AN ABSTRACT OF THE THESIS OF

Christopher E. Holm for the degree of Master of Science in Oceanography presented on December 8, 2006.
Title: Development of an Autonomous In-Situ Instrument for Long-Term Monitoring of Cu(II) in the Marine Environment.

Abstract approved:

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Zanna Chase

An autonomous, in-situ instrument was developed to detect dissolved copper in seawater, suitable for deployment on time scales from weeks to months. A commercially available in-situ nitrate analyzer (YSI 9600) was adapted to measure copper (II) in seawater by chemiluminescence. Modifications included construction of a photomultiplier (PMT) based detector and flow-cell, the use of more chemically resistant plastics for parts in contact with the reagents, addition of an in-line acidification step and optimization of the method and flow parameters. Filtration to 0.45μm and acidification online (pH ~1.7) produces a measurement of total dissolved Cu(II). Calibration is achieved by periodically analyzing ligand-stabilized seawater standard and blank solutions stored at pH 8 and acidified online. Micro solenoid pumps take in sample and dispense reagent, standard, and blank solutions, which are stored in 1L plastic bags. All waste is collected in two 5L bags. In-situ, the instrument has an average detection limit of 0.8(3) nM, a sample precision of 7%, and an accuracy, assessed over all deployments, of 17%. The instrument is capable of functioning autonomously for 25 days sampling every hour and calibrating every six hours, with reagent consumption being the limiting factor.

Development of an Autonomous In-Situ Instrument for Long-Term Monitoring of Cu(II) in the Marine Environment.

by
Christopher E. Holm

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APPROVED:

Major Professor, representing Oceanography

Dean of the College of Oceanic and Atmospheric Sciences

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Christopher E. Holm, Author

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figure Page

Chapter 1: Introduction

1.1 Management of Marine Pollution

Coastal ecosystems are some of the most productive and yet stressed areas of the ocean. In 2003, the value added to the gross domestic product (GDP) from commercial and recreational fishing alone was 43.5 billion dollars (NOAA, 2005). Unfortunately, population growth and increased development inevitably leads to increased pressure on the coastal environment. Marine pollution is one of these inevitable pressures. For example, approximately 44% of tested U.S. estuaries and 12% of the nation's shorelines are considered unfit for swimming, fishing, or supporting aquatic life, and there were over 16,000 beach closings and swimming advisories in 2001 alone all due to pollution (NOAA, 2005).

Mitigating these pressures is the task of every person charged with managing the coastal environment, and accurate information is the essential currency of effective environmental management. All too often however, coastal managers are asked to operate in spite of potential unknowns and a lack of accurate information. Collecting complete and accurate information is a daunting task given the natural complexity of environmental systems, and the general lack of funding for widespread environmental monitoring and assessment programs. One of the primary advantages of in-situ instrumentation is the ability to collect long term high frequency data sets with significantly lower cost than traditional sampling methods. This ability to collect reliable long term data sets is vital to gathering the information necessary to effectively manage the rivers, estuaries, and coastal waters which comprise the foundation of our coastal economies.

1.2 Toxic Trace Metals

The release of toxic trace metals (TTM), such as copper, into estuaries and coastal oceans is a growing problem worldwide (Newman and Unger, 2003). Over the past century, the dominant paradigm controlling the perception of marine pollution has shifted several times. In the 1960s and '70s, the discovery of methyl mercury poisoning from seafood in Japan (Minamata disease) brought attention to the dangers of assuming "dilution is the solution to pollution." This discovery of bioaccumulation quickly shifted the dominant environmental school of thought into the "boomerang" paradigm, and highlighted the importance of quantifying the variability, concentration, and bioavailability of TTM in the coastal oceans. To date, significant effort has been devoted to quantifying the current levels of copper and other toxic trace metals, identifying their major sources and sinks, and documenting their effect on marine organisms. Unfortunately, the cost of trace metal analysis has limited the current work to discrete sampling, and the use of bio-indicators in an attempt to understand the complex and dynamic cycles which ultimately determine the fate of metals in the coastal ocean. This approach is limited in its temporal and/or spatial coverage, which has hindered the understanding of trace metal cycling in the marine environment.

Copper (Cu) is a longstanding pollutant of concern in the coastal zone. Dissolved copper is on the EPA's primary pollutant list for drinking water and is an environmental concern in many coastal systems around the world. Copper acts both as an essential micronutrient and a toxicant within the range of concentrations

measured in the present day ocean (Coale, 1991; Donat and Bruland, 1995; Sunda and Huntsman, 1998).

While copper has many natural sources, anthropogenic sources have now exceeded background levels by several orders of magnitude and are the leading cause of trace metal mobilization into the biosphere (Nriagu and Pacyna, 1988). Copper typically enters coastal waters through industrial and domestic waste, atmospheric deposition, storm-water runoff, and through its use in anti-fouling paints. Increasing evidence suggests that many coastal ecosystems have elevated concentrations of dissolved metals when compared to unaltered regions (Sañudo-Wilhelmy and Flegal, 1991).

Within most open ocean and coastal surface waters, complexation results in free Cu^{2+} concentrations that are well below those thought to be toxic to even the most sensitive marine organisms (10^{-13}-10^{-12} M). However, total dissolved copper concentrations in polluted estuaries and harbors such as the Elizabeth River (Sunda et al., 1990), Chesapeake Bay (Donat, 1994) and San Francisco Bay (Donat et al., 1994) have been shown to exceed the concentrations of these strong complexing ligands and the resulting concentration of free Cu^{2+} (10^{-11}-$10^{-9.7}$ M) has been shown to be toxic to some more sensitive phytoplankton species (Brand et al., 1986; Sunda, et al., 1990; Moffett et al., 1997).

Consequently, copper concentrations have reached dangerous levels in some coastal ecosystems and have degraded the health of those areas (Moffett et al., 1997). The accumulation of copper has been documented in commercially harvested species, and in some areas, is endangering human health (Chou et al.,

2000). Therefore, it is critical that we understand how these trace metals interact within the marine environment, and it is clear that any effective long term environmental monitoring program should include copper among its list of priority pollutants.

1.3 Advantages of In-Situ Sampling

One of the greatest challenges in understanding and regulating the levels of toxic trace metals in the coastal ocean is quantifying non-point source pollution. While non-point source pollution may contribute up to half of coastal metals pollution in the U.S., the difficulty in identifying and quantifying such a diffuse and variable source has translated into inadequate data collection and consequently inadequate regulation (Kennish, 1998). In part, this lack of scientific data is due to the expense of trace metal analysis and the difficulty of obtaining and analyzing samples without introducing contamination (Benoit et al., 1997).

The methods traditionally available to environmental managers to implement copper monitoring programs in the coastal zone can be generalized into two broad categories: the direct analysis of copper through collection of discrete samples, and the collection of time averaged proxies for the biological availability of copper using synthetic films and marine organisms such as mussels (Mussel Watch).

The collection of discrete samples for direct analysis of copper has advantages in that it yields an accurate direct measure of copper concentration at a particular location and time. This method is time consuming and expensive however, as collection of trace metal clean samples and analysis of copper in

seawater usually involves significant infrastructure and instrumentation. This high cost severely inhibits the practical use of this method for long term monitoring of copper.

The implementation of biological monitoring programs (Mussel Watch) is one way in which environmental managers have tried to work around this cost limitation. Mussel watch programs are attractive for long term monitoring of pollutants like copper in that a time averaged assessment of the biologically available fraction of the pollutant can be made by collecting existing organisms and analyzing their tissues.

Organisms tend to bioconcentrate copper allowing for a less sensitive analytical method to be utilized (lower cost) and a decreased chance of sample contamination. Bioindicators have been successfully utilized for long term monitoring in Taiwan (Jeng et al., 2000) and in the Bay of Fundy, Canada (Chou, 2003). These programs are complicated by species selection (Chou, 2003) and a number of environmental factors. For example, long term temporal trends can be obscured by variables other than the dissolved copper concentration including: mussel physiology, water temperature, salinity, and dissolved organic carbon content (DOC) (O'Connor, 2005). An inexpensive direct measurement of copper concentrations is therefore still ideal for effective management of this pollutant in aquatic systems.

By enabling long-term, high-frequency, in-situ datasets, autonomous instrumentation is capable of providing this relatively inexpensive monitoring tool. The development of an autonomous in-situ analyzer for copper in the marine

environment will address the needs of these programs as they relate to copper specifically. The instrument developed here is capable of autonomous hourly monitoring of copper for periods of up to a month (25 days). The compact size allows for single person deployments of this instrument in a wide range of environments.

For example, this instrument could be deployed from a river bank, off of a dock or other structure in an estuarine system, or off a mooring in the coastal ocean. With monthly maintenance the instrument could provide long term data sets which allow for an accurate assessment of trends. Moreover, a network of instruments could be deployed to accurately pinpoint the dominant inputs of copper into an estuary, river, or coastal ocean, and to identify non-compliant point sources, or diffuse non-point source inputs. Ideally, the instrument will be made commercially available through YSI, and the accompanying documentation will allow a technician with limited experience to quickly become proficient at maintaining and deploying this analyzer. This will allow environmental managers nationally and internationally to begin implementing long term copper monitoring and input assessment programs for significantly less cost than the options currently available.

1.4 Current In-Situ Methods

Several attempts have been made to automate trace metal analysis in-situ. For example, the osmosampler (Jannasch et al., 1998, 2004) and the diffusion gradient in thin film hydrogel technique (Denney et al., 1999; Twiss and Moffet, 2002) have been used to automate the collection of samples, whereas molecular biosensors (Zeng et al., 2003), electrochemical sensors (Wang et al., 1995; Tercier

et al., 1998; Howell et al., 2003), and flow-through chemical analyzers with optical detection (Chapin et al., 2002, Callahan et al., 2004) have been used for in-situ trace metal detection. Molecular biosensors and electrochemical sensors offer promising new approaches to trace metal detection, but they have not been shown to function for more than a few days in the field. In addition, many of these sensors suffer from a broad range of interferences, which limits their use in a complex matrix like seawater. For these reasons, submersible flow-through chemical analyzers with optical detection have proven to be the most reliable and effective systems for long-term autonomous in-situ trace metal monitoring.

1.5 In-Situ Chemical Analyzers

In-situ chemical analyzers have been developed and successfully deployed for periods of up to a year for macronutrients such as nitrate and nitrite (Chapin et al., 2004; Daniel et al., 1995), and trace metals such as iron, zinc, and copper (Fe: Chapin et al., 2002; Laës et al., 2005; Zn: Chapin and Wanty, 2005; Cu: Callahan et al., 2004). These analyzers generally use continuous flow analysis (CFA) or flow injection analysis (FIA) coupled to a simple optical detector. While this platform has proven its versatility in the realm of long-term in-situ chemical monitoring only one such system currently exists to analyze copper (Callahan et al., 2004). This system is promising for analysis in areas with higher copper concentrations and redox active environments like hydrothermal vents and anoxic sediments where the measurement of both Cu(II) and Cu(I) would be potentially valuable. However, the method lacks the sensitivity (Detection Limit (DL)= 3.0nM, Callahan et al., 2004) to be useful in many coastal and open ocean systems (Windom, et al. 1991; Town

and Filella, 2000). Furthermore, published trials of the Callahan et al. (2004) SEAS instrument lasted only one day, and it is not clear that the instrument is suitable for extended (weeks to months) autonomous deployment.

1.6 The DigiSCAN (YSI 9600) Nitrate Analyzer

The DigiSCAN in-situ analyzer was developed at the Monterey Bay Aquarium Research Institute (MBARI) for detection of nitrate and phosphate. Designed for long-term deployments using micro-solenoid pumps (Weeks and Johnson, 1996), which have more flexibility than osmotically powered pumps (Jannach et al., 1994) and a low power requirement, DigiSCAN offers significant potential as a platform for autonomous, in-situ, toxic trace metal detection. The environmental instrument company, YSI, has commercialized the DigiSCAN and is currently selling the instrument as a nitrate monitor. The YSI 9600 nitrate monitor is essentially a miniaturized, submersible version of classic wet chemical bench-top methods. Reagents are housed in plastic bags, propelled through a manifold using solenoid pumps, and mixed to produce color that is detected by a miniature colorimetric detector. In the work described here, we adapted the YSI 9600 platform for autonomous in-situ chemical analysis of total (pH<1.7) dissolved (<0.45μm) copper by chemiluminescence detection. In contrast to the bathocuproine disulfonate (BDS) absorbance method employed by Callahan et al. (2004), we employ a chemiluminescence method for Cu(II) in seawater, using 1,10 phenanthroline as the luminescent complex, which is capable of reliably measuring concentrations below 1.0nM (Coale and Johnson, 1992; Zamzow et al., 1998). The instrument has been successfully deployed for up to two weeks.

Chapter 2: Material and Methods

2.1 Trace Metal Analysis and Contamination

Copper, like other trace metals, is present in exceedingly low concentrations in the oceans. For example, the average oceanic concentration is ~ 2nM, which represents taking a copper penny and dissolving it in the equivalent of ~49 Olympic swimming pools. Consequently, making an accurate measurement of these low concentrations is an analytical challenge, and sample contamination becomes a serious problem. Contamination can come from skin, dust, tap water, sample bottles, filters, and many other sources. Therefore, it is critical that steps are taken at each stage of the analysis to prevent unnecessary contamination. These steps include rigorous cleaning of any laboratory materials that come in contact with the sample, and preventing inadvertent exposures of a sample to contaminated gear or surfaces during sample collection and processing (Benoit et al., 1997). In the work described here, every attempt was made to limit sample contamination, and current trace metal sample handling procedures were followed throughout (Benoit et al., 1997).

2.2 Cleaning Procedures

Sample and reagent bottles were made of low density polyethylene. Reagent preparation, standard preparation and sample manipulations were carried out in a class 100 laminar flow hood. All new and used bottles, syringes, and 1L YSI Flexboy bags were cleaned according to the following method. First, bottles were soaked for at least 24 hours in a 5 gallon bucket (HDPE) containing a 1% solution of Micro-90. The same solution of Micro-90 was re-used multiple times

and was changed out after approximately 200 bottles. Next, bottles were rinsed with tap water multiple (8-10) times and filled (no headspace) with 3N trace metal grade HCl (J.T. Baker). Filled bottles were stacked in another 5 gallon bucket, and allowed to sit for at least seven days. Then the bottles were emptied back into the acid carboy, rinsed with Milli-Q H_2O 3 to 5 times and filled with 2N trace metal grade HNO_3 (J.T. Baker) leaving no headspace. Acid was re-used multiple times and replaced after approximately 200 bottles. Again bottles were stored in a 5 gallon bucket for at least 7 days. Finally, bottles were emptied back into the filling carboy and rinsed multiple (5 to 10) times with Mill-Q H_2O. Bottles designated for sampling were filled with 0.1% Ultrex® II HNO_3 (J.T. Baker), individually wrapped in a polyethylene glove, and stored in groups of eight in two large Ziploc bags. Reagent and standard bottles were dried in a class-100 laminar flow hood and stored with the caps on in two large Ziploc Bags.

Pall Acropak 0.2μm poly-ester-sulphone membrane filters were cleaned using a MasterFlex Peristaltic pump. Up to five capsule filters were placed in line inside a class-100 laminar flow hood and Milli-Q H_2O was flushed through each filter making sure to open the upper vent valve on each filter to allow all of the air to escape before closing the vent. Care was taken in each of the subsequent steps to limit the introduction of air into the system. Filters were then filled with 10% methanol (Fisher, optima grade) and allowed to soak for 24 hours. Milli-Q was then pumped through and allowed to sit for 24 hours, followed by 1N HCl (J.T. Baker, trace metal grade) for 3 days, Milli-Q H_2O for 24 hours, 1N HNO_3 (J.T. Baker, trace metal grade) for 3 days, Milli-Q H_2O for 24 hours, and finally 0.1N

Ultrex® II HCl (J.T. Baker). Filters were unhooked and the inlet and outlet of the filter was connected with a piece of tubing to keep the 0.1N Ultrex® II HCl contained and were stored in two Ziploc bags.

Smaller 0.45, 5 and, 10 μm syringe filters were cleaned in a similar manner as the acropack filters, except that a Rainin peristaltic pump was used with 0.056mm tubing and Upchurch syring fittings. Approximately four to five filters can be connected and cleaned in a series before backpressure becomes an issue. Finally, syringe filters were stored after rinsing with water rather than 0.1N Ultrex® II HCl.

