An assessment of the biodiversity and bioremediation potential of distromatic Ulva spp (Chlorophyta) in the Great Bay Estuarine System of New Hampshire and Maine, USA

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AN ASSESSMENT OF THE BIODIVERSITY AND BIOREMEDIATION POTENTIAL OF DISTROMATIC ULVA SPP. (CHLOROPHYTA) IN THE GREAT BAY ESTUARINE SYSTEM OF NEW HAMPSHIRE AND MAINE, U.S.A.

BY

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THESIS

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ABSTRACT

AN ASSESSMENT OF THE BIODIVERSITY AND BIOREMEDIATION POTENTIAL OF DISTROMATIC ULVA SPP. (CHLOROPHYTA) IN THE GREAT BAY ESTUARINE SYSTEM OF NEW HAMPSHIRE AND MAINE, U.S.A.

by

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Eutrophication of coastal ecosystems is a global problem, and algae have become an important resource for bioremediation. The goals of this study were (1) to assess the biodiversity of Ulva spp. in the Great Bay Estuarine System (GBES) of New Hampshire and Maine, and (2) to assess which Ulva populations are most appropriate for bioremediation by determining if environmental nutrient history and/or taxonomic differences affect ammonium uptake. Molecular analysis of the internal transcribed spacer nrDNA regions of Ulva spp. revealed four distinct distromatic taxa: Ulva lactuca Linnaeus, Ulva rigida C. Agardh, Ulva compressa Linnaeus, and U. pertusa Kjellman. The latter three are new reports for New Hampshire. Intra- and interspecific comparisons revealed that nutrient history influences substrate affinity, whereas taxonomy influences uptake rate. Consequently, there is a clear need for reassessment of global Ulva populations, and both nutrient history and taxonomy should be considered when using Ulva for bioremediation.
INTRODUCTION

In a world where climate change, energy crisis, and eutrophication of coastal ecosystems are issues at the forefront of scientific research, algae have become an important potential resource for the prevention of ecological degradation and for bioremediation of environmental health. Studies regarding the ability of algae to sequester excess carbon from the atmosphere, provide alternative energy sources, and absorb excess nutrients in coastal systems and aquaculture facilities have been conducted within the last decade (Neori et al., 1996; Chopin et al., 2001; Porrello et al., 2003; Zhou et al., 2006). Some of these research areas show greater potential than others and may become economically and environmentally feasible bioremediation solutions.

The use of macroalgae as a biofilter for eutrophic coastal environments and aquaculture effluents is a promising field of bioremediation research that has received a lot of attention (Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Chung et al., 2002; Mata & Santos, 2003; Marinho-Soriano et al., 2008; Day, 2008). Coastal ecosystems around the world are heavily influenced by anthropogenic nutrient inputs and many suffer greatly from the impacts. Major sources of anthropogenic nutrients to coastal systems include sewage treatment plants, housing and industrial development, agriculture, and aquaculture
Nutrients are carried from terrestrial environments into rivers and streams by heavy rains and eventually into coastal environments.

**Eutrophication and Macroalgal Blooms in Estuaries**

Estuaries are coastal regions where rivers meet the sea and can be severely impacted by eutrophication. The complex coastal processes coupled with diverse flora and fauna make estuaries unique ecosystems that support many ecologically important and economically valuable organisms. Oysters, fish, submerged aquatic vegetation (SAV), and other invertebrates exist in an intricate web of competitor-predator-prey interactions within these systems (Dayton, 1975). The primary producers inhabiting estuaries are at the base of these interactions and are important monitors of water quality and providers of habitat and food (Dayton, 1975; Wilson et al., 1990; Valentine & Heck, 1999; Williams & Heck, 2001; Moore, 2004). For example, blue crabs and waterfowl use seagrass beds as nurseries and as a food source, respectively (Wilson et al., 1990; Rivers & Short, 2007), and macroalgal community structure is often dictated by grazing pressure from invertebrates (Lubchenco, 1978). In pristine temperate estuaries, seagrasses are often the dominant primary producers because of nutrient limitation and muddy substratum less favorable to macroalgal attachment (Lobban & Harrison, 1997; Valiela et al., 1997). By contrast, estuaries subjected to anthropogenic nutrient input can become eutrophied and undergo an altered ecosystem balance in which opportunistic macroalgae become the dominant
primary producer. Usually these opportunistic algae have annual life histories and appear as filaments or thin blades. They compete with seagrasses for light by becoming epiphytic or free floating macroalgal canopies (Hauxwell et al., 2001; 2003; Valiela et al., 1997).

A transition from seagrasses dominance to opportunistic macroalgae in estuaries occurs when high nutrient loads enter the estuary (Twilley et al., 1985; Short et al., 1995; Short and Burdick, 1996). Macroalgae are able to quickly absorb and assimilate these nutrients into biomass (Valiela et al., 1997). Seagrasses are generally light limited and get their nutrients through roots and shoots from sediments and the water column, respectively, whereas macroalgae are often nutrient limited and absorb nutrients through their cells from the water column (Duarte, 1995). Thus, seagrasses are often unable to compete with macroalgae under high nutrient conditions, and the result is often shading of seagrasses and subsequent loss of their beds in eutrophic estuaries (Valiela et al., 1997). Under conditions where nutrient inputs, water residence time, salinity, estuarine bathymetry and other as yet unknown factors are favorable, macroalgal blooms can become extensive and cover very large areas (Fletcher, 1996; Valiela et al., 1997). In the transition from a seagrass to a macroalgal dominated estuary, the impact on associated fauna can be severe because macroalgal mats do not provide habitat or protection for fish and invertebrates as effectively as seagrass beds (Deegan et al., 2002). In addition, macroalgal mats can create prolonged anoxic conditions due to high respiration rates and decomposition of accumulated algal detritus (D'Avanzo & Kremer, 1994; D'Avanzo et al., 1996).
Macroalgal blooms can be dominated by a single or very few species of algae (Fletcher, 1996). Members of the genus *Ulva* are common algae that cause "green tides" in eutrophic temperate coastal environments (Fletcher 1996; Valiela *et al.* 1997). *Ulva* spp. are green algae with morphologies consisting of distromatic (two cell layers) flat blades or monostromatic hollow tubes (Sears, 2002; Hayden *et al.*, 2003). Although extensive macroalgal blooms can have harmful effects on estuaries, there is potential for using them as tools for bioremediation. Macroalgal use as a biofilter to improve water quality in polluted environments exposed to fertilizer runoff, waste water and aquaculture effluents has received a great deal of attention and this research is very promising. Cohen and Neori (1991) showed that *Ulva lactuca* Linnaeus (sea lettuce) was an effective biofilter for marine fish pond effluents because it was able to take up almost all of the ammonium produced by *Sparus aurata* L (Gilthead seabream). El-Sikaily *et al.* (2007) documented further evidence for the use of macroalgae as a biofilter in reporting that *U. lactuca* could effectively remove chromium from wastewater.

**Nutrient Uptake in Macroalgae**

The effectiveness of macroalgae as biofilters is dependent on their physiological and morphological characteristics. Macroalgae take up essential nutrients directly from the water column into their cells by a number of methods. Nutrient uptake mechanisms in macroalgae are complex processes initialized by exposure to nutrients and followed by assimilation and metabolism. When a seaweed is first exposed to a nutrient medium, there is an initial rapid influx of
ions into the free space of its thallus, which includes its cell walls and intercellular spaces. Because inorganic forms of nitrogen are charged ions, they do not freely diffuse across a cell membrane. As a result, they are carried across the cell membranes through a passive process of facilitated diffusion, which involves a carrier protein embedded in the cell membrane that allows diffusion of ions across the membrane along an electrochemical gradient. Although no energy is used in this process, facilitated diffusion allows cells to be ion selective, and it is also a process that can become saturated when external ion concentrations are high (DeBoer, 1981). However, under environmental conditions where nutrients may be limiting, macroalgae must exert energy to take up nitrogen. Under such circumstances, macroalgae can use active uptake mechanisms that involve the transport of ions against an electrochemical gradient. Active nutrient uptake, like facilitated diffusion, can be ion specific and is crucial for algae when essential nutrients are in low concentrations (DeBoer, 1981).

The specificity and saturation properties of ion uptake in macroalgae provide a way to describe the kinetics of the uptake process. Nutrient uptake in algae can be described by the Michaelis-Menten kinetics of enzyme-substrate complexes and illustrated by a substrate saturation curve (MacIissac & Dugdale, 1969). When examining nutrient uptake kinetics in algae, this curve illustrates the relationship between the concentration of the nutrient of interest and its uptake rate by an alga. Because algae can become nutrient saturated, uptake rate increases with increasing substrate concentration up to the saturation point. The maximum uptake rate that occurs when cells become saturated is described
by the $V_{\text{max}}$ value. The substrate concentration at which uptake rate is half of the maximum, or $K_s$, can be used in combination with the $V_{\text{max}}$ to assess nutrient uptake kinetics. A high $V_{\text{max}}$ indicates that an alga does not become saturated until nutrient concentrations reach very high concentrations, whereas a low $K_s$ indicates high nutrient uptake efficiency. Fast growing, ephemeral algae that produce “green tides” often have both characteristics of uptake kinetics which allows them to take advantage of nutrient pulses in the water column (Pedersen & Borum, 1997; Runcie et al., 2003; Cohen & Fong, 2004b; Dongyan et al., 2004).

Macroalgae are able to inhabit a variety of marine, estuarine, and to a lesser extent, freshwater habitats. Depending on the habitat they occupy, the particular nutrient that limits macroalgal growth may differ. Nutrient limitation in marine algae is a well studied topic, but there is considerable debate over declaring the nutrient that is generally most limiting. In the open ocean, particularly the Pacific, phosphorous was initially believed to be the most limiting nutrient to microalgae (Redfield, 1958). However, more recently, iron has been accepted as the more limiting nutrient in the open ocean (Falkowski, 1997). On the other hand, in temperate coastal environments where macroalgae are more prevalent, nitrogen is growth limiting (Ryther & Dunstan, 1971; DeBoer, 1981; Hanisak, 1983; Wheeler & Bjornsater, 1992; Taylor et al., 1995; Pedersen & Borum, 1996). Nitrogen limitation in macroalgae is confirmed by the extensive macroalgal blooms that form in eutrophied areas where an abundant supply of nitrogen becomes available. Because anthropogenic input has altered the
nutrient balance in many coastal systems, much of the research examining nutrient relationships in marine macroalgae has focused on how the nitrogen history of the environment influences their uptake physiology (Littler & Littler, 1980; Fujita, 1985; Pedersen, 1994; Pedersen & Borum, 1997; Taylor et al., 1998; Jing-Wen & Shuang-Lin, 2001; Barr & Rees, 2003; Runcie et al., 2003).

Environmental Influence on Ammonium Uptake in Ulva spp.

Most macroalgae show an uptake preference for ammonium-N over nitrate-N and nitrite-N, as the latter forms require energy to be reduced to ammonium for assimilation into amino acids and other nitrogen-containing molecules (Cohen & Fong, 2004a). However, growth rates are similar whether algae are grown with ammonium or nitrate, unless the alga is energy deficient (Lewin, 1962). Ammonium is also a common waste product from aquaculture (Cohen and Neori 1991) and fertilizer runoff (Ayoub, 1999), so it is often the most prevalent form of dissolved inorganic nitrogen in eutrophied coastal environments. Previous research has shown that Ulva lactuca grown under ammonium rich conditions takes up nutrients at a slower rate than specimens grown under low ammonium conditions. Fujita (1985) found that U. lactuca grown under ammonium enriched conditions showed slower uptake rates than U. lactuca that had been starved. In addition, U. lactuca collected from the field showed much higher uptake rates than those grown under enriched laboratory culture conditions. The results of Fujita’s (1985) work support the hypothesis that algae growing in coastal marine environments are nitrogen limited, which results in higher affinity for ammonium (low Ks) and higher maximum uptake rates (high
$V_{\text{max}}$) compared to algae growing under eutrophic conditions. Thus, there is strong evidence that environmental factors, particularly nutrient history, affect ammonium uptake kinetics of macroalgae growing in those environments. The influence of environmental factors on ammonium uptake kinetics is important to investigate when determining the bioremediation potential of algae from different nutrient environments.

Environmental history affects physiological parameters other than nutrient uptake kinetics. Tissue nitrogen and pigment are important factors to investigate, as they are closely related to ammonium uptake. Tissue nitrogen of seaweed has been shown to follow seasonal patterns mirroring those of ambient nitrogen concentrations (Hardwick-Witman and Mathieson, 1986; Penniman and Mathieson, 1987; Wheeler and Björnsäter, 1992; Naldi and Viaroli, 2002). Ambient ammonium influences ammonium uptake kinetics both directly by a concentration gradient and indirectly through its relationship to internal tissue nitrogen (Fujita, 1985; Pedersen, 1994; Jing-Wen and Shuang-Lin, 2001; Barr and Rees, 2003). Early research on ammonium uptake kinetics concluded that total nitrogen content within algal cells determined nitrogen uptake kinetics (Fujita 1985, Rosenberg, Probyn and Mann 1984). However, recent evidence suggests that smaller nitrogen pools within cells are more important regulators of nitrogen uptake kinetics than total tissue nitrogen (Pedersen 1994, Jing-Wen and Shuang-Lin 2001a, Jing-Wen and Shuang-Lin 2001b).

Jing-Wen and Shuang-Lin (2001b) found that initial ammonium uptake in *Ulva pertusa* was more closely related to small intracellular inorganic nitrogen
pools than total nitrogen. Their conclusion was based on an observation of increased ammonium uptake rates on the first day following nitrogen starvation when the total nitrogen content of the algal tissue was still 4.42%. The critical nitrogen tissue content for sustained growth in *U. lactuca* is 2.17% (Pedersen and Borum, 1997). Because the total nitrogen content of algal tissue was above the critical level for growth, Jing-Wen and Shuang-Lin (2001) concluded that small internal nitrogen pools with fast turnover rates, such as small peptide and amino acid pools, controlled the surge in ammonium uptake during the first 2 hours of exposure to the substrate. However, variations in assimilation uptake rates after 2 hours of exposure to the substrate occurred over much longer time periods (days to weeks), indicating that they were regulated by a nitrogen pool with a slower turnover rate, possibly the incorporation of amino acids into proteins.

