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*Ulva* spp. Bloom Dynamics in a Hyper-Eutrophic Estuary: Jamaica Bay, New York

Annesia Lamb

*The Graduate Center, City University of New York*

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Ulva spp. bloom dynamics in a hyper-eutrophic estuary: Jamaica Bay, New York

by

Annesia L. Lamb

A dissertation submitted to the Graduate Faculty in Earth and Environmental Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York
2018
Ulva spp. bloom dynamics in a hyper-eutrophic estuary: Jamaica Bay, New York

by

Annesia L. Lamb

This manuscript has been read and accepted for the Graduate Faculty in Earth and Environmental Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

Date

Brett Branco
Chair of Examining Committee

Date

Cindi Katz
Executive Officer

Supervisory Committee:

Juergen Polle
Charles Yarish
Chester Zarnoch

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

*Ulva spp.* bloom dynamics in a hyper-eutrophic estuary: Jamaica Bay, New York

By Annesia L. Lamb

Advisor: Brett Branco

In this dissertation, I present three studies that further our understanding of macroalgae identity, growth, and proliferation. Eutrophication is prevalent in shallow coastal ecosystems world-wide. One of the ecosystem consequences is the development of a bloom forming green marine macroalgae, *Ulva* spp. *Ulva* can have negative effects such as *Zostera* spp. degradation, fish, and shellfish declines. I performed assessments of (1) identity of the bloom-forming *Ulva* and other macroalgae assemblage, (2) physical, chemical, and biological drivers of *Ulva* bloom growth and proliferation, and (3) optimal irradiance and temperature requirements for early growth stages in *Ulva linza*.

The first study is a comprehensive survey of the bloom-forming *Ulva* and other macroalgae. I performed *in situ* observations in a shallow coastal estuary, Jamaica Bay, New York. Jamaica Bay is a eutrophic estuary that receives excessive (>11,500 kg N day⁻¹) nitrogen loads. This study utilized nuclear ribosomal DNA, Internal Transcribed Spacer Region (ITS), and chloroplast elongation factor, *tuf*A DNA Barcoding techniques. The dominant blade forming species of *Ulva* were *U. compressa*, *U. lactuca*, and *U. laetevirens*. Other observed *Ulva* were *U. cf. clathratioides*, *U. prolifera*, and *U. stipitata*. Some of the other macroalgae observed were *Gracilaria vermiculophylla*, *Fucus vericulosus*, and *Porphyra* spp. The findings of this study can aid in the control of *Ulva* blooms. Knowing the species is the first step in determining the growth requirements and can build upon other studies world-wide.
In the second study of this work I analyzed the in situ standing biomass of Ulva spp., Gracilaria vermiculophylla, and determined the drivers of bloom-forming Ulva. I also compared 1995/1996 Ulva spp. standing biomass in Jamaica Bay to my field analysis and aimed at understanding the reasons for differences between two decades of time. Ulva spp. biomass was collected at two locations over the field season of 2015 in Jamaica Bay. Tissue nitrogen was measured during the season in Ulva spp. to determine if Ulva was nutrient limited. Water column nutrients, salinity, and temperature were measured to understand the drivers of Ulva spp. blooms. The Ulva spp. biomass at Norton Basin and Marine Park began to increase in May and lasted through September at Norton Basin with the average peak standing biomass at 232 g dry weight m$^{-2}$. At Marine Park the bloom lasted through October with an average peak standing biomass at 139 g dry weight m$^{-2}$. At Marine Park Gracilaria vermiculophylla biomass was larger than Norton Basin throughout the season with a peak standing biomass in July at 15.53 g dry weight m$^{-2}$. The average nitrogen tissue contents of Ulva spp. ranged between 2 to 4.5 percent. The lowest tissue nitrogen content was during the Ulva spp. bloom months, June and July. Ulva spp. was not nutrient limited any time during the season. Water column ammonium was the most abundant nutrient and it appears that Ulva is growing on for most of the season. Nitrate was low during the bloom months at almost 0 mg N L$^{-1}$. The occurrence of high ammonium in the water column is most likely from wastewater treatment discharge and remineralization in the sediments.

The third study of this work I performed a controlled laboratory experiment to determine the optimal growth at different irradiance and temperatures in the developmental stages of Ulva linza. The optimal growth at an irradiance of 200-µmol photons m$^{-2}$ s$^{-1}$ was at 25°C and at 100-
μmol photons m$^{-2}$ s$^{-1}$ was 21°C. Previous studies have identified similar growth results in other species of *Ulva* including the closely related *U. prolifera*.

Overall this work has management implications because we know the abundance and nitrogen storage potential of *Ulva* spp. from this major bay in metropolitan New York City coastal waters. Modelling the storage of nitrogen in *Ulva* spp. could be useful for optimal harvesting purposes to manage *Ulva* blooms. These harvests can remove nitrogen from eutrophic estuarine systems. Since nitrogen can only be removed by denitrification harvesting allows managers to purposefully remove nitrogen.
ACKNOWLEDGEMENTS

I’d like to first thank my advisor, Brett Branco, for his support in this scientific process. I’d also like to thank my committee, Jürgen Polle, Charles Yarish, and Chester Zarnoch. Their comments and suggestions made this document richer. They also taught me a lot about algae and the nitrogen cycle. Two people helped me with my preliminary drafts, Simona Augyte and Maria Jerskey. Without their help I would still be stuck in my early drafts fighting for freedom. Brian Wysor, Jang K. Kim, and Yaunzi Huo are my colleagues in algae crime and their help should not go without thanks. They helped me with genetic work, culturing, and general Ulva questions that I had. I want to also thank Debaki Chakrabarti, Alan Hack, and Henrik Zakari for emotional support during my graduate studies and dissertation writing days. Their support helped keep me balanced and mindful.

Many people helped me with field and lab work that was a necessity for this research and I wish to thank these people many of which had their own research projects that were happening at the same time. These seaweed friends are Katja Knoll, Jolene Willis–Lujan, Heghnar Skyian, Jack Lin, Kristine Erskine, Aleksandra Kravtsova, Danielle Hagans-Alexander, Janel Chap, Emily Alexander, Emily Bell Dinan, Christine Halloran, Hermine Huot, Jane Hauptman, Tatiana Morin, LaToya Anderson, Lena Apostol, Lisa Bloodgood, Chris Cassone, David Cham, Liz Clark, Ronniecia Hackshaw, Heather Sioux, Tricia Bhatia, Adriana Alvarado, Varvana Budetti, Alisha Bunting, Cosette Davis, Max Edeson, Kevin Gnai, Leslie Toohey, Julie Yaish, and Tasfia Tabassum.

I want to also thank Ken Karol for teaching me how to properly store and archive my herbarium specimens at the New York Botanical Garden. Thank you to my EES classmates, in
particular Patrick Alexander, Charuta Kulkarni, and Stephanie Devries for sharing the day to day grind of being a graduate student. Thank you to my friends and family for their support.
PREFACE

The following document contains the collection of work completed during my PhD. Each work has a topic that is related to the overall theme of this study, the *Ulva* spp. dynamics in a hyper-eutrophic estuary. Each work stands on its own as a publishable article. The topics covered include:

a. Identification of the bloom forming *Ulva* spp. and macroalgae in Jamaica Bay
b. Abundance and environmental drivers of *Ulva* spp. growth
c. Optimal irradiance and temperature controls *Ulva linza*

For topics a-c, the corresponding chapter is presented in the form of a journal article, each having a separate introduction, methods, and discussion sections. To summarize the introductory material for the reader’s convenience, an additional introduction section is added at the beginning of this dissertation containing a brief introduction. And to summarize all the concluding material a separate discussion is added at the end of this dissertation containing a brief summary from each topic.
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Chapter 1

INTRODUCTION

Eutrophication is a widespread problem in coastal environments due to external nutrient loading from anthropogenic sources. Eutrophication contributes to poor water quality, loss of biodiversity, saltmarsh loss and blooms of harmful macroalgae (Valiela 2009; Duarte 2009; Nixon 2009). Macroalgae growth further alters ecological processes by creating hypoxia, shading seagrasses and salt marsh grasses, as well as increasing hydrogen sulfide-production (Valiela 1997; Kracauer-Hartig et al. 2002; Tang and Gobler 2011; Newton and Thornber 2012).

In many eutrophic shallow estuaries such as Waquoit Bay, Massachusetts, USA and Venice Lagoon, Italy macroalgae becomes a dominant primary producer group under high nitrogen loading (Sfriso 1992; Valiela et al. 1997; Brush and Nixon 2010).

The Green Algal Genus *Ulva*

*Ulva*, a common macroalgae (also known as a “sea lettuce”), forms large distromatic sheet or tube-like structures up to 1 m in length and is found in eutrophic shallow estuaries in the Mid-Atlantic to New England regions of the United States (Figure 1.1; Mathieson and Dawes 2017). They also occur in warm temperate estuaries around the world. *Ulua* prefers sheltered intertidal and subtidal marine environments, where it often generates large algal blooms (Figure 1.2; Mathieson and Dawes 2017).

*Ulva* is capable of rapid growth and colonization during the spring and summer months (Valiela et al. 1997; Mathieson & Dawes 2017). In warm months, *Ulva* growth occurs near the upper sublittoral zone to the near mean low water in thick narrow bands. Zoospores from the water column settle, germinate, and produce multinucleate rhizoids from a basal cell that
Figure 1.2 Image of 2013 *Ulva* bed in October at Norton Basin during low tide. Photo by ALamb.
develops into tube-like or sheet-like blades. The cells contain cup-shaped or plate-shaped chloroplasts. The tubular morphology may be due to occurrence of its bacteria microbiome (Provasoli 1958; Bonneau 1977; Nakanishi et al. 1996; Matsuo et al. 2005; Marshall et al. 2006). Spores attach to sediment or shells or any hard substrate until they reach adulthood in 6-8 weeks and then become free-floating. *Ulva* has a complex isomorphic life cycle that has diploid and haploid stages. Mshigeni and Kajumulo (1979) determined that *Ulva* gametophytes and sporophytes alternate every year and wave action affects polymorphism.

The adult fronds usually detach and float to various positions along the intertidal zone throughout the season, being transported into salt-marsh pools and the fringes of the marine environment (Rhyne 1973; Harlin and Miller 1981; Sfriso et al. 1992). Salmonsen et al. (1999) determined that transport of *Ulva* was mainly due to water flow velocity and wind speed and rarely exited the estuarine environments.

Thalli of the Ulvophyceae class attach to substrate by multinucleate rhizoidal branches. The blade forms from zoospores that develop from uniseriate, pluriseriate, and tubular stages. If *Ulva* is grown in axenic cultures the tubular state collapses, is disrupted and uniseriate filaments are produced, forming circular branched plants and pincushion like colonies (Provasoli 1958). The Cytophaga-Flavobacterium-Bacteroides group grows on the surface of *Ulva* and produces vitamins, and a morphogenetic factor called thallusin. Addition of bacteria from natural *Ulva* collections will restore “normal” development and blade-like morphology if grown in the laboratory cultures (Bonneau 1977; Marshall 2006).

Species of *Ulva* have been traditionally identified by morphological anatomical, and cytological characteristics. Initially there were three separate genera: *Ulva*, *Enteromorpha*, and *Chloropelta*. However numerous studies have shown that genetically non-distinct algae exhibit
morphological changes within the species according to environmental factors such as bacteria abundance and association, temperature, nutrient availability, salinity, light availability, and grazing organisms (Tan et al. 1999; Hayden et al. 2003; Shimada et al. 2003). A previous nuclear ribosomal DNA analysis also showed that these three genera do not have evolutionary distinction, and *Enteromorpha* has been previously synonymized with *Ulva* (Hayden et al. 2003). It is therefore necessary to sequence parts of the genome of the algae for accurate identification of the species.

**Climate and Its Impact on *Ulva***

Climate change is caused by increasing carbon dioxide in the atmosphere, which drives both ocean acidification and global temperature rise (IPCC 2007). Studies analyzing *Ulva* spp. response to increasing carbon dioxide are conflicting (Drechsler and Beer 1991; Björk et al. 1992, 1993; Dreschsler et al. 1993; Sharkia et al. 1994; Rautenberger et al. 2015). Rautenberger et al. (2015) states that *Ulva* spp. will become saturated and growth rates will stay the same. Scoma et al. (2016) analyzed the growth rates of *Ulva lactuca* and reported increased growth with increasing carbon dioxide. Furthermore, Latimer et al. (2013) showed that under just a 1-2 ºC change in temperature many temperate species could replace cold-temperate species.

**Jamaica Bay**

Jamaica Bay, New York, USA is a shallow estuary receiving saltwater from the Hudson-Raritan Estuary and the Atlantic Ocean. An extensive portion of the Bay is defined by hardened shorelines but it also contains salt marsh islands, muddy and sandy coasts. It is part of the Gateway National Recreation Area (GNRA) and being within the limits of New York City it is the nation’s largest urban national park. The Hudson-Raritan estuary receives 2.5 billion gal day$^{-1}$ of freshwater effluent (Hydroqual Inc. 1991). Jamaica Bay receives 15 million gal day$^{-1}$ of
freshwater from a combined sewer system and wastewater treatment plants that serve inhabitants of Brooklyn and Queens. For comparison, the Bronx River, Bronx and Manhattan, NY, receives 250 million gal day$^{-1}$ from Wards Island WWTP (Protopapas 1999).

Jamaica Bay experiences seasonal primary producer blooms, high nutrient loads (>15,000 kg N day$^{-1}$), and harmful bacteria episodes (Mark Ringenary, Water Resources Specialist, National Park Service, personal communication; Franz and Friedman 2002; Benotti et al. 2007; Wallace and Gobler 2015). Jamaica Bay receives double of the nitrogen loading than most eutrophic estuaries in North America or Europe (Table 1.1). The Bay has a long history of industrial use and has many pollution related issues such as raw sewage overflows, anoxic events, fishkills, degraded shellfish and seagrass populations (Waldman 2012).

Currently, there is no documentation when *Ulva* appeared or if it is a native genus to the East Coast U.S. *Ulva* beds have existed in Jamaica Bay at least 50 years (Charles Yarish, Professor, University of Connecticut, personal communication). *Ulva* has existed in New York Harbor since at least the 1840s. We know this from the Durant (1850) publication because it contains original macroalgae specimens collected from many sites around New York Harbor (Figure 1.1).
Table 1.1 Nitrogen loads to shallow lagoons per year. Jamaica Bay loads highlighted in bold.

