ABSTRACT

Title of Thesis: THE INVESTIGATION OF MERCURY REDOX CHEMISTRY IN NATURAL WATERS AND THE DEVELOPMENT OF A NEW METHOD FOR INCUBATION EXPERIMENTS

Lindsay Whalin, Master of Science, 2005

Thesis directed by: Prof. Robert P. Mason

Marine Estuarine and Environmental Sciences

The redox processes that control Hg speciation in natural waters are poorly understood and study results often disagree, primarily a consequence of varied and often flawed methodologies. An incubation method was developed utilizing PFA Teflon® bag reaction vessels to reduce sources of error, and additions of isotopically labeled Hg to quantify rate constants. With lowmeasures of error and duplicate bag reproducibility , this method was applied via incubations of natural waters in ambient sunlight to test three theories; 1) Hg oxidation and reduction are photochemically mediated, 2) Hg reduction is correlated to [DOC], and 3) Hg oxidation is enhanced by halides. The former was proven through the detection of redox chemistry during daylight and its absence in the dark. Results indicate the importance of both redox processes in natural waters, but failed to prove the latter two theories, potentially due to greater than expected [DOC] in one experiment.

THE INVESTIGATION OF MERCURY REDOX CHEMISTRY IN NATURAL WATERS AND THE DEVELOPMENT OF A NEW METHOD FOR INCUBATION EXPERIMENTS

by

Lindsay Whalin

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
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2005

Advisory Committee:

Professor Robert. P. Mason

Professor Neil V. Blough

Assistant Professor Ronald L. Siefert

DEDICATION

This work is dedicated to my parents,
John and Denise Whalin, and to Jamil Adler.

ACKNOWLEDGMENTS

This work was funded by the National Science Foundation. I would like to thank my advisor Dr. Robert Mason, and my committee members Dr. Ronald Siefert and Dr. Neil Blough, for their guidance. I would also like to thank the members of the Mason Lab for their help, especially Andrew and Debby Heyes, Fabien Laurier and Carrie Miller. Additionally, I am endlessly grateful to Heather Soulen and Jamil Adler for sweating their weekends away to help me with my experiments. Finally, I could not have achieved my academic goals without my family, especially my parents John and Denise Whalin, and fiancée, Jamil Adler, who have supported me in every way imaginable.

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LIST OF ABBREVIATIONS

199	Spiked isotope $^{199}Hg(OH)_2$
202	Spiked isotope $^{202}Hg^0$
ATC	Site of water collection, coastal seawater near Atlantic City
Ave.	Average
CBL	Site of water collection, brackish water from Chesapeake Biological Lab
DGHg	Dissolved gaseous Hg, operational definition of Hg^0 in solution
DI	Deionized water
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
HgC	Complexed Hg
HgT	Total Hg, all Hg in solution
ICP-MS	Inductively coupled plasma – mass spectrometer
LMCT	Ligand to metal charge transfer
MMHg	Monomethyl Hg
No fit	Data did not fit curve described by integrated reversible reaction rate equation
P	P value, probablility
PAX	Site of water collection, freshwater from Patuxent River
Red.	Reduction
Redox	Oxidation – reduction
RGHg	Reactive Gaseous Hg, atmospheric ionic Hg
RHg	Reactive Hg, ionic Hg not strongly complexed, operationally defined
Ox.	Oxidation
SE	Standard error
t_{calc}	Calculated t value
t_{crit}	critical t value (3.747)
UHP	Ultra-high purity (argon gas)

CHAPTER 1. INTRODUCTION: BACKGROUND, OBJECTIVES AND APPROACH

1.1 INTRODUCTION

1.1.1 BACKGROUND

Awareness of the immense toxicity of mercury (Hg) was heightened in the beginning of the mid-century with reports of a village in Minimata Bay, Japan plagued with disease due to contamination of the fish supply. Since then, scientists have discovered an abundance of aquatic systems which suffer from Hg contamination (Fitzgerald and Clarkson 1991). The atmosphere has been recognized as a significant reservoir of Hg, and as the major source of the element to natural waters (Fitzgerald, *et al.* 1991; Hurley, *et al.* 1998). Long distance transport of Hg in the atmosphere can lead to deposition great distances from the source, as is evident from the contamination of remote lakes (Fitzgerald, *et al.* 1998; Lucotte, *et al.* 1995). The most toxic form commonly found in aquatic systems is monomethylmercury (MMHg), formed by the methylation of the reactive, ionic form, principally Hg(II) (Morel, *et al.* 1998). This organometal readily bioaccumulates and biomagnifies (Fitzgerald and Clarkson 1991), posing a serious threat to aquatic ecosystems and human populations.

Figure 1.1 is a simple schematic of processes that affect the speciation of Hg in the atmosphere and natural waters. The two inorganic forms of Hg are elemental, Hg^0, and ionic Hg, principally present as Hg(II) (ionic Hg will be referred to as Hg(II)). Both exist in the gas phase in the atmosphere, but with greatly differing properties. $Hg^0_{(g)}$ is relatively inert with low solubility ($H = 0.11\ M\ atm^{-1}$ at 20 ˚C (Morel and Hering 1993)), reactivity and deposition velocity. The lifetime of $Hg^0_{(g)}$ in the atmosphere is extensive, approximately 1 year (Mason, *et al.*1994) due to these properties. In contrast, ionic Hg (Hg(II)) exists as a variety of complexes and overall this fraction has been termed reactive gaseous mercury (RGHg). This fraction is highly soluble (e.g. for one species, $HgCl_2$, H = 2.78x10^6 M atm^{-1} (Schroeder, *et al.*1991)) and reactive, which results in a high deposition velocity and short lifetime in the atmosphere. There are several processes which can lead to RGHg deposition. It can be deposited directly, absorbed by atmospheric water, or adsorbed to atmospheric particles (aerosols). If absorbed by water, it can be removed from the atmosphere when it rains. This is known as wet deposition. RGHg can also be adsorbed to an aerosol which can also be deposited directly, known as dry deposition, or removed by wet deposition. In the latter case, the aerosol itself is absorbed into atmospheric water or serves as condensation nuclei for the creation of a new cloud droplet (Finlayson-Pitts and Pitts 2000; Hedgecock, *et al.*2003) . Due to the long lifetime of Hg^0, it is the dominant form of Hg in the atmosphere (Lindberg and Stratton 1998). Still, the presence of RGHg is greatly important, since it can significantly increase the deposition of Hg to land and water surfaces (Laurier, *et al.* 2003).

Hg^0 and Hg(II) are also found in aqueous droplets in the atmosphere and in natural water bodies. Hg^0 exists as a dissolved gas and is referred to as dissolved gaseous Hg (DGHg), while the Hg(II) exists in the aqueous phase and is referred to as reactive Hg (RHg). DGHg and RHg are the operational definitions of elemental and ionic Hg based on the method of measurement. Again, Hg^0 is relatively inert, while ionic Hg is reactive and readily forms complexes (HgC) with dissolved and particulate constituents. The weakest of the known dissolved complexes is $Hg(OH)_2$. Slightly stronger are complexes with halides, mainly chloride, e.g. $HgCl_2$ and bromide, e.g.$HgBr_2$. Stronger complexes are formed with organic matter and sulfides. Complexation with particulate matter can lead to storage of the metal if it is incorporated into the structure, or reactions may continue if the complex is surficial (Morel and Hering 1993).

Ionic Hg can be methylated to form the highly toxic and bioaccumulative MMHg. Thus, the processes which control the conversion between Hg^0 and Hg(II), known as redox processes, play a critical role in determining the toxicity and environmental availability of Hg in natural waters. This is because reduction of the labile Hg(II) to the inert Hg^0 and possible subsequent evasion can remove Hg from the aquatic system before it can be taken up by organisms as Hg(II) or, after methylation, as MMHg.

1.1.2 LITERATURE REVIEW : FINDINGS OF PREVIOUS STUDIES

The focus of the research presented here is on the abiotic redox processes which affect Hg speciation in natural waters. While some studies suggest the importance of biotic oxidation and reduction in natural waters (Mason, *et al.*1995; Siciliano , *et al.* 2002), most recent studies have focused on abiotic processes, and primarily those that are photochemically mediated.

Oxidation of Hg has been documented in freshwater (Hines and Brezonik 2004; Amyot, *et al.*1997a; Lalonde , *et al.*2001) and seawater (Mason, *et al.*2001; Amyot , *et al.* 1997a; Lalonde, *et al.* 2001; Lalonde, *et al.* 2004). It has been proposed that the hydroxyl radical, $OH^{\bullet}$ is the primary oxidant of Hg in natural waters (Hines and Brezonik 2004; Gardfeldt, *et al.*20 01c), and it has been suggested the halides play an important role in enhancing oxidation (Mason, *et al.* 2001; Lalonde, *et al.*2001; Hines and Brezonik 2004). Some processes which produce $OH^{\bullet}$ in natural waters are the photolysis of nitrate and nitrite (Brezonik and Fulkerson-Brekken 1998), ligand-metal charge transfer reactions (Vaughan and Blough 1998), and photo-Fenton chemistry (Southworth and Voelker 2003). Thus, the oxidation of Hg in natural waters is highly dependent upon photochemistry as has been demonstrated by Lalonde, *et al.*(2001) and Lalonde, *et al.* (2004). This is evident in the dependence of the Hg oxidation rate found in the brackish waters of the St. Lawrence Estuary on UV radiation (Lalonde, *et al.*2004) (Fig. 1.2), in the coastal waters of the Gulf of Mexico (Amyot, *et al.* 1997a), and in the diel pattern of DGHg identified in the freshwater of the Florida Everglades (Krabbenhoft, *et al.* 1998). Second order rate constants for Hg oxidation by $OH^{\bullet}$ agree well for artificial and natural waters: $1x10^9$ M^{-1} s^{-1} (Hines and Brezonik 2004), $1x10^9$ M^{-1} s^{-1} (Lin and Pehkonen 1999), and $2.4x10^9$ M^{-1} s^{-1} (Gardfeldt, *et al.*2001c) (Table 1.1).

Preliminary experiments in this research suggest the predominance of oxidation over reduction in seawater. This may be due to the phenomenon in which the presence of halides, for instance chloride and bromide, enhance the Hg oxidation rate (Mason, *et al.* 2001; Lin and Pehkonen 1999), for which two mechanisms have been proposed. The first is the production of additional oxidants such as OCl^- (Lin and Pehkonen 1999; Mason, *et al.* 2001) or aqueous halide radicals like dichloride ($Cl_2^{\bullet-}$) and dibromide ($Br_2^{\bullet-}$) (Mason, *et al.* 2001). Lin and Pehkonen (1999) state that OCl^- is responsible for up to 90% of the Hg oxidation in nighttime marine atmospheres, with the rate constant of $2.1x10^6$ M^{-1} s^{-1}. Mason, *et al.* (2001) suggests that it might additionally oxidize Hg in surface seawater. The second mechanism relates to the stabilizing capacity of chloride on Hg ions, Hg(I) and Hg(II). Complexation between the two after oxidation can minimize the back reaction, or the reduction of the Hg ion (Pehkonen and Lin 1998; Mason, *et al.* 2001).

Reduction of Hg in natural waters is better characterized, and has been identified in freshwater (Amyot, *et al.* 2000; Amyot, *et al.*1994; Amyot , *et al.*1997b) , seawater (Rolfhus and Fitzgerald 2004; Amyot, *et al.*1997a) , and in brackish waters (Cossa and Gobeil 2000). Again, photochemical processes have been identified as dominating the reduction of Hg in freshwaters (Amyot, *et al.* 1997b), the brackish waters of the Florida Everglades (Krabbenhoft, *et al.* 1998), as seen in the diurnal pattern to the reduction rate, and in seawater (Rolfhus and Fitzgerald 2004; Amyot, *et al.*1997a) with the positive correlation between reduction rates and solar irradiance. Figures 1.3 and 1.4, taken from Gardfeldt, *et al.* (2001b), represent this dependency on sunlight.

Reduction in natural waters has also been correlated with organic matter content (Xiao, *et al.*1995) . Hg forms strong complexes with DOM/DOC (dissolved organic matter/carbon), and the value of the stability constants (given as log K) for these complexes are estimated as low as 10.6 (Benoit, *et al.* 2001) and as high as 24 (Lamborg, *et al.* 2002). If the latter is correct, the majority of Hg in both freshwater and coastal seawater will be organically complexed. Additionally, it has been found that some DOM acts in a photosensitizing manner (Spokes and Liss 1995). This DOM contains chromophores which can absorb light and each absorbed photon can initiate reactions (Spokes and Liss 1995). There are two suggested mechanisms for photoreduction of Hg. The first is direct reduction by chromophoric material via a ligand to metal charge transfer (LMCT) (Allard and Arsenie 1991; Xiao, *et al.* 1995). The second is reduction by the formation of reactive intermediates; reactants produced by the reaction of DOM and an oxidized substrate. The most abundant of these intermediates, formed by DOM photolysis, is the superoxide radical ($O_2^{\bullet-}$) (Zhang and Lindberg 2001), which can also be produced by the photolysis of organic acids such as oxalate (Pehkonen and Lin 1998). While very short-lived, it has been shown to reduce Hg in aqueous solutions with a rate constant of $1.7x10^4 M^{-1} s^{-1}$(Pehkonen and Lin 1998). Interestingly, this rate constant decreases to $1.1x10^4 M^{-1} s^{-1}$ in the presence of chloride (Table 1.1), most likely a result of enhanced reoxidation from the formation of additional oxidants, or due to the stabilization of the ionic form of Hg in the chloride complex (Pehkonen and Lin 1998).

1.1.3 LIMITATIONS IN PREVIOUS STUDIES

Studies of the redox chemistry of Hg in natural waters have been hampered in several ways. First, the materials and techniques that have been used introduce significant error, producing large uncertainties in the rate constants calculated by these measurements. Second, recent evidence suggests that a basic assumption that has been applied in the calculation of rate redox rate constants is false, and that the failure to measure oxidation and reduction simultaneously has resulted in the calculation of net rather than actual rate constants. The development of a method which reduces sources of error and that allows for the concurrent measurement of oxidation and reduction was therefore a key goal of this research.

An extensive search of the literature indicates that studies involving incubation and successive sampling of waters have large uncertainties, or neglect to report measures of error at all (Fig. 1.5) (Mason, *et al.*2001; Lalonde , *et al.*2001; Lalonde , *et al.*2004) . Certain error is expected owing to the nature of the experiments; reaction rates are fast, on the order of 10^5 to $10^9 M^{-1} s^{-1}$, while sample collection and analysis times are slow, on the order of minutes. However, the high levels of error found in these experiments severely decreases confidence in the measurement, and makes discerning trends difficult.

Inspection of incubation experiments, incubations of sample water to identify changes in speciation, found in the literature and the results of several preliminary incubations for this research reveal that much of the error can be attributed to the techniques used, namely the creation of headspace in reaction vessels, which allows for Hg^0 evasion, and the use of several reaction vessels, creating the “bottle effect”. Some error may also be introduced by the type of material used as reaction vessels in these experiments.

Evasional losses of Hg^0 in incubation experiments though inevitable, represent a loss of Hg from a closed system. It is therefore critical to minimize evasion. This fact was discovered belatedly in some studies such as Lalonde, *et al.*(20 01), whose incubation experiments utilized one large bottle, which was sub-sampled at set timepoints. As sample water was removed from the bottle, headspace was created, allowing for the evasion of Hg^0. DGHg measurements and any calculations based on them were therefore inaccurate. This is true for the measurement of both reduction and oxidation. In the case of oxidation, the calculated oxidation rate based upon the disappearance of DGHg would be erroneously high because it would count DGHg evaded to the headspace as DGHg oxidized. In contrast, the calculation of the rate of reduction would be erroneously low due to the loss of newly created DGHg by evasion. Even if RHg rather than DGHg measurements were used in the calculation, oxidation and reduction rates would be inaccurate because the loss of DGHg would alter the redox equilibrium. The low Hg^0 concentration in the water would decrease oxidation, while the lack of back reaction would increase reduction.

Other incubation studies utilized several small bottles rather than one large one (Rolfhus and Fitzgerald 2004; Mason, *et al.*2001; Amyot , *et al.*1994; Amyot , *et al.*

1997a; Amyot, *et al.*1997b; Hines and Brezonik 2004) . Each bottle received the same treatment, and all were deployed for incubation simultaneously. Instead of subsampling from the large bottle at set time points, one of the smaller bottles was simply removed for analysis. The assumption in this method was that because each bottle was treated identically, the same processes would occur in all. Large measures of error and scatter in the data of these experiments prove this assumption incorrect, most likely due to differences among the bottles and their placement in the incubation chamber. This phenomenon has been coined "the bottle effect" (Mason, *et al.*2001) . Figure 1.6 is an example of the scattered data which result from the use of this method.

Another cause of error in Hg incubation experiments can be ascribed to the use of FEP Teflon® bottles. Several experiments utilizing these bottles have seen Hg losses which could not be attributed to evasion to bottle headspace (Rolfhus and Fitzgerald 2004; Mason, *et al.*2001; Lalonde , *et al.* 2001). Preliminary experiments of this research indicate that FEP Teflon® absorbs DGHg. This finding is supported by a study conducted to test various Hg sample storage methods, which determined that storage of solutions containing DGHg in (FEP) Teflon® was futile due to rapid loss (Parker and Bloom 2005). Results indicate that the material is porous and suggest that varying wall thickness as well as the age and frequency of use alter the materials porosity, making DGHg absorption vary between bottles.

Incubations investigating Hg redox chemistry in natural waters have suffered from one other serious limitation, as both oxidation and reduction have not been measured simultaneously. To simplify the calculations, a spike is added of one form of Hg and it is assumed that initially back reaction is negligible, allowing us to use the rate equation for an elementary reaction rather than the reversible reaction that characterizes redox processes. The detection of both net reduction and net oxidation of Hg in natural waters suggests that this assumption is false (Mason, *et al.*2001; Amyot , *et al.* 1997a; Gardfeldt, *et al.*2001b; Hines and Brezonik 2004; Cossa and Gobeil 2000; Amyot , *et al.* 1997b; Amyot, *et al.*1994; Rolfhus and Fitzgerald 2004) . Consequently, previous work has calculated net rather than actual rate constants for Hg redox chemistry. For example, if the rate of reduction is larger than that of oxidation in a system, what is measured is “net reduction”, the rate of the forward reaction, or reduction, minus the back reaction, oxidation. Conversely, if the rate of oxidation is larger than that of reduction in a system, “net oxidation” is measured, where the forward reaction is oxidation and the back reduction. The inability to measure Hg oxidation and reduction simultaneously and the subsequent failure to determine their specific rates impedes the ability to ascribe a mechanism to these reactions, limiting the knowledge and information gained from these experiments.

1.2 OBJECTIVES

The previous work highlighted above has suggested the importance of sunlight, organic carbon and halides in the oxidation and reduction of Hg in natural waters. It is the aim of this research to test these theories via the determination of actual (rather than net) rates of oxidation and reduction in fresh, brackish and seawater, which span the spectrum of organic carbon and halide concentrations. A new method was developed which eliminated several sources of error found in our previous experiments, and that allowed for the simultaneous measurement of oxidation and reduction.

In order to accomplish this, the development of a new method which would decrease error and allow for the simultaneous measurement of oxidation and reduction was necessary, and therefore a primary aim of this research.

1.2.1 HYPOTHESES

1) Both oxidation and reduction of Hg in natural waters are predominately photochemically mediated.

2) Photochemical reduction of Hg(II) is controlled by the concentration of DOC in the medium, thus reduction will be greater in waters of terriginous origin .

3) Photochemical oxidation rates will be enhanced in seawater compared freshwater, due to reactions involving reactive halogen species in solution.

1.3 APPROACH

In order to investigate the primary processes which control the redox chemistry of Hg in aquatic systems, incubation experiments with natural water samples were performed. The effects of sunlight and water type were investigated by varying the timing of the experiments, and type of water used. A new method was developed to address the sources of error identified in previous incubations and to measure the concurrent processes of oxidation and reduction. Error in the method was reduced with the use of deflatable bags composed of an inert material for reaction vessels. To measure both redox processes simultaneously it was necessary to add traceable spikes of inorganic Hg to the sample water. Thus, isotopically labeled spikes of both inorganic Hg species were used.

1.3.1 METHOD DEVELOPMENT

1.3.1.1 INTRODUCTION

In the research presented here, two significant modifications were made to the traditional incubation methodology found in the literature. To reduce error introduced by headspace and the bottle effect, custom 5L PFA Teflon® bags, manufactured by American Durafilm Inc., were designed for use as reaction vessels. These bags were filled and deployed in the incubation chamber in lieu of either several small bottles with the same treatment, or one large bottle. Furthermore, the two species of Hg added as spikes were isotopically labeled, ^{199}Hg(II) and ^{202}Hg^0. In short term kinetics experiments, reduction and oxidation could thus be identified by changes in the speciation of the 199 and 202 isotopes.

1.3.1.2 BAGS

The advantages to the utilization of bags over bottles as reaction vessels are twofold. Like the use of one large bottle, the bag can be sub-sampled from, reducing error associated with the bottle effect. In contrast to the large bottle however, the bag is deflatable, such that no headspace is created as sample water is removed. Coupled with the use of glass collection bottles and rapid analysis after collection, a minimum of DGHg is lost from evasion.

It was quickly discovered that in order to be effective, several other properties were required of the bags. They must be thick enough to withstand repeated acid cleanings as well as the pressure of large volumes of water. They must be thin enough, however, to properly deflate upon the removal of water, and to allow for maximum light penetration. Finally, they must be inert, and not react with or absorb any species of Hg on its surfaces. After several ineffective trials, a bag was designed which met all of these requirements. The foremost properties of these bags are that they are made of PFA Teflon® (FEP is not inert), hold 5 L of water, and are 1mL thick, allowing for 99% quantity and wavelength light penetration (American Durafilm, Inc.).

