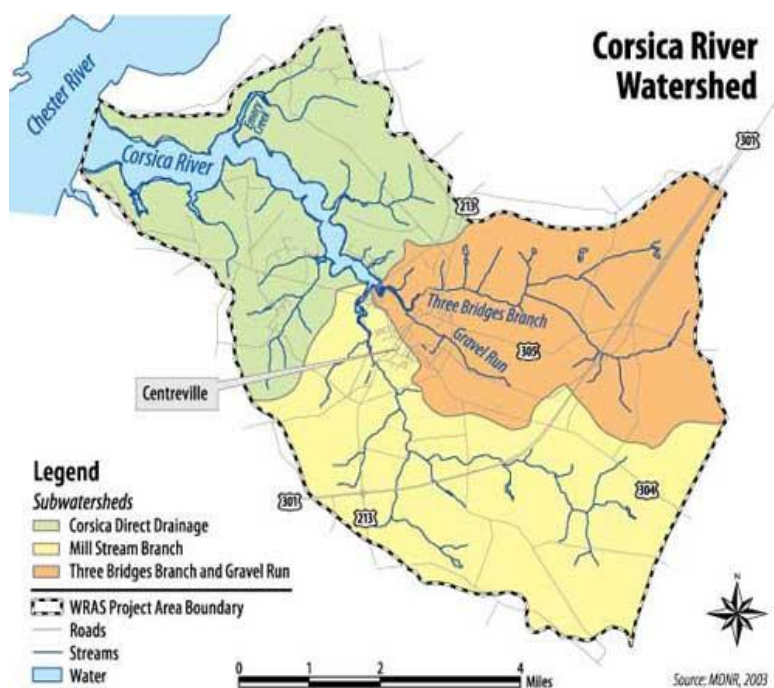


Figure 3 Map of the Corsica River and three sub watersheds. Reprinted from: [http://water.epa.gov/polwaste/nps/success319/md\\_corsica.cfm](http://water.epa.gov/polwaste/nps/success319/md_corsica.cfm).

push to find methods for reducing diffuse source nitrogen in estuaries.

## Current Research

Current research exploring nutrients on water quality often incorporates the simulation of ecosystem functions under diverse environmental parameters often through the use of ecosystem models. The Farm Aquaculture Resource Management (FARM) model is one such example. FARM is an extensive model developed for the European Union to measure water quality impact and scenarios to maximize harvest and profit for designated sites and shellfish species (Ferreira et al., 2009). A model such as FARM is a comprehensive approach to minimizing an aquaculture's impact while also providing the highest economic return for the shellfish harvest. However, ecosystem models are time consuming to construct and have often been created to set water quality standards by quantifying anthropogenic actions in the watershed and shallow waters on estuarine system dynamics. Models assembled for the Chesapeake Bay have not included interspecies aquaculture assemblages for collaborated nitrogen remediation purposes.

The use of biofiltration to reduce diffuse nutrient loading is gaining support as a viable restoration tool for degraded estuaries. It is known that marine bivalves and macroalgae assimilate nitrogen into their tissue and the use of mariculture could potentially have significant impacts on regional water quality (Nelson, Leonard, Posey, Alphin & Mallin, 2004; Neori et al., 2004; Tyler & McGlathery, 2006; Higgins, Stephenson & Brown, 2011; Carmichael, Walton & Clark, 2012; Woods Hole Group, 2012; He, Zhang, Chai, Wen & He, 2014; Rose et al., 2014 and others). Rose et al. identifies the importance of bivalve nitrogen assimilation and ecosystem services to estuarine and coastal waterways and assert shellfish aquaculture as a cost effective tool for comprehensive programs to reduce nitrogen enrichment (2014). It has recently been observed that some species, such as the soft shell clam *Mya arenaria*, may have higher nitrogen tissue composition when compared to other organisms in the system and assimilation may be dependent on ambient nitrogen concentrations (Carmichael, Shriver & Valiela, 2012).



Despite unanimous agreement acknowledging marine aquaculture's role in nitrogen cycling, there is insufficient laboratory and field experiments measuring the direct nitrogen removal potential by marine species leading to underexplored uses of this natural process for nitrogen management (Carmichael et al., 2012b; Ray, Terlizzi & Kangas, 2014).

## **1.2 POLY CULTURE AS A TOOL FOR NITROGEN MANAGEMENT**

### **Objectives**

The objective of this study is to identify the amount of nitrogen that can be removed from a nutrient enriched estuary through polycultures with high nitrogen assimilation capacities (Chopin et al., 2010). The overall goal of the polyculture is to attain water quality standards, restore habitats and aquatic populations and revive the shellfish industry in the Corsica River.

### **Methodology**

To estimate the potential nitrogen removed by multispecies' harvests, culturing techniques and cultured species must be identified.

- ❖ Site: The Corsica River was chosen for its listing as an impaired water (303(d)) and location in the upper Chesapeake Bay where oyster restoration projects have been less successful. Impairment is strongly linked with intense fertilizer application for agriculture and other diffuse sources of nutrient pollution.
- ❖ Culturing Techniques:
  - Bivalves were utilized to assimilate organic nitrogen and regulate phytoplankton blooms through consumption, while macroalgae were chosen to uptake inorganic nitrogen and compete with opportunistic algae for nutrients.

- Bottom culture techniques were applied for bivalves, whereas floating rope cultures were considered for *G. tikvahiae*.
  - *U. lactuca* harvests were only considered when biofouling occurred on bivalve predation netting.
- ❖ Species:
- *M. arenaria* was chosen due to its historic population in the Corsica River along with the ability to assimilate organic nitrogen, improve water clarity through suspension feeding and an established market demand.
  - *G. tikvahiae* was chosen as a native macroalgae species to the Chesapeake Bay with high nitrogen uptake rates, high tolerance for changing environment, easy propagation and a strong market for agar products.
  - *U. lactuca* was chosen as a common biofouling organism which would normally be discarded back into the system, but instead will be harvested and removed.

Nitrogen removal for *M. arenaria* was calculated from established nitrogen concentrations in marine organisms as well as current research identifying higher assimilation rates for *M. arenaria* compared to other bivalve species. Nitrogen removal in two algal species was determined from a macroalgae sub model that was modified for harvest and Corsica River environmental parameters. Nitrogen harvests for all three species were then added to a nitrogen nutrient budget created for the Corsica River to demonstrate the magnitude of nitrogen removal by the polyculture in comparison to nitrogen inputs and existing nitrogen sinks.

Finding solutions to water quality degradation requires multifaceted and innovative approaches to tackle diffuse pollution. The United States Environmental Protection Agency's Office of Water distinguishes water quality trading as an efficient means to meet watershed restoration goals by

using improved water quality in one sector to offset undermanaged pollution sources from alternative sources to meet total maximum daily loads for nutrients (2003). This strategy would primarily address the need to reduced diffuse pollution loading, such as sediment and agricultural nutrient runoff, which occurs in countless watersheds throughout the Chesapeake Bay. Polyculture may provide a regional solution to meet watershed restoration goals by trading nutrient credits from aquaculturalists to offset nutrient inputs from the Corsica River subwatersheds while also providing funding to initiate new algal markets and possible incentives to watermen.

### **1.3 STRUCTURE OF THESIS**

This thesis is divided into five chapters. The second chapter is a more detailed examination of past and current research to provide background and motivation behind this analysis addressing relevant policy and regulations. The third chapter contains a thorough explanation of all methods used to complete the analysis. Chapter four is a presentation of relevant results and model outputs. The last chapter is a discussion of the results and how this study can contribute to the overall health and restoration of the Corsica River.

## 2 LITERATURE REVIEW

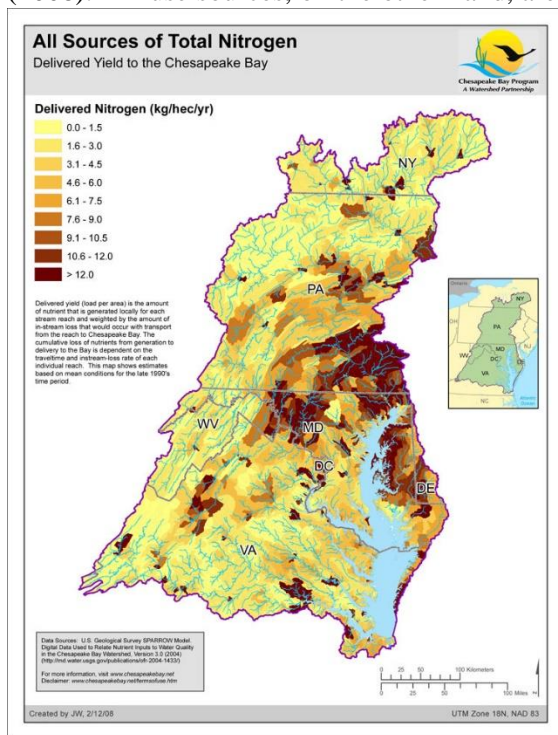
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### 2.1 ENVIRONMENTAL OVERVIEW

Over the past several centuries, human induced changes to the Chesapeake Bay watershed have led to declining water quality and drastic losses to native aquatic populations. Activities such as deforestation, intensive fertilization of crops, expansion of impervious surfaces and airshed contamination by the combustion of fossil fuels have all negatively impacted the waters of the Chesapeake Bay (Cloern, 2001). The Chesapeake Bay's ratio of watershed area to water volume is quite high ( $2.2 \text{ m}^{-1}$ ), indicates that the watershed has a profound influence on the estuary (Kemp et al., 2005). Sediment and nutrient movement from the watershed to the estuary have been linked to the loss of seagrasses and benthic algae, declines in dissolved oxygen in the benthos and the proliferation of phytoplankton biomass including harmful algal blooms (HABs) (Cooper & Brush, 1993; Bartoli, Cattadori, Giordiani & Viaroli, 1996; Boynton, Hagy, Murray, Stokes & Kemp, 1996; Cloern, 2001; Kemp et al., 2005).

Sediment is typically introduced to surface waters when there is significant soil erosion on agricultural land or in areas of deforestation. During large rain events, water runs over the land at a substantial velocity that can carry away sediment to surface waters and eventually coastal environments. When high sediment loads reach the bay, it significantly increases turbidity and prevents light penetration through the water column which is vital to aquatic primary producers.

Nutrients are introduced to the Chesapeake Bay through point sources and diffuse sources (i.e. non point sources). The National Oceanic and Atmospheric Administration [NOAA] identifies point sources as sites of pollution where the pollution can be traced back to the site of discharge and can range from a smoke stack, sewage overflows or even concentrated animal feeding operations (2008). Diffuse sources, on the other hand, are extremely hard to regulate since no single polluter



*Figure 4 Nitrogen loading from all sources. Loads represent amount generated in individual watersheds and amount of generated nitrogen that is “lost” to the Chesapeake Bay (higher losses indicate higher loading to the Bay). Reprinted from Chesapeake Bay Program.*

can be identified. Pollution from these sources typically enters the surface waters via rainwater runoff and can be from many sources including road residue and crop fertilizers (NOAA, 2008). Federal legislation has been successful at mitigating point source pollutants through strict standards and permitting process to the point that the facilities have upgraded to the bounds of feasible technology for further reduction of pollution loading. A majority of sediment, phosphorus and nitrogen loading to the Chesapeake Bay is draining from either agricultural land, particularly on the Eastern Shore, or urban communities with large areas of impervious surfaces for water runoff.

Nitrogen and phosphorus are categorized as pollutants when concentrations stimulate algal blooms that adversely affect living resources and water quality. Both of these elements naturally enter estuarine nutrient cycles as a result of weathering and nutrient recycling, however the large increase of nitrogen and phosphorus reaching the Bay today can be traced back to anthropogenic sources

predominantly in the forms of nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ). Nutrient introduction to the aquatic environments are sourced from various activities in the watershed and are retained in certain systems where the nutrients can severely impact ecosystems (Bukaveckas & Isenberg, 2013).

### **2.1.1 Eutrophication**

Eutrophication is defined as the increase in the rate of supply of organic matter to an ecosystem (Nixon, 1995). Eutrophication is known to plague coastal margins and estuaries as a result of their close boundaries with human development and as the major mixing point for terrestrial runoff as it meets the marine environment. Smayda distinguishes phytoplankton blooms as the growth during nutrient replete conditions temporarily uninhibited from grazing pressures (1997), where growth is dependent on nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) availability (Bruland, Rue & Smith, 2001).

As seen in Figure 5, if a nutrient is not limiting, phytoplankton will continue to propagate and reduce water clarity at the surface until alternative factors limit growth or a nutrient store is depleted. Once the algal cells die off, they settle out of the water and begin to decompose as a result of the oxygen consuming process of denitrification. Eventually the bottom water becomes hypoxic which drives out grazing benthic macrofauna which regulate phytoplankton blooms, or benthic producers can no longer recover from the limited light availability and nutrient competition is eliminated. For the Chesapeake Bay and the Corsica River, nitrogen is identified as the limiting nutrient for phytoplankton in marine systems (Fisher, Peele, Ammerman & Harding Jr., 1992). As oxygen becomes unavailable, denitrification is restricted and remineralization allows  $\text{NH}_4^+$  to be recycled into the water column and made available to the phytoplankton (Kemp et al., 2009). Typically, if nitrogen is not reduced, the system will see no relief and will continue to degrade.



Hypoxia is defined as water where oxygen levels cannot support indigenous organisms (or  $< 2 \text{ mg l}^{-1}$ ) and anoxia as waters lacking oxygen (Boesch, Brinsfield & Magnien 2001; Kemp et al., 2009). This is the most severe consequence of eutrophication and can devastate fisheries and ecosystems. Low dissolved oxygen disrupts the metabolism of macro organisms and if prolonged can lead to mortality. The Chesapeake Bay experiences “persistent seasonal” and stratified hypoxia which is further amplified by the bay’s loss of key habitats such as oyster reefs, seagrass beds and tidal marshes that regulate and support sedimentation and nutrient cycling (Kemp et al., 2009).

