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A Multiscale Analysis of the Factors Controlling Nutrient Dynamics and Cyanobacteria Blooms in Lake Champlain

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A MULTISCALE ANALYSIS OF THE FACTORS CONTROLLING NUTRIENT DYNAMICS AND CYANOBACTERIA BLOOMS IN LAKE CHAMPLAIN

A Dissertation Presented

by

Peter D. F. Isles

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The Faculty of the Graduate College

do

The University of Vermont

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ABSTRACT

Cyanobacteria blooms have increased in Lake Champlain due to excessive nutrient loading, resulting in negative impacts on the local economy and environmental health. While climate warming is expected to promote increasingly severe cyanobacteria blooms globally, predicting the impacts of complex climate changes on individual lakes is complicated by the many physical, chemical, and biological processes which mediate nutrient dynamics and cyanobacteria growth across time and space. Furthermore, processes influencing bloom development operate on a variety of temporal scales (hourly, daily, seasonal, decadal, episodic), making it difficult to identify important factors controlling bloom development using traditional methods or coarse temporal resolution datasets. To resolve these inherent problems of scale, I use 4 years of high-frequency biological, hydrodynamic, and biogeochemical data from Missisquoi Bay, Lake Champlain; 23 years of lake-wide monitoring data; and integrated process-based climate-watershed-lake models driven by regional climate projections to answer the following research questions: 1) To what extent do external nutrient inputs or internal nutrient processing control nutrient concentrations and cyanobacteria blooms in Lake Champlain; 2) how do internal and external nutrient inputs interact with meteorological drivers to promote or suppress bloom development; and 3) how is climate change likely to impact these drivers and the risk of cyanobacteria blooms in the future? I find that cyanobacteria blooms are driven by specific combinations of meteorological and biogeochemical conditions in different areas of the lake, and that in the absence of strong management actions cyanobacteria blooms are likely to become more severe in the future due to climate change.
CITATIONS

Material from this dissertation has been published in the following form:

DEDICATION

I would like to dedicate this dissertation to my parents, who instilled a love of lakes in me early on, and who have supported me wholeheartedly through all of the twists and turns that it took to get me to this point. I would also like to dedicate this to my dog Trout, who kept me sane throughout this process and made sure that I took breaks from the computer screen once in awhile.
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CHAPTER 1: MULTIPLE FACTORS CONTROL HARMFUL ALGAL BLOOMS IN A WARMING WORLD

The past several decades have seen substantial increases in the incidence and severity of harmful algal blooms (HABs) worldwide (Paerl and Huisman 2009). The increases are generally attributed to increases in nutrient loads to water bodies resulting from human activities (eutrophication) and to increases in global temperature (Wagner and Adrian 2009). HABs can affect freshwater, estuarine, or marine ecosystems, and may be composed of members of diverse phytoplankton orders. The negative (“harmful”) consequences of HABs are generally defined as direct toxic effects on humans (Cheung et al. 2013); however, HABs and eutrophication can have a variety of detrimental effects on aquatic ecosystems through their effects on dissolved oxygen concentrations (Havens 2008) and changes to aquatic food webs (Landsberg 2002). The increases in HABs worldwide have made them one of the most important threats to water quality worldwide (Brooks et al. 2015), and problems related to HABs are likely to become more frequent as global populations increase and there is greater stress placed on aquatic resources (Smith and Schindler 2009).

Most HABs in lakes are composed primarily of cyanobacteria (Paerl et al. 2001). Cyanobacteria themselves are a diverse group of organisms found in every natural environment where light is present for photosynthesis, including soils, deserts, streams, lakes, and oceans. Cyanobacteria can symbiotically exist in lichens and plant root nodules, and are the precursors of chloroplasts found in eukaryotic autotrophs. Under normal conditions, cyanobacteria are a critical component of most aquatic ecosystems;
however, under some conditions runaway growth of colonial cyanobacteria may form dense blooms which impair human recreational and economic activities (Anderson et al. 2000; Hoagland et al. 2002), produce acute liver and neurotoxins (Van Dolah 2000; Codd et al. 2005), release noxious taste and odor compounds (Cheung et al. 2013), and change energy pathways and trophic structures in lakes (Scheffer et al. 1993; 1997). Toxins produced by cyanobacteria blooms have also been implicated in neurodegenerative disease such as ALS (Cox et al. 2005; Brand et al. 2010).

**Drivers of cyanobacteria blooms in lakes**

There is a general consensus that increases in external nutrient inputs are the primary cause of most recent increases in cyanobacteria concentrations in lakes (Heisler et al. 2008; Schindler 2012), and changes in climate are an important secondary cause (Paerl and Huisman 2008), but beyond this general consensus a number of hypotheses as to the specific factors which promote blooms of cyanobacteria exist. The direct controls on phytoplankton blooms that have been proposed include phosphorus (P) (Schindler 1977; Schindler et al. 2008), nitrogen (N) (Blomqvist et al. 1994; Herrero et al. 2001; Sterner 2008; Scott and McCarthy 2010), silica (Si) (Tilman et al. 1982; Levine et al. 1997), light availability (Sterner et al. 1997; Huisman et al. 1999b), temperature (Butterwick et al. 2005; Paerl and Huisman 2008), zooplankton grazing (Carpenter et al. 1985; Jeppesen et al. 1999), iron availability (Fe) (Xu et al. 2012; Molot et al. 2014), flushing rates (Elliott et al. 2005; Jones et al. 2011), and infection by viruses or fungal pathogens (Brussaard 2004; Ibelings et al. 2004). The proposed controls may be mediated by a wide variety of secondary factors, including geochemical conditions influencing
nutrient release from lake sediments (Jensen and Andersen 1992; Davison 1993; Reynolds and Davies 2001; Smith et al. 2011; Schroth et al. 2015), competitive dynamics among phytoplankton species which mediate nutrient and light availability (Tilman et al. 1982; Brauer et al. 2012), competition for nutrients with aquatic vascular plants (Scheffer et al. 1993; Scheffer 1999) or bacteria (Caron 1994; Jansson et al. 1996), nutrient recycling by higher trophic levels (Elser and Urabe 1999), thermal stability of lakes (Huisman et al. 2004; Huber et al. 2012), meteorological controls (Gerten and Adrian 2000; Jöhnk et al. 2008), dissolved organic carbon (Weyhenmeyer et al. 2014), and filtration by bivalves (Miller and Watzin 2007).

While many of the above factors are likely to be important in some or all lakes, a thorough treatment of each is beyond the scope of this dissertation. I focus primarily on “bottom-up” controls—the availability of the basic chemical elements, as well as energy in the form of light and heat, which phytoplankton need to grow. The three key elements that are most important from this perspective are carbon, nitrogen, phosphorus (silica is also important for diatoms and chrysophytes, but will not be discussed in depth here). C, N, and P are the critical building blocks for the majority of phytoplankton biomass (the other two most abundant elements in phytoplankton biomass, hydrogen and oxygen, are both present in water) (Sterner and Elser, 2002). Phytoplankton require C, N, and P in relatively constant proportions of 106:16:1, respectively (Redfield 1934), although specific requirements can vary within and among algal divisions (Klausmeier et al. 2004; Loladze and Elser 2011). The supply of C, N, and P and their relative proportions can control the maximum biomass achievable in an ecosystem, mediate competition between
different phytoplankton groups, and structure food webs in lakes (Paerl and Huisman 2009; Hessen et al. 2013). C, N, and P also have different chemical properties, which help to determine their role in the structure and function of lake ecosystems.

Carbon is the key element forming the structural elements of all living things (Sterner and Elser, 2002). Carbon is also the most common energy source for most heterotrophic organisms, which access that energy through fermentation and cellular respiration. Due to its ability to form four stable covalent bonds with other elements, C can be organized into a wide variety of structures, many of which are very complex. Indeed, C is an important component of all biological macromolecules. Inorganic C is available in the environment in the form of atmospheric CO$_2$, and in mineral form as carbonate (CO$_3^{2-}$) or bicarbonate (HCO$_3^-$). The atmospheric pool is the ultimate source of most organic C comprising living biomass, and this C is only available to the biosphere through photosynthesis. In lakes, C may be delivered from external sources in the form of dissolved and particulate organic C fixed by terrestrial plants (termed allochthonous C), or may be fixed directly from atmospheric CO$_2$ by in-lake primary producers (autochthonous C). The relative importance of allochthonous v. autochthonous C may be important in determining the net C balance of lake ecosystems (Pace et al. 2007; Brett et al. 2009; Wagner and Adrian 2009).

For phytoplankton, C availability is closely linked to light availability. While some phytoplankton are mixotrophic, and thus able to assimilate organic C from their environments (Reynolds 2006), all phytoplankton by definition get a portion of their C from photosynthesis. As a result, light limitation in phytoplankton can result in carbon
starvation and low C:N and C:P ratios (Sterner et al. 1997). Different phytoplankton groups have a number of mechanisms to maximize access to light and photosynthetic capacity, including a wide range of accessory pigments to harvest light at different wavelengths (Huot and Babin 2010) and the ability to regulate vertical position through density changes or active migration (Gervais 1997; Salonen and Rosenberg 2000). Different adaptations with respect to light harvesting are appropriate to different lake types and thermodynamic conditions, particularly the degree of epilimnetic mixing and thermocline depth; changes in these conditions may therefore result in changes to phytoplankton community composition (Huisman et al. 2004).

In addition to optimizing light conditions, phytoplankton differ in their ability to assimilate inorganic carbon. One factor that may mediate competition for C is the ability of phytoplankton to use bicarbonate in addition to CO₂. When CO₂ dissolves in water, it can combine with water to form carbonic acid and bicarbonate (HCO₃⁻). At high pH, which frequently results from high photosynthetic activity, most available inorganic C may be in the form of bicarbonate. Different phytoplankton groups have different abilities to take up bicarbonate, which requires active transport across cellular membranes (Reinfelder 2011). For example, chrysophytes are unable to use bicarbonate (Bhatti and Colman 2005), and are rarely found in bloom conditions, whereas cyanobacteria are frequently able to use it effectively, which may contribute to the dominance of cyanobacteria during productive periods when pH is high and CO₂ is scarce (Kaplan et al. 1982; Badger and Price 2003). In addition to bicarbonate uptake, phytoplankton groups have evolved a variety of carbon-concentrating mechanisms to increase the efficiency of
the photosynthetic apparatus (Badger and Price 2003; Reinfelder 2011). This is important because aqueous CO$_2$ concentrations are generally low (relative to atmospheric concentrations) due to slow diffusion across the air-water interface, and because high intra-cellular CO$_2$ concentrations greatly increase the efficiency of the enzymes which catalyze photosynthesis, particularly Rubisco (Reinfelder 2011). Photosynthetic enzymes are highly N-rich and energetically costly to produce, so increasing their efficiency constitutes a major competitive advantage (Sterner and Elser, 2002). One important carbon concentrating mechanism in cyanobacteria is the carboxysome (Meyer et al. 2008), an organelle into which intra-cellular carbon is pumped and which is the site of the dark reactions of photosynthesis. While carbon is generally not considered to be a major limiting nutrient in lakes, C limitation has been shown to reduce growth rates during bloom conditions (O’Neil et al. 2012).

Nitrogen is also a key element for lake phytoplankton. Nitrogen is a major component of proteins and nucleic acids, as well as other biological compounds. Like C, most N in the biosphere was originally fixed into organic mater from atmospheric sources. Nitrogen is abundant in the atmosphere as a divalent molecule, N$_2$; however, it is inaccessible to most organisms because of the strong triple bonds holding N$_2$ together. As a result, all naturally fixed N is produced by a subset of cyanobacteria and heterotrophic bacteria that have developed enzymatic pathways to catalyze N fixation. This group includes a number of common bloom-forming cyanobacteria in lakes. Nitrogen-fixing organisms have a competitive advantage over other organisms under conditions of N scarcity; however, N-fixation is costly due to the high level of energy needed to break the...
N-N triple bond, so even those organisms capable of fixing atmospheric \( \text{N}_2 \) only do so when fixed \( \text{N} \) is unavailable in the environment (Herrero et al. 2001; Muro-Pastor et al. 2005), and N-fixation is often suppressed when light availability is low (De Nobel et al. 1998). Like C, N can be lost from lakes in gaseous forms. At low oxygen concentrations denitrification occurs, converting \( \text{NO}_3^- \) to \( \text{N}_2 \) gas (by way of several intermediate steps). Denitrification is responsible for large losses of N from many lake systems (Binnerup et al. 1992; Burgin and Hamilton 2007; James et al. 2011; Small et al. 2013). Largely as a result of denitrification, lakes are usually net sinks for N, and denitrification in lakes may benefit downstream coastal waters, where N is more likely to be limiting (Piña-Ochoa and Álvarez-Cobelas 2006; Elser et al. 2007; Finlay et al. 2013).

While N fixation is the only natural source of N to the biosphere, human activities have altered the global N cycle dramatically through the discovery of the Born-Haber process, which has roughly doubled the rate of input of N into the terrestrial N cycle (Vitousek et al. 1997). Furthermore, fossil fuel combustion and industrial activity has dramatically increased rates of atmospheric N deposition to many parts of the world, and has likely resulted in changes to N:P ratios and nutrient limitation in many lakes (Crowley et al. 2012; Canham et al. 2012; Hessen 2013). Changes to precipitation regimes may also result in changes to the timing and magnitude of N inputs to lakes, as well as to the retention rate of N within lakes (Jeppesen et al. 2010; Finlay et al. 2013).

Of the three elements discussed in detail here (C, N, and P), P has received perhaps the most attention in lakes because it most frequently limits phytoplankton production in fresh water (Guildford and Hecky 2000). While it is a smaller component
of living biomass than C or N, P is a critical element for a number of biological macromolecules. In cells, P is a major component of nucleic acids, and is the main energy currency for enzymatic processes within cells in the form of ATP. The largest pool of P in cells is usually ribosomal RNA; as a result, P is critical for protein synthesis, and intracellular P concentrations are often tied to growth rates (Loladze and Elser 2011). Phosphorus is also a major component of biological phospholipid membranes (Sterner and Elser 2002). Because of the importance and relative scarcity of P, a number of primary producers are able to sequester P in storage molecules such as polyphosphate (Hupfer et al. 2004) or inositol phosphates (Turner et al. 2002; Giles et al. 2015).

In the environment, P is almost always found oxidized to PO$_4^{3-}$, or in organic compounds linked to phosphate groups by ester linkages (Reynolds and Davies 2001). Unlike C and N, P has no gaseous phase, so is not accessible from atmospheric reserves and must be delivered from lake catchments or atmospheric deposition (Schindler 1977). Phosphate is naturally produced by the gradual weathering of minerals in catchments, and is ultimately transported downstream to lakes and oceans, where it is eventually buried and reincorporated into minerals. When it reaches lake sediments, negatively charged PO$_4^{3-}$ often complexes with minerals containing metals such as Fe, Al, and Ca, becoming inaccessible to primary producers (Davison 1993). This may lead to P sequestration, and ultimately burial, if the P remains in solid phase. However, sediment-bound P bound to iron oxyhydroxides may be released into sediment porewaters under reducing conditions when Fe is reduced and solubilized. This process is extremely important in shallow lakes, where large increases in summer water column P concentrations can occur frequently.
when sediment respiration consumes oxygen and promotes P release (Nürnberg and Peters 1984; Jensen and Andersen 1992; Søndergaard et al. 1999).

The importance of P as a limiting nutrient in lakes was established in the 1970s, largely as a result of experiments by Schindler and others in the Experimental Lakes Area in Ontario, Canada (Schindler 1971; 1977). Through nutrient addition experiments to natural lakes, these researchers demonstrated that only with the addition of P could runaway algal bloom conditions be induced, and that additions of C or N alone produced only modest increases in phytoplankton biomass. These striking (and photogenic) experimental results were supported by a compelling logic: because C and N were both accessible to lakes from atmospheric pools, over time lakes should be able to compensate for deficiencies of C or N through fixation of atmospheric nutrients. By contrast, in natural conditions P was only available from the slow weathering of minerals in catchments feeding lakes, so there was no endogenous mechanism by which lake ecosystems could increase P stores; the result should therefore be P limitation over long timescales. As a result of the Canadian experiments and later studies, P quickly became the focus of most water quality research and management actions (Smith and Schindler 2009). The heavy focus on P dynamics in lakes is sometimes referred to as the “phosphorus limitation paradigm” (Sterner 2008).

While few researchers dispute the importance of P in lake systems, the P limitation paradigm has been criticized because it minimizes the role of other limiting resources. Nitrogen, in particular, is frequently an important limiting nutrient in lakes, particularly for cyanobacteria (Blomqvist et al. 1994). In the past decade, debate over the
relative importance of N and P has intensified, as increasing evidence has emerged that N is frequently an important limiting resource on timescales relevant to ecology and management (Schindler et al. 2008; Lewis and Wurtsbaugh 2008; Sterner 2008; Thad et al. 2013). Meta-analyses of whole lake manipulation experiments, including those in the Canadian experimental lakes which gave rise to the phosphorus limitation paradigm, that combined additions of both N and P give rise to the much greater increases in phytoplankton biomass than either N or P alone (Lewis and Wurtsbaugh 2008). Laboratory assays measuring in-situ nutrient limitation also confirm that dual nutrient additions have the greatest effect on growth, and frequently find evidence of N limitation (Elser et al. 2007; Sterner 2008), although this does not necessarily contradict the assumptions of the P limitation paradigm, which emphasizes nutrient limitation on long-term rather than short-term timescales (Schindler 1977). The relative importance of N and P in lakes may also be changing as a result of anthropogenic stressors. Like the N cycle, the P cycle has been dramatically accelerated by human activities (Filippelli 2008; Liu et al. 2016). By some estimates, the amount of P being introduced into aquatic ecosystems has doubled as a result of anthropogenic inputs (Filippelli 2008). The dramatic increases in P inputs may increase the likelihood of N limitation, although the increases in P may be offset by corresponding increases in N inputs in some systems. Climate change may also affect the relative importance of N vs. P limitation through changes to external nutrient inputs or internal processing of nutrients in lakes (Weyhenmeyer et al. 2007; Nürnberg and LaZerte 2015). In general, while P is still
considered the most important nutrient in lake ecosystems, recent research makes clear that a number of other factors must also be considered.

**The importance of multiple scales**

The many drivers which impact nutrient and cyanobacteria bloom dynamics operate at a variety of temporal scales (Carpenter and Kitchell 1987; Jennings et al. 2012; Kara et al. 2012). At a sub-daily timescale, photosynthesis-driven fluctuations of pH and dissolved oxygen may lead to corresponding changes in redox conditions at the sediment water interface which promote internal nutrient loading (Spears et al. 2008; Smith et al. 2011). At a daily to weekly timescale, phytoplankton growth rates (which are frequently on the order of 0.5 d\(^{-1}\) to 2 d\(^{-1}\) (Reynolds et al. 1984; Reynolds and Irish 1997) may respond to nutrient or light limitation (Sterner et al. 1997; Bhatti and Colman 2005), doubling or collapsing in the space of several days. At seasonal scales, algal succession is sometimes relatively predictable (Blenckner et al. 2002), but intra-annual differences in meteorological conditions or changes to external nutrient loads may lead to a large differences between years (Salmaso 2005; Anneville et al. 2005; Søndergaard et al. 2015).

Recent literature has highlighted the role of episodic weather events in driving phytoplankton dynamics. Storm events have been recognized as important controls on phytoplankton biomass, particularly in shallow lakes or lakes of intermediate depth (Cook et al. 2010; Klug et al. 2012; Jennings et al. 2013). Storms may impact lakes either through mass inputs of water or nutrients as a result of heavy precipitation (Klug et al. 2012), or through the direct effects of wind mixing on lake thermal structure. Wind
mixing events may alter competition for light between phytoplankton groups (Huisman et al. 1999b; a; 2004), by eroding lake thermal structure, leading to changes in average epilimnetic irradiance. Mixing may also affect the accessibility of hypolimnetic nutrients (Jennings et al. 2012). In addition to storm events, meteorological events such as droughts (Grantz et al. 2014) and summer heat waves (Jöhnk et al. 2008; Wagner and Adrian 2009) may be extremely important in mediating phytoplankton dynamics. The effects of episodic events are particularly difficult to observe in environmental data due to their unpredictability and the difficulty of sampling during storms. Only with the advent of automated sampling approaches have limnologists come to fully recognize the important roles that these discrete events play.

The diversity of factors controlling blooms and the multiple timescales on which they operate presents challenges to studying the mechanisms driving bloom development. To account for these multiple factors, field data must be collected at temporal scales appropriate to the process of interest. Furthermore, many variables cannot be conveniently measured at high temporal resolution, so it is frequently necessary to compare data taken at different temporal scales (Carpenter and Kitchell 1987; Levin 1992; Kara et al. 2012). As an added complication, the growth of phytoplankton populations may show lagged responses to controlling variables. Similar challenges present themselves with respect to spatial scale (Gikuma-Njuru et al. 2013; Soranno et al. 2015). Accounting for lagged responses and temporal autocorrelation, particularly in data collected across a range of temporal and spatial scales, requires a diversity of approaches

Large datasets of high-frequency or long-term measurements present significant problems because of spatial and temporal autocorrelation, and because p-values become difficult to interpret with large datasets. A number of statistical methods have been applied to study lake processes using large datasets to find ways to get the most value out of existing data. Improvements on linear regression such as quantile regression may provide relatively simple improvements over established techniques, and allow researchers to make more nuanced inferences about factors controlling cyanobacteria blooms at the extremes of their distribution (Xu et al. 2015a; b). Artificial neural networks (ANNs) have been applied to forecast cyanobacteria blooms in several studies (Recknagel et al. 1997; 2006) with promising results, although the models can be difficult to use for process-based inferences. Self-organizing maps, a neural-network based clustering method, have been applied in at least two studies to identify ecologically significant patterns in complex lake data (Rimet et al. 2009; Pearce et al. 2013). Genetic programming has also been tested in bloom forecasting applications with some promising results (Muttil and Chau 2006). Time-series techniques such as ARIMA or dynamic regression which take advantage of the temporal resolution of high-frequency buoy data may account for the lagged effects of bloom drivers (Mantua 2004; Arhonditsis et al. 2004), but may be difficult to apply if data gaps are present. Wavelet analysis can identify periodic signals in noisy data and has been applied to long-term lake-monitoring datasets (Carey et al. 2016). Wavelet analysis may be useful in high-frequency analysis
of water quality data as well, but has not yet been widely applied. Bayesian network models also have been useful for identifying the drivers of phytoplankton dynamics across systems (Rigosi et al. 2015). All of these methods show promise, but none have yet emerged as established methods for handling lake ecological data. The majority of new papers continue to rely heavily on traditional parametric methods or process-based ecological models (Mooij et al. 2010; Hipsey et al. 2015).

The role of lake type in determining response to stressors

Phytoplankton growth may be influenced by different factors within and among lakes. Lakes vary along several gradients, including oligotrophic – eutrophic (Xu et al. 2015a), clear—colored (Weyhenmeyer et al. 2014; Strock et al. 2014), deep—shallow (Moss et al. 1997), large—small (Tessier and Woodruff 2002), or turbid—clearwater (Scheffer et al. 1993). Lake type with respect to any of these gradients may mediate responses to external stressors (Huber et al. 2008; 2012). Thus lake characteristics are important to consider when making inferences about lake processes.

One of the most important variables controlling the physical, chemical, and biological structure of lakes is depth (Moss et al. 1997). Production in deep monomictic or dimictic lakes with stable thermal stratification during summer is strongly influenced by mixing depth and duration of the stratified season (Magnuson et al. 1997; Williamson et al. 2009). Deep oligotrophic lakes tend to have nutrient-poor eplimnia; bottom waters in these deep lakes remain oxygen rich throughout the stratified period, preventing both water-column denitrification and the release of sediment-bound phosphorus (Nürnberg and Peters 1984; Nürnberg 1984; Foley et al. 2011). As deep lakes become more
productive along a trophic gradient, increased sedimentation of particulate organic matter results in increased oxygen consumption in the hypolimnion. If bottom water oxygen is sufficiently depleted, reductive dissolution of iron oxyhydroxides may lead to increased release of sediment P to the water column (Schindler 1977; Nürnberg 1984; Davison 1993). Sediment-released P may support increased production in deep chlorophyll maxima if the photic zone extends below the thermocline (Fee 1976), or may support increased epilimnetic nutrient concentrations through diffusion of P across the thermocline (Reynolds and Davies 2001), active migrations of motile phytoplankton or zooplankton (Reynolds et al. 1987; Xu et al. 2009), or deepening of the thermocline (Cantin et al. 2011; Jennings et al. 2013). In addition to influencing sediment P release, decreasing hypolimnetic oxygen concentrations may promote denitrification, reducing available nitrogen and promoting further sediment P release (Saunders and Kalff 2001; Piña-Ochoa and Álvarez-Cobelas 2006; Burgin and Hamilton 2007).

In shallow polymictic lakes, similar oxygen-dependent processes may occur, but they operate at much shorter timescales (Wilhelm and Adrian 2008; Huber et al. 2012). Shorter periods of stable stratification allow less time for the development of bottom-water hypoxia relative to deep lakes. Higher temperatures and generally higher nutrient concentrations, however, promote rapid respiration and depletion of oxygen in sediments and the water column (Giles et al. 2016). Consequently, shorter periods of stratification are required to promote sediment nutrient release (Isles et al. 2015). In addition, the proximity of the sediments to surface waters provides a site for denitrification which is not available in deep sites (Fennel et al. 2008; James et al. 2011). Polymictic lakes may
vary greatly with respect to productivity, duration of stratification events, and sensitivity to mixing; these variations may produce highly dynamic systems adapted to different conditions of nutrient and light availability (Huber et al. 2008; Wilhelm and Adrian 2008; Cook et al. 2010; Jennings et al. 2012; Klug et al. 2012). In addition, the penetration of light to surface sediments in shallow lakes allows the growth of rooted macrophytes, which may compete with phytoplankton, increase zooplankton populations, and promote well-documented alternate stable states in these systems (Scheffer et al. 1993; Jeppesen et al. 1999; Scheffer 1999; Moss et al. 2012).

The effects of climate change on cyanobacteria blooms

The responses of individual lakes to climate change are likely to vary as a result of site-specific factors (Wagner and Adrian 2009; Kosten et al. 2009). However, a growing consensus is emerging that climate change may tend to exacerbate cyanobacteria blooms in the future (Paerl and Huisman 2008; Schindler 2009; Carey et al. 2012). Climate change can impact lakes either directly through inputs of energy to lakes in the form of wind, temperature, or solar radiation, or indirectly through mass fluxes to lakes mediated by a watershed filter (Magnuson et al. 1997; Leavitt et al. 2009; Vogt et al. 2011). Changes to either energy or mass inputs to lakes may result in changes in lake biogeochemical conditions and cyanobacteria abundance.

Increased energy inputs to lakes are likely to favor cyanobacteria under future climate scenarios. The most direct mechanism by which energy inputs may exacerbate cyanobacteria blooms is by increasing surface water temperatures. Many bloom-forming cyanobacteria have higher temperature optima than other phytoplankton species (Robarts
and Zohary 1987; Butterwick et al. 2005; Carey et al. 2012), although high diversity within each phytoplankton division suggests caution when making broad generalizations (Taranu et al. 2012; Lurling et al. 2012). In many temperate lakes, temperatures within the optimal range for cyanobacteria such as *Microcystis, Planktothrix, Anabaena,* and *Aphanizomenon* (above 25 °C) are relatively rare and usually short-lived (Read et al. 2014). Cyanobacteria in temperate lakes may therefore respond strongly to moderate warming that makes these conditions more prevalent. In addition to direct effects of warming, a number of indirect ways by which warming may increase cyanobacteria dominance have been hypothesized. Warmer temperatures are expected to increase the strength of thermal stratification (De Stasio et al. 1996) and the development of microstratification in shallow lakes or epilimnia of deep lakes (Imberger 1985; Hanson et al. 2008; Pernica et al. 2013). Increases in thermal stability are likely to favor cyanobacteria over other phytoplankton (Huisman et al. 2004), in part because buoyant, gas vacuolate cyanobacteria are capable of maximizing access to light during these conditions (Reynolds et al. 1987).

The effects of climate change on mass inputs to lakes are difficult to predict, due in part to the variability of precipitation predictions from global climate models (Guilbert et al. 2014). In general terms, total precipitation is likely to increase and be delivered in more intense storm events (Dokken 2012; Guilbert et al. 2015). At the same time, the likelihood of droughts and heat waves is also projected to increase (Jöhnk et al. 2008). This combination of trends (increased total mass inputs, and longer hot, stable periods) is likely to favor cyanobacteria, although there is likely to be a great deal of variation
between locations (Paerl et al. 2011b). Stronger storms will likely lead to higher total loads of nutrients to lakes. High-energy storms are capable of transporting higher concentrations of nutrients, particularly nutrients in the particulate phase (Vanni et al. 2001; Rao et al. 2009). At the same time, the pulsed delivery of nutrients in fewer larger storms may favor phytoplankton species that are able to store P and N in internal nutrient storage forms. Many cyanobacteria possess strong nutrient storage capabilities, particularly for phosphorus (Reynolds, 2006) (Droop 1974). Nutrient stores allow cyanobacteria to maintain high growth rates for extended periods when other phytoplankton are P limited.

**Study site: Lake Champlain**

Lake Champlain is a large, natural lake located in a continental rift valley between the Green Mountains of Vermont, the Adirondack Mountains of New York, and the St. Lawrence River valley in Quebec, CA. Lake Champlain is an important lake for the region historically, culturally, and economically (Albers, 2000). Ecologically, Lake Champlain is notable for its large ratio of watershed area to lake area (19:1) (Facey et al. 2012), making the lake highly susceptible to mass inputs of water and nutrients from its watershed. The lake is also characterized by complex bathymetry, consisting of 5 largely independent basins (South Lake, Main Lake, Malletts Bay, Northeast Arm, Missisquoi Bay), which are partially isolated hydrodynamically due to natural and man-made barriers, and which vary widely with respect to depth and surface area. Partly as a result of its complex geography, Lake Champlain has a wide range of ecological states, ranging
from oligotrophic-mesotrophic deep areas to highly eutrophic shallow bays (Xu et al. 2015a).

Paleolimnological analyses suggest that sediment and nutrient loading to Lake Champlain have increased in recent decades, beginning in the mid-19th century. Increases in sedimentation were the result of agricultural development and deforestation in the catchment. By 1870, close to 70% of the forests had been cleared for logging and agricultural activities (Levine et al. 2012); these forests gradually returned through the 20th century as agricultural activities declined. After the 1960s, however, a variety of land use changes occurred as a result of population increases, leading to increased delivery of storm-water and sediment to the lake. At the same time, agricultural intensification resulted in a shift from small family farms to larger facilities resulting in increased imports of feed and fertilizer (Albers, 2000). In addition to changes in sediment and nutrient imports, Lake Champlain has been heavily impacted by non-native species introductions in the late 1900s and early 2000s (Marsden and Hauser 2009), resulting in substantial changes to the lake ecosystem.

Cyanobacteria blooms have become more frequent in Lake Champlain in recent years (Smeltzer et al. 2012), and have received a great deal of attention from policy makers and the media. As a result of high total phosphorus levels associated with these blooms, a recent lawsuit by the Conservation Law Foundation resulted in a review of the Lake Champlain Total Maximum Daily Load (TMDL) for phosphorus (EPA 2015). In 2015, new regulations were passed to limit phosphorus inputs into the lake and signed into law (Act 64;
http://legislature.vermont.gov/assets/Documents/2016/Docs/ACTS/ACT064/ACT064%20As%20Enacted.pdf). Act 64 set aggressive P reduction targets to improve water quality in Lake Champlain, particularly with the goal of reducing cyanobacteria blooms. While my dissertation is intended to be relevant to research on the impacts of climate on lake ecosystems worldwide, it is also intended to be relevant in the context of local decision-making. While current legislative proposals give consideration to climate change effects on watershed processes, the direct effects of warming on internal lake processes have not yet been incorporated into nutrient loading targets. In the remaining chapters of my dissertation, I provide a comprehensive look at the factors controlling cyanobacteria blooms in Lake Champlain. I then provide both conceptual and quantitative frameworks for assessing the likely impacts of climate change on future water quality.

