Nutrient sources for excessive growth of benthic algae in Lake Ontario as inferred by the distribution of SRP

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Total phosphorus concentrations in the open waters of the Laurentian Great Lakes are currently at or beneath target concentrations set by international agreement. Despite the success of phosphorus loading controls in remediating nearshore eutrophication problems in the past, nuisance growth of *Cladophora* has recently returned to the lower Great Lakes. This thesis examines soluble reactive phosphorus (SRP) in a northwestern segment of Lake Ontario to assess whether allochthonous or autochthonous sources of phosphate lead to localized areas of PO_4^{3-} enrichment that may help to explain the seemingly paradoxical resurgence of *Cladophora.* As SRP is often an overestimate of PO_4^{3-} in P-limited waters, measures of SRP made with the standard method were compared with measures of SRP made with modified methods (i.e., using dialysis and magnesium-induced co-precipitation) designed to more accurately measure phosphate when it was expected to be at low concentrations. Measures of SRP made with standard and modified methods did not differ, however, SRP was 1 to 3 orders of magnitude higher than a more sensitive steady-state radiobioassay for PO_4^{3-} used for comparison in offshore waters. Although the utility of SRP is limited when phosphate concentrations are very low, SRP is useful to measure localized areas of phosphate enrichment, and its relative concentrations can be compared in time and space.

To quantify the degree to which allochthonous inputs and dreissenids contribute to PO_4^{3-} concentrations that permit *Cladophora* growth, intensive sampling for SRP was carried out prior to, during and following the *Cladophora* growing season. SRP was higher in the nearshore than offshore and near the mouth of a large tributary and a treated wastewater outfall than in samples from other locations along the shoreline, but only in the spring and autumn. Phosphate turnover times indicated lower P-limitation in the nearshore and near local inputs versus the offshore. Higher concentrations of SRP were measured in samples taken 15 cm and 50 cm above dreissenid mussel-beds than in those obtained at corresponding depths over other substrata and from higher up in the water column through the *Cladophora* growing season, while Chl *a* concentrations displayed the reverse trend. These results suggest that PO_4^{3-} excreted by dreissenids could be more important in time and space than external inputs in supporting nuisance *Cladophora* growth in the current nearshore environment. Continued research and monitoring of P dynamics in the nearshore combined with model approaches should better predict whether more stringent P controls would be effective in managing *Cladophora* growth.

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Chapter 1 Introduction

1.1 Nearshore Eutrophication in the Laurentian Great Lakes

During the 1960's to the early 1980's, the Laurentian Great Lakes suffered from eutrophication under the strain of a growing population. Excess nutrients, including phosphorus (P), were loaded into the lakes through wastewater effluents, industrial effluents, and as a result of poor agricultural practices. This led to the production of excessive algal biomass, particularly in the lower Great Lakes. In undisturbed temperate lakes, P concentrations are generally maintained at extremely low levels. P-containing minerals in the earth's crust are scarce and generally have a low solubility (Griffith et al., 1973) and dissolved inorganic P ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, i.e., "orthophosphate", or just "phosphate") has a strong tendency to adsorb onto a solid surface (Föllmi, 1996), or to be rapidly taken up by organisms. Thus, the amount of phosphate entering lakes from external sources is very small in relation to the amount of phosphate regenerated through internal cycling. In fact, P is the least naturally abundant of the major nutrients required by organisms for growth (others are carbon, hydrogen, nitrogen, oxygen and sulfur) and its low ambient concentration in lake water is a factor limiting primary production (Edmondson, 1970; Schindler, 1974; Schindler, 1977; Edmondson & Lehman, 1981; Flint & Stevens, 1989; Bentzen & Taylor, 1991; Cotner & Wetzel, 1992; Nausch et al., 2004; Schindler & Vallentyne, 2008). This is why the large anthropogenic P loads of half a century ago led to increased phytoplankton biomass and cyanobacterial blooms in the open waters, and excessive abundances of the benthic alga *Cladophora* along the littoral lake-bottoms of the lower Great Lakes (Bellis & McLarty, 1967; Herbst, 1969; Shear & Konasewich, 1975; Auer et al., 1982; Millner & Sweeney, 1982; Millner et al., 1982; Painter & Kamaitis, 1987; Sweeney, 1993). Other associated issues also plagued the lakes, including taste and odour problems in drinking water, elevated levels of cyanobacterial toxins, deepwater anoxia, and the deterioration of fish-spawning shoals (Smol, 2002).

In an effort to remediate eutrophication issues, P-loading controls were mandated in 1972 as part of the Great Lakes Water Quality Agreement (GLWQA) between Canada and the United States (much to the despair of detergent companies, whose products then contained large amounts of P). Specific targets for total phosphorus (TP) concentrations were set for each lake in 1978, and were updated in 1987 by the International Joint Commission's Water Quality

Board. Over nine billion dollars have been spent by the Canadian and US governments over the past three decades controlling loading primarily from point-sources, often through improved practices of sewage treatment (Smol, 2002). Fortunately, the P-loading reductions have been successful in yielding a decline in basin-wide TP concentrations (e.g., Stevens & Neilson, 1987; Nicholls et al., 2001; Dolan & McGunagle, 2005). Current reports indicate the open waters of all of the lakes are attaining their respective target concentrations, with the exception of localized exceedances in Lake Erie (Env. Can. & US EPA, 2009). In particular, TP in offshore Lake Ontario is consistently below its target concentration of 10 µg P L^{-1}, and appears to still be in decline (Env. Can. & US EPA, 2009; Malkin, et al., 2010a). Though there are few baseline data on Great Lakes phytoplankton and zooplankton productivity from the past, overall productivity appears to have decreased since P controls were implemented (Danforth & Ginsburg, 1980; Makarewicz, 1993; Nicholls & Hopkins, 1993; Johengen et al., 1994; Millard et al., 1996; Johannsson et al., 1998; Nicholls, 1999; Munawar, 2003; Holeck et al., 2008). The most apparent result in the years immediately following P-control mandates was a reduction of *Cladophora* growth in the lower Great Lakes (Painter & Kamaitis, 1987), marking an improvement in water quality (Parker & Maberly, 2000). As problems associated with eutrophication appeared to be largely remediated by the early 1980's, P-management strategies were deemed successful (Stevens & Neilson, 1987; Charlton et al., 1993; Munawar, 2003). Unfortunately, the decline of nearshore eutrophication issues corresponded with a decline in research, so there is little information available regarding *Cladophora* biomass and nearshore phosphorus concentrations after the early 1980's (Higgins et al., 2005b; Auer et al., 2010).

Despite the progress of past P management actions, there has been a perceived resurgence of *Cladophora*, with nuisance fouling in the littoral zones of the lower Great Lakes over the past 10 to 15 years (Higgins et al., 2005b; Higgins et al., 2008b; Malkin et al., 2008). At present, *Cladophora* growth generally occurs wherever hard substrate and sufficient light are available along the coasts of Lake Ontario, Lake Erie, western Lake Michigan, and in some parts of Lake Huron near nutrient sources (Byappanahalli et al., 2003; Higgins et al., 2005b; Malkin et al., 2008; Env. Can. & US EPA, 2009). *Cladophora glomerata* (L.) Kützing has recently been confirmed as the dominant filamentous alga in these lakes (e.g., Ross et al., 2006) and will be referred to herein as simply, *Cladophora.* Although *Cladophora* is found in many systems,

nuisance abundances signal cultural eutrophication (Whitton, 1970; Pitcairn & Hawkes, 1973; Neil & Jackson, 1982; Dodds & Gudder, 1992; Parker & Maberly, 2000).

Recent evaluations of the trophic status of each of the Great Lakes point to the importance of spatial distributions of nutrients and algae (Agreement Review Committee, 2007; Env. Can. & US EPA, 2009). The reports state that although the efforts that began in the mid 1970's to reduce P entering the lakes have been successful in reducing nutrient concentrations and Chl *a* in offshore waters, high total phosphorus (TP) concentrations still occur locally in some embayments, harbours and nearshore areas. Whether or not nearshore conditions are improving or degrading remains undetermined at present, but elevated P concentrations in nearshore areas likely contribute to the nuisance growth of *Cladophora* and toxic cyanophytes such as *Microcystis* (Env. Can. & US EPA, 2009). The return of *Cladophora* has triggered public complaints regarding the negative aesthetic impact it imposes on coastlines after it senesces, sloughs off of the lake bottom, and washes ashore (Higgins et al., 2005b). The clumps of rotting algae are unsightly, foul-smelling, and lead to the perception of poor water quality in the eyes of the public. Detached *Cladophora* mats can also affect industry located along the shores of the lower Great Lakes. For example, blockage of the cooling water intake at Ontario Power Generation's Pickering Nuclear Generating Station on the north shore of Lake Ontario led to its partial shutdown in August, 2005 and August, 2007. A similar event occurred to the east at OPG's Darlington Nuclear Generating Station in September, 2005. In fact, OPG estimates clogging due to *Cladophora* has resulted in a loss of $30 million over the past 12 years. The public attention surrounding Great Lakes *Cladophora* overgrowth in recent years has prompted researchers and managers to reassess their understanding of nearshore eutrophication dynamics. A collection of primary research articles on the topic have been published (e.g., Higgins et al., 2005b; Higgins et al., 2006; Higgins et al., 2008b; Malkin et al., 2008; Ozersky et al., 2009; Auer et al., 2010; Malkin et al., 2010a; Malkin et al., 2010b; Tomlinson et al., 2010) indicating renewed scientific attention to the problem. The effectiveness of the GLWQA was recently reviewed by the Canadian and US governments, more than 20 years after its last revisions. Although successful in the past, the GLWQA was reported to be outdated and unable to address current threats to Great Lakes water quality, with specific reference to nearshore eutrophication issues (Agreement Review Committee, 2007). As of 2010, negotiations of the necessary amendments to the Agreement are underway.

Issues surrounding water quality and algal fouling in the Great Lakes do not stand alone in their ecological history over the past half-century. Other significant changes in the Great Lakes ecosystems have taken place, including the introduction of an array non-indigenous species, frequently from the Ponto-Caspian region, such as the spiny water flea (*Bythotrephes cederstroemi*) and zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*, respectively). Interestingly, the invasion and widespread establishment of dreissenid mussels in the Great Lakes is perceived to have coincided with the re-emergence of shoreline fouling by *Cladophora* (Mills et al., 2003; Hecky et al., 2004). Dreissenids are known to be ecosystem engineers, causing changes in physical, chemical, and biological properties of their invaded habitats (e.g., Lowe & Pillsbury, 1995; Vanderploeg et al., 2002; Hecky et al., 2004; Higgins & Vander Zanden, 2010) and may be at least partially accountable for the unforeseen return of excessive abundances of benthic algae that are widespread along the shores of the oligotrophic (L. Ontario, L. Michigan) and mesotrophic (L. Erie) Great Lakes (Hecky et al., 2004; Higgins et al., 2005b). Their filter-feeding actions can extend habitat for *Cladophora* by promoting water clarity (Zhu et al., 2006; Malkin et al., 2008; Auer et al., 2010) and may enhance phosphate concentrations directly available to benthic algae (Hecky et al., 2004; Ozersky et al., 2009). The *Dreissena – Cladophora* relationship will be discussed in further detail later in this Chapter.

1.2 *Cladophora* and Phosphorus

Cladophora is widely distributed in marine and freshwater systems (Blum, 1956) and its physical and chemical requirements have been described by many others (e.g., Bellis & McLarty, 1967; Whitton, 1970). *Cladophora* growth and metabolic requirements specific to the Great Lakes ecosystem have also been described (e.g., Gerloff & Fitzgerald, 1976; Canale & Auer, 1982b; Higgins et al., 2006; Higgins et al., 2008b). *Cladophora* is a filamentous green alga that grows attached to hard substrate in alkaline temperate and tropical freshwater environments (Sheath & Cole, 1992). *Cladophora* requires a relatively high light environment, some degree of water motion (Whitton, 1970), and its optimal temperature for growth in the Great Lakes occurs between 13 to 31 C (Higgins et al., 2008b). In north-temperate climates, this alga typically displays a bi-modal pattern of growth that involves a summer biomass peak followed by senescence and detachment, then a period of slow growth before peaking and detaching again in the autumn (Whitton, 1970). In the Great Lakes, the spring/summer biomass

peak is higher than the autumn peak (Bellis & McLarty, 1967; Lorenz & Herdendorf, 1982; Higgins et al., 2008b). Where *Cladophora* growth is extensive, the midsummer senescence and detachment may be a major "sloughing" event, as filaments are torn from their holdfasts or broken under the stress of water turbulence (Bellis & McLarty, 1967; Whitton, 1970; Canale & Auer, 1982a) and transported horizontally. *Cladophora* mats are considered a public nuisance because they degrade coastal aesthetics when they are carried to shore and they can clog water intakes (Higgins et al., 2005b). The mechanisms responsible for the weakening of *Cladophora* filaments leading to sloughing are not fully understood, but temperature stress (Bellis & McLarty, 1967; Whitton, 1970; Dodds & Gudder, 1992; Tomlinson et al., 2010), nutrient deficiency (Mantai et al., 1982), and metabolic imbalance between growth and loss rates at the base of filaments due to self-shading (Higgins et al., 2008a; Malkin et al., 2010b) are some proposed explanations. Research into the factors leading to extensive *Cladophora* biomass accrual in the Great Lakes has focused largely on phosphorus and light availability (e.g., Auer & Canale, 1980; Canale & Auer, 1982b; Higgins et al., 2006; Hiriart-Baer et al., 2008; Malkin et al., 2008; Auer et al., 2010; Tomlinson et al., 2010).

Like all living things, *Cladophora* requires phosphorus for metabolism and growth. Phosphorus is a component of nucleic acids and cellular structure (e.g., membrane phospholipids) and is necessary for cellular function (providing energy in the form of ATP, mediating signal transduction and protein synthesis through phosphorylation, etc.). In aquatic plants, growth limitation occurs when internal nutrient concentrations approach a certain minimum cell concentration. Although there are reports of nitrogen limitation (e.g., Millner et al., 1982), nearly all studies of *Cladophora* in freshwaters report phosphorus limitation (Canale & Auer, 1982b; Freeman, 1986; Painter & Kamaitis, 1987; Malkin, 2007). *Cladophora* filaments do not root into the sediment but rather fasten to hard substrate and must derive their nutrition from P in the water column. Indeed, *Cladophora* growth responds to enhanced P concentrations in surrounding water (Pitcairn & Hawkes, 1973; Neil & Jackson, 1982), and past studies have documented the importance of water column P in supporting nuisance level occurrences in aquatic systems (Wong & Clark, 1976; Parker & Maberly, 2000), including the Great Lakes (Auer & Canale, 1980; Painter & Jackson, 1989; Higgins et al., 2006).

Cladophora can access only a very small fraction of total phosphorus in the water column. While particulate P can be ingested by animals and phagotrophic protists, this form of P is not available to *Cladophora* until it is remineralized. Both benthic algal and phytoplankton species can only utilize dissolved P, and still only the inorganic portion of it directly. With column chromatography, Lean (1973) and others (e.g., Stainton, 1980; Bentzen & Taylor, 1991; Fisher & Lean, 1992) found that most of the dissolved P pool in lake water is "colloidal" (MW > 5000) and is likely dissolved organic P (DOP) that is actually greater than 70,000 MW. The smallest anionic group (250 MW) termed "XP" by Lean is DOP thought to be composed of nucleotides and other small organic molecules (Taylor, 2010). Thus, the actual pool of P directly available to *Cladophora* (i.e., dissolved inorganic P; PO_4^{3-}) is very small. When available, *Cladophora* can take up PO_4^{3-} directly from the water column and store it in its tissues where (under sufficient light and temperature) it is converted into biomass. When $PO_4^{3-}$is limiting, *Cladophora* might also be able to access "XP" with the help of phosphatases and other exo- and ectoenzymes (Bentzen & Taylor, 1991; Bentzen et al., 1992; Taylor, 2010). Extracellular alkaline phosphatase enzyme activity (APA) allows bacteria and algae to access organic P (e.g., monophosphate esters) when PO_4^{3-} supply is diminished (Healey & Hendzel, 1979). Although AP is known to be used by *Cladophora* for P acquisition (Lapointe & O'Connell, 1989; Young et al., 2010), the relationship between PO_4^{3-} supply, tissue P and AP expression in *Cladophora* is complex and not yet fully understood (Higgins et al., 2008b; Young et al., 2010). Another potential source of DOP available to *Cladophora* may be the phosphonate pool. Naturally occurring phosphonates are rare but they are important pesticides. Marine algae have enzymes to access phosphonate P (Dyhrman et al., 2006), and a gene enabling phosphonate utilization has been detected and expressed in freshwater picocyanobacteria from Great Lakes samples (Ilikchyan et al., 2009). The use of phosphonates for nutrition by both benthic and pelagic algae in the Great Lakes requires further examination.

When discussing the relationship between *Cladophora* and phosphate, it is important to consider how phosphate is measured in natural waters. Accurate measurement of ambient phosphate is inherently difficult because, in P-limited systems such as the Great Lakes, it is rapidly incorporated into the particulate pool and therefore exists in very low concentrations (Lean, 1973; Dodds, 1993). Colourimetric methods are the most commonly employed for routine monitoring of P. Soluble reactive phosphorus (SRP) is used as the fraction of total P to

represent PO_4^{3-}. SRP is defined as the fraction in <0.2µm-filtrate that reacts with molybdate in the molybdenum blue method originally developed by Murphy and Riley (1962). Unfortunately, orthophosphate is often not a major component of SRP in temperate lakes in the summer epilimnion (Rigler, 1966; Stainton, 1980; Bentzen & Taylor, 1991; Taylor & Lean, 1991; Fisher & Lean, 1992; Dodds, 1993; Hudson et al., 2000; Dodds, 2003). The discrepancy between SRP and phosphate is believed to occur because, in addition to PO_4^{3-}, SRP includes a portion of colloidal DOP originating from the particulate fraction that is released as a result of cell breakage during filtration and hydrolyzed by the acidic reagents in the assay (e.g., Stainton, 1980; Taylor & Lean, 1991; Taylor, 2010). The method's high detection limit also hinders the ability of SRP to accurately represent truly available orthophosphate concentrations. The drawbacks associated with using the traditional SRP assay are discussed in more detail in Chapter 2, as are some potential modifications to the assay (i.e., dialysis and magnesium-induced co-precipitation) to improve its ability to measure phosphate.

Despite the well-known inadequacies of SRP as a reliable metric in P-limited systems, most studies of *Cladophora* growth in response to PO_4^{3-} in the Great Lakes use SRP. In fact, SRP has proven to be a successful operational measure of P available to *Cladophora* in model simulations of its ecology in the Great Lakes (e.g., Canale & Auer, 1982b; Higgins et al., 2006; Tomlinson et al., 2010). It is possible that although SRP overestimates true phosphate concentrations in surface waters during the *Cladophora* growing season, it is actually an accurate measure of phosphate in proximity to the benthos or during local input events because of the high fraction of available P in animal excreta (e.g., from dreissenid mussels) and in wastewater and stormwater. The SRP procedure is also easy to conduct, especially compared to more sophisticated methods to estimate phosphate concentrations, such as those involving the use of radio-tracers (e.g., Hudson et al., 2000). Further, radioassay methods are based on phosphate kinetics in near steady-state conditions of pelagic bacteria and plankton communities and are not applicable to nearshore areas where P dynamics are influenced by the benthos. For these reasons, water column SRP is currently the accepted means for assessing P available to *Cladophora*. My study is based largely on water column and SRP concentrations obtained with and without modifications to the standard SRP method.

If elevated levels of SRP are responsible for *Cladophora* blooms (Herbst, 1969; Auer & Canale, 1980), it seems logical to elucidate the critical range of ambient SRP that will control *Cladophora* growth from reaching nuisance densities. Unfortunately, there is little information available on past nearshore SRP concentrations or *Cladophora* in the Great Lakes in the period after nuisance growth was largely controlled (early 1980's, Higgins et al., 2005b), making it difficult to compare present conditions with past conditions supporting (and not supporting) large *Cladophora* biomass. Modeling has therefore become a principle means to obtain information on past conditions (hindcasting) and to predict current and future conditions of *Cladophora* growth in response to SRP concentrations. The first such mass balance model, the "Auer and Canale Model," was constructed to predict macroalgal biomass density in relation to a high nutrient point-source in Lake Huron. Its key findings were that *Cladophora* growth rates, production, and biomass increase linearly with increasing internal phosphorus content, eventually saturating with no further increase in growth (Auer & Canale, 1982), and that maintaining SRP below a threshold of 2 µg P L^{-1} should be sufficient to maintain non-nuisance levels (defined as < 50 g DW m^{-2}) in the Great Lakes (Canale & Auer, 1982b).