In addition to oxygen diminution resulting from increased biological oxygen demand at the resulting from phytoplankton biomass settling on the sediment, eutrophication also increases light attenuation and restricts benthic primary production. Benthic primary production includes photosynthesis from submerged aquatic vegetation (SAV), benthic diatoms and benthic macroalgae (seaweeds), which not only supply oxygen near the sediment, but also provides critical habitat for benthic communities (e.g. blue crabs, sponges, sea squirts, barnacles, zooplankton, white perch and American shad) whether it be for foraging, refuge or as a nursery for juveniles. As a result, areas of the bay and tributaries with recurring eutrophication are seeing shifts from a strong benthic community to primarily pelagic and/or opportunistic species (Kemp et al., 2005; Sowers & Brush, 2014). Species tolerant of low dissolved oxygen can drastically change dynamics of food web structures by extending foraging and refuge territory (Nestlerode & Diaz, 1998)

Seasonal eutrophication is common in the Chesapeake Bay and its tidal tributaries, but when nutrients are supplied in excess it can drastically alter estuarine communities and change the trophic level dynamics.



### **2.1.2 Impact of Eutrophication and Sedimentation on Seagrass Beds**

Seagrasses are marine flowering plants commonly belonging to the *Zostera*, *Thalassia* and *Posidonia* genera (McRoy & McMillan, 1977 as cited in Waycott et al., 2009). Seagrass beds support commercial fisheries (Watson, Coles & Lee Long, 1993), while also providing essential ecological functions including sediment stabilization (Orth et al., 2006), nutrient cycling and carbon sequestration (Romero, Lee, Pérez, Mateo & Alcoverro, 2006; McGlathery, Sundbäck & Anderson, 2007).

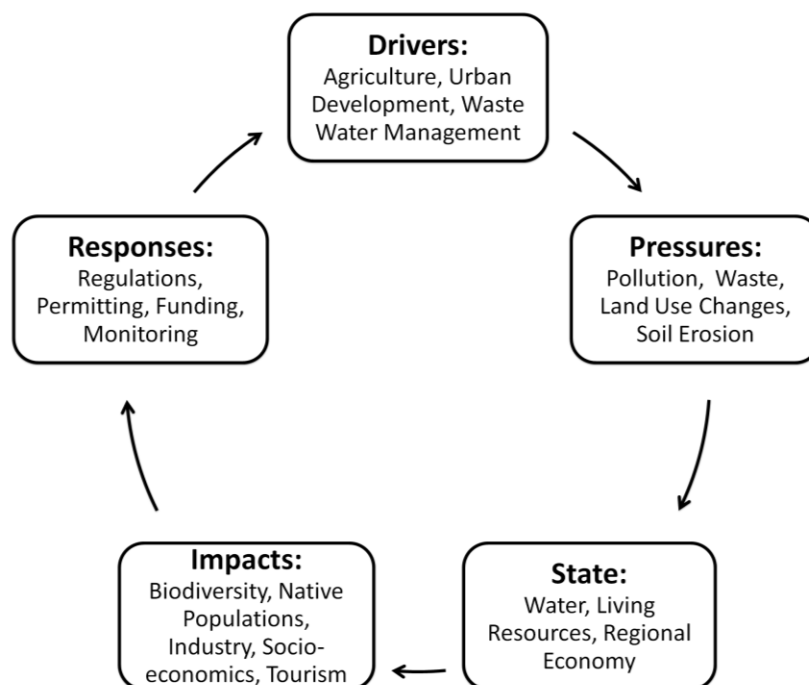
Sixteen species of bay grasses are common to the Chesapeake Bay and are vital food and habitats for fish, invertebrates and waterfowl (Chesapeake Bay Program, 2005). Bay grasses, or SAV, provide protection from storm surges while also improving water quality through oxygen production, sediment stabilization and nutrient filtration. Although historic bay grass beds were once as large as 200,000 acres, by 1984 they were reduced to only 38,000 and by 2013 were averaging 68,000 acres, only 37% of the 2010 restoration goal. SAVs are sensitive to environmental changes and growth is limited primarily by light availability, which is currently being reduced by sediment suspension and nutrient enrichment.

Orth and Moore identify the periods of seagrass losses over the past 80 years, preceded by limited recovery, as a result of climate change and anthropogenic pressures including high sediment and nutrient inputs (1983; Neff et al., 2000). Even short exposures to heightened turbidity or increased temperature can have profound effects on seagrass meadows even after only a few days (Moore, Shields & Parish, 2013).

*Zostera marina* (eelgrass) is the most abundant seagrass in the western North Atlantic including lower regions of the Chesapeake Bay with smaller populations found in the northeastern Bay

(Moore & Short, 2006; Maryland Department of Natural Resources [MD DNR], 2010). The Chesapeake Bay marks the southern range of *Z. marina* in the western Atlantic and therefore favors water of cooler temperatures in the Bay (MD DNR, 2010 ). Jarvis, Brush and Moore have demonstrated that the collective stress of warming water temperatures and low light availability have resulted in significant declines in *Z. marina* production in the York River of Chesapeake Bay (2014). It has also been proposed that *Z. marina* beds do not recover unless there is enough improvement to water quality to support existing beds, which would augment rising water temperatures (2009). If water quality does not improve, these “severely threatened” seagrass beds may be completely eradicated in certain parts of the Bay (CBP, 2005; Moore et al., 2013).

### 2.1.3 Summary of Pressures, Impacts and Responses of the Chesapeake Bay



*Figure 6* DPSIR diagram. Identifies the major drivers, pressures, states, impacts and responses that the Chesapeake Bay is experiencing due to excess nitrogen inputs from the watershed.

Many factors influence water quality in such a large system such as the Chesapeake Bay, but it is evident that human induced changes within the watershed are increasing the amount of nutrients entering the bay, which stimulate eutrophication and subsequent hypoxic conditions (D'Elia, Harding, Leffler & Mackiernan, 1992). Fig 6 illustrates the drivers, pressures, state and impacts previously discussed in this chapter so far. Diffuse sources of nitrogen, such as fertilizer leachate into ground water from agriculture, create pressure on estuaries in the form of pollution. The system reacts to the nitrogen with prolific algal blooms which reduce water clarity and increase biological oxygen demand. This alters the water quality state of the estuary into an oxygen deprived and turbid environment. Many trophic levels are impacted resulting in loss of benthic primary production, hypoxic bottom waters, and overabundance of opportunistic species. Habitat loss such as sea grass beds reduce spawning or recruitment of some native species which in turn can collapse fisheries and human livelihood.

These processes are well observed in the Chesapeake Bay and as a result it was the first estuary in the United States to be chosen for major restoration efforts designated for incorporated watershed and ecosystem management (Chesapeake Bay Program, 2012).

## **2.2 WATER QUALITY MANAGEMENT OF THE CHESAPEAKE BAY**

The Chesapeake Bay is the largest estuary in the United States and is supplied by a 165,760 square kilometer watershed divided over seven jurisdictions consisting of Virginia, Maryland, Delaware, Pennsylvania, New York, West Virginia and the District of Columbia (Sowers & Brush, 2012). Creating and implementing policies between states to minimize transboundary pollutants is extremely difficult and has resulted in federal intervention for many surface waters throughout the

nation (e.g. the Clean Water Act). In the case of the Chesapeake Bay, very few water quality and ecosystem goals established by federal legislation have been attained, while cultural eutrophication, seasonal hypoxia and critical habitat degradation remain persistent. Over the past century, federal and state agencies have layered multitudes of management and regulatory policies to restore the Bay's water quality and ecosystems by identifying sustainable, yet equitable, management practices.

### **2.2.1 Federal Water Pollution Control Act: Clean Water Act (CWA)**

The Federal Water Pollution Control Act of 1948 was a major milestone for creating federal funding and research incentives to reduce the influx of pollution into national water bodies (New Hampshire Department of Environmental Services [NHDES], n.d.). However, it was not until major amendments were instated in 1966, 1972, 1977 and 1987 that the legislation made considerable advances for setting water quality standards and implementation strategies to meet standards and became commonly known as the Clean Water Act (CWA) (NHDES, n.d.). Two key targets for the CWA, was to “eliminate the discharge of pollutants into navigable waters by 1985” and for the protection and propagation of fish, shellfish and wildlife populations while maintaining the recreational uses of water bodies by July 1, 1983 (Clean Water Act, 1972). The CWA also delegated management planning to each state to control pollutant sources within their jurisdictions and to create programs to address non-point sources of pollution (Clean Water Act, 1972). The 1972 amendments also gave rise to the National Pollution Discharge Elimination System (NPDES) which requires permits to discharge pollutants into surface waters by point sources and is credited as drastically reducing point source pollution such as waste water facilities and concentrated animal production to name a few (NHDES, n.d.).

The Federal Water Pollution Control Act and all subsequent amendments pertain to overarching water quality standards and grants authority to the Environmental Protection Agency (EPA) for enforcing such regulations on ALL United States domestic surface waters (NHDES, n.d.). In order to maintain state sovereignty, states and local jurisdictions are allowed to create and implement individual management strategies, with EPA oversight, to meet federal requirements.

### **2.2.2 Chesapeake Bay Agreements**

In response to mounting public concern of Chesapeake Bay water quality despite implementation of the CWA, Senator Charles Mathias of Maryland sought and received congressional funding in the mid 1970's for a five year study of the Bay estuary to account for the observed declines in living resources (United States Environmental Protection Agency [USEPA], 2010). Following this initial evaluation, the 1983 Chesapeake Bay Agreement was created as a formal but voluntary contract to begin restoration efforts of Chesapeake Bay ecosystems and water quality, with signatories from cabinet secretaries of Maryland, Virginia, Pennsylvania, Washington D.C., the administrator of the EPA and the Chesapeake Bay Commission chairman (USEPA, 2010). Five years later, a 1987 Chesapeake Bay Agreement was established which identified nutrient loading as the primary contributor to estuarine biotic decline, and the agreement called for a 40% reduction of “controllable” nitrogen and phosphorus loads to mainstream Chesapeake Bay by the year 2000 (Chesapeake Executive Council, 1987; USEPA, 2010; Linker, Batiuk, Shenk & Cerco, 2013). Although it was recognized in 1987 that both point sources and nonpoint sources contributed to nutrient loading in the bay, sources such as atmospheric deposition and forest loading would not be accounted for due to implementation feasibility (Linker et al., 2013).

Two benchmark evaluations took place in 1991 and 1997 to assess the progress of the two agreements as the 2000 target date approached. The 1991 appraisal led to an amendment to the Chesapeake Bay Agreements, which included tributary restoration and a goal for the greater distribution of submerged aquatic vegetation (SAV) (USEPA, 2010). The 1997 evaluation reveals that between 1985 and 1996, phosphorus loads had decreased by 6 million pounds annually and that nitrogen loads decreased by 29 million pounds annually (USEPA, 2010). The USEPA observed that by 1997, wastewater plants lessened their phosphorus loads by 51% and nitrogen by 15%, with nitrogen improvements primarily attributed to biological nutrient removal and facility upgrades (2010). However, despite progress made at point sources, nonpoint sources only reduced their phosphorus and nitrogen loads by 9% and 7% respectively (USEPA, 2010).

The Chesapeake 2000 Agreement used the momentum of previous agreements by maintaining the 40% reduction goal from 1987 nutrient levels, but redirected the development water quality standards to be based on restoring the Bay's living resources (USEPA, 2010; Linker et al., 2013). The Chesapeake Executive Council (2000a) stated four primary goals to meet water quality standards:

- ❖ Restore, enhance and protect the finfish, shellfish and other living resources, their habitats and ecological relationships to sustain all fisheries and provide for a balanced ecosystem (pg. 2)
- ❖ Preserve, protect and restore those habitats and natural areas that are vital to the survival and diversity of the living resources of the Bay and its rivers (pg. 4)
- ❖ Achieve and maintain the water quality necessary to support the aquatic living resources of the Bay and its tributaries and to protect human health (pg. 6)

- ❖ Develop, promote and achieve sound land use practices which protect and restore watershed resources and water quality, maintain reduced pollutant loadings for the Bay and its tributaries, and restore and preserve aquatic living resources (pg. 8)

The 2000 Agreement not only mitigates nutrient loading into the bay, but also the consequences of land use changes on aquatic life. For instance, sediment import from deforestation can increase turbidity and negatively impact primary producers such as SAVs, which in turn decreases dissolved oxygen necessary for sustaining life in both the water column and the benthos. This document also emphasizes the importance of good stewardship, such as education and best management practices, to uphold the integrity of Chesapeake Bay's watershed management strategies.

Soon after the Chesapeake 2000 Agreement was finalized, a Memorandum of Understanding (MOU) was signed between the six watershed states (Delaware, Maryland, Virginia, Pennsylvania, New York and West Virginia), Washington D.C. and the EPA to collaborate restoration efforts between all jurisdictions of the watershed (Chesapeake Executive Council, 2000b). The MOU allowed for the synergistic establishment of nutrient and sediment caps by all seven jurisdictions in 2003. These limits were created from modeled projections of dissolved oxygen levels, chlorophyll *a* concentrations and water clarity that were determined detrimental to living resources (Link et al., 2013; USEPA, 2010).

### **2.2.3 Chesapeake Bay Protection and Restoration Executive Order 13508**

By 2009, it was clear that the Chesapeake Bay not meet designated use goals determined by the CWA. Therefore, on May 12, 2009, President Barack Obama prepared the Executive Order 13508: Chesapeake Bay Protection and Restoration (Exec. Order No. 13508; USEPA, 2010). Under order

13508, the Federal Leadership Committee was created and granted authority to manage restoration strategies as well as organize data reporting efforts from various agencies within the watershed. After four decades of mounting water quality policy, insufficient progress was made resulting in the implementation of total maximum daily loads (TMDL) for nitrogen, phosphorus and sediment input into the Bay (Linker et al., 2013; USEPA 2010).

### **2.2.42010 Total Maximum Daily Loads (TMDL)**

The USEPA reports that 97% of the Bay's segments are listed as "impaired" resulting from excess nutrients and total suspended solids (TSS) (2010). Excess nutrients can drive systems towards eutrophication and a subsequent increase in biological oxygen demand (BOD), hypoxia and eventually aquatic animal death. Large algal blooms can also drive other primary producers out of a system by increasing light attenuation at the surface and preventing light penetration to benthic algae and SAV. In order to protect the health Chesapeake Bay's living resources and ecosystems, the largest and most complex US TMDL system was established (USEPA, 2010).

