The Effects of an Imidazolium Ionic Liquid on Net Primary Productivity in Lakes Across a pH Gradient

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ABSTRACT

The effects of treatment with an imidazolium ionic liquid, 1-butyl-3-methylimidazolium bromide (bmimBr) and the natural ecosystem effects on net primary production were investigated in this dual manipulative and observational study. Because scientific knowledge regarding the influence of ionic liquid waste on organisms is largely limited to toxicity tests in the laboratory, this study sought to examine the direct effects of bmimBr on a variable characteristic of limnetic ecosystems—aquatic net primary productivity, using water samples from lakes on the property of the University of Notre Dame Environmental Research Center. For the IL manipulations, dissolved oxygen concentrations and pH in air-tight bags with sample water from six lakes (controls), and sample lake water with low or high bmimBr concentrations were measured before and after a six-hour period of deployment in a lake mesocosm. The IL treatments were found to have significant effects on hourly changes in dissolved oxygen, the trends of which often differed with lake pH. For the natural observations, the same setup was employed with sample lake water, but no bmimBr was added to the bags, and the six-hour period of deployment occurred in the eight source lakes. Several lakes were found to significantly differ in hourly changes in dissolved oxygen, often along a pH gradient, and varying with weather conditions. Divergence from a tight correlation with pH is likely due to the endogenous trophic levels within each lake that affect algal abundance. The results indicate that bmimBr affects aquatic net primary productivity differently due to varying ecosystem characteristics, which can be explained, in part, by lake pH. At the concentrations studied, bmimBr is detrimental to algal primary production rates (or microbial respiration rates), depending on the specific limnetic system, suggesting that further studies of ionic liquid effects on ecosystems should be considered in the design of safer alternatives to industrial solvents.

Key-words: Net primary production, imidazolium ionic liquid, 1-butyl-3-methylimidazolium bromide

INTRODUCTION

With the emerging applications of green chemistry, corporate, academic, and governmental laboratories may soon begin substituting room-temperature, non-volatile ionic liquids (ILs) for traditional, volatile industrial solvents such as toluene and benzene (Kulacki and Lamberti 2008). However, knowledge of the toxicity and biodegradability of these compounds is limited at best, and longer-carbon chain, biodegradable ILs can still be highly toxic to environmental trophic levels (Docherty, et al. 2007). Incomplete biodegradation forms toxic metabolites in addition to carbon dioxide, biomass, and water (Kulacki et al. 2007). Aquatic organisms can be adversely affected by the interaction of ILs with both abiotic and biotic components of their habitat, necessitating detailed investigation of these interactions to aide in the chemical design of ILs that prevent or minimize damage to the environment.

Primary productivity refers to "the rate of formation of organic matter from inorganic carbon by photosynthesis," and is an important ecosystem measure that affects all trophic levels (Bott 2006). Thus, the effects of ILs on net primary productivity, primary productivity less respiration, should be studied in order to make comprehensive predictions about the environmental consequences of ILs. Oxygen production is facilitated by photosynthesizers, while autotrophs, heterotrophs, bacteria, and decomposers all contribute to respiration via metabolism and decomposition in aquatic environments (Bott 2006).

Within the past year, a study at the University of Notre Dame examined the effects of three imidazolium ILs of increasing carbon substituent length (and thus increasing toxicity) on two genera of freshwater algae (Kulacki and Lamberti 2008). All ILs tested were found to stunt the growth of the algae to varying degrees, based on the toxic interaction of ILs with specific structural differences of the algae. In addition, the study found that the exposure of one genera of algae to a nutrient media lessened the IL damage to growth rate, suggesting the importance of considering abiotic factors in determine the ecological effects of ILs. A measure of pH could be studied in relation to IL-treated water samples to investigate for a potential difference in IL effects along a pH gradient.

In light of the current relevance of IL use in an industrial capacity, and the need to design effective, environmentally benign IL forms, my principal objective was to test the effects of an imidazolium ionic liquid, 1-butyl-3-methylimidazolium bromide (bmimBr), on changes in dissolved oxygen concentration, the measure of net primary productivity, in phytoplankton-containing limnetic water samples from lakes in Northern Wisconsin and the Upper Peninsula of Michigan. A 2003 study found that the toxicity of metals such as copper, present in limnetic systems, increases with increasing pH, suggesting that the toxicity of other chemicals such as IL's could also change with differing lake pH's (De Schamphelaere et al. 2003). Thus, study lakes were selected on the basis of a pH gradient (UNDERC Lakes Appendix I) to investigate the effects of bmimBr on changes in dissolved oxygen concentration in differing water chemistry--acidic, neutral, and basic. In addition, because of the need to expand the study of ILs to include their effects on complex ecosystems, a host of lakes, including those from which limnetic samples were used in the IL manipulations, were also studied to determine changes in dissolved oxygen concentration along the pH gradient of the lakes under natural conditions.

To address both my IL manipulation and natural observation studies, my hypothesis was twofold: (1) Both low and high concentrations of the imidazolium ionic liquid, bmimBr, will affect the hourly change in dissolved oxygen concentration in treatment bags with respect to the control bags in a lake mesocosm. (2) Natural control of hourly changes in dissolved oxygen concentrations will also change across lakes along a pH gradient, according to the relative balance between algal primary producers and respiring microbes and decomposers. Knowledge of the relationship between net primary productivity and pH could aide in predictions of IL effects on natural Northern Wisconsin and UP of Michigan lakes, and, by extension, other aquatic habitats.

MATERIALS AND METHODS

Lake Study: Ionic Liquid Manipulation

Prior to IL experimentation, a stock solution of 26.82 mg/L bmimBr was prepared from 13.41 g in 500 mL de-ionized water. On each morning of IL testing, ten liters of water from a sample lake, filtered through a 250 micrometer sieve, were collected. The location from which each sample of lake water was collected for observational study or IL manipulation, and the chronology of collection is detailed in Table 2 of “Tables and Figures.” The lake dissolved oxygen concentration and temperature were measured 10 cm from the surface using a YSI Environmental DO200 Oxygen Probe, and pH was measured with a Hanna pH probe at the same level. The air temperature was also measured and recorded. In the laboratory, 1 L and 0.25 L volumetric flasks were filled three-quarters full with lake water, the appropriate volume of bmimBr stock solution was added to the flask to achieve the desired IL concentration level—50 mg/L for the “low” treatment, and 500 mg/L for the “high” treatment, and the remaining flask volume was filled with lake water. The added volumes of bmimBr are specified in Table 1, specific to flask volume and IL concentration. All flasks were mixed thoroughly.

