ABSTRACT

MILLER, WILLIAM JEROME. Redox Chemistry of Six Anaerobic Swine Lagoons in Eastern North Carolina. (Under the direction of Wayne Robarge.)

Emissions of ammonia (NH_3) from swine lagoons have been well documented, but recently the emission of di-nitrogen gas (N_2) has also been reported. Formation of di-nitrogen gas affords a possible mechanism to lessen the impact of swine lagoons on the environment. Currently there are no known mechanisms for di-nitrogen production in swine lagoon systems as used for swine production in North Carolina. The production of di-nitrogen from either organic nitrogen or ammonium (NH_4^+) must involve a change in the redox state of nitrogen. There are few studies of swine lagoons as redox systems, and this research was concerned with detailing the redox processes active in swine lagoons with the goal of identifying a possible mechanism for di-nitrogen formation.

Five lagoons in the eastern North Carolina were monitored weekly from August 2nd, 2000 until February 1st, 2001, and one lagoon from June 7th, 2000 until February 1st, 2001. Three measurements were carried out in the field: Eh, pH, and lagoon temperature. Lagoon fluid samples were collected while field measurements were taken. Fluid samples were analyzed for total and total dissolved (<0.4 micron) concentrations of various elements including manganese, iron, copper, zinc, phosphorous, sulfur, calcium, magnesium, sodium, and potassium.

Measured redox potential (Eh) values ranged from –27 to –220 mV, with a range of mean values for individual lagoons of –87 to –154 mV. Measured pH values ranged

from 7.11 to 8.34. Total iron concentrations ranged from 0.3 to 5.3 mg L^{-1}. Dissolved iron concentrations ranged from 0.1 to 1.0 mg L^{-1}, with a range in mean values for individual lagoons of 0.4 to 0.7 mg L^{-1}. Ammonium concentrations ranged from 135 to 815 mg NH_4-N L^{-1}.

Overall there was no significant stratification of measured lagoon parameters within the lagoon water column. Observation of the data as a function of time suggests that there are temporal trends to the data which seem to occur across all the lagoons studied, implying that changes in weather affect the chemical processes occuring within the lagoons.

Plotting measured Eh and pH values on pe/pH phase diagrams constructed using redox couples assumed to be present in the swine lagoons suggest the presence of an active iron (Fe^{2+}/Fe^{3+}) redox couple. In addition, using chemical speciation software (MinteqA2) and analytical data, calculated equilibrium Eh values compared favorably to measured Eh values when the iron redox couple was considered the potential determining reaction.

Ferric iron (Fe^{3+}) represents an electron acceptor that can be used to drive the oxidation of ammonium to nitrate/nitrite, ultimately resulting in the production of di-nitrogen gas. Ferric iron, produced by the oxidation of dissolved ferrous iron (Fe^{2+}) by oxygen diffusing through the lagoon surface, could exist as a separate solid phase in the lagoon effluent or possibly bound to a microbial surface

Thermodynamically the conversion of ammonium to nitrite via oxidation by ferric iron could occur according to the following reaction:

$$Fe(OH)_3 + 1/6\ NH_4^+ + 5/3H^+ = Fe^{2+} + 1/6NO_2^- + 8/3H_20$$

The equilibrium constant for this reaction is $10^{0.7}$, which although small results in a negative Gibbs free energy of reaction of 1.6 kcal/mol.

Alternatively ammonium could be oxidized to form nitrate with hydroxylamine formed as in an intermediate according to the two following reactions:

$$NH_4^+ + 2Fe(OH)_3 + 3H^+ = 2Fe^{2+} + NH_2OH + 5H_2O$$

$$1/6NH_2OH + Fe(OH)_3 + 11/6H^+ = 1/6NO_3^- + Fe^{2+} + 8/3H_2O$$

The equilibrium constant for the oxidation of ammonium is $10^{-5.2}$, while the equilibrium constant for the oxidation of hydroxylamine is $10^{4.5}$.

Nitrate/nitrite produced by these two processes could be used to oxidize organic matter while generating di-nitrogen gas, referred to as the denitrification process, liberating a significant amount of energy for microbial growth.

Redox Chemistry of Six Anaerobic Swine Lagoons in Eastern North Carolina

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Soil Science

Raleigh
2002

APPROVED BY:

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Chair of Advisory Committee

Biography

William Jerome Miller was born in Staten Island, NY on the morning of April 30, 1977. Mr. Miller was raised in central New Jersey until the age of 14, when he and his family moved to Chesapeake, VA. He attended James Madison University in Harrisonburg, VA and graduated with a B.S. degree in Geology with a minor in Chemistry. From there he moved to Currituck, NC., before moving to Raleigh, NC. to attend North Carolina State University.

Acknowledgements

It gives me great satisfaction to be able thank the many people who have helped me in the prepartion and execution of this project. I have deep appreciation for the scientific knowledge and guidance provided by my graduate committee members Dr. Wayne Robarge, Dr. John Classen, and Dr. Dean Hesterberg. I would also like to thank Guillermo Ramirez and Lisa Lentz for their technical support. Finally, I would like to thank my family for the support and love they have always shared with me.

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Chapter 1

Introduction to Swine Production in North Carolina: Economics and Environmental Issues

According to the North Carolina Department of Agriculture and Consumer Services and the Department of Environment and Natural Resources, North Carolina ranks as the second largest pork producing state in the nation, second to Iowa, producing about 1/6 of the nations pork (**3,5**). For the 2000 fiscal year, swine production ranked 1st among major North Carolina farm commodities, with a gross income of 1.65 billion dollars (**3**). As of March 2002, all hogs and pigs on North Carolina farms totaled 9.6 million head, with 8.6 million head consisting of market hogs and 1 million head of breeding stock. U.S. hog inventory as of March 2002 totaled 58.7 million head (**3**).

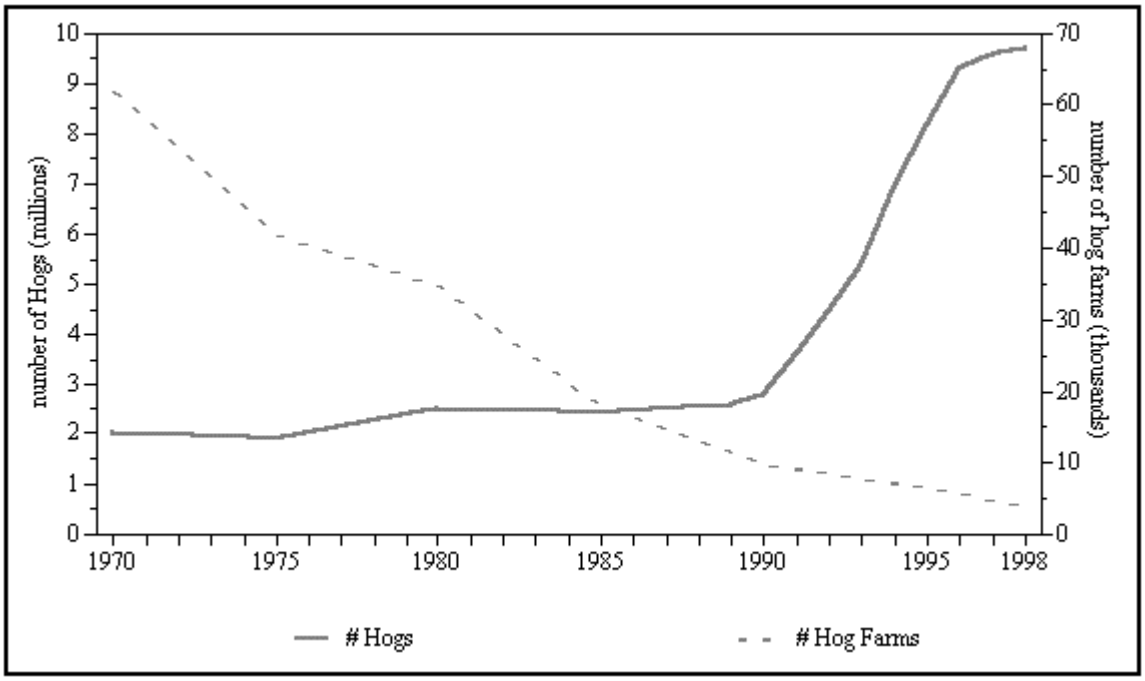
The growth of the swine industry in North Carolina has been rapid over the past two decades. In 1985 there were 2.35 million animals in the state, in 2002 approximately 9.6 million (**3**). During this period of growth there has been a decrease in the number of hog farms operating (See Figure 1-1, taken from **5**).

For the year 2000 there were 2,000 hog operations having 499 or less animals, representing 0.5 % of the total state hog inventory. In contrast, there were 640 operations having 5,000 or more animals, representing 76% of the total state hog inventory (**3**).

The trend associated with this growth has been away from what could be considered traditional farming to modern confined animal feeding operations (CAFOs). According to the U.S. EPA an animal feeding operation (AFO) is an operation where animals are confined and maintained for a total of 45 days or more in any 12 month

period, and crops, vegetation, forage growth, or post harvest residues are not sustained in the normal growing season over any portion of the facility. An AFO becomes a CAFO if

Figure 1-1: Growth in the North Carolina swine herd and swine farms from 1970 through 1998.



it either has more then 1,000 animal units, or if it has more then 300 animal units and discharges pollution (waste) into surrounding surface waters. Alternatively, the U.S. EPA can designate a facility a CAFO, regardless of size, if it decides the facility "is a significant contributor of pollution to the waters of the U.S." (**6**).

Waste generated from swine confinement houses collects in pits under the houses, which is then gravity fed or pumped into a lagoon system. Lagoon systems function both to store waste and reduce the waste biochemical oxygen demand (BOD) through

biological decomposition. Lagoon liquids are removed and applied to adjacent fields as fertilizer for agronomic crops or pasture land during the growing season (**15**).

Further growth in the swine industry has been halted by a law passed in 1997 by the North Carolina Legislature, the Clean Water Responsibility and Environmentally Sound Policy Act. This law imposed a moratorium on the construction of new and expanded swine operations with 250 or more hogs using lagoon systems and spray fields for waste treatment and disposal. The purpose of the moratorium was to ultimately develop a plan to phase out the use of anaerobic swine lagoons and spray fields, and also to allow time for research on the environmental impact of using this system (**2**).

The culmination of this moratorium to date has resulted in the Smithfield and Premium Standard agreement. This agreement has made 17.5 million dollars available for development and demonstration of an environmentally and economically sustainable alternative to lagoon systems for the treatment of swine waste (**1**).

Concern regarding CAFOs and lagoon systems can be grouped into 4 issues: water quality, air quality, nutrient loading in manure application fields, and nuisance (odors) and health effects (pathogens) of aerial emissions (**52**). The issue of nuisance and health effects of emissions concerns the release of odors and possible pathogens into communities and waters surrounding CAFOs. Nutrient loading is a concern when land application of manure or lagoon effluent exceeds the crop utilization potential in the receiving field, and nutrients accumulate, particularly phosphorous (P) and trace metals (Cu and Zn). Water quality concerns are based on two potential vectors for contamination: surface and ground water. Surface waters can become contaminated due to runoff of manure application, erosion of manure laden soils, and overland flow of

lagoon fluids during catastrophic events. Groundwater can become impacted due to seepage through lagoon liners and from infiltration of nutrients from manure laden fields. Air quality concerns focus on the emissions of ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), volatile organic compounds (VOCs), pathogens, odor, and particulates (dusts) from CAFO houses and from lagoon systems (**52**).

Recently while monitoring gas emissions from lagoons in eastern North Carolina and Georgia the emission of di-nitrogen gas (N_2) was observed (**19**). Currently there are no known mechanisms for di-nitrogen production in lagoon systems. The production of di-nitrogen (a compound where nitrogen is in its ground state) from either organic nitrogen or ammonium (NH_4) (where both are electron rich nitrogen species) must involve a change in the redox state of nitrogen. There are few studies of lagoons as redox systems, although lagoons have a unique juxtaposition of redox extremes, with a reduced methane producing environment in direct contact with the oxygen laden atmosphere. This research was concerned with detailing the redox processes occurring in lagoon environments

A Brief Overview of Lagoon Treatment Systems Used in N.C. Swine Production

In North Carolina swine waste is primarily stored and treated using lagoon treatment systems. Due to high waste loading rates lagoon systems in North Carolina function anaerobically, and as such are commonly referred to as anaerobic swine lagoons. As of 1998, when the moratorium on lagoon construction was enacted, there were approximately 2,400 major swine operations in North Carolina with approximately 4,000 active lagoon systems (**5**). A swine lagoon can be defined as an earthen lined man-made

lake used for the containment and treatment of waste generated during hog production. Wastes from the animal houses are flushed or pumped with water from the buildings and are stored in lagoons. For swine operations in North Carolina, liquid is drawn from lagoons and applied to adjacent fields to fertilize pastures or crops.

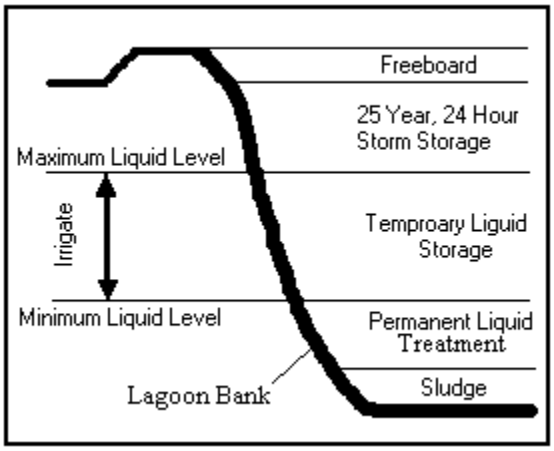
Lagoon Design

Lagoons are excavated pits with the excavated soil used to build embankments and, if clay rich, used as a liner material to prevent seepage. Waste that is collected under the swine houses is flushed out with lagoon fluid and is either gravity fed or pumped to the lagoons. Included with this recycled lagoon effluent is any waste as well as spilled drinking water. Another fluid input to lagoon environments is through precipitation.

Lagoons are lined to decrease the permeability of the sides and bottom of the lagoon. This reduces or prevents the loss of fluid from the lagoon environment. Seepage of lagoon fluid could result in the contamination of the underlying subsoil and groundwater (**47,50**). Typically liners are constructed of compacted clay layers. As mentioned, soil excavated during lagoon construction is used if there is sufficient clay content. If excavated soil is not suitable for lining material, bentonite is usually imported and blended with the soil (**47**).

For operational purposes the lagoon system can be divided into five stratifications (see Figure 1-2) which are based on storage volumes (**14**).

Figure 1-2: Vertical lagoon stratifications. Note: Diagram not to scale.



The sludge layer is an accumulation of settleable organic solids that settle to the bottom of the lagoon. Above the sludge layer is the permanent liquid treatment zone, where there is the largest amount of biological activity and waste degradation. This zone is designed to hold the minimal amount of water necessary to promote biological activity. The temporary liquid storage zone, located above the treatment zone, is effectively the water available for irrigation. It is a volume based on the amount of wastewater, rainfall, and extra wash water that will enter the lagoon during periods when liquids cannot be irrigated onto a growing crop. Above the storage zone is a volume designated the storm storage zone. It is designed to hold twice the most rainfall likely to occur in 25-year period over a 24 hour duration, which for North Carolina is estimated to be from 10 to 18 inches. Finally there is the freeboard zone, which is the difference between the lowest point of the base of the lagoon embankment and the highest level the liquid should be

allowed to accumulate. There is a 1 foot minimum in design of the freeboard zone in North Carolina.

A well designed lagoon provides 3 specific functions (**32**):

1) It provides an environment for microorganisms to decompose the organic constituents of the waste into simpler organic compounds;
2) They act as storage for waste water until the effluent can be disposed of through land application;
3) They provide a facility to capture manure contaminated runoff from adjacent fields This last benefit is generally not applicable to farms in North Carolina, especially in eastern North Carolina, as most lagoons are built above ground to avoid contact with the water table.

The objectives of the anaerobic biological treatment of swine waste water are two-fold (**16**):

1) To coagulate and remove non-settleable colloidal solids
2) To stabilize the organic matter constituent of swine waste

Coagulation- An important benefit of the lagoon treatment of waste is nutrient and particulate BOD reduction in the effluent through settling. Microorganisms accumulate nutrients and carbon into cell tissue during growth, and these cells function to form biological flocs composed of the cells and inorganic colloidal matter. These flocs are then removed from the water column through gravity settling (**16**).

Stabilization of waste- Lagoons are designed to operate as a biological reactor. This process occurs through anaerobic digestion (**16**). Anaerobic digestion is a process in

which microorganisms are used under anaerobic conditions to convert biodegradable organic materials to odorless gases, non-biodegradable solids, and cell tissue. There are three steps to this process: hydrolysis, acidogenesis, and methanogenesis (**54**).

The first step, hydrolysis, consists of the hydrolysis of organic polymers and lipids by a group of organisms into simpler building blocks such as monosaccharides, amino acids, and other compounds suitable as a source of energy and cell carbon. The second step consists of the anaerobic fermentation of these building blocks to simple organic acids, the most common of which is acetic acid. The organisms responsible for this step are facultative and obligately anaerobic bacteria. Collectively these organisms are referred to as acid formers or acidogens (**16**). The third and final step in anaerobic digestion consists of the conversion of the acetic acid formed in step two to methane and carbon dioxide. The bacteria responsible for this process are strictly anaerobic and are referred to as methanogens or methane formers (**16**).

Lagoon Microbiology

As mentioned above, anaerobic treatment is carried out by a wide variety of bacteria that can be categorized into 2 groups, the acid formers and the methane formers (**16**).

Acid Formers- The acid forming bacteria are facultative or obligately anaerobic heterotrophs. The production of energy is through the conversion of complex organic matter into simple alcohols and acids, primarily acetic, propionic and butyric acid. These metabolic end products then form the substrate for the methane formers. Optimum

growth of these bacteria occurs in thermophilic (49-57 ^{o}C) and mesophilic (30-38 ^{o}C) temperature regimes (**47**).

Methane Formers- Methanogens are a group of bacteria responsible for methane production in anaerobic environments. Methane formers thrive in anaerobic conditions rich in organic matter and are strictly anaerobic organisms. Most methanogens require thermophilic conditions (49-57 ^{o}C) to achieve optimum growth although growth occurs under mesophilic conditions (30-38 ^{o}C) (**54**). Stabilization of swine waste during anaerobic treatment occurs with the production of methane and carbon dioxide. Methane gas is highly insoluble and is volatilized from solution (**16**). The methanogens are considered the rate limiting factor in the anaerobic digestion of waste, usually because of the high temperature dependence for optimum growth. As such, whether an anaerobic treatment process can successfully stabilize the waste depends largely on the function of the methanogens (**47,54**).

Sulfur Bacteria- Another group of microorganisms active in swine lagoon systems are the sulfur bacteria. As we have mentioned anaerobic digestion of waste results in the reduction and removal of carbonaceous material. In contrast to the carbon flux from the system, as a result of the flow of carbon from the acid formers to the methanogens, the flow of sulfur in anaerobic environments is cyclical. This is the result of the activities of the desulfurizing (sulfur reducing bacteria) and the purple sulfur bacteria (sulfur oxidizing bacteria). Sulfate is reduced to sulfide via the sulfur reducing bacteria, while sulfide and reduced sulfur compounds are oxidized to sulfate by the sulfur oxidizing bacteria (**49**).

Purple Sulfur Bacteria- Purple sulfur bacteria grow anaerobically in the presence of light and are responsible for the conversion of reduced sulfur compounds to elemental sulfur or sulfate (**47**). This process is used not for energy production but rather as a means of reducing carbon dioxide, which is used as the terminal electron acceptor, for cell synthesis. Purple sulfur bacteria have a limited ability to utilize organic compounds as carbon sources for phototrophic growth, with most using acetate or pyruvate. A few species will grow chemolithotrophically with thiosulfate as the electron donor, and *Thiocapsa* will grow chemoorganotrophically on acetate. When sulfide concentrations in the environment are high, these bacteria will internally store elemental sulfur (**13**)

Sulfate Reducing Bacteria- With this morphologically diverse group of bacteria sulfate is used as a terminal electron acceptor under anaerobic conditions for the oxidation of a broad range of electron donors, such as organic acids, fatty acids, alcohols, and hydrogen gas. All sulfate reducing bacteria are obligate anaerobes. This diverse collection of organisms can be divided into two groups. One group utilizes lactate, pyruvate, ethanol, or certain fatty acids as carbon and energy sources with the concomitant reduction of sulfate to hydrogen sulfide. The other group specializes in the oxidation of fatty acids, particularly acetate, while reducing sulfate to sulfide (**13**).

Environmental Issues Involving Lagoon Systems

Environmental concerns with respect to the use of lagoon systems can be divided into four categories: concerns related to the soil (nutrient accumulation), water quality (surface water and groundwater contamination), air quality (ammonia emissions, odors), and human pathogens (**5,23,52**).

When excessive land application of waste exceeds the crop utilization potential of the receiving field, inorganic minerals such as phosphorous, trace metals, and nitrogen accumulate in the soil. This poses a potential risk for plant growth and human health (**23,52**).

To maintain the large number of animals associated with CAFOs feed must be imported to the facility. Swine, like all animals, do not have 100% efficiency in food digestion. It is reported that swine excrete approximately 75% of the nitrogen and phosphorous consumed in their feed (**52**). Swine lagoon effluent contains considerable amounts of these and other nutrients (Table 1-1).

Table 1-1 : Mean characteristic of anaerobic swine lagoon liquid. Data reported from the Biological and Agricultural Engineering Department NCSU, 1994.

Nutrient	lbs nutrient/acre-inch
TKN	128
P_2O_5	51
Zn	0.70
Cu	0.27

Over-application of this waste to agronomic crops becomes an issue when application exceeds nutrient removal. Using a reported example as illustration, in 1993 590, 000 sows and 10 million top hogs in North Carolina combined to produce approximately 9 million tons of fresh manure. Accordingly, 11.5 tons of fresh manure applied as a fertilizer would be enough to provide a one inch cover on 100,000 acres. This amount of fresh manure would suffice to provide all of the fertilizer needs for North Carolina's 6 largest agricultural counties: Johnston, Sampson, Pitt, Wilson, Nash, and Robeson (**4**).

Nitrogen Accumulation- There is concern over the fate of nitrogen in land applied effluent. Once effluent is applied nitrification of the deposited NH_4^+ in the soil environment can lead to soil acidification. Nitrate, which is the product of the nitrification of ammonium, is highly mobile in the soil profile and can be transported with groundwater from land where effluent is applied.

One study attempted to evaluate the effect of swine lagoon effluent on the soil chemical properties of a receiving field. Effluent was applied to research fields of loamy sand containing hybrid Bermuda grass and annual ryegrass at loading rates of 560, 1120, and 2240 kg N ha^{-1} yr^{-1} (**27**). The results of the study show an accumulation of KCl extractable NO_3-N to a depth of 120 cm, while there was no significant accumulation of NH_4-N. Extractable soil phosphorous (Mehlich 3 solution extractable) accumulated mainly in the upper 40 cm of the soil profile, with an initial concentration of 15 mg kg^{-1} in the upper 20 cm and a final concentration of 45 – 110 mg kg^{-1} for the 1 and 4 times the regular effluent rate respectively. There was no significant increase of soil carbon (C), extractable soil copper, zinc, and manganese (Cu, Zn, and Mn), while there was a general increase in soluble salt accumulation (Ca, Mg, K) (**27**).

Phosphorous Accumulation- Phosphorous accumulation in the surface layer of agricultural land is associated through erosion and surface runoff with the contamination of surface waters. Soils in the coastal plain region of North Carolina are generally coarse textured and are highly permeable, with low P-fixation capacity, making them susceptible to subsurface and surface movement of phosphorous. In order to evaluate the movement of phosphorous on land receiving swine waste 16 swine farms in North Carolina were studied (**18**). It was observed that at the surface of land receiving swine waste the average

total Mehlich III extractable phosphorous concentration was greater then 250 mg P kg^{-1}. The average natural background concentration for the soil studied was determined to be 10 mg kg^{-1}. Significant vertical phosphorous movement was also observed, with an average subsurface P concentration of 50 mg P kg^{-1}. No phosphorous fertilizer recommendations for agronomic purposes are given when the P concentration exceeds approximately 100 mg P kg^{-1}.

Trace Metal Accumulation- The accumulation of trace metals is also of concern, especially copper and zinc, which are usually present in swine manure exceeding the nutrient requirements of receiving crops. On a coastal plain field of hybrid Bermuda grass pasture receiving swine lagoon effluent accumulation of Cu and Zn, as well as P and K was observed (**31**). Soil samples were taken in 1990 and again in 1992 at intervals of 0-15, 15-30,30-60, and 60-90 cm. In the top layer of soil (0-15 cm) copper and zinc increased from an average of 0.9 to 5.3 and 2.6 to 10.5 kg ha^{-1} respectively. Accumulation in the deepest subsoil (60-90 cm) was also observed, with copper and zinc concentration increasing from an average of 0 to 2.5 and 0.5 to 2 kg ha^{-1} respectively.

Surface Water Eutrophication- Concerns related to swine waste impact on water quality can be divided into two issues: surface and groundwater contamination. Potential pathways for surface water contamination involve runoff from manure application fields, erosion of manure applied soil, and direct discharge from lagoon systems. Groundwater contamination can result from seepage through lagoon liners, and nutrient infiltration on manure applied fields (**5,52**).

In the soil environment ammonium applied with lagoon effluent is oxidized to nitrate, which is highly mobile and readily leached from the soil profile. This is especially

a problem with sandy soils, which have little anion exchange capacity for fixing nitrate. That is the case with most swine operations in North Carolina, as 90% of North Carolina hogs are raised in the coastal plain region (**4**). Increased nitrogen deposition in nutrient sensitive areas in coastal and marine ecosystems, through atmospheric deposition or contaminated groundwater, stimulates new growth that can lead to harmful algae blooms.

Nitrogen movement from land applied effluent has been evaluated using isotopic nitrogen as a tracer (**41**). The ratio of $^{15}N/^{14}N$ nitrogen in nitrogen containing compounds is dependent on the source of the compound. This ratio is distinctly higher for swine waste lagoon effluent then for naturally occurring nitrogen compounds in the environment. Two farms, each with 1000 sow farrow to feeder animals, with typical anaerobic lagoon systems and spray irrigation of effluent on fields of Bermuda grass pasture were investigated (**41**). Soil samples were taken from the waste application fields, on the edge of the field, in the riparian buffer zone, and in a stream located adjacent to the spray field. These samples were then analyzed for nitrate concentration. All samples showed an increase in nitrate concentration over background levels, and this surplus of nitrate had an average $^{15}N/^{14}N$ isotopic ratio identical to that of the lagoon liquid. This indicated that nitrate which was accumulating under the application field was moving through the surface water into the adjoining stream. It was noted that the riparian zone reduced the concentration of nitrate 25 to 100 times the concentration found in the application field (**41**).

Besides the dangers associated with the use of lagoon effluent, there is also the inherent danger of using lagoon systems. Storing large amounts of waste in earthen lined lakes there is always the possibility of lagoon overflows during catastrophic events, and

leakage due to failure of lagoon integrity (**32**). Lagoon failures have released millions of gallons of waste directly into surface waters resulting in eutrophication, with concomitant fish kills and high pathogen loading (**15**). In 1995 a breach of a hog lagoon resulted in the release of approximately 2 million gallons (7.6 million liters) of waste into surrounding surface waters (**28**). The spill caused high turbidity, low to negligible dissolved oxygen concentrations, high nutrient loading, and high fecal coliform concentrations in receiving waters. Ammonium concentrations remained high for a period of 14 days after the spill, with a maximum concentration of 47 mg NH_4-N L^{-1}. The phosphorous concentration in the receiving waters reached a high of 11.5 mg P L^{-1}. Some effects of the spill were seen in elevated nutrient parameters at a station 18 km downstream from the site.

Groundwater Eutrophication- The effect of spray irrigation of lagoon effluent on a receiving field, a riparian buffer zone, and an adjacent stream in the coastal plain region of North Carolina was evaluated in order to assess groundwater impacts (**43**). During the study the farm in question expanded, with an increase of 3,300 head to 14,000 head. Seven wells were placed: three at the edge of the receiving field and four in the riparian zone. Of the seven wells four, three in the receiving field and one in the riparian zone, showed an increase in groundwater nitrate-nitrogen (NO_3-N). Two wells exceeded the drinking water standard of 10 mg NO_3-N /L, with concentrations of 11.1 and 12.1 mg NO_3-N /L. Stream water NO_3-N showed no increase in warm months, presumably due to vegetative uptake in the riparian zone, but showed significant increase during cold months. NH_4-N concentration in samples showed no dependence on the time of year, but showed a significant increase after the expansion of the operation.

Seepage through lagoon lining has also been documented (**50**). Seepage of lagoon effluent was observed to be prevalent in older lagoons with no liner, even 3.5 to 5 years after receiving waste. Before the implementation of liners in lagoon construction waste storage in lagoons was believed to naturally seal lagoon systems through chemical reactions and clogging at the soil pore level. Although lagoons with clay liners were determined to be effective when managed properly, there is always the danger of damaging the liner.

Atmospheric Contamination

Lagoons are also emission sources of many gases that are considered potential health and environmental hazards. Four areas of concern are the emissions of ammonia (NH_3), hydrogen sulfide (H_2S), methane (CH_4), and odorous compounds.