More recently, Higgins et al. (2005a and 2006) and Malkin et al. (2008) applied a "*Cladophora* Growth Model" (CGM) to evaluate the effects of P management, enhanced water clarity by dreissenid mussels, and climate change on *Cladophora* growth. The CGM found *Cladophora* growth to be highly sensitive to spatial and temporal variation in SRP. Site-to-site differences in SRP concentrations resulted in a 2× difference in depth-integrated biomass, and maximum growth rates were strongly influenced by SRP concentrations during periods of rapid biomass accrual (mid-June to mid-July). Further, inter-annual differences in SRP concentrations (~ 1 µg P L^{-1}) during the spring period resulted in up to a 3.5× difference in depth-integrated biomass (Higgins et al., 2006). Increased water transparency was also found to be important; spatial differences in water clarity resulted in a 2× difference in depth-integrated biomass between sites, with the greatest effect at intermediate depths (2-6 m) where growth is not light saturated (Higgins et al., 2006). Hindcasting simulations with the CGM demonstrated that an enhancement of light availability following dreissenid mussel invasion has been responsible for enabling *Cladophora* growth at greater depths than in pre-dreissenid conditions (Malkin et al., 2008). The CGM also predicted that higher surface water temperatures in the future could extend the *Cladophora* growing season (Malkin et al., 2008).

Tomlinson et al. (2010) have since revised and modified the "Canale and Auer Model" to the "Great Lakes *Cladophora* Model" (GLCM), intended for application along the gradients of light and nutrient status experienced by the Great Lakes. As P is the only environmental forcing condition amenable to management, the main goal of the GLCM was to quantify the relationship between ambient P and *Cladophora* growth. The authors advise that *Cladophora* should respond to P management strategies rendering SRP concentrations below 1 μg P L^{-1}, and note that this prediction is consistent with observations of SRP levels in Great Lakes waters not supporting *Cladophora* growth (i.e. Lake Huron and Lake Superior). Most recently, Auer et al. (2010) simulated P-light-*Cladophora* interactions in Lake Ontario, Lake Erie, and Lake Michigan using the CGM and the GLCM. These simulations brought to light the potential for enhanced water clarity (as a result of dreissenid filter-feeding) to effectively offset reductions in *Cladophora* growth potential through P management strategies, especially in Lake Ontario and Lake Michigan. Further, the authors acknowledge that although *Cladophora* appears to be largely driven by whole-lake SRP concentrations (model SRP data used in simulations were offshore, spring values), the lack of comprehensive nearshore SRP data limited the model's ability to account for potential contributions from local inputs and from dreissenid mussels. Whether mass-balance models that present the Great Lakes as well-mixed systems controlled mainly by pelagic conditions are still acceptable is an important management question. Research focused on the nearshore is required to examine the impact of littoral sources of phosphate.

Natural pathways for P in the Great Lakes include the waste products of organisms, decaying organisms, weathering of P-containing rocks, atmospheric deposition, groundwater, and tributary inputs. P is also supplied via anthropogenic sources, such as municipal wastewater and industrial effluents, and agricultural and urban runoff. Indeed, high algal productivity is supported by P from allochthonous and littoral sources (Wetzel, 1983). Although *Cladophora* fouling in the 21st century is not associated with the high point source loading of half a century ago, surface drainage is often a major contributor of P to receiving waters (Wetzel, 1983). Perhaps excess phosphate is entering nearshore waters through more diffuse, non-point allochthonous sources, which are inherently more difficult to manage (Makarewicz, 2009). The Great Lakes are surrounded by more than 30 million people, with growth occurring in urban areas. The population in metropolitan regions of the Great Lakes Basin increased by 16.3% from 1996 to 2006 in Canada and by 7.6 % from 1990 to 2000 in the United States (Env. Can. & US

EPA, 2009). Unfortunately, urbanization generally results in increased P loading in proportion to population densities (Wetzel, 1983). Developed lands have higher percentages of impervious surfaces and enhanced hydraulic connectivity to coastlines (Walsh et al., 2005). These features negatively affect the ability of the substrate to buffer both the magnitude and composition of urban runoff, which may contain high concentrations of dissolved P from fertilizers applied to residential lawns, parks or golf courses (Bernhardt & Palmer, 2007). A recent study reported significantly higher *Cladophora* tissue enrichment in both nitrogen and phosphorus in urban sites versus rural sites in Lakes Ontario and Erie (Houben, 2007). Analyses of *Cladophora* biomass along the northern coastline of Lake Ontario indicated that sites adjacent to highly urbanized areas supported biomass levels 2-3× higher than sites adjacent to areas with low urbanization (Higgins & Howell, 2010).

Tributaries are a source of phosphate and particulate P (some of which can be later remineralized) to lakes. Fertilizer use and other land management practices in agriculture and forestry affect P loading through these pathways (Wetzel, 1983). In the 1970's, land use changes were responsible for about 50% of nutrient inputs to the lower Great Lakes (Schindler, 2006). More recently, Crosbie and Chow-Fraser (1999) found that the trophic state of wetlands located primarily in the catchment areas of Lakes Ontario and Erie was determined by the percentage of agricultural land in their watersheds. At present, most of the land around the lower Great Lakes is used for agriculture. The implications of this are not fully understood, though the impact of agricultural runoff on water quality is generally believed to have been reduced through improved land-use practices. Agricultural best management practices (BMP's) targeting nutrient runoff have been recently shown to effectively reduce filamentous algal growth in the littoral zone (Bosch et al., 2009). Indeed, long-term data-sets indicate that the amount of TP and the percent of TP that is SRP loaded through some tributaries have declined since the 1960's (e.g., Malkin et al., 2010a). On the other hand, there is also evidence that, despite a balance achieved between phosphorus inputs (commercial fertilizer and animal waste) and phosphorus removal as crops, loading of SRP through certain tributaries has increased, likely due to changes in agriculture practices affecting the delivery of SRP to the lakes (e.g., Strickland et al., 2010). Enhanced delivery of SRP from agricultural lands may be caused by, for example, changes to tillage practices (e.g., minimum tillage and no tillage), changes in drainage practices (e.g., subsurface tiles), and changes to methods and timing of fertilizer application (Strickland et al., 2010).

Further, in large lakes, spring thermal bars (~4 C sinking isotherm) can trap coastal waters, reduce mixing with the open lake (Csanady, 1972a), and may lead to a retention of nutrients in nearshore areas during the period when tributaries and surface runoff are most likely to bring in P (Flint & Stevens, 1989). The potential retention of phosphate in nearshore waters would coincide with the initial *Cladophora* growing period.

Another important potential pathway for *Cladophora* to access phosphate is through internal recycling in the lake via invasive dreissenid mussels. Dreissenid mussels were first reported from the lower Great lakes in the late 1980's (Griffiths et al., 1991) and currently cover substantial areas throughout Lakes Erie, Michigan and Ontario. They also grow in the upper Great Lakes, but at much lower densities. Like *Cladophora*, *Dreissena* uses hard substrate for attachment, and is often found to co-occur with nuisance densities of the algae. In fact, *Cladophora* grows directly on the mussels, which thereby can increase the surface area available for algal attachment (Hecky et al., 2004; Ozersky et al., 2009). *Dreissena* also effectively clears the water column of plankton and other particles, enhancing light penetration (Fahnenstiel et al., 1995; Dobiesz & Lester, 2009), and increasing habitat for *Cladophora* by extending it to greater depths (Zhu et al., 2006; Malkin et al., 2008; Auer et al., 2010). In addition to improving water transparency through filter-feeding, dreissenid mussels mediate the conversion of particle P to available P in near-bottom waters. Mussels excrete SRP at high rates (e.g., Arnott & Vanni, 1996; Conroy et al., 2005) potentially relaxing P-limitation for *Cladophora.* A study by Ozersky et al. (2009) in Lake Ontario demonstrated that the SRP in dreissenid excreta was enough to sustain *Cladophora* growth and actually exceeded its demands within a defined area. Mussels also egest feces and release pseudofeces that may be remineralized to phosphate. The hypothesis that mussel-beds promote *Cladophora* growth through an enhanced interception, retention and recycling of nutrients in the littoral zone of the Great Lakes is referred to as the "nearshore phosphorus shunt" (Hecky et al., 2004). *D. bugensis* successfully colonize both coastal and profundal sediments, but their impact is likely greater in the warmer and well-mixed nearshore, especially where the lake bottom is rocky (Hecky et al., 2004).

1.3 Thesis Overview

This thesis examines soluble reactive phosphorus in a nearshore segment of Lake Ontario. It was undertaken to evaluate potential sources of littoral phosphorus enrichment supporting excessive benthic algal production. It is composed of two data chapters (Chapters 2 and 3), each written as a discrete study. The objective of Chapter 2 was to test the ability of soluble reactive phosphorus (SRP) to measure phosphate because SRP is often an overestimate of PO_4^{3-} in P-limited waters. This was accomplished by comparing measures of SRP made by the standard assay (with gentle filtration) with those made by modified versions designed to more accurately measure phosphate when concentrations were expected to be low (i.e., dialysis, preconcentration). The SRP results were then compared with a sensitive $ssPO_4$ radiobioassay for orthophosphate (Hudson et al., 2000), performed for this purpose in offshore waters. Based on the results of Chapter 2, the aim of Chapter 3 was to assess the relative impacts of allochthonous inputs and *Dreissena* on local phosphate concentrations supporting *Cladophora* growth. This was achieved with intensive sampling for SRP in time and space within the Lake Ontario study area. The turnover time of phosphate was also measured in surface waters on some occasions and Chl *a* was included in water column analyses.

Chapter 2 The utility of SRP as a measure of phosphate available to *Cladophora*

2.1 Introduction

The return of nuisance *Cladophora* growth in the lower Great Lakes has instigated a call for a revision of the Great Lakes Water Quality Agreement to better address nearshore eutrophication issues. Amendments to the GLWQA will be based on a sound understanding of nearshore P dynamics, which appear to have changed since the Agreement was last updated over twenty years ago. Despite oligotrophic conditions in the open-lake, nearshore areas may be experiencing reduced P-limitation, either intermittently as PO_4^{3-} is delivered by storms, or continuously from point sources or from the benthos, where invasive dreissenid mussels are hypothesized to be enhancing the interception, retention, and recycling of P (Hecky et al., 2004). Monitoring programs and model approaches predicting *Cladophora* growth in relation to measured ambient P concentrations generally do not take into account the effect of benthic activity or any distinctive cycling pathways in the nearshore (Hecky et al., 2004) and these assessments rely on soluble reactive P (SRP) as a measure of P directly available to *Cladophora*, despite the tendency of SRP to overestimate true phosphate concentrations in P-limited systems (Rigler, 1966; Stainton, 1980; Bentzen & Taylor, 1991; Taylor & Lean, 1991; Fisher & Lean, 1992; Dodds, 1993; Hudson et al., 2000; Dodds, 2003).

In the Murphy and Riley molybdenum blue method, PO_4^{3-} reacts with ammonium molybdate under acidic conditions and is reduced to produce the phosphomolybdenum blue complex which is determined by absorption spectrophotometry. However, most of measured SRP in P-deficient environments is believed to be derived from colloidal P liberated from plankton upon sample filtration (e.g., Hudson et al., 2000; Taylor, 2010) that undergoes hydrolysis by the acidic reagents used in the assay (Stainton, 1980; Tarapchak et al., 1982). The method's high detection limit and the potential for interference by arsenate (e.g., Chamberlain & Shapiro, 1969) and silicate (e.g., Neal et al., 2000) also hinder the ability of SRP to accurately measure phosphate. Estimating P availability for *Cladophora* solely from SRP in P-limited systems under the assumption that it represents phosphate may lead to inaccuracies in models. However, these interferences, including interferences by other dissolved forms of phosphorus, are more likely to be significant when PO_4^{3-} is present at nanomolar or subnanomolar concentrations. It may be that SRP overestimates true phosphate concentrations in open-lake surface waters during much

of *Cladophora*'s growing season, but is a good approximation of phosphate in waters influenced by the benthos or during local input events because of the high fraction of phosphate in animal excreta (e.g., from dreissenid mussels) and in wastewater and stormwater. If this is the case, standard SRP sampling procedures from surface waters during the growing season may actually underestimate phosphate available to *Cladophora.* This could also hinder the ability of model simulations to accurately predict its response to P management strategies.

Identifying how SRP indicates PO_4^{3-} in the current nearshore environment should shed light on the issues described above. Much of the value of using SRP lies in the relative ease of the analytical procedure, especially compared to more sensitive methods to estimate phosphate concentrations, such as those involving the use of radiotracers (e.g., Hudson et al., 2000). While radioassay methods are necessary for accurate interpretation of P-cycling in lakes and can provide estimates of orthophosphate concentrations orders of magnitude lower than SRP in P-limited waters, a drawback specific to the study of this problem is that they are based on phosphate kinetics in the near steady-state conditions of pelagic communities. This means they are not applicable to nearshore areas where P dynamics are influenced by the benthos. Modifications to the standard SRP assay may be enough to better understand P dynamics in the Great Lakes. Dialysis membranes to passively separate the particulate pool from the dissolved pool offer a means of measuring orthophosphate for comparison with conventional SRP determinations. Dialysis membranes are often used to study dissolved P in pore water studies (e.g., Hesslein, 1976) and have been used to study dissolved P species in lake water (Fisher & Lean, 1992; Taylor, 2010). Taylor (2010) studied dissolved P in filtrates and dialyzates from lake water in which PO_4^{3-} was low and turning over quickly, and found that the colloidal P fraction present in < 0.2 µm-filtrates did not exist in < 100, 000 MW cutoff dialyzed fractions. Taylor (2010) also found low-molecular weight DOP (XP) consisting of nucleotides and other small biological molecules in both fractions, but at concentrations lower than PO_4^{3-}. Thus, the advantage of dialysis is that the filtration artifact of PO_4^{3-} associated with the traditional SRP method can be avoided. Another modification to the standard SRP assay involves concentrating dissolved P in the filtrate (or dialyzate) before colourimetry to achieve lower method detection limits. In a method known as "MAGIC" (i.e., magnesium-induced co-precipitation), dissolved P is quantitatively removed from solution by in vitro formation of brucite [$Mg(OH)_2$], initiated by the addition of NaOH. The sample is then centrifuged and the pellet is dissolved in weak HCl

and analyzed for SRP. The technique was developed for seawater (Karl & Tien, 1992) but has recently been modified for use in freshwaters with the addition of a source of Mg (Anagnostou & Sherrell, 2008). Dialysis and MAGIC may provide relatively simple means of obtaining measurements of SRP that more accurately reflect PO_4^{3-} in P-limited waters. Other modifications to the standard SRP method not utilized in this study include reducing sample exposure time to the acidic reagents in order to avoid hydrolysis of DOP, using long-path liquid-waveguide capillary cell techniques to lower detection limits (e.g., Li & Hansell, 2008a), and applying corrections for potential interferences (e.g., Koroleff, 1983).

This Chapter attempts to identify how well SRP indicates PO_4^{3-} in the study area. This was done by comparing measures of phosphate made by the traditional SRP assay with those made by modified versions designed to more accurately measure phosphate when concentrations were expected to be low. I hypothesized that estimates of phosphate made by the traditional SRP assay would be higher than those made by modified SRP methods that substitute dialysis for filtration and use MAGIC to lower method detection limits. I predicted that the modified versions would yield estimates more comparable to those made by the steady-state PO_4 radioassay (Hudson et al., 2000), performed for comparison with SRP results in offshore waters.

2.2 Methods

2.2.1 Site description

This study was carried out over two field seasons and in three different study areas. In 2009, samples were obtained from a number of nearshore sampling stations (2 m to18 m depth) and an offshore station (42 m depth) within a segment of northwestern Lake Ontario, located to the east of Toronto, near Pickering, Ontario (43.80°N, 79.07°W; Figure 2-1; Figure 3-1). Details of the study area are described in full in Chapter 3. In 2010, Lake Ontario water was collected from an offshore station (68 m depth) near Burlington, Ontario (43.29°N, 79.28°W; Figure 2-1). On one occasion in 2010, lake water was collected from Lake of Bays (45.18°N, 79.00°W), which is an oligotrophic shield lake located approximately 190 km north of Toronto.

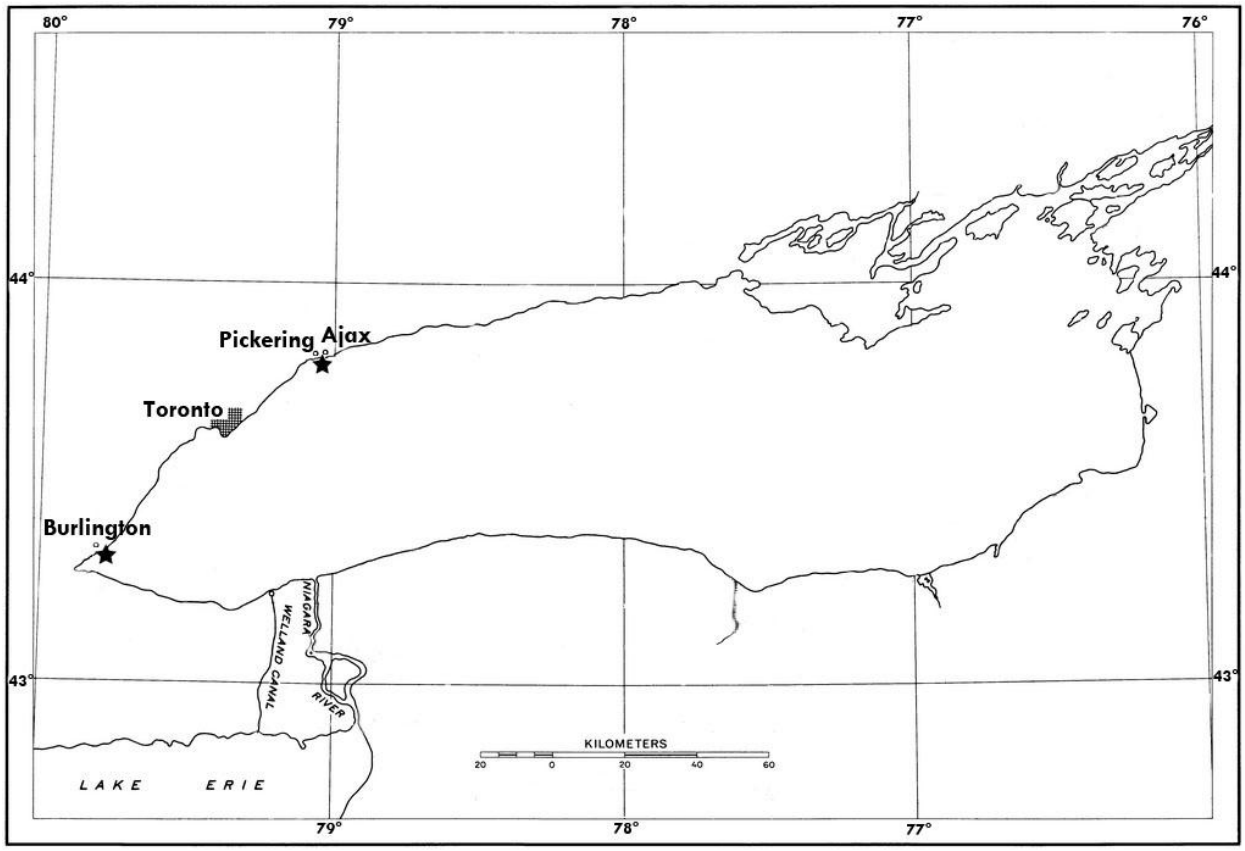


Figure 2-1. Map of Lake Ontario showing sampling locations (adapted from the National Oceanic and Atmospheric Administration).

2.2.2 2009 Sampling procedures

Sample collection for filtered and dialyzed samples occurred once in May, twice in June, and once in October at one or more of the seven stations within the study area, from various depths in the water column (e.g., from 3 m below the surface to 15 cm above the lake bottom). Sample collection for the steady-state PO_4 radiobioassay occurred once a month from June to August from 3 m at the offshore station only. Triplicate (for filtrate SRP) and duplicate (for ssPO_4) samples were removed from the lake with Niskin casts or pumped through tubing, depending on the depth, and were transferred to 60-mL or 100-mL (for filtrate SRP) or 1-L (for ssPO_4) 10 % HCl-washed HDPE-bottles that had been rinsed with sample water, and were stored at ambient temperature.

At stations and depths corresponding with those collected with Niskin casts or pumped through tubing, the *in situ* dialysis procedure was conducted in the following way. Triplicate lengths (30 cm) of dialysis membrane (Spectra/Por Biotech cellulose ester, 100,000 MW cutoff, 32-mm flat width) were filled with de-ionized water, closed at both ends and suspended in open aluminum and plexi-glass frames. They were stored submerged in de-ionized water in a cooler to be transferred to the lake, where they were incubated for 3 to 4 h. This length of time was determined to be sufficient for internal orthophosphate in the dialysis tubing to equilibrate with ambient conditions in laboratory experiments where tubing was placed in beakers or carboys of KH_2PO_4-enriched lake-water. After incubation in the lake, the resulting dialyzed samples were carefully decanted into 60-mL 10 % HCl-washed HDPE bottles and stored at ambient temperature.