Pigment composition of seaweeds is also related to environmental nutrient concentrations (Rosenberg and Ramus, 1982; Pinchetti *et al.*, 1998; Barr and Rees, 2003). Pinchetti *et al.* (1998) found that chlorophyll a:b ratios in nitrogen enriched *U. rigida* remained the same regardless of nitrogen conditions, but pigment content increased with nitrogen enrichment resulting in dark green blades. Pigment contents in algae, particularly chlorophyll and carotenoids, indirectly affect nutrient uptake due to their influence on photosynthetic rates and subsequently the amount of carbon available for nitrogen assimilation (Precali and Falkowski, 1983; Falkowski, 1992). The intricate relationship between environmental nitrogen availability, tissue nitrogen, pigment content, and blade
color make these physiological parameters important to observe when assessing the ammonium uptake kinetics of macroalgae.

**Genetic Differences in Ammonium Uptake**

Previous studies have investigated nutrient uptake kinetics of *Ulva* spp. and compared them to macroalgae in other taxa. Jing-Wen and Shuang-Lin (2001) observed faster uptake rates in *Ulva pertusa* Kjellman than *Gracilaria tenuistipitata* C. F. Change and B. M. Xia after a period of nitrogen starvation, but nitrogen reserves in *Ulva* were depleted more quickly than those of *G. tenuistipitata*. The rapid nitrogen depletion was apparent in the more dramatically increasing rates of uptake in *U. pertusa* as the algae became more nitrogen starved. It has been proposed that nutrient uptake characteristics put fast-growing, ephemeral algae at higher risk for nitrogen limitation when water column concentrations are low because they are unable to store as much nitrogen as slower growing species. Pedersen and Borum (1997) found similar results to those of Jing-Wen and Shuang-Lin (2001) in comparing ammonium uptake rates of fast-growing algae like *U. lactuca* to slower growing algae like *Fucus vesiculosus* Linnaeus. Consequently, Pedersen and Borum (1997) concluded that *U. lactuca* would become nitrogen limited under low nitrogen conditions, while *F. vesiculosus* and *Codium fragile* (Suringar) Hariot would endure better under nitrogen stress. However, in environments consistently exposed to high nutrient concentrations or frequent pulses, fast-growing species are able to take advantage of available nitrogen more quickly than larger, slower-growing species due to their high $V_{max}$ values.
The physiological difference in nutrient uptake between *Ulva* spp. and slow-growing perennial seaweed has been attributed to morphology, particularly surface area to volume ratios. Algae with high surface area to volume ratios, including the distromatic blade morphology of some *Ulva* spp., have more efficient nutrient uptake than algae with low surface area to volume ratios (Rosenberg and Ramus, 1984, Taylor *et al.*, 1998). The physiological differences in nutrient uptake efficiency between ephemeral macroalgae and slower growing algae have been hypothesized to account for the dominance of *Ulva* spp. and other opportunistic species in eutrophic environments (Rosenberg *et al.*, 1984; Rosenberg & Ramus, 1984; Fujita, 1985) and are the characteristics that can be exploited for bioremediation purposes.

The superiority of *Ulva* spp. as a biofilter for improving water quality over slower growing perennial species has been made clear by studies comparing *Ulva* spp. to other algal genera. However, there have been few investigations of possible differences in ammonium uptake kinetics between *Ulva* spp., resulting in a lack of knowledge concerning whether some taxa are better suited for bioremediation than others. Fujita (1985) compared sheet-like to tube-like *Ulva* spp. (previously *Enteromorpha*) and reported that the former took up ammonium more rapidly than the latter at various nitrogen concentrations evaluated (10-60 μM NH₄⁺). However, no studies have directly compared different distromatic *Ulva* spp. collected from sites with different nutrient histories; such a comparison would indicate if genetic differences between *Ulva* spp. influence ammonium uptake kinetics. Carpenter and Guillard (1971) compared phytoplankton strains...
of three different species from eutrophic and oligotrophic waters and found that the $K_s$ for nitrate was higher in eutrophic than oligotrophic strains of all three species. Furthermore, they discovered that these strains maintained their distinctive uptake kinetic parameters through several generations of culture under identical conditions. The results from their study indicated that there may be a genetic influence on nutrient uptake kinetics in marine algae through transfer from generation to generation. Comparisons have not been made between ammonium uptake kinetics of different Ulva generations to determine if uptake kinetics are conserved across generations, regardless of environmental conditions.

**Morphological and Molecular Identification of Ulva spp.**

Prior to the development of molecular tools for distinguishing between macroalgal species, differentiating among Ulva spp. was very troublesome. Generally cellular characteristics such as cell shape, arrangement, and pyrenoid number were used as distinguishing characters (Bliding, 1968; Koeman & van den Hoek, 1981; Hoeksema & van den Hoek, 1983), but recent evidence has shown that these characteristics are variable depending on thallus age, seasonality, salinity, and whether the blades are attached or free floating (Malta et al., 1999) and may therefore not be good indicators of species (Loughnane et al., 2008). Consequently, a number of genes have been identified that can be used to successfully differentiate between species of algae within the Ulvales. The most common genes used for species identification in Ulva spp. are the large subunit of the chloroplast encoded ribulose-bisphosphate carboxylase gene.
and the two nuclear encoded internal transcribed spacer regions (ITS1 and ITS2) plus the 5.8S gene (Blomster et al., 1998; Coat et al., 1998; Malta et al., 1999; Tan et al., 1999; Hayden & Waaland, 2002; Hayden et al., 2003; Shimada et al., 2003; Hayden & Waaland, 2004; Loughnane et al., 2008).

Because of the high mutation rates of noncoding ITS1 and ITS2 compared to the rbcL gene, the ITS regions have also been used to investigate population differences within Ulva spp. (Leskinen et al., 2004). Using molecular data to confirm species identification, it is possible to characterize the nutrient uptake kinetics of different Ulva species inhabiting the same areas. Such information will enable comparison of nutrient uptake efficiency in different Ulva species. Repeating the study for high and low nutrient locations can provide insight into how environmental nutrient conditions affect ammonium uptake kinetics.

However, the utility of molecular data only goes as far as our taxonomic knowledge of a species. In cases where species differentiation based on morphology is difficult, it is essential to have sequences from the type material of each species so that there is no question regarding the validity of sequences that correspond to each species.

The holotype material of a species is the single specimen that represents the original organism first described and named as a new species by the author. It is essential for our understanding of the species concept in taxonomic studies. In molecular systematic research, a database containing molecular sequences of diagnostic genes from type specimens is an invaluable tool for avoiding misidentifications, especially in taxonomic research involving species that are
morphologically simple and/or variable. GenBank is a database of gene sequences that is useful for species identification, but can only be used with complete confidence if sequences from the type material are available. In cases were holotype sequences are not available, it is important to attempt contacting researchers who may have sequenced holotype material of the species of interest.

Concrete species identification based on type material is essential for comparing the uptake kinetics of Ulva spp. from different areas, and it is the only way to make sure that the same specimens of a particular species are being compared rather than a collection of unknown species. It is also important to know the species present in different areas for the sake of biodiversity and conservation. There has been an increase in the number of introduced species across all macroalgae taxa in the past two decades. Although a proportion of the increase can be attributed to more frequent surveying, most of the introduced species result from anthropogenic activities such as heavy ship traffic and aquaculture activity (Ruiz et al., 2000; Ribera Siguan, 2002; Siguan, 2002). Introduced species often threaten biodiversity and ecosystem community dynamics (Carlton, 2002). Therefore, surveying and identifying species composition is essential for monitoring ecosystem health and implementing conservation strategies. It is also important to identify what Ulva spp. occur in a particular area. If there are differences in uptake kinetics, it is be important to determine the species or environmental conditions that provide the most efficient source of bioremediation.
**Purpose**

The goals of this study were four fold: (1) to assess the biodiversity of *Ulva* spp. in the Great Bay Estuarine System of New Hampshire and Maine, (2) to determine if genetic differences influence ammonium uptake by comparing uptake kinetics of field collected material to cultured offspring and by comparing uptake kinetics of different *Ulva* spp., (3) to determine if environmental nutrient history affects ammonium uptake kinetics in *Ulva* spp., and (4) to determine what *Ulva* species or populations, based on genetics, environmental nutrient history or both factors, are best suited for bioremediation. The biodiversity of *Ulva* spp. was investigated using molecular tools to differentiate between species. Based on molecularly verified species identification, intraspecific comparisons between the ammonium uptake kinetics of *Ulva* spp. exposed to different nitrogen environments were made to determine if nutrient history affects ammonium uptake kinetics of *Ulva* spp. Furthermore, interspecific comparisons between the uptake kinetics of *Ulva* spp. found in the same location within the Great Bay Estuarine System of New Hampshire and Maine, USA were made to determine if genetic differences between species influenced ammonium uptake kinetics.
Site descriptions

The Great Bay Estuarine System is located on the east coast of North America at the border of New Hampshire and Maine, USA (Figure 1). Two main bays: Great Bay and Little Bay, plus the Piscataqua River, Portsmouth Harbor and eight tributaries make up the Great Bay Estuarine System. The system contains 100 miles of shoreline and its substratum is dominated by mud but becomes rockier in the outer estuary near the open coast (Hardwick-Witman & Mathieson, 1983). Temperature, salinity, and water clarity vary within the estuary. Generally, temperature and salinity are more variable in the inner than the outer estuary close to the mouth (Emerich Penniman et al., 1985). For example, the seasonal temperature ranges at Hilton Park (-2°C-24°C) and Adams Point (-2°C-27°C), two sites 8-12 miles from the coast, are wider than at the coastal Fort Stark site (-1°C-19°C). In addition, salinity remains fairly constant at Fort Stark (27-32 psu) whereas salinity varies greatly at Adams Point (7–31 psu) and Hilton Park (1-30 psu). Water clarity also varies throughout the estuarine system. Clarity is low inland and improves closer to the open coast. Overall, salinity and water clarity decrease, but temperature range increases from the outer estuary near the open coast to sites further inland.

The present study included six sites (Figure 1) within the Great Bay Estuarine System from which Ulva spp. specimens were collected for determination of ammonium uptake kinetics. The names, locations, GPS coordinates, distances from the coast, and a short description of each site are shown in Table 1. The six sites were chosen as three pairs: Chapman's
Landing-Adams Point; Hilton Park-Oyster River; Fort Stark-South Mill Pond. Site pairs were based primarily on ammonium history and secondarily on distance from the coast. The first pair consisted of a riverine and mid-estuarine site located 12-17 miles inland, the second pair represented a riverine and mid-estuarine site 8-12 miles inland, and the third pair consisted of an outer estuarine (3 miles from the coast) and a coastal site. Within each pair, one site was considered a relatively high ammonium site and the other a low ammonium site. Site characterizations were based on nutrient data from the NOAA Office of Ocean and Coastal Resource Management National Estuarine Research Reserve System (2004) and the New Hampshire Estuaries Project (Phil Trowbridge pers. comm.; Trowbridge, 2006; Trowbridge and Jones, 2007).

*Ulva* spp. samples were collected during low tide of the six sites. When possible, each uptake experiment was conducted on samples that were collected the same day from one pair of sites. Otherwise, ammonium uptake of samples from a single site was measured, and samples from the corresponding paired site were measured soon after. The seaweed samples were collected in plastic bags and kept on ice during transport. Water samples were also collected in triplicate at each site. Seaweed samples were returned to the University of New Hampshire, rinsed in Instant Ocean, tentatively identified until further molecular verification, and exposed to different levels of ammonium concentrations for analysis of uptake kinetics.
MATERIALS AND METHODS

**Morphological and Molecular Identification of *Ulva* spp.**

Because all specimens of *Ulva* spp. collected from the six sites looked morphologically similar, tentative identifications were made at the cellular level by determining the number of pyrenoids per cell, the number and organization of starch grains, cell size and chloroplast structure. However, because within species morphological variation was almost as great as between species morphological variation, especially for material collected from different sites, molecular analysis of DNA was necessary to differentiate between species and to determine what species were being used for ammonium uptake assessment. Methods for molecular identification of *Ulva* spp. are included below.

DNA was extracted using a Puregene Tissue Extraction Kit (Gentra Systems Minneapolis, MN) from *Ulva* spp. specimens that, shortly after collection, were dried in silica gel. A 4 to 16 mm² piece of *Ulva* tissue was ground in 300 μL of Cell Lysis Solution (Puregene D-5002) in a 1.7 ml microcentrifuge tube using a pinch of sand and a disposable pellet pestle. Samples were heated at 60°C for one hour in a heat block (Fisher Scientific 2-Block Dry Bath Incubator or VWR Analog Heatblock, Henry Troemnar LLC, USA)
with an intermediate inversion step (10 times) after 30 minutes. After the samples cooled to room temperature, 100 µL of Protein Precipitation Solution (Puregene D-5003) was added to each sample. Samples were then inverted for two minutes and chilled in a freezer for one hour.

When samples thawed, they were centrifuged for 5 minutes at 13,000 rpm in a Fisher Scientific Marathon 16KM microcentrifuge (for this protocol, all centrifugation steps occurred at 13,000 rpm). The supernatant was poured into a fresh 1.7 ml tubes containing 300 µL of 100% isopropanol, which was then inverted 50 times and centrifuged for 5 minutes. The supernatant was discarded, and the pellet was washed in 300 µL of 70% alcohol. After 3 minutes of centrifugation the supernatant was again discarded and the pellet left to air dry. Samples were rehydrated overnight at room temperature (or for 1 hour at 65°C) in 25 µL of DNA Hydration Solution (Puregene D-5004). The following day (or one hour later after incubation), samples were centrifuged for 5 minutes in preparation for a polymerase chain reaction (PCR).

Following the last centrifugation setp, a 4 µL sample of rehydrated extracted DNA was added to a 46 µL mixture of Polymerase Chain Reaction (PCR) reagents (Promega Madison, WI, USA) for a final reaction volume of 50 µL (Table 2). The PCR amplification was conducted in an Eppendorf Mastercycler 5333 (Hamburg, Germany). The primers and PCR reaction profile used for amplification of the internal transcribed spacer (ITS) nrDNA region including the 5.8S gene were identical to those used by Hayden et al. (2003) and Blomster et al. (1998) and are shown in Table 3. For confirmation of the
reliability of the ITS sequences to differentiate between *Ulva spp.*, the chloroplast-encoded *rbcL* gene was also amplified in some samples. The primers used were developed by Jeremy Nettleton (pers. comm.) and are shown in Table 3.