**Dissertation outline**

In the following chapters, I use multiple approaches to address several overarching questions: 1) To what extent do external nutrient inputs or internal nutrient processing control nutrient concentrations and cyanobacteria blooms in Lake Champlain? 2) How do internal and external nutrient inputs interact with meteorological drivers to drive bloom development? 3) How is anthropogenic climate change likely to impact these drivers and the risk of cyanobacteria blooms in the lake? Because of the multiple processes driving cyanobacteria blooms and the broad scope of my questions, I use a variety of approaches to resolve different temporal and spatial scales. I address my questions using (1) high-frequency biological, hydrodynamic, and biogeochemical data collected over 4 years of intensive field work in a shallow, eutrophic bay of Lake
Champlain; (2) 23 years of long-term monitoring data from 15 stations throughout the lake and 18 tributaries to the lake; and (3) integrated climate-watershed-lake models driven by regional climate projections.

The second chapter, Dynamic internal drivers of a historically severe cyanobacteria bloom in Lake Champlain revealed through comprehensive monitoring, focuses on characterizing the study system of Missisquoi Bay, Lake Champlain, using comprehensive high-frequency data from 2012 when the bay experienced its most severe bloom on record. The bloom year is partitioned into ecologically relevant stages of initiation, growth, decline, and senescence, and the factors associated with each of these stages are described, and placed in historical context using long-term monitoring data.

The third chapter, Buoyancy regulation and wind mixing destabilize lake metabolism: a novel application of high-frequency data, will provide an in-depth look at the role of wind mixing in controlling blooms in Missisquoi Bay by mediating light limitation. Wind-mixing has been found to be important in mediating competition for light among phytoplankton taxa in shallow lakes, with cyanobacteria typically dominating during calm, stable conditions (Nürnberg and Peters 1984; Nürnberg 1984; Huisman et al. 2004; Foley et al. 2011). In this study, I developed a novel approach to using high-frequency profiling buoy data to estimate the interacting roles of transient thermal stratification and buoyancy regulation in driving net ecosystem production during a strong cyanobacteria bloom in 2012. During turbid peak bloom conditions, calm stratified periods are necessary for cyanobacteria to overcome light limitation through buoyancy regulation and sustain bloom conditions.
In the fourth chapter, Temporal dynamics of wind mixing events control inter-annual variability in cyanobacteria bloom severity in a large, shallow bay of Lake Champlain, I continue to explore the role of wind mixing and transient thermal stratification on metabolism in Lake Champlain using three years of high-frequency data. I use time series analysis, correlation analysis, and a supervised self-organizing map to identify the critical physical thresholds which facilitate periods of high primary production and high net respiration in Missisquoi Bay. I find that the temporal structure, as well as the absolute frequency of wind mixing events is critical for bloom development, and that subtle differences in meteorological conditions can promote dramatically different bloom conditions.

In the fifth chapter, Climate-driven changes in energy and mass inputs systematically alter nutrient concentration and stoichiometry in deep and shallow sites in Lake Champlain, the focus expands beyond Missisquoi Bay to the entire lake. Using 23 years of long-term monitoring data from 15 sites within the lake, in addition to nutrient loading data from 18 monitored tributaries and meteorological data, I examine factors controlling long term changes in N and P concentrations at different sites. N:P declined lake-wide over the monitoring period, but the causes of these declines were different in deep and shallow areas. In deep sites, trends in N and P were driven by dissolved nutrient loads, which were strongly influenced by increases in precipitation. In shallow sites, declines in N:P were driven by decreases in summer wind speeds, increases in summer temperatures, and dissolved P inputs. A conceptual model is developed to generalize these findings and predict likely responses of N:P in different lakes to changing climate.
In both shallow and deep areas of the lake, falling N:P which is likely to result from climate warming may predispose the lake to more severe cyanobacteria bloom in the future.

In an appendix, Modelling the impacts of projected changes in climate and nutrient loading on cyanobacteria blooms in a eutrophic shallow bay of Lake Champlain using coupled watershed-lake models, I present the design and calibration of a watershed hydrology model and 3-D coupled hydrodynamic and water quality model of Missisquoi Bay which can be used to predict the responses of lake phosphorus concentrations and cyanobacteria blooms to future climate conditions. The hydrodynamic and water quality models are calibrated to 3 years of high-frequency monitoring data and 22 years of long-term monitoring data. I also present the integration of this lake model into a larger integrated assessment model capable of simultaneously simulating changes in hydrology, land use, nutrient transport, and lake water quality using a suite of downscaled climate projections for the years. The lake model reveals spatial and temporal patterns of water quality variables that are consistent with monitoring data, and the model highlights the importance of temperature in driving inter-annual variability in cyanobacteria concentrations. High-emissions climate scenarios result in higher average cyanobacteria concentrations and longer bloom seasons than low-emission scenarios, but all climate projections show an increase in TP and cyanobacteria concentrations driven primarily by temperature effects.
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CHAPTER 2: DYNAMIC INTERNAL DRIVERS OF A HISTORICALLY SEVERE CYANOBACTERIA BLOOM IN LAKE CHAMPLAIN REVEALED THROUGH COMPREHENSIVE MONITORING

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Abstract

The shallow bays of Lake Champlain have experienced increasingly severe algal blooms over recent decades, but the drivers of inter- and intra-annual variability of bloom severity are poorly understood. Disentangling the relative importance of multiple processes driving cyanobacteria blooms is difficult, in part because traditional monitoring programs often lack adequate temporal resolution, and in part because many studies seek to identify a single dominant mechanism. In this study, a holistic approach was used to identify multiple drivers of a strong cyanobacterial bloom over time in a shallow, eutrophic bay of Lake Champlain utilizing high-temporal-resolution meteorological, water quality, and biogeochemical sensor data; an intensive field sampling program; and a long-term monitoring dataset. In contrast to studies in similar systems, spring runoff and nutrient loads could not explain the severity of the 2012 bloom. Instead, internal nutrient loading and hydrodynamic conditions mediated shifts in resource limitation. The cyanobacteria growth phase was associated with the depletion of available nitrogen and sediment phosphorus release. The peak bloom stage was characterized by multiple limiting resources and stable water column conditions. The decline phase of the bloom was associated with light limitation brought about by wind mixing, and was punctuated by a major storm event, which brought a major biogeochemical shift in the lake system. This study illustrates the complexity of cyanobacteria resource limitation in response to internal and external forcing over time, and also the power of comprehensive monitoring approaches in disentangling complex drivers of eutrophic ecosystem function that vary across temporal and spatial scales.
Keywords: “Cyanobacteria bloom”, “internal phosphorus loading”, “Lake Champlain”, “resource limitation”, “high-frequency data”
Introduction

Cyanobacteria blooms in the northeastern arm of Lake Champlain (Vermont and New York, USA; Quebec, Canada) have increased over the past 50 years (Smeltzer et al. 2012), particularly in the shallow, eutrophic bays. This mirrors a global increase in the occurrence of toxic and nuisance cyanobacteria blooms in recent decades resulting from land use changes and climate warming (Dokulil and Teubner 2000; Paerl and Huisman 2009). Shallow lakes and bays are particularly sensitive to these stressors because factors such as large ratios of catchment area to lake volume, relatively warm temperatures and the accessibility of benthic nutrients may all predispose them to cyanobacterial dominance (Søndergaard et al. 2003). There is general consensus that inputs of nutrients are the most important long-term driver of increases in blooms, as well as the most practical target of management interventions (Smith and Schindler 2009; Schindler 2012); however, the effects of changes in the temporal patterns of nutrient inputs are incompletely understood (Vanni et al. 2006), and nutrient effects may be mediated by the effects of physical drivers (Huisman et al. 1999a; 2004) or food web structure (Carpenter et al. 1985; Jeppesen et al. 1997).

Recent research has focused on the importance of winter and spring nutrient inputs in driving phytoplankton dynamics during the bloom season (Gerten and Adrian 2000; Pierson et al. 2013). Strong positive correlations have been identified between spring river discharge (as well as spring inputs of total phosphorus and reactive phosphorus) and the magnitude of summer cyanobacteria blooms in western Lake Erie (Stumpf et al. 2012; Michalak et al. 2013). Lake Champlain lies within the same
geographic region as the Laurentian Great Lakes and shares many of their geological, physical and ecological characteristics (Facey et al. 2012), suggesting that algae blooms in Lake Champlain may be controlled by similar factors. In addition to the effects of spring runoff, external river inputs during the summer growing season may have complex effects on lake phytoplankton dynamics at varying timescales dependent on the antecedent nutrient and phytoplankton conditions in the receiving waters and the degree to which discharge events are accompanied by lake mixing (Jennings et al. 2012).

The paradigm of phosphorus (P) as the key nutrient limiting production in lakes has guided research and management for several decades and has resulted in significant improvements in water quality in many systems (Schindler 1977; Litke 1999); however, recent papers highlighting the importance of co-limitation by nitrogen (N) and P have produced renewed interests in the role of N limitation at ecologically meaningful timescales (Sterner 2008; Scott and McCarthy 2010). In western lake Erie, recent research has found evidence for N limitation in late summer cyanobacteria blooms (Chaffin et al. 2013). This echoes findings from lakes in Germany, which have found increasing incidence of N-limitation late in the summer (Kolzau et al. 2014).

The timing of internal nutrient processing may vary greatly between lakes, and can dramatically alter water column nutrient stoichiometry (Søndergaard et al. 2005). The mechanisms that control the release of benthic P are fairly straightforward in stratified lakes with hypoxic hypolimnia, where the reductive dissolution of iron and manganese oxyhydroxides leads to the release of adsorbed P to the water column. The mechanisms are more complex in shallow systems where thermal stratification occurs
periodically or not at all and where oxygen is typically abundant throughout the water column, but where high fluxes of sediment P are observed nonetheless (Jensen and Andersen 1992; Søndergaard et al. 1999; Hupfer and Lewandowski 2008). In these systems, benthic P release may be driven by reducing conditions resulting from temporary hypoxia during periods of transient thermal stratification (Burger et al. 2008), or by other factors such as high pH (Boers 1991; Seitzinger 1991; Jin et al. 2006) or nitrate (Jensen and Andersen 1992). In addition to differences in P cycling, N transformations also differ between shallow and deep waters. Warmer sediment temperatures and complete water column mixing may accelerate denitrification in shallow bays relative to deeper systems, (Van Luijn et al. 1999; McCarthy et al. 2013), and shallow bays may have increased availability of reduced inorganic N due to the breakdown of organic N compounds in the sediments or through dissimilatory or assimilatory nitrate reduction when oxygen is absent (Burgin and Hamilton 2007). Nitrogen fixation may also be important to the nitrogen budget of both deep and shallow lakes, and the magnitude of N fixation may vary in response to multiple factors, particularly ammonium concentration (Muro-Pastor et al., 2005).

The multiple internal and external mechanisms that impact the balance of N and P suggest that the factors driving this balance may operate at multiple temporal scales. Some processes such as photosynthesis-driven shifts in dissolved oxygen occur at short temporal scales (hourly to daily) (Staehr et al. 2011a), some processes such as wind mixing or peaks in river discharge are episodic and may cause rapid ecosystem responses when they do occur (Jennings et al. 2012), some such as denitrification or diagenesis of
detrital nutrients occur gradually on seasonal scales, and some such as anthropogenic nutrient inputs and climate changes are the result of both multi-annual trends and seasonal patterns (Piña-Ochoa and Álvarez-Cobelas 2006; Reitzel et al. 2012). This multiplicity of scales may help to explain divergent interpretations of basic ecosystem processes, because these interpretations may vary greatly depending on measurement intervals (Carpenter and Kitchell 1987; Levin 1992; McGill 2010).

The advent of automated sensor technology in combination with long-term monitoring data and robust field sampling programs has the potential to address incongruities in interpretations of trends in resource limitation which arise from processes operating at multiple timescales (Anderson et al. 2012). High-frequency sensors enable researchers to observe rapid ecosystem shifts in response to episodic events and to identify drivers of physical and biological processes that cannot be resolved using the weekly or bi-weekly data produced by traditional monitoring programs. In this study, we use data from an automated monitoring platform moored in a temperate shallow eutrophic bay in combination with an intensive field-sampling program and long-term monitoring data to 1) assess the severity of the 2012 bloom in Missisquoi Bay relative to other years; 2) assess the importance of external nutrient and water inputs in driving the severity of summer blooms; 3) quantitatively describe internal nutrient dynamics across the initiation, propagation, and senescence of an algal bloom; and 4) mechanistically describe the drivers of these dynamics across these stages of the bloom.
Methods

Study site

The study was conducted at a monitoring platform moored in Missisquoi Bay, Lake Champlain, which spans the border between Vermont, USA and Quebec, Canada (Fig. 2.1). Missisquoi Bay is eutrophic and has experienced regular blooms of toxic cyanobacteria over the past two decades composed primarily of Microcystis (mostly M. aeruginosa, with smaller populations of M. weisenbergii), Dolichospermum (predominantly D. circinalis, D. flos-aquae, and smaller populations of D. crassa), and Aphanizomenon flos-aquae (Levine et al. 2012). The bay is large (75 km²), uniformly shallow (max depth 5m, mean depth 2.8m) and can be considered to be isolated hydrodynamically from the main body of Lake Champlain, the only outflow being a 200 m opening through a causeway in the southwest. Our monitoring platform was moored in the southeastern portion of the bay at N 44˚ 59.503’, W 73˚ 06.798’. Depth at the study site ranges from 3 to 4 meters in 2012. The main tributary is the Missisquoi River, which accounts for 79% of total discharge into the bay (Limnotech 2012). The Missisquoi River drains a large forested (62%) and agricultural (25%) watershed (Levine et al. 2012) which spans the US-Canada border and is heavily impacted by nonpoint source agricultural pollution (Smeltzer and Simoneau 2008). Two other rivers, the Pike and the Rock, account for 18% and 3% of total discharge into the bay, respectively (Limnotech 2012).
Historical water quality data

Water quality data for total phosphorus, total nitrogen, chlorophyll-a, and surface pH from 2006-2012 were obtained from the Lake Champlain Long Term Monitoring Program website (http://www.watershedmanagement.vt.gov/lakes/htm/lp_longterm.htm) for station 51 (VT DEC and NY DEC, 2014). Spatial surveys have found water quality parameters at this location to be more similar to the site of the current study than the other long-term monitoring site in Missisquoi Bay (Isles, unpublished data).

Automated data collection

Water quality and meteorological data were collected using a modified YSI vertical profiling system (YSI systems, Yellow Springs OH) equipped with a YSI 6980 Controller Assembly, a YSI 6955 Winch Assembly, a meteorological (MET) station, and a depth sounding unit. The controller assembly housed independent CR1000 dataloggers
(Campbell Scientific, Logan UT, USA) for the sonde and MET stations, which were linked to a single modem via a PakBus configuration. LoggerNet Pro v. 4 software (Campbell Scientific, Logan UT, USA) was used to communicate with the winch and MET dataloggers. Power was supplied to the station by a 12 V battery, interfaced to two solar panels by solar charger/regulator.

The winch assembly was equipped with a YSI 6600V2 sonde containing probes for temperature (°C), conductivity (mS cm\(^{-1}\)), pH, dissolved oxygen (mg L\(^{-1}\)), phycocyanin fluorescence (PC; Relative Fluorescence Units, RFU), chlorophyll \(a\) fluorescence (Chl-\(a\); RFU), and turbidity (nephelometric turbidity units; NTU). The sonde was calibrated weekly following the manufacturer’s recommendations. Phycocyanin is a pigment specific to cyanobacteria and has been shown to be a good predictor of cyanobacteria biovolume in Missisquoi bay, while Chl-\(a\) measured using a YSI 6025 sensor has been shown to be a poor predictor of cyanobacteria biovolume (McQuaid et al. 2011; Zamyadi et al. 2012), and can be seen as primarily reflecting the abundance of eukaryotic phytoplankton, although there may be some contribution from phycocyanin-containing cryptophyte species. Changes in the ratio of PC to Chl-\(a\) can therefore be interpreted as shifts in the relative proportions of cyanobacteria and eukaryotic phytoplankton. Sonde profiles were programmed to occur hourly at 0.5 m increments throughout the water column. The bottom 1 m of the water column was excluded to prevent collision of the probes with the sediment surface during periods of high wave activity. The meteorological station included sensors for wind speed and direction, air temperature, relative humidity, pressure, and solar, as well as an internal
compass to correct for buoy orientation. Meteorological data was logged at 30 min intervals. The platform was deployed on 29 June 2012 and removed for the season on 6 November 2012. Sonde and met station data collection came online on 18 July 2012. Two ISCO automated water samplers (Teledyne ISCO, Lincoln NE) were mounted on the deck of the platform with intake hoses at 0.5 m and 2 m below the water surface and set to collect samples for total nitrogen (TN) and total phosphorus (TP) analysis every 8 hours at 5:00 h, 13:00 h, and 21:00 h daily (EDT). ISCO bottles were pre-acidified using concentrated sulfuric acid (0.1%) to preserve samples for nutrient analysis and collected weekly.

*Weekly field sampling and water column measurements*

Weekly field sampling was conducted at the site of the automated buoy throughout the season starting on 29 June and continuing through 26 October. Duplicate water samples were collected at each of 5 depths as measured from the water surface (<0.1, 1, 2, 2.5, 3 m) using a Van Dorn bottle to capture vertical gradients of nutrients and phytoplankton within the water column. All samples were collected in 1 L acid-rinsed bottles and kept on ice for transport back to the lab. Samples for dissolved metals were collected in separate acid-washed polyethylene bottles. Photosynthetically active radiation (PAR; \(\mu\text{mol m}^{-2}\text{s}^{-1}\)) profiles of the water column were taken weekly at 0.5 m increments using a LI-193 spherical quantum sensor (LI-COR Biosciences, Lincoln NE).

*Sample processing*

Within 24 h of collection, lake and river samples (1 L) were sub-sampled for nutrient, metals, and phytoplankton analyses. Total N and P samples were acidified to pH
< 2 (0.1% H$_2$SO$_4$) and stored at room temperature. Soluble reactive P (SRP) and dissolved inorganic N (DIN; NO$_3^-$/NO$_2^-$, NH$_4^+$) samples were filtered (0.45 mm PES) and stored at 4$^\circ$C (<24 h) and -20$^\circ$C, respectively. Dissolved metals samples were prepared by filtration through 0.45 mm PES followed by acidification (0.1% hydrochloric acid) and storage at room temperature. Phytoplankton samples were preserved in 1% Lugol’s solution with glass beads to prevent degradation of silica in diatoms and synurophytes.

**Analysis of nutrients and metals**

Soluble reactive P was determined by molybdenum colorimetry with ascorbic acid modification (USEPA 1995) using a Shimadzu 1601 UV-Vis spectrophotometer (10cm path length; Shimadzu Scientific Instruments Corp., Columbia MD, USA). Dissolved NO$_3^-$/NO$_2^-$ was measured using EPA method 353.2 (USEPA 1993) and NH$_4^+$ concentrations were determined using EPA method 350.1 (USEPA 1993b) and measured using an AQ2 Autoanalyzer (Seal Analytical, Mequon WI). Following persulfate digestion (Std. Method 4500 P-J.; APHA 2005), Total N and Total P were determined using EPA methods 353.2 and 365.1, respectively (USEPA 1993c). Throughout this study, nutrient concentrations are reported based on elemental mass (eg., P or N rather than PO$_4^{3-}$ or NO$_3^-$), and all nutrient ratios are expressed as molar ratios. Dissolved metals concentrations as well as total dissolved phosphorus (TDP) were measured by inductively-coupled-plasma-mass-spectroscopy (ICP-MS; Element 2, Thermo Scientific) at the Woods Hole Oceanographic Plasma Facility. The protocols of Shiller (2003) were followed for dissolved trace metal sampling.
Phytoplankton samples

Surface phytoplankton biovolume was measured using the FlowCAM© imaging-in-flow system (Fluid Imaging Technologies, Yarmouth, ME). All samples were imaged in autoimage mode at 200x, 100x, and 40x magnification. 40x magnification was found to be sufficient to estimate >90% of total biovolume during the bloom, and was used to quantify large and colonial phytoplankton >25 µm in diameter (measured as areal-based diameter, ABD). These estimates did not capture populations of small unicellular phytoplankton, which may make up a majority of total biovolume during some non-bloom periods. FlowCAM estimates have been found to provide similar results to microscope counts, particularly with respect to the relative proportions of different groups (Álvarez et al., 2013). A subset of samples was counted using the Utermöhl inverted microscope technique for validation of FlowCAM estimates, and FlowCAM-estimated cyanobacteria biovolume was also compared to sonde phycocyanin estimates (Fig. S1). All microscope counts were conducted at 400x, and all counts contained at least 100 grids or 100 natural units of the most abundant phytoplankton species. Species identifications followed Prescott (1962; 1978), Wehr and Sheath (2002), and Komarek and Zapomelová (2007), with considerable assistance from the online key Phycockey (Baker 2014). Additional net phytoplankton cell count and biovolume data were acquired from the Lake Champlain Long-Term Monitoring Program for station 51 (VT DEC and NY DEC, 2014).
Estimation of tributary nutrient fluxes

USGS stream gage data was obtained for the Missisquoi River at Swanton, VT, station number 04294000. TN and TP concentration data for the Missisquoi River from 2000 through 2013 were obtained from the Lake Champlain Long-Term Monitoring Program through the Vermont Department of Environmental Conservation website (http://www.anr.state.vt.us/dec/waterq/lakes/htm/lp_longterm.htm).

Tributary fluxes throughout the sampling season were estimated for the Missisquoi River using the EGRET R package v.1.2.4 (Hirsch 2013). This package computes regression surfaces of concentration-discharge relationships weighted by season (Hirsch et al. 2010). Fluxes at the gaging station were scaled to reflect the proportion of the watershed captured by the station (0.98) as well as the proportional contribution of the Missisquoi River to total hydrologic inputs to the bay (0.79) (Limnotech 2012).

Euphotic depth estimation

Euphotic depth was defined as 1% of irradiance immediately below the water surface, and was estimated from weekly PAR profiles by fitting a curve of Beer’s Law:

\[ I_z = I_0 \cdot e^{-k_z} \]

Where \( I_z \) is irradiance at depth \( z \), \( I_0 \) is surface irradiance, \( k \) is the light extinction coefficient, and \( z \) is depth. In order to interpolate between weekly measurements, stepwise multiple regression (forward and backward) was used to find the best model for the estimation of euphotic depth based on sonde variables.
Statistics

All statistical analyses were carried out using R statistical software (R Core Team 2013). Smoothing of PC and Chl-a data for visualization was carried out using a smoothing spline with the function `smooth.spline`. Autocorrelation analyses were carried out using the `acf` function, correlations were carried out using the function `cor.test` with Pearson’s product-moment correlation \( \alpha < 0.05 \), linear and multiple least-squares regressions were carried out using the function `lm` \( \alpha < 0.05 \), and model selection was carried out using AIC with the functions `step` and `lm`. Curve fitting was carried out using the function `optim`.

Results and Discussion

2012 in historical context

Comprehensive analysis of the historical water quality dataset suggests that in 2012, Missisquoi Bay experienced the strongest bloom within recent years (Figure 2.2). To illustrate this point, Figure 2.2 shows several metrics of bloom severity from long-term monitoring data from a site close to the sampling location for the current study (Station 51; VT DEC and NY DEC, 2014). Total phosphorus in 2012 was consistently higher throughout the growing season than in any of the previous years. Chl-a reached its highest observed concentration in August of 2012, although concentrations during July were somewhat lower than the previous years. pH, an integrated signal of total primary production, was also consistently higher in August of 2012 than in any other year on record. In contrast to these other indicators, total nitrogen was relatively low from June-July, though it increased in August to approach its highest values for the year 2012.
Collectively, our analysis of multiple parameters within historical water quality data set from Missisquoi Bay clearly demonstrates that the bloom of 2012 was particularly severe relative to other years.

Figure 2.2: Comparison of bloom metrics and external loads from long-term monitoring data in Missisquoi Bay (A-D) and the Missisquoi River (E-F), 2006-2012. A: total phosphorus (µg L⁻¹). B: Chlorophyll-a (µg L⁻¹). C: total nitrogen (mg L⁻¹). D: pH. 2012 is shown with the red filled circles. E: Cumulative discharge from the Missisquoi River (m³). F: cumulative TP loading (kg). Blue dashed horizontal line on panel F represents a rough estimate of total water column TP needed to sustain peak bloom conditions (see text).
While recent research in western Lake Erie has found strong correlations between the magnitude of spring discharge and the severity of summer blooms (Stumpf et al. 2012), Missisquoi Bay seems to show a contrasting response. The strong bloom in 2012 occurred during the driest year in the period of record. From May onwards, cumulative discharge from the Missisquoi River was lower than that of any previous calendar year (Figure 2.2 E). Total phosphorus loading from the Missisquoi River, which is well correlated with discharge (Smeltzer and Simoneau 2008) was also lower in 2012 than in other years (Figure 2.2 F). Snowpack in the Missisquoi watershed was also at a historically low level during the winter (http://www.jaypeakresort.com/skiing-riding/the-mountain/snowfall-charts/), suggesting that spring snowmelt and associated P and N loading was also historically low. In contrast, the year 2011 experienced the highest spring TP loading on record due to extensive spring floods (Fig. 2.2 F), but experienced only a modest cyanobacteria bloom (Watzin et al. 2012), and water column concentrations of TP, TN, and Chl-a were all unremarkable (Fig. 2.2). Similarly, the year 2007 which had the highest cumulative spring discharge for the years on record, was also the only year in the past decade in which cyanobacteria did not dominate the phytoplankton community in Missisquoi Bay (Watzin et al. 2008). Assuming maximum water column concentrations of 100 µg P L⁻¹ during a strong bloom year (Fig. 2.2 A), uniform concentrations throughout the bay, and bay volume of 0.22 km³ (Levine et al. 2012), the total mass of P in the water column required to sustain a bloom was calculated to be approximately 22,000 kg (horizontal blue line on Fig. 2.2, panel F). This is well below previous records of total spring loading from the Missisquoi River alone, although
this does not account for removal pathways. However, in 2012, the highest recorded summer TP in the bay occurred in a year with the lowest spring TP load. This suggests that variation in the internal loading of phosphorus within the bay has had a larger influence on the interannual variability of TP concentrations and bloom dynamics than previously thought.

2012 bloom progression

While useful to provide some historical context of bloom severity and for identification of a primarily internally-driven biogeochemical system in 2012, the composition and temporal resolution of the DEC monitoring dataset limits its utility for describing the mechanisms responsible for bloom dynamics. It was serendipitous, then, that the historical bloom of 2012 also coincided with the deployment of our comprehensive high-frequency, vertically resolved monitoring effort. Indeed, the fact that 2012 was remarkable both for the strength of the summer cyanobacteria bloom and for the lack of external inputs makes it an ideal year for studying the internal drivers of bloom progression in Missisquoi Bay, and the framework and components of our comprehensive monitoring effort provided a powerful tool to study these internal dynamics.

To establish context for the 2012 bloom dynamics, a combined plot of Chl-a and PC fluorescence data provides an illustration of the progression throughout the deployment of our monitoring initiative (Fig. 2.3). Sensor data show PC rising from near background levels at the middle of July, and reaching sustained levels of ~30 RFU during the middle of August. PC fell during the last week of August and first week of
September, but remained fairly high until a storm on 5 September. Following that storm, PC remained low. Chl-a largely paralleled PC for most of July and the first half of August, but they became decoupled in late August and early September, with Chl-a continuing to increase until the 5 September storm. Following the storm, Chl-a rebounded to its highest levels of the season, suggesting increased dominance by eukaryotic algae. The clear trajectory of the bloom as revealed by Chl-a and PC fluorescence data allowed us to separate the season into distinct stages of development for discussion and analysis purposes (Fig. 2.3; table 2.1). Six stages are herein discussed: pre-bloom, growth phase, peak bloom, late bloom, chlorophyll bloom, and post-bloom. Of these, the first four occurred with minimal external inputs of nutrients with the exception of the 5 September storm at the end of the late bloom stage, so nutrient dynamics during these stages are considered to be driven by internal processes, which are occurring in conjunction with meteorological forcing (eg temperature, light, wind). All of the bloom stages will be discussed with respect to physical drivers, phytoplankton dynamics, nutrient dynamics, and potential limiting resources.
Figure 2.3: Spline-smoothed concentrations of chlorophyll-$a$ and phycocyanin fluorescence showing the phases of the 2012 bloom. Area with blue diagonal hatching represents phycocyanin, area with green horizontal hatching represents chlorophyll-$a$. Units are ratio fluorescence units, and are not directly comparable between chlorophyll-$a$ and phycocyanin. Letters denote bloom phases: Pre = ‘pre-bloom’, Growth = ‘growth phase’, Peak = ‘peak bloom’, Late = ‘late bloom’, CHL = ‘chlorophyll-$a$ bloom’, Post = ‘post-bloom’. These intervals are also used in subsequent plots.

Table 1: Phases of the 2012 Bloom Season. Meteorological and water quality data show mean (st. dev) of the data for each variable for the interval described. River loads are presented as total aggregate load for the bloom stage. All limnological variables are depth-averaged unless otherwise noted. ND indicates no data.
<table>
<thead>
<tr>
<th>pH (surface)</th>
<th>ND</th>
<th>9.09 (0.43)</th>
<th>9.76 (0.28)</th>
<th>9.37 (0.48)</th>
<th>8.28 (0.70)</th>
<th>7.67 (0.15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphotic Depth (m)</td>
<td>ND</td>
<td>3.76 (0.36)</td>
<td>2.7 (0.57)</td>
<td>2.56 (0.43)</td>
<td>2.98 (0.52)</td>
<td>4.18 (0.21)</td>
</tr>
<tr>
<td>TN mg/L</td>
<td>0.41 (0.08)</td>
<td>0.64 (0.28)</td>
<td>1.40 (0.55)</td>
<td>1.39 (0.39)</td>
<td>0.88 (0.17)</td>
<td>0.64 (0.13)</td>
</tr>
<tr>
<td>TP mg/L</td>
<td>0.039 (0.0093)</td>
<td>0.078 (0.029)</td>
<td>0.127 (0.044)</td>
<td>0.137 (0.038)</td>
<td>0.087 (0.019)</td>
<td>0.046 (0.017)</td>
</tr>
<tr>
<td>TN:TP</td>
<td>25.1 (7.6)</td>
<td>18.2 (4.3)</td>
<td>25.1 (6.8)</td>
<td>23.4 (4.6)</td>
<td>22.5 (2.3)</td>
<td>32.8 (9.0)</td>
</tr>
<tr>
<td>River TP load (kg)</td>
<td>970.6</td>
<td>586.2</td>
<td>206.0</td>
<td>7609.6</td>
<td>4142.4</td>
<td>13061.0</td>
</tr>
<tr>
<td>River TN load (kg)</td>
<td>23386.7</td>
<td>17874.6</td>
<td>5660.4</td>
<td>32787.1</td>
<td>40086.5</td>
<td>120419.1</td>
</tr>
</tbody>
</table>

**Pre-Bloom (21 June through 17 July)**

The pre-bloom stage of development coincided with the deployment of our equipment, but logistical challenges associated with sensor deployment precluded automated data collection during this period. As such, we primarily rely on meteorological data from a nearby station and discrete weekly grab sampling to describe the dynamics associated with this phase. The pre-bloom was characterized by low and declining discharge from the Missisquoi River, warm air temperatures, and long days. Water temperature data were not available for this period, but trends from other years indicate that water temperatures were warm and rising through this period (Fig. 2.4). Met data from a nearby monitoring station on the lake indicates that air temperatures were warmer in the pre-bloom period than in the subsequent bloom stages (mean air temperature 22.9°C during the pre-bloom, compared to 22.7°C during the growth phase, 22.1°C during the peak bloom, 21.6°C during the late bloom, and 18.0°C during the Chl-a bloom) and wind speeds were lower (mean wind speed 3.6 m s⁻¹ during the pre-bloom, compared to 4.8 m s⁻¹ during the growth phase, 3.5 m s⁻¹ during the peak bloom, 5.5 m s⁻¹ during the late bloom, and 6.1 m s⁻¹ during the Chl-a bloom; http://www.uvm.edu/vmc/project/colchester-reef-meteorological-monitoring-38-m).