2.2.3 2010 Sampling procedures

Once in June and twice in July, water from offshore Lake Ontario was pumped from 3 m into 10 % HCl-washed 20-L polyethylene containers and stored at ambient temperature. Once in June, water from Lake of Bays was removed from 1 m below the surface with a Van Dorn sampler and transferred into a 20-L (for SRP) and two 4-L (for ssPO4) 10 % HCl-washed polyethylene containers and stored at ambient temperature.

2.2.4 2009 Laboratory procedures

SRP and MAGIC

Subsamples (30 mL) from lake water collected with Niskin casts or pumped through tubing were filtered (<12 h of collection) at low pressure (<100 mm Hg), through 0.2-µm cellulose-ester filters to separate dissolved P from particulate P. Approximately 60-mL to100-mL of sample was added to the filter manifold so the sample was not filtered entirely; this was an effort to reduce cell breakage and overestimation of SRP. Filtrates were maintained at 4 C until the analysis (<24 h of collection) with the molybdenum-blue method of Murphy and Riley (1962) and Stainton et al. (1977). The reagent blank was prepared with Milli-Q distilled, de-ionized water and its absorbance was subtracted from all samples. Milli-Q distilled, de-ionized water was used to zero the instruments and to measure the range of instrumental noise. Most samples were analyzed with a Cary spectrophotometer in a 10-cm path length, reduced-volume cell.

However on some occasions, an Ultrospec spectrophotometer with a 5-cm path length cell was used instead, which raised the detection limit. The detection limits, taken as equal to 2 × the SD of the average instrumental noise (measured several times on separate occasions using samples of Milli-Q distilled, de-ionized water) and using the orthophosphate absorbance coefficient, were 0.3 µg P L^{-1} (10 nM) and 0.6 µg P L^{-1} (20 nM) for the two spectrophotometers respectively.

Dialyzed samples were not filtered (as they already contained only dissolved species <100,000 MW) and were frozen in the laboratory at approximately –16 C until analysis. Dialyzed samples were predicted to contain SRP below the analytical detection limit and were pre-concentrated before colourimetry using a magnesium-induced coprecipitation (MAGIC) procedure modified from Anagnostou and Sherrell (2008). The MAGIC procedure used in this study is as follows. Samples were poured into acid-leached, 50-mL centrifuge tubes followed by the addition of 1-mL MAGIC-cleaned artificial seawater (Instant Ocean) supernatant (Mg source) and 150-µL 1 M NaOH. The tubes were mixed vigorously, allowed to stand for ~25 min, and centrifuged for 5 min at ~7000 g. The supernatant was immediately decanted and the resulting brucite pellets were dissolved in 400-µL to 1-mL of 0.2 M HCl. The dissolved pellets were then vortex-mixed and brought to 5 mL with distilled, de-ionized Milli-Q H_2O. Samples were promptly processed using SRP colourimetry following Murphy and Riley (1962) and Stainton et al. (1977). For some samples (those from October), the MAGIC step was performed on subsamples of the dialyzates so that non-preconcentrated dialyzates could be used in a comparison analysis. The MAGIC procedural blank was assumed equal to the SRP determination on MAGIC pellets precipitated from Milli-Q water. Determined in this way, the blank included the reagents of both MAGIC and SRP colourimetry. Its absorbance was subtracted from all samples. Milli-Q distilled, de-ionized water was used to zero the instruments. All dialyzed samples were analyzed with a Cary spectrophotometer in a 10-cm path length, reduced-volume cell. The detection limit of the MAGIC method, taken as equal to 2× the SD of dissolved MAGIC pellets precipitated from Milli-Q water (measured several times on separate occasions), corrected for the concentration factor (~10), and using the orthophosphate absorbance co-efficient, was: 0.04 µg P L^{-1} (1.25 nM).

Steady – state radiobioassy

A steady-state estimate of phosphate ($ssPO_4$) can be calculated from the uptake constant and regeneration rate of dissolved P by the planktonic communities in lake water (Hudson et al., 2000). These were measured in three summertime samples from the offshore station. The uptake constant (*k*) was determined as follows. Samples (50 mL) from the original 1-L of lake water were injected with carrier-free $^{32}PO_4$ and subsamples (1 mL) were then filtered through 0.2-µm, 25-mm polycarbonate filters at successive times following isotope addition. Scintillation fluor was added and the radioactivity of the plankton on the filters was measured, as was the radioactivity of unfiltered samples. The natural logarithm of the percentage of isotope left in solution was regressed against time and the initial slope was used to approximate the rate constant (*k*) for PO_4^{3-} uptake. To determine the regeneration rate (R) of dissolved P, the technique described by Hudson and Taylor (1996) was followed. Carrier-free $^{32}PO_4$ was added to 500-mL of sample water to label the plankton and the sample was incubated for 24 h. Subsamples were taken to determine total ^{32}P. After incubation, uptake of ^{32}P was stopped by adding 1 mg P L^{-1} as $^{31}PO_4$, competitively inhibiting further $^{32}PO_4$ uptake and marking the start of the postincubation period. Subsamples were filtered by drawing 1 mL aliquots through 0.2 µm nylon cartridge filters and assayed for dissolved ^{32}P every hour for 5 h. The slope of dissolved ^{32}P radioactivity plotted against post-incubation time was used as an estimate of the release rate of dissolved ^{32}P, which was converted to an estimate of the phosphorus regeneration rate (R) when multiplied by the concentration of total phosphorus and divided by the total radioactivity. The $ssPO_4$ was calculated from the regeneration rate and uptake constant, i.e. $ssPO_4 = R/k$. The units for $ssPO_4$ are the same as those used for total phosphorus.

2.2.5 2010 Laboratory procedures

SRP (in filtrate and dialyzate) and MAGIC

Triplicate-lengths (30 cm) of dialysis membrane (Spectra/Por Biotech cellulose ester, 100,000 MW cutoff, 32-mm flat-width diameter; rinsed and soaked for 30 min in Milli-Q distilled, de-ionized water) were filled with de-ionized water and placed in the 20-L polyethylene containers of lake water. After a four-hour incubation period, the resulting dialyzates were carefully poured from the tubing so that each sample was divided into two 50-mL centrifuge tubes (one for SRP with MAGIC, one for SRP without MAGIC). Triplicate samples (200 mL) were removed from the 20-L polyethylene containers and subsamples (30 ml, 50 mL) were filtered through 0.2-µm cellulose-ester filters under low pressure (<100 mm Hg) into 50-mL centrifuge tubes for standard SRP colourimetry and for SRP colourimetry with MAGIC-preconcentration. The MAGIC procedure was based on that by Anagnostou and Sherrel (2008) and is described above for 2009 samples. Analysis for SRP was carried out on all samples according to Murphy and Riley (1962) and Stainton (1977) with a Cary Bio 100 spectrophotometer in a 10-cm path-length, reduced-volume cuvette, with a detection limit of 0.3 µg P L^{-1} (10 nM) for non-preconcentrated samples and 0.04 µg P L^{-1} (1.25 nM) for MAGIC preconcentrated samples determined as described above for 2009 analyses.

Steady – state radiobioassy

$ssPO_4$ was estimated in Lake of Bays water as described above for 2009.

2.2.6 Statistical analyses

Statistical analyses were performed with SPSS v. 17 (IBM, Chicago, Il, USA). The effects of method (and site and date interactions) on SRP concentrations were determined with three-way analyses of variance. Differences were deemed statistically significant at an alpha level of 0.05. SRP below the detection limit, including values less than 0.0 µg P L^{-1} (when absorbance was measured as negative) were not truncated to zero because to do so would bias the results. However, caution must be taken when interpreting values below detection limit.

2.3 Results

2.3.1 SRP in filtered and dialyzed lake water

The SRP concentrations measured in filtrate (<0.2 µm) and in dialyzate (<100,000 MW) collected from nearshore and offshore Lake Ontario (and once from Lake of Bays) were similar, although SRP replicates in dialyzed samples were often more variable (Figure 2-2). There was no significant difference between SRP concentrations obtained with the two methods ($F = 0.084$, $P = 0.773$; three-way ANOVA for method, date and station). However, a significant date-method interaction term ($F = 3.891$, $P = 0.003$) indicated differences between methods varied with date. There was also heterogeneity of variance among dates and sites. A number of high outliers (> 3 SE from the mean of the other two replicates of a triplicate-set of samples) were encountered in the 2009 dialyzate samples and were removed before statistical analyses were performed.

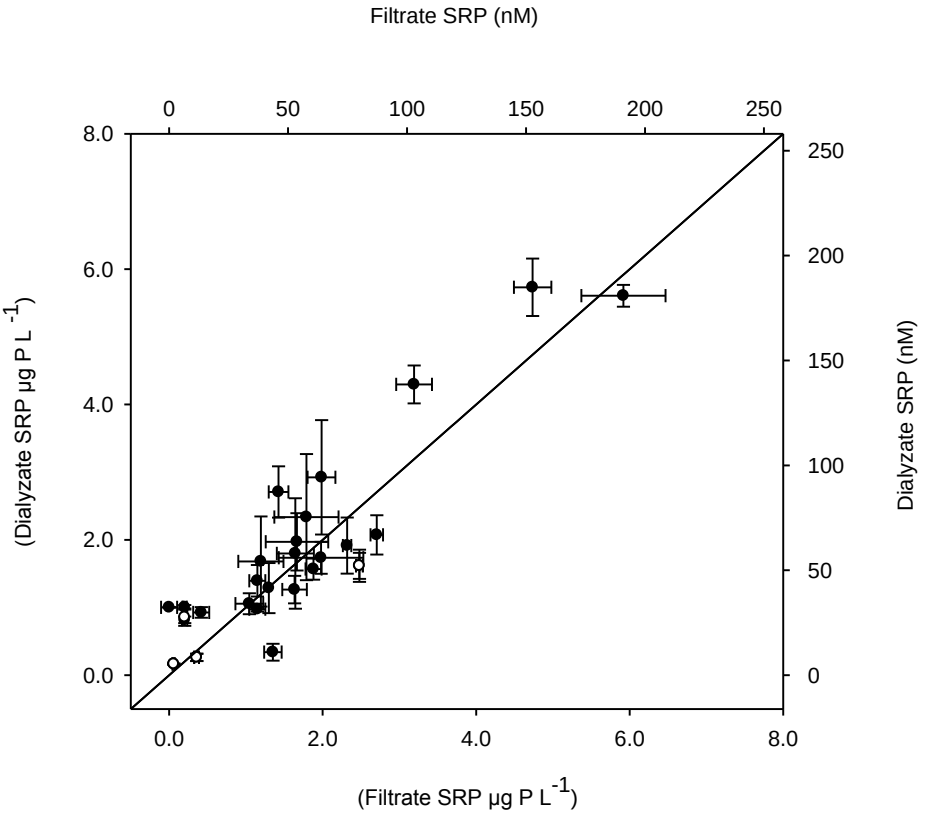


Figure 2-2. Comparison of mean SRP concentrations in filtered and dialyzed samples. The solid circles are 2009 samples when dialysis membranes were incubated in nearshore and offshore Lake Ontario and the open circles are 2010 samples when dialysis membranes were incubated in a 20-L container of offshore Lake Ontario (or Lake of Bays) water. ── is the 1:1 line. Error bars represent standard error.

2.3.2 SRP in lake water with and without MAGIC preconcentration

SRP concentrations measured in water samples preconcentrated with MAGIC before colourimetry were not different from SRP concentrations measured in water samples without the MAGIC preconcentration step on most occasions. Both methods displayed higher error in 2009 than in 2010 (Figure 2-3 and Figure 2-4). Three-way ANOVAs (for method, date and station) found no significant difference between SRP concentrations measured by the two methods in 2009 ($F = 0.018$, $P = 0.895$) or in 2010 ($F = 0.057$, $P = 0.813$), though there was heterogeneity of variance among dates and sites for both years.

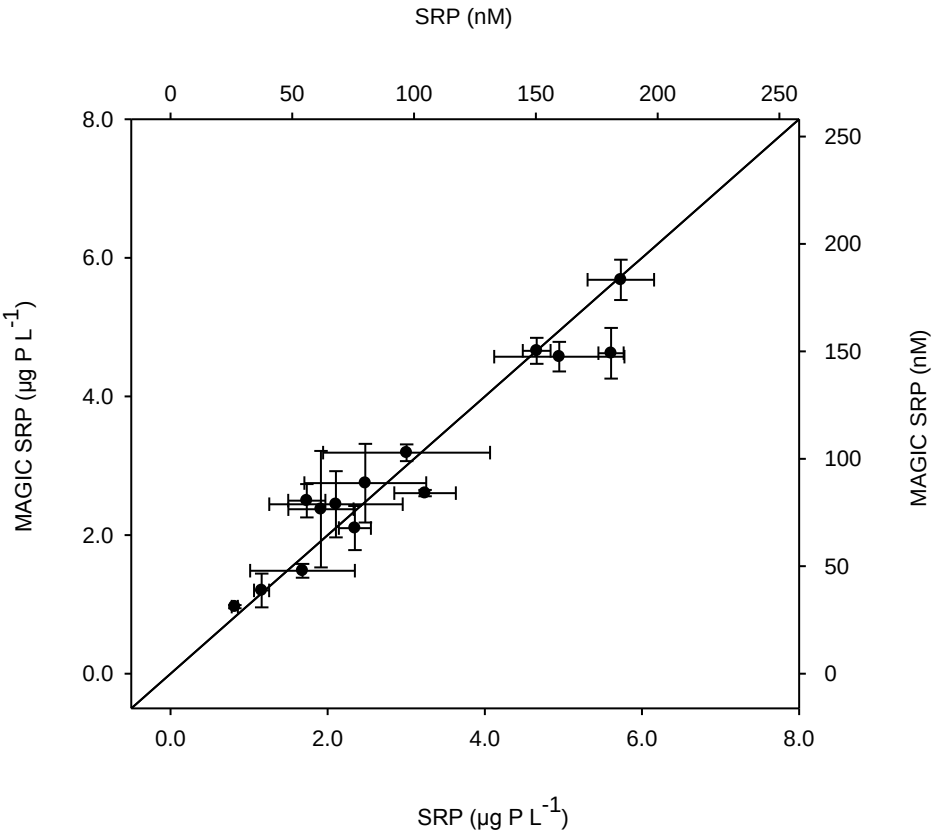


Figure 2-3. Comparison of mean SRP concentrations in samples with and without MAGIC preconcentration prior to colourimetry. Data are dialyzed samples incubated at nearshore and offshore stations in Lake Ontario on October 4th, 2009. ── is the 1:1 line. Error bars represent standard error.

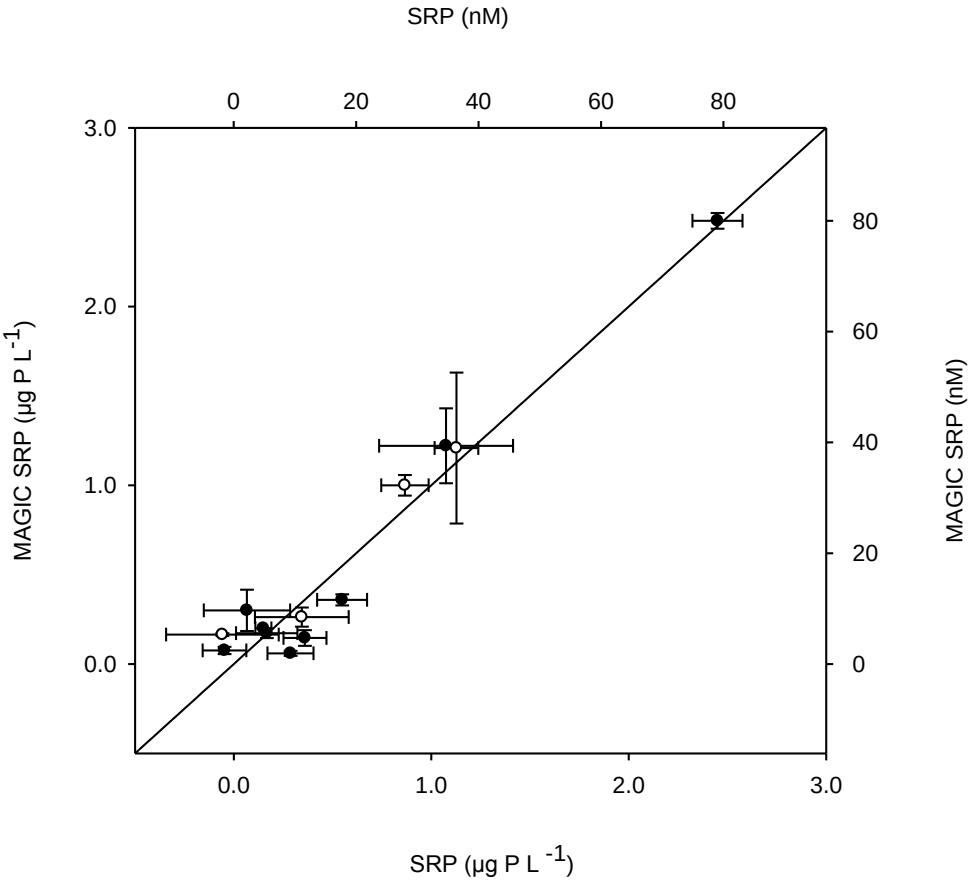


Figure 2-4. Comparison of mean SRP concentrations in samples with and without MAGIC preconcentration prior to colourimetry. The solid circles are filtered samples and the open circles are dialyzed samples from a 20-L container of offshore Lake Ontario (or Lake of Bays) water. ── is the 1:1 line. Error bars represent standard error.

2.3.3 SRP and the steady-state estimate of PO_4 in offshore lake water

Phosphate concentrations measured as SRP in filtered and dialyzed samples were 1-3 orders of magnitude higher than $ssPO_4$ at the offshore station, except on August 19th, 2009 (but SRP in filtrate was below the method detection limit). SRP in filtered samples without MAGIC-preconcentration was below detection limit on all dates (Table 2-1).

Table 2-1. Comparison of mean SRP ± 1SD in dialyzed and filtered samples and mean $ssPO_4$ ± 1SD for corresponding 2009 offshore Lake Ontario samples and a 2010 Lake of Bays sample. F = filtered samples, D = dialyzed samples, $_{MAGIC}$ = MAGIC preconcentrated, nd = no data.

Date Lake	Units	**SRP** F	F_{MAGIC}	D	D_{MAGIC}	**$ssPO_4$**
		n = 2			n = 2	n= 2
22/06/09	µg P L^{-1}	0.418 ± 0.181***	nd	nd	0.864 ± 0.135*	0.012 ± 0.000
Lake Ontario	nM	13.484 ± 5.389			27.871 ± 4.032	0.387 ± 0.000
		n = 3				n = 2
21/07/09	µg P L^{-1}	0.105 ± 0.181***	nd	nd	nd	0.013 ± 0.004
Lake Ontario	nM	3.387 ± 5.389				0.419 ± 0.129
		n = 3				n = 2
19/08/09	µg P L^{-1}	0.042 ± 0.151**	nd	nd	nd	0.064 ± 0.010
Lake Ontario	nM	1.355 ± 4.871				2.065 ± 3.226
		n = 2	n = 2	n = 3	n = 3	n = 2
30/06/10	µg P L^{-1}	0.152 ± 0.001**	0.202 ± 0.040*	0.857 ± 0.222**	1.002 ± 0.059*	0.005 ± 0.000
Lake of Bays	nM	4.903 ± 0.032	6.516 ± 1.290	27.625 ± 7.161	32.323 ± 1.903	0.161 ± 0.000

*detection limit = 0.04 µg P L^{-1}, ** detection limit = 0.3 µg P L^{-1}, *** detection limit = 0.6 µg P L^{-1}

2.4 Discussion

It was important to identify how well soluble reactive phosphorus represents PO_4^{3-} available to *Cladophora*, which is P-limited in the Great Lakes. The modified versions of the SRP assay used in this study were expected to improve the ability of SRP to measure phosphate. However, significantly different concentrations of SRP were not encountered with the use of dialysis in lieu of filtration or by preconcentrating samples prior to SRP colourimetry. Although all methods were capable of measuring low concentrations of SRP in offshore waters, most of these measures of SRP were below its detection limit and still 1-3 orders of magnitude higher than the steady-state estimate of PO_4 when chemical and radiobioassay methods were compared directly. These results suggest that if care is taken to filter gently and if samples are not filtered to dryness, SRP is an adequate measure of phosphate when concentrations are high. However, SRP is a poor measure of phosphate when its concentrations are below the SRP method detection.