To prepare the five control bags, 250 mL of lake water was poured from a bucket to a graduated cylinder, and subsequently poured into each labeled Whirl Pak bag. The initial dissolved oxygen concentration and temperature was measured with the DO200 probe, and then the bags were sealed without any air bubbles. The same pouring, measuring, and sealing procedure was repeated for the five low-IL concentration treated bags and five high-IL concentration treated bags. Each labeled bag was tied to a clay tile using fishing line, and deployed in an outdoor lake mesocosm, a circular black tub filled with well water, approximately 0.5 m high and 0.75 wide. The bags were anchored to float approximately 10 cm from the surface. Weather conditions, air temperature, and the water temperature and dissolved oxygen concentration of the mesocosm were recorded. After six hours, all bags were removed from the mesocosm, and individually opened to measure their dissolved oxygen concentration, temperature, and pH. Air temperature and weather conditions were again recorded. All IL-containing lake water was disposed in an IL waste container.

Lake Study: Natural Observation

On each morning of observational study of the UNDERC lakes, the lake dissolved oxygen concentration and lake temperature were measured approximately 10 cm from the surface using a YSI Environmental DO200 Oxygen probe. The lake pH was measured using a waterproof Hanna pH probe, and air temperature was measured using a thermometer. All measurements were recorded, along with a detailed weather observation. One bucket of water was collected from each sample lake, and filtered through a 250 micrometer sieve. 250 mL of lake water was carefully poured, minimizing air bubbles, into each of six labeled Whirl Pak bags. The initial dissolved oxygen concentration and temperature was measured with the DO200 probe, and sealed without air. Each labeled bag was tied to a clay tile using fishing line, and deployed two to four meters offshore, anchored to float about 10 cm from the surface. After six hours, all bags were removed from the lake, and individually opened to measure their dissolved oxygen concentration and temperature. Air temperature and weather conditions were again recorded.

Unfiltered water samples were collected in 1L glass jars from each lake and returned to the laboratory. There, water was passed through a Type AE glass fiber filter (1µm pore size). Filtrate was frozen and filters were stored in film canisters in a freezer, for soluble reactive phosphorus and chlorophyll *a* analysis, respectively. At the end of the sampling period, SRP analysis was conducted using a Spectronic® Genesys™ 2 spectrophotometer according to standard procedures (Steinman and Mulholland 2006). Chlorophyll *a* analysis was conducted on filters using the methanol extraction method on a Quantech™ fluorometer (American Public Health Association). The change in dissolved oxygen concentrations between initial and final data collection times was calculated for each bag, and the hourly change in oxygen concentration was calculated based on the time allotted for production in the lake or mesocosm.

All statistical analyses were conducted using SYSTAT® 12. An ANOVA was run for each lake water sample manipulated with bmimBr to determine the effects of bmimBr treatments on hourly changes in dissolved oxygen concentration with respect to controls. With the expectation that low and high concentration treatments of bmimBr would differ significantly from controls in hourly change in dissolved oxygen, Tukey's post-hoc analysis was used to determine which treatments were significantly different. An ANOVA was also run for each lake that was sampled for both IL manipulation and natural observation under the same weather conditions to test for significant differences in the hourly change of oxygen concentration between natural lake water bags and control, low bmimBr, and high bmimBr experimental bags. In addition, an ANOVA was conducted across all lakes naturally observed under sunny conditions, and all lakes observed under overcast conditions, to determine differences in hourly changes in oxygen concentration between lakes under natural conditions. In the absence of preconceived notions about differing hourly changes in oxygen concentration, Fisher's Least-

Significant-Difference Test was used in post-hoc analysis to determine which lakes were significantly different under sunny or overcast conditions.

Finally, paired t-tests were conducted to identify the effect of sunny versus overcast conditions on mean hourly change in oxygen concentration for the lakes sampled for natural observation on days of both weather conditions.

RESULTS

Lake Study: Ionic Liquid Manipulation

In the IL manipulations, the three lakes with the most basic pH's -- Morris, Plum, and Brown Lakes (Fig. 2) -- demonstrated a similar trend in which the control bags were characterized by the least negative change in hourly oxygen concentration (in the cases of Morris and Brown) or the most positive change in hourly oxygen concentration (in the case of Plum). Either respiration rates or diminished production rates depressed the dissolved oxygen concentrations in the low IL treatment bags, and to an even greater extent in the high IL treatment bags. In contrast, Bog Pot and Roach—lakes with acidic pH—demonstrated a different trend in which the control bags were characterized by the most negative change in hourly oxygen concentration, and the low IL and high IL treatment bags were characterized by a less negative change in hourly oxygen concentration, with no significant difference between the two treatments. Finally, Cranberry Lake, the most acidic lake tested, demonstrated third trend in which the low IL treatment bags were characterized by the most negative change in hourly oxygen concentration, followed by the high IL treatment bags' less negative change in hourly oxygen concentration that was not significantly different from the control bags. Statistical results of IL effects are listed in Table 3 and depicted in Figure 1 of "Tables and Figures," with significant differences (ANOVA followed by Tukey's test) in comparison with controls starred.

In addition, with the exception of Bog Pot, all natural bags of lake water were significantly different (all $p<.00001$) from control bags in the IL manipulation experiments (under the same weather conditions).