While met data from this station are not directly comparable to data at our monitoring site...
(Table 2.1) due to higher average wind speeds and lower air temperature at the main lake station, relative trends between bloom stages are similar. Mean daily solar radiation was also highest during this pre-bloom period (238 W m⁻², compared to 211 W m⁻² during the growth phase, 173 W m⁻² during the peak bloom, 177 W m⁻² during the late bloom, and 156 W m⁻² during the Chl-α bloom). Taken together, the temperature, wind speed and solar radiation data allow us to infer that the internal physical drivers that would facilitate nutrient release from the sediment water interface (transient thermal stratification) and increasing phytoplankton productivity (high light and warm water temperatures) were progressively developing during this phase. Indeed, SRP increased over the course of the pre-bloom, while DIN declined (Fig. 2.5). Higher concentrations of SRP in the bottom water and lower concentrations at the surface were consistent with internal sediment phosphorus loading. The decreases in DIN are likely the result of both uptake by diatoms and denitrification, which has been shown to be highest in Missisquoi Bay in the late spring (McCarthy 2011). Rising SRP and falling DIN resulted in falling ratios of both DIN:SRP and TN:TP (Figs. 2.5 and 2.6).
Figure 2.4: \( \log_{10} \) Missisquoi River Discharge (m\(^3\) s\(^{-1}\)), 3 hour running average of wind speed (m s\(^{-1}\)), water temperature at 0.5m and 2m (°C), pH at surface and 2m, and DO (mg L\(^{-1}\)) at 0.5m and 2m (with 100% saturation shown in dotted line). Shaded regions represent phases of the bloom (see figure 3).
Figure 2.5: Dissolved inorganic nitrogen (µg L⁻¹), SRP (µg L⁻¹), and DIN:SRP measured at different depths. Several values of DIN:SRP with DIN below detection limits are omitted due to the logarithmic scale. Dotted line represents the Redfield N:P ratio (16:1). Shaded regions represent phases of the bloom (see figure 2.3).
Figure 2.6: Total N mg L$^{-1}$ (top), TP mg L$^{-1}$ (middle) and TN:TP molar ratio (bottom) at an 8-hour timescale, all presented as means of samples from 0.5m and 2m depth. Solid red lines represent regressions estimating rate of increase of water column TN and TP concentrations during bloom development. Dotted line indicates the Redfield ratio of 16:1. Shaded regions represent phases of the bloom (see fig. 2.3).

The phytoplankton community during this period was initially dominated by the diatom *Aulacoseira ambiguа*, which declined over the course of this period coincident with increasing populations of *Microcystis aeruginosa* and *Dolichospermum circinalis* (Fig. 2.7, Supplemental Fig. 2.2), with smaller populations of the morphospecies *Microcystis weisenbergii, Dolichospermum flos-aquae* and *Dolichospermum crassa*. Populations of smaller phytoplankton may not counted by FlowCAM may have contributed to phytoplankton biovolume during this stage as well. While it is difficult to infer resources limiting production unequivocally at the start of the pre-bloom due to a
lack of comprehensive data, as time progressed and internal loading increased water column SRP concentrations while DIN decreased, the system trended towards N limitation. By the end of the pre-bloom, conditions were well primed for the growth of cyanobacterial populations.

Figure 2.7: Biovolume (mm$^3$ m$^{-3}$) and proportional biovolume of large (>25µm) phytoplankton in Missisquoi Bay from surface samples during the 2012 bloom season.

_Growth Phase (18 July through 6 August)_

The growth phase consisted of continued warm air and water temperatures and frequent thermal stratification, which was now measured directly from the automated sensor system (location-Figure 2.1, e.g. Figure 2.3 and subsequent plots). Water temperatures approached the growth optima for _Microcystis_ and _Dolichospermum_ during this period (25-30°C; (Paerl et al. 2011b). Indeed, populations of cyanobacteria rose throughout the growth phase, while proportions of eukaryotic phytoplankton declined.
Aphanizomenon flos-aquae and Microcystis aeruginosa dominated during this phase, with populations of Dolichospermum circinalis increasing during the first week of August. River discharge was low and wind speeds were light and variable throughout this period (Fig. 2.4). Intermittent calm periods coupled with strong solar radiation contributed to frequent thermal stratification. Factors influencing lake hydrodynamics (river discharge, thermal dynamics, wind speed, solar radiation) all suggest that the system was physically conducive to the continued release of sediment-bound nutrients (Burger et al. 2007; 2008).

The onset of the growth phase coincided with the depletion of available nitrogen and the onset of N-limiting conditions. This is indicated by DIN concentrations, which were very low, by DIN:SRP ratios well below the Redfield ratio, and by TN:TP ratios which were the lowest of the season, and well below 20 which has been cited as a value below which N limitation becomes dominant (Guilford and Hecky, 2000). Further support comes from the emergence of nitrogen-fixing cyanobacteria during this period. The emergence of N-limiting conditions at the onset of bloom development supports the theory that low available N favors the dominance of cyanobacteria with low nitrogen requirements, and particularly N-fixing cyanobacteria (Blomqvist et al. 1994; Herrero et al. 2001).

Despite the scarcity of DIN, TN increased throughout the growth phase. This indicates that there was an additional source of N fueling bloom development. Comparison of loading data and water column concentrations during the growth period make it clear that the rise in TN cannot be explained by river inputs alone. River TN
loads during the growth period (18 July through 20 August, the peak of PC fluorescence) could account for a maximum increase of 3.045 µg L$^{-1}$ d$^{-1}$, while the average linear rate of increase observed over this period was 35.25 µg L$^{-1}$ d$^{-1}$. Other possible sources could have been N release from lake sediments or N fixation by cyanobacteria. Previous research in Missisquoi Bay has found the rate of sediment N release to be of a similar order of magnitude to the observed increases in water column TN (McCarthy 2011), and have found low rates of N-fixation even with large populations of N-fixing cyanobacteria (McCarthy et al. 2013). Heterocyst counts in Nostocales species are generally low in Missisquoi Bay (Angela Shambaugh, Vermont Agency of Natural Resources, personal communication) but the prevalence of Aphanizomenon and Dolichospermum during the 2012 bloom suggests that N-fixation may have played at least some role in the observed increase of N, although research in other productive systems suggests that this fixation was likely unable to balance N deficiency (Grantz et al. 2014).

The occurrence of N-limiting conditions during the growth phase was in part due to high levels of internal P loading. Total P continued to increase throughout the growth phase, before reaching maximum values in the middle of the peak bloom period (Fig. 2.6). As with TN, the increase in TP observed during the growth phase and the early part of the peak bloom cannot be explained by river inputs during this period. The linear rate of increase in TP from the onset of the growth phase on 18 July through the highest observed algal concentrations on 20 August (calculated as the mean of TP at 0.5 m and 2 m) was 2.66 µg L$^{-1}$ d$^{-1}$ (Fig. 6, regression line shown in red). During this same period, the maximum potential increase that could be accounted for by river TP flux was 0.102 µg L$^{-1}$.
1 d⁻¹, assuming a bay volume of 0.22 km³ (Levine et al. 2012). This suggests that while spring loads may be sufficient to explain maximum summer TP concentrations (Fig. 2.2 F), the low TP concentrations during the pre-bloom coupled with the low external flux during the growing season make clear that before river P is made available to phytoplankton it must first be deposited in the sediments. Therefore, the water column P availability during the bloom period is mediated by conditions driving the re-mobilization of sediment P.

The strongest evidence of sediment P mobilization can be seen in the water column SRP concentrations, which remained high during most of the growth phase. High SRP concentrations in the bottom waters and relatively low concentrations in surface waters was consistent with the development of vertical gradients, which would result from the upward diffusion of P from sediments (Fig. 2.5). The mobilization of sediment P has generally been attributed to the depletion of oxygen at the sediment-water interface (Søndergaard et al. 1999). Dissolved oxygen in Missisquoi Bay generally increased throughout the growth phase of the bloom, reflecting increased primary production in the water column (Fig. 2.4), and surface concentrations often approached 200% saturation during the day.

Despite the increase in primary production, DO showed transient depth gradients, with oxygen concentrations at 2 m often decreasing during the course of daytime photosynthesis. This stratification of DO was dependent on the development of thermal stratification. The difference in DO between the surface and 2 m (ΔDO_{surf–2m}) for all bloom stages excluding the post-bloom was highly correlated with ΔT_{surf–2m} during the
same period ($R^2 = 0.569, p<0.0001, n=1542$) (Fig. 8). The slope of the relationship between $\Delta DO_{surf-2m}$ and $\Delta T_{surf-2m}$ was higher during bloom stages with high phytoplankton biomass, indicating that increased water column respiration and decreased light available for photosynthesis allowed increasingly rapid oxygen depletion in the lower water column as the bloom developed (Fig. 2.8). Dissolved oxygen concentrations at 2 m were often only slightly below 100% saturation on days when the oxygen profile showed stratification; however, manual vertical profiles of dissolved oxygen from 2013 which provide better measurements of $O_2$ in the bottom water show strong oxygen gradients in the bottom 1 m of the water on calm days (Supplemental Fig. 2.3). Even these profiles likely underestimate true oxygen concentrations at the sediment-water interface; previous research has found sharp oxygen gradients on the millimeter scale near the sediment-water interface (Smith et al., 2011), particularly under calm conditions. As a result, we interpret periods of stratification between the surface and 2 m as a proxy for conditions facilitating the development of hypoxia at the sediment-water interface.

![Graphs A, B, C](image)

**Figure 2.8:** Panel A: Thermal stratification between 0.5 and 2 m ($^\circ$C) vs. stratification in DO (mg L$^{-1}$). Point types represent samples from different stages of the bloom: circles = growth phase, triangles = peak bloom,
crosses = late bloom, and exes = chl-a bloom. Lines represent linear regressions for each bloom stage.

Panel B: Thermal stratification (˚C) vs. stratification in pH. Panel C: Thermal stratification (˚C) v. 3-hour running average of wind speed (m s\(^{-1}\)). Vertical line is at 4.5 m s\(^{-1}\).

The relationship between \(\Delta\text{DO}_{\text{surf} - 2\text{m}}\) and \(\Delta T_{\text{surf} - 2\text{m}}\) supports the conclusion that the internal flux of phosphorus was driven by transient thermal stratification resulting in bottom water oxygen depletion and reductive dissolution of Fe and Mn oxyhydroxides. This is further supported by the geochemical data. Dissolved Fe and Mn in water taken at 3 m were tested for correlations with total dissolved P at the same depth. No significant correlations were found for Fe, but dissolved Mn was significantly positively correlated with total dissolved P as measured by ICP-MS (\(R^2 = 0.42, p = 0.030; n=11\)). Manganese is more easily reduced than Fe, which may make it a more sensitive indicator of the occurrence of transient stratification than dissolved Fe in Missisquoi Bay (Pearce et al. 2013). The presence of dissolved Mn in the lower water column is another indicator that reducing conditions were present in the bottom water even when DO concentrations at 2 m depth were close to saturation. Taken together, the DO, metals, and SRP data confirm the hypothesis that internal loading is driven by transient thermal stratification and resulting bottom water reducing conditions. Furthermore, the effects of transient stratification are magnified during periods of high biomass, which may constitute a positive feedback for maintaining bloom conditions. The frequent thermal stratification and the rise in water column TP observed during the growth phase suggest that these processes were particularly important in fueling the rapid growth of cyanobacteria populations in 2012.
Peak Bloom (8 August through 24 August)

During the peak bloom, river discharge was at a minimum and average wind speeds were consistently low, rarely rising over 4.5 m s\(^{-1}\) (Fig. 2.4; Table 2.1). These conditions resulted in frequent periods of thermal stratification during daylight hours and provided a thermal structure, which was conducive to the continued release of sediment bound P. During the peak bloom, phytoplankton biovolume reached the maximum of the season. The phytoplankton community was largely comprised of Dolichospermum and Microcystis spp. (Fig. 2.7), suggesting that ecosystem demands for both N and P would also be extremely high during this phase.

A high biological demand for nutrients would explain the rapidly declining SRP concentrations that occurred during the peak bloom. The drop in SRP concentrations below detection limits coincided with the peak of algal biovolume. TN and TP were high and were correlated with PC, suggesting that most of the water column nutrients were associated with cyanobacterial biovolume. TP continued to rise for most of the peak bloom, which must be fueled by sediment P release (as indicated by the lack of external inputs) but fell at the end of the period paralleling the decline in cyanobacteria populations.

In addition to the role of reductive dissolution, elevated pH due to high photosynthetic activity may have also played a role in promoting release of sediment P during the peak bloom. Previous research has found that pH above 9 can accelerate sediment P flux (Boers 1991; Seitzinger 1991; Jensen and Andersen 1992; Jin et al. 2006). In Missisquoi Bay, surface pH remained between 9 and 10.5 throughout this
The effects of this high pH on sediment nutrient release may be mitigated by the steep pH gradients that occur near the sediment water interface during calm conditions (Smith et al. 2011), but high pH may help to sustain P flux during mixed conditions when boundary layers are less pronounced.

Total N tracked PC concentrations closely, rising for most of the peak bloom but declining towards the end. As described for the TP trends, this indicates that most of the nutrients in the system were incorporated into algal biomass during the peak bloom period. The decrease in cyanobacteria biovolume at the end of the peak bloom period coincided with marked increases in DIN, consistent with the remineralization of algal N. The TN:TP ratio rose throughout the growth phase and peak bloom. TN:TP (calculated using an average of concentrations at 0.5m and 2m) was positively correlated with cyanobacterial abundance during the growth period, from the start of automated data collection through the period of highest PC concentrations on 25 August ($R^2 = 0.20, p < 0.0001, n=90$). This rise in TN:TP sheds light on the debate about the relationship of TN:TP with cyanobacterial dominance (Smith 1983). In this dataset, low TN:TP coincided with the onset of cyanobacterial growth, but during the bloom itself, TN:TP is positively correlated with PC. The rise in TN:TP during bloom development may be partly attributable to light limitation, which has been shown to affect algal N:P through the increasing allocation of cellular resources to nitrogen-rich photosynthetic machinery (Sterner et al. 1997; Sterner and Elser, 2002), rather than to the easing of N-limitation. It may also reflect the fact that heterocystous cyanobacteria such as Dolichospermum tend to have higher optimal internal N:P content than other colonial cyanobacteria.
(Klausmeier et al. 2004), and that these populations represented an increasing proportion of the phytoplankton community during bloom development.

The peak bloom was characterized by multiple scarce resources including DIN, SRP, and light. Light scarcity resulted from high cell concentrations and was reflected in shallow euphotic depths (Table 2.1). The generally low wind speeds during the peak bloom likely favored dominance by positively-buoyant cyanobacteria by allowing them to access limited light resources despite these shallow euphotic depths (Reynolds et al. 1987; Huisman et al. 2004). Low DIN serves as an indication of continued N scarcity, particularly under light limited conditions when N fixation may be suppressed (de Tezanos Pinto and Litchman, 2010), while the depletion of SRP at the peak of cyanobacteria biovolume suggests that cyanobacteria were approaching carrying capacity relative to benthic P supply, and that P limitation played a role in the subsequent decline of cyanobacteria populations.

Late Bloom (25 August through 5 September)

During the late bloom, river discharge remained low, indicating that nutrient dynamics were still being driven primarily by internal dynamics. Cyanobacteria biovolume was still high but decreasing during the late bloom, and there were increased proportions of eukaryotic phytoplankton (evidenced by decreased PC:Chl-a; Table 2.1, Fig. 2.7). Water temperature declined gradually throughout this period, and water temperatures were rarely stratified due to greater winds. Figure 2.9 C shows that above a threshold of 4.5 m s\(^{-1}\), thermal stratification is inhibited by wind mixing. While wind speeds during the peak bloom generally remained below this threshold (Fig. 2.4), wind
speeds were above this threshold for most of the late-bloom period. Since thermal and related DO stratification of the water column was clearly limited due to wind mixing, conditions during the late bloom were not conducive to redox-driven benthic nutrient release. However, both SRP and DIN were present in moderate and increasing quantities during the late bloom, suggesting that another internal source and or mechanism was responsible for their availability (Figure 2.5). These moderate dissolved nutrient concentrations were likely due to the recycling of cell-derived P and N due to remineralization of peak bloom algal nutrients as the peak bloom died off. It is likely that the biogeochemical feedbacks in this system fundamentally changed in response to a more mixed and turbulent water column. Additionally, desorption of P at the sediment-water interface could provide a source of SRP during these mixed conditions if pH at the SWI was raised above ~9 (the zero point of charge of Mn/Fe (oxy) hydroxides), which may be possible if bottom turbulence was sufficient to erode highly buffered boundary layers (Thomas and Schallenberg 2008; Smith et al. 2011). The moderate available concentrations of DIN and SRP also suggest that another factor was limiting phytoplankton populations during the late bloom.

Indeed, our sensor data reveals that the euphotic depth was at its shallowest of the season during the late bloom (Table 2.1). The combination of shallow euphotic depth with frequent mixed conditions may have overcome the potential for buoyancy regulation by cyanobacteria, which may have contributed to net population growth during the peak bloom despite shallow euphotic depths by bringing the cells closer to the surface and available light to stimulate growth (Ganf and Oliver 1982; Reynolds et al. 1987). The
ample concentrations of available N and P, in combination with the evidence of light scarcity and the prolonged period of wind mixing, suggest that light was the primary limiting resource during this period. This conclusion is further supported by the occurrence of DO concentrations which were below saturation at all depths during the first half of this period (Fig. 2.4), suggesting that primary production was suppressed despite large populations of phytoplankton and that respiratory losses and decomposition were sufficient to overcome reaeration. DO rose at the end of this period as wind speeds subsided and primary production increased (Fig. 2.4), and there was a short-lived increase in PC during these calm conditions (Fig. 2.3), further supporting the conclusion that light limitation induced by wind mixing was responsible for the declines during the early part of the late-bloom period. These highly dynamic shifts in production and wind mixing were only observable as a result of our high-frequency, vertically resolved sensor array, highlighting the power of our approach in identifying and quantifying dynamics that would likely be missed or mischaracterized using a conventional monitoring approach.

*Chl-a Bloom (6 September through 22 September)*

The first major storm of the summer occurred on 4 September through 5 September (Figs. 2.3, 2.8), and had dramatic impacts on the progression of the bloom, marking the end of the late bloom and the beginning of the Chl-a bloom. The Chl-a bloom was given that name because it was characterized by high Chl-a fluorescence but low PC fluorescence, indicating a shift in relative abundance from cyanobacteria to eukaryotic phytoplankton (predominantly *Aulacoseira*, Fig. 2.7, Supplemental Fig. 2.2),
although cyanobacteria remained the largest component of total phytoplankton biovolume (and may have been underrepresented in the FlowCAM data if colonies had broken into small fragments during senescence). Smaller eukaryotes not measured by the FlowCAM may also have increased during this period. The increases in *Aulacoseira* may be partly attributable to the resuspension of sedimented cells with increased turbulence during the storm. Following the storm event, the physical drivers of the system changed relative to the previous bloom stages. The Chl-a bloom stage was physically characterized by relatively high winds and river discharge coupled with progressively declining air and water temperature (Fig. 2.4). Thermal stratification was infrequent during this period due to cooling and hydrodynamic disturbance from wind and river discharge, indicating another change in the physical drivers of nutrient and ecosystem dynamics.

PC and Chl-a fluorescence declined sharply and almost instantaneously in response to the storm event, suggesting that flushing and dilution displaced the extant phytoplankton populations within the bay. The chemical environment was also strongly impacted by the storm event. Remarkably, in the space of 2 days pH dropped by close to 3 units and DO dropped and remained relatively low throughout the rest of the monitoring period (Fig. 2.4), likely driven by dramatic decreases in water column photosynthetic activity and coupled with the influx of more acidic river water. After the storm moved through, DO and pH were rarely stratified due to lower primary production and reduced thermal stratification. Total N and TP dropped immediately following the storm corresponding to the drop in phytoplankton biovolume and related cell-bound
nutrients; these drops were likely due to sedimentation of senescing cells as well as flushing effects. Following the storm, TN and TP continued to decline throughout the Chl-\(\alpha\) bloom period. In contrast to the drop in TN and TP, both SRP and DIN were high immediately following the storm, which provided a dramatic influx of soluble available nutrients, likely associated with nonpoint source pollution from the Missisquoi Bay’s highly agricultural watershed (Smeltzer and Simoneau 2008). Subsequently, SRP declined over the course of the period, while DIN remained high, resulting in DIN:SRP ratios well above 16.

The storm that occurred at the beginning of the Chl-\(\alpha\) bloom period appeared to shift the competitive balance between cyanobacteria and eukaryotic phytoplankton by delivering available nutrients and increasing water clarity and light availability. While this process was likely underway already as a result of decreasing water temperatures and decreasing day length (which may trigger akinete formation in Nostacales), the storm event appeared to accelerate this transition and prevent the occurrence of late-season cyanobacteria blooms, which sometimes occur in Missisquoi Bay. Observing the impact of this storm would have been difficult without the combination of a high-frequency monitoring array and an adjacent USGS gage site; using only bi-weekly monitoring data that did not resolve the immediacy of the storm’s impacts, it would have been difficult to attribute changes in the phytoplankton community and chemical environment to the storm event rather than gradual successional processes.
Post-Bloom (23 September through 26 October)

The post-bloom period was characterized by low water temperatures, high winds, and high river discharge. During this time frame, water temperatures dropped from 17˚C to 10˚C, making them sufficiently low to depress growth rates for most bloom-forming phytoplankton in Missisquoi Bay (Butterwick et al. 2005). PC and Chl-a were low during this period, confirming that phytoplankton biomass was low. The ratio of TN:TP was at its highest during the post-bloom with a mean value of 32 (Table 2.1), suggesting that the system was stoichiometrically P-limited, but the high levels of DIN and SRP during this period demonstrate the neither nutrient was the limiting resource of the bay’s phytoplankton population. The low and progressively decreasing water temperature and solar radiation were likely the key factors limiting growth rates during this period.

Conclusions

The strongest bloom in recent years in Missisquoi Bay occurred in 2012 when tributary discharge was near historic lows, making it an ideal year for studying cyanobacteria bloom progression as a function of internal processes in this system. We were well poised to study these internal nutrient dynamics because of a comprehensive, cutting-edge monitoring effort that coincidentally began in early summer 2012, and many details would have been missed or mischaracterized using only data from traditional water quality monitoring efforts. High-frequency data were used to partition the bloom into distinct stages of growth, maintenance, and decline, and allowed us to describe the physical, chemical, and biological processes driving bloom dynamics during these stages. Many of the internal processes that affected the balance of resource limitation were
influenced by the effects of wind on water column stability. On a seasonal scale the onset of N limitation was dependent on the release of sediment P, and the benthic oxygen depletion that fueled this release was dependent on calm, stratified periods. As algal biovolume and respiration increased, shorter periods of thermal stratification were sufficient to induce stratification in dissolved oxygen (Fig. 2.8), which likely resulted in the more rapid development of reducing conditions at the sediment water interface (Supplemental Fig. 2.3) constituting a feedback that may have helped to sustain bloom conditions. In addition to its effects on sediment nutrient release, wind mixing also mediated competition for light in this system by overcoming cyanobacterial buoyancy regulation in the later stages of the bloom.

We demonstrate that, contrary to expectations from recent studies in western Lake Erie (Stumpf et al. 2012), high spring discharge does not appear to be the dominant factor controlling interannual variability in the severity of summertime cyanobacteria blooms in Missisquoi Bay. If anything, the data suggest that Missisquoi Bay may have the opposite relationship to spring discharge, with years of low external loads corresponding to stronger blooms. We hypothesize that this may be a function of the N:P balance. In 2012, the growth period for cyanobacteria was initiated during a period with low N:P. While P loading from the sediments is driven largely by internal processes, TN concentrations in every year are highest in the spring following the snowmelt period, suggesting that external loading may control nitrogen availability more than P availability. As a result, low spring discharge in combination with spring populations of N-consuming phytoplankton may actually promote cyanobacteria blooms in Missisquoi bay by
inducing N-limited conditions in which cyanobacteria are more likely to dominate (Smith 1983). This is consistent with long-term monitoring data, which show very low TN concentrations in 2012 during the pre-bloom period relative to other years, and warrants further study.

While this paper highlights the role of internal cycling, it is critical to note here that the ultimate source of the enrichment of benthic P that drove the bloom was the watershed, and the key factor responsible for eutrophic conditions ultimately remains historical and current loading from the agricultural catchment. The dramatic impact of the 5 September storm provides a further illustration of the importance of watershed processes on the lake system. In the space of several days the storm resulted in a drop in pH of almost 3 units, large increases in the concentrations of available nutrients in the system, and a shift in dominance from cyanobacteria to eukaryotic phytoplankton. Storms may have particularly strong influences late in the summer, when decreasing water temperatures depress cyanobacteria growth rates. Missisquoi Bay may be particularly susceptible to the effects of discharge due to the high ratio of watershed area to lake volume, and studying the effects of similar storms requires high-frequency data capable of capturing the rapidly evolving response of the system to storm events.

This work illustrates the power of synthesizing long-term water quality monitoring data with high-frequency sensor data and intensive sampling programs to allow for more detailed and process-based monitoring studies. Similar efforts to describe these processes across multiple time-scales in this and other lakes may ultimately yield a deeper understanding of lake processes. This study is by nature limited by its lack of
spatial resolution; while Missisquoi Bay is largely homogeneous with respect to depth and sediment composition, the deployment of more limited sensor arrays at additional sites would help to quantify the extent of spatial variability in the future. Findings in this study may be applicable to shallow bays of the Great Lakes and elsewhere. While the individual mechanisms described herein may not be novel, the ability to examine many aspects of this system using a unique dataset in a historically strong bloom year with a clear trajectory of growth and decline provides an unusually clear picture of how these mechanisms may interact to drive cyanobacteria bloom progression.

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Supplemental Figure 2.1: Surface FlowCAM biovolume estimates (µm³/mL) for all cyanobacteria genera (points) plotted with sonde phycocyanin data at 0.5m.
Supplemental Figure 2.2: Phytoplankton cell biovolumes (µm³/L) from 63µm net samples taken by the Long-Term Monitoring Program, station 51.
Supplemental Figure 2.3: Vertical profiles of DO and Temperature taken on two calm days (A, B), and 2 windy days (C, D) in 2013 showing temperature and oxygen gradients in the lower water column.
CHAPTER 3: BUOYANCY REGULATION AND WIND MIXING DESTABILIZE LAKE METABOLISM: A NOVEL APPLICATION OF HIGH-FREQUENCY DATA

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Running Head: “Cyanobacterial Buoyancy Regulation and Lake Metabolism”

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Summary

1. The ability of many colonial gas-vacuolate cyanobacteria to regulate their buoyancy allows them to access limited light resources in eutrophic surface waters, but this may subject cyanobacteria to rapid population collapses when hydrodynamic conditions overcome their capacity to maintain buoyancy and push large populations below the euphotic zone. Mixing conditions may lead to rapid drawdowns in dissolved oxygen, resulting in fish kills or other adverse ecological impacts.

2. While the mechanisms controlling cyanobacteria buoyancy have been described previously, quantifying the effects of these mechanisms on the balance of production and respiration at seasonal scales has been difficult due to the wide range of factors (both hydrodynamic and physiological) that can influence the efficiency of buoyancy regulation.

3. High-frequency monitoring data are used to demonstrate the roles that wind mixing and buoyancy regulation play in causing a fish kill during a strong cyanobacteria bloom in a shallow, eutrophic bay of Lake Champlain. Buoyancy regulation is estimated using mean residence depth calculated from automated sensor data, and is found to be highly sensitive to wind mixing.

4. A novel application of high-frequency vertical profiler data is proposed to estimate the realized benefit of buoyancy regulation on total cyanobacterial primary production. The method is tested against net ecosystem production estimates calculated using the diel oxygen technique.
5. Results demonstrate that buoyancy regulation enables most of the gains in net cyanobacteria biomass observed during the bloom period, and that bloom conditions could not be sustained under persistent mixed conditions.
Introduction

A great deal of attention has focused on the role of phosphorus and nitrogen as the resources limiting production in freshwater systems (Schindler 1977; Paerl et al. 2011a). However, in many eutrophic systems where nutrient loads and phytoplankton biomass are high, light limitation mediated by hydrodynamic conditions may constitute the major driver of harmful algal bloom dynamics (Huisman et al. 1999b; a; 2004). Light availability is a function of phytoplankton cell density; as populations increase due to nutrient loading an increasing proportion of the mixed layer becomes aphotic. At sufficiently high nutrient loads light becomes the primary limiting resource. Under such conditions, strategies to maximize access to light constitute major competitive advantages and may shape phytoplankton community composition (Reynolds et al. 1987; Brauer et al. 2012).

One of the most common strategies used by phytoplankton to access limited light resources is buoyancy regulation. Buoyancy regulation is present in a number of taxa, ranging from flagellated eukaryotes such as dinoflagellates (Regel 2004; Xu et al. 2009), raphidophytes (Salonen and Rosenberg 2000) and cryptomonads (Gervais 1997) to colonial cyanobacteria. Here we focus on buoyancy regulation in cyanobacteria, which are the major component of harmful algal blooms in eutrophic freshwater systems. In the paradigmatic view (Walsby 1971), buoyancy regulation is a mechanism to exploit the separation of light available at the surface and nutrients available in the metalimnion or at the sediment surface. Buoyancy regulation in cyanobacteria is controlled by three complementary mechanisms. The first is the production of gas vesicles, which provide
positive buoyancy to the cells. Building and breaking down these vesicles is used primarily to regulate long-term buoyancy in *Microcystis* rather than to mediate short-term changes in position in the water column (Reynolds et al. 1987). The second mechanism is the accumulation of carbohydrate ballast during photosynthesis. Carbohydrate ballast is the primary method by which diel changes in buoyancy regulation are achieved. As cells acquire carbohydrate stores in the form of glycogen during photosynthesis (primarily in the morning hours) they become negatively buoyant and sink to the metalimnion or the sediment surface where they can access phosphate or ammonium. As they deplete the carbohydrate ballast through respiration in the dark waters they rise again to the surface later in the afternoon or overnight. The third mechanism is variation in colony size, which mediates the effects of the other two factors (Reynolds et al. 1987). Single cells or small colonies are difficult to disentrain from the water column even if their densities are considerably lower (or higher) than water. Changes in colony size are directly related to either sinking or floating rates via Stokes’ Law. The efficiency of buoyancy regulation is mediated by the degree of turbulence, which is usually a result of wind mixing and thermal stability (George and Edwards 1976; Ganf and Oliver 1982). In a system dominated by buoyant cyanobacteria, wind mixing events may mediate competition for light (Huisman et al. 1999b; 2004).

While the mechanisms described above have been demonstrated, the effects of buoyancy regulation on cyanobacterial metabolism at seasonal scales has been difficult to quantify due to the limitations of data collection using traditional methods. Targeted diurnal sampling events provide proof of concept for these theories (Walsby 1997;
Mitrovic et al. 2001), but are unable to quantify the advantage which buoyancy regulation confers over a broad range of observed environmental conditions. Modern technologies have the potential to overcome these limitations through the use of in-situ high-frequency sensors, which are capable of measuring light, oxygen concentrations, and proxies for phytoplankton abundance.