The 2010 TMDL allocations were formed as a mediation strategy to satisfy the 2009 executive order. This watershed management scheme is a step towards meeting the policy goal of having all pollution control procedures in place by 2025 to ensure full restoration of the Bay and tidal rivers, where 60% of the implementation completed by 2017 (USEPA, 2010). One of the largest watershed management predicaments is the transboundary contribution of nutrients and sediments to the main channel of Chesapeake Bay from seven distinct jurisdictional sources, each with their own legislative, hydrological, geophysical and chemical processes. To resolve this complexity, allocations set for major river basins within each jurisdiction (USEPA, 2010). These allocations enforce the *polluter pays principle* so that watersheds contributing the most to the degradation of



mainstream Chesapeake Bay's water quality, will have to employ even greater control measures for both point source and diffuse pollution to surface waters (USEPA, 2010; Linker et al., 2013).

The 2010 allocations are significant in that they are the first TMDLs to introduce atmospheric nitrogen deposition as well as federal land and federal facilities which constitute 6.2% of the Chesapeake Bay watershed (Linker et al., 2013). The airshed was represented using the Community Multiscale Air Quality Model (CMAQ) along with a regression model to account for "wet fall nitrogen deposition" while a third model, the Watershed Model Phase 5.1, was created for the Chesapeake watershed dynamics (Linker et al., 2013). Linker et al. then took several model scenarios representative of the airshed and watershed, and fed them into the Water Quality and Sediment Transport Model (WQSTM) to evaluate potential dissolved oxygen, presence of SAV, water clarity and chlorophyll *a* (2013). As a result of these models and simulation, allocations were biologically determined for each major river basin based on their fulfillment of accumulated policy to protect living resources from degradation and preserve the designated uses of the Chesapeake Bay's water segments, which can be recreational, commercial or ecological uses (Linker et al., 2013; USEPA, 2010). It is also critical that river basin allocations reflect the magnitude of influence of each river basin on the estuary, meaning that larger polluters will have to do more to meet water quality standards (Table 1).

Table 1 Final nutrient and sediment allocations for the Chesapeake Bay. Data sourced from the Chesapeake Bay TMDL (USEPA, 2010).

Jurisdiction	Nitrogen Allocations (million lbs/year)	Phosphorus Allocations (million lbs/year)	Sediment Allocations (million lbs/year)
Pennsylvania	73.93	2.93	1,983.78
Maryland	39.09	2.72	1,218.10
Virginia	53.42	5.36	2,578.90
District of Columbia	2.32	0.12	11.16
New York	8.77	0.57	292.96
Delaware	2.95	0.26	57.82
West Virginia	5.45	0.59	310.88
<b>Total Basin Allocation</b>	<b>185.93</b>	<b>12.54</b>	<b>6,453.61</b>
<b>Atmospheric Deposition Allocation</b>	<b>15.70</b>	<b>N/A</b>	<b>N/A</b>
<b>Total Basin wide Allocations</b>	<b>201.63</b>	<b>12.54</b>	<b>6,453.61</b>

In order to reach the appointed allocations, watershed implementation strategies (WIPs) are created by each jurisdiction with final approval of the EPA (USEPA, 2010). A mandatory 45 day public comment period is required, from which final revisions are made to the WIP and the ultimate TMDL is created to meet established allocations. Mitigation strategies in place to meet WIPs include, but are not limited to, planting of riparian buffers, best management practices (BMP) for soil conservation (such as no-till) and strict permitting and controls at point sources such as wastewater treatment facilities. However, throughout the Chesapeake Bay, very few water quality

and ecosystem goals established by federal legislation have been attained, while cultural eutrophication, seasonal hypoxia and critical habitat degradation remain persistent.

### **2.2.5 Corsica River Management**

The Corsica River was placed on the “impaired waters” list in 1996 and was classified as a Category 1 watershed, indicating that the watershed was not meeting water and other natural resource standards detailed by the EPA and is in need of restoration (Town of Centreville, 2004). After being designated as a category 1 watershed, the town of Centreville signed a memorandum of understanding with the State of Maryland to create a watershed restoration action strategy (WRAS) under the CWA framework for storm water management, sewer and water regulations and comprehensive land use.

The Corsica River WRAS included an action plan for denitrification technology in septic systems, forest buffers, restoration of wetland and submerged aquatic vegetation and over 4000 acres of cover crops (McCoy, 2003). Restoration projects also include the restoration of native shellfish for turbidity reduction via biofiltration, primarily focusing on historic oyster populations for their high water column filtration rates (Town of Centreville, 2004).

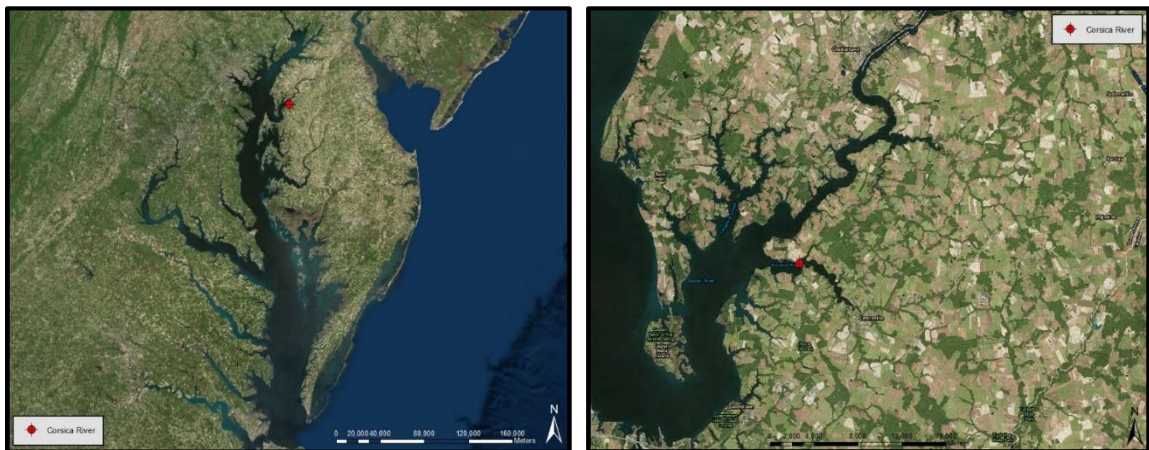
In 2000, the EPA approved and set TMDLs for the Corsica River stating that nitrogen loads should not exceed 130,485 kg of nitrogen a year (Town of Centreville, 2004). WRAS projects have been successful at reducing nitrogen input to the estuary, but it is often the seasonal spikes of dissolved nitrogen in the late winter and early spring that produce profuse algal blooms (MD DNR, 2003).

Resource managers are now looking towards ecosystem services to utilize the natural assimilative capacities of organisms for nitrogen bioremediation.

## 2.3 OVERVIEW OF STUDY

Despite efforts by policy to set standards and restoration strategies for nutrient impacted watersheds, many of the nutrients entering the waterways are from poorly regulated sources such as agricultural runoff and groundwater contamination. Once the nutrients enter the tributaries it is up to the inherent processes of the estuary to move, transform or eliminate the excess nutrients.

### 2.3.1 Site Selection



*Figure 7 Map of the Corsica River in the Chesapeake Bay*

The Corsica River was chosen for several reasons. First, this river is located in the upper Chesapeake Bay (Fig 7), which supports large expanses of agricultural lands on the Delmarva Peninsula, which are a considerable supply of diffuse nitrogen. Second, the estuary is of a practical size to support aquaculture and to be impacted in return. Finally, the Corsica River community is forthcoming in its desire to restore the river and is currently implementing several components of a WRAS.

The Corsica River receives high inputs of nutrients from its agriculturally dominated watershed and is at an ecological “tipping point” (Boynton, Testa & Kemp, 2009). High nutrient loads coupled with sediment transport result in light only penetrating to 10% of the estuary bottom to sustain submerged aquatic vegetation and only 28% which can support benthic algae. Although by 2006 nitrogen inputs were only reaching about 119,000 kg of nitrogen per year (well below the allotted 130,000 kg nitrogen per year TMDL), the estuary still experiences frequent nutrient induced algal blooms resulting in hypoxia and subsequent fish kills.

Of five water quality monitoring stations located in the Corsica River (Fig 8), at least three sites show seasonal spikes of dissolved inorganic nitrogen (DIN) that fall between “fair” and “poor” water quality standards with one station far exceeding the limit for “poor” quality as set by the United States Environmental Protection Agency (US EPA) (Fig 9) (2012). In addition, Figure 10 demonstrates that the chlorophyll a trends between April and October at all five stations exceed fair water quality standards. Diffuse sources of nutrients are the primary contributors to nitrogen loading in the Corsica River, while also being the most difficult to reduce. This watershed and estuary would greatly benefit from a nitrogen remediation strategy.

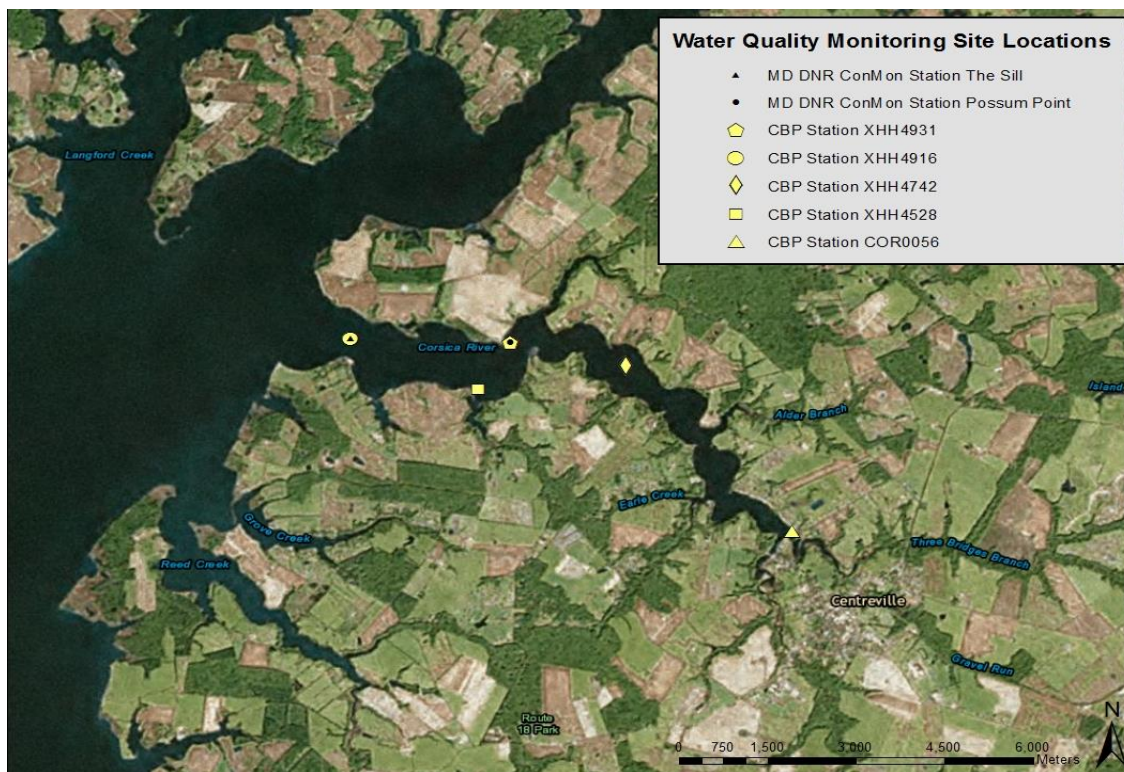


Figure 8 Map of environmental monitoring stations in the Corsica River

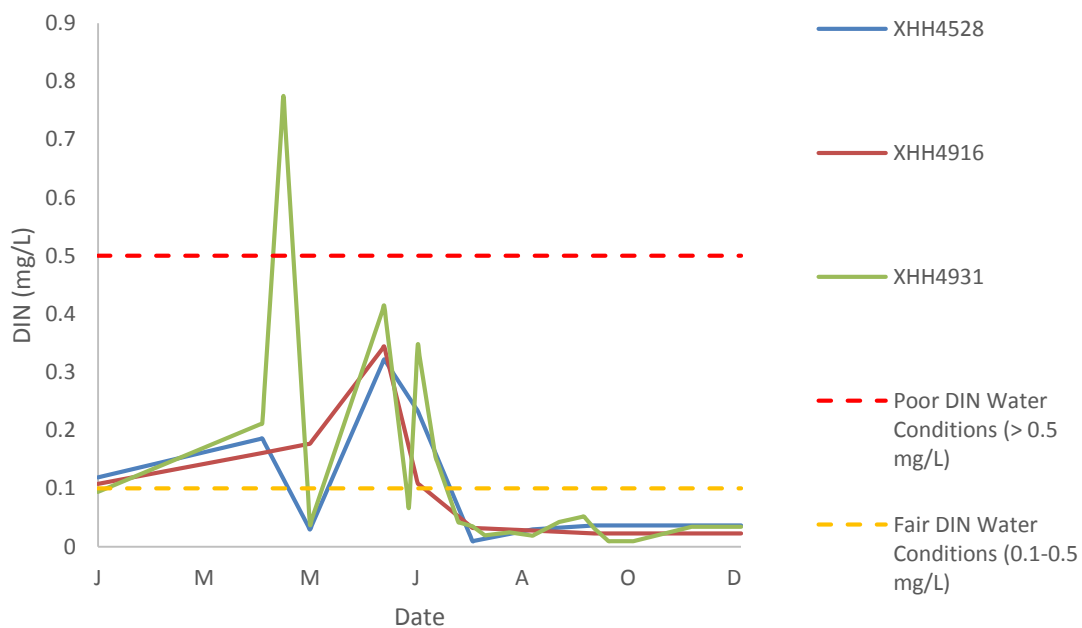
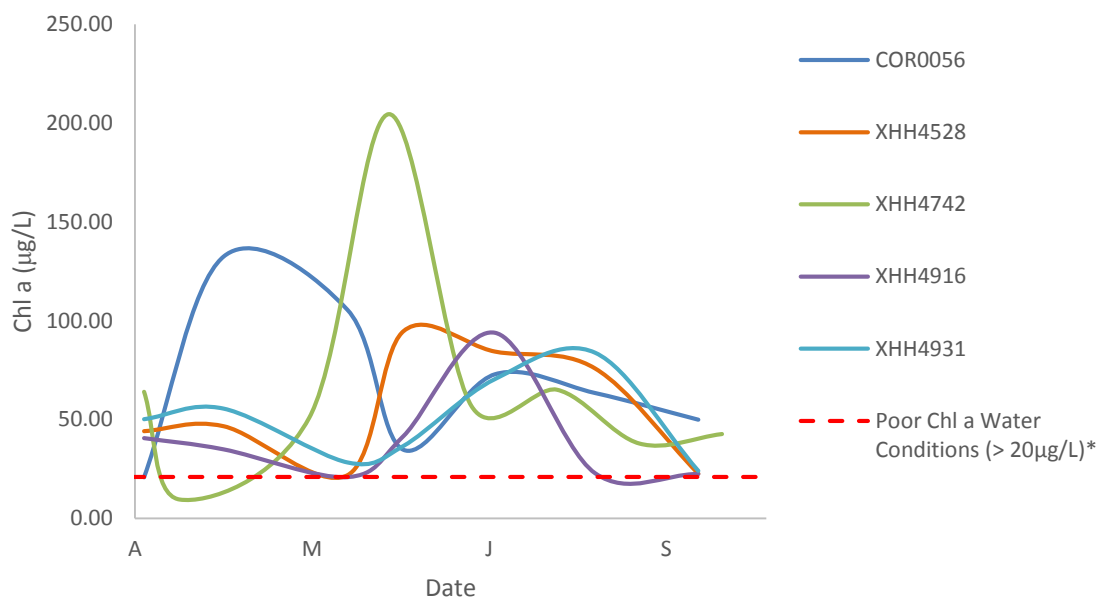