The PCR profile consisted of a 5 minute denaturation step at 94°C, followed by a cycle of 94°C for 1 minute and 60°C for 3 minutes repeated 30 times. A 10 minute extension at 60°C took place at the end of the cycle. The PCR profile for amplification of the *rbcL* gene consisted of an initial denaturation period of two minutes at 95°C followed by a cycle that was repeated 30 times and contained the following steps: 95°C for 30 seconds, 51.5°C for 30 seconds, 72°C for 1 minute. An elongation period of 5 minutes at 72°C was the last step. Gel isolation was used to purify DNA for sequencing. Each PCR reaction was run on a 0.8% UltraPure Low Melting Point Agarose (Invitrogen Corporation, Carlsbad, CA, USA) gel in 0.5x nTBE Buffer (54 g L⁻¹ Trisbase, 27.5 g L⁻¹ boric acid, 0.2% 0.5 M solid EDTA at pH 8.0) at 100 V for 30 minutes to one hour. A BenchTop 100bp DNA ladder (Promega Madison, WI, USA) was used for size comparison. DNA bands in the agarose gel were cut out on a UV Transilluminator (model VWR M-20E, VWR Scientific, USA) using a glass coverslip and the samples were incubated at 65°C to melt the agarose and then cooled to 37°C. The amplified DNA samples were incubated with 1-1.5 μL of agarase per 100 μL of DNA at 37°C overnight.

Following DNA purification, the samples were quantified via flourometry using an Invitrogen Qubit Fluorometer (Turner BioSystems) and reagents from a
Quant-iT dsDNA BR assay kit (Invitrogen, Carlsbad, CA). Based on the DNA quantity, samples were diluted to the appropriate concentration for sequencing, which was done by the University of New Hampshire Hubbard Center for Genome Studies Sequencing Core Facility using Applied Biosystems BigDye Terminator Cycle Sequencing Kits (v1.1 and v3.1) and an ABI 3130 DNA Analyzer. The DNA sequences received from the sequencing facility were edited and made contiguous using SeqMan Pro 7.2.1 and aligned using Megalign 7.2.1 (both from DNASTAR Lasergene, Inc, Madison, WI, USA). Sequences obtained in this study were aligned with those retrieved using the Blast feature in Megalign 7.2.1 that extracted sequences that were the closest match from the National Institute of Health (NIH) genetic sequence database, GenBank. The sequences used for comparison are shown in Table 4. Sequence data were analyzed by the maximum likelihood and neighborjoining methods in PAUP *4.0b10 (Sinauer Associates, Inc. Sunderland, MA) as well as by the baysian analysis method using the downloadable software MrBayes version 3.1.1. The tree was rooted with two outgroups using *Umbraulva olivascens* (P. J. L. Dangeard) G. Furnari and *Blidingia minima* (Nägeli ex Kützing) Kylin. The strength of the phylogeny was assessed using posterior probability values in the baysian analysis and bootstrap values from 500 sample replications for maximum likelihood, and 1000 replications for neighborjoining at a 50% confidence level.
Non-Specific Ammonium Uptake

Prior to molecular analysis of *Ulva* spp., ammonium uptake experiments were conducted on collected material that most likely consisted of multiple species of *Ulva* because the presence of multiple species was not realized at the time. Following sample collection, circular disks 2 cm in diameter were cut from *Ulva* blades and allowed to acclimate for 1-3 hours prior to the experiment in ammonium-free water with turbulent bubbling at 18°C and 150 μmol·m⁻²·s⁻¹ light intensity. Two disks were placed into 1.7 ml centrifuge tubes immediately after specimens were collected for protein and pigment analysis (see below). Using circular disks in growth, ecological, and reproductive studies of *Ulva* spp. is common (Burrows, 1971; Malta *et al.*, 1999; Hernández *et al.*, 2008; Lüning *et al.*, 2008), but an acclimation period was allotted in the present study to allow for wound recovery after cutting. Thus, the contents from those cells along the periphery of the disks that were lysed were able to leech into the medium and away from the disks prior to analysis of ammonium uptake.

The experimental design for the initial non-specific ammonium uptake experiments consisted of 24 (125 ml) treatment flasks filled with sterilized, ammonium-free seawater that was spiked with ammonium to one of four concentrations (25, 50, 150, 250μM NH₄⁺) and enriched with nitrogen-free Von Stosch medium (Table 5). Twelve flasks were designated for algae from each of the two paired sites examined, and each of the four ammonium concentrations
were replicated three times. All flasks were illuminated at 150 \( \mu \text{mol.m}^{-2}.\text{s}^{-1} \) light intensity with fluorescent bulbs. The initial ammonium concentration in each flask was determined by taking a 1 ml water sample prior to the addition of algae. Then, ten \textit{Ulva} disks from multiple blades were added to each flask and a 1 ml water sample was taken immediately after the algae was added and constituted the time 0 water sample. Flasks were shaken at 195 rpm so that the disks were continuously moving throughout the water column, thereby reducing the boundary layer. In early experiments, a 1 ml water sample was taken from each flask at 6 minute intervals for 30 minutes.

Uptake rates were calculated using the following equation, as adopted from (Pedersen, 1994),

\[
V = (S_0 \times v_0) - (S_t - v_t) \\
\times t \times A
\]

where \( V \) = uptake rate (\( \mu \text{mol NH}_4^+ \cdot \text{cm}^{-2} \cdot \text{hr}^{-1} \)), \( S_0 \) = substrate concentration of the initial sample (\( \mu \text{M} \)), \( v_0 \) = initial sample volume (L), \( S_t \) = substrate concentration of successive sample, \( v_t \) = volume of successive sample, \( t \) = time elapsed between successive samples (hr), and \( A \) = surface area of \textit{Ulva} spp. tissue in the sample (cm\(^2\)).

**Species-Specific Ammonium Uptake**

The realization of the presence of multiple species of \textit{Ulva} in the Great Bay Estuarine system lead to modified ammonium uptake experiments as described below so that comparisons could be made between known \textit{Ulva} spp. after molecular analyses. Blades of \textit{Ulva} spp. were collected during the fall of
2008 from only one site per experiment. If possible, individuals occupying
different habitats were collected in order to increase the chance of collecting
more than 1 species. Blades were rinsed in Instant Ocean and observed for
morphological differences. Six blades were chosen for each uptake experiment.
The blades were chosen based on morphological variations that indicated the
presence of different species and allowed for replication of different species
present. Four disks were cut from each blade and a small sample was dried in
silica gel for molecular determinations. Herbarium specimens were made with
the remaining part of each blade after the disks were extracted.

Four disks from each specimen were acclimated in 400 ml of nitrogen-free
Von Stosch artificial sea water for approximately one hour under 100 \text{ \mu mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}
light intensity and at 5°C. Following acclimation, each disk was placed into a
separate flask containing 50 ml of aerated artificial seawater enriched with
nitrogen-free Von Stosch medium at one of four ammonium concentrations (25,
50, 150, 250 \text{ \mu M NH}_4^+). A 1 ml water sample was taken immediately following
addition of the disks to each flask. \textit{Ulva} spp. disks were exposed to these
ammonium treatments for two hours, after which a final 1 ml water sample was
taken for ammonium determination. Water samples were frozen for later
ammonium analysis using the phenol-hypochlorite method of ammonium
determination and ammonium uptake rates were calculated as described above.

Once molecular analysis of the ITS1 and ITS2 genes indicated the
species of each blade, data from the same species were combined to create an
ammonium uptake curve for each species. The ammonium uptake curves were
fitted to the Michaelis-Menten equation \[ V = \frac{V_{\text{max}} \cdot S}{K_s + S} \] for substrate uptake kinetics by nonlinear regression analysis in SYSTAT version 10 (Systat Software Inc., Chicago, IL, USA), and the \( V_{\text{max}} \), \( K_s \), and \( \alpha \) (\( V_{\text{max}} : K_s \) ratio) values were used to describe the ammonium uptake kinetics of \( Ulva \). Intraspecific and interspecific comparisons between ammonium uptake curves of \( Ulva \) from paired sites (high and low ammonium) were made using an F-test for comparing two nonlinear regressions that consisted of the following equation:

\[
F = \frac{\{SSE_1 \& 2-(SSE_1+SSE_2)\}/((m+1)*(k-1))}{(SSE_1+SSE_2)/((n1-m-1)+(n2-m-1))}
\]

where SSE = sum of squares of the residual for each model, \( m = \# \) of parameters in the model, \( k = \# \) of models, \( n = \# \) of observations within each group (Zar, 2009). The same method was used to make interspecific comparisons between ammonium uptake curves of different \( Ulva \) spp. found at the same site.

**Culture Development and Ammonium Uptake**

Development of \( Ulva \) spp. in culture was attempted for comparison of ammonium uptake kinetics of cultured material to field collected material as an additional method for determining if there was a genetic influence on ammonium uptake kinetics. To initiate reproductive tissue development, \( Ulva \) spp. specimens were placed between two damp paper towels and left in the refrigerator for 24-48 hours (Levine and Wilce, 1980). Circular disks 2 cm in diameter were then cut from several blades and placed in petri dishes containing
sterilized artificial seawater (ASW) enriched with Von Stosch enrichment media (Ott, 1966; Levine and Wilce, 1980; Sahoo and Yarish, 2005). High ammonium concentrations were added to induce swarmer release (Chapman, 1973). The petri dishes were shaken at 18°C on a long day light cycle until swarmers were released.

A light source was administered to the surface of the water containing swarmers to encourage their movement towards the water surface. A Pasteur Pipet (thinned with a Bunsen burner) was touched to the surface of the water to facilitate capillary transfer of a small water sample containing swarmers to another petri dish filled with fresh enriched SW (Chapman, 1973; Kawai et al., 2005). Swarmer transfer was repeated several times to ensure enough were collected. Glass cover slips were placed in the petri dishes containing swarmers to facilitate settling and development. Once germlings developed to a transferable size, cultures were thinned to prevent self-shading and transferred to 125 ml flasks with turbulent bubbling. Enriched ASW was changed weekly. Ammonium uptake of cultured material was measured as described above in the nonspecific ammonium uptake section.

**Water and Tissue Analysis**

Determination of ammonium concentration in water samples collected during ammonium uptake experiments was conducted via the method described by Grasshoff et al. (1983). The following prepared reagents were added to 1 ml water samples collected from treatment flasks in 1.7 ml centrifuge tubes: 40 μL
phenol reagent, 20 μL tri-sodium citrate solution, and 40 μL hypochlorite reagent. The water samples were mixed thoroughly, incubated for 30 min. at 37-40°C in a Lab-Line Imperial II Incubator (Lab-Line Instruments Inc., Melrose Park, IL, USA) and then cooled for 30 min. at room temperature. Nitrogen-free artificial seawater was used as a reference for all samples. Ammonium concentrations were determined colorimetrically at 630 nm using an Unicam Helios α UV-VIS Spectrophotometer (Cambridge, UK).

Physiological characteristics of *Ulva* spp., including protein and pigment content, and blade color were assessed in order to compare algae collected from sites with high and low ammonium histories. Circular disks 2 cm and 8 mm in diameter were cut from fresh *Ulva* blades and frozen in 1.7 ml centrifuge tubes for protein and pigment analysis, respectively. Tissue protein content in six *Ulva* spp. blades from each site was determined using Bradford Reagent (Sigma-Aldrich Co. St. Louis, MO, USA) and the standard Bradford Protein Assay Protocol (Bradford, 1976). Approximately 50-75 g (fresh weight) of algal tissue was ground in 0.1M PO₄³⁻ buffer (4.5 g NaH₂PO₄ anhydrous, 22.4 g Na₂HPO₄·12H₂O per liter) using a pinch of sand and a porcelain mortar and pestle. Samples were ground in 500 μL of 0.1M PO₄³⁻ buffer and then rinsed twice to ensure that all material was recovered, resulting in a final extraction volume of 1500 μL. Samples were centrifuged for 1 min. in a Fisher Scientific micro-centrifuge model 235C. A 100 μL aliquot of supernatant was removed from each extraction sample followed by the addition of 3 ml of Bradford Protein Solution to each. After waiting 5 min. for the reaction to proceed, the absorbance
of each sample was read at 595 nm in an Unicam Helios α UV-VIS Spectrophotometer (Cambridge, UK). Standards were prepared in 0.1M PO₄³⁻ buffer with the following amounts of Bovine Serum Albumin (Sigma-Aldrich Co. St. Louis, MO, USA): 0, 10, 20, 30, 40, and 50 μg. Protein contents of Ulva spp. specimens from high and low ammonium sites were compared to determine if there was a correlation between ammonium history and protein tissue content. Mean tissue protein content of Ulva spp. was compared between sites using a one-way ANOVA in SYSTAT 10 (Systat Software Inc., Chicago, IL, USA).

The chlorophyll and carotenoid contents in Ulva spp. tissue was determined via ethanol extraction. Ulva spp. disks 8 mm in diameter were ground in 2 ml of 95% ethanol with a pinch of sand. Samples were centrifuged for one minute in a Fisher Scientific micro-centrifuge model 235C and the supernatant was decanted to a 5 ml PLASTIBRAND standard disposable cuvette (Sigma-Aldrich) for absorbance measurements at three separate wavelengths (470, 649, 664 nm) using a Unicam Helios α UV-VIS Spectrophotometer. Total chlorophyll (a + b), chlorophyll a, chlorophyll b, and carotenoid content with respect to tissue surface area were calculated from the following equations, as presented by (Lichtenthaler, 1987).