Pipettes were cleaned immediately prior to use with three rinse bottles. Bottles 1 and 2 contain 6N Ultrex® II HCl, and bottle 3 contains Milli-Q H_2O. Three full volumes of bottle 1, 2 and 3 were drawn and dispensed as waste before moving on to the next bottle. Then 1 to 3 complete volumes of the intended solution were thrown out as waste before collecting the final volume.

2.3 Reagent Preparation and Sources

The first reagent solution contains 10% (v/v) hydrogen peroxide and is made by diluting 30% H_2O_2 (Stabilized A.C.S Reagent grade, VWR) with Milli-Q® purified H_2O. The second reagent solution (R-2) contains 60mM 1,10 phenanthroline, 0.02 M Triethylenetetramine (TEPA), 0.075M NaOH, and 0.02 M Ethylhexadecyldimethyl-ammonium bromide (CDAB) in Milli-Q® purified H_2O. This reagent is made by adding 8g of CDAB (Reagent Grade, Sigma), and 3g of NaOH (A.C.S. Reagent grade, J.T. Baker) to 1L of Milli-Q® purified H_2O, and allowing for complete dissolution. Then 100μL of a 4mM stock solution of TEPA

(97%, Fluka), and 5mL of a 12mM stock solution of 1,10 Phenanthroline (99%, Aldrich) is added. A solution of 0.036N HCl is used to acidify the incoming sample stream and contains 6mL of a 6N stock solution of Ultrex® II HCl (J.T. Baker) in 1L of Milli-Q® purified H_2O.

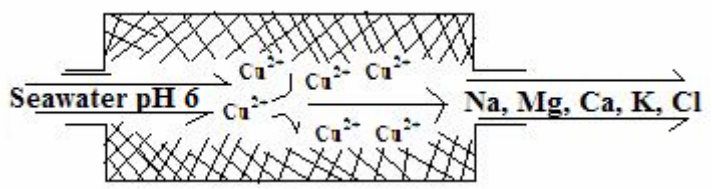
A carrier solution (pH 3 Milli-Q H_2O) is used in the bench top FIA method. This solution is made by filling a 1L HDPE bottle up to the shoulder and then adding 250µL of 6N Optima HCl.

2.4 Creation of Chelated Standards and Blanks at pH 1.7 and 8

All standard and blank solutions are made from "copper free" seawater. Coastal seawater is filtered using a Pall Acropak 0.2µm poly-ester-sulphone membrane filter. The filtered water is brought to pH ~6 with ~300µL/L of 6N Ultrex® II HCl and passed through a 5mL iminodiacetic acid column (HiTrap Chelating HP, Amersham Biosciences) at a flow rate of < 5mL/min. The iminodiacetic acid is covalently bonded, via a hydrophilic eight to twelve carbon spacer arm, to a highly cross-linked agarose matrix. This matrix efficiently binds to Cu^{2+} ions at pH 6 (Figure 2.1). The column is prepared by first washing it with 50mL of 0.1N HCl, then 100mL of Milli-Q H_2O, and finally with 50mL of seawater before collection. After collection of the effluent, the copper-free seawater is returned to pH 8 with ~80-100µL/L of 6N NaOH (A.C.S. Reagent grade, J.T. Baker), 50µL/L of 4mM TEPA (200nM) is added and the seawater is used to make standard and blank solutions. A stable chelating agent, TEPA, is added at no less than a 2x excess relative to the copper present in order to maintain standard stability and to prevent loss of copper to the walls of the flexible (LDPE) reagent bags

(log(k)=23.1 vs. 18.7 for EDTA, Smith and Martell, 1975). Acidified (pH ~1.7) standards are made up in pH 6 seawater and then acidified by adding 3.7mL/ L of 6N Ultrex® II HCl.

Figure 2.1: Iminodiacetic acid column used to separate copper ions from seawater prior to preparation of standard and blank solutions. As pH 6 seawater is pumped through the column pictured below, copper ions are bound by the iminodiacetic acid matrix, while major ions pass through unaltered. The column's efficiency decreases at ambient (pH 8) hydrogen ion activities, most likely due to competition with in-situ organic ligands and a lower binding efficiency of the iminodiacetic acid matrix itself. At higher hydrogen ion activities (< pH 3) copper ions are stripped from the column matrix and eluted in the effluent stream.

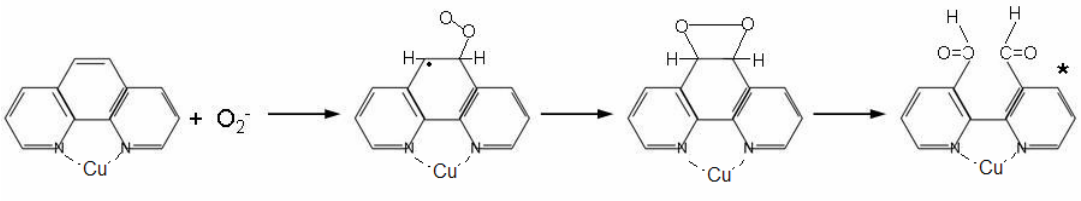


Generally six pH 8 standards, at 0.5, 1.0, 5, 10, 20, and 40nM, were used to ensure linearity pre-deployment, and then one standard was run throughout the deployment along with a blank solution. The concentration of the on-board standard was chosen such that it would be as close as possible to the expected sample concentration, while still bracketing with the blank the entire dynamic range of the samples. Sample concentrations were calculated using the slope of the closest standard and blank reading after a correction factor was applied to reflect the difference between blank, standard, and sample pump volumes. Acidified standards (pH 1.7) were used with a bench top flow injection system for the analysis of acidified discrete samples. All standard solutions were prepared gravimetrically using two primary standards at 12μM and 400nM made from a commercial (1000ppm ± 1%) reference standard (Fisher).

2.5 Analytical Method

A chemiluminescence method for flow-through analysis of Cu(II) in seawater using 1,10-phenanthroline (Coale et al., 1992) was adapted for use in-situ. Chemiluminescence is produced by oxidative destruction of 1,10-phenanthroline during the catalytic decomposition of hydrogen peroxide by the copper-1,10-phenanthroline complex (figure 2.2), which occurs in a basic pH ~9.5 medium (Yamada and Suzuki, 1984). The sensitivity of this reaction is enhanced by the presence of surfactant micelles in solution from the addition of cetylethyldiethylammonium bromide (CEDAB) (Yamada and Suzuki, 1984). In addition, a low baseline measurement is achieved by adding tetraethylenepentamine (TEPA), a stable complexing agent for copper(II), directly to the reagents in order to remove the background signal attributed to copper impurities in the reagents themselves (Yamada and Suzuki, 1984). Although this method originally used an 8-hydroxyquinoline column to pre-concentrate the sample, modifications by Zamzow et al. (1998) improved the sensitivity sufficiently to measure coastal ocean concentrations without the need for pre-concentration.

Figure 2.2: Proposed mechanism of 1-10 phenanthroline-copper chemiluminescence reaction (Xiao et al., 2002).



The chemiluminescence reaction is generally considered to be sensitive only to the labile or "free" Cu(II) ion as evidenced by comparison with ASV and CSV methods (Sunda and Huntsman, 1991). Therefore, in order to make a measurement of "total" copper consistent with the operational definition used by other researchers (Sunda and Huntsman, 1991; Coale and Johnson, 1992; Morel and Hering, 1993), the sample stream is acidified in line to a pH of ~1.7. Blank and standard solutions kept at pH 8 and stabilized with 200nM TEPA are also acidified in line in order to keep sample, blank, and standard treatment as consistent as possible. Finally, filtration through a 10μm pre-filter (UHMWPE solvent Filter, Upchurch Scientific) and a 0.45 μm polyethersulfone syringe filter (25mm, VWR) allows for a measurement of total dissolved (<0.45μm) copper.

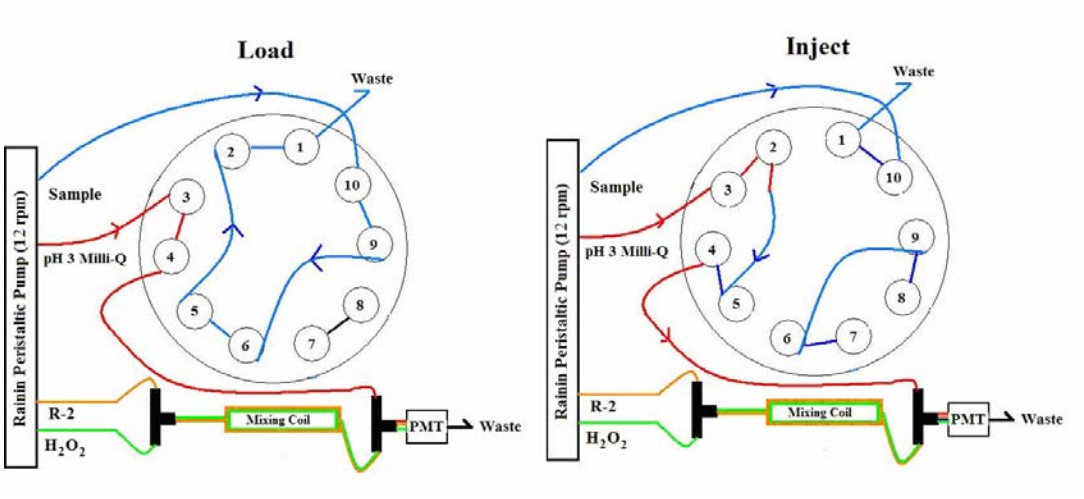
2.6 Desk Top FIA Set-up and Operation

A bench-top flow-injection analysis (FIA) version of the Zamzow et al. (1998) method was used for method development and for running discrete samples. The average limit of detection (3σ of the blank) from all of the discrete sample calibrations was 0.25±0.07nM. The method was generally linear to ~40nM. A certified reference sample (NASS-5, NRC) was run during all of the discrete sample analysis runs and was within the analytical error of the certified value (Avg. value 4.8±0.3nM, certified 4.7±0.7nM). The precision of replicate analyses was generally better than 5% relative standard deviation.

The bench top copper flow injection chemiluminescence system consists of a Rainin peristaltic pump, a two way selection valve, and a photomultiplier tube (PMT) mounted in a plastic flow cell. The Rainin peristaltic pump has four tubing

mounts which supply sample, carrier fluid (pH 3 Milli-Q), R-2, and 10% hydrogen peroxide. Pump tubing (Fisher, Standard) with an I.D. of 0.056mm is used on all four lines which maintains equal flow rates between sample, carrier and reagents. The pump is normally run at 12 rpm, giving a flow rate of 2.11 mL per minute with 0.056mm tubing. Sample, carrier, and reagents are stored in a class 100 laminar flow hood with holes drilled in the side to allow tubing to be routed outside to the peristaltic pump, selection valve, and PMT. Reagents are combined using a T-junction and run through a 2m and 1m mixing coil in series (Global Fia). A second T-junction combines the reagent and sample/carrier flow streams which then flow directly into the PMT (Figure 2.3). The 1-10 phenanthroline-copper chemiluminescence reaction is extremely fast, therefore the length of tubing between the T-junction and the PMT is kept at a minimum. An injection valve is used to inject a constant volume of sample into a carrier stream. The valve has two positions, a "load" position which rinses and fills two sample loops, and an "inject" position which allows carrier to push the slug of sample out of the injection loop and into the reagent stream (Figure 2.3). The photomultiplier is mounted over a section of coiled 0.056mm tubing.

Figure 2.3: Layout of bench top copper FIA system. In the "load" position sample is pumped into an injection loop while carrier (pH 3 Milli-Q) is sent to mix with the reagents and on to the PMT. Once the valve is switched into the "inject" position, carrier solution clears the sample loop and mixes sample with the reagents where the light produced by the chemiluminescence reaction is measured by the PMT.

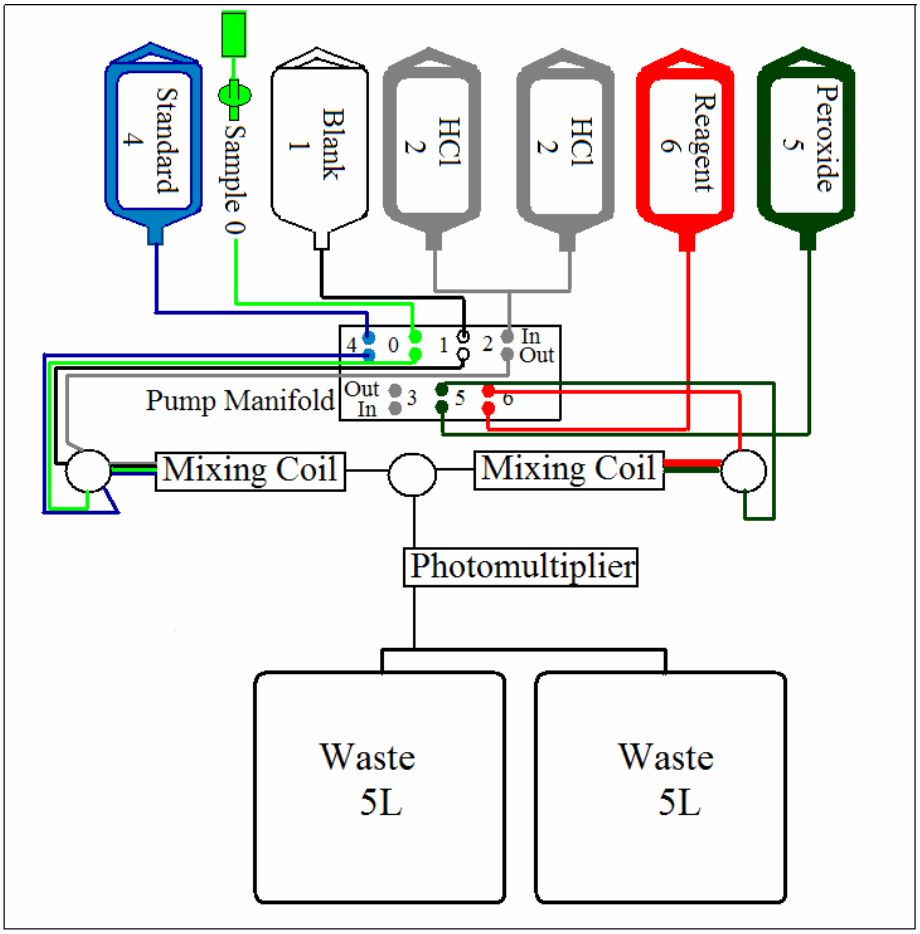


2.7 Instrument Layout and Operation

We used the commercially-available, YSI 9600 nitrate monitor as the base platform for our chemiluminescence based copper analyzer. Major modifications to the instrument included replacing the colorimetric detector with a photomultiplier tube (PMT, Hamamatsu, H7360-02) and custom flow-cell, and constructing a more chemically-resistant manifold. A further description of these modifications can be found below. The YSI 9600 instrument package consists of a watertight housing containing the controller, data logger, and battery which is attached to an oil-filled pressure compensated housing containing the micro-solenoid driven pumps (Lee Company) and flow manifold. The housing is secured on an inner frame with reagent and waste bag supports, and PMT. The entire package is protected by a water permeable outer case which measures 0.32 x 0.7m. The copper modified

instrument has a simple polyvinylchloride pump manifold with ¼-28" threaded inlet and outlet ports for each of the 7 micro-solenoid pumps (Figure 2.4). Headless nuts (Upchurch) are used to attach 1.6mm OD x 0.8mm ID Teflon tubing (Omnifit) to each of the inlet and outlet ports of the pump manifold. Each of the reagent, blank, and standard inlet ports are attached via Teflon tubing to 1L Flexboy® (LDPE, Stedim) reagent bags (Figure 2.4). The sample inlet port is attached to a 10µm solvent filter mounted on the top of the instrument, and a 0.45µm filter was added in-line. The outlet ports of the reagent pumps are routed via Teflon tubing to a T-junction (Upchurch) where the two reagents are combined and flow into a 0.5m knitted mixing coil (Global Fia). Similarly the blank, standard, sample, and acid pumps are routed to a 5 port junction (Upchurch), where blank, standard, or sample solutions are mixed with 0.036N HCl and then continue into a 38cm piece of Teflon tubing with 4 knit coils in the center. The mixture is then sent into another T-junction where it mixes with the reagents (Figure 2.4) and then continues on to the PMT. The 1,10-phenanthroline chemiluminescence reaction is extremely fast so the amount of tubing between the T-junction combining the reagents and sample, and the PMT interface, is minimized in order to optimize the signal. The effluent stream from the PMT is collected and stored in two 5L Flexboy® waste bags. Two 1L bags of acid are normally attached via a T-junction to the same pump, in order to extend the instrument's run duration. One of the instrument's 7 pumps is normally not used for long term deployments. The seventh pump can be used to add another standard solution in order to increase the accuracy of the calibration at the expense of a shortened deployment lifetime.