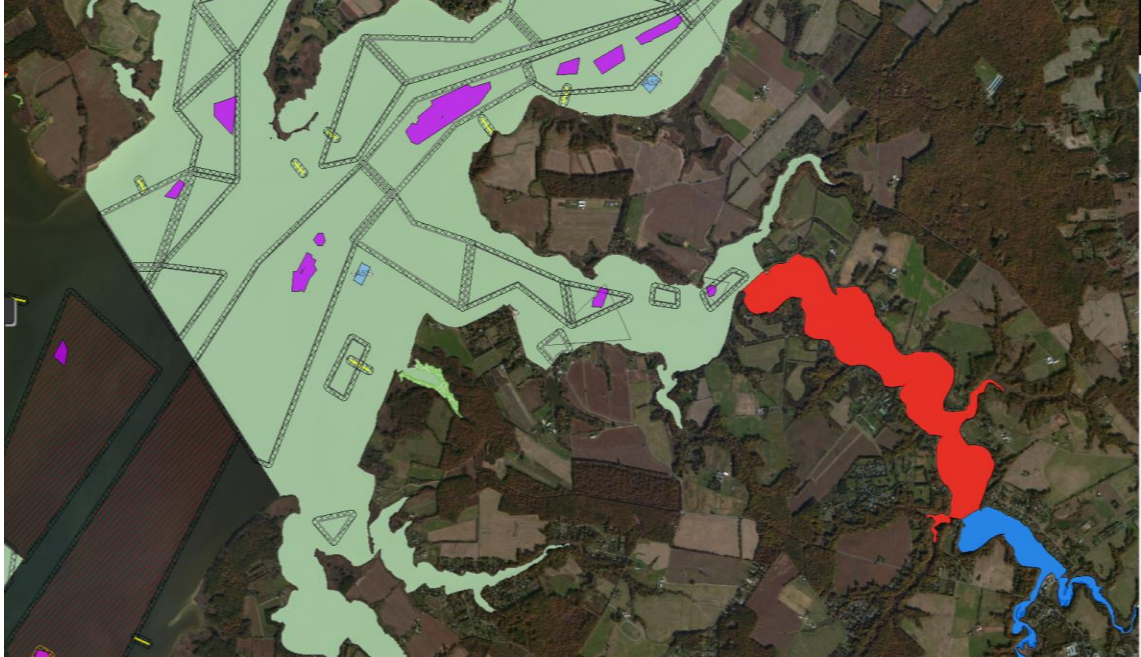
Figure 9 Annual dissolved inorganic nitrogen concentrations in the lower Corsica River (2013). Data was obtained from the Chesapeake Bay Program at three different monitoring stations. \*US EPA (2012)



*Figure 10* Chlorophyll a concentrations in the Corsica River (2013). Monthly data from April through October were collected at five monitoring stations. Data obtained from the Chesapeake Bay Program. \*US EPA (2012)

The lower Corsica River, along with the upper and lower regions of the Chester River, is currently designated as an oyster sanctuary with Yates Bars running throughout the channels (Fig 11). These Yates Bars are currently being restored, but the lower Corsica River is still open to shellfish harvesting. In 2011 the Maryland Department of Natural Resources allowed a select number of aquaculture leases to be awarded in the sanctuary (Davidsburg, 2011). Such opportunities are developed to promote stewardship of the sanctuary and the enhancement of substratum through sediment stabilization and habitat provisioning (Davidsburg, 2011). This act demonstrates that the MD DNR is willing to improve estuary and living resource health through the implementation of aquaculture and aligns well with this study to use polycultures for nutrient remediation.





*Figure 11 Corsica River fishery zoning and restoration sites. Grey, cross-hatched line segments represent Yates Bars, purple areas represent active restoration sites and red shading indicates areas closed to shellfish harvesting (<http://gisapps.dnr.state.md.us/Aquaculture/index.html>).*

### 2.3.2 Species Selection

#### *Mya arenaria*

Shellfish require nitrogen as an essential component in both tissue and shell proteins and is dominantly sourced from the consumption of phytoplankton (Woods Hole Group, 2012). Bivalve shells contain small amounts of nitrogen also in the form of protein, on which calcium carbonate crystals are deposited to develop a shell matrix. Although the metabolic processes are similar amongst shellfish species, the nitrogen content contained within the tissue vary slightly between bivalve species (Fig 12).

*M. arenaria* were chosen as a polyculture species for their historic presence in the upper Chesapeake Bay, preference for soft sediment, commercial value, established hatchery practices,



sediment stabilization and moderate to high nitrogen assimilation in tissue (Forster & Zettler, 2004; Woods Hole Group, 2012; Carmichael et al., 2012a; Maryland Department of Natural Resources [MD DNR], n.d.).

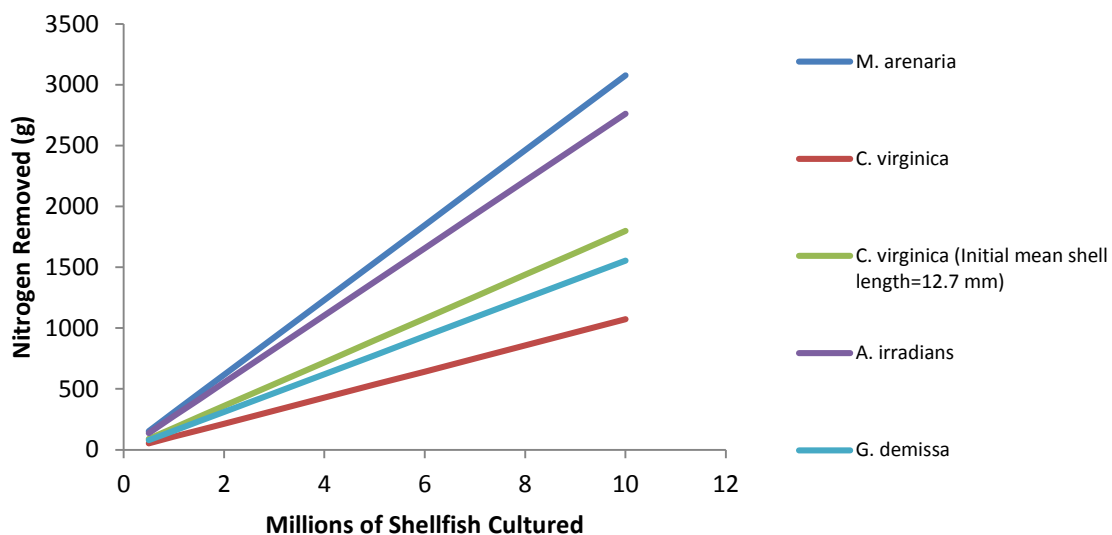


Figure 12 Potential nitrogen removal by various bivalves. Study was developed by the Woods Hole Group for an assessment of bivalve nitrogen remediation. *M. arenaria* demonstrates the highest nitrogen removal while *C. virginica* removes the least during the same growing period (May-September, 150 days) and the same initial shell length. (Woods Hole Group, 2012).

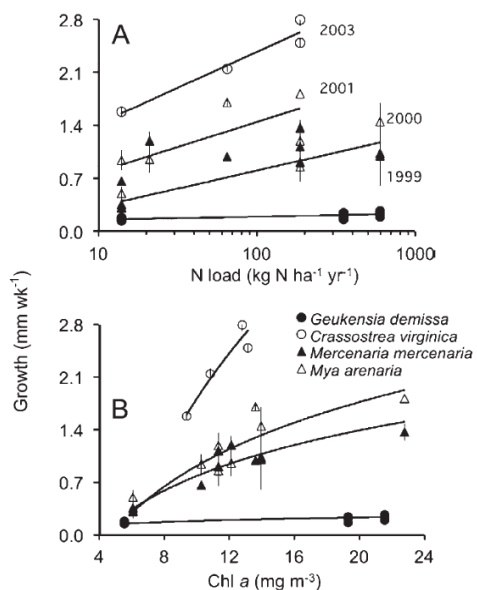


Figure 13 Bivalve growth rates. Bivalves include *Geukensia demissa*, *Crassostrea virginica*, *Mercenaria mercenaria* and *Mya arenaria* (A:  $y=1.23\ln(x)-1.92$ ,  $R^2=0.80$ ,  $P<0.001$ ; B:  $y=0.41\ln(x)+0.47$ ,  $R^2=0.94$ ,  $P=0.03$ ). Reprinted from Carmichael

*M. arenaria* experienced a fishery collapse in 1968 and the current population is only 10% of its original size (MD DNR, n.d.). *M. arenaria* not only prefers fine sediment which allows for burrowing, but also shallow habitats. The highest filtration impacts have been recorded at depths less than 5 meters below the surface (Forster & Zettler, 2004). The MD DNR lists *Mya* as having filtering rates higher than that of *Crassostrea virginica* (eastern oyster) where

1,500 juveniles only 5mm long and in an area of 1 m<sup>3</sup> can filter 2.5 m<sup>3</sup> of water a day, where filtration rates are highly dependent on food availability, water temperature and size of the individual clam (n.d.). *M. arenaria* also demonstrate increased growth rate in waters with high nutrient loading (Fig 13) and this relationship is closely linked with the proliferation of phytoplankton in the presence of nitrogen (Carmichael et al., 2012a) and due to fast growth rates can reach market size in less than two years (Baker & Mann, n.d.). This is ideal for aquaculture as it indicates probable food supply and prompt grow out season before harvest.

Today *M. arenaria* is being studied for its potential to assimilate nitrogen as compared to other bivalve species. Out of six marketable bivalves analyzed by the Woods Hole Group, *M. arenaria* is reported to have the highest nitrogen removal capacity over a single harvest season (Fig 12). This species also survives well in shallow waters of varying sediment type providing predation nets are available in muddy substrate (Baker & Mann, n.d.).

### ***Gracilaria tikvahiae***

*G. tikvahiae* was incorporated into the polyculture due to its established commercial value, high tolerance for significant changes in temperature, euryhaline adaptability and ability to uptake inorganic nitrogen during photosynthesis and store the nitrogen as biomass (Ray et al., 2014).

*G. tikvahiae* is a red algae that can survive temperatures ranging from 15-30° C and salinities between 10-40 ppt (Yarish, Redmond & Kim, 2012). This alga prefers shallow and nitrate/ammonia rich environments and easily propagates by reproductive spores or vegetative propagation. Using vegetative propagation is an easy, asexual method to accumulate biomass for planting and ensuring consistent morphologies.

In order to reduce the waste stream and prevent moving the nitrogenous biomass from the water to a landfill, the harvest needs to be recycled in an alternative system. Macroalgae aquaculture accounts for 24% of global aquaculture and is primarily produced in China, Korea, Japan and Chile (FAO, 2012) Gracilaria is the most cultivated macroalgae in the world due to its common use in food, medicine and the microbiological industry for products such as agar (He et al., 2014) Despite the massive cultivation in other parts of the world, production does not yet meet the global demand and is heavily imported into the United States (Yarish et al., 2012). Due to its easy cultivation on floating rope lines and tolerance for changing environments, planting and harvesting of this crop goes well beyond just nitrogen.

### *Ulva lactuca*

When waters are loaded with high levels of dissolved inorganic nitrogen, *U. lactuca* proliferates quickly and is often a contributor to seasonal eutrophication. *Ulva* is also known to grow in mats over aquaculture gear and prevent adequate water flow through bivalve cages. Biofouling material is scraped from the gear and brushed back into the water, which returns organic waste to the system and ultimately increases benthic BOD. By providing an incentive for watermen to collect the algal discards could result in an additional nitrogen sink for the Chesapeake Bay and its tidal tributaries.

*U. lactuca* is commonly found as a food product in many countries and could potentially be used for biofertilizers and many cosmetic products.

Both *U. lactuca* and *G. tikvahiae* species have demonstrated competitor interactions with phytoplankton over nutrient supplies and ultimately reduce phytoplankton biomass (Brush & Nixon, 2010). *M. arenaria*, on the other hand, act as pelagic-benthic couplers which removes nitrogen and sediment from the water column and transfers energy and materials to the benthos. By

increasing the dominance of benthic primary production and reducing turbidity, the nutrient enhanced system can ultimately be restored to a dynamic and healthy environment for more than just opportunistic species while also reaching federal and state water quality standards.