We investigated the role of buoyancy regulation in mediating primary production using high-temporal-resolution vertical profile data for the 2012 bloom season from a monitoring buoy moored in Missisquoi Bay, Lake Champlain (Vermont, USA and Quebec, Canada). Missisquoi Bay is a shallow eutrophic embayment of Lake Champlain which has experienced annual summer blooms of cyanobacteria for the past two decades (Levine et al. 2012) driven by high levels of non-point source nutrient inputs from a large watershed dominated by agricultural land uses and internal nutrient loading. The bloom during 2012 was strong, due in part to a relatively warm, dry summer (Isles et al. in review), and a major fish kill event occurred in the bay. Our objectives in this study were to 1) look for evidence of cyanobacterial buoyancy regulation using automated profiler data; 2) investigate factors controlling the observed changes in buoyancy; 3) calculate the effects of these changes in buoyancy regulation on phytoplankton growth and primary productivity; and 4) to identify the roles that buoyancy regulation and wind mixing played in the occurrence of the fish kill event in 2012.
Methods

Study site

Missisquoi Bay is the northeastern most segment of Lake Champlain, and spans the border of Vermont, USA and Quebec, Canada. The bay is shallow (max depth 5m, mean depth 2.8m; (Levine et al. 2012)) and largely isolated from other parts of the lake. The only outflow is a narrow opening in a causeway in the southwest part of the bay. Missisquoi Bay has the highest light-attenuation coefficients in Lake Champlain due to the high concentrations of organic detritus of terrigenous origin and comparatively high concentrations of phytoplankton (Effler et al. 1991). The study site was in the southeast quadrant of Missisquoi Bay, with depth ranging from 3-4 meters over the course of the monitoring period.

Buoy data collection

Data were collected at a modified YSI profiling platform equipped with a YSI 6600 V2 multiparameter sonde (Yellow Springs Instruments, Yellow Springs OH). The sonde was mounted on a winch and was raised and lowered through the water column hourly at 0.5 m increments throughout the bloom season, excluding the bottom 1 m of water to prevent collision of the sonde with the sediment during periods of high wave activity. The sonde contained probes for phycocyanin, chlorophyll-\(a\), dissolved oxygen (DO), turbidity, temperature, pH, and conductivity. The platform was also equipped with a meteorological station measuring wind speed, wind direction, solar radiation, relative humidity, atmospheric pressure, and air temperature at hourly increments. The sonde was calibrated weekly.
**Weekly field sampling**

Weekly profiles of photosynthetically active radiation (PAR) were taken at 0.5m increments using a Licor LI-193 spherical quantum sensor (LiCor Biosciences, Lincoln NE, USA). Water samples were also collected weekly for phytoplankton counts and nutrient analyses. Water temperature profiles were also measured using a chain of HOBO temperature sensors (Onset Computer Corporation, Bourne MA, USA) mounted at 0.5m increments which allowed measurement of the entire temperature profile, including the bottom 1 m. Phytoplankton samples were collected weekly at 5 depths and preserved in Lugol’s solution. Phytoplankton biovolumes were estimated using a benchtop FlowCAM imaging-in-flow system (Fluid Imaging Technologies, Yarmouth ME, USA). All samples were analyzed in autoimage mode at 40x magnification using a 300 µm flow cell; this was sufficient to observe all particles greater than 30 µm, including cyanobacterial colonies. A subset of the samples were counted using the Utermöhl inverted microscope technique for validation of the FlowCAM results. All imaged particles were identified to the lowest possible taxonomic level (usually genus or species).

**External data sources**

To fill gaps in the solar radiation dataset from the monitoring platform, solar radiation data were downloaded from the Vermont Monitoring Cooperative for a nearby long-term monitoring station on Lake Champlain at Colchester Reef (http://www.uvm.edu/vmc/project/colchester-reef-meteorological-monitoring-38-m/dataset).
Mean residence depth

Mean residence depth (MRD), a metric describing the center of mass of a population in the water column, was calculated from the sonde phycocyanin data for each hour as:

\[ \frac{\sum (PC_i \cdot D_i)}{\sum PC_i} \]  
(Equation 1)

where PC=phycocyanin RFU at depth i, and D = depth in meters.

Due to the lack of measurements in the bottom 1 m of the water column, MRD should be interpreted as a relative indicator of vertical position rather than an absolute estimate of the center of mass of phytoplankton in the water column. Because MRD was calculated from 4 consistent depth records from 0.5 to 2 m, MRD of 1.25 (the mean of measured depths) represents no evidence of buoyancy regulation. To test for lagged relationships between MRD and daily thermal stability, the temperature difference between surface and bottom water (ΔTemp) was calculated. Cross correlation analysis of MRD and Temp was carried out using the R function ccf.

Reconstruction of euphotic depth and light attenuation

Euphotic depth was reconstructed by fitting a light extinction curve to weekly PAR profiles of the water column using Beer’s Law to estimate the light extinction coefficient:

\[ I_z = I_0 e^{kz} \]  
(Equation 2)

where \( I_z \) = irradiance (µmol m\(^{-2}\) s\(^{-1}\)) at depth z, \( I_0 \) = irradiance immediately below surface, k = light extinction coefficient, e is the base of the natural log, and z = depth (m).

Equation 2 was then solved for 1% surface irradiance to estimate euphotic depth. A
stepwise multiple regression variable selection (forward and backward) was then conducted to identify the best model to predict measured euphotic depth from turbidity, phycocyanin, and chlorophyll using the R functions \textit{lm} and \textit{step} (R Core Team 2014). This regression model was then used to estimate euphotic depth and \( k \) at each timestep. Surface irradiance \( (I_0) \) was estimated from hourly shortwave solar radiation using the simplifying assumptions that 1 watt m\(^2\) \( \approx \) 0.47 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) (Britton and Dodd 1976), and \( \sim 6\% \) of incident solar radiation is reflected at the lake surface (Jöhnk et al. 2008). A light profile of the water column at 10 cm depth increments was generated for each timestep using the estimates of \( I_0 \) and \( k \).

\textit{Diel O\(_2\) technique}

The diel \( O_2 \) technique was used to estimate NEP for validation of photosynthetic yield estimates using methods described in detail in (Staehr et al. 2010). Briefly, this technique solves the equation of Odum (1956):

\[
\frac{\delta O_2}{\delta t} = GPP - R - F - A \\
\text{(Equation 3)}
\]

where \( GPP = \) gross primary productivity, \( R = \) respiration, \( F = \) atmospheric flux, \( A = \) other oxygen sources or sinks (units are mg O\(_2\) L d\(^{-1}\) for all variables). \( \delta O_2/\delta t \) is measured, \( A \) is ignored and \( F \) is calculated from dissolved oxygen, water temperature, and wind speed, allowing the calculation of \( GPP - R \), or net ecosystem production (NEP). These NEP estimates were used validate estimates of phytoplankton metabolism estimated using photosynthetic yields from equation 4.

In this study, the effects of transient thermal stratification on estimates from the diel \( O_2 \) technique were ignored. (Staehr et al. 2012) suggest methods to better estimate
metabolic rates in a stratified system by modeling $\delta O_2/\delta t$ for each depth layer, but this is complicated in a shallow system with a rapidly changing diurnal mixed layer. Additionally, the lack of data in the bottom 1 m of the water column made accurate estimations of bottom water metabolism impossible (Coloso et al. 2010). Instead, only surface oxygen concentrations and temperature were used in atmospheric flux calculations. While this would complicate estimates of GPP or R, estimates of NEP should be reliable over the course of the period of analysis, although error in estimates for any specific day is possible (Staehr et al. 2012).

**Photosynthetic yield**

Previous research in Missisquoi Bay has shown that chlorophyll-α fluorescence as measured by a YSI 6600 sonde to be a poor predictor of both cyanobacteria biovolume and extracted chlorophyll-α concentrations, while phycocyanin has been shown to be a strong predictor of both (Zamyadi et al. 2012). Before calculating photosynthetic yield, chlorophyll equivalents were estimated from phycocyanin concentrations (PC) using a regression model previously developed for Missisquoi Bay ($Chl-a = 1.61 \times PC + 1.338$; (Zamyadi et al. 2012). Photosynthetic oxygen production per unit chlorophyll ($P_c; \mu$mol O$_2$ (mg Chl)$^{-1}$ h$^{-1}$) was then calculated for the peak bloom period with the equation of Platt et al. (1980), using an adaptation of the daily integral method developed by Walsby (1997) (hereafter referred to as the integral method):

$$P_c = P_m\left(1 - e^{(-\alpha I/P_m)}\right) + R + \beta I$$

(Equation 3.4)

where $P_m =$ maximum oxygen yield (μmol O$_2$ (mg Chl)$^{-1}$ h$^{-1}$), $\alpha =$ initial oxygen response coefficient at low irradiance, $\beta =$ photoinhibition coefficient, and $I =$ irradiance (μmol m$^{-2}$
Parameter estimates for $P_m$, $\alpha$ and $\beta$, and $R$ were taken from literature values collected for similar taxa under similar temperature conditions (Walsby 1997; Mitrovic et al. 2001; Suggett et al. 2001). The irradiance profiles generated above were then used with these parameters to calculate photosynthetic yield per unit chlorophyll for each 10 cm increment at each timestep.

Linear interpolation of phycocyanin fluorescence between measured 0.5 m intervals was used to estimate vertically-resolved phycocyanin concentrations in 10-cm increments. Interpolated concentrations at each depth were then multiplied by the corresponding $P_e$ to give vertically-resolved cyanobacterial oxygen production estimates at 10 cm increments throughout the water column. These estimates were then summed to provide estimates of hourly oxygen production. Concentrations were assumed to be consistent between 0.5 m and the surface, and between 2 m and the lake bottom (3.0 m). Surface cyanobacteria concentrations (particularly when surface scums are present) were likely underestimated and bottom water cyanobacteria concentrations likely overestimated. To create a hypothetical fully mixed scenario to test the benefit conferred by buoyancy regulation, photosynthetic oxygen production was calculated for the same total concentration phycocyanin vertically averaged over the water column.

The equation used to calculate net $O_2$ production by the integral method (Equation 3.4) ignores sources of respiration not correlated with cyanobacteria biomass. While pelagic bacterial respiration often covaries with phytoplankton (Currie 1990; Coveney and Wetzel 1995), benthic respiration is not considered. Sediment oxygen demand in offshore areas of Missisquoi Bay has been estimated as roughly 1.5 mmol $O_2$ m$^{-2}$ h$^{-1}$, and
has been found to be fairly consistent across seasons (McCarthy 2011). This constant value was added to the reconstructed photosynthetic yield for both the observed ‘buoyant’ scenario and the hypothetical ‘mixed’ scenario to allow more accurate comparison to NEP estimates by the diel O$_2$ technique.

**Results**

*Phytoplankton bloom progression*

Missisquoi Bay had a strong algae bloom in 2012, with peak phytoplankton biovolume of 580 mm$^3$ m$^{-3}$. Daytime surface O$_2$ concentrations and surface pH frequently exceeded 200% saturation and 10, respectively, for the peak bloom in August. The bloom was dominated by *Aphanizomenon flos-aquae*, *Microcystis aeruginosa* and *Dolichospermum circinalis*, with *Microcystis* and *Dolichospermum* making up > 95% of the phytoplankton biovolume during the peak bloom. The progression of the bloom as reflected in phycocyanin fluorescence and was split into three periods corresponding to growth, peak and decline stages of the bloom (Fig. 3.1a).
Figure 3.1: A. Phycocyanin fluorescence measured in relative fluorescence units (RFU) during the growth phase (GP), peak bloom (PB), and late bloom (LB) periods of the 2012 bloom season. B. Wind speed (m s\(^{-1}\)) during the same periods. C. Aphotic zone defined as <1% surface irradiance (shown in red).

Mean residence depth was calculated for each hour and aggregated by bloom stage for the ‘growth phase’, ‘peak bloom’, and ‘late bloom’ periods (Fig. 3.2). There was no evidence of diel changes in MRD during the growth or late bloom periods, but a strong diel signal was apparent during the peak bloom (Fig. 3.2). MRD declined in the afternoon as cyanobacteria moved towards the surface, and increased in the evening as cyanobacteria moved deeper in the water column. Cyanobacteria did not appear to migrate to the sediments as average MRD did not exceed the neutral depth of 1.25 m at any time of day (Fig. 3.2).
Physical drivers

Wind speed was variable but relatively high during the growth phase, low during the peak bloom, and high during the late bloom (Fig. 3.1b). Wind speed during the peak bloom generally remained below 4.5 m s\(^{-1}\). Euphotic depth was close to 4 m at the start of the growth phase, but became progressively shallower throughout that period, and was generally between 2 and 3 m during the peak and late blooms (Fig. 3.1c). Light suppression of photosynthetic rates can occur well above the euphotic depth, and optimal photosynthetic depths (calculated as \(\frac{1}{2}\) surface irradiance) were close to 0.5 m throughout the bloom period.

Because strong evidence for buoyancy regulation was only found during the peak bloom, subsequent analyses will focus on that period. The temperature profile showed a pattern of strong diurnal mixed layer development (Imberger 1985) during the first half of the peak bloom. During this period thermal stratification setting up in the morning, deepened throughout the day, and broke down during the evening hours (Fig. 3.3).
Thermal stratification had a similar temporal pattern to MRD. The strongest gradients thermal corresponding to the most stable water column, tended to appear during mid-afternoon, which corresponded to the period when cyanobacteria were closest to the surface (Fig. 3.2). MRD was negatively correlated with the temperature difference between surface and bottom (\( \Delta \text{Temp} \)) \( (r = -0.57, p < 0.0001) \). Cross-correlation analysis of MRD and \( \Delta \text{Temp} \) showed that the two were largely synchronous, although the decline in MRD preceded the daily maximum of \( \Delta \text{Temp} \) by about 1 hour (Fig. 3.4).

![Figure 3.3: Temperature contour plot of Missisquoi Bay during the peak bloom.](image-url)
A mixing event occurred in the middle of the peak bloom, corresponding to a high winds associated with a cold front. Solar radiation was low for this date due to dense cloud cover (Fig. 3.5a). The cold front, which reduced water temperature in the bay by several degrees (Fig. 3.3) also corresponded to the date of the fish kill event. Dissolved oxygen decreased at all depths with this mixing event (Fig. 3.5b), dropping to a minimum around 6 mg L\(^{-1}\). The white perch *Morone americana*, the most abundant fish in Missisquoi Bay by biomass (Gearhart, *pers. communication*), has a dissolved oxygen threshold of 5 mg L\(^{-1}\) (Setzler-Hamilton 1991). Patchy spatial distribution of phytoplankton in the bay likely resulted in lower concentrations of dissolved O\(_2\) in parts of the bay with higher phytoplankton populations and/or deeper water, resulting in O\(_2\) concentrations below this threshold.
Figure 3.5: Top: Solar radiation (watts m\(^{-2}\)) during the peak bloom period. Bottom: Dissolved oxygen (mg L\(^{-1}\)) at the surface (black line) and bottom (blue line) during the peak bloom. Dotted line represents 100% saturation, and red star represents the fish kill event.

**Diel O\(_2\) technique**

The diel O\(_2\) technique showed positive net oxygen production for the peak bloom period of 6332 mmol O\(_2\) m\(^{-2}\) (Fig. 3.6). NEP estimates were noisy, likely resulting from variability in the wind data as well as movement of the diurnal mixed layer. The date of the fish kill, 17 August, had negative net oxygen production of -413 mmol O\(_2\) m\(^{-2}\), lower than that on any other date during the peak bloom. Every other day during the peak bloom had net oxygen production with the exception of the last day, 25 August, when populations of cyanobacteria began to decline to the lower levels that prevailed during the late bloom (Fig. 3.1).
Figure 3.6: Top: Hourly estimates of NEP (µmol m\(^{-2}\) h\(^{-1}\)) estimated by the integral method for the observed buoyant scenario (black), the hypothetical mixed scenario (blue), and the diel oxygen technique (gray). Bottom: Daily estimates of NEP from the same scenarios.

**Integral method**

Parameter values for equation 4 were estimated as: \(P_m = 270.4\), \(a = 1.34\), \(B = -0.18\), \(R = -31.55\). When combined with estimated chlorophyll-\(a\) concentrations and irradiances, as well as sediment oxygen demand estimated from literature values, NEP calculated from phytoplankton data was very similar to results from the diel O\(_2\) technique (Fig. 3.6).

In the hypothetical mixed scenario, where cyanobacteria concentrations were homogenized throughout the water column, total O\(_2\) production was considerably lower
than that calculated using the observed vertical distribution of cells. The difference between these two scenarios is an estimate of the realized benefit of buoyancy regulation. The area under the surface in Figure 3.7, Panel A represents oxygen production in the observed ‘buoyant’ scenario for the day preceding and during the fish kill event, whereas the area under the surface in panel B represents production by the same concentration of cyanobacteria under the ‘mixed’ scenario. The hypothetical mixed scenario showed net oxygen production of 1087.3 mmol O$_2$ m$^{-2}$ during the peak bloom, in contrast to net oxygen production of 6201.5 mmol O$_2$ m$^{-2}$ from the observed ‘buoyant’ scenario and 6747 mmol O$_2$ m$^{-2}$ from the diel oxygen technique.

![Observed Buoyancy Regulation, Aug 16–17](image1.png) ![Even cell distribution, Aug 16–17](image2.png)

Figure 3.7: Oxygen production for the day before and during the fish-kill event under the observed ‘buoyant’ scenario (left) and hypothetical ‘mixed’ scenario (right) illustrating the metabolism model used for this study.
Discussion

Evidence for buoyancy regulation and light limitation

Cyanobacteria demonstrated a clear diel pattern of vertical migration during the peak bloom period when wind speeds remained below 4.5 m s$^{-1}$, a threshold above which turbulent mixing is able to overcome transient thermal stratification in Missisquoi Bay (Fig. 3.2b; Isles et al. 2015). The observed threshold is similar to thresholds observed in other shallow eutrophic lakes above which cyanobacterial buoyancy regulation is unable to overcome turbulent mixing (Ganf and Oliver 1982). The absence of evidence for buoyancy regulation in the growth and late bloom periods suggests that the ability of cyanobacteria in Missisquoi Bay to regulate buoyancy is contingent on low wind speeds and a stable water column.

The diel changes in MRD observed here were inconsistent with the classical picture of buoyancy regulation (Reynolds et al. 1987). Cyanobacteria in Missisquoi Bay appeared to rise towards the surface in the late morning-early afternoon and sink to a neutral position in the evening. By contrast, the classical model of buoyancy regulation predicts that cyanobacteria should be near the surface in the morning, sink in the late morning or early afternoon as they accumulate carbohydrate ballast, and rise again in the evening as carbohydrate reserves are exhausted. The classical model suggests that diel migration is an active process to take advantage of vertical separation of light resources at the surface and nutrients in the bottom waters. Cyanobacteria in Missisquoi Bay did not appear to actively migrate downward to capture nutrient resources; during no period was MRD less than a neutral depth of 1.25 m. There was clear evidence, however, that cyanobacteria
migrated towards the surface to access light (Fig. 3.2), suggesting that light was likely the limiting resource driving diel migration during this period.

The shallow estimated euphotic depths highlight the severity of light limitation (Fig. 3.1), and bottom water dissolved oxygen dynamics provide further evidence of light scarcity. On days with strong diurnal mixed layer development, dissolved oxygen concentrations at 2 m began to decline as soon as thermal gradients developed in the morning, even on days with high solar irradiance (Fig. 3.5). This was true even on days with estimated photic depths of close to 3 m (Fig. 3.1), suggesting that photic depth may have been underestimated. The evidence of light scarcity in the bay suggests a large potential benefit for cyanobacteria that are able to remain in surface waters through buoyancy regulation.

The coupling between MRD and ΔTemp evidenced by the cross correlation plot (Fig. 3.4) suggests that diurnal patterns of MRD in Missisquoi Bay were driven by stabilization of the water column brought about by development of the diurnal mixed layer (Imberger 1985). This mechanism has been previously described in other light-limited systems (Tilzer 1987) and can be conceptualized as a passive process, relative to the active migration hypothesized by the classical view. When thermal gradients set up due to solar heating, positively buoyant cyanobacteria cells were able to overcome mixing and rise towards the surface. When thermal gradients broke down in the evening hours, the cells were once again evenly dispersed. This suggests a tight coupling between primary production and water column stability.
Wind mixing and the fish kill

The high-frequency physical and chemical sensor data highlight the powerful effects of wind mixing on a cyanobacteria bloom that had developed under prolonged calm conditions. On 17 August, as the cold front passed through the system, the thermal structure of the lake eroded and a well-mixed, cooler water column persisted for the course of about a day. Furthermore, the dense cloud cover associated with the cold front exacerbated the light limitation brought on by water column mixing. The combined impact of the abiotic drivers associated with the frontal passage was a dramatic drawdown of dissolved oxygen throughout the water column – in stark contrast to pre and post frontal passage calm periods, when low dissolved O$_2$ was observed in bottom waters but surface waters remained highly oversaturated. On calm, cloudy days with low irradiance but no mixing (such as 10 August; Fig. 3.5), similar drawdowns of dissolved O$_2$ were not observed, suggesting that buoyancy regulation allows high cell densities to persist despite low solar irradiance when the water column is stable. Together, the temperature, wind, solar irradiance, and O$_2$ data provide strong evidence that mixing was responsible for O$_2$ depletion during the fish kill event, and illustrate the power of high-frequency sensor data to describe system dynamics during discrete events such as frontal passage.

Metabolism estimates and algal production

NEP estimates from the integral method over the entire peak bloom were generally consistent with those calculated using the diel O$_2$ technique. Some of the variation in day-to-day estimates of production under the ‘buoyant’ scenario and diel O$_2$
methods may be attributed to delayed signals of primary production from the diel O$_2$
technique resulting from diurnal mixed layer deepening and the timing of overnight
mixing (Coloso et al. 2010). A few dates, such as 20 August, show major deviations in
production estimates between methods, with production strongly negative under the
integral method, but strongly positive by the diel oxygen technique. High fluorescence
values resulted in very shallow estimated euphotic depths for this date, which may have
resulted in unrealistically low estimates of photosynthetic yield by the integral method. In
addition, fluorescence data used in this study cannot account for production by surface
scums of cyanobacteria, because the uppermost sonde measurement was taken at 0.5 m.
High surface populations could therefore result in high O$_2$ production that is not reflected
in yield calculations if conditions were sufficiently calm to promote scum development.

Comparison of the ‘buoyant’ and ‘mixed’ scenarios supports the conclusion that
wind mixing significantly reduces total primary production in Missisquoi Bay (Fig. 3.6),
and wind may limit cyanobacteria growth in this system by overcoming cyanobacteria
buoyancy regulation. Total production for the peak-bloom period under the mixed
scenario was one sixth of that under the ‘buoyant’ scenario or the diel O$_2$ technique. On
several days, particularly during the end of the peak bloom when cell concentrations were
highest, net production under the mixed scenario was negative, which would result in
population declines under mixed conditions (Fig. 3.6b).

Our analysis shows that positive buoyancy confers a major benefit to
cyanobacteria cells in Missisquoi Bay under light-limited conditions, and that the erosion
of these benefits under mixed conditions are largely responsible for the observed fish-kill
event. The fish-kill in Missisquoi bay seemed to be driven by different mechanisms than those postulated for other fish-kill events. Fish kill events are frequently preceded by collapses of cyanobacteria populations that remain low following the event (Jeppesen et al. 1989BC); these events are consistent with the gradual decomposition of algal biomass. Other accounts of wind-driven fish-kill events in persistently stratified eutrophic lakes have been attributed to the entrainment of anoxic hypolimnetic water into the epilimnion with mixed layer deepening (Pabst et al. 1980). In this study, the integral method successfully predicted net O₂ consumption during the fish kill event despite including no estimates of bacterial respiration. This suggests that the drawdown of oxygen was driven by suppression of photosynthesis coupled with high ongoing respiration, rather than a dramatic increase in respiration brought about by the collapse of the bloom.

*A conceptual model of a wind-unstable system*

Using a dataset with high temporal and vertical resolution, we constructed a novel conceptual model in which the combination of buoyancy regulating cyanobacteria and prolonged periods of calm weather may give rise to an unstable system highly susceptible to disturbances in the form of wind (Fig 3.8). During prolonged calm periods, high cell densities accumulate in an increasingly shallow photic zone. The biomass, however, may not be sustainable in fully mixed conditions due to light limitation and carbon starvation. Calm conditions can therefore allow the development of large populations that exceed carrying capacity with respect to light under mixed conditions, resulting in net oxygen consumption in the lower water column and resulting increases in sediment nutrient release. Prolonged calm periods prime the system for disturbance by wind that can lead to
rapid depletion of oxygen in the water column due to high cyanobacterial respiration and low photosynthesis. In shallow polymictic lakes where the entire water column may be mixed during these events, oxygen depletion may result in positive feedbacks whereby low oxygen combined with increased boundary layer turbulence may promote further release of sediment-bound phosphate (Thomas and Schallenberg 2008), fueling further cyanobacterial growth when calm conditions return.

Figure 3.8: Conceptual diagram of the feedbacks between vertical migration and lake metabolism in shallow systems. Left, a clear water system, with light available throughout the water column promoting net oxygen production at all depths, oxidizing surficial sediments and suppressing release of P from sediment. Middle: a bloom state under calm conditions. Low wind speeds allow positively buoyant cyanobacteria to sustain high levels of oxygen production in the surface waters, while light limitation results in net oxygen consumption in the deeper part of the water column. Low oxygen in bottom waters contributes to sediment phosphorus release, constituting a positive feedback. Right: Bloom state with a mixing event. Wind mixing redistributes cyanobacteria cells evenly throughout the water column, resulting in net oxygen consumption through the water column and rapid, ubiquitous drawdown of oxygen concentrations, potentially resulting in fish kill events or other adverse ecological consequences.
To the best of our knowledge, our study represents the first application of the daily integral technique to high-frequency profiling buoy data. Application of our approach to other sites may yield insights into the relationship between diurnal mixed layer development and lake metabolism, and the role of buoyancy regulation across diverse lake systems. Parameter estimates used in the photosynthetic yield calculations include a number of sources of uncertainty, so care should be taken in interpreting the results quantitatively. However, the conclusion that buoyancy regulation is important for the maintenance of high cell densities is robust. Further studies should 1) corroborate these results with in-situ measurements of primary production using bottle incubation or isotopic techniques, 2) attempt to characterize temporal variation in respiration more fully, and 3) incorporate the effects of temperature changes to model oxygen evolution across seasonal scales. The ability of phytoplankton to acclimate to changing light environments should also be considered when attempting to scale these processes to a wider range of environmental conditions, as should changes in the fluorescence signal associated with shifts in the phytoplankton community.

While the focus of the present study is the impact of buoyancy regulation on oxygen dynamics and phytoplankton bloom development, our conceptual model also has important implications for nutrient loading processes in shallow eutrophic systems. Transient thermal stratification is important for internal nutrient loading processes (Burger et al. 2008; Isles et al. 2015), but internal loading has rarely been considered in the context of interacting effects of buoyancy regulation and wind mixing. Many of these effects may not be adequately captured by existing models which do not include relevant
parameters for cyanobacteria colony size and density changes. Such factors may be important in creating feedbacks that sustain bloom conditions in shallow systems.

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CHAPTER 4: TEMPORAL DYNAMICS OF WIND MIXING THRESHOLDS
CONTROL INTER-ANNUAL VARIABILITY IN CYANOBACTERIA BLOOM SEVERITY IN A LARGE, SHALLOW BAY OF LAKE CHAMPLAIN

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Running head: Interannual Variability in Primary Production

Keywords: Cyanobacteria bloom, Self-Organizing Map, Lake Metabolism, High-frequency Data, Temperature Thresholds
Abstract

Cyanobacteria blooms in shallow lakes have been heavily researched, yet the drivers of year-to-year variability in bloom severity remain unclear. Critical thresholds of abiotic drivers such as wind, irradiance, air temperature, and tributary inputs may control the development and collapse of blooms, and these thresholds may exist with respect to time as well as the magnitude of the event. In this study, we compare high-frequency estimates of gross primary production, respiration, and net ecosystem metabolism in a shallow bay of Lake Champlain to concurrent measurements of physical and chemical parameters over three years with very different bloom dynamics. We use supervised and unsupervised self-organizing maps to identify the drivers of cyanobacteria bloom development during key stages of growth and decline. We then use threshold analysis to identify critical thresholds of thermal stratification associated with bloom development. The results suggest that wind mixing and periods of transient thermal stratification are critical for the development of bloom conditions in our study site, and the frequency and timing of these events has a large influence on inter-annual variability in bloom severity. While specific thresholds related to water column stability are likely to vary between sites, the central role of these thresholds may be widespread, and the methods developed here can be widely applied to other lake systems to identify the drivers of key bloom stages and site-specific thresholds controlling net ecosystem production.
Introduction

Cyanobacteria blooms are a major and increasing threat facing water quality in lakes worldwide (Brooks et al. 2015), and threaten aquatic ecosystems through the production of toxins (Cheung et al. 2013) and the depletion of dissolved oxygen when blooms collapse (Havens 2008). Cyanobacteria blooms have been extensively studied (Paerl et al. 2011b; Berdalet et al. 2014), and there is a general consensus that over long timescales anthropogenic nutrient inputs are the primary driver of deteriorating water quality (Heisler et al. 2008). There is also an emerging consensus that climate change is likely to make blooms more frequent and severe (Paerl and Huisman 2009; O’Neil et al. 2012; Carey et al. 2012). At shorter timescales, however, there is frequently a great deal of inter-annual variability in primary production and cyanobacteria bloom severity that cannot be explained by these long-term stressors (Sass et al. 2008; Søndergaard et al. 2015). The controls of this inter-annual variability in bloom severity remain poorly understood, particularly in shallow lakes where the effects of annual external nutrient loads are frequently less important than internal nutrient loading (Søndergaard et al. 1999).

Understanding the sources of inter-annual variability in primary production is critical for predicting responses of cyanobacteria blooms to future climate changes (Staehr et al. 2011a). In shallow eutrophic lakes where nutrients are frequently plentiful, inter-annual variability is found to be sensitive to storm events (Jennings et al. 2012; Klug et al. 2012), heatwaves (Jöhnk et al. 2008) and critical thresholds related to thermal stability (Huber et al. 2008; 2012). Erosion of lake thermal structure by high wind speeds
or major hydrologic inputs may affect lake nutrient and dissolved oxygen concentrations (Jennings et al. 2012; Strock et al. 2016) and mediate light availability (Huisman et al. 2004). The timing of mixing events may also be critical for determining bloom severity (Jöhnk et al. 2008; Wilhelm and Adrian 2008; Huber et al. 2012). Critical thresholds related to thermal stability are likely present in many lakes; however, specific threshold values are likely to vary between lakes due to lake size, lake depth, trophic status, and other factors (Huber et al. 2008). These thresholds are likely to interact with a wide variety of other variables to determine bloom progression. Therefore, identification of critical thresholds requires both comprehensive datasets capable of controlling for multiple predictor variables and statistical methods capable of identifying non-linear relationships between variables (Andersen et al. 2009).

In addition to the inherent difficulty of identifying critical thresholds in complex datasets, identifying mechanisms controlling bloom development is complicated by hysteresis effects. Different environmental factors may be associated with the same concentration of cyanobacteria during stages of growth and decline; this reduces the power of correlation- and regression-based methods to identify controlling variables during key growth periods. Explicit consideration of gross primary production (GPP) and ecosystem respiration (R) in addition to cyanobacteria biomass may help to resolve these differences, because the relative magnitudes of these parameters are likely to differ during bloom development and bloom collapse. High-frequency sensor data have become more widespread in recent years, allowing high-frequency estimates of phytoplankton biomass as well as GPP, R, and net ecosystem production (NEP) (Coloso et al. 2008;
Staehr et al. 2010; 2011b; Laas et al. 2012). Even with well-resolved data, however, it can be difficult to identify the drivers of metabolic parameters and bloom development (Coloso et al. 2011) due to the wide variety of factors that may influence lake metabolism, including nutrients, light, temperature, carbon inputs, and food web structure (Staehr et al. 2011a). Many of these factors are highly correlated with each other and are likely to interact, making it difficult to interpret regression coefficients (Zuur et al. 2009).

A number of statistical techniques have been applied to lake datasets in recent years to try to overcome the limitations of linear statistical methods and to take advantage of increasingly large datasets. Some examples include Bayesian network models (Rigosi et al. 2015), ARIMA models (Arhonditis et al. 2004), wavelet analysis (Carey et al. 2016), genetic programming (Muttil and Chau 2006), artificial neural networks (Recknagel et al. 1997; Maier and Dandy 2000; Muttil and Chau 2006), and self-organizing maps (Rimet et al. 2009; Pearce et al. 2013). In this study we employed the latter, the self-organizing map (SOM). The SOM is an artificial neural network that can be used for non-parametric clustering, visualization, and prediction with complex datasets (Kohonen 1990; Kalteh et al. 2008). Because the SOM is fundamentally a clustering algorithm, it may help to resolve differences in phytoplankton growth, maintenance, and decline periods that are difficult to identify using linear models. SOMs have been found to outperform traditional clustering methods with water quality data that are highly correlated and show hysteresis (Kalteh et al. 2008), and perform well on datasets with irrelevant variables, high dispersion, outliers, and heterogeneous cluster densities (Mangiameli et al. 1996). Like other artificial neural networks, SOMs may outperform
linear methods on data that respond to thresholds rather than continuous predictors (Melssen et al. 2006). In addition to their computational advantages, SOMs provide powerful visualization capabilities that are useful for data exploration.