Lake Study: Natural Observation

In the natural observation of the eight test lakes, differentiated by weather conditions, hourly change in oxygen concentration mirrored the pH gradient across the lakes. Under sunny conditions, lakes with neutral or basic pH demonstrated positive changes in oxygen concentrations. In contrast, lakes with increasingly acidic pH's demonstrated increasingly more negative changes in hourly oxygen concentration. Experimental measures of pH across lakes are depicted in Figure 2, and natural control of primary production under sunny conditions is depicted in Figure 3.

Under overcast conditions, more basic lakes demonstrated positive hourly changes in dissolved oxygen concentration, whereas more acidic lakes demonstrated negative hourly changes in dissolved oxygen concentration. Morris, with fairly neutral pH of 6.7, demonstrated an hourly change in oxygen concentration that changed from positive to negative under overcast, rather than sunny conditions. Bog Pot and Roach, both with a pH of 5.2, did not demonstrate significant differences from each other in hourly change in oxygen concentration. Natural control of primary production under overcast conditions is depicted in Figure 4.

The three lakes tested on two separate instances of different weather conditions reflected the action of more primary production under sunny versus overcast conditions. Bog Pot (pH of 5.2) demonstrated a less negative change in hourly oxygen concentration under sunny conditions, while still decreasing overall—indicating net respiration. Plum (pH of 7) demonstrated a more positive change in hourly oxygen concentration under sunny conditions, while increasing overall—

indicating net production. Morris (pH of 6.7) demonstrated a change in hourly oxygen concentration from positive to negative corresponding to the change in weather conditions from sunny to overcast. Weather effects on primary production are depicted in Figure 5.

Chlorophyll *a* concentrations in parts per billion from each of the test lakes indicated Bog Pot with the highest chlorophyll *a* concentration (2.306 ppb), and Roach and Crampton with the lowest (0.301 ppb and 0.368 ppb, respectively), as depicted in Figure 6. Chlorophyll *a* content showed no observable correlation with hourly change in dissolved oxygen concentration (Fig. 7), as suggested by an r^2 value of 0.007.

The relative soluble reactive phosphorus (SRP) concentrations in micrograms per liter of water samples from each of the test lakes indicated basic Brown with the highest SRP concentration (4.507 µg/L) and acidic Roach with the lowest SRP concentration (nearly 0 µg/L), as depicted in Figure 8. SRP concentration showed no observable, strong correlation with hourly change in dissolved oxygen concentration (Fig. 9), as suggested by an r^2 value of 0.273.

DISCUSSION

Lake Study: Ionic Liquid Manipulation

In accordance with part (1) of the alternative hypothesis, bmimBr treatments did have a significant effect on the hourly change in dissolved oxygen concentration for five of the six lakes sampled for the IL manipulation study (Fig. 1). Three different trends were observed for the six lakes, corresponding to a natural lake pH gradient. Control bags in the neutral to basic lakes—Morris, Plum, and Brown—demonstrated the most production (and/or least respiration). In addition, the low bmimBr concentration treatment bags demonstrated less production (and/or more respiration), and the high bmimBr treatment bags demonstrated the least production (and/or most respiration). As presented in Ranke et al.'s review of ionic liquids, cationic surfactants such as bmimBr are often more toxic toward algae than anionic varieties, and would hinder algal growth in the limnetic systems tested (2007). The low (50 mg/L) and high (500 mg/L) treatments greatly exceeded bmimBr's aquatic toxicity values, which range between 5 and 50 mg/L (Ranke et al. 2007). Thus, the IL manipulation proved fatal or severely stunted the growth of algae in the low and high bmimBr treatment bags, as correlated with their concentrations.

Plum Lake was the only case which demonstrated positive net production (for control bags and low bmimBr treatment bags) in the lake mesocosm for IL manipulations. It is plausible that the relative abundances of species in this limnetic system's food web facilitated a preponderance of algae. In accordance with the Trophic Cascade Hypothesis (Carpenter et al. 1987), the presence of many planktivores such as bluegill in Plum would depress zooplankton populations, which would in turn maintain high algal populations in the water column. Alternatively, a limited population of planktivores could seek refugia in the littoral regions of Plum to avoid predation by Plum piscivores such as smallmouth bass, walleye, northern pike, and muskellunge (Carpenter et al. 1987). Zooplankton populations would be depressed only in the littoral regions of concentrated planktivores, facilitating algal growth in the littoral region—the area where water was sampled for this study. The Whirl Pak bags with lake water from Plum could thus contain high levels of algae.

No significant differences in hourly change in dissolved oxygen concentration were evident between bmimBr-treated bags and control bags for Brown Lake, suggesting that its nature as a highly productive, eutrophic lake supports a preponderance of algal species, some of which may not be as affected by ionic liquids (Williamson et al. 1999).

The second trend, present in the moderately acidic lakes—Bog Pot and Roach—indicated control bags with the least production (and/or most respiration). The low and high bmimBr treatment bags demonstrated about the same hourly change in oxygen concentration, which reflected more production (and/or less respiration) than controls. Ranke et al. reported in 2007 that IL's can have damaging effects on microbial metabolism. Thus, bmimBr treatments likely proved fatal and/or severely stunted the growth of the microbes and decomposers present these acidic, dystrophic environments to a greater extent than the algal species, thus depressing respiration rates or damaging respiration more greatly, relative to production. IL effects on microbes could have important implications for bacteria that decompose human and other wastes (Ranke et al. 2006). It should be noted that oxygen levels in Bog Pot and Roach lake water samples never fell to anoxic conditions, ruling out the possibility of oxygen as a limiting resource for competing respirers.

The final trend, present in Cranberry--the strongly acidic lake—indicated the least production (and/or most respiration) in the low concentration bmimBr treatment bags. The control bags and high bmimBr treatment bags demonstrated about the same hourly change in oxygen concentration, which reflected less respiration (and/or more production) than controls. Toxic damage of bmimBr at low levels likely proved fatal and/or stunted the growth of the algal species present in Cranberry's bog-like environment, which depleted dissolved oxygen concentrations, and may explain this trend. However, toxic damage of bmimBr at high levels affected the microbes and decomposers as well as the algae, balancing the production and respiration rates back to control levels, which were less negative than the low bmimBr treatment. Further study of the unique algal and microbial species present in each limentic system could provide a greater understanding of bmimBr's species-specific effects at different concentrations.