**Total chlorophyll** a + b = 22.24 x Abs₆₄₉ + (5.24 x Abs₆₆₄)

**Chlorophyll a** = 13.36 x Abs₆₆₄ - (5.19 x Abs₆₄₉)

**Chlorophyll b** = 27.43 x Abs₆₄₉ - (8.12 x Abs₆₆₄)

**Total Carotenoids** = 4.8 x Abs₄₇₀ - (12.7 x Abs₆₄₉) + (3.65 x Abs₆₆₄)
Mean chlorophyll a:b ratios and chlorophyll:carotenoid ratios of *Ulva* specimens were compared between sites using a one-way ANOVA in SYSTAT 10 (Systat Software Inc., Chicago, IL, USA).

Blade color of field collected *Ulva sp.* blades was measured with an X-Rite Color Digital Swatchbook and Color Shop 2.6 (San Rafael, CA) soon after collection. Blade color was determined according to the CIE Lab color scale, and samples were compared based on their location on the three dimensional Lab axis. The “L” dimension ranged from 0-100, with 100 = white and 0 = black. The “a” dimension represented the spectrum between green (more – a) and red (more + a), whereas the “b” dimension represented the spectrum between blue (more – b) and yellow (more + b). Comparisons of blade color of *Ulva* from paired sites based on the three dimensional CIE Lab color scale were made by discriminate analysis in SYSTAT 10 (Systat Software Inc., Chicago, IL, USA).
RESULTS

Morphological and Molecular Identification of *Ulva* spp.

Molecular analysis of the ITS regions, (ITS1 and ITS2) including the 5.8S rDNA gene, from field collected *Ulva* spp. specimens revealed four distinct haplotypes that matched sequences on Genbank that had been identified as four separate species: *Ulva lactuca*, *U. pertusa*, *U. rigida*\(^1\) C. Agardh, and *U. compressa*\(^2\) Linnaeus (Figure 5, Table 4). Cellular characteristics supported the molecular identification of four different taxa and correspond with the matching sequences on GenBank. Maximum likelihood phylogenetic analysis of representative sequences from each collection site resulted in a phylogenetic tree with four distinct groups strongly supported by bootstrap values (97-100%; Figure 5).

\(^1\) *Ulva rigida* and *U. scandinavica* are synonyms. The sequences used for comparison with sequences collected in the present study were labeled as *U. scandinavica*, but *U. rigida* is the older name so it is used when describing specimens collected during the present study.

\(^2\) ITS sequences obtained in the present study matched sequences in Genbank labeled *Ulva pseudocurvata* Koeman & Hoek and *Ulva compressa* (Table 4). However, evidence suggests the former was from a specimen mistakenly identified as *U. pseudocurvata* that was actually a distromatic morphotype of *U. compressa*, which is the name assigned to the sequences collected in the present study.
The morphological variation between specimens collected from The Great Bay Estuarine System was large, so it was difficult to make accurate identifications at the cellular level. Figures 2 and 3 show the variability of blade morphology and cell structure, respectively, among all Ulva spp. collected in this study. Figures 4-7 show representative specimens for each of the four taxa found in the Great Bay Estuarine System, and all specimens collected during the present study are described in the Appendix. Some cellular characteristics were helpful for species differentiation, mainly pyrenoid number and chloroplast structure, but molecular analysis was required for definite identification of Ulva spp. due to the high morphological variability within species.

*Ulva compressa*³ blade morphology was extremely variable (Figures 2, 4). Its chloroplasts filled the entire cell, but the presence of obvious starch grains sometimes prevented observation of the chloroplast and the determination of pyrenoid number (Figure 3). When pyrenoids were visible, I found many cells with multiple pyrenoids. *Ulva rigida* thalli were generally irregularly shaped, dark green in color and had a relatively rigid texture (Figures 2, 5). *Ulva rigida* specimens were difficult to differentiate from *U. compressa* because their blade morphology was similar. *Ulva rigida* cells were relatively large and contained parietal chloroplasts with multiple pyrenoids (Figure 3). The characteristic “teeth”

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³ *Ulva compressa* specimens resembled what is described as *Ulva pseudocurvata* in Maggs et al. (2007), hence mistaken identification if the distromatic morphotype of *U. compressa* as *U. pseudocurvata* described in footnote 2.
on the blade margin of this species were not observed. Usually, *U. lactuca* Linnaeus specimens were round with ruffled margins (Figures 2, 6) and had the characteristic lobed parietal chloroplast with a single pyrenoid, but multiple pyrenoids were often present (Figure 3). In surface view, *Ulva pertusa* looked similar to *U. lactuca* because the chloroplasts were confined to one side of the cell, but most cells were larger and had multiple pyrenoids (Figure 3). *Ulva pertusa* blades were often perforated with many holes (Figures 2, 7), and I encountered reproductive *U. pertusa* cells shown in Figure 8 that formed a wide reproductive margin.

The distribution of different *Ulva* spp. varied geographically. *Ulva lactuca* and *Ulva pertusa* were the most widely distributed, occurring at Adams Point, Hilton Park, Fort Stark, and South Mill Pond (Figures 9 & 10). Both species were always found coexisting. *Ulva rigida* was found at Adams Point, South Mill Pond, and Oyster River. *Ulva compressa* was found only at two sites: Oyster River and Adams Point (Figures 9 & 10). Adams Point, a low ammonium site, had the highest species diversity, while South Mill Pond, a high ammonium site, had the second highest species diversity. No material was found at Chapman’s Landing when collections were made for molecular identification.

**Environmental Influence on Ammonium Uptake Kinetics**

A control experiment conducted with flasks containing water spiked to four ammonium concentrations and no *Ulva* spp. blades showed no ammonium depletion over time (data not shown), while its depletion occurred when blades
were present. Because there may have been more than one *Ulva* spp. in the non-specific ammonium uptake experiments, only ammonium uptake data from Fort Stark and South Mill Pond compared with ammonium uptake data from South Mill Pond culture material are reported. Results showed a significant difference in ammonium uptake kinetics for the non-specific ammonium uptake experiments only between Fort Stark and South Mill Pond (*p*=0.044; Figure 11). The $V_{\text{max}}$ and $K_s$ for *Ulva* spp. from Fort Stark (0.60 ± 0.17 μmol N·cm$^{-2}$·hr$^{-1}$, 110.2 ± 54.6 μM) were higher than for *Ulva* spp. from South Mill Pond (0.31 ± 0.036 μmol N·cm$^{-2}$·hr$^{-1}$, 40.7 ± 12.97 μM). The remaining sites did not show a significant difference in ammonium uptake kinetics curves and therefore are not shown.

Modified ammonium uptake experiments that accounted for the presence of multiple species (i.e. species-specific ammonium uptake experiments) allowed for intraspecific comparisons of the ammonium uptake kinetics of the same *Ulva* spp. from sites with different nutrient histories. A summary of ammonium uptake kinetics values for all *Ulva* spp. and sites evaluated is shown in Table 6. Fort Stark and South Mill Pond were the only two paired sites in which the same species (*U. lactuca* and *U. pertusa*) were found. Hence, the ammonium uptake kinetics of these two species could be compared between the two sites. Curve comparison of the nonlinear regression analysis showed that there was no significant difference between the ammonium uptake kinetics of *U. lactuca* from Fort Stark and South Mill Pond (Figure 12 *p*=0.65). The $V_{\text{max}}$ and $K_s$ for *U. lactuca* from Fort Stark were 0.34 ± 0.284 μmol N·cm$^{-2}$·hr$^{-1}$, 72.1 ± 208.88 μM.
and 0.19 ± 0.01 μmol N·cm⁻²·hr⁻¹, 27.6 ± 8.7 μM for South Mill Pond. There was a large discrepancy between the $K_s$ values for *Ulva* spp. and *U. lactuca* from South Mill Pond, suggesting that there may have been multiple species present in the initial ammonium uptake experiment.

Comparison between ammonium uptake curves of *U. pertusa* from Fort Stark and South Mill Pond showed no significant differences in uptake kinetics (Figure 12). Fort Stark specimens had a $V_{\text{max}}$ of 0.26 ± 0.076 μmol N·cm⁻²·hr⁻¹ and a $K_s$ of 93.8 ± 77.6 μM, compared to specimens from South Mill Pond that had a $V_{\text{max}}$ of 0.15 ± 0.31 μmol N·cm⁻²·hr⁻¹ and a $K_s$ of 18.9 ± 22.9 μM.

**Genetic Influence on Ammonium Uptake Kinetics**

The ammonium uptake experiments conducted with single *Ulva* spp. blades (i.e. species-specific ammonium uptake) also allowed for interspecific comparisons to be made between uptake kinetics of algae from the same study site. No significant difference was found between uptake kinetic curves of *U. lactuca* and *U. pertusa* from Fort Stark (Figure 13).

Three species of *Ulva* were found at South Mill Pond: *U. lactuca*, *U. pertusa*, and *U. rigida*. The $V_{\text{max}}$ was 0.19 ± 0.01 μmol N·cm⁻²·hr⁻¹ for *Ulva lactuca*, 0.15 ± 0.031 μmol N·cm⁻²·hr⁻¹ for *U. pertusa*, and 0.11 ± 0.005 μmol N·cm⁻²·hr⁻¹ for *U. rigida*. $K_s$ values were 27.6 ± 8.7, 18.9 ± 22.9 and 7.8 ± 4.0 μM NH₄⁺, respectively. An F test comparing the three uptake curves showed no significant difference ($p=0.441$), but when *U. rigida* and *U. lactuca* were
compared independently of *U. pertusa*, there was a significant difference between the two ammonium uptake curves (Figure 14; p=0.011).

*Ulva pertusa* and *U. lactuca* plants from Hilton Park showed no significant difference in ammonium uptake curves (Figure 15; p=0.33). The $V_{\text{max}}$ and $K_s$ values for *U. pertusa* were 0.21 μmol N·cm$^{-2}$·hr$^{-1}$ and 19.2 μM, respectively. The $V_{\text{max}}$ and $K_s$ values for *U. lactuca* from Hilton Park were 0.30 μmol N·cm$^{-2}$·hr$^{-1}$ and 20.3 μM, which were very similar those of *U. pertusa* from Hilton Park. Only one species of *Ulva* was collected from the Oyster River site, so no interspecific comparisons can be made. The $V_{\text{max}}$ and $K_s$ values for *U. compressa* from Oyster River were 0.14 μmol N·cm$^{-2}$·hr$^{-1}$ and 35.2 μM, respectively (Figure 16). Although no similar species of *Ulva* were found at Hilton Park and Oyster River, there was a significant difference in ammonium uptake kinetics between *U. lactuca* from Hilton Park and *U. compressa* from Oyster River (p=0.023). *Ulva lactuca* had a $V_{\text{max}}$ twice that of *U. compressa* (0.30 vs. 0.14 μmol N·cm$^{-2}$·hr$^{-1}$) and a lower $K_s$ (20.3 vs. 35.2 μM).

*Ulva rigida* and the distromatic blade form of *U. compressa* were both found at Adams Point and they showed significant differences in ammonium uptake curves (p=0.035). The $V_{\text{max}}$ and $K_s$ values for *U. rigida* from Adams Point were $0.12 \pm 0.034$ μmol N·cm$^{-2}$·hr$^{-1}$ and $10.9 \pm 27.6$ μM, respectively, verses $0.16 \pm 0.014$ μmol N·cm$^{-2}$·hr$^{-1}$ and $14.2 \pm 11.8$ μM for *U. compressa* from Adams Point (Figure 17). As no *Ulva* spp. were present at Chapman’s Landing during the collection period for the single blade ammonium uptake experiments, no uptake kinetics data were summarized from this site. Despite the variability in
ammonium uptake kinetics among different species and between different sites, all four taxa consistently showed decreased ammonium uptake rates at substrate concentrations greater than 200 μM, indicating that ammonium toxicity occurred beyond this concentration.

Culture Development and Ammonium Uptake

South Mill Pond specimens were the only algae successfully grown in culture to a large enough size for measuring ammonium uptake. The ammonium uptake curve for South Mill Pond cultures is shown in Figure 18. The $V_{\text{max}}$ was 0.24 μmol ± 0.045 N·cm⁻²·hr⁻¹ and the $K_s$ was 33.1 ± 20.96 μM. Molecular analysis of the culture specimens indicated that most of them were *U. lactuca*, but some were *U. rigida*. As a result, the ammonium uptake data were a combination of *U. lactuca* and *U. rigida* data.

Comparison of the ammonium uptake kinetics of South Mill Pond cultures and the field collected material showed no significant difference based upon an F test for nonlinear regression analysis (p=0.55). There was also no significant difference between South Mill Pond cultures and Fort Stark specimens (p=0.11). The culture studies were unsuccessful for all other sites.

Tissue Analysis

The protein content in *Ulva* spp. tissue varied seasonally between June to September (Figure 19). Typically, protein content was greatest in August and lowest in September with the exception of South Mill Pond where maximum values occurred in June. Some of the paired sites showed a significant
difference in tissue protein content depending on months of collection. In June, the average tissue protein content of *Ulva* spp. from South Mill Pond (5639.5 ± 418.6) was significantly greater (p = 0.000043) than that from Fort Stark (2081.6 ± 418.6) (Figure 20). In addition, samples from South Mill Pond had the highest tissue protein content compared to all other sites. There were no collections made from Hilton Park or Chapman's Landing in June, so comparisons between the other two site pairs could not be made.

The tissue protein contents at different sites were most variable in July (Figure 21). Adams Point samples had higher protein contents (3243.0 ± 354.6) than Chapman's Landing samples (1708.6 ± 354.6), but the trend was not significant (p = 0.054). The other paired sites did not show significant differences in protein content, but specimens from South Mill Pond had the highest tissue protein content compared to all other sites. No significant difference was found in protein tissue content between any sites during the month of August, although South Mill Pond samples continued to have the highest protein levels (Figure 22). September samples of *Ulva* spp. also showed no significant difference between any of the paired sites, and this was the only month in which South Mill Pond samples had lower tissue protein than those from Fort Stark, which showed the highest protein content for September compared to the other sites (Figure 23).