The results from the dialysis-filtration comparisons are consistent with those from recent laboratory studies of natural waters containing high levels of SRP. In lake water with SRP concentrations ranging from near detection to > 100 µg P $L,^{-1}$ no difference was found between dialyzed samples, vacuum filtered samples, or syringe filtered samples (J.M. Sereda, University of Saskatchewan, pers. comm.). A similar study compared filtered and dialyzed water collected from different tributaries of Lake Simcoe and demonstrated that, while lower SRP was measured in dialyzed samples, the difference in SRP concentrations between the two methods was very small (R.L. North, University of Trent, pers. comm.). One difference between the results of these studies and my data is that I measured higher SRP concentrations in dialyzed samples than in filtered samples on some days. One explanation for this unexpected result may be that when dialysis membranes were incubated in the lake, dialyzates were not collected simultaneously with the water that was to be filtered; there was often a span of one to three hours in between the collection of each sample type at a given station. Further, filtration generally occurred between 8-12 hours following collection. Ambient P in these samples may have been biologically assimilated in the time prior to filtration, rendering SRP lower than in dialyzed samples, in which phosphate was separated from organisms *in situ*. Another explanation is the possibility of interference or contamination by substances on the dialysis tubing to contribute to measured SRP in dialyzate. The potential for the dialysis method to lead to higher measures of SRP requires

further examination. Overall, it appears that gentle filtration and not filtering samples to completion was sufficient to achieve the lowest measures of SRP in this study.

Offshore SRP was very low, generally within the range of 2 to 40 nM when MAGIC preconcentration was applied. These measurements are comparable to those made in ultraoligotrophic systems, such as western Lake Superior and the Sargasso Sea, where SRP has been found to be between <1 nM and 10 nM (Wu et al., 2000; Cavender-Baresa et al., 2001; Anagnostou & Sherrell, 2008; Li & Hansell, 2008b) and the oligotrophic North Pacific, where SRP is often measured on the order of 20–50 nM (Karl, 2000) using MAGIC or long-path liquid-waveguide capillary cells. Still, offshore SRP measured with both traditional and modified methods overestimated $ssPO_4$ by 1-3 orders of magnitude in all cases except in on August 19th, 2009, though SRP was well below its detection limit on this date. The corresponding steady-state estimate of PO_4^{3-} on August 19th, 2009 was also the only value over the range measured in the original application of the steady-state radiobioassay (i.e., 0.027- 0.885 nM) by Hudson et al. (2000). The authors surveyed 14 lakes and found SRP to be 2-3 orders of magnitude higher than $ssPO_4$, but that they were correlated. In a subsequent survey of 7 different lakes, Nowlin et al. (2007) measured $ssPO_4$ from 0.087 to 0.611 nM and also found that SRP was 2–3 orders of magnitude higher, but that there was no correlation between the two estimates. SRP and $ssPO_4$ were not correlated in this study either, indicating that, while SRP overestimated PO_4^{3-}, it did not consistently overestimate PO_4^{3-}.

Chemical methods and radiotracer methods are very difficult to compare, even with modifications that lower detection limits. It has been known for more than 40 years that SRP assays do not measure only PO_4^{3-} (e.g., Rigler, 1966; Bentzen & Taylor, 1991; Hudson et al., 2000). What the SRP measurement represents, however, has not been well documented. The hypothesis that the SRP pool is largely composed of DOP generated by filtration was not supported by the results of this study, likely because samples were filtered at low pressure and not to dryness. The steady-state method (Hudson et al., 2000) assumes that dissolved P regenerated by the plankton community is mostly in the form of phosphate or low molecular weight organic compounds that are quickly hydrolyzed by phosphatases (Lean & Nalewalko, 1979; Taylor & Lean, 1991). As dialysis membranes permit the entry of P species under ~100,000 MW, it could be suggested that dialyzate also contains DOP that does not get labeled during ^{32}P-based analyses, but contributes to SRP. However, as DOP is labeled by ^{32}P in

filtrates, this seems unlikely, and even if it is the case, SRP is still an overestimate of PO_4, whether in dialyzate or filtrate. $ssPO_4$ may underestimate PO_4^{3-} if the ^{32}P released does not contain the slowly turning over P that chemical methods detect. While this is likely true to some extent, it would not be able to explain the differences between $ssPO_4$ and SRP observed in this study.

The method detection limit prevents the standard SRP method from measuring concentrations as low as can be measured as $ssPO_4$. MAGIC-concentrated SRP was not used in two of the four SRP- $ssPO_4$ comparisons (July 21st and August 19th, 2009) but improving the detection limit by a factor of 10 with MAGIC did not lead to lower SRP than obtained with the standard method, and MAGIC-concentrated SRP was not in the range of estimates of phosphate made by the steady-state radiobioassy in offshore waters on the days they were compared directly. Other than the fact that $ssPO_4$ was still below the MAGIC detection limit, the reasons for this are unclear. It is possible that MAGIC pellets were concentrating some forms of PO_4^{3-} contamination that were not accounted for by analytical blanks as it is very difficult for blanks to encompass all potential sources of contamination. The proper application of a procedural blank should account for all phosphate contamination from the field (equipment, sample bottles, atmosphere, etc.) and in the laboratory (filters, sample bottles, reagents, etc.). The procedural blanks in this study accounted for the reagents of SRP colourimetry (and MAGIC when applicable) and some procedural phosphorus contamination. However, they would have not included contamination from filters, dialysis membranes, or contamination derived from field procedures. Such contamination may have contributed to measured SRP when MAGIC was used, but it is less likely it was problematic beyond the standard SRP detection limit.

Another possible explanation for the disparity between chemical and radiotracer methods is the potential for interferences by arsenate, silicate or coloured dissolved organic matter to contribute to SRP. The freshwater MAGIC method described by Anagnostou and Sherrell (2008) underwent considerable revisions to reduce these interferences in samples from Lake Superior waters. Arsenate reacts with molybdate in a similar way as phosphate and the reaction rate of the arsenomolybdenum complex is catalyzed by the presence of orthophosphate ions (Anderson & Bruland, 1991). Arsenate interference can be overcome by reduction of arsenate ($H_2AsO_4^-$) to arsenite ($As(OH)_3$) with thiosulphate, but this step also increases precipitation of colloidal sulfur in lake water samples (Koroleff, 1983). Recent investigations of arsenate

interference in seawater demonstrated that arsenate interference increases linearly with phosphate concentrations and that an arsenate concentration of 20 nM would lead to an overestimation of ~4.2 to 4.6 % by SRP (Patey et al., 2010). Arsenate has been measured in Lake Superior at 15 nM (Anagnostou & Sherrell, 2008) and in Lake Ontario at ~7 nM (Anderson & Bruland, 1991). According to Patey et al. (2010), if arsenate concentrations were 20 nM, a sample containing 5.0 nM PO_4^{3-} would be measured as ~5.3 nM SRP, and a sample containing 50.0 nM PO_4^{3-} would be measured as ~52.2 nM SRP. According to these estimates, though it is possible arsenate contributed to a slight overestimation of SRP relative to $ssPO_4$, it would be not be able to account for the difference between the two methods, especially when SRP in offshore waters was measured to be on the order of 30 to 40 nM, much higher than $ssPO_4$.

Silicate ($Si(OH)_4$) can also form complexes with molybdenum. According to Koroleff (1983), silicate interference is negligible for silicate concentrations up to 200 µM (15.20 mg L^{-1}), if absorption is measured within 10 to 30 min of the reagent addition. However, silicate concentrations of 100 µM (7.60 mg L^{-1}) have been shown to increase the measured SRP concentration by 36 ± 19 nM (Patey et al., 2010), and silicate concentrations of 42 µM (~3.20 mg L^{-1}) in Lake Superior were found to contribute 17 nM to the SRP signal (Baehr & McManus, 2003). A recent lake-wide assessment in Lake Ontario by Holeck et al. (2008) reported a mean silicate concentration during the summer of 3.5 µM (0.27 mg L^{-1}), with a range of 1.3 to 13.4 µM (0.10-1.02 mg L^{-1}), and did not find significant differences in silicate concentrations in nearshore and offshore waters. This wide range is identical to the range of silicate measured from May to September, 2008 in the same nearshore region where this study took place, although the mean concentration of 7.4 µM (0.56 mg L^{-1}) was higher (S.Y. Malkin, University of Waterloo, pers. comm.). According to the relationship between silicate interference and SRP described in Patey et al. (2010), silicate at its lower range in Lake Ontario (1.3 µM) would increase SRP by 0.68 nM ± 0.13 nM, the mean silicate concentration at the nearshore study station (7.4 µM) would contribute an additional 3.8 ± 0.74 nM to SRP, and silicate at the upper range (13.4 µM) would raise measured SRP by 7.0 ± 1.34 nM. Further, only 10% of silicate is recovered in MAGIC pellets (Anagnostou & Sherrell, 2008). Like arsenate, silicate may have interfered with the SRP signal in this study, although it is unlikely that it was contributing to SRP beyond its detection limit when samples were not preconcentrated. When MAGIC was applied, silicate interference would not account for the difference between SRP and $ssPO_4$.

Coloured dissolved organic matter (CDOM) may cause optical interferences. Anagnostou and Sherrel (2008) encountered CDOM in MAGIC pellets precipitated from coastal samples from Lake Superior, thought to originate from terrestrial/ anthropogenic sources. I did not investigate absorbance of filtered nearshore water samples without colourimetric reagents, so coastal SRP measurements in this study may have been affected by CDOM. However, it is less likely that CDOM contributed to the SRP signal in summer offshore Lake Ontario samples when SRP and $ssPO_4$ were compared. Filtered lake water without colourimetric reagents (including samples precipitated in MAGIC pellets) had no observed absorbance above distilled, de-ionized Milli-Q water. Overall, the variability in the size of the discrepancies between $ssPO_4$ and SRP make it difficult to attribute overestimation of PO_4^{3-} by SRP to any given interference, especially when their potential effects can only be inferred. It may be that there is simply no analytical method that would be practical when PO_4^{3-} is small and turning over quickly.

Putting these findings together, I suggest SRP in gently-filtered lake water is useful to indicate phosphate concentrations, but only when phosphate is at high levels. What is "high" may vary across systems, but it is unlikely that measures < 1 µg L^{-1} by SRP are accurate estimates of phosphate. As the range of SRP concentrations to which *Cladophora* growth is thought to be responsive are also as low as ~1.0 µg P L^{-1}, there are limitations to using SRP to study this problem. Still, SRP is useful to measure localized areas of phosphate enrichment and its relative concentrations can be compared spatially. Informed by these conclusions, the next Chapter will attempt to identify sources of phosphate for C*ladophora* through its growing season by measuring SRP over a fine spatial scale within a nearshore segment of Lake Ontario.

Chapter 3 The distribution of SRP in nearshore Lake Ontario: Evidence for the roles of allochthonous sources and dreissenids in supplying phosphate for *Cladophora*

3.1 Introduction

Despite the success of phosphorus loading controls in remediating cultural eutrophication problems in the past, nuisance levels of the filamentous green alga *Cladophora glomerata* have returned to the nearshore zones of the lower Laurentian Great Lakes. *Cladophora* grows attached to hard substrate in alkaline waters at temperatures between 13 to 31 C, requires a relatively high light environment with some degree of water motion, and is considered to be P-limited in the Great Lakes (Whitton, 1970; Sheath & Cole, 1992; Higgins et al., 2008b). Although *Cladophora* is present in many temperate freshwater systems (Blum, 1956), problem growths are associated with nutrient enrichment (e.g., Dodds & Gudder, 1992). As the widespread occurrences of excessive benthic algae in the Great Lakes during the 1960's and 1970's were largely attributable to P loading by wastewater treatment plants and industry, regulation of P levels in effluents mandated by the Great Lakes Water Quality Agreement (1972, 1978) proved to be effective (Painter & Kamaitis, 1987).

The resurgence of *Cladophora* over the past 10 to 15 years, however, has occurred while point-source loading of P is managed and total P (TP) concentrations in the open waters of the lakes are at or beneath target concentrations set by international agreement, with the exception of some localized exceedances in Lake Erie (Env. Can. & US EPA, 2009). Lake Ontario has experienced the most dramatic lake-wide declines in both TP and SRP and its offshore waters are consistently below the 10 µg P L^{-1} target for TP (Kwiatkowski, 1982; Lean, 1987; Stevens & Neilson, 1987; Flint & Stevens, 1989; Johengen et al., 1994; Nicholls et al., 2001; Auer et al., 2010; Malkin et al., 2010). Yet, carpets of *Cladophora* cover much of the littoral lake bottom and sloughing events often lead to clumps of decaying algae on beaches and shorelines, or clogging water intakes (Higgins et al., 2005b; Malkin et al., 2008). The unanticipated return of *Cladophora* in the lower Great Lakes has brought about a surge of primary research on the topic (i.e., Higgins et al., 2005b; Higgins et al., 2006; Higgins et al., 2008b; Malkin et al., 2008; Ozersky et al., 2009; Auer et al., 2010; Malkin et al., 2010a; Malkin et al., 2010b; Tomlinson et

al., 2010) and a recent bi-national review of the GLWQA has called for its amendment to more adequately address nearshore eutrophication issues.

Allochthonous inputs (i.e., point sources, tributaries, surface runoff, atmospheric inputs, and groundwater) are the ultimate source of P to the Great Lakes. However, P entering the lakes through municipal wastewater is better managed now than in the past, and long-term data-sets (e.g., Malkin et al., 2010a) suggest P-loading from tributaries (including Duffins Creek, Lake Ontario) has declined since the 1960's, as has the percentage of that P that is SRP. Still, there is also evidence (e.g., Strickland et al., 2010) that the quantity of SRP loaded to the lakes by certain tributaries (e.g., Maumee River, Lake Erie) has actually increased, possibly due to land-use changes that enhance SRP delivery (e.g., minimum tillage and no tillage, subsurface drainage, and changes to methods and timing of fertilizer application). The ability of both point and non-point sources to direct phosphate to the littoral zone should not be overlooked. Further, diffuse allochthonous sources of phosphate such as urban stormwater runoff, more difficult both to quantify and to manage, could be underestimated suppliers of P available to *Cladophora*. In a recent survey along the northern coastlines of Lake Ontario, Houben (2007) reported higher P content in *Cladophora* filaments in proximity to urban areas versus rural areas. Higgins and Howell (2010) recently found sites adjacent to highly urbanized areas supported biomass levels 2-3× higher than sites adjacent to areas with low urbanization, suggesting that elevated nutrient concentrations associated with urbanization can lead to nuisance levels of growth. Finally, spring thermal bars (~4 C sinking isotherm) can reduce mixing with the open lake (Csanady, 1972a) and may lead to a retention of nutrients in the nearshore when tributaries and surface runoff are likely to deliver high P loads (Flint & Stevens, 1989), and coinciding with the initial *Cladophora* growing period.

Another hypothesis to explain excessive growth of *Cladophora* in the current nearshore environment is that the benthic alga benefits from the ability of invasive dreissenid mussels to transform particulate P to phosphate. *Dreissena* grows on the same type of lakebed as *Cladophora* and has colonized much of the lake bottom in the lower Great Lakes, covering 47% of the bottom of Lake Ontario in 2008 (Howell & Makarewicz, 2010). The high densities of dreissenids in these lakes (e.g., mean 3674 ± 2233 SD mussels m^{-2} between 2 m to 15 m depths within a northwestern segment of Lake Ontario, Halton Region, Ontario; Ozersky et al., 2009) could alter nutrient distributions by removing suspended particulate matter through filter feeding,

then excreting dissolved P as well as releasing P-rich feces and pseudofeces in the benthos (Hecky et al., 2004). The shifting of nutrients and the enhancement of light penetration to the littoral benthos is a process termed "benthification" (Zhu et al., 2006), and may be an explanation for the seemingly contradictory observations of nuisance *Cladophora* growth in increasingly oligotrophic systems (Hecky et al., 2004). Elevated dissolved nutrients have been measured above mussel-beds in marine systems. For example, Pfister (2007) found the presence of mussels to locally enhance dissolved phosphorus concentrations and primary production, and a study by Aquilino et al. (2009) demonstrated the ability of mussels to facilitate the growth of seaweed by enhancing nutrient concentrations in the nearby water column on exposed, wave-swept, rocky shores. In Lake Ontario, SRP in dreissenid excreta alone has been shown to exceed P demands for *Cladophora* in Lake Ontario, even during its peak growth period (Ozersky et al., 2009). In terms of management strategies, this could mean that SRP in dreissenid excreta at the lake bottom may negate the benefits of loading reduction and necessitate even more rigorous regulations for P inputs (Auer et al., 2010).

The results of recent modeling studies indicate that *Cladophora* should be responsive to management regimes that maintain SRP concentrations < 1 μg P L^{-1} SRP (Tomlinson et al., 2010). Although models have been successful at predicting *Cladophora* growth, they generally run on a broad scale and do not necessarily take into account the effect of benthic activity or any distinctive cycling pathways in the nearshore (Hecky et al., 2004) due to the lack of detailed SRP data in coastal zones. Nearshore areas may experience P-enrichment, at least in localized areas as a result of enhanced PO_4^{3-} from anthropogenic sources or supplied through dreissenid excreta, but it is unlikely these contributions are accounted for with traditional sampling methods. Understanding of the *Cladophora* – PO_4^{3-} relationship should therefore benefit from detailed spatial and temporal investigations of SRP concentrations within the nearshore.

The goal of this Chapter was to assess the degree to which local inputs and internal cycling by dreissenid mussels contribute to elevated concentrations of PO_4^{3-} in a nearshore region of Lake Ontario that supports nuisance levels of *Cladophora* growth. I hypothesized that SRP concentrations would be higher and phosphate turnover times would be longer in samples obtained from the nearshore than from the offshore as a result of local point and diffuse inputs of P to the study area. Concomitantly, I predicted that SRP would be higher and phosphate turnover would be longer in water samples obtained near a river mouth (Duffins Creek) and in

proximity to a point source (Duffins Creek Water Pollution Control Plant) than from stations further away from these inputs. Due to the known ability of *Dreissena* to excrete SRP, I also predicted elevated concentrations of SRP would be measured in samples obtained from near-bottom waters above mussel-beds versus those obtained from higher up in the water column. A corollary was that dreissenid grazing would lead to lower levels of Chl *a* in near-bottom water samples versus surface water samples. I did not expect to find evidence of these mussel-induced trends in water overlying areas of lake-bottom not colonized by *Dreissena*.

3.2 Methods

3.2.1 Site description

The study was carried out in 2009 along an 8 km portion of the north shore of Lake Ontario, located to the east of the City of Toronto, near the City of Pickering and the Town of Ajax in the Regional Municipality of Durham (Figure 2-1; Figure 3-1). This site was chosen because of reports of increasing *Cladophora* fouling in the area, including expensive algal interferences in the cooling water intakes of Ontario Power Generation's Pickering Nuclear Generating Station located in the centre of the site (43.80°N, 79.07°W). The northwestern shoreline of Lake Ontario is a highly dynamic environment and lake water at this site experiences exchanges with the surrounding nearshore as well as with the open lake. The prevailing winds are from the west and southwest and are commonly 10 to15 km h^{-1} in the summer months, but are more variable in both speed and direction in the spring and autumn. Winds of 15 to 25 km h^{-1} are not uncommon with corresponding waves reaching 1 to 2 m.

The Durham shoreline is almost completely urbanized and includes a number of storm drains, a major tributary (Duffins Creek), a wastewater treatment plant (Duffins Creek Water Pollution Control Plant) discharge, and two discharges of heated water from the Nuclear Generating Station within the study area. The Duffins Creek catchment is >250 km^{-2} and is primarily agricultural and secondarily forested (Malkin et al., 2010a). The Duffins Creek Water Pollution Control Plant (WPCP) treats sewage flows from both the York and Durham Regions, with approximately 80 % originating from York Region. There are plans for further expansion of the WPCP scheduled for completion in April, 2012, to accommodate the demands of intense population growth in these regions. At present, the effluent is released from a diffuser approximately 1 km from shore at a depth of approximately 9 m. The diffuser consists of a series

pipes fitted with dispersion nozzles on the last ~100 m of the outflow and these serve to diffuse the effluent from an initial SRP concentration of 300 to 400 µg P L^{-1} to 15 to 20 µg P L^{-1} once it enters the lake (L.F. Leon, Environment Canada, pers. comm.).