In this study, we use both supervised and unsupervised self-organizing maps to model within season differences in lake biogeochemical conditions. We use estimates of GPP, R, and NEP, in addition to comprehensive high-frequency biogeochemical and meteorological monitoring data, to isolate conditions associated with periods of bloom development and bloom collapse. These data were collected in Missisquoi Bay, Lake Champlain during three years with very different bloom conditions. Our goals were 1) to develop reproducible methods to identify fundamentally distinct stages of cyanobacteria bloom progression that can facilitate comparisons between years with different bloom dynamics; 2) to determine the environmental parameters that control cyanobacteria growth and decline during these stages; and 3) to identify key thresholds of these environmental parameters that determine ecosystem response. Because previous research demonstrated that external nutrient loads do not control cyanobacteria bloom intensity in Missisquoi Bay on a year-to-year basis (Isles et al. 2015, Isles et al. *Chapter 5 of this dissertation*), we predicted that primary production in this highly eutrophic bay would be controlled by abiotic drivers such as temperature, wind, and tributary inputs, and the inter-annual variability in these drivers would explain differences in cyanobacteria bloom severity between years.
Methods

Study site

Lake Champlain is a large, temperate lake located between the states of Vermont and New York, USA, and Quebec, Canada. Missisquoi Bay, located at the northeastern tip of Lake Champlain, is large (77.5 km$^2$), shallow (max depth 4 m, mean depth 2.8 m at annual low water), and eutrophic (average TP 48 µg L$^{-1}$) (Levine et al. 2012). It is largely isolated from the main body of the lake, the only outlet being a 100 m opening in a causeway in the southwestern corner of the bay. Missisquoi Bay experiences regular cyanobacteria blooms. Blooms usually peak in August and early September, but bloom severity varies greatly between years (Smeltzer et al. 2012; Isles et al. 2015). Missisquoi Bay has a high ratio of catchment area to lake area (40:1), and the large catchment consists of mostly forested and agricultural land uses (Levine et al. 2012). There are three major tributaries to the bay, the Missisquoi, Pike, and Rock rivers, which contribute 79%, 18% and 3% of total discharge into the bay, respectively.

Data collection

Data for this study were collected using a YSI profiling buoy (Yellow Springs Instruments, Yellow Springs OH) moored in the southeast quadrant of the bay. Depth at the study site ranged from 3 – 4.5 m over the monitoring period. The buoy was programmed to take vertical profiles hourly at 0.5 m increments. Consistent data were collected for the upper 2 m of the water column; data from greater depths were collected when the lake level was high enough. The profiling buoy was equipped with a YSI 6600 series sonde with probes for temperature, conductivity, pH, phycocyanin fluorescence...
(BGA), chlorophyll-a fluorescence (Chl-a), turbidity, and dissolved oxygen (DO). A meteorological station on the buoy collected hourly data on wind speed, wind direction, air temperature, RH, pressure, and shortwave solar radiation. There were several gaps in the solar radiation data due to instrument malfunctions that were filled using the nearby Colchester Reef met station data available through the Vermont Monitoring Cooperative (http://www.uvm.edu/vmc/project/colchester-reef-meteorological-monitoring-38-m/dataset). In addition to sensor measurements, Isco automated water samplers were moored on the buoy collecting water samples every 8 hours for analysis of total nitrogen (TN) and total phosphorus (TP). Nutrient samples were analyzed on an AQ2 autoanalyzer (Seal Analytical, Mequon WI) using standard methods (USEPA 2014a; b). Daily and 15 minute tributary discharge data (Q) for the Missisquoi River were acquired from the USGS (gage station 04294000). Daily averages were calculated for all variables for comparison to metabolic parameters, which are calculated on daily timescales.

Metabolism calculations

The basic ecosystem metabolic parameters, gross primary production (GPP), ecosystem respiration (R), and net ecosystem metabolism (NEP) (Odum 1956), were estimated from diel changes in surface water DO concentrations after accounting for atmospheric gas exchange using hourly surface water temperature, wind speed, relative humidity, and irradiance. Metabolic parameters were calculated using the metab.mle function in the LakeMetabolizer R package (Winslow et al. 2015). The gas exchange coefficient k600 was estimated using the “Crusius” method (Crusius and Wanninkhof 2003). Prior to metabolism calculations, small gaps in the raw data (≤ 3 hours) were filled
using linear interpolation. GPP, R, and NEP are expressed as mg O$_2$ L$^{-1}$ d$^{-1}$. By convention, R is expressed as negative.

To examine inter-annual differences in the persistence of growth periods, which appeared to be different based on visual inspection of the data, autocorrelation functions (ACFs) were calculated for GPP, R, and NEP for each year. For ACF calculations, data from each year were truncated to the range of Julian dates represented in the shortest monitoring season (2012) before analysis to allow comparisons between seasons.

**Correlation analysis**

Pair-wise correlations of all daily averaged independent variables as well as GPP, R, and NEP were plotted to facilitate visual comparisons of relationships among variables using the R package corrplot (Wei, 2013). Because of the recognized importance of temperature stratification in the bay (Isles et al. 2015; Giles et al. 2016), a metric of thermal stratification, ΔTemperature, was defined as the temperature difference between the surface and 2 m (the deepest consistent temperature reading across the three years), and included in both the correlation and SOM analyses.

**Self-Organizing Map**

An unsupervised SOM artificial neural network approach was used to cluster the data into fundamentally different bloom stages using the R package “kohonen” (Wehrens and Buydens 2007). Briefly, a grid of nodes (usually limited to one, two or three-dimensions) is selected, and each node is assigned a random vector of values (weights). These weight vectors are equal in size to the input vectors in the training dataset. In an iterative process, vectors of observations from the training data are matched to the nearest
weight vector associated with nodes on the grid based on shortest Euclidean distance; weight vectors are then updated to more closely resemble the new data based on a learning rate, $\alpha$, as are weights of the surrounding nodes within a defined neighborhood. As the training process progresses through multiple iterations, the neighborhood shrinks and $\alpha$ becomes smaller. If a two-dimensional grid of nodes is selected, the result is a two-dimensional mapping where observation data cluster to nodes in the 2-D grid, and adjacent nodes are more similar to one another than to distant nodes. This map can be used for identification and visualization of underlying structures in the data (Kohonen 1990).

To prepare the data for the SOM, all variables were tested for skewness, and several highly skewed variables were log-transformed (BGA, ChlA, TP, TN, and Q). Dissolved oxygen and pH were excluded from the SOM analyses because of non-independence with relation to metabolic variables, and turbidity was excluded because it was highly correlated with BGA. Following the transformation of skewed variables, all variables (GPP, R, NEP, $\log_{10}$ BGA, $\log_{10}$ Chl-a, TN, $\log_{10}$ TP, solar radiation, wind speed, temperature stratification, water temperature, and $\log_{10}$ Q) were scaled to z-scores. Weights for each variable were calculated using principal component analysis. For each variable, the Euclidean distance from the origin was calculated for a vector of the loadings on each of the first 6 principal components for that variable and used as the weight. The first 6 principal components accounted for 85.2% of the variance in the transformed dataset. This is similar to methods used in (Pearce et al. 2011), where input variables were weighted based on CCA X-coefficients rather than PC loadings.
In this study, a 10 x 10 non-toroidal hexagonal grid was used for the unsupervised SOM based on two forms of topographical error: average distance between pairs of most similar codebook vectors, and average distance between the best 2 matching nodes for each data point. To identify the optimal number of clusters for further analysis, hierarchical clustering was applied to the matrix of SOM weights over a range of 2-100 clusters. An adjusted $R^2$ was calculated for each of these cluster numbers using a linear model with the cluster as a categorical predictor variable and the matrix of weights as response variables. The lowest number of clusters at the first plateau of the resulting plot (cluster numbers against $R^2$) was used to optimize the number of clusters for further analysis. All standardized data points were assigned to each of these clusters based on SOM node assignments, and group means of each cluster were plotted to compare the general characteristics of the clusters.

Supervised Self-Organizing Map

Following the selection of relevant clusters in the data using the unsupervised SOM, a supervised self-organizing map (SSOM) was used to further investigate the drivers of metabolic parameters associated with phytoplankton growth (NEP, GPP, and R). A SSOM is similar in structure to an unsupervised SOM, but the mapping of independent variables is updated based on a second map of dependent variables. Given the use of predictor and response variable matrices, the SSOM technique has functional similarities to canonical correlation analysis (CCA) and can be used for prediction, but does not rely on linear transformations. There are several types of SSOMs; in this study we apply a bi-directional Kohonen map (Melssen et al. 2006), an algorithm that alternates
between training the features in the dependent matrix based on the independent matrix and updating the latter based on the dependent variables. This method is implemented in the R package “kohonen” (Melssen and Buydens 2007). To weight the SSOM input variables, we first performed CCA using the measured variables as predictors (X matrix), and NEP, GPP, and R as response variables (Y matrix). The loadings of the CCA X matrix were used to weight the input variables based on distance of the vector of loadings to the origin, similar to the PCA approach used in the unsupervised SOM. We trained the SSOM with a 12 x 12 non-toroidal, hexagonal grid using the “bdk” function in the R package “kohonen” (Wehrens and Buydens 2007) to predict a matrix of GPP, R, and NEP (the Y matrix) given the following independent variables (the X matrix; log_{10} BGA, log_{10} Chl-a, log_{10} TN, log_{10} TP, solar radiation, wind speed, temperature stratification, water temperature, and log_{10} Q) using the 2012 and 2013 datasets. The resulting mapping was then used to predict NEP, R, and GPP in 2014 to validate model results; and correlation and root-mean-square-error (RMSE) were calculated. Following the SSOM validation, the component planes were analyzed visually for patterns corresponding to trends in metabolic parameters. Hierarchical clustering was applied to the SSOM codebook vectors of the Y matrix to identify important clusters of nodes representing significant endogenous groupings of metabolic parameters.

Threshold analysis

Following identification of wind and thermal stratification as key variables controlling ecosystem metabolism, thresholds of thermal stratification controlling GPP and phycocyanin concentrations were identified. To evaluate the best threshold models,
R² was calculated for linear models predicting GPP, BGA, and Chl-a as a function of thermal gradient thresholds from 0 to 4°C at 0.01°C intervals. R² for this range of thresholds was calculated separately over smoothed windows of thermal stratification for 1-4 days (leading averages), to determine the effect of different periods of persistently calm weather in driving primary production in the system. Selection of “best” threshold was that with the highest R² for both BGA and GPP.

Results

Inter-annual variability in bloom severity

The three study years had dramatically different bloom dynamics (Fig. 4.1). An extremely strong cyanobacteria bloom occurred in 2012. BGA began to increase at the start of data collection in mid-July, and reached extremely high densities by mid-August. By contrast, 2013 was an extremely weak bloom year with BGA beginning to increase at approximately the same day of year as in 2012, but never achieving sustained growth. There was an unusual late-season spike in BGA at the end of September that lasted for about a week. This period had the strongest bloom conditions observed during 2013 based on visual observations during field sampling. Conditions in 2014 were intermediate between 2012 and 2013. BGA again began to increase at roughly the same date in late July, but then declined before reaching the levels achieved during 2012, and remained comparatively low for the remainder of the summer. Despite the differences in bloom severity between years, the period of high cyanobacteria activity was similar in each of the three years, with phycocyanin increasing in early-mid July, peaking in August, and declining through early September. Chl-a fluorescence during the three years had similar
patterns of inter-annual variability as BGA but differed seasonally (Fig. 4.1), frequently reaching maximal concentrations in September after BGA had declined. In 2012, Chl-a was high throughout the summer, and had several distinct peaks in late August and September, which corresponded to periods of declining PC and likely represented periods of eukaryotic phytoplankton growth. Chl-a remained low throughout 2013 until late September when there was the late-season bloom occurred following an extended dry period. Chl-a in 2014 was similar to 2013 during July and August, but increased in September to greater than 10 µg L$^{-1}$ while BGA remained low, suggesting dominance by eukaryotic phytoplankton.
Abiotic conditions

There were important differences in abiotic conditions between the three years. 2012 was characterized by a very dry spring and early summer and an unusually warm August, resulting in low Q and high August water temperatures (Fig. 4.1). Precipitation
was greater in 2013 than 2012, resulting in much higher Q in spring, throughout the summer, and in early autumn. There was a dry period during September 2013, and Q declined before the late-season bloom event in 2013 (Fig. 4.1). Water temperature was high in July 2013, but was relatively low throughout August. In 2014, conditions again fell between the other two years.

**GPP, R, and NEP**

There were large inter-annual differences in GPP that corresponded to the variability of bloom severity in the three years (Supplemental Fig. 4.1). While 2012 had strong sustained high primary productivity throughout the first two months of the monitoring period, particularly during August, the primary production in 2013 and 2014 was much more erratic, with multiple peaks each year and fewer periods of sustained bloom activity. Although R varied between years, the variation was less than that of GPP (Supplemental Fig. 4.2). In general R was more negative in 2012 than in other years, particularly during August when GPP was high. Interannual differences in GPP and R were reflected in estimates of NEP, which was generally higher during bloom periods (Supplemental Fig. 4.3). NEP tended to be positive during the summer months, and slightly negative in the late season; however, annual estimates of the lake net oxygen balance cannot be estimated due to gaps in the dataset.

Interannual differences in the persistence of cyanobacteria production are reflected in the autocorrelation function of GPP (Fig. 4.2). In 2012, autocorrelation of GPP was high and lagged values were significantly correlated for 19 days, reflecting sustained periods of growth. Autocorrelation in 2013 was considerably lower, though
lags were significant for 14 days. Autocorrelation was lowest in 2014, when and lags were only significant through 2 days. Inter-annual differences in ACFs for R and NEP were less pronounced than for GPP; while 2012 had somewhat more autocorrelation in these variables at most lags, the differences were relatively minor.

![Graphs of GPP, R, and NEP autocorrelation functions (ACFs) for 2012-2014.](image)

Figure 4.2: Autocorrelation functions (ACFs) of GPP, R, and NEP in 2012-2014.

Correlations between all measured variables and calculated metabolic parameters were visualized (Fig. 4.3); GPP and R were both more strongly correlated with pH than any of the other monitored variables. NEP, while still strongly correlated with pH, was more strongly correlated with DO. Phycocyanin fluorescence (BGA) was more strongly correlated with GPP, R, and NEP (r = 0.63, -0.36, and 0.37, respectively) than was Chl-a fluorescence (r = 0.36, -0.16, and 0.25, respectively). BGA was also more strongly correlated with variables associated with total phytoplankton biomass (TN, TP, and turbidity) than Chl-a, suggesting that phycocyanin fluorescence was a better proxy for phytoplankton abundance than chlorophyll fluorescence for the summer phytoplankton
community in Missisquoi Bay. This is consistent with findings from previous studies in the bay (McQuaid et al. 2011; Zamyadi et al. 2012).

![Figure 4.3](image)

**Figure 4.3:** Correlogram showing pairwise correlations between all measured variables and calculated metabolic parameters.

**Unsupervised SOM**

The SOM effectively clustered the data into distinct areas on the two-dimensional map (bottom panel of Fig. 4.4) with low topographical error (mean distance between most similar nodes was 1.42 map units, mean distance between best and second-best matching nodes for each data point was 1.72 map units). The 12 SOM component planes (Fig. 4.4) provide a means to visualize the variation of different variables projected onto the same, two-dimensional plane. While the x- and y- dimensions in these plots have no
specific meaning, smooth gradients in the two-dimensional space indicate that variation in that variable is similar to the major gradients of variation in the entire dataset; sharp gradients in a variable may represent thresholds with respect to that variable, and groups of nodes with similar values represent important clusters in the data. The SOM component planes indicate that the variation in the dataset was generally organized on a gradient of productivity with variables associated with bloom conditions lower on the left side of the map, and higher on the right (Fig. 4.4). Vertically in the map space, a clear separation was apparent between calm, stratified conditions on the top of the map, and windy, unstratified conditions on the bottom of the map.
Figure 4.4: Component planes of the unsupervised SOM, and locations of resulting clusters (bottom panel) in the SOM space. All values shown are z-scores of transformed variables.
Four distinct clusters of nodes were selected based on analysis of the $R^2$ over the full range of possible cluster numbers (Fig. 4.5). The means of data assigned to each cluster were calculated and based on these mean values, the clusters were defined as “Cold Wet”, “Windy”, “Peak Bloom”, and “Stratified” (Fig. 4.6). The Julian dates of these clusters were visualized on the time-series of BGA to visually assess the relationship to bloom stages (Fig. 4.6). It is immediately apparent that the “Stratified” cluster is associated with periods of increasing BGA. The key variables distinguishing this cluster from others are very low wind speeds and strong thermal stratification. Despite relatively low Chl-a and BGA, this cluster was associated with high NEP, suggesting that this stratified cluster represents periods of high growth rates. The “Peak Bloom” cluster was characterized by very high nutrients, BGA, and Chl-a, and by very low river discharge. This cluster occurred mostly in 2012, although there were several points during the strongest blooms experienced in 2013 and 2014. The “Peak Bloom” cluster had slightly lower wind speeds than average, and slightly higher $\Delta$Temperature. The “Windy” cluster differed from the others mostly with respect to much higher wind speeds than average and much lower $\Delta$Temperature. This cluster was also associated with the lowest NEP of any of the clusters, suggesting an association with periods of bloom decline, which is consistent with the locations of this cluster on the BGA plots for each of the 3 years (Fig. 4.6). The “Windy” cluster was the most common cluster during the summer of 2013 (the weak bloom year), and was common during August 2014 as well, but was infrequent in the summer of 2012 when a very strong bloom occurred. The last cluster was the “Cold-Wet” cluster, which was characterized by high river discharge, low
temperature and solar radiation, and low phytoplankton concentrations. This cluster was mostly represented late in the year, and also during strong storm events in 2012 and the spring of 2013.

Figure 4 5: Adjusted $R^2$ of SOM weights as a function of clusters. The number of clusters chosen was the first plateau of the curve (vertical dashed line).
**Supervised SOM**

The SSOM trained on data from 2012 and 2013 predicted GPP in 2014 reasonably accurately, given the high variability in the dataset ($r = 0.556$, RMSE=1.7 mg O$_2$ L$^{-1}$ d$^{-1}$). The SSOM predictions were less accurate for R ($r = 0.178$, RMSE = 2.346 mg O$_2$ L$^{-1}$ d$^{-1}$) and NEP ($r = 0.265$, RMSE=2.045 mg O$_2$ L$^{-1}$ d$^{-1}$). The SSOM component planes clearly illustrate the partitioning of GPP, R and NEP on the two-dimensional map (Fig. 4.7). The trained SSOM had a general gradient of increasing productivity, with low GPP at the right-hand side of the map, and higher values on the left. The environmental variables that mapped most closely to high GPP throughout the SSOM space were low
BGA and high \(\log_{10}Q\). Chl-a loosely followed the right-left gradient of GPP, but the pattern was much less pronounced than for BGA. Other variables highly correlated to cyanobacteria biomass (TN, TP; Fig. 4.7) mapped similarly to BGA, but these variable gradients were less smooth.

Using hierarchical clustering on the SSOM weights of the Y map, three important clusters were identified. Periods of extremely high productivity were focused in clusters at both the bottom and top left of the SSOM (Fig. 4.7). Both of these clusters had high BGA concentrations and represented bloom conditions; however, the “Growth” cluster in the bottom left was associated with moderate R and positive NEP, whereas the “Decline” cluster in the top left was associated with extremely negative R values and negative NEP (Fig. 4.7, 4.8). Most of the environmental variables associated with eutrophic conditions (BGA, Chl-a, TP, TN, temperature) fell similarly between “Growth” and “Decline” clusters. However, these two clusters differed dramatically with respect to wind speed (which was high in the “Decline” cluster, and low in the “Growth” cluster) and \(\Delta\text{Temperature}\) (which was high in the “Growth” cluster and low in the “Decline” cluster).
Figure 4.7: Component Planes of Supervised Self-Organizing Map. Independent variables are pictured in the top three rows; response variables are plotted on the bottom row. Dark black lines represent cluster boundaries; clusters are more clearly indicated in the “Clusters” panel.
Both supervised and unsupervised SOM approaches suggested that wind speed and thermal stratification were key variables controlling primary production and the growth or decline of blooms, but the regression of ΔTemperature against BGA was not significant ($p = 0.19, R^2 = 0.0040$), and the regression of ΔTemperature against GPP, while significant, did not explain a large proportion of the variation ($p < 0.0001, R^2 = 0.0932$). Analysis of thermal thresholds, however, revealed that a thermal stratification threshold of 0.2°C, based on ΔTemperature averaged over a 2-day leading window, was able to increase the proportion of variation explained in GPP from 0.09 to 0.2, and in BGA from 0.004 to 0.08 (Fig. 4.9). This threshold represents the 67th percentile of ΔTemperature. In contrast to BGA and GPP, no ΔTemperature thresholds were able to explain even 1% of the variance in Chl-a (data not shown). Superimposing the 0.2°C threshold on the time-series of BGA, temperature stratification thresholds appear to be strongly related to periods of growth in all years; and the frequency of stratified days
during the key bloom period of July 15-September 15 appears to be strongly related to interannual differences in bloom severity (Fig. 4.10). There were stratified periods associated with declining blooms; however, some of these stratified decline periods are associated with high river discharge (such as the period from J.D. 248-252 in 2012), suggesting that distinct processes may have been at work during these periods. However, there were no sustained periods of growth during prolonged periods with ΔTemperature below the 0.2°C threshold in any of the three years.

Figure 4.9: \( R^2 \) of different threshold temperatures v. BGA (triangles) and GPP (circles), based on a 2-day leading average of ΔTemperature. Vertical dashed line represents best threshold value. Solid horizontal line represents \( R^2 \) of GPP as a function of ΔTemperature (without threshold). Dotted horizontal line represents \( R^2 \) of BGA as a function of ΔTemperature (without threshold).
Discussion

Missisquoi Bay experienced extreme inter-annual variability in cyanobacteria bloom severity and primary production from 2012-2014 (Fig. 4.1). Phycocyanin
fluorescence (BGA) was the strongest indicator of this inter-annual variability, and was much more strongly correlated with eutrophic indicator variables (TN, TP, turbidity) than was Chl-a fluorescence (Fig. 4.3). Our analysis suggests that inter-annual differences in BGA were strongly influenced by subtle thresholds of wind speed and thermal stratification (Figs. 4.9, 4.10), particularly during phytoplankton growth periods. These thresholds of thermal stratification partially controlled the balance of GPP and R during the summer bloom season, and periods of persistent weak stratification were associated with the accumulation of phytoplankton biomass, whereas persistent mixed periods were associated with the decline of blooms (Fig. 4.10). The methods developed in this study are widely applicable to studies of cyanobacteria bloom progression in other lake systems.

The unsupervised SOM clustering approach was very effective for identifying patterns in environmental parameters driving variability in lake metabolism (Figs. 4.4, 4.6). The clear differences with respect to wind speed and thermal stratification associated with clusters representing cyanobacteria growth and decline periods provided a foundation for further analyses. Both clusters with high NEP and phytoplankton growth (“Stratified” and “Peak Bloom” clusters) were associated with greater-than-average ΔTemperature and lower-than-average wind speeds (Fig. 4.6). This is particularly true for the “Stratified” cluster, which had very high ΔTemperature and NEP despite relatively low BGA and Chl-a concentrations. By contrast, clusters associated with bloom decline (“Windy” and “Cold Wet”) were associated with higher than average wind speeds, and with periods of high river inputs.
After identifying general patterns of GPP, R, NEP and environmental variables associated with cyanobacteria growth and decline stages using the SOM, the SSOM clustering approach was able to explicitly model the drivers of NEP, GPP and R (Figs. 4.7, 4.8). Drivers of these parameters have been difficult to isolate with multiple linear regression models in previous studies (Coloso et al. 2011) because of the high correlation among potential explanatory variables. Furthermore, the high level of autocorrelation apparent in some of the metabolic parameters at daily timescales (Fig. 4.2) and the non-independence of the environmental parameters (many of which are correlated with phytoplankton biomass; Fig. 4.3) would make it difficult to interpret regression coefficients (Céréghino and Park 2009). By identifying distinct clusters of productive periods associated with different levels of R and NEP (Fig. 4.7), the SSOM was able to highlight not only the role of bloom intensity indicators such as BGA and TP (which were important for structuring the map along a productivity gradient; Fig. 4.7), but also the role of weak stratification which separated growth and decline periods. The robust performance of the SSOM on the 2014 validation dataset, particularly for GPP, provides confidence that the processes identified in 2012 and 2013 were generalizable to other years for this system.

The finding that low winds and stable water column conditions stimulate phytoplankton production in Missisquoi Bay is consistent with other studies of large shallow lakes (Jöhnk et al. 2008; Kosten et al. 2011b; Huber et al. 2012). However, unlike previous studies which identified critical thresholds using extreme events such as major storms (Jennings et al. 2012) or historic heat waves (Jöhnk et al. 2008), the
thresholds that influenced cyanobacteria dynamics in Missisquoi Bay were much lower and within the range of normal variability in wind and temperature conditions (67th percentile of thermal stratification). While the 0.2°C threshold identified here may seem small, the same threshold was identified for both BGA and GPP (Fig. 4.9), providing increased confidence in the result. This result is also supported by a recent study of circulation patterns in the bay, which independently identified 0.2°C as a critical threshold above which stable 2-layer circulation dynamics can become established, suggesting that there is a physical basis for this threshold (Manley et al. In review). There is no reason to think that Missisquoi Bay should be unique in its sensitivity thermal stratification thresholds, although there are likely to be substantial differences in the ∆Temperature thresholds among shallow polymictic lakes, where mixing events may occur anywhere from several times during a year (Jennings et al. 2012) to almost daily (Laas et al. 2012). However, even in deeper lakes, which experience prolonged periods of stable stratification, diel fluctuations in surface temperature within the upper mixed layer are widespread and may be large (Woolway et al. 2016), and these fluctuations are found to be important for gas exchange and metabolism (Pernica et al. 2013). This suggests that subtle ∆Temperature thresholds controlling bloom development may be widespread and underappreciated.

There are several mechanisms that may account for the profound influence of thermal stability on cyanobacteria bloom development and primary production. The first and most obvious is the direct effect of temperature on cyanobacteria growth rates. Warmer water temperatures promote higher growth rates for many bloom-forming
cyanobacteria (Butterwick et al. 2005), including *Dolichospermum*, *Aphanizomenon*, and *Microcystis* species which usually dominate summer blooms in Missisquoi Bay. Diel thermal stratification occurs through heating of the upper mixed layer (Imberger 1985), which results in substantially warmer temperatures at the surface (Woolway et al. 2016) even if vertically averaged water temperatures are somewhat cooler. These warmer surface temperatures may have a profound effect on cyanobacteria growth rates for buoyant cyanobacteria that can remain in warm surface waters (Robarts and Zohary 1987).

Wind mixing may also control blooms by mediating light availability in turbid shallow systems such as Missisquoi Bay (Huisman et al. 2004). In Missisquoi Bay, light extinction coefficients are often high (Effler et al. 1991), Isles et al., *Chapter 3 of this dissertation*), frequently resulting in light levels below the compensation point (1% surface irradiance) in bottom waters despite the bay’s shallow depth. During peak bloom conditions, average water column light availability may be too low to sustain phytoplankton growth, particularly on cloudy days when irradiance is low (Isles et al., *Chapter 3 of this dissertation*). Cyanobacteria are efficient competitors in turbid systems, in part because of their ability to regulate their buoyancy to access surface light resources (Reynolds et al. 1987; Walsby 1997; Mitrovic et al. 2001), and tend to dominate turbid nutrient-rich systems because of their superior competitive abilities for light (Brauer et al. 2012). The ability of phytoplankton to migrate vertically, however, is mediated by turbulent mixing; under turbulent conditions this competitive advantage is erased (Mitrovic et al. 2001). Therefore, the thermal thresholds identified in this study may
affect GPP by facilitating buoyancy regulation in cyanobacteria, enabling them to overcome light limitation. Evidence for buoyancy regulation has been observed in Missisquoi Bay, where during calm periods cyanobacteria rise to the surface in the afternoon following the development of diurnal mixed layers (Isles et al., Chapter 3 of this Dissertation). In contrast to BGA, no ΔTemperature thresholds were found with respect to Chl-a fluorescence, which is more reflective of eukaryotic phytoplankton than cyanobacteria (Zamyadi et al. 2012). Eukaryotic phytoplankton are frequently dominated by diatoms in Missisquoi Bay (Shambaugh et al. 1999), which cannot regulate their buoyancy and are expected to perform better under mixed water column conditions (Huisman et al. 1999b).

In addition to direct effects on primary production, wind mixing may also be critical for mediating sediment nutrient release, providing an indirect control on phytoplankton growth and primary production (Burger et al. 2007; Zilius et al. 2013). In Missisquoi Bay, bottom water DO has been found to decline rapidly as soon as diel thermal gradients become established (Isles et al., Chapter 3 of this dissertation), and bottom water DO becomes depleted more quickly under bloom conditions (Isles et al. 2015). Sediment nutrient release in Missisquoi Bay has been shown to be controlled by redox fluctuations near the sediment water interface (Smith et al. 2011); and these fluctuations are highly dependent on bottom water dissolved oxygen concentrations (Giles et al. 2016). When the redox front for iron and manganese reduction moves above the sediment-water interface due to oxygen depletion in the bottom centimeters of the water column, reductive dissolution of Fe and Mn oxyhydroxides leads to rapid release of
sorbed P to the overlying waters. Previous studies in the bay have found that small thermal gradients in the upper water column are a strong indicator of the development of reducing conditions in the bottom water near the sediment-water interface (Isles et al. 2015; Giles et al. 2016). In addition to P release, bottom water DO depletion can also facilitate fluxes of ammonium from sediments (McCarthy et al., In Press), so the development of thermal stratification may increase supply rates for both of the critical limiting nutrients in Missisquoi Bay. Conversely, persistently mixed conditions may induce nutrient limitation and bloom decline.

In this study we developed a robust method to identify ecologically distinct stages of cyanobacteria bloom progression, and to identify the key factors controlling bloom development during each of those stages. By overcoming hysteresis effects, the SOM clustering method was able to isolate distinct abiotic features associated with bloom development and decline, allowing us to identify critical thresholds of thermal stratification that explained much of the inter-annual variability in bloom severity observed in our system. While the specific thresholds controlling bloom development are likely to vary between sites, the methods developed in this study are applicable to a wide variety of lake systems experiencing cyanobacteria blooms. With the increasing availability of high-frequency monitoring data, the opportunity exists to use these methods to develop a comprehensive understanding of the drivers associated with stages of bloom development in lakes worldwide.

The existence of subtle ΔTemperature thresholds within the normal range of variation may make shallow eutrophic lakes highly sensitive to long-term changes in
average wind speeds. Wind speeds have declined throughout the contiguous United States in recent decades, and these declines have been on the order of 1% y⁻¹ in the northeastern United States (Pryor et al. 2009). Declining trends in wind speed are evident in data from the Burlington, VT airport, where there has been a 20% decline in mean growing season wind speed over the past 23 years (Isles et al., Chapter 5 of this dissertation). The decline in wind speed has likely resulted in more frequent incidence of transient stratification over the 0.2°C threshold identified here, and may have contributed to increases in cyanobacteria bloom severity observed in Missisquoi Bay over recent decades. If similar thresholds exist in other shallow lakes, declining wind speeds may have contributed to the widespread increases in cyanobacteria blooms that have been observed in recent years (Paerl and Huisman 2009). Projections of future trends in wind speeds are highly uncertain (Pryor et al. 2006), but if average summer wind speeds continue to decline blooms are likely to become more intense in the future. To improve global predictions of lake responses to climate change, we recommend that future research in shallow lakes should incorporate the role of wind, and focus on systematic methods of identifying key hydrodynamic thresholds controlling cyanobacteria abundance across gradients of lake size, depth, and trophic status. More attention should also be given to describing the specific biogeochemical mechanisms linking transient thermal stratification to bloom development.