Both chlorophyll and carotenoid content were measured in *Ulva* spp. during August and September. Chlorophyll a and b content were both higher in August than in September for Hilton Park, whereas only Chlorophyll a differed between August and September for Adams Point (Figure 24). Hilton Park and
Oyster River showed a significant difference in chlorophyll a:b and carotenoid content, but this was only significant in September (Figure 25). *Ulva* spp. from Hilton Park had significantly higher chlorophyll a:b ratios (1.39 ±0.06; p<<0.05) and total carotenoids (0.29 μg·cm⁻² ± 0.07; p=0.03) than specimens from Oyster River in September (1.12 ± 0.06; 0.16 μg·cm⁻² ± 0.07). Monthly differences were apparent between *Ulva* spp. from Fort Stark for chlorophyll a:b ratios (p=0.004), but not for total carotenoid content. Chlorophyll a:b was higher in August (1.47 ± 0.04) than in September (1.16 ± 0.06). In addition, *Ulva* spp. from Adams Point showed significantly higher carotenoid contents in August (0.43 μg·cm⁻² ± 0.06; p=0.03) than September (0.09± 0.07).

When chlorophyll and carotenoid content data were related to each other, a different pattern was observed. There was a significant difference (p = 1.7 E -10) in mean chlorophyll:carotenoid ratios between *Ulva* spp. collected from the sites farthest from the coast (Chapmans Landing, Adams Point, Oyster River) verses the mid and outer estuarine sites close to the coast (Hilton Park, South Mill Pond, Fort Stark). The inland estuarine sites had significantly higher chlorophyll:carotenoid ratios than the outer estuarine sites (Figure 26).

Blade color analysis of *Ulva* spp. using the X-Rite Color Digital Swatchbook was consistent with the pigment analysis. A MANOVA conducted on the blade color analysis data revealed that there was a significant difference in blade color between specimens from Oyster River and Hilton Park (p=0.0000; Figure 27). Hilton Park specimens were lighter in color (higher on the “L” axis) and more yellow-green (more positive on the “b” axis, more negative on the “a”
axis, respectively) than the Oyster River samples. No other paired sites showed significant differences in blade color.
DISCUSSION

**Morphological and Molecular Identification of *Ulva* spp.**

Several studies have reported that morphological features are not reliable characteristics to use for species identification in *Ulva* (Coat *et al.*, 1998; Malta *et al.*, 1999; Woolcott & King, 1999; Shimada *et al.*, 2003; Loughnane *et al.*, 2008). The use of ITS regions for species identification has been proposed as a solution for this identification problem (Tan *et al.*, 1999; Hayden *et al.*, 2003; Shimada *et al.*, 2003; Lopez *et al.*, 2007). In the present study, *Ulva* specimens were successfully differentiated using ITS sequences. The combination of morphological and molecular characteristics employed here revealed four different *Ulva* taxa from The Great Bay Estuarine System. *Ulva lactuca* was the only distromatic species previously known from this area (Mathieson & Hehre, 1986; Mathieson & Penniman, 1986; Bohnsack, 2003). Thus, two new taxa, *U. rigida* and *U. pertusa*, and the foliose morphology of *U. compressa* are confirmed from this study. Although *U. lactuca* is a common species found in the estuary, the concept of *U. lactuca* is presently changing, and the species identified in this study will probably be renamed in the near future (C. Maggs and F. Mineur, pers. com.).
The tubular form of *U. compressa* is common within The Great Bay Estuarine System. The distromatic blade morphotype has previously been reported in other areas and is apparently not as rare as formerly thought (F. Mineur, pers. comm.). Previously the distromatic form of *U. compressa* has been confused with *U. pseudocurvata* due to similarities in morphology and cellular characteristics. Because of this confusion, the ITS sequences on GenBank from Hayden *et al.* (2003) and Hayden and Waaland (2004) are misidentified as *U. pseudocurvata* when they are actually sequences from a distromatic *U. compressa* (F. Mineur, pers. comm.). Due to misidentification of distromatic *U. compressa*, the species was at one point synonymized with *U. pseudocurvata* (Tan *et al.*, 1999; Hayden *et al.*, 2003; Hayden and Waaland, 2004). However, despite the morphological similarities between *U. pseudocurvata* and the distromatic *U. compressa*, the two species are not closely related (Maggs *et al.*, 2007).

The factors controlling the development of *Ulva compressa* into one morphological form or the other are unknown. Blomster *et al.* (1998) reported that the morphologies of *U. compressa* and *U. intestinalis* (reported as *Enteromorpha* spp.) varied depending on the salinity of their environment. Low salinity or salinity shock was correlated with branch development in *U. intestinalis*, which complicates differentiation between the two species because the tubular form of *U. compressa* is also branched. Furthermore evidence for the influence of salinity on morphological development in *Ulva* spp. is provided by Tan *et al.* (1999) who suggested that salinity may influence morphological
development in *U. compressa*. They reported that the *U. pseudocurvata* specimens (probably distromatic *U. compressa*) were collected from the upper Ythan Estuary (Scotland) close to the freshwater source, whereas the tubular *U. compressa* specimens occurred in more coastal sites near the mouth of the estuary. In this study, the distromatic form of *U. compressa* was found at the Adams Point and the Oyster River sites, both of which are more than 10 miles inland from the coast and have relatively low salinity compared to coastal sites. No samples of *U. compressa* were collected from the lower estuarine sites, and this may be because the species was present in its tubular form at higher salinity sites and not collected for this study.

Mathieson and Hehre (1986) and Mathieson and Penniman (1986) reported *U. compressa* (reported as *Enteromorpha comrpessa*) as a cosmopolitan species in the Great Bay Estuarine system as it occurred both in open coastal and estuarine sites. The species was recorded at Colony cove, Crommett Creek, and the Oyster River tidal headwater in Durham, NH. The salinity range of all three sites is wide, and both Crommett Creek and the Oyster River sites can exhibit freshwater conditions. The distromatic morphotype of *U. compressa* was also found near the tidal headwater of the Oyster River in the present study, so it is possible that the two morphotypes are coexisting, in which case the influence of salinity on morphological development might not be the best explanation for the existence of the two morphotypes. However, due to the fact that *U. intestinalis* can exhibit branching under low salinity conditions (Blomster *et al.*, 1998), those species reported as *U. compressa* from the Oyster
River tidal headwater (Mathieson and Hehre; Mathieson and Penniman, 1986) may have been branched forms of *U. intestinalis*. Molecular investigation of freshly collected as well as herbarium specimens should be conducted following this study in order to determine whether or not the two morphotypes of *U. compressa* are co-inhabiting low salinity sites in the Great Bay Estuarine System. If they do not coexist, the variable morphology found within *U. compressa* provides further support for Tan *et al*.’s (1999) hypothesis that there may be reversible genetic switches controlling morphological development in *Ulva*, and they may be influenced by salinity in some cases.

*Ulva rigida* is a common species found on the east coast of North America from Newfoundland, Canada to Long Island Sound, New York. However, it has not been reported from the coasts of Massachusetts or New Hampshire (Sears, 2002). In the present study, it was found at several sites in the lower and upper Great Bay Estuarine System. The ITS sequences of *Ulva rigida* specimens collected in this study matched exactly the *U. scandinavica* sequence recorded by Shimada *et al*. (2003) (Accession # AB097659), but they differed by five base pair substitutions compared to the *U. rigida* sequences submitted by Hayden *et al*. (2003) and Tan *et al*. (1999). Previous studies have shown analogous differences between the ITS sequences of *U. rigida*, *U. scandinavica*, and *U. armoricana*, which are three very closely related and morphologically similar taxa that have been synonymized with *U. rigida* (Maggs *et al*., 2007). Coat *et al*. (1998) found six base pair substitutions between *U. armoricana* and *U. rigida*. Lougnane *et al*. (2008) and Malta *et al*. (1999) found small differences in ITS
sequences in rbcL and ITS sequences between the three taxa, but both showed that they were very closely related. Maggs et al. (2007) reported that the intraspecific variation in ITS sequences is similar to the interspecific variation between the three groups, so the three taxa are considered synonymous. There was little to no intraspecific variation in ITS sequences among the specimens collected in this study.

*Ulva pertusa* specimens collected in this study usually contained holes that were useful in distinguishing it from other *Ulva* spp. The holes are described as resulting from morphological development rather than grazing, a common cause of holes in other *Ulva* spp. (Shimada et al., 2003; Lopez et al., 2007). The holes in *U. pertusa* specimens observed here were usually relatively small and not as extensive as those reported for older specimens. *Ulva pertusa* is a species of Asian origin with a distribution that includes the North and South Pacific and the coasts of South Africa and Europe (Guiry & Guiry, 2009). It is recently reported in Europe by López et al. (2007). The method of introduction is unknown, but is assumed to have been from Asian oyster aquaculture (Verlaque, 2001; Verlaque et al., 2002; Lopez et al., 2007). The species was reported off the cost of Brittany, France and in the Mediterranean Sea in 1994 (López et al., 2007 found it was misidentified as *U. rotundata* in Coat et al., 1998; Varlaque, 2002) and on the Iberian Peninsula in 2007, but it is thought to have been there since at least as far back as 1990 (López et al., 2007). It has also been reported in the Netherlands (Stegenga et al., 2007).
The present study provides the first report of *U. pertusa* from the Northwestern Atlantic Ocean. Mathieson et al. (2008) clarified and molecularly verified the presence of 20 introduced seaweed species in the Northwest Atlantic, two of which were green algae. The presence of *U. pertusa* in the Great Bay Estuarine System adds an additional introduced species to the list, resulting in the presence of 3 introduced species of green algae out of 21 total species in the Northwest Atlantic.

*Ulva pertusa* was found at four sites in The Great Bay Estuarine System, always in association with *U. lactuca*. Despite its wide ecological range, it seemed to be more successful in the lower than the upper estuary. Figure 28 shows *U. pertusa* and *U. lactuca* growing epiphytically on the same blade of *Chondrus crispus* Stackhouse from Hilton Park, revealing that the two species share similar niches but can still coexist. Thus *U. pertusa* has a wide estuarine distribution and can tolerate a broad range of salinities, but it does not seem to be outcompeting *U. lactuca* at Hilton Park based on frequent collections of both species. Nevertheless, more thorough and continuous investigations of the biodiversity of *Ulva* spp. should be made in the future to monitor the distribution and potential spread of *U. pertusa* in the Northwest Atlantic due to the fact that it is a non-native species.

The results of this study, in conjunction with several others (Malta et al., 1999; Tan et al., 1999; Loughnane et al., 2008) provide further evidence that morphological characteristics are not always reliable to make definitive species identifications of *Ulva* thalli with distromatic blades. Thus, molecular data are
essential for ensuring correct species identification, which are crucial for accurate biodiversity measurements, monitoring of introduced species, and sound ecological and physiological studies.

For molecular data to be most significant every effort must be made to sequence type material or at least samples from type locations. Taxonomic research relies on holotype specimens for the delineation of current and new taxa throughout the world. GenBank provides sequences for reference, but some are misidentified due to the lack of holotype sequences. The confusion caused by misidentified sequences is a clear indication that sequencing holotype material is an important pursuit that should be continued because it alleviates much confusion in the taxonomic research world.

**Environmental Influence on Ammonium Uptake Kinetics**

The ammonium uptake rates observed in this study were comparable to those documented by Taylor *et al.* (1998) who measured uptake rates of *Ulva* spp. relative to surface area rather than weight as in the present study. The mean ammonium uptake rate for *Ulva* spp. exposed to 10 μM ammonium reported in their study was 0.025 μmol·cm⁻²·h⁻¹. The lowest ammonium treatment used in my study was 25 μM, with uptake rates generally two to three times higher than those reported by Taylor *et al.* (1998) at 10 μM ammonium. Because the relationship between ammonium uptake and ammonium concentration is basically linear at low ammonium concentrations, the higher
ammonium uptake rates observed in this study at 25 μM ammonium are comparable to those reported by Taylor et al. (1998).

The range of $K_s$ previously reported for Ulva spp. was variable, while the values observed in my study were at the high end of this range. Runcie et al. (2003) also observed high $K_s$ values for U. lactuca relative to other studies (Pedersen, 1994; Campbell, 1999), but they reported that the lower $K_s$ values were a result of longer incubation times. In contrast to this claim, my study consisted of relatively long incubation times (2 hours) and high $K_s$ values were observed. Runcie et al. (2003) also found that $K_s$ values varied depending upon incubation time; therefore comparing $K_s$ between studies that used different incubation times may not provide useful or dependable information. The relatively long incubation time used here was necessary for observing measurable decreases in ammonium due to the relatively low macroalgal tissue:water volume ratios employed.

According to Pedersen (1994), ammonium uptake consists of three phases: surge, internally controlled, and externally controlled uptake. Surge uptake is controlled by initial adsorption of molecules to the cell wall and the status of small inorganic nitrogen and amino acid pools. Internally controlled uptake rates decrease after the initial surge uptake and are regulated by the assimilation of ammonium into amino acids and their subsequent incorporation into macromolecules. Externally controlled uptake occurs at very low ammonium concentrations and is dependent upon the ammonium available in the medium.
In Pedersen's (1994) study, he found that incubation periods on the scale of hours incorporated both the initial surge uptake rates and the internally controlled uptake rates, resulting in an overestimate of uptake relative to the rates controlled by internal nitrogen content. The 2 hour incubation period used here may have contributed to the high $K_s$ values observed, but since all specimens were exposed to the same treatments, it should not affect comparisons. The values I found are unlikely to be highly overestimated, as the plants employed were not nitrogen starved. Thus, surge uptake was most likely short-lived compared to previous studies that employed nitrogen starved algae in uptake experiments. The goal of this study was to compare ammonium uptake kinetics between different populations based on their environmental nutrient history; hence, ammonium uptake was measured as quickly as possible after being collected and with a short acclimation period. The uptake rates measured here probably represent the internally controlled ammonium uptake rates that are dependent upon their environmental nutrient history.

Although *Ulva* spp. from various sites throughout the estuary were subject to different salinities, it has been reported that salinity does not significantly affect ammonium uptake kinetics in *Ulva lactuca* (Lartigue *et al.*, 2003). Therefore, the salinity differences from various sites were probably not a cause for concern when comparing the ammonium uptake kinetics of different populations.