The lake bottom in the study area includes soft and hard substrata, best described as "patchy." In general, the westernmost segment of the site is composed of fine sediments and sand, increasingly interspersed with coarser material and bedrock towards the east. Bottom substrate in the eastern half of the study site consists of cobble and boulders between depths of 3 m to 15 m and is dominated by shale at greater depths (E.T. Howell, Ontario Ministry of Environment pers. comm.). The dominance of hard substrata in the eastern portion of the study site's littoral zone supports dense coverage of dreissenid mussels and *Cladophora.* The abundance of these species was surveyed by researchers at the Ministry of Environment (MOE) twice in the summer of 2008 (June 10th and July 17th), along a depth transect (3 m, 6 m, 10 m, and 18 m). High densities of mussels (exclusively *D. bugensis* or quagga mussels) were found to have colonized all depths usually within the range of 2,000 to 7,000 mussels m^{-2}, but sometimes reaching > 13,000 mussels m^{-2} (E.T. Howell, Ontario Ministry of Environment, pers. comm.). *Cladophora* coverage was observed to be almost always 100 % on both survey days, except at 18 m, where was it found covering 40 to 50% of the lake bottom. *Cladophora* biomass was more variable, ranging from 1.5 to 160 g DW m^{-2}, with the highest biomass measured at 3 m depth in June (E.T. Howell, Ontario Ministry of Envrionment, pers. comm.). Average *Cladophora* biomass along the transect was 56 ± 52 SD g DW m^{-2} in June and 35 ± 36 SD g DW m^{-2} in July. Nuisance conditions in the Great Lakes are generally considered to be >50 g DW m^{-2} (Canale & Auer, 1982b; Higgins et al., 2005b) and it should be noted that *Cladophora* coverage in 2008 was less dense than it had been in previous years (Higgins & Howell, 2010; Howell & Makarewicz, 2010; Leon et al., 2010). In both 2008 and 2009, co-occurrence of *Dreissena* and *Cladophora* was typical on stable substrate, with *Cladophora* growing on mussel shells. *Cladophora* and dreissenid coverage were not quantified in the study year (2009). However, abundant mussels and mats of *Cladophora* were noted between depths of 5 m to 17 m (personal observation by snorkeling and via underwater video camera).

3.2.2 Sampling procedures

The sampling period ran from late April to mid-October of 2009, involving a total of 13 sampling trips. Water was sampled from seven stations located along the shoreline and along a nearshore-offshore transect. The sandy station (43.48.63°N, 79.04.86°W; 5 m depth) was chosen to represent shoreline lake water over sandy and therefore mussel-free substrate; the WPCP station (43.48.38°N, 79.02.46°W; 12 m depth) was chosen to represent surface water influenced by the outfall diffuser of the Duffins Creek Water Pollution Control Plant; and the Duffins station (43.48.92°N, 79.02.08°W; 2 m depth) was chosen to represent the influence of Duffins Creek. The nearshore transect included 3 stations over hard substrate but removed from point sources: a shallow station (43.49.04°N, 79.00.79°W; 5 m depth), a mid station (43.48.87°N, 79.00.73°W; 9 m depth) and a deep station (43.48.33°N, 79.00.77°W; 17 m depth). These latter three stations correspond to MOE's 2008 benthic survey stations. Finally, an offshore station (43.46.47°N, 79.00.65°W; 42 m depth) was chosen to represent offshore lake water.

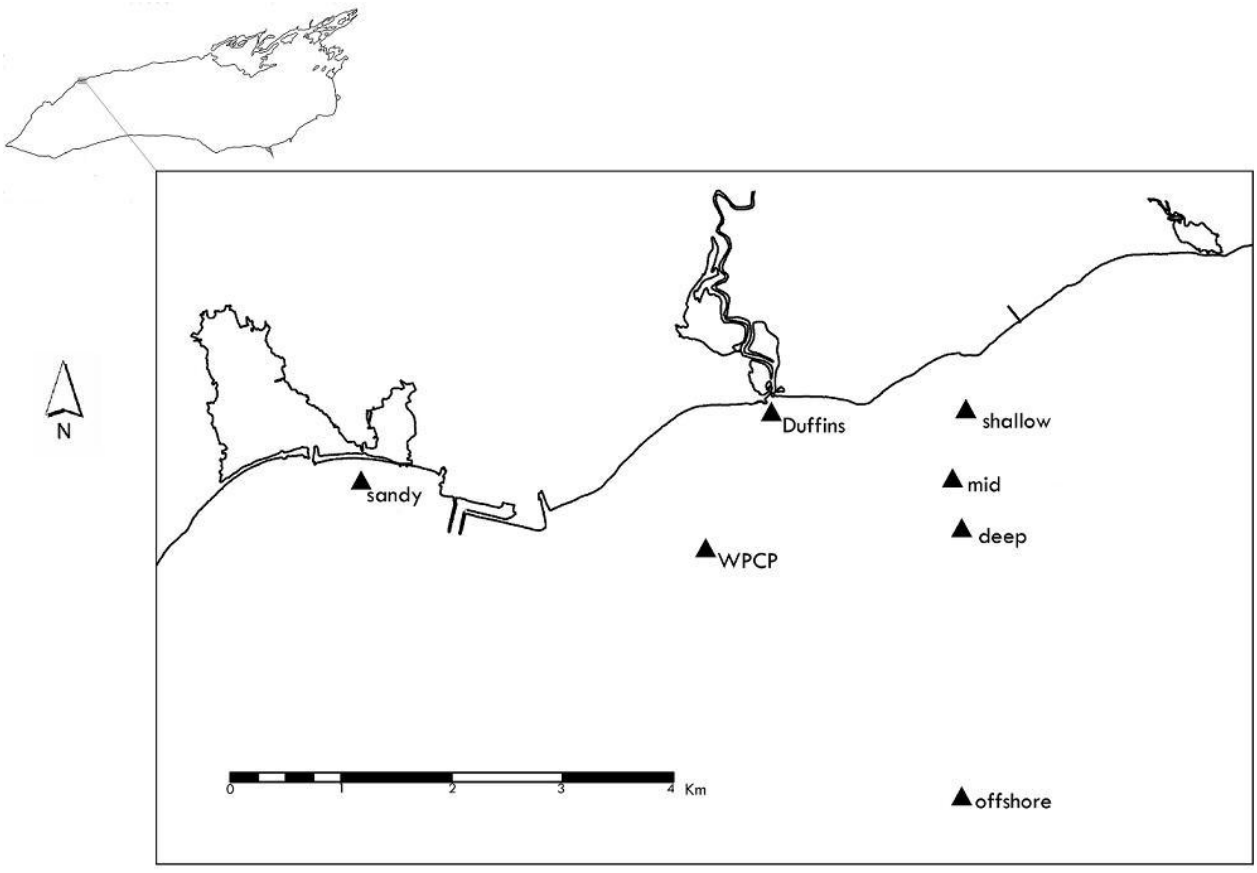


Figure 3-1. Map of study area in Lake Ontario near the Regional Municipality of Durham, Ontario.

On all sampling dates, Niskin bottles were used to obtain near-surface (3 m) water samples from all stations except the Duffins station, which was sampled at 1.5 m. Additional samples were obtained 2 m above the bottom at the mid and deep stations. On some occasions, a horizontal Van Dorn sampler was used to collect water from 50 cm or 100 cm above the bottom at the sandy and shallow stations. Triplicate samples (obtained with separate casts) were obtained for SRP and Chl *a* analyses, and duplicates were taken for phosphate turnover time. Samples were stored in 100-mL (for SRP) and 1-L (for phosphate turnover time and Chl *a*) HDPE-containers pre-washed with 10% HCl and rinsed with sample water. They were stored at ambient temperature.

In addition, on most sampling dates, a weighted aluminum frame was lowered to the bottom of the sandy, shallow, mid, and deep stations to pump water through attached tubing outfitted with 100 µm screening from 15 cm, 50 cm, and 100 cm above the bottom. A video camera attached to the unit allowed for observation of its orientation, the bottom substrate where it was deployed, and for the occurrence of re-suspended materials. After deployment, conditions were allowed to settle for 10 min before the onset of pumping. The tubing was purged and bottom conditions were allowed to settle for 5 min in between sampling from different depths. Samples from the mid and deep stations were often filtered on board through 0.2 µm polycarbonate filters. Otherwise, water was pumped directly into 60-mL or 100-mL (for SRP) and 1-L (for Chl *a*) HDPE containers pre-washed as above. Triplicate samples were stored at ambient temperature.

On selected sampling dates (May 10th, June 10th, June 22nd and October 4th), I collected samples using dialysis rather than filtration to gauge the impact on SRP estimates. Triplicate lengths (30 cm) of dialysis membrane tubing (Spectra/Por Biotech cellulose ester, 100,000 MW cutoff, 32 mm flat-width diameter) were filled with de-ionized water, closed at both ends, encased in open aluminum and plexi-glass frames, and stored in a cooler submerged with de-ionized water prior to incubation in the lake for 3 to 4 hours. This length of time was determined to be sufficient for internal orthophosphate in the dialysis tubing to equilibrate with ambient conditions in laboratory experiments where tubing was placed in beakers of KH_2PO_4-enriched lake-water. On most days, this sampling method was employed at only some stations (e.g., from 3 m depth or 15 cm, 50 cm and 100 cm above the bottom at sandy and shallow stations). Only on October 4th were all stations and depths sampled using dialysis. The resulting dialyzates were carefully decanted into 60-mL HDPE containers pre-washed and stored as above.

3.2.3 Laboratory procedures

Soluble reactive phosphorus (SRP)

From water samples collected with Niskin bottles or pumped through tubing, 30-mL subsamples were filtered within 12 h at low pressure (<100 mm Hg), through 0.2-µm cellulose-ester filters and maintained at 4 C until analysis <24 h after collection. Approximately 60-mL to 100-mL of sample water was added to the filter manifold so the sample was not filtered entirely; this was an effort to reduce cell breakage and overestimation of SRP. Samples collected with dialysis tubing did not require filtration and were immediately frozen at approximately -16 C until analysis. Phosphomolybdic acid methodology was employed according to Murphy and Riley (1962) and Stainton et al. (1977) for all samples, but with an additional magnesium-induced co-precipitation (MAGIC) concentration step for dialyzed samples, based on the freshwater method by Anagnostou and Sherrell (2008) and as described in Chapter 2, in anticipation of SRP concentrations below the detection limit. Samples were analyzed with a Cary 100 Bio spectrophotometer in a 10-cm path-length, reduced-volume cuvette or with an Ultrospec spectrophotometer in a 5-cm path-length cuvette. Using the 5-cm cuvette raised the detection limit; it was used to analyze the following samples: all benthic profile samples from the mid and deep stations, plus filtered surface samples from June 10th, June 22nd, July 10th and July 21st. In this study, detection limits for SRP were determined as described in Chapter 2 to be 0.3 µg P L^{-1} (10 nM) and 0.6 µg P L^{-1} (20 nM) for the two spectrophotometers respectively. The use of MAGIC on May 10th June 10th, and June 22nd dialyzates lowered the detection limit to 0.04 µg P L^{-1} (1nM) for those samples.

Phosphate turnover times

Phosphate turnover times were measured three times in the summer. The uptake constant (*k*) was determined as described in Chapter 2. The reciprocal of the rate constant is phosphate turnover time.

Chl a

Samples were filtered onto glass fiber filters (Whatman GFF, 0.7 µm pore size, 47 mm) and stored frozen at -16 C until analysis. Filters were then incubated in 90% acetone in a freezer at approximately -16 C for 18 to 24 hr. The extracts were quantified by fluorometry according to Strickland and Parsons (1968) and Stainton et al. (1997) before and after acidification on a fluorometer (Turner Designs) that was calibrated annually with pure Chl *a*.

3.2.4 Statistical analyses

Statistical analyses were performed with SPSS v. 17 (IBM, Chicago, Il, USA) to determine the effects of station or height above bottom on SRP concentrations, phosphate turnover time, and Chl *a* concentrations where applicable. Means for the sampling period were compared with two-way ANOVAs for the effect of station or height above bottom and time. If there was a significant date-station or date-height interaction term, one-way ANOVAs were computed for each individual date. Significant differences were determined using Tukey HSD post-hoc comparisons, or Games-Howell comparisons when transforming the data did not resolve unequal variances. Differences were deemed statistically significant at an alpha level of 0.05. Post-Hoc comparisons could not be performed on single value SRP concentrations; single values were considered significantly different from SRP means at other stations/heights if they did not fall within the 95% confidence intervals for the means. Values below the method detection limits are reported but must be interpreted with caution. I did not truncate SRP values below detection limits to zero, including negative SRP values (encountered when negative absorbance was measured), because to do so would bias the results.

3.3 Results

3.3.1 Seasonal SRP at the surface

Soluble reactive phosphorus (SRP) concentrations in surface samples were generally low throughout the sampling period, but displayed seasonal variation in samples taken from nearshore (shoreline and transect) stations. SRP concentrations in nearshore samples were highest in April and October and lowest in July and August. SRP concentrations in offshore samples were below method detection limits (0.3 µg P L^{-1} or 0.6 µg P L^{-1}) and were sometimes negative (Figure 3-2).

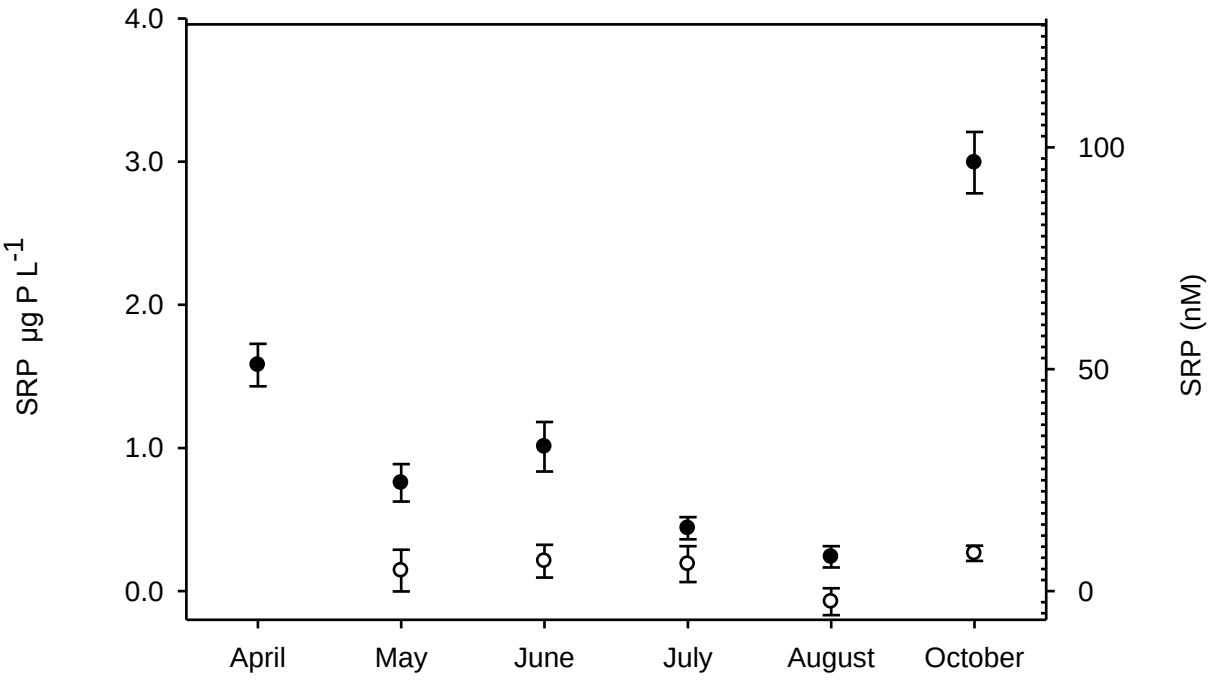


Figure 3-2. Mean monthly SRP concentrations in water samples collected from nearshore waters (solid circles) and offshore waters (open circles). Data are from surface (3 m) samples taken twice each month at all stations. April is an exception as sampling occurred only once and did not include the offshore station. Nearshore is the mean of the SRP concentrations in samples from all stations except the offshore station, while offshore is the mean of the SRP concentrations in samples from the offshore station only. Data using dialysis are not included. Error bars represent standard error.

3.3.2 SRP and phosphate turnover time along the nearshore-offshore transect

SRP concentrations were higher in samples taken from nearshore stations than in those from the offshore station (F = 24.529, $P < 0.001$; two-way ANOVA; Figure 3-3). However, a significant date-station interaction was encountered (F = 3.828, $P < 0.001$). Significant differences between stations occurred on some individual sampling dates, with mean SRP concentrations in nearshore water samples higher than in samples obtained from the offshore station (Table 3-1). SRP values were often below the detection limit.

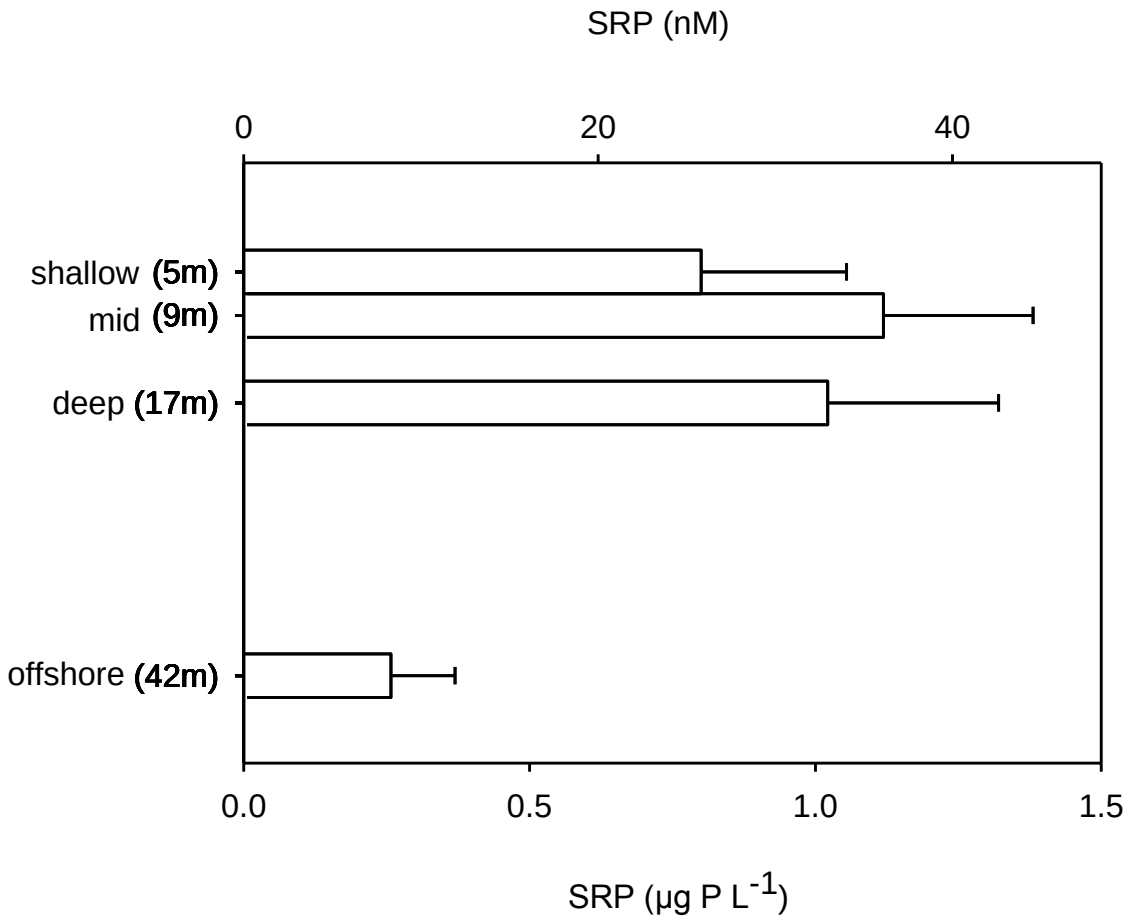


Figure 3-3. Mean SRP concentrations in surface (3 m) samples collected from stations located along the nearshore-offshore transect for all sampling days in 2009. Data using dialysis are not included. Error bars represent standard error.

Table 3-1. Mean SRP concentrations in surface (3 m) water samples collected from stations located along the nearshore-offshore transect through the spring, summer, and autumn of 2009 compared by one-way analyses of variance or independent t-tests. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote sites determined significantly different from each other using Tukey HSD comparisons. shallow = 5 m, mid = 9 m, deep = 17 m, offshore = 42 m. na = not applicable, nd = no data.

Date Station	**n**	**SRP µg P L^{-1} ± 1SD**	**F (or t)**	***P***
April 28				
shallow	na	nd		
mid	3	1.89 ± 0.24	1.531	0.200
deep	3	1.08 ± 0.89		
offshore	na	nd		
May 12				
shallow	na	nd		
mid	3	0.60 ± 0.41	4.011	0.091
deep	3	1.18 ± 0.10		
offshore	2	0.38 ± 0.42		
May 29				
shallow	3	0.89 ± 0.15		
mid	3	0.69 ± 0.44	4.042	0.051
deep	3	0.07 ± 0.59		
offshore	3	0.00 ± 0.15		
June 10				
shallow	3	0.42 ± 0.54 ^{BC}		
mid	3	1.99 ± 0.31 ^{A}	19.905	0.000*
deep	3	1.15 ± 0.18 ^{AB}		
offshore	3	0.00 ± 0.18 ^{C}		
June 10 dialyzate				
shallow	na	nd		
mid	2	3.76 ± 0.23 ^{A}	437.766	0.000*
deep	3	0.98 ± 0.06 ^{B}		
offshore	2	1.00 ± 0.01 ^{B}		
June 22				
shallow	2	0.47 ± 1.12		
mid	2	0.63 ± 0.43	0.456	0.723
deep	3	0.21 ± 0.96		
offshore	3	0.42 ± 0.18		
June 22 dialyzate				
shallow	2	0.92 ± 0.04		
mid	na	nd	0.642	0.616
deep	na	nd		
offshore	2	0.86 ± 0.11		

Table continues on following page.