Figure 2.4: The layout of the copper-modified YSI 9600 instrument. Six 1 L reagent bags and a sample inlet are connected to seven micro-solenoid pumps which propel the reaction mixture into a photomultiplier. The YSI polycarbonate pump manifold was replaced by a modified, more chemically resistant version constructed of PVC.



2.8 YSI 9600 Alteration

A water tight photomultiplier tube (PMT) based detector and flow cell was designed and built at the Monterey Bay Aquarium Research Institute (MBARI). In addition, modifications to the circuitry of the YSI 9600 necessary for communication with the PMT based detector were also completed at MBARI. Finally, a more chemically resistant end-cap and pump manifold made from PVC were designed and built at MBARI.

2.9 Instrument Programming

Microsoft Hyper-Terminal was used to communicate with the instrument through a RS-232 serial connection on a laptop computer. The instrument was re-programmed using the existing programming language written by YSI. Command files generally consist of a main "schedule" consisting of several "batch" files. Each batch file is composed of specific instrument commands. Two schedules are used throughout each deployment, a sampling schedule, and a calibration schedule, and are run on a user defined interval. The sample (table 2.1) and calibration schedules generally follow the same basic protocol with minor modifications. Each schedule begins by flushing the mixed reagents from earlier measurements out of the reagent mixing coil. Then sample, blank, or standard is flushed through and mixed with acid. The flow is stopped, the instrument is placed into a low power sleep mode, and the acidified mixture is allowed to sit for 90 seconds. The sample, acid, H_2O_2 and R-2 pumps are then activated and the acidified sample, blank, or standard is then mixed with the reagents and sent to the PMT. The PMT is powered on, and a reading is taken as the middle portion of the reagent and sample segment reaches the PMT interface. The process is then repeated twice more beginning with the sample, blank, or standard acidification step in order to get three replicate measurements. In the case of the calibration schedule, blank and standard solutions are analyzed in triplicate beginning with the blank. Finally, the system is "cleaned" using either blank (sample schedule) or acid solution (calibration schedule) in preparation for the next measurement, and the instrument is placed back into a low power sleep mode. This cleaning step is designed to flush the reagents through to

the waste and reduce chemical corrosion along the flow path. Blank and acid solutions were used interchangeably in order to increase the deployment lifetime by balancing the blank and acid solution requirements. An additional filter clearing step is added to the beginning of the sample schedule, just after the reagent flush, in order to ensure a fresh sample is being analyzed.

Table 2.1: The sampling sequence used during the Moss Landing and Elkhorn Slough deployments. A longer, less efficient sequence was used during the Yaquina Bay (OR) deployment. The same sequence was used for sample, standard, and blank analyses with minor modifications. During the calibration sequence, steps 1-14 (without step 2)[1] are carried out with the blank and then followed immediately by steps 3-15 with the standard.

Step	Event	Pump #	Solution Used	Time (sec)	# of Pulses	Volume Pumped (mL)
1	Reagent Flush	5,6	H_2O_2, R-2	22.5	15	0.75
2	Filter Flush[1]	0	Sample[2]	210	120	6
3	Acidification	0,2	Sample, Acid	35	20	1
4	Acidification Delay			90		
5	Sample Displaced	0,2,5,6	Sample, Acid, H_2O_2, R-2	3.5	2	0.1
6	Replicate 1	0,2,5,6	Sample, Acid, H_2O_2, R-2	1.75	1	0.05
7	Acidification	0,2	Sample, Acid	3.5	2	0.1
8	Acidification Delay			90		
9	Sample Displaced	0,2,5,6	Sample, Acid, H_2O_2, R-2	3.5	2	0.1
10	Replicate 2	0,2,5,6	Sample, Acid, H_2O_2, R-2	1.75	1	0.05
11	Acidification	0,2	Sample, Acid	3.5	2	0.1
12	Acidification Delay			90		
13	Sample Displaced	0,2,5,6	Sample, Acid, H_2O_2, R-2	3.5	2	0.1
14	Replicate 3	0,2,5,6	Sample, Acid, H_2O_2, R-2	1.75	1	0.05
15	Cleaning	1	Blank[3]	35	20	1

1) Filter Flushing step is not present in calibration sequence
2) Here and elsewhere the sample pump is replaced by standard or blank in the calibration sequence.
3) Final cleaning step is accomplished with Acid in the calibration sequence.

Chapter 3: Results and Discussion

3.1 Introduction

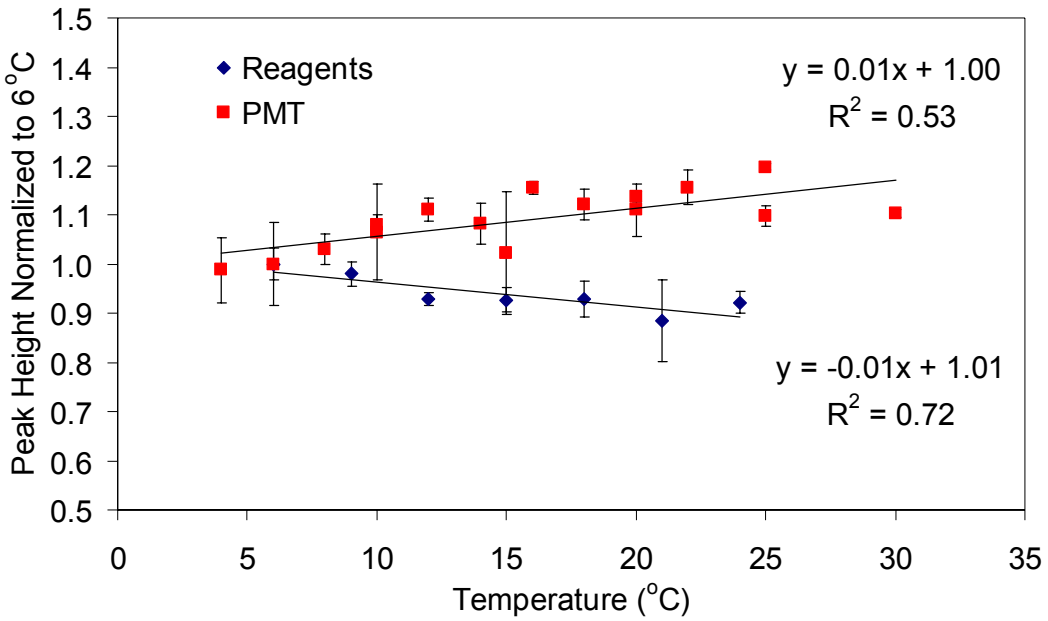
Laboratory and field studies were undertaken to test the performance of the in-situ copper analyzer in accordance with the goals of the project, mainly to precisely (<10% RSD) and accurately (<10%) measure total dissolved copper in estuarine and coastal ocean environments. In addition to initial development and testing under laboratory conditions, the instrument was deployed in-situ on seven separate occasions. The instrument was deployed in a freshwater testing tank from June 30th to July 3 2005, in Yaquina Bay (OR) from July 25th to Aug. 1st 2005, in the Gulf of Aqaba, Eliat Israel from Sept. 5th to the 8th 2005, in Yaquina Bay (OR) from Jan. 27th to Feb. 8th 2006, in the Newport Marina (OR) on July 20th 2006, in Moss Landing Harbor (CA) from Aug. 2nd to the 10th 2006, and finally in Elkhorn Slough (CA) from Aug. 14th to the 17th 2006. Initial deployments were vital to making further improvements to the instruments performance, and the later deployments allowed for an assessment of its performance in-situ.

3.2 Instrument Alteration

3.2.1 Chemical Corrosion of the Pump Manifold- The original YSI polycarbonate manifold was used during the first few deployments, but over time, chemical corrosion of the flow channels lead to leaking and inconsistent pumping. This manifold had been used in the lab as well, but because it had been flushed with purified water after each use, corrosion was not observed up to that point. The manifold was replaced with the PVC version described in Chapter 2 in early January of 2006 and used for all subsequent deployments.

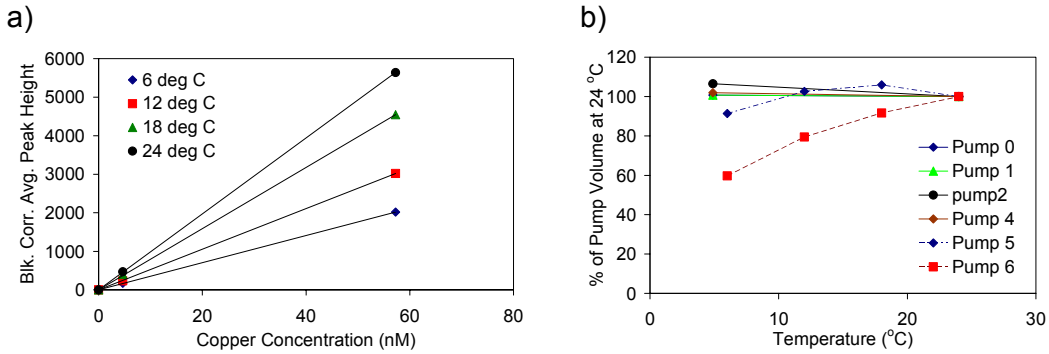
3.2.2 Effect of Temperature on Instrument Performance- A strong temperature effect was observed in the standard measurement during the second (Jan. 27 to Feb. 8th 2006) Yaquina Bay deployment. Upon immersion in the bay a decrease of approximately two fold was observed in the blank and standard signal in response to a drop in temperature from ~25 to ~10°C. This drop in signal led to poor sample precision and a high detection limit. In order to determine the source of this temperature sensitivity, both the PMT and the reagents were tested separately in a temperature controlled water bath. From the results of these experiments (figure 3.1) it is obvious that neither the PMT nor the reagents could produce a drop in sensitivity of the magnitude observed in-situ.

Figure 3.1: Instrument response to independent temperature variation of the reagents and PMT. The ratio of peak height to the peak height at 6°C is shown to allow comparison of the reagent and PMT response. Error bars represent the standard deviation of three replicate measurements.



Further testing was performed by placing the instrument in a temperature controlled incubator. The pump-volumes of the micro-solenoid pumps (nominally 50μL each stroke) were tested across a range of temperatures and flow conditions. While most of the pumps were found to function consistently over a broad temperature range (5-25^{o}C), the two reagent pumps (5 and 6) were found to be sensitive to temperature (Figure 3.2, a). The effect this change in pump volume had on instrument sensitivity was found to correspond well to the temperature sensitivity observed in the field (Figure 3.2, b). The temperature sensitivity of these two pumps was determined to be due to worn out pumps, mostly likely caused by over-use and excessive backpressure caused by a much longer mixing coil present in the early version of the instrument. In response, the reagent mixing coil was shortened by a factor of two to reduce backpressure, and the instrument program was adjusted temporarily to avoid using the malfunctioning pumps during the Moss Landing and Elkhorn Slough deployments. No temperature effect was observed in either of those deployments.

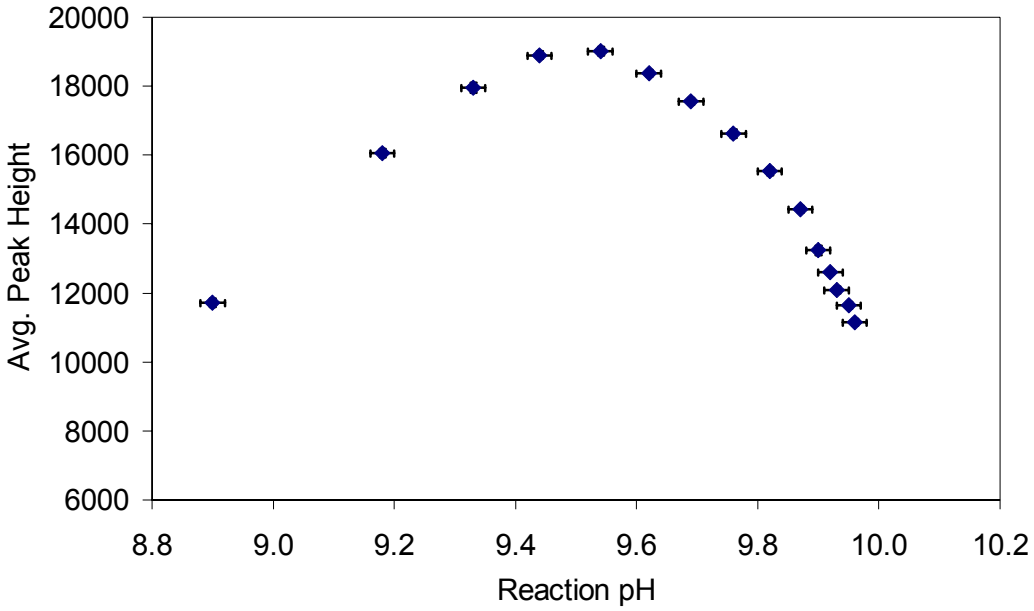
Figure 3.2**:** Effect of temperature on the volume of fluid displaced by the micro solenoid pumps. The effect of this change in pump efficiency on the intensity of the chemiluminescence reaction was measured using 5 and 60nM standards (a). Only pump 6, and to a lesser extent, pump 5, were affected by a change in temperature (b).



3.3 Method Alteration

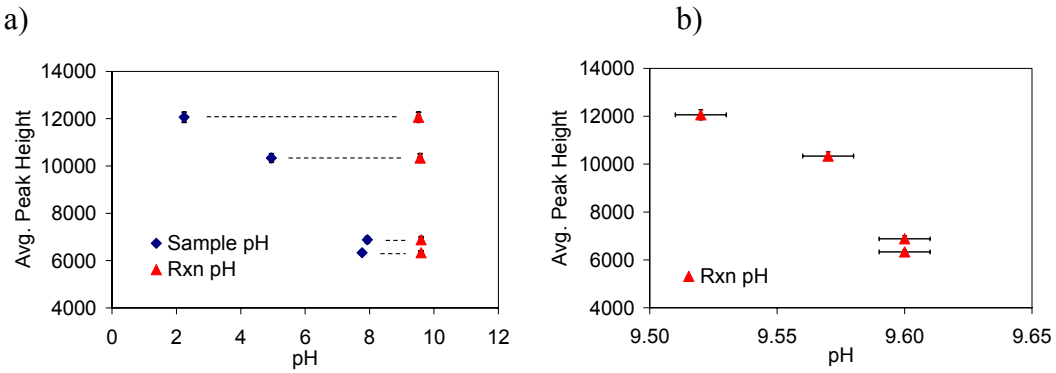
3.3.1 pH sensitivity- The 1,10 phenanthroline chemiluminescence reaction is sensitive to both the initial sample pH and the final "reaction pH," which is a function of the H_2O_2 concentration, the NaOH concentration, as well as the pH and buffering capacity of the sample. Experiments were carried out in order to assess the extent to which this sensitivity would complicate the in-situ method. A 10nM acidified (pH 1.7) seawater standard was run multiple times while the pH (NBS) of the R-2 reagent solution was varied by adding small amounts (<1mL/500mL) of 6N Ultrex II HCl. After the chemiluminescence response was measured the effluent was collected and the pH was measured using a pH electrode (Thermo, Orion 9157BN). The chemiluminescence was highly sensitive to small pH changes with a small plateau at a pH of 9.5 (figure 3.3).

Figure 3.3: Chemiluminescence response from the 1-10 phenanthroline method as a function of reaction pH. Reaction pH was manipulated using small (<1mL/500mL) additions of trace metal clean NaOH to the R-2 reagent. An acidified (pH 1.7) 10nM copper standard was analyzed with each reagent mixture and the pH of the effluent was measured. Error bars represent the standard deviation of three replicate measurements.



Based on these results, the NaOH concentration in the R-2 was adjusted in order to place the final reaction pH as close to 9.5 as possible. The reagents appeared to be buffered adequately since the reaction pH was relatively insensitive to large changes in sample pH (Figure 3.4, b). On-board acidification insured that the sample pH would vary only slightly (pH 1.7± 0.5) over the range of ambient pH values encountered in a typical estuary or coastal ocean.