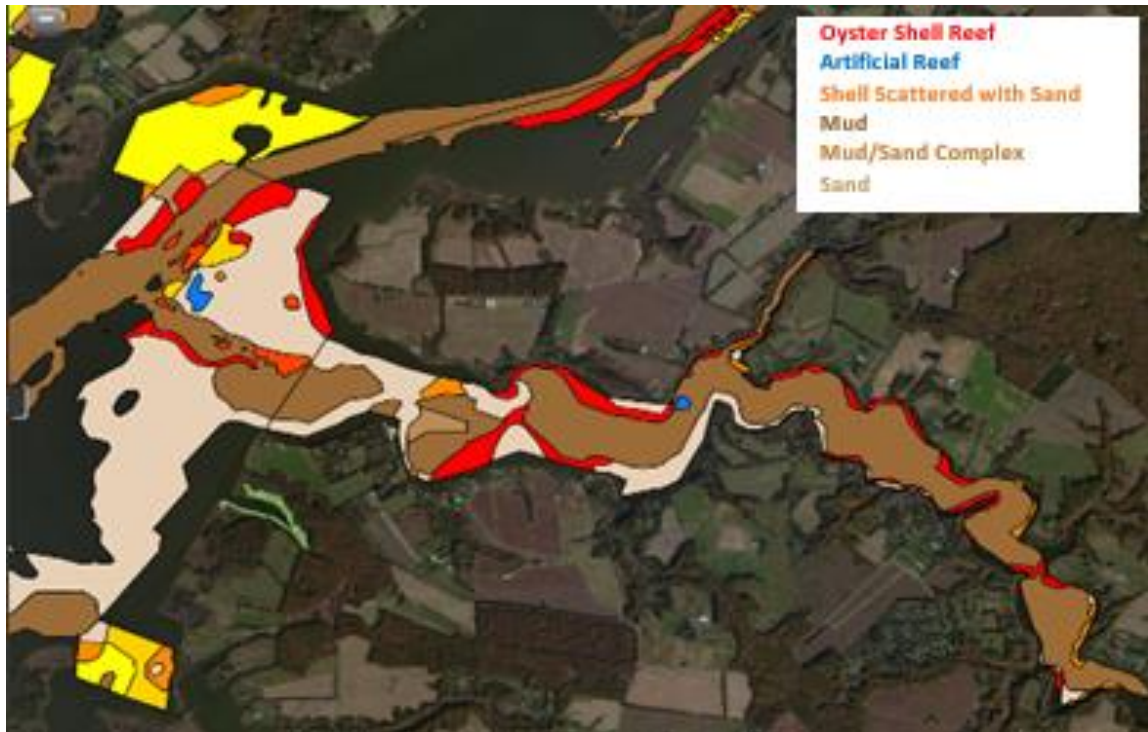
### 2.3.3 Polyculture Design

*M. arenaria* will be planted in the sediment with predation material over the planting area. Predation is the leading cause of mortality for young soft shell clams and predation netting is necessary for survival of juvenile clams. This material will undoubtedly become a suitable habitat for *U. lactuca* to propagate and will serve as a dual source of harvest for the same amount of materials and space traditionally used just for clams. Bottom cultures will be maintained regularly and as *U. lactuca* collects as biofouling material, it will be removed from the system as harvest.

Adjacent to the bottom culture will be a series of floating rope cultures with *G. tikvahiae*. Since the Corsica River has relatively high turbidity, the algae will need to be suspended at 0 meters from the surface, which will also promote high rates of gross primary production. The rope cultures should be easy to access during the growing season with limited disturbance to the surrounding area and oyster reefs.

The Corsica River is home to historic oyster bars and it is important that the polycultures are divided into smaller plots to avoid disturbing the shell reefs. It is also ideal to strategically place the plots at different locations to maximize exposure of the water column to the polycultures, while allowing clams to burrow in the fine sand-mud sediment along the shores. Figure 14 shows the transition from mud at the head of the river to a mud/sand mixture in the lower Corsica and predominantly sand coverage at the mouth and the southeastern side of the Chester River (mud=brown,

sand=beige). Areas in red indicate oyster reef that would be avoided so as to not disturb oyster restoration.



*Figure 14 Sediment composition of the Corsica River. Reprinted from: MD DNR, <http://gisapps.dnr.state.md.us/Aquaculture/index.html>.*

### 2.3.4 Corsica River Nitrogen Budget

Boyton, Testa and Kemp constructed a nutrient budget for the Corsica River to trace the movement and transformations of nutrients in the system, while also ensuring all significant nutrient inputs and outputs are accounted for (2009). This tool can provide project managers a framework from which dominant nutrient contributors can be identified and addressed.

The nutrient budget was computed from 2006 and 2007 and nitrogen loads and exports were broken into the following sources (Fig 15):

**Diffuse in Stream:** Primarily agricultural runoff and leachate from corn, soybean and wheat cultivation

**Storm Water Runoff:** Sourced from the urban center of Centerville, Maryland

**Atmospheric Deposition:** Deposited through rainfall directly on the surface waters of the Corsica

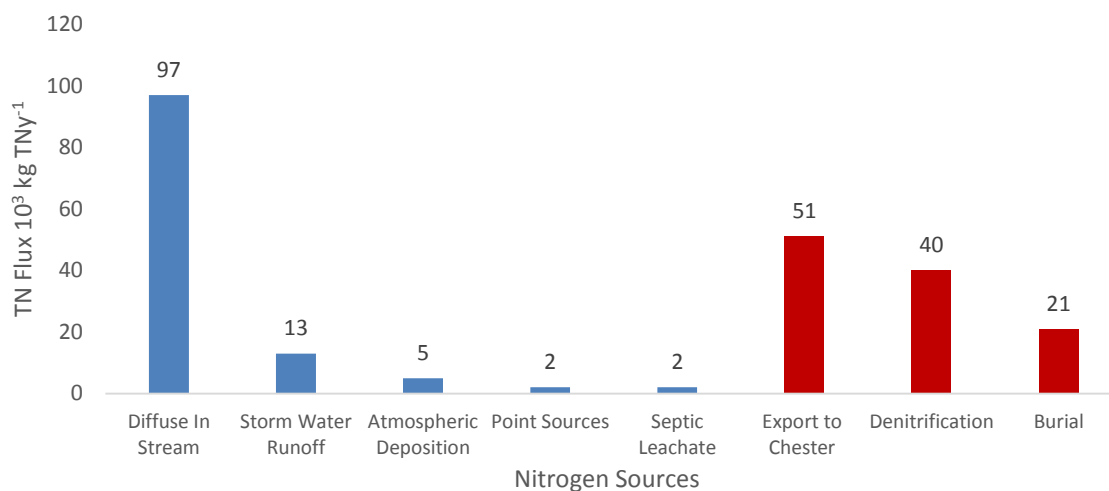
**Point Source:** Sourced from the Centerville sewage treatment plant

**Septic Leachate:** Only includes septic tanks within a close proximity to the tidal shores of the river (non-tidal range septic systems contribute to the “diffuse” nitrogen load into ground water)

**Export to Chester:** Nitrogen lost to the Chester River and eventually the main channel of the Chesapeake Bay

**Denitrification:** Includes denitrification process in both estuarine and marsh sediments

**Burial:** Nitrogen buried in the sediment, typically from shore erosion and watershed land use change



*Figure 15 Nitrogen Budget for the Corsica River (2006). Blue bars represent nitrogen inputs while the red bars correspond to nitrogen losses. Graph adapted from Boynton, Testa and Kemp (2009).*

Diffuse inputs contribute the largest share of nitrogen, whereas point sources only account for 2,000 kg N per year. The Corsica River also has three main routes of nitrogen losses including export to the Chester River, denitrification and burial. Of particular concern is the annual export of nitrogen to the already nutrient enriched waters of the Chester River.

After the nitrogen assimilation values have been determined, they will be added to the nitrogen budget to assess the impact the polycultures may have on system as well as their ability to be incorporated into a watershed restoration action strategy.

### 3 METHODS

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#### 3.1 CULTURE SITE

Nitrogen removal potential was evaluated for three sizes of planting areas covering 1%, 3% and 5% of the total bottom area (BA) of the Corsica River. Of each area designated for the polycultures, a third will be portioned for the *G. tikvahiae* floating rope cultures. The rope cultures would ideally be set upstream from the bivalves so algal detritus will flow with the current over the bivalve plot for possible consumption. Since the demand for consumable shellfish is high, more benthic area is devoted to the bivalves.

*Table 2 Designation of plot sizes for different species of polyculture*

<b>Percent of BA for polyculture</b>	<b>Total BA for polyculture (km<sup>2</sup>)</b>	<b>Total BA for Mya/Ulva culture (km<sup>2</sup>)</b>	<b>Total SA for Gracilaria culture (km<sup>2</sup>)</b>
<b>1%</b>	56.45	37.64	18.82
<b>3%</b>	169.36	112.91	56.45
<b>5%</b>	282.27	188.18	94.1

Nitrogen removal for the entire polyculture site will be assessed from three different designations of bottom area (BA) for the entire Corsica River. For instance, a 1% BA polyculture uses 1% of the entire river's BA, which is then further divided so the *M. arenaria* plot consists of 2/3 of the designated BA and *G. tikvahiae* uses the other 1/3 of the plot area (Table 1).

#### 3.2 POTENTIAL NITROGEN ASSIMILATION

##### **Mya arenaria**

*M. arenaria* will be grown by bottom culture with predation netting to ensure survival (MD DNR, n.d.) To assess nitrogen content assimilated in *M. arenaria* tissue, upper and lower potential



nitrogen removal was calculated for each of the three culture scenarios and the average was used for final evaluation. Since planting and harvesting of clams is measured by shell length, it was important to establish shell length to tissue dry weight (DW) ratio to calculate nitrogen composition in tissue. This data was collected by Glaspie at Virginia Institute for Marine Science and was analyzed using linear regression (unpublished).

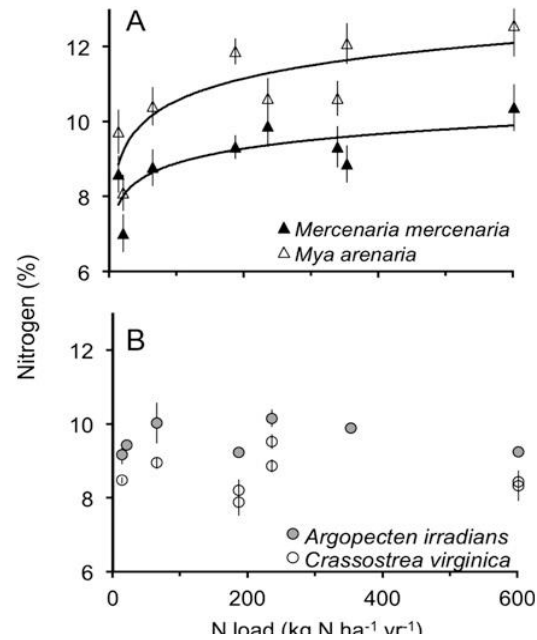
Lower nitrogen values were estimated from literature grams of carbon to dry weight ratios and converted to grams of nitrogen using a standard literature ratio for C:DW (Chauvaud, Thompson, Cloern & Thouzeau, 2003) and Redfield Ratio of 16 mol N:106 mol C (Eq 1) (Redfield, Ketchum & Richards, 1963). Calculations were made for an ideal clam at time of planting with a shell length of 10 mm (Beal, Lithgow, Shaw, Renshaw & Ouellette, 1995).

Eq 1: Lower estimate of nitrogen in tissue for a 10mm shell length clam

$$\text{Nitrogen content (gN)} = (xgDW) \left( \frac{0.4gC}{1gDW} \right) \left( \frac{1molC}{12.011gC} \right) \left( \frac{16molN}{106molC} \right) \left( \frac{14.007gN}{1molN} \right)$$

Maximum values were calculated using field data collected by Carmichael et al. for several bivalve species in an estuarine system in Cape Cod, Massachusetts (2012a). This study found that nitrogen assimilation by *Mya arenaria* is positively correlated with nitrogen loading rates and provides higher than Redfield Ration assimilation rates of nitrogen (Fig 16) (2012a; Woods Hole Group, 2012). Calculations were made for clams harvested at 42 mm at a stocking density of 197 clams per square meter under the assumption that the clams were reared in the same system (Beal et al., 1995). It is assumed that the shellfish are reared in the Corsica River so all nitrogen in tissue was

derived from the Corsica River at time of harvest. Nitrogen assimilated into clam shells were not evaluated based on inadequate data and variable reporting.



**Figure 16** Increased nitrogen assimilation by clams. Both *Mya arenaria* and *Mercenaria mercenaria* exhibit positive correlation between nitrogen loading and nitrogen content in clam tissue, while no relationship was determined for *Argopecten irradians* and *Crassostrea virginica* [*M. arenaria*:  $y = 0.86 \ln(x) + 6.59$ ;  $R^2 = 0.69$ ;  $P = 0.01$ ]. Percent nitrogen was determined by mass spectrometry (Reprinted from Carmichael et al., 2012a).

Eq 2: Upper estimate of nitrogen in tissue

$$\text{Nitrogen in Tissue} = 0.86 \ln(x) + 6.58$$

Where x is the N load ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ )

Nitrogen removal values for *M. arenaria* were reported as the average between the high and low estimates for each of the three polyculture plot sizes. Total biomass harvested was also recorded.

### **Macroalgae**

An ecosystem model for Greenwich Bay, a shallow sub-estuary in Narragansett Bay Rhode Island, was amended to only include macroalgae relationships for *Ulva lactuca* and *Gracilaria tikvahiae* (Brush & Nixon, 2010). STELLA software was used to modify and run the model under conditions appropriate for the Corsica River. Both *G. tikvahiae* and *U. lactuca* were amended to include a harvest converter and harvest biomass stock to collect harvested algae in carbon units ( $\text{gC m}^{-2}$ ) as well as depth conversions so only kD values at the appropriate culture depths were used.

The forcing functions were modified to be applicable to the Chesapeake Bay by using QA/QC data collected in or within close proximity to the Corsica River and missing data was interpolated with MATLAB.

**I<sub>0</sub>:** 2008 PAR data from Horn Point Laboratory, UMCES (Cambridge, MD) (Fisher, 2008).

**Temperature:** 2013 temperature data from the National Data Buoy Center; QA/QC (NOAA, 2013).

**DIN/ kD:** 2013 nutrient and kD data from the CBP monitoring station XHH4528 in the Corsica River; QA/QC (Chesapeake Bay Program, 2013). This site was chosen for providing recent 2013 data, as well as being located in a region open for shellfish harvest. It was also important that this water quality monitoring station not be located at a tributary exchange interface.

Water temperature data imported in the model must be continuous data for the entire year. Since the Corsica River has very little data publicly available, monthly samples taken at station XHH4528 were compared with the daily averages for station 44043 located in the upper Chesapeake Bay (39.152 N, 76.391 W) and was accepted as a substitute data station for the model (Fig 17).