1.3.1.3 STABLE ISOTOPES

The last decade has witnessed the proliferation of the use of stable isotopes in many types of scientific endeavors, for instance, in the source identification of pollutants. It is a natural progression that isotopes be used to investigate important and poorly understood reactions, especially reversible reactions, which affect the toxicity of such pollutants. With this in mind, the research presented here utilized the stable isotopes of Hg to investigate the factors controlling the redox chemistry of Hg in aquatic surfaces.

This was accomplished with the addition of isotopically labeled mercury as ^{199}Hg(II) and ^{202}Hg^0 to sample waters in short term kinetic incubation experiments. By measuring for RHg and DGHg isotopes, this study was able to differentiate and follow the changes in Hg speciation, and calculate rates of both oxidation and reduction as they occurred simultaneously.

1.3.2 INCUBATION EXPERIMENTS

All experiments conducted for this research were incubations of ^{202}Hg^0 and ^{199}Hg(II) spiked natural waters. A large volume of sample water (20 to 40 L) was filtered upon collection, redistributed amongst the bags, and deployed in the sunlight via a flow through incubation chamber (to regulate temperature). The bags were sub-sampled from at set time-points, and the water collected measured for DGHg and RHg to assess changes in speciation and for rate constant calculation, and for total Hg (HgT) to identify complexation and or losses.

1.3.2.1 EXPERIMENTAL DESIGN

Individual experiments conducted for this research differed in the following ways depending upon the process being investigated. The influence of water type and constituents was examined by incubating waters from three aquatic systems which bracket the major properties of interest: fresh river water containing high concentrations of DOC and low concentrations of halides, coastal seawater containing low [DOC] and high [halides], and brackish bay water with high concentrations of both (Fig. 1.7).

To investigate role of photochemistry an experiment was conducted to measure changes in Hg redox rate constants throughout the day. This was accomplished by filling and deploying into the incubation chamber four unspiked bags simultaneously before sunrise. Individual kinetics experiments were then performed in each of the reaction vessels at four different times of the day, spanning sunlight conditions. Each kinetics experiment began with the addition of the Hg spikes to one of the previously deployed reaction vessels. Several sub-samples were then taken within a short time, up to 7 within 1 to 2 hours. Measurements of the change in Hg speciation through this short period of time allowed for the calculation of oxidation and reduction rate constants. Changes in these rates were then assessed for dependence on time of day and light intensity.

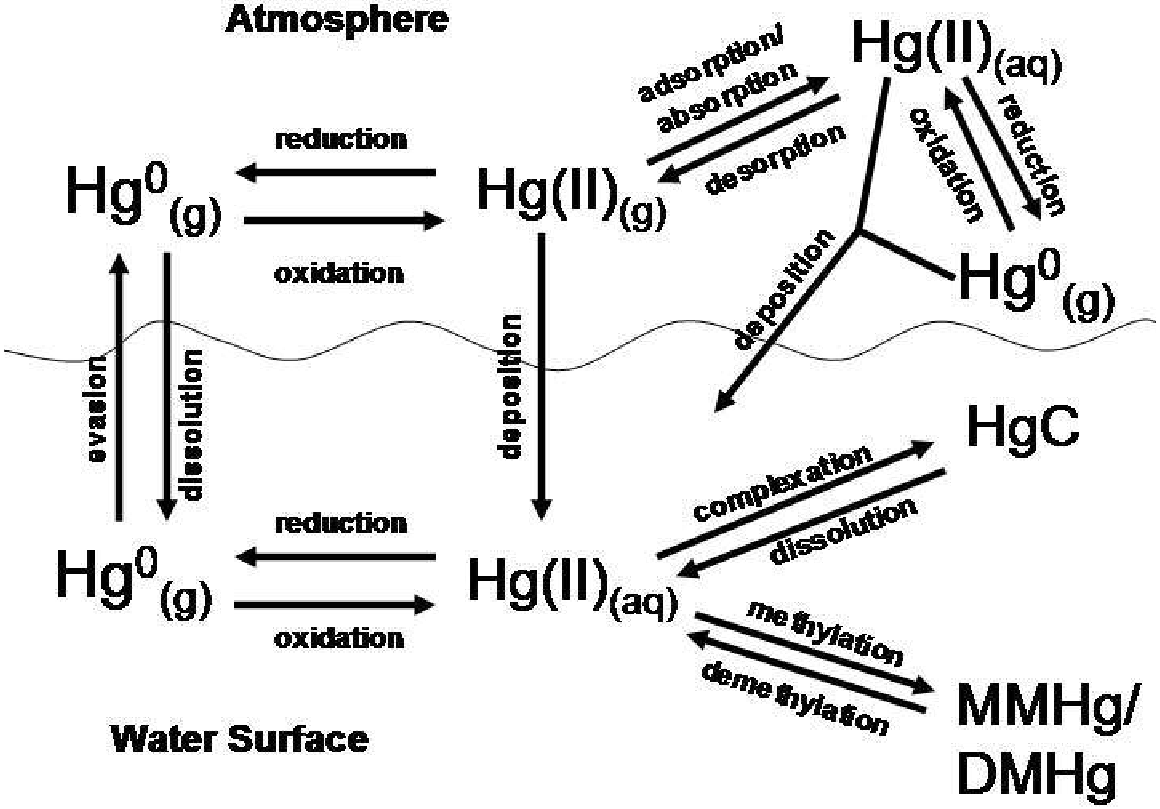


Figure 1.1. Schematic of the major processes which affect the speciation of Hg in the atmosphere and water column.

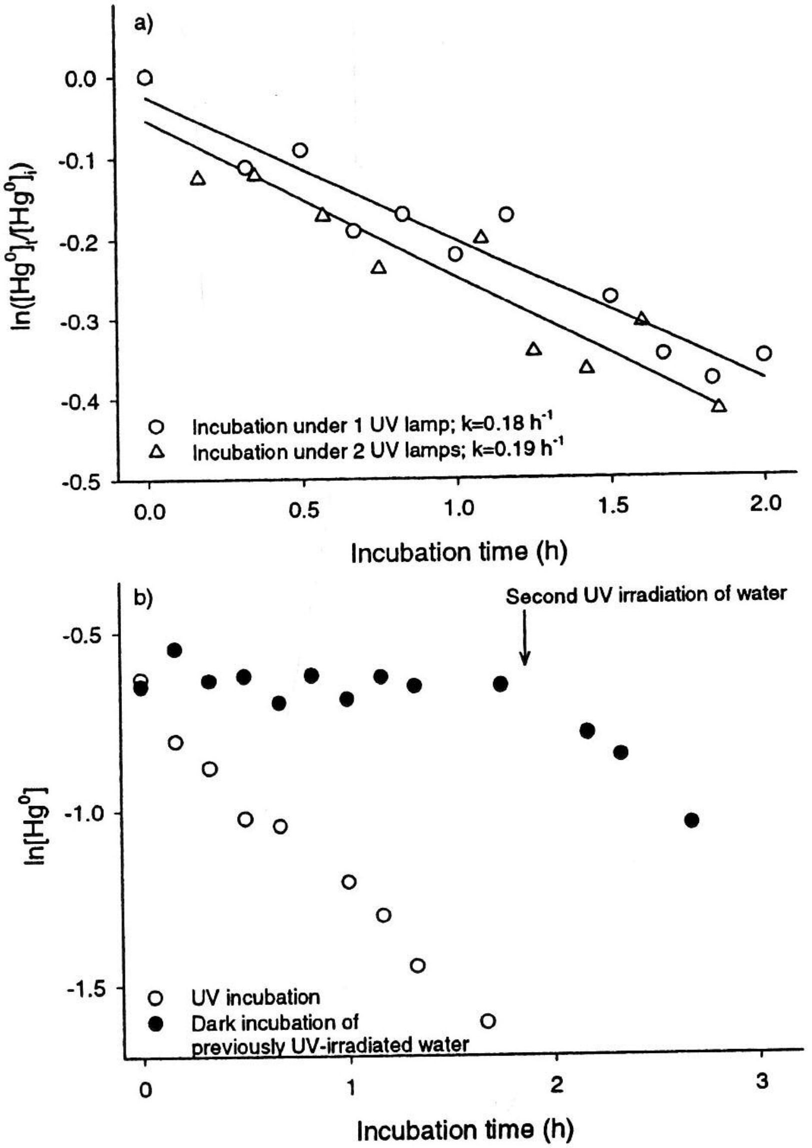
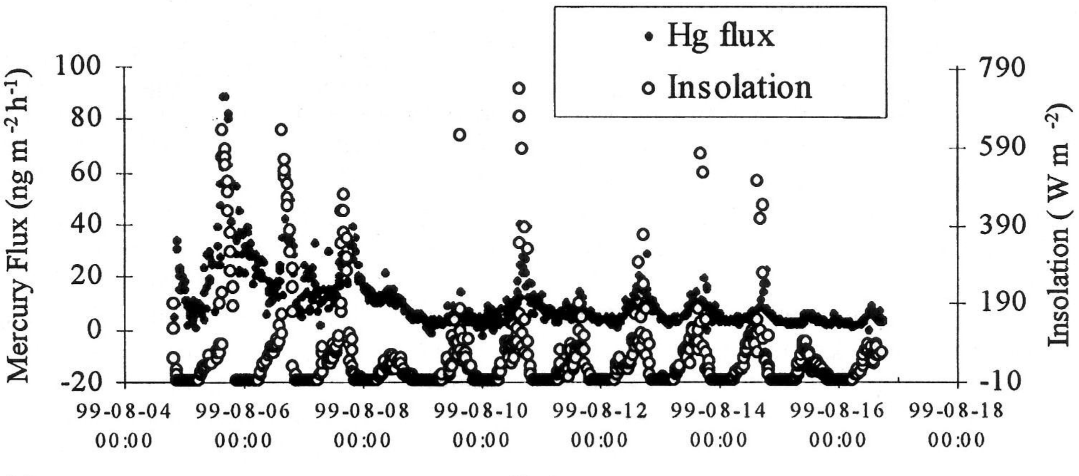


FIGURE 2. (a) Hg^0 photooxidation in natural brackish water spiked with Hg^0(aq) and incubated under one UV-B lamp (circles) or two UV-B lamps (triangles). (b) "Direct" Hg^0 photooxidation in natural brackish water spiked with Hg^0(aq) and incubated under a UV-B lamp (open circles), and "indirect" Hg^0 photooxidation where previously UV-irradiated brackish water was removed from the UV rays, spiked with Hg^0(aq), and kept in the dark (closed circles) or incubated under the UV-B lamp again (after the arrow).

Figure 1.2. Photooxidation of Hg^0 in brackish waters.

Lalonde *et al*. (2004)



(a)

Figure 1.3. Diel pattern of Hg flux over river water surface.

Gardfledt, *et al.* (2001b)

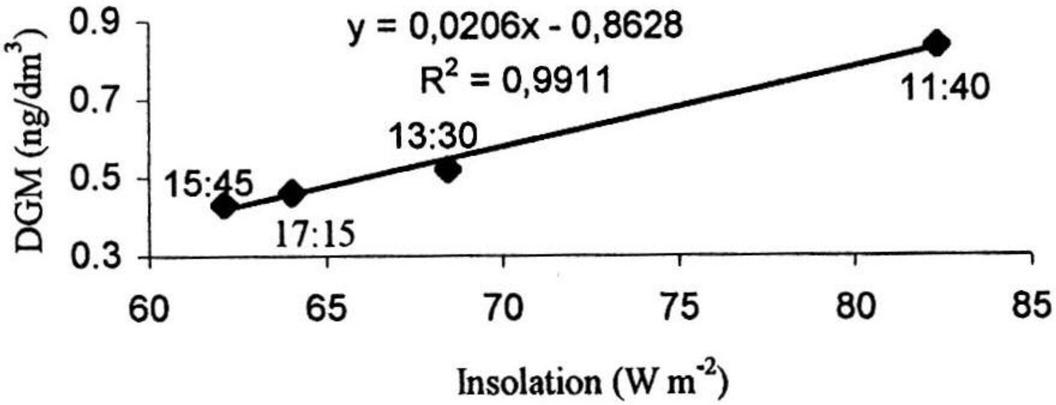


Figure 1.4. Correlation between DGHg concentration and sunlight in surficial river water. Gardfeldt, *et al.* (2001b)

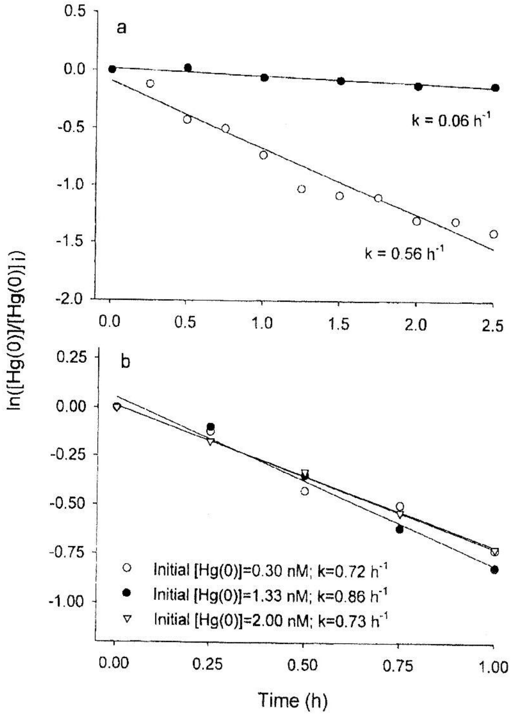


FIGURE 5. **Kinetics of the oxidation of Hg(0) in saline surface waters of Baie Saint-Paul spiked with Hg(0)(aq): (a) Hg(0) photooxidation (open circles, initial [Hg(0)] = 0.33 nM) and dark oxidation (closed circles, initial [Hg(0)] = 0.21 nM); (b) Hg(0) photooxidation rates in samples of varying intial Hg(0)(aq) spike assuming a first-order kinetics. The Hg(0) photooxidaiton rates were obtained from irradiation of water samples to a UV-B lamp.**

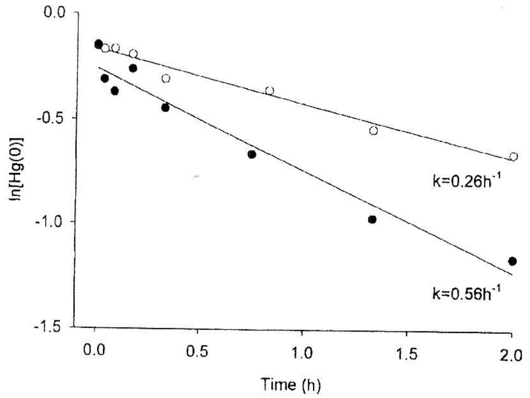


FIGURE 6. **Reaction rates in surface freshwater from Cap Rouge spiked with Hg(0)(aq) with and without KCl (0.5 M).**

Figure 1.5. Results of photooxidation studies with no error bars, or other measure of uncertainty. Note also the use of a concentration ratio in Fig. 5, which serves to reduce variability in the data. Lalonde, *et al.* (2001)

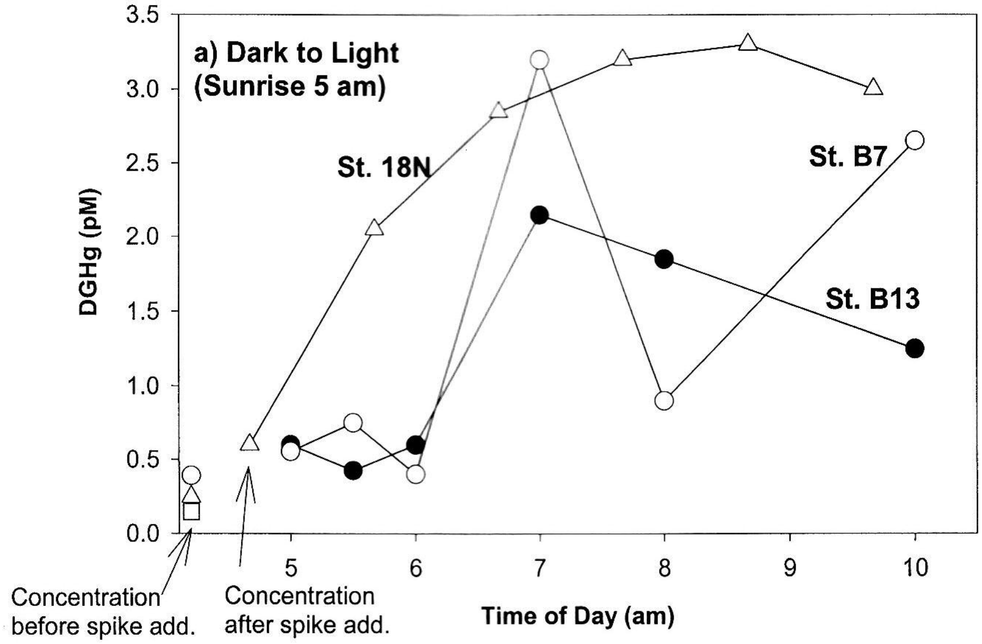


Figure 1.6. Variability in results of an incubation experiment, attributed to the “bottle effect”. Note again the lack of error bars. Mason *et al*. (2001)

FW	BW	SW
Freshwater	Brackish water	seawater
River	Bay	Coastal

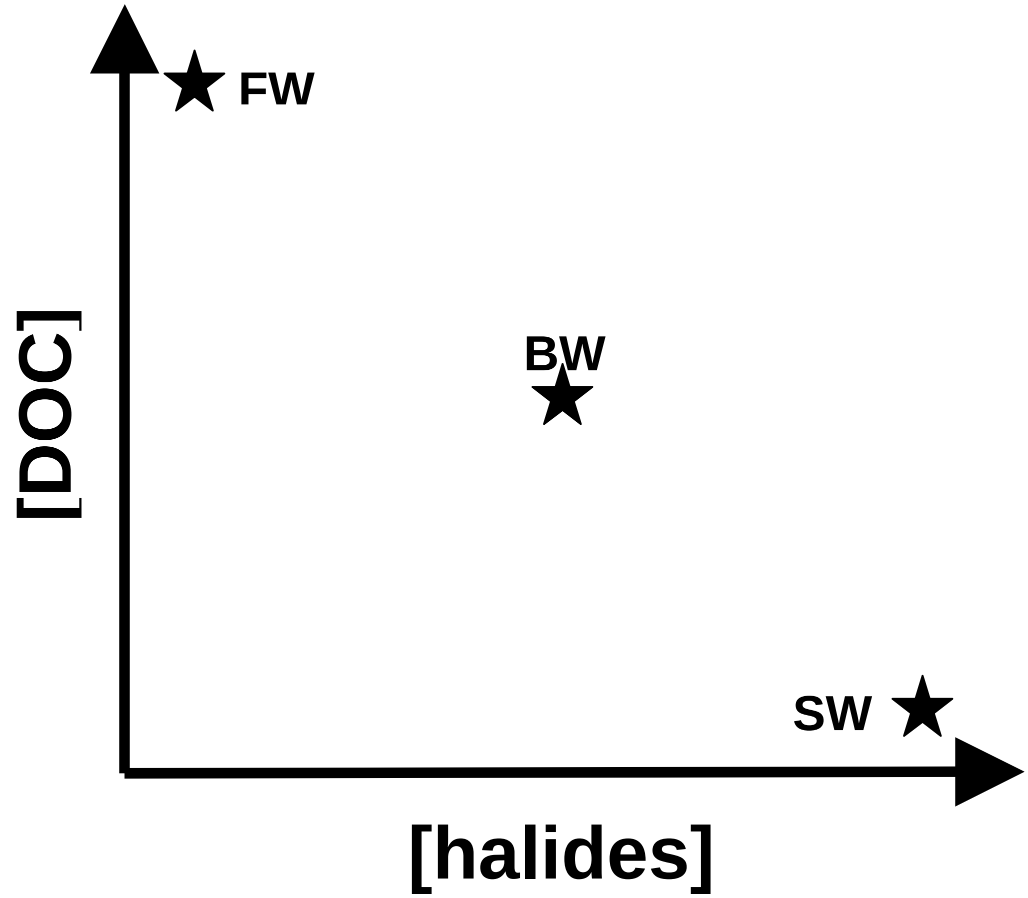


Fig. 1.7. Schematic of [DOC] and [halide] variation for the aquatic systems sampled.

Cited Reference	**Summary of Study**	**Water Type**	**2^{nd} Order Rate Constant Determined ($M^{-1}s^{-1}$)**
Pehkonen and Lin (1998)	Role of $O_2^{\bullet -}$ in the photochemical reduction of Hg.	DI with $O_2^{\bullet -}$	Red. $1.7x10^4$
Pehkonen and Lin (1998)	Addition of Cl^- to above expt. decreases net reduction. Mechanism, whether hampering reduction of enhancing oxidation, unknown.	DI with $O_2^{\bullet -}$ and Cl^-	Red. $1.1x10^4$
Zhang and Lindberg (2001)	Determination of net reduction in sunlight exposed lake water spiked with Fe(III)	FW with Fe(III)	1 to $6x10^7$
Lin and Pehkonen (1999)	Role of $OH^\bullet$ in the photochemical oxidation of Hg.	DI with $OH^\bullet$	$1x10^9$
Hines and Brezonik (2004)	Role of $OH^\bullet$ in the photochemical oxidation of Hg.	DI with $OH^\bullet$	$2x10^9$
Gardfeldt, *et al.* 2001a	Role of $OH^\bullet$ in the photochemical oxidation of Hg.	DI with $OH^\bullet$	$2.4x10^9$

Table 1.1. Summary of experiments and results found in the literature investigating the redox of Hg in water, and the role of water type/constituents.