For example, a recent study has shown that other imidazolium salts are toxic to some aquatic organisms in the concentration ranges used in our study such as *Scenedesmus* *quadricauda* and *Daphnia magna*, but not to others, such as *Dreissena polymorpha* and *Pimephales promelas* (Kulacki and Lamberti 2008).

Lake Study: Natural Observation

In full sunlight, the most favorable condition for primary production, hourly changes in dissolved oxygen concentration varied across the natural pH gradient of the lakes (Fig. 3). Positive hourly changes in oxygen concentration (net productivity) for the neutral to basic lakes (Morris, Plum, and Brown) suggest the high level of productive activity of these eutrophic lakes. Negative hourly changes in oxygen concentration (net respiration) for the acidic lakes (Cranberry, Bog Pot, and Crampton) suggest the decreased productive activity of these dystrophic or oligotrophic lakes relative to eutrophic (Todd Crowl, personal communication). Acidic aquatic environments facilitate higher decomposition rates by increasing the abundance of microbes and decomposers, which increases particulate and dissolved organic matter. The resultant increased turbidity of the water and light obstruction could diminish the productivity of such environments, and justifies the net respiration witnessed in Cranberry and Bog Pot (Goldman 1988).

Crampton, an oligotrophic lake with low production due to minimal algae but high dissolved oxygen content, was not significantly different in hourly changes in dissolved oxygen from Brown (pH of 8.2) or Bog Pot (pH of 5.2) (Todd Crowl, personal communication). The oligotrophic nature of Crampton is supported by its very low chlorophyll *a* concentration of 0.368 ppb. Thus, despite its acidic pH of 5.4 and absence of an established, productive algal community, the relative balance between oxygen producers and consumers prevented significant divergence from either eutrophic Brown or dystrophic Bog Pot. However, it should be noted that Brown and Bog Pot differ significantly in hourly change in oxygen concentration.

Neutral to basic aquatic environments can facilitate greater algal diversity and production rates than strongly acidic environments, changing the production-respiration balance in favor of production (Williamson et al. 1999). Detritus and other organic matter from decomposition are absorbed as nutrients that cycle through the productive populations of algae and other organisms. The clearer water column (relative to dystrophic systems) facilitates light penetration, which in turn promotes aquatic photosynthesis.

Under overcast weather conditions, a less favorable condition for primary production, hourly changes in dissolved oxygen concentration also varied across the natural pH gradient of the lakes (Fig. 4). Positive hourly changes in oxygen concentration (net productivity) for Tenderfoot again suggest the productivity of this eutrophic lake, and negative hourly changes in oxygen concentration (net respiration) for Bog Pot and Roach (same pH of 5.2 and not significantly different), suggest the decreased productivity of these dystrophic lakes relative to eutrophic (Williamson et al. 1999). The hourly change in dissolved oxygen concentration in neutral Morris (pH of 6.7) was negative and for Plum (pH of 7) was positive. This opposing trend for these fairly neutral and eutrophic lakes suggests that the slight acidity of Morris may have caused respiration of microbes to dominate photosynthesis in overcast conditions—stressful to algae. Alternatively, this result could be attributed to biotic factors such as the different trophic cascades based on fish species present in Morris and Plum.

In testing the same lake under both sunny and overcast conditions, it was found that weather can affect production rates (Fig. 5). The effect of weather was significant only for Morris and Plum, the neutral lakes. Too much variation was present in the testing of Bog Pot under the two different weather conditions, likely due to heavy precipitation preceding the first sampling dates. Morris best demonstrates the effects of weather conditions on primary productivity in comparison with the other lakes. In full sun, sufficient light causes positive hourly changes in oxygen concentration by net productivity, but under overcast conditions, insufficient light leads to negative hourly changes in oxygen concentration by net respiration.

More lakes should be studied under diverging weather conditions to make any conclusive claims about the specific effects of weather, beyond the trend of greater production relative to respiration under sunny conditions. In addition, rain may either dilute or add nutrients to limnetic systems, depending on the specific identities of the dissolved matter. Because several sampling days were preceded by days of rain, results could have been affected. Heavy rain or wind associated with rain could oxygenate the water column and/or mix the water to make it more turbid and less transparent, preventing light penetration necessary for aquatic photosynthesis. Thus, a continuation of this study should also investigate the specific effects of rain and wind on days prior to sampling.

Chlorophyll *a* concentration, reflecting algal abundance in limnetic systems, did not prove to be a valuable indicator of net productivity (Fig. 7), in line with the 1971 finding that changes in algal biomass are not proportional to changes in photosynthetic activity, with the exception of periods of intense productivity (Allen 1971). It should be noted that acidic lakes such as Roach and Cranberry had low chlorophyll *a* concentrations, while very basic Brown had a high chlorophyll *a* concentration, perhaps attesting to pH preferences of algal species present at UNDERC. Though Roach and Bog Pot have the same lake pH, the latter possessed a much higher chlorophyll *a* concentration, which suggests that the presence of piscivores in Roach depresses algal populations through a trophic cascade, whereas their absence in Bog Pot promote a preponderance of algae (Carpenter et al. 1987).

Similarly, soluble reactive phosphorus concentration did not tightly correlate with hourly changes in dissolved oxygen content (Fig. 9), but higher concentrations of the phosphorus nutrient indicative of highly eutrophic systems such as Tenderfoot and Brown possessed positive hourly changes in oxygen concentration (net production). In contrast, lower SRP concentrations indicative of dystrophic systems such as Cranberry and Roach possessed negative hourly changes in oxygen concentration (net respiration).