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**Eutrophication Management and Ulva**

A eutrophication model can help understand the function of an ecosystem and the nutrient retention/release, consumption of dissolved oxygen, and supply of organic carbon to the benthic environment (Brush and Nixon 2010). Shallow, photic system models including macroalgae have been developed in Europe and for Narragansett Bay, RI (Coffaro and Sfriso 1997; Solidoro et al. 1997; Martins and Marques 2002; Aveytua-Alcazar et al. 2008; Brush and Nixon 2010). However, majority of eutrophication models biotic component focus on seagrass and nutrient interception (Bricker et al. 1999; Bricker et al. 2003; Brush and Nixon 2010). There is a need to include Ulva and other macroalgae in eutrophication models to better understand their impact on nutrient cycling in a eutrophic estuary. However, the metrics of Ulva abundance, species composition, and optimal irradiance and temperature need to be considered first for a particular estuary.

There is also a need to account for Ulva and other macroalgae in eutrophication management of estuaries. McGlathery et al. (2007) proposed that future management of eutrophication in shallow estuaries would rely heavily on the macroalgae. They hypothesized that in the future macroalgae may act as coastal filters for nitrogen, because salt marsh and seagrass communities will be degraded. McGlathery et al. (2007) also identified understanding the influence of macroalgae on nitrogen retention as a gap in ecosystem function knowledge. They summarized two important concepts involving macroalgae as eutrophication proceeds in shallow coastal estuaries (1) mass transport of previously plant bound nutrients will increase because seagrasses will be replaced by unattached bloom forming macroalgae that move with the water; and (2) denitrification will be an unimportant sink for nitrogen because primary producers typically outcompete bacteria for available ammonium and nitrate (Figure 1.3).
Figure 1.3 Denitrification patterns as nitrogen increases in an estuary. From McGlathery et al. (2007).
The research in this dissertation was motivated by the following research questions:

1. What are the main contributors to macroalgae blooms in Jamaica Bay and at what macroalgal abundance?

2. How might the blooms be affecting nitrogen pools in Jamaica Bay?

Answering these questions will help us to predict the response of Jamaica Bay to changes in nutrient loading and climate.

To answer the research questions, I have the following specific objectives:

• identify the bloom-forming *Ulva* species in Jamaica Bay
• identify the assemblage of macroalgae in Jamaica Bay
• quantify the macroalgal abundance in Jamaica Bay
• quantify irradiance and temperature responses of *Ulva* in Jamaica Bay
Chapter 2

Identification of the bloom forming Ulva and macroalgal assemblage in Jamaica Bay, NY USA

A. L. Lamb, J. K. Kim, C. Yarish, and B. F. Branco

The content of this chapter is in press, Rhodora, Volume 120 (984)

ABSTRACT

Eutrophication is prevalent in shallow water ecosystems world-wide. Ulva is a genus of bloom forming macroalgae that occur in shallow estuaries. Ulva have ecosystem consequences such as Zostera spp. degradation, fish and shellfish declines. The presented study describes a comprehensive survey of Ulva spp. distributed in Jamaica Bay, NY, USA. Using ITS and tufA DNA Barcoding and cytological techniques, we identified the dominant species of Ulva at 8 sites in Jamaica Bay and 1 site in Long Island Sound, CT to match Ulva compressa, U. cf. clathratioides, U. prolifera, U. stipitata, U. laetevirens, and U. lactuca with other sequences world-wide. All samples collected had <1% divergence between species. Ulva stipitata, a compressed tubular species, was found in Jamaica Bay and is the second known occurrence of the species in the Northwest Atlantic. The presented study has management implications because we know the nitrogen storage potential of Ulva spp. from this major bay in metropolitan New York City coastal waters. Modelling the storage of nitrogen in Ulva spp. could be useful for optimal harvesting purposes to manage Ulva blooms.

Key Words: Ulva, macroalgal blooms, green tide, ITS, tufA, eutrophication, Jamaica Bay
INTRODUCTION

Some species of *Ulva* (Chlorophyta, Ulvophyceae) are opportunistic bloom forming seaweeds that take advantage of nitrate- and ammonia-rich estuaries and contribute to a cascade of ecosystem changes (Bliding 1969; Valiela et al. 1997; Cohen & Fong 2006; Hu et al. 2010; Zhang et al. 2014; Huo et al. 2016). The excess input of particulate carbon from decomposing *Ulva* promotes heterotrophic dominated ecosystems that are limited in both plant and animal diversity at all trophic levels (Franz and Friedman 2002; McGlathery et al. 2007; Newton and Thornber 2012). *Ulva* blooms are problematic as their respiration promotes low oxygen environments due to their ability to grow in large amounts, blanket the water column, and prevent mixing of atmospheric oxygen into the water column (Tyler and McGlathery 2003; Watson et al. 2015).

*Ulva* blooms can harm ecosystems and cause a cascade of negative effects; therefore, management must be informed of *Ulva* species and abundance. It becomes necessary to harvest the *Ulva* biomass and use it for other purposes such as fish feed and fertilizer. Harvesting biomass can also have positive outcomes for water quality through nutrient bioextraction (Yarish et al. 1991; Merrill et al. 1992; Kim et al. 2014, 2015; Rose et al. 2015). Nutrient bioextraction is the practice of harvesting seaweed for the purpose of nitrogen removal from natural water bodies (Rose et al. 2015). Efforts to control nitrogen sources have been made with the reduction of wastewater treatment discharge. However, with nitrogen discharges from sewage treatment plants in Brooklyn and Queens, NY, remediation is limited. Therefore, nutrient bioextraction may be another way to remove nitrogen from the system once it has been discharged.

The nutrient cycling impacts and biomass changes of macroalgae, including *Ulva*, are often incorporated into numerical eutrophication models (Fitzpatrick 2002; Fitzpatrick et al.
Therefore, it is important that modelers know what species should be included, particularly if they have different nitrogen: phosphorus ratios and growth rates. Identification of different *Ulva* species is necessary because they can take up and store nitrogen and carbon at different rates and therefore have different responses to nitrogen loading (Fong et al. 2001; Kamer et al. 2001). For example, *Ulva lactuca* and *U. curvata* have different uptake rates of 390 and 250 µmol L\(^{-1}\) h\(^{-1}\) respectively in nutrient starved thalli (Rosenberg and Ramus 1981; Cohen and Neori 1991). In addition, *Ulva* can have different transport rates with attached tubular species being sedentary and free-floating blade species having more movement around bays and embayments (Flindt et al. 1997; Salomonsen et al. 1999).

Jamaica Bay is a shallow hypereutrophic embayment along the densely populated coast of New York. The Bay receives a large portion of freshwater discharges from wastewater treatment plants that equal approximately 970 x 10\(^6\) L day\(^{-1}\) (Benotti et al. 2007). For decades, the Bay has had excessive phytoplankton and *Ulva* blooms and fish and shellfish kills (Franz 1982; Wallace and Gobler 2015). The need to model the Bay’s response to nitrogen reduction starts with the identification of dominant primary producers.

Initial identification of *Ulva* in Jamaica Bay began with Durant (1850) who identified *U. compressa* Linnaeus, *U. latissima* Gunnerus (1766) = a brown alga *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders (2006), *U. linza* Linnaeus (1753), *U. intestinalis* Linnaeus, and *U. ramulosa* Smith (1809-1810) = *U. clathrata* (Roth) Agardh (1823), by morphological techniques. Rhoads et al. (2001) while completing a water quality study in the Norton Basin/ Little Bay area mentioned *U. lactuca* blooms as a nuisance. Their identification of *U. lactuca* was based on a USFWS (1997) report completed in the New York Bight. Since then, all reports of *Ulva* were identified as *U. lactuca* until a study completed in the middle portions of
the Bay (Franz and Friedman 2002; Kracauer-Hartig et al. 2002; Continental Shelf Associates 2004; Potts et al. 2012; Wallace and Gobler 2015). Based upon DNA barcoding studies Wallace and Gobler (2015) identified *U. rigida* Agardh (1823) as the dominant bloom forming *Ulva* with sites in the middle portion of the Bay.

In identifying morphological variability in individual species DNA barcoding has become critical for species identifications (Saunders and Kucera 2010). Closely related species of red and green algae were first differentiated by using the chloroplast-encoded large subunit RuBisCO (*rbcL*) DNA sequences as a barcode (Freshwater et al. 1995; Millar and Freshwater 2005; Shimada et al. 1999; Lane et al. 2007; Freshwater et al. 2010). Generally, a sequence divergence of <1% signifies that organisms are the same species while divergences of >1-2% are different (Freshwater and Rueness 1994; Freshwater et al. 1995; Millar and Freshwater 2005). In recent years the use of successful DNA barcoding with *Ulva* has been completed with the nuclear ribosomal internal transcribed spacer 1 and 2 regions (ITS1 and ITS2), the chloroplast *rbcL*, and the chloroplast elongation factor *tufA* (Guidone et al. 2013; Kirkendale et al. 2013; Saunders and Kucera 2010; Hofmann et al. 2010; Mao et al. 2014). Saunders and Kucera (2010) have shown that *tufA* can be used as a barcode gene for chlorophytes (Nielsen et al. 2013; Mao et al. 2014). Variability of the *tufA* and ITS biomarkers allows species discrimination and conserved regions to amplify at the gene 5’ and 3’ end primers (O’Kelly et al. 2004).

In this study our goals were two-fold as follows: (1) to identify the dominant blade and tubular species of *Ulva* blooming in Jamaica Bay using DNA Barcoding techniques with *tufA* and ITS; (2) to determine the intertidal macroalgal assemblage in the Bay. Our hypothesis was that ITS and *tufA* genes would produce complimentary results and confirm cytological observations identifying either *U. compressa* or *U. rigida* as the dominant *Ulva* species.
MATERIALS AND METHODS

Specimen collection. Ulva specimens were collected from intertidal areas in Jamaica Bay, NY (40°36’20.39”N, 73°52’16.71”W). Collections were made at a total of eight sites at five bloom-impacted sites including Motts Basin, Norton Basin, West Pond at the Wildlife Refuge, Marine Park, and Big Egg Marsh and three non-bloom impacted sites, Bayswater Point, Plum Beach, and Cross Bay Bridge northeast (Figures 2.1 and 2.2). The definition of a bloom-impacted site is a 100 percent Ulva spp. cover. A non-bloom impacted site is <100 percent Ulva spp. cover. During 2013, all sites contained Ulva at 25-100% coverage with these being either floating or detached from hard substrata (Table 2.1). Ulva collected in 2015 was both floating (detached from its substrata) and attached to a substrata. Two sites were sampled for macroalga and collected during all months of the year to achieve greater detail. The two sites were sampled for Ulva tube-like and blade-like structures. Samples in 2013 were collected at low tide and selected to represent the dominant morphology (blade with no ruffles, light green, no perforations and free-floating) and found during the bloom period, May-October (Figure 3). Non-dominant Ulva were seen at the same time as the dominant Ulva and were small blades with ruffles, dark green, perforations and no perforations, and attached to substrata. Ulva spp. (CT_USA 1C) was collected at Penfield Reef near Bridgeport, CT in western Long Island Sound. A small piece was taken from the mother thallus that was over 1 m in diameter, and spores were isolated and developed for a culture strain at the University of Connecticut, Stamford, CT.
Figure 2.1 A map of the eight sampling sites in Jamaica Bay, New York. These include Plum Beach (PB), Big Egg Marsh (BE), West Pond (WP), Cross Bay Bridge (CB), Elders Island East (EE), Norton Basin (NB), and Bayswater Point (BP), Marine Park (MP). The sites are intertidal mud-flats with adjacent *Spartina alterniflora* salt marsh. 40°36'20.39''N, 73°52’16.71’’W.
Figure 2.2 Norton Basin Jamaica Bay, New York field site with adjacent salt marsh (left). *Ulva* spp. bloom at Norton Basin Jamaica Bay, New York (right). Both images taken in July 2015.
Table 2.1 Collection sites and distribution of *Ulva compressa* blades within Jamaica Bay, New York and western Long Island Sound. Taxa abbreviation: UC = *Ulva compressa*.