Table 3-1. *Continued.*

Date **Station**	**n**	**SRP µg P L^{-1} ± 1SD**	**F (or t)**	***P***
July 10				
shallow	3	0.52 ± 0.36		
mid	3	0.94 ± 0.54	2.346	0.159
deep	3	0.10 ± 0.18		
offshore	2	0.44 ± 0.31		
July 21				
shallow	2	1.15 ± 0.00 ^{A}		
mid	3	0.31 ± 0.18 ^{B}	16.545	0.001*
deep	3	0.42 ± 0.18 ^{B}		
offshore	3	0.10 ± 0.18 ^{B}		
August 12				
shallow	3	-0.15 ± 0.09		
mid	4	0.23 ± 0.44	0.983	0.443
deep	4	0.62 ± 0.31		
offshore	2	-0.25 ± 0.12		
August 19				
shallow	3	0.09 ± 0.16		
mid	4	0.29 ± 0.15	0.651	0.600
deep	4	0.27 ± 0.48		
offshore	3	0.05 ± 0.15		
October 4				
shallow	3	2.32 ± 0.10 ^{A}		
mid	3	2.71 ± 0.14 ^{A}	56.989	0.000*
deep	3	2.97 ± 0.43 ^{A}		
offshore	2	0.15 ± 0.21 ^{B}		
October 4 dialyzate				
shallow	2	2.17 ± 0.95 ^{B}		
mid	2	2.07 ± 0.41 ^{B}	18.643	0.002*
deep	3	4.66 ± 0.31 ^{A}		
offshore	2	0.82 ± 0.06 ^{B}		
October 14				
shallow	2	1.50 ± 0.08 ^{AB}		
mid	3	2.03 ± 0.09 ^{A}	84.092	0.000*
deep	3	2.56 ± 0.32 ^{A}		
offshore	3	0.33 ± 0.05 ^{B}		

Notes: 1. A *t* statistic not assuming homogeneity of variance was computed for June 22 dialyzate.

The mean phosphate turnover times in surface samples were longer in those from nearshore stations than from the offshore ($F = 9.475$, $P = 0.002$; two-way ANOVA), but the date-station interaction was also significant ($F = 7.161$, $P = 0.003$). Significant differences between turnover times among stations occurred on July 21st, though there was no consistent trend. Turnover time was very long at the shallow station on August 19th (Table 3-2). Phosphate turnover time was longer in the nearshore than offshore on June 22nd, but not significantly so. Turnover time was shortest in July and longest in June and August.

Table 3-2. Mean phosphate turnover times in surface (3 m) water samples collected from stations located along the nearshore-offshore transect through the summer of 2009 compared by one-way analyses of variance. * Denotes significant P-values (< 0.05). Different capital letters in superscript denote sites determined significantly different from each other using Tukey HSD post-hoc comparisons. na = not applicable, nd = no data.

Date Station	**n**	**Turnover Time (min) ± 1SD**	**F**	***P***
June 22				
shallow	2	45 ± 7		
mid	2	42 ± 22	1.337	0.128
deep	na	nd		
offshore	2	16 ± 0		
July 21				
shallow	2	15 ± 3 ^{AB}		
mid	2	12 ± 1 ^{B}	11.777	0.019*
deep	2	23 ± 1 ^{A}		
offshore	2	12 ± 1 ^{B}		
August 19				
shallow	2	125 ± 9 ^{A}		
mid	2	75 ± 7 ^{B}	12.368	0.017*
deep	2	54 ± 9 ^{AB}		
offshore	2	80 ± 13 ^{AB}		

Notes: Welch's robust F statistic not assuming homogeneity of variance was computed for June 22.

3.3.3 SRP and phosphate turnover time along the Pickering / Ajax shoreline

Mean SRP concentrations for the sampling period were higher in surface samples from the Duffins and WPCP stations than from the shallow and sandy stations ($F = 15.174$, $P < 0.001$; two-way ANOVA; Figure 3-4), but the date-station interaction was significant ($F = 7.024$, $P = 0.002$). SRP was significantly higher in water samples from the Duffins station than in those from all other stations in April (Table 3-3). SRP in surface samples obtained near the WPCP were significantly higher than those from all other shoreline stations on one day in June. SRP was also significantly higher in samples from both the Duffins and the WPCP stations than in those obtained from sandy and shallow stations in October. SRP values below detection limit, including negative SRP values, were encountered from late May to early August in samples taken along the shoreline.

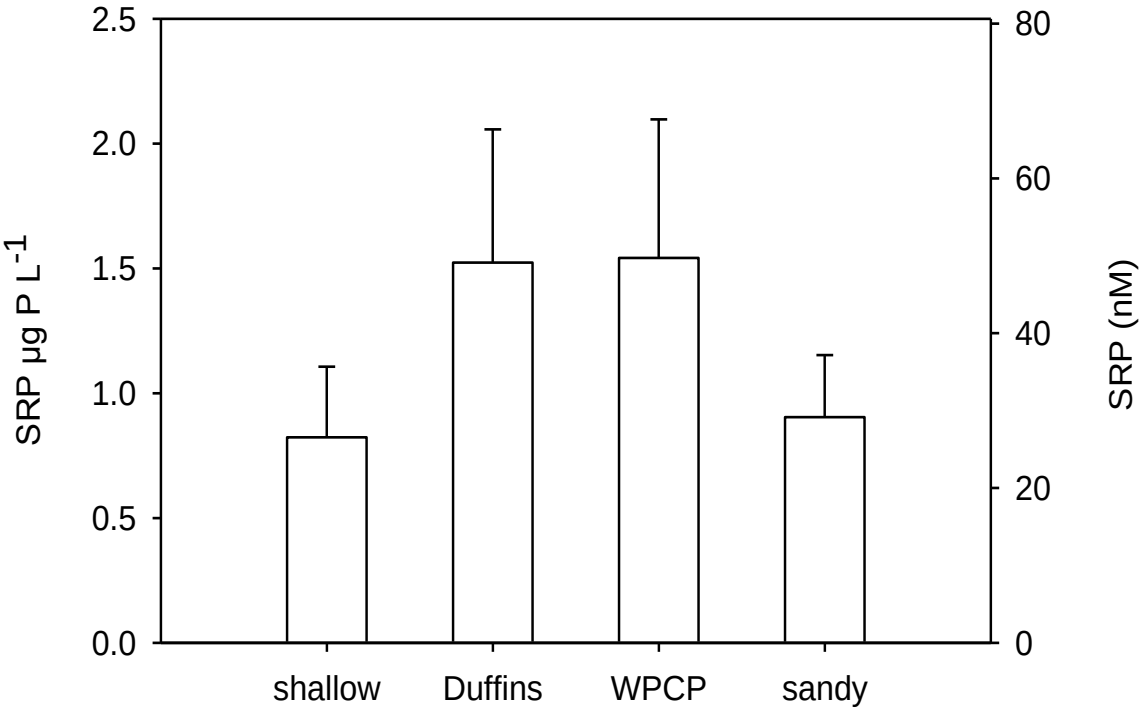


Figure 3-4. Mean SRP concentrations in surface (3 m) water samples collected from stations located along the Pickering / Ajax shoreline for all sampling days in 2009. Data using dialysis are not included. Error bars represent standard error.

Table 3-3. Mean SRP concentrations in surface (3 m) water samples collected from stations located along the Pickering/Ajax shoreline through the spring, summer, and autumn of 2009 compared by one-way analyses of variance. * Denotes significant P-values (< 0.05). Different capital letters in superscript denote stations determined significantly different from each other using Tukey HSD or Games-Howell post-hoc comparisons. shallow = 5 m, Duffins = 2 m, WPCP = 12 m, sandy = 5 m, na = not applicable, nd = no data.

Date Station	**n**	**SRP µg P L^{-1} ± 1SD**	**F**	***P***
April 28				
shallow	na	nd		
Duffins	3	2.13 ± 0.28 ^{A}	8.992	0.016*
WPCP	3	1.18 ± 0.23 ^{B}		
sandy	3	1.62 ± 0.32 ^{AB}		
May 12				
shallow	na	nd		
Duffins	3	2.00 ± 1.43	2.753	0.144
WPCP	3	0.87 ± 0.28		
sandy		0.83 ± 0.11		
May 29				
shallow	3	0.89 ± 0.15		
Duffins	3	0.65 ± 0.58	0.538	0.669
WPCP	3	0.25 ± 0.96		
sandy	3	0.29 ± 0.88		
June 10				
shallow	3	0.42 ± 0.54		
Duffins	3	0.94 ± 0.36	2.326	0.161
WPCP	2	0.57 ± 0.22		
sandy	3	1.15 ± 0.18		
June 22				
shallow	2	0.47 ± 1.12 ^{AB}		
Duffins	2	0.94 ± 0.44 ^{AB}	15.397	0.044*
WPCP	2	3.76 ± 0.44 ^{A}		
sandy	3	0.42 ± 0.48 ^{B}		
July 10				
shallow	3	0.52 ± 0.36		
Duffins	3	0.42 ± 0.48	0.421	0.743
WPCP	3	0.21 ± 0.36		
sandy	3	0.52 ± 0.36		
July 21				
shallow	2	1.15 ± 0.00		
Duffins	3	-0.10 ± 0.00	3.104	0.110
WPCP	3	0.63 ± 0.79		
sandy	2	0.37 ± 0.22		

Table continues on following page.

Table 3-3.*Continued.*

Date Station	**n**	**SRP µg L^{-1} ± 1SD**	**F**	***P***
August 12				
shallow	3	-0.15 ± 0.09		
Duffins	3	0.04 ± 0.23	1.360	0.323
WPCP	3	-0.02 ± 0.08		
sandy	3	0.14 ± 0.08		
August 19				
shallow	3	0.09 ± 0.16		
Duffins	3	0.57 ± 0.35	2.369	0.147
WPCP	3	0.25 ± 0.11		
sandy	3	0.16 ± 0.26		
October 4				
shallow	3	2.32 ± 0.10 ^{A}		
Duffins	2	5.92 ± 0.78 ^{A}	82.600	0.000*
WPCP	2	4.74 ± 0.34 ^{A}		
sandy	3	1.61 ± 0.28 ^{B}		
October 4 dialyzate				
shallow	2	2.17 ± 0.95 ^{B}		
Duffins	3	5.61 ± 0.28 ^{A}	62.574	0.012*
WPCP	2	5.73 ± 0.60 ^{A}		
sandy	2	1.26 ± 0.29 ^{B}		
October 14				
shallow	3	1.50 ± 0.08 ^{C}		
Duffins	3	3.26 ± 0.22 ^{B}	19.062	0.001*
WPCP	3	4.52 ± 0.67 ^{A}		
sandy	3	2.77 ± 0.46 ^{BC}		

Notes: 1. Welch's robust F statistic not assuming homogeneity of variance was computed for June 22 and October 4 dialyzate.

Phosphate turnover times in surface waters at stations along the Pickering / Ajax shoreline were measured only once, on August 19th. The phosphate turnover time in samples from the Duffins station was significantly longer than the phosphate turnover time in water samples from the shallow and sandy stations, but not longer than in those obtained near the WPCP outfall (Games-Howell post-hoc comparisons; Table 3-4).

Table 3-4. Mean (n = 2) Phosphate turnover times in surface (3 m) water samples collected from stations located along the Pickering/Ajax shoreline on August 19th, 2009 compared by a one-way analysis of variance.

Station	Turnover Time (min) ± 1SD	F	*P*
shallow	125 ± 10	627.099	0.004
Duffins	504 ± 63		
WPCP	160 ± 90		
sandy	133 ± 1		

Notes: Welch's robust F statistic not assuming homogeneity of variance was computed.

3.3.4 SRP and Chl *a* through the water column at the shallow station

Although there was a trend toward higher SRP in samples from near-bottom layers compared to SRP in samples taken from higher above the bottom at the shallow station (Figure 3-5; Table 3-5), differences were not significant (F = 2.639, *P* = 0.060; two-way ANOVA).

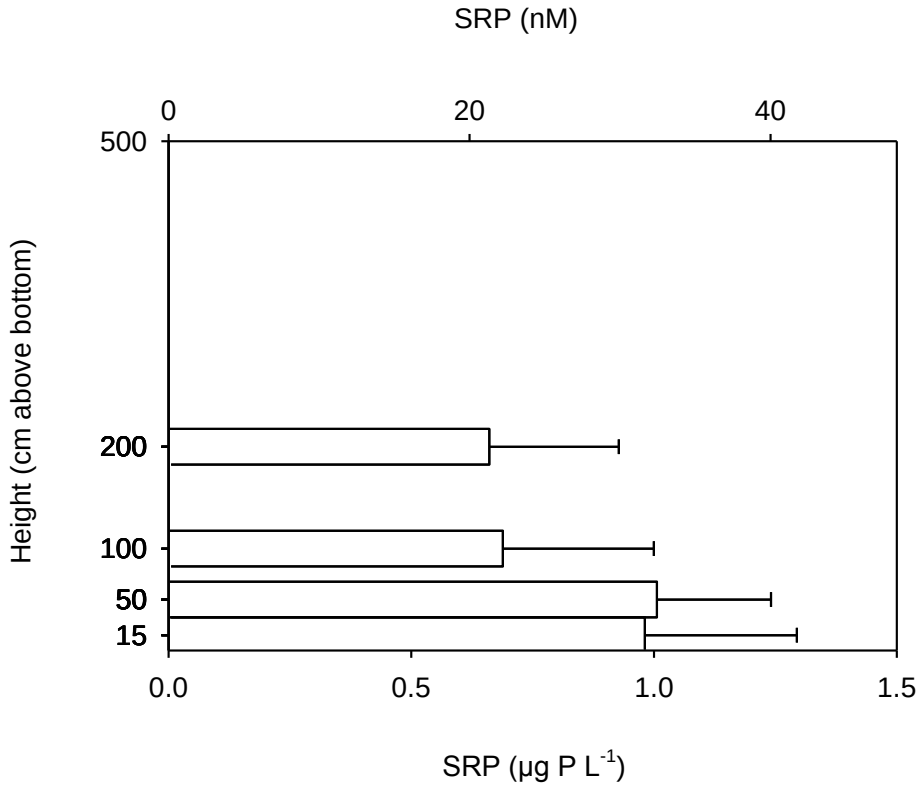


Figure 3-5. Mean SRP concentrations in water samples collected from a benthic profile over dreissenid mussel-beds at the shallow (5 m) station in the 2009 sampling period. Data are from filtered and dialyzed samples. Error bars represent standard error

Table 3-5. Mean SRP concentrations in samples collected from a benthic profile over dreissenid mussel-beds at the shallow (5 m) station in the late spring to mid-autumn of 2009. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**
May 29 dialyzate		
200	3	0.89 ± 0.15
100	3	0.69 ± 0.08
50	2	1.23 ± 0.59
15	2	1.15 ± 0.01
June 10 dialyzate		
200	3	0.42 ± 0.54
100	3	1.42 ± 0.37
50	3	1.05 ± 0.27
15	3	1.13 ± 0.72
June 22		
200	2	0.47 ± 1.11
100	na	nd
50	3	1.31 ± 0.72
15	na	nd
July 10		
200	3	0.52 ± 0.36
100	na	nd
50	3	0.84 ± 0.36
15	na	nd
July 21		
200	3	1.15 ± 0.00
100	na	nd
50	3	0.63 ± 0.46
15	na	nd
August 12		
200	3	-0.15 ± 0.09
100	3	-0.14 ± 0.21
50	3	0.05 ± 0.23
15	3	0.19 ± 0.19
August 19		
200	3	0.09 ± 0.16
100	3	0.14 ± 0.06
50	3	0.62 ± 0.33
15	3	0.47 ± 0.20
October 4		
200	3	2.32 ± 0.10
100	3	1.27 ± 0.09
50	3	1.64 ± 0.42
15	3	1.98 ± 0.96
October 4 dialyzate		
200	2	2.17 ± 0.95
100	2	2.60 ± 0.06
50	3	2.75 ± 0.98
15	3	2.50 ± 0.42

Mean Chl *a* concentrations were measured only once, on October 4th at the shallow station. Chl *a* was very low throughout the water column with no significant differences between depths (one-way ANOVA; Table 3-6).

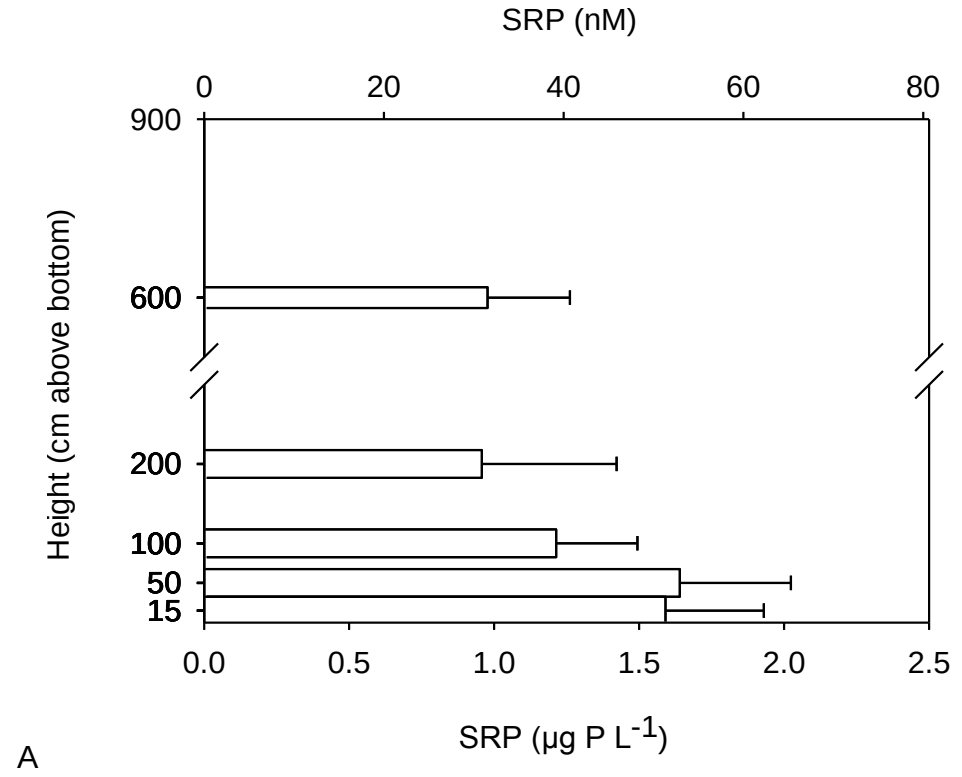
Table 3-6. Mean (n = 3) Chl *a* concentrations in water samples collected from a benthic profile over dreissenid mussel-beds at the shallow (5 m) station on October 4th, 2009.

Date Height (cm above bottom)	**Chl *a* µg L^{-1} ± 1SD**
October 4	
200	0.10 ± 0.14
100	0.08 ± 0.01
50	0.09 ± 0.01
15	0.10 ± 0.01

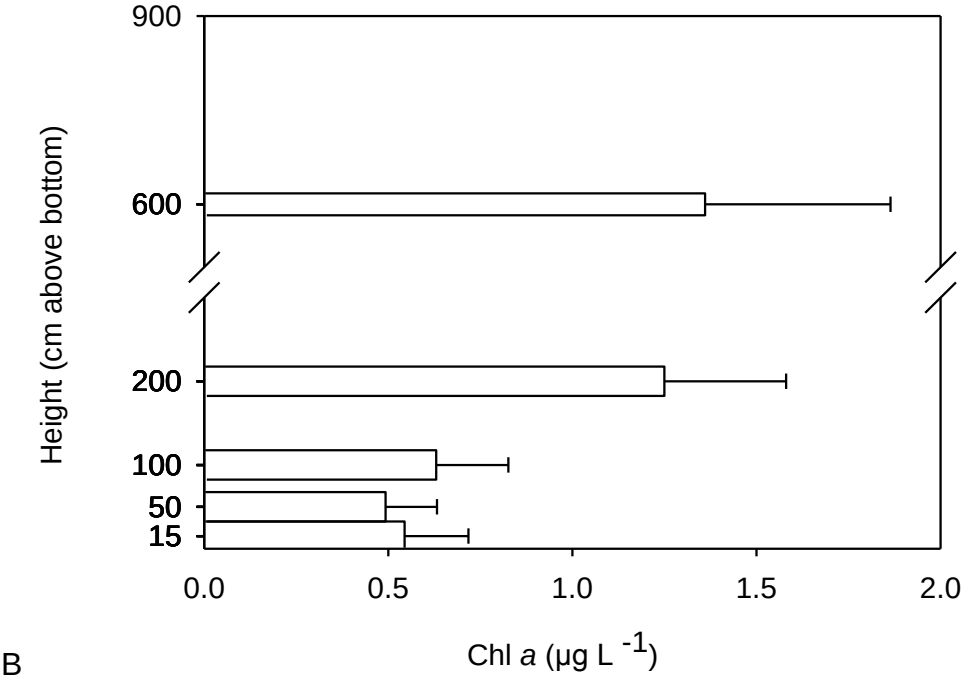
3.3.5 SRP and Chl *a* through a benthic profile and at the surface of the mid station

SRP concentrations were high in 2009 water samples obtained near mussel-beds and low in surface water samples at the mid station (F = 13.332, $P < 0.001$; two-way ANOVA; Figure 3-6). However, the date-height interaction term was significant (F = 5.720, $P <$ 0,001). On four of eight sampling dates, mean SRP concentrations in near-bottom water samples were significantly higher than those in samples from upper layers. On October 14th, however, mean SRP concentrations were significantly higher in near-surface waters than in water 100 cm above the bottom (Table 3-7).