In addition, most of the hourly changes in dissolved oxygen concentration in the natural bags of lake water were significantly different from hourly changes in the control bags for the IL manipulation experiments (under the same weather conditions), suggesting that the lake mesocosm did not adequately mimic natural limnetic conditions. Other biotic and abiotic factors characteristic of the lakes are therefore likely influencing production rates. The lake mesocosm promoted a higher temperature than natural lake temperatures, and often shaded the bags near the end of the day, potentially affecting production rates. In addition, the mesocosm was filled with well water, rather than the sample lake water. To improve this study, a much larger lake mesocosm with regulated temperature and filled with source lake water could be constructed to better mimic natural light, temperature, and water quality conditions of test lakes. Alternatively, part of the source lake could be blocked off, providing a region in which IL-treated bags could be deployed under original conditions.

CONCLUSIONS

Thus, the imidazolium ionic liquid, bmimBr, affects aquatic net primary productivity differently according to the algal, microbial, and decomposing communities present, which vary with lake pH. Additional future studies should test a broader spectrum of lakes with additional replicates, more finely defined by specific weather conditions, including wind, which may oxygenate the water column through mixing. The effects of different kinds of IL's of varying biodegradability and toxicity should also be tested to gain a more holistic understanding of IL effects on ecosystems. Also, the specific abundances of piscivores, planktivores, and zooplankton should be determined to gain a greater understanding of the trophic cascade characteristic of each test lake, which will, in turn, affect the rate of primary production and IL effects on this rate. In all, these additional further studies will support the design of environmentally benign ionic liquids to replace harmful industrial solvents. The use and disposal of such ionic liquids will be possible only through the diligent, concerted efforts of toxicologists, ecologists, chemical engineers, and environmentally conscious corporations.

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TABLES AND FIGURES

Table 1 Preparation of high and low concentration treatments of bmimBr during IL manipulation

Volume Preparation for IL Treatments		
Treatment	**Flask Volume**	**bmimBr Volume Added**
Low (50 µg/L)	250 mL	466.07 µL
Low (50 µg/L)	1 L	1864.28 µL
High (500 µg/L)	250 mL	4660.70 µL
High (500 µg/L)	1 L	18642.80 µL

Table 2 Source lakes and date of sampling for both natural observation and IL manipulation studies

Location and Chronology of Lake Sampling		
Date	**Study**	**Lake**
6-7	IL Manipulation	Roach
6-30	Natural Observation	Bog Pot
6-30	Natural Observation	Morris
7-1	IL Manipulation	Bog Pot
7-2	Natural Observation	Bog Pot
7-2	Natural Observation	Morris
7-3	IL Manipulation	Morris
7-4	IL Manipulation	Cranberry
7-6	Natural Observation	Tenderfoot
7-6	Natural Observation	Roach
7-12	Natural Observation	Brown
7-13	IL Manipulation	Plum
7-13	Natural Observation	Plum
7-14	Natural Observation	Crampton
7-14	IL Manipulation	Brown
7-14	Natural Observation	Plum
7-15	Natural Observation	Cranberry

Table 3 Statistical results of ANOVA's conducted for each lake tested in IL manipulations

Results of Ionic Liquid Effects on Hourly Changes in Oxygen Concentration		
Lake	**p-value**	**Treatments Significantly Different from Controls**
Cranberry	.0079	Low
Bog Pot	<.0001	Low and High
Roach	<.0001	Low and High
Morris	.0027	High
Plum	<.0001	Low and High
Brown	.0993	None

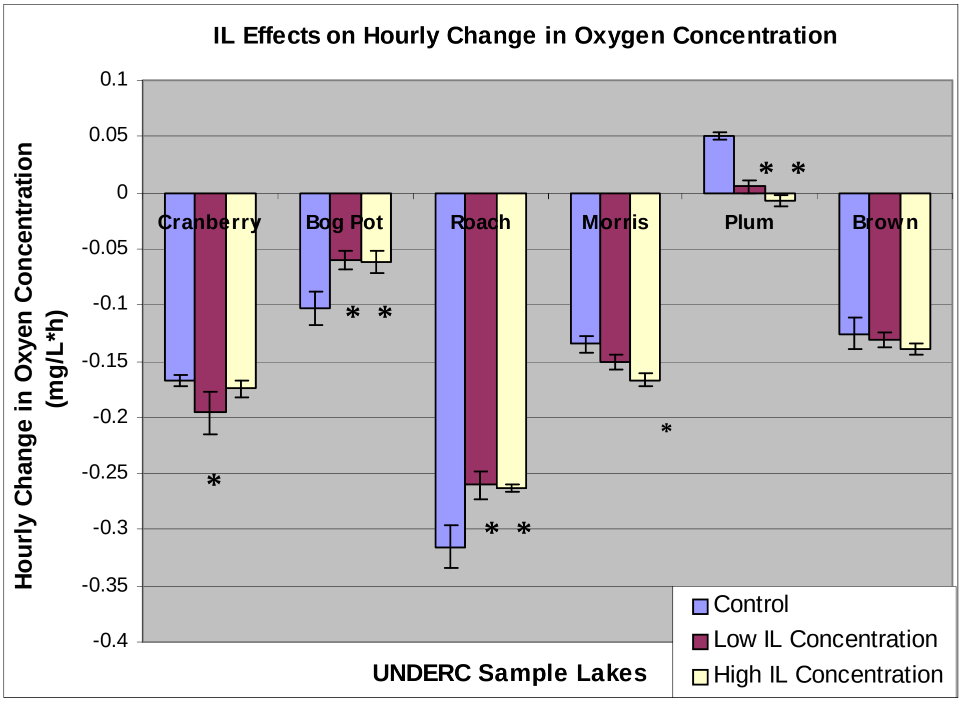

Fig. 1 Effects of low (50 µg/mL) and high (500 µg/mL) concentrations of bmimBr on the hourly change in oxygen concentration in Whirl Pak bags of sample lake water deployed in mesocosm. Bars represent ± 1 SD for each control or treatment mean. Asterisks represent significant differences in hourly change in oxygen concentration between treatment bags and control bags.