Odor- Anaerobic digestion results in the production of a variety of volatile fatty acids whose odor people often find offensive. These odorous volatile compounds are the end products or intermediate products of the fermentative breakdown of fecal matter by anaerobic bacteria (**54**). There have been some 150 volatile organic compounds that have been identified and isolated from swine waste. These compounds are the result of biological processes and include organic acids, alcohols, aldehydes, fixed gases, carbonyls, esters, amines, mercaptans, and nitrogen heterocycles (**4**). Contact with these compounds may cause irritation to the eye, nose, and throat, can affect respiratory volume, and can induce inflammatory responses (**15**).

Methane- The emission of methane gas is an issue, as methane has been indicated as a contributor to global warming. Methane is a product of the breakdown of fatty acids and simple organic compounds by methanogenic bacteria in anaerobic lagoons.

Measurements of biogas production (CO_2 & CH_4) from 3 anaerobic swine lagoon in North Carolina were taken to determine the rate of biogas production (**38**). The mean apparent biogas productivity for all three lagoons was 0.04,0.03,and 0.03 $m^3m^{-3}day^{-1}$. The composition of the biogas collected was determined to be between 95-86% CH_4 and 5-14% CO_2.

Hydrogen Sulfide- Hydrogen sulfide has been identified as a gaseous product of the microbial decomposition of methionine and other sulfur compounds present in manure (**9**), as well as a product of bacterial sulfate reduction (**8**). Hydrogen sulfide at high concentrations is an eye and respiratory tract irritant and can cause instant respiratory arrest and death (**15**). Chronic exposure to low concentrations can lead to airway inflammation and a decrease in lung function (**4**). In fresh swine manure incubated under anaerobic conditions the production of sulfur gases has been observed (**9**). Most of the sulfur volatilized was in the form of H_2S and CH_3SH, and the total amount of sulfur volatilized after one month of incubation at 23 ^oC represented less then 1% of the total sulfur content of the manure.

Measurements from 3 swine operations in North Carolina documented emissions of H_2S from lagoon surfaces (**38**). For all three lagoons the mean biogas productivity was 0.03 $m^3m^{-3}day^{-1}$. This collected gas displayed a range of H_2S concentrations from 30 to 850 mg H_2S/L.

Ammonia- Ammonia from in swine lagoons originates from the large input of urea in swine urine (**23**). Urea is readily hydrolyzed into ammonium, which volatilizes from solution as ammonia. Deposition of ammonia in nutrient sensitive areas is suspected of contributing to eutrophication of surface water, and has deleterious health effects

including irritation to the eyes, skin, mucous membrane, and upper respiratory system (**15**). The North Carolina Division of Air Quality estimates that 90,000 metric tons of NH_3 was volatilized from swine farms in the state of North Carolina in 1995 (**41**). Emissions of ammonia from two lagoons in North Carolina averaged 10.5 and 6.2 kg NH_3-N ha^{-1} d^{-1} (**20**). Both lagoons were of similar size, 2.67 and 2.37 hectares respectively, but the lagoon with the higher ammonia flux had a higher average lagoon ammonium concentration, 636.8 mg NH_4 L^{-1} as opposed to 201.0 mg NH_4 L^{-1}.

There is also concern about gas emissions, particularly ammonia (NH_3) and nitrous oxide (N_2O), during spray application. In an experiment attempting to quantify the emission of these gases during sprinkler application of swine lagoon effluent large nitrogen losses where observed. During the irrigation process, approximately 13% of the ammonium-nitrogen (NH_4-N) in the effluent was lost through NH_3 volatilization before reaching the crop or soil surface. In the 24 hour period following irrigation approximately 82% of the NH_4-N in the applied effluent was lost. Total N_2O loss during the measurement period was 4.7 kg N_2O-N ha^{-1}, which accounted for approximately 13% of the total N applied (**40**).

In order to evaluate the air quality in the vicinity of a swine production facility, air samples where collected from outside a swine house and analyzed for ammonia and hydrogen sulfide (**28**). Air samples were collected at a distance of 60 meters from the swine houses at a height of 2 meters. The mean outdoor ammonia concentration for a large confinement operation, which had three manure lagoons on site, was 0.251 ppm while a control, non swine producing farm had undetected ammonia concentrations (< 0.004 ppm). H_2S was not detected in any of the samples.

Dry deposition of ammonia in areas surrounding an anaerobic swine lagoon was evaluated using throughfall and bulk deposition collectors. Emissions from a swine operation housing 11,000 head and using a 2.5 hectare lagoon were found to significantly enhance dry deposition of ammonia in adjacent forest canopies (**37**).

Pathogens- Recently the potential impact of contact with human pathogens present in swine waste has become an important issue. Swine wastes contain numerous pathogenic microorganisms (bacterial, viral, protozoan) that could potentially infect humans. Analysis of raw swine manure found fecal colliform bacteria concentrations of up to 100 million organisms per gram of waste (**15**).

Another concern is contact with pathogenic organisms through aerosols or dust particles. Dusts from swine facilities can contain molds, bacteria, insect parts, pollen, grain particles, mineral ash, animal feed, animal fluid, dander, and excreta (**4**). Respiration of pathogenic organisms represents a possible vector for human infection.

Aqueous Redox Chemistry

Fundamentals of Redox Chemistry

A chemical reaction that involves the transfer of an electron from one species to another is referred to as a redox reaction (**39**)**.** The term redox is taken from the terms reduction and oxidation which are used to describe redox reactions. A reduction is defined as the gain of an electron, while an oxidation is defined as the loss of an electron. Species that lose electrons in a redox reaction are referred to as reducing agents or reductants. Species that gain electrons during a redox reaction are referred to as oxidizing

agents or oxidants (**46**). A conceptual model for a generalized redox reaction can be represented by the following equation (**12,46**):

$$\mathbf{Bo + ne^- = Br} \qquad \mathbf{(1)}$$

Where Bo represents the oxidized species of component B, ne^- refers to n number of electrons (e^-) being transferred, and Br represents the reduced form of component B. Equation 1 represents the reduction of a chemical species labeled B. Such a reaction is referred to as a half reaction. A redox reaction therefore involves the coupling of two half reactions, a reduction and an oxidation.

The tendency of a chemical species to lose or gain electrons when in contact with another body is an inherent property of the species. Redox reactions occur when one species has a greater tendency to attract electrons then another, and electrons are transferred between the two species. The propensity for a chemical species to either lose or gain electrons relative to other chemical species can be measured as an electrical potential (**39**). The term used to describe this electrochemical potential for chemical species is referred to as the redox potential or ORP (Oxidation/Reduction Potential) and is symbolized by the letter E or Eh. In order to quantify the electrochemical potential of a species, it being a relative quantity, a point of reference was established in order to define a zero point for redox potential measurements. The point of zero potential was established as the potential established at the half reaction equilibrium between hydrogen ions and hydrogen gas molecules (**21**). This half reaction is represented by the following reaction:

$$\mathbf{0.5H_2 = H^+ + e^-} \qquad \mathbf{(2)}$$

Where the activity of the hydrogen ion and the electron is unity and the partial pressure of hydrogen gas is 1 atm at 298.15 K.

Species with excess positive charge relative to this zero potential are defined as having a positive potential while species with excess negative charge are defined as having a negative potential. The tendency in nature is for electrons to move from bodies with negative potential to bodies with positive potential.

Half reactions represent independent chemical reactions in themselves. As such there exists a state of equilibrium between the two oxidation states of a chemical component, referred to as B above, where the gain of electrons by species Bo is equal to the loss of electrons by species Br. This equilibrium is an inherent property of the component, B. Therefore for every redox reaction that goes to equilibrium there can be defined a unique equilibrium constant, analogous to the approaches used for dissolution or dissociation reactions. The only new consideration is for the electron transfer (**42**). The equation representing the ratio of redox species at thermodynamic equilibrium can be stated as (**46**):

$$\mathbf{K = (Br)/(Bo)(e^-)^n} \qquad \mathbf{(3)}$$

Where K is the equilibrium constant, and (Br), (Bo), and $(e^-)^n$ represent the activities of each species.

Since the equilibrium between redox reactive species also has an inherent potential, the redox potential (Eh) can be related to the thermodynamic equilibrium constant by the following equation (**46**):

$$Eh = RTlnK/ nF \quad (4)$$

Where Eh is the redox potential in volts, R is the gas constant, T is the temperature in Kelvin, K is the equilibrium constant, n equals the number of electrons transferred between species, and F is the Faraday constant.

As mentioned before, free electrons cannot exist as independent species in solution. However by rearranging the equilibrium expression to isolate the electron species, and for simplicity assuming a one electron transfer (n = 1), a useful relationship can be developed (**46**):

$$pe = pK – log[(Br)/(Bo)] \quad (5)$$

Where pe = -log (e-) and pK = -log K. The parameter pe represents the hypothetical equilibrium activity of an electron in solution and can be used to determine the tendency for a solution to either accept or transfer electrons, similar to hydrogen ion activity. Reactions with a high pe indicate a high tendency for oxidation, while reactions with a low pe indicate a high tendency for reduction (**46**). Although pe is a theoretical concept it has a quantifiable analog, the electrochemical redox potential (Eh), which can be related by the following equation (**46**):

$$pe = [F/(2.3)(R)(T)]Eh \quad (6)$$

Where F is the Faraday constant, R is the gas constant, and T is the temperature in Kelvin.

Another useful relationship that can be derived for redox reactions involves the relationship of the measured redox potential, Eh, with the composition of the solution and is referred to as the Nernst equation (**46**). This relationship describes how the potential

measured for a given redox reaction changes as the activities of the species involved changes. The mathematical representation is as follows:

$$\mathbf{Eh = Eh^o + (2.3\ RT/nF)log\ [(ox)/(red)]} \qquad \mathbf{(7)}$$

Where Eh is the measured redox potential, Eh^o is the standard redox potential (all activities at unity and all partial pressures at 1 atm), (ox) is the product of the activities of the reactants (with the reaction written as a reduction half reaction), and (red) is the product of the activities of the products.

According to the laws of thermodynamics the tendency for a process to occur is towards a reduction in the free energy of the system. Understanding that the free energy of the system can be thought of in terms of electrical potential, a measurable quantity, we can use the following relationship to predict if it is likely that a redox process will occur (**46**):

$$\mathbf{\Delta G_r^o = -nF(Eh)} \qquad \mathbf{(8)}$$

Where ΔG_r^o = the change in free energy of a reaction under standard conditions.

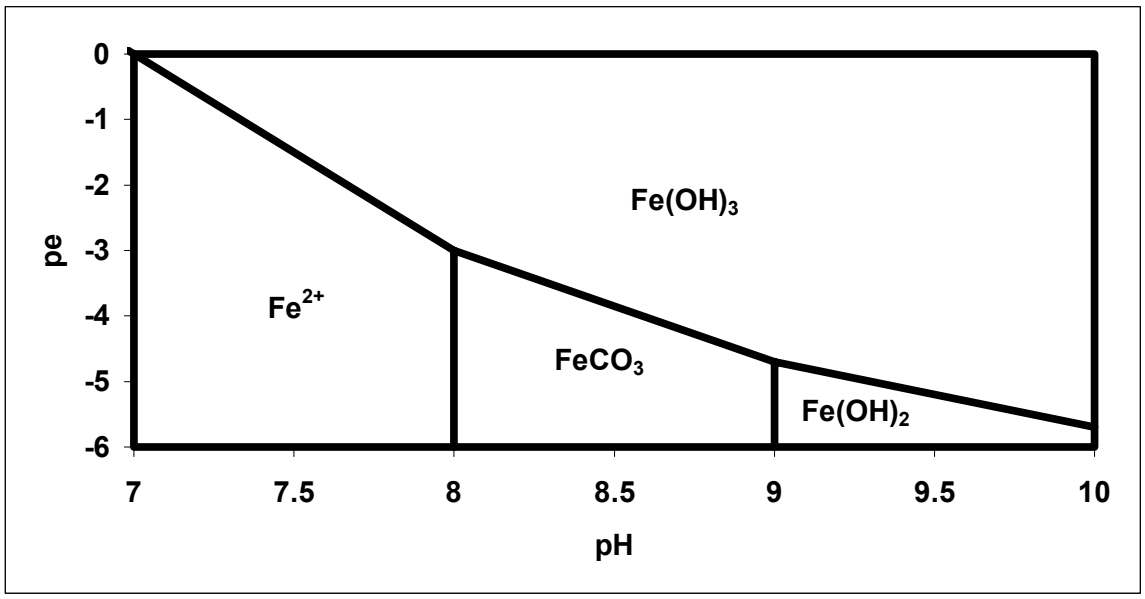
Therefore if the coupling of two half reactions results in a positive net redox potential (Eh), that reaction would be considered thermodynamically possible.

pe/pH Phase Diagrams- The hydrogen ion and electron activity in solution are considered master variables in that by knowing the activity of these two, and the total concentration of a specific component in solution, the speciation of the component can be determined (**10,45**). A pe/pH diagram exemplifies this concept. A pe/pH diagram is essentially a graphical representation of the equilibria between the chemical species in a redox couple (**46**).

Phase diagrams can be used to evaluate the relative speciation of different redox species of the same chemical component. A phase diagram consists of pe plotted as a dependent variable and pH plotted as an independent variable. The body of the phase diagram is divided into regions bounded by the equilibrium conditions for plotted half reactions, mathematically represented by the linear expression of pe (equation 5: $pe = pK - \log[(Br)/(Bo)]$). The interior of the regions bounded by these lines represent regions of stability of a particular aqueous species or a solid phase (**42**).

Figure 1-3 is an example of a pe/pH phase diagram of common iron species. As mentioned, the lines bounding the separate regions of each species represent the linear expression of the equilibrium conditions between the two species. The regions labeled with particular iron species represent conditions where that particular species would be the predominant iron species found.

Figure 1-3: Example of a pe/pH phase diagram using common iron species.



Redox Chemistry in Natural Environments

In natural waters only a few elements are active in redox reactions. Those elements are carbon, oxygen, nitrogen, sulfur, iron, and manganese (**45**). Each one of these elements, considering their respective redox species, has it own unique half reaction potential. The predominance of a particular redox couple follows a progression in the natural environment that reflects the environmental conditions. These conditions relate to the function of the oxidized species of a redox couple as an electron acceptor.

In natural environments electron acceptors are reduced preferentially. When all electron acceptors are available oxygen (O_2) is preferentially reduced first, followed in order by nitrogen (NO_3^- & NO_2^-), manganese (MnO_2), iron (Fe^{3+}), sulfur (SO_4^{2-}), and then carbon (CO_2) (**42**). As illustration, for a closed system containing organic matter, oxidation of organic matter first occurs with reduction of O_2. When oxygen is depleted oxidation of organic matter will then occur through the reduction of NO_3^- and NO_2^-. Reduction of MnO_2 should also occur near the same pe activity as the reduction of nitrate. This is followed by the reduction of ferric iron (Fe^{3+}, FeOOH(s), $Fe(OH)_3$) to ferrous iron (Fe^{2+}). After this, fermentation of organic matter occurs with simultaneous reduction of SO_4^{2-} and CO_2 (**46**).

In order to relate measured redox potentials to environmental conditions, various terminologies have been developed. In terms related to soil science there have been several schemes proposed to describe redox conditions in the soil environments. One such scheme relates redox potential to the water content of soil (**53**). Aerated soils are considered those with soil solution redox potentials between +400 to +700 mV, and waterlogged soils are considered those with potentials as low as –250 to – 300 mV.

Another scheme related to soil redox potential is based on the predominance of a particular oxidant (**42**). Oxic soils are considered to have pe greater then 7 (at pH 7), suboxic soils are considered to have pe values between 2 and 7, and anoxic soils have pe levels lower then 2. In this scheme suboxic soils differ from oxic soils in that the pe levels are low enough to deplete oxygen but not low enough to deplete sulfate ions.

In aquatic environments a similar scheme was developed based on the availability of specific electron acceptors (**24**). Aerobic environments are waters where there are measurable amounts of dissolved oxygen. Anoxic environments are waters where there the oxygen concentration is undectectable and the nitrate concentration is greater then 0. Aquatic environments are considered anaerobic when both the oxygen and nitrate concentrtations are undetectable (**24**).

Measuring Redox Potential

Redox potential in aqueous systems is usually measured using an electrode pair consisting of an inert metal electrode, the sensing element, and an electrode of known potential used as a reference (**12**). Consider equation 1, an example of a reduction half reaction:

$$\mathbf{Bo + ne^- = Br} \qquad \mathbf{(1)}$$

When the net current flow for the oxidation reaction equals the net current flow for the reduction reaction the redox couple is considered to be at equilibrium (**45**). Although at equilibrium the net current for the redox couple is zero, there is a current associated with both the oxidation and the reduction reactions. This current is referred to as the exchange current (**12,45**).

If the metal electrode behaves ideally when it is inserted in a medium containing a redox couple both the oxidation and reduction reactions would occur at the interface of the electrode and the solution. In order for this to occur two assumptions must be valid. First the electrode must be inert, meaning it serves only as an electron donor or acceptor and does not participate in the reaction (**12**). Second the species of interest must be electroactive with the electrode metal, meaning the species is able to transfer electrons through the metal electrode (**45**). If these assumptions are valid then the electrode acts as an electron exchanger for the oxidized and reduced species, and assumes the value of the equilibrium potential for the couple (**12**).

There are no known materials that behave ideally in the aforementioned manner. However the noble metals and some other materials come close to approximating these conditions in limited environments. All of the noble metals, especially gold and platinum, have been used to make electrodes as well as graphite and boron carbide (**12**). Generally electrodes made of platinum are used most frequently and are considered to be the most reliable and inert (**10,51**), especially in systems dominated by the ferrous/ferric iron couple with ionic strengths greater then 0.005 mol/L (**28**). If the electrode does react with species in solution the properties of the electrode may change and the measured potential may be an artifact of the electrode rather then the system being investigated (**51**).

The redox potential of aqueous systems is measured by inserting an electrode, usually platinum, coupled with a reference electrode into the medium being investigated. The measured potential is then corrected for the difference between the potential of the reference electrode and the standard hydrogen electrode, which is considered as the zero

potential point. This calculated potential, as compared to the standard hydrogen electrode, is reported as the redox potential of the system (**12**).

Electrode Calibration- In order for redox potential to be a useful parameter for describing a natural system, measurement of the potential must be made via an accurate and reproducible method. Redox electrodes differ from ion specific electrodes, for example a pH electrode, in that it is not possible to calibrate the electrodes over the wide range of potentials encountered in natural systems (**21**). With ion specific electrodes calibration is achieved by evaluating and correcting the performance of the electrode against standards of known activity for the analyte of interest. Redox electrode measurements respond to electrical potential, a property possessed by a wide variety of chemical species, so calibration is only possible in systems where the active redox couples are already known. However there are a number of solutions that can be used to evaluate the functioning of the electrode and identify mechanical malfunctions.

In order for a calibration solution to be useful it must be simple to prepare, be well poised (i.e. stable against oxidation from atmospheric contamination), be stable against temperature changes, and sensitive enough to distinguish between mechanically defective and working electrodes (**53**). Three solutions are widely used for the calibration of redox electrodes : Light's solution, Zobell's solution, and solution saturated with quinhydrone (**46**). Light's solution gives a potential based on the ferric/ferrous redox couple using an aqueous mixture of ferrous and ferric iron sulfate. It was developed by Truman Light (**26**). Zobell's solution is a solution also based on the ferrous/ferric iron couple using a mixture of ferric and ferrous cyanide (**29**). Saturated quinhydrone solution

is a suspension of the organic molecule quinhydrone in a pH buffer, where the pH of the buffer determines the solution potential (**34**).

The stability of Light's and quinhydrone solution while stored in a stoppered glass bottles at room temperature (21 ^{o}C) was evaluated as a function of time (**53**). Light's solution was observed to be stable at a constant potential for up to 30 days, while the measured potential of quinhydrone solution decreased significantly over the first three days. Based on this data the life span of quinhydrone solution was effectively under 2 hours, and daily preparation of solution was recommended. Quinhydrone appeared to possess a greater sensitivity for mechanical defect based on the observed data, but statistical tests showed no significant difference (**53**).

Another similar test compared the performance of Light's versus Zobell's solutions stored in stoppered glass bottles (**29**). Light's solution was observed to have greater stability, with a potential change of only 4.8 mV observed over a 34 day period, while Zobell's solution had a potential drift of 13.7 mV over a period of 35 days. In addition to solution stability the probe stabilization time, or time needed for the probe to equilibrate with the solution, was evaluated. After 20 minutes, stable readings were observed in Zobell's solution in 79% of the readings, compared to 39% for Light's solution. It was also concluded that Zobell's solution was less sensitive to mechanical defects between electrodes (**29**).

The above experiment also evaluated several other factors that influence the measurement of redox potential. Stirring of the solution in contact with the electrode was not observed to effect measurement precision, but resulted in slightly lower stabilization times in both solutions studied (**29**). Cleaning of the platinum electrode also resulted in a

decrease in the stabilization time for electrodes in calibration solutions. A number of cleaning procedures have been recommended, but many were observed to damage the electrode through direct attack on the platinum surface. The use of acid to clean the electrode surface was observed to have a positive effect on lowering stabilization time, but the recommended method was polishing with a mild abrasive such as jewelers rouge or 400-600 grit wet/dry carborundum paper (**12**).

Limitations of the Electrode Approach- In spite of the fact that there are reliable methods to collect redox potential measurements, there is concern over the use of the measured potentials to quantitatively define chemical species in natural environments. For most systems the use of measured potentials to quantitatively define chemical species in natural environments is considered unjustifiable (**12,45,46,51**). Concerns as to the limitations of the direct measurement of redox potential using electrodes can be summarized as follows (**51**):

1) Alteration of the system studied by disturbance of the sample (i.e. the release or absorption of redox active gases, e.g. O_2, and H_2S)
2) Defects associated with the junction between the reference electrode and the solution
3) Low exchange current densities at the electrode surface
4) The possibility of mixed potentials
5) The ability of the sensing electrode to remain inert, i.e. alteration of the chemical properties of the platinum electrode
6) Assumptions concerning the reversibility of the potential determining redox couple (**12**)

7) The assumption that the potential determining species is electroactive (electrically interacts, i.e. exchanges electrons) with the sensing electrode (**29**)

Junction Potential- Electrical continuity between the test solution and the reference electrode is made through a liquid junction in the electrode. Any time two dissimilar electrolyte solutions are in contact, a potential develops at the interface between the two (**12**). This potential, called the junction potential, develops due to the different mobility's of ions in solution and in the reference internal solution. The separation of charge as ions diffuse through the liquid junction develops as an electrical potential (**21**). The measured potential for an unknown solution is thus the sum of the potential of the system and the junction potential (**12**) :

$$\mathbf{E_{observed} = E_{system} + E_{junction}} \qquad \mathbf{(9)}$$

To correct for or minimize this junction potential, electrolyte solutions are chosen so that both the cation and anion have similar mobilitys, and are designed so that the junction allows for an adequate rate of flow (**12**). Another problem associated with liquid junctions is clogging of the porous plug, which results in a sluggish and unstable response (**21**). Obstructions of the junction occur for various reasons, usually due to reactions at the surface such as precipitation of metal sulfides or from interaction or coagulation of suspended matter (**51**). To ameliorate this problem junctions are designed to incorporate free flow of solution from the junction, or designed to allow fresh solution to be forced through the junction prior to use (**21**).

Electrode Poisoning- A problem associated with both the reference and sensing electrodes is poisoning of the electrode surfaces with substances in the test solution (**12,29**). Using electrodes for long periods of time in solutions containing reactive

substances, such as sulfides and bromides, tends to lead to deterioration of electrode performance (**12**). These components diffuse into the internal volume of the reference electrode and can react with species of the redox couple, thereby altering the equilibrium conditions and altering the reference potential. The effect of this problem can be minimized by replacing the internal fill solution frequently or using an electrode designed to have a second fill reservoir separating the internal elements from the test solution (**12**).

The condition of the platinum surface of the sensing electrode can also be altered through reaction with dissolved species in the test solution, such as dissolved oxygen and sulfide (**29**). Alteration of the platinum surface inhibits the ability of the electrode to act as an inert electrode, which can effect the interaction between the probe and redox species in the test solution (**12,51**). A thermodynamic analysis of the function of the platinum electrode over the pH and pe range of the natural environment suggests that the platinum surface is susceptible to chemical attack in high and low pe environments (**51**). At high pe levels the platinum electrode is susceptible to formation of platinum oxides, the nature of which is debated. When this occurs the platinum electrode seems to act as an oxide surface in well aerated environments and respond to pH. At low pe levels the platinum is susceptible to attack by sulfide ions, which would alter the properties of the platinum electrode and could add a spurious potential associated with the new surface complex coating the electrode.

Mixed Potential- Relating measured redox potentials to specific species in solution requires that a given couple is solely responsible for the exchange current at the sensing electrode's surface (**10**). Frequently it is possible to have several different redox couples individually equilibrating, but not equilibrating with other redox couples. The balancing

anodic and cathodic exchange current may be equal and hence give a stable potential, but may not correspond to one redox couple but rather to 2 or more couples (**45**). Such potentials are referred to as mixed potentials and have little quantitative value (**46**). An example of this is the coupling of a ferrous/ferric iron couple with a O_2/H_2O couple (**10,45**). In iron dominated systems when a trace of dissolved oxygen ($\geq$ 0.01 mg/L) is introduced the measured potential is equilibrated at the point where the rate of O_2 reduction is equal to the rate of ferrous iron oxidation. However ferric iron is produced at the electrode and slowly shifts the measured potential. Thus the measured stable potential has no quantitative bearing on the activities of iron species in solution (**45**).

Thermodynamic Reversibility- One of the fundamental assumptions involved with interpreting measured redox potentials is that the exchange current associated with the sensing element involves a thermodynamically reversible reaction (**45**). Under certain conditions this may be valid, but in general quantitative interpretation of measured potentials is considered impossible (**12,45,46,51**). The reason for this is that most of the important redox processes in the natural environment, with the exception of iron and manganese, involve species of carbon, hydrogen, nitrogen, oxygen, and sulfur which generally form species that are not electroactive (i.e. gases and molecules) at the surface of the electrode (**10,12**). It is accepted that no reversible electrode potentials are established in systems dominated by the NO_3/NH_4, SO_4/H_2S, and CH_4/CO_2 redox couples (**45,46**).

Exchange Current- The establishment of a potential at the surface of the platinum electrode involves the equilibration of an anodic and cathodic current (**12**). At equilibrium the net current is zero, but the current associated with either the oxidation

(anodic) or reduction (cathodic) reaction represent unidirectional current flows called the exchange current. The larger the exchange current the more stable the measured potential. For example, redox electrodes will not respond to the O_2/H_2O couple because over a large range of potential the exchange current at the electrode is so low it is essentially zero (**12**). As a result, in the absence of other dominant couples the measured potential will not stabilize but rather drift continuously over a range of potentials.

A problem is encountered with measuring redox potential in natural environments when the dominant redox species of interest are present in low concentrations (**10,12,29**). In these cases potential determining redox species may be present but in such a low concentration that a stable potential is not established at the electrode surface. As a result the measured potential will be unstable and affected by spurious potentials. In order for the establishment of a stable potential at the electrode surface redox species must be at activities $\geq 10^{-5}$ M (**10**).

Electrode Limitations- It is also important to address limitations associated with the technique of measuring redox potential. The potential observed using metal based reference electrodes is dependent on temperature. As such it is essential that the electrode and test solution be in thermal equilibrium, or that the temperature of the test solution is measured. In addition the use of well poised solutions such as Light's and Zobell's solution may mask defects in the electrode (**12**). Electrodes tested in such solutions may read the correct potential even though in less poised systems the observed potential may differ by as much as 100 mV.

All of the above problems limit the use of redox measurements for the precise quantitative determination of redox species in observed systems. However redox

potential measurements are still useful in defining natural systems as an operational parameter (**51**). And in aqueous systems where iron and manganese species are abundant, it is possible to make useful quantitiative redox measurements (**12**).

Possible Mechanisms for Di-nitrogen Production in Lagoon Systems

A recent study of emissions from two lagoons in North Carolina treating swine wastewater noted significant production of di-nitrogen gas (N_2) (**19**). Emissions were reported for a system of four lagoons located on a swine farm in Georgia and for two farms examined during the course of this research, referred to as Farm 10 and Farm 20. During the period from July to November 1998 emission rates of di-nitrogen gas averaged 120 and 9 kg N_2-N ha^{-1} day^{-1} for Farms 10 and 20 respectively. This is contrary to expected nitrogen emissions from swine lagoon systems. Swine lagoons are considered anaerobic systems where aqueous nitrogen is mainly in the form of ammonium, and ammonia is the major nitrogenous emission. Several mechanisms for the phenomenon of di-nitrogen emission have been described (**22**). Recent research has shown the existence of ammonium oxidation and gaseous N_2 production in anaerobic and micro-aerobic condition. One mechanism is termed the Anammox process, and is the anaerobic oxidation of ammonium with nitrate as electron acceptor resulting in di-nitrogen gas production (**33**). Another mechanism is termed the OLAND process, for Oxygen Limited Autotrophic Nitrification/Denitrification, and is the simultaneous nitrification and denitrification of nitrogen by nitrifying bacteria under oxygen limited conditions (**7**).