Chl *a* concentrations displayed the opposite trend to SRP; they were low in near-bottom water samples but high in samples from the upper layers (F = 75.383, $P < 0.001$; two-way ANOVA; Figure 3-6), but again, the date-height interaction was also significant (F = 12.047, $P < 0.001$). Chl *a* was higher in surface water samples and in samples from 200 cm above the bottom than in samples collected from underlying layers on all individual sampling dates (Table 3-8).



A



B

Figure 3-6. Mean A) SRP concentrations and B) Chl *a* concentrations in water samples collected from a benthic profile over dreissenid mussel-beds and near the surface at the mid (9 m) station in the 2009 sampling period. Error bars represent standard error.

Table 3-7. Mean SRP concentrations in samples collected from a benthic profile over dreissenid mussel-beds and near the surface at the mid (9 m) station through the spring, summer, and autumn of 2009 compared by one-way analyses of variance or independent t-tests. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote heights determined significantly different from each other using Tukey HSD or Games-Howell post-hoc comparisons. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F (or *t*)**	***P***
April 28				
600	3	1.89 ± 0.24		
200	na	nd		
100	3	2.18 ± 0.60	0.771	0.483
50	na	nd		
15		nd		
May 12				
600	3	0.60 ± 0.41 ^{B}		
200	na	nd		
100	3	1.40 ± 0.27 ^{A}	2.850	0.046*
50	na	nd		
15	na	nd		
July 30				
600	na	nd		
200	3	- 0.38 ± 0.21 ^{B}		
100	3	0.68 ± 0.74 ^{AB}	22.613	0.004*
50	3	1.03 ± 0.20 ^{A}		
15	3	0.92 ± 0.20 ^{A}		
August 6				
600	1	0.55 ± na ^{C}		
200	1	1.80 ± na ^{BC}		
100	3	1.49 ± 0.31 ^{B}	8.669	0.011*
50	3	2.33 ± 0.18 ^{A}		
15	3	1.80 ± 0.31 ^{AB}		
August 12				
600	4	0.23 ± 0.44 ^{B}		
200	1	0.55 ± na ^{B}		
100	3	2.22 ± 0.36 ^{A}	28.643	0.000*
50	3	2.64 ± 0.48 ^{A}		
15	3	2.85 ± 0.18 ^{A}		
August 19				
600	4	0.29 ± 0.15		
200	1	0.63 ± na		
100	3	0.11 ± 0.36	1.894	0.201
50	3	0.63 ± 0.31		
15	3	0.84 ± 0.72		

Table continues on following page.

Table 3-7. *Continued.*

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F**	***P***
August 26				
600	1	1.25 ± na		
200	1	2.20 ± na		
100	3	0.31 ± 0.31		
50	3	0.73 ± 0.72	2.372	0.165
15	3	0.94 ± 0.53		
October 14				
600	3	2.03 ± 0.09 ^{A}		
200	na	nd		
100	3	1.33 ± 0.63 ^{B}		
50	3	2.49 ± 0.45 ^{AB}	36.681	0.002*
15	3	2.21 ± 0.46 ^{AB}		

Notes: Welch's robust F statistic not assuming homogeneity of variance was computed for July 30 and Oct. 14.

Table 3-8. Mean Chl *a* concentrations in water samples collected from a benthic profile over dreissenid mussel-beds and near the surface at the mid (9 m) station through the spring, summer, and autumn of 2009 compared by one-way analyses of variance. * Denotes significant *P*-values (< 0.05) Different capital letters in superscript denote heights determined significantly different from each other using Tukey HSD post-hoc comparisons. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**Chl *a* µg L^{-1} ± 1SD**	**F**	***P***
July 30				
600	na	nd		
200	3	1.81 ± 0.34 ^{A}		
100	3	0.73 ± 0.35 ^{B}	12.780	0.002*
50	3	0.43 ± 0.19 ^{B}		
15	3	0.70 ± 0.28 ^{B}		
August 6				
600	1	0.67 ± na ^{A}		
200	1	0.55 ± na ^{AB}		
100	3	0.46 ± 0.06 ^{BC}	15.295	0.003*
50	3	0.33 ± 0.02 ^{D}		
15	3	0.36 ± 0.05 ^{CD}		
August 12				
600	4	1.48 ± 0.44 ^{A}		
200	1	1.13 ± na ^{A}		
100	3	0.32 ± 0.10 ^{B}	67.152	0.000*
50	3	0.41 ± 0.08 ^{B}		
15	3	0.32 ± 0.04 ^{B}		
August 19				
600	4	1.42 ± na ^{A}		
200	1	1.52 ± na ^{A}		
100	3	0.74 ± 0.04 ^{B}	29.615	0.000*
50	3	0.63 ± 0.17 ^{BC}		
15	3	0.46 ± 0.06 ^{C}		
August 26				
600	1	3.10 ± na ^{A}		
200	1	2.34 ± na ^{A}		
100	3	1.46 ± 0.09 ^{B}	251.433	0.000*
50	3	1.09 ± 0.04 ^{C}		
15	3	1.32 ± 0.04 ^{B}		
October 14				
600	3	0.12 ± 0.01 ^{AB}		
200	3	0.15 ± 0.04 ^{A}		
100	3	0.07 ± 0.00 ^{BC}	10.408	0.001*
50	3	0.07 ± 0.01 ^{C}		
15	3	0.08 ± 0.02 ^{BC}		

3.3.6 SRP and Chl *a* through a benthic profile and at the surface of the deep station

SRP concentrations were higher in samples from benthic waters overlying mussel-beds and lower in samples from the surface (3 m) at the deep station ($F = 25.344$, $P < 0.001$; two-way ANOVA; Figure 3-7), but the date-height interaction was also significant ($F = 3.371$, $P < 0.001$). SRP concentrations were significantly higher in samples from the bottom layers than in samples taken near the surface on all sampling days, except for July 30th when there was no sample collected from near surface waters (Table 3-9).

Chl *a* concentrations at the deep station were very low in water collected from directly above the lake bottom and high in samples taken from near the surface ($F = 850.848$ $P < 0.001$; two-way ANOVA; Figure 3-7). However, the date-height interaction was significant ($F = 50.873$, $P = 0.003$). Chl *a* was lower at the bottom than near the surface on all days except July 30th, but samples were not taken from surface waters on this day (Table 3-10).

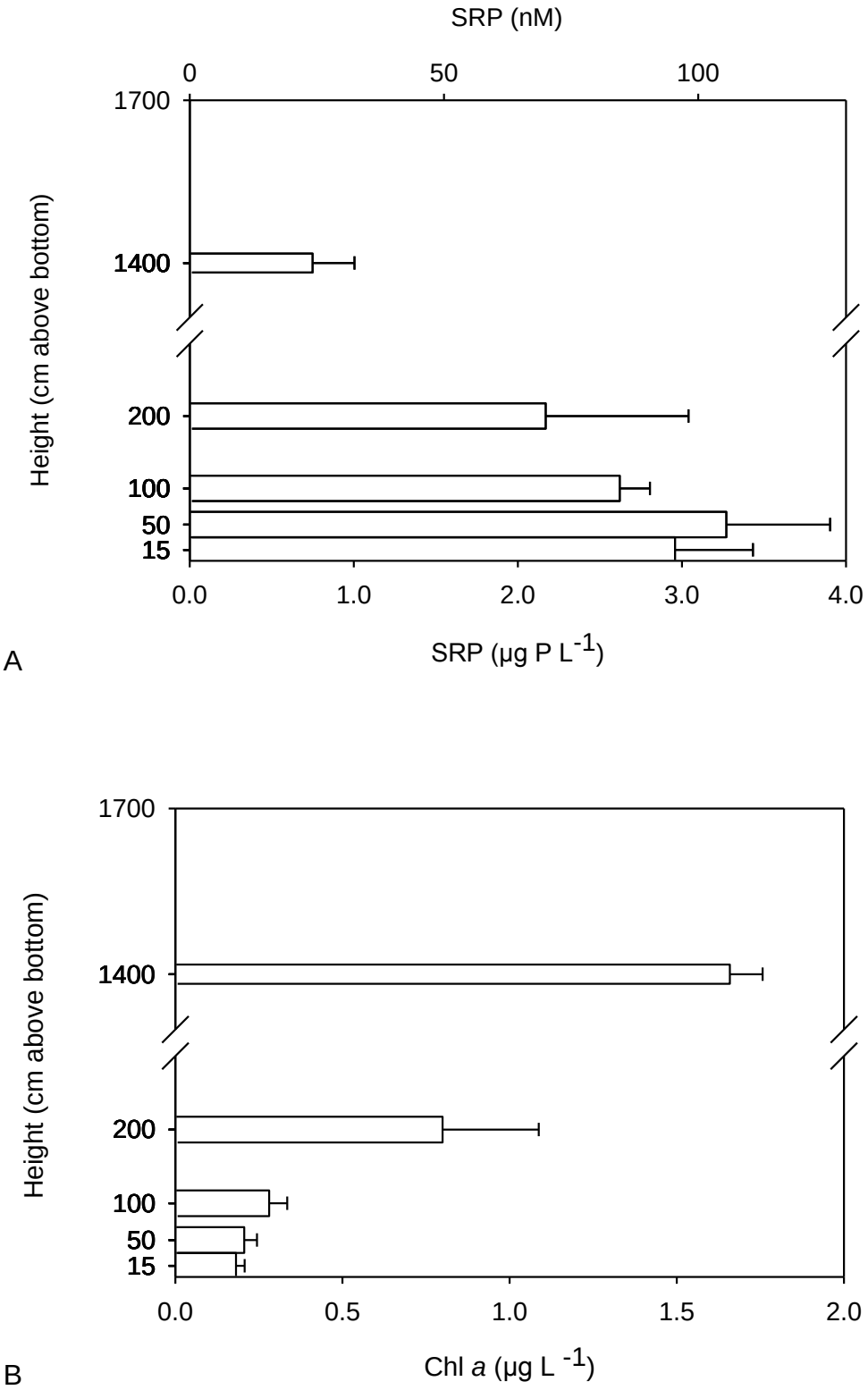


Figure 3-7. Mean A) SRP concentrations and B) Chl *a* concentrations in samples collected from a benthic profile over dreissenid mussel-beds and near the surface at the deep (17 m) station in the 2009 sampling period. Error bars represent standard error.

Table 3-9. Mean SRP concentrations collected from a benthic profile over dreissenid mussel-beds and near the surface at the deep (17 m) station through the spring and summer of 2009 compared by one-way analyses of variance or independent t-tests. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote heights determined significantly different from each other using Tukey HSD comparisons. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F (or t)**	***P***
April 28				
1400	3	1.08 ± 0.98 ^{B}		
200	na	nd		
100	3	3.18 ± 0.81 ^{A}	3.028	0.039*
50	na	nd		
15	na	nd		
May 12				
1400	3	0.18 ± 0.10 ^{B}		
200	na	nd		
100	3	1.48 ± 0.09 ^{A}	3.552	0.038*
50	na	nd		
15	na	nd		
June 22				
1400	3	0.21 ± 0.96 ^{B}		
200	na	nd		
100	3	2.35 ± 0.63 ^{AB}	24.494	0.001*
50	na	nd		
15	3	4.02 ± 0.18 ^{A}		
July 30				
1400	na	nd		
200	3	4.46 ± 0.41 ^{A}		
100	3	2.81 ± 0.20 ^{B}	11.559	0.003*
50	3	5.64 ± 1.08 ^{A}		
15	3	4.58 ± 0.20 ^{A}		
August 6				
1400	1	-0.08 ± na ^{C}		
200	1	0.86 ± na ^{BC}		
100	3	1.80 ± 0.31 ^{AB}	7.295	0.017*
50	3	2.01 ± 0.48 ^{A}		
15	3	2.01 ± 0.36 ^{A}		
August 12				
1400	4	0.25 ± 0.62 ^{B}		
200	1	1.18 ± na ^{B}		
100	3	3.48 ± 0.48 ^{A}	30.389	0.000*
50	3	3.37 ± 0.31 ^{A}		
15	3	3.16 ± 0.36 ^{A}		

Table continues on following page.

Table 3-9. *Continued.*

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F**	***P***
August 19				
1400	4	0.27 ± 0.48 ^{C}		
200	1	0.94 ± na ^{BC}		
100	3	2.50 ± 0.54 ^{A}	13.092	0.001*
50	3	2.51 ± 0.54 ^{A}		
15	3	1.67 ± 0.36 ^{AB}		
August 26				
1400	1	1.88 ± na ^{C}		
200	1	4.08 ± na ^{A}		
100	3	2.40 ± 0.18 ^{B}	13.800	0.003*
50	3	2.51 ± 0.32 ^{BC}		
15	3	2.30 ± 0.18 ^{BC}		

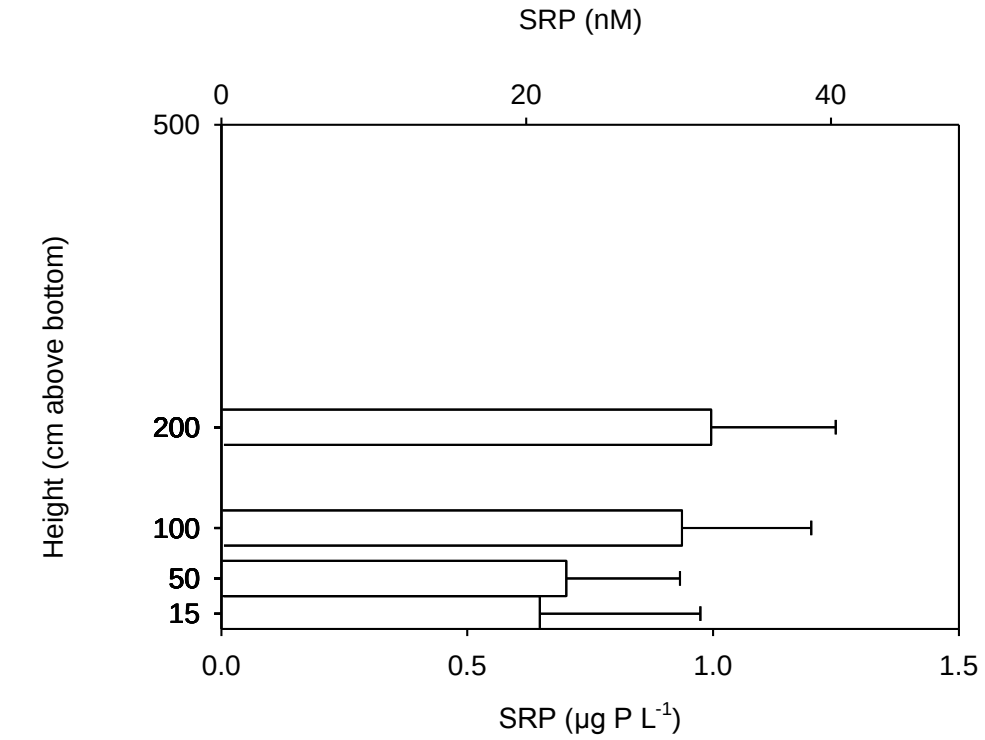
Table 3-10. Mean Chl *a* concentrations in samples collected from a benthic profile over dreissenid mussel-beds and near the surface at the deep (17 m) station through the summer of 2009 compared by one-way analyses of variance. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote heights determined significantly different from each other using Tukey HSD post-hoc comparisons. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**Chl *a* µg L^{-1} ± 1SD**	**F**	***P***
July 30				
1400	na	nd		
200	3	0.32 ± 0.07		
100	3	0.36 ± 0.06	3.438	0.072
50	3	0.20 ± 0.13		
15	3	0.18 ± 0.06		
August 6				
1400	1	1.93 ± na ^{A}		
200	1	0.50 ± na ^{A}		
100	3	0.39 ± 0.03 ^{B}	252.683	0.000*
50	3	0.31 ± 0.03 ^{BC}		
15	3	0.25 ± 0.07 ^{C}		
August 12				
1400	1	1.50 ± na ^{A}		
200	1	1.49 ± na ^{A}		
100	3	0.19 ± 0.02 ^{B}	984.379	0.000*
50	3	0.14 ± 0.02 ^{B}		
15	3	0.15 ± 0.04 ^{B}		
August 19				
1400	1	1.54 ± na ^{A}		
200	1	1.49 ± na ^{A}		
100	3	0.35 ± 0.06 ^{B}	535.750	0.000*
50	3	0.28 ± 0.01 ^{BC}		
15	3	0.23 ± 0.01 ^{C}		
August 26				
1400	1	1.67 ± na ^{A}		
200	1	0.17 ± na ^{A}		
100	3	0.12 ± 0.00 ^{B}	61335.119	0.000*
50	3	0.11 ± 0.00 ^{C}		
15	3	0.11 ± 0.00 ^{C}		

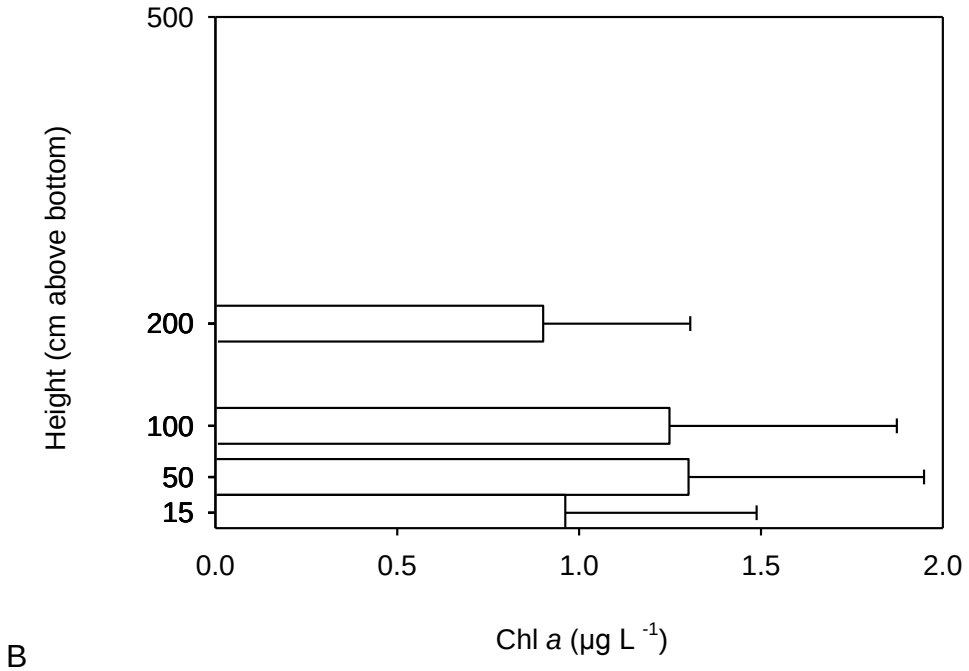
3.3.7 SRP and Chl *a* through the water column at the sandy station.

SRP concentrations were higher in water samples collected closer to the surface than in water samples collected from lake water overlying mussel-free substrate at the sandy station ($F = 7.798$, $P < 0.001$; two-way ANOVA; Figure 3-8), but a significant date-height interaction was also encountered ($F = 2.054$, $P = 0.019$). Significant differences in SRP among heights occurred on August 26th and October 14th, with higher SRP concentrations in samples from 200 cm above the bottom than in samples from water below (Table 3-11).

Mean Chl *a* concentrations were variable in samples taken from a benthic profile over the sandy lake bottom (Figure 3-8). Height above bottom was found to significantly affect Chl *a* ($F = 29.482$ $P < 0.001$), however, the date-height interaction was also significant ($F = 16.122$, $P < 0.001$; two-way ANOVA). Differences in Chl *a* concentrations among heights displayed no consistent trend (Table 3-12).



A



B

Figure 3-8. Mean A) SRP concentrations and B) Chl *a* concentrations in samples collected from a benthic profile over mussel-free substrate at the sandy (5 m) station in the 2009 sampling period. Data using dialysis are not included. Error bars represent standard error.

Table 3-11. Mean SRP concentrations in samples collected from a benthic profile over mussel-free substrate at the sandy (5 m) station through the spring, summer, and autumn of 2009 compared by one-way analyses of variance or independent t-tests. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote heights determined significantly different from each other using Tukey HSD or Games-Howell post-hoc comparisons. na = not applicable, nd = no data.