CHAPTER 2. THE DEVELOPMENT OF A NEW METHOD FOR THE STUDY OF AQUEOUS OXIDATION AND REDUCTION OF Hg

2.1 INTRODUCTION

Redox chemistry of Hg in natural waters is poorly understood. Several types of studies have attempted to characterize it and to identify the dominant mechanisms which control the oxidation and reduction of Hg in natural waters, but they have only been partially successful due to existing experiment methodology (Mason, *et al.* 2001; Lalonde, *et al.*2001; Lalonde , *et al.*2004; Amyot, *et al.*1994; Amyot , *et al.* 1997b; Amyot, *et al.*1997a; Rolfhus and Fitzgerald 2004; Hines and Brezonik 2004; Xiao , *et al.* 1995; Allard and Arsenie 1991; Ferrara, *et al.*2000; Gardfeldt , *et al.*2003) . Many of these studies have been incubations; experiments involving the incubation of sample water in reaction vessels from which sub-samples are periodically taken to assess the changes in Hg speciation. These studies have been limited in two important ways. Current methodologies lead to large errors in Hg measurements, and have been unable to measure both oxidation and reduction though they occur simultaneously. It was therefore a principal aim of this research to develop a method which addressed these issues in order to obtain a more thorough understanding of the redox chemistry of Hg in natural waters.

2.1.1 ERROR IN PREVIOUS WORK

Measures of error in scientific experiments represent uncertainties in the data. When error is large relative to the measurement, confidence in that measurement decreases, and trends are difficult to identify and prove. It is thus important to eliminate as many sources of error as possible. As we presently execute them, incubation studies of the redox of Hg in water introduce significant error, attributable to the type of reaction vessels used. There are two methods found in the literature which employ two different reaction vessels, but both of which produce large amounts of error. They are the single large bottle method and the several small bottles method. In addition to these sources of error, this research suggests that the reaction vessel materials most often used in incubation experiments of this type, FEP Teflon® and PETG plastics, can introduce uncertainties and error.

2.1.1.1 SINGLE LARGE BOTTLE METHOD

This method, used in experiments conducted by Lalonde, *et al.*(2001), involves the collection of sample water into one large bottle (generally several liters), which is deployed in an incubation chamber. Water is periodically sub-sampled from the bottle and analyzed to assess changes in Hg speciation. Headspace is created in the bottle upon removal of water, and given the low vapor pressure of Hg^0 in aqueous solutions (Morel and Hering 1993), DGHg evades from the water into this headspace. The evaded DGHg is not measured or otherwise accounted for, and the result is that measurements of DGHg in the water are inaccurate as are subsequent calculations based on either DGHg or RHg measurements, including calculations of redox rates. This is because equilibrium between the two reactions, oxidation and reduction, is dependent upon DGHg and RHg concentrations. Measured DGHg concentrations are less than the actual DGHg created by reduction because of the loss due to evasion. The lowered Hg^0 concentration reduces oxidation, resulting in inaccurate RHg concentrations. Finally, the reduction rate changes due to the alteration of the RHg concentration (see 1.1.3). The consequence of DGHg evasion is therefore that DGHg and RHg measurements, as well as the calculated rates of oxidation and reduction based on those measurements, are erroneous.

2.1.1.2 SEVERAL SMALL BOTTLES METHOD

This method, used in experiments conducted by Mason, et al. (2001), Rolfhus and Fitzgerald (2004), Amyot, et al. (1994), Amyot, et al. (1997a), Amyot, et al. (1997b), and Hines and Brezonik (2004), involves the simultaneous deployment of several small bottles, treated identically, into the incubation chamber. Rather than sub-sampling at set time points, as in the above method, one bottle is simply removed from the incubation chamber and analyzed. This method assumes that because the sample in each bottle is treated identically, the same processes will occur in both, and error will be minimal. The assumption of less error was proven false however, in an experiment conducted by Mason, *et al.* (2001). Error was compounded due to disparity among the bottles and their placement. In other words, conditions could not be identical for all of the bottles. Examples of potential differences include the cleanliness of the bottles or variation during the addition of spikes. Some bottles might receive less light because of their position in the incubation chamber, or due to greater wall thickness or opaqueness of the bottles. For instance, light penetration was found to be altered in FEP Teflon® bottles, the most commonly used reaction vessels, in studies conducted by Rolfhus and Fitzgerald (2004) and Amyot, *et al.* (1994). This is important considering that photochemistry has been suggested as an important mediator of Hg redox reactions. The error associated with this method has been dubbed “the bottle effect”.

2.1.1.3 REACTION VESSEL MATERIAL

Aside from the error introduced from the number and size of reaction vessels used, it has been determined that the most commonly used material, FEP Teflon®, also introduces error to Hg speciation measurements. This is because the material absorbs

DGHg. Most likely, removal of DGHg from the water occurs via absorption into the pores of the material. The obvious concern is the removal of DGHg from the sample water, however this could potentially be measured and accounted for. More worrisome is that porosity varies between bottles, attributed to natural variation of the material during manufacturing, as well as changes occurring through repetitive acid cleaning and use. This variation makes accounting for the DGHg loss in each bottle extremely difficult.

2.1.2 A NEW REACTION VESSEL

To reduce the error associated with the two methods described above, a reaction vessel was needed that is inert and large enough to subsample from, but which does not create headspace in the process. The result is a PFA Teflon® bag, which deflates upon the removal of water. Each bag holds 5 L of water such that several sub-samples can be taken.

All four sides of the bag are completely sealed except for one corner in which a “lay flat valve” is installed (Fig. 2.1), consisting of a tubular piece of the bag material, approximately 1” wide and 6” long, that is inserted and sealed into the corner of the bag. Approximately 1” of this tube is in the interior of the bag, the rest is exterior, serving as the entrance. Pressure from the liquid in the bag pinches the lay flat valve closed, thereby sealing the entrance, though a clamp is attached during an experiment to ensure against leaks. In order to open the valve, a rigid Teflon® tube, ⅛” or ¼” in diameter, is inserted into the lay flat valve. The pressure of this tube on the opposite side of the water pressure opens the valve so that fluid can exit the bag. A similar rigid tube is used to open the bag to pump in sample water, and the 25% nitric (HNO_3) and hydrochloric (HCl), and Milli-Q water used for cleaning.

In order to assess the competency of these new bags, incubation experiments were conducted. Specifically, it was necessary to test 1) that the bags were inert (several pilot bags were found to absorb DGHg) and 2) that error associated with their use was significantly small.

2.1.3 NET REDOX RATES

Heretofore, investigations of the redox processes which control Hg speciation in natural waters have not measured oxidation and reduction separately. It was assumed that if one form of Hg was added as a spike at higher concentration that in the initial stages of the experiment that the back reaction was negligible so that the rate equation for elementary reactions could be used to calculated redox rate constants. This method is detailed in figure 2.2. The data from the experiment, namely the values for the inorganic species concentration at the subsample timepoints, are used in the plot of the line described by the integrated rate equation for an elementary reaction. To determine rate constants using this method the data is plotted in the following manner: the natural log is taken of the concentration of RHg or DGHg determined at each subsample time. These values are then plotted against that subsample time, often resulting in a straight line interpreted to be the rate constant for the change in speciation (oxidation or reduction) over time. In the case of reduction, for instance, a plot might be created for the natural log of RHg concentrations over time, producing a straight line. This would indicate an exponential decay of RHg concentrations over time, suggesting a decrease in the rate of reduction over the course of the experiment. Such an occurrence was often interpreted as depletion in the ionic Hg concentration available for reaction, known as substrate limitation. It is also possible however that back reaction, in this case oxidation, was significant, and becoming increasingly important as concentrations of Hg^0 increased.

Indeed, the assumption of negligible back reaction is probably often false, as is evident by the detection of both net reduction and net oxidation in all measured types of natural waters (Mason, *et al.* 2001; Amyot, *et al.*1997a; Gardfeldt , *et al.*2001b; Hines and Brezonik 2004; Cossa and Gobeil 2000; Amyot, *et al.*1997b; Amyot , *et al.*1994; Rolfhus and Fitzgerald 2004). An important consequence of using the rate constant determination method based on an elementary reaction when back reaction is significant is that only net redox rate constants can be determined. For example, if reduction outweighs oxidation in a particular water sample, the net rate of reduction, and the only rate actually determined in these experiments, is the actual rate of reduction minus the actual rate of oxidation. Experiments have thus only identified which redox process dominates in a particular water sample. Moreover, the inability to determine actual rates severely limits our understanding of the mechanisms which drive oxidation and reduction in natural waters, because we cannot separate and accurately quantify the impact of each mechanism. This indicates the importance of the ability to simultaneously measure reduction and oxidation of Hg.

2.1.4 STABLE ISOTOPES

In incubation experiments, the solution to the above problem is to calculate rate constants based on the rate equation for reversible reactions. This requires the addition of traceable Hg. In this experiment, the sample water was spiked with isotopically labeled Hg. Measurement of DGHg or RHg by ICP-MS allows for the assessment of the changes in speciation in such a way that both oxidation and reduction can be tracked separately, and rate constants calculated for both. In the incubation experiments conducted for this research, sample waters were spiked with $^{199}Hg(II)$ and $^{202}Hg^0$ (see 1.3.1.3). Rate constants could be determined for both oxidation and reduction from the disappearance of $^{199}Hg(II)$ or $^{202}Hg^0$. This was accomplished using the method described in figure 2.3, in which the rate equation is for a reversible reaction. This method will be described in more detail in later sections. Both species were spiked, such that two values were determined for each rate constant. Comparison of the two provided an additional measure of success of the method.

2.2 MATERIALS AND METHODS

2.2.1 STUDY SITES

Several experiments were conducted in this research. Two of these are presented in Chapter 2 to aid in the discussion of the development of the new method. Water for these experiments was collected in the Fall of 2004 from two sites on the east coast of the U.S., from the Patuxent River (PAX), located in the Chesapeake Bay watershed in Maryland, and off the shores of Brigantine Island, near Atlantic City New Jersey (ATC) (Fig. 2.4). Collection occurred in the upper reaches of the Patuxent River, above tidal forces, where the water is fresh and of terriginous origin. In contrast, water collected from Brigantine Island was seawater, dominantly of marine origin.

2.2.2 SOLUTION PREPARATION

Clean techniques devised by Patterson and Settle (1976) and Gill and Fitzgerald (1985) were used at all stages of the experiment including preparation, water collection, and during the incubations. Materials to which the sample water was exposed were rigorously cleaned by submersion and/or filling, first with nitric (25% Baker instra-analyzed reagent, J.T. Baker, Phillsburg) and then hydrochloric (25% Baker instra-analyzed reagent, J.T. Baker, Phillsburg) acids for 3 days, followed by liberal rinsing with Milli-Q reagent ultrapure (~18 MΩ) deionized water after each. Filters (0.45 μm AquaPrep 600 certified trace metal clean, PALL Corporation, East Hills, NJ), were filled with 10% HCl for one day, to ensure cleanliness while keeping their fragility in mind. Powder free nitrile gloves (N-Dex Free, Best Manufacturing Co., Menlo, GA) were worn at all times.

To perform Hg speciation analysis, the following solutions were prepared in the lab, using Milli- Q reagent ultrapure (~18 MΩ) deionized water and Instra-analyzed reagent grade hydrochloric acid (J.T. Baker, Phillsburg), where indicated. Stannous chloride was used to reduce RHg for analysis, and was prepared by the addition of 200 g of $SnCl_2$-$2H_2O$ (Fisher Certified A.C.S., Fisher Scientific, Fair Lawn, NJ) to 100 mL concentrated HCl, which was added to 1.0 L water. After RHg analysis, approximately 1% Bromine Monochloride was added to the sample in its bottle to eliminate Hg complexation and completely reduce the sample for HgT analysis (note that because the sample is first purged of DGHg, this measurement is actually HgT-DGHg). The BrCl solution was prepared by the dissolution of 27 g reagent grade KBr (Baker analyzed A.C.S., J.T. Baker, Phillsburg) (muffled overnight at 500°C) in 2.5 L HCl, which stirred for at least one hour in a fume hood. 38 g $KBrO_3$ (Baker analyzed A.C.S., J.T. Baker, Phillsburg) (muffled overnight at 500 °C) was slowly added to the solution which remained in the fume hood in a loosely capped bottle, continuously stirring for an additional hour. To eliminate excess reductant (BrCl), hydroxylamine hydrochloride was added to the sample after one hour. This solution was created by the dissolution of 300 g

NH_2OH-HCl (Fisher Certified A.C.S., Fisher Scientific, Fair Lawn, NJ) in 1 L water, to which 1 mL $SnCl_2$ was added for Hg purification. All of the above solutions were purged overnight with ultra-high-purity argon gas (UHP Ar) at 500 mL min^{-1} to remove Hg^0.

A standard curve for the instrument used in isotopic Hg analysis, an Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (Hewlett-Packard 4500 quadrapole), was created using a NIST certified 10,000 ppm Hg oxide solution (NIST 3133). A working standard was created via serial dilutions with reagent water and 0.5% BrCl solution. The ^{199}RHg standard was created from the serial dilution of $^{199}Hg(OH)_2$ (Oak Ridge National Laboratory) stock solution with reagent water, to which 0.5% HCl was added to stabilize it. The ^{202}DGHg stock standard was created by the insertion of a small drop (barely visible) of elemental $^{202}Hg^0$ (Oak Ridge National Laboratory) into a 4” section of permeable silicon tubing (Cole Parmer, Vernon Hills, NJ). The tubing was then capped at both ends with Teflon® plugs, and submerged into a nearly full (to minimize headspace) bottle of reagent water. The bottle was set overnight in a dark place too allow diffusion while discouraging oxidation, after which the tubing was removed. Evasion was minimized by opening the bottle as little and for as short a time possible. The ^{202}DGHg standard was measured for DGHg and RHg before every use to determine concentration and detect oxidation. The working ^{202}DGHg standard was created by serial dilution minutes before use, as degradation via evasion is rapid. All standards were stored refrigerated in the dark.

2.2.3 CLEANING PROTOCOLS

Any material to which sample water was exposed (except for the pump and filters) was rigorously cleaned using techniques designed for the elimination of trace metals as well as organics. Each piece went through 2 stages of acid cleaning; 1) 25% HNO_3 (trace metal grade) to remove trace metals, as well as to breakdown and remove organics, and 2) 25% HCl (trace metal grade) to remove trace metals, but also to eliminate nitrates which HNO_3 can introduce. Sample lines, bottles, and carboys were rinsed and then filled with the acid. All other pieces were carefully filled and immersed in the acid. The pieces were exposed to the acid for at least 3 days.

Liberal rinsing with Milli-Q water occurred before and after each acid step. Both the exterior and interior of each piece was rinsed in the following pattern; 3 times as soon as possible (within one day) after use in an experiment, 3 times before the HNO_3 step, and 5 times each after both acid steps. All pieces except the bags and carboys, which were conditioned upon filling, were set to dry in a trace metal clean laminar flow hood.

The pump was not filled with acid due to its fragility, though copious amounts of each acid (also 25%) were pumped through, followed by a liberal rinse with Milli-Q water. The surface area of the pump to which the sample water is exposed is small (a few cubic inches), and is unlikely to contaminate the sample, especially considering the volume of sample water which is pumped through it (approximately between 30 and 60 L). To be sure however, several liters of sample water was pumped through and wasted before the flow was deemed usable for the sampling.

The filters used in these experiments are fragile and degradable by strong acids. The filters are manufactured in a low particle environment, however, such that rigorous acid cleaning is not critical. Instead, filters were rinsed copiously (~5 L) with Milli-Q water, filled with 10% trace metal grade HCl, set to soak for one day, and rinsed again with Milli-Q water (~10 L).

2.2.4 SAMPLE COLLECTION

Sample water was collected from both sites several hours past sunset, commencing at 11pm and 2am at ATC and PAX respectively. Water was pumped (The Silencer, Shurflo, Elkhart, NJ) through Teflon$^{®}$ lined (Tygon SE200, Cole Parmer, Vernon Hills, NJ) tubing (dubbed the sample line). The water was filtered in situ (0.45 μm AquaPrep 600 certified trace metal clean, PALL Corporation, East Hills, NJ) and collected into 1 or 2 acid cleaned 20 L LDPE carboys, filled entirely to eliminate headspace. Vials were also filled for ancillary analysis, consisting of DOC and chloride concentrations. Carboys were kept in a black bag (to block light) and on ice in order to minimize reactions, especially redox chemistry. The water was then distributed amongst the bags at in the lab at CBL as soon as possible.

2.2.5 EXPERIMENTAL DESIGN

The experiments presented here were incubations using the two types of natural water collected, freshwater and seawater. They are used to demonstrate the typical error found to be associated with the incubation bags and with the method overall. Additionally, both experiments effectively represent the benefit of using stable isotopes to simultaneously measure oxidation and reduction of Hg in water.

2.2.5.1 INCUBATION CHAMBER

Incubations in this research involved the collection of water samples into bags, and deployment into an incubation chamber. This apparatus was designed to mimic conditions just below the surface of the water column. The incubation chamber is simply a four chambered plexi-glass box with no lid, which was placed in direct sunlight (Fig.

2.5). The bottom and sides of each chamber were painted flat black to ensure that only light from above reached the bags. This is a conservative way to mimic the water column where the great majority of light is received from above, substantially decreasing down the water column such that oblique exposure is minimal. DI water was pumped into the chamber to create a slow flow of water throughout in order to ensure consistency in temperature. DI over natural water was chosen for its optical properties, in that light penetration is maximal, as it would be just below the surface of the water column.

2.2.5.2 KINETICS EXPERIMENTS

Other than the type of water, the two experiments were virtually identical; both were “short term kinetics experiments”. Two bags were filled and deployed in the incubation chamber before sunset. The assumption here is that each bag will undergo the same processes, including photochemistry, throughout the day. Bags were approximately 4 inches in depth when full, and the water was clear enough that light properties would not change significantly with depth in the bag. The kinetics experiment began mid-day, in which the two bags were treated as duplicates undergoing the same treatment.

The kinetics experiments were initiated by the addition of ^{199}Hg(II) and ^{202}Hg^0 each (1 to 5 pM) to the deployed bags, accomplished through the insertion of a ⅛” rigid Teflon® tube into the lay flat valve of the bag. This concentration of spike is small, yet background Hg concentrations were less than 10% of the spike concentrations? in all experiments, such that interference was minimal, and could be neglected. The exterior end of the tube was attached to a 10 mL HDPE syringe which was filled with the ^{199}Hg(II) spike solution. Sample water was pulled in to fill the syringe to maximum capacity, followed by a careful plunge to completely empty the contents of the syringe into the bag. The filling and plunging of the syringe was repeated 2 times to ensure the maximum transfer of the spike. The syringe was then detached from the tube to allow air in to empty the water from the tube into the bag, and the tube carefully removed. The bag was then vigorously agitated to induce mixing, while the next spike was prepared (~0.5 to 1 min). This spiking procedure was repeated for the $^{202}Hg^0$ spike.

Immediately after the addition of the second spike, the first subsample was taken from the bag. The protocol for subsampling was as follows. The bag was agitated for 1 min to mix. The clamp was removed from the lay flat valve, and a ¼” rigid Teflon® tube inserted. 5 to 10 mL of water was flushed through the tube to rinse, after which a sub-sample bottle (1 L amber colored glass) was inserted into the stream. When the bottle was 5% full, the tube was lifted upward to stop flow, and the bottle swirled to condition. Each bottle was only conditioned once due to the limited volume of sample, however bottles were dried after cleaning such that rigorous rinsing with sample water was unnecessary. The bottle was then filled completely to avoid headspace which would encourage evasion.

To quench chemistry in the sample so that measurement reflects the concentrations of Hg at the time of sub-sampling, the sample bottle was placed in a dark, ice-filled cooler immediately after it was taken. The bag was then clamped closed and placed into the incubation chamber. Again, to ensure that as little change as possible occurred in the sample from ongoing chemistry, the sample was literally run to the lab for DGHg purging (less than 5 min).

2.2.6 SAMPLE ANALYSIS

Three measurements were performed on each subsample, DGHg, RHg and HgT, all of which were analyzed for isotopic character by ICP-MS (Hewlett-Packard 4500 quadrapole). The basis of each measurement was to reduce the fraction to be analyzed in the sample to Hg^0 (obviously not necessary for DGHg), and then to purge the sample with argon gas for adsorption onto gold, followed by desorbtion and measurement. Purging took place in a trace metal clean area, using two different types of reaction vessels for DGHg, and RHG and HgT respectively. This method is a modified version of that devised by Gill and Fitzgerald (1987).

The overarching objective of the ATC and PAX experiments was to determine Hg redox rates in natural waters during the peak of daily sunlight activity. However, the results provide important information regarding the validity of the method. This was measured by the error in the method and between the bags, and in the successful use of the stable isotopes to simultaneously measure oxidation and reduction. The method error is reported as the standard error, or coefficient of variation for replicate runs of the same sample. The bag error is reported as the standard deviation of measurements from the duplicate bags.

2.2.6.1 DGHg ANALYSIS

The initial purge of DGHg was conducted in the bottle that the sub-sample was collected in. This was accomplished by attaching a modified cap to the bottle (after wasting approximately 150 mL of sample to create headspace) which contained attachments for a sparger and soda lime and gold traps. The sparger was attached to a gas line which was submerged into the sample. Ultra-high purity (UHP) argon gas was pumped into the bottle and dissipated by the sparger. As the bubbles of argon gas moved through the sample DGHg was entrained. The gas then surfaced to the headspace and exited through an opening in the cap to which a soda lime trap was connected, followed by a gold trap. It is necessary to purge with 10 times the volume of sample water with gas in order to completely strip the sample of DGHg. In this case, samples were purged at 200 mL min^{-1} for 1 hour for a 1 L sample. To determine the $^{202}Hg^0$ spike concentration, the spike was added to Milli-Q in a clean bottle and purged. A second purge was then performed to check for residue. Residue of standards and samples were determined to be negligible, below the detection limit.

Soda lime traps consisted of 0.9 cm ID by 10 cm Teflon® heat shrink tubing, filled with 2-3 grams of 8-14 mesh indicating soda lime (Baker®), capped on either side by quartz wool (to eliminate escape of soda lime particles into gold trap) and Teflon® stoppers which have a small hole drilled in them. The purpose of the soda lime was to absorb moisture from the exiting gas, which can create false readings upon desorption.