The ammonium uptake experiments conducted with unknown *Ulva* spp. showed significantly higher ammonium uptake rates for plants from Fort Stark than South Mill Pond. The higher $V_{max}$ for Fort Stark specimens supports the
hypothesis that macroalgae from oligotrophic environments have higher uptake capacity than those from eutrophic environments. Barr and Rees (2003) found that *U. intestinalis* specimens from eutrophied areas had relatively high tissue nitrogen, and therefore lower ammonium assimilation rates, as they are influenced by the rate of amino acid synthesis and incorporation into macromolecules (Pedersen, 1994; Jing-Wen & Shuang-Lin, 2001). When nitrogen is abundant in an aquatic environment, algae are able to assimilate it into amino acids and subsequently macromolecules. Thus, when tissue reserves are high, there is no need for the algae to take up ammonium at high rates. In fact, some algae, including *Ulva lactuca*, are unable to accumulate ammonium in their cells, so it must be assimilated immediately, or it will not be absorbed if the internal nitrogen pools are full (Runcie et al., 2003). As a result, algae from high nutrient areas generally have slower uptake and assimilation rates than those from low nutrient areas.

The $K_s$ values for Fort Stark and South Mill Pond *Ulva* did not support the same hypothesis noted above. South Mill Pond specimens had lower $K_s$ values than Fort Stark specimens, suggesting that the former had a higher affinity for ammonium than the latter. Low $K_s$ values indicate more efficient nutrient uptake (higher affinity) because algal cells can take up the substrate faster at lower substrate concentrations. I expected to find lower $K_s$ values for Fort Stark specimens based on their nutrient history that would result in more efficient nutrient absorption from the water column. Although the $K_s$ values did not support this hypothesis, an additional uptake kinetics parameter can be used for
making comparisons between macroalgal affinities for a substrate: the $V_{\text{max}}:K_s$ ratio (also represented as $\alpha$). The ratio is useful because $K_s$ is dependent on $V_{\text{max}}$. Hence, the ratio of the two parameters can be used as a more accurate measurement of affinity when $V_{\text{max}}$ values differ greatly between two species or populations (Lobban and Harrison, 1997). In the case of *Ulva* spp. from Fort Stark and South Mill Pond, their $\alpha$ values were very similar (0.0055 and 0.0076 respectively) despite the fact that their $K_s$ values differed greatly. Observations of the relationship between ammonium uptake rate and ammonium concentration for *Ulva* spp. from these two sites revealed that the initial slope of each curve was similar (Figure 11), which was in agreement with the $\alpha$ values and suggested that these specimens had similar affinities for ammonium. Hence, this was a case where the $V_{\text{max}}:K_s$ ratios provided more accurate indications of *Ulva* spp. affinity for ammonium than the $K_s$ values.

When *Ulva lactuca* from Fort Stark and South Mill Pond were compared, the ammonium uptake kinetics showed the same pattern as reported above, but they were not significantly different. With more replication, there would probably be a significant difference between the two uptake curves because the $V_{\text{max}}$ and $K_s$ of *Ulva lactuca* from these two sites were dissimilar. Although there was no significant difference between the uptake kinetics curves for *U. pertusa* from Fort Stark and South Mill Pond, specimens from Fort Stark had a higher $V_{\text{max}}$ and $K_s$ than those from South Mill Pond. The $\alpha$ values confirmed that *U. pertusa* from South Mill Pond had a higher affinity for ammonium than *U. pertusa* from Fort Stark, which again did not support the hypothesis that *Ulva* spp. from eutrophic
sites would have higher affinities for ammonium than *Ulva* spp. from oligotrophic sites.

The ammonium uptake curves of specimens from Fort Stark did not follow Michaelis Menten uptake kinetics as closely as specimens collected from other sites. In fact, the uptake rates for *Ulva lactuca* and *U. pertusa* from Fort Stark were almost linear with respect to ammonium concentration until concentrations reached 200 μM, at which point the uptake rates dropped. Michaelis Menten enzyme kinetics may not actually provide an accurate model for Fort Stark specimens, which would explain why the substrate affinity did not follow the expected patterns for this site compared to South Mill Pond. Fujita (1985) also reported linear ammonium uptake in *U. lactuca* collected from the field, but only concentrations up to 60 μM were investigated in his study. He suggested that uptake may be non-saturable at higher concentrations than those he investigated, which was the case in the present study for Fort Stark specimens. In the range used here (25-250), uptake rates did not become saturated, but when concentrations reached 200 μM, uptake rates decreased indicating that ammonium may have become toxic beyond this concentration.

Although Fort Stark *Ulva lactuca* and *U. pertusa* specimens showed almost linear uptake rates, the slope of the uptake curves for Fort Stark specimens were not higher than those from South Mill Pond as was expected due to the ammonium histories of the two sites. Water motion is a possible explanation for the similar α values observed in Fort Stark and South Mill Pond *Ulva* spp. Parker (1981) reported that *U. lactuca* exposed to water motion had
enhanced ammonium uptake versus *U. lactuca* exposed to still water at most ammonium concentrations examined. Fort Stark is an exposed coastal site, and therefore has higher water motion compared to most of the other sites examined. Consequently, the ammonium availability for *Ulva* spp. growing at Fort Stark may be high despite the low ambient ammonium concentrations. The consistent availability of ammonium for the Fort Stark *Ulva* spp. may account for those specimens exhibiting affinities for ammonium that were similar to specimens from South Mill Pond, a high ammonium site.

In conclusion, comparison of ammonium uptake kinetics between *Ulva* spp. from sites with different ammonium histories did not necessarily coincide with previously reported results, which found that enriched macroalgae showed lower uptake affinities for ammonium than unenriched or starved macroalgae (Carpenter and Guillard, 1971; Fujita, 1985; Pedersen and Borum, 1997). However, these studies usually altered the nitrogen status of the macroalgal tissue prior to measuring ammonium uptake rates by enriching or starving the plants, which exaggerates their ammonium uptake response and may not be comparable to natural responses. Furthermore, the nitrogen status of a plant may not always be correlated with the nitrogen history of a site. Barr and Rees (2003) compared three populations of *U. intestinalis* from sites with different nitrogen histories and found that the concentration of inorganic nitrogen in the seawater did not necessarily correlate with the nitrogen status of the plants. They hypothesized that there may be other nitrogen sources, particularly ammonium sources, such as invertebrate excretion that may be episodic and/or
immeasurable. In comparison, the ammonium uptake kinetics of populations measured in this study also did not always fit the expected patterns, and might be due to other nutrient sources or environmental factors as hypothesized by Barr and Rees (2003). However, based on the results from the experiments described above, it would be more appropriate to collect Ulva lactuca and U. pertusa from oligotrophic rather than eutrophic environments if they were to be used as ammonium biofilters because despite their lower affinity for ammonium at Fort Stark, they did have high affinity for ammonium when collected from Hilton Park (a low ammonium site) and consistently had higher $V_{\text{max}}$ values than their counterparts from eutrophic sites indicating a higher uptake efficiency for ammonium.

**Genetic Influence on Ulva spp. Ammonium Uptake Kinetics**

There was no significant difference between the ammonium uptake curves for South Mill Pond cultured and *in situ* South Mill Pond and Fort Stark materials. However, the uptake kinetics of cultured specimens showed more resemblance to South Mill Pond than Fort Stark specimens. Thus, my results suggest that there may be a genetic influence on ammonium uptake kinetics since the cultured materials had uptake kinetics similar to their parents, despite varying culture conditions and *in situ* field collections. Unfortunately, material from other sites was not successfully grown in culture, so similar comparisons cannot be made.
*Ulva lactuca* and *U. pertusa* from Fort Stark had similar uptake kinetics and would therefore be equally efficient as ammonium biofilters; the same was true for both species from Hilton Park. Of the three species from South Mill Pond (*U. lactuca*, *U. pertusa*, and *U. rigida*), *U. rigida* had the lowest $K_s$ and thus the greatest affinity for ammonium. However, its uptake capacity was lower than the other two species from South Mill Pond. The differences between these three species from South Mill Pond were small and insignificantly different; hence, they seem to be equally efficient at taking up ammonium from their environment. It should be noted that the most polluted site, South Mill Pond, had the second highest diversity of *Ulva* spp. after Adams Point, a low ammonium site.

The significant difference in ammonium uptake kinetics between *Ulva rigida* and *U. compressa* from Adams Point showed that *U. rigida* had a lower $K_s$ than *U. compressa*, indicating a higher affinity for ammonium and a slightly lower uptake capacity (i.e. lower $V_{\text{max}}$). However, the $V_{\text{max}}$:$K_s$ ratios for these two species from Adams Point were identical (0.011), indicating that they had a similar affinity for ammonium. It was somewhat surprising to find that *U. rigida* was not significantly better at ammonium uptake versus other *Ulva* spp. since it is a very common bloom-forming species in Europe (Sfriso *et al.*, 1992; Viaroli *et al.*, 1993; Viaroli *et al.*, 1996).

Although Adams Point and the Oyster River site were not directly compared, they were the only two sites where distromatic *Ulva compressa* was found. Observation of the uptake kinetics of *U. compressa* from these two sites revealed similar $V_{\text{max}}$ values, but the $K_s$ for Oyster River material was more than
twice that from Adams Point. In addition, the $V_{\text{max}}:K_s$ ratio for *U. compressa* from Oyster River (0.004) was much lower than the same species from Adams Point (0.011), suggesting that *U. compressa* from Adams Point had a higher affinity for ammonium and may therefore be slightly better suited for bioremediation than Oyster River material.

Overall, there was no clear pattern between ammonium uptake kinetics, environmental nutrient history, and species of *Ulva*. However, both environmental history and taxonomic differences between populations seemed to influence ammonium uptake kinetics. Generally, the low ammonium sites consistently showed high $V_{\text{max}}$ and $V_{\text{max}}:K_s$ ratios (with the exception of Fort Stark) across all species sampled, which is consistent with the hypothesis that algae from oligotrophic areas have higher uptake capacity and affinity than those from eutrophic environments. Hilton Park and Fort Stark samples showed the highest $V_{\text{max}}$ values for *U. lactuca* and *U. pertusa* populations. Furthermore, Hilton Park and Adams Point specimens showed consistently high affinities for ammonium across all species (Table 6). As a result, it seems that *U. lactuca* and *U. pertusa* from low ammonium environments, particularly Hilton Park, have the most efficient ammonium uptake kinetics. At the highest nutrient site, South Mill Pond, these two species had lower $V_{\text{max}}$ values than the low nutrient sites, but they still had higher uptake capacity than *U. rigida* specimens from the same site. However, *U. rigida* had a higher affinity for ammonium at South Mill Pond than *U. lactuca* and *U. pertusa*, so it is not clear which of the three species from South Mill Pond would be better for bioremediation.
In conclusion, environmental nutrient history influences ammonium uptake kinetics of *Ulva* populations, and these influences cross species boundaries. In general, algae from low ammonium sites had relatively high \(\alpha\) values indicating a high affinity for ammonium. However, some species consistently showed more efficient ammonium uptake kinetics than others regardless of environmental nutrient history. Generally, *U. lactuca* and *U. pertusa* retained higher \(V_{\text{max}}\) values than all other species evaluated. Therefore, I conclude that environmental nutrient history strongly affects substrate affinity, but some genetic factor maintains relative uptake rates within individual taxa, resulting in a sort of hierarchy between species with respect to maximum uptake rate. As a consequence, the *Ulva* spp. examined in this study that would be the most efficient ammonium bioremediators are *U. lactuca* and *U. pertusa* from low ammonium areas.

*Ulva pertusa* is a species of Asian origin, and therefore its efficiency of ammonium uptake on the western Atlantic coast may be a cause for concern. Although *U. pertusa* is currently coexisting with *U. lactuca* and *U. rigida* at Hilton Park and South Mill Pond, there may be a potential for *U. pertusa* to outcompete other *Ulva* spp. in the future, especially if this species is a recent introduction and has not yet stabilized its interactions with other species. An investigation of historical records of *Ulva* spp. for this area is important in order to determine the approximate time of introduction for *U. pertusa*, and should be conducted in the near future.
Tissue Analysis

The protein content of *Ulva* spp. from South Mill Pond was the highest for all months except September, when the protein content at all sites declined relative to previous months. In July, the protein content corresponded to the relative ammonium levels at the paired sites, except for Chapman’s Landing samples (high ammonium site) that had lower protein than the Adams Point samples (low ammonium site). In August, protein contents in all samples were relatively equal, and no differences were observed between sites. In September, overall protein content decreased and did not correspond well to ammonium concentration patterns at the paired sites.

In conclusion, protein was not a good representative of the history of ammonium concentration at any of the sites evaluated. The reason may be that macroalgae at high ammonium sites are using the available nitrogen for growth rather than protein production, so the assimilated ammonium is not concentrated as protein in their cells. Hardwick-Witman and Mathieson (1986) reported that tissue nitrogen levels in *Ascophyllum nodosum* followed the same pattern as ambient inorganic nitrogen levels, but its growth rate did not. Instead, growth occurred during the summer when ambient nitrogen concentrations are lowest in the Great Bay Estuarine System (Mathieson *et al.*, 1976). Because *Ulva* spp. are unable to store ammonium in their tissue like perennial species, most of the nutrients they absorbed during the summer may have gone towards growth as light was not a limiting factor. Instead of protein, the free amino acid (FAA) pool may have been a better indication of nutrient history in the environment because
ammonium is assimilated more quickly into amino acids than proteins. Furthermore, FAA has been shown to provide feedback inhibition in ammonium uptake and assimilation (Pedersen, 1994; McGlathery et al., 1996; Jing-Wen & Shuang-Lin, 2001). Unfortunately, attempts to quantify amino acid content in Ulva spp. tissue were not successful in this study.

Chlorophyll a:b ratios for Ulva spp. in this study were slightly lower than those reported in the literature. Chlorophyll a:b ratios for green algae are generally low compared to higher plants. Nakamura et al. (1976) found that chlorophyll a to b ratios ranged from 1.5 to 2.2 in marine green algae. My results showed a range of 1.2 to 1.4, which is near the low end of previous findings. Time of year and environmental conditions can affect the chlorophyll a to b ratios in marine algae, and they may account for the low values observed in this study.