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F (or *t*)**	***P***
April 28				
200	3	1.63 ± 0.32	-1.055	0.351
100	3	1.36 ± 0.31		
50	na	nd		
15	na	nd		
May 12				
200	3	0.83 ± 0.11	1.368	0.862
100	3	0.96 ± 0.11		
50	na	nd		
15	na	nd		
June 10				
200	3	1.15 ± 0.18	-2.214	0.232
100	na	na		
50	2	0.42 ± 0.44		
15	na	nd		
June 10 dialyzate				
200	na	nd	1.027	0.427
100	3	0.99 ± 0.38		
50	3	1.40 ± 0.40		
15	2	1.00 ± 0.34		
June 22				
200	3	0.60 ± 0.46	1.147	0.315
100	na	nd		
50	3	1.10 ± 0.60		
15	na	nd		
July 10				
200	3	0.52 ± 0.36	-0.4180	0.148
100	na	nd		
50	3	0.11 ± 0.18		
15	na	nd		
July 21				
200	2	0.37 ± 0.22	-0.447	0.712
100	na	nd		
50	2	0.21 ± 0.44		
15	na	nd		

Table continues on following page.

Table 3-11. *Continued.*

Date Height (cm above bottom)	**n**	**SRP µg P L^{-1} ± 1SD**	**F**	***P***
August 12				
200	3	-0.14 ± 0.08		
100	3	-0.27 ± 0.14	0.635	0.613
50	3	-0.14 ± 0.18		
15	3	-0.14 ± 0.18		
August 19				
200	3	0.16 ± 0.26		
100	3	0.15 ± 0.02	0.306	0.820
50	3	0.30 ± 0.27		
15	3	0.26 ± 0.24		
August 26				
200	1	2.20 ± na ^{A}		
100	3	0.84 ± 0.18 ^{B}	5.144	0.043*
50	3	0.94 ± 0.63 ^{AB}		
15	3	0.31 ± 0.31 ^{B}		
October 4				
200	3	1.63 ± 0.28		
100	3	1.35 ± 0.20	0.937	0.467
50	3	1.43 ± 0.22		
15	3	1.20 ± 0.51		
October 4 dialyzate				
200	2	1.94 ± 0.39		
100	3	2.13 ± 0.21	2.114	0.217
50	2	2.10 ± 0.45		
15	2	1.43 ± 0.14		
October 14				
200	3	2.71 ± 0.46 ^{A}		
100	3	2.13 ± 0.12 ^{AB}	6.754	0.014*
50	3	1.95 ± 0.30 ^{AB}		
15	3	1.62 ± 0.24 ^{B}		

Table 3-12. Mean Chl *a* concentrations in samples collected from a benthic profile over mussel-free substrate at the sandy (5 m) station in August and October of 2009 compared by one-way analyses of variance. * Denotes significant *P*-values (< 0.05). Different capital letters in superscript denote sites determined significantly different from each other using Tukey HSD or Games-Howell post-hoc comparisons.

Date Height (cm above bottom)	**n**	**Chl *a* µg L^{-1} ± 1SD**	**F**	***P***
August 19				
200	1	1.26 ± na ^{A}		
100	3	1.87 ± 0.06 ^{A}	92.837	0.000*
50	3	2.01 ± 0.07 ^{A}		
15	3	0.94 ± 0.13 ^{B}		
August 26				
200	1	1.88 ± na		
100	3	2.71 ± 0.24	4.337	0.060
50	3	2.77 ± 0.07		
15	3	2.46 ± 0.32		
October 4				
200	3	0.27 ± 0.02 ^{A}		
100	3	0.22 ± 0.02 ^{B}	4.687	0.036*
50	3	0.23 ± 0.01 ^{AB}		
15	3	0.24 ± 0.01 ^{AB}		
October 14				
200	3	0.21 ± 0.01		
100	3	0.20 ± 0.01	3.794	0.058
50	3	0.19 ± 0.00		
15	3	0.22 ± 0.02		

3.4 Discussion

The distribution of SRP in this segment of Lake Ontario indicated localized areas of PO_4^{3-} enrichment occurring near external sources and at the lake bottom in proximity to dreissenids. Elevated SRP concentrations were measured in nearshore surface water samples in the spring and fall while offshore SRP was consistently low. SRP was high in samples collected at the mouth of Duffins Creek and near the Duffins Creek WPCP discharge, though generally not during the growing season. The turnover time of phosphate was longer in the nearshore and near local inputs than in the offshore. Higher concentrations of SRP measured in samples taken near dreissenid mussel-beds than over other substrata and from higher up in the water column support the hypothesis that *Dreissena* excretion generates locally-elevated SRP concentrations. The low levels of Chl *a* measured in samples taken near mussels-beds compared to those in surface water samples are consistent with the notion that *Dreissena* transforms particulate P to phosphate at the benthos. Dreissenid mussels appear to be an important source of phosphate for *Cladophora* through its main growing season.

One interesting finding of this study was that the influence of allochthonous sources on SRP concentrations in the nearshore was most often not apparent in surface water samples taken through the summer. Both the WPCP and Duffins Creek are undoubtedly sources of SRP, and my data support that they cause locally elevated SRP concentrations in the spring and the fall. However, although the WPCP supplies a continuous load of SRP to receiving waters, June 22nd was the only day within April to August in which elevated SRP concentrations were measured at this station versus other stations along the shoreline. Others have measured concentrations of SRP between 2 and 4 µg P L^{-1} SRP and even higher in water sampled directly beside the diffuser (L.F. Leon, Environment Canada, pers. comm.). It is possible that the impact of the WPCP during the growing season was masked by rapid assimilation of SRP either in the water column or in samples taken near this source in the time between their collection and filtration in the laboratory. The hypothesis that SRP was being biologically assimilated in sample bottles is supported by the dialysis data. Although dialysis membranes were not used at either Duffins Creek or the WPCP stations during the summer, SRP in dialyzed samples was often higher than in filtered samples along

the nearshore-offshore transect. Further, maximum PO_4^{3-} uptake rates measured for surface water at a mid-lake station in Lake Ontario were about 0.8 µg P L^{-1} h^{-1}(Lean et al., 1987). If we assume the half saturation constant is 20 nM or 0.62 µg P L^{-1} (Bentzen & Taylor, 1991) then the predicted uptake rate for PO_4^{3-} at 2 µg P L^{-1} would be about 0.6 µg P L^{-1} h^{-1}. This may explain why other research conducted in nearshore Lake Erie and Lake Ontario (e.g., Houben, 2007) did not report elevated SRP concentrations in water sampled near wastewater treatment plant outfalls. Another possibility is that samples from this station may not have been obtained directly from the effluent plume. The treated wastewater effluent is diffused from the outfall at approximately 9 m below the surface, and may not immediately mix with receiving waters (L.F. Leon, Environment Canada, pers. comm.).

The influence of Duffins Creek is likely more temporally dependent than the WPCP, as SRP is delivered intermittently by storm events and especially in the spring. A storm in April was documented to inject a high load of sediment from the Creek into the study area (Booty & Bowen, 2010), and I measured high concentrations of SRP in proximity to the mouth of Duffins Creek on April 28th and May 12th. Still, it is possible that rapid assimilation of SRP in samples taken near this source between collection and filtration or in the water column could have masked its contribution to SRP in the study area through the growing season. For example, despite a large, visible plume at the mouth of the Creek on May 29th, water samples from this station did not contain elevated SRP relative to other stations along the shoreline, with SRP < 1µg P L^{-1} at all stations. Subsequently, SRP concentrations measured near Duffins Creek remained low throughout the summer, and did not differ from other shoreline stations. Phosphate turnover time however, was very long (> 8 h) at the mouth of the Creek on August 19th, significantly longer than at the sandy and shallow stations, possibly indicating that planktonic communities near the mouth of the creek were less P-limited. Discrepancies between SRP concentrations and phosphate turnover time may occur because turnover time represents supply relative to demand, while SRP measures supply (but will often overestimate it). When PO_4^{3-} is very low, SRP is a poor indicator of PO_4^{3-} (Chapter 2). It may be that because true PO_4^{3-} was below the method detection limit, SRP was unable to account for actual differences in phosphate along the shoreline. Still, it is also possible that plankton communities were limited by light at the mouth of the creek due to increased turbidity. As comparisons of phosphate turnover time

were only done once along the shoreline, this study would have benefited from more measurements through the season to better address the possibility that the WPCP and the Creek locally reduce P-limitation through the growing season. Overall, the data support the hypothesis that the WPCP and the Creek lead to localized areas of elevated SRP in the nearshore, at least in the spring and autumn. Their impact in the summer may be masked by the rapid assimilation of SRP and the inability of SRP to measure low concentrations of PO_4^{3-}.

Although the development of a spring thermal bar can behave as a boundary between nearshore and offshore waters (Csanady, 1972a), Malkin et al. (2010) suggest that the observation of lower SRP in the nearshore during the spring is due to earlier growth of algae in the nearshore and consequent consumption of PO_4^{3-}. Nearshore waters warm sooner and, being shallower, have a more favourable mean irradiance for algal growth than deep, cold offshore waters. The spring SRP data in this study are indicative of a trend of elevated nearshore SRP relative to the offshore, but not to the degree that was observed in October. Other work has documented low and constant coastal and pelagic SRP concentrations during the summer in Lake Ontario (e.g., Makarewicz, 1991; Houben, 2007; Holeck, 2008). In addition to enhanced biological uptake as described above, the mixing between the nearshore and the offshore during the stratified period characteristic of this region (Csanady, 1972b; Blanton, 1975) may help to explain why differences in SRP concentrations between nearshore and offshore waters were not observable in the summer months. Thus, both biological uptake and hydrodynamic forces may have contributed to the lack of strong differences observed between nearshore and offshore waters during the spring and summer and may also help to explain why the localized influences of allochthonous inputs on SRP concentrations in the nearshore were not always apparent in water samples.

Higher concentrations of SRP were measured in water samples taken in close proximity to dreissenid mussel-beds compared to those over sandy substrate and those taken higher up in the water column through the *Cladophora* growing season. Interestingly, the trend of near-bottom SRP enrichment over mussel-beds compared to surface waters was most consistent and most pronounced at greater depths. It may be that the water column at the shallow station is too mixed to allow for observation of the vertical structure of SRP to the same degree as at deeper stations. Demand for PO_4^{3-} by *Cladophora* at shallower depths is

also likely greater than demand by the light-limited *Cladophora* stands at the deep station. Still, the measurements of SRP through the water column at the deep, mid and shallow stations are evidence for the hypothesis that *Dreissena* can lead to higher SRP near the bottom where *Cladophora* grows. While this study reported very low SRP concentrations in surface waters throughout the growing season, SRP was usually higher in samples obtained from waters overlying mussel-beds. These data suggest that dreissenids could make PO_4^{3-} available to *Cladophora* when its demand for P is highest, an effect less apparent by external inputs. As dreissenids have colonized large areas of the nearshore zones of the lower Great Lakes, their impact relative to allochthonous inputs may be more substantial in time and space.

These results are in agreement with other work demonstrating the potential for *Dreissena* to be an important source of SRP in the current nearshore lake environment. Ozersky et al. (2009) studied *D. bugensis* excretion at a different northwestern segment of Lake Ontario. The authors reported ambient SRP over *D. bugensis* mussel-beds (20 cm above the bottom at stations of 1.5 m depth) from 0.9 to 2.8 µg P L^{-1}, with an average of 1.9 µg P L^{-1} across their study dates, and found that SRP increased to a range of 1.9 to 4.2 µg P $L,^{-1}$ averaging of 3.0 µg P L^{-1} in chamber incubations of lake water over mussel-beds cleared of *Cladophora*. The chamber experiments by Ozersky et al. (2009) also indicated increased SRP concentrations in chambers with mussels present compared to incubations on mussel-free rock. The authors also calculated excretion rates, reporting that mussels excreted SRP at an average rate of 7.02 µg SRP g DM^{-1} $h,^{-1}$ and concluded that P released by *D. bugensis* was in excess of the demands of *Cladophora* in their study area, and the total supply by mussels was greater than SRP supplied by a large tributary and a waste water treatment plant outfall during *Cladophora'*s main growing season. There are no other studies to my knowledge that have investigated SRP concentrations directly over dreissenid mussels in lake environments, but laboratory studies have also demonstrated high P excretion by dreissenids. For example, excretion rates reported by Conroy et al. (2005) were similar to excretion rates measured by Ozersky (2009), estimating that *D. bugensis* can supply 6.83 µg SRP g DM^{-1} $hr.^{-1}$ Arnott & Vanni (1996) estimated even higher rates by *D. polymorpha*, at 13.75 to 31.58 g SRP µg DM^{-1} hr^{-1}.

SRP was useful for making comparisons of relative concentrations within the nearshore to investigate potential sources leading to localized areas of phosphate enrichment. However, a number of shortcomings of this study should be noted. Sampling earlier in the season would have provided more insight into initial SRP conditions prior to significant biological assimilation. Filtration immediately following sample collection or using dialysis at external sources through the summer may have improved my ability to accurately assess their impact during the growing season. Another drawback is the uncertainty of the SRP values beneath or approaching the detection limit. While the elevated concentrations of SRP in April, October, and over dreissenid mussel-beds made with the standard assay may be indicators of available PO_4^{3-}, the low concentrations measured in surface waters through the growing season are not likely representative of PO_4^{3-} concentrations (Chapter 2).

Other physical measurements, such as temperature, light attenuation, and conductivity, would have provided context for the patterns of SRP concentrations observed. Measurements of total phosphorus would have been helpful near Duffins Creek to evaluate the true P load to the area, as much of the P delivered by the Creek is likely not in dissolved form but might have been convertible to available P by mussels. Possible improvements to sampling procedures would include triplicate measurements for SRP at all times and more samples from 15 cm above the bottom at the shallow and sandy stations. Further, it is not certain that the elevated concentrations of SRP found over mussel-beds at the deepest station were not also an effect of depth. Higher SRP measured in hypolimnetic waters is not unexpected due to reduced mixing, a lesser demand of PO_4^{3-} by algae, and the remineralization of settled organic matter at the bottom of the lake. An additional station, possibly over a sandy bottom, but at a greater depth, or even a midlake station would have served as a better control for the possible effects of depth on SRP concentrations. Finally, quantifying mussel density and *Cladophora* biomass in the study year would have made the results of this study more applicable to other systems and perhaps more useful for modelers and managers.

Uptake, external inputs, internal re-cycling and hydrodynamic process control the seasonal pattern of ambient SRP concentrations in nearshore waters. Recent model simulations predict that the SRP domain over which *Cladophora* growth will be responsive to P management is on the order of 0.2 to 1.0 μg P L^{-1} (Tomlinson et al., 2010), which is less

than, or approaching, the detection limit of most chemical techniques. Model approaches also document the importance of SRP concentrations during the period of rapid *Cladophora* biomass accrual (i.e., mid-June to mid-July) (Higgins et al., 2006). In this study, SRP concentrations in the nearshore were often greater than 1 µg P L^{-1} from April to June, but were < 1 µg P L^{-1} in July and August in waters not in proximity to dreissenids. PO_4^{3-} concentrations measured below this critical threshold are likely even lower because of the inability of SRP to represent phosphate at low concentrations (Chapter 2).

The results from this Chapter support the hypothesis that allochthonous sources cause locally elevated SRP concentrations, though generally not when demand for P by *Cladophora* is highest. Their impact in the summer may be masked by the rapid assimilation of SRP, or the inability of SRP to measure low concentrations of phosphate. The data presented here are also evidence for the hypothesis that dreissenid mussels increase phosphorus availability for *Cladophora* (Hecky et al., 2004), and it appears they supply phosphate to the benthic alga through its period of maximum biomass accrual. Dreissenids cover extensive areas of the lake bottom, beyond those directly affected by external inputs. The wide spatial distribution of *Dreissena* and its ability to continuously excrete SRP during the summer may mean that P-recycling by dreissenids is the most important sustainer of *Cladophora* during its growing season, especially if acting in conjunction with the mussels' ability to improve the underwater light climate (e.g., Malkin et al., 2008; Auer et al., 2010). In fact, the results from this study suggest dreissenid mussels can maintain elevated SRP in near-bottom waters, even while SRP concentration in surface waters and near external inputs are very low, and could therefore be responsible for the return of nuisance levels of *Cladophora* in the lower Great Lakes. If this is the case, the influence of dreissenids on nearshore P dynamics may necessitate even more stringent phosphorus controls in order to effectively manage *Cladophora* growth. Further research and monitoring focused on the nearshore zones of all the lower Great Lakes is required to obtain SRP data-sets representative of current conditions. As *Cladophora*'s response to P management is also dependent on its internal P stores, programs would benefit from measurements of tissue P concentrations from *Cladophora* stands through the season and at different depths. Such endeavors concentrated on the nearshore combined with model simulations should be helpful in predicting *Cladophora*'s response to further P mitigation strategies.

Chapter 4 Summary of Conclusions and Future Directions

Both allochthonous sources and internal cycling by dreissenid mussels appear to give rise to locally elevated phosphate concentrations in nearshore Lake Ontario, as indicated by higher levels of soluble reactive phosphorus in samples obtained in proximity to these sources. Indeed, when water samples are not filtered to completion and if filtration is conducted at low pressure, SRP is likely an adequate indicator of phosphate when it is at elevated concentrations. What is "high" will vary across systems, but it is unlikely that concentrations below 1 µg P L^{-1} in P-limited waters are accurate indicators of PO_4^{3-}. However, elevated concentrations of SRP were generally not measured near external sources during the period of maximum *Cladophora* biomass accrual, while a trend of higher SRP above mussel-beds than in surface waters was observed through the *Cladophora* growing season. *Dreissena* has colonized large areas of the nearshore zones of the lower Great Lakes and its ability to enhance water transparency has extended *Cladophora* habitat to greater depths. The results of this study indicate that dreissenid excretion may sustain high SRP concentrations directly available to *Cladophora*, at times well over the 1 µg P L^{-1} ambient concentration of SRP predicted to support nuisance abundances of the benthic alga. Phosphate excreted by dreissenid mussels may be a more substantial source for *Cladophora* in time and space than phosphate provided by allochthonous inputs. If this is the case, nuisance *Cladophora* growth in the current nearshore environment could be ascribable to P-recycling by invasive dreissenid mussels.

SRP is useful for making comparisons of relative concentrations within the nearshore when they are high. However, SRP values below ~ 1.0 µg P L^{-1}, i.e., at or below the detection limit, should be interpreted with caution. As the range of SRP concentrations to which *Cladophora* growth is thought to be responsive are also as low as ~1.0 µg P L^{-1}, there are limitations to using SRP to study this problem. Another factor that may affect the utility of SRP in P-limited conditions is that phosphate introduced by point sources and tributaries may be rapidly sequestered by biota. In such situations, SRP could underestimate the contribution of these sources to the local nutrient supply. For example, allochthonous sources are undoubtedly sources of SRP, and my data support that these inputs cause locally elevated SRP concentrations in the spring and autumn. However, their contribution of SRP

to the nearshore may have been masked during the summer because of their rapid assimilation by benthic algae, by seston in the water column, or even by seston in samples prior to filtration.

The influence of dreissenids on nearshore P cycling may necessitate even more rigorous phosphorus controls in order to effectively manage *Cladophora* growth. Further P management would be ambitious and would likely require a concerted lake-wide effort. Whether or not the results of such actions would be effective or even desirable requires further attention. Continued research focused on phosphorus dynamics in space and time within the nearshore zones of the lower Great Lakes is required to better inform managers and modelers concerned with this problem. Research and monitoring programs should include measurements of total phosphorus concentrations supplied by tributaries because of the potential for particulate P to be converted to P available to *Cladophora* by dreissenids. Sampling for SRP early in the season is necessary to assess concentrations in the nearshore prior to significant biological assimilation. Filtration (with measures taken to reduce artifacts) immediately following sample collection may be necessary to demonstrate the impact of external inputs on SRP concentrations during the growing season. Dialyzed samples incubated in proximity to local inputs during the summer may be useful to test if phosphate is sequestered in samples before filtration, as higher SRP was often measured in dialyzed samples than in filtered samples during the growing season. Dialysis membranes are also helpful to test for filtration artifacts. However, the use of dialysis is not appropriate for routine monitoring and the potential for contamination or interference by substances on the tubing should be investigated. Sample collection very close to dreissenid mussel-beds (i.e., 15 cm to 50 cm above the lake bottom) is necessary to account for their impact on local SRP concentrations. However, as the highest concentrations of SRP measured over dreissenid mussel-beds were obtained near the bottom at the deepest station (17 m), further work involving SRP measured over dreissenid mussel-beds should include SRP data collected from corresponding depths at other locations in the lake. Finally, as *Cladophora*'s response to P management is dependent on its internal P stores, programs would benefit from measurements of tissue P through the season and at different depths. *Cladophora* growth models combined with research and monitoring efforts focused on the nearshore should be better able to predict *Cladophora*'s response to further P mitigation programs.

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