References


Supplemental Figure 4.1: Daily gross primary production (GPP) estimates for 2012-2014
Supplemental Figure 4.2: Ecosystem respiration (R) estimates for 2012-2014.
Supplemental Figure 4.3: Net ecosystem production (NEP) estimates for 2012-2014.
CHAPTER 5: CLIMATE-DRIVEN CHANGES IN ENERGY AND MASS INPUTS
SYSTEMATICALLY ALTER NUTRIENT CONCENTRATION AND
STOICHIOMETRY IN DEEP AND SHALLOW REGIONS OF LAKE CHAMPLAIN

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Keywords: total nitrogen, total phosphorus, N:P, climate change, Lake Champlain
Running Head: Climate-driven declines in TN:TP in Lake Champlain
Abstract

The balance of nitrogen (N) and phosphorus (P) is a key factor controlling phytoplankton community composition in lakes and the prevalence of cyanobacteria. Concentrations of N and P in lakes may be differentially impacted by nutrient loading from watersheds (mass inputs) and by changes in rates of internal nutrient cycling driven by physical conditions such as temperature and stratification (energy inputs), all of which are projected to change with climate warming. We mined 23 years of monitoring data to compare long-term and intra-annual trends in total N (TN), total P (TP) and TN:TP at 15 sites in Lake Champlain to concurrent measurements related to lake-wide mass and energy inputs including: tributary discharge, watershed nutrient inputs, wind speed, air temperature, bottom water temperature and dissolved oxygen. TN:TP declined sharply lake-wide, particularly in the past decade, yet the drivers of this trend varied based on site depth. In deep sites, declines were driven by changes in watershed loading of dissolved P and N and (in some cases) by decreases in hypolimnetic dissolved oxygen. In shallow sites, declines in TN:TP were primarily driven by long-term increases in temperature and decreases in wind speed, and exhibited systematic intra-annual variability in TN:TP due to the seasonal progression of sediment P loading, N removal processes, and external nutrient inputs. Findings suggest that while different climate drivers have affected nutrient dynamics in shallow and deep sites, both are likely to experience declines in N:P and increases in cyanobacteria dominance if recent climate trends continue.
Introduction

Nitrogen (N) and phosphorus (P) are the primary nutrients limiting phytoplankton production in lakes and structuring lake food webs (Guildford and Hecky 2000). The ratio of N to P (N:P) is often cited as an important factor determining phytoplankton community composition and the likelihood of cyanobacteria blooms (Smith 1983; Guildford and Hecky 2000), although this relationship is not straightforward and a number of studies suggest that the absolute concentrations of nutrients (Downing et al. 2001; Brauer et al. 2012) or the forms of those nutrients (Blomqvist et al. 1994; Reynolds and Davies 2001; Hessen 2013) may be more important in determining community structure. Changing patterns of temperature and precipitation are expected to alter patterns of nutrient delivery from lake catchments (Jeppesen et al. 2009; Leavitt et al. 2009; Jones et al. 2011) and internal cycling of nutrients (Grantz et al. 2014); therefore, the potential exists for N and P to respond differently to climate change. Consequently, lake N:P ratios may change in the future, resulting in corresponding shifts in biological communities (Elser et al. 2000).

The impacts of changing climate on lake N:P are difficult to resolve using long-term datasets due to the multiplicity of factors which influence lake N and P concentrations. A number of large lakes have experienced increases in the ratio of dissolved inorganic nitrogen (DIN) to TP (DIN:TP) over the period from 1970-2010 as a result of decreases in anthropogenic P loading and climate-mediated changes in water residence time (Finlay et al. 2013). In contrast, TN:TP has declined recently in Lake Erie (Scavia et al. 2014) and Lake Winnipeg (Schindler et al. 2012), and shallow northern
European lakes have experienced falling N concentrations in recent decades as a result of decreasing N deposition and increasing temperature (Weyhenmeyer et al. 2007). As a result of the multiple interacting factors controlling N and P concentrations in lakes, predicting the influences of climate change on lake N:P requires consideration of both watershed processes and in-lake nutrient cycles (Bleckner 2005).

Climate change may affect lake N:P indirectly through changes in mass fluxes of water and nutrients to lakes mediated by lake catchments, or directly through changes in fluxes of energy to lakes (air temperature, wind, or solar radiation) (Leavitt et al. 2009). External mass inputs of N and P may respond to changes in seasonal patterns of temperature and precipitation. While long-term studies of catchment loading are often complicated by land-use changes, some studies suggest that external loads of P have increased as a result of changing climate patterns, despite reductions in anthropogenic inputs (Jeppesen et al. 2009). Modeling studies using projected changes in climate predict substantial changes in N and P loading (Andersen et al. 2006) and in water inputs and flushing rates (Jones et al. 2011) with future climate warming. Changes to tributary nutrient loading may be manifest in changes in the concentrations or loads of nutrient inputs (Jeppesen et al. 2009), or in the timing of these inputs (Pierson et al. 2013). The ratio of dissolved to particulate nutrients delivered to lakes may also differ with changing climate (Vanni et al. 2001). Many climate models predict an increase in storm intensity under warming scenarios (Guilbert et al. 2015); higher energy storms are expected to lead to increased transport of particulate nutrients, while the effects on dissolved nutrient transport are less clear (Sharpley et al. 2008). In many systems, P transport is dominated
by the particulate fraction while most N is transported in dissolved form. The result may be a decrease in N:P loading ratios under warming scenarios.

Internal processing of nutrients and N:P ratios may be influenced by changes in temperature, wind mixing, and solar radiation, and responses may vary from deep to shallow lakes. Deep temperate lakes are generally expected to experience longer periods of summer stratification under warmer climates (Wagner and Adrian 2009; Williamson et al. 2009; Foley et al. 2011), while thermal structure in shallow polymictic lakes may have complex responses to climate drivers (Kosten et al. 2011a). In most lakes, higher surface water temperatures are likely to contribute to increased cyanobacteria dominance, particularly if climate changes result in increased incidence of summer heat waves (Jöhnk et al. 2008; Paerl and Huisman 2008; Kosten et al. 2011b). In deep lakes, longer periods of stratification may lead to hypolimnetic oxygen depletion and increased P release from sediments (Soranno et al. 1997; Jankowski et al. 2006). In shallow lakes warmer sediment temperatures are likely to boost sediment respiration rates and lower oxygen concentrations near the sediment-water-interface, leading to increased denitrification as well as enhanced release of P adsorbed to reducible Fe and Mn oxyhydroxides (Davison 1993; Hupfer and Lewandowski 2008; Smith et al. 2011). Lakes of intermediate depth may show responses characteristic of both deep and shallow lakes, depending on specific features of individual lakes and catchments.

In this study, we compared decadal and intra-annual trends in TN, TP, and TN:TP at 15 long-term monitoring sites in Lake Champlain over 23 years to inputs of dissolved and particulate nutrients from tributary inputs, and to meteorological drivers which may
influence internal nutrient cycling. We focused on contrasts between deep and shallow lake sites, and examined trends in other variables (water temperature, dissolved oxygen, and cyanobacteria abundance) that help to explain or corroborate trends in N and P. The monitoring sites in Lake Champlain span a wide range of depths and trophic conditions. Additionally, the lake has several distinct basins with limited exchange of water, making the long-term dataset useful for comparisons of trends across a range of conditions while retaining the consistency of a single dataset collected and analyzed using the same methods and equipment. Lake Champlain is also notable for its large ratio of watershed area to lake area (19:1), making it an ideal site for studying the interacting effects of climate and watershed loading on lake processes. Our objectives in this study were to: 1) determine whether lake N and P concentrations and stoichiometry have changed over the 23-year monitoring period, 2) identify characteristic seasonal patterns of nutrient concentration and stoichiometry in shallow and deep sites, 3) mechanistically interpret long-term trends in lake nutrient dynamics as a function of changes in energy and mass inputs to the system, and 4) develop a broadly applicable conceptual model based on our results to explain the drivers of nutrient behavior across diverse lake environments and project the response of these environments to climate-driven changes in energy and mass fluxes.
Methods

Site description

Lake Champlain is a large natural lake located between the Adirondack Mountains in New York, the Green Mountains in Vermont, and the St. Lawrence valley in Quebec (Fig. 5.1). Lake Champlain is 193 kilometers long and 19 kilometers wide at its widest point, with a maximum depth of 122 m and an average depth of 20 m. Lake Champlain contains five major basins separated by human-made and natural barriers, and different areas of the lake experience a wide range of trophic conditions (Xu et al. 2015) (Table 1). Parts of Lake Champlain have had increased incidence of cyanobacteria blooms in recent years (Smeltzer et al. 2012; Levine et al. 2012).
Figure 5.1: Map of Lake Champlain showing the major lake basins (shaded regions of the lake), major tributaries, and the locations of the long-term monitoring sites in the lake (pentagons) and watershed (circles). Lake basins are coded by pattern: Missisquoi Bay, northeast; Northeast Arm, east-northeast; Malletts Bay, east central; Main Lake, west; South Lake, south. Burlington Airport, where weather data were collected, is marked with an airport symbol.

Table 5.1: Locations, station ID’s and trophic state of lake monitoring sites

<table>
<thead>
<tr>
<th>Station Name</th>
<th>Station ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Depth (m)</th>
<th>Trophic State</th>
<th>Lake Basin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missisquoi Bay</td>
<td>50</td>
<td>44˚ 00.80’</td>
<td>-73˚ 10.43’</td>
<td>4</td>
<td>Eutrophic</td>
<td>Missisquoi</td>
</tr>
<tr>
<td>Missisquoi Bay Central</td>
<td>51</td>
<td>45˚ 02.50’</td>
<td>-73˚ 07.78’</td>
<td>5</td>
<td>Eutrophic</td>
<td>Missisquoi</td>
</tr>
<tr>
<td>South Lake B</td>
<td>2</td>
<td>43˚ 42.89’</td>
<td>-73˚ 22.98’</td>
<td>5</td>
<td>Eutrophic</td>
<td>South Lake</td>
</tr>
<tr>
<td>Location</td>
<td>Lat</td>
<td>Long</td>
<td>Depth</td>
<td>Trophic State</td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>--------</td>
<td>------------</td>
<td>-------</td>
<td>---------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Isle LaMotte (off Rouse’s Pt.)</td>
<td>46</td>
<td>44˚ 56.90'</td>
<td>-73˚ 20.40'</td>
<td>7</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>St. Alban’s Bay</td>
<td>40</td>
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<td>-73˚ 09.73'</td>
<td>7</td>
<td>Eutrophic</td>
<td></td>
</tr>
<tr>
<td>South Lake A</td>
<td>4</td>
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<td>-73˚ 24.47'</td>
<td>10</td>
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<td></td>
</tr>
<tr>
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<td>-73˚ 25.09'</td>
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<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
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<td>44˚ 29.49'</td>
<td>-73˚ 13.90'</td>
<td>15</td>
<td>Mesotrophic</td>
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<tr>
<td>Shelburne Bay</td>
<td>16</td>
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<td>-73˚ 13.92'</td>
<td>25</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>Malletts Bay</td>
<td>25</td>
<td>44˚ 34.92'</td>
<td>-73˚ 16.87'</td>
<td>32</td>
<td>Oligotrophic</td>
<td></td>
</tr>
<tr>
<td>Port Henry Segment</td>
<td>7</td>
<td>44˚ 07.56'</td>
<td>-73˚ 24.77'</td>
<td>50</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>Northeast Arm</td>
<td>34</td>
<td>44˚ 42.49'</td>
<td>-73˚ 13.61'</td>
<td>50</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>Isle LaMotte (off Grand Isle)</td>
<td>36</td>
<td>44˚ 45.37'</td>
<td>-73˚ 21.30'</td>
<td>50</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>Otter Creek Segment</td>
<td>9</td>
<td>44˚ 14.53'</td>
<td>-73˚ 19.75'</td>
<td>97</td>
<td>Mesotrophic</td>
<td></td>
</tr>
<tr>
<td>Main Lake</td>
<td>19</td>
<td>44˚ 28.26'</td>
<td>-73˚ 17.95'</td>
<td>100</td>
<td>Mesotrophic</td>
<td></td>
</tr>
</tbody>
</table>

* Trophic state designated by the Lake Champlain Basin Program based on TP criteria.

**Data sources**

Epilimnetic TP, epilimnetic TN, bottom water dissolved oxygen (DO), and bottom water temperature collected fortnightly from 1992-2014 were obtained from the Lake Champlain Long Term Monitoring Program ([http://www.watershedmanagement.vt.gov/lakes/htm/lp_longterm.htm](http://www.watershedmanagement.vt.gov/lakes/htm/lp_longterm.htm)) for all 15 lake monitoring sites (Table 1; (VT DEC and NY DEC, 2014). Phytoplankton cell count data from 63-µm plankton net vertical plankton samples were obtained from the Vermont Department of Environmental Conservation by request. Tributary nutrient load data (daily and annual) for TN, TP, and DP from each of the 18 monitored tributaries to the lake for 1992-2012 were taken from (Medalie 2013). TN:TP in the lake and tributaries
are expressed as TN:TP$_{\text{lake}}$ and TN:TP$_{\text{river}}$ to avoid ambiguity. Meteorological data were obtained from Weather Underground for the Burlington International Airport from 1 January 1992 through 31 December 2014 (http://www.wunderground.com/history/airport/KBTV/). River discharge data were acquired from USGS gage stations for the nine largest tributaries to Lake Champlain (USGS gage stations 04275500, 04276500, 04271500, 04292500, 04294000, 04282500, 04280000, 04273500, and 04290500).

Additional tributary nutrient data were collected from two major tributaries to Lake Champlain, the Missisquoi River and the Winooski River (Fig. 5.1), and used to supplement the long-term monitoring data, which lacked samples for total dissolved nitrogen (DN). Isco automated water samplers (Teledyne Isco, Lincoln NE) were installed at downstream gage sites on the Missisquoi and Winooski rivers, and collected water samples at regular intervals when river stage crossed a minimum threshold. Water samples were retrieved from the Iscos within 24 hours of sampling and analyzed for concentrations of DN, TN, DP, and TP. Samples for DN and DP were filtered immediately following collection using 0.45µm PES filters. Following persulfate digestion (Std. Method 4500 P-J.; APHA 2005), TN and TP were determined using EPA methods 353.2 and 365.1, respectively (USEPA 1993a, b). All nutrient analyses were conducted on an AQ2 Autoanalyzer (Seal Analytical, Mequon WI).

*Statistical methods*

Lake TN and TP data were aggregated into shallow (< 15 m) Lake TN and TP data were aggregated into shallow (< 15 m) and deep (≥ 15 m) sites for analysis of long-term
and within-year trends. Data from three sites where data collection began in 2001
(Missisquoi Bay Central, Shelburne Bay, Otter Creek Segment) were omitted for analysis
of long-term trends, but included for analysis of within-year trends. Segmented
regression was used to fit linear models with breakpoints to both long-term and intra-
annual nutrient data against date or Julian date using the R package “segmented”
(Muggeo 2003, Muggeo 2008) for sites where Davies’ test was significant (α = 0.01)
(Davies 2002). Slopes during the main growing season (July and August) were used to
investigate within-year trends in nutrients. Where Davies’ test was not significant, slopes
were determined with least-squares regression. All regressions of N:P data were
conducted on log_{10}-transformed N:P; however, plots are presented with untransformed
axis labels to allow for easier interpretation of the data. All nutrient ratios are presented
as molar ratios.

Long-term changes in annual river loads of TN, TP, TN:TP and DN:DP were also
analyzed using least-squares regression and segmented regression. Because trends in
annual loads did not match trends in lake nutrient concentrations (see results), average
annual concentrations of river nutrients were calculated as the total annual load divided
by the total annual discharge, and were compared to lake nutrient concentrations. Further
analyses were conducted for two sites representative of shallow and deep bays with major
tributary inputs (Missisquoi Bay and Malletts Bay, respectively). For these sites,
regressions were conducted of TN_{river}, TP_{river}, TN:TP_{river}, and DN:DP_{river} of tributary
nutrient inputs against TN_{lake}, TP_{lake}, and TN:TP_{lake} of the receiving bays, respectively.
Dissolved N (DN) for DN:DP_{river} estimates was estimated as a constant fraction of TN
based on the mean and median ratios of $\text{DN:TN}_{\text{river}}$ measured in the two tributaries where DN data were available (Missisquoi and Winooski Rivers). Similar regressions were also conducted for the aggregate annual loads of all monitored tributaries against nutrient concentrations at the central Main Lake station.

Long-term trends were determined for air temperature and wind speed using averages for the lake monitoring season (April-November), and long-term trends in aggregate tributary discharge were determined using annual averages. In addition, monthly changes in meteorological drivers, river discharge, and lake nutrients from 1992-2014 were estimated using least-square regressions against time for each variable subsetted to each month. The slopes of the resulting regression lines were used to estimate the total change for each month from 1992-2014. All regressions were evaluated for statistical significance at $\alpha = 0.05$.

To test the combined influence of external inputs and climate drivers, stepwise multiple regression model selection (forward and backward) was used to identify the best models to explain $\text{TN}_{\text{lake}}$ and $\text{TP}_{\text{lake}}$ in the representative deep and shallow bay where direct comparisons to tributary loads were possible (see above). Separate regression models were evaluated for lake nutrients for spring (April-June), summer (July-August), fall (September-October), and annual (monitoring season). Because of the importance of winter and spring discharge in determining annual nutrient budgets (Stumpf et al. 2012), tributary nutrient concentrations and discharge for seasonal models were calculated as cumulative values for the calendar year through the season being modeled.
In deep lake sites displaying stable thermal stratification each summer, quantile regression was used to test for significant trends in the maxima of bottom water temperature and minima of bottom water DO over time (Cade and Noon 2003). All quantile regression analyses were conducting using the R package “quantreg” (Koenker 2015). The upper end of the temperature distribution was modeled as the 80th percentile, while the lower end of the DO distribution was modeled using the 20th percentile. Quantile regression was also used to evaluate trends in peak cyanobacteria abundance (cells mL\textsuperscript{-1}) for the three most abundant genera (Anabaena, Aphanizomenon, and Microcystis). Tau of 0.9 was used for these regressions, and regressions were conducted on log (n+1) transformed abundance data.

**Results**

*Long-term trends in TN\textsubscript{lake}, TP\textsubscript{lake}, and TN:TP\textsubscript{lake}*

Over the course of the monitoring period, TN\textsubscript{lake} decreased significantly in both deep and shallow sites within the lake (p < 0.00001) at an average annual rate of 3.5 µg L\textsuperscript{-1} y\textsuperscript{-1} (deep and shallow sites; Fig. 5.2). TN\textsubscript{lake} declined steadily over time in deep sites, whereas TN\textsubscript{lake} remained constant for the first decade in shallow sites but then declined following 2002 (although there were substantial differences between individual sites; Supplemental Fig. 5.1). On a seasonal basis, shallow and deep sites showed similar patterns with respect to TN\textsubscript{lake}, with the largest declines during July and August, and the most modest declines in the fall (Fig. 5.3).
Figure 5.2: Long-term trends in lake TN (mg L\(^{-1}\)), TP (µg L\(^{-1}\)), log\(_{10}\)(TN:TP) (molar), daily discharge from all tributaries (m\(^3\) s\(^{-1}\), annual average), air temperature (°C, April-November average) and wind speed (m s\(^{-1}\), April-November average).
Figure 5.3: Average changes from 1992-2014 for each month in: lake TN (mg/L), TP (µg/L), and log_{10}(TN:TP) (molar), aggregate daily discharge rates from the nine largest tributaries to the lake (log scale, cubic meters per second), and air temperature and wind speed from the Burlington airport. Significant changes are marked with an asterisk (p < 0.05).

TP_{lake} showed the opposite trend to TN_{lake}, increasing significantly lake-wide throughout the monitoring period (p < 0.00001) at average annual rates of 0.30 µg L^{-1} y^{-1} (shallow sites) and 0.20 µg L^{-1} y^{-1} (deep sites; Fig. 5.2). On a monthly basis, the largest increases in TP_{lake} in shallow sites occurred in October, with significant increases in June and September as well (Fig. 5.3). In deep sites, increases in TP_{lake} were significant every month, with the largest increases in September. As with TN_{lake}, substantial differences in
trends between individual sites were evident, particularly in the shallow sites (Supplemental Fig. 5.1).

As a result of decreasing TN$_{lake}$ and increasing TP$_{lake}$, TN:TP$_{lake}$ decreased sharply during the monitoring period at all sites, particularly in the second half of the monitoring period (Fig. 5.2). On a monthly basis, the declines were especially steep during spring and fall in shallow sites, and in summer and early fall in deep sites (Fig. 5.3). The deep sites had significant breakpoints in TN:TP$_{lake}$ in 2002-2003 for all sites within the main lake basin ($\alpha < 0.01$; Supplemental Fig. 5.1), with relatively flat trends before this point, and sharper declines following. Shallow sites showed a similar long-term trend, but did not have significant breakpoints.

_Intra-annual trends in TN, TP, TN:TP_

Most monitoring sites experienced seasonal declines in TN$_{lake}$ from spring through the summer, and these seasonal declines tended to be greater in shallow sites (Supplemental Figs. 5.2, 5.3). The rate of decline also appeared to be related to initial spring TN; sites with higher spring TN showed steeper seasonal declines (Missisquoi Bay), while the sites with lowest spring TN showed slight increasing trends over the monitoring season (St. Albans Bay and Northeast Arm) (Supplemental Fig. 5.2).

In contrast to TN$_{lake}$, TP$_{lake}$ increased over the course of the monitoring season in shallow sites, and tended to decline slightly in deep sites (Supplemental Fig. 5.2, 5.3). TP$_{lake}$ in the shallow sites generally increased until late summer, when the trajectory reversed (Supplemental Fig. 5.2). The slopes of TP$_{lake}$ during the main growing season were negatively correlated with log$_{10}$ depth, and the regression of slope against depth was
highly significant ($p = 0.00089$; Supplemental Fig. 5.3). Sites with depths $\leq 11$m had positive slopes, while sites $\geq 15$m had slopes close to 0 (Supplemental Fig. 5.3). All but one of the deep sites showed slightly decreasing $\text{TP}_{\text{lake}}$ over the course of the monitoring season. The exception was the Northeast Arm site, which had substantial increases in $\text{TP}_{\text{lake}}$ late in the season.

Different depth-dependent intra-annual patterns of $\text{TN}_{\text{lake}}$ and $\text{TP}_{\text{lake}}$ resulted in sharply contrasting seasonal patterns of $\text{TN}:\text{TP}_{\text{lake}}$ in shallow v. deep sites. Strong and systematic intra-annual patterns of $\text{TN}:\text{TP}_{\text{lake}}$ were apparent in the shallow site time series, with $\text{TN}:\text{TP}_{\text{lake}}$ initially high, but decreasing throughout the growing season, and rising again in the autumn following breakpoints in late August or September (Fig. 5.4). Deep sites had consistent $\text{TN}:\text{TP}_{\text{lake}}$ across the growing season with only slight increases or decreases and no significant breakpoints, with the exception of the Northeast Arm site, which had an initial increase in $\text{TN}:\text{TP}_{\text{lake}}$ in spring, and a subsequent decline throughout the growing season, particularly in late fall.
Figure 5.4: TN:TP plotted against Julian date at the 15 monitoring sites showing seasonal trends and significant breakpoints if present (black symbols), separated into shallow sites (< 15m) and deep sites (≥ 15m). If no significant breakpoint is present, the slope of the least-squares regression is used. Slopes are calculated for log_{10} transformed TN:TP, but untransformed values are shown on axes here for easier interpretation of results.

**Dissolved and total nutrient inputs**

$\text{TP}_{\text{river}}$ loads were dominated by particulate nutrients in the long-term monitoring data and the data collected for the current study, with $\text{DP}_{\text{river}}$ loads representing, on average, 26% of $\text{TP}_{\text{river}}$ loads from all tributaries (Medalie 2013). $\text{DP}_{\text{river}}/\text{TP}_{\text{river}}$ was higher during periods of low discharge, with $\text{DP}_{\text{river}}$ often contributing 50% or more of $\text{TP}_{\text{river}}$. Annual loads, however, were dominated by high flow events when particulate nutrients dominated. In contrast, $\text{N}_{\text{river}}$ loading was dominated by the dissolved fraction, with close to 100% of $\text{N}_{\text{river}}$ delivered in dissolved form much of the time (Supplemental...
Fig. 5.4). Because long-term monitoring data were not available for $D_{N\text{river}}$, $D_{N\text{river}}$ was estimated as $0.85 \times TN_{\text{river}}$ for estimates of $D_{N\text{river}}:D_{P\text{river}}$. The estimate for $D_{N\text{river}}$ reflects both the mean (0.867) and median (0.891) ratios of $D_{N\text{river}}:TN_{\text{river}}$ for the two monitored tributaries.

Aggregate $TN_{\text{river}}$ loads for all major tributaries to the lake did not change significantly over the monitoring period (Fig. 5.5), in contrast to the $TN_{\text{lake}}$ declines observed lake-wide (Fig. 5.2). $TP_{\text{river}}$ loads were highly variable among years and showed no trend over time. Flow-normalized concentrations of $TN_{\text{river}}$ and $TP_{\text{river}}$ both decreased significantly during the monitoring period (Medalie 2013); as a result, the constant or increased loading of $TN_{\text{river}}$ and $TP_{\text{river}}$ can be attributed to increases in total river discharge over the monitoring period.

$\log_{10} TN:TP_{\text{river}}$ of aggregate annual river nutrient loads showed no significant trend with time (Fig. 5.5). By contrast, $D_{N\text{river}}:D_{P\text{river}}$ had a clear breakpoint, increasing slightly during the 1990s and decreasing sharply following 2001-2002. $TN:TP_{\text{lake}}$ in deep stations within the main lake basin, which increased slightly before breakpoints in 2002-2003 and fell sharply in subsequent years (Fig. 5.2, Supplemental Fig. 5.1), followed a similar pattern to $D_{N\text{river}}:D_{P\text{river}}$. 

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Figure 5.5: Long-term trends in external nutrient loading from all monitored tributaries to Lake Champlain (data from Medalie 2013). Regression lines are shown for $\text{TN}_{\text{river}}$, $\text{TP}_{\text{river}}$, and $\text{TN}:\text{TP}_{\text{river}}$, and segmented regression line with breakpoint is shown for $\text{DN}:\text{DP}_{\text{river}}$.

Comparisons of lake and tributary nutrients

Annual $\text{TN}:\text{TP}_{\text{lake}}$ of the representative deep bay (Malletts Bay) was not significantly correlated with annual $\text{TN}:\text{TP}_{\text{river}}$ loads from its major tributary ($p = 0.23$, $R^2 = 0.074$), but was strongly positively correlated with annual $\text{DN}:\text{DP}_{\text{river}}$ loads ($p < 0.0001$, $R^2 = 0.659$), and the regression line was close to the 1:1 line (Figure 5.6). By contrast, annual $\text{TN}:\text{TP}_{\text{lake}}$ of the representative shallow bay (Missisquoi Bay) had no significant correlation with either $\text{TN}:\text{TP}_{\text{river}}$ or $\text{DN}:\text{DP}_{\text{river}}$ from its tributary. Annual $\text{TN}:\text{TP}_{\text{lake}}$ of the deep Main Lake was significantly correlated with annual $\text{DN}:\text{DP}_{\text{river}}$ of all tributary inputs combined ($p = 0.00733$, $R^2 = 0.322$), but not correlated with $\text{TN}:\text{TP}_{\text{river}}$ of aggregate annual tributary loads.
Annual loads of DN\textsubscript{river} to the deep bay were not correlated with TN\textsubscript{lake}; however, average annual concentrations of DN\textsubscript{river} were highly significant predictors of TN\textsubscript{lake} in the deep bay ($p = 0.00015$, $R^2 = 0.51$) and TN\textsubscript{lake} was distributed close to the 1:1 line (Fig. 5.6). Concentrations of DP\textsubscript{river} of tributary inputs were also highly significant predictors of TP\textsubscript{lake} concentrations in the deep bay ($p = 0.0023$, $R^2 = 0.39$), and the regression line was very close to the 1:1 line (Fig. 5.6). The concentration of DN\textsubscript{river} was not a significant predictor of TN\textsubscript{lake} in the shallow bay ($p = 0.95$, $R^2 = 0$). The concentration of DP\textsubscript{river} was a marginally significant predictor of TP\textsubscript{lake} ($p = 0.045$, $R^2 = 0.15$) in the shallow bay. However, the average annual concentrations of TP\textsubscript{lake} were roughly twice the average annual concentrations of DP\textsubscript{river} (Fig. 5.6).

![Figure 5.6: Comparison of tributary and lake nutrient ratios and concentrations in selected deep (Malletts) and shallow (Missisquoi) bays, each with a major tributary input. Points represent annual averages of lake](image-url)
and tributary nutrients for 1992-2012. Regression lines (dotted and dashed) are only shown for significant regressions. Solid red line represents 1:1.

Meteorological drivers

Aggregate annual river discharge from 1992-2014 from the 9 largest tributaries to the lake increased over the monitoring period, and average discharge increased for each month of the growing season (May-October) (Fig. 5.2, 5.3). Increases in log_{10} discharge were largest in June and July (Fig. 5.3). Air temperature increased over the monitoring period (Fig. 5.2), with the highest increases in April and May (Fig. 3). Wind speed declined significantly over the monitoring period (Fig. 5.2), and in each month with the exception of April, with the greatest decreases in September and October (Fig. 5.3).

Multivariate models of both nutrient and meteorological drivers revealed that different meteorological variables controlled lake nutrients in the deep and shallow bay and in different seasons (Table 5.2). In the deep site, no significant model to predict TN_{lake} was identified for spring, likely as a result of highly variable nutrient concentrations during the snowmelt period; however TN_{lake} in the deep site was strongly influenced by TN_{river} concentrations during summer and fall, and on an annual basis. In addition to the influence of external loading, high temperatures during the summer and high wind speeds during the fall were associated with low TN_{lake}. In the shallow site, only the spring TN_{lake} had a significant model and this model had a relatively low R^2 (0.33). The spring TN_{lake} model had a negative coefficient for TN_{river} concentrations, and a positive coefficient for spring discharge.
TP\textsubscript{lake} in the deep site was most strongly influenced by discharge, DP\textsubscript{river} concentrations, and wind speed (Table 5.2). In spring and on an annual basis, discharge was the most important predictor of TP\textsubscript{lake}, with years of high discharge associated with high TP\textsubscript{lake}. Temperature was also a predictor for spring and annual TP\textsubscript{lake}, although the slope was not significant. In summer and fall in the deep bay, TP\textsubscript{lake} was positively influenced by DP\textsubscript{river} concentrations and negatively influenced by wind speed. TP\textsubscript{lake} in the shallow bay only had significant models for summer and on an annual basis. For the summer and annual models, TP\textsubscript{lake} was strongly positively influenced by DP\textsubscript{river} concentrations. In the summer, TP\textsubscript{lake} was also very strongly negatively influenced by wind speed, and was higher in years with lower discharge. Although DP\textsubscript{river} was frequently an important predictor for TP\textsubscript{lake}, in no model for either shallow or deep sites was there a significant coefficient for TP\textsubscript{river} concentrations, and in those sites where TP\textsubscript{river} was selected in the best model, the coefficients were always negative.