<table>
<thead>
<tr>
<th>Site Abbreviation</th>
<th>Locality</th>
<th>Date Collected</th>
<th>Voucher</th>
<th>Habitat Description</th>
<th>Location</th>
<th>ITS accession</th>
<th>Taxa Found</th>
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<tr>
<td>NB</td>
<td>Norton Basin, Far Rockaway, Queens</td>
<td>2013.10.18</td>
<td>NYBG 2335065</td>
<td>Sheltered deep embayment on mudflat with nearby sand shoals. The specimen was attached to substrate containing pavement and shell fragments.</td>
<td>18T 604592.33 m E, 4496402.81 m N</td>
<td>NY USA 1</td>
<td>UC</td>
</tr>
<tr>
<td>NB</td>
<td>Norton Basin, Far Rockaway, Queens</td>
<td>2013.10.18</td>
<td>--</td>
<td>Sheltered deep embayment on mudflat with nearby sand shoals. The specimen was free floating with substrate containing pavement and shell fragments.</td>
<td>18T 604592.33 m E, 4496402.81 m N</td>
<td>NY USA 3</td>
<td>UC</td>
</tr>
<tr>
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<td>2013.10.18</td>
<td>NYBG 2335067</td>
<td>Sheltered deep embayment on mudflat with nearby sand shoals. The specimen was free floating with substrate containing pavement and shell fragments.</td>
<td>18T 604592.33 m E, 4496402.81 m N</td>
<td>NY USA 5</td>
<td>UC</td>
</tr>
<tr>
<td>BP</td>
<td>Bayswater Point at Motts Basin, Far Rockaway, Queens</td>
<td>2013.10.18</td>
<td>NYBG 2335066</td>
<td>Sheltered shallow embayment adjacent to freshwater discharging power plant, has sand and pebble substrate. The specimen was free floating.</td>
<td>18T 603804.29 m E, 4496310.45 m N</td>
<td>NY USA 6</td>
<td>UC</td>
</tr>
<tr>
<td>BP</td>
<td>Bayswater Point at Motts Basin, Far Rockaway, Queens</td>
<td>2013.10.18</td>
<td>--</td>
<td>Sheltered shallow embayment adjacent to freshwater discharging power plant, has sand and pebble substrate. The specimen was free floating.</td>
<td>18T 603804.29 m E, 4496310.45 m N</td>
<td>NY USA 7</td>
<td>UC</td>
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<td>WP</td>
<td>West Pond at Jamaica Bay Wildlife Refuge, Broad Channel, Queens</td>
<td>2013.07.19</td>
<td>--</td>
<td>Sheltered shallow embayment containing mostly sand is adjacent to salt marsh islands and a fresh water pond. The specimen was free floating.</td>
<td>18T 599039.42 m E, 4496636.12 m N</td>
<td>NY USA 18</td>
<td>UC</td>
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<td>WP</td>
<td>West Pond at Jamaica Bay Wildlife Refuge, Broad Channel, Queens</td>
<td>2013.07.19</td>
<td>NYBG 2335057</td>
<td>Sheltered shallow embayment containing mostly sand is adjacent to salt marsh islands and a fresh water pond. The specimen was free floating.</td>
<td>18T 599039.42 m E, 4496636.12 m N</td>
<td>NY USA 19</td>
<td>UC</td>
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<tr>
<td>PB</td>
<td>Plum Beach, Gerritsen, Brooklyn</td>
<td>2013.10.05</td>
<td>NYBG 2335062</td>
<td>Exposed mudflat near Rockaway Inlet. The specimen was attached to shell material.</td>
<td>18T 591462.32 m E 4492971.16 m N</td>
<td>NY USA 12</td>
<td>UC</td>
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<tr>
<td>BE</td>
<td>Big Egg Marsh East, Broad Channel, Queens</td>
<td>2013.05.04</td>
<td>NYBG 2335056</td>
<td>Exposed mudflat adjacent to salt marsh islands near Beach Channel. The specimen was free floating.</td>
<td>18T 599383.60 m E 4494511.30 m N</td>
<td>NY USA 17*</td>
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<td>EE</td>
<td>Elders Island East</td>
<td>2013.08.16</td>
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<td>Exposed mudflat adjacent to salt marsh islands near North Channel. The specimen was free floating.</td>
<td>597070.87 m E, 4498459.19 m N</td>
<td>NY USA 20</td>
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<tr>
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<td>South east of Crossbay Bridge, Broad Channel, Queens</td>
<td>2013.09.15</td>
<td>--</td>
<td>Sheltered mudflat adjacent to inland salt marsh near North Channel. The specimen was attached to shell material.</td>
<td>598946.34 m E, 4499187.67 m N</td>
<td>NY USA 21</td>
<td>UC</td>
</tr>
<tr>
<td>CB</td>
<td>South east of Crossbay Bridge, Broad Channel, Queens</td>
<td>2013.09.15</td>
<td>--</td>
<td>Sheltered mudflat adjacent to inland salt marsh near North Channel. The specimen was free floating.</td>
<td>598946.34 m E, 4499187.67 m N</td>
<td>NY USA 22</td>
<td>UC</td>
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<tr>
<td>CB</td>
<td>South east of Crossbay Bridge, Broad Channel, Queens</td>
<td>2013.08.09</td>
<td>Sheltered mudflat adjacent to inland salt marsh near North Channel. The specimen was free floating.</td>
<td>598946.34 m E, 4499187.67 m N</td>
<td>NY USA</td>
<td>UC</td>
<td></td>
</tr>
<tr>
<td>CT_USA</td>
<td>Near Penfield Reef, Bridgeport, CT</td>
<td>2012.08</td>
<td>Open water near Bridgeport, CT. The specimen was free floating.</td>
<td>649800.84 m E, 4556795.77 m N</td>
<td>CT_USA</td>
<td>UC</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.3 Recent herbaria of *Ulva compressa* collected in 2013 from Jamaica Bay. The variation in sheet forming morphology ranges in tissue color and shape of entire blade. (A) Bayswater Point, scale=10.2 cm (left), 5.1 cm (middle), 10.2 cm (right); (B) Cross Bay Bridge, scale=5.1 cm (left), 10.2 cm (middle), 10.2 cm (right); (C) Elders Island, scale=10.2 cm (left), 10.2 cm (right); (D) Norton Basin, scale=7.6 cm (left), 10.2 cm (right); (E) Plum Beach, scale=10.2 cm; and (F) West Pond, scale=10.2 cm (left), 10.2 cm (right).
**Morphological characterization.** In the laboratory, the morphology of each sample collected in 2013 was identified using thallus type, blade (distromatic) or tubular, size, color, and perforation (Figure 2.3). Samples were cleaned with a cotton swab containing deionized water and betadine solution. For the Jamaica Bay samples four small tissue pieces, 4 mm², from each organism was torn and placed into four 2 mL vials, one with silica gel and three in glycerol storage solution and put in the freezer at -80°C for long-term storage. Each organism was cleaned with sterile deionized natural seawater (NSW) and pressed on acid free herbarium paper for morphological preservation. All herbarium samples were photographed using a Nikon D5100 digital SLR for archiving in the algae herbarium at The New York Botanical Garden, Bronx, NY (Figures 2.3 and 2.4).
Figure 2.4 Herbaria of *Ulva* specimens from Jamaica Bay. (A) 20150529MP_Ulva04 (*U. stipitata*) (B) 20150617MP_Ulva06 (*U. compressa*) (C) 20150617MP_Ulva05 (*U. compressa*) (D) 20150617MP_Ulva03 (*U. stipitata*) and 20150617MP_Ulva04 (E) 20150617MP_Ulva01 (*U. stipitata*) (F) 20150504MB_Ulva04 (*U. prolifera*) (G) 20150504MB_Ulva03 (*U. compressa*) (H) 20150504MB_Ulva02 (I) 20150504MB_Ulva01 (J) 20150529MP_Ulva03 (*U. compressa*) (K) 20150529_MP_Ulva05 (*U. stipitata*) (L) 20150529MP_Ulva02 (*U. lactuca*) (M) 20150529MP_Ulva01 (*U. laetevirens*) (N) 20150411MP_Ulva02 (*U. stipitata*), 20150411MP_Ulva03 (*U. prolifera*), 20150411MP_Ulva04 (*U. stipitata*), 20150411MP_Ulva05 (*U. prolifera*), and 20150411MP_Ulva06 (*U. stipitata*) (O) 20150411MP_Ulva01.
Cytological characterization. We identified cellular structures using rehydrated tissues from herbarium material when fresh specimens were not available. We hydrated the herbarium material with sterile natural seawater, by letting it soak for 30 min and then mounted on a glass microscope slide and cover slip. We viewed the sample on an inverted microscope with 50x magnification. In all 2013 samples we determined cell size, shape, arrangement, chloroplast location and shape, and pyrenoid size and number based on Hofmann et al. (2010) and Guidone et al. (2013).

Extraction of DNA and PCR Amplification. In 2013 we sequenced ITS and tufA. However, in 2015, we chose not to sequence tufA because many studies have found that OTUs (operational taxonomic unit) are recovered in both markers (O’Kelly et al. 2010; Saunders and Kucera 2010; Guidone et al. 2013). DNA was extracted from organisms rinsed with sterile deionized H₂O and cut with a sterile exact-o-knife to an approximate size of 4 cm². Using a BigDye 3.0 Kit (PE Applied Biosystems Inc.), we amplified the tufA region using primers tufGF4 (5’ GGNGCNGCNCAAATGGAYGG 3’) and tufAR (5’CCTTNCNGAATMGCRRAWCGC 3’) (Fama et al. 2002; Saunders and Kucera 2010), and the ITS region using primers 1763-18S, 1505-18S combined with ENT26S (Hayden et al. 2003). Polymerase Chain Reaction (PCR) amplification was carried out by adding the following quantities in a 1 mL test tube: 2 µL of template (Ulva DNA); 5 µL of 10x buffer; 5 µL of 10 mM dNTPs; 2 µL of 10mM solution of tufGF4 and tufAR primers; 0.2 µL of Taq DNA polymerase; and sterile deionized H₂O add up to 50 µL (Kirkendale et al. 2013). Reactions were run according to the procedure in Kirkendale et al. (2013) and included 38 cycles in the thermo cycler, 4 min denaturation at 94°C, 1 min denaturation at 94°C, annealing for 30 sec at 45°C, and
extension for 1 min at 72°C, and final elongation for 7 min at 72°C (Saunders and Kucera 2010). The product of the PCR amplification was combined with ethylene bromide stain and electrophoresed on a 2% agarose gel. PCR products were cleaned using a PCR Purification Kit (Qiagen Inc., Hilden, Germany) and sequenced with primers on an Applied Biosystems 3130 XL automated sequencer (Saunders and Kucera 2010).

Our sequences were blasted in GenBank for comparative phylogenetic analysis of tufA and ITS data. Gene alignments were subjected to Muscle alignment and cut using Mega v.6 software. The phylogenetic trees were generated in R, a programming language and free software, using maximum likelihood (ML), nearest joining neighbor (NJ rooted) and maximum likelihood tree analysis using “ape”, “phangorn”, and “ggtree” packages (Paradis et al. 2004; Schliep 2011; Yu et al. 2017).

RESULTS

Molecular identification. One clade matched our 2013 Jamaica Bay and Long Island Sound samples and is identified with the following support: *Ulva compressa*, ML=100% (Figures 2.5 and 2.6). All 13 Jamaica Bay samples collected had <1% divergence between species and showed 100% ML similarity. Based on ML distances the genotypes match the foliose *Ulva compressa* in green tides from Rhode Island and worldwide. Our tufA phenology matches sequences from Australia, JN029296.1 (Kirkindale et al. 2013), Australia, KF195530.1 and KF195551.1 (Lawton et al. 2013), and New Brunswick Canada, HQ610284.1 (Saunders and Kucera 2010). The tufA nrDNA (ML) based on uncorrected p-distances is shown in Figure 2.5. One clade matched our Jamaica Bay and Long Island Sound samples and is identified as *Ulva compressa* (ML = 100%). All 13 Jamaica Bay samples collected in 2013 had <1% divergence (p
distance = 0.0025) and showed 100% ML similarity. The ITS ML similarity <1% divergence (p
distance = 0.005) occurred between our samples and those of Rhode Island USA, KC582333.1
(Guidone et al. 2013), China, HM584736 (Duan et al. 2012), and Great Britain, AF013982 (Tan
et al. 1999). The ITS nrDNA (ML) based on uncorrected p-distances is shown in Figure 2.6.
Figure 2.5 *Ulva* maximum likelihood of the *tuf*A marker based on uncorrected p-distances for comparison between sequences and *Ulva* spp. from Jamaica Bay, shown in percent. All Jamaica Bay sequences are labeled above as NY USA.
Figure 2.6 *Ulva* maximum likelihood tree of the ITS marker based on uncorrected p-distances for comparison between sequences and *Ulva* sp. from Jamaica Bay, shown in percent. Jamaica Bay and Long Island Sound sequences are NY USA and CT USA. 2015 sequences are labeled with the date and number.
For specimens collected in 2015, multiple *Ulva* clades matched our Jamaica Bay ITS nrDNA ML based on uncorrected p-distances. In April of 2015, 20150411MP *Ulva* 02, 20150411MP *Ulva* 03, 20150411MP *Ulva* 04, and 20150411MP *Ulva* 05 matched sequences from AJ234305 (Tan et al. 1999) and KC582305 (Guidone et al. 2013) *Ulva prolifera* Müller (1778) (Figure 2.6; Table 2.3). The specimens had <1% divergence between species and showed 100% ML similarity. Specimens 20150411MP *Ulva* 06 matched sequences from KC582316.1 (Guidone et al. 2013) *Ulva stipitata* Areschoug (1850). This sequence had <1% divergence between species and showed 100% ML similarity. In May of 2015, 20150504MB *Ulva* 04 and 20150504MB *Ulva* 06 matched sequences from AB830485 (Ogawa et al. 2013) *Ulva prolifera*. These specimens had <1% divergence between species and showed 100% ML similarity. 20150504MB *Ulva* 05 matched the *Ulva prolifera* sequences with a 51% ML similarity.

20150504MB *Ulva* 03 matched sequences from KC582333 (Guidone et al. 2013), HM584736 (Duan et al. 2012), and AF013982 (Tan et al. 1999) *Ulva compressa* (Figure 2.6; Table 2.3). This sequence had a <1% divergence between species and showed 100% ML similarity. In late May, 20150529 *Ulva* 01 matched sequences from EU933970.1 (Kraft 2008), KC582319 (Guidone et al. 2013), KF683443 (Wallace and Gobler 2015), and AY260565 (Hayden et al. 2003) *Ulva laetevirens* and *Ulva rigida*. These sequences had <1% divergence between species and showed 100% ML similarity. For 20150529 *Ulva* 04 and 20150529 *Ulva* 05 they matched sequences from KC582316.1 (Guidone et al. 2013) *Ulva cf. stipitata* (Figure 2.6; Table 2.3). These sequences had <1% divergence between species and showed 100% ML similarity. 20150529 *Ulva* 06 matched sequences from KC582303 (Guidone et al. 2013) *Ulva cf. clathratiodies*. This sequence had a <1% divergence between species and showed 100% ML similarity. 20150529 *Ulva* 02 matched sequences from KC582323 (Guidone et al. 2013) and AB097651 (Shimada et
Ulva lactuca. These sequences had <1% divergence between species and showed 100% ML similarity. In June, when Ulva bloom was at its peak, 06/01/2015, 20150601MB Ulva 03 and 20150601MB Ulva 04 matched sequences from KC582316.1 (Guidone et al. 2013) Ulva cf. stipitata (Figure 2.6; Table 2.3). These sequences had <1% divergence between species and showed 100% ML similarity. For 06/17/2015 20150617MP Ulva 02 and 20150617MP Ulva 03 matched sequences from KC582316.1 (Guidone et al. 2013) Ulva stipitata (Figure 2.6; Table 2.3). These sequences had <1% divergence between species and showed 100% ML similarity. 20150617MP Ulva 05 and 20150617 Ulva 06 matched sequences from HM584736 (Duan et al. 2012), AF013982 (Tan et al. 1999), KC582333 (Guidone et al. 2013) Ulva compressa. These sequences had <1% divergence between species and showed 100% ML similarity.

Table 2.2 Collection sites and distribution of Ulva sp. blades and tubes within Jamaica Bay, New York. Taxa abbreviation: UC = Ulva compressa, UP = Ulva prolifera, US = Ulva stipitata, ULA = Ulva laetevirens, UL = Ulva lactuca, UCL = Ulva clathratioides.