Gold traps consisted of 0.6 cm ID by 10 cm quartz tubing filled with 3 cm of gold coated glass beads (Brooks-Rand, Seattle, WA), held in the center of the tube with quartz wool. As the exiting gas moved through the trap, Hg^0 amalgamated to the gold. This trap was then placed in the UHP argon gas line leading into the ICP-MS, where it was wrapped with a Nichrome heating coil and rapidly heated via variac to desorb the trapped Hg^0 into the instrument. Again, this is a modified version of the commonly used method for Hg analysis (Gill and Fitzgerald 1987), with analytical measurement performed by ICP-MS rather than cold vapor fluorescence spectroscopy. Unlike the latter method, an analytical trap was not used, only the sample trap was desorbed.

2.2.6.2 RHg AND HgT ANALYSIS

A similar method as above was used for the analysis of RHg and HgT (also modified version of the method devised by Gill and Fitzgerald (1987)), with two major differences. First the reaction vessel was not the sub-sample bottle, but a 500 mL Pyrex Bubbler into which an aliquot of the sample was poured. The Bubbler was affixed with a sparger attached to an UHP argon gas source, as well as the requisite soda lime and gold traps. The necessity of using a separate reaction vessel comes from the second major difference in the method, that it is necessary to reduce RHg and HgT to DGHg for measurement via the gold amalgamation and desorption method.

RHg, also known as easily reducible Hg, is operationally defined. It is Hg that is present as a free ion, Hg(II) for instance, or exists in a weak complex, $Hg(OH)_2$ and $Hg(Cl)_2$ for example. The complex is weak enough such that reduction occurs upon the addition of stannous chloride ($SnCl_2$). To measure RHg, the Bubbler was prepared by attaching the gas lines and soda lime and gold traps and adding 500 μL $SnCl_2$ solution. An aliquot (100-500 mL depending upon the concentration) of one sample was poured into the Bubbler which was then tightly closed. The sample was then purged at a flow rate of 300 mL min^{-1} with UHP argon gas for 15 minutes. It is necessary to be consistent in an experiment with both the volume and the period of the purge, as reduction occurs continuously and a small amount of reduction may still occur if a sample is purged longer than the set time period. Analysis follows the desorption method described above.

Analysis for HgT requires the complete reduction of all Hg present in the solution. This requires the break down of all Hg complexes, accomplished with the addition of a strong reductant. Bromine monochloride (BrCl) was the reductant used, as in the method developed and described by Gill and Fitzgerald (1987) and Bloom and Crecelius (1983). Depending upon the concentration of DOC, 2 to 5 mL (generally 1% of the sample), was added to every liter of sample water directly into the sub-sample bottle once satisfactory DGHg and RHg measurements had been executed. BrCl digestion occurred for 1 hour, after which an equal aliquot of hydroxylamine hydrochloride was added to halt reduction and neutralize left over reductant, which is damaging to the traps and instrument. Tin chloride solution was then added and the sample was purged and analyzed following the procedure described for RHg analysis.

2.2.7 DATA ANALYSIS

Each sample was analyzed for 199, 201 and 202 isotopes. 199 and 202 concentrations were calculated by their ratios to 201 against a standard curve, given known ratios for the $^{199}Hg(OH)_2$, $^{202}Hg^0$ and stock Hg standards.. Oxidation and reduction were determined by the disappearance of ^{202}DGHg and ^{199}RHg, respectively. Pseudo first order rate constants (s^{-1}) for each were determined by fitting this data to the curve described by Fig. 2.3 equation a, in which the disappearance of [^{199}RHg] is used as an example. If the fit was significant, individual rate constants for oxidation and reduction were determined using Fig. 2.3 equation b and the values of $[A]_{eq}$ and (k_1+k_2) determined by the curve fit. Error was determined for $(k_1 + k_2)$ from the curve fit, and then divided between the two rate constants according to its percentage of the sum. This method of rate constant calculation will be referred to as the reversible reaction method. In addition, a p-value was generated for the fit of the dataset to the curve, which was used to determine the goodness of fit. A high p-value represented a high probability that there was no relationship. This was denoted as “no fit”.

T tests were employed to compare these rates to assess similarity, and thus reproducibility among the bags. Input data consisted of three values each for the rate constants of oxidation and reduction at all three sites. These values were 1) the constant determined from the average of both bags and both methods of determination, i.e. the constant determined from ATC bags 1 and 2 using both ^{202}DGHg and ^{199}RHG datasets, 2) this value minus the average standard error, and 3) the averaged value plus the average standard error. The t values returned from this test (t_{calc}) were compared to the critical t value ($t_{crit} = \pm 3.747$), determined from 4 degrees of freedom and 99% confidence ($\alpha = 0.01$). If t_{calc} was not less than $-t_{crit}$ or greater than $+ t_{crit}$, the hypothesis that they are equal cannot be rejected, or the values are statistically similar.

2.3 RESULTS AND DISCUSSION

2.3.1 DGHg LOSS IN TRIAL BAGS

As mentioned in the introduction, a dominant factor contributing to the large uncertainties and scattered data seen in many of the Hg redox incubation experiments identified in the literature, was the use of reaction vessels composed of materials which absorb DGHg. This phenomenon was confirmed when identified in two trial incubation bags used in preliminary experiments for this research. In one trial, the bags were composed of xx mm thick FEP Teflon®, the material of bottles most often used in Hg incubations. The bag was determined to be insufficient for use as a reaction vessel due to the highly variable results obtained during an incubation experiment. Moreover, the concentration of ^{202}Hg in the sub-sampled water of this experiment was measured to be over an order of magnitude less than the spike concentration, suggesting a loss of DGHg.

A separate experiment was conducted to determine the cause of the DGHg loss. A clean FEP Teflon® bag was filled with Milli-Q water, spiked with 5 pM ^{202}DGHg, and deployed in the incubation chamber. The bag was sub-sampled at 0, 10 and 30 minutes after the spike, returning values of 5.0, 2.6, and 2.1 pM respectively (detection limit 0.003 pM). This confirmed the loss of DGHg. The bag was then emptied of its contents and rinsed with a dilute HCl solution which was subsequently measured at 4.6 pM. The recovery of the lost Hg suggests that, within the experimental error of the measurements, that it was either adsorbed to the walls of the FEP Teflon® bag as Hg^{II} complexes, or absorbed as Hg^0 into its pore spaces. Both effectively remove DGHg from solution, but the latter is most likely considering that rapid reduction of DGHg in Milli-Q water is unlikely.

Another trial incubation bag, manufactured from 0.5 mm PETG film, also suffered from DGHg loss. The following experiment was performed to determine the method of loss. Two clean bags were filled with Milli-Q water, spiked with 15pM ^{202}DGHg, and deployed in the incubation chamber. After one hour, the bags were removed and sub-sampled. Analysis of this water produced measurements of 4 and 0.01 pM, confirming the loss of DGHg. Both were then emptied of their contents and rinsed with a dilute HCl solution similar to the experiment described above. Measurement of these solutions returned values below the detection limit of the instrument (0.005 pM). The failure to recover the lost ^{202}Hg suggests that DGHg was not only sequestered into the walls of the bag, but channeled through it entirely during the duration of the experiment and lost to the water flowing through the incubation chamber.

Though PETG bottles are often used in the collection of water samples for mercury analysis, this phenomenon has not been observed or otherwise presented previously. The disparity is most likely due to the difference in the wall thickness of the vessels. The bottles are several millimeters thick while the bags used in these experiments were only 0.5 mm. The thickness of the bottle walls would make the creation of channels passing completely through the walls less likely. Still, the PETG bottles probably do absorb DGHg into its pore spaces, much like the FEP Teflon® material. These results suggest that neither FEP Teflon® nor PETG materials should be used in experiments involving the analysis of DGHg. Indeed, in most studies which have used FEP or PETG bottles, samples have been acidified and this would reduce the loss as the Hg^0 would be oxidized to Hg^{II} under the acidic conditions.

2.3.2 INCUBATION BAG

The use of the PFA Teflon® incubation bags as reaction vessels, however, proved successful, demonstrated by low measures of error in the two experiments. This is true for the overall method error, measured as the average coefficient of variation of replicate analysis, and for the error associated specifically with the bags, measured as the standard deviation between similar measurements performed on two bags (duplicates). These measures of error are reported in Tables 2.1 and 2.2. Method error for Hg speciation analysis in the ATC experiment ranged from 0.032 to 0.068 pM, representing between 2.1% and 11.1% of the average of the respective measurement. The highest percentage was for the measurement of ^{202}DGHg, which is used to calculate oxidation. However, when compared to the average percent change in ^{202}DGHg concentration as a result of oxidation, 36% (Table 2.3), 11% is more than acceptable.

Method error for the PAX experiment was on the same order, between 0.056 and 0.087 pM. This represented between 1.2% and 10.0% of the average measurement. The highest percentage for the PAX experiment was for the measurement of ^{199}RHg, which was used to calculate reduction. Again, when compared to the average percent change in ^{199}RHg concentration however, 32%, 10% is reasonable. More disputable, however is the 5% of sample method error for the measurement of ^{202}DGHg in the PAX experiment, because the average percent change in its concentration was only 7%. In cases such as this, where overall reduction or oxidation is small, the error becomes important.

Error between the bags was low for the ATC experiment (table 2.2), and measurements for the 199 and 202 isotopes were of similar order and comparable to the overall method error. The percentages of error were high for the ^{202}RHg and ^{199}DGHg measurements compared to the other measurements in this experiment (table 2.3). Considering that their presence in the sample first requires their production, this error is attributable to the low concentrations of these two forms of Hg found in most of the samples. The percentage error for ^{199}RHg is also relatively large in the ATC experiment, 14%, though the absolute error is small. This is because the spike of ^{199}RHg was small, less than 2 pM. Additionally, the average change in ^{199}RHg concentration in this experiment was 46%, such that 14.6% error is acceptable. In this context, it is apparent that error was significantly small in the ATC experiment, for the method overall and for the bags specifically, validating the use of the PFA Teflon® incubation bags.

Similar results were found in the PAX experiment, with bag error of the same order as in ATC. The exception is the ^{202}DGHg measurement, which was larger because the spike was larger at approximately 10 pM. Note that the percentage of this error for this measurement in general was low however. Additionally, a pattern of higher percentages for the ^{202}RHg and ^{199}DGHg measurements was seen in PAX as in ATC. Similar to the method error for the PAX experiment, the 8.2% bag error for the measurement of ^{199}RHg is reasonable considering the average percent change of its concentration, 32%. However, the 4.6% bag error for the ^{202}DGHg measurements is more questionable considering the small change in its concentration, an average of only 7%. Results from PAX do indicate that the incubation bags can be successfully used for reaction vessels in incubations. However, they also show that error can become significant when the change in absolute concentrations is small.

The low measures of uncertainty produced in these experiments are a direct result of the minimization of DGHg losses, a consequence of the incubation bags. Evidence of this can be seen in Figures 2.6 and 2.7, plots of HgT for the spiked isotopes. If significant Hg losses occurred, HgT would decrease drastically through the experiment, however no significant drop in HgT was seen for either isotope in either experiment. ATC HgT measurements remained between 1.1 and 1.5 pM. The duplicate 202 measurements do slope down slightly, but not to thedegree that would be expected as a result of loss from the bag. If this negative slope is due to DGHg loss, it is most likely a result from evasion during the switching of bottle lids for the DGHg purge. The 199 measurements appear to have a slightly positive slope, however indicating that these small slopes do not represent losses or gains, but are due to analytical error, a consequence of the calculation of HgT. Recall that HgT analysis was performed subsequent to purging for DGHg, resulting in an actual measurement of HgT-DGHg. The values for HgT in plots 2.6 and 2.7 are a summation of DGHg and HgT-DGHg, with cumulative error (calculated as the square root of the sum of the squared individual measurement errors measurements) of 0.055 pM and 0.083 pM for ATC, and 0.097 pM and 0.11 pM for PAX, for the measurement of 199 and 202 HgT, respectively.

HgT results were generally similar in the PAX experiment, however there were two obvious differences. The first is that the 202 measurements produce both positive and negative slopes, again suggesting that these small slopes are the result of the inherent error, and do not represent a systemic loss of DGHg. The second difference is that the 202 spike in the PAX experiment was greater than the 199 spike in PAX, closer to 8 pM than 1 pM. This is because the Hg spikes in preliminary experiments of this research were all between 5 and 10 pM. The PAX experiment was the first attempt to decrease the spike to determine if error could be kept small in a system more representative of a natural environment where concentrations can be low. In the PAX experiment, just the 199 spike was decreased because error in the RHg, but not DGHg, measurements were previously consistently low. Both spikes were decreased in the ATC experiments. As discussed above, results indicate that spikes of less than 2 pM rendered low measures of error, indicating that the method is sound.

2.3.3 SIMULTANEOUS MEASUREMENT OF OXIDATION AND REDUCTION

The second objective of the new method, the simultaneous measurement of oxidation and reduction, was also successful, allowing for the calculation of rate constants for both. Concurrent measurement of reduction and oxidation can be seen in Figures 2.8 and 2.9, which show a general pattern of exponential decay of ^{199}RHg and ^{202}DGHg, respectively. This data was fit to a curve described by the equation in figure 2.3 equation a. Figure 2.10 is an example of this curve fit. If the fit was significant (low p value), rate constants for oxidation and reduction were then determined by solving the two equations in figure 2.3 by substitution given the curve fit values for $[A]_{eq}$ and (k_1+k_2). Using datasets tracking the disappearance of both ^{199}RHg and ^{202}DGHg, two values could be determined for both processes, oxidation and reduction. These values are listed in table 2.4, and the t values for the comparison of those rates can be found in table 2.5.

Several important details are revealed in these tables. First, constants determined using the ^{202}DGHg and ^{199}RHg datasets were in general agreement. This can also be said for constants determined from the duplicate bags. Comparisons between all ATC oxidation data sources returned t_{calc} values which passed the t-test, except for one. In other words, this test determined that all of the rate constants determined for ATC oxidation are statistically similar except for the comparison of the ^{202}DGHg and ^{199}RHg datasets from bag 2 ($t_{calc} = -4.68$). Considering that these constants are statistically similar to the others determined for oxidation in the ATC water, it is safe to assume the constants are valid. The low p values for the fit of these data to the curve, ranging from 0.0258 to 0.0933, indicate that there was no interference by processes other than Hg redox. The average rate constant for Hg oxidation in ATC waters is $4.1(\pm0.89)\times10^{-4}\ s^{-1}$.

Rate constants calculated for Hg reduction in ATC waters also agreed fairly well. Four out of the six comparisons returned t_{calc} values within the t_{crit} range, so that they did pass the t-test. The two values that did not pass the t-test resulted from the comparison of the ^{199}RHg dataset in bag 1 with both isotopic datasets in bag 2 (tcalc = 4.53 and 6.07, respectively). There is agreement between the constants determined from both ^{202}DGHg and ^{199}RHg datasets in bag 2, and between them individually and other constants. Thus, the constant determined from the 199 isotopic data in bag 1 is potentially an outlyer. This value did agree with the ^{202}DGHg data from the same bag, but the error in the latter measurement was relatively large. The excellent agreement among the ^{202}DGHg datasets in both bags and ^{199}RHg dataset in bag 2, and the considerably small error of the latter constant suggest that the value is most likely on the order of 10^{-4} s^{-1}. Though 2 out of 6 comparisons suggested that the Hg reduction rate constants for ATC water were statistically different, a review of their errors and fit to the curve, as well as the comparisons with other datasets, suggests that the values agree reasonably well. These results suggest that the use of the new method in the ATC experiment was successful.

Comparison such as that performed above on the results of the ATC experiments is more difficult for PAX. This is because rate constants could not be determined for either bag from the ^{202}DGHg data., thus the “no fit” designated by the high p-values. Note in figure 2.9 the appearance of a linear rather than exponential decay trend for the PAX data. This is a result of an incomplete dataset; 2 measurements from each bag are missing, a consequence of a leak in the setup used for the DGHg purge. Results of the t tests suggest that the rates calculated for Hg oxidation are similar, while those for reduction are not. Considering the relatively large error associated with the Hg reduction rate constant determined from bag 1 (P =0.1960), it is likely the actual value is closer to that determined from bag 2.

An important point is that the rate of both reactions is rapid, with rate constants on the order of 10^{-3} - 10^{-4} s^{-1}. Thus, steady state between the production and consumption of the measured Hg species is reached rapidly in these experiments with characteristic times approximating one hour. Therefore, in order to determine accurate rate constants, the change in speciation before the Hg species approaches steady state must be assessed. This implies an additional flaw in the methods thathave been previously used for Hg incubations, where measurements were made over a relatively long time scale, or additional Hg was not added to the water. In the former case, the Hg species may have reached steady state before or shortly after the second time point was taken. An example of such an experiment is illustrated in figure 1.6 in where subsamples were taken every hour. In the latter case, background Hg had already reached steady state. Therefore, the changes in speciation measured in both types of experiments simply reflect changes in the steady state concentration of the Hg species with time due to changes in the amounts of photochemical reactants.

Considering the large errors reported in studies discussed from the literature, and those studies where no measure of error was reported at all, the results of these experiments are excellent. Moreover, they signify success in the use of this new method in the ATC and PAX experiments. This is substantiated in two ways. First, the similarities indicate good reproduction of Hg redox processes between the bags. This suggests that there is no “bottle effect” associated with the use of these bags. Second, rate constants for both Hg oxidation and reduction are quantifiable. Furthermore, the rate constants determined from both ^{202}DGHg and ^{199}RHg datasets were similar, indicating that both Hg oxidation and reduction rate constants can effectively be determined from the addition of just one isotopically labeled inorganic Hg species, though the use of both is suggested to aid in the identification of the role of complexation, which will be discussed in detail in chapter 3. Finally, rate constants for Hg oxidation and reduction at both sites were on the same order, suggesting that both processes can be important in natural waters. This implies the importance of the use of the rate equation for a reversible reaction in the calculation of rate constants and of the addition of traceable Hg. Clearly, this approach confirms the need for the approach taken here, with the new method.

2.4 CONCLUSIONS AND RECOMMENDATIONS

The results from these two experiments uphold the validity of this new method for the investigation of Hg redox in water through incubations. The low occurrence of error and small measures of uncertainty between duplicate bags suggests that the incubation bags are excellent reaction vessels in this context. The redox rate constant results also suggest that the method produced good replication of the processes between bags. The implication is that the incubation bags are an excellent solution to the limitations found in some methods, and consequently that the bags can be used successfully in the investigation of Hg redox processes using incubations. Similarly, these results indicate that the method as a whole was sound.

Results of these experiments also indicated that the addition of isotopically labeled inorganic Hg species aided in the identification and quantification of Hg oxidation and reduction. The spikes allowed for the calculation of first order rate constants for Hg oxidation and reduction, while other methods have only measured the net rate of the two processes. Most datasets in the experiments for this research produced a good fit to the rate equation, suggesting that there was no interference by processes other than redox. This fact will be expanded upon in chapter 3. Finally, the similarity between rate constants calculated from the ^{202}DGHg and ^{199}RHg datasets suggest that both processes, Hg oxidation and reduction, can be identified through the spike addition of either Hg species, and that both can be important in natural waters.

The successes of these experiments indicate that the method can be used for Hg redox incubation experiments. Specifically, the method reduces uncertainty, thereby improving confidence in the results, facilitating the identification of trends. In addition, the method allows for more precise quantification of Hg redox processes, increasing our capacity to understand the mechanisms which control Hg redox in natural waters, and aiding in the comparison of different water types and bodies.

This new method can be used in incubations of natural waters to identify the Hg redox properties of a specific water body, and it can be used in the incubation of artificially produced waters to study the specific mechanisms controlling Hg redox chemistry. Similarly, it can aid in the investigation of other important processes which affect the fate of Hg in aquatic systems, for instance methylation and demethylation. Finally, a similar method can be created to study other complementary reactions in aqueous solutions, for example to investigate important redox pathways of other metals.

An important recommendation for the future use of the method developed in this research is the alteration of the Hg spike addition methodology. As can be seen by the initial concentrations in figures 2.8 and 2.9, these additions were not always equivalent or consistent. Potential loss pathways are adsorption or absorption to the walls of the HDPE syringes used to transfer the spike to the bag, or, in the case of the ^{202}DGHg spike, evasion during preparation for the spike addition. Though the discrepancy in initial spike concentrations did not appear to affect the results of these experiments, this may not be the case for other processes.