Table 5.2: Best models explaining variation in TN\textsubscript{lake} (top) and TP\textsubscript{lake} (bottom) in different seasons as a function of both external inputs and atmospheric variables for a representative deep bay (Malletts Bay) and shallow bay (Missisquoi Bay). Initial models for TN\textsubscript{lake} and TP\textsubscript{lake} were: $TN\textsubscript{lake} = f(TN\textsubscript{river} + \text{AirTemperature} + \text{WindSpeed} + \text{Discharge})$, and $TP\textsubscript{lake} = f(TP\textsubscript{river} + DP\textsubscript{river} + \text{AirTemperature} + \text{WindSpeed} + \text{Discharge})$. Models with the lowest AIC were chosen using stepwise multiple regression model selection (forward and backward). “No model” indicates that the best model selected excluded all variables. Significant coefficients are shown in bold ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>TN\textsubscript{lake} (mg/L)</th>
<th>Best Model</th>
<th>$R^2$ (adjusted)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Bay (annual)</td>
<td>1.201 $TN\textsubscript{river} - 0.01136\text{AirTemp} - 0.03591$</td>
<td>0.5403</td>
<td>0.00036</td>
</tr>
</tbody>
</table>
### Bottom water temperature and DO (deep sites)

Maximum bottom water temperature in deep, stratified sites increased from 2006-2013 by 2-4°C, and minimum bottom water DO decreased by roughly 3 mg L\(^{-1}\) over the same period (Supplemental Fig. 5.5). These patterns were similar within each of the deep basins, and were statistically significant at a majority of sites. In all cases, the highest bottom water temperatures and lowest bottom water DO were observed in late summer (early September), before the breakdown of thermal stratification. Frequent bottom water hypoxia was observed in Malletts Bay and the Northeast Arm, two separate basins largely isolated from the main body of Lake Champlain. Although the slopes were negative,
decreasing trends in bottom water DO were not significant in these basins as a result of the high intra-annual variability.

**Phytoplankton data**

Cell count data show an increase in the peak abundance of the N-fixing cyanobacterial genus *Anabaena* in Lake Champlain from 2006-2012 when data were available (p < 0.0001) (Supplemental Fig. 6). The average change over the 7-year period was roughly 1 order of magnitude lake-wide. The N-fixing *Aphanizomenon* did not increase significantly (p = 0.17), but the non N-fixing cyanobacteria *Microcystis* did increase (p = 0.027).

**Discussion**

Our results suggest that Lake Champlain has experienced relatively dramatic changes in the magnitude and seasonality of energy (air temperature, wind speeds) and mass inputs (discharge, N, and P) over recent decades, and that these changes have resulted in systematic changes in lake nutrient concentrations and ratios which are mediated by watershed processes and lake depth. Within-year trends in nutrient concentrations indicate that deep and shallow areas of the lake respond differently to changes in mass and energy inputs, but in both cases changes in these drivers have resulted in increased TP\text{lake}, decreased TN\text{lake}, and decreased TN:TP\text{lake} (Fig. 5.3, 5.4). Together, external nutrient loads and climate trends can explain much of the variation in TN\text{lake} and TP\text{lake} concentrations observed throughout Lake Champlain over the monitoring period (Table 5.2). Here we present a conceptual model (Fig. 5.7) that
synthesizes these observations and propose mechanisms by which $\text{TN}_{\text{lake}}$ and $\text{TP}_{\text{lake}}$ are likely to respond to ongoing climate change in deep and shallow portions of the lake and implications for pollution mitigation opportunities under climate change. Furthermore, considering the diverse range of hydrodynamic, biogeochemical and ecological environments that span the 15 sites of the Lake Champlain monitoring network (Xu et al. 2015a; b), the developed conceptual framework is likely broadly applicable to a wide array of lakes experiencing similar climate driven changes in energy and mass fluxes.

![Conceptual model of external influences on TN:TP in shallow and deep lake sites](image)

Figure 5.7: Conceptual model of external influences on TN:TP in shallow and deep lake sites (top) showing intra-annual drivers of nutrient variability (middle) and possible responses to changing climate (bottom). A: Response of shallow sites to dissolved and particulate nutrient inputs. B: Response of deep sites to dissolved and particulate nutrient inputs. C: Intra-annual patterns of TN:TP in shallow lakes related to
tributary inputs and temperature. D: Seasonal depletion of hypolimnetic oxygen in deep sites leading to late-season sediment P loading. E: Potential effects of climate change on TN:TP in shallow sites. F: Potential effect of climate change on deep sites. See text for a more detailed description of model.

Deep sites

The seasonally consistent concentrations of $\text{TN}_{\text{lake}}$, $\text{TP}_{\text{lake}}$ and $\text{TN:TP}_{\text{lake}}$ from May-October in most deep lake sites (Fig. 5.4, Supplemental Fig. 5.2) lead us to conclude that nutrients from tributary inputs, which are primarily delivered in the spring, are efficiently recycled within the epilimnion throughout the stratified period of each year. Slight losses of both $\text{TN}_{\text{lake}}$ and $\text{TP}_{\text{lake}}$ were observed over the growing season at most sites (Supplemental Figs. 5.2, 5.3) (Fig. 5.7, panel B), which are most likely the result of sedimentation of algal detritus as phytoplankton productivity increases during the growing season (Finlay 2013). The one deep site that had strong intra-annual variability in lake nutrient concentrations was the Northeast Arm site. In the Northeast Arm, which frequently experiences bottom water hypoxia (Supplemental Fig. 5.5), $\text{TP}_{\text{lake}}$ increased and $\text{TN:TP}_{\text{lake}}$ decreased in late summer (Fig. 4, Supplemental Fig. 5.2), consistent with what would be expected from sediment P release late in the season when DO is lowest (Nürnberg 1984; Foley et al. 2011). In general, however, although internal loading may modify late-season $\text{TN:TP}_{\text{lake}}$ in some deep sites, the dominant seasonal trends in $\text{TN}_{\text{lake}}$ and $\text{TP}_{\text{lake}}$ of deep lake sites were driven by early season mass inputs from tributaries, and subsequent tight cycling within the epilimnion.

The very strong correlations between $\text{DN:DP}_{\text{river}}$ and $\text{TN:TP}_{\text{lake}}$ in the representative deep bay (Fig. 5.6) and in the Main Lake lead us to conclude that the most
important drivers of long-term declines in $TN_{\text{lake}}$ and increases in $TP_{\text{lake}}$ in deep sites were changes in watershed inputs of dissolved nutrients. Furthermore, the average annual concentrations, rather than the loads, of dissolved nutrients in tributary inflows were the primary determinants of $TN_{\text{lake}}$, $TP_{\text{lake}}$, and $TN:TP_{\text{lake}}$ (Fig. 5.6, Table 5.2). Because P is disproportionately delivered in this particulate fraction, while most N is delivered in the dissolved fraction (Supplemental Fig. 5.4), $DN:DP_{\text{river}}$ is much higher than $TN:TP_{\text{river}}$. This contributes to the higher $TN:TP_{\text{lake}}$ in deep sites relative to shallow sites where sediment nutrients are potentially available to surface water column (Figure 5.7, A and B).

External loads of $TN_{\text{river}}$ and $DP_{\text{river}}$ were level or increased over time as a result of increasing precipitation and discharge (Medalie 2013), but $TN_{\text{river}}$ loads became more dilute, resulting in lower $TN_{\text{river}}$ concentrations and ultimately lower $TN_{\text{lake}}$. Rivers draining forested catchments had substantial declines in $TN_{\text{river}}$ concentrations (Medalie 2013), which is likely due in part to decreases in atmospheric acid and N deposition in the northeastern United States (Canham et al. 2012). In contrast to the declines in $TN_{\text{river}}$, $DP_{\text{river}}$ loads became more concentrated in more developed catchments, likely as a result of changes in agricultural practices (Ghebremichael et al. 2010), resulting in increases in $TP_{\text{lake}}$. These increases in $TP_{\text{lake}}$, in combination with increased epilimnetic temperatures, likely contributed to the declines in $TN_{\text{lake}}$ by stimulating increased phytoplankton growth (Smeltzer et al. 2012), resulting in increased N removal through sedimentation (Finlay et al. 2013). This may account for the relatively large declines in $TN_{\text{lake}}$ during the peak summer months of July and August in deep sites (Fig. 5.3). This increased productivity
may have also contributed to increases in late-summer sediment P release driven by declining bottom water oxygen concentrations (Supplemental Fig. 5.5), resulting in the relatively high increases in mean TP$_{\text{lake}}$ of all deep sites in early fall (Fig. 5.3). Increases in temperature and decreases in average wind speed in late summer and autumn (Fig. 5.3) may also have resulted in longer duration of stratification and subsequent increases in internal P loading. Data to quantify duration of stratification are not available in this long-term monitoring dataset; increased duration of stratification has, however, been widely reported for north temperate lakes and is expected to continue in the future (Magnuson et al. 1997; Jankowski et al. 2006; Stainsby et al. 2011; Foley et al. 2011). In general, the synthesis of seasonal and long-term nutrient dynamics in the deep portions of the lake clearly demonstrate that changes in nutrient concentrations and ratios over time have been driven primarily by changes to mass inputs of dissolved nutrients to the lake, but have likely been compounded by changes in climatic drivers.

Shallow sites

In stark contrast to deep sites, TN:TP$_{\text{lake}}$ had a remarkably consistent pattern of intra-annual variability in all shallow sites, and this pattern supports the conclusion that internal nutrient transformations were critical in determining lake nutrient concentrations (Fig. 5.4). Similar to deeper sites, TN:TP$_{\text{lake}}$ of shallow lake sites was close to DN:DP$_{\text{river}}$ in spring (Figs. 5.4, 5.5) when tributary inputs were high and the water was cool, confirming that spring runoff mass inputs drive early season nutrient concentrations and ratios. However, during the summer, when river inputs were generally low, TP$_{\text{lake}}$ dramatically increased in all shallow sites as a result of sediment P loading
(Supplemental Fig 5.2). Internal P loading is well-documented in shallow lakes, where warm sediment temperatures result in high sediment oxygen demand and sediment P release (Søndergaard et al. 1999). Water column concentrations also respond more steeply to a given areal flux of sediment P in shallow sites than in deep sites because of low ratios of lake volume to sediment surface area, giving rise to the strong correlation of seasonal rate of TP increase with lake depth (Supplemental Fig. 5.3). At the same time as P increases during early summer, N is gradually removed in shallow sites during the dry summer months when river discharge is low (Supplemental Fig. 5.2), most likely by denitrification (Hayes et al. 2015). Denitrification rates are often controlled by nitrate concentrations in shallow lakes (Kolzau et al. 2014; McCarthy et al. 2011), which may help to explain the rapid seasonal declines of $\text{TN}_{\text{lake}}$ at Missisquoi Bay relative to other sites (Supplemental Fig. 5.2). Missisquoi Bay had the highest spring $\text{TN}_{\text{lake}}$ concentrations in Lake Champlain, and denitrification rates have been found to exceed the ability of the system to compensate through N fixation or sediment ammonium fluxes to the water column (McCarthy et al. 2011). In contrast to Missisquoi Bay, St. Albans Bay has relatively minor tributary inputs and, along with the adjacent Northeast Arm (which also lacks major river inputs), has the lowest spring $\text{TN}_{\text{lake}}$ of the monitoring sites (Supplemental Fig. 5.3). St. Albans Bay and Northeast Arm were the only monitoring sites where $\text{TN}_{\text{lake}}$ increased over the monitoring season, which may indicate increased importance of N fixation at these relatively N-poor sites. St. Albans Bay experiences severe cyanobacteria blooms which are usually dominated by N fixing *Anabaena* during the summer months, supporting our interpretation of the observed seasonal TN trends at
this site (Angela Shambaugh, Vermont Department of Environmental Conservation, personal communication). Taken together, the declining TN$_{lake}$ during late spring and summer coupled with the increases in TP$_{lake}$ over the same period result in the broadly consistent systematic intra-annual progression observed in TN:TP$_{lake}$ in the shallow sites (Fig. 5.4) similar to seasonal patterns observed in other shallow lakes in recent studies (Kolzau et al. 2014; Grantz et al. 2014; Hayes et al. 2015).

The strong influence of internal P loading and denitrification on TN:TP$_{lake}$ evidenced by seasonal changes in nutrient concentrations suggests that long-term declines in TN:TP$_{lake}$ in shallow sites have been strongly impacted by meteorological drivers that facilitate internal nutrient transformations (Fig. 5.3, Table 5.2), although shallow sites are clearly impacted by riverine dissolved nutrient inputs as well, particularly in spring (Fig. 5.6, Table 5.2). Of particular importance in the shallow bay investigated in detail here is the strong relationship between low summer wind speed and high summer TP$_{lake}$ (Table 5.2); this is consistent with recent studies using high-frequency data in this bay (Smith et al. 2011; Isles et al. 2015; Giles et al. 2016) and elsewhere (Kleeberg and Dudel 1997; Huber et al. 2008; Burger et al. 2008) which have revealed that low summer wind speeds facilitate the development of transient thermal stratification, bottom water oxygen depletion, and internal nutrient loading during summer months. The long-term increases in temperature and decreases in wind speed observed during the monitoring period, particularly in September and October (Fig. 5.3), likely resulted in increased late season internal P loading, as evidenced by the dramatic increases in TP$_{lake}$ during these months in shallow sites (Fig. 5.3). In general, our analysis of the long-term trends in lake
nutrients and physical drivers, when coupled with previously discussed analyses of seasonal trends, suggests that changing energy fluxes that facilitate (or suppress) internal nutrient loading have had the greatest impact on shallow lake system nutrient dynamics over the monitoring period.

Climate impacts on TN, TP, and TN:TP

Our integrated analysis of seasonal and long-term nutrient dynamics allow us to develop a conceptual model of the processes controlling TN:TP_{lake} in deep and shallow sites, and this model suggests ways in which TN:TP_{lake} is likely to respond to future climate changes (Fig. 5.7). We preface our conceptual model with the caveat that it does not address several important factors that may impact TN_{lake} and TP_{lake}, including changes in ice cover (Schroth et al. 2015), lagged effects of off-season climate drivers on summer production (Pierson 2009), invasive species introductions (Smeltzer et al. 2012), and impacts on trophic cascades (Carvalho and Kirika 2003; Winder and Schindler 2004), which certainly warrant further study. However, the strong and spatially consistent trends that we have discussed and the high amount of variance explained by external loads and climate drivers (Table 5.2) suggest that our model accounts for much of the observed variation in TN:TP_{lake} in Lake Champlain, and is useful for understanding and projecting the impacts of climate change on the diverse environments of Lake Champlain as well as similar systems elsewhere.

In deep lake sites, future changes are likely to be influenced primarily by changes in the dissolved nutrient concentrations of tributary inputs (Fig. 5.6), and secondarily by increases in bottom water temperature, decreases in hypolimnetic DO, and subsequent
increases in sediment P release. Dissolved nutrient concentrations are likely to change in the future due to changes in precipitation regimes (Guilbert et al. 2014), phenology of catchment processes (Bernal et al. 2012), and anthropogenic activities (Michalak et al. 2013). Furthermore, future climate warming may lead to longer stratified periods and more frequent incidence of hypolimnetic anoxia, resulting in increased sediment P release and declines in TN:TP\text{lake} late in the summer in deep sites (Fig. 5.7F), particularly if concentrated external dissolved nutrient loads coupled with increased water temperatures continue to support increasing epilimnetic production throughout the early summer (Foley et al. 2011).

While long-term trajectories of lake nutrients in shallow sites are likely to be driven by external nutrient loads (Søndergaard et al. 2003; Jeppesen et al. 2007), our results suggest that intra-annual variability in shallow sites is driven by meteorological and hydrodynamic conditions which promote sediment P release. Changes in these climate drivers (energy inputs) are likely to disproportionately impact nutrient dynamics in shallow sites. In future warming scenarios, when spring discharge and N loading peak earlier due to earlier snowmelt, TN\text{lake} in shallow sites is likely to peak earlier in the season (Grantz et al. 2014; Guilbert et al. 2014). At the same time, earlier onset of warm temperatures is likely to result in earlier onset of internal P loading, resulting in increased TP\text{lake} (Nürnberg and LaZerte 2015). When taken together, earlier declines in TN\text{lake} and earlier increases in TP\text{lake} are likely to result in earlier declines in TN:TP\text{lake} and lower annual average TN:TP\text{lake} with future climate warming (Fig. 5.7, panel E). Declines in TN:TP\text{lake} in shallow sites over the monitoring period have been strongest in the spring.
and fall (Fig. 5.3), which may reflect a lengthening summer bloom season over the past two decades, suggesting that these processes are already occurring.

The long-term declines in TN:TP lake wide, in combination with increases in water temperature, have likely contributed to increases in cyanobacteria throughout Lake Champlain over the monitoring period, and particularly the increases in N-fixing cyanobacteria (Smith 1983; Butterwick et al. 2005; Paerl and Huisman 2008) Supplemental Fig. 5.6). In our conceptual model (Fig. 5.7), all of the observed climatic trends during the monitoring period (increased temperature, decreased wind speeds, increased discharge; Fig. 5.2) are consistent with the increased development of N-limiting conditions. Climate projections suggest that these trends are likely to continue (Guilbert et al. 2014), and that further increases in the dominance of N-fixing cyanobacteria will occur. While the trends of declining N:P do not match those of some large lakes where point-source pollution controls resulted in large declines in P loading over the historical period (Finlay et al. 2013), they are similar to recent trends in large lakes heavily influenced by non-point-source agricultural pollution (Schindler et al. 2012; Scavia et al. 2014; Nürnberg and LaZerte 2015), where synergistic effects of climate warming and diffuse nutrient loading have resulted in increases of both external and internal loading.

Several useful insights for lake managers dealing with eutrophication and climate change emerge from this analysis. For deep systems, management actions should emphasize controlling dissolved nutrient inputs, and particularly dissolved P. The importance of tributary dissolved nutrient concentrations (rather than loads) for deep lake
sites suggests that management actions may be able to achieve targets for lake water quality despite the increase in nutrient loads expected with projected increases in precipitation (Jeppesen et al. 2009; 2010). Control of external dissolved nutrients is particularly important given the potential for increased late-season anoxia and sediment P release in deep basins; reductions in eplimnetic nutrient concentrations early in the season will reduce production in surface waters, thereby reducing sedimentation of algal carbon to the hypolimnion to support respiration and oxygen depletion (Foley et al. 2011). In shallow basins where particulate P is available to the water column through internal loading, lake recovery is likely to be difficult, and managers should target particulate as well as dissolved nutrient fractions. Because the majority of P is delivered in particulate form, efforts to control erosion and sediment transport should be emphasized in catchments feeding shallow lakes, as well as efforts to control dissolved nutrient loads. This is particularly important because climate change is predicted to continue to increase the incidence of the strongest storm events in many areas (Guilbert et al. 2014), leading to corresponding increases in particulate phase nutrient transport that we demonstrate disproportionately impact shallow systems.

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Supplemental Figure 5.1: Long-term trends in TN, TP, and TN:TP (molar ratio) at 15 monitoring sites in Lake Champlain separated by lake basin, showing segmented regression lines or least square regression lines. Breakpoints (black bold symbols) are shown for sites with significant differences in slope across the series. For sites with no significant difference in slope, least squares regression lines are shown and large red symbols mark the range of the monitoring period for each site. Segmented regression was only used on sites with data available for the full monitoring period (1992-2014).
Supplemental Figure 5.2: Intra-annual trends in TN, TP, and TN:TP (molar ratio) at 15 monitoring sites in Lake Champlain separated by lake basin, showing segmented regression lines or least square regression lines. Breakpoints (black bold symbols) are shown for sites with significant differences in slope across the
series. For sites with no significant difference in slope, least squares regression lines are shown and large red symbols mark the range of the monitoring period for each site.

Supplemental Figure 5.3: Slopes of $T_{N_{lake}}$ and $T_{P_{lake}}$ vs. Julian date over the main growing season for each of the 15 monitoring sites. Slopes were identified using segmented regression (see supplemental figure 5.2 for illustration of method).
Supplemental Figure 5.4: Total Nitrogen (TN) vs. total dissolved nitrogen (DN) for the Winooski and Missisquoi Rivers (right), showing sampling dates superimposed on the river hydrographs (left). Red filled circles on graphs show location of points with low TDN/TN during the rising limb of the hydrograph in discrete storm events.
Supplemental Figure 5.5: Bottom water temperature (°C, left) and dissolved oxygen (mg L⁻¹, right) at the six deepest, most consistently stratified monitoring sites separated for the three deep basins of the lake from 2006-2013. Red lines show quantile regression lines for the upper 80th percentile (temperature) and the lower 20th percentile (dissolved oxygen) for each site. Significant trends are marked with (*).
Supplemental Figure 5.6: Increases in the peak abundance of the cyanobacteria genera Anabaena, Aphanizomenon, and Microcystis for all stations in Lake Champlain, 2006-2012. Cell counts are log_{10} transformed before analysis. Red line shows quantile regression for the 90th percentile (tau = 0.9). p-values are shown for the quantile regression fits.
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Xu, Y., A. W. Schroth, P. D. F. Isles, and D. M. Rizzo. 2015b. Quantile regression


APPENDIX A: DESIGN AND CALIBRATION OF AN INTEGRATED MODELING FRAMEWORK FOR A SHALLOW EUTROPHIC BAY OF LAKE CHAMPLAIN USING LONG-TERM AND HIGH-FREQUENCY DATA

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ABSTRACT:

Changing climate may impact lake ecosystems through changes to watershed nutrient inputs, residence time, internal nutrient cycling, phytoplankton physiology, ecosystem respiration, and other mechanisms. Deterministic numerical models simulating lake and watershed processes are powerful tools for integrating the effects of multiple changes, and can be useful for predicting the effects of climate change on lake processes when sufficient data are available to validate model performance. In this study, we present the integration of a 3-D coupled hydrodynamic, water quality, and sediment diagenesis model of Missisquoi Bay, Lake Champlain, into an integrated assessment model simulating the impacts of climate change on watershed hydrology and lake water quality. The lake model was calibrated against 23 years of bi-weekly monitoring data, and against 2 years of high-temporal resolution sensor data and comprehensive field sampling data. The model accurately reproduced both the magnitude and inter-annual variability of temperature, circulation patterns, cyanobacteria bloom magnitude and timing, chlorophyll-a concentrations, and total phosphorus during the calibration period at sub-annual timescales, but did not fully represent nitrogen dynamics within the bay. Analysis of model results suggests that temperature is critical in driving inter-annual variability in cyanobacteria growth rates, and that spatial variability in bloom severity is likely impacted by dissolved nutrient concentrations in different tributaries and hydrodynamic circulation patterns. Projections of future changes in lake temperatures from the hydrodynamic model suggest that the lake will become substantially warmer by
mid-century, particularly in late spring and early autumn, resulting in longer potential bloom seasons.
Introduction

Climate change is expected to increase average annual temperatures in the Lake Champlain basin by roughly 3°C by mid century (Guilbert et al., 2014). Precipitation is expected to increase as well, particularly the incidence of the strongest storm events (Guilbert et al., 2015). These changes are already underway (Isles et al. *Chapter 5 of this dissertation*), and are likely to have profound impacts on lake thermal structure (De Stasio et al., 1996), and biological and chemical aspects of lake systems (Adrian et al., 2009; Magnuson et al., 1997; Vogt et al., 2011). A variety of possible impacts of climate change on lake water quality are expected, many of which may result in increased dominance by cyanobacteria and increased likelihood of harmful algal blooms (HABs) (Carey et al., 2012; Paerl and Huisman, 2008; Wagner and Adrian, 2009). The responses of specific lakes to climate change, however, may vary widely as a function of catchment characteristics, lake morphology, trophic state, and local variations in climate (Havens et al., 2001; Huber et al., 2008; Kosten et al., 2009; Read et al., 2014; Wilhelm and Adrian, 2008; Isles et al., *Chapter 4 of this dissertation*). This may be particularly true in shallow lakes, where threshold effects are particularly important in determining lake responses to both nutrient inputs and climate (Jöhnk et al., 2008; Scheffer and Nes, 2007; Wilhelm and Adrian, 2008). To account for the site-specific impacts of multiple controlling factors, process-based models calibrated using robust datasets may provide reasonable projections of future ecological states and allow researchers to test the likely effects of mitigation strategies (Couture et al., 2014; Hipsey et al., 2015; Jeppesen et al., 2009).
A variety of mechanistic model frameworks have been applied to study the effects of climate change on shallow lakes (Burger et al., 2008; Havens et al., 2001; Hipsey et al., 2015; Mooij et al., 2010). The choice of model framework depends on many factors, including the size and complexity of the lake being modeled, the availability of data for calibration and validation, and the study objectives. Shallow lake models frequently highlight ecological interactions between phytoplankton, zooplankton, and macrophytes, which are important for stabilizing alternate stable states in shallow lakes (Mooij et al., 2009; Scheffer et al., 1993); however, these processes may be relatively less important in large shallow lakes, where wind-driven turbulence often prevents the establishment of macrophyte beds (Janssen et al., 2014). Furthermore, data on macrophytes and upper trophic levels to calibrate models are frequently lacking in lake monitoring programs. In larger shallow lakes, spatial and temporal variability in sediment nutrient fluxes of P and N are critical for predicting ecosystem response (Havens et al., 2001; James et al., 2011; Trolle et al., 2009), particularly over long timescales when sediment flux rates are likely to respond to long-term loading rates. Shallow lakes with large catchments may also be highly sensitive to changes in flushing rates due to high ratios of watershed area to lake volume (Jones et al., 2011). Accurate representation of hydrologic inputs may be particularly important in these sites.

Missisquoi Bay is a large, shallow, eutrophic freshwater bay of Lake Champlain with a large catchment, and has been the site of considerable research due to the regular occurrence of severe cyanobacteria blooms in recent decades (Isles et al., 2015; Levine et al., 2012; Smeltzer et al., 2012). The importance of internal nutrient loading has been
well-documented in Missisquoi Bay (Giles et al., 2016; Isles et al., 2015; Limnotech, 2012; Schroth et al., 2015; Smith et al., 2011) and other shallow lakes (Hupfer and Lewandowski, 2008; Søndergaard et al., 1999). Previous research in the bay has demonstrated that periods of transient thermal stability drive bottom water redox conditions and sediment nutrient release (Courtney D Giles et al., 2016; Smith et al., 2011). It has also been demonstrated that multiple resources (N, P, and light) control phytoplankton dynamics across years and seasons (Isles et al., 2015), Isles et al., *Chapter 5 of this dissertation*). As a result of deteriorating water quality throughout Lake Champlain, and in Missisquoi Bay in particular, policymakers have recently developed new targets for reducing phosphorus loads to the bay (VT Act 64; http://legislature.vermont.gov/bill/status/2016/H.35). The load reduction targets consider the potential impacts of climate change on watershed nutrient loading, but the potential response of in-lake processes to changing climate has not been assessed in detail.

In this study, we present the calibration and integration of a 3-D coupled hydrodynamic and lake model into an integrated assessment model (IAM) of the lake-watershed system, which can be used to predict the response of temperature, total phosphorus and phytoplankton blooms in Missisquoi Bay, Lake Champlain to future changes in climate and nutrient loading. The modeling framework chosen for this study consisted of a 3-D hydrodynamic model (Environmental Fluid Dynamics Code, EFDC; Hamrick 1997), and a water quality model containing an integrated sediment diagenesis sub-model (Row Column AESOP, RCA; Fitzpatrick 2004) capable of tracking changes in sediment nutrient pools (Di Toro, 2001). The coupled EFDC-RCA model was calibrated
using 23 years of monitoring data at two long-term monitoring sites within the bay, and two years of comprehensive high-frequency biological, chemical, and hydrodynamic data collected as part of this study. Our goals were (1) to assess the accuracy of the model representation of lake physical, chemical, and biological processes, (2) to gain mechanistic insights into spatial processes driving bloom development in Missisquoi Bay, (3) to present the integration of the coupled hydrodynamic-water quality model into a larger IAM framework, and (4) to present results of the hydrodynamic model projections for Missisquoi Bay through mid-century.

Methods

Site Description

Missisquoi Bay is a large (77.5 km²) eutrophic bay at the northeastern end of Lake Champlain. Missisquoi Bay is shallow (max depth 4 m, mean depth 2.8 m at mean low water) and experiences annual lake level fluctuations of approximately 1.6 m. Missisquoi Bay is largely isolated from the main body of the lake, the only outlet being a narrow opening in a causeway (100 m) in the southwestern end of the bay. Missisquoi Bay has a large ratio of watershed area to lake area (40:1), and a much larger ratio of catchment area to lake volume than Lake Champlain as a whole (Levine et al., 2012), resulting in net flow of water out of the bay. The catchment feeding the bay is drained by three major rivers: the Missisquoi River, Pike River, and Rock River, which contribute 79%, 18%, and 3% of discharge to the bay, respectively. The watershed is comprised mostly of forested (62%) and agricultural (25%) land uses. Missisquoi Bay has
experienced increasingly severe cyanobacteria blooms over the past two decades driven by increasing loads of N and P, increasing temperature, decreasing wind speed, and falling N:P ratios (Isles et al., 2015; Smeltzer et al., 2012; Isles et al., Chapter 5 of this dissertation).

Field Data Collection

Comprehensive hydrodynamic and biogeochemical data were collected from 2012-2014 and used to calibrate and validate model outputs. At the main biogeochemical monitoring station, temperature was recorded hourly at 0.5 m increments using a YSI 6600 series sonde (Yellow Springs Instruments, OH) on an automated profiling buoy taking concurrent measurements of biogeochemical parameters (see below). Additional water temperature data were measured every 15 min at 1 m intervals at several stations around the bay using strings of HOBO temperature loggers (locations of temperature loggers varied from year to year). In addition to temperature sensors, a network of Acoustic Doppler Current Profilers (ADCPs) and water level sensors were deployed around the bay to measure water circulation patterns (Manley et al., in review).

Biogeochemical data were collected at a high-frequency monitoring site located in the southeastern quadrant of the bay (Fig. A.1). The YSI profiling buoy collected hourly measurements of chlorophyll-a fluorescence, phycocyanin fluorescence, turbidity, pH, dissolved oxygen (DO), and conductivity at hourly intervals from June-October (exact dates varied among years). In addition to sensor measurements, water samples for total nitrogen (TN) and total phosphorus (TP) analysis were collected at 8-hour intervals using Isco automated water samplers (Teledyne Isco, Lincoln NE). Isco bottles were pre-
acidified using concentrated sulfuric acid to a final sample pH of 2. Weekly field sampling and maintenance trips were also conducted throughout the monitoring season, and samples were taken for soluble reactive P (SRP), NO$_3^-$, NH$_4^+$, TN, TP, and phytoplankton community composition. More detailed descriptions of field sampling and analysis methods can be found in Isles et al. (2015) and Giles et al. (2016).

Meteorological data were collected at the profiling buoy, which collected ½ hourly measurements of wind speed, wind direction, air temperature, relative humidity, atmospheric pressure, and shortwave solar radiation during the monitoring season. A second meteorological station on a hydrodynamic monitoring buoy moored in the bay provided redundancy for measurements of air temperature, wind speed, and RH; data from this site was used to fill gaps in data from the profiling buoy to drive EFDC during the high-frequency calibration period.

Long-term monitoring data from 1992-2014 for temperature, TP, and Chl-a were acquired from the Lake Champlain Long-Term Monitoring Program (http://www.watershedmanagement.vt.gov/lakes/htm/lp_longterm.htm) for the two monitoring sites within Missisquoi Bay (Stations 50 and 51).

Lake Hydrodynamic Model

The Environmental Fluid Dynamics Code (EFDC; Hamrick, 1994) is a widely used hydrodynamic model maintained by the U.S. Environmental Protection Agency (https://www.epa.gov/exposure-assessment-models/environment-fluid-dynamics-code-efdc-download-page). EFDC uses a finite volume solution scheme for hydrostatic primitive equations on a staggered grid. The Missisquoi Bay Model consisted of 420
horizontal cells, with up to 5 vertical layers at each horizontal location (Fig. A.1). There
are 3 open boundary cells to the main lake, and 5 tributary input cells (three for the
Missisquoi River, 1 for the Pike River, 1 for the Rock River). Meteorological boundary
conditions for the long-term monitoring period (1992-2012) were acquired from the
North American Regional Reanalysis dataset (NARR; https://www.ncdc.noaa.gov/data-
access/model-data/model-datasets/north-american-regional-reanalysis-narr). NARR wind
speed and solar radiation data were corrected to match the range of in-situ data using
regressions against data from the monitoring buoy. For the high-frequency calibration
period in 2012-2013, data from the monitoring buoy were used when data were available,
and NARR data were used for the late fall through early spring.