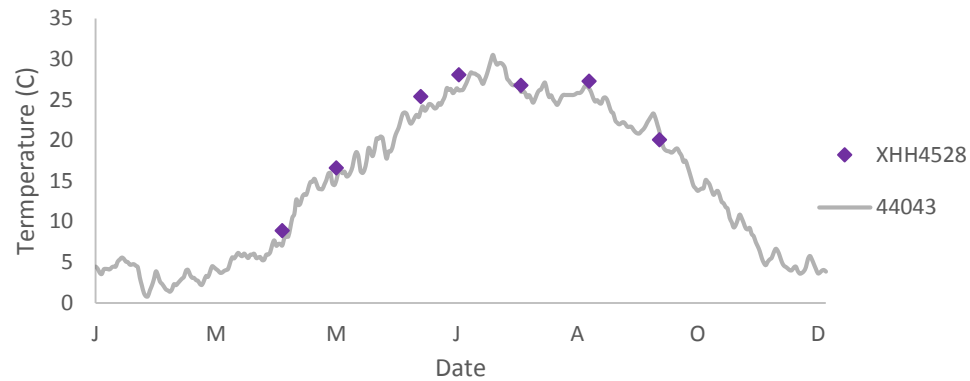


Figure 17 Water temperatures for the Corsica River and the upper Chesapeake Bay

The theoretical *G. tikvahiae* culture would be grown on 0.025m thick polyethylene rope suspended directly on the surface of the river by floats. Since the Corsica River has such high turbidity, macro algae would grow the best at 0 m depth for high gross primary production. The rope would be 137 meters long and the amount of ropes is dependent on the polyculture plot size. The 1%, 3% and 5% BA plots have 69, 206 and 343 culture ropes, respectively. Total surface area for *Gracilaria* referred to the total surface area of the culture ropes in the plot since this is the only substratum on which algae can collect and be harvested. Initial biomass for the culture assumed that each rope would be planted with about 20 grams of wet weight (WW) every 0.1m (Lindell et al. 2013). Initial biomass must be in the form of grams carbon per square meter. To calculate this value, the *G. tikvahiae* 0.2 DW to WW ratio was used (Food and Agriculture Organization [FAO], 1976) and a 0.26 carbon to DW ratio (Brush & Nixon, 2010).

Eq 3: Wet weight to carbon conversion for input of initial biomass into the model

$$Gracilaria \left( \frac{gC}{m^2} \right) = (xgWW) \left( \frac{0.2gDW}{1gWW} \right) \left( \frac{0.26gC}{1gDW} \right) \left( \frac{1}{GracSA(m^2)} \right)$$

*U. lactuca* depth converter was set to the same depth and area as the clam cultures to account for a biofouling harvest. Due to the poor water clarity of the Corsica River, the model was run at three different depths for the bivalve/green alga culture to compare potential *U. lactuca* harvests with different rates of primary production. The depths were 1.0, 0.5 and 0.25 meters below the river surface.

To calculate accumulated harvest for each species of macroalgae, a harvest function was created using the following “IF-THEN-ELSE” statements:

$$\text{Grac\_Harvest} = \text{IF } (\text{GRAC\_C [S1]} > (\text{Grac\_Init} * \text{Grac\_Harvest\_Threshold})) \text{ THEN} \\ (\text{GRAC\_C [S1]} - \text{Grac\_Init}) \text{ ELSE } 0$$

$$\text{Ulva\_Harvest} = \text{IF } (\text{ULVA\_C [S1]} > (\text{Ulva\_Init} * \text{Ulva\_Harvest\_Threshold})) \text{ THEN} \\ (\text{ULVA\_C [S1]} - \text{Ulva\_Harvest\_quantity}) \text{ ELSE } 0$$

Where 1.5 is the harvest threshold that the algal mass must reach before it is “harvested”. All biomass after this threshold is moved to a harvest stock that accrue mass over time until the original algal stock can no longer support harvest.

For these biomasses to be useful in this study, they must be converted from grams of carbon per square meter to total grams of nitrogen using a nitrogen to carbon minimum ratio built into the model by Brush & Nixon (2010).

Eq 4: Carbon per square meter conversion to total nitrogen

$$\text{Gracilaria N (g)} = \left(\frac{xgC}{1m^2}\right) \left(\frac{1molC}{12.011gC}\right) \left(\frac{1molN}{6molC}\right) \left(\frac{14.007gN}{1molN}\right) (\text{Grac Rope SA } (m^2))$$

$$Ulva N (g) = \left(\frac{xgC}{1m^2}\right) \left(\frac{1molC}{12.011gC}\right) \left(\frac{1molN}{5molC}\right) \left(\frac{14.007gN}{1molN}\right) (Ulva BA (m^2))$$

Finally, a total harvest biomass was calculated by converting the harvest model output from grams of carbon back to grams of DW using carbon to DW ratios (Brush & Nixon, 2010).

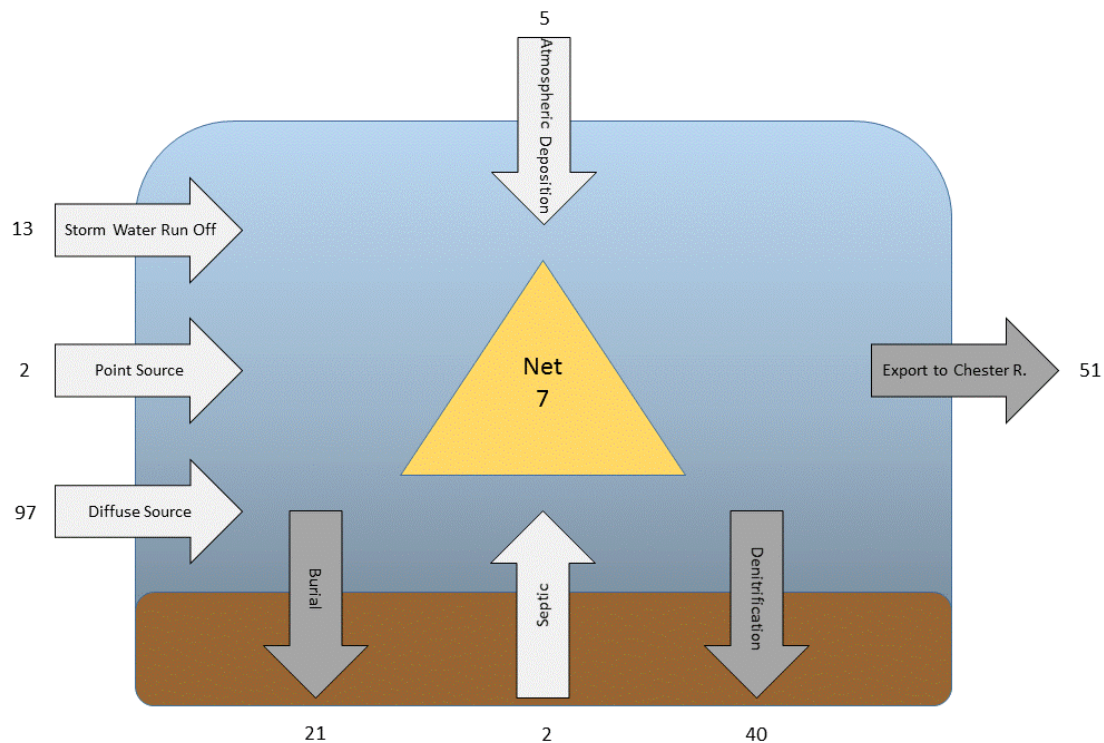
Eq 5: Carbon to DW conversion

$$Gracilaria Harvest (gDW) = \left(\frac{xgC}{m^2}\right) (RopeSA(m^2)) \left(\frac{1gDW}{0.26gC}\right)$$

$$Ulva Harvest (gDW) = \left(\frac{xgC}{m^2}\right) (UlvaBA(m^2)) \left(\frac{1gDW}{0.28gC}\right)$$

### 3.3 CORSICA RIVER NUTRIENT BUDGET

Nitrogen assimilation values were incorporated into the existing Corsica River nitrogen budget for a holistic perspective and to provide a scale to which the values can be compared. The addition of the polyculture will contribute additional nitrogen “loss” for the system for which there is a current net stock of nitrogen (Fig 13).



*Figure 18* Diagram of the Corsica River nitrogen budget. Nitrogen loading is represented by the light grey arrows and is dominated by diffuse nitrogen loading, whereas nitrogen leaves the system primary through denitrification and export to the Chester River and ultimately the Chesapeake Bay. All values represent  $\text{kg } 10^3 \text{ N year}^{-1}$  and there is an annual net pool of 7000 kg of nitrogen in the system (Boynton, Testa & Kemp, 2009).

## 4 RESULTS

### 4.1 POTENTIAL NITROGEN ASSIMILATION

#### ***Mya arenaria***

Nitrogen removal for *M. arenaria* was calculated for an ideal clam of 10 mm shell length, which is the approximate size used for planting. A ratio of shell length to dry weight (DW) of tissue was calculated to obtain the grams of DW for the 10 mm ideal clam (Fig 17 and 18).

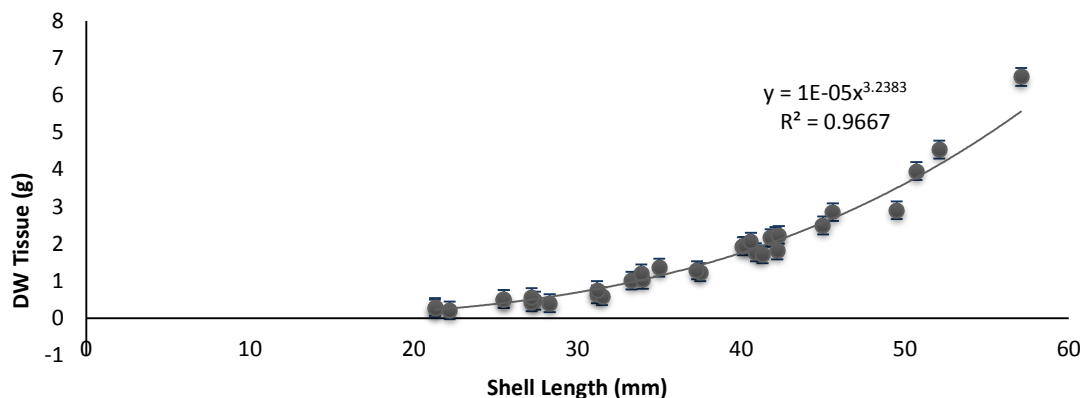


Figure 19 *M. arenaria* shell length vs dry weight. Data represents a small spring sample of *M. arenaria* organisms collected at five sites throughout Chesapeake Bay ( $M=1.63$ ,  $SD=1.37$  at 95% confidence) (Glaspie, unpublished data).

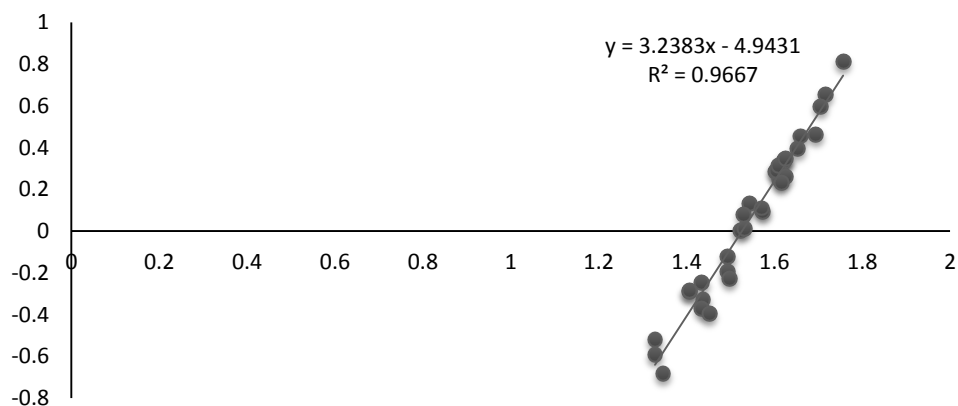


Figure 20 Logarithmic transformation of *M. arenaria* shell length vs dry weight. Parameters have a strong positive relationship and were evaluated by regression analysis ( $R^2=0.97$ ;  $p < 0.001$ ).



Shell length to dry weight conversion when shell length is 10 mm

$$y = 1E-05x^{3.2383}$$

$$y = 1E-05(10)^{3.2383}$$

$$y = 0.0173g \text{ DW}/10 \text{ mm shell length}$$

Dry weight was then used to calculate the lower estimate of percent nitrogen assimilated into clam tissue.

Eq 1: Lower estimate of nitrogen in tissue for a 10mm shell length clam

$$\text{Nitrogen (g)} = \left( \frac{0.0173gDW}{10mmShell} \right) \left( \frac{0.4gC}{1gDW} \right) \left( \frac{1molC}{12.011gC} \right) \left( \frac{16molN}{106molC} \right) \left( \frac{14.007gN}{1molN} \right)$$

$$\text{Nitrogen} = \left( \frac{0.0012gN}{0.0173gTissue} \right)$$

7.04% N in tissue

An upper estimate of percent nitrogen in tissue was calculated from field measurements of percent nitrogen in *M. arenaria* as a function of nitrogen loading in the Corsica River (Eq 2). Field data was collected for *M. arenaria* by Carmichael et al. (2012a) and 2006 Corsica River nitrogen loading was calculated by Boynton, Testa and Kemp (2009).

Eq 2: Upper estimate of nitrogen in tissue

$$y = 0.86\ln(x) + 6.59$$

$$x = 210.79 \text{ kg N ha}^{-1}\text{yr}^{-1} \text{ (Boynton, Testa \& Kemp, 2009)}$$

$$y = 11.19\% \text{ N tissue}$$

The nitrogen percent range in tissue is between 7.04% and 11.19%. The largest culture plots have the highest mass of nitrogen and total harvest (Table 3 and 4). These values identify potential nitrogen removal through harvest and does not include clam mortality or movement of nitrogen from clams to sediment as biodeposits.