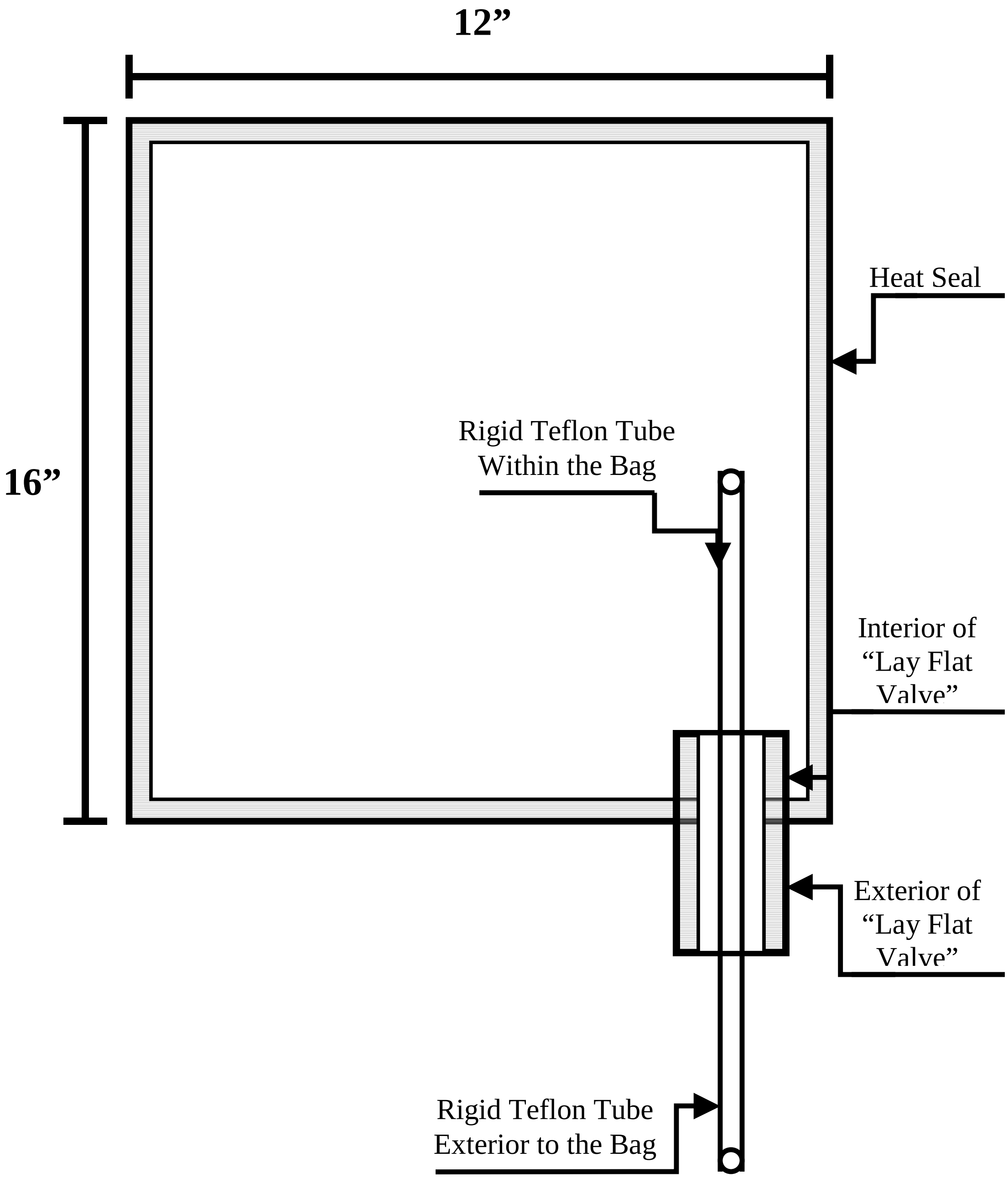


Fig 2.1 Diagram of the incubation bag with lay flat valve. The rigid Teflon® tube used to open the valve to allow outflow, or to fill and spike the bags is also shown.

a.

$$Hg^0 \xrightarrow{k_1} Hg(II)$$

b.

$$\frac{d[Hg^0]}{dt} = -k[Hg^0]$$

integrate and plot

c.

$$Ln\frac{[Hg^0]}{[Hg^0]_0} = -kt$$

where equation c describes a line in which k is the slope

Fig. 2.2 Equations used in the elementary reaction method of redox rate constant determination.

a.

$$A \overset{k_1}{\underset{k_2}{\Leftrightarrow}} B$$

$$[A]_t = [A]_{eq} + \left([A]_0 - [A]_{eq}\right)e^{-(k_1+k_2)t}$$

b.

$$\frac{k_1}{k_2} = \frac{[B]_{eq}}{[A]_{eq}} = \frac{\left([A]_0 - [A]_{eq}\right)}{[A]_{eq}}$$

where if A = ^{199}RHg and B = ^{199}DGHg,

$[A]_t$ is the concentration of ^{199}RHg at time t

$[A]_{eq}$ is the concentration of ^{199}RHg at steady state

$[A]_0$ is the concentration of ^{199}RHg at time zero

k_1 is the reduction rate constant

k_2 is the oxidation rate constant and

$[B]_{eq}$ is the concentration of ^{199}DGHg at steady state

Figure 2.3. Equations for the calculation of oxidation and reduction rate constants. Note that the example given here uses the disappearance of [^{199}RHg] to calculate rate constants, but the ^{202}DGHg dataset can be used as well, in which case [A] = ^{202}DGHg and [B] = ^{202}RHg.

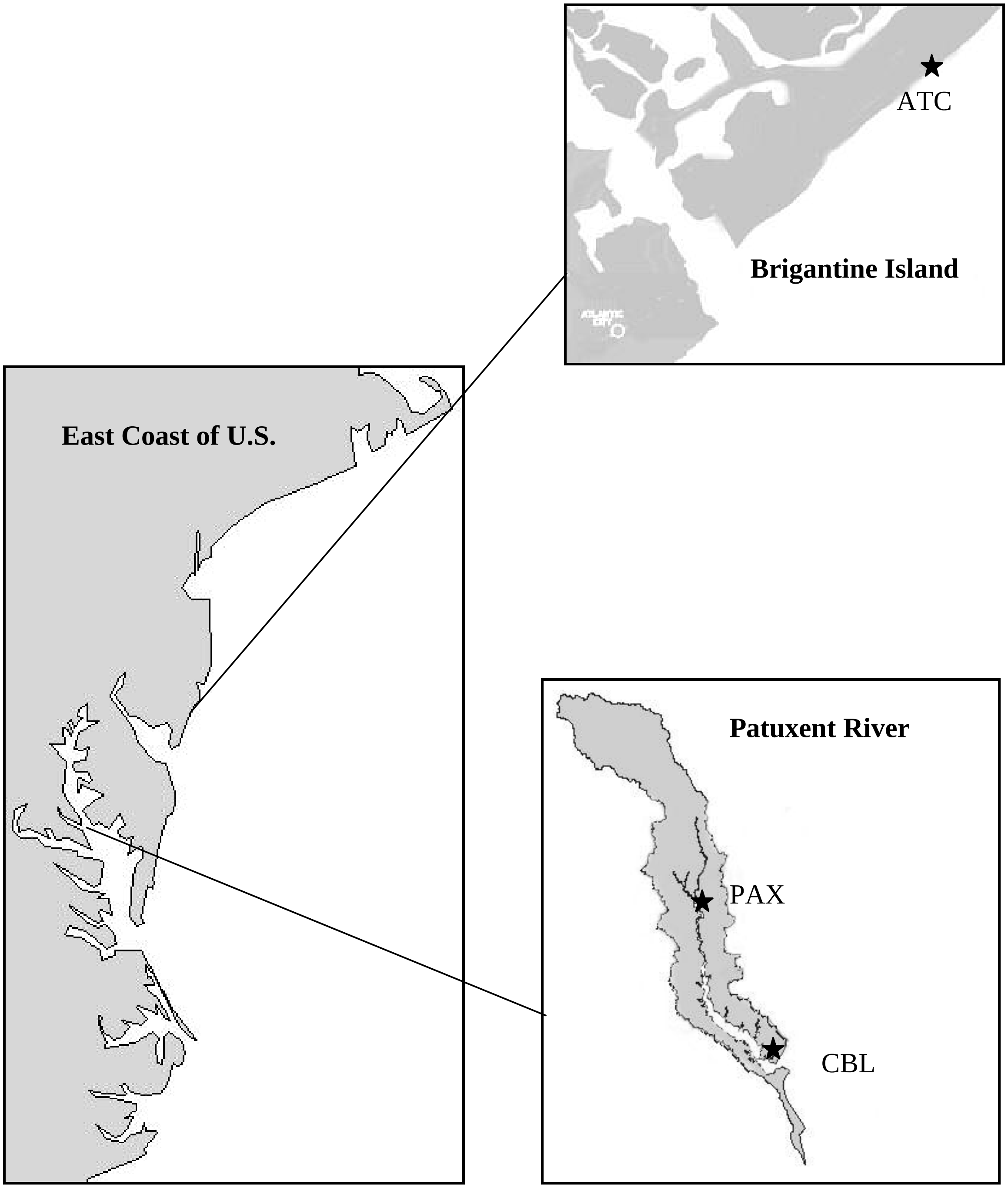


Fig. 2.4. Map water collection sites on the Patuxent River (PAX),at Brigantine Island (ATC), and at the Chesapeake Biological Laboratory (CBL).

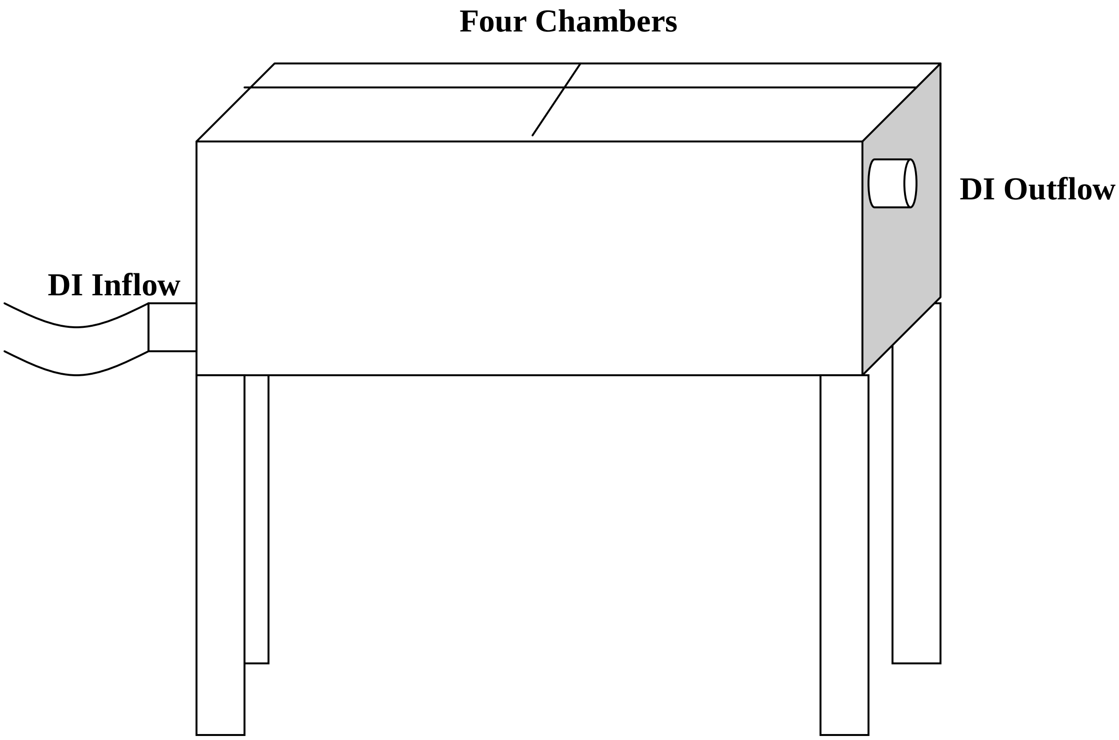


Fig. 2.5 Diagram of incubation Chamber, deployed in direct sunlight on the CBL Seawater Pad in Solomons, MD.

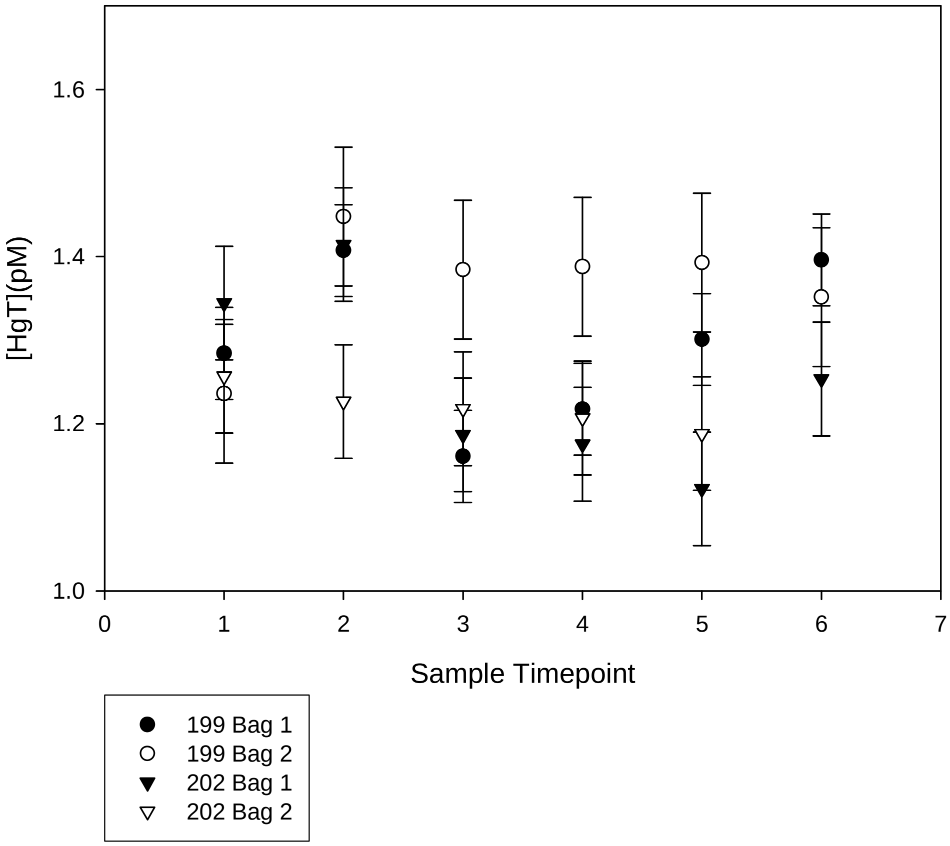


Fig. 2.6 Plot of total HgT measurements throughout the experiment. Demonstrates that there was no significant DGHg loss during the ATC experiment.

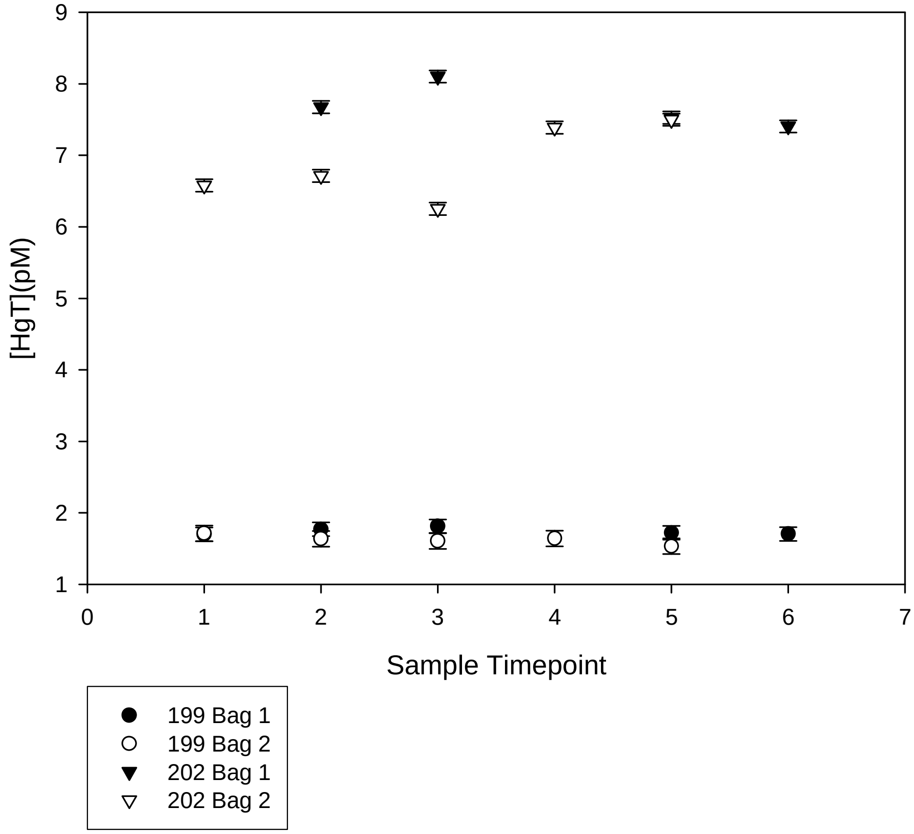


Fig. 2.7 Plot of total HgT measurements throughout the experiment, Demonstrates that there was no significant DGHg loss during the PAX experiment.

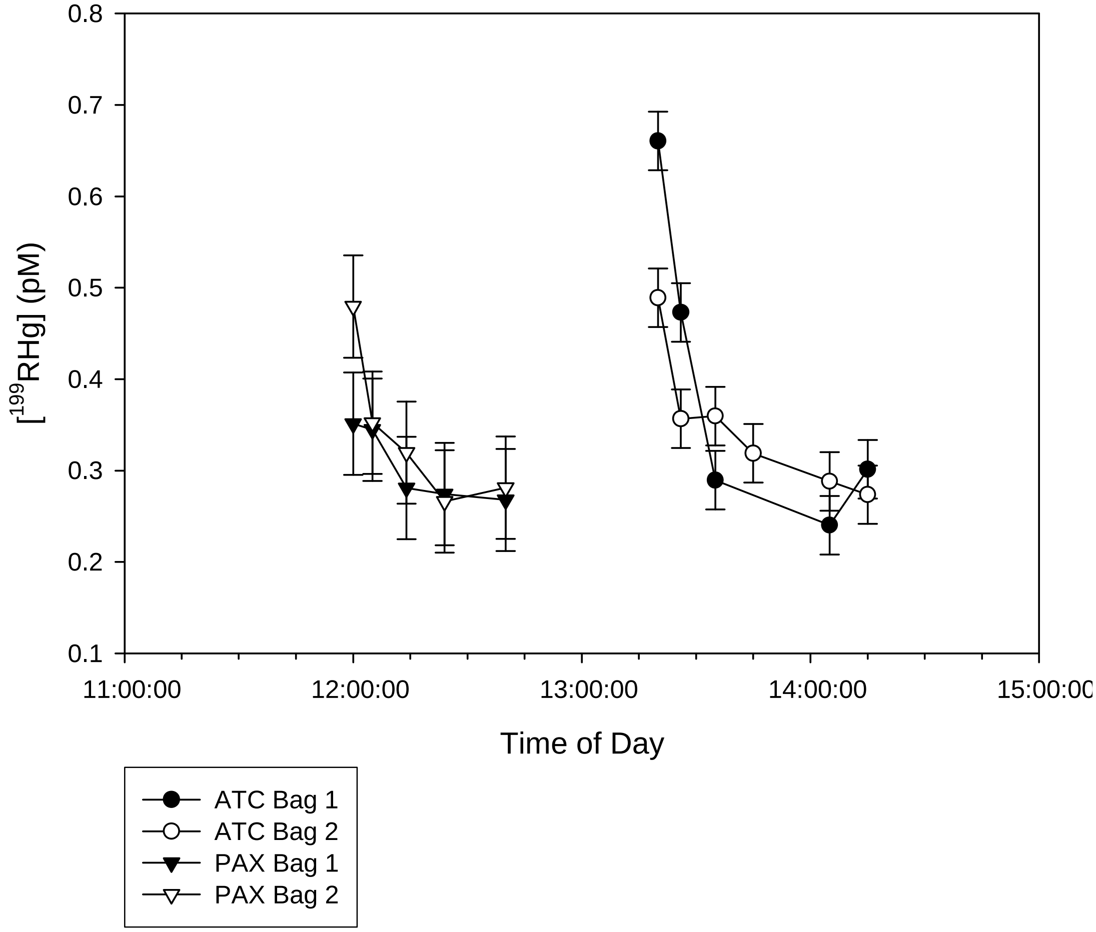


Fig. 2.8 Measurements of ^{199}RHg over time in duplicate bags for short term kinetics experiments performed at ATC and PAX.

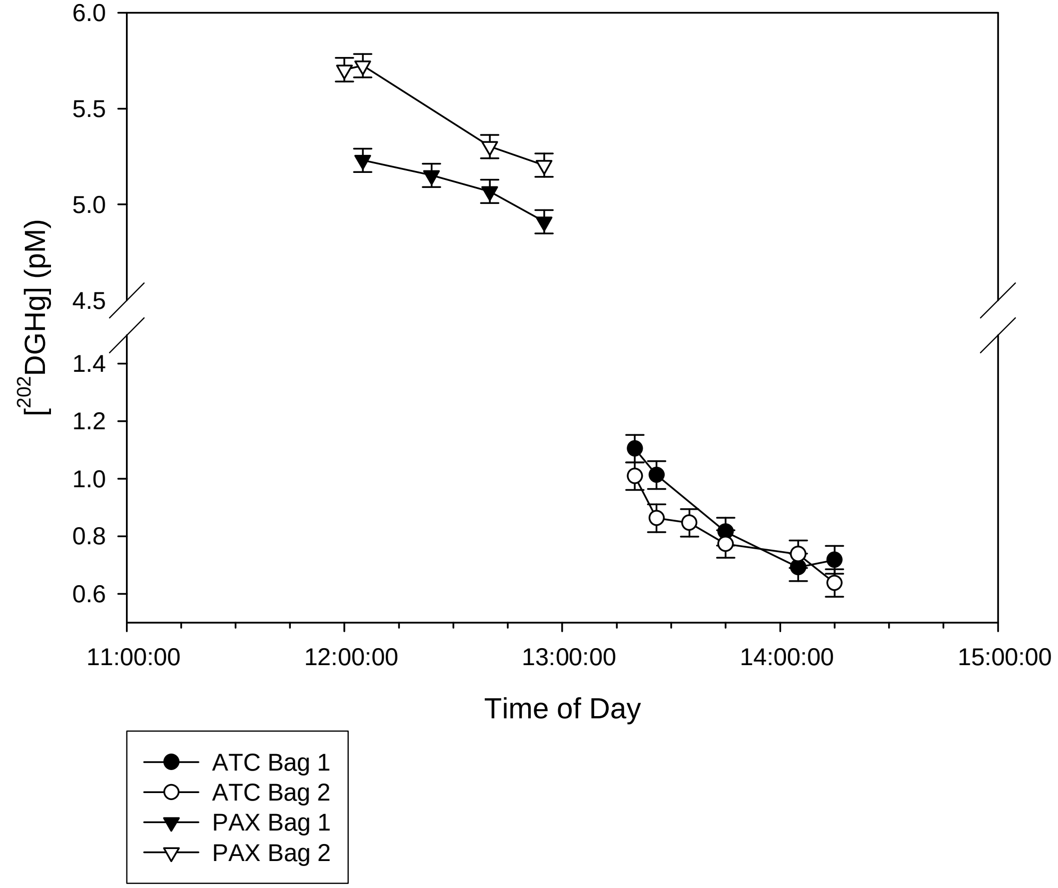


Fig. 2.9 Measurements of ^{202}DGHg over time in duplicate bags for short term kinetics experiments performed at ATC and PAX.