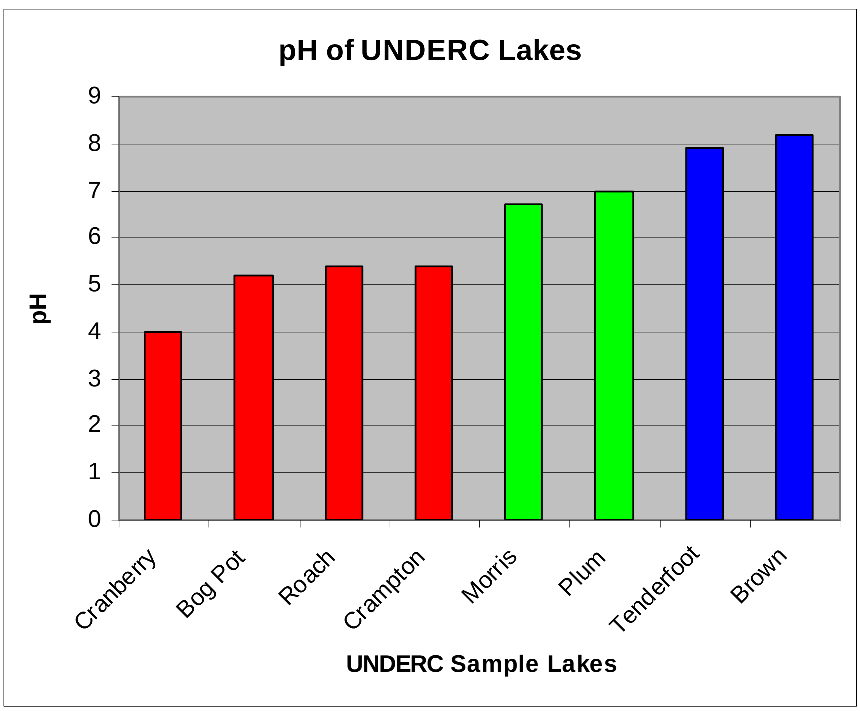

Fig. 2 Measurements of mean natural pH across the eight UNDERC test lakes of this study. Vertical bar represents ± 1 SD for the natural pH mean. Color gradations from red to green to blue reflect acidic, neutral, and basic pH means, respectively.

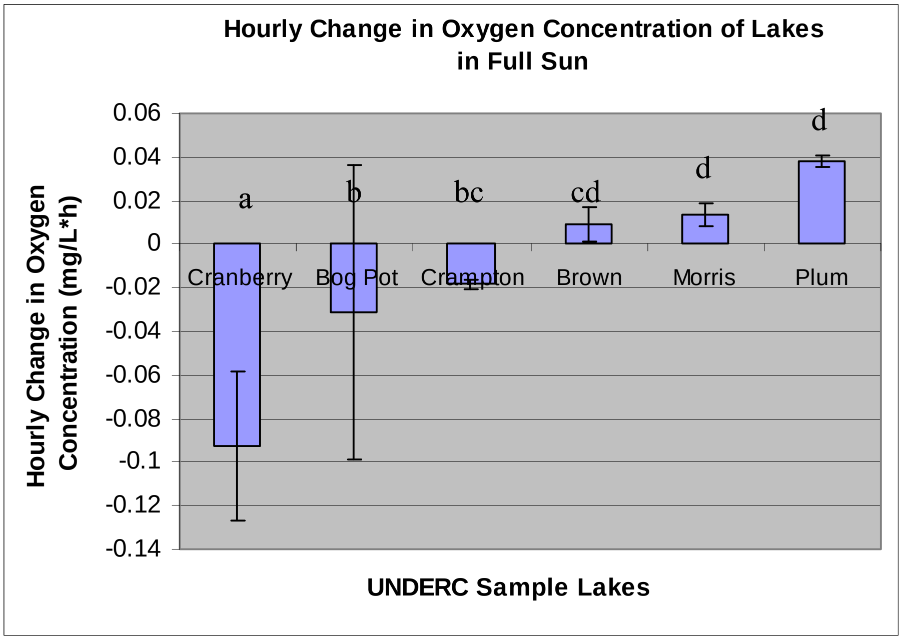

Fig. 3 Natural control of hourly change in oxygen concentration in bags of sample lake water across six different UNDERC lakes, deployed in full sunlight. Vertical bars represent ± 1 SD for each natural mean. Different letters represent significant differences in hourly change in oxygen concentration between bags of water from different lakes.

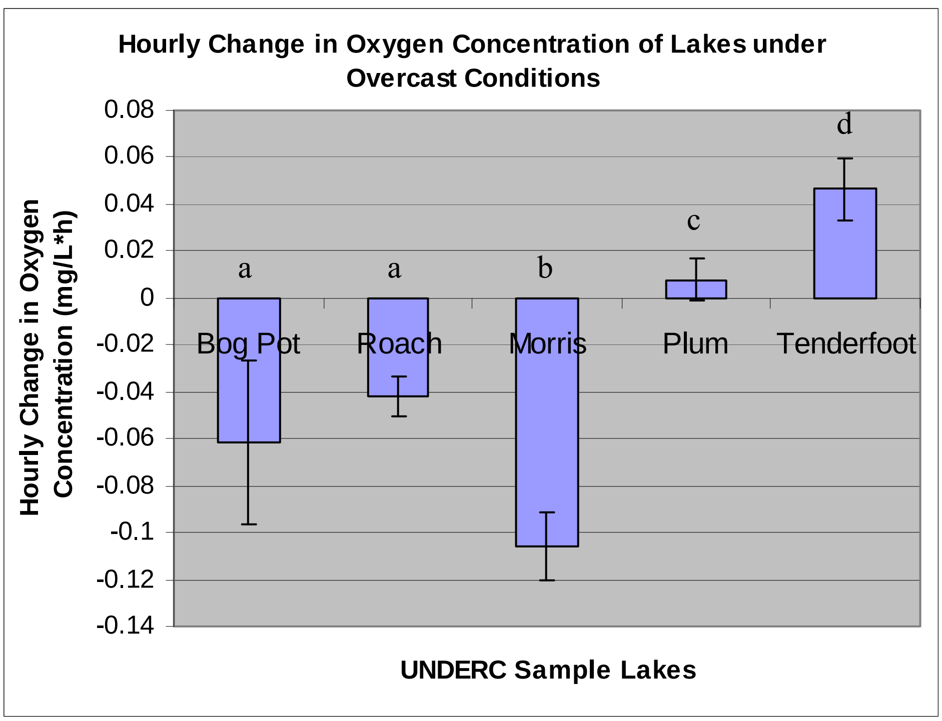

Fig. 4 Natural control of hourly change in oxygen concentration in bags of sample lake water across six different UNDERC lakes, deployed in overcast weather conditions. Vertical bars represent ± 1 SD for each natural mean Different letters represent significant differences in hourly change in oxygen concentration between bags of water from different lakes.