*Table 3 Planting density and total potential nitrogen removal through Mya arenaria harvest at 1%, 3% and 5% bottom plantings. Upper and lower nitrogen values are calculated using nitrogen content in tissue from Eq 1 and Eq 2.*

<b>PLANTING</b>	<b>Shell Length (mm)</b>	<b>Total Planting Area (km<sup>2</sup>)</b>	<b>Number of Clams Planted (10<sup>6</sup>)</b>
<b>1% Bottom Area</b>	8-15	37.64	7.41
<b>3% Bottom Area</b>	8-15	112.91	22.24
<b>5% Bottom Area</b>	8-15	188.18	37.01

<b>HARVEST</b>	<b>Shell Length (mm)</b>	<b>Total Biomass Harvested (kg DW)*</b>	<b>Potential N Removed (kg yr<sup>-1</sup>)*</b>
<b>1% Bottom Area</b>	>42	14,457.79	1,018-1,618
<b>3% Bottom Area</b>	>42	43,373.38	3,053-4,853
<b>5% Bottom Area</b>	>42	72,288.96	5,089-8,089

\*Calculations are for a 42 mm clam

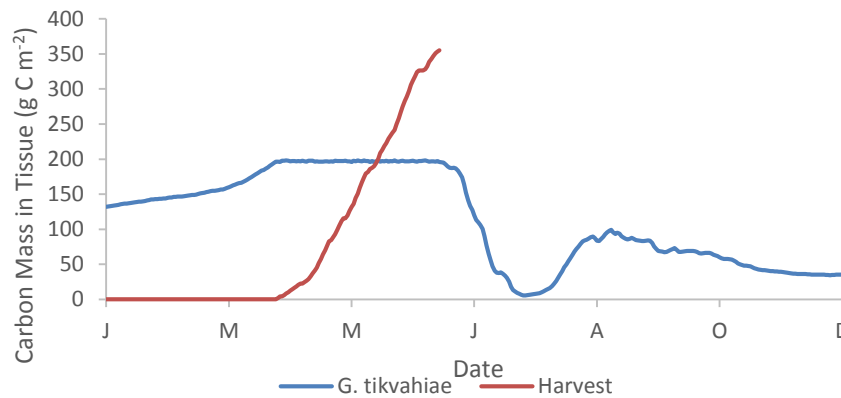
### Macroalgae

Temperature data from monitoring station 44043 was statistically analyzed to determine if the data was significantly different from the data collected in the Corsica River at the same monitoring station used for other data parameters (kD and DIN).

$H_0$ : There is no significant difference between the two stations  
 $H_1$ : There is a significant difference between the two stations

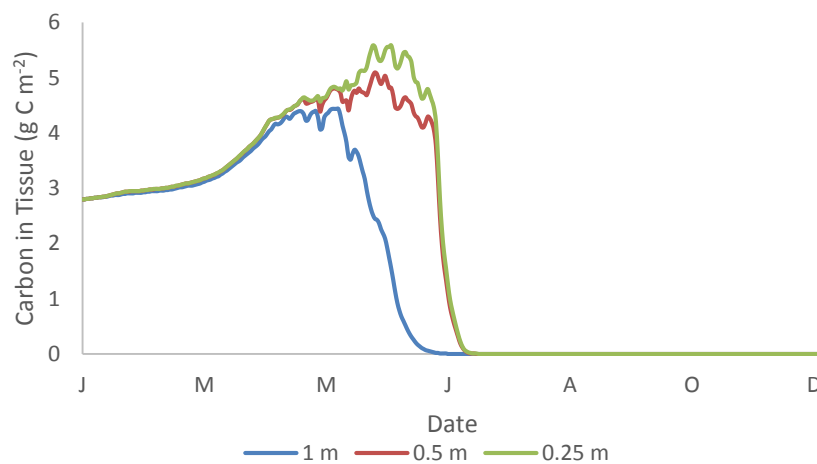
A two sample T-test (equal variance) was performed and a high p-value indicates that the null hypothesis is not rejected and a statistical difference cannot be determined [ $t(12) = 0.29$ ;  $p > 0.05$ ]. Temperature data was then determined suitable for the model.

Once the harvest begins for *G. tikvahiae*, the biomass plateaus and then drops off in June and July with the highest production occurring in early spring and summer (Fig 12).



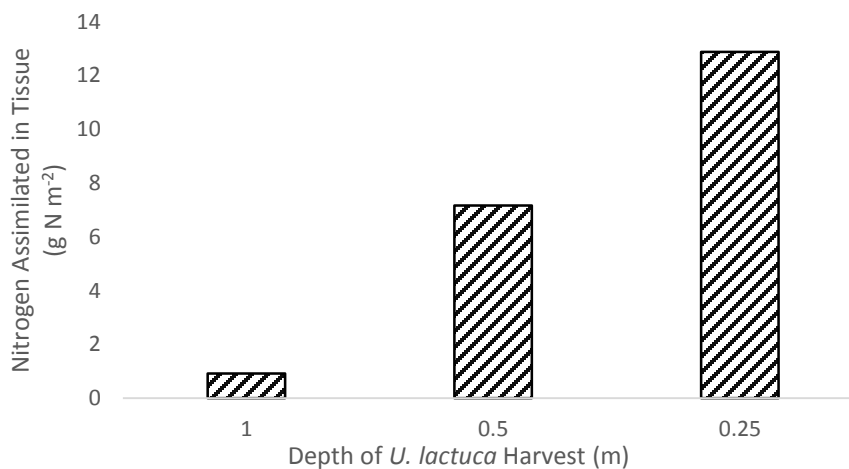
*Figure 21* Response of floating *G. tikvahiae* culture to regular harvest. Carbon mass equal between the three plot sizes since they were planted at the same density. Harvest ends when algal biomass drops below initial planting as algae begins to decline with the onset of summer.

The model for *U. lactuca* was run for three depths that correspond to the planting depth of the *M. arenaria* (Fig 22). At the most shallow depth, a larger biomass of *U. lactuca* was produced and at the deepest depth, the least amount of biomass was produced.



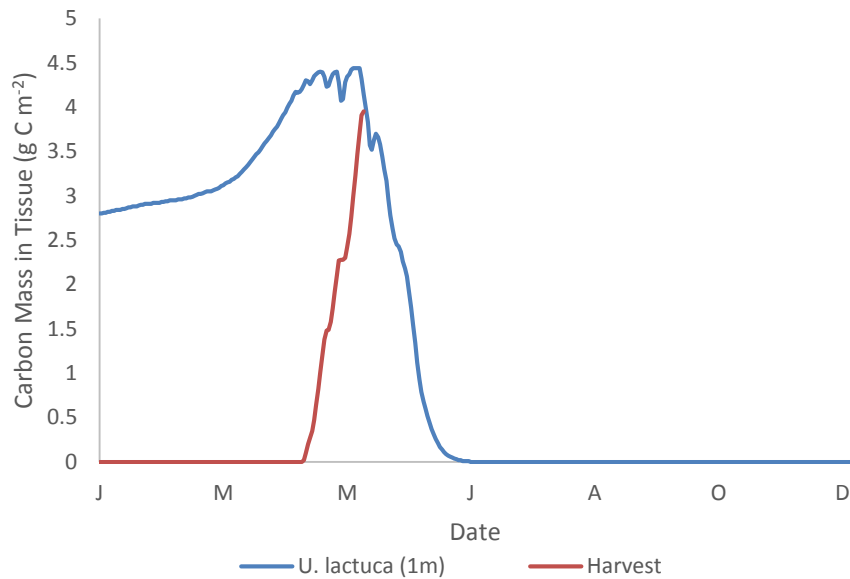
*Figure 22 Comparison of U. lactuca that grows on M. arenaria aquaculture. U. lactuca grows more densely 0.25 m below the surface. U. lactuca grown at 1 m below the surface grows less dense and has a shorter production season than the shallower depths.*

As biomass increases, so does the mass of nitrogen per square meter (Fig 23). Therefore, shellfish grown at shallow depths will produce larger amounts of biofouling mass and nitrogen harvested.



*Figure 23 Nitrogen Density in U. lactuca harvests at three different depths. At the shallowest depth (0.25m), there is more nitrogen per square meter than at the deepest depth (1m).*

At the 1 meter depth, *U. lactuca* is harvested after it surpasses the harvest threshold and continues to be harvested until the density drops below the harvest threshold (Fig 24). Once harvesting begins, the biomass does not recover after the summer and biofouling is not present for the remainder of the year.



*Figure 24* Response of *U. lactuca* culture to regular harvest over time. Harvest ends when culture can no longer remain above the biomass harvest threshold.

### Summary of Total Polyculture

*U. lactuca* had the highest nitrogen removed and total biomass harvested at 0.25 meters from the surface. Yet, it was the nitrogen removed from the 1 meter plot area that was used in the budget analysis.

Table 4 Macroalgae model data. Identifies the nitrogen density, nitrogen removed and total harvest of *U. lactuca* at three depths and *G. tikvahiae* cultured at the surface.

Species	Depth (m)	Nitrogen Density (g N m <sup>-2</sup> )	Polyculture BA	Total N Removed (kg N yr <sup>-1</sup> )	Total Biomass Harvested (kg DW yr <sup>-1</sup> )
<i>U. lactuca</i>	0.25	12.87	1%	484.46	7,418.28
			3%	1,453.38	22,254.85
			5%	2,422.30	37,091.42
	0.5	7.16	1%	269.49	4,126.50
			3%	808.46	12,379.49
			5%	1,347.43	20,632.48
	<b>1</b>	<b>0.92</b>	<b>1%</b>	<b>34.67</b>	<b>530.93</b>
			<b>3%</b>	<b>104.02</b>	<b>1,592.80</b>
			<b>5%</b>	<b>173.37</b>	<b>2,654.67</b>
<i>G. tikvahiae</i>	0	69.05	1%	51.30	1,015.22
			3%	153.08	3,029.27
			5%	254.86	5,043.33

Table 5 Total nitrogen and harvest removed from 1% BA

Species	Potential N Removed (kg yr <sup>-1</sup> )	Harvested Biomass (kg DW yr <sup>-1</sup> )
<i>M. arenaria</i> *	1,318	14,458
<i>G. tikvahiae</i>	51	1,015
<i>U. lactuca</i> **	35	531
<b>Total</b>	<b>1,404</b>	<b>16,004</b>

Table 6 Total nitrogen and harvest removed from 3% BA

Species	Potential N Removed (kg yr <sup>-1</sup> )	Harvested Biomass (kg DW yr <sup>-1</sup> )
<i>M. arenaria</i> *	3,953	43,373
<i>G. tikvahiae</i>	153	3,029
<i>U. lactuca</i> **	104	1,593
<b>Total</b>	<b>4,210</b>	<b>47,995</b>

Table 7 Total nitrogen and harvest removed from 5% BA

Species	Potential N Removed (kg yr <sup>-1</sup> )	Harvested Biomass (kg DW yr <sup>-1</sup> )
<i>M. arenaria</i> *	6,589	72,289
<i>G. tikvahiae</i>	255	5,043
<i>U. lactuca</i> **	173	2,655
<b>Total</b>	<b>7,017</b>	<b>79,987</b>

\*Average N Removed

\*\*Values are 1 (m) harvest

## 4.2 MODIFIED CORSICA RIVER BUDGET

It is evident that the primary nitrogen sink for the polyculture is the *M. arenaria* harvests and the algal co-harvests only make minimal contributions to net nitrogen (Fig 16-18). Fig 18 shows that a 5% BA of polyculture can remove nearly all of the net nitrogen in the system and produces a substantial harvest for all species.

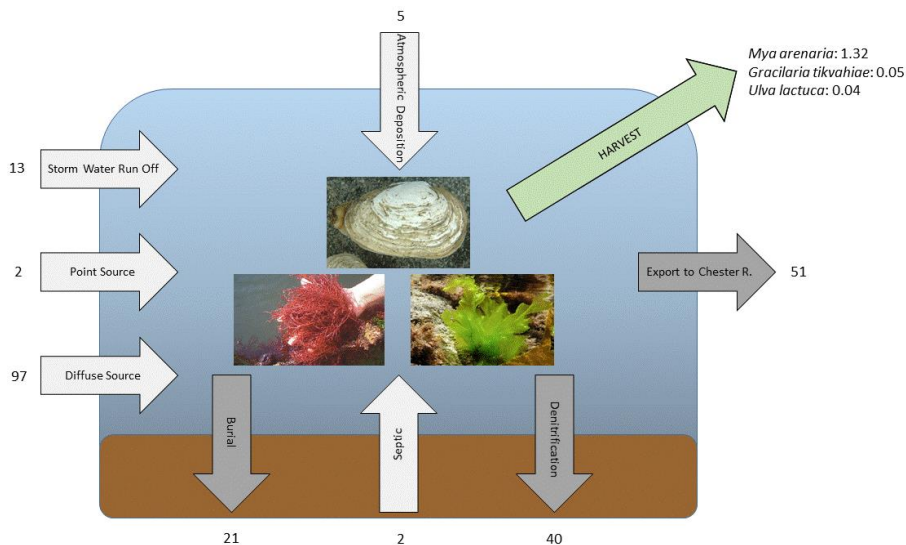
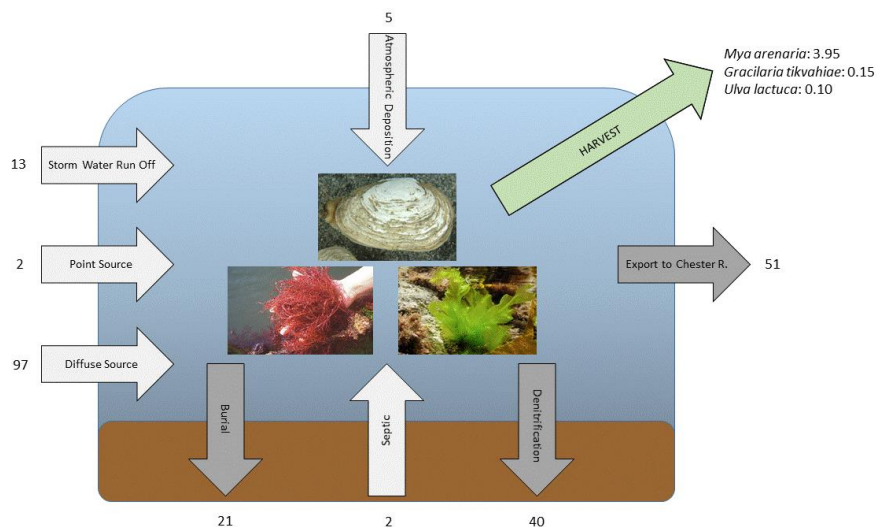
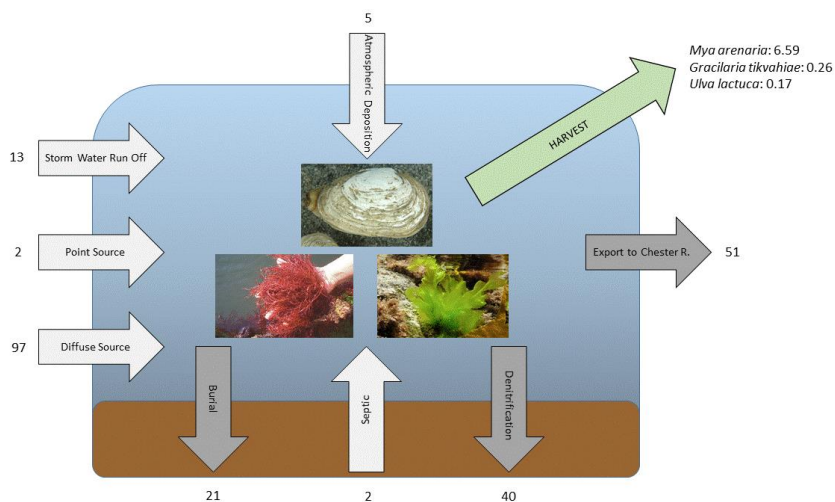


Figure 25 Modified Corsica River nitrogen budget with 1% bottom area harvest. *M. arenaria* could potentially remove 1,320 kg N yr<sup>-1</sup>, *G. tikvahiae* 50 kg N yr<sup>-1</sup> and *U. lactuca* 40 kg N yr<sup>-1</sup>. (Photos: D. Cowles, Rosario Beach Marine Laboratory [*M. arenaria*]; K. Peters, Wikipedia [*U. lactuca*]; J. Preston, Connecticut Sea Grant [*G. tikvahiae*]).