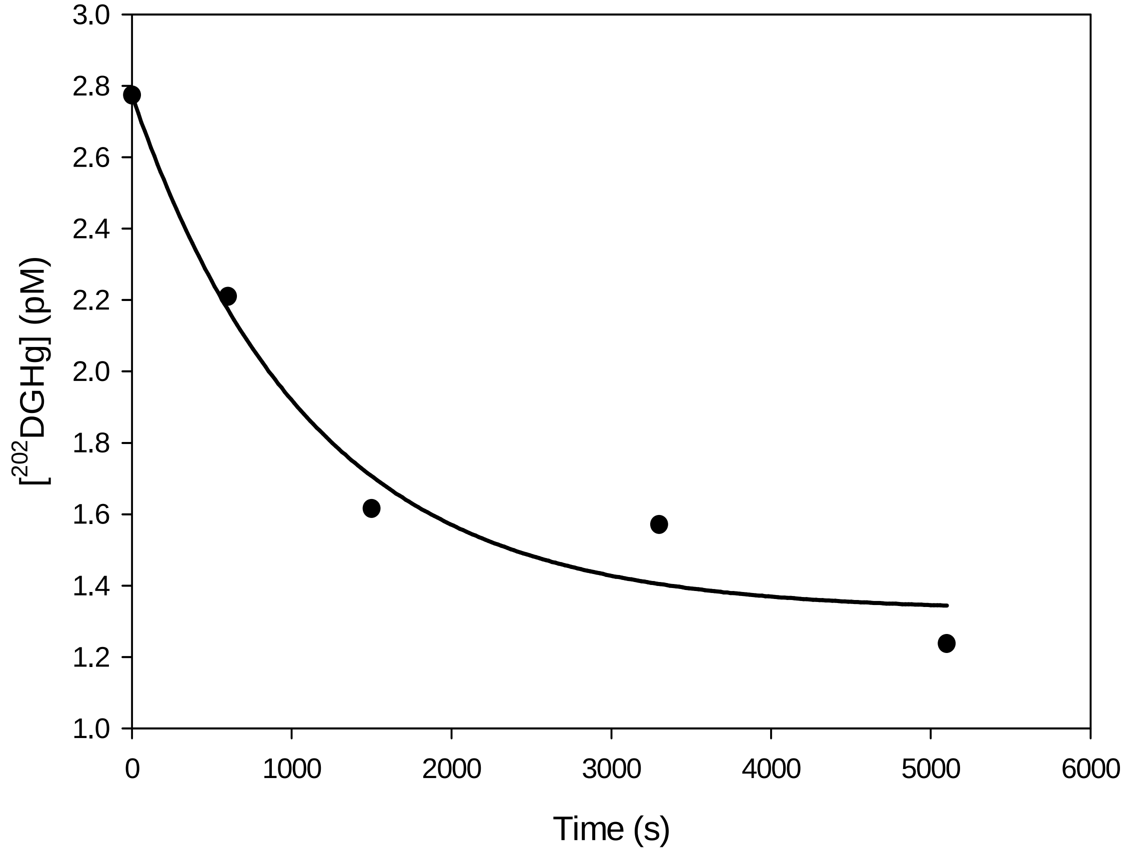


Fig. 2.10 Non-linear least squares regression curve fit to CBL 1pm 202 isotope data.

Site	**^{202}DGHg (pM)**	**% of ave. sample**	**^{199}RHg (pM)**	**% of ave. sample**	**^{199}HgT-DGHg (pM)**	**% of ave. sample**	**^{202}HgT-DGHg (pM)**	**% of ave. sample**
ATC	0.048	11.1	0.032	2.1	0.045	3.4	0.068	5.1
PAX	0.061	5.5	0.056	10.0	0.079	4.7	0.087	1.2

Table 2.1. Method error, presented as the coefficient of variation between replicate analysis of the measurement. Also presented, the percentage that error is of the average measurement concentration in pM.

Site	Measurement	199(pM)	% of ave.	202(pM)	% of ave.
ATC	DGHg	0.026	12.6	0.059	7.1
	RHg	0.053	14.6	0.066	15.9
	HgT - DGHg	0.072	6.5	0.036	8.4
PAX	DGHg	0.027	13.3	0.241	4.6
	RHg	0.027	8.2	0.056	13.4
	HgT - DGHg	0.063	4.2	0.067	4.0

Table 2.2. Error associated with the bags, presented as the average error for each type of measurement. Each was calculated as the standard deviation of that measurement between the duplicate bags

Site	**ATC**	**PAX**
Ave. % Change in ^{202}DGHg (Oxidation)	36	7
Bag Error - % of Ave. Sample	7	4
Method Error - % of Ave. Sample	11	5
Ave. % Change in ^{199}RHg (Reduction)	46	32
Bag Error - % of Ave. Sample	14	8
Method Error - % of Ave. Sample	2	10

Table 2.3 Comparison of the average change in isotopic Hg speciation due to oxidation and reduction with the average error for the measurements to determine if that error is acceptable

Rate Constants (s^{-1})									
		Oxidation				Reduction			
Expt	**Bag**	**202**		**199**		**202**		**199**	
ATC	**1**	4.4x10^{-4}		4.2x10^{-4}		6.2x10^{-4}		1.1x10^{-3}	
SE	P	1.8x10^{-4}	0.0933	7.0x10^{-5}	0.0258	2.6x10^{-4}	0.0933	1.8x10^{-4}	0.0258
ATC	**2**	2.6x10^{-4}		5.3x10^{-4}		4.4x10^{-4}		4.4x10^{-4}	
SE	P	1.0x10^{-4}	0.0600	1.6x10^{-6}	0.0521	1.7x10^{-4}	0.0600	1.3x10^{-6}	0.0521
PAX	**1**	no fit		8.0x10^{-4}		no fit		2.9x10^{-4}	
SE	P		0.9999	4.8x10^{-4}	0.0605		0.9999	1.8x10^{-4}	0.1960
PAX	**2**	no fit		1.5x10^{-3}		no fit		1.1x10^{-3}	
SE	P		0.3792	3.3x10^{-4}	0.0218		0.3792	2.4x10^{-4}	0.0218

Table 2.4 Comparison of rate constants determined from both isotopes for the ATC and PAX experiments. SE is the standard error and P is the P value for fit of the data.

Oxidation		ATC	ATC	ATC	PAX
		1 199	2 202	2 199	2 199
ATC	1 202	0.18	1.51	-1.87	
ATC	1 199		2.27	-2.72	
ATC	2 202			-4.68	
PAX	1 199				-2.09
Reduction					
ATC	1 202	-2.62	1.00	1.20	
ATC	1 199		4.53	6.07	
ATC	2 202			0	
PAX	1 199				-4.85

Table 2.5 T- values for one tailed t-test between rates determined from both datasets (^{202}DGHg and ^{199}RHg) and duplicate bags 1 and 2. For 4 degrees of freedom and 99% confidence ($\alpha = 0.01$), t_{crit} (critical) = ± 3.747. If t_{calc} (calculated) < $-t_{crit}$ or > $+ t_{crit}$, the hypothesis that the two values are equal cannot be rejected, or the values are similar.

CHAPTER 3. INVESTIGATING Hg REDOX CHEMISTRY IN NATURAL WATERS

3.1 INTRODUCTION

Theories concerning the abiotic mechanisms which control the redox chemistry of Hg in natural waters abound, yet few have been proven through repeated testing. This is due to the limitations of the incubation methods used in many of the studies investigating Hg redox processes. These theories can now be rigorously tested utilizing the new method designed in this research. Therefore, the aim of this research was to test three theories commonly found in the literature using the new method outlined in chapter 2. While the experiments performed do not conclusively substantiate mechanisms for Hg redox chemistry, they do prove that such assessments can be performed using the new method. The three hypotheses tested are 1) that both Hg reduction and oxidation in natural waters are photochemically mediated; 2) that reduction will be greater in freshwater due to higher concentrations of organic matter; and 3) that oxidation will be greater in seawater due to higher concentrations of halides.

3.1.1 Hg OXIDATION

Oxidation of Hg^0 has been identified in seawater (Mason, *et al.* 2001; Lalonde, *et al.* 2001; Amyot, *et al.* 1997a; Lalonde, *et al.* 2004) and freshwater (Hines and Brezonik 2004; Lalonde, *et al.* 2001). Studies suggest that the dominant oxidant of Hg in natural waters is $OH^\bullet$ (Hines and Brezonik 2004; Gardfeldt, *et al.* 2001c) (see 1.1.2). The processes which produce $OH^\bullet$ in natural waters have been found to be photochemically mediated (Vaughan and Blough 1998; Brezonik and Fulkerson-Brekken 1998; Southworth and Voelker 2003). Consequently, it has been suggested that the oxidation of

Hg^0 is photochemically mediated (Lalonde, *et al.* 2001; Lalonde, *et al.* 2004). In incubation experiments conducted by Lalonde, *et al.* (2001) and Lalonde, *et al.* (2004), spiked Hg^0 concentrations were observed to significantly decrease in natural saline waters upon irradiation with UV light, while no decrease occurred in dark controls. However, there are two problems associated with the rate constant determined for the oxidation in this research. First, only the net oxidation rate constant could be determined because of the inability to separate the forward and reverse reactions. Second, the method used to calculate the rate constant used elementary rather than reversible (or complementary) reactions. The resulting rate constants are inaccurate because the method assumes that Hg^0 is completely consumed during the experiment. Figure 2.2 describes the equations to describe redox kinetics used in these experiments, heretofore referred to as the elementary reaction method of rate constant calculation.

In an experiment conducted by Amyot, *et al.* (1997)(1997a), incubations of unspiked seawater under ambient sunlight resulted in a decrease of DGHg after an initial increase. The rate constant determined for this oxidation was approximately $2.8 \times 10^{-5}\ s^{-1}$. Unfortunately, the method of rate constant determination was not reported, such that its accuracy could not be assessed.

In order to test the theory that Hg oxidation is photochemically mediated, this study employed incubations of isotopically labeled Hg(II) and Hg^0 spikes of natural waters. Short term kinetics experiments were performed throughout the day to determine oxidation and reduction rate constants which could be compared to assess the impact of sunlight.

It has also been suggested that halides, such as chloride and bromide, may enhance the oxidation of Hg in natural waters (Mason, *et al.* 2001; Lalonde, *et al.* 2001; Hines and Brezonik 2004). Several mechanisms have been proposed. The first is that additional oxidants are formed by the reaction of the halides with $OH^{\bullet}$; for example with chloride to produce OCl^-. It has been suggested that this mechanism dominates Hg oxidation in the aqueous solutions of the marine atmosphere (Lin and Pehkonen 1999).

The second proposed mechanism is that halides form complexes with Hg ions, Hg(I) and Hg(II), stabilizing them such that the back reaction, or reduction, is decreased. In this case, oxidation is not directly enhanced, rather the back reaction is reduced which causes greater net oxidation. A third process may also be responsible for enhanced Hg oxidation in seawater; the production of dichloride ($Cl_2^{\bullet -}$) and dibromide ($Br_2^{\bullet -}$) radicals in seawater (Mason, *et al.* 2001), however the mechanisms controlling the production of these radicals is poorly understood. Experiments with simple artificial solutions have proven that these mechanisms potentially occur (Lin and Pehkonen 1999), but studies in natural waters have been unable to confirm any theory because of the previous inability to simultaneously measure Hg oxidation and reduction.

In order to understand which mechanism, if any, enhances the oxidation of Hg in natural waters, mechanistic studies are required. While this research does not attempt to answer this question, the methodology developed by this research may aid in investigating these mechanisms in future studies. This research does however propose to test the overarching theory that the presence of halides in natural waters enhances Hg oxidation. This was accomplished by comparing similar incubation experiments performed on three different natural water types; saline coastal water, brackish water, and fresh river water. The rate constants for oxidation can be compared to determine if oxidation was greater in saline waters over fresh.

Thus, Hypothesis1 states that: Photochemical oxidation rates are enhanced in seawater over freshwater due to reactions involving reactive halogen species in solution.

3.1.2 Hg REDUCTION

Hg reduction has also been identified in seawater (Rolfhus and Fitzgerald 2004; Amyot, *et al.* 1997a) and freshwater (Amyot, *et al.* 1994; Amyot, *et al.* 1997b; Amyot, *et al.* 2000). It has also been suggested that Hg reduction is highly photochemically mediated. For example, in incubation experiments conducted by Amyot, *et al.* (2000) and Amyot, *et al.* (1994) using unspiked natural waters, a positive correlation was identified between DGHg production and UV radiation. In addition, in a study conducted by Gardfeldt, *et al.* (2001a), a diurnal pattern in Hg^0 evasion over both seawater and river surfaces was observed, with maximum evasion during daylight hours.

In addition, it has been suggested that Hg reduction in natural waters is correlated with organic matter content (Costa and Liss 1999; Xiao, *et al.* 1995; Allard and Arsenie 1991), which can act in a photosensitizing manner (as a chromophore) (Spokes and Liss 1995) (see 1.1.2). There are two suggested mechanisms regarding the link between DOC/DOM and Hg reduction. First, is the direct reduction of Hg(I) or Hg(II) via ligand metal charge transfer by chromophoric material (Allard and Arsenie 1991; Spokes and Liss 1995). Second, is the formation of reactive intermediate reductants, such as $O_2^{\bullet -}$ (Voelker, *et al.* 1997; Zhang and Lindberg 2001), formed via the photolysis of DOC.

The theory that Hg reduction and [DOC] are correlated can also be tested using the method designed for this research. Rate constants can be determined and then compared between incubation experiments performed on the three water types. If this theory is correct, it is expected that the reduction rate constant would be greater in freshwater, which generally has higher concentrations of organic matter over seawater. It is also expected that the rate constant for brackish water would be between these two end members.

Thus, Hypothesis 2 states that: Photochemical reduction of Hg(II) is controlled by the concentration and form of DOC in the medium, thus reduction will be greater in waters of terriginous origin.

3.1.3 PHOTOCHEMICALLY DRIVEN Hg REDOX CHEMISTRY

As described in sections 3.1.1 and 3.1.2, it has been suggested that both oxidation and reduction of Hg in natural waters are photochemically mediated. This hypothesis can be tested using the method designed for this research and described in chapter 2. The rate constants for oxidation and reduction can be determined from short term incubation experiments conducted on the same water through different stages of ambient sunlight.

Thus, Hypothesis 3 states that: Both oxidation and reduction of Hg in natural waters are predominately photochemically mediated.

3.2 MATERIALS AND METHODS

The two experiments performed to test the validity of the new method, described in chapter 2, were also used for this research. Therefore, section 2.2 should be referred to for the materials and methods for this section of the study, with the following exceptions and additions.

3.2.1 STUDY SITES

In addition to the two study sites described in 2.2.1, water was also collected off of the research pier at the Chesapeake Biological Laboratory (CBL), located where the mouth of the Patuxent River meets the Chesapeake Bay, in late summer of 2004 (Fig. 2.4). This water is a mix of freshwater from the river and brackish water from the bay.

3.2.2 EXPERIMENTAL DESIGN

In addition to the midday experiments described in 2.2.5, a diel experiment was performed to compare rates of oxidation and reduction between times of day and light conditions. It was an incubation which spanned almost 24 hours, in which 4 separate short term kinetics experiments were performed on 4 bags. In mid-August of 2004, 40L of filtered water was collected off of the CBL pier to fill four 5L bags. All four bags were treated identically and deployed simultaneously before sunrise at 4am. Each bag was then used in a separate, short term kinetics experiment conducted at four different times of the day chosen to span the days light activity.

The first experiment began just before sunrise, at 6am. At this time in the morning it is expected that light activity and concentrations of long lived oxidants and reductants produced the day before would be minimal (Fig. 3.1). The second experiment commenced at 10am, when light activity was rapidly increasing, whereas it was near its peak during the third experiment, performed at 1pm. The final experiment began at 10pm, 3 hours after sunset, when light activity was again at a minimum, but also when long lived oxidants and reductants might have still been present. Similar to the other experiments described, spike additions consisted of $^{199}Hg(OH)_2$ and $^{202}Hg^0$.

3.2.3 RATE CONSTANT DETERMINATION

As described briefly in section 2.2.7, the rate constants for Hg oxidation and reduction were determined using the reversible reaction method which fits the data to a curve describing equation a in figure 2.3 using the Sigma Plot 8.0 computer program. Specifically, the input data consisted of the multiple values of [^{199}RHg] or [^{202}DGHg] at time t, its original concentration, and the time in seconds (s) that the respective sub-sample was taken from the initial. The derivation of this equation and a more thorough explanation of this method can be found in figure 3.2. The most important features of figure 3.2 are the reaction type, reversible, and the resultant rate equation which includes the rate constants for both Hg oxidation and reduction. The integrated version of this equation was transformed to include the concentration of the spiked Hg species at steady state, $[Hg]_{eq}$. This value and the value for (k_1+k_2) were the unknowns in the equation, defined by the fit of the data to the curve. If a significant fit was obtained, such as the fit of the ^{202}DGHg data set for the 1pm CBL experiment (p value = 0.0303) shown in figure 2.10, the values returned for $[^{199}RHg]_{eq}$ or $[^{202}DGHg]_{eq}$, and for (k_1+k_2) became the inputs into the equilibrium, or steady state equation (Fig. 2.3 eqn. b) in order to determine the individual rate constants for oxidation and reduction.

A high p-value indicates that the dataset does not fit the curve described by the reversible reaction rate equation, and in this situation, the result is denoted as “no fit”. This p-value is generated by the SigmaPlot program to describe the fit of the dataset to the equation applied. There are several reasons a data set might fail to produce a significant fit. The first is that the process being described by the equation did not occur. Such a dataset would most likely appear scattered, with no indication of any trend. It is also possible to fail to obtain a significant fit when the process did occur. There are at least two situations when this might be the case. The first is when the data set, here representing the change in Hg speciation, is influenced by processes other than the one being studied. The data in this case might appear scattered as in the latter case. The second can occur if the timescale of the experiment was too short to capture the changes in speciation required to apply the equation. In contrast to the other examples, the data in this case would not be scattered, but would appear to adhere to the initial portion of the curve. Such a process would be slow enough to be considered negligible for the timescales in this research.

Comparison of rate constants was substantiated by t-tests. Two different methods were used in the photochemistry and Hg redox experiments. Photochemistry experiment input data consisted of three values each for the rate constants of oxidation and reduction at all three sites. These values were 1) the constant determined from the average of both bags and both methods of determination, i.e. the constant determined from ATC bags 1 and 2 using both ^{202}DGHg and ^{199}RHG datasets, 2) this value minus the average standard error, and 3) the averaged value plus the average standard error. Input data for the Hg redox experiments consisted of the rate constants of oxidation and reduction determined by all methods at all three sites, i.e. those determined from ATC bags 1 and 2 using both ^{202}DGHg and ^{199}RHG datasets for ATC, etc. T-values revealed the larger of the two inputs, and p values returned the probability that the values are the same.

3.3 RESULTS AND DISCUSSION

3.3.1 PHOTOCHEMICALLY MEDIATED Hg REDOX CHEMISTRY

3.3.1.1 Hg PHOTOREDUCTION

Consistent with reduction, ^{199}RHg was observed to decline over time in several of the CBL incubation bags. Figure 3.3 illustrates that there was no visible decrease in ^{199}RHg at 6am or 10am, some at 10 pm, and that the maximum decay of ^{199}RHg during the CBL experiment was observed at 1pm. In order to confirm the occurrence of Hg reduction and to quantify it, the rate constants for this process must be defined. Those determined for Hg reduction in the four CBL experiments are listed in table 3.1.

There was no significant change in ^{199}RHg or ^{202}DGHg concentrations during the 6am experiment, and neither isotope dataset could be fitted to the curve described in figure 3.2. As mentioned in section 3.2.3, there are several possible explanations for an absence of fit. In this case the data were scattered and showed no overall change in concentration with time, and therefore did not result in a significant fit. This suggests that there was simply no identifiable Hg reduction at 6am. In other words, either photoreduction was not occurring, or the rate of reaction was insufficient to produce a measurable change in concentration, given the analytical error associated with the method.

As in the 6am experiment, the 10am ^{199}RHg data was scattered and did not produce a significant fit to the curve. In contrast, an excellent fit was obtained with the ^{202}DGHg data from this experiment, returning a p value of 0.0157. A reduction rate constant of 4.8(± 1.0) $\times 10^{-5}$ s^{-1} was determined, suggesting that Hg reduction did occur in the CBL water at 10am. Greater Hg reduction rate constants were calculated with both isotopic data sets for the 1pm experiment (t-test p values between 202 10am and 202 1pm and 199 1pm are 0.0039 and 0.1077 that they are equal). The values determined for 1pm from both data sets were similar (t-test p value = 0.8013 that they are equivalent), averaging $6.5(\pm 2.6)\times 10^{-4}$ s^{-1}. Results from the 10pm experiment were analogous to those at 6am, scattered and without a significant fit by either of the isotopic datasets, suggesting that no significant Hg reduction occurred at 10pm.