Figure 3.4**:** Chemiluminescence response as a function of sample pH. The pH of a 10nM standard was manipulated with small additions of clean HCl. Panel (a) shows sample pH values (blue) and the corresponding reaction pH measurements (red). Panel (b) shows an expanded view of the reaction pH measurements in panel (a). Error bars indicate the standard deviation of three replicate measurements.



The effect of sample pH was investigated using a seawater sample spiked with 10nM copper. The sample pH was varied by adding small amounts of 6N Ultrex II HCl to the spiked sample and making a series of measurements using the bench top FIA system (figure 3.4, a). Again, after each measurement the effluent stream was collected and the reaction pH was measured (figure 3.4, b). The luminescent response increased approximately two fold from a sample pH of ~8 to ~2 (figure 3.4, a). While it is expected that some portion of this signal will be due to the effect of sample pH on the reaction pH, this effect alone can not account for the change in intensity that was observed (figure 3.4, b). Clearly the chemiluminescence response is sensitive to sample pH even when the reaction pH is unchanged.

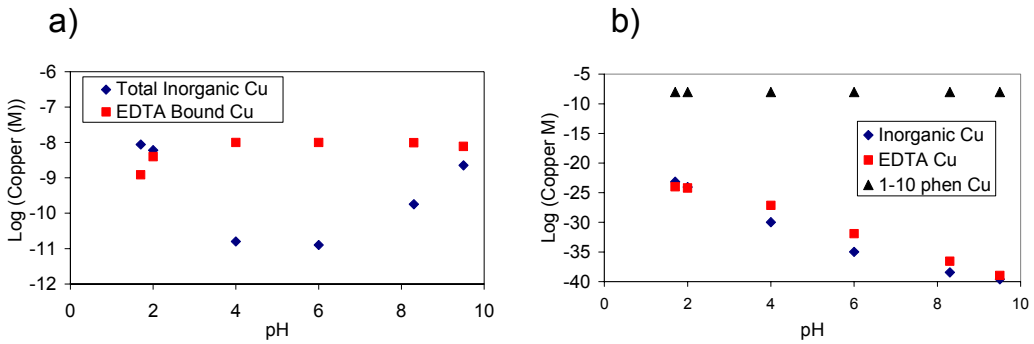
One possible explanation for this pH relationship is the effect of CO_2 on the 1,10-phenanthroline chemiluminescence reaction. CO_2(aq) is thought to quench the chemiluminescence reaction between the 1,10-phenanthroline-copper complex and

free radical oxygen through the formation of peroxycarbonate radical (Xiao et al., 2002). However, if only CO_2(aq) is active in the quenching reaction, then as the sample becomes more acidic an increasing fraction of the available TCO_2 should be present as CO_2 (aq). This reaction should drive the trend in the opposite direction of what is observed in figure 3.4 (a). It is possible that other carbonate species are involved in the quenching reaction although it is unlikely that the TCO_2 of the sample changed dramatically over the time scales of this experiment (90 seconds between measurements).

Another potential control on the sample response is the role of organic ligands. It is well established that in the marine environment copper occurs predominantly bound in organometallic complexes. Field studies have shown that 93-99% of Cu in surface waters is complexed by strong organic ligands (van den Berg, 1984; Buckley, and van den Berg, 1986; Moffett and Zika, 1987; Donat et al., 1986; Coale and Bruland, 1988, 1990; Moffet et al., 1990; Donat and Bruland, 1992). The copper-1,10-phenanthroline reaction is sensitive only to free copper or copper in the ionic form (Sunda and Huntsman, 1991). Because organic complexation decreases in importance as the pH becomes more acidic (Morel and Hering, 1993), it is possible that the sample pH trend observed is a reflection of the extent of organic ligand binding in the sample. This possibility was investigated by calculating the equilibrium speciation of a model seawater system containing 10nM copper and 100nM EDTA using MINEQL$^+$ (figure 3.5a). The calculated total inorganic copper concentrations are similar to the experimental results suggesting this is potentially an accurate model (figure 3.5a). Equilibrium concentrations were

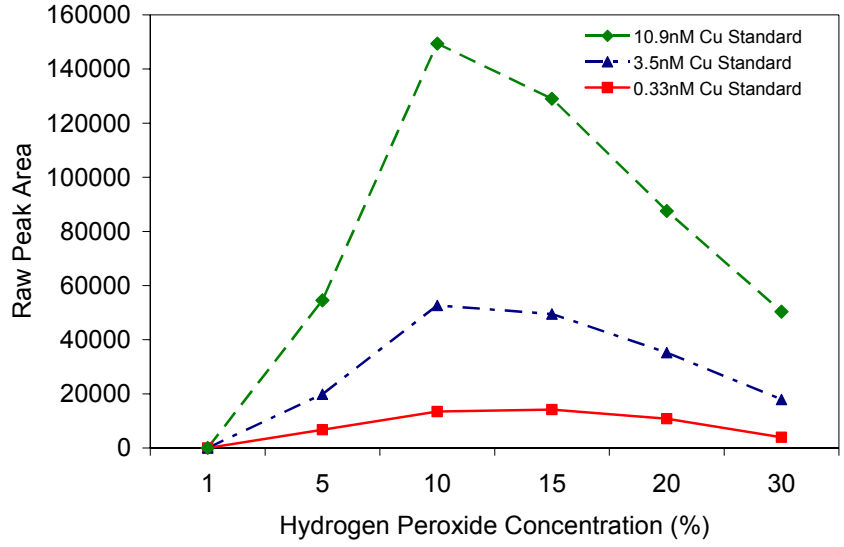
also calculated for a model seawater system containing 10nM Cu, 100nM EDTA, and 60mM 1-10 phenanthroline using ligand stability constants from Smith and Martell (1982). The results of this calculation show that the 1-10 phenanthroline copper complex dominates the copper speciation over a pH range of 1.7 to 9.5 (Figure 3.5b). Therefore, it is likely that thermodynamic equilibrium is not reached during the short period of time between the mixing of sample with reagents, and the luminescence measurement. This would suggest that the sample pH effect observed is due to slower dissociation rates of the organic bound copper, and the relative abundance of the organic bound fraction. This kinetic disequilibrium could explain some of the discrepancies between the equilibrium concentrations calculated (Figure 3.5a,b) and the experimental results (Figure 3.4a). Further research using organic-free seawater and ligand-amended seawater is necessary in order to confirm this hypothesis. Fortunately, the effect of sample pH on the accuracy of the work described here is minimal as long as sample acidification is kept consistent.

Figure 3.5: Copper speciation in a model seawater matrix calculated using MINEQL^{+}, and supplemented with binding constants from Smith and Martell (1982). (a) System contains total concentrations of 10nM Cu, and 100nM EDTA. (b) System contains total concentrations of 10nM Cu, 100nM EDTA, and 60mM 1-10 phenanthroline.



3.3.2 Optimum H_2O_2 and 1-10 phenanthroline Concentrations- The optimal hydrogen peroxide concentration was determined by setting the R-2 concentration constant, and then running a range of standards with varying concentrations of hydrogen peroxide (Figure 3.6). The optimal concentration was determined to be 10%. This number is in agreement with that cited by Zamzow et al. (1998), although several other researchers have found 5% to be the optimal concentration (Coale and Johnson, 1992; Tyrrell et al., 2004). Some of these differences are likely due to changes in the reaction pH as a function of changing the hydrogen peroxide and sodium hydroxide concentrations.

Figure 3.6**:** Results of running three separate copper standards (10.9nM, 3.5nM, and 0.33nM) and a blank in Milli-Q H_20 using 6 different concentrations of H_2O_2 (1,5,10,15,20,30%) as reagent. Final reaction pH was not measured in these experiments. The highest luminescent intensity of the copper-1,10-phenanthroline system is seen with a hydrogen peroxide concentration of ~10%.

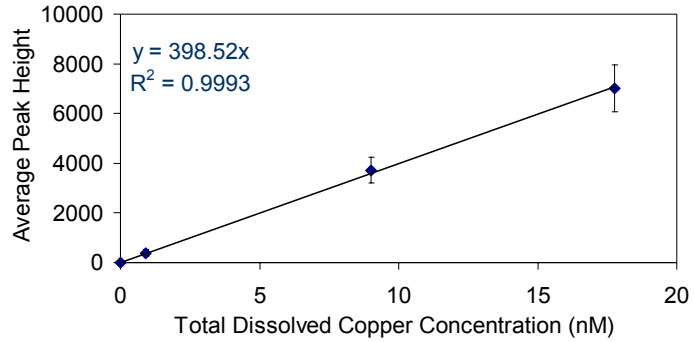


The optimal 1-10 phenanthroline concentration is not as straightforward as the optimal hydrogen peroxide concentration. In fact, the 1-10 phenanthroline

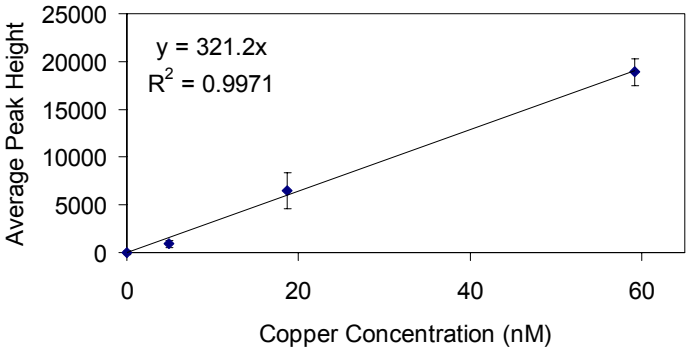
concentration can be varied to optimize the linear range of the method depending on what is expected at any particular sampling location. For example, copper concentrations in Yaquina Bay, OR generally range from less than 1nM to around 2nM, therefore a 1-10 phenanthroline concentration of 60mM was used to achieve a linear range of ~0.8nM to 20nM Cu (Figure 3.7, a). In contrast, the expected concentration of copper in Moss Landing Harbor was between 5nM and 60nM so a concentration of 10mM 1-10 phenanthroline was used to achieve a linear range of 5 to 60nM (Figure 3.7, b).

Figure 3.7: Instrument response as a function of dissolved copper concentration in pH 8, TEPA stabilized seawater standards acidified in-line. a) The Yaquina Bay (OR) pre-deployment. b) Moss Landing Harbor (CA) pre-deployment. This test allows for a comparison of the slope derived from a series of standards to that derived from the analysis of the on-board standard only. Error bars represent the standard deviation of three replicate measurements.

a)



b)



3.3.3 Salinity Corrections- The effect of salinity on the 1-10 phenanthroline chemiluminescence method can be handled in two ways. Ideally, if working in a location where salinity does not vary significantly, then a water sample near the sampling site can be collected, cleaned and used to make the standard and blank matrix. This was the method employed during all of the deployments in this study. This is by far the most accurate method of correcting for analytical variations due to salinity, and at salinities higher than 20 the chemiluminescence method is fairly invariant to salinity (Figure 3.8).

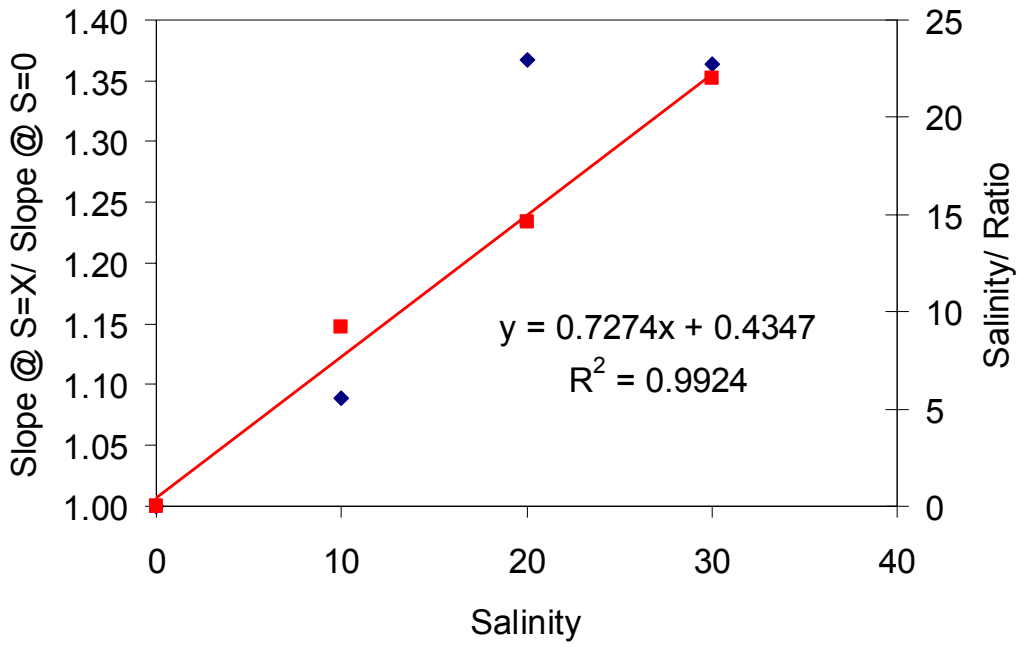
Unfortunately at salinities below 20, the method will be affected. The maximum variation introduced by salinity in the slope of the calibration curve is 1.365±0.002 times the slope at a salinity of zero. Therefore, if the instrument is deployed in a location with large variations in salinity, and the salinity drops below 20, then a salinity correction should be used. Standard and blank solutions would then need to be made in a Milli-Q H_2O matrix, and the slope of the calibration curve would be corrected according to equation 3.1. This equation is experimentally derived using altered matrix standards (Figure 3.8). Due to the inconsistencies in the slopes of the calibration curves produced using the 1-10 phenanthroline method, it was necessary to determine the correction for salinity from the ratio of the slope at a given salinity (S=X) to the slope of a calibration curve in Milli-Q H_2O.

$$\text{Slope } (S=X)=\text{Slope } (S=0)*\left(\frac{S}{0.7274*S+0.4347}\right) \qquad \text{Eq. 3.1}$$

Where S= Salinity

This method of salinity correction is not ideal and should be avoided where possible, as it produces a potential uncertainty in the slope of up to 20%.

Figure 3.8: The effect of salinity on the 1-10 phenanthroline chemiluminescence method. The variation in slope at a given salinity (S=X) is normalized to the slope at (S=0) and shown in blue diamonds. Equation 3.1 was derived by comparing salinity over the slope ratio to salinity (red squares), and performing a linear regression.



3.3.4 In-Line Sample Acidification- Considering the importance of consistent sample, blank, and standard acidification, all blank and standard solutions were kept at a pH similar to that of the incoming sample (~pH 8). Solutions were stabilized at pH 8 by adding 200nM of TEPA, a strong copper binding ligand. Proper sample, blank, and standard acidification was ensured by measuring the kinetics of copper ion dissociation from a range of natural and synthetic organic ligands. Time trial experiments were conducted with seawater from an oligotrophic region of the N Pacific, from south beach in Newport, OR just

beyond the surf zone, and with a synthetic ligand system using TEPA and EDTA in Milli-Q. An acidification time of 90 seconds was determined to be equivalent to conventional acidification techniques with an equilibration period of at least a month (figure 3.9). The minimum acidification time was found to vary inversely with temperature and directly with copper concentration (Figure 3.10). A waiting period of 90 seconds was found to be adequate for an 80nM standard at 5°C, which represents a worst case scenario for the conditions in which the instrument was deployed.

Figure 3.9: Kinetics of Cu release from organic ligands following acidification of a sample collected from Yaquina Bay, Oregon. Half of the 1L sample was acidified (pH 1.7) immediately after collection, while the other half was left at ambient pH (8.3). A small aliquot of the pH 8 sample was then analyzed using a continuous flow system until the signal was stable. The aliquot was acidified and again measured until a plateau was reached. This plateau was compared with the acidified sub-sample and no significant difference was observed between a sample acidified for 90 seconds and a sample acidified for 1 month.