**Figure 26** Modified Corsica River nitrogen budget with 3% bottom area harvest. *M. arenaria* could potentially remove 4,000 kg N yr<sup>-1</sup>, *G. tikvahiae* 150 kg N yr<sup>-1</sup> and *U. lactuca* 100 kg N yr<sup>-1</sup>. (Photos: D. Cowles, Rosario Beach Marine Laboratory [*M. arenaria*]; K. Peters, Wikipedia [*U. lactuca*]; J. Preston, Connecticut Sea Grant [*G. tikvahiae*]).



**Figure 27** Modified Corsica River nitrogen budget with 5% bottom area harvest. *M. arenaria* could potentially remove 7,000 kg N yr<sup>-1</sup>, *G. tikvahiae* 260 kg N yr<sup>-1</sup> and *U. lactuca* 170 kg N yr<sup>-1</sup>. (Photos: D. Cowles, Rosario Beach Marine Laboratory [*M. arenaria*]; K. Peters, Wikipedia [*U. lactuca*]; J. Preston, Connecticut Sea Grant [*G. tikvahiae*]).



## 5 DISCUSSION

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### 5.1 SUMMARY OF RESULTS

The Corsica River has a long history of high nutrient and sediment loading resulting from land use changes throughout the Corsica watershed. Great efforts have been made from federal, state and local communities to implement nutrient management strategies for water quality and living resource restoration. Progress includes upgrades to waste water treatment plants and septic systems as well as riparian buffers and cover crops at agriculture sites. Yet, despite drastic reductions to point sources nitrogen discharges, diffuse sources are the primary source for nitrogen loading and loading rates are 50 times that of point sources. Diffuse sources are the most difficult to manage as it often requires a concerted effort across a suite of sources ranging from septic leaching to fertilizer applications. Once contaminants enter a body of water they are nearly impossible to remove, thereby necessitating a preventative approach. While policy and technology work together to impede inorganic nitrogen movement from the watershed to estuaries, many regions need an immediate method of nitrogen remediation for water quality restoration.

#### **Mya Summary**

This study indicates that *Mya arenaria* can potentially remove between 1,000-7,000 kg of nitrogen when harvested from three different plot areas. Harvest can account for some or all of the net nitrogen residing in the system or, if combined with another nitrogen sink such as algae, can significantly diminish excess nitrogen with smaller clam plantings. In this analysis, nitrogen mineralized from biodeposits was not included in the calculations as it would require a clear knowledge of sediment and microbial processes, but may lower the estimate for total nitrogen removed. On the other hand, nitrogen content in the shells was also not accounted for which could ultimately raise the nitrogen removal estimate. If this is the case, this species of clam mariculture could potentially provide a much larger nutrient sink than was previously estimated.

This species of clam is a great candidate for restoration projects, not just for its nitrogen integration capacity, but for all of the additional ecosystem services they can provide. Bivalves are considered the “benthic-pelagic coupler” for many ecosystems as they filter particles from the water column and deposit them on the sediment and act as an intermediary between trophic level predation. This provides the benthic community with critical materials for growth. Clams are burrowing bivalves and *M. arenaria* prefer to burrow vertically in the sediment and only migrate up and down through the riverbed. It is speculated that this process of bioturbation allows for oxygenated water to reach microbial communities, which is a necessary constituent for denitrification. Burrowing macroinvertebrates have also been attributed with bank stabilization and reduced sediment loss to erosion forces.

Despite their other qualities, bivalves are most known for their biofiltration and ability to improve water clarity. As with most bivalves, *M. arenaria* filter not only plankton from the water, but also sediment which is then redeposited on the river bed. This is important for the Corsica River since one of the watershed restoration action strategies was to promote biofiltration to reduce turbidity. The planning commission for this project identified oysters as a primary tool for this endeavor, but *M. arenaria* would be a worthwhile investment as well.

### **Gracilaria Summary**

*G. tikvahiae* made moderate reductions to nitrogen in the studied polyculture arrangement, where the largest contribution to nitrogen removal came to only 225 kg nitrogen a year. The Corsica River is progressing towards attaining water quality standards and the lower river has less frequent spikes in nitrogen concentrations from the subwatersheds. Therefore, the lower Corsica may benefit from small reductions to nutrient concentrations at a localized scale.

The model simulated a peak in growth during the spring and early summer followed by a quick decline in growth by mid-summer. Nitrogen loading is at its highest in winter and spring as the water table is relatively high and is easily penetrated with dissolved surface nitrates and nitrites. As the months get increasingly drier leading into the summer, the water table drops and deeper groundwater with less surface leachate feeds into the rivers. It is then advantageous that *Gracilaria* thrive when nitrogen is at its peak as it allows *G. tikvahiae* to compete for nutrients directly with phytoplankton.

Furthermore, this species of algae is in high demand for its agar and hydrocolloid producing capabilities. Not only by using this species for aquaculture can watersheds work towards water quality standards, but also turn a profit from harvest production. In 2009 *Gracilaria* supplied up to 80% of the world's agar production, whereas only a decade earlier the species accounted for only 63% of production (Santelices, 2014). There is a growing need for *Gracilaria* cultivation and it can provide both monetary value for the region's watermen while also providing a water quality service for the estuary.

### **Ulva Summary**

Despite only being harvested as a biofouling agent, *U. lactuca* generated a large amount of harvested biomass and a comparable nitrogen removal to *G. tikvahiae*. The model was run at three different depths for *M. arenaria* plantings and the shallowest depth provided ideal light conditions for high primary production. However, the budget only considered nitrogen removed at the deepest depth to reflect watermen's preference for reduced biofouling. In this study, *M. arenaria* is the foundation species in the polyculture and also provides a large nitrogen sink for the system, whereas *U. lactuca* grows in mats on the culture materials and reduces adequate water flow over the clam cultures. Therefore, it is better for the viability of the *Mya* population that as little *U. lactuca* is grown as possible. As the market for this algae expands in the United States, it may be more

advantageous to culture the clams at shallower depths to harvest more algae. Currently, excessive biofouling maintenance is not cost or time effective for the watermen.

## **5.2 FURTHER RESEARCH**

This study was performed to assess the potential nitrogen stored and removed by increasing an available nitrogen sink in the Corsica River via polyculture. Analysis methods were developed from current literature and agency published data to represent the impact of these cultures on nitrogen stocks in the system. However, based on resources and data available, different quantitative procedures were used for the *M. arenaria* and macroalgae with different limitations. Ideally, this assessment would be an integrated analysis and thus requires a single model where waste streams are cycled into the available nitrogen stores. If bivalve and macroalgae polycultures were implemented as a bioremediation strategy, additional data and collaborations would be required.

### **Comprehensive Model**

In order to make the *M. arenaria* calculations more accurate, data for nitrogen assimilation into the shell would need to be available. In addition, remineralization rates of biodeposits would need to be accounted for. Although ecosystem models have taken great strides to incorporate the environmental impacts of bivalve aquaculture on a regional scale, there is still discrepancy as to the magnitude and consistency of these results to other systems. Much of this has been attributed highly variable denitrification rates of sediment and prevents the wide spanning use of particular models by resource managers.

This study would be most effective if an integrated ecosystem model was created to incorporate all three polyculture species. For this to be attainable, a sediment analysis for denitrification rates as well as continuous monitoring data for all potential aquaculture sites would be needed. This data

would ideally include water temperature, PAR, salinity, dissolved oxygen, total nitrogen, total phosphorus and current velocity. It would also be interesting to design this model with specific converters based on aquaculture best management practices and ideal harvest schedules.

### **Stakeholder Analysis**

After this preliminary study, it would be crucial to involve all relevant stakeholders at all stages of the project's development. The Corsica River watershed is known for strong community activism when it concerns Corsica River restoration projects. Since aquaculture can be met with mixed opinions it would be beneficial to have residents involved early in the project's design to prevent resistance against future implementation.

If the river is opened to polycultures, watermen would need to be consulted to determine if *M. arenaria* and *G. tikvahiae* cultures are desirable harvests. Watermen would need to embrace the concept of polycultures and be willing to apply for the leases if the zoning is granted. It is also imperative to learn what would make this venture appealing to them enough so they can become sound stewards of the estuary.

State and federal agencies would also need to be consulted for the new establishment of aquaculture leases in the Corsica River. The river was opened to aquaculture in 2011 by Maryland Department of Natural Resources to boost stewardship and productivity. The U.S. Environmental Protection Agency will also need to be referenced for potential grants or rebates to accredit towards TMDLs and potential nutrient trading schemes as part of the Corsica River WRAS.

### **Pilot Study**

Finally, a pilot study would be used to determine best management practices or techniques specific to each culture site. Methods identified in this study were created for a generic site in the lower

Corsica River from a diverse set of literature and may not be practical if the site is moved upstream or into another tidal tributary. A field study is required to determine location of plot sites in coordination with zoning and aquaculture leasing agencies without impacting water channel traffic and other designated water uses.

### 5.3 CONCLUSIONS

Enhanced eutrophication in estuarine systems has long been documented as a leading cause in habitat and water quality degradation. Fortunately, great strides have been made to reduce nutrient loading including massive upgrades to waste water treatment plants and septic systems in close proximity to tidal waters as well as nutrient and water retention on agricultural land. However, diffuse sources of nutrients are still the largest contributors to nutrient pollution and are also the most difficult to prevent and regulate. Using the natural ecosystem services of organisms to assimilate or fix nitrogen is a critical method to restore eutrophic systems.

Seasonal eutrophication increases turbidity, which reduces light penetration to the benthos and destroys submerged aquatic vegetation while contributing to hypoxic conditions. If watermen are permitted to culture even small areas of the Corsica River with *M. arenaria* and *G. tikvahiae*, a substantial amount of nitrogen can be removed through harvest. An even larger nitrogen sink can be created if biofouling macroalgae, such as the common *U. lactuca*, were also harvested and removed from the system.

Integrated cultures have the opportunity to provide many ecosystem services beyond nitrogen assimilation. The extractive capacity of the macroalgae compete with phytoplankton for available resources and prevent algal blooms, while also providing dissolved oxygen and habitat for fish and invertebrates. The shellfish aquaculture stabilizes the sediment through *M. arenaria* burrowing

activity as well as introduced materials and netting. The suspension feeding also pulls sediment out of the water column and redeposits the particles on the benthos to add material nutrients. Burrowing animals like clams provide the sediment with oxygenated water, which drives the denitrification cycles and reduces remineralization. Aquaculture material also provide needed hard substrate for oyster larvae recruitment and other filter feeders. Additional habitat such as aquaculture materials boost local biodiversity and amplify water conditioning by supporting various ecosystem services from associated flora and fauna.

This analysis is meant to show the potential nitrogen removal capacity that biofiltration can accomplish, but also requires a much more rigorous investigation to fully understand the environmental impact an infrastructure such as this would create. First, a complete model incorporating all three harvestable species would need to be completed using continuous monitoring data (CONMON). Second, it would be beneficial to know the denitrification rates of the sediment to ensure proper fixation of bivalve biodeposits to prevent remineralization. Finally a pilot study and cost analysis would be critical to determine best management practices for the project and potential markets for the new macroalgae harvests. It is also imperative that there would be strong stakeholder involvement at all stages to promote stewardship for the restoration effort.

Waterman may be incentivized to culture soft shell clams and macro algae if the state participates in a nutrient trading scheme from which waterman can be credited for their nitrogen sink harvests. States such as Maryland may also be willing to negotiate startup costs or rebates if cultures can assist with meeting watershed restoration action strategies (WRAS). Not only are the clams and macroalgae assimilating nitrogen, but clams can filter the water column while macroalgae compete with phytoplankton for nutrients. This reduces turbidity allowing more light can reach the sediment, thereby supporting SAV restoration goals.

When shellfish and/or macroalgae aquaculture are recognized and implemented for their nutrient remediation capabilities, this project can be adapted for alternative ecosystems. For instance, less marketable species such as *Ischadium recurvum* can be cultured in regions with high concentrations of fecal coliforms where shellfish harvests are prohibited. Additionally, since the highest nutrient concentrations and eutrophication events occur at the head of the tributaries, fresh water species can be used in such locations. If water quality trading is implemented to include shellfish and algal aquaculture and public stock enhancement, credits can offset the loss of harvest profits in contaminated sites or ecosystems that support nonmarketable species.

Although this study only examines one method for nitrogen management, which may not be suitable for all tidal tributaries, it can assist with regional water quality restoration and provide buffers to prevent tributary degradation from transferring to the main channel of Chesapeake Bay. Polycultures offer ecosystem based management to capitalize on natural ecosystem services to improve environmental health and provide economic stability for a historic industry and, when coupled with watershed policy, can mitigate the detrimental impacts of nutrient enrichment.



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