<table>
<thead>
<tr>
<th>Site Abbreviation</th>
<th>Locality</th>
<th>Date Collected</th>
<th>Voucher</th>
<th>Habitat Description</th>
<th>Location</th>
<th>ITS accession</th>
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<td>NYBG2335103</td>
<td>Sheltered tidal channel in salt marsh pool. The specimen was attached to Spartina stems and intermixed with Gracilaria vermiculophylla.</td>
<td>18T 590639.86 m E 4494994.9 3 m N</td>
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Morphological and cytological features. According to cytological features all 13 samples collected in 2013 in Jamaica Bay and 4 samples collected in Long Island Sound, CT were identified as *Ulva compressa*. Figure 3 shows the morphological diversity of the distromatic blades in our *Ulva* samples. All samples found were distromatic blades either round (Figure 2.3B, both) or thin (Figure 2.3A, far right). Blades found at the end of summer had perforations probably due to low oxygen and high sulfide concentrations that are found in *Ulva* beds after the summer bloom or detachment of tissue or substrate. Another possibility is that amphipod and snail grazing may have contributed to the perforations. The thalli in our samples did not have perforations during early growth periods. Thin blades, 5 mm in length, were found at unprotected habitats, Bayswater Park (BP) and Plum Beach (PB) that had higher water flushing rates. Reproductive material or males of *Ulva compressa* were noted as clear or light tan fringe tissue. The cell structure of all samples collected from Jamaica Bay were similar to Hofmann et al. (2010) cell observations from New Hampshire and Maine USA (Figure 2.7 B-E).
The cells of *Ulva compressa* samples were 10 µm in size, angular to round in shape, had a hooded chloroplast in half of the cells and were distributed on the same side in most cells, while the other half was distributed throughout the entire cell (Figure 2.7A and E). Usually the cell had one large pyrenoid and a cell size of 10µm x 8µm (Figure 2.7A).
Figure 2.7 Cytological observation of Jamaica Bay *Ulva compressa* compared to *Ulva* spp. identified by Hofmann et al. (2010). (A) *Ulva compressa* from Jamaica Bay; (B) *Ulva lactuca* (C) *Ulva laetevirens*; (D) *Ulva pertusa*; (E) *Ulva compressa*. All scale bars are 20 µm.
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**Macroalgae observed.** *Ulva* spp. was observed at both field sites, Marine Park and Norton Basin during March-December of 2015 (Table 2.2). *Gracilaria vermiculophylla* (Ohmi) Papenfuss (1967) was also present at both field sites every month except during April at Norton Basin. The latter site had greater species diversity with occurrences of *Fucus vesiculosus* Linnaeus (1753), *Pyropia* spp. *Agardhiella subulata* (Agardh) Kraft, and M.J. Wynne (1979), *Chondrus crispus* Stackhouse (1797), *Chorda filum* Stackhouse (1797), and *Dasya baillouviana* Montagne (1841). Marine Park had occurrences of *F. vesiculosus*, *A. subulata*, *C. crispus*, and
Spermothamnion repens Magnus (1873). The only occurrence of bloom-forming macroalgae was bladed Ulva spp.

DISCUSSION

Ulva compressa was the only species identified during 2013, regardless of whether we used ITS or the tufA genetic markers. This validates our choice not to use a second genetic marker, tufA, for analysis in 2015. Most striking is that there was no difference in diversity amongst the Ulva samples we collected in 2013. However, in 2011, Wallace and Gobler (2015) sequenced Ulva from central sites in Jamaica Bay and all of their specimens matched an U. rigida and an U. laetevirens, accession number KC582319 (Figure 2.6). Ulva laetevirens was not identified in their study, however we believe that it could be the species they found was not U. rigida. Both Kraft et al. (2008) and Mao et al. (2014), conducted a detailed cytological and phylogenetic study of Ulva laetevirens. The differences between our identification and those of Wallace and Gobler (2015) may be due to different sampling methods and/or different sampling sites or that the Ulva was actually different in 2013 than 2011. Wallace and Gobler (2015) sampled exclusively in the subtidal zone from a ship using a non-selective grab sampler. In contrast, we conducted our study exclusively in the intertidal zone.

In 2015, we identified five different Ulva species using the ITS genetic marker, four of the species, Ulva clathratioides, U. compressa, U. laetevirens, U. prolifera, and U. stipitata, have never been identified in Jamaica Bay prior to this work. Using a genotype-matching approach adopted in Hoffman et al. (2010) we found specimens with genotypes that were identical to green tides reported elsewhere around the world. For example, those specimens found in Fukui, Japan bloom (Ogawa et al. 2013) matched our specimens collected on
05/04/2015 at Norton Basin. Our other specimens (05/04/2015 and 06/17/2015) exhibited sequences that matched the *Ulva linza-procera-prolifera* clade from Qingdao, China, the world’s most famous *Ulva* bloom (Duan et al. 2012). We did not find any tubular *Ulva* morphologies at any of our sites during the 2013. The 2013 bloom in Jamaica Bay consisted of blades, unlike those in other areas of the world such as China, Japan, Australia, and Northeast USA where both blades and tubes occurred together.

Ecologists, biologists, and resource managers can be certain that the *Ulva* blooms in Jamaica Bay are a combination of *U. compressa*, *U. rigida*, and *U. laetevirens*. Previous studies in the Bay have identified *Ulva* as the common “sea lettuce,” *U. lactuca* (Franz and Friedman 2002; MacKenzie Jr. 2005; Potts et al. 2012). Based on our study in Jamaica Bay and LIS, the diversity of the 2013 bloom forming *Ulva* was limited to *U. compressa*. A study completed by Guidone et al. (2013) suggests that *U. compressa* is not new to Narragansett Bay, RI because it’s often misidentified as *U. lactuca*. We think this misidentification stretches beyond Narragansett Bay up and down the east coast of USA.

Currently in Jamaica Bay and the northeast region of the USA we see *Ulva compressa* as foliose (Hofmann et al. 2010). The type morphology for *Ulva compressa* was first identified by Linnaeus (1753:1163) and was described as, “tubulosa ramosa” or containing branched tubes. In 1850, Durant completed a macroalgae survey of New York Harbor and found (*U. ramulosa=**U. clathrata*), *U. linza*, and *U. compressa* tubes in Jamaica Bay (Figures 2.8 and 2.9). Durant described the morphology and location of *Ulva compressa* as, “elongated, branched, subcompressed, and gradually enlarged to very obtuse apices. *Ulva compressa* occurred during the summer below low water, being mostly parasitical, and not frequent”. We know that *U.*
compressa can occur in both foliose or tubular form; hence the fact that Durant did not find foliose U. compressa is predictable because the type herbarium specimen is tubular.

The foliose species that Durant found are Ulva latissima (=Saccharina latissima) and U. lactuca (Figure 2.8 and 2.9). Ulva latissima (=Saccharina latissima) was characterized as, “Broadly ovate, flat.” and occurring, “May to February, very abundant, on rocks, stones, shells, and parasitical; 3 inches to 5 feet long; the largest specimens grow on flats at Gowanus and Communipaw: this plant bleaches in decay to a cream color or white, and some specimens are exceedingly beautiful in that state.” It appears that the bloom forming species in 1850 was Ulva latissima and U. lactuca was found only during the summer attached to Fucus or other algae and not in high abundance. Ulva latissima bloomed at the Gowanus Canal, Brooklyn, NY where, in 1850, human influences were high and nitrogen loading was probably occurring. The reason we are now identifying Ulva compressa as a foliose plant is because of molecular methodologies.

In conclusion, we provide the first comprehensive molecular characterization of Jamaica Bay Ulva sp. using the molecular marker ITS and tufA. We add to the macroalgae assemblage that was performed by Durant in 1850. Thanks to DNA Barcoding we know the species composition of Jamaica Bay better but there are still unanswered questions. For example, has the species composition in Jamaica Bay changed since 1850? One way to answer this question is to verify differences by barcoding in Durant’s (1850) herbarium specimens.
**Figure 2.8** *Ulva compressa*. L. Durant 1850. No. 221. Photos taken from the Durant (1850) collection owned by the New York Public Library.
Figure 2.9 *Ulva latissima*. L. Durant 1850. No. 230. Photos taken from the Durant (1850) collection owned by the New York Public Library.
Since we know the species of *Ulva* in Jamaica Bay, modeling and predictions about nitrogen reduction impacts can be assessed. Reducing nitrogen loading and eutrophication requires significant investment in infrastructure in the catchment area and can take years to take effect (Smetacek and Zingone 2013). There are many cases world-wide in which eutrophication has been reduced but ulvoid “green tides” still exist (Sfriso and Marcomini 1996; Yabe et al. 2009; Facca et al. 2011). Jamaica Bay’s load reduction program that aims to reduce nitrogen loads by 50% by 2020 may reduce eutrophication but take many years to see positive effects (NYCDEP 2010). Nitrogen can be stored in various pools for periods of time in an estuary (Herbert 1999). *Ulva* could uptake nitrogen from these pools and bloom after the water column load has been reduced.

**Acknowledgements**

We would like to thank Dr. Brian Wysor for processing samples and molecular advice. We wish to thank the Aquatic Research and Environmental Assessment Center (AREAC) for lab space. We thank Dr. Juergen Polle and Dr. Zaid McKie-Krisberg at Brooklyn College for phylogenetic tree advice. Dr. Ken Karol at NYBG is also acknowledged for helping the first author to organize various herbarium specimens. Thanks to Dr. Simona Augyte for reviewing the early manuscript and offering her advice. Thank you to the two reviewers that took their time to review and comment on this manuscript. This research was conducted under permits distributed by the NYC Parks Dept. and Gateway National Recreation Area – NPS.
Chapter 3

Seasonal *Ulva* spp. bloom and nitrogen dynamics in a eutrophic urban estuary, Jamaica Bay, NY USA

Annesia L. Lamb

This chapter is in preparation for submission to *Estuaries and Coasts*

**ABSTRACT**

*Ulva* spp. are dominant bloom-forming macroalgae that can cover the benthos and negatively affect benthic marine organisms. This study aimed to understand the seasonal and decadal bloom dynamics of *Ulva* spp. by quantifying: 1) standing biomass of macroalgae including *Ulva* and *Gracilaria vermiculophylla* across 12 months in 2015 and relating to dissolved inorganic nitrogen (DIN) in the macroalgal beds as well as tissue nitrogen content in *Ulva*; and 2) standing *Ulva* biomass in 1995/1996 and 2012, in Jamaica Bay, New York. *Ulva* standing biomass was collected at four locations during the growing seasons in 2012 and 2015. Peak biomass occurred in June/July and ranged from 138 to 232 g dry wt m\(^{-2}\). Blooms persisted through October. The average nitrogen tissue content of *Ulva* ranged between 1.96 to 5.14%. The highest percent nitrogen concentrations were observed in August and September and the lowest was observed in June. Ammonium was the abundant nutrient in the water column during the 2012 and 2015 season. In 2015, nitrate was low in the water column during the summer, when macroalgae blooms were at highest biomass. *Ulva* biomass in 2012 was 94% lower than the 1995/1996 biomass levels. The decrease in *Ulva* biomass over the 20-year period may be related to significant wastewater treatment nitrogen load reductions and associated decreases in water column DIN. The study results show that *Ulva* biomass is related to interactions of
temperature, light, and DIN availability on a seasonal basis and that long-term changes in
nitrogen inputs may be reducing *Ulva* biomass on a decadal scale. Collectively this study can aid
in policy decisions regarding nuisance macroalgal blooms including long-term reductions in
nitrogen inputs and a seasonal schedule for harvest of *Ulva* to maximize nitrogen removal.

Keywords: Macroalgae, dissolved inorganic nitrogen, *Ulva* spp., nitrogen,

**INTRODUCTION**

In temperate estuaries, the introduction of dissolved inorganic nitrogen (DIN) from point
and non-point sources contributes to eutrophication (Conley et al. 2000; Tedesco et al. 2014;
Rose et al. 2015). Primary producers, such as macroalgae, tend to respond to increased DIN by
assimilating it at high rates and blooming when temperatures and light becomes favorable
(Fletcher 1996; Valiela et al. 1997; Raffaelli et al. 1998; Neori et al. 2004; Morand and Merceron
2005; He et al. 2008; Abreu et al. 2011a, 2011b). In turn, macroalgae will form mats that blanket
the water column, preventing oxygen exchange and limiting light (Nelson et al. 2008). Shellfish,
fish, seagrasses, and salt marsh grass can become oxygen deprived due to excessive macroalgal
respiration (Nelson et al. 2008).

Jamaica Bay (New York, USA) is an urban estuary that has hyper-eutrophic conditions as
indicated by chlorophyll-*a* concentrations > 60 µg L⁻¹, during the winter-spring and summer
phytoplankton blooms from 2009-2013 (NYCDEP New York Harbor Survey 1996; Bricker et al.
1999; Scavia and Bricker 2006; Hoellein and Zarnoch 2014). The eutrophication in Jamaica Bay
is largely due to effluent coming from four wastewater treatment plant outfalls (WWTP; Benotti
et al. 2007) that provides ~90% of the freshwater entering the estuary. Stable isotopes studies
indicate that nitrogen found in primary producers comes from human-derived sources (Wallace and Gobler 2015; Watson et al. 2018).

*Ulva* spp. (Chlorophyta, Ulvophyceae) are opportunistic macroalgae that can form blooms in temperate estuaries (Thomsen et al. 2006; Saunders 2009; Thomsen et al. 2009; Guidone and Thornber 2013; Nettleton et al. 2013). When these algae are in excess, they may affect other primary producers, biogeochemical cycling, trophic interactions, and other environmental conditions (Cuomo et al. 1997; Thomsen et al. 2009; Zertuche-González et al. 2009). Wallace and Gobler (2015) surveyed subtidal *Ulva* and found >90% bottom coverage in Jamaica Bay. They did not however quantify *Ulva* biomass and therefore current system-wide standing biomass is unknown in Jamaica Bay.

*Ulva* can grow using nitrate and/or ammonium (Cohen and Neori 1991). Ammonium can be utilized by *Ulva* especially when nitrate is low in heterotrophic systems, like Jamaica Bay, which may be very important in this system where ammonium tends to be found in higher concentrations than nitrate (NYCDEP Harbor Survey 2016). Thus, it is possible that ammonium is the important driver of *Ulva* biomass in systems with high wastewater nitrogen.