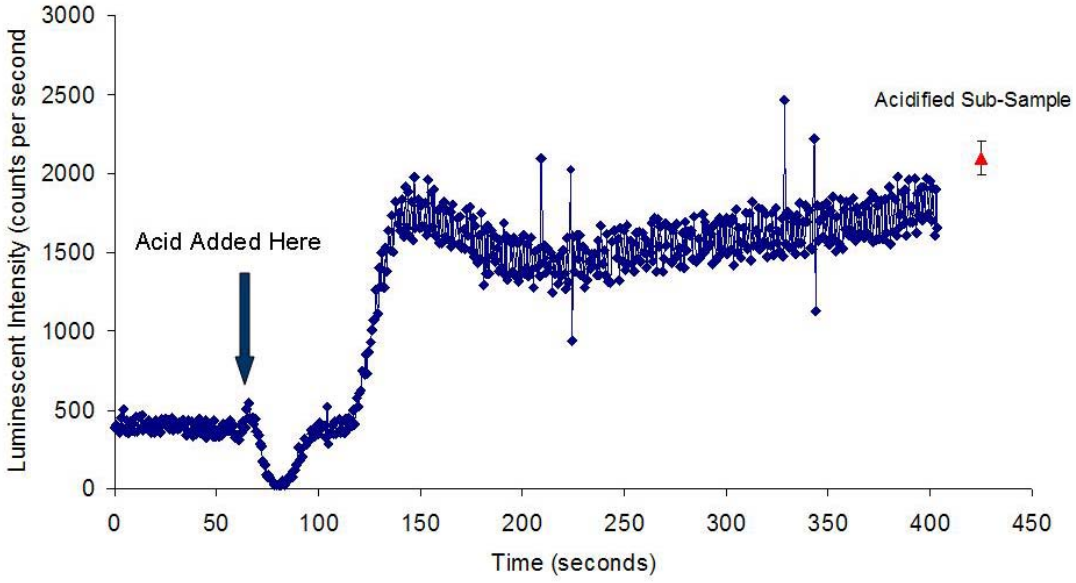
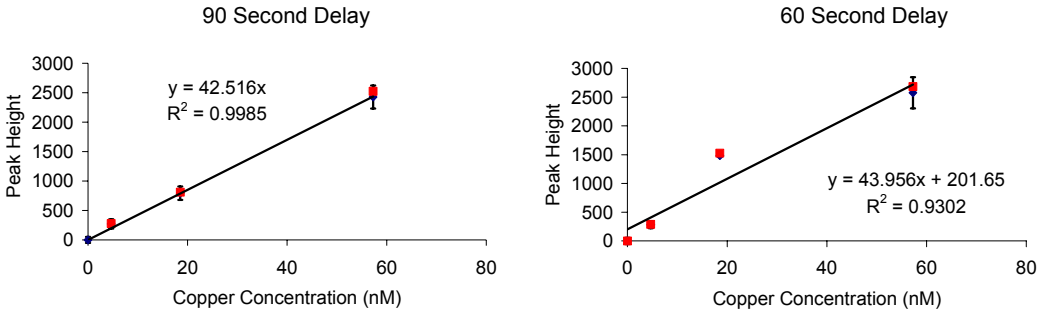


Figure 3.10: Instrument calibrations performed at 5°C using a 90 second acidification time and a 60 second acidification time. At room temperature a 60 second acidification time is adequate to achieve a linear calibration out to 60nM.



3.4 Instrument Programming

In order to use the 9600 for copper analysis, it was necessary to reprogram the pumping sequence for optimum performance with the copper chemiluminescence method. Experiments were run to test the optimal flow rate, amount of flushing and amount of cleaning necessary, and to optimize the flow path and timing. In addition, the program was optimized to use as little reagent and battery power as possible in order to maximize the deployment length.

The optimal flow rate was found to be ~ 30µL /sec although due to the segmented flow of the micro-solenoid pumps the method was not very sensitive to flow rate. Flow rate is controlled by setting the pump on and pump off timing delays. The pump on delay sets the amount of time that the pump is energized keeping the plunger open. After the pump is turned off and the plunger drops the pump off delay sets the amount of time before the cycle can start again. The optimal flow rate of ~ 30µL /sec corresponds to pump on and pump off delay times of 500ms and 1250ms respectively. This flow rate was determined by

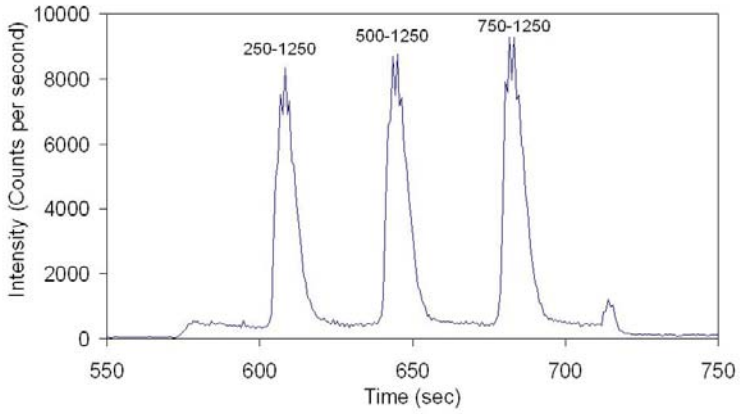
independently varying the pump on (Figure 3.11, a) and pump off (Figure 3.11, b and c) delay times and measuring the effect on the chemiluminescence response of a 10nM standard.

The minimum amount of flushing required for each step was determined by analysis of a blank, followed by a 10nM standard, and then tracking the blank signal until it returned to the initial intensity. The length of reagent and sample mixing coils were determined by balancing the benefits (increased sample precision), against the negative effects of the mixing coils (higher backpressure and increased reagent consumption due to increased flushing). The minimum amount of flushing was determined each time the flow path was changed. Finally, the minimum amount of flushing needed to clear the 10μm and 0.45μm filter and feeder line was determined (Figure 3.12).

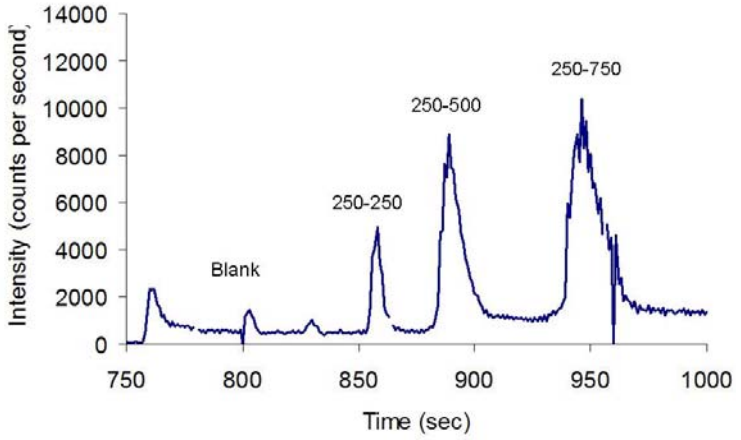
Power consumption could be minimized only by decreasing the amount of time the instrument power was on. Unfortunately, there is no separate command to turn on and off the PMT, so whenever power is supplied to the pumps, power is also supplied to the PMT. Therefore, an attempt was made decrease the overall length of each step.

Figure 3.11: Three tests of the optimal flow rate (a,b,c). The optimal flow rate was determined by systematically changing the pump on and pump off delay times. The numbers listed above each set of peaks are in the following format: pump on-pump off (ms). The individual peak numbers in panel (c) represent the number of pumps of standard injected.

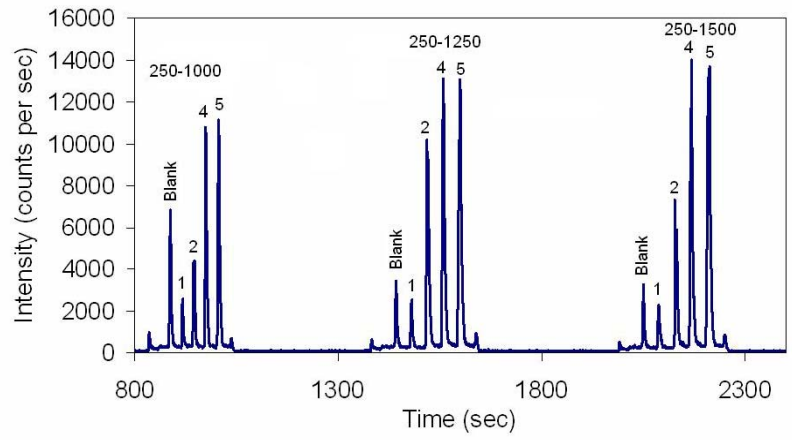
a)



b)

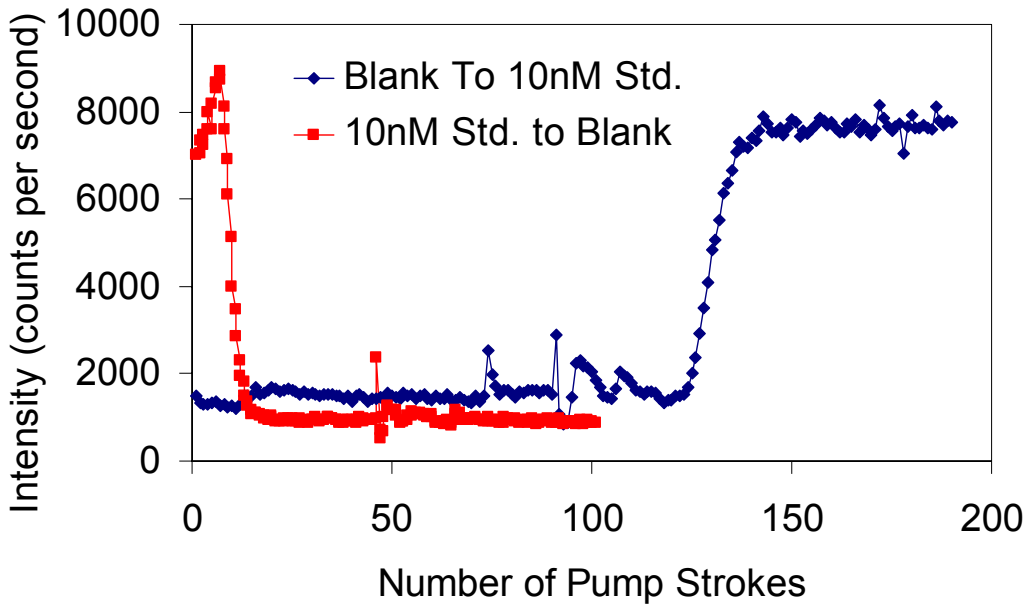


c)



Early on in the method development the timing of the sample peak was highly irregular. In order to accommodate the shifting sample peak a longer program with a higher reagent consumption than the program detailed in the methods section was needed. These inconsistencies were traced to two malfunctioning pumps. Adjustments were made to fix the problem and the shorter program detailed in the methods section was used for the Moss Landing and Elkhorn Slough deployments.

Figure 3.12: Filter flushing tests designed to determine the minimum number of pump strokes needed to clear the 10µm and 0.45µm filters. This was determined by analyzing a blank sample and then following it with a 10nM standard (blue line) until it reached a plateau. Then the sample line was returned to a blank solution and the signal was again traced until it returned to the baseline (red line).



3.5 Field Deployments

Prior to each deployment a series of pH 8 seawater standards was run to ensure linearity over the expected range (Figure 3.7). The blank, standard, and sample intake lines were placed in the same standard solution to normalize each of the individual pumps. The pump calibration was done with the sample filters in-line before and after each deployment to determine if any drift occurred due to filter clogging. A correction factor was then applied to the sample intensities to reflect the difference in sample pump volumes from that of the standard and blank pumps.

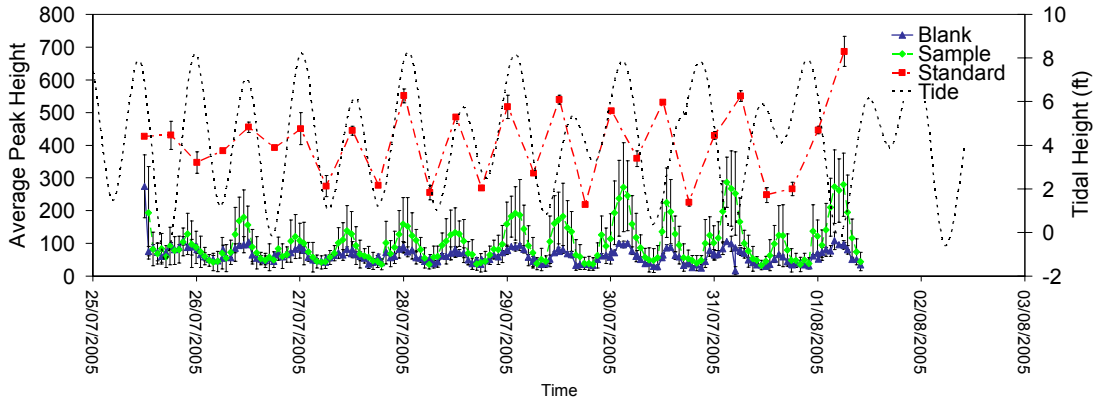
Initial in-situ testing was done in a freshwater tank with the sample line connected to a reagent bag containing a pH (3) 15nM standard, and the standard line connected to a pH (8) 15nM standard stabilized with EDTA. This initial test served the purposes of testing over an extended period while submersed and ensuring the stability of a standard at pH (8) with 200nM EDTA. The instrument ran flawlessly until it ran out of reagents after 3 days. The relative standard deviation of the 15nM standard over the entire 3 days was 10%. This initial testing contained some unnecessary use of reagents which limited the longevity of the run to 3 days sampling every 15 minutes. Minimization of the schedule post deployment extended the lifetime of the instrument to 15 days if running every hour and calibrating every six hours, without compromising the accuracy and precision of the measurement. Further testing in the lab showed inconclusive results for the stability of EDTA stabilized standards over a period of 2 months (Data not shown).

The instrument was next deployed in Yaquina Bay, Oregon from July 25th to Aug. 1st 2005 (Figure 3.13), off the OSU dock in ~5m of water, with a ~3m tidal

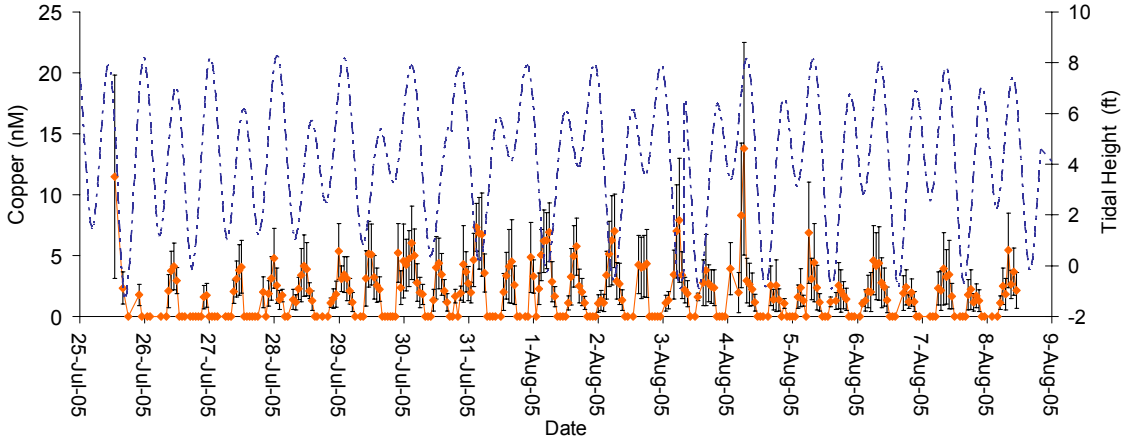
exchange. The instrument was moored to a floating platform, just below the surface. An underwater cable was used to supply power and to check instrument performance throughout the deployment. Unfortunatly, due to chemical corrosion which occurred when the reagents sat in the pump manifold over extended periods of time, the results of this deployment were highly irregular (Figure 3.13).

Figure 3.13: Data from a deployment in Yaquina Bay, OR in July of 2005. (a) Raw standard (red), sample (blue) and blank (orange) peak heights are plotted with tide in the background (black). (b) Blank and standard corrected sample copper concentrations (orange) with tidal heights relative to MLLW (blue). Values below the detection limit (1.04nM) are defined as zero and error bars represent the standard deviation of replicate (n=3) measurements.

a)



b)



The instrument was deployed in the Gulf of Aqaba, Eliat Israel from Sept. 5th to the 8th 2005, off of the Israeli ocean research center dock in 4m of water with a ~0.5m tidal exchange. However, no useful data was collected during this period due to further chemical corrosion of the pump manifold. Corrosion during this deployment became extremely pronounced and visible leading to a complete failure in instrument function. In addition, several of the micro-solenoid pumps were pumping irregularly either due to overuse and excessive backpressure, or internal chemical corrosion. All of the malfunctioning pumps, the pump manifold, and the instrument end cap were replaced after this deployment.