In summary, the results of this experiment indicate that no measurable Hg reduction occurred in CBL water at 6am and 10pm, both times when light was absent (Fig. 3.1). Significant Hg reduction was identified however, during the daylight hours of 10am and 1pm. Furthermore, the Hg reduction rate constant at 1pm was at least 5 times faster than at 10am, correlating to the sharp increase in light intensity (Fig. 3.1). The indication is that Hg reduction in these waters was photochemically mediated.

3.3.1.2 Hg PHOTOOXIDATION

^{202}DGHg was observed to decrease with time in some of the four CBL experiments (Fig. 3.4). Specifically, no decline was evident at 6am or 10pm, however a significant decrease in ^{202}DGHg was observed at 10am and 1pm. The shape of the curves at 10am and 1pm do reveal important information in that both clearly indicate exponential decay, a sign of oxidation. Moreover, the 10am curve is less steep than that for 1pm, and does not appear to reach the same degree of plateau. This would suggest that the steady state of ^{202}DGHg production and loss was achieved more rapidly at 1pm. Thus, the rate of Hg oxidation was higher at 1pm than at 10am. To confirm this observation, the rate constants for Hg oxidation were determined for all four bags in the CBL experiment. They are listed in table 3.1.

As mentioned in section 3.3.1.1, neither 6am isotopic data set produced a significant fit to the curve due to scatter. This indicates that no measurable Hg oxidation occurred in the CBL water at 6am. The ^{202}DGHg data set for 10am does indicate significant Hg oxidation, returning a rate constant of 2.1(±0.4)x10^{-4} s^{-1}. Hg oxidation rate constants for 1pm were of the same order and averaged of 7.2(±2.9) x10^{-4} s^{-1}, also indicating that there was significant Hg oxidation at 1pm. Once more, analogous to the results for 6am, neither 10pm isotopic data set could be fit to the curve because of scatter, indicating that no measurable Hg oxidation occurred in the CBL water at this time.

The temporal pattern of Hg oxidation in the CBL water was similar to that of its reduction, namely that the process occurred during daylight hours, at 10am and 1pm, and was absent in the dark hours of 6am and 10pm (Fig. 3.1). Similar to the results for reduction, the rate of Hg oxidation at 1pm was greater than at 10am, though the increase in rate was not as large as that for Hg reduction. These results indicate that Hg oxidation in the CBL water was photochemically mediated.

3.3.1.3 PHOTOCHEMICALLY MEDIATED Hg REDOX CHEMISTRY CONCLUSIONS

The finding that both Hg oxidation and reduction are photochemically mediated in CBL waters upholds the stated hypothesis that both oxidation and reduction of Hg in natural waters are predominately photochemically mediated. Of course, it is incorrect to extrapolate from CBL water to all natural waters, however two facts suggests that such an argument is plausible. First, as mentioned in section 3.2.1, CBL water is a fairly representative mix of the general types of water bodies. There is freshwater input from the Patuxent River and brackish input from the Chesapeake Bay, itself a mix of freshwater from its many tributaries and coastal Atlantic seawater.

Secondly, this data supports the many other studies found in the literature which suggest the importance of photochemistry to Hg redox. Gardfeldt, *et al.* (2001a) detected maximal Hg^0 evasion during the daytime over fresh and seawater surfaces. Krabbenhoft, *et al.* (1998) found that DGHg concentrations in the brackish waters of the Florida Everglades peaked around noon, while its production halted in the dark. Lalonde, *et al.* (2001) and Lalonde, *et al.* (2004) observed a systematic decrease in DGHg upon irradiation with UV light in fresh and brackish waters, respectively. The oxidation rate constants determined in both of these experiments were similar, approximating $1.7 \times 10^{-4}\ s^{-1}$, and were on the same order as those determined in this experiment. Comparison of Hg oxidation rate constants is hampered however because the constants were calculated using the elementary reaction method in the former study, and the latter did not report their method of rate constant determination (see section 3.1.2).

Amyot, *et al.* (1994) detected a correlation between UV_B radiation and sunlight on DGHg production in lake waters, however rate comparison was not possible because none were reported. Finally, Amyot, *et al.* (2000) detected a peak in DGHg concentrations in freshwater immediately following sunrise, and found a correlation between UV radiation and DGHg production. The rate of Hg reduction was determined to be approximately $6.1 \times 10^{-4} s^{-1}$, similar to the results of this research, though this rate was determined from unfiltered water. These studies, and the results of this research indicate that Hg oxidation and reduction are predominately mediated by photochemistry in natural waters.

Although this study was not designed to determine the specific mechanisms of Hg redox in natural waters, the results might shed some light. For example, at 10am the rate of Hg oxidation (~7.4×10^{-4} pM s^{-1}) was more than five times faster than the rate of Hg reduction (~1.2×10^{-4} pM s^{-1}). Yet, at 1pm the rates were on the same order, ~2.0×10^{-3} pM s^{-1} and 3.3×10^{-3} pM s^{-1} for Hg oxidation and reduction respectively. Explanations for such a discrepancy include the prospect that Hg oxidation in these waters required less light. It is also feasible that the processes require different wavelengths of light, as suggested in the Hg reduction experiments by Amyot, *et al*. (1994) and Amyot, *et al*. (2000). Another explanation for greater Hg oxidation over reduction at lower light levels is the possibility that oxidation relies more heavily on secondary processes, such as the formation of reactive intermediates as suggested by Mason, *et al.* (2001). Such a discrepancy points to the necessity for more detailed studies aimed at identifying the mechanisms which cause Hg redox.

3.3.2 Hg REDOX CHEMISTRY: SITE AND WATER TYPE COMPARISON

3.3.2.1 Hg OXIDATION AND REDUCTION RESULTS

Hg reduction was evident in both duplicate bags for ATC and PAX, as can be seen by the exponential decay of ^{199}RHg for all four bags in figure 2.8. In order to quantify Hg reduction and to more precisely compare the process between the two sites, it was necessary to determine their rate constants. A thorough discussion of all of the rate constants determined in this experiment can be found in section 2.3.3, but for the sake of simplicity in comparison, the rates were averaged and listed in table 3.2. The average Hg reduction and oxidation rate constants for the midday CBL experiment was also included in this table. In this way, an additional water type, brackish and spanning [DOC] and [halide], can be used in the site comparison. Hg reduction rate constants were similar at all three sites (t-tests produced p-values of 0.9013, 1.0 and 0.9311 for the probability that the values were the equivalent between ATC and PAX, ATC and CBL and PAX and CBL, respectively), and were on the order of $7x10^{-4}s^{-1}$.

Hg oxidation was also detected in both duplicate bags for ATC and PAX. This fact is not well represented by figure 2.9, the measurements of ^{202}DGHg overtime for these two experiments, due to the incomplete dataset for the PAX experiment. The trend in this data appears linear rather than the expected exponential decay. Unfortunately, a leak in the setup for the sample DGHg purge was discovered too late and four of these samples (two from each bag) were lost. Of course, the rate could also be determined from the ^{199}RHg dataset which was not affected by the leak. The ^{199}RHg dataset for the ATC experiment not only fit the model for exponential decay, but appears to approach steady state. The averaged rate constants of Hg oxidation for these experiments can be found in table 3.2. The highest value was determined for PAX, $1.2(\pm0.4)x10^{-3}$ s^{-1}, followed by CBL, and then ATC, $4.1(\pm0.9x10^{-4}$ $s^{-1})$.

It should be noted that these results are in conflict with those expected given the hypotheses that the rate of reduction would be greater in PAX than in ATC waters, and that the rate of oxidation would be greater in ATC waters than those of PAX. These results indicate that reduction rates were similar at all sites, and that the rate of oxidation was greater in PAX than ATC water (t-test p-value of 0.0328 that they are equal).

3.3.2.2 Hg REDUCTION

The occurrence of reduction in these waters, and the similarity in rate constants at all sites indicates the importance of this redox process in these waters specifically, and suggests its importance in natural waters in general. In addition, these results imply that Hg reduction is important in seawater, especially considering that the rate constant for reduction was greater than that of oxidation in this water (t-test $p = 0.2018$). A review of studies found in the literature reveals a plethora of data which support this finding. For instance, the large ratios of [Hg^0] to [Hg(II)] (greater than expected from thermodynamic calculations) found in open ocean water (Mason, *et al.* 1995; Mason, *et al.* 1998; Mason and Sullivan 1999; Mason and Fitzgerald 1993), and in brackish waters (Cossa and Gobeil 2000), as well as the significant evasion of Hg^0 detected over seawater surfaces (Gardfeldt, *et al.* 2001a; Ferrara, *et al.* 2000) suggest significant Hg reduction activity can occur in saline waters. Moreover, an incubation experiment performed by Rolfhus and Fitzgerald (2004) on Hg(II) spiked coastal water detected net reduction.

Considering the results of these experiments, and more generally that typical concentrations of DOC in open ocean waters approximate 100μM (Stumm and Morgan 1996), the suggestion that significant reduction occurs in open ocean waters necessitates a mechanism for Hg reduction other than the photolysis of DOC. For instance, some of the Hg reduction implicated or observed in the above studies may have been biotic in nature. Indeed, it was suggested that 20% of the photoreduction identified in the above incubation experiment could be attributed to microbial reduction (Rolfhus and Fitzgerald 2004). Furthermore, several studies which identified Hg reduction by heterotrophic bacteria support this finding (Mason, *et al.* 1995; Siciliano, *et al.* 2002; Barkay, *et al.*

1989). Studies have also implicated the importance of Hg reduction by algae as a means of detoxification (Ben-Bassat and Mayer 1977; Ben-Bassat and Mayer 1978).

It is less likely that biotic processes explain the Hg reduction in these experiments considering the sample waters were filtered. While it is feasible that some bacteria could have passed through the 0.45μm filter used, it is doubtful that significant microbial reduction occurred from such a small concentration of bacteria. Other proposed mechanisms for Hg reduction not linked to DOC photolysis include the direct photolysis of certain Hg complexes, such as those with OH^- and Cl^- (Nriagu 1994).

There is also the potential that there exist other mechanisms involved in the production of the superoxide radical ($O_2^{\bullet -}$) which may be responsible for significant Hg reduction in natural waters. For example, Zhang and Lindberg (2001) propose that the photolysis of Fe(III) in the presence of natural organic ligands may create highly reducing organic free radicals as well as $O_2^{\bullet -}$. Of course, this process requires the presence of DOC and is in fact an additional method of reductant production via DOC photolysis. It should therefore be noted that DOC photolysis may have significantly contributed to Hg reduction in ATC waters as well as PAX. As is evident in figure 3.5, DOC concentrations were low in both waters, but more importantly, they were only slightly larger in PAX (519μM) versus ATC (158μM) waters. The DOC concentration in the ATC water was higher than typically found for the open ocean (<100μM).

Calculations performed to determine the potential complexation of Hg(II) in both of these waters suggest that 45 to 100% of the ^{199}RHg spiked into the ATC waters was organically complexed. These values are based on the assumption that the dominant Hg complexes in these waters are with Cl^-, OH^- and DOM. [Cl^-] was determined by major ion analysis, [OH^-] was determined from an estimated pH of 8, and [DOM] was estimated from [DOC] assuming a molecular weight of 1000 g mol^{-1} and approximating that DOM was 50% by mass DOC (Benoit, *et al.* 2001). The inclusion of [Br^-], estimated as 0.35% [Cl^-] (Stumm and Morgan 1996), did not affect the results, thus it was left out of this discussion. Stability constants for Hg complexes with OH^- and Cl^- were obtained from Stumm and Morgan (1996), and two stability constants for organic complexation of Hg(II) were taken from the literature, log K = 10.6 (Benoit, *et al.* 2001) and log K = 24 (Lamborg, *et al.* 2002). These values represent the end-members of the Hg(II)-DOM complex stability constants defined in literature studies, and were chosen in an attempt to span the breadth of DOM affinity for Hg(II) given the undefined nature of the DOC in these studies experimental solutions. Estimates of Hg(II) complexation by organic matter using both constants were high (45% from log K = 10.6 and 100% from log K = 24), indicating that DOC complexation and photolysis was important in ATC waters. The fraction of the total ionic Hg as RHg ({RHg]/[Hg_T – DGHg]) also gives some measure of the degree of complexation as the the non-RHg fraction is mostly of iorganically-bound Hg. For the ATC water, this fraction was xx, suggesting significant organic complexation. Such high organic complexation could potentially explain the large reduction rate constant determined for this water. Alternatively, the high reduction rate constants in both waters may also indicate simply that ligand to metal charge transfer is more important to the reduction of Hg than indirect photochemical processes, such as DOC photolysis.

It was also necessary to confirm the validity of using the change in RHg concentration to determine rate constants for reduction. Such a method assumes that reduction of the complexed fraction is negligible. Therefore, the same method of rate constant calculation was applied using the concentration of all the ionic Hg in the sample for the ATC and PAX experiments. This was accomplished by using the HgT-DGHg measurement determined from every sample. Briefly, no fit was obtained for either bag in either experiment. One significant difference existed however. The plot data appeared scattered for the ATC experiment, while the PAX data produced a straight line. This indicates that no significant reduction of the complexed fraction occurred during the ATC experiment, while reduction of this fraction occurred, but was slow during the PAX experiment. The data for the latter experiment represented the initial portion of a curve that might fit the reversible reaction rate equation, so that if the experiment ran longer, a fit may have been produced identifying a slow and in this experiment negligible rate of reduction. This suggests that applying the change in RHg concentration to determine the reduction rate constant was valid in this case.

Recall that rate constants for Hg reduction calculated from the ^{202}DGHg and ^{199}RHg datasets were similar. This fact is significant in that it provides information about the kinetics of complexation, in this case that it was rapid and much faster than those of Hg redox processes. If complexation was slow, one would expect to see interference in the reduction of ^{202}RHg (directly after it is produced by the oxidation of ^{202}DGHg), thereby changing its rate. Not that this information can only be obtained by the use of two inorganic Hg tracers.

To determine if it is feasible that $O_2^{\bullet -}$ was responsible for the reduction in these experiments, second order rate constants were calculated using typical concentrations of $O_2^{\bullet -}$ in natural waters, 1×10^{-11} M (Goldstone and Voelker 2000). The rate constant values for bags 1 and 2 of the ATC experiment, $1x10^4$ and $1x10^5$ M^{-1} s^{-1}, [are these calculations correct – my quick calculations suggest that the values should be higher] respectively agree well with values found in the literature (table 1.1). This suggests that the superoxide radical may have been the dominant reductant during the ATC experiment.

3.3.2.3 Hg OXIDATION

The detection of oxidation in the waters of all three sites tested, and the finding that rate constants for Hg oxidation were on the same order as that for its reduction, indicates the importance of this redox process in these waters specifically, and suggests its importance in natural waters in general. This is especially significant given the previous assumption that Hg oxidation was negligible, a theory based on the inert properties of Hg^0. Another implication of the results of these experiments, specifically that the rate constant for oxidation in the PAX water was greater than in ATC (t-test p = 0.0328), and that the rate of oxidation was equal to or greater than that of reduction in PAX (t-test p =0.4848), is that oxidation is important in freshwater.

Hg oxidation rate constants increase in magnitude from ATC to CBL to PAX. Considering the hypothesis of this research regarding Hg oxidation, this order is the reverse of expected. Furthermore, these results are peculiar because there is little in the literature to support it. One example of findings that do support those found in this research are those from a study conducted by Hines and Brezonik (2004), who detected oxidation in lake water and determined first order loss rates for Hg^0 in the range of 1.1 to $2.1x10^{-4}$ s^{-1}. This rate constant is lower than that determined for PAX, however it is likely their value would increase if the actual rather than the net rate was measured. The same study also concluded that the net rate of oxidation increased upon the addition of chloride to the freshwater sample however, a result inconsistent with those of this research.

Lalonde, *et al.* (2001) also identified a systematic Hg^0 loss in their unspiked freshwater incubations, following an initial increase. The oxidation rate constant determined from this rate of loss (6.9×10^{-5} s^{-1}) is smaller than that in the PAX experiment, ($1.2(\pm 0.4) \times 10^{-3}$), again potentially attributable to the calculation of a net rate. Compounding the difficulty in evaluating this data is the fact that the method of rate constant determination was not described in the article (though the data plot axes insinuate the use of the elementary reaction method), and the data appeared scattered, most likely due to the measurement of very small changes in small concentrations. Finally, the apparent loss of Hg^0 was larger in the seawater tested ($k = 1.86 \times 10^{-4}$ s^{-1}) than the freshwater tested in these experiments.

There is more data in the literature that are inconsistent with the results of the three experiments in this research. Most notable are the incubation experiments performed on unspiked freshwater by Amyot, *et al.* (1994; 1997b), Amyot, *et al.* (1997) and Amyot, *et al.* (2000) where DGHg production was observed. It should be noted that during the latter two experiments the concentration of Hg^0 reached a plateau. This phenomenon was attributed to substrate limitation, a definite possibility considering the small (fM) concentration of Hg(II) in these waters, but it is also possible that the plateau was indicative of Hg oxidation. First order rate constants determined for Hg reduction in the 2000 experiment, 2.8 to 5.6×10^{-4} s^{-1}, appear to agree fairly well with those calculated for PAX. Both methods of rate constant calculation (elementary and reversible) were performed in this study, returning similar results, however the rate constants for oxidation, which are intrinsically determined in the reversible reaction method, were not reported.

It is necessary to consider mechanisms that potentially explain the results obtained for Hg oxidation in the three waters tested in this research, especially considering their incongruity with the findings of some studies. For instance, the larger oxidation rate constants determined for the PAX and CBL waters over those of ATC (t-test p values of 0.0328 and 0.1533 and negative t values indicated that ATC<PAX and ATC<CBL) may be a consequence of greater concentrations of the constituents that produce $OH^{\bullet}$ upon photolysis in PAX and CBL. Several such mechanisms have been identified, including the photolysis of nitrite and nitrate (Brezonik and Fulkerson-Brekken 1998). Mason et al. (2001) showed that Hg^0 could be reduced in distilled water solutions spiked with nitrite. The redox cycle of iron has also been implicated in the production of $OH^{\bullet}$ via Fenton's reagent (Kwan and Voelker 2003; Southworth and Voelker 2003).

Perhaps most importantly, considering that concentrations of DOC are typically much higher in freshwater than seawater, Vaughan and Blough (1998) proved that $OH^{\bullet}$ can be produced by the photolysis of DOM when they detected its production (via probe loss) upon irradiation of Suwannee River fulvic acid stripped of nitrites, nitrates and metal ions. As described in section 3.2.2.2 however, the difference in [DOC] between the PAX and ATC may not have been significant in regard to complexation, thus photolytic production of oxidants was potentially similar in the two waters.

Second order rate constants were calculated for the ATC experiment for the hypothetical oxidation of Hg by $OH^{\bullet}$ given typical steady state concentrations in natural waters, $1x10^{-15}$ M (Brezonik and Fulkerson-Brekken 1998; Hines and Brezonik 2004). Rate constant values of $2x10^{11}$ M^{-1} s^{-1} and $9x10^{10}$ M^{-1} s^{-1} for bags 1 and 2 respectively, do not compare well with those in the literature (table 1.1). They are much higher and therefore suggest that $OH^{\bullet}$ may not have been the predominant oxidant in the ATC experiment.

A simple calculation was performed using the second order rate constants for Hg oxidation by $OH^{\bullet}$ taken from the literature in Table 1.1 to determine the expected steady state concentration of the oxidant given the rates of oxidation found in the experiments for this research. The purpose of this estimate was to ascertain if the hydroxyl radical could be responsible for the majority of Hg oxidation seen in these waters. Figure 3.6 illustrates that using approximations of $1x10^{9}$ $M^{-1}s^{-1}$ and $1x10^{-4}$ s^{-1} for that rate constants for Hg oxidation for the second order reaction with $OH^{\bullet}$ and the pseudo first order reaction respectively, the steady state concentration of $OH^{\bullet}$ would have to be on the order of 10^{-13} M. Considering that typical concentrations are between 10^{-17} and 10^{-15} M, it seems unreasonable that $OH^{\bullet}$ could be dominantly responsible for the Hg oxidation seen in the ATC, PAX and CBL experiments.

A feasible explanation for these results is that CBL and PAX waters contained higher concentrations of other oxidants such as singlet oxygen ($^{1}0_2$), or the peroxyl radical ($ROO^{\bullet}$). Other common oxidants in natural waters are hydrogen peroxide (H_2O_2) (Amyot, *et al.* 1997b) and ozone (O_3) (Hall 1995; Munthe 1992), though the rates of Hg oxidation by these two oxidants have been determined to be relatively slow. Alternatively, there may be other oxidants present that have not been characterized in detail. For example, Mason et al. (2001) showed that the rate of oxidation was enhanced in NaCl or NaBr solutions in the presence of nitrite, suggesting that there may be some catalytic reactions occurring in the solutions that provide reactive halogen oxidants. Species such as Cl, Br and BrO have been shown to be important in Hg oxidation in the atmosphere and further studies should investigate the role of such potential reactions in surface seawater.

An alternative explanation for these results is that there may have been greater consumption of oxidants by constituents other than the spiked Hg^0 in the ATC water than in the waters of PAX and CBL. However, this is not likely given that the major sinks for many of these oxidants are characteristically found at higher concentrations in freshwater. For example, the dominant sinks for $OH^\bullet$ are probably DOC and carbonate/bicarbonate (Voelker, *et al.* 1997; Brezonik and Fulkerson-Brekken 1998). Finally, these differences may result in varying light properties between the two experiments, since they were not conducted simultaneously, but on two different days.

3.3.2.4 Hg REDOX CHEMISTRY CONCLUSIONS

The results of these experiments prove that both redox processes are important in natural waters. Furthermore, the rates of oxidation and reduction are of similar magnitude. This is especially important in the case of oxidation which was previously thought to be negligible in natural waters. A review of the literature regarding Hg redox in natural waters for the purposes of comparison makes apparent two limitations in our present understanding of the subject. First, there is severe paucity of data. Second, comparison between data from different studies is severely hampered by the lack of consistency among methodologies, which may explain the many discrepancies in the results of those studies.