Nitrogen storage in *Ulva* tissue ranges between 2-5% in temperate estuaries (Fujita et al. 1989). The amount of nitrogen storage depends on *Ulva*’s ability to uptake nutrients. If nutrients are high in the system internal nitrogen storage will also be high. If nutrients are low or thalli are focused on reproduction, nitrogen storage will be low. The hyperbolic relationship between cell nutrient content and growth rate has been defined in microalgae (Droop 1974). Critical tissue levels occur when thalli are limited in terms of maximum growth rate (Hanisak 1979). Fujita et al. (1989) reported that *Ulva rigida* exhibited critical tissue levels at 3% nitrogen when grown on ammonium alone and 2.4% when grown on nitrate alone.
Drivers of seasonal biomass of *Ulva* need to be understood to incorporate *Ulva* and other macroalgae in shallow, photic ecosystem models and for coastal management (Brush and Nixon 2010). Furthermore, verification of macroalgae biomass is necessary for understanding the role of nuisance macroalgae within ecosystem models. Recent studies have shown that macroalgal blooms may contribute to coastal acidification in eutrophic ecosystems (Wallace and Gobler 2014; Breitburg et al. 2015) but greater understanding of spatial and temporal bloom dynamics is needed to inform modelling efforts. There is also a need to account for macroalgae in long-term management plans for estuaries (McGlathery et al. 2007).

The objectives of this study were to: (1) determine the seasonal *in situ* biomass pattern of *Ulva*; (2) identify factors driving changes in seasonal biomass; (3) identify seasonal changes in *Ulva* nitrogen storage; and (4) identify long-term trends in *Ulva* biomass by comparing 1995/1996 biomass and nutrient data with data collected in this study.

**MATERIALS AND METHODS**

*Biomass Collection.* *Ulva* standing biomass was collected in 2012 and 2015. *Gracilaria vermiculophylla* standing biomass was also collected in 2015 (Figure 3.1). A two-month *Ulva* biomass survey was performed at Ruffle Bar and Big Egg in June and September 2012. These sites are islands in the center of Jamaica Bay (Figure 3.2). A twelve-month macroalgal biomass survey was conducted at Marine Park and Norton Basin, with monthly sampling beginning in January and ending in December 2015. These sites were chosen based on their proximity to Rockaway Inlet (Marine Park) and wastewater treatment outfalls (Norton Basin) (Figure 3.2). There is a gradient of nitrogen concentrations in Jamaica Bay going from low near the inlet to high in the eastern portion of the Bay (Benotti et al. 2007; Hoellein and
Zarnoch 2014). The study sites were selected to capture this gradient. The Marine Park site (40°36′5.553″W, 73°55′50.334″N) is located near Rockaway Inlet and the entrance to Jamaica Bay and was expected to have low water column nitrogen. The Norton Basin site (40°36′37.015″W, 73°46′12.938″N) is located at the eastern end of the bay and is adjacent to a combined sewer outfall (Hoellein and Zarnoch 2014). It was expected to have higher water column nitrogen concentrations.
Figure 3.1 Clockwise: Norton Basin field site in July 2013. Assemblage of *Gracilaria vermiculophylla* and *Ulva* spp. at Marine Park field site in July 2015, students collecting the *Ulva* spp. bloom at Norton Basin field site in July 2015, *Gracilaria vermiculophylla* herbarium collected at Norton Basin.
Figure 3.2 Map of Jamaica Bay, New York sampling sites (black dots) and wastewater treatment plant outfalls (grey dots).
In each study the macroalgal biomass was collected along two 70 m transects, which were 30 m apart. The transects were perpendicular to the shoreline from the low water mark to the high-water mark, thus capturing the entire intertidal zone. Samples were collected every 10 m along the transect for a total of 16 samples per site. The transect locations were fixed so sampling location was consistent for each monthly sampling. *Ulva* biomass was collected within a 0.25 m² quadrat. The biomass samples were transported to the laboratory on ice, rinsed with tap water, excess water removed with a salad spinner (Oxo, Chambersburg, PA) then dried to a constant weight at 60°C to determine final dry weight.

*Ulva* biomass was collected by EEA Inc. in 1995/1996, from Aug-Oct in 1995 and May-July in 1996, using a 0.1 m² quadrat at Ruffle Bar and Little Egg Marsh (EEA, Inc. 1996). Little Egg marsh is adjacent to Big Egg marsh which was sampled in 2012 for this study. A 91 m transect was established parallel to the shoreline at each location and biomass was collected every 9 m. The biomass samples were transported to the laboratory in plastic bags where the wet and dry weights were recorded.

**Tissue Nutrients.** Dried *Ulva* tissue samples were ground with a 0.35 L Magic Bullet homogenizer (Homeland Housewares, LLC, Los Angeles, CA), and analyzed on a 2400 CHS/O Series II Perkin Elmer (Shelton, CT) at Baruch College (Hauxwell et al. 1998; Kim et al. 2014) to determine tissue C and N. C:N ratios were determined by the mass ratio of % carbon and % nitrogen.

**2015 Water Quality.** Temperature and salinity data for Bergen Basin (adjacent to Norton Basin)) were obtained from 2015 published data by NYCDEP (NYCDEP Harbor Survey
Ammonium, nitrate, TKN, TKP, and orthophosphate were collected in the water column at both 2015 field sites. Five replicate water samples were collected at 1 m depth during each month, filtered with a 0.7 µm pore diameter filter, transported to the laboratory on ice, and run on an AQ2 discrete nutrient analyzer (Seal Analytical Inc., Mequon WI) using colorimetric methods (Strickland and Parsons 1972). Ammonium was determined using the phenol-hypochlorite methods (EPA-103-A). Nitrate+nitrite was found using the reduction of nitrate to nitrite using copperized cadmium and sulfanilamide methods (EPA-127-A). Orthophosphate was measured using the ammonium molybdate-antimony potassium tartrate approach (EPA-118-A). Total Kjeldahl-nitrogen was determined by the digestion in sulfuric acid solution and alkaline salicylate-hypochlorite methods (EPA-135-A). Finally, sulfuric acid solution digestion and ammonium molybdate-antimony potassium tartrate methods were used for total Kjeldahl-phosphorous (TKP; EPA-111-A). Water samples for DIN in 1995 were collected at 1 m depth by boat and analyzed according to the EPA water quality standards mentioned above. Dissolved organic nitrogen (DON) was calculated by: $TKN - DIN$. Organic phosphorus was calculated by: $TKP - OP$.

**Statistical Analysis.** A multiple linear regression and a two-factor ANOVA were performed on the Ulva biomass data from each month and site (month-biomass*site) to determine if there were differences in biomass between sites. Changes in Ulva dry weight collected at Marine Park and Norton Basin were assessed by a multiple linear regression and a one-factor ANOVA (month-biomass). A paired one tailed student’s t-test was used to determine differences among 2012 and 1995/1996 on Ulva biomass. A one-factor ANOVA (month-nutrient concentration) determined differences in nutrients over the 2015 season. Statistical tests were
performed in R software with lmtest and fBasics packages (Zeileis and Hothorn 2002; Wuertz et al. 2017; R Core Team 2018).

RESULTS

Macroalgae Biomass. The dominant macroalgae at the two sites in Jamaica Bay, New York surveyed in 2015 and 2012 was *Ulva* spp. (Figure 3.3). In 2015, the *Ulva* biomass was greater at Norton Basin than at Marine Park (Figure 3.3). *Ulva* biomass at Norton Basin began to bloom during May and lasted through September with the average peak biomass in June at 231.65 g dry wt m$^{-2}$ (Figure 3.3). The biomass differed significantly from May through the summer months and then stayed constant from October to December. At Marine Park *Ulva* biomass began to bloom in May and lasted through October with the average peak biomass at 138.31 g dry wt m$^{-2}$ (Figure 3.3). The biomass was constant with the exception of the peak in July and diminished during December. The *Ulva* biomass at Norton Basin was greater than the biomass at Marine Park during the summer months (Figure 3.3).

*Gracilaria vermiculophylla* biomass was significantly lower than *Ulva* biomass during the 2015 season. *Gracilaria* had greater biomass at Marine Park than Norton Basin throughout the season. Peak biomass was in August at 15.53 g dry wt m$^{-2}$ (Figure 3.4). At Norton Basin, the *Gracilaria* biomass was at or close to zero during the entire season.
Figure 3.3 Standing 2015 seasonal biomass of *Ulva* spp. at field sites. Marine Park is represented with black circles and solid line. Norton Basin is represented with black triangles and dashed line. Error bars are standard error. * = p value < 0.05.
**Figure 3.4** Standing 2015 seasonal biomass of *Gracilaria vermiculophyla* at field sites. Marine Park is represented with black circles and solid line. Norton Basin is represented with black triangles and dashed line. Error bars are standard error. * = p value < 0.05.
Tissue Nutrients. The nitrogen tissue contents of *Ulva* from May-Sept ranged between 1.96 and 5.14 % (Figure 3.5). At Norton Basin the percent nitrogen was lower in June compared to Marine Park (Figure 3.5). The C:N ratio of *Ulva* ranged between 5 and 17 from May-Sept (Figure 3.6). The C:N ratio was higher at Norton Basin during June and July due to a reduction in percent nitrogen (Figure 3.6).

2015 Water Quality. Salinity ranged from 21-27 with the lowest recording in September. The temperature ranged from 2-28 °C with the highest values in July and August (Figure 3.7). The DIN concentration at Norton Basin in the *Ulva* bed showed a peak at 1.4 mg N L⁻¹ in June. The DIN decreased in July when the *Ulva* biomass was highest (Figure 3.8). The ammonium concentration at Norton Basin in the *Ulva* bed showed a peak at 1.3 mg N L⁻¹ in June (Figure A1). Ammonium decreased to 0.4 mg N L⁻¹ in July, then increased in August and again in September to 1.0 mg N L⁻¹. Ammonium concentration at Marine Park was 0.7 mg N L⁻¹ in April and increased until June when it was 1.0 mg N L⁻¹. The nitrate plus nitrite concentrations at Norton Basin and Marine Park in the *Ulva* bed was 0.1-0.13 mg N L⁻¹ respectively in April (Figure A2). Nitrate plus nitrite decreased 50% in May at Marine Park then was below detection in August. In September, the concentration went up to 0.05-0.11 mg N L⁻¹. The orthophosphate at Norton Basin was 0.06 mg P L⁻¹ in April then began to increase over the season until August at 0.23 mg P L⁻¹ (Figure 3.9). The orthophosphate in the *Ulva* bed at Marine Park was lower than Norton Basin in August and similarly increased over the season. The average TKN ranged between 1.24 and 2.44 mg N L⁻¹ at Marine Park (Table 3.1). The highest value for TKN was in April and the lowest in June. The average TKN at Norton Basin ranged between 1.39 and 2.31 mg N L⁻¹. The highest value for TKN was in August and the lowest was in May. TKN for June...
was not measured. DON at Marine Park ranged from 0.17 to 1.98 mg N L$^{-1}$ with the highest value in June and the lowest value in July. DON at Norton Basin ranged between 0.25 and 1.86 mg N L$^{-1}$. The highest value was in July and the lowest value was in May. The average TKP ranged between 0.21 and 1.1 mg P L$^{-1}$ at Marine Park (Table 3.1). The highest value for TKP was in May and the lowest value was in July-August. Organic P values were found to range between 0.14 and 1.05 mg P L$^{-1}$. The highest value was in May and the lowest value was in August. Organic P values for Norton Basin were found to range between 0.1 and 0.68 mg P L$^{-1}$. Similarly, the highest value was in May and the lowest in August.
Figure 3.5 Comparison of 2015 percent nitrogen concentration in *Ulva* spp. at Norton Basin and Marine Park. Norton Basin is represented with black triangles. Marine Park represented with black circles. Error bars are standard error. * p value < 0.005.
Figure 3.6 Comparison of 2015 C:N values in *Ulva* spp. at Norton Basin and Marine Park. Norton Basin is represented with black triangles. Marine Park is represented with black circles. Error bars are standard error.
Figure 3.7 2015 water salinity and temperature at Bergen Basin. Temperature is represented with dashed lines. Salinity is represented with solid lines. NYCDEP (2016) Harbor Survey Report.
Figure 3.8 Comparison of sites in water column DIN within the *Ulva* spp. bed in 2015. Marine Park is represented with circles and Norton Basin is represented with triangles. Error bars are standard error.
Figure 3.9 Comparison of sites in water column orthophosphate within the Ulva spp. bed in 2015. Marine Park is represented with black circles and Norton Basin is represented with black triangles. Error bars are standard error. * p value < 0.05.
Table 3.1 Mean physiochemical measurements for sampling sites in the water column Ulva bed during the summer (Apr.-Aug.) 2015. Units are in mg N and P L⁻¹. A notation of nm means parameter was not measured.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Norton Basin</th>
<th>Marine Park</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
<td>May</td>
</tr>
<tr>
<td>NOₓ</td>
<td>0.102±</td>
<td>0.099±</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.020</td>
<td>0.011</td>
</tr>
<tr>
<td>OP</td>
<td>0.058</td>
<td>0.558</td>
</tr>
<tr>
<td>DIN</td>
<td>0.852</td>
<td>1.133</td>
</tr>
<tr>
<td>TKN</td>
<td>2.263±</td>
<td>1.386±</td>
</tr>
<tr>
<td>DON</td>
<td>1.512</td>
<td>0.253</td>
</tr>
<tr>
<td>TKP</td>
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<td>0.770±</td>
</tr>
<tr>
<td>OrgP</td>
<td>0.437</td>
<td>0.675</td>
</tr>
</tbody>
</table>

Comparison of 1995/1996 to 2012 Ulva spp. Biomass. Standing Ulva biomass in 1995/1996 was found to be significantly higher than the biomass of Ulva in 2012 at all sites compared (Figure 3.10). This includes a comparison of Big Egg to Little Egg in June and September and a comparison of Ruffle Bar in September of each year. Bay wide means for temperature and salinity were similar in 1995 and 2012. Similarly, Bay wide means for DIN, orthophosphate, ammonium, nitrate+nitrite in 1995 were not significantly different from 2012 (Table 3.2) despite reductions in total nitrogen loadings from wastewater treatment plants over this time period.
**Figure 3.10** 2012 survey of *Ulva* spp. biomass compared to 1995/1996 *Ulva* spp. biomass surveys. 1995/1996 biomass is represented with black bars. 2012 survey represented with hatched bars. EEA, Inc. (1996). Error bars are standard error. * p value < 0.05.
Table 3.2 Mean baywide nitrate+nitrite and ammonium measurements for years 2012 and 1995 in the water column (Mar.-Dec.) 2015. Units are in mg N L\(^{-1}\). A notation of nm means parameter was not measured.