The instrument was deployed in Yaquina Bay, Oregon from Jan. 27th to Feb. 8th 2006, off the OSU dock in ~5m of water, with a ~3m tidal exchange. The instrument was moored to a floating platform, just below the surface. An underwater cable was used to supply power and to check instrument performance throughout the deployment.

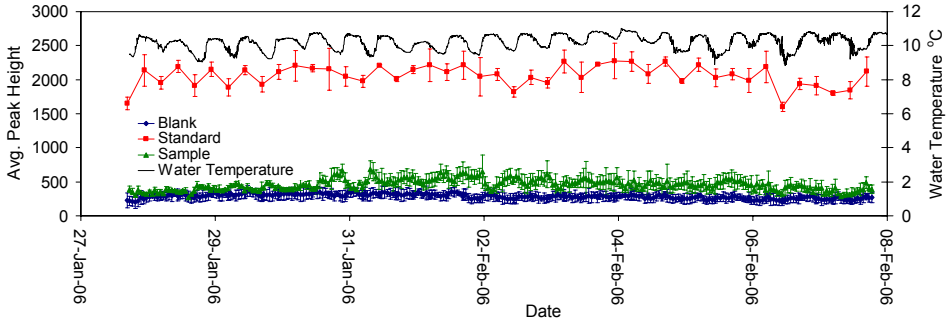
The 10μm filter used in the second Yaquina Bay deployment was not soaked in methanol before being used. Consequently, some upward drift in the sample pump volume was observed relative to the standard and blank pumps during the deployment, due to the hydration of the hydrophobic filter. A linear interpolation was used to estimate the time dependence of the filter correction. The instruments duration was limited to 12 days due to a shortage in acid.

Average sample precision was 23%, and accuracy, assessed by comparison with the discrete samples was 24% averaged over the deployment. The precision of the on board 10nM standard was markedly better at 8% over the entire deployment.

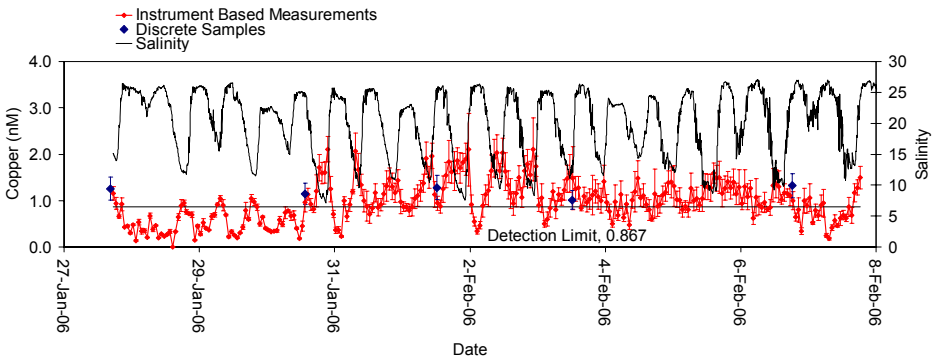
Discrete samples generally were within the precision of sample measurements as evident by visual inspection (Figure 3.14), and by the comparability of accuracy and precision estimates. No statistical difference was observed between the discrete and instrument based measurements using a t-test. The detection limit was high at 0.9nM, (Figure 3.14) which is more than twice what is normally observed in the lab (~0.4nM), and the instrument was linear to ~25nM.

Figure 3.14: Data from a deployment in Yaquina Bay, OR, in February 2006. The instrument was deployed off the OSU dock in ~5m of water, just below the surface. (a) Raw luminescent intensities of Blank, Standard, and Sample are plotted over the 12 day deployment period. For comparison water temperatures from a CTD deployed with the instrument are also shown. (b) Blank and standard corrected total dissolved (pH 1.7, <0.45µm) copper concentrations from the bay. Salinity data from the same CTD is also plotted along with the method detection limit ((3σ of the Blank)/slope) and periodic discrete bottle samples analyzed using the bench top FIA method. Each sample, standard, and blank data point is an average of three measurements with the error bars representing the standard deviation of those measurements.

a)



b)



After reorganization of the pump order, the addition of a shorter mixing coil, a reduction in the 1,10 phenanthroline concentration, and further optimization of the method, the instrument was deployed in Moss Landing Harbor, CA from the 2nd to the 10th of August 2006, and in Elkhorn Slough, CA from the 14th to the 17th of August 2006. In Moss Landing Harbor, the instrument was deployed in approximately 3.5m of water with a 2m tidal exchange. It was moored to a floating platform approximately 1m below the surface. The harbor is highly stratified, with sharp gradients in nutrient concentrations above 1m in depth (Joe Needoba, per. Comm.). In Elkhorn Slough the instrument was moored to station L01 of the MBARI LOBO network. Station L01 is located in the main channel of Elkhorn Slough near the Moss landing Harbor entrance to Monterey Bay. Although the instrument was only deployed for eight days due to time constraints, the optimized schedule, described in the methods section, extends the lifetime of the instrument to 25 days sampling every hour and calibrating every six hours, with the limiting factor being the reagent volume.

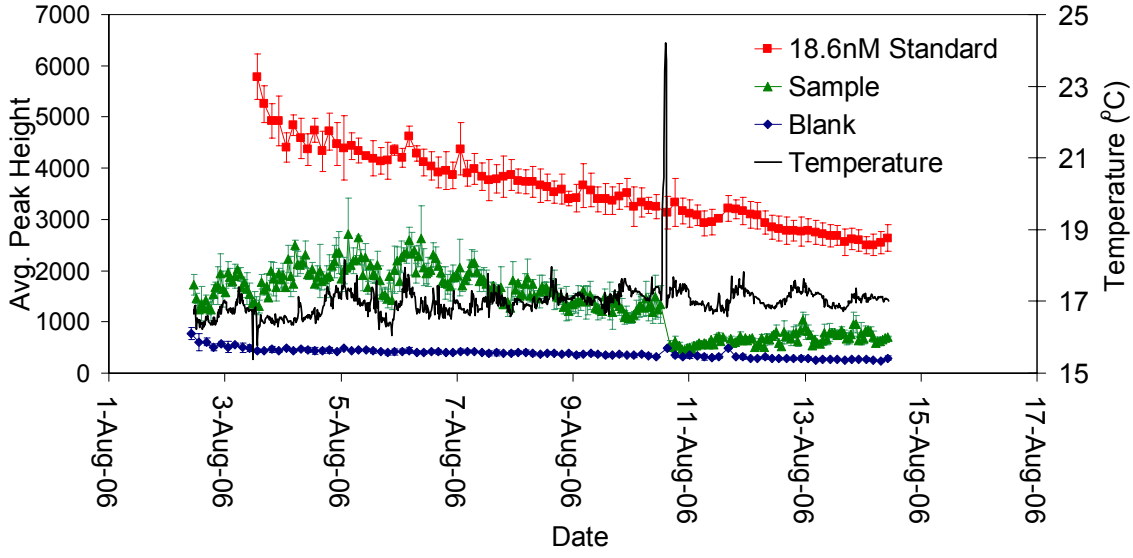
During these deployments the average sample precision was 2.0%, and accuracy assessed by comparison with discrete samples was 15%. However, some of this "low accuracy" could be due to the inherent difficulties in getting discrete samples that are collected at exactly the same time and location as the instrument, and free of contamination. Seven out of nineteen discrete samples were determined to be significantly different from the instrument based measurements using a t-test. The average detection limit (3σ of the blank) for this deployment was 0.9nM.

Before this deployment the 1,10 phenanthroline concentration was reduced from 60 to 10mM in order to increase the linear range of the instrument out to 60nM.

Discrete samples were collected every hour over one complete tidal cycle on August 8th in order to asses the variability in the harbor at the same resolution as the instrument's sampling frequency (figure 3.15). Although the overall accuracy is fairly low (15%), a visual comparison of this high frequency sampling with what is recorded by the instrument suggests the variability recorded by the instrument is real. For example, the average hourly percent change over the 12 hours, recorded by the discrete samples, was 11±13% versus 9±6% as recorded by the instrument. In addition, while both the Moss Landing Harbor data (Figure 3.15) and the Elkhorn Slough data (Figure 3.16) are dominated by a tidal signal, a comparison of the two records suggests that the harbor has a high degree of subtidal variability. Strong, short term variability is present in the Harbor record that does not appear in the Elkhorn Slough data, which again suggests that the variability in Moss Landing Harbor is real, and not simply an instrumental artifact.

Figure 3.15**:** Data from an in-situ deployment in Moss Landing Harbor (CA) in August 2006. (a) Raw standard, sample, and blank measurements plotted with temperature measurements from a YSI 600XLM Sonde deployed along with the instrument. On August 10th, 2006 the instrument was removed from the water to check the filter performance (large spike in temperature) and an air bubble was trapped in the filter during re-emersion contributing to the drop in sample signal. (b) Total (pH 1.7) dissolved (<0.45µm) copper concentrations are plotted along with salinity data from the sonde and discrete samples measured using the bench top FIA method. Discrete samples that are statistically different from the instrument are plotted with a yellow diamond. Error bars represent the standard deviation of three replicate measurements.

a)



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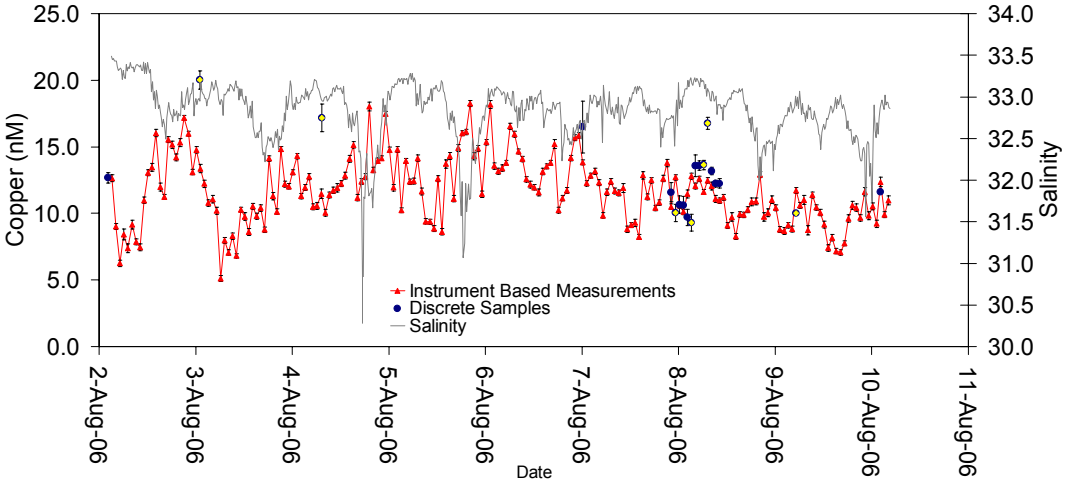
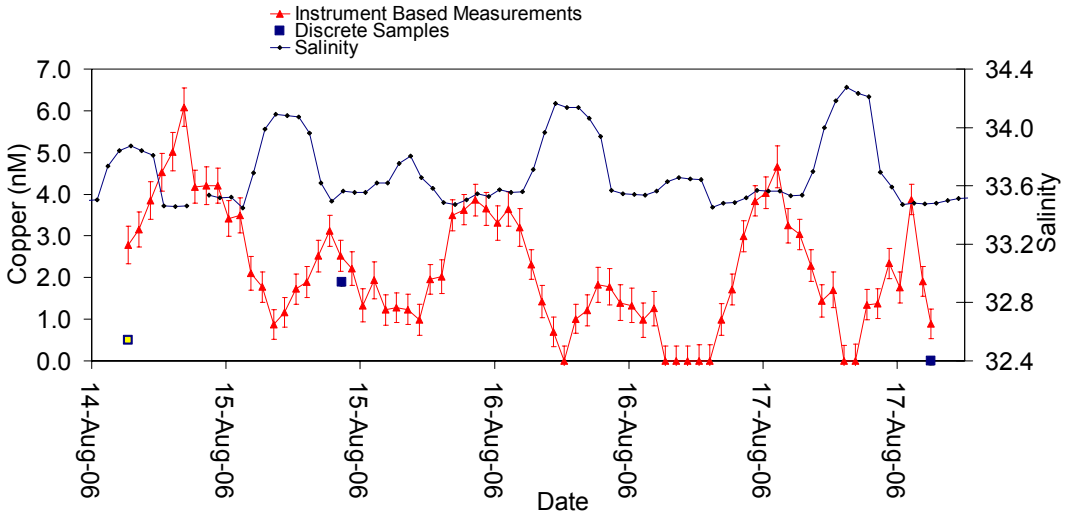


Figure 3.16: Total (pH 1.7) dissolved (<0.45µm) copper concentrations from an in-situ deployment in Elkhorn Slough (CA) in August 2006. Salinity data from the L01 MBARI mooring and discrete samples analyzed using the bench top FIA method are also shown. Discrete samples that are statistically different from the instrument are plotted with a yellow diamond. Error bars represent the standard deviation of three replicate measurements.



3.6 In-Situ Results

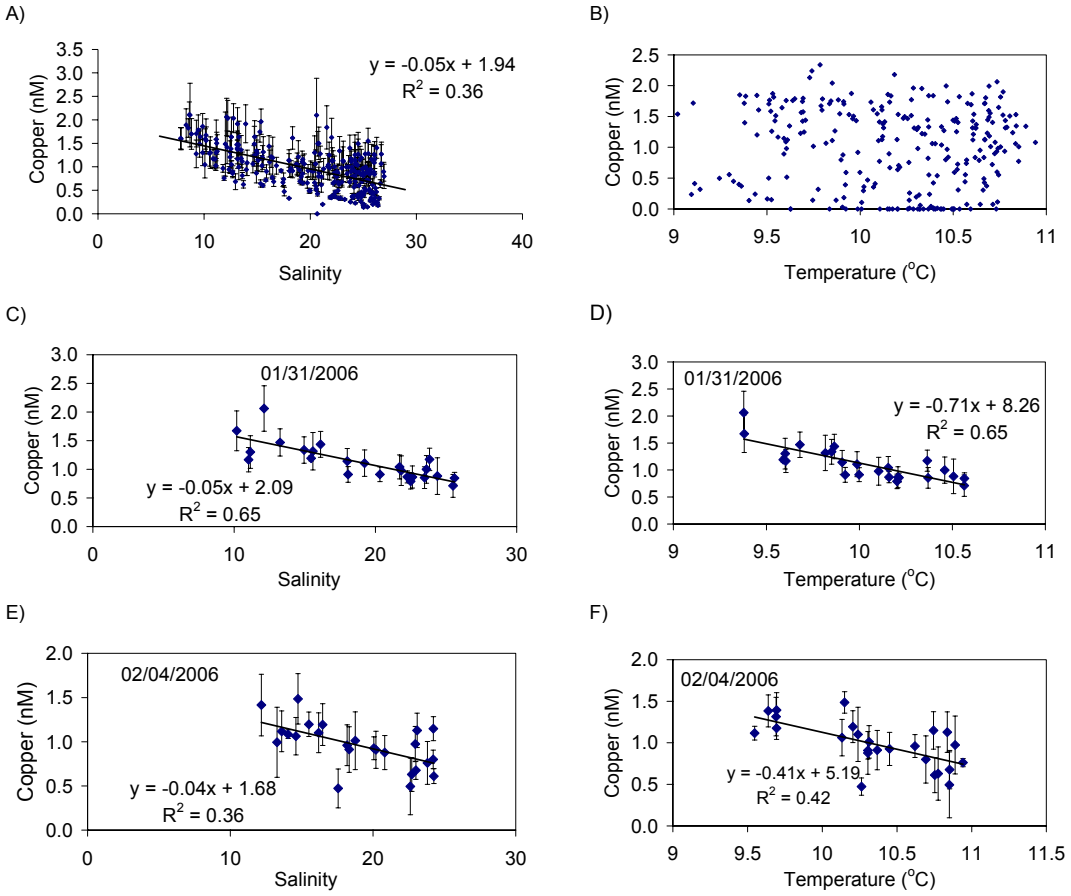
Yaquina Bay, Moss Landing Harbor, and Elkhorn Slough each represent a different mix of environmental controls on copper. Further investigation of copper cycling at these sites would be necessary to fully constrain all of the copper sources and sinks and their relative importance. However, using these initial results some general descriptions of copper cycling at these sites during the time of each deployment can be made.

The dominant source of copper at the OSU dock in Yaquina Bay from the 27th of January to the 8th of February 2006 was the Yaquina River. Copper concentrations at this site show an overall negative correlation with salinity (Figure 3.17) indicative of a riverine source. This overall correlation suggests the Yaquina

River has a total dissolved copper concentration of ~1.9nM assuming the river has zero salinity.