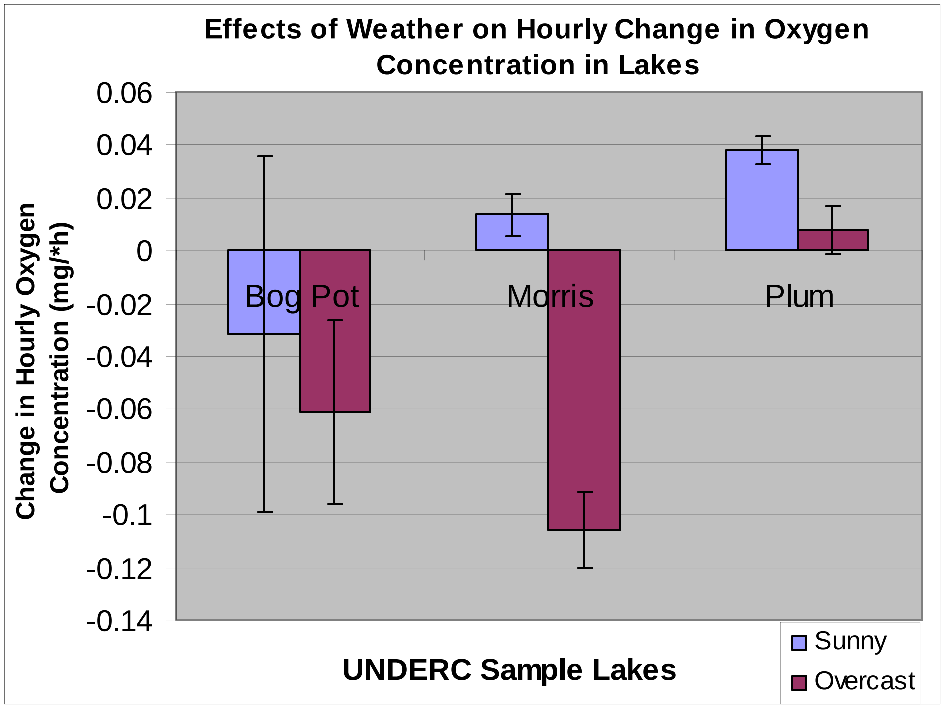

Fig. 5 Differences in hourly change in oxygen concentration in bags of sample lake water across three different UNDERC lakes, deployed in source lakes under weather conditions of both sunny and overcast on two different days. Vertical bars represent ± 1 SD for each natural mean.

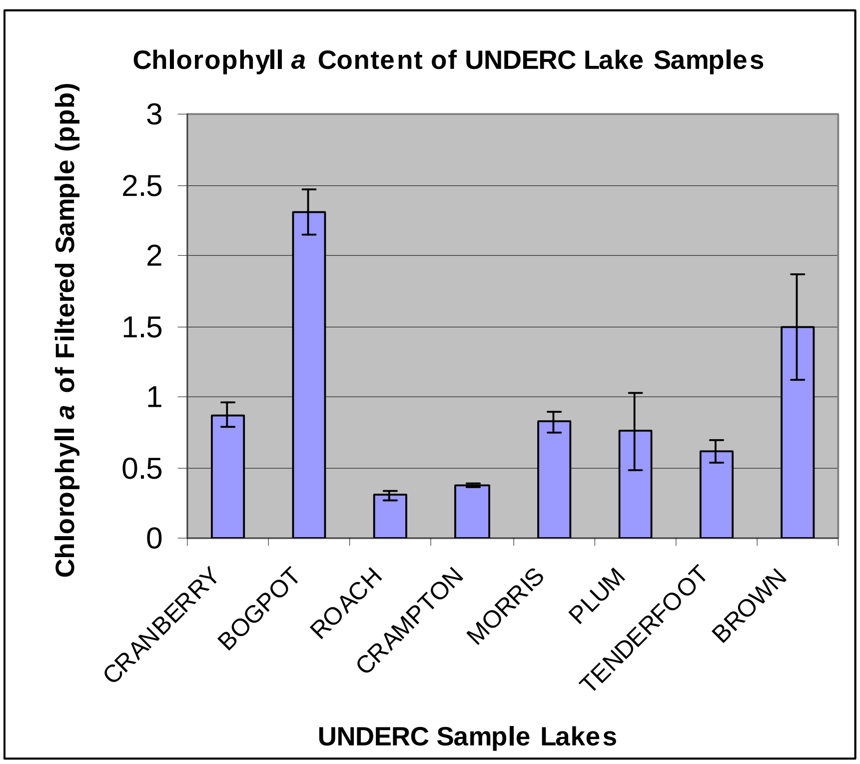

Fig. 6 Measurements of mean chlorophyll *a* concentration from the eight UNDERC test lakes of this study. Vertical bar represents ± 1 SD.