Pigment contents showed a similar seasonal pattern to protein content, and generally they were higher in Ulva spp. tissue in August than September. Protein and pigment content are related because of the chlorophyll-protein complexes present in the photosynthetic apparatus (Beale & Weinstein, 1991). Furthermore, chlorophyll biosynthesis involves the precursor glutamate, which is an amino acid product of nitrogen assimilation. Therefore, chlorophyll and protein biosynthesis essentially compete with each other (Precali & Falkowski, 1983) and nitrogen is thought to be transferred from the chlorophyll synthesis pool to the protein synthesis pool under low nitrogen conditions (McGlathery & Pedersen, 1999; Menendez et al., 2002). Since the sites used in this study had relatively high nitrogen availability, chlorophyll and protein synthesis were not
competing heavily. Hence, it is not surprising that these two physiological parameters showed similar seasonal patterns. However, chlorophyll a:b ratios were significantly different between Fort Stark and South Mill Pond in September, which was a result of decreased chlorophyll a content in Fort Stark specimens. A corresponding decrease in protein and carotenoid content was observed in September collected Fort Stark specimens. The decrease in both protein and chlorophyll a content suggest that there may have been unusually low nitrogen availability at Fort Stark in late August or September.

Pigment content and blade color analysis in *Ulva* spp. did not correspond well with relative ammonium history of study sites, but blade color analysis could be used to detect differences between chlorophyll:carotenoid ratios. Blade color of *Ulva* spp. from Hilton Park was more yellow-green according to the color analysis, which corresponded with higher chlorophyll:carotenoid ratios in Oyster River samples and suggested they had lower carotenoid content than Hilton Park samples. The results are consistent with the fact that changes in the relative concentration of chlorophyll and carotenoids in plant tissue affect tissue color. Haxo & Clendenning (1953) found a chlorophyll to carotenoid ratio of 8.3 in vegetative thalli of *U. lactuca* var. *rigida* Le Jolis (=*U. rigida*), but the reproductive margins of the blade had much higher carotenoid content and thus lower chlorophyll:carotenoid ratios. The chlorophyll:carotenoid ratios of *Ulva* spp. vegetative thalli measured in this study varied from 6.8 and 15.4, which is a large range but includes the values previously reported by Haxo and Clendenning.
A distinct difference between the chlorophyll:carotenoid ratios was
observed for the inland sites compared to the outer estuarine and coastal sites. The inner estuarine sites: Chapman’s Landing, Adams Point and Oyster River had significantly higher chlorophyll:carotenoid ratios compared to the mid to outer estuarine and coastal sites (Hilton Park, South Mill Pond, and Fort Stark).

Distinct salinity differences exist between these sites, as well as differences in substratum and water clarity (Mathieson and Hehre, 1986; Mathieson and Penniman, 1986). The sites farthest inland (> 10 miles from coast) are less saline, have muddy substrata, and low water clarity, while the outer estuarine and coastal sites (< 10 miles from coast) are more saline, have rocky substratum, and better water clarity. Water clarity may be the most influential factor for chlorophyll:carotenoid ratios since low light conditions lead to higher pigment production or altered pigment ratios (Ramus et al., 1976). Henley and Ramus (1989) reported that carotenoid:chlorophyll ratios were positively related to light exposure in Ulva rotundata. The Ulva spp. specimens from inner estuarine sites in the Great Bay Estuarine System had higher chlorophyll:carotenoid ratios most likely because of the poor water clarity and muddy substrata characteristic of the inner most estuarine sites, which reduced the amount of light exposure to those algae. As a result, the carotenoid content was relatively low. In contrast, the three sites closest to the coast were characterized by rocky substrata and fair water clarity, and specimens from those sites had low chlorophyll:carotenoid ratios due to relatively high carotenoid concentrations in response to higher light exposure. Furthermore, lower chlorophyll:carotenoid ratios in the three sites
closest to the coast may be a result of generally lower nitrogen availability compared to the three inner estuarine sites, because it has been documented that carotenoid:chlorophyll a ratios increase when nitrogen is limiting (Plumley *et al.*, 1989).

**Conclusion**

In conclusion, my results document two newly recorded species of *Ulva* present in New Hampshire (*U. pertusa* and *U. rigida*), with the first also being new to the Northwestern Atlantic coast. The occurrence of a distromatic morphotype of *U. compressa* is also newly described for this geography and primarily found in upper estuarine sites. Thus, the biodiversity of *Ulva* spp. in The Great Bay Estuarine System is double that previously documented (Mathieson and Hehre, 1986). Both environmental nutrient history and taxonomy influence ammonium uptake kinetics, but each affects a different uptake parameter. If *Ulva* spp. are collected from the field with the intended use of filtering ammonium from polluted waters, they should be collected from environments with relatively low ammonium concentrations due to their higher ammonium affinities relative to those from highly eutrophic environments. The same is true for *Ulva* spp. that were grown in culture for the purpose of bioremediation. They should be grown under oligotrophic conditions so that their uptake kinetics will be most efficient. The two most appropriate species for this purpose are *U. lactuca* and *U. pertusa*.
In investigating the biodiversity of *Ulva* spp. it is important to use molecular as well as morphological assessment. My results suggest that the introduced species *U. pertusa* may be more efficient at ammonium uptake than some native *Ulva* species. Its high uptake efficiency combined with other physiological or environmental factors could make the introduced species more competitive than native species. Considering that native *Ulva* spp. blooms can have negative impacts on eutrophic estuaries (Valiela *et al.*, 1997; Hauxwell *et al.*, 2001; Deegan *et al.*, 2002; Hauxwell *et al.*, 2003), the potential for even more severe blooms from introduced “green tide” species could be even more detrimental to estuarine ecosystems. Exploiting the physiology of *Ulva* spp. for bioremediation is a good way to turn a nuisance macroalgal bloom into a useful solution, but the introduction of new species into already polluted environments can create a whole new difficult problem. Therefore, strict management regulation is essential for stopping the introduction of new species around the world in order to prevent further deterioration of our coastal ecosystems.
Table 1. List of collection sites used in this study and their location within the Great Bay Estuarine System.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Coordinates</th>
<th>Ammonium Content</th>
<th>Miles from the Coast*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapman’s Landing</td>
<td>Stratham, NH</td>
<td>70°55'43&quot;W, 43°02'24&quot;N</td>
<td>H</td>
<td>17.5</td>
<td>On the Squamscott River, salt marsh area, substratum muddy with scattered shingle, low water clarity, Point in narrow straight between Great Bay and Little Bay, substratum muddy with scattered shingle, salt marsh abundant</td>
</tr>
<tr>
<td>Adams Point</td>
<td>Durham, NH</td>
<td>43°05'43&quot;N, 70°51'08&quot;W</td>
<td>L</td>
<td>12.3</td>
<td>At tidal headwater, substratum muddy, low water clarity, salt marsh and shingle along river bank</td>
</tr>
<tr>
<td>Oyster River</td>
<td>Durham, NH</td>
<td>43°07'57&quot;N, 70°54'58&quot;W</td>
<td>H</td>
<td>14.2</td>
<td>At the mouth of Bellamy River, strong tidal currents, substratum rocky</td>
</tr>
<tr>
<td>Hilton Park</td>
<td>Dover, NH</td>
<td>43°07'07&quot;N, 70°49'42&quot;W</td>
<td>L</td>
<td>8.6</td>
<td>Sheltered pond with tidal restriction, heavily polluted, substratum rocky</td>
</tr>
<tr>
<td>South Mill Pond</td>
<td>Portsmouth, NH</td>
<td>43°04'19&quot;N, 70°45'20&quot;W</td>
<td>H</td>
<td>3.0</td>
<td>Exposed open coastal site, substratum rocky</td>
</tr>
<tr>
<td>Fort Stark</td>
<td>Newcastle, NH</td>
<td>43°03'32&quot;N, 70°42'45&quot;W</td>
<td>L</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*From Mathieson and Hehre, 1986
Table 2. Volume of PCR reagents used for each extracted DNA sample listed in the order added.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume used (µL)</th>
</tr>
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<tbody>
<tr>
<td>diH₂O</td>
<td>27.6</td>
</tr>
<tr>
<td>Taq Buffer</td>
<td>5</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>7</td>
</tr>
<tr>
<td>dNTP's</td>
<td>4</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>1</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>1</td>
</tr>
<tr>
<td>Taq Polymerase</td>
<td>0.4</td>
</tr>
<tr>
<td>DNA sample</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
</tr>
</tbody>
</table>
Table 3. Primer sequences used to amplify the ITS1 and ITS2 nrDNA sequence and the rbcL gene of *Ulva* spp.

<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 (Forward)</td>
</tr>
<tr>
<td>18S1763</td>
<td>5' GGTGAACCTGCGGAGGGATCATT 3'</td>
<td>ITS1 (Forward)</td>
</tr>
<tr>
<td>5.8S142</td>
<td>5' TATTCCGACGCTGAGGCAG 3'</td>
<td>ITS1 (Reverse)</td>
</tr>
<tr>
<td>5.8S30</td>
<td>5' GCAACGATGAAGAAACGCAGC 3'</td>
<td>ITS2 (Forward)</td>
</tr>
<tr>
<td>ENT26S</td>
<td>5'GCTTATTGATATGCTTAAGTTCAGCGGAT 3'</td>
<td>ITS2 (Reverse)</td>
</tr>
<tr>
<td>UF1</td>
<td>5' CCACAAACAGGAAACTAAACGC3'</td>
<td>rbcL (Forward)</td>
</tr>
<tr>
<td>UR398</td>
<td>5' GCTGGTGAATACGTAATC3'</td>
<td>rbcL (Reverse)</td>
</tr>
<tr>
<td>UF361</td>
<td>5'CGTGCTTTACGTTTAGAAG3'</td>
<td>rbcL (Forward)</td>
</tr>
<tr>
<td>UR599</td>
<td>5'GGTTGAGTTACCTTACGTTGC3'</td>
<td>rbcL (Reverse)</td>
</tr>
<tr>
<td>UF526</td>
<td>5'GGACGCTGCTGTATGATGAGC3'</td>
<td>rbcL (Forward)</td>
</tr>
<tr>
<td>UR788</td>
<td>5'GCAAAATGACCACGTGTC3'</td>
<td>rbcL (Reverse)</td>
</tr>
<tr>
<td>UF723</td>
<td>5'GGAACGTTGCTGATGTC3'</td>
<td>rbcL (Forward)</td>
</tr>
<tr>
<td>UR970</td>
<td>5'TACCTACTACTGTTCCG3'</td>
<td>rbcL (Reverse)</td>
</tr>
<tr>
<td>UF879</td>
<td>5'TCAAGCTGACGCTCAGGCAGC3'</td>
<td>rbcL (Forward)</td>
</tr>
<tr>
<td>UR1354</td>
<td>5'ATACTTTCACAAGCTGCAGC3'</td>
<td>rbcL (Reverse)</td>
</tr>
</tbody>
</table>
Table 4. Source and accession number for GenBank sequences used for comparison with the sequences in the present study.

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Gene</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan et al., 1999</td>
<td>U. lactuca</td>
<td>ITS1 and ITS2</td>
<td>AJ234309</td>
</tr>
<tr>
<td>Tan et al., 1999</td>
<td>U. pseudocurvata*</td>
<td>ITS1 and ITS2</td>
<td>AJ234312</td>
</tr>
<tr>
<td>Leskinen et al., 2004</td>
<td>U. compressa</td>
<td>ITS1 and ITS2</td>
<td>AJ550765</td>
</tr>
<tr>
<td>Shimada et al., 2003</td>
<td>U. pertusa</td>
<td>ITS1 and ITS2</td>
<td>AB097654</td>
</tr>
<tr>
<td>Shimada et al., 2003</td>
<td>U. scandinavica**</td>
<td>ITS1 and ITS2</td>
<td>AB097659</td>
</tr>
</tbody>
</table>

* Ulva pseudocurvata was misidentified, and actually represents a distromatic form of Ulva compressa.

** Ulva scandinavica is synonymous with U. rigida and U. armoricana (Maggs et al., 2007)
Table 5. Recipe for Von Stosch Enrichment Medium Stock Solution (Ott, 1966).
Enrichment medium was used in 1:1000 ratio to seawater.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount added to 1 L Stock Solution (g)</th>
<th>Concentration in Final ASW (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃*</td>
<td>42.5</td>
<td>5.00 x 10⁻³</td>
</tr>
<tr>
<td>Na₂HPO₄·12H₂O</td>
<td>10.75</td>
<td>3.00 x 10⁻²</td>
</tr>
<tr>
<td>FeSO₄·H₂O</td>
<td>0.278</td>
<td>1.00 x 10⁻⁵</td>
</tr>
<tr>
<td>MnC₁₂·H₂O</td>
<td>0.0198</td>
<td>1.00 x 10⁻⁴</td>
</tr>
<tr>
<td>Na₂EDTA·2H₂O</td>
<td>3.72</td>
<td>1.00 x 10⁻⁴</td>
</tr>
<tr>
<td>Thiamine-HCl</td>
<td>0.2</td>
<td>5.93 x 10⁻⁶</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.001</td>
<td>4.09 x 10⁻⁹</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.001</td>
<td>7.38 x 10⁻⁷</td>
</tr>
</tbody>
</table>