In addition to the tidally dominated copper signal, a larger overall rise and fall in copper concentrations is observed and is likely contributing to the variability in the copper salinity relationship (Figure 3.18, A). This rise in total dissolved copper from approximately the 31st of January to the 3rd of February is coincident

Figure 3.17: Copper, salinity, and temperature measurements from the Yaquina Bay deployment (Jan. 27-Feb. 8, 2006). An overall negative correlation with salinity (A) was observed and no overall correlation with temperature (B). Results from comparing salinity to copper concentrations during two 12 hour periods: January 31, 2006 (C), and Febuary 4, 2006 (E). Results of comparing temperature to copper during two 12 hour periods: January 31, 2006 (D), and Febuary 4, 2006 (F)

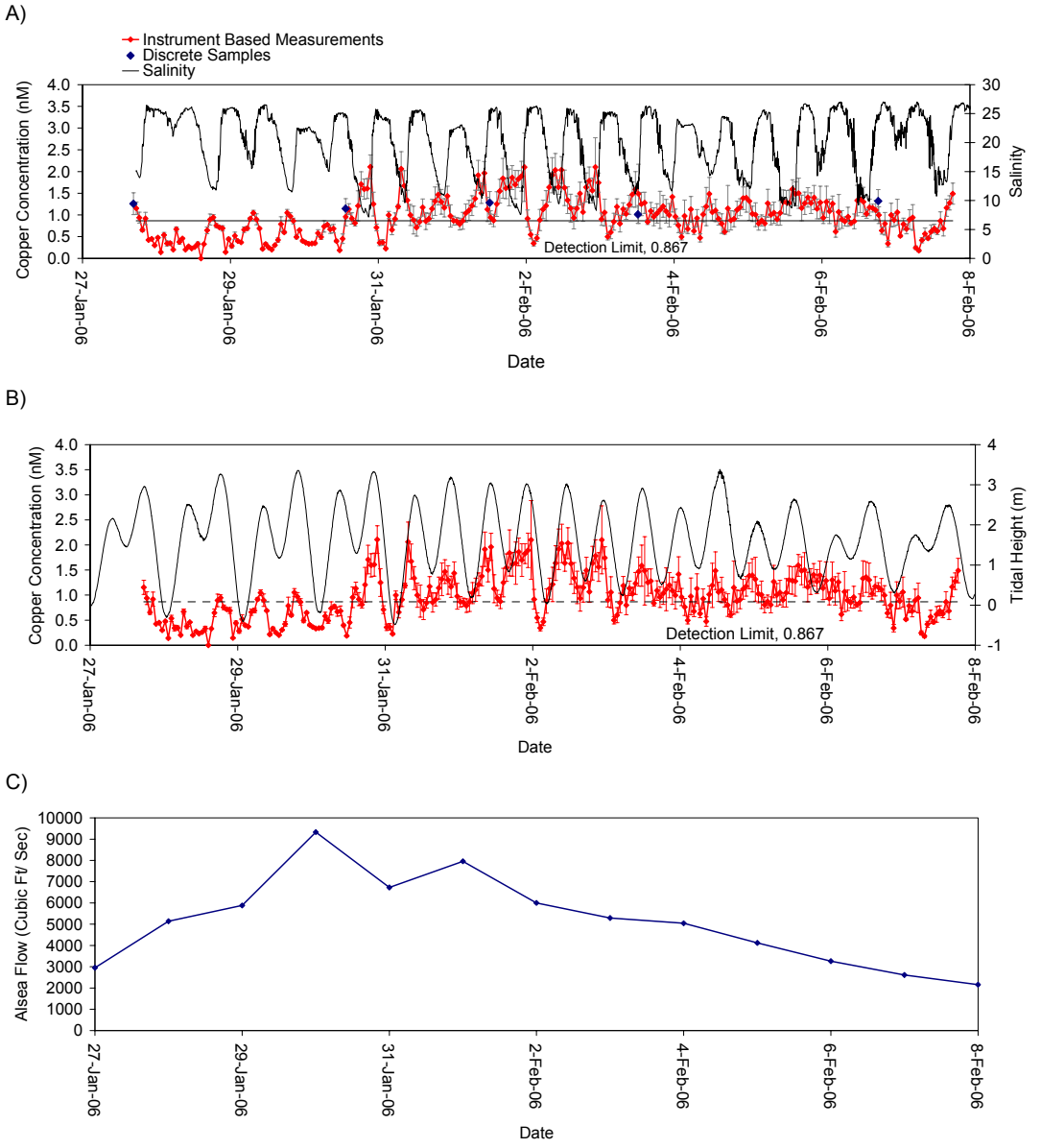


with the lowest recorded salinities during the deployment suggesting a higher proportion of river water reaching this site during that time period (Figure 3.18, A). This feature is not a function of the tide as the tidal signal is transitioning from a stronger spring tide to a weaker neap tide during the same period (Figure 3.18, B).

Several days of high precipitation in the watershed likely contributed to a higher stream flow, evidence for which can be seen by looking at a nearby watershed, the Alsea River, for which stream flow data is available (Figure 3.18, C). The Yaquina and Alsea River flows are well correlated and the timing of the increase in flow in the Alsea River is likely similar to that of the Yaquina River. The beginning of the peak in copper and minimum in salinity occurs on January 31st, one day after the peak in stream flow in the Alsea (Jan. 30, 2006), which is consistent with an estimate of the residence time of water in Yaquina Bay of ~1 day at high river flow (Callaway et al., 1988).

A comparison of copper concentrations in the river calculated at higher flow (2.1nM, Figure 3.17, C) and at lower flow (1.7nM, Figure 3.17, E) suggest that the concentration in the river did not change much if at all over this time period. Therefore, the change in concentration at the deployment site is simply due to a higher proportion of river water present at the site. However, this would suggest that the variability present in the overall copper-salinity correlation is due to some other process beyond simply a variable river concentration.

Figure 3.18: In-Situ data collected from Yaquina Bay, Oregon from Jan. 27 to Feb. 8, 2006. (A) Instrument derived copper concentrations plotted along with salinity from a CTD and discrete sample measurements of copper during the deployment. (B) Instrument derived copper concentrations plotted along with measured tidal heights relative to MLLW. (C) Alsea Stream Flow data collected from USGS station 14305500.



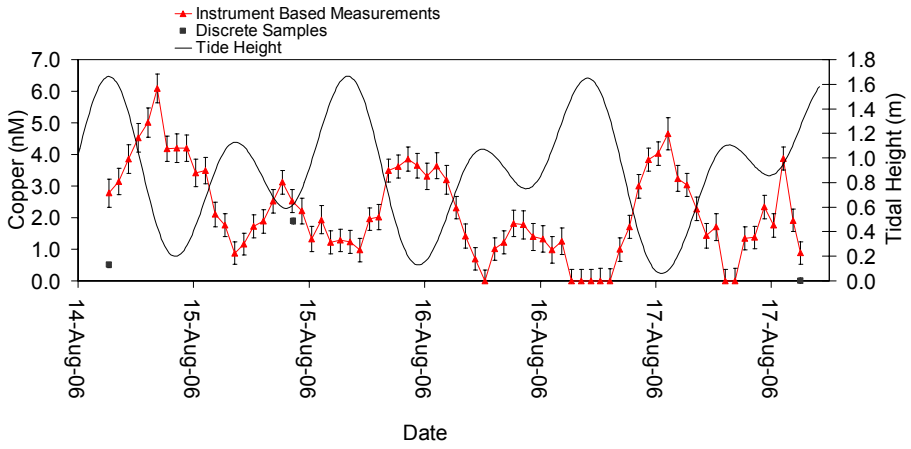
In contrast to Yaquina Bay, the dominant source of copper in Moss Landing Harbor from the 2nd to the 10th of August 2006 was most likely diffusion from a local source. Potential sources include the surrounding sediments, wastewater pipelines, anti-fouling paints on the ships in the harbor, or the treated wood pilings. Total dissolved copper concentrations in Moss Landing Harbor were much higher than those observed in Yaquina Bay and were also highly variable. No correlation between salinity and copper was observed, although a very narrow range in salinities was sampled during the deployment (30-33.5, Figure 3.15, b). The variability of the copper signal in Moss Landing Harbor was predominantly on the order of the tides, consistent with the residence time of water in the harbor which is also on the order of the tides (6hrs) (Chapin et al., 2004). However, shorter scale (1hr) variability in the salinity, temperature, dissolved oxygen, and copper concentrations suggest a high degree of heterogeneity in the water mass at this site (Salinity and Copper, Figure 3.15, b: Temperature and dissolved oxygen data not shown).

Elkhorn Slough also presents an interesting contrast to Yaquina Bay in that no significant fresh water input is present during the summer months (Chapin et al., 2004). In fact, due to evaporation, the water in the upper slough often has higher salinity than the water coming in from Monterey Bay. (Chapin et al., 2004). The residence time of water in Elkhorn Slough is also on the order of the tide and the water column at site L01 is well mixed and much more homogeneous than that found in Moss Landing Harbor (Chapin et al., 2004). A strong correlation exists between measured copper concentrations and tidal height, suggesting the dominant

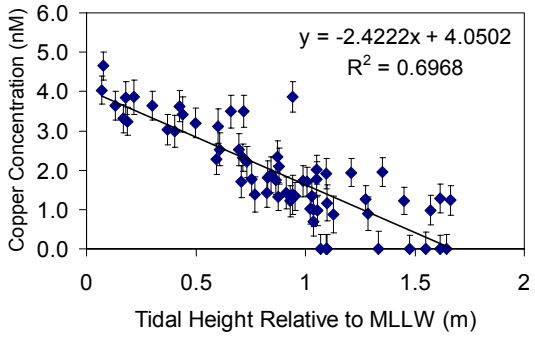
source of copper in Elkhorn Slough at station L01 from the 14th to the 17th of August 2006 was tidal flushing of surrounding slough water enriched with copper by diffusion from the sediments (Figure 3.19). The peak in copper concentrations generally occurs as the tide is ebbing and is higher when the exchange is from higher high water to lower low water. This suggests that at high tide copper diffusion from the sediments enriches water in the shallow edges of the slough and as the tide ebbs that water is brought into the main channel and past the instrument at L01.

Figure 3.19: Correlation of copper concentrations and tidal heights from an in-situ deployment in Elkhorn Slough (CA) in August 2006. (A) Total (pH 1.7) dissolved (<0.45µm) copper concentrations and tidal heights relative to MLLW. (B) Comparison of tidal height relative to MLLW and copper concentrations.

A)



B)



3.7 Barriers to long term Deployments

Four main factors control the maximum duration of any potential deployment: power consumption, reagent consumption, filter life, and reagent and standard stability. To date power consumption and battery life have not been explicitly tested. All but the 3-day Elkhorn Slough deployment were run using shore power. It is known that the pumps will begin to malfunction below ~9V (YSI, per comm.) and the PMT will not function below ~7V. The use of lithium batteries instead of alkaline batteries could extend the deployment times in cold waters. With the schedule detailed in the methods, the H_2O_2 and R-2 solutions will run out after 25 days when sampling every hour and calibrating every six hours.

To test the filter life a pump test was run before and after each deployment. This test was carried out by placing the unfiltered standard and blank lines into the same standard solution as the sample line with both filters in-line. These tests illustrate that filter performance was unchanged after 10 days in Moss Landing Harbor (ratio of sample to standard pre deployment 1.7± 0.3, post deployment 1.4±0.4). In addition, another filter remained unchanged after three days in Moss Landing Harbor and four days in Elkhorn Slough (pre 1.5±0.3, post 1.5±0.5). The pre and post calibrations for the Yaquina Bay deployment are also encouraging as the standard to sample ratio actually decreased over the 12 day period presumably as the hydrophobic 10μm filter became more efficient due to hydration (pre 1.4±0.3, post 0.6±0.1). Filter duration is untested past 12 days, but based on these results, which showed no trend of decreased filtration over time, we expect the filters to remain operational for at least 25 days when sampling every hour.

The final consideration is reagent and standard stability. The R-2 and H_2O_2 reagents are fairly stable under the in-situ conditions (~10-15^oC, dark) tested, however explicit testing of reagent duration was only performed for the H_2O_2. Under laboratory conditions (25^oC, dark bottle), the H_2O_2 is stable for at least 27 days (Ratio of fresh 10% H_2O_2 to that stored in laboratory: day 0, 1.0±0.3; day 27, 1.0±0.4, Figure 3.20). The stability of an ambient pH, TEPA-stabilized standard was also tested by creating a 1L 10nM seawater standard, splitting it into two fractions, and acidifying one. The two fractions were analyzed together over the period of a month, and the ratio of the response (pH 8 std / pH 1.7 std.) was recorded. The results (figure 3.21) indicate that there is no change in standard response after 27 days for a 10nM standard kept at pH 8 and stabilized with 200nM TEPA. Given these results, it is unclear why the standard signal dropped 47% during the 8 day deployment in Moss Landing Harbor (Figure 3.15a). One possible explanation is that the standard pump may have slowly weakened, although this wouldn't be expected to yield a consistent decline over time, but still remains possible. The 1-10 phenanthroline may have also degraded over time. While this is also unexpected given the consistency of the Yaquina Bay standard signal, it is possible that the warmer water and greater surface irradiance, especially with respect to UV light, contributed to the faster degradation rate of 1-10 phenanthroline reagent in Moss Landing relative to the Yaquina Bay deployment.

Figure 3.20: A 10% H_2O_2 solution was stored in a dark bottle on the bench top (25°C) and used to make measurements of an acidified seawater standard, which were then compared to measurements of the same standard using a fresh solution of 10% H_2O_2.

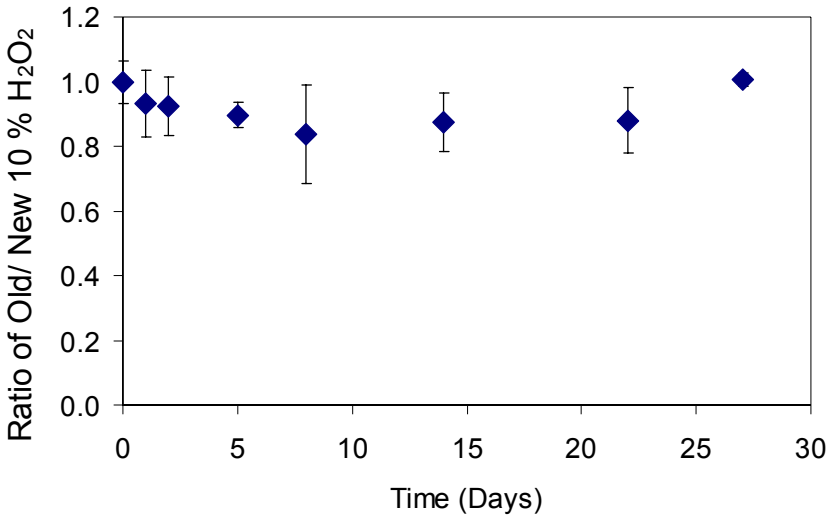
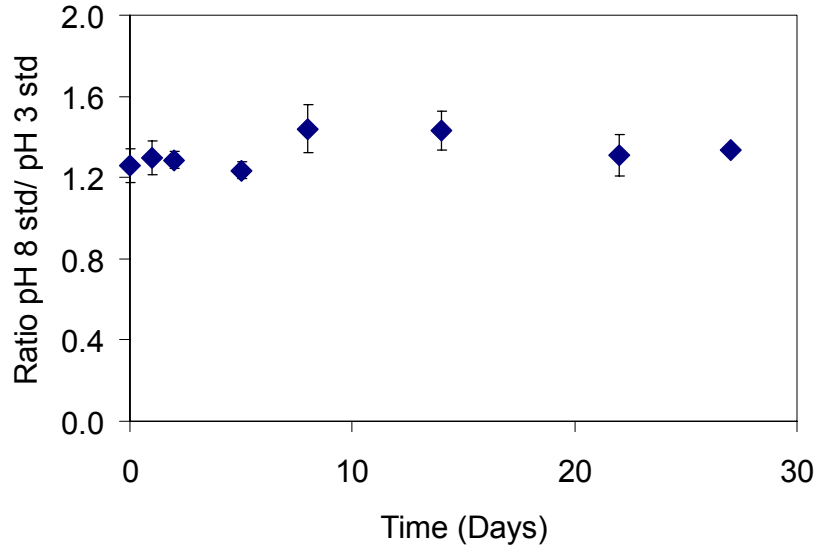


Figure 3.21: Time-series measurements to test the stability of a pH 8, TEPA stabilized seawater standard. Each point represents the ratio of 4 measurements of a pH 8 standard and 4 measurements of a pH 1.7 version of the same standard. Error bars represent the standard error of those measurements.