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mar</td>
<td>Apr</td>
<td>May</td>
<td>Jun</td>
<td>Jul</td>
<td>Aug</td>
<td>Sep</td>
<td>Oct</td>
<td>Nov</td>
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<td>May</td>
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<td>Aug</td>
</tr>
<tr>
<td>NO(_x)</td>
<td>0.179±</td>
<td>0.106±</td>
<td>0.180±</td>
<td>0.160±</td>
<td>0.097±</td>
<td>0.109±</td>
<td>0.211±</td>
<td>0.147±</td>
<td>0.315±</td>
<td>0.396±</td>
<td>0.250±</td>
<td>0.152±</td>
<td>0.262±</td>
<td>0.093±</td>
<td>0.198±</td>
<td>0.160±</td>
</tr>
<tr>
<td>NH(_4)†</td>
<td>0.075</td>
<td>0.027</td>
<td>0.025</td>
<td>0.029</td>
<td>0.020</td>
<td>0.023</td>
<td>0.026</td>
<td>0.020</td>
<td>0.042</td>
<td>0.039</td>
<td>0.241±</td>
<td>0.307±</td>
<td>0.464±</td>
<td>0.343±</td>
<td>0.232±</td>
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<tr>
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<td>0.151</td>
<td>0.207</td>
<td>0.112</td>
<td>0.156</td>
<td>0.080</td>
<td>0.152</td>
<td>0.133</td>
<td>0.056</td>
<td>0.155</td>
<td>0.113</td>
<td>0.151</td>
<td>0.083</td>
<td>0.191</td>
<td>0.122</td>
<td>0.067</td>
<td>0.164</td>
</tr>
</tbody>
</table>

**DISCUSSION**

*Ulva* biomass in 2015 followed a distinct seasonal pattern. Biomass was low in January through April, increased in May, peaked in June and July, and then declined through late summer and fall at our study sites (Figure 3.3). This pattern is likely due to variation in temperature and DIN. Optimal growth temperatures for *Ulva* are between 20-23 °C although *Ulva* have different thermal tolerances (Nordby and Hoxmark 1972; Yokohama 1973; Rosenberg and Ramus 1981; Wang et al. 2007). Rhyne (1973) found that a two temperature stimuli were required for induction of reproductive activity of *Ulva curvata* and *Ulva rotundata*. The first stimulus temperature was 7-9 °C and the second was 17-20 °C. Rhyne (1973) also found that *Ulva* growing on oyster shells grew the best at temperatures between 20-30 °C. Thus, the optimal temperature for *Ulva* growth in Jamaica Bay occurred from mid-May through mid-September (Figure 3.7).

DIN and daylength are thought to be correlated with macroalgal biomass in estuarine systems (Duarte 1995; Valiela et al. 1997). Nelson et al. (2003) identified daylength, temperature, and DIN as having an effect on ulvoid growth in the coastal waters of Washington State, USA. In this study, irradiance appears to be an important factor driving *Ulva* biomass
(Table 3.1). The peak *Ulva* biomass coincides with the greatest irradiance in Brooklyn, NY. Pederson and Borum (1996) showed that DIN was the main limiting nutrient in *Ulva lactuca* in lab and field studies in Denmark. In Jamaica Bay, patterns of ammonium ranged from 0.3-1.3 mg N L\(^{-1}\) and the nitrate ranged from <0.01-0.13 mg N L\(^{-1}\). Ammonium never reached zero during the season but decreased during July, suggesting that ammonium was taken up by *Ulva*. Nitrate reached zero during the season in August and below 0.02 in July. Nelson et al. (2003) found DIN to be negatively correlated with ulvoid biomass in Washington State, USA. Although we did not find this relationship at our sampling sites with monthly surveys, we did note that peak biomass coincided with lowest observed DIN values. Peak *Ulva* biomass also overlaps with the summer phytoplankton bloom in Jamaica Bay (Hoellein and Zarnoch 2014; Wallace and Gobler 2015), which will contribute to the lower observed DIN. Collectively, this suggests that water column DIN may be available in excess of *Ulva*’s physiologically needs throughout the growing season with the exception of months during peak biomass.

Tissue nitrogen was depleted in June and July indicating that reserves of nitrogen were low (Figure 3.5) when biomass was greatest (Carpenter and Capone 1983; Fujita et al. 1989; Pederson and Borum 1997; Hurd et al. 2014). Lavery and McComb (1991) found that percent nitrogen < 2% is indicative of nitrogen limitation in *Ulva rigida*. The results indicate that *Ulva* did not experience %N at (or lower) than that level. Thus, *Ulva* may have experienced reduced tissue N content when biomass was highest, competition was greatest, and lowest observed DIN levels but had access to enough nitrogen to avoid being limited.

How much nitrogen is enough for *Ulva* to grow optimally? Fan et al. (2014) showed *Ulva prolifera* to have uptake rates of 9.1 mg N g\(^{-1}\) DW\(^{-1}\) d\(^{-1}\) when exposed to 2.74 mg N L\(^{-1}\) of nitrate. When that medium was reduced to 1.1 mg N L\(^{-1}\), the growth rate dropped significantly to 4.0 mg
N g$^{-1}$ DW$^{-1}$ d$^{-1}$. Pedersen and Borum (1996) identified that a culture of 5.6 mg N L$^{-1}$ was needed to support *Ulva lactuca* maximum growth at 7.4 mg N g$^{-1}$ DW$^{-1}$ d$^{-1}$. In Jamaica Bay the *Ulva* may not be at their optimal growth, since the maximum concentration of nitrogen found in the *Ulva* bed was 1.4 mg N L$^{-1}$ (Figure 3.8). However, due to *Ulva*’s ability to uptake nitrogen in the water column much of the nitrogen from the WWTPs may have been taken up before it was measured. *Ulva* may also uptake nitrogen from sediment organic matter remineralization, non-point fluxes such as atmospheric deposition, and groundwater discharge. The non-point fluxes and atmospheric deposition of nitrogen in Jamaica Bay are 3.67 mg N m$^{-2}$ day$^{-1}$ and 6.67 mg N m$^{-2}$ day$^{-1}$ respectively (Benotti et al. 2007). Cornwell (1999) found sediment DIN fluxes exceeding 168 mg N m$^{-2}$ day$^{-1}$ in Jamaica Bay, which could be a significant portion of nitrogen assimilated by *Ulva*. The amount of nitrogen from sediment DIN fluxes is 94%.

In June and July *Ulva* biomass was the higher at Norton Basin than the other sites. These site differences may be due to the residence times at each site and/or greater availability of nutrients. Norton Basin is located in the eastern portion of the Bay where residence time is between 10-22 days (Marsooli et al. 2018) while Marine Park is closer to Rockaway Inlet where residence time would be much shorter (Benotti et al. 2007). Greater nutrient availability in Jamaica Bay could also be due to the proximity of Norton Basin to WWTP outfalls and water column nitrogen tends to be higher in the eastern part of the Bay (Benotti et al. 2007). Lastly, the differences in *Ulva* biomass abundance between Marine Park and Norton Basin might be because the *Ulva* found at each site belongs to different species. Lamb et al. (2018) reported the bloom-forming *Ulva* species in Jamaica Bay are *compressa* and *laetevirens*. Growth rates among *Ulva* species are known to differ significantly (Fortes and Lüning 1980; Neori et al. 1991).
The large population density of Brooklyn and Queens results in DIN concentrations from WWTPs between 1-22 mg N L\(^{-1}\) and are most certainly the source of nutrients for macroalgae in the area (NYCDEP Harbor Survey 2012). Wallace and Gobler (2015) in Jamaica Bay analyzed \(\delta^{15}N\) values for *Ulva rigida* and found the source of growth was from WWTPs. We found lower *Ulva* biomass in 2012 as compared to biomass recorded at the same locations in 1995/1996. Reductions in nitrogen loads starting in the 1990s may have contributed to lower biomass of the *Ulva* spp. blooms occurring throughout the summer. The total nitrogen load to Jamaica Bay in 1995 was 19,000 kg N day\(^{-1}\) compared to 14,000 kg N day\(^{-1}\) in 2005 (Benotti et al. 2007). The New York City Department of Environmental Protection (NYCDEP) began upgrading WWTPs, which included expanding capacity and adding aeration at all WWTPs (Bronsan and O’Shea 1996). A total maximum daily load (TMDL) mandate was created in 2010 requiring the city to reduce nitrogen loads by 50% by 2020 (NYCDEP 2012).

We found biomass differences between *Ulva* and *Gracilaria* at both sites (Figure 3.3 and 3.4). At Norton Basin the biomass of *Gracilaria* was low except in December. As indicated in Figure 3.7, water temperatures in the Bay were between 7 and 10.5°C in December. Optimal temperature for *Gracilaria* was determined between 29 and 34 °C (Gorman et al. 2017). The low *Gracilaria* biomass may be due to the high biomass of *Ulva* during the summer, which can limit the light available for *Gracilaria* (Figure 3.4). *Gracilaria* may have been limited by light for most of the season because of the large blades of *Ulva* inhabiting the area. The morphology of *Gracilaria* is stringy therefore it becomes entangled in large benthic mats (personal observation). At Marine Park *Gracilaria* has a different pattern, with biomass increasing from April to the peak in July, coinciding with the *Ulva* bloom. At this site the *Gracilaria* may have been less light limited because of the lower *Ulva* abundance. A study completed by Nejrup and Pedersen (2010)
in Denmark found the greatest *Gracilaria vermiculophylla in situ* growth rates to occur in June and July. We suggest the *Gracilaria vermiculophylla* bloom at Marine Park was caused by mild temperatures and a less intense *Ulva* abundance.

*Ulva* blooms can harm the ecosystem and cause a cascade of negative effects therefore managers need to understand the bloom dynamics of the species of *Ulva* in their estuary (Cuomo et al. 1997; Thomsen et al. 2009; Zertuche-González et al. 2009). The *Ulva* biomass can be harvested and reused as part of a bioremediation program. Harvesting biomass can have positive outcomes for water quality (Merrill et al. 1992; Kim et al. 2014, 2015; Rose et al. 2015). Efforts to control nitrogen sources have been made with the reduction of wastewater treatment plant discharge (NYCDEP 2012). However, with such large nitrogen discharges from sewage treatment plants in Brooklyn and Queens, NY, remediation is limited. Therefore, bioremediation may be another way to remove nitrogen from the system once it has been discharged.

The harvest of *Ulva* spp. or other macroalgae biomass for nutrient extraction can be optimized with site specific data on abundance and tissue nitrogen concentration. For example, in Jamaica Bay harvesting *Ulva* in June when average biomass is 231 g DW m$^{-2}$ and tissue nitrogen is at a 2% would yield a nitrogen removal of 4.62 g N m$^{-2}$. However, ~8% more nitrogen (4.98 g N m$^{-2}$) could be removed with a 50% reduction in effort by harvesting in September when average *Ulva* spp. biomass is less (111 g DW m$^{-2}$) but tissue nitrogen is much higher (4.5%).

In conclusion, the dominant seasonal macroalgae in Jamaica Bay, NY was *Ulva* spp. Seasonal and long-term abundance may be related to DIN availability as well as temperature. Macroalgae biomass in Jamaica Bay is likely to decrease as WWTPs reduce their nitrogen discharges. Efforts to use bioremediation to mitigate nitrogen loadings would benefit from site-
specific analyses of bloom dynamics as shown in this study. It is essential to manage harvests of *Ulva* to maximize nutrient removal in Jamaica Bay.

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Chapter 4

Optimal irradiance and temperature in the early growth stages of *Ulva linza* (Chlorophyta, Ulvophyceae)

**ABSTRACT**

The development of a bloom-forming green marine macroalga, *Ulva* spp. can be detrimental to coastal ecosystems. We examined the optimal growth irradiance and temperature in the early growth states of *Ulva linza* by using a growth chamber in controlled conditions. The growth chamber conditions for irradiance were 50, 100, and 200-µmol photons m\(^{-2}\) s\(^{-1}\) and for temperature ranged from 15-27 °C. We used the nuclear internal transcribed spacer ITS gene to identify *Ulva linza* as the species collected. The optimal irradiance and temperature for *Ulva linza* was 25 °C at 200-µmol photons m\(^{-2}\) s\(^{-1}\). At 21°C growth was highest at 100 µmol photon m\(^{-2}\) sec\(^{-1}\). This study was similar to other optimal temperature requirements in other studies with an optimal range of 21 and 25 °C in *Ulva linza*.

Keywords: optimal conditions, *Ulva* blooms, eutrophication
INTRODUCTION

*Ulva* spp. blooms are a world-wide problem in ecosystems impacted by eutrophication (Valiela et al. 1997; Liu et al. 2013). In recent years, the impact of *Ulva* spp. growth has been studied in response to eutrophication in the Yellow Sea, China (Huo et al. 2015; Zhang et al. 2017; Wu et al. 2018). Coastal eutrophication is caused by wastewater treatment discharge, atmospheric deposition, groundwater leaching, and agriculture (Valiela et al. 1997). *Ulva* blooms can cause oxygen depletion in both the water column and benthic environment which can negatively affect the benthic fauna (Valiela et al. 1997; Franz and Friedman 2002).

There are a number of physical, chemical, and biological factors that have an impact on *Ulva* spp. growth and nutrient uptake (Hurd et al. 2014). The physical factors are light, temperature, water motion, and tolerance to desiccation (Hurd et al. 2014). The chemical factor is nutrient concentrations (Pedersen 1994). The biological factors include the surface-area to volume ratio (or SA:V), age classes, and parts of the seaweed (Hurd et al. 2014). *Ulva* spp. are often dominant because of their morphological characteristics, fast growth rates, and ability to assimilate both dissolved inorganic nitrogen (DIN) and organic nitrogen (ON; Fletcher 1996; Pedersen and Borum 1996; Pedersen and Borum 1997). Adaptations, such as their ability to assimilate and store nutrients for future growth make them opportunistic organisms that dominate over other aquatic macroalgae and plants in eutrophic conditions (McGlathery et al. 2007).

Nutrient availability is a key factor in regulating growth and species composition of aquatic plant communities (Duarte 1992; Hurd et al. 2014). Macroalgae are distributed along nutrient gradients and are characterized by slow-growing species in nutrient poor regions, while fast-growing opportunistic species dominate under nutrient rich conditions (Kautsky et al. 1986;
Macroalgae with thin thalli and simple morphologies take up nitrogen at faster rates per unit area of biomass and have higher affinities for uptake at low nitrogen concentrations (Fujita 1985; Hein et al. 1995; Pedersen and Borum 1997).