Figure A.1: Map of Missisquoi Bay showing lake model grid.

Due to the large fluctuations of lake level in Lake Champlain, lake surface
elevation at the opening to the main lake was an important boundary condition for the
hydrodynamic model. For the calibration period, this boundary elevation was taken from
the lake level gage at Burlington, VT (USGS gage 04294500). Lake temperatures were
calibrated against long-term water temperature measurements at the two long-term
monitoring sites from 1992-2012 (Station 50) and 2006-2012 (Station 51) for the long-
term calibration period. During the high-frequency calibration period, hourly EFDC
outputs were compared to high-frequency, vertically resolved temperature measurements
at several sites in the bay.

Lake Water Quality Model

Row Column AESOP (RCA; Fitzpatrick 2004) is a water quality modelling code
that has been applied in a number of studies in lakes, rivers, and estuaries to support
management decision making (Hu and S. Li, 2009; M. Li et al., 2016; Limnotech, 2012;
Lung and Larson, 1995; Verhamme et al., In review). Four phytoplankton groups were
represented, approximating spring diatoms, summer eukayotes, non N-fixing
cyanobacteria, and N-fixing cyanobacteria. RCA does not simulate N fixation explicitly;
however, an N-fixing class was parameterized with a low half-saturation constant for N
and variable C:N stoichiometry allowing biomass accumulation at high C:N when N was
limiting. In addition to phytoplankton dynamics, the model simulates multiple G classes
of particulate and dissolved organic C, N, and P, with different diagenesis rates, as well
as inorganic C, N, and P species and suspended sediment. Comprehensive optimization
techniques could not be used to calibrate RCA due to the intensive computational time
required and large numbers of parameters, so the model was calibrated manually. Most
parameter estimates followed the recommendations in the model documentation
Fitzpatrick, 2004); phytoplankton growth parameters were adjusted in the calibration process using parameter estimates similar to those in previous modeling studies (Burger et al., 2008; Trolle et al., 2011b) and using parameter data aggregated by the Aquatic Ecosystem Modeling Network (AEMON; Trolle et al., 2011a). The most sensitive parameters for the water quality model were maximum growth rates for the phytoplankton groups, temperature optima for the phytoplankton groups, $\frac{1}{2}$ saturation constants for nutrient uptake for the phytoplankton groups, and phytoplankton respiration rates. Full RCA water quality model parameters can be found in supplementary table 2.

RCA has an integrated sediment diagenesis subroutine based on the three G-class model of Di Toro (Di Toro, 2001). The sediment model consists of a 2-layer representation of the sediment, with a variable-depth oxygenated surface layer, the depth of which is driven by modeled sediment oxygen demand. Particulate organic carbon, nitrogen, and phosphorus degrade to $\text{PO}_4^{3-}$, $\text{NH}_4^+$, and $\text{CH}_4^+$ at different temperature-dependent rates for labile (G1), semi-labile (G2), and refractory (G3) classes. Sediment nutrients can be lost to the water column or to burial. Burial rate is controlled by mass settling from the water column. The sediment model simulates partitioning of $\text{PO}_4^{3-}$ between dissolved and particulate phases as a function of sediment oxygen concentrations. Nitrification and denitrification are represented within the aerobic and anaerobic layers using a solution scheme described in Di Toro (2001). The most sensitive parameters of the sediment submodel were sediment solid concentrations in the aerobic and anaerobic layers (M1 and M2), phosphate partitioning coefficients in the aerobic and anaerobic layers ($\pi_{\text{PO}_4}$), minimum porewater diffusion coefficient, critical oxygen
concentration for phosphate partitioning \((O_{2,\text{crit}})\) and diagenesis rates of G2 (partially reactive) organic compounds. These parameters were the focus of much of the calibration process. Full sediment model parameters used in this model can be found in supplementary table 3.

For the high-frequency calibration period, tributary nutrient fluxes were estimated using the R package EGRET (Hirsch and De Cicco, 2013; Hirsch et al., 2010; Medalie, 2013). EGRET uses a regression surface weighted by time, discharge, and season to estimate nutrient concentrations and fluxes based on historical monitoring data and daily discharge measurements. Tributary TP, DP, and TN data for the Missisquoi, Pike, and Rock Rivers were obtained from the Lake Champlain long-term tributary monitoring program (http://www.watershedmanagement.vt.gov/lakes/htm/lp_longterm.htm), along with corresponding river gage data (USGS gage station 4294000 for the Missisquoi River, and Quebec MDDEP gage stations 30424 and 30425 for the Pike and Rock rivers, respectively). Total N, TP, and DP were then partitioned into the organic and inorganic N and P fractions used by the model using constant proportions. For the long-term calibration period, daily load estimates which had been previously estimated using similar methods for 1992-2012 were taken from (Medalie, 2013).

Daily averages of phycocyanin and chlorophyll fluorescence were compared to modeled cyanobacteria populations and chlorophyll-a concentrations, respectively. While cyanobacteria do contain chlorophyll, previous research in Missisquoi Bay has found that chlorophyll fluorescence measured using a YSI sonde responds only weakly to cyanobacteria populations, and that phycocyanin is much more effective at tracking
cyanobacteria biovolume (McQuaid et al., 2011; Zamyadi et al., 2012). Because the fluorescence units were not directly comparable to modeled carbon equivalents, correlation but not RMSE or bias was calculated for these comparisons. Daily averages of TN and TP were compared to daily model outputs for 2012-2014. In addition, TN, TP, and Chl-a data from the long-term monitoring program were compared to model estimates for the years 1992-2012 (station 50) or 2006-2012 (station 51). Coefficients of determination, mean bias, and RMSE were calculated with each of these variables matched to model outputs by date. Sediment nutrient concentrations and flux rates were estimated from a previous field study (Limnotech, 2012), from published values (Levine et al., 2012), and from sediment cores collected bi-weekly during 2013 and 2014 (Giles et al., 2015; Giles et al., 2016).

*Integrated Assessment Model*

An integrated assessment model (IAM) was developed as part of the Research on Adaptation to Climate Change project of Vermont EPSCoR. The modeling framework consisted of downscaled global circulation model (GCM) projections for the Lake Champlain basin representing the range of regional climate projections (Winter et al., 2016); the watershed hydrology model RHESSys (Tague and Band, 2004); the lake hydrology model EFDC; the lake water quality model RCA; and several components programmed to translate inputs and outputs and estimate boundary conditions between models (Fig. A.2). All of these models were tied together within a Pegasus workflow (Deelman et al., 2005), which tracked intermediate outputs, optimized computing
resources, and enabled the efficient execution of many more model scenarios than would be possible without a similar framework (Hamed et al., In Review).

Figure A.2: General design of the Integrated Assessment Model.

**Future Climate Scenarios**

Five Global Climate Models (GCM) were chosen for this study (IPSL-CM5A-MR.1, MIROC-ESM-CHEM.1, MRI-CGCM3.1, NORESM1-m.1) which represented the range of precipitation (high and low) and temperature (high and low) from previous analysis of the downscaled GCM outputs for our region (Guilbert et al., 2014; Guilbert et al. 2016). Because of the mountainous landscape in the Missisquoi River watershed, GCM outputs needed to be downscaled further to accurately reflect temperature and precipitation at high elevations. These data were downscaled in two steps. First, data that
had been intermediately downscaled to 1/8˚ resolution were acquired from the Coupled Model Intercomparison Project (CMIP5) dataset. These intermediately downscaled data were then further refined to 30” resolution using topographic downscaling. Detailed methods and validation of downscaling methods are described in Winters et al. (2016).

To estimate meteorological variables required by EFDC but not included in GCM outputs, a weather generator was developed to resample historical observations. Briefly, for each set of temperature and precipitation observations from the GCM output, the weather generator selected a subset of 20 days from the historical NARR climate dataset which most closely matched observed values and were within 10 Julian days of the GCM observation. A vector of values from a single date corresponding to the full suite of climate variables (wind speed, wind direction, relative humidity, atmospheric pressure, shortwave solar radiation, and cloud cover) was then selected from this subset of data by random sampling.

**Tributary discharge modeling**

Watershed hydrology was simulated for the Missisquoi River basin using the Regional Hydrological-Ecological Simulation System (RHESSys; Tague and Band, 2004), after calibration of streamflow estimates to the USGS Missisquoi River gage station at Swanton, VT (USGS gage station 04294000). RHESSys simulates both surface and sub-surface runoff at the hillslope scale. A detailed description of the RHESSys model for a nearby watershed using similar methods can be found in (Mohammed et al., 2015). Estimates of discharge entering the bay were scaled for the proportion of the watershed captured by the gage station (98%).
Lake model integration

To integrate the lake model into the IAM, several important boundary conditions that were not directly available from the GCM or RHESSys outputs (lake level, river temperature, river nutrient concentrations) had to be estimated and incorporated into lake-model input files dynamically based on available model outputs and historical data.

Because RHESSys only simulated flows for the Missisquoi River, future discharges for the Pike and Rock rivers were estimated as constant proportions of Missisquoi River discharge based on proportional discharges of the three tributaries over the historical period. While this distorted the shape of the hydrograph for these tributaries, total annual inputs should be consistent with proportional loads during the historical period.

A bias-corrected multiple regression model was developed to estimate lake level from air temperature (available in future GCM projections) and Missisquoi River discharge (available from RHESSys) in future climate simulations. The lake level model was trained on daily lake level monitoring data from the USGS gage station in Burlington VT, historical daily discharge data from the USGS gage station at Swanton (USGS gage station 04294000), and daily air temperature data from the Burlington Airport (http://www.wunderground.com/history/airport/KBTV/) from 1992-2012. The lake level model used average discharge over multiple lagged windows (1 day, 7 days, 30 days, 60 days) as well as current air temperature as predictor variables in a multiple regression model. This model was further refined by subtracting predictions of a quadratic regression of the residuals against discharge from the multiple regression model.
predictions to correct for overestimation during periods of low lake level and underestimation during periods of high lake level (Supplemental Fig. A.1).

River temperatures were estimated from projected air temperatures using a logistic regression model (Mosheni et al., 1999):

\[ T_s = \mu + \frac{\alpha - \mu}{1 + e^{\gamma(\beta - T_a)}} \]

Where \( T_s \) is stream temperature, \( T_a \) is the average air temperature for the preceding 2 weeks, \( \mu \) is the lower bound water temperature, \( \alpha \) is the upper bound stream temperature, \( \gamma \) determines the steepness of the transition, and \( \beta \) determines the inflection point. In this study, we used \( \alpha = 29.6, \beta = 13, \gamma = 0.16 \), and \( \mu = 0.1 \).

Nutrient concentrations in tributary inputs for future climate scenarios were estimated using regression models of concentration against discharge fit to the historical monitoring period. Separate models were developed for TP, TN, and the ratio of dissolved P (DP) to TP as functions of discharge. RHESSys streamflow estimates were then used to predict future nutrient concentrations based on these models. Nutrients were then partitioned into the organic and inorganic nutrient classes using constant proportions based on best estimates from observed partitioning in the available monitoring data.

Results

Boundary conditions

The most important boundary conditions for EFDC were meteorological drivers, lake level, and tributary water inputs. Significant trends in boundary conditions observed over the historical monitoring period include increased temperature (\(+0.095 \, ^\circ C \, y^{-1}\) ),
decreased wind speed \((-0.048 \, \text{m s}^{-1} \, \text{y}^{-1})\), and increased tributary discharge \((+0.071 \, \text{m s}^{-1} \, \text{y}^{-1})\). Seasonal trends in meteorological drivers are described in greater detail in Isles et al. \textit{(In review, Chapter 5 of this dissertation)}. No clear trend in lake level over the monitoring period was evident. The bias-corrected lake level model reproduced lake levels accurately over the historical period \((R^2 = 0.852, \text{RMSE} = 0.199 \, \text{m}; \text{Supplemental Fig. A.2})\), despite only incorporating data from one of the many tributaries to Lake Champlain (the Missisquoi River).

Annual TN and Dissolved P (DP) loads increased over the long-term monitoring period, particularly in the first decade (Medalie, 2013). Total P loads from the Missisquoi River did not increase significantly, but the slope of the regression line was positive. Increases in nutrient loads were due primarily to increases in precipitation and river discharge; flow-normalized nutrient concentrations remained constant for TP and TN over the historical period, while flow normalized concentrations of DP increased by approximately 6 \, \mu \text{g L}^{-1} from 1992-2010 (Medalie, 2013).

\textit{High-frequency calibration}

During the high-frequency calibration period, model outputs matched observed seasonal and vertical temperature distributions from high-frequency data closely at the main monitoring site (Fig. A.4), as well as additional monitoring sites throughout the bay (Supplemental Figs. 2-4). EFDC was able to reproduce the magnitude and frequency of transient vertical temperature gradients observed in the field data (Fig. A.4), which was critical given the central role that these temperature gradients have been found to play in lake biogeochemical (Courtney D Giles et al., 2016; Smith et al., 2011) and
hydrodynamic (Manley et al., In review) processes. EFDC also reproduced observed circulation patterns in the bay measured using ADCP data. Modeled flow patterns were dominated by counterclockwise circulation in the northern portion of the bay, with the development of a gyre in the northern lobe of the bay (Fig. A.3). The southwestern side of the bay generally has north-south flow, with water leaving the bay through the causeway in the southwest (although there were variable circulation patterns under different wind and discharge conditions).

Figure A.3: Modeled mean flow direction in Missisquoi Bay during August 2012.
The water quality model accurately reproduced within and between-year trends in phytoplankton dynamics, particularly for cyanobacteria (Fig. A.5). The timing of bloom onset was similar in model and field data in both 2012 and 2013. The model accurately reproduced the strong bloom in 2012 and the relatively weaker bloom in 2013, although the strength of the 2013 bloom was overestimated, particularly during August (Fig. A.5). The model also represented the relative dominance of N-fixers and non N-fixers accurately, with the N-fixers dominating in 2012, and non-N-Fixers dominating in 2013 (Isles et al., 2015). The contrast in inter-annual bloom severity is apparent in the spatially resolved average August and September chlorophyll concentrations during the two years (Fig. A.6). In addition to inter-annual variability, the model revealed consistent patterns
of spatial variability in bloom severity. For each year of the high-frequency (Fig. A.6) and long-term (Supplemental Fig. A.5) calibration periods, modeled August chlorophyll was highest in the eastern and northern areas of the bay, and in the center of the main basin. The lowest modeled concentrations of chlorophyll were consistently in the southwestern arm of the bay, near the outflow to the main lake (Fig. A.6). These are also the areas of the bay with the largest proportion of water derived from the nutrient-rich Pike river (Fig. A.7).

Figure A.5: High-frequency comparison of observed v. modeled cyanobacteria (both N-fixers and non-N fixers). Phycocyanin probe units are not easily convertible to model cyanobacteria units.
Figure A.6: Spatial and temporal variability in bloom severity. Panels show mean August (top) and September (bottom) Chl-a in 2012 (left), and 2013 (right).
Figure A.7: Modeled spatial distributions of August PO$_4^{3-}$ (top), August N-fixer concentrations (middle), and dye tracing Pike River water (bottom) during the 2012 high-frequency calibration scenario.
The model reproduced observed TP concentrations very well during the high-frequency calibration period in both 2012 and 2013 (Fig. A.8). Total P concentrations were controlled primarily by internal P loading driven by low bottom-water O$_2$ concentrations (Fig. A.9). Bottom water oxygen was much higher in July and August 2013 than 2012, resulting in lower summer TP. A period of low O$_2$ in late September 2013 promoted P release in the autumn, which corresponds to the timing of the late-season bloom which occurred in Missisquoi Bay in that year (Giles et al., 2016; Fig. A.5).

![Modeled v. Observed TN and TP](image)

**Figure A.8:** Observed v. modeled total phosphorus (top) and total nitrogen (bottom).

In contrast to the strong performance with TP, TN was not well represented in the model, particularly during the strong bloom in 2012 (Fig. A.8). This may be partly due to the model’s inability to represent N-fixation during a year dominated by N-fixing.
cyanobacteria (Isles et al., 2015), and partly due to underestimation of sediment ammonium fluxes (McCarthy et al., *In press*). The rate of decline of TN during the summer of 2013 was similar to that in the observed data, but initial concentrations of TN were drastically underestimated. If the external N loading estimates used in this study are accurate (Medalie, 2013), the model results suggest that there is an important internal N source which is not being modeled; this may be atmospheric N fixation or sediment N release, or some combination of the two.

![Graph](image)

Figure A.9: Bottom water daily minimum dissolved oxygen concentrations.

The model reproduced observed the range and inter-annual variability of inorganic P and N concentrations (Fig. A.10). In 2012, the ranges of SRP concentrations were similar in modeled v. observed results, although the model did not reproduce the high degree variability observed in the field data (Fig. A.10). In 2013, the model performed very well for SRP, capturing the low concentrations throughout the summer, and the increase in autumn following a major storm event (Fig. A.10). The model reproduced general trends in observed NO$_3^-$ and NH$_4^+$ accurately in 2012, although it failed to fully capture the high concentrations of both compounds following an early
September storm and the collapse of the bloom. The model also reproduced NO$_3^-$ well in 2013, although again it underrepresented NO$_3^-$ concentrations in the aftermath a September storm event. The model substantially underestimated NH$_4^+$ concentrations in 2013.

*Figure A.10: Modeled v. observed inorganic nutrients*

*Long-term calibration*

EFDC represented temperature accurately over the long-term monitoring period. There was a tendency to slightly over-estimate observed temperatures (Fig. A.11), but RMSE was low (1.70°C at LTMP station 50), and long-term monitoring data for temperature were relatively scarce (Fig. A.11). The largest underestimates were in several consecutive years in 1996-1999; model fits for the historical period excluding these years were better (RMSE < 1°C). The model indicated an overall warming trend during the
historical monitoring period, consistent with the long-term monitoring data (Figs. A.11, A.12, A.13).

Figure A.11: Modeled results (black lines) versus long-term monitoring observations for chlorophyll-a (top), total phosphorus (middle), and water temperature (bottom) at LTMP station 50. On right, scatterplots of modeled v. observed variables matched by date, showing root mean squared error and mean bias. Red line is 1:1.
Figure A.12: Modeled results (black lines) versus long-term monitoring observations for chlorophyll-a (top), total phosphorus (middle), and water temperature (bottom) at LTMP station 51. On right, scatterplots of modeled v. observed variables matched by date, showing root mean squared error and mean bias. Red line is 1:1.
Figure A.13: Observed and modeled bloom season average concentrations (June – September) of temperature (top), chlorophyll (middle), and TP (bottom) at LTMP station 50. Error bars represent standard deviation.
The model had a tendency to slightly underestimate TP relative to the historical period (mean bias = $-7 \mu g \text{L}^{-1}$; Fig. A.11, A.12) and individual point estimates frequently deviated from observed values (RMSE = $9 \mu g \text{L}^{-1}$), but the model reproduced the overall trend of long-term increases in lake TP (Fig. A.13). Seasonally, the model tended to underestimate TP in the spring and early summer, but represented average TP in late
summer and early fall closely (Fig. A.14). Model TP frequently overestimated lake TP in the immediate aftermath of storm events, particularly in spring (Fig. A.11, A.12).

Chlorophyll concentrations over the long-term monitoring period were slightly underestimated (mean bias = -3.4 μg L\(^{-1}\)) but were generally close to observed values during August and September (the peak bloom months; Fig. A.14). Chlorophyll was slightly under-estimated in May-June, and in October. The model reproduced the general increasing trend in Chl over the long-term monitoring period, but did not capture the year-to-year variability in the field data. Long-term increases in chlorophyll were driven primarily by increases in abundance of the N-fixer class, suggesting increasing N limitation in the bay (Fig. A.15). This modeled increase in N-fixers is consistent with increasing abundance of N-fixing cyanobacteria in cell counts from Missisquoi Bay in recent years (Angela Shambaugh, VT Agency of Natural Resources, personal communication).

Figure A.15: Modeled phytoplankton classes over the long-term monitoring period.
Future scenarios

Growing season air temperature (May-October) increased in all future climate scenarios, but there was no clear difference between for low and high-emissions scenarios due to the relatively short projection window and high variability between GCM projections (Fig. A.16). Most GCMs predicted increased precipitation, but there was a great deal of variability between models. There were significant changes in winter and spring temperature and precipitation which may also impact nutrient loading and summer bloom conditions; GCM results are presented in more detail in Guilbert et al. (2015) and Winter et al. (2016). Estimated shortwave solar radiation was slightly lower than historical means, and estimated wind speed was similar to historical means (Fig. A.16), but did not extrapolate the declining trend in wind speed from the last 20 years (Isles et al. Chapter 5 of this dissertation).
Water temperatures increased significantly by 2040 in all future climate scenarios under all greenhouse gas emission scenarios (Fig. A.17). Increases in water temperature were not distributed uniformly across seasons. The largest increases in lake water temperature are projected in the spring (May-June), and late fall (October-November). Temperature increases by mid century are somewhat higher in high carbon emission scenarios (RCP 8.5) than low carbon emission scenarios (RCP 4.5). The greatest difference between the RCP scenarios is in the early spring. April temperatures increase much more dramatically in RCP 6.0 and RCP 8.5 than in RCP 4.5, likely as a result of earlier snowmelt periods in high-emission scenarios. The most modest temperature
increases were in late summer (August-September), where temperatures are projected to increase by about 1°C in all emissions scenarios.

Figure A.17: Long-term changes by month in water temperature at the model cell corresponding to the high-frequency monitoring site. Temperatures are aggregated by decade, and boxes show monthly values for each of the three greenhouse gas emission scenarios (RCP 4.5, RCP 6.0, RCP 8.5). Temperatures are normalized to the monthly means of the first decade (2001-2010).
Discussion

The lake model reproduced long-term and within-year patterns of phosphorus and phytoplankton dynamics reasonably accurately. Although there were a number of areas in which the model had shortcomings, on the whole it reproduced long-term and within-year trends in key water quality variables (TP and Chl), and the accuracy of the model estimates reported here is similar to results reported in previous studies (Couture et al., 2014; Trolle et al., 2011b). As such, this modeling framework is likely to yield plausible estimates of future ecological state when driven with data from future climate scenarios.

A major advantage of the model outputs is the high spatial resolution, which is not available from the field monitoring data. The model results displayed clear spatial variability in cyanobacteria bloom severity during both the high-frequency (Fig. A.6) and long-term (Supplemental Fig. A.6) periods, and this spatial pattern was consistent across almost all years. In all cases, the most intense blooms were found in the northeastern and eastern part of the bay. This area of the bay tended to have the highest PO$_4^{3-}$ concentrations as well, and this may be partially the result of the influence of nutrient-rich water from the Pike river (Fig. 6). The Pike river drains a heavily agricultural catchment, and dissolved P concentrations from this river are roughly twice those of the Missisquoi River (Medalie, 2013). While annual P loads from the Pike are much smaller than loads from the Missisquoi due to its smaller catchment, the high concentrations of dissolved nutrients may accelerate phytoplankton growth rates in areas where the Pike river water is concentrated. Furthermore, the increased dissolved P in the northern portion of the bay is likely retained in the bay’s northern gyre (Fig. A.3). Gyres have been shown
to promote the growth and accumulation of cyanobacteria blooms in other systems (Ishikawa et al., 2002). If Pike river inputs are having a disproportionate impact on the development of blooms in the bay, this would be analogous the situation in western lake Erie, where concentrated nutrient loads from the Maumee River have been found to have disproportionate influence on cyanobacteria bloom development (Daloglu et al., 2012; Michalak et al., 2013). However, considerably more spatially explicit field data are needed to corroborate this hypothesis. If nutrient-rich water from the smaller tributaries (Pike and Rock rivers) does play a major role in cyanobacteria bloom development in Missisquoi Bay, the failure to simulate hydrological processes in those catchments accurately introduces an element of uncertainty to projections from the integrated assessment model.

Inter-annual variability in P dynamics was controlled both by external inputs in the spring, which fueled initial phytoplankton growth in late spring and early summer, and by internal loading which began in the mid-late summer, and was the primary source of nutrients during the cyanobacteria bloom period. While the model reproduced long-term trends of increasing P, inter-annual variability appeared to be less well-represented over this timeframe than in the high-frequency calibration period (Fig. A.7, A.11). The strong performance during the high-frequency calibration period and weaker performance during the long-term monitoring period suggests that in-situ climate data may be critical for accurate representation of processes in the bay. Missisquoi bay is highly sensitive to meteorological thresholds which control internal P loading (Giles et al., 2016; Isles et al., Chapter 4 of this dissertation). The NARR dataset used for historical runs, despite being
statistically corrected to approximate the range of *in-situ* data, may not accurately represent lake wind conditions, particularly with respect to critical thresholds controlling internal nutrient release (Isles et al. *Chapter 4 of this dissertation*). This problem may compounded in future climate scenarios by the fact that wind conditions, which drive the development of transient thermal stratification, are not well-represented in GCMs (Pryor et al., 2006). Wind speeds have declined considerably in the northeastern United States over the past 20 years by roughly 1% y\(^{-1}\) (Pryor et al., 2009). The resampling-based weather generator approach employed here does not project this trend into the future; as a result, if ongoing trends in wind speed continue this IAM may underrepresent the development of transient thermal stratification and internal P loading in the future.

The model substantially underestimated TN concentrations (Fig. A.8); however, broad seasonal trends in TN concentrations were reproduced by the model. Seasonally, TN was highest in winter and early spring, following major tributary inputs, and declined over the summer months as a result of algal sedimentation and denitrification. These general patterns match observed seasonal patterns in field data (Isles et al. *Chapter 5 of this dissertation*). Underestimates of lake TN were likely due to failure to accurately represent sediment N fluxes, and to the inability of the model to simulate N fixation. Sediments were a large N sink in the model, with denitrification greatly exceeding fluxes of ammonium produced from the diagenesis of particulate organic nitrogen. While denitrification has been found to be high in Missisquoi Bay, sediment N fluxes have been also been found to be high, particularly in spring in sites close to river mouths (McCarthy, 2011). The model was unable to reproduce the early-season N fluxes, likely
because the temperature dependence of the model equations favors sediment N release during warm periods when sediment oxygen demand is high and the oxygenated sediment layer (which is the principal site of nitrification) is thin (Fitzpatrick, 2004). While field studies have found relatively low rates of N fixation in Missisquoi Bay (McCarthy et al., 2013), N fixation may be highly variable as a result of ambient ammonium concentrations (Herrero et al., 2001; Muro-Pastor et al., 2005), and it may be a significant source of N to Missisquoi Bay under some conditions. This may be particularly important in 2012 (Fig. A.8), when the strong bloom was dominated by N-fixing Dolichospermum.

Despite the weakness with respect to TN dynamics, the model performed better with inorganic N species (Fig. A.10). The model also reproduced PO$_4^{3-}$ accurately, suggesting that phytoplankton nutrient limitation may have been reasonably well simulated. The increasing abundance of N-fixing cyanobacteria class in the model output over the long-term calibration period (Fig. A.15) is consistent with increases in N-fixing cyanobacteria observed throughout Lake Champlain, and with declining N:P in Missisquoi Bay (Isles et al., Chapter 5 of this dissertation). The increase in N-limiting conditions in the model appears to be driven primarily by increases in sediment P loading during the summer bloom season, which is the result of increasing temperature (Fig. A.11) and gradually increasing sediment P concentrations. The strong N limiting conditions and dominance by N-fixing cyanobacteria during the extremely dry year in 2012 is consistent with field observations (Isles et al., 2015), and with studies in other shallow sites, which have linked drought periods to N limitation in shallow lakes (Grantz
et al., 2014; Kolzau et al., 2014). Changing seasonal patterns of precipitation may have also played a role in long-term increases in N limitation. Modeled NO$_3^-$ showed a clear seasonal progression, starting high in the spring, and gradually declining through the summer months (Fig. A.10). Earlier average snowmelt resulting from increasing spring temperatures may have contributed to earlier NO$_3^-$ peaks and lower available N during the growing season.

While the lake model generally performed well with respect to the key phytoplankton and phosphorus variables, it did have a number of limitations. EFDC and RCA do not simulate ice cover dynamics endogenously, although ice cover can be imposed as a boundary condition in both models. As a result of this, winter conditions cannot be approximated realistically in future climate scenarios. Winter has been shown to be an important season for sediment nutrient release in Missisquoi Bay (Schroth et al., 2015; Joung et al., In preparation). Changing seasonality of snowmelt inputs may also result in changes in summer bloom conditions which are not captured by this or other modeling frameworks due to the poor representation of cold-water biogeochemical processes (Pierson et al., 2013). While it is not clear what role winter internal or external P loading has on long-term P mass balances in Missisquoi Bay, winter processes will likely change substantially with future climate warming (Guilbert et al., 2014), and the effects of winter processes on long-term P storage should be studied in greater detail. The Missisquoi Bay model also fails to represent top-down control of phytoplankton by higher trophic levels, which may be extremely important for controlling phytoplankton populations and nutrient recycling (Jeppesen et al., 2003; Levine et al., 1999). This may
partially account for underestimation of water column NH4+, particularly following periods of bloom decline (Fig. A.10); NH4+ in lakes is frequently strongly influenced by nutrient regeneration driven by zooplankton (Elser and Urabe, 1999). Future shifts in food webs may therefore result in changes in lake biogeochemical processes which cannot be simulated by this model.

The hydrodynamic model projections under future climate scenarios suggest that blooms in Missisquoi Bay are likely to become more widespread (Fig. A.17). Cyanobacteria are known to have higher temperature optima than other phytoplankton groups (Butterwick et al., 2005), and are expected to benefit disproportionately from warming conditions. The relatively large increases in spring and fall suggest that the bloom season may become longer, resulting in earlier bloom onset and greater persistence of blooms late into the season. Future RCA simulations using these scenarios are expected to corroborate these predictions.

Overall, the integration of the Missisquoi Bay Model into the integrated assessment model was a success. We were able to automate coupled simulations with a complex set of models using the Pegasus framework, and to develop methods for estimating the key boundary conditions required by the lake model. There are several potential pitfalls in the modeling framework that need to be considered in future applications of the model; in general, however, the Missisquoi Bay Model performed well in its current form, and it is likely to be a powerful tool for addressing the impacts of future climate changes on water quality.
References


Giles, Courtney D, Isles, P.D.F., Manley, T., Xu, Y., Druschel, G.K., Schroth, A.W.,
2016. The mobility of phosphorus, iron, and manganese through the sediment-water continuum of a shallow eutrophic freshwater lake under stratified and mixed water-column conditions. Biogeochemistry 127, 15–34. doi:10.1007/s10533-015-0144-x


in shallow lakes. Ecological Applications 19, 1791–1804.


Michalak, A., Anderson, E., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B.,


Supplemental Table A.2: RCA phytoplankton parameters.

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<p>| Phytoplankton Detritus Diagenesis Fractions                                                                 |
|---------------------------------------------------|---------------------------------------------------|
| KMPHY T                                           | All Groups                                        |
| Half-saturation constant for phytoplankton         | mg-C/L                                           | 0.5     |
| FRPOP                                             | Refractory particulate organic phosphorus          | 0.1     |
| FLPOP                                             | Labile particulate organic phosphorus              | 0.25    |</p>
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Supplemental Table A.3: RCA General water quality parameters

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<td>KADSI</td>
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Supplemental Table 4: RCA sediment model parameters

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<th>G2 (semi-labile)</th>
<th>G3 (refractory)</th>
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<td>FRPPH1</td>
<td>Fractions of algal P going to G1, G2, G3 sediment OP for algal group #1</td>
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Supplemental Figure A.1: Lake level model calibration results. Top: Un-corrected multiple regression model. Middle: plot of lake level against residuals, with blue-dots representing quadratic regression fit used for bias correction. Bottom: Bias-corrected lake level model.
Supplemental Figure A.2: Observed V. Modeled temperature profiles from thermistor chain at main site (Summer 2013).
Supplemental Figure A.3: Observed v. Modeled temperatures at west-central monitoring site, summer 2013.
Supplemental Figure A.4: Observed v. modeled temperatures for north delta monitoring site, summer 2013.
Supplemental Figure A.5: Modeled annual average August chlorophyll for long-term monitoring period.
Supplemental Figure A.5: continued from previous.