* Nitrogen source was omitted from the enrichment stock solution used in the ammonium uptake experiments
Table 6. Summary of ammonium uptake kinetics values for all *Ulva* spp. and all sites evaluated. $V_{\text{max}}$ values for *U. lactuca* and *U. pertusa* are in bold to show their consistently high values, and $\alpha$ values from low ammonium sites with the exception of Fort Stark are italicized to emphasize the influence of environmental nutrient history on ammonium affinity in *Ulva* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site</th>
<th>$V_{\text{max}}$ (µmol N·cm$^{-2}$·hr$^{-1}$)</th>
<th>$\alpha$ (L·cm$^{-2}$·hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. lactuca</em></td>
<td>Fort Stark (L)</td>
<td>0.34</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Hilton Park (L)</td>
<td>0.30</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>South Mill Pond (H)</td>
<td>0.19</td>
<td>0.007</td>
</tr>
<tr>
<td><em>U. pertusa</em></td>
<td>Fort Stark (L)</td>
<td>0.26</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Hilton Park (L)</td>
<td>0.21</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>South Mill Pond (H)</td>
<td>0.15</td>
<td>0.008</td>
</tr>
<tr>
<td><em>U. compressa</em></td>
<td>Adams Point (L)</td>
<td>0.16</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Oyster River (H)</td>
<td>0.15</td>
<td>0.004</td>
</tr>
<tr>
<td><em>U. rigida</em></td>
<td>Adams Point (L)</td>
<td>0.12</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>South Mill Pond (H)</td>
<td>0.11</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Figure 1. Map of the Great Bay Estuarine System of New Hampshire and Maine with labeled sites showing the location of Ulva spp. collections. Satellite map courtesy of Google Earth Pro 2008.
Figure 2. Blade morphologies of the four Ulva spp. found in the Great Bay Estuarine System. Scale bars represent 4 cm. Arrows point to parts of the thalli where experimental disks were cut for ammonium uptake analysis.
Figure 3. Four cell morphologies representing the four different *Ulva* spp. found in the Great Bay Estuarine System: a) *Ulva compressa* with multiple starch grains b) *U. rigida* showing large cells with multiple pyrenoids c) stained *U. lactuca* cells showing parietal chloroplast with single pyrenoid d) *U. pertusa* showing parietal chloroplast with multiple pyrenoids. All scale bars are 20 \( \mu m \) in length.
Figure 4. Morphological variation in Ulva compressa specimens collected from the Great Bay Estuarine System. Refer to Table 1 of Appendix for descriptions of specimen collections. A. Oyster River #78603, B. Oyster River #78553, C. Oyster River #78592, D. Oyster River #78596. Scale bars represent 4 cm.
Figure 5. Morphological variation in Ulva rigida specimens collected in the Great Bay Estuarine System. Refer to Table 1 of Appendix for descriptions of specimen collections. A. Oyster River #78555, B. Adams Point #78552, C. Adams Point #78571, D. Adams Point #78551, E. Oyster River #78554, F. Adams Point #78576. Scale bars represent 4 cm.
Figure 6. Morphological variation in *Ulva lactuca* collected from the Great Bay Estuarine System. Refer to Table 1 of Appendix for descriptions of specimen collections. A. South Mill Pond #78588, B. South Mill Pond #78587, C. Fort Stark #78548, D. Fort Stark #78566, E. Hilton Park (Dover Point) #78589, F. Fort Stark #78565. Scale bars represent 4 cm.
Figure 7. Morphological variation in *Ulva pertusa* specimens collected from the Great Bay Estuarine System. Refer to Table 1 of Appendix for descriptions of specimen collections. A. Hilton Park (Dover Point) #78595, B. South Mill Pond #78584, C. Adams Point #78550 D. Hilton Park (Dover Point) #78547 E. Adams Point #78579. Scale bars represent 4 cm.
Figure 8. *Ulva pertusa* cells from Hilton Park showing delineation of reproductive margin with unicellular propagules. Vegetative cells are in the bottom right corner, reproductive cells are in the upper left corner.
Figure 9. Phylogenetic tree containing sequences representing four distromatic Ulva taxa found in the Great Bay Estuarine System that are labeled with site abbreviations and collection number. The sequences labeled with accession numbers are from GenBank. The sample labeled "U. pseudocurvata" from Tan et al., 1999 actually represents the distromatic morphotype of U. compressa. Posterior probability values from MrBayes analysis and bootstrap values from neighborjoining and maximum likelihood analysis are shown at branch nodes.
Figure 10. Map of the Great Bay Estuarine System showing the distribution of the four Ulva taxa collected.
Figure 11. Ammonium uptake kinetics curve of a) Ulva spp. from Fort Stark and b) Ulva spp. from South Mill Pond; p=0.044.
Figure 12. Ammonium uptake kinetics curve of A) *Ulva lactuca* and B) *Ulva pertusa* from Fort Stark and South Mill Pond. An F test comparing the nonlinear regressions showed that the uptake curves are not significantly different for either species between these two sites (p > 0.05).
Figure 13. Ammonium uptake kinetics of *Ulva* spp. cultures from South Mill Pond. The material in these cultures was a mixture of *Ulva lactuca* and *Ulva rigida*.
Figure 14. Ammonium uptake kinetics curve of *U. lactuca* and *U. pertusa* from Fort Stark. An F test comparing the nonlinear regressions showed that the uptake curves are not significantly different (p>0.05).
Figure 15. Ammonium uptake kinetics curve of *Ulva lactuca*, *Ulva pertusa* and *Ulva rigida* from South Mill Pond. An F test comparing the nonlinear regressions showed that there was no significant difference between the three curves (p=0.441). However, when *U. lactuca* and *U. rigida* were compared separately, there was a significant difference between the two uptake curves (p=0.011).
Figure 16. Ammonium uptake kinetics curve of *Ulva lactuca* and *Ulva pertusa* from Hilton Park. An F test comparing the nonlinear regressions showed that the uptake curves are not significantly different (p = 0.33).
Figure 17. Ammonium uptake kinetics curve of *Ulva compressa* from Oyster River. \( V_{\text{max}} = 0.14 \, \mu\text{mol N} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) and \( K_{m} = 35.2 \, \mu\text{M} \).
Figure 18. Ammonium uptake kinetics curve of *Ulva compressa* and *Ulva rigida* from Adams Point. An F test comparing the nonlinear regressions showed that there was a significant difference between ammonium uptake curves for these two species (*p* = 0.035).
Figure 19. Tissue protein content of *Ulva* spp. from all six sites in the Great Bay Estuarine System from June to September (mean±SE).
Figure 20. Tissue protein content of *Ulva* spp. from Adams Point (AP), Oyster River (OR), Fort Stark (FS), and South Mill Pond (SMP) collected in June 2008. Shared letters above the bars represent values that are not significantly different (mean±SE; p=0.000043).
Figure 21. Tissue protein content of *Ulva* spp. collected from all six sites in the Great Bay Estuarine System in July 2008. Shared letters above the bars represent values that are not significantly different (mean±SE; p<0.05). * p=0.05 for pairwise comparison between CL and AP mean protein content.
Figure 22. Tissue protein content in *Ulva* spp. collected from the Great Bay Estuarine System in August, 2008. There were no significant differences between these means (mean±SE).
Figure 23. Tissue protein content in *Ulva* spp. from five sites in the Great Bay Estuarine System in September, 2008. Shared letters above the bars represent values that are not significantly different (p>0.05).
Figure 24. A. Chlorophyll a content in *Ulva* spp. tissue collected from the Great Bay Estuarine System during August and September 2008 (mean±SE). Two way ANOVA showed a significant interaction between site and month (p=0.00002). B. Chlorophyll b content in *Ulva* spp. tissue (mean±SE). A two way ANOVA indicated there was a significant interaction between site and month (p=0.0008). Asterisks in both A and B represent sites where there was a significant difference in chlorophyll between August and September.
Figure 25. Pigment content for each site in Great Bay Estuary during August and September; top: chlorophyll a:b, bottom: total carotenoids. Different letters represent significant differences within the same month; asterisks represent significant differences within the same site between the two different months (mean±SE).
Figure 26. Mean chlorophyll:carotenoid ratios (±SE) for *Ulva* spp. at all sites in the Great Bay Estuarine System in August and September combined. Different letters above bars indicate a significant difference (mean±SE; p<<0.05).
Figure 27. Three-dimensional representation of blade color analysis of *Ulva* spp. in the Great Bay Estuarine System. Each graph represents a different pair of sites: A) Adams Point and Chapman’s Landing, B) Hilton Park and Oyster River, C) Fort Stark and South Mill Pond. Only *Ulva* spp. from Hilton Park and Oyster River showed a significant difference in blade color (p<<0.0000). Lines from points to axes shown for reference.
Figure 28. *Ulva lactuca* and *U. pertusa* growing on *Mastocarpus stellatus* from Hilton Park. Large, round holes in *U. lactuca* are from disks cut for ammonium uptake experiment, whereas all holes in *U. pertusa* are natural. Blade margin of *U. pertusa* shows lighter reproductive region.
WORK CITED


Bohnsack, M. V. 2003. *Illustrated key to the seaweeds of New England*. The Rhode Island Natural History Survey, Kingston,


PERSONAL COMMUNICATIONS

Christine Maggs, School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, Belfast, UK

Frédérick Mineur, School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, Belfast, UK

Jeremy Nettleton, Department of Biological Sciences, University of New Hampshire, Durham, NH

Phil Trowbridge, New Hampshire Department of Environmental Services: Watershed Management Bureau, Concord, NH.
Table 1. Description of all *Ulva* spp. specimens collected during the present study. All specimens are deposited in the Albion Hodgdon Herbarium (NHA) at the University of New Hampshire, Durham, NH, U. S. A. Specimens have been molecularly identified unless labeled as *Ulva* sp. Names in parenthesis indicate tentative identification without molecular verification.

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<thead>
<tr>
<th>Taxon Name</th>
<th>Site Location</th>
<th>Locality</th>
<th>Habitat</th>
<th>GPS</th>
<th>General Notes</th>
<th>Collector</th>
<th>NHA#</th>
<th>Date</th>
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Footnote: Laurie Hofmann
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- *Ulva compressa* is collected from Adams Point, Durham, NH. The habitat is epiphytic on *A. nodosum* at Jackson Lab, with GPS coordinates 43°05'43"N 70°51'08"W. The general notes indicate that circular disks are cut from the thallus. The collector is Laurie Hofmann with NHA# 78574 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from Adams Point, Durham, NH. The habitat is Drift on mudflat near boat launch, with GPS coordinates 43°05'43"N 70°51'08"W. The general notes indicate that circular disks are cut from the thallus. The collector is Laurie Hofmann with NHA# 78575 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from Adams Point, Durham, NH. The habitat is Attached to boat docks at Jackson Lab, with GPS coordinates 43°05'43"N 70°51'08"W. The general notes indicate that circular disks are cut from the thallus. The collector is Laurie Hofmann with NHA# 78576 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from Adams Point, Durham, NH. The habitat is Attached on mudflat cove near boat docks at Jackson Lab, with GPS coordinates 43°05'43"N 70°51'08"W. The general notes indicate that circular disks are cut from the thallus. The collector is Laurie Hofmann with NHA# 78577 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from Adams Point, Durham, NH. The habitat is Drift on mudflat near boat launch, with GPS coordinates 43°05'43"N 70°51'08"W. The collector is Laurie Hofmann with NHA# 78578 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from Adams Point, Durham, NH. The habitat is Drift on mudflat near boat launch, with GPS coordinates 43°05'43"N 70°51'08"W. The collector is Laurie Hofmann with NHA# 78579 and collected on 14-Nov-2008.

- *Ulva pertusa* is collected from Adams Point, Durham, NH. The habitat is Drift on mudflat near boat launch, with GPS coordinates 43°05'43"N 70°51'08"W. The collector is Laurie Hofmann with NHA# 78579 and collected on 14-Nov-2008.

- *Ulva rigida* is collected from South Mill Pond, Portsmouth, NH. The habitat is adjacent to tidal constriction in park, with GPS coordinates 43°04'19"N 70°45'20"W. The general notes indicate that circular disks are cut from the thallus. The collector is Laurie Hofmann with NHA# 78580 and collected on 15-Nov-2008.
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<td>Epiphytic on <em>A. nodosum</em></td>
<td>43°07'09&quot;N 70°49'42&quot;W</td>
<td>Circular disks cut from thallus</td>
<td>Laurie Hofmann</td>
<td>78589</td>
<td>23-Nov-2008</td>
</tr>
<tr>
<td><em>Ulva pertusa</em></td>
<td>Dover Point, Dover, NH</td>
<td>Adjacent to old Rt 4 bridge</td>
<td>Attached to rock</td>
<td>43°07'09&quot;N 70°49'42&quot;W</td>
<td>Circular disks cut from thallus; reproductive margins white</td>
<td>Laurie Hofmann</td>
<td>78590</td>
<td>23-Nov-2008</td>
</tr>
<tr>
<td><em>Ulva lactuca</em></td>
<td>Dover Point, Dover, NH</td>
<td>Adjacent to old Rt 4 bridge</td>
<td>Epiphytic on <em>M. stellatus</em> with <em>U. pertusa</em></td>
<td>43°07'09&quot;N 70°49'42&quot;W</td>
<td>Circular disks cut from thallus</td>
<td>Laurie Hofmann</td>
<td>78591</td>
<td>23-Nov-2008</td>
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<tr>
<td><em>Ulva pertusa</em></td>
<td>Dover Point, Dover, NH</td>
<td>Adjacent to old Rt 4 bridge</td>
<td>Epiphytic on <em>M. stellatus</em> with <em>U. pertusa</em></td>
<td>43°07'09&quot;N 70°49'42&quot;W</td>
<td>Reproductive margins white</td>
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<td><em>Ulva lactuca</em></td>
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<td>43°07'09&quot;N 70°49'42&quot;W</td>
<td>Circular disks cut from thallus</td>
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<td>23-Nov-2008</td>
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<td>Locality</td>
<td>Habitat</td>
<td>GPS</td>
<td>General Notes</td>
<td>Collector</td>
<td>NHA#</td>
<td>Date</td>
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<td><em>Ulva lactuca</em></td>
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<td>Epiphytic on <em>M. stellatus</em></td>
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<td>Circular disks cut from thallus</td>
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<td><em>Ulva pertusa</em></td>
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<td>Epiphytic on <em>Ascophyllum nodosum</em></td>
<td>43°07'09&quot;N</td>
<td>Circular disks cut from thallus</td>
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<td><em>Ulva</em> (pertusa)</td>
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<td>Epiphytic on <em>Ascophyllum nodosum</em></td>
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<td>Laurie Hofmann</td>
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<tr>
<td><em>Ulva compressa</em></td>
<td>Oyster River, Durham, NH</td>
<td>Old Landing Park near floating pier; low intertidal</td>
<td>Attached to mudflat</td>
<td>43°07'56&quot;N</td>
<td>Circular disks cut from thallus of A</td>
<td>Laurie Hofmann</td>
<td>78597</td>
<td>5-Dec-2008</td>
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<td><em>Ulva sp.</em></td>
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<td>Attached to mudflat</td>
<td>43°07'56&quot;N</td>
<td>Circular disks cut from thallus</td>
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<td>General Notes</td>
<td>Collector</td>
<td>NHA#</td>
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<td>Ulva sp.</td>
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<td>5-Dec-2008</td>
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