Optimal growth in Ulvoid green algae can be stimulated by irradiance, temperature, and nutrient conditions (Taylor et al. 2001). Compared with other macroalgae, ulvoids exhibit high rates of photosynthesis, nutrient uptake, and growth (Duarte 1995; Merceron et al. 2007; Nelson et al. 2008; Choi et al. 2010). There are many abiotic and biotic factors that may limit ulvoid species abundance and productivity, however, irradiance and temperature are the most important factors (Nelson et al. 2008).

*Ulva* spp. blooms are important in determining the seasonal evolution of the coastal ecosystem (Marinov et al. 2007). Growth patterns are affected by a combination of physical, chemical, and biological factors. The full extent of the optimal *Ulva* spp. response of the tube morphology to irradiance and temperature is not known (Hurd et al. 2014). The objective of this study was to test the optimal conditions of irradiance and temperature for growth of *Ulva* spp.

**MATERIALS AND METHODS**

*Extraction of DNA, PCR Amplification, and Sequencing.* To confirm which *Ulva* species, we collected, DNA was extracted from *Ulva* spp. in January 2016, rinsed with sterile diH$_2$O, and cut with a sterile exact-o-knife to an approximate size of 4 cm$^2$. Using a BigDye 3.0 Kit (PE Applied Biosystems Inc.) we amplified the ITS region using primers 1763-18S, 1505-18S combined with ENT26S (Hayden et al. 2003; Table 4.1). Polymerase Chain Reaction (PCR) amplification and reaction was carried out by using the methods from Kirkendale et al. (2013)
and Saunders and Kucera (2010). To quantify the PCR product, we performed gel electrophoresis. The product of the PCR amplification was combined with ethylene bromide stain and electrophoresed on a 2% agarose gel. PCR products were cleaned using a PCR Purification Kit (Qiagen Inc.) and sequenced with primers on an Applied Biosystems 3130 XL automated analyzer (Carlsbad CA, USA) according to Saunders and Kucera (2010).

Table 4.1 Primers used in this study for PCR amplification and sequencing. Primers used are from Hayden et al., 2003.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S1505</td>
<td>5’ TCTTTGAAACCGTATCGTGA 3’</td>
<td>ITS1</td>
</tr>
<tr>
<td>18S1763</td>
<td>5’ GGTGAACCTGCGGAGGGATCATT 3’</td>
<td>ITS1</td>
</tr>
<tr>
<td>ENT26S</td>
<td>5’ GCTTATTGATATGCTTAAGGTTAGCGGG 3’</td>
<td>ITS2</td>
</tr>
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</table>

**Phylogenetic Analysis.** Our sequences were blasted in GenBank (NCBI) for comparative phylogenetic analysis of ITS and tufA sequences world-wide. Gene alignments were subjected to maximum likelihood (ML) Muscle analysis and conducted using Mega v.6 software (Tamura et al. 2011). The phylogenetic trees were generated in R, a programming language and free software, using ML and nearest joining neighbor (NJ) rooted analysis using “ape”, “phangorn”, and “ggtree” packages (Paradis et al. 2004; Schliep 2011; Yu et al. 2017).

**Biomass Collection and Preparation.** In January 2016 *Ulva* spp. tubes were collected from Marine Park, Brooklyn, New York at low tide. The specimens were wrapped in wet paper towels then mailed to the Seaweed Biotechnology Laboratory at the University of Connecticut, Stamford, CT, for initiation of cultures. After one day spores were allowed to release from the
mother plants by setting them in 1 L Erlenmeyer flasks with aeration at room temperature and light >100 µmol photon m\(^{-2}\) sec\(^{-1}\) for three days until the culture turned dark green. These cultures were filtered through an 80 micron Nitex mesh screen and rinsed with natural seawater (NSW) to remove sporulation inhibitor chemicals SI1and SI2 (Wichard and Oertel 2010). The Nitex mesh was placed under high light >200 µmol photons m\(^{-2}\) sec\(^{-1}\) for 3 hours then 50-100 mL was rinsed into a beaker to further aid in the development of reproductive Ulva. The spore solutions were pipetted onto glass microscope slides approximately 2 x 2 cm, allowed to settle overnight in a moisture chamber then filled with Von Stosch Enrichment (VSE) media.

**Experimental Setup.** To investigate the optimal temperature and irradiance (photon fluence levels) conditions we took the cultivated strain (F1) and allowed spore release from the parental Ulva spp. plant (F2; Figure 4.1). Spores were filtered through an 80 µm Nitex mesh filter with VSE media into a 150 mL beaker. Spores were then examined under the microscope at 200x and quantified using a hemocytometer (Figure 4.2). Two droplets of this spore solution (1.2e\(^{06}\) cells) were placed on glass microscope slides in five replicates and then placed into a moisture chamber for one day to settle. The next day the microscope slides were placed into Corning 3250 crystallizing Petri dishes with 200 mL of media on a temperature x and light gradient table for the duration of the 7 day experiment (Figure 4.3; Yarish and Edwards 1982; Egan et al. 1989). On days 1, 2, 3, and 7 sporlings were examined by removing one microscope slide at a time with a microscope equipped with “PixelLINK digital camera (PixelLINK, Ottawa, Ontario Canada) to observe and record germination and growth. At the end of the experiment, one microscope slide of sporlings was left in the culture chamber to observe morphology of the plant after one month of growth.
To calculate the specific growth rate in the optimal temperature and irradiance experiment the following formula was used:

\[ \text{SGR} = \frac{\ln(w_2) - \ln(w_1)}{t_2 - t_1} \]

Where \( w_2 \) = length of sporophyte at the end time interval (microns), \( w_1 \) = length of sporophyte at the start time interval (microns), \( t_2 \) = time at the end interval (days), \( t_1 \) = time at the start interval (days).

**Statistical Analysis.** To examine the effects of nutrients and time on *Ulva* spp. growth a two-way ANOVA was performed. We examined the effects of temperature and irradiance on *Ulva* spp. growth using a two-way ANOVA and a post-hoc Tukey multiple comparisons of means with a 95% family-wise confidence level test. Positive significance was based on \( p=0.05 \). Statistical tests were completed in R standard package software version 3.4.
Figure 4.1 Image of the *Ulva linza* gametophyte parental taken at 200x magnification. Scale is 1 µm.
Figure 4.2 Image of zoospores taken at 200x magnification. Scale is 1 µm.
Figure 4.3 Gradient table setup for the irradiance and temperature experiment.
RESULTS

Identification of Ulva spp. One clade matched our Jamaica Bay samples and is identified with the following support: *Ulva linza* (ML = 99%; Figure 4.4). All samples collected had <1% interspecific divergence. Based on maximum likelihood (ML) distances the genotypes match the tubular *Ulva linza* in green tides from China. The ITS ML 1% divergence in *Ulva linza* occurred between our samples and Qingdao, China, EU888138 (Zhang et al. 2008) and HM584731 (Duan et al. 2012).

Zoospore Ontogeny. The zoospores attached to microscope slides after day 1 and formed a spherical body. Germination of the zoospores was recorded on the second day. The formation of the primary rhizoid formed after day two and continued growth (Figure 4.5A and Figure 4.5B). Development of the uniseriate thallus by successive transverse division followed (Figure 4.5C and Figure 4.5D). On day seven development of the extensive rhizoidal system and multicellular thallus continued (Figure 4.6A and Figure 4.6B).

Optimal Irradiance and Temperature Experiment. The length of *Ulva linza* from spore to day 7 was 0.436 mm and was longest at 200 µmol photons m\(^{-2}\) s\(^{-1}\) and at 25°C (Figures 4.7 and 4.8). The length of *Ulva linza* at day 7 was 0.433 mm and was second highest at 100 µmol photons m\(^{-2}\) s\(^{-1}\) and at 21°C. The 50 µmol photons m\(^{-2}\) sec\(^{-1}\) growth was minimal at 0.020 mm after 7 days. After performing a two-way ANOVA on the effects *Ulva linza* growth on irradiance and temperature we found that 100 and 200 µmol photons m\(^{-2}\) sec\(^{-1}\) were different from each other and the 50 µmol photons m\(^{-2}\) sec\(^{-1}\) treatment (Tables 4.2 and 4.3). The specific growth rate (SGR) of *Ulva linza* was highest at 100 µmol photons m\(^{-2}\) s\(^{-1}\) at 27°C and 21°C with
a SGR of 3.60 (Figure 4.9). *Ulva linza*’s total growth after 30 days was 84±38 mm with 15 replicates (Figure 4.10).
Figure 4.4 *Ulva* maximum likelihood tree of the ITS I and ITS II markers based on uncorrected p-distances for comparison between 17 sequences and 1 *Ulva* sp. from Jamaica Bay, shown in percent. Jamaica Bay sequence is *Ulva* sp. 2U.
Figure 4.5 Images of thallus ontogeny in *Ulva linza*. A. Elongation of cell forming primary rhizoid, scale = 1 µm. B. Further elongation of cell forming primary rhizoid, scale = 1 µm. C. Development of uniseriate thallus by successive transverse division, scale = 1 µm. D. Further development of rhizoid system and uniseriate thallus, scale = 1 µm.
Figure 4.6 A and B. Development of extensive rhizoidal system and multicellular thallus by longitudinal divisions. A, scale = 20 µm. B, scale = 1 µm.
Figure 4.7 Growth of *Ulva linza* at five temperatures and three light intensities during 1-7 days of cultivation. Data means with standard error (n=15).
Figure 4.8 Growth of *Ulva linza* at five temperatures and three light intensities after seven days of cultivation. Data means with standard error (n=15).
Table 4.2 A two-way ANOVA on the effects of *Ulva linza* growth on temperature and light.

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<th>Df</th>
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<th>Mean Sq</th>
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<td>4968952</td>
<td>1656317</td>
<td>1221.54</td>
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<tr>
<td>Temp</td>
<td>4</td>
<td>569575</td>
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</tr>
<tr>
<td>Light</td>
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<td>579121</td>
<td>289561</td>
<td>213.55</td>
<td>&lt;0.001</td>
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<tr>
<td>Day X Temp</td>
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<td>159336</td>
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<td>Day X Light</td>
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<td>1981983</td>
<td>330330</td>
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<td>Day X Temp X Light</td>
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<td>812419</td>
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<td>&lt;0.001</td>
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<td>Residuals</td>
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<td>1356</td>
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</table>

Table 4.3 A post hoc Tukey test on the two-way ANOVA.

<table>
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<th>Upr</th>
<th>P adj</th>
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<td>42.467</td>
<td>56.903</td>
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<td>200-50</td>
<td>59.060</td>
<td>51.848</td>
<td>66.271</td>
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<tr>
<td>200-100</td>
<td>9.375</td>
<td>2.188</td>
<td>16.561</td>
<td>0.006</td>
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Figure 4.9 Mean specific growth rate (SGR) of *Ulva linza* sporophytes at three temperatures and three light intensities after seven days of cultivation.
Figure 4.10 Image of experimental *Ulva linza* tubes after 30 days of cultivation. Scale is 1 cm.
DISCUSSION

In this study growth was highest at 25 °C at 200 µmol photon m⁻² sec⁻¹. At 21 °C growth was highest at 100 µmol photon m⁻² sec⁻¹. This suggests that *Ulva linza* will thrive between 21 °C and 25 °C. At temperatures lower than 21°C *Ulva linza* will probably not grow optimally. Our findings were similar to other *Ulva* spp. studies that examined irradiance and temperature. However, studies characterizing *Ulva linza* optimal temperatures are not available. Taylor et al. (2001) found *Ulva linza* to have an optimal growth rate at 72 µmol photons m⁻² sec⁻¹ and tubular *Ulva compressa* at 175 µmol photons m⁻² sec⁻¹. The optimal light range in this study for *Ulva linza* was 100-200-µmol photon m⁻² sec⁻¹. *Ulva* spp. can grow during the spring/summer when they bloom at photon intensities at 2000 µmol photons m⁻² sec⁻¹ (Hurd et al., 2014). *Ulva linza* is well below its compensation point at 50 µmol photons m⁻² sec⁻¹, suggesting that this is the reason why *Ulva* become dormant in the winter.

*Ulva linza* had a broad tolerance to temperatures and are consistent with other studies in other species world-wide. Zhang et al. (2016) found *Ulva prolifera* had an optimal growth temperature at 20 °C. Wu et al. (2000) found similar results and Wang et al. (2007) found an optimal growth for *Ulva prolifera* at 20-25 °C. Rosenberg and Ramus (1981) found the optimal growth temperature to be 20 °C for *Ulva curvata*. While Nordby and Hoxmark (1972) found the optimal temperature of 21 °C for *Ulva lactuca*. Other studies have found optimal temperatures for *Ulva pertusa* between 20-23 °C (Yokohama 1973; Wang et al. 2007). The tubular morphology of *Ulva linza* exhibited rapid growth rates at both 100 and 200 µmol photons m⁻² sec⁻¹ but little growth at 50 µmol photons m⁻² sec⁻¹. Morphology is driving growth rates of the species and is explained by having tubular structures. These structures have a high surface area: volume ratio allowing all cells to be activated by sunlight (Taylor et al. 2001; Hurd et al. 2014).
Our samples matched *Ulva linza* from Zhang et al. (2008) and Duan et al. (2012). Duan et al. (2012) reported a very detailed cytological study of *Ulva linza*. The cytology was very similar compared with our samples. The chloroplasts are evenly distributed, and cell shape is oblong with varied sizes throughout. We can be certain that phenology matches were correct because of the cytological comparisons.

It is essential to combine environmental and ecological issues that consider compliance with environmental policies and development plans. Macroalgae dynamics can support decision-making in coastal systems (Sfriso and Marcomini 1996; Brush and Nixon 2010). Brush and Nixon (2010) created an estuarine model for *Ulva lactuca*. In their model the temperature-dependent maximum attainable rate of gross primary production is set to exponentially proceed at 20-22 °C. The sharp decline in *Ulva* biomass is set at 25 °C and greater (Brush and Nixon 2010). Our study confirms this use of temperature with a slightly broader range for *Ulva linza* at 21-25 °C. Irradiance used in the model was close to field replications for Narragansett Bay at >2000 μmol photons m⁻² sec⁻¹ and is similar for Jamaica Bay. In Jamaica Bay, a 3-D time-variable hydrodynamic and water quality model used to inform management decisions has been developed (Hydroqual 2002). Some of the parameters incorporated into the Jamaica Bay Eutrophication Model (JEM) for *Ulva* spp. are nutrient availability, maximum growth rates with PO₄³⁻, NH₄⁺, and NO₃⁻, optimal temperature growth rates, and saturating irradiance. We suggest that the *Ulva linza* optimal temperatures be incorporated into the JEM because this is one of the species found in abundance in Jamaica Bay. Another species optimal temperature to consider incorporating into the model would be *Ulva compressa* due to its bloom-forming presence in Jamaica Bay (Lamb et al. 2018).
In conclusion, this study provides evidence that optimal growth temperatures are consistent with temperatures in the field when *Ulva* spp. blooms. This study also showed the minimum irradiance for *Ulva linza* growth was >50 µmol photon m\(^{-2}\) sec\(^{-1}\) but greatest growth (bloom-forming) would occur at >100 µmol photon m\(^{-2}\) sec\(^{-1}\) and optimal temperatures between 21-25 ºC.