The lack of data regarding Hg redox processes in natural waters is especially true in the case of oxidation. In large part this is because Hg^0 was considered inert, such that it was only recently considered that Hg oxidation in natural waters might be significant. Moreover, it appears that since this recognition the investigation of Hg oxidation has been focused on seawater. Whatever the reason, it is obvious that more information is greatly needed in order to better understand Hg redox in freshwater.

Additionally, the numerous methodologies used in the studies have produced results that are difficult to confirm and compare. Incubation experiments appear to provide the most information on the redox of Hg, however there is significant divergence in the performance of such studies which make even comparison between these problematic. These differences include the use of the single large bottle versus the several small bottle methods and the absence or application of inorganic Hg spikes. Finally another feature of most of these studies which makes inter-comparison difficult, and makes comparison with the results from this research even more difficult is that these studies measured net rates rather than actual rates. The latter is preferred as it provides added and more specific information, the individual rates for both oxidation and reduction.

3.4 CONCLUSIONS AND RECOMMENDATIONS

Inconsistencies among the results of studies found in the literature, and between some of these studies and the findings of this research, highlight the many discrepancies that exist in our theories regarding Hg redox processes in natural waters and in the methods we use to test those theories. An important outcome of this research was the acknowledgement that more data is necessary, that some consistency in methodology would greatly enhance our ability to quantifiably compare the results of different studies, and that more controlled mechanistic studies are crucial.

No contradiction was found between the results of this study and those found in the literature regarding the role of photochemistry in Hg redox. Significant oxidation and reduction was identified during the daylight hours of 10am and 1pm, while none was observed in the dark hours of 6am and 10pm. The indication is that both Hg oxidation and reduction are predominately photochemically mediated. This finding is well supported by the literature.

The similarity of Hg reduction rate constants at all three sites failed to uphold the hypothesis that reduction would be greater at PAX than ATC due to a greater concentration of DOC. However, the overall range in DOC studied in these experiments was small, and differences may occur over the much larger range of DOC found in natural waters. A review of the literature supports these findings however in that net reduction has been identified in both seawater and freshwater.

The comparison of Hg oxidation at the three sites yielded the opposite of the expected result given the stated hypothesis that oxidation would be greater at ATC than PAX due to greater concentrations of halides. Results indicate that Hg oxidation rate constants increased between ATC and CBL, and then between CBL and PAX. A review of the literature revealed some support for these findings, as well as studies which contradicted these results. Several suggestions were devised regarding the mechanisms of Hg oxidation in order to establish theories which might explain the results.

The assessment of redox in seawater and freshwater revealed the large diversity in theories and methodologies for Hg redox investigations, and exposed the breadth of the information we have yet to understand. In large part the latter is a consequence of the attempt to understand Hg redox in natural waters when there is still little known about redox processes in these waters in general. An important outcome of this study was that it also lead to improvements of the incubation methods which, if used by others, might greatly enhance and accelerate our knowledge of the subject.

The impediments that consistently interfered with this research were discrepancies between the many studies which made comparison with my results difficult. These impediments manifest themselves in several ways. First, there is a glaring paucity of data, especially with regard to Hg oxidation. Second, few studies have been performed which address the investigation of a single mechanism in a controlled environment. Finally, as is common with studies that attempt to gather information directly from or to replicate the natural environment, there existed too many variables; different water types and bodies, varying light conditions and temperatures, and different experimental methods used. This became most evident in the attempt to compare the rate constants determined in this research with those found in the literature.

In many studies there were understandably large uncertainties, especially in incubation experiments performed without spike additions. None of the studies reported rate constants for both oxidation and reduction determined from the same water. Finally, the rate constants that were reported were usually determined using a method which assumes the total consumption of the reactant that may not occur. There is an obvious need for some uniformity in the calculation of these rate constants such that comparison between studies is possible.

There are several ways to greatly improve our understanding of Hg redox in natural waters as well as our ability to effectively compare studies. First, more data are needed in general, but information regarding oxidation in natural waters is especially lacking, thus more studies are required. Second, mechanistic experiments performed on artificial solutions would greatly help constrain and quantify the dominant processes which control Hg redox chemistry. For instance, more mechanistic experiments investigating the roles of $OH^{\bullet}$, $O_2^{\bullet -}$ and halides in Hg redox are necessary. Though it is difficult to assess if this information translates to the natural environment considering the complex interactions among the constituents of natural waters, it would be helpful to better constrain and to quantify the specific affect of these oxidants and reductants on Hg redox. Furthermore, the information gleaned from mechanistic studies can be applied to a model, such as the Aqueous Phase Kinetic Model devised by Dr. Siefert (1998), which can incorporate the kinetics of all those processes we currently understand, in order to assess how they interact. These results can then be compared to those of natural waters and potentially explain the important processes occurring in them.

Finally, in order to compare the results of these studies, a consistency in methodology is important. This includes the termination of the use of FEP Teflon®, the application of the reversible reaction method for the calculation of rate constants, and if possible, the addition of isotopically labeled inorganic Hg species.

To review, the most important themes exposed in a review of the results of this part of my research and of the available literature are these. First, more Hg speciation data are necessary in all types of waters. The lack of information, a consequence of the paucity of data, severely limits our ability to compare results and our understanding of

Hg redox in general. 2) Some consistency regarding methodology is needed, especially regarding the calculation of rate constants. 3) Finally, experiments like those conducted for this research provide important information that can be directly applied to the system being studied. However, more studies are necessary which investigate the specific mechanisms which control the redox of Hg. The number of variables introduced through the use of natural waters and ambient sunlight impede the constraint of mechanisms, thus more Hg redox mechanism studies in controlled environments are required.

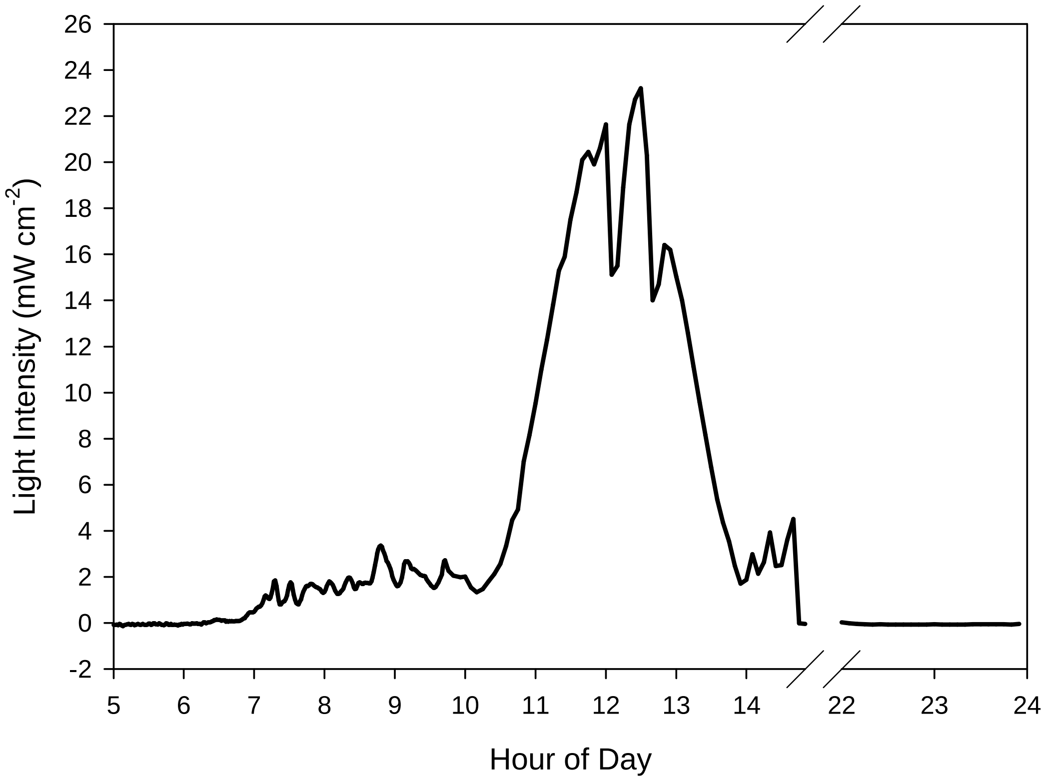


Fig. 3.1 Light intensity during the CBL diel experiment.

$$A \underset{k_2}{\overset{k_1}{\Leftrightarrow}} B$$

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B] \qquad [A]_1 + [B]_t = [A]_0 + [B]_0 = T \qquad \frac{d[A]}{dt} = -(k_1 + k_2)[A] + k_2 T$$

$$[A]t = [A]_0 \left(\frac{k_2 + \left(k_1 e^{-(k_1+k_2))t}\right)}{k_1 + k_2} \right) \qquad [A]_t = \frac{[A]_0 k_2}{k_1 + k_2} + \frac{[A]_0 k_1}{k_1 + k_2} e^{-(k_1+k_2)t}$$

$$\frac{[A]_0 k_2}{k_1 + k_2} = [A]_{eq} \qquad \frac{[A]_0 k_1}{k_1 + k_2} = [B]_{eq} = [A]_0 - [A]_{eq}$$

$$[A]_t = [A]_{eq} + \left([A]_0 - \{A]_{eq}\right) e^{-(k_1+k_2)t}$$

Fit data to the above equation where $[A]_t$, $[A]_0$ and t are known,

and $[A]_{eq}$ and the slope (k_1+k_2) are determined by the fit

at steady state

$$\frac{k_1}{k_2} = \frac{[B]_{eq}}{[A]_{eq}} = \frac{\left([A]_0 - [A]_{eq}\right)}{[A]_{eq}}$$

The individual rate constants are then determined from solving

the two equations with two unknowns

Fig. 3.2 Description of oxidation and reduction rate constant determination, reversible reaction method. The process defines reduction when A = ^{199}RHg and oxidation when A = ^{202}DGHg.

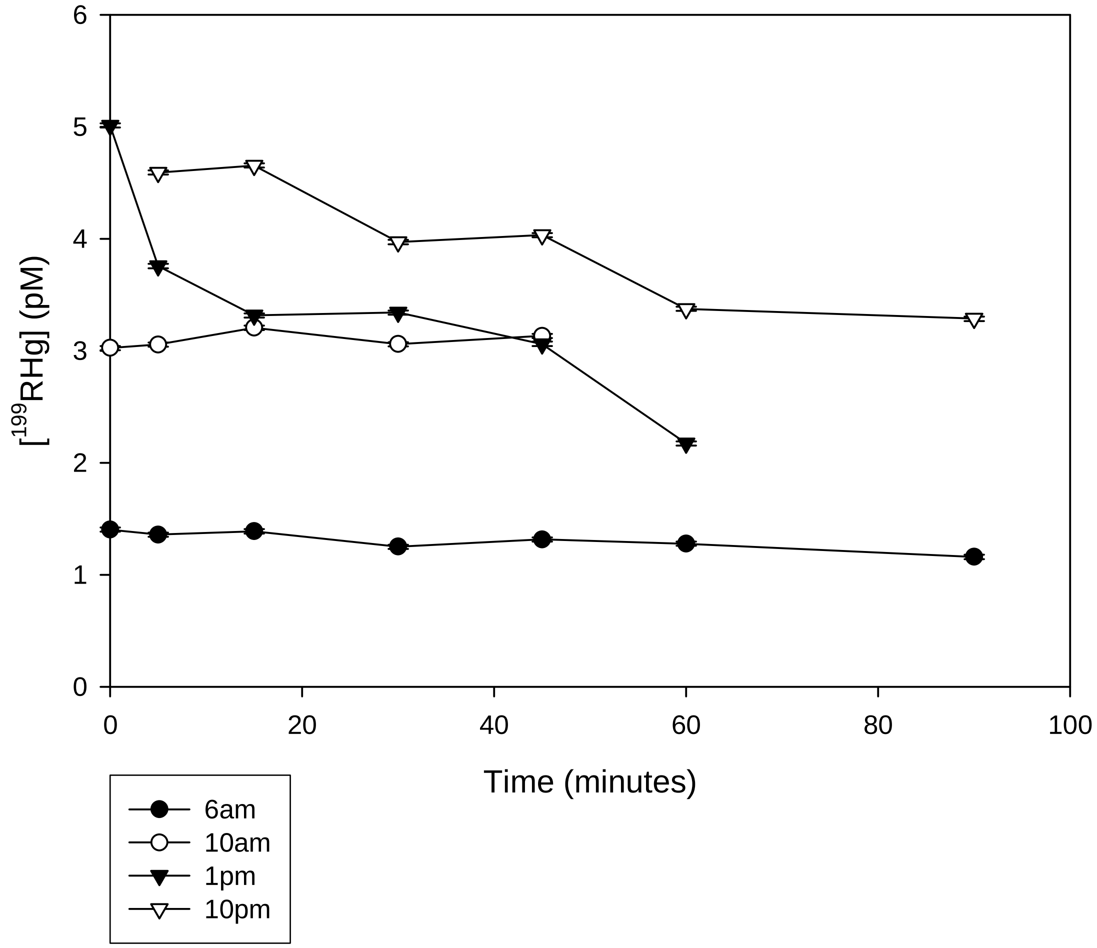


Fig. 3.3 Measurments of ^{199}RHg for the four short term kinetics experiments performed throughout one day at CBL.

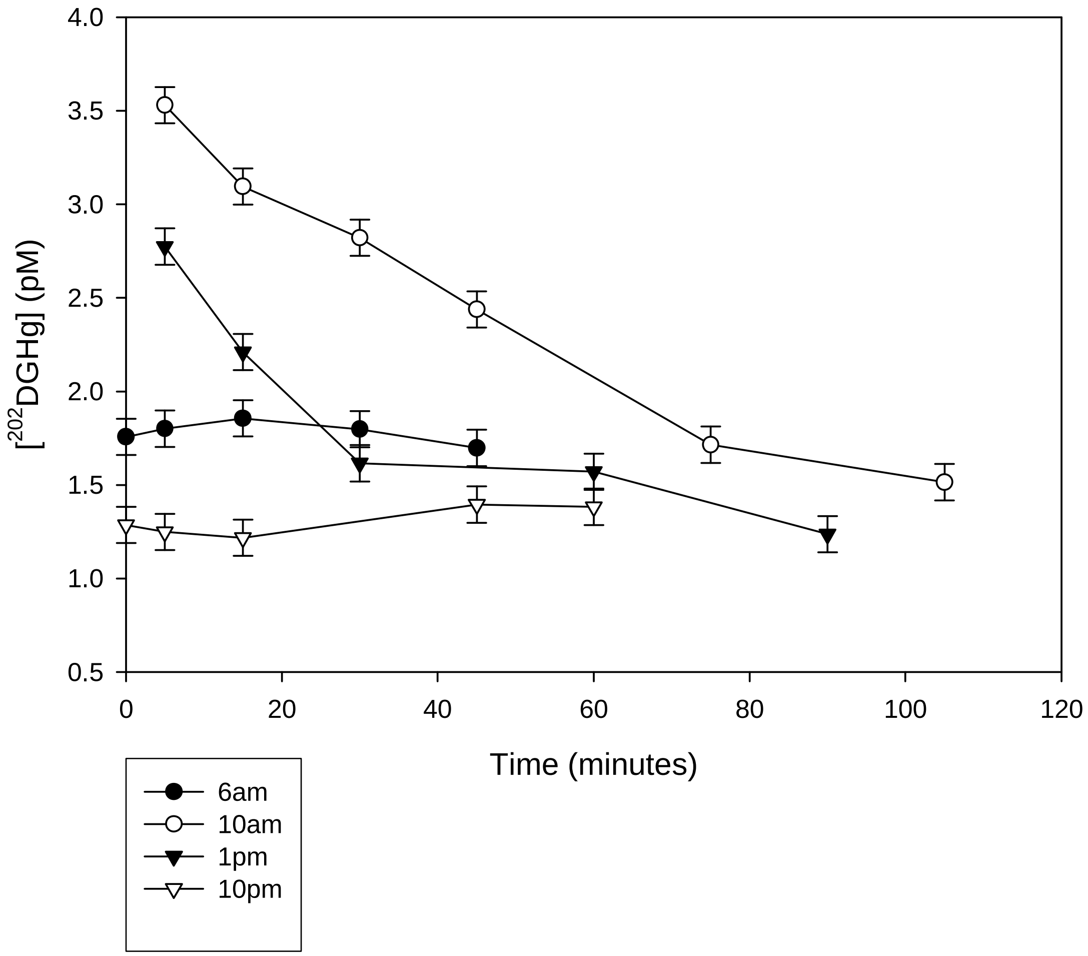


Fig. 3.4 Measurments of ^{202}DGHg for the four short term kinetics experiments performed throughout one day at CBL.

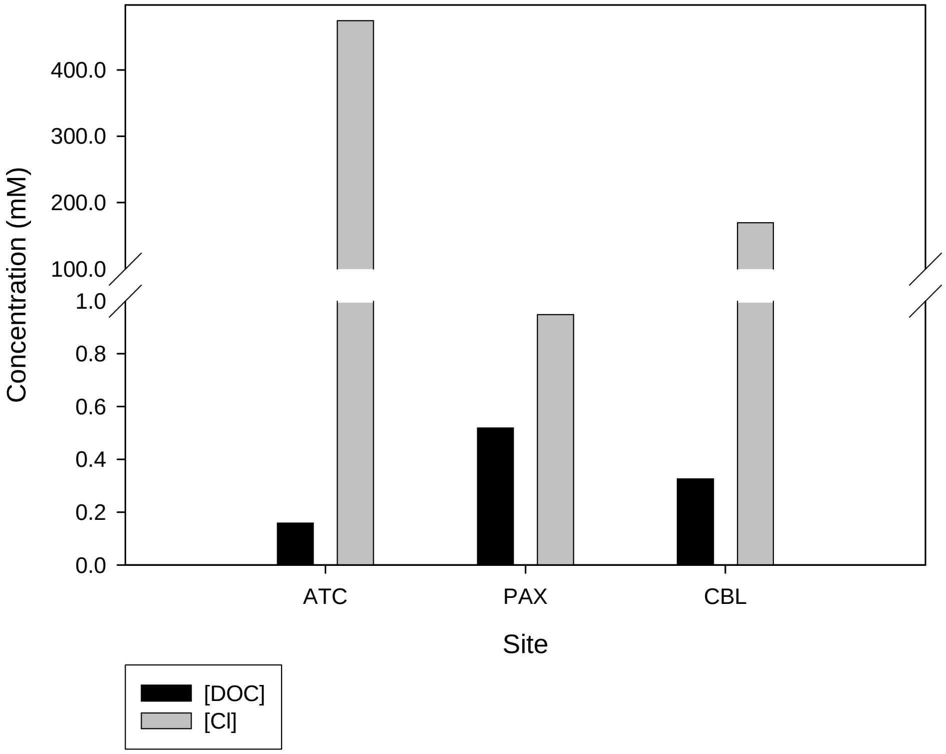


Fig. 3.5 Concentrations of DOC and chloride at the three sites.

First order rate equation for the oxidation of Hg

$$\frac{d[Hg^0]}{dt} = -k_a[Hg^0]$$

approximate rate constant for Hg oxidation in ATC, PAX and CBL experiments

$$k_a \sim 1x10^{-4}\ s^{-1}$$

Second order steady state rate equation for the oxidation of Hg by $OH^\bullet$

$$\frac{d[Hg^0]}{dt} = -k_b[Hg^0][OH^\bullet]_{ss}$$

approximate rate constant for Hg oxidation by $OH^\bullet$

$$k_b \sim 1x10^{-9}\ M^{-1}\ s^{-1}$$

substitute

$$k_a[Hg^0] = k_b[Hg^0][OH^\bullet]_{ss}$$

rearrange

$$\frac{k_a}{k_b} = [OH^\bullet]_{ss}$$

insert approximated values for k_a *and* k_b

$$\frac{1x10^{-4}s^{-1}}{1x10^{9}M^{-1}s^{-1}} = 1x10^{-13}M$$

Figure 3.6 Calculation of estimated steady state $OH^\bullet$ concentration necessary to explain Hg oxidation seen in ATC, PAX and CBL.

Rate Constants (s^{-1})									
Time		Oxidation				Reduction			
		202		**199**		**202**		**199**	
6 am		no fit		no fit		no fit		no fit	
	P		1.0000		0.8586		1.0000		0.8586
10 am		$2.1x10^{-4}$		no fit		$4.8x10^{-5}$		no fit	
SE	P	$4.0x10^{-5}$	0.0157		0.5975	$1.0x10^{-5}$	0.0157		0.5975
1 pm		$4.7x10^{-4}$		$9.7x10^{-4}$		$4.3x10^{-4}$		$8.7x10^{-4}$	
SE	P	$1.2x10^{-4}$	0.0303	$4.5x10^{-4}$	0.0605	$1.1x10^{-4}$	0.0303	$4.0x10^{-4}$	0.1198
10 pm		no fit		no fit		no fit		no fit	
	P		1.0000		0.9998		1.0000		0.9998

Table 3.1 Comparison of rate constants determined for the CBL experiment. No fit indicates that the data set did not fit the curve describe in figure 3.3. SE is the standard error and P is the P value for fit of the data.

Site	**Average Oxidation Rate Constant (s^{-1})**	**Average Reduction Rate Constant (s^{-1})**
ATC	$4.1\text{x}10^{-4}$	$6.5\text{x}10^{-4}$
SE	$8.9\text{x}10^{-5}$	$1.5\text{x}10^{-4}$
PAX	$1.2\text{x}10^{-3}$	$7.0\text{x}10^{-4}$
SE	$4.1\text{x}10^{-4}$	$2.1\text{x}10^{-4}$
CBL	$7.2\text{x}10^{-4}$	$6.5\text{x}10^{-4}$
SE	$2.9\text{x}10^{-4}$	$2.6\text{x}10^{-4}$

Table 3.2 Averaged rate constants at midday for Hg oxidation and reduction between the three sites ATC, PAX and CBL. SE is the averaged standard error.

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