Anaerobic Ammonium Oxidation (Anammox)

Up until the discovery of the Anammox process the oxidation of ammonium to nitrite was considered to be an aerobic process, based on the fact that the microorganisms responsible for this reaction were considered to be obligately aerobic. However a group of researchers noted the disappearance of ammonium (NH_4^+) from a denitrifying fluidized bed reactor treating effluent from a methanogenic reactor (**33**). The effluent contained nitrate from an earlier treatment which they were attempting to denitrify. However both the nitrate and ammonium concentrations in the effluent were observed to decrease with the production of di-nitrogen gas. The removal of the two species was proposed to occur via the following reaction (**33**):

$$3\ NO_3^- + 5\ NH_4^+ \rightarrow 4\ N_2 + 9\ H_2O + 2\ H^+$$

This reaction became evident in that the measured decrease in nitrate and ammonium agreed with calculated values only if the stoichiometric amounts of both species involved were included. It was concluded that anaerobic ammonium oxidation was a previously unobserved process by which ammonium could be oxidized using nitrate as the electron acceptor under anaerobic conditions (**33**).

Anammox as a Biological Process- After these initial observations a study was conducted to confirm the biological nature of the Anammox process (**48**). In control experiments with activated sludge samples which were sterilized either through gamma irradiation or autoclaving no changes in ammonium and nitrate concentration were observed. This was proposed as evidence that the process is not the result of a chemical interaction. Furthermore the addition of antibiotics and microbial inhibitors completely

stopped the Anammox activity. Another focus of this study was to determine the fate of ammonium nitrogen using ^{15}N labeled ammonium and $^{14}NO_3$. The major end product of the process was observed to be $^{15\text{-}14}N_2$ (98.2%) and only 1.7 % $^{14\text{-}14}N_2$. No change in sulfate concentration was observed. Therefore it was proposed that the reaction consumed equimolar amounts of nitrite and ammonium, via the following reaction:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$$

Effects of Oxygen on Anammox- Since nitrate is a required reactant for the Anammox process to occur, research into the effects of oxygen on the Anammox process were conducted (**44**). The presence of oxygen, taken down to a concentration of 0.5% saturation in this study, was observed to inhibit the Anammox process, albeit reversibly. Therefore it was proposed that Anammox can not occur in micro- or macro-aerobic conditions. The researchers also tried to ascertain if nitrifying bacteria were responsible for Anammox activity. No ammonium oxidation was observed during periods of aerobic conditions in the Anammox sludge. Furthermore, samples were enriched in oxygen and colonies of ammonium oxidizers were allowed to exponentially increase in population. These organisms were then subjected to conditions conducive to Anammox activity but significant ammonium oxidation was not observed. Based on these observations it was concluded that the possibility of nitrifier populations being responsible for Anammox was highly improbable (**44**).

Oxygen Limited Autotrophic Nitrification/Denitrification

Another possibility as to the mechanism of di-nitrogen production in lagoon systems involves the metabolism of nitrifying bacteria. Nitrite metabolism of

Nitrosomonas europaea has been observed even in anaerobic conditions (**7**). Nitrifying bacteria, considered obligate aerobes, are proposed to actively oxidize ammonia and respire nitrate and nitrite under oxygen limited conditions. The metabolic process was proposed to involve simultaneous nitrification and denitrification, and is termed oxygen-limited autotrophic nitrification-denitrification (OLAND). In 1980, gaseous nitrogen production (N_2O) from nitrifying bacteria was observed under conditions of low oxygen (< 3 mg/L). The organisms responsible were shown to be nitrifying bacteria, *Nitrosomonas sp* (**17**).

Autotrophic conversion of ammonium to gaseous N products has been proposed to involve 2 steps (**25**). The first is the oxidation of ammonium, which occurs in aerobic or oxygen limited conditions, with nitrite or possibly nitrate as the end product. This reaction was described as follows:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$$

The second step was the reduction of nitrite or nitrate with ammonium acting as the electron donor according to the following reaction:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$$

Measured data for reactant consumption and product formation agreed with expected values based on the stoichiometry of the above reaction. Di-nitrogen production was not directly measured, as the system studied was an open system. Based on electron and mass balances of the assumed reactions, di-nitrogen production was estimated to account for 80% of the total nitrogen removed, as nitrous oxide was detected and accounted for 20% of the expected nitrogen removal. The operative organisms were considered to be nitrifiers that were able to denitrify under conditions of oxygen stress.

The nitrifiers were in turn considered to be dominated by ammonium oxidizers. No evidence was observed of significant losses due to ammonia volatilization (**25**).

OLAND and Di-nitrogen Production Through Denitrification- Research conducted in order to evaluate whether denitrification or nitrification was responsible for the OLAND process suggested that it was indeed denitrification responsible for the gaseous nitrogen production (**36**). It was observed in this experiment that a nitrifying bacterium, *Nitrosomonas europaea* , produced N_2O only during conditions of limited oxygen supply. The observed results agreed with values calculated from the theoretical kinetics of this reaction taken as a denitrifying process. Nitrite in solution was reduced to N_2O directly while ammonium was oxidized, with nitrite acting as the terminal electron acceptor. Thus the concentration of oxygen must be negligible as it effectively competes with nitrite as an electron acceptor. Although an organism would obtain no net energy from the denitrification process, it was suggested that such a process would benefit the organism in that it conserved oxygen for the initial steps of ammonium oxidation while removing a toxic product (nitrite). This further had advantage by decreasing the competition for oxygen by consuming the energy source for nitrite oxidizers (**36**).

Effects of Oxygen on OLAND- Under limited oxygen conditions it was proposed that both *N. europaea* and *N. eutropha* used ammonium as an electron donor and oxygen or nitrate as an electron acceptor, with nitrate consumption increasing as the oxygen concentration in solution decreased. Under anoxic conditions, ammonium and nitrate were observed to be removed at a molar ratio of 1:1. When samples were introduced into a controlled oxygen setting, it was observed that upon lowering the dissolved oxygen concentration from 0.4 to 0.2 mg/L there was an increase in gaseous N loss, from 14% -

60% total N . Furthermore it was observed that when cultures were grown under oxygen limited conditions for several months, they retained and even enhanced their ability to use nitrate as an electron acceptor and contribute to N loss (**11**).

It was also shown in this study that under oxygen limitation mixed cultures of both nitrifying and denitrifying bacteria enhanced nitrogen loss as compared to pure cultures. In pure cultures 19% of ammonium nitrogen was converted to gaseous nitrogen loss. Under mixotrophic conditions 36% of ammonium N was converted to gaseous nitrogen loss (**11**).

Passive Surface Aeration

The Anammox and OLAND processes represent two microbially mediated processes that could account for production of di-nitrogen gas as observed in lagoons treating swine wastewater (**19**). The only issue of concern is the obvious need for nitrite or nitrate in both processes. Recalling that nitrite and nitrate concentrations are negligible in lagoon effluent, ammonium oxidation must depend on at least an initial input of oxygen. Swine lagoons are considered to be anaerobic to depth, except for the possibility of a thin aerobic layer at the air-liquid interface. Indeed diffusion of oxygen through and into anaerobic environments is considered to be a means of oxygen enrichment, albeit as a rather thin zone in anaerobic environments (**22,30,36**). However it is important to note that in dynamic systems the absence of oxygen could mean only that the input of oxygen is less then the need for oxygen. Therefore oxygen could play a role in anaerobic environments although it is not detected in significant amounts. In anoxic and anaerobic environments, which are defined as anaerobic when both oxygen and nitrate

concentrations are undetectable and anoxic when oxygen concentration is undetectable and nitrate concentration is greater then 0, oxygen input and subsequent nitrate production could result in aerobic respiration and denitrification (**24**).

Research Objectives

From the review of the published literature as detailed above, several mechanisms may exist for the conversion of ammonium to di-nitrogen gas in the highly anaerobic environment of lagoons, which are commonly used as an integral part of large-scale swine production in North Carolina. The conversion of ammonium to di-nitrogen gas in anaerobic swine lagoons will require a change in the oxidation state of nitrogen [NH_4^+ with N(-III) to N_2 with N(0)] and thus must be coupled with other redox reactions which may be present. In addition, of the published mechanisms for the potential conversion of ammonium to di-nitrogen gas, all require the formation of either nitrite or nitrate before final conversion to di-nitrogen gas. Swine lagoons are known to emit methane and can be sources of hydrogen sulfide emissions as well. Thus there are at least two redox reactions already known to be active in swine lagoons. However, it is not immediately clear how the formation of methane via methanogenesis, nor the redox couple between sulfide and sulfate, could be coupled with redox reactions that would result in the formation of nitrite or nitrate leading to the eventual formation of di-nitrogen gas. Therfore, to further investigate the possibility of the conversion of ammonium to di-nitrogen gas in anaerobic swine lagoons the following hypothesis was proposed: that there exists in swine lagoons at least one additional redox couple that can interact with ammonium to lead to the formation of nitrite or nitrate, and that the chemical species that compose this redox

couple exist in sufficient amounts within anaerobic swine lagoons to result in the formation of significant (measurable) amounts of di-nitrogen gas. To test this hypothesis the following research objectives were established:

1) To characterize swine lagoons located on typical large-scale swine production facilities in eastern North Carolina with regard to their redox status using traditional measurement techniques (i.e. platinum probe and reference electrode) as a function of time and depth within the water column of each lagoon.
2) To supplement the field measurements of the redox status of the swine lagoons with chemical characterization data as a function of time and depth within the water column of each lagoon.
3) To use traditional techniques (i.e. pe-pH phase diagrams) to identify potential redox couples that could account for the field measurements of redox potential. To use the chemical characterization data in conjunction with a chemical speciation computer model (MINTEQA2) to provide supplementary information regarding potential redox couples that could account for the field measurements of redox potential.
4) Upon identification of the presence of one or more redox couples in the swine lagoons based on interpretation of the field redox measurements (with supporting information from the chemical characterization data), speculate as to potential redox reactions between the chemical species associated with the identified redox couples and ammonium that could result in the formation of nitrite or nitrate. To use the chemical characterization data to determine

whether the chemical species assumed associated with the identified redox couples are present in sufficient amount to result in the formation of significant amounts of di-nitrogen gas.

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Chapter 2

Materials and Methods

Site Description

Five lagoons in the eastern coastal plain region of North Carolina were monitored from August 2, 2000 until February 1, 2001, and one lagoon from June 7, 2000 until February 1, 2001. Figure 2-1 is a map of the location of all six lagoon sites. Included in the map is the location of the Clinton Horticultural Research Center, from which precipitation data for the sampling period was available. The six lagoons will be named after their respective farm designations, which is given in Table 2-1 along with the number of swine and herd type.

Figure 2-1: Location of lagoons sampled in eastern North Carolina.

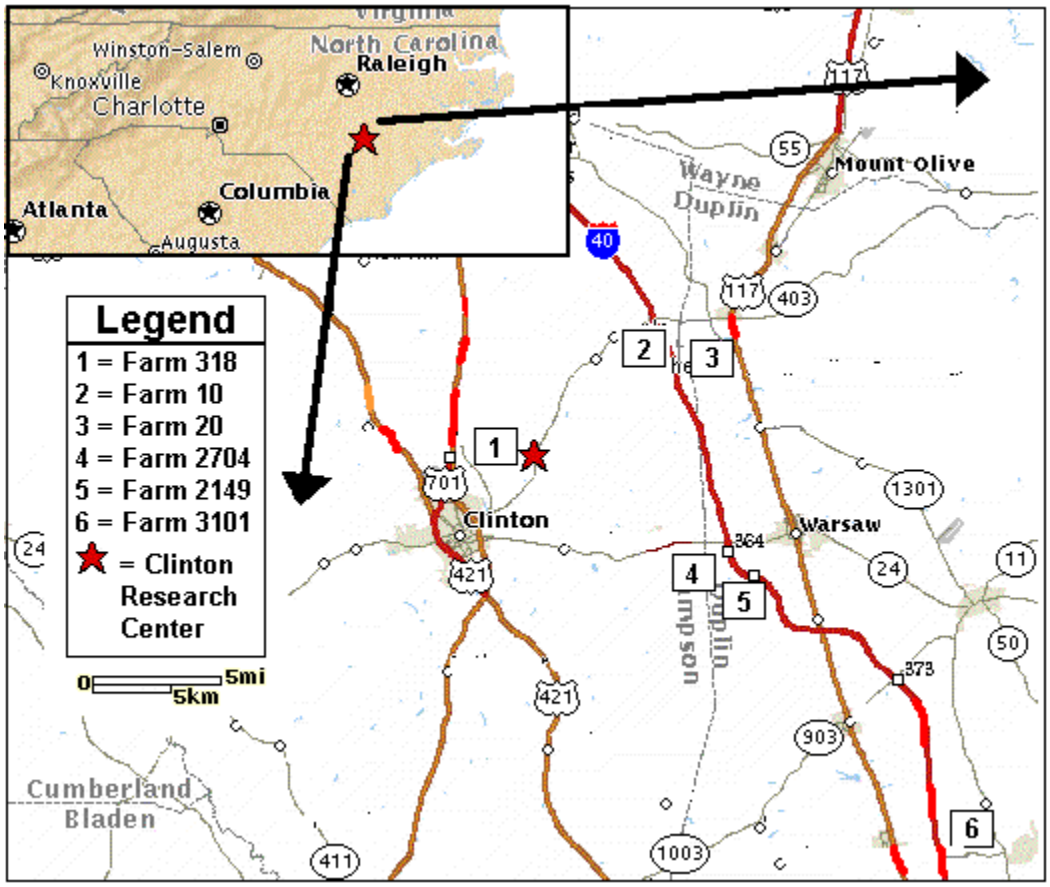


Table 2-1 : Farm ID, number of swine at farm, and herd type.

Farm ID	Number of Swine	Herd Type
10	1200	Farrow to Finish
20	2000	Farrow to Wean
318	2940	Finish
2174	1350	Farrow to Feeder
2704	3600	Farrow to Wean
3101	3320	Finish

Sample collection was discontinued after February 1st, 2001, due to concern over contamination and transfer of swine disease at the farms sampled. During this time outbreaks of hoof and mouth disease among livestock herds in the United Kingdom resulted in the decimation of many Eastern European swineherds. As a result of this outbreak, swine farmers in North Carolina developed biological protection protocols for limiting the introduction of disease vectors on their farms. Due to the need to implement the biological protection procedures mandated by the swine farmers, time constraints made it logistically impractical to continue data collection. The decision was made to halt continued measurement and sample collection, and the data already collected was considered sufficient to complete the project goals.

Sample Collection

Three measurements were carried out in the field; Eh, pH, and lagoon temperature. Lagoon liquid (refered to as effluent) samples were collected while field measurements were taken to correlate sample analytical data with field measured chemical parameters.

Eh- Redox potential measurements were obtained using an Accumet ® AP62 portable hand held meter and a Dynaprobe II ® model Eh electrode. Dynaprobe potential measurements were referenced against a built in Ag/AgCl reference electrode. Calibration of the redox electrode was carried out in the lab before and after sampling. Calibration protocol for assessing the performance of the redox electrode involved a two step process. The first step involved mechanically separating the electrical outputs from the reference and sensing electrodes. The reference electrode from the field probe was then compared to a working Dynaprobe lab reference electrode. The two reference electrodes were placed in a saturated KCl solution and compared using the Accumet meter. Potential measurements other then 0 mV were taken as indication of possible probe failure. Suspect probes were then evaluated using saturated quinhydrone solution according to the protocol established by Braodley James (**2**). Enough quinhydrone solid was dissolved in both pH 4 and 7 buffers to provide for a saturated solution of quinhydrone. The electrochemical potential of quinhydrone is effected by pH, so the buffers provide two different redox potentials for probe evaluation.

Probes that indicated failure were treated first by cleaning the liquid junction with a soft toothbrush in an attempt to mechanically remove any detritus obstructing fluid flow through the liquid junction. In the event this procedure was ineffective in resolving probe

failure, the probe was then boiled in a solution saturated with KCl. Malfunctioning probes were placed in a beaker of saturated KCl solution on a heating plate and allowed to boil for approximately 1 hour. After the boiling period the probe was kept in the KCl solution overnight. All malfunctioning probes responded to this treatment and were restored to proper working order.

pH- pH measurements were taken using a Corning® G-P Combo model pH electrode and an Accumet ® AP62 portable hand held meter. Calibration of the pH electrode was carried out in the field at the beginning of the sampling day and in the lab at the end of the sampling date. The calibration protocol developed by Corning (**3)** involved a two-point calibration using pH 7 and 10 buffers. Failure of the pH probe was found to involve clogging of the liquid reference junction. The pH electrode model used was designed with replaceable ceramic junctions and treatment of probe failure was accomplished by removal of the clogged junction and replacement with a new junction.

Temperature- Temperature measurements were taken using the Accumet® AP62 portable hand held meter and accompanying Accumet temperature probe. Temperature measurements were used to calculate standardized Eh measurements. Temperature adjusted potentials are given in Table 2-2.

Table 2-2 : Temperature dependent potentials for Ag/AgCl reference electrode.

Temperature (^{o}C)	Eh (mV)
15	209
20	206
25	202
30	198
35	195
38	193
40	191

Sample Collection/Handling

Data and lagoon samples were collected at three depths: 30.5 cm (1 ft) below the air/water interface, at 61-91.5 cm (2-3 ft) below the air/water interface, and 61-91.5 cm (2 to 3 ft) from the bottom of the lagoon. These three depths are referred to as the Surface, Storage, and Treatment measurements respectively. Data and samples were collected using an apparatus that is diagramed in Figure 2-2.

Figure 2-2 : Lagoon sampling apparatus a) Schematic of boat and fluid collection device/probe b) Schematic of fluid collection device/probe tip.

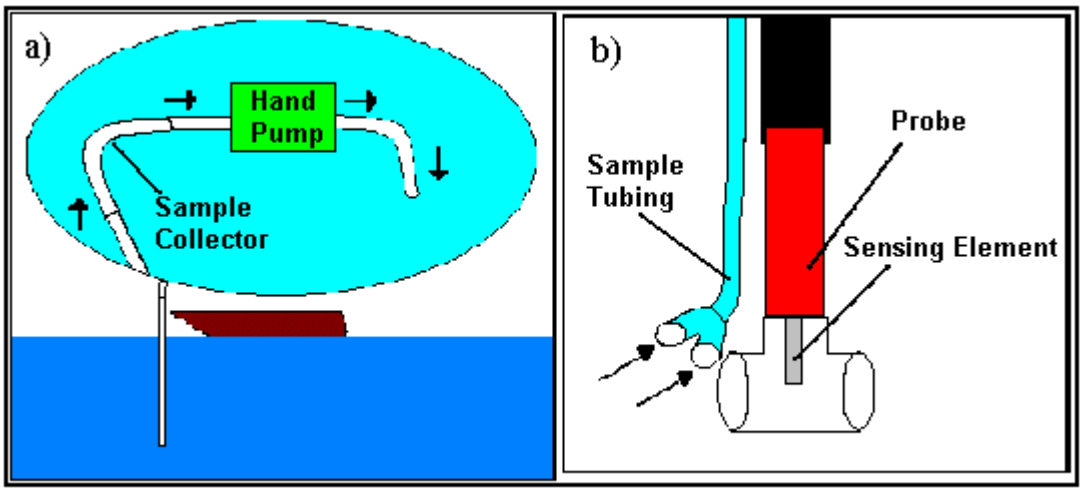


Figure 2a represents the sampling boat out on a swine lagoon. A length of PVC pipe marked at 30.5 cm (1 ft) intervals for 304.8 cm (10 ft) was suspended over the side of the boat. At the tip of this pipe was attached the probe, as diagramed in Figure 2b. Attached to the probe was also a length of 1/8 in (3.175 cm) outer diameter Tygon© tubing, with an opening for sample intake attached to the probe tip.

Fluid samples were pumped up from depth using a Guzzler ™ hand pump located in the boat. A portion of the lagoon fluid was collected in a beaker and the pH and temperature were immediately recorded.

In order to collect a sample of the lagoon liquid a small section of the Tygon© tubing isolated from contact with air was connected, via quick-disconnect couplings (Colder Products Co., Cole-Parmer Cat. No. U-31303-10), prior to the hand pump. This Tygon© tubing was removed when sample was to be collected and replaced with a

similar section of tubing for the next sample. Samples were then placed in an ice chest in the field and stored overnight in a refrigerator, which is set at approximately 11 ^{o}C. The following day samples were transferred to 150 mL Nalgene© HDPE bottles and frozen until analysis.

Sample Preparation and Analysis

Total Concentrations- Total concentrations of selected elements were determined using 25 ml subsamples of collected frozen lagoon fluid that was thawed. The subsample was placed in a glass beaker and dried overnight at 104^{0}C in a convection oven. Samples were then removed and ashed in a muffle furnace at 500^{0}C. Ashed samples were then digested using 6 N HCl and resuspended in 0.5 N HCl. This fraction was analyzed for manganese, iron, copper, zinc, phosphorous, sulfur, calcium, and magnesium. Ion Coupled Plasma (ICP) spectroscopic techniques using a Perkin-Elmer® Optima 2000 DV spectrometer were used to analyze ashed lagoon samples for total elemental analysis. Analysis was conducted according to methods developed from the Standard Methods text (**4**).

Dissolved Species- For determination of total dissolved cations and dissolved anions, a subsample of 15 mL was removed from the thawed sample bottles after thorough shaking. This subsample was then centrifuged in a refrigerated centrifuge at 30,600 x g (16,000 rpm's) for 20 minutes. The resulting supernatant was then filtered though a 0.4 micron polycarbonate membrane (Millipore © HTTP membrane filters). The resulting sample fraction was then placed in a 60 mL Nalgene© HDPE bottle and frozen until analysis.

The filtered solution was analyzed for total dissolved iron, manganese, copper, zinc, phosphorous, sulfur, calcium, magnesium, sodium, and potassium using Ion Coupled Plasma (ICP) spectroscopic techniques on a Perkin-Elmer® Optima 2000 DV spectrometer. Analysis was conducted according to methods developed from the Standard Methods text (**4**).

Analysis for dissolved orthophosphate, nitrate, and ammonium in the filtered sample was conducted using colorimetric techniques. Analysis was carried out on the Lachat® QuickChem 8000 autoanalyzer using standard protocols (**5**).

Total dissolved chloride and sulfate were determined on the filtered solution using chromatographic techniques. Analysis was conducted using a Dionex® DX 500 ion chromatograph, with a model AG-14 guard column and a model AS-14 separation column. Analytical methods were established according to methods developed from the Standard Methods text (**4**).

Statistical Analysis

Statistical analysis was conducted using software developed by the SAS Institute. (**6**). The complete set of data for each parameter at each depth for each lagoon, and the complete set of data averaged over depth for each lagoon was statistically analyzed using a Randomized Complete Block ANOVA test, with data blocked by day of year. Results from the ANOVA test were then tested using the Tukey Multiple Comparison procedure. The ANOVA test was used to determine whether depth or lagoon had a significant effect on mean parameter value, and the Tukey test was used to attempt to delineate where the differences occurred.

Chemical Speciation- The chemical speciation of processed analytical data was conducted using MinteqA2, a chemical speciation program developed by the United States Environmental Protection Agency (US EPA). MinteqA2 software was downloaded from the U.S. EPA website and was installed to run off the MS DOS interface of a personal computer. Included with this software were the thermodynamic databases containing the information needed to complete the required calculations (**1**).

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Chapter 3

Overall Data Summary

Eh, pH, and Lagoon Temperature Measurements- Mean values (averaged over depth and sampling period) and standard deviations for Eh, pH, and temperature per lagoon are recorded in Table 3-1. Minimum and maximum values for each parameter are also included.

Table 3-1 : Eh, pH, and lagoon temperature data averaged over depth and sampling period (8/2/00-2/1/01) for each lagoon. Farm 10 sampling period from 6/7/00-2/1/01.

	FARM					
	10	**20**	**318**	**2149**	**2704**	**3101**
	Eh (mV)					
Mean	**-121**	**-87**	**-154**	**-134**	**-113**	**-126**
Std.Dev.(+/-)	*39*	*40*	*27*	*23*	*36*	*29*
Minimum	-217	-220	-207	-193	-195	-183
Maximum	-32	-27	-84	-95	-31	-43
	pH					
Mean	**7.78**	**7.60**	**8.09**	**7.73**	**7.95**	**7.72**
Std.Dev.(+/-)	*0.17*	*0.20*	*0.11*	*0.17*	*0.13*	*0.14*
Minimum	7.50	7.11	7.85	7.52	7.64	7.46
Maximum	8.18	8.00	8.34	8.14	8.28	8.14
	Temperature (^{o}C)					
Mean	**22.9**	**18.3**	**20.0**	**20.1**	**19.5**	**20.9**
Std.Dev.(+/-)	*7.8*	*6.6*	*7.1*	*7.1*	*7.2*	*7.2*
Minimum	7.5	6.8	8.3	8.1	7.1	8.6
Maximum	33.3	29.1	34.7	33.2	34.2	33.2

Redox potential measurements across all six lagoons ranged from –220 mV to –27 mV, with a mean redox potentials ranging from –154 mV to –87 mV. For each lagoon there is a wide range in measured redox potential, with the smallest difference between

maximum and minimum observations being approximately 100 mV and the largest difference nearly 200 mV. Observed pH measurements across all six lagoons ranged from 7.11 to 8.34, with mean pH values ranging from 7.60 to 8.09. pH values within each lagoon also demonstrated a wide range of values, from 0.5 to nearly 1 pH units. Measured temperature across all six lagoons ranged from 6.8 to 34.7 ^{o}C. Mean temperature values ranged from 18.3 to 22.9 ^{o}C. The progression in mean temperature values from minimum to maximum mirrors the progression in which the farms where visited on sampling dates. Farm 20 was the first lagoon sampled, approximately around 9:00 a.m., followed by Farms 2704, 2149, 3101, 318, and finally Farm 10 at approximately 4:00 p.m..

Trace Metals (Mn, Cu, Fe, Zn) - Mean values (averaged over depth and sampling period) and standard deviations for the measurement of manganese, copper, iron, and zinc (Mn, Cu, Fe, and Zn) for each lagoon are recorded in Table 3-2. Minimum and maximum values for each parameter are also included. Trace metal data is separated into two fractions: total and total dissolved. "Total" represents a total concentration of a specific analyte found in lagoon liquid. "Total Diss." represents the total concentration of a specific analyte in the portion of liquid passing through a 0.4 micron filter. Total concentration includes the contribution from the dissolved fraction.

Of all trace metals analyzed iron appears to be the most abundant, with mean total iron concentrations ranging from 0.912 to 1.562 mg/L. Iron is also the trace metal with the greatest total dissolved concentration, ranging from 0.186 to 0.673 mg/L. Among the trace metals analyzed copper and zinc show the widest range in mean values, with total

copper ranging from 0.058 to 0.451 mg/L and total zinc ranging from 0.099 to 0.633 mg/L. Such large differences between lagoons are most likely related to properties of the individual farm, such as type of herd and feed type. Copper and zinc are traditionally added to feed for young piglets in excess of daily dietary needs for health benefits (**1**).

The large difference between the amount of total and dissolved trace metal concentration indicates that a significant fraction of these metals are bound in a solid phase of some nature, either organic or inorganic, which is separated during centrifugation and/or retained on the 0.4 micron filter. All farms, with the exception of Farm 318, contain mean dissolved manganese levels at or near the detection limit for the analytical procedure used. For mean dissolved copper, Farms 20, 2704, and 3101 contain trace levels at or below the detection limit. All farms contain mean dissolved zinc concentrations near or below the detection limit, with Farm 2149 having a mean zinc concentration only slightly above the detection limit.

The fraction of each metal, given as a % of the total concentration, that exists in the total dissolved fraction (fraction passing through 0.4 micron filter) is given in Table 3-3. From this table it is observed that, not considering farms with detection limit values, both zinc and manganese exist mostly in the fraction of sample separated during centrifugation or retained on the 0.4 micron filter.

Table 3-2: Trace metal (Mn, Cu, Fe, Zn) concentration (in mg/L) averaged over depth and sampling period (8/2/00-1/25/01) for each lagoon. "Total" represents total acid extractable concentration and "Total Diss." represents total concentration in sample passing through 0.4 micron filter. A "[1]" indicates a value representing the detection limit for a specific analyte.