**ACKNOWLEDGEMENTS**

I would like to thank Dr. Brett F. Branco, Dr. Juergen Polle, Dr. Charles Yarish, and Dr. Chester B. Zarnoch for offering their advice for this research and making edits to the written document. Thank you to Dr. Charles Yarish for utilization of the Seaweed Biotechnology Laboratory at the University of Connecticut. I would also like to thank Yuanzi Huo for *Ulva* advice and help with the specifics of the research. Thanks to Dr. Simona Augyte for comments to the early manuscript. This research was conducted under a New York City Parks Department permit.
Chapter 5

DISCUSSION

This research is the first comprehensive study of *Ulva* spp. in Jamaica Bay, NY, USA. Our findings suggest that the *Ulva* species composition are *Ulva clathratioides*, *U. compressa*, *U. lactuca*, *U. laetevirens*, *U. linza*, *U. prolifera*, and *U. stipitata* (Figures 2.5 and 2.6). Moreover, the bloom-forming species in the Bay is *Ulva compressa* occurring in large distromatic sheets (Figures 2.3 and 2.4). Using the genotype-comparison approach adapted by Hoffman et al. (2003) we found similarities among *Ulva* spp. around the world. Most notably that specimens (05/04/2015 and 06/17/2015) exhibit sequences that match the *Ulva linza-procera-prolifera* clade from Qingdao, China – the world’s most famous *Ulva* bloom (Duan et al. 2012).

The peak *Ulva* spp. biomass was in June and July of 2015 (Figure 3.3). Concurrently, the *Gracilaria vermiculophylla* peak biomass was in July and August but only at Marine Park, Brooklyn. The low *Gracilaria vermiculophylla* biomass at Norton Basin, Queens may have been due to high blanketing *Ulva* spp. biomass in the water column. The seasonal pattern of *Ulva* spp. appears to be controlled by irradiance, temperature, and nitrate. The optimal temperature for *Ulva* spp. occurred from June-mid Sept (Figure. 3.6; Nordby and Hoxmark 1972; Yokohama 1973; Rosenberg and Ramus 1981; Wang et al. 2007). In Washington State, USA Nelson et al. (2003) identified daylength, temperature, and DIN as having an effect on ulvoid growth. DIN composed of ammonium and nitrate ranged from 0.3-1.4 mg N L⁻¹ (Figure 3.7). Nitrate was <0.02 mg N L⁻¹ in July and August. Ammonium was abundant all season. Furthermore, *Ulva* spp. tissue nitrogen was depleted near 2 % in June and July indicating that reserves of nitrogen were low (Figure A2). This suggests that *Ulva* spp. was not nutrient limited during most of the
season even though nitrogen loads have decreased in Jamaica Bay (Carpenter and Capone 1983; Fujita et al. 1989; Pederson and Borum 1997; NYCDEP 2012; Hurd et al. 2014).

*Ulva linza* showed optimal growth at 100 and 200 µmol photons m\(^{-2}\) sec\(^{-1}\) but little growth at 50 µmol photons m\(^{-2}\) sec\(^{-1}\) (Figures 4.4 and 4.5). The morphology may be driving the high growth rates of the species and is explained by having tubular structures. Tubular structures have a high surface area: volume ratio allowing cells to be activated by sunlight (Taylor et al. 2001; Hurd et al. 2014). *Ulva linza* had a broad tolerance to temperatures (Figure 4.4). The temperature range for optimal growth of *Ulva linza* was 21-25ºC. Zhang et al. (2016) found *Ulva prolifera* had an optimal growth temperature at 20ºC while Wu et al. (2000) found similar results. Wang et al. (2007) found an optimal growth for *Ulva prolifera* at 20-25ºC.

Latimer et al. (2013) has addressed many of the management issues in the Long Island Sound (LIS). These issues are eutrophication, hypoxia, habitat degradation, invasion of non-native species, ocean acidification, and climate change. The study is the first to understand the factors controlling biological processes and seasonal occurrence in the Bay. Irradiance, temperature, and day length are the primary factors controlling distribution and productivity of *Ulva* spp. (Lüning 1990; Hurd et al. 2014). Other factors that control intertidal and sublittoral distributions are nutrient availability, competition for substrate, and predation (Pederson et al. 2008; Hurd et al. 2014). Modeling is one approach that can be used to predict where *Ulva* spp. blooms occur (Sfriso et al. 1996; Brush and Nixon 2010). These efforts could further reduce *Ulva* biomass and lower the effects of eutrophication before the occurrence of further ecosystem consequences in Jamaica Bay (Solidoro et al. 1997). The findings of this study can be used in management decisions.
The biomass of *Ulva* spp. blooms occur because of optimal conditions, mainly due to nitrogen loading, irradiance, and temperature presence in Jamaica Bay. This study found that *Ulva linza* had optimal temperatures between 21 °C and 25 °C with the highest growth at 100-200 µmol photon m⁻² sec⁻¹ (Figure 4.4). In the field during 2013 temperatures in May ranged between 17-18.5 °C. By mid-June, temperatures were well over 20°C making May an optimal month for *Ulva* growth. Even when temperatures were not optimal, *Ulva* spp. still grew in 2015 probably due to abundance of light. The best time for harvesting would be in September, before the during summer decline in temperature and when the *Ulva* is on the verge of decay. This time period is further supported by the *Ulva compressa* biomass percent nitrogen values being high in the month of September (Figure 3.4).

Previous studies have quantified macroalgal biomass, the relationship of macroalgal growth with nutrients, and external factors such as temperature and irradiance but with no clear mathematical or conceptual model capable of predicting macroalgae biomass (Martins and Marques 2002; Trancoso et al. 2005). Conceptual models of macroalgae biomass dynamics that directly link to the estuarine eutrophication problem have been proposed (Cloern 2001). Cloern (2001), in his conceptual model of eutrophication specifically states that macroalgae biomass becomes a direct response to increased nutrient loading (Table 5.1). I propose a conceptual model that is capable of understanding the various factors that control *Ulva* spp. blooms (Figure 5.1). These factors include irradiance, temperature, nutrients, biomass, and competition with other macroalgae species.
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<td>Food Web Structure</td>
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Figure 5.1 Conceptual model of *Ulva* spp. internal and external drivers in a eutrophic estuary. Adapted from Berger et al. (2004).
My conceptual model begins with increased WWTP discharge of freshwater and nutrients. Given the optimal nitrogen concentrations (2.74 mg N L$^{-1}$) and external factors, light (100-200 µphotons m$^{-2}$ sec$^{-1}$) and temperature (21-25 °C) Ulva spp. growth rates should be at a maximum (Figure 5.1; Fan et al. 2014). Factors that may limit the growth of Ulva spp. are salinity, phytoplankton abundance, Gracilaria vermiculophylla abundance, and herbivory. Salinity stress was shown in Ulva lactuca by Xia et al. (2004) to affect the photosystem II receptor after exposed to a salinity greater than 48 ppt. In Ulva pertusa growth rates are affected at a salinity of 5 ppt, which brought growth rates down at the optimal growth salinity of 20 ppt from 0.03 day$^{-1}$ to 0.02 day$^{-1}$ (Choi et al. 2010). In Jamaica Bay phytoplankton blooms occur in April and August (Wallace and Gobler 2015). Phytoplankton can increase turbidity and sedimentation of organic carbon therefore decreasing depth penetration and increasing compensation for nutrients in adult Ulva thalli (Figure 5.1 and 5.2). Phytoplankton can also disrupt settlement of juveniles (Berger et al. 2004). Gracilaria vermiculophylla can increase shading and competition for nutrients in adult thalli of Ulva spp. As nutrients increase in the system the palatability of Ulva spp. can increase therefore increasing grazers (Berger et al. 2004).
Figure 5.2 Hypothesized nitrogen cycle in an *Ulva* bed throughout the season in a eutrophic estuary.
My model explains why *Ulva* spp. has decreased over the last 20 years (Chapter 3). Along with a decreased nutrient load, phytoplankton has thrived, limiting the amount of nutrients *Ulva* spp. can uptake. We can see the sustained growth of phytoplankton in chlorophyll-*a* measurements (Figure 5.3). The water clarity, shown by measurements of turbidity and secchi depth, due to phytoplankton in the water column has also made germination and juvenile settlement suboptimal for *Ulva* spp. (Figures 5.4 and 5.5). Both light and competition for space may be responsible for the lack of settlement. *Gracilaria vermiculophylla* has thrived, competing with *Ulva* for light and nutrients.
Figure 5.3 Bi-monthly chlorophyll $a$ measurements in Jamaica Bay at all sites from 1996-2015. NYCDEP (2015) and NPS (2015).
Figure 5.4 Bi-monthly turbidity measurements in Jamaica Bay at all sites from 1996-2015. NYCDEP (2015) and NPS (2015).
Figure 5.5 Bi-monthly secchi disk measurements at all sites in Jamaica Bay from 1996-2015. NYCDEP (2015) and NPS (2015).
Ulva spp. in Jamaica Bay may have detrimental effects on the ecosystem. Too much Ulva can have negative effects on key functional groups of invertebrates (Franz and Friedman 2002). Green et al. (2014) found macroalgal abundances as low as 110 to 120 g dry wt. m\(^{-2}\) that produced significant and rapid effects on microbenthic invertebrates. They also proposed that macroalgae are a reliable indicator of eutrophication due nutrient load responsiveness. Models should encompass nutrient regulation of WWTPs, stoichiometry of algal biomass production including dominant species, functional groups and algal community structure (Bricker et al. 2003; Ferreira et al. 2011). In this study, we found that peak Ulva biomass was 470 g dry wt. m\(^{-2}\) in Jamaica Bay, over four times more than Green et al. (2014) found to cause adverse effects in Upper Newport Bay, California. It could mean that many areas in Jamaica Bay are experiencing detrimental effects from Ulva blooms. Kracauer-Hartig et al. (2002) found that Spartina alterniflora salt marshes in Jamaica Bay are disappearing at a fast rate and proposed that sea-level rise and Ulva blooms may be a contributing factor. The Ulva spp. blooms can have negative impacts on Spartina alterniflora by blanketing the salt marsh peat as well as increase the hydrogen sulfide in the peat (Kolker 2006; Watson et al. 2015).

World-wide Ulva spp. blooms have created ecosystem consequences with high biomass presence. Guidone and Thronber (2013) found in Narragansett Bay the peak biomass was 450 g dry wt. m\(^{-2}\). While Liu et al. (2013) found in the Yellow Sea, China the peak biomass was 2100 g dry wt. m\(^{-2}\) and Schramm (1999) found in the Venice Lagoon, Italy the peak biomass to be 3000 g dry wt. m\(^{-2}\). Clearly Jamaica Bay’s Ulva spp. blooms are not quite as high as China and the Venice Lagoon with a peak Ulva biomass of 232 g dry wt. m\(^{-2}\). However, these blooms could reach this state in Jamaica Bay with rapid human population growth. Given failure to upgrade and build new WWTPs nitrogen could reach record highs that would fuel Ulva blooms.
*Ulva* spp. blooms may be associated with ecological thresholds in estuaries. Ecological thresholds are defined as, “the point at which there is an abrupt change in an ecosystem quality, property or phenomenon, or where small changes in an environmental driver produce large responses in the ecosystem” (Groffman et al. 2006). Since *Ulva* spp. blooms have been in existence Jamaica Bay has experienced shellfish die-off and a degradation in salt marshes. These responses in the ecosystem may have been cause in part from *Ulva* blooms and a degradation in water quality. Based on a model McGlathery et al. (2007) created the phytoplankton will out compete macroalgae for nutrients and light. Their hypothesis states there is a distinct tipping point in the ecology of macroalgae verses phytoplankton under which phytoplankton will win given high nutrient loads. Sfrsio and Marcomini (1996) found that phytoplankton blooms out competed *Ulva* spp. for nutrients and irradiance and saw a decline in *Ulva* spp. biomass in the Venice Lagoon.

Looking toward the future, scientists are increasingly trying to understand the role of increasing carbon dioxide in the atmosphere, which drives both ocean acidification and global temperature rises, in *Ulva* spp. growth. Ocean acidification is the reduction of ocean water pH (Takahashi et al. 2014). This process is happening due to the ocean being a sink for CO$_2$. The excess CO$_2$ in the atmosphere is from anthropogenic processes (IPCC 2007). Within the next 100 years the atmospheric CO$_2$ concentrations are projected to increase from 411 ppm to a maximum of 1142 ppm in 2100 (June 2018; [www.co2.earth.org](http://www.co2.earth.org); Stocker et al. 2013). The increase will reduce the surface ocean pH by 0.4 units (Meehl et al. 2007). The response of *Ulva* spp. to ocean acidification and increased CO$_2$ in the world’s oceans is unclear at this time. There are many studies that report HCO$_3^-$ as the primary source of carbon for *Ulva* spp. (Drechsler and Beer 1991; Björk et al. 1992, 1993; Drechsler et al. 1993; Sharkia et al. 1994). Therefore, *Ulva* spp.
would be unaffected by increases in CO\(_2\) and the main driver of growth is light (Rautenberger et al. 2015). Rautenberger et al. (2015) suggest that *Ulva* rigida is already both CO\(_2\) and HCO\(_3^-\) saturated. However, Scoma et al. (2016) proposed that *Ulva lactuca* growth rates increase with increasing CO\(_2\). Under a warmer climate, just a 1-2°C change, warm-temperate species such as the red weed *Gracilaria vermiculophylla* and *Gracilaria tikvahiae* and *Ulva* spp. could replace the cold-temperate species such as rockweed *Fucus* spp. (Latimer et al. 2013).
Figure A1 Water column ammonium within the *Ulva* spp. bed in 2015. Marine Park is represented with black circles and Norton Basin is represented with black triangles. Error bars are standard error.
Figure A2 Water column nitrate + nitrite within the Ulva spp. bed in 2015. Marine Park represented with black circles and Norton Basin is represented with black triangles. Error bars are standard error.


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