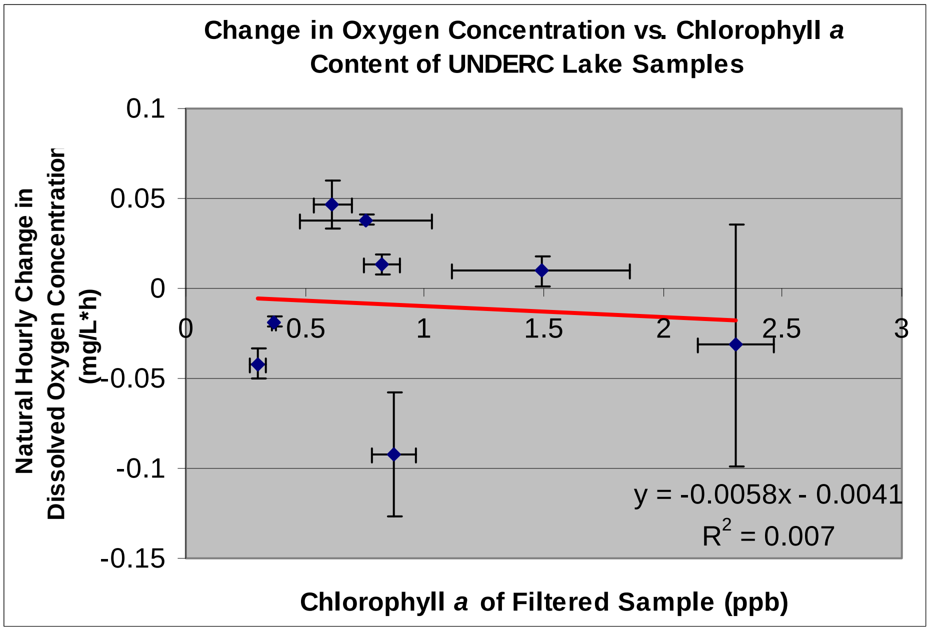

Fig. 7 Plot of the natural hourly change in dissolved oxygen concentration and the chlorophyll *a* content in UNDERC lakes. Vertical bars represent ± 1 SD for each natural mean of hourly change in oxygen concentration. Horizontal bars represent ± 1 SD for each lake's mean chlorophyll *a* content across replicates.

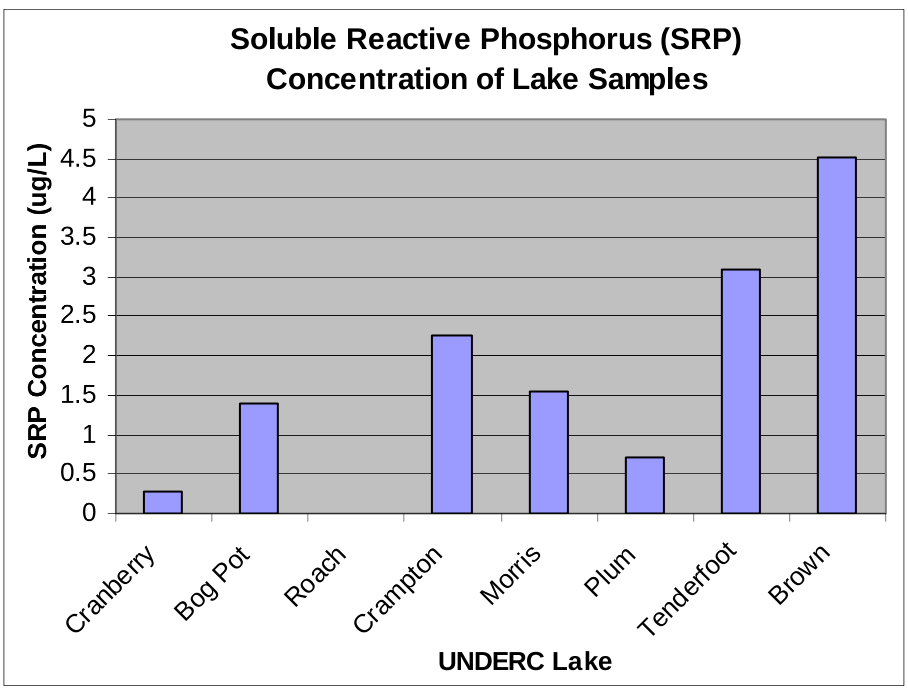

Fig. 8 Measurements of soluble reactive phosphorus (SRP) from the eight UNDERC test lakes of this study.

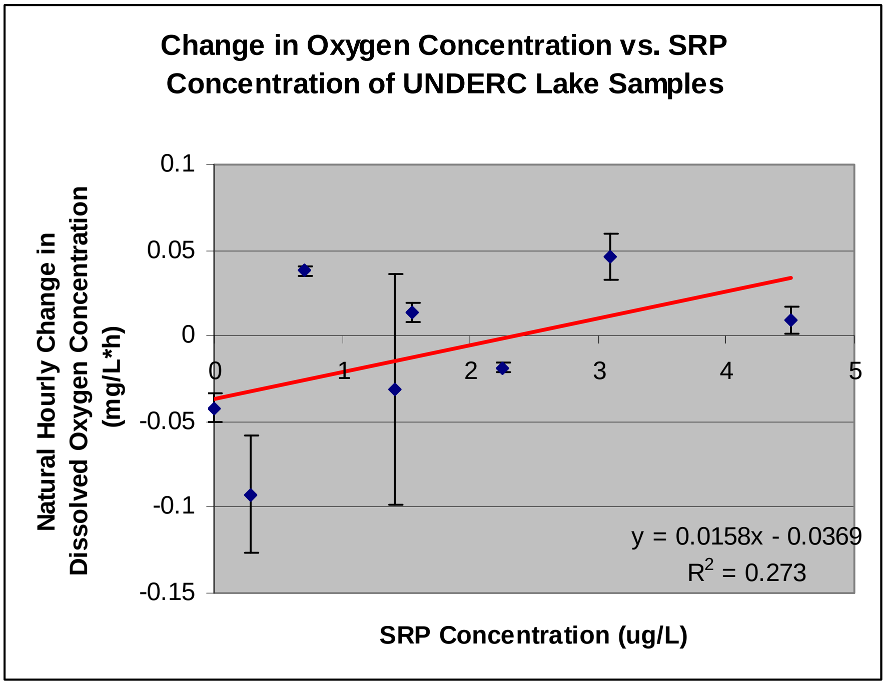

Fig. 9 Plot of the natural hourly change in dissolved oxygen concentration and the soluble reactive phosphorus concentration in UNDERC lakes. Vertical bars represent ± 1 SD for each natural mean of hourly change in oxygen concentration.