		FARM					
		10	20	318	2149	2704	3101
		Manganese					
Total	Mean	0.120	0.133	0.307	0.081	0.062	0.083
	Std.Dev.(+/-)	*0.045*	*0.037*	*0.108*	*0.027*	*0.024*	*0.048*
	Minimum	0.051	0.068	0.079	0.041	0.023	0.021
	Maximum	0.228	0.239	0.581	0.162	0.146	0.233
Total Diss.	Mean	< 0.010 [1]	0.011	0.027	0.012	0.014	0.014
	Std.Dev.(+/-)	*0.011*	*0.009*	*0.034*	*0.012*	*0.010*	*0.013*
	Minimum	< 0.010 [1]	< 0.010 [1]	< 0.010 [1]	< 0.010 [1]	< 0.010 [1]	< 0.010 [1]
	Maximum	0.046	0.039	0.126	0.059	0.046	0.061
		Copper					
Total	Mean	0.351	0.057	0.451	0.183	0.058	0.095
	Std.Dev.(+/-)	*0.155*	*0.071*	*0.163*	*0.066*	*0.029*	*0.097*
	Minimum	0.140	0.012	0.094	0.076	0.020	0.026
	Maximum	0.756	0.477	0.964	0.378	0.146	0.650
Total Diss.	Mean	0.134	< 0.050 [1]	0.191	0.091	< 0.050 [1]	< 0.050 [1]
	Std.Dev.(+/-)	*0.105*	*0.010*	*0.081*	*0.047*	*0.010*	*0.019*
	Minimum	< 0.050 [1]	< 0.050 [1]	0.064	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
	Maximum	0.558	< 0.050 [1]	0.400	0.208	0.051	0.085
		Iron					
Total	Mean	1.361	0.912	1.562	1.511	0.844	1.422
	Std.Dev.(+/-)	*0.724*	*0.346*	*0.550*	*0.490*	*0.342*	*1.079*
	Minimum	0.504	0.354	0.601	0.830	0.331	0.328
	Maximum	3.820	2.120	3.350	3.180	1.972	5.300
Total Diss.	Mean	0.673	0.186	0.411	0.589	0.416	0.458
	Std.Dev.(+/-)	*0.199*	*0.063*	*0.095*	*0.158*	*0.096*	*0.121*
	Minimum	0.312	0.073	0.216	0.220	0.170	0.185
	Maximum	1.042	0.387	0.590	0.889	0.624	0.675

Table 3-2 (continued): Trace metal (Mn, Cu, Fe, Zn) concentration (in mg/L) averaged over depth and sampling period (8/2/00-1/25/01) for each lagoon. "Total" represents total acid extractable concentration and "Total Diss." represents total concentration in sample passing through 0.4 micron filter. A "[1]" indicates a value representing the detection limit for a specific analyte.

		Zinc					
Total	**Mean**	**0.625**	**0.099**	**0.338**	**0.633**	**0.155**	**0.213**
	Std.Dev.(+/-)	*0.245*	*0.037*	*0.201*	*0.191*	*0.050*	*0.131*
	Minimum	0.233	0.033	0.098	0.312	0.038	0.059
	Maximum	1.336	0.177	0.908	1.264	0.256	0.704
Total Diss.	**Mean**	**0.051**	**< 0.050 [1]**	**< 0.050 [1]**	**0.061**	**< 0.050 [1]**	**< 0.050 [1]**
	Std.Dev.(+/-)	*0.023*	*0.004*	*0.017*	*0.021*	*0.011*	*0.008*
	Minimum	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
	Maximum	0.110	< 0.050 [1]	0.088	0.110	0.074	< 0.050 [1]

Table 3-3: Fraction of total dissolved metal concentration given as a percentage of total metal concentration (total dissolved/total*100). A "[1]" indicates the maximum amount possible, determined by using detection limit values for dissolved fraction concentration.

	Farm 10	Farm 20	Farm 318	Farm 2149	Farm 2704	Farm 3101
Zn	8	50 [1]	14 [1]	10	32 [1]	23 [1]
Cu	38	66 [1]	42	50	86 [1]	53 [1]
Mn	8 [1]	8	9	15	23	17
Fe	49	20	26	39	49	32

Nitrogen, Phosphorous, and Sulfur- Mean values (averaged over depth and sampling period) and standard deviations for the concentrations of nitrogen (as ammonium-N), phosphorous, and sulfur (N,P, and S) species for each lagoon are shown in Table 3-4. Minimum and maximum values are also included in the table. Again, "Total" represents a total concentration of a specific analyte found in lagoon liquid. "Total diss." represents the total concentration of a specific analyte in the portion of liquid passing through a 0.4 micron filter. Total concentration includes the contribution from the dissolved fraction. The phosphorous fraction is divided into total P, total dissolved P, and orthophosphate-P (OP-P). OP-P represents the concentration of total dissolved, reactive phosphorous that is found as the orthophosphate species. Ammonaical nitrogen was determined on the dissolved fraction of lagoon liquid. Sulfur data is divided into three fractions, total S, total dissolved S, and sulfate-S. Sulfate-S represents the concentration of total dissolved sulfur that is found specifically as the sulfate ion.

Nitrogen (ammonium-N)- Mean ammonium-nitrogen (NH_4-N) values varied across all six lagoons, with Farm 20 having the lowest mean value at 183 mg NH_4-N/L and Farm 10 having the highest value at 653 mg NH_4-N/L (Table 3-4). Among the six lagoons there was also considerable range to the data within each lagoon, from around 100 to 400 mg NH_4-N/L. Analysis for dissolved nitrate was conducted on all dissolved fraction samples for the first month and a half of the sampling period. However, no samples contained detectable amounts of nitrate and analysis for nitrate was discontinued.

Table 3-4: Nitrogen, phosphorous, and sulfur concentration (in mg/L) averaged over depth and sampling period (8/2/00-1/25/01) for each lagoon. "Total" represents total acid extractable concentration and "Total Diss." represents total concentration in sample passing through 0.4 micron filter. Ammonium (NH_4-N), orthophosphate (OP-P), and sulfate (SO_4-S) concentration determined in the dissolved sample fraction.

		FARM					
		10	**20**	**318**	**2149**	**2704**	**3101**
		Nitrogen					
NH_4-N	**Mean**	**653**	**183**	**500**	**453**	**391**	**465**
	Std.Dev.(+/-)	*65*	*33*	*106*	*53*	*42*	*68*
	Minimum	547	135	221	390	390	375
	Maximum	805	273	815	599	599	663
		Phosphorous					
Total	**Mean**	**91.2**	**68.9**	**12.8**	**49.8**	**36.4**	**41.0**
	Std.Dev.(+/-)	*6.6*	*11.7*	*4.8*	*5.7*	*6.1*	*7.1*
	Minimum	79.7	49.3	4.7	33.2	22.8	27.4
	Maximum	111.7	96.8	26.4	59.9	51.6	57.4
Total Diss.	**Mean**	**81.9**	**39.4**	**5.0**	**35.6**	**28.7**	**28.6**
	Std.Dev.(+/-)	*6.7*	*8.5*	*3.6*	*5.3*	*5.4*	*6.7*
	Minimum	64.5	27.9	1.8	23.5	19.7	14.2
	Maximum	96.1	59.0	18.5	45.7	42.2	45.4
OP-P	**Mean**	**75.3**	**34.9**	**5.2**	**33.4**	**25.8**	**26.6**
	Std.Dev.(+/-)	*9.7*	*11.6*	*3.0*	*8.0*	*6.3*	*7.7*
	Minimum	60.6	20.5	1.7	16.9	17.0	10.7
	Maximum	101.9	60.7	16.3	53.3	42.1	43.4
		Sulfur					
Total	**Mean**	**32.5**	**37.3**	**23.9**	**22.9**	**33.8**	**17.3**
	Std.Dev.(+/-)	*7.2*	*5.3*	*6.1*	*3.6*	*5.0*	*3.3*
	Minimum	20.8	26.9	15.6	15.5	21.7	11.3
	Maximum	55.9	50.6	36.8	32.2	51.8	25.1
Total Diss.	**Mean**	**18.9**	**31.2**	**14.5**	**15.7**	**24.3**	**12.3**
	Std.Dev.(+/-)	*5.8*	*10.8*	*4.0*	*7.1*	*10.1*	*5.8*
	Minimum	10.0	13.1	10.8	8.3	7.7	2.5
	Maximum	29.6	50.2	28.2	30.8	42.6	23.6
SO_4-S	**Mean**	**8.6**	**22.9**	**3.2**	**7.7**	**17.7**	**6.7**
	Std.Dev.(+/-)	*6.3*	*10.8*	*3.4*	*7.0*	*10.6*	*5.5*
	Minimum	0.3	5.6	0.3	0.3	0.6	0.2
	Maximum	18.0	38.2	16.0	21.5	34.5	16.7

Phosphorous- All three phosphorous species; total, total dissolved, and dissolved orthophosphate phosphorous, showed considerable range in concentration within each lagoon over the sampling period. Statistical analysis of the mean total and total dissolved phosphorous values indicates that the mean total phosphorous concentration is significantly greater (tested at the P= 0.05 level) then the mean total dissolved phosphorous concentration at all six lagoons. The statistical analysis conducted was a one tailed t-test to determine if mean total phosphorus was significantly greater then the mean total dissolved phosphorous. Table 3-4 indicates that there is a sizable amount of phosphorous, around 10-30 mg P/L, separated out during centrifugation or retained on the 0.4 micron filter. The data suggests that orthophosphate is the predominant dissolved phosphorous species, and that the total dissolved phosphorous ≈ orthophosphate phosphorous.

Sulfur- The results of a one tailed t-test conducted on the mean total and total dissolved sulfur concentrations indicate that the mean total sulfur concentration is significantly greater (tested at the P=0.05 level) then the total dissolved sulfur concentration in all six lagoons. The sizable difference in concentration, around 10 mg S/L, between total sulfur and total dissolved sulfur for all six lagoons indicates that a significant fraction of sulfur is separated during centrifugation or retained on the 0.4 micron filter. The same statistical analysis conducted on mean total dissolved sulfur and dissolved sulfate-sulfur concentrations indicates that the mean total dissolved sulfur concentration is significantly greater (at the P=0.05 level) then the mean dissolved sulfate-sulfur concentration.

Based on the data, the fraction of sulfur comprising total dissolved sulfur can be divided into sulfate-sulfur and non sulfate-sulfur. It is likely that this non-sulfate fraction

of dissolved sulfur includes sulfide species as well as sulfur associated with soluble organic matter, given that hydrogen sulfide has been observed in swine lagoon emissions.

Base Cations (Ca,Mg,Na,K) and Chloride (Cl) - Mean values (averaged over depth and sampling period) and standard deviations for the measurement of calcium, magnesium, sodium, potassium, and chloride (Ca, Mg, Na, K, and Cl) for each lagoon are recorded in Table 3-5. Minimum and maximum values for each element are also included. Again, "Total" represents a total concentration of a specific analyte found in lagoon liquid. "Total Diss." represents the total concentration of a specific analyte in the portion of liquid passing through a 0.4 micron filter. Total concentration includes the contribution from the dissolved fraction.

According to Table 3-5 and Table 3-3, the difference between mean total Ca and Mg values and dissolved Ca and Mg values indicates that a portion of these elements are bound in a solid phase separated out during centrifugation or retained on the 0.4 micron filter. This is true across all lagoons with the singular exception of magnesium values for Farm 318. Statistical analysis of the data using a one tailed t-test indicates that indeed the mean total calcium and magnesium concentrations are significantly greater (tested at the P=0.05 level) then the mean total dissolved calcium and magnesium concentrations, with the exception of magnesium values for Farm 318. Most, if not all, the magnesium in the lagoon fluid at Farm 318 exists as a dissolved species. It is also apparent from Table 3-5 that mean values for every parameter are sizably greater for Farm 318 then for the other

Table 3-5: Base cation (Ca, Mg, Na, K) and anion (Cl) concentration (in mg/L) averaged over depth and sampling period (8/2/00-1/25/01) for each lagoon. "Total" represents total acid extractable concentration and "Total Diss." represents total concentration in sample passing through 0.4 micron filter. Sodium (Na) and potassium (K) values based on analysis of only 3 sampling dates.

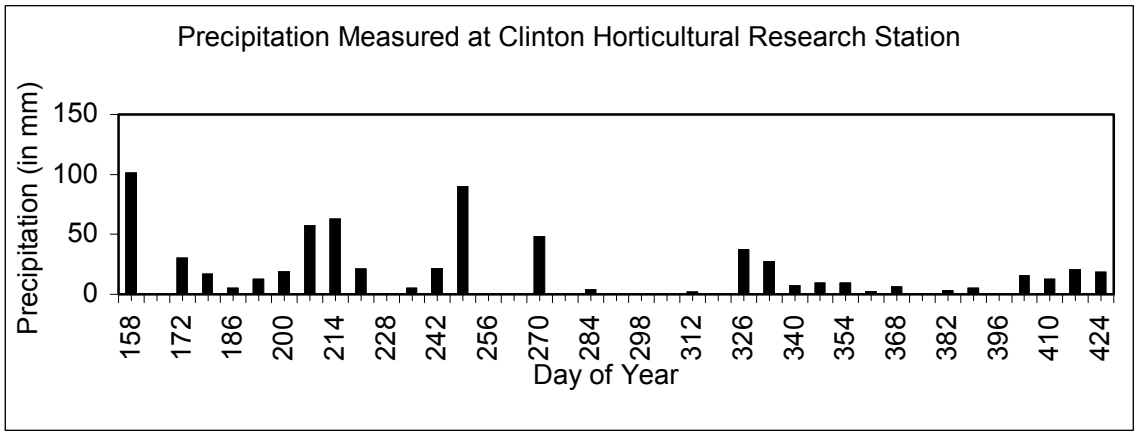
		FARM					
		10	**20**	**318**	**2149**	**2704**	**3101**
		Calcium					
Total	**Mean**	**38.5**	**51.1**	**71.5**	**42.9**	**41.6**	**43.1**
	Std.Dev.(+/-)	*12.2*	*8.1*	*24.9*	*10.1*	*10.8*	*10.2*
	Minimum	17.5	32.8	31.8	22.5	16.4	17.6
	Maximum	75.4	72.6	130.8	68.0	60.4	68.4
Total	**Mean**	**9.8**	**18.9**	**9.1**	**14.8**	**11.0**	**13.9**
Diss.	*Std.Dev.(+/-)*	*5.3*	*6.2*	*6.0*	*6.6*	*6.9*	*9.7*
	Minimum	3.0	7.9	2.5	4.8	1.0	3.2
	Maximum	22.0	31.6	29.9	36.0	31.8	39.6
		Magnesium					
Total	**Mean**	**7.9**	**25.6**	**64.2**	**15.6**	**11.9**	**15.8**
	Std.Dev.(+/-)	*2.4*	*6.3*	*17.9*	*5.5*	*5.0*	*5.2*
	Minimum	3.7	10.9	38.6	5.4	3.8	5.6
	Maximum	14.4	35.4	85.4	27.0	27.0	24.7
Total	**Mean**	**3.5**	**13.7**	**62.5**	**6.9**	**8.3**	**8.0**
Diss.	*Std.Dev.(+/-)*	*1.7*	*5.3*	*11.4*	*3.6*	*3.4*	*3.8*
	Minimum	1.3	3.9	34.0	2.1	3.7	3.9
	Maximum	8.8	25.4	81.2	18.5	17.9	18.5
		Sodium					
Tota	**Mean**	**308**	**125**	**409**	**155**	**152**	**118**
Diss.	*Std.Dev.(+/-)*	*27*	*8*	*37*	*9*	*7*	*11*
	Minimum	278	117	364	142	142	104
	Maximum	348	139	463	170	162	134
		Potassium					
Total	**Mean**	**1076**	**510**	**1527**	**781**	**723**	**562**
Diss.	*Std.Dev.(+/-)*	*99*	*29*	*115*	*42*	*33*	*51*
	Minimum	964	464	1342	722	664	510
	Maximum	1232	550	1677	834	766	652
		Chloride					
Totall	**Mean**	**657**	**222**	**756**	**383**	**361**	**248**
Diss.	*Std.Dev.(+/-)*	*58*	*14*	*47*	*24*	*21*	*38*
	Minimum	560	193	655	335	315	169
	Maximum	745	256	840	432	405	324

lagoons. Mean values for Na, K, and Cl for Farm 10 as well as Farm 318 are approximately 2 times greater then values found in the other lagoons. Total concentrations of Na and K were not attempted as these elements were assumed to both be soluble. For Na and K analysis only three sampling dates were analyzed because of analytical limitations, with the assumption that these elements would be conserved temporally.

Temporal Trends

An important parameter to consider when evaluating temporal trends in swine lagoon chemistry is the effect of rainfall. Rainfall is usually associated with storm events and is also a source of chemical inputs (e.g. sulfate, nitrate, oxygen) into lagoon systems. Rainfall data for the sampling period was retrieved from the database at the Clinton Horticultural Crops Research Station in Clinton, NC. Figure 3-1 is a plot of rainfall (in mm) over the sampling period, where each data point represents total rainfall (in mm) for the seven day period ending on the day plotted.

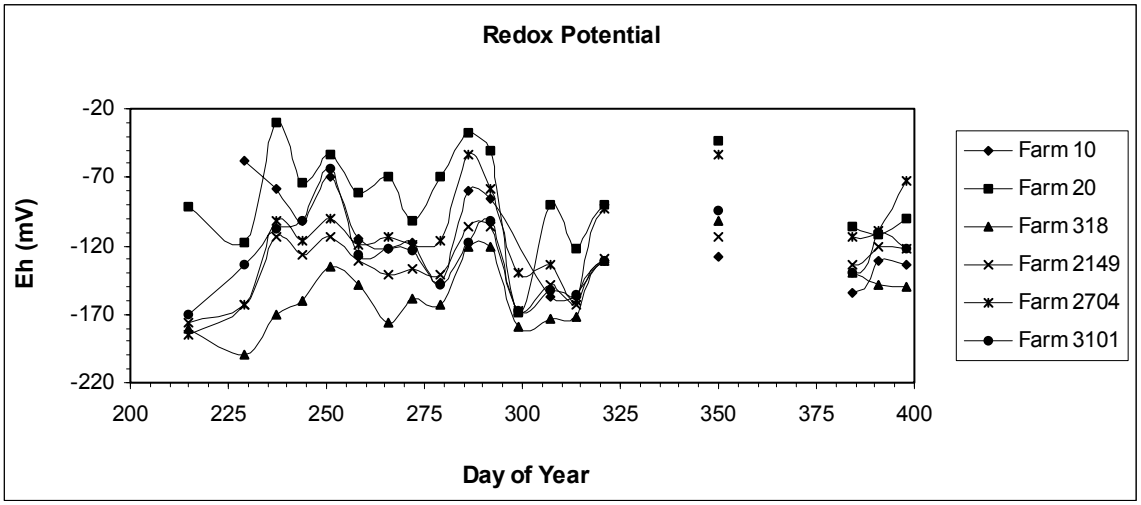
Figure 3-1: Rainfall (in mm) measured at the Clinton Horticultural Crops Research Station in Clinton, NC. Each data point represents total rainfall (in mm) for the seven day period ending on the day plotted. Day of year 215 represents 8/2/00, and day of year398 represents 2/1/01.

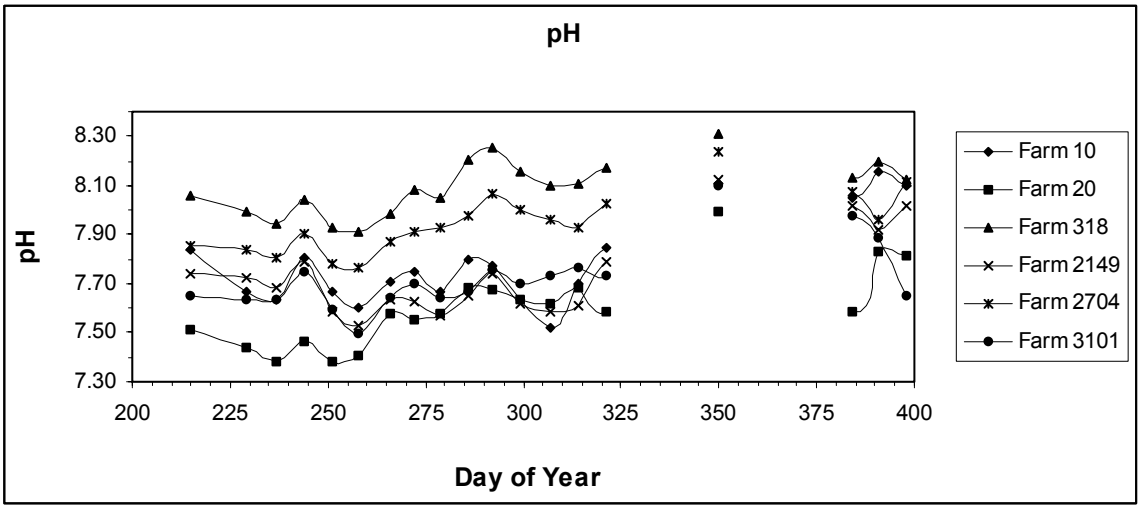


Observing temporal plots of data over the sampling period facilitates evaluation of the wide range in concentration values for most analytes. Figure 3-2a is a plot of Eh, pH, and lagoon temperature data averaged over depth for each sampling date and plotted over the entire sampling period. For Farm 10, where the sampling period for field measurements began on 6/7/00, field measurement data were included in the plot with all six farms to facilitate comparison. However plots of field measurement data solely for Farm 10 for the sampling period from 8/2/00 - 1/25/01 are plotted in Figure 3-2b. Explanation as to possible reasons for trends in the temporal data will be discussed later in Chapter 4, section 4.3.

Eh- The plots of measured Eh suggest periodic and dramatic fluctuations in measured potential which are consistent across all six lagoons. All six lagoons tend to follow the same pattern of increasing and decreasing Eh, but to differing absolute values. With the exception of Farm 10, at all lagoons there appears to be a significant increase in measured potential around day 229, an increase of up to 70 mV for some lagoons. All six lagoons also appear to show significant increases in potential on day 286, some increase by almost 50 mV. Another smaller increase possibly occurs around day 251. There also might be evidence of the beginning of an increase on day 321, but without data immediately following this date there can be no conclusive decision.

Figure 3-2a : Mean Eh, pH, and lagoon temperature data averaged over depth and plotted as a function of the sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 398 represents 2/1/01.





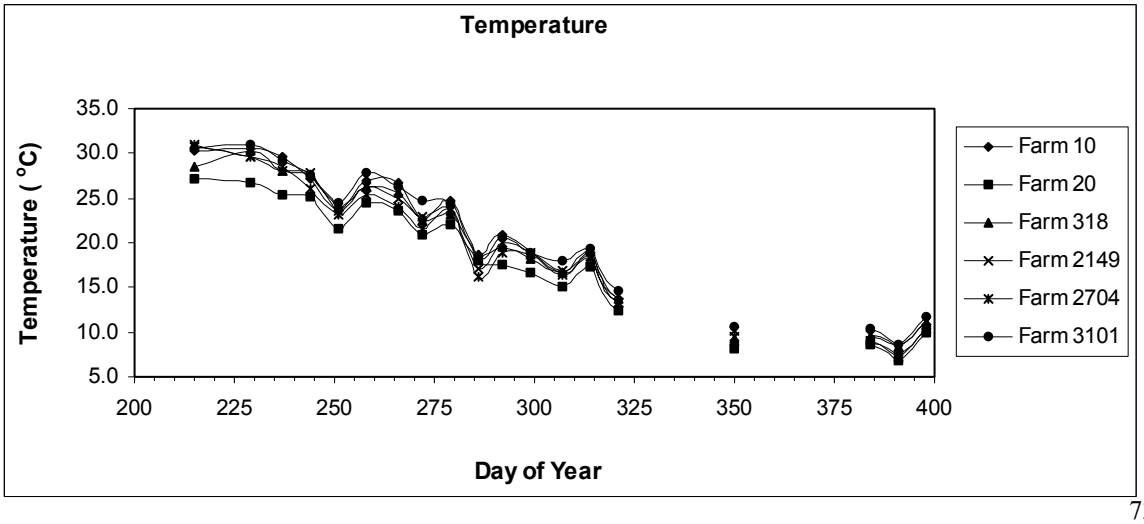
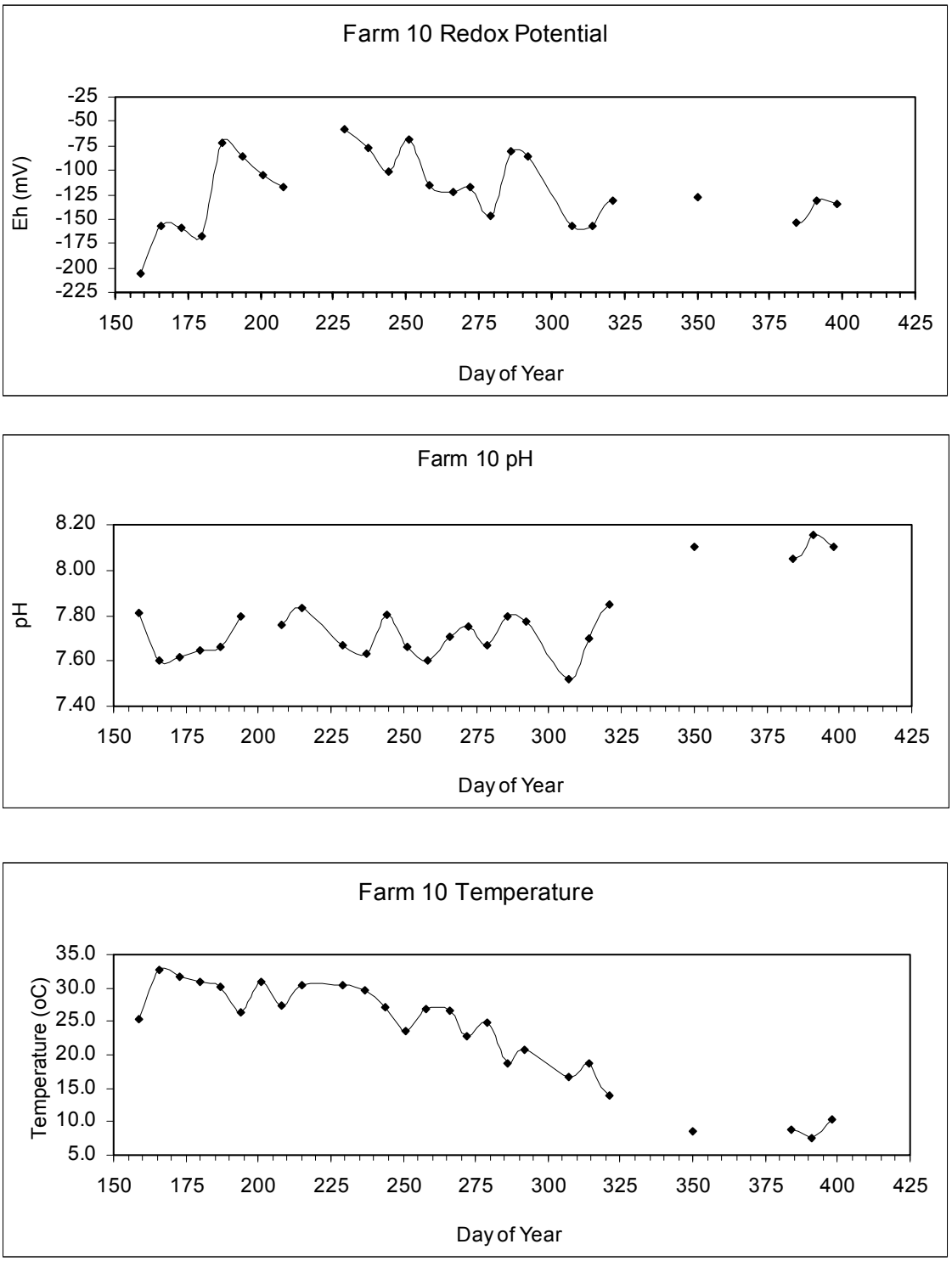


Figure 3-2b: Farm 10 mean Eh, pH, and lagoon temperature data averaged over depth and plotted as a function of sampling period (6/7/00-2/1/01). Day of year 159 represents 6/7/00, and day of year 398 represents 2/1/01.



pH - Examination of the plot of pH for each farm in Figure 3-2 suggests there might be a slight increase in measured pH toward the end of the sampling period. However without more data points near that end of the sampling period it is difficult to make a conclusive decision as to whether there is a significant trend to the data or not. However, similar to Eh values, pH values suggest periodic fluctuations. There appears to be periods where changes in pH are consistent across all lagoons, all increasing and decreasing in the same fashion but differing in the magnitude of change. A small increase in pH across all six lagoons occurs around day 244, and another period of pH increase occurs from around day 286 through day 292.

Lagoon Temperature- Lagoon temperature data in Figure 3-2 reflects the trend of decreasing ambient temperature from the summer months through the fall into the winter season. The slightly undulating nature of the plots suggests local climate fluctuations, which all six lagoons appear to respond to equally. There appears to be two significant decreases in lagoon temperature, one on day 244 and the other on day 286, with a smaller decrease occurring on day 272. There also appears to be a slight temperature increase in all six lagoons on day 314.

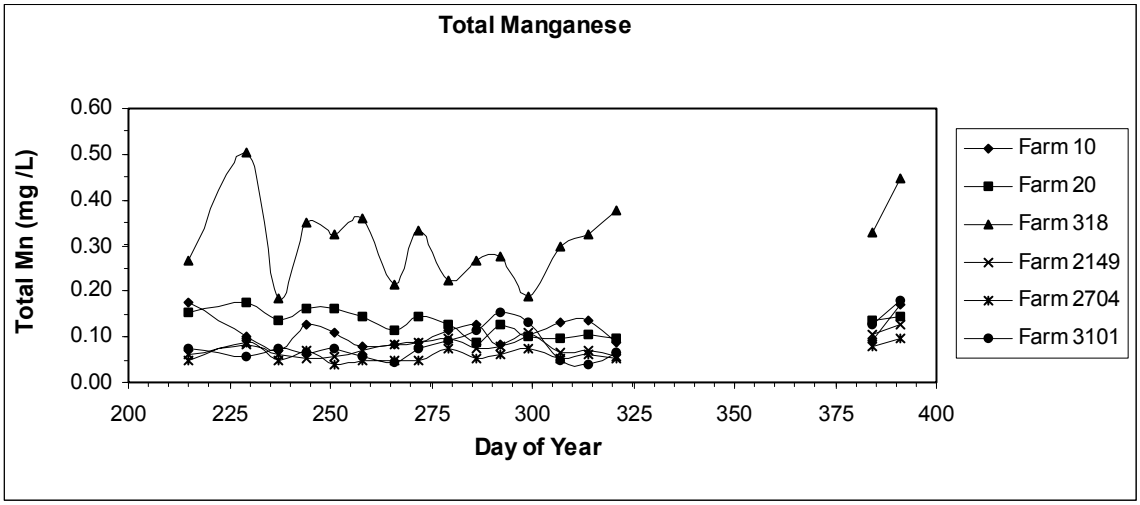
The time of day when sampling occurred also appears to effect lagoon temperature measurement. As mentioned, the daily sampling procedure started at Farm 20 at approximately 9:00 a.m., followed by Farms 2704, 2149,3101,318, and finally Farm 10 at approximately 4:00 p.m.. This progression is evident in Figure 3-2, as Farm 20 has the coldest lagoon temperature with farms sampled later in the day increasing in temperature.

Figures 3-6 contain plots of manganese, copper, iron, and zinc data averaged over depth for each sampling date and plotted for each farm over the entire sampling period (8/2/00-1/25/01).

Manganese- Examining the plots of total manganese for each lagoon there is no significant trend to the data, indicating that for most lagoons total manganese concentration remains constant. However Farm 318 displays wide variation in both total and dissolved manganese concentrations. There appears to be a minor increase in dissolved manganese concentration around day 279 that occurs in all lagoons with the exception of Farm 318. Data from the final sampling date indicates there might be a significant increase in dissolved manganese, but without additional data an evaluation of this data is impractical.

Copper- Concentrations of total copper for lagoons on Farms 3101, 20, and 2704 remain relatively constant, indicating that there is no apparent overall trend in total copper concentration. There appears to be a slight increase in total copper at the end of the sampling period. Total copper concentration for Farms 10, 2149, and 318 appear to fluctuate sporadically, but show no significant overall trend. As with total copper, dissolved copper on Farms 3101, 20, and 2704 remain relatively constant, while on Farms 10, 2149, and 318 values fluctuate sporadically. However for Farms 10, 2149, and 318 fluctuations in dissolved copper concentrations appear to synchronize periodically. There appears to be an initial sharp decrease at the beginning of the sampling period, with two sharp increases beginning around day 272 and again on day 292.

Figure 3-3: Mean manganese concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



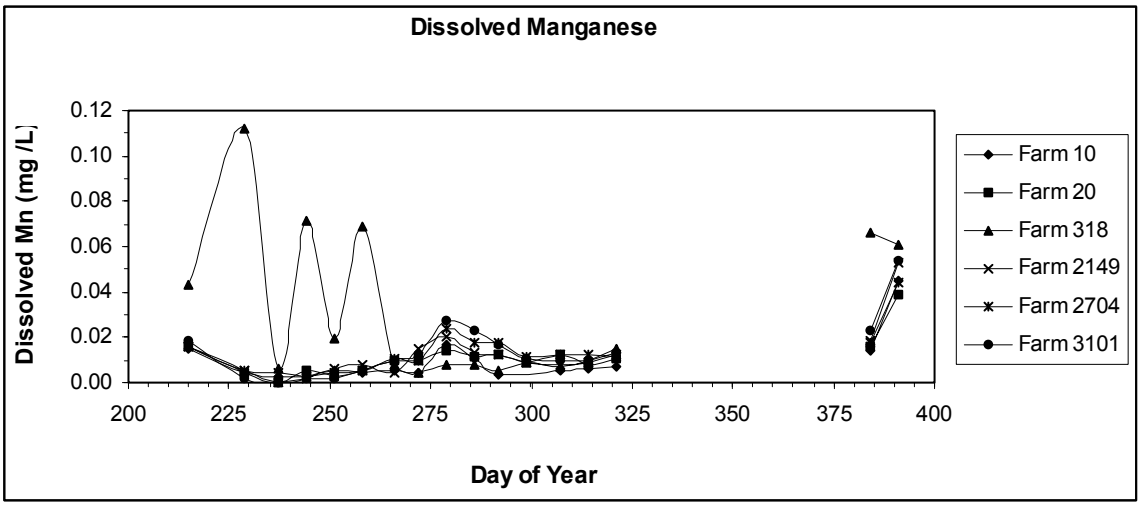
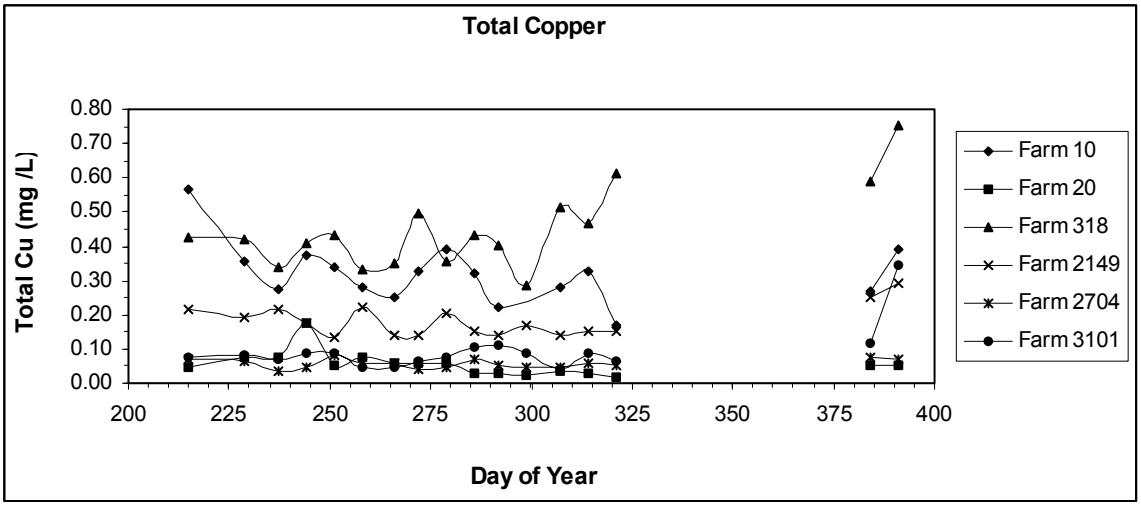


Figure 3-4: Mean copper concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



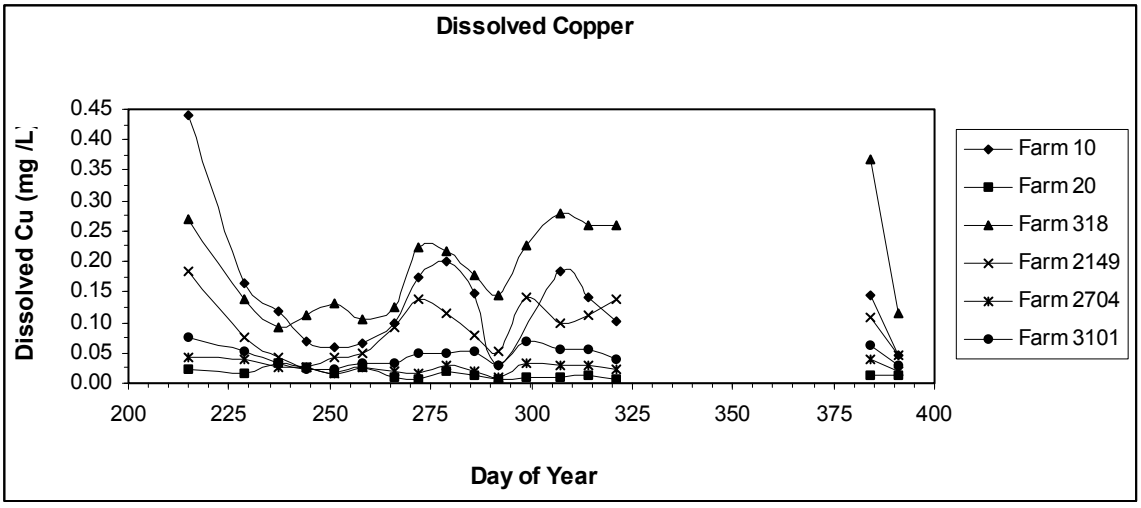
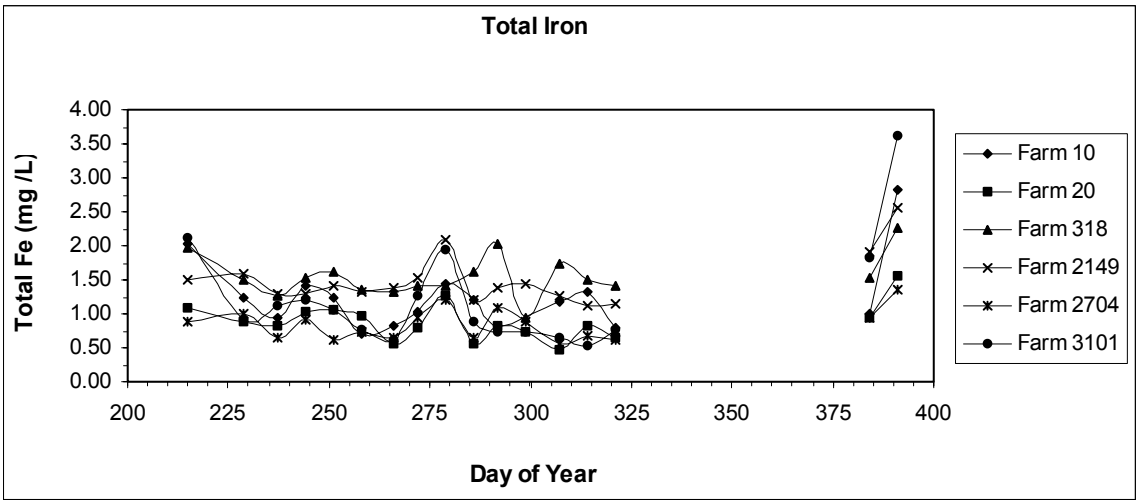


Figure 3-5: Mean iron concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



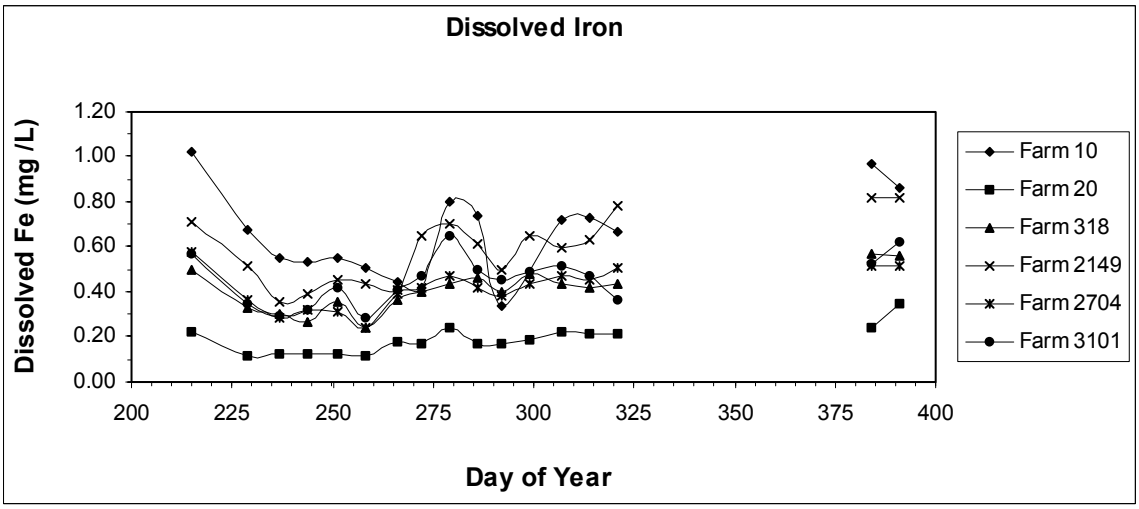
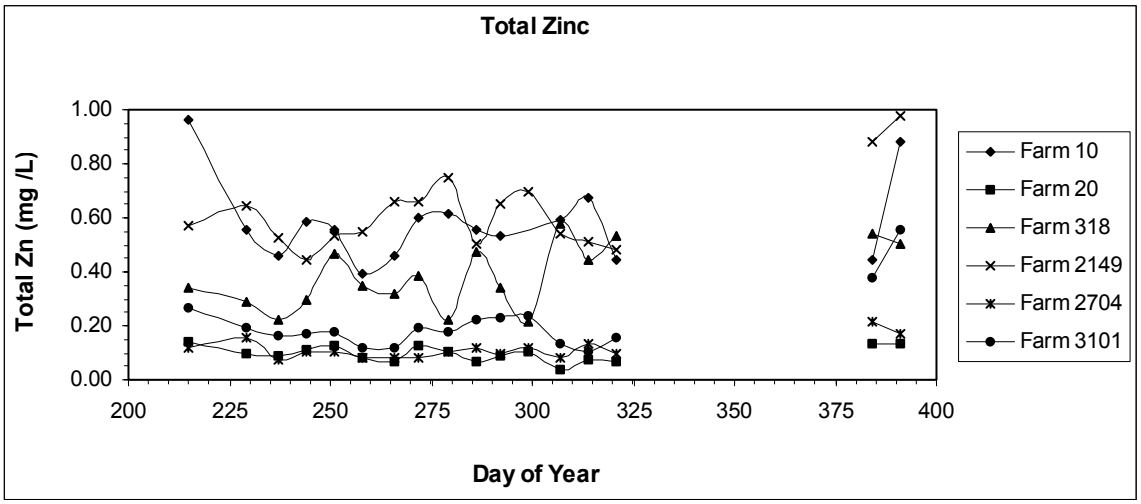
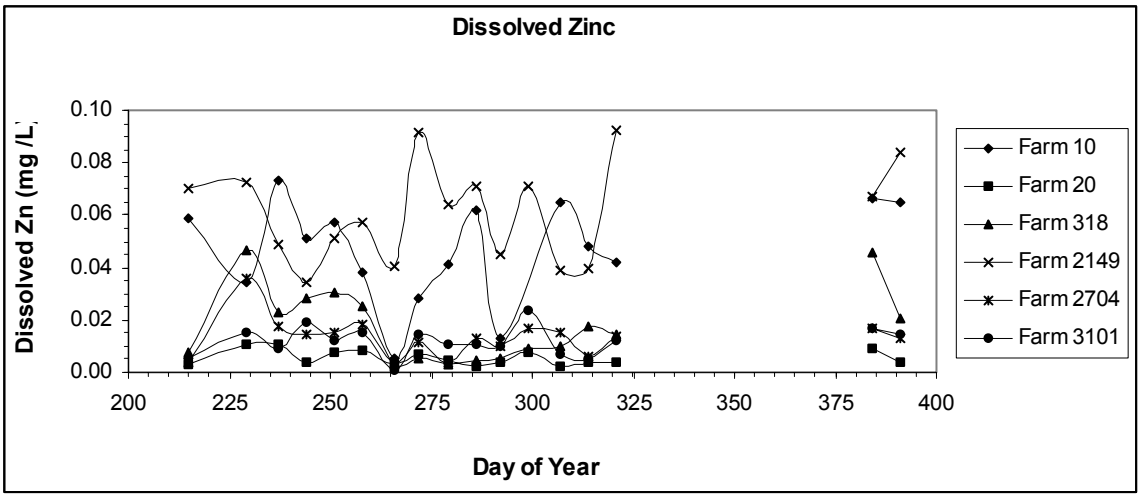


Figure 3-6: Mean zinc concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.





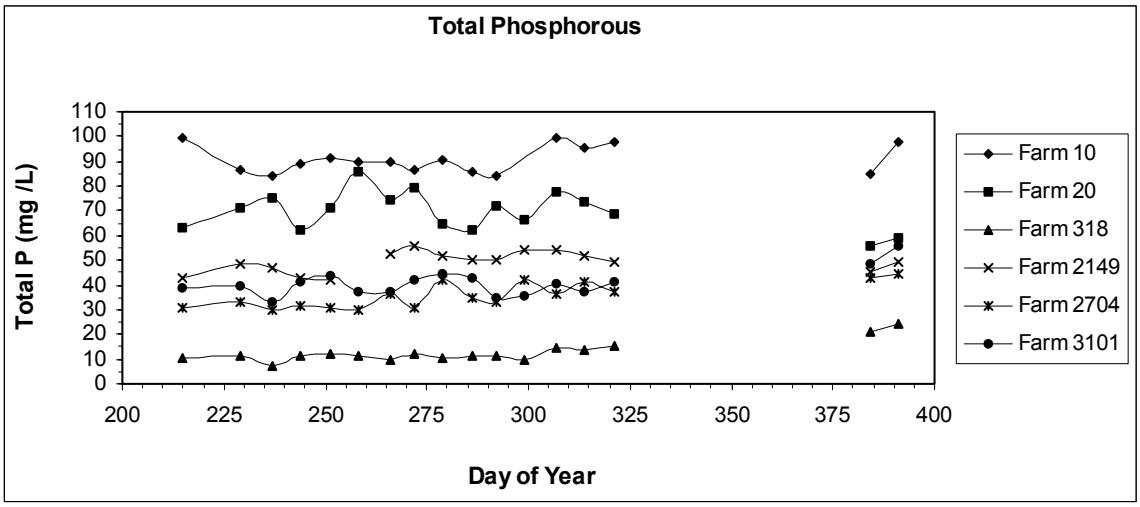
Iron- Aside from the data points at the end of the sampling period, there does not appear to be any large changes in total or dissolved iron. An initial decrease in dissolved iron concentration is evident across all six lagoons at the beginning of the sampling period, although only Farms 10,318, and 3101 show a concomitant decrease in total iron concentration. There appears to be an event causing a dramatic increase in both total and dissolved iron around day 279. This fluctuation in iron concentration occurs across all lagoons with the exception of Farm 318.

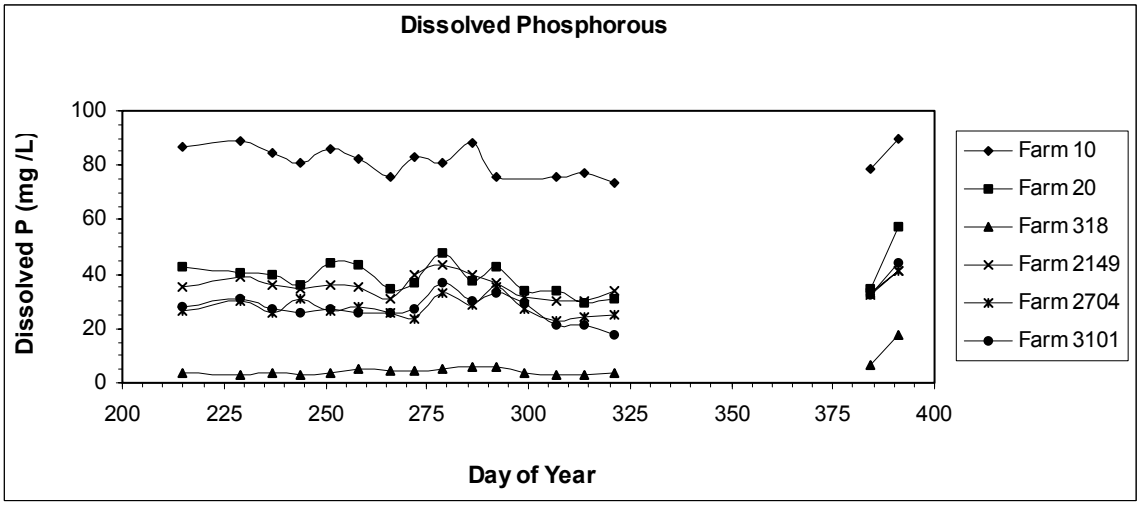
Zinc- According to Figure 3-6, plots of total and dissolved zinc concentration for each lagoon display no significant trend. Across the sampling period Farms 3101, 20, and 2704 appear to maintain relatively stable concentrations of both zinc fractions, while on Farms 10, 2149, and 318 both total and dissolved zinc concentrations appear to fluctuate around a mean value.

Figures 3-7 through 3-9 contain plots of phosphorous, sulfur, and ammonium nitrogen data averaged over depth for each sampling date and plotted for each farm over the entire sampling period (8/2/00-1/25/01).

Phosphorous- For all phosphorous fractions (Total, total dissolved, and orthophosphate-P) the data appears to remain relatively constant throughout the sampling period, indicating that phosphorous in the lagoon is maintained at a relatively stable concentration. Plots of total phosphorous (P) for all farms fluctuate periodically, with no apparent pattern to the fluctuations. On Farms 10,20,3101, and 2704 there appears to be an

Figure 3-7: Mean phosphorous concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.





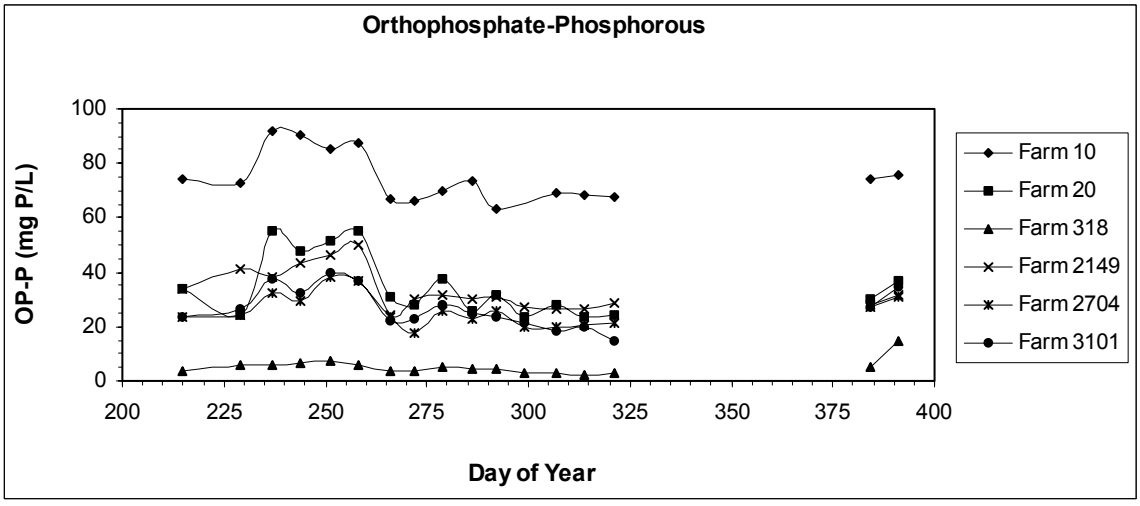


Figure 3-8: Mean sulfur concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.

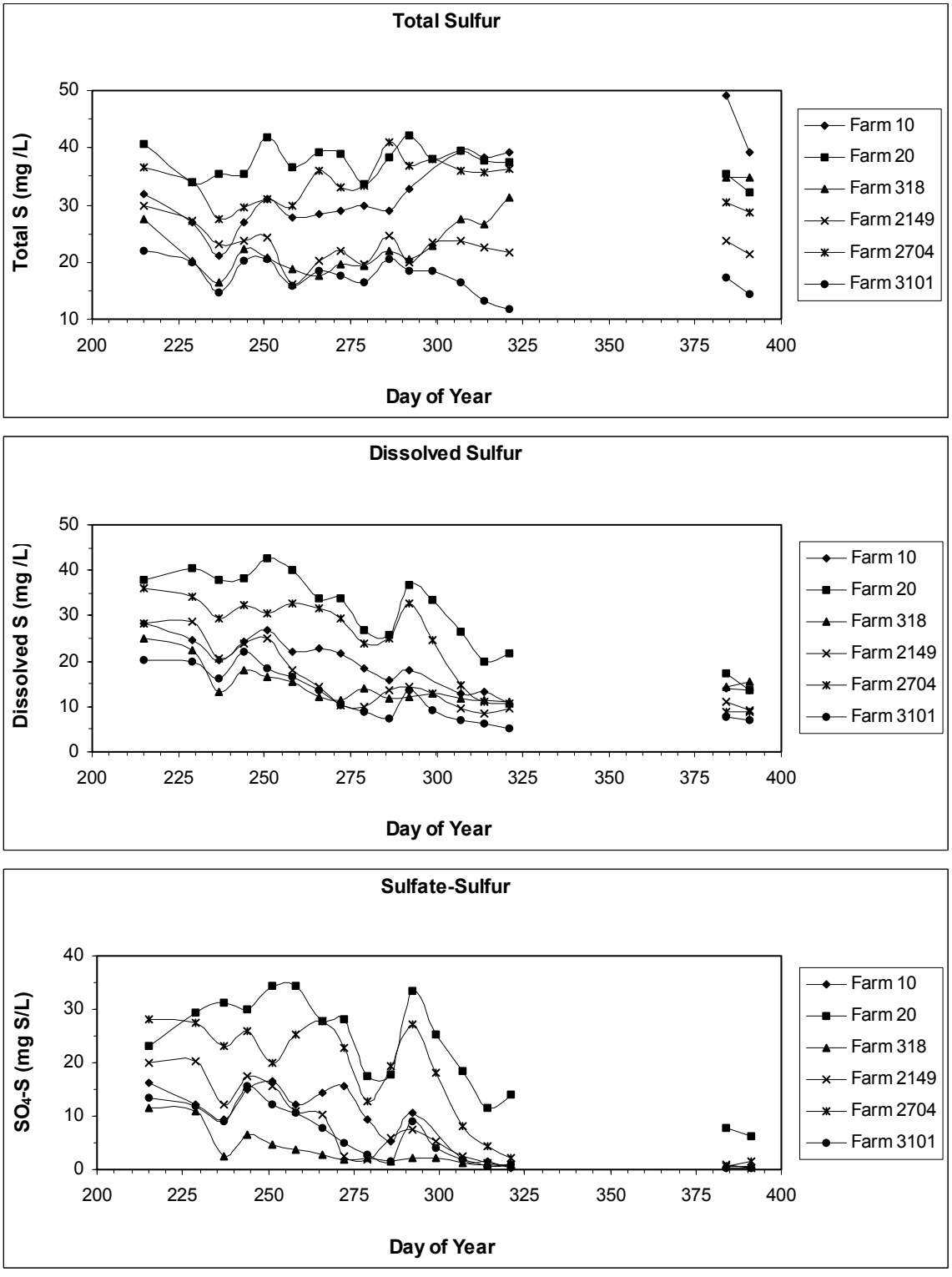
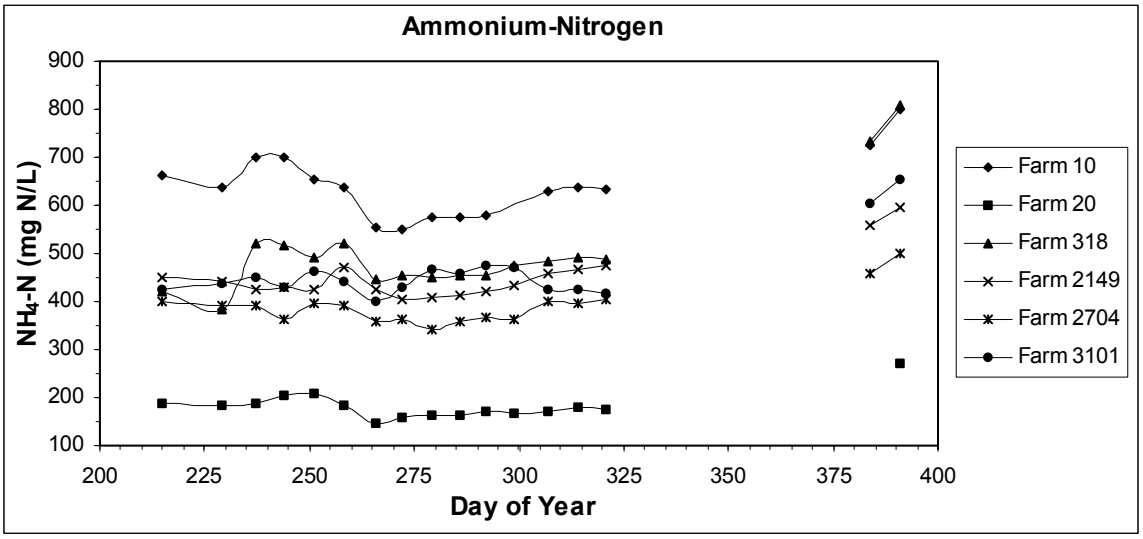


Figure 3-9: Mean dissolved ammonium nitrogen concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



event around day 279 causing an increase in dissolved P of around 10 mg/L, with another smaller fluctuation appearing across these lagoons, with the exception of Farm 10, around day 292. On farms 10,20,2704, and 3101 there appears to be an event around day 237 causing an increase in orthophosphate (OP) of almost 20 mg/L in some cases, with another fluctuation around day 258.

However, it can be observed that during these fluctuations in orthophosphate concentration, orthophosphate-phosphorous concentration exceeds values for total

dissolved phosphorous. This discrepancy indicates analytical error in the data. The data was included with the assumption that there is no real difference between the values for these sampling dates, and that any difference is due to error in the analysis of the sample. Significant sample dilution was necessary for orthophosphate determination, and to a lesser extent dissolved phosphorous, with the result that any analytical error is magnified by the dilution effect.

Sulfur- Plots of total sulfur concentration for Farms 318 and 10 seem to show a slight increase in sulfur over the sampling period. All other lagoons appear to maintain a relatively steady concentration of total sulfur over the sampling period. At some points in time it appears that total sulfur and total dissolved sulfur overlap, suggesting there were periods where few sulfur containing particles larger then 0.4 microns were suspended in solution.

Total dissolved sulfur and sulfate sulfur both appear to decrease over the sampling period. Furthermore there appear to be periods where the sulfate sulfur and total dissolved sulfur concentrations increase and "peak". This phenomenon appears to be correlated across all six lagoons. One large peak occurs around day 292, with a possible smaller peak around day 244.

For all lagoons total dissolved sulfur and sulfate-sulfur concentration decreased over the sampling period and, with the exception of Farm 20 (at 6 mg SO_4 –S/L), approached the detection limit. Total dissolved sulfur decreased as well and stabilized at a non-zero value around day 307. Presumably this reflects localized climate change

affecting all six lagoons. Water temperatures for all six lagoons during this period drop to 20^{o}C and below.

Considering the three inputs for sulfate in the lagoon environment (rain water, water and waste from the houses, and microbial conversion), it seems possible that the drop in lagoon temperature contributed to a metabolic slowdown in microbial sulfide oxidation. This suggests that there is a microbially mediated sulfur cycle, and therefore the possibility of a sulfate/sulfide redox couple, active in swine lagoon environments.

Nitrogen- Ammonium levels in all six lagoons remain relatively stable throughout the sampling period, displaying no apparent temporal trend. At the end of the sampling period on days 384 and 391 all six lagoons showed an increase in ammonium concentration of around 100 mg NH_4-N/L. An increase in ammonium concentration during the cold winter months would be expected, as ammonia volatilization is a temperature dependent process. Alternatively, a decrease in biological activity during cold months could have resulted in a decrease in microbial assimilation of nitrogen.

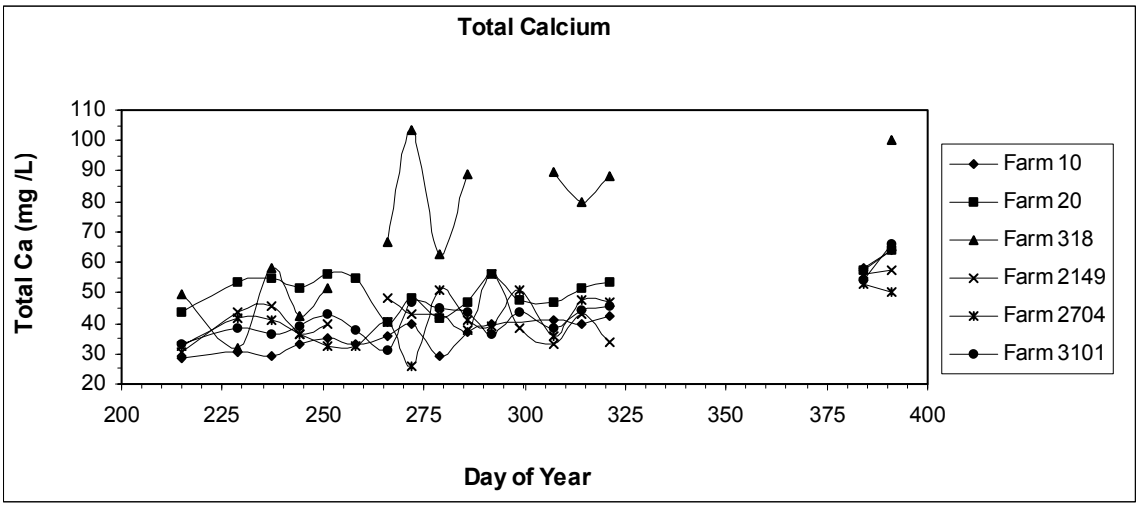
It is possible that the temperature decrease in lagoon fluid resulted in a decrease in ammonia volatilization, with the end result being an elevated ammonium concentration. Considering that ammonia is a basic species, where protonation results in formation of ammonium ion, a decrease in ammonia volatilization should coincide with an increase in pH of lagoon effluent [pK = 9.3 for $NH_z^+ + H_2O = NH_3 + H^+$ (**3**)]. With the exceptions of Farms 2704 and 318 there appears to also be an increase in lagoon pH around days 384 and 391 (Figure 3-2).

Base Cations (Ca,Mg,) and Chloride (Cl)- Figures 3-10 through 3-12 contain plots of calcium, magnesium, and chloride concentrations averaged over depth for each sampling date and plotted for each farm over the entire sampling period (8/2/00-1/25/01).

Overall there does not appear to be any significant trend to the data for calcium, magnesium, and chloride (Ca, Mg, Cl). Not enough data on sodium and potassium concentration was collected to discern temporal trends. Chloride concentration shows a possible slight trend, increasing towards the end of the sampling period.

Data for total and dissolved calcium and magnesium show periodic fluctuations. With the exception of Farm 318, there appears to be two increases in dissolved calcium concentration that are apparent across all lagoons. The first increase occurs around day 229, with a possible smaller increase around day 251.

Figure 3-10: Mean calcium concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



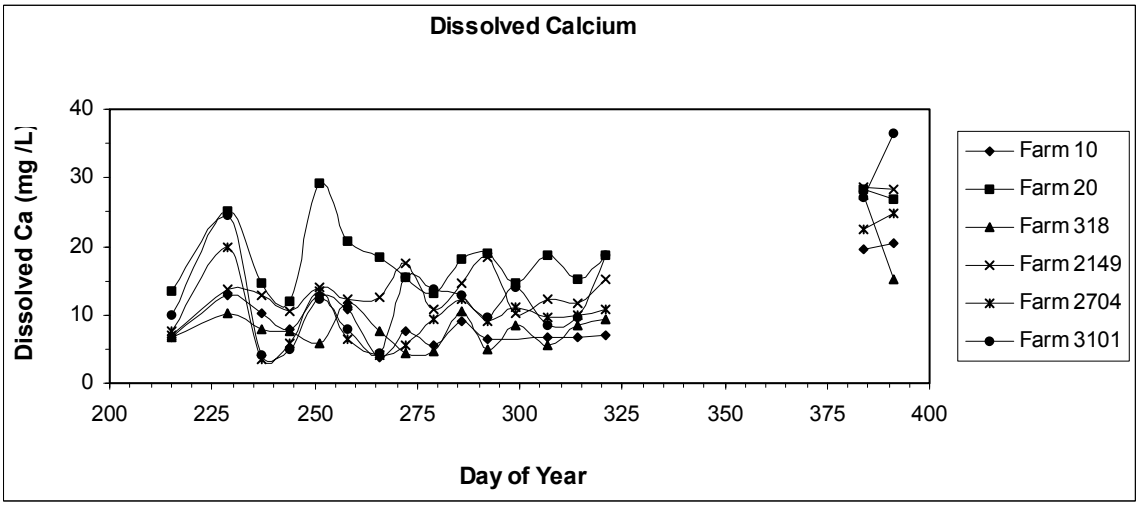
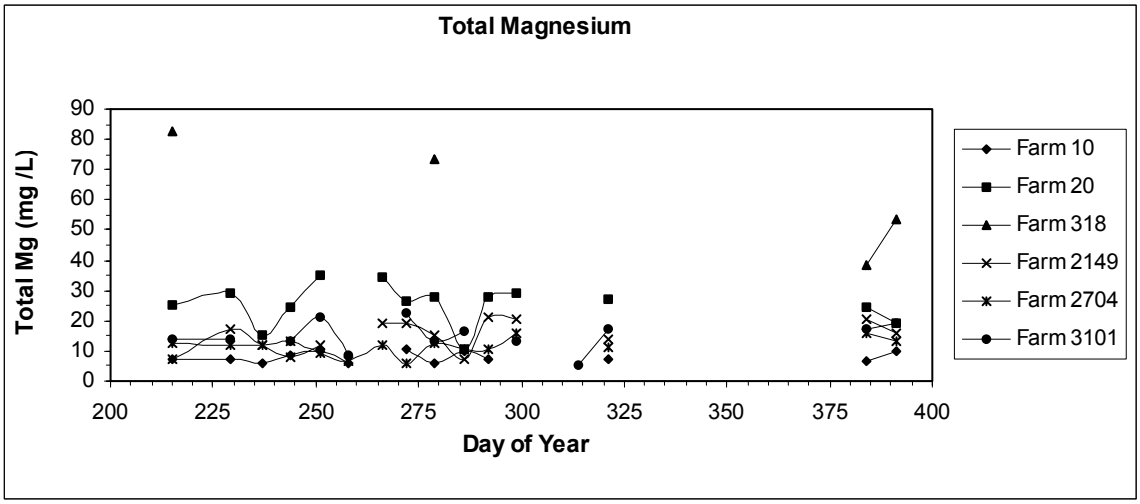


Figure 3-11: Mean magnesium concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.



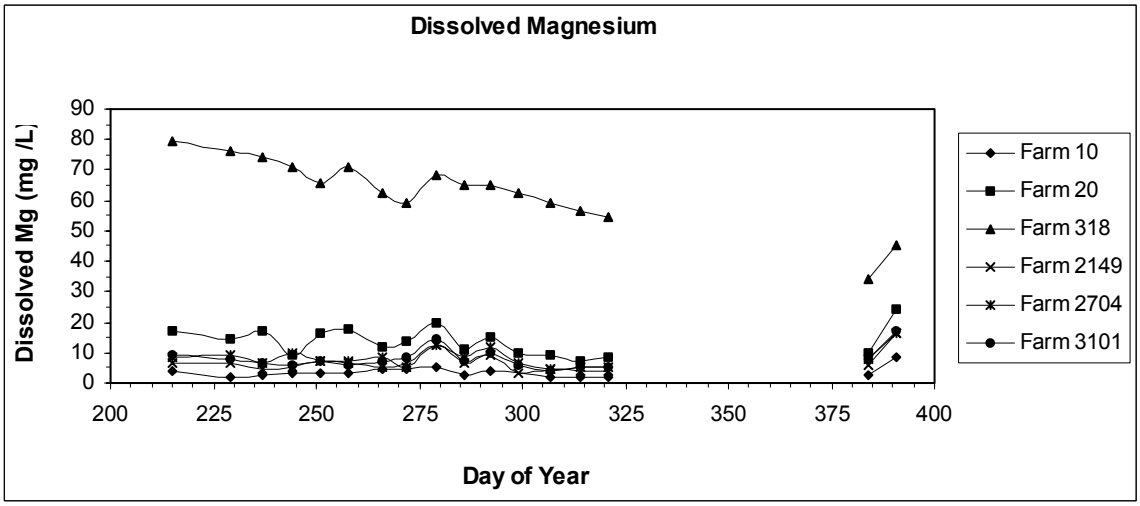
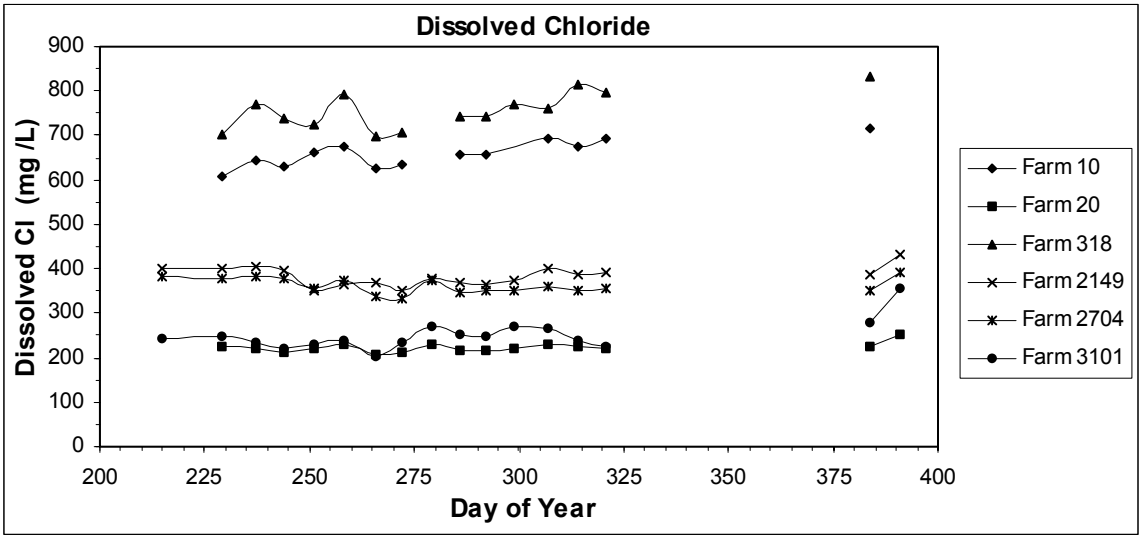


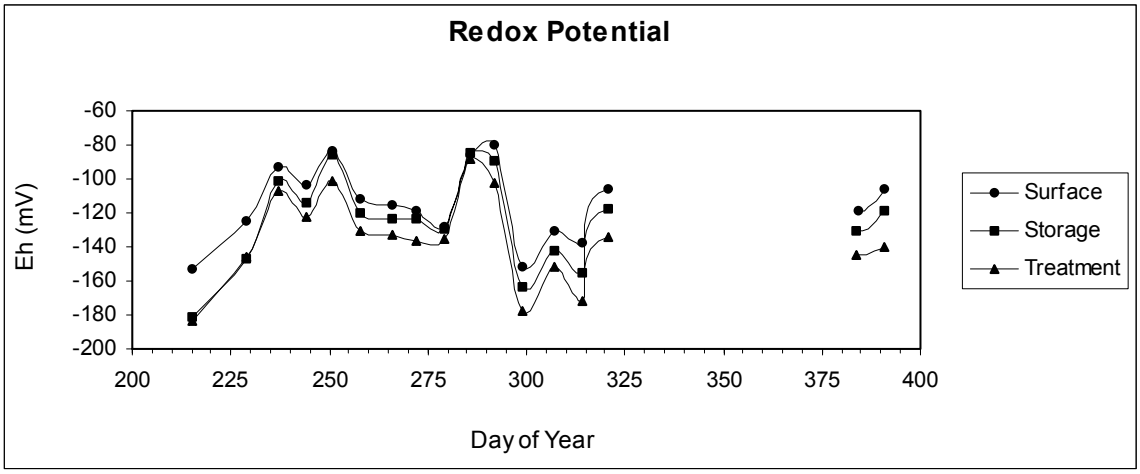
Figure 3-12: Mean dissolved chloride concentration averaged over depth and plotted as a function of sampling period (8/2/00-1/25/01) for each lagoon. Day of year 215 represents 8/2/00, and day of year 391 indicates 1/25/01.

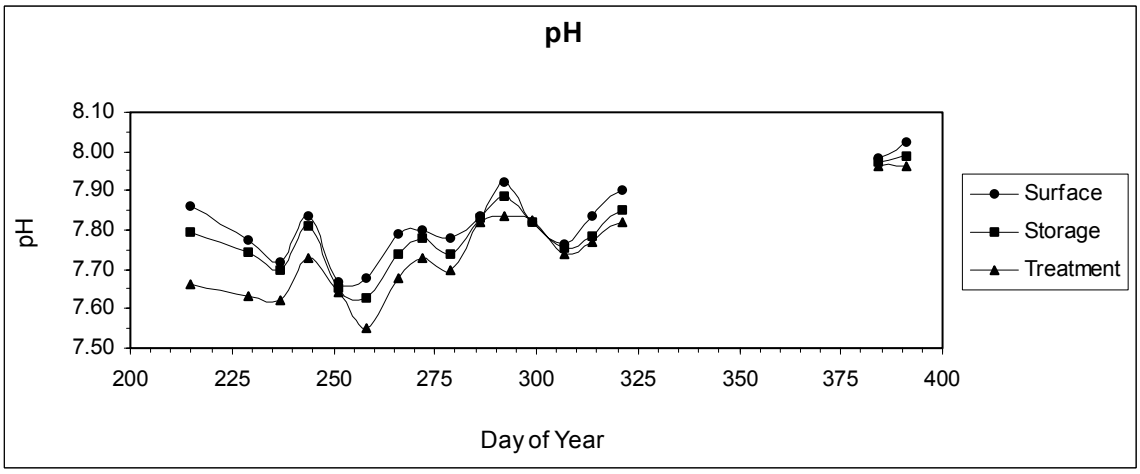


Variation with Depth

Figure 3-13 contains plots of Eh, pH, and lagoon temperature data averaged at each depth over all six lagoons. It is apparent that there is some stratification to the data, and that the stratification is consistent. Surface measurements across all field measurements appear to be consistently higher then storage values, which in turn appear higher then treatment values. From the plots it is evident that on some days the readings indicate a lack of stratification in measured values, while on some days stratification is apparent. It is also evident that there is no correlation in stratification across the field measurements, in that when one measurement shows a lack of stratification the other measurements do not necessarily demonstrate a lack of stratification.

Figure 3-13: Eh, pH and lagoon temperature data averaged over all six lagoons and plotted as a function of depth. Day of year 215 represents 8/2/00, and day of year 398 indicates 2/1/01. "Surface" indicates depth of 0-0.3 m below the lagoon surface, "Storage" indicates a depth of 0.6-0.9 m below the lagoon surface, and "Treatment" indicates a depth of 0.6-0.9 m from the bottom of the lagoon.





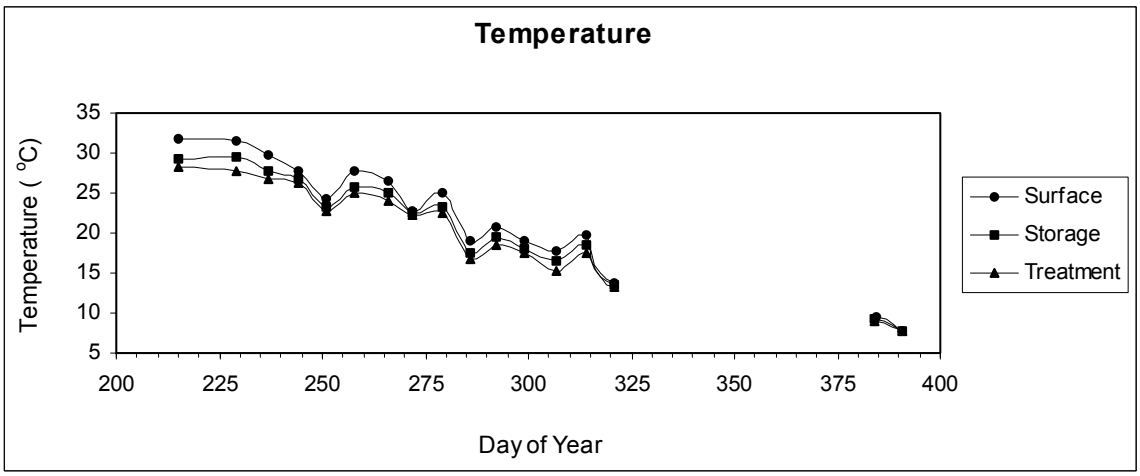


Table 3-6 contains data for each lagoon averaged over the sampling period for each depth sampled (Surface, Storage, and Treatment).

The complete set of data for each parameter at each depth for each lagoon was statistically analyzed using a Randomized Complete Block ANOVA test, with data blocked by day of year (**2**). Results from this test were then tested using the Tukey Multiple Comparison procedure. The ANOVA test was used to determine whether depth had a significant effect on mean parameter value, and the Tukey test was used to attempt to delineate which depths were significantly different (**4**). Table 3-7 illustrates the results of these tests. The mean value of the parameters indicated in Table 3-7 were found to be significantly effected by depth, and subsequently their depth relationship is defined.

Eh- All six farms showed a significant effect of depth on measured redox potential although the absolute magnitude of the difference is rather small. One would assume that any depth variation found in a lagoon environment would be related to distance from the atmosphere-lagoon interface. Results from the statistical analysis seem to lend credence to this hypothesis, as all farms showed a decrease in redox potential with distance from the lagoon surface. This suggests that lagoon chemistry is impacted by the continuous diffusion of oxygenated air into and through the lagoon surface.

Table 3-6a : Mean Eh, pH, and lagoon temperature data averaged over sampling period (8/2/00-2/1/01), and mean measured parameter values averaged over sampling period (8/2/00-1/25/01) given by depth sampled for all lagoons. Data values expressed as mg/L unless otherwise noted. A "1" represents the detection limit value for a specific analyte. "Surface" indicates depth of 0-0.3 m below the lagoon surface, "Storage" indicates a depth of 0.6-0.9 m below the lagoon surface, and "Treatment" indicates a depth of 0.6-0.9 m from the bottom of the lagoon.

	Farm 10			Farm 20		
	Surface	**Storage**	**Treatment**	**Surface**	**Storage**	**Treatment**
Eh (mV)	-110	-122	-131	-76	-83	-95
pH	7.80	7.76	7.76	7.68	7.63	7.49
Temp. (^{o}C)	23.9	23.0	21.7	18.9	18.5	17.5
Mn tot	0.118	0.122	0.119	0.173	0.124	0.138
Mn diss	< 0.010 [1]	< 0.010 [1]	< 0.010 [1]	0.012	0.011	0.011
Cu tot	0.344	0.367	0.341	0.095	0.088	0.044
Cu diss	0.122	0.131	0.151	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
Fe tot	1.270	1.488	1.326	0.960	0.932	0.850
Fe diss	0.653	0.654	0.713	0.182	0.181	0.195
Zn tot	0.610	0.612	0.652	0.100	0.092	0.106
Zn diss	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
NH_4-N	646	660	653	169	178	199
P tot	92	89	92	68	65	73
P diss	82	82	82	40	40	38
OP-P	73	77	76	35	34	36
S tot	33	32	32	38	39	35
S diss	20	19	17	34	34	26
SO_4-S	9	8	7	25	26	18
Ca tot	40	36	39	49	51	53
Ca diss	10	10	10	19	19	18
Mg tot	8	7	8	25	25	27
Mg diss	4	3	4	13	15	13
Na diss	315	311	299	125	124	126
K diss	1090	1099	1039	513	503	513
Cl diss	665	657	658	222	223	222

Table 3-6b : Mean Eh, pH, and lagoon temperature data averaged over sampling period (8/2/00-2/1/01), and mean measured parameter values averaged over sampling period (8/2/00-1/25/01) given by depth sampled for all lagoons. Data values expressed as mg/L unless otherwise noted. A "1" represents the detection limit value for a specific analyte. "Surface" indicates depth of 0-0.6 m below the lagoon surface, "Storage" indicates a depth of 0.6-0.9 m below the lagoon surface, and "Treatment" indicates a depth of 0.6-0.9 m from the bottom of the lagoon.

	Farm 318			Farm 2149		
	Surface	**Storage**	**Treatment**	**Surface**	**Storage**	**Treatment**
Eh (mV)	-146	-154	-162	-125	-134	-144
pH	8.11	8.09	8.07	7.75	7.73	7.72
Temp. (^{o}C)	21.7	19.5	18.8	20.9	19.9	19.4
Mn tot	0.279	0.334	0.308	0.077	0.080	0.086
Mn diss	0.028	0.027	0.025	0.013	0.012	0.013
Cu tot	0.472	0.462	0.416	0.183	0.161	0.205
Cu diss	0.186	0.185	0.202	0.090	0.092	0.090
Fe tot	1.491	1.771	1.419	1.519	1.463	1.554
Fe diss	0.407	0.400	0.429	0.584	0.582	0.602
Zn tot	0.406	0.405	0.355	0.624	0.631	0.646
Zn diss	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	0.058	0.061	0.064
NH_4-N	502	501	498	452	453	455
P tot	12	14	13	50	52	48
P diss	5	5	5	34	35	38
OP-P	5	5	5	33	34	33
S tot	24	24	23	23	22	24
S diss	15	14	14	15	16	16
SO_4-S	4	2	3	8	7	8
Ca tot	65	76	70	45	43	41
Ca diss	9	10	9	14	14	16
Mg tot	57	66	75	15	18	14
Mg diss	62	63	60	6	7	8
Na diss	406	409	413	157	154	154
K diss	1491	1542	1547	800	761	781
Cl diss	755	768	746	383	381	385

Table 3-6c : Mean Eh, pH, and lagoon temperature data averaged over sampling period (8/2/00-2/1/01), and mean measured parameter values averaged over sampling period (8/2/00-1/25/01) given by depth sampled for all lagoons. Data values expressed as mg/L unless otherwise noted. A "1" represents the detection limit value for a specific analyte. "Surface" indicates depth of 0-0.3 m below the lagoon surface, "Storage" indicates a depth of 0.6-0.9 m below the lagoon surface, and "Treatment" indicates a depth of 0.6-0.9 m from the bottom of the lagoon.

	Farm 2704			Farm 3101		
	Surface	**Storage**	**Treatment**	**Surface**	**Storage**	**Treatment**
Eh (mV)	-105	-113	-121	-115	-124	-140
pH	7.98	7.96	7.91	7.75	7.72	7.69
Temp. (^{o}C)	20.2	19.5	18.7	21.9	20.8	20.0
Mn tot	0.061	0.069	0.056	0.085	0.087	0.075
Mn diss	0.014	0.012	0.015	0.015	0.012	0.017
Cu tot	0.046	0.060	0.068	0.081	0.112	0.091
Cu diss	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
Fe tot	0.826	0.958	0.761	1.647	1.422	1.162
Fe diss	0.416	0.408	0.424	0.436	0.446	0.492
Zn tot	0.110	0.123	0.111	0.212	0.226	0.197
Zn diss	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]	< 0.050 [1]
NH_4-N	387	395	390	463	471	463
P tot	35	36	38	39	43	41
P diss	29	28	29	28	29	29
OP-P	26	26	26	26	26	28
S tot	35	34	33	18	18	16
S diss	26	26	22	13	12	12
SO_4-S	20	18	15	8	6	6
Ca tot	40	43	42	44	44	39
Ca diss	11	11	11	15	16	11
Mg tot	12	13	11	16	17	14
Mg diss	8	8	9	8	8	8
Na diss	157	152	147	118	119	116
K diss	732	730	707	558	570	557
Cl diss	365	359	360	261	247	247

Table 3-7 : Measured lagoon parameters that show a statistically significant (p= 0.05) effect of depth on mean parameter value. "Surface", "Storage", and "Treatment" refer to the mean parameter balue (averaged over sampling period) at specified depth. "Lagoon(s)" column designates for which lagoon the depth effect applies.

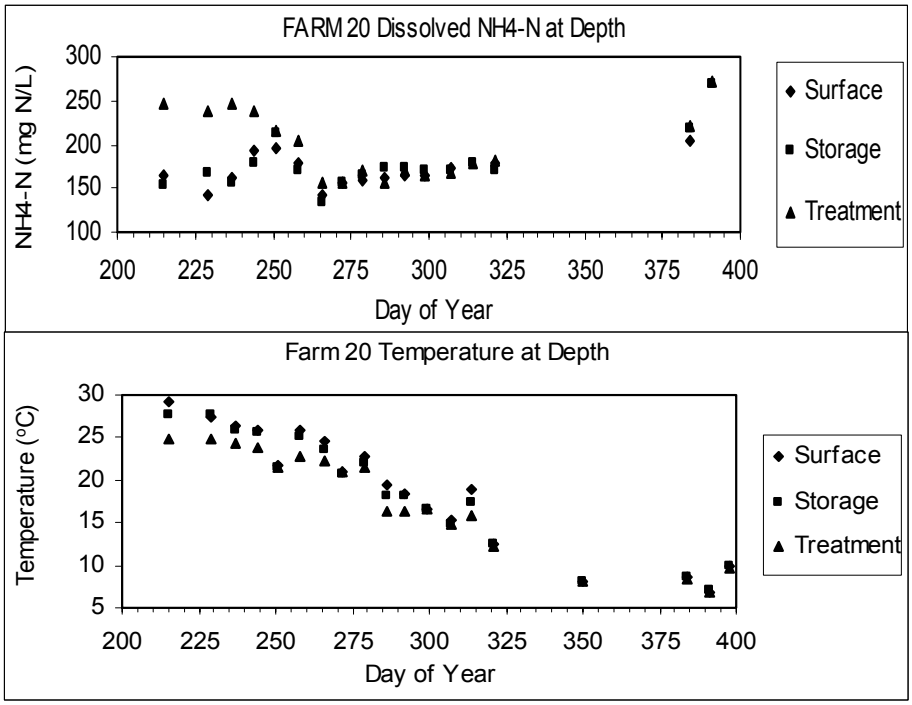
Parameter	Lagoon(s)	Depth Effect
Eh	all	Surface>Storage>Treatment
PH	10,2149	Surface>Storage=Treatment
PH	3101,318	Surface>Storage>Treatment
PH	2704,20	Surface=Storage>Treatment
Temp.	10,3101,2704	Surface>Storage>Treatment
Temp.	2149,318	Surface>Storage=Treatment
Temp.	20	Surface=Storage>Treatment
SO_4	20	Surface=Storage>Treatment
SO_4	10	Surface>Treatment,Storage indeterminable
SO_4	20	Cannot discern difference
NH_4	20	Surface>Treatment,Storage indeterminable
P diss	2149	Surface>Treatment,Storage indeterminable
Mg diss	318	Storage>Treatment,Surface indeterminable
Mn diss	3101	Treatment>Storage,Surface indeterminable

Sulfur- Sulfate concentration, another species that is redox sensitive, was also effected by depth. Of all six lagoons, three lagoons (those on Farms 10, 20, and 2704) showed a significant effect of depth on mean sulfate concentration although again the absolute magnitude of the difference is rather small. The results as to the nature of the depth effect were not the same for all three lagoons. For Farm 10, the results imply that mean sulfate concentration in the surface (9 mg SO_4-S/L) and storage zone (8 mg SO_4-S/L) were equal to each other and greater then the mean sulfate concentration in the treatment zone (7 mg SO_4-S/L). On Farm 20, mean sulfate concentration was determined to be equal in the storage (26 mg SO_4-S/L) and surface zones (25 mg SO_4-S/L), with these areas having a greater mean sulfate concentration than the treatment zone (18 mg SO_4-S/L). For Farm

2704 the Tukey test was not able to distinguish where the difference occurred (Surface = 20 mg SO_4-S/L, Storage = 18 mg SO_4-S/L, Treatment = 15 mg SO_4-S/L). This is possible because the ANOVA test is considered more powerful in detecting differences in the data set then the Tukey test (**4**). Hence a slight effect of depth on parameter value was determined, but the nature of it was indeterminable.

Ammonium- Statistical analysis of the data found no significant depth effect on ammonium concentration, with the exception of Farm 20. Results from the Tukey test showed a clear difference between the ammonium concentration in the surface and the treatment zone, although where the storage zone fell in relation to these two zones was indeterminable. Figure 3-14 is a plot of ammonium-nitrogen and temperature at depth over the sampling period for Farm 20.

Figure 3-14 : Farm 20 mean ammonium nitrogen concentration and lagoon temperature data at depth for sampling period 8/2/00-1/25/01.



From Figure 3-14 we can see the disparity between depths is likely due to the phenomenon observed on the first four sampling dates. Observation of the temperature plot shows a difference in lagoon temperature between the treatment zone and the overlying zones. This suggests that the stratification of ammonium evident in the first sampling dates could possibly be the result of a density stratification developed in the lagoon in response to the temperature differential. Density stratification within the lagoon would result in distinct layers of differing temperature with minimal mixing. Therefore it is possible that ammonia volatilization in the lower, colder lagoon fluid would be retarded and ammonium would accumulate.

Other Species- All six farms showed a significant effect of depth on both pH and lagoon temperature. As evidenced in Table 3-7 the effect of depth on each parameter varied for each lagoon and was not consistent for all lagoons. On Farm 318 statistical analysis determined that depth had a significant effect on dissolved magnesium concentration. According to the results of the statistical analysis of mean dissolved magnesium concentration, the storage zone concentration (~63 mg Mg/L) was greater then the treatment zone concentration (~60 mg Mg/L) with the surface layer concentration (~ 62 mg Mg/L) indeterminable. Observation of the data suggests that there is effectively no real difference between the three means, considering they only differ by approximately 5%. On Farm 2149 depth was determined to have an effect on dissolved phosphorous concentration but not orthophosphate-P. This suggests that the observed effect was an artifact of the chemical analysis, as mentioned earlier, and not a real phenomenon.

Variation Among Lagoons

In order to evaluate the relationships between lagoons, data averaged over depth for each lagoon was statistically analyzed with the data blocked by day (**2**). Results from the RCB ANOVA test for each parameter were then tested using the Tukey Multiple Comparison procedure. Table 3-8 illustrates the results of the Tukey Multiple Comparison test for each lagoon.

Table 3-8 : Results of Tukey Multiple Comparison showing relationships between lagoons for each lagoon parameter. Numbers denote statistically different populations, with 1 being the population with the largest mean (for population means see Tables 1-4).

	Farm					
	10	**20**	**318**	**2149**	**2704**	**3101**
Eh	2,3	1	4	3	2	2,3
pH	3	4	1	3	2	3
Temp.	2	4	2,3	2,3	3	1
Mn tot	2,3	2	1	3	3	3
Cu tot	2	4	1	3	4	4
Fe tot	2	3	1	1,2	3	2
Zn tot	1	4	2	1	3,4	3
NH_4-N	1	5	2	3	4	3
P tot	1	2	6	3	5	4
S tot	2	1	3	3	1,2	4
Ca tot	3	2	1	2,3	2,3	2,3
Mg tot	2	2	1	2	2	2
Na diss	2	3	1	3	3	3
K diss	2	4	1	3	3	4
Cl diss	2	4	1	3	3	4
Sum	29-31	45	28-29	38-41	43-46	44-46

The mean value for each parameter (taken from Tables 3-1 through 3-4) was evaluated and ranked in reference to all lagoons. A value of 1 represents the parameter

with the greatest mean population, with increasing values representing parameters with decreasing mean populations. Parameters with two number designations, for example (2,3), represent populations that could not be definitely distinguished as belonging to either population. At the bottom of each lagoon column is a ranking determined by adding up the integers for each parameter. Rankings with a range, for example (43-46), represent lagoons where one or more parameters showed properties belonging to more then one population. The ranking was included just as an empirical way to observe similarities among lagoons, it does not have statistical significance.

Looking at Table 3-8 and Tables 3-1 through 3-3 suggests that Farms 2704, 3101, and 2149 seem to be similar in most categories. Farm 10 and Farm 318 seem to be overall distinctly different environments then the other farms. Farm 10, and more evidently Farm 318, seem to have a higher mean concentration for most parameters. This could be the result of engineering differences between the lagoons. Farm 20, although with an overall rank similar to Farms 2149, 2704, and 3101, appears to be different from all six lagoons in many chemical parameters. Referring to Tables 3-1 through 3-3, Farm 20 appears to have a lower concentration for most parameters, with the exception of sulfur.

Observation of the temporal plots of lagoon components further validates the differences between the lagoon systems suggested by this statistical analysis. For most parameters, lagoons on Farms 318 and 20 appear to "bracket" the other lagoons, with Farm 318 having the highest concentration of most analytes and Farm 20 having the lowest. Farm 10 also appears to be on the high end of some components, notably ammonium, iron, and phosphorous.

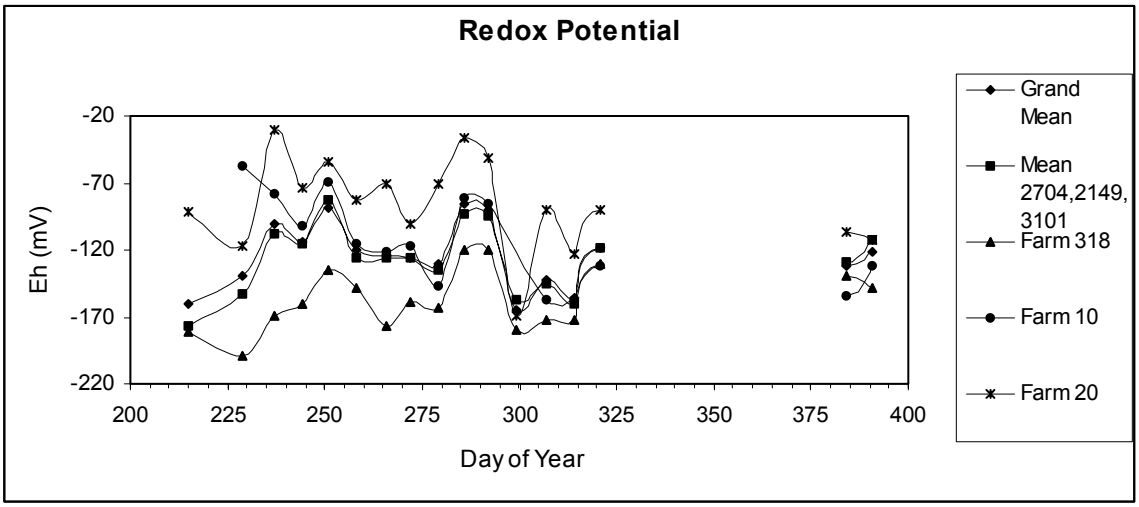
Figure 3-15 is a plot of field measurement data averaged over all six lagoons and averaged over depth, referred to as the Grand Mean data. Included with this plot is field measurement data averaged over depth for Farm 10, Farm 318, Farm 20, and the combined mean of Farms 2704, 2149, and 3101. These plots were intended to further elaborate on whether there is a significant chemical difference between lagoon environments sampled. Observing the plots for Eh and pH in Figure 3-15 distinct differences are noted among lagoons.

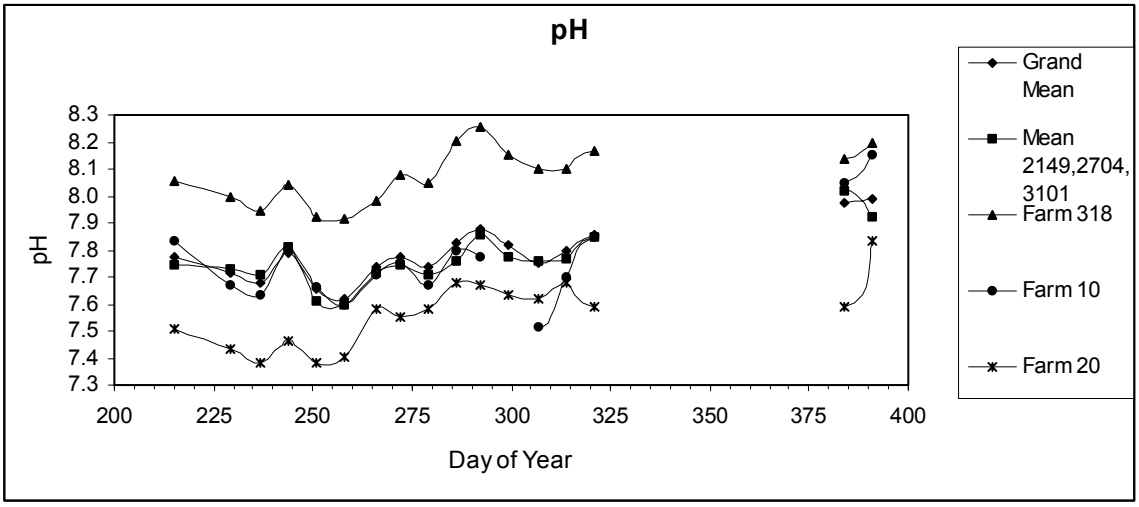
It appears that both Farm 318 and 20 as lagoon systems are the extremes within the six lagoons studied having high and low conditions for both pH and Eh. It is interesting to note that plots of Eh and pH for these lagoons follow the general axiom concerning the inverse relationship between Eh and pH, that decreases in redox potential tend to occur with increases in pH. Farm 10, although seeming to be chemically different then Farms 2149, 2704, and 3101, appears to closely follow the combined mean trend of these farms. This suggests that Farm 10 functions similar to these lagoons, and perhaps the difference in elemental concentrations noted in Tables 3-2 through 3-5 is due to waste overloading.

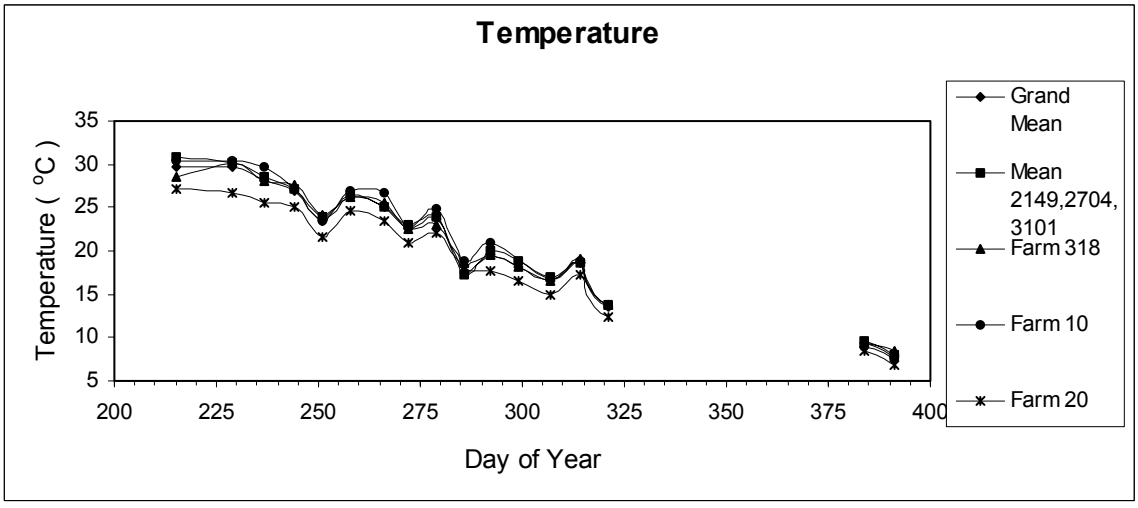
Data Set for Evaluation of Redox Chemistry

In order to facilitate the evaluation of the redox chemistry of the lagoons studied, the data collected needed to be consolidated into a more manageable dataset. Based on the statistical analysis conducted we combined the data in the following manner. Considering the results of the statistical analysis on depth effect, further treatment of the data values for

Figure 3-15: Eh, pH, and lagoon temperature data plotted as Grand Mean data, Farm 20, Farm 318, Farm 10, and mean of Farms 2149, 2704, and 3101. Day of year 215 represents 8/2/00, and day of year 398 indicates 2/1/01.







all measured parameters, unless otherwise noted, will be averaged over all three depths. Although statistical analysis of measured Eh values indicated a depth effect on measured potential, Eh values will be averaged over depth. This might appear to be counterproductive, considering that redox chemistry is the focus of this research, however mean Eh values at depth differed in most cases by no greater then approximately 10-20 mV. Differences this small would be relatively insignificant in any calculations attempted. Therefore in order to create a workable dataset it was concluded that Eh values would be averaged over depth for further redox chemistry evaluation.

Based on the results of the analysis of lagoon effect on parameter value, the data will be handled in the following way. Assuming that all the lagoons behave similarly, and can be considered similar, data for all parameters will be averaged over all six lagoons. This data will be referred to as the Grand Mean Data. Assuming that Farm 10, 20, and 318 are distinctly different systems then the other farms, data for Farms 10, 20, and 318 will be separated and handled individually. Farms 2149, 2704, and 3101, which appear to be similar environments, will have their data averaged and the resulting mean values used as a single data set. Tables 3-9 through 3-13 contain the data that will be used for the evaluation of redox chemistry.

Table 3-9: Mean data values averaged over depth for measured parameters per each sampling date for all farms (Grand Mean Data). Day of year 215 represents 8/2/00, and day of year 391 represents 1/25/01. Note: Data in mg/L unless otherwise noted. A "[1]" indicates a detection limit value for specific analyte. A "[2]" indicates data not available.

	DAY OF YEAR																
	215	**229**	**237**	**244**	**251**	**258**	**266**	**272**	**279**	**286**	**292**	**299**	**307**	**314**	**321**	**384**	**391**
Eh (mV)	-161	-139	-100	-114	-89	-120	-124	-126	-131	-86	-91	-165	-142	-155	-118	-131	-122
pH	7.77	7.72	7.68	7.79	7.65	7.62	7.74	7.77	7.74	7.83	7.88	7.82	7.75	7.80	7.86	7.97	7.99
Temp.(°C)	29.7	29.6	28.1	26.9	23.4	26.2	25.2	22.5	23.6	17.8	19.6	18.2	16.6	18.5	13.6	9.3	7.8
Mn tot	0.131	0.167	0.096	0.138	0.128	0.126	0.144	0.130	0.120	0.121	0.130	0.120	0.114	0.123	0.123	0.145	0.194
Mn diss	0.021	0.022	<0.01 [1]	0.014	<0.01 [1]	0.016	<0.01 [1]	<0.01 [1]	0.018	0.014	0.011	0.010	<0.01 [1]	<0.01 [1]	0.011	0.026	0.049
Cu tot	0.234	0.199	0.169	0.211	0.187	0.169	0.151	0.188	0.190	0.185	0.159	0.122	0.176	0.187	0.178	0.226	0.363
Cu diss	0.172	0.081	0.057	<0.05 [1]	<0.05 [1]	0.051	0.064	0.102	0.105	0.082	<0.05 [1]	0.096	0.109	0.101	0.095	0.122	<0.05 [1]
Fe tot	1.595	1.186	1.013	1.227	1.167	0.974	0.891	1.157	1.554	1.022	1.154	1.346	0.979	0.990	0.896	1.357	2.366
Fe diss	0.599	0.390	0.318	0.326	0.369	0.303	0.366	0.421	0.549	0.483	0.377	0.445	0.493	0.486	0.496	0.607	0.622
Zn tot	0.402	0.324	0.256	0.285	0.326	0.262	0.284	0.341	0.328	0.325	0.323	0.273	0.328	0.323	0.295	0.433	0.537
Zn diss	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]
NH_4	546	532	574	569	567	568	501	507	516	522	529	493	551	558	558	795	780
P tot	48	48	46	46	48	51	50	51	51	48	48	42	54	52	52	50	55
P diss	37	39	36	35	37	37	33	36	41	38	38	25	31	31	31	36	49
OP	98	100	134	128	137	139	87	86	101	93	92	58	84	82	82	98	115
S tot	31	27	23	26	28	24	27	27	25	29	28	28	30	29	30	32	28
S diss	29	28	23	26	27	24	21	19	17	16	21	19	14	12	12	12	11
SO_4	56	56	44	55	52	48	45	38	23	26	45	33	17	10	10	5	5
Ca tot	36	40	44	40	43	40	44	51	45	49	46	41	48	51	52	56	67
Ca diss	9	18	9	8	15	12	8	11	10	13	11	12	10	10	13	26	25
Mg tot	25	16	11	14	17	7	22	17	25	11	17	24	NA [2]	NA [2]	15	21	22
Mg diss	21	19	19	17	18	19	17	16	22	17	19	18	14	13	13	11	21
Na diss	198	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	205	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	232
K diss	818	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	835	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	937
Cl diss	NA [2]	427	442	429	425	446	406	412	NA [2]	431	429	398	452	449	448	465	NA [2]

Table 3-10: Farm 2149, 2704, and 3101 data averaged across all three farms. Mean data values averaged over depth for measured parameters per each sampling date. Day of year 215 represents 8/2/00, and day of year 391 represents 1/25/01. Note: Data in mg/L unless otherwise noted. A "[1]" indicates a detection limit value for specific analyte. A "[2]" indicates data not available.

	DAY OF YEAR																
	215	**229**	**237**	**244**	**251**	**258**	**266**	**272**	**279**	**286**	**292**	**299**	**307**	**314**	**321**	**384**	**391**
Eh (mV)	-177	-153	-108	-115	-82	-126	-126	-127	-135	-92	-95	-158	-145	-160	-118	-129	-113
pH	7.75	7.73	7.71	7.81	7.61	7.60	7.72	7.75	7.71	7.76	7.86	7.77	7.76	7.77	7.85	8.05	7.92
Temp.(^{o}C)	30.7	30.1	28.5	27.2	23.9	26.5	25.1	23.1	24.0	17.1	19.9	18.8	17.1	18.7	13.7	9.7	8.0
Mn tot	0.062	0.075	0.061	0.063	0.081	0.059	0.058	0.071	0.087	0.081	0.097	0.104	0.054	0.058	0.059	0.104	0.134
Mn diss	0.017	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	0.012	0.024	0.018	0.015	0.010	0.010	0.010	0.012	0.020	0.050
Cu tot	0.122	0.115	0.108	0.103	0.185	0.110	0.083	0.083	0.110	0.109	0.102	0.101	0.075	0.099	0.088	0.149	0.235
Cu diss	0.101	0.056	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	0.068	0.065	0.051	<0.05 [1]	0.080	0.061	0.066	0.066	0.069	<0.05 [1]
Fe tot	1.499	1.166	1.020	1.130	1.230	0.945	0.885	1.234	1.740	0.914	1.070	1.690	0.823	0.768	0.833	1.559	2.513
Fe diss	0.618	0.408	0.310	0.343	0.475	0.317	0.401	0.512	0.606	0.509	0.447	0.522	0.528	0.517	0.554	0.621	0.653
Zn tot	0.320	0.333	0.256	0.239	0.421	0.249	0.287	0.311	0.342	0.282	0.325	0.350	0.252	0.248	0.243	0.491	0.569
Zn diss	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]
NH_4	548	547	543	526	662	559	509	514	523	530	542	545	551	554	558	697	752
P tot	38	40	37	39	59	33	42	43	46	42	39	44	44	43	43	46	50
P diss	30	33	30	30	50	30	27	30	38	33	35	29	25	25	25	32	42
OP	83	94	110	107	175	127	71	72	87	80	82	70	66	68	66	84	100
S tot	29	27	22	24	25	21	25	24	23	29	25	27	25	24	23	25	21
S diss	28	28	22	26	23	22	20	17	14	15	20	16	10	9	9	9	8
SO_4	62	60	45	59	44	47	46	30	18	27	44	28	12	6	14	2	2
Ca tot	32	41	41	37	39	35	40	38	46	41	44	44	36	45	42	54	58
Ca diss	8	19	7	7	13	9	7	13	11	13	12	12	10	10	15	26	30
Mg tot	11	14	12	12	14	8	16	16	14	11	16	17	NA [2]	NA [2]	14	18	16
Mg diss	8	8	6	7	6	7	7	7	13	8	10	5	4	5	5	7	17
Na diss	135	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	137	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	152
K diss	659	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	668	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	738
Cl diss	342	342	339	331	416	327	303	305	340	324	320	332	343	327	325	340	368

Table 3-11: Farm 10 mean data values averaged over depth for measured parameters per each sampling date. Day of year 215 represents 8/2/00, and day of year 391 represents 1/25/01. Note: Data in mg/L unless otherwise noted. A "[1]" indicates a detection limit value for specific analyte. A "[2]" indicates data not available.

	DAY OF YEAR																
	215	**229**	**237**	**244**	**251**	**258**	**266**	**272**	**279**	**286**	**292**	**299**	**307**	**314**	**321**	**384**	**391**
Eh (mV)	NA [2]	-58	-78	-101	-69	-115	-122	-117	-147	-80	-86	NA [2]	-157	-157	-132	-154	-131
pH	7.84	7.67	7.63	7.80	7.66	7.60	7.71	7.75	7.67	7.80	7.78	NA [2]	7.52	7.70	7.85	8.05	8.15
Temp.(°C)	30.4	30.4	29.6	27.1	23.6	26.9	26.7	22.7	24.7	18.7	20.9	NA [2]	16.7	18.8	13.8	8.9	7.5
Mn tot	0.177	0.102	0.072	0.125	0.111	0.077	0.082	0.087	0.112	0.127	0.084	NA [2]	0.131	0.136	0.086	0.091	0.170
Mn diss	0.015	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	0.017	0.011	<0.01 [1]	NA [2]	<0.01 [1]	<0.01 [1]	<0.01 [1]	0.014	0.045
Cu tot	0.565	0.358	0.277	0.374	0.336	0.279	0.250	0.326	0.393	0.320	0.220	NA [2]	0.282	0.324	0.170	0.267	0.393
Cu diss	0.439	0.165	0.117	0.070	0.059	0.064	0.099	0.175	0.200	0.149	0.031	NA [2]	0.184	0.140	0.102	0.143	0.047
Fe tot	2.035	1.227	0.935	1.413	1.230	0.696	0.810	1.032	1.435	1.201	0.836	NA [2]	1.191	1.323	0.802	0.997	2.837
Fe diss	1.024	0.673	0.548	0.535	0.555	0.509	0.448	0.422	0.803	0.736	0.341	NA [2]	0.717	0.732	0.668	0.971	0.863
Zn tot	0.964	0.552	0.458	0.585	0.554	0.390	0.460	0.603	0.616	0.556	0.533	NA [2]	0.594	0.676	0.441	0.444	0.881
Zn diss	0.059	<0.05 [1]	0.073	0.051	0.057	<0.05 [1]	<0.05 [1]	<0.050 [1]	<0.05 [1]	0.062	<0.05 [1]	NA [2]	0.065	<0.05 [1]	<0.05 [1]	0.067	0.065
NH_4	661	636	702	702	652	639	556	551	574	576	580	NA [2]	627	636	631	725	801
P tot	99	86	84	89	91	90	90	87	91	86	84	NA [2]	100	95	98	85	98
P diss	87	89	84	81	86	82	76	83	81	89	76	NA [2]	76	77	74	79	90
OP	227	224	282	276	262	268	206	202	213	226	195	NA [2]	211	209	207	228	231
S tot	32	27	21	27	31	28	28	29	30	29	33	NA [2]	40	38	39	49	39
S diss	28	25	20	24	27	22	23	22	18	16	18	NA [2]	13	13	11	14	13
SO_4	49	37	28	45	49	37	43	47	28	16	32	NA [2]	6	5	1	1	2
Ca tot	28	31	29	33	35	33	35	39	29	37	40	NA [2]	41	40	42	58	64
Ca diss	7	13	10	8	13	11	4	7	6	9	7	NA [2]	7	7	7	19	21
Mg tot	7	7	6	9	10	6	NA [2]	11	6	10	7	NA [2]	NA [2]	NA [2]	8	7	10
Mg diss	4	2	3	3	3	3	4	5	5	2	4	NA [2]	2	2	2	3	8
Na diss	284	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	299	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	342
K diss	993	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	1037	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	1199
Cl diss	NA [2]	608	642	628	663	675	625	635	NA [2]	658	655	NA [2]	695	675	695	717	NA [2]

Table 3-12: Farm 20 mean data values averaged over depth for measured parameters per each sampling date. Day of year 215 represents 8/2/00, and day of year 391 represents 1/25/01. Note: Data in mg/L unless otherwise noted. A "[1]" indicates a detection limit value for specific analyte. A "[2]" indicates data not available.

	DAY OF YEAR																
	215	**229**	**237**	**244**	**251**	**258**	**266**	**272**	**279**	**286**	**292**	**299**	**307**	**314**	**321**	**384**	**391**
Eh (mV)	-92	-118	-31	-74	-54	-82	-70	-101	-70	-37	-51	-169	-90	-122	-90	-106	-112
pH	7.51	7.44	7.38	7.46	7.38	7.41	7.58	7.55	7.58	7.68	7.67	7.63	7.62	7.68	7.59	7.59	7.83
Temp.(°C)	27.2	26.7	25.5	25.1	21.6	24.6	23.5	20.9	22.1	18.0	17.6	16.6	15.0	17.3	12.5	8.5	6.9
Mn tot	0.151	0.173	0.137	0.163	0.162	0.144	0.391	0.143	0.125	0.089	0.129	0.100	0.096	0.106	0.098	0.136	0.145
Mn diss	0.016	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	0.010	0.010	0.014	0.011	0.012	<0.01 [1]	0.013	<0.01 [1]	0.011	0.016	0.039
Cu tot	<0.05 [1]	0.073	0.075	0.175	0.052	0.076	0.059	0.058	0.060	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	0.052	<0.05 [1]
Cu diss	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]
Fe tot	1.077	0.891	0.823	1.038	1.072	0.969	0.572	0.793	1.263	0.571	0.835	0.723	0.473	0.820	0.656	0.946	1.545
Fe diss	0.221	0.117	0.128	0.125	0.120	0.118	0.182	0.171	0.237	0.171	0.173	0.183	0.221	0.210	0.210	0.236	0.348
Zn tot	0.143	0.099	0.089	0.113	0.124	0.084	0.065	0.125	0.106	0.070	0.088	0.103	<0.05 [1]	0.077	0.067	0.136	0.130
Zn diss	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]
NH_4	188	183	188	204	208	185	144	157	164	164	169	167	171	179	177	NA [2]	271
P tot	63	71	75	62	71	86	75	79	65	62	72	66	78	74	69	56	59
P diss	43	40	40	36	44	44	35	36	48	37	42	34	34	29	31	35	57
OP	132	124	122	111	135	134	106	112	147	115	130	105	104	89	94	107	175
S tot	41	34	35	35	42	36	39	39	34	38	42	38	39	38	37	36	32
S diss	38	41	38	38	43	40	34	34	27	26	37	33	27	20	22	17	13
SO_4	69	88	94	90	103	103	83	84	52	54	100	76	55	35	42	24	19
Ca tot	44	53	55	51	56	55	41	48	42	47	56	48	47	51	54	57	64
Ca diss	14	25	15	12	29	21	18	15	13	18	19	14	19	15	19	28	27
Mg tot	25	29	15	25	35	NA	34	26	28	11	28	29	NA [2]	NA [2]	27	24	19
Mg diss	17	14	17	9	16	18	12	14	19	11	15	10	9	7	9	10	24
Na diss	118	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	123	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	135
K diss	483	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	501	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	545
Cl diss	NA [2]	226	222	212	220	229	205	211	231	217	216	223	228	226	221	224	252

Table 3-13: Farm 318 mean data values averaged over depth for measured parameters per each sampling date. Day of year 215 represents 8/2/00, and day of year 391 represents 1/25/01. Note: Data in mg/L unless otherwise noted. A "[1]" indicates a detection limit value for specific analyte. A "[2]" indicates data not available.

	DAY OF YEAR																
	215	**229**	**237**	**244**	**251**	**258**	**266**	**272**	**279**	**286**	**292**	**299**	**307**	**314**	**321**	**384**	**391**
Eh	-181	-199	-170	-160	-135	-149	-176	-159	-163	-120	-121	-180	-173	-172	-131	-140	-148
pH	8.06	7.99	7.94	8.04	7.92	7.91	7.98	8.08	8.05	8.21	8.26	8.15	8.10	8.10	8.17	8.14	8.20
Temp.	28.6	30.3	28.1	27.7	24.1	26.3	25.6	22.5	23.0	18.6	19.5	18.1	16.6	19.0	13.8	9.5	8.4
Mn tot	0.269	0.502	0.186	0.352	0.322	0.360	0.217	0.335	0.223	0.267	0.276	0.189	0.297	0.324	0.379	0.329	0.447
Mn diss	0.043	0.112	<0.01 [1]	0.071	0.019	0.069	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	<0.01 [1]	0.015	0.066	0.061
Cu tot	0.428	0.420	0.336	0.407	0.433	0.331	0.352	0.494	0.355	0.435	0.403	0.287	0.515	0.468	0.613	0.593	0.755
Cu diss	0.269	0.138	0.091	0.112	0.133	0.107	0.125	0.222	0.216	0.179	0.146	0.227	0.279	0.258	0.259	0.368	0.116
Fe tot	1.962	1.500	1.260	1.520	1.620	1.343	1.313	1.418	1.406	1.615	2.043	0.938	1.743	1.495	1.418	1.523	2.276
Fe diss	0.494	0.325	0.300	0.264	0.354	0.239	0.366	0.396	0.438	0.464	0.404	0.478	0.433	0.420	0.434	0.568	0.559
Zn tot	0.343	0.293	0.225	0.297	0.467	0.351	0.320	0.386	0.220	0.476	0.344	0.214	0.576	0.443	0.534	0.543	0.503
Zn diss	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]	<0.05 [1]
NH_4	419	383	520	516	491	520	447	453	448	454	453	474	484	493	487	735	807
P tot	11	11	7	11	12	12	9	12	10	11	11	9	14	14	15	21	24
P diss	4	3	4	3	4	5	4	4	5	6	6	4	3	3	3	6	18
OP	12	9	12	10	12	15	13	13	15	17	17	12	9	8	10	19	54
S tot	27	20	16	22	21	19	18	20	19	22	20	23	27	26	31	35	35
S diss	25	22	13	18	17	15	12	12	14	12	12	13	12	11	11	14	16
SO_4	35	33	7	20	14	11	9	6	7	5	7	7	3	3	2	1	2
Ca tot	50	32	58	42	52	NA [2]	67	104	63	89	NA [2]	24	90	80	89	NA [2]	100
Ca diss	7	10	8	8	6	12	8	4	5	10	5	8	5	8	9	27	15
Mg tot	83	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	74	NA [2]	NA [2]	39	NA [2]	NA [2]	NA [2]	39	54
Mg diss	79	76	74	71	66	71	62	59	68	65	65	63	59	57	55	34	46
Na diss	377	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	394	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	456
K diss	1453	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	1466	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	NA [2]	1661
Cl diss	NA [2]	700	770	740	727	790	698	708	NA [2]	742	743	772	760	813	797	833	NA [2]

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2) **SAS Institute.** 1999. The SAS system for Windows, version 8.2. Cary, NC, 27513

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Chapter 4

Identifying Active Redox Couples

The data summarized in Chapter 3, Tables 3-9 through 3-13, will form the basis for an analysis to identify potential determining redox couples active in the swine lagoons studied. The analysis was carried out using two different approaches. The first approach involved the plotting of measured Eh and pH values on pe/pH phase diagrams constructed using redox couples considered to be possible in the swine lagoons based on chemical data. The second approach involved using analytical data to calculate an expected Eh of a lagoon at equilibrium and comparing this value to measured values. The results generated using these two approaches were then used to predict the potential determining redox couple sensed by the redox probe in the swine lagoons studied.

pe/pH Phase Diagrams

A pe/pH phase diagram is a thermodynamic representation of the reaction between two species of a particular redox couple. The generalized equation for the transfer of electrons between species of a redox couple can be expressed as follows:

$$A_{(ox)} + e^- + H^+ = A_{(red)} \quad (1)$$

Where $A_{(ox)}$ represents the oxidized species of component A and $A_{(red)}$ represents the reduced species of component A. For equation 1 the equilibrium relationship is represented by a constant ,K, and can be related to the activity of the species involved according to the following equation:

$$K = (A_{(red)})/ [(A_{(ox)}) (e^-)(H^+)] \quad (2)$$

By converting equation 2 to a base 10 logarithmic form and rearranging the values, equation 2 can be expressed as a function of pe (**5**). Assuming that the redox couple is at

equilibrium equation 2 reduces to a linear expression of pe:

$$pe = -pH + C \quad (3)$$

Where C is a constant representing the value = logK – log $(A_{(red)})/ (A_{(ox)})$. By plotting values of pe and pH using equilibrium redox reactions solved in this manner a graphical representation of the equilibrium between redox couples is generated, which is called a pe/pH phase diagram (e.g. Figure 4-1).

A pe/pH phase diagram can be used to identify active redox couples in natural environments if pH and Eh measurements are collected. Plotting measured Eh and pH values on phase diagrams containing various redox couples can elucidate what may be the redox couple active in that environment, and what is the relative ratio of oxidant to reductant based on conditions measured.

Figure 4-1 is an example of a pe-pH phase diagram including redox couples for iron, sulfur, and carbon half reactions. Each line represents equilibrium conditions for each of the redox couples indicated in Table 4-1. For the ferrous/ferric iron couple poorly crystaline ferrihydrite ($Fe(OH)_3$) was considered to be the solid phase controlling the solubility of ferric iron. Poorly crystaline ferrihydrite has a higher solubility product constant than crystalline ferrihydrite and other crystalline ferric oxides, and although it is not the most stable it would be the first ferric oxide solid phase to form (**5**). The amorphous solid phase values were used based on the assumption that the oxidation and reduction cycle of iron would occur rapidly and conversion of amorphous ferrihydrite to a more stable crystalline iron oxide would not occur.

Figure 4-1: Example of a pe-pH phase diagram template including selected redox couples for iron, sulfur, and carbon. Circled numbers denote the redox couple listed in Table 4-1.

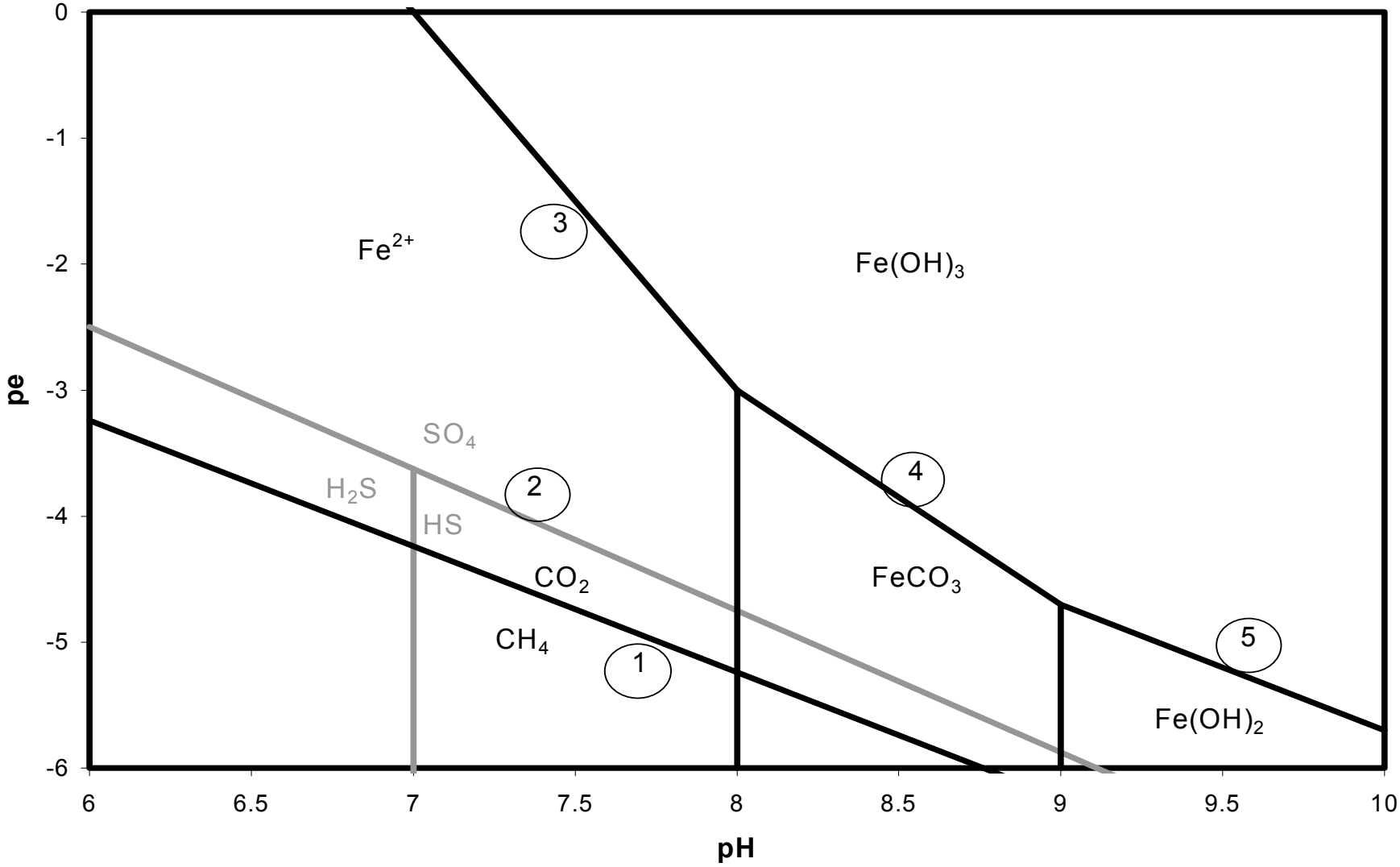


Table 4-1 : Equilibrium reactions represented by phase boundaries in Figure 4-1.

	Log K[1]
Carbon	
1) $H_2CO_3^* + 8H^+ + 8e^- = CH_{4(aq)} + 2H_2O$	21.6
Sulfur	
2) $SO_4^{2-} + 9H^+ + 8e^- = HS^- + 4H_2O$	34.0
Iron	
3) $Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	16.2
4) $Fe(OH)_3 + 2H^+ + HCO_3^- + e^- = FeCO_3 + 3H_2O$	16.6
5) $Fe(OH)_3 + H^+ + e^- = Fe(OH)_2 + H_2O$	3.3

[1] Values taken from Pankow (**2**), and Stumm and Morgan(**3**)

Equilibrium equations for the reactions in Table 4-1 were converted to expressions of pe according to guidelines taken from Pankow (**4**) and Stumm and Morgan (**5**). The development of the equations used for the construction of Figure 4-1 are outlined in Table 4-2.

In order to construct a phase diagram without information regarding the chemical species involved several assumptions must be made. For the iron couple, the values stated for the carbonate ion were based on data accepted as representative of water in equilibrium with atmospheric carbon dioxide (**4,5**). The value set for the concentration of soluble iron species represents data considered representative of natural waters(**4,5**). In addition the total soluble iron concentration was considered to consist solely of free ionic ferrous iron, and was established as 10^{-5} M. For the sulfur couple the concentration of sulfate and sulfide were assumed to be equal in order to facilitate simplification of the pe expression. For the methane/ carbon dioxide couple the concentration of methane and dissolved carbon dioxide were assumed to be equal, again to facilitate the calculation of the pe expression.

Table 4-2 : pe expressions of reactions used in construction of Figure 4-1.

For the reaction $Fe(OH)_3 + 2H^+ + HCO_3^- + e^- = FeCO_3 + 3H_2O$: $pe = 16.6 + \log[HCO_3^-] - 2pH$ Where: $[HCO_3^-] = \alpha_1 C(IV)_T$ $C(IV)_T = 10^{-3}$ M $\alpha_1 = ([H^+]/K_1 + 1 + K_2/[H^+])^{-1}$ pK1= 6.4 pK2= 10.3
For the reaction $H_2CO_3^* + 8H^+ + 8e^- = CH_4 + 3H_2O$: $pe = 2.7 - 1/8\log([CH_4]/[H_2CO_3^*]) - pH$ Where $[H_2CO_3^*] = \alpha_0 C(IV)_T$ $\alpha_0 = (1 + K_1/[H^+] + K_1K_2/[H^+]^2)^{-1}$ pK1= 6.4 pK2= 10.3 When $[CH_4] = C(IV)_T$ $pe = 2.7 - 1/8\log(\alpha_0) - pH$
For the reaction $SO_4^{2-} + 9H^+ + 8e^- = HS^- + 4H_2O$: $pe = 4.25 + 1/8\log([SO_4^{2-}]/[HS^-]) - 9/8pH$ Where $[SO_4^{2-}] = \alpha_2 S(VI)_T$ $[HS^-] = \alpha_1 S(-II)_T$ $\alpha_1 = ([H^+]/K_1 + 1 + K_2/[H^+])^{-1}$ $\alpha_2 = ([H+]^2/K_1K_2 + [H^+]/K_2 + 1)^{-1}$ For HS^- pK1= 7 pK2= 14 For SO_4^{2-} pK1= -3 pK2= 2 When $S(VI)_T = S(-II)_T$ $pe = 4.25 + 1/8\log(\alpha_2/\alpha_1) - 9/8pH$

It is important to note that changing estimated values would result in a change in the appearance of the phase diagram. Equilibrium lines will shift horizontally or vertically according to which values are changed. As an example, by increasing the concentration of dissolved ferrous iron by a factor of ten the equilibrium between ferrous iron and ferrihydrite will shift downward as shown in Figure 4-2. Considering the

aforementioned effect that variation between estimated parameters and real values can have on phase diagrams, the assumptions stated will be used as a first attempt to identify the redox couples active in the swine lagoons studied. This approach is taken knowing that measurement of all parameters involved in the construction of a pe/pH phase diagram must be known in order to definitively use phase diagrams to identify active redox couples.

Plotting data on pe/pH phase diagrams - Figure 4-3 is a pe-pH phase diagram containing data points for all of the data collected at each depth, for each lagoon, over the sampling period. Included in this diagram is the line representing redox equilibria between ferrous iron and ferrihydrite using the maximum and minimum dissolved iron concentrations measured (1.8×10^{-5} M and 1.5×10^{-6} M). The data points appear to scatter around the line representing the equilibrium conditions for the ferrihydrite ($Fe(OH)_3$) and aqueous ferrous iron (Fe^{2+}) redox couple. This suggests the existence and measurement of a Fe^{2+}/Fe^{3+} couple, and the existence of a stable iron oxide species in the water column. The wide scatter of data points around the iron phase boundary line supports the assumption that redox measurements appear to fluctuate widely around a mean measured potential. This could be explained by concluding that redox conditions in lagoon systems at any given time do not necessarily correspond to stable equilibrium conditions. Events occur where the measured potential fluctuates, and measurement of redox potentials at this time would not correspond to equilibrium potentials of the dominant redox couple. Some data points, which appear skewed towards the sulfur phase boundaries, indicate the possible influence of the SO_4/HS couple on measured potential.

Figure 4-2 : pe/pH phase diagram demonstrating the effect of changing dissolved iron concentration on phase boundary lines. Values cited are concentration values for dissolved iron in moles per liter. Auxillary conditions noted in Table 4-2.

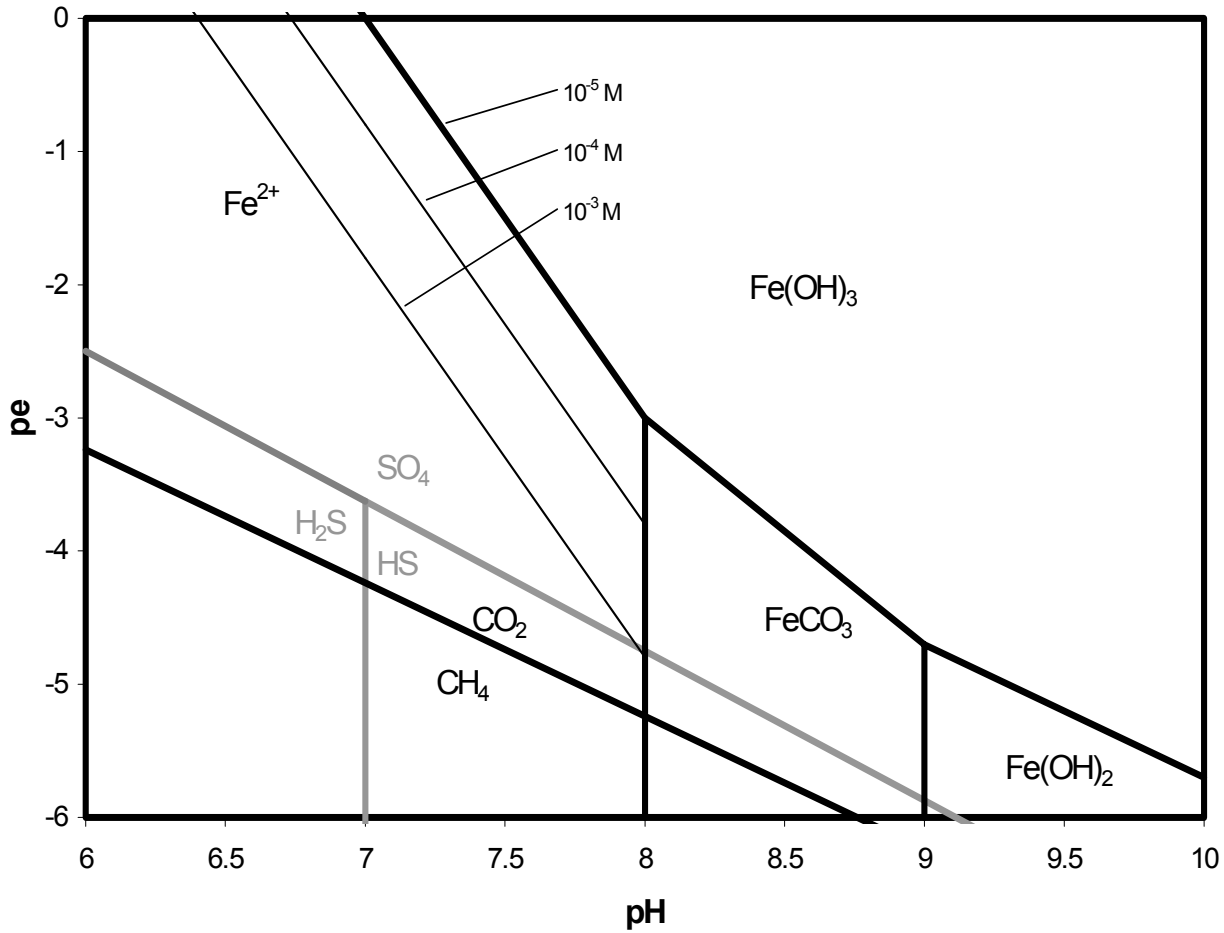


Figure 4-4 is a phase diagram containing data taken from Chapter 3, Tables 3-9 through 3-13. Data for Figure 4-4 includes the combined mean of Farms 2149,2704,and 3101 and data from Farms 10, 20, and 318 independently (Tables 10, 11, 12, and 13 respectively). This plot again demonstrates the variability of redox conditions found between the lagoon environments sampled. For the lagoons sampled, Farm 20 and Farm 318 appear at opposite ends of the spectrum of measured redox potentials. All the data points appear to scatter along the line representing the $Fe^{2+}/Fe(OH)_3$ equilibria, and the differences in measured pH and Eh between lagoons is readily apparent. Figure 4-4 also suggests that Farm 318 might best be represented by the ferrous carbonate/ferrihydrite couple. This indicates that at Farm 318 the activity of free ferrous iron in the lagoon is controlled by the dissolution of ferrous carbonate.

Conclusions on the use of pe/pH phase diagrams

The evaluation of active redox couples using field measured Eh and pH values in conjunction with pe/pH phase diagrams resulted in two conclusions. First, the data suggests that the Fe redox couple was measured and is the potential determining redox couple based on using an electrode method for potential measurement. The assumption used in the construction of the pe/pH phase diagram in regard to the ferrous iron/ferrihydrite redox couple, that dissolved ferrous iron concentration was in the order of 10^{-5} M, coincidentally agrees with dissolved iron concentrations measured in the lagoons studied. Based on this conclusion this couple will be used in the next section to calculate Eh values using data from the chemical analysis of lagoon effluent. Second, although the sulfur and carbon couples do not appear to be potential determining

Figure 4-3: pe-pH phase diagram containing data points for all collected data. Auxillary conditions noted in Table 4-2.

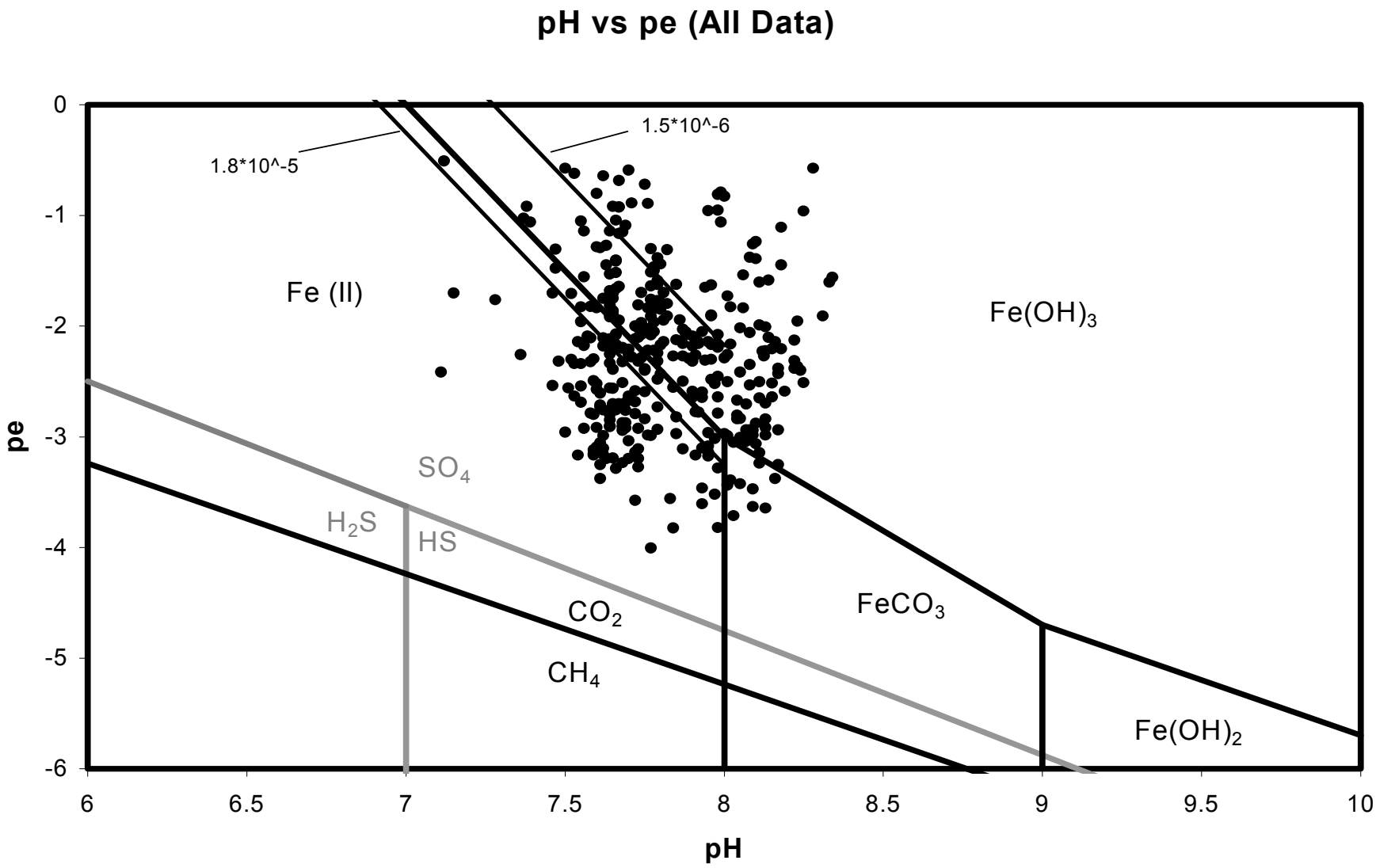
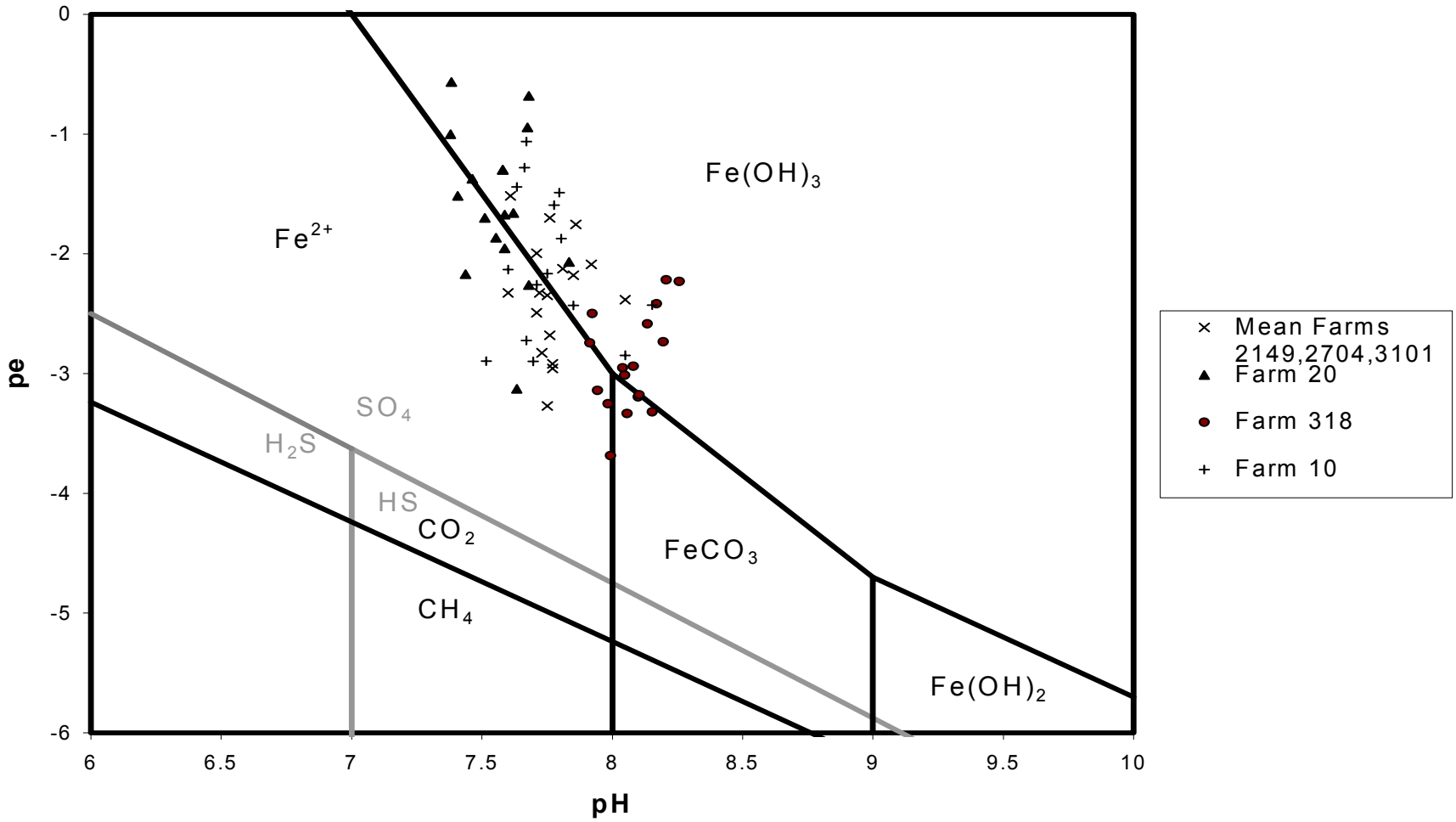


Figure 4-4: pe/pH phase diagram containing data from Chapter 3, Tables 10-13 (Table 10 = Mean Farms 2149, 2704, 3010; Table 11 = Farm 10; Table 12 = Farm 20; Table 13 = Farm 318). Auxillary conditions noted in Table 4-2.



reactions, they will be included in the calculation of Eh values since they are known to be active in swine lagoons.

Seasonal trend in lagoon redox chemistry - An interesting aside to the use of pe/pH phase diagrams to identify active redox couples is the use of these diagrams to visualize temporal trends in lagoon redox chemistry. Figure 4-5 is a phase diagram containing data points for all of the data collected at Farm 10 averaged over depth and grouped by season. Farm 10 was plotted because field measurements were taken over the longest sampling period (6/00-2/01). Season was defined according to the following calendar designations for the year 2000:

Spring Equinox = March 20, 2000

Summer Solstice = June 21, 2000

Autumnal Equinox = September 22, 2000