Chapter 4: Conclusions

Overall, the instrument's precision in-situ was 7%, and accuracy assessed over all of the deployments was 17%. Eight out of twenty-seven discrete samples were determined to be significantly different from the instrument derived measurements using a t-test. The average detection limit in-situ was 0.8nM, however with further optimization of the method, this could be as low as 0.4nM based on its performance in the laboratory. In addition, the instrument's true accuracy may be better than 17%, considering some error may have resulted from the collection of discrete samples rather than instrumental error.

While the instrument generally performed according to our expectations some important shortcomings were noted. Most significantly, the micro-solenoid pumps tended to be difficult to work with since they are extremely sensitive to backpressure and air bubble formation. For example, all of the air must be cleared from both filters immediately prior to deployment otherwise the sample pump will not operate. Additionally, before each deployment every line was checked for proper pump performance and often small adjustments to fittings could mean the difference between a completely functional and a completely non-functional pump. Finally, the pumps tend to wear out quickly and operate sporadically when exposed to moderately high back-pressure. This problem can be difficult to pinpoint without measuring the volume of fluid displaced by each pump under a range of environmental conditions mainly temperature and pressure.

Even with these drawbacks the DigiSCAN (commercially available as YSI 9600) instrument has proven to be a versatile platform for autonomous in-situ

detection of copper(II), and as long as discrete samples are used to monitor instrument performance, this instrument could be used reliably to gather long-term copper data sets for a variety of purposes. Given the potential difficulty of working with and deploying this instrument, the development of electrochemical sensors that are capable of long-term deployment is still advantageous. This is true however, only if these sensors approach the simplicity seen with current in-situ pH, dissolved oxygen, and salinity probes. Currently, this technology does not exist, and given the importance of collecting accurate long-term copper records, this instrument represents an important and practical advancement in in-situ trace metal instrumentation which should be utilized where appropriate.

Moreover, further development of the instrument could allow it to be used for the detection of other species by chemiluminescence, including Co, Mn, and Fe. In addition, copper speciation could be determined with a modified 9600 by using the method published by Zamzow et al. (1998). This is accomplished by titration of copper binding ligands at natural pH with added free copper, which could be performed on the 9600 by adding an additional reagent bag of copper and modifying the control code accordingly. This is an important adaptation from a toxicological perspective since it is free copper, not organically bound copper, that is taken up by aquatic organisms (Sunda and Guillard, 1976).

There is a growing need to understand the cycling of toxic trace metals in the marine environment, and this can only be done through collection of data at greater temporal and spatial resolution than is possible with conventional sampling. The copper modified DigiSCAN instrument (YSI 9600) has the potential to greatly

improve our ability to collect copper data at greater spatial and temporal resolution and is a step toward similar instruments for a suite of other metals. This improved data set could give researchers the ability to further quantify not only the flux of metals from non-point source pollution and anthropogenic sources but also the effect of physical concentrating factors like advection and upwelling on the cycling of toxic species in the marine environment. Furthermore, the commercialization of this instrument by YSI will allow environmental management agencies to add long-term monitoring and input assessment programs for copper to existing marine pollution assessment programs for significantly lower cost than what is currently available. The development of this instrument and others like it represents an important field of research from a basic research perspective, a human health perspective, and a regulatory perspective.

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APPENDIX

Appendix A: Instrument Operations Manual

Introduction

This operations manual is designed as a supplement to the YSI 9600 Nitrate Monitor Operations Manual. Specific information about the parts of the instrument, filling of the reagent bags, and assembling the instrument can be found in the YSI operations manual and will not be repeated here. Reagent preparation and instrument calibration are also not covered as that information can be found in the methods section of the thesis. What will be covered: instrument communication and programming, assembly of the PMT housing, deployment preparation, and general troubleshooting.

Instrument Programming

Communication with the instrument was achieved using a RS-232 serial connection on a laptop computer and Microsoft Hyper-Terminal. A communication profile was set up in Hyper-Terminal using the following settings: connection port= Comm 1, baud rate = 9600, data bits = 8, parity = none, stop bits = 1, flow control = none. Note: in order for this connection to work, the instrument needs to have an updated 9600.hex file, which can be downloaded from YSI (ftp://uploads.sontek.com/pub/outgoing/ysi) following these steps:

To upgrade firmware:
1.) download and install the ysi code updater application
2.) replace the 9600.hex file in the ysi code updater directory (C:\Program Files\YSI Code Updater) with the new .hex file
3.) Run Updater.exe with the 9600 connected

You can check the version of your firmware by running NUview --> System --> Setup. The firmware version will be listed under "Brains Version".

Once a connection is established, communication with the instrument can be achieved using a unique set of commands. A list of those commands can be seen by typing in "list cmd." The instrument is run using an executable "schedule" which is composed of "batches" of specific commands. A list of the available commands and their corresponding definitions can be obtained from the Nitrate Monitor Code Documentation written by David E. Garigen at YSI. The help command can also be useful in defining a command. To use the help command, type "help" followed by a space and then the corresponding command. Schedules can be run in two different ways, using the run or log command. The run command will simply execute the schedule loaded into the RAM and will not save any data associated with the run. This is useful when filling reagent lines, troubleshooting, or when testing instrument performance. The log command and its imbedded commands, will allow the schedule to be run on a prescribed interval, and the data will be saved in a "raw" file. For example, to set up the instrument to run the copper method every hour calibrating ever six hours, starting in 1 hour, and saving the data to file name "deployment. raw" the following commands would be entered:

log sampfile Copper_SAMP (press enter)

log calfile Copper_CAL

log output both

log logfile deployment

log delay 3600

log sampint 3600

log sampspercal 6

Then to check that everything was entered properly, enter,

log

This is what will be displayed:

Sample Schedule = Copper_SAMP
Cal Schedule = Copper_CAL
Log Output = both
Log File = deployment
Instrument ID = 04J15057 AA
Site Name = Yaquina Bay
Delay until start = 3600
Sample Interval = 3600
Samples per Cal = 6
Sample/Standard = 1.0000
Standard (mg/L) = 2.00
OK

Note that time is displayed here in seconds, therefore, 3600 seconds=1 hour.

A list of available schedules can be seen by entering:

dir sch

07/28/06 18:03:34 2484 133400 pumps23
07/28/06 18:36:38 4881 134000 Copper_s_SAMP
07/28/06 21:10:37 5076 137900 Calibrate
07/29/06 12:05:35 5864 139000 Copper_CAL
07/29/06 12:07:00 4904 13AA00 Copper_SAMP
07/29/06 12:08:47 5864 13C000 Copper_CAL_3pt
07/29/06 12:10:06 4904 13DA00 Copper_SAMP_3pt
01/23/06 16:08:26 4382 161C00 pH Test
02/28/05 11:18:19 2484 1B2700 pump0
02/28/05 11:19:47 2484 1B3300 pump1
02/28/05 11:21:19 2484 1B3F00 pump2
02/28/05 11:22:09 2484 1B4B00 pump3
02/28/05 11:22:52 2484 1B5700 pump4
02/28/05 11:24:19 2484 1B6300 pump5
02/28/05 11:36:38 2484 1B7B00 pump6
07/18/06 13:48:18 4382 20E700 pH_TEST_3
01/06/06 11:33:11 1778 275A00 FILL
06/01/05 13:50:24 1801 34E800 Flush

The content of each schedule and its intended purpose is given here:

Copper_SAMP- Used to make sample measurements during deployment

```
Batch Name        #
 0 base           15
 1 filterprep     120
 2 sampprep       20
 3 delay          1
 4 sampflush      2
 5 sample         1
 6 sampprep       2
 7 delay          1
 8 sampflush      2
 9 sample         1
10 sampprep       2
11 delay          1
12 sampflush      2
13 sample         1
14 clean          20
OK

BATCH - base
 0 power pump on
 1 pumps 5 6
 2 delay 500
 3 pumps
 4 delay 1000
 5 power pump off

BATCH - filterprep
 0 power pump on
 1 pumps 0
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - sampprep
 0 power pump on
 1 pumps 0 2
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - delay
 0 delay 90000

BATCH - sampflush
 0 power pump on
 1 pumps 0 2 5 6
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - sample
 0 power pump on
 1 pumps 0 2 5 6
 2 delay 500
 3 pd 1
 4 battery
 5 pumps
 6 measure sig start
 7 delay 1250
 8 measure sig stop
 9 datalabel sample
10 power pump off

BATCH - clean
 0 power pump on
 1 pumps 1
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off
```

Copper_CAL- Used to calibrate the instrument during deployments

```
 # Batch Name      #
 0 base            15
 1 blankprep       20
 2 delay            1
 3 blkflush         2
 4 blank            1
 5 blankprep        2
 6 delay            1
 7 blkflush         2
 8 blank            1
 9 blankprep        2
10 delay            1
11 blkflush         2
12 blank            1
13 stdprep         20
14 delay            1
15 stdflush         2
16 standard         1
17 stdprep          2
18 delay            1
19 stdflush         2
20 standard         1
21 stdprep          2
22 delay            1
23 stdflush         2
24 standard         1
25 clean
20
OK

BATCH - base
 0 power pump on
 1 pumps 5 6
 2 delay 500
 3 pumps
 4 delay 1000
5 power pump off

BATCH - blankprep
 0 power pump on
 1 pumps 1 2
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - delay
 0 delay 90000

BATCH - blkflush
 0 power pump on
 1 pumps 1 2 5 6
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

 BATCH - blank
 0 power pump on
 1 pumps 1 2 5 6
 2 delay 500
 3 pd 1
 4 battery
 5 pumps
 6 measure sig start
 7 delay 1250
 8 measure sig stop
 9 datalabel blank
10 power pump off

BATCH - stdprep
 0 power pump on
 1 pumps 4 2
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - stdflush
 0 power pump on
 1 pumps 2 4 5 6
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off

BATCH - standard
 0 power pump on
 1 pumps 2 4 5 6
 2 delay 500
 3 battery
 4 pd 1
 5 pumps
 6 measure sig start
 7 delay 1250
 8 measure sig stop
 9 datalabel standard
10 power pump off

BATCH - clean
 0 power pump on
 1 pumps 2
 2 delay 500
 3 pumps
 4 delay 1250
 5 power pump off
```

Copper _s_SAMP- used to test sample line performance without the filters in-line. This schedule is exacty the same as Copper_SAMP, except that it has no filter flushing step.

Calibrate- Used to run a calibration curve pre-deployment. The schedule is similar to Copper_CAL, except that it has an additional flushing period to allow standards to be changed out between measurements.

# Batch Name	Counts
0 base	10
1 blankprep	20
2 delay	1
3 blank	4
4 stdprep	60
5 delay	1
6 standard	6
7 stdprep	2
8 delay	1
9 standard	6
10 stdprep	2
11 delay	1
12 standard	6
13 clean	25

Batches are the same as in Copper_CAL

FILL- Used to fill all of the feeder lines pre-deployment.

# Batch Name	Counts
0 FILL0124	40
1 FILL356	40
2 FLUSH2	40

BATCH - FILL356	BATCH - FLUSH2	BATCH - FILL0124
0 power pump on	0 power pump on	0 power pump on
1 pumps 3 5 6	1 pumps 2	1 pumps 0 1 2 4
2 delay 250	2 delay 250	2 delay 250
3 pumps	3 pumps	3 pumps
4 delay 500	4 delay 500	4 delay 500
5 power pump off	5 power pump off	5 power pump off

Flush- Used to clean out all of the feeder lines post deployment

#	Batch Name	Counts
0	FILL356	100
1	FILL0124	100
2	FILL356	100
3	FILL0124	100

Batches are the same as in FILL

Pump(0-6)- used to check each individual pump for proper operation

#	Batch Name	Counts
0	pumptest	1
1	loop2top	10

BATCH – pumptest	BATCH - loop2top
0 power pump on	0 repeat 0
1 pumps (0-6)	
2 delay 500	
3 pumps	
4 delay 1000	
5 power pump off	

Preparing for a Deployment

Prior to each deployment the instrument is calibrated using 6 seawater standards and the Calibrate schedule. This ensures that the instrument is performing properly and that the method is linear over the expected range of concentrations. Next, all air is cleared from the filters by placing them in a beaker of water, and drawing in fluid using a 10mL syringe. This step is crucial, as any air left in the filters will create enough surface tension that the microsolenoid pumps will not be able to pump fluid through. The filters are then placed on the sample line and feeder lines for the blank and standard lines are placed in the same standard as the filters. The instrument is then run, to allow a comparison of each pump's performance relative to the others. Each of the reagent bags are then closed using

the clips on the outflow of the bag, and the instrument is packed up for transport. On site, the reagent bags are unclipped, and each pump is run to ensure that it is functioning properly. The filters are re-primed with the 10mL syringe, and the filter apparatus is pulled through the top and put in a beaker of water to check that the sample pump is indeed functioning. Once all the pumps have been checked the instrument is placed back in the outer cover, and the filter holder is screwed back into place. The instrument is then programmed and deployed. After the deployment is over, the instrument is given the stop command, the bags are re-sealed using the clips, if available, fresh water is used to rinse the instrument off and again the instrument is packed up for transport. Once back in the lab, the bags are unclipped, and the sample line is again calibrated to the blank and standard lines using the same method. If sufficient reagent is left over, the calibration is also performed to ensure the instrument remained linear throughout the deployment.

In summary:

Pre-Deployment-

In Lab:
PMT and battery housings are properly greased and water tight
Calibration curve is run
Sample, blank, and standard lines are calibrated with filters on
On Site:
Open all reagent and waste bags
Check that all flow lines are intact
Check all pumps
Prime filters and check sample line operation
Attach filter housing
Program log information
Deploy

Post Deployment-

On Site:
Close all waste and reagent bags
Upload Data
Clean off instrument with fresh water
In Lab:
Open waste and reagent bags,
Calibrate sample, blank, and standard lines with filters
Run calibration curve
Upload data
Clean instrument, reagent bags, and waste bags

Raw Data is uploaded by typing in command "upload raw (filename) "
Then quickly clicking on Transfer-Receive File-specify path-use Kermit- hit receive. The raw file is converted using a conversion program which converts file from .raw to ASCII.

Disassembling and Assembling the PMT

Disassembly

1) Remove one of the brown ¼-28 fittings in order to break vacuum.
2) Remove end-cap by removing Phillips head screws.
3) Remove other brown ¼-28 fitting and remove tubing from light maze.
4) Using threaded end of Upchurch headless drive, screw into the maze and pull maze out.
5) Remove other end-cap
6) Using blunt object, push on the backside of the PMT to unseat it and push out flow cell.

Assembly

1) Replace all o-rings, and grease
2) Reset PMT
3) Line up flow cell with silver pins and push flow cell into position
4) Pull tubing through light maze holes, but do not thread into the maze.
5) Line up Maze with holes in outer housing and silver pins and install light maze, keeping tubing tight.
6) Thread tubing into light maze and install one ¼-28 fitting.
7) Install end-cap, taking care not to over tighten screw and strip threads.
8) Install other ¼-28 fitting.
9) Install other end-cap again making sure to not over tighten screws.
10) Using electrical tape, tape over all holes and seams.
11) Attach to instrument frame.
12) Attach plug after applying electrical grease.

Troubleshooting

Problem- Cannot Communicate with Instrument

Possible solutions

1) Check serial connection on computer, and/or restart computer.
2) Check Instrument has sufficient power
3) Check Hyper Terminal settings
4) Disconnect all power (including battery) sources from instrument, and reconnect power (reboot instrument).
5) Install new 9600.hex file onto instrument

Problem- Pump is not functioning

Possible Solutions

1) Unscrew all fittings in-line and re-tighten
2) Fill lines with water using a syringe before re-pumping
3) Remove feeder lines and check for backpressure.
4) Add light positive pressure on pump using a syringe.
5) Replace pump.

Problem- Instrument is not tracking concentration

Possible Solutions

1) Check that all pumps are functioning visually
2) Check all pump volumes displaced
3) Check for leaks
4) Check reagents
5) Check that background is not high due to contamination in acid
6) Extend schedule to measure a wider range of points to see whether the PMT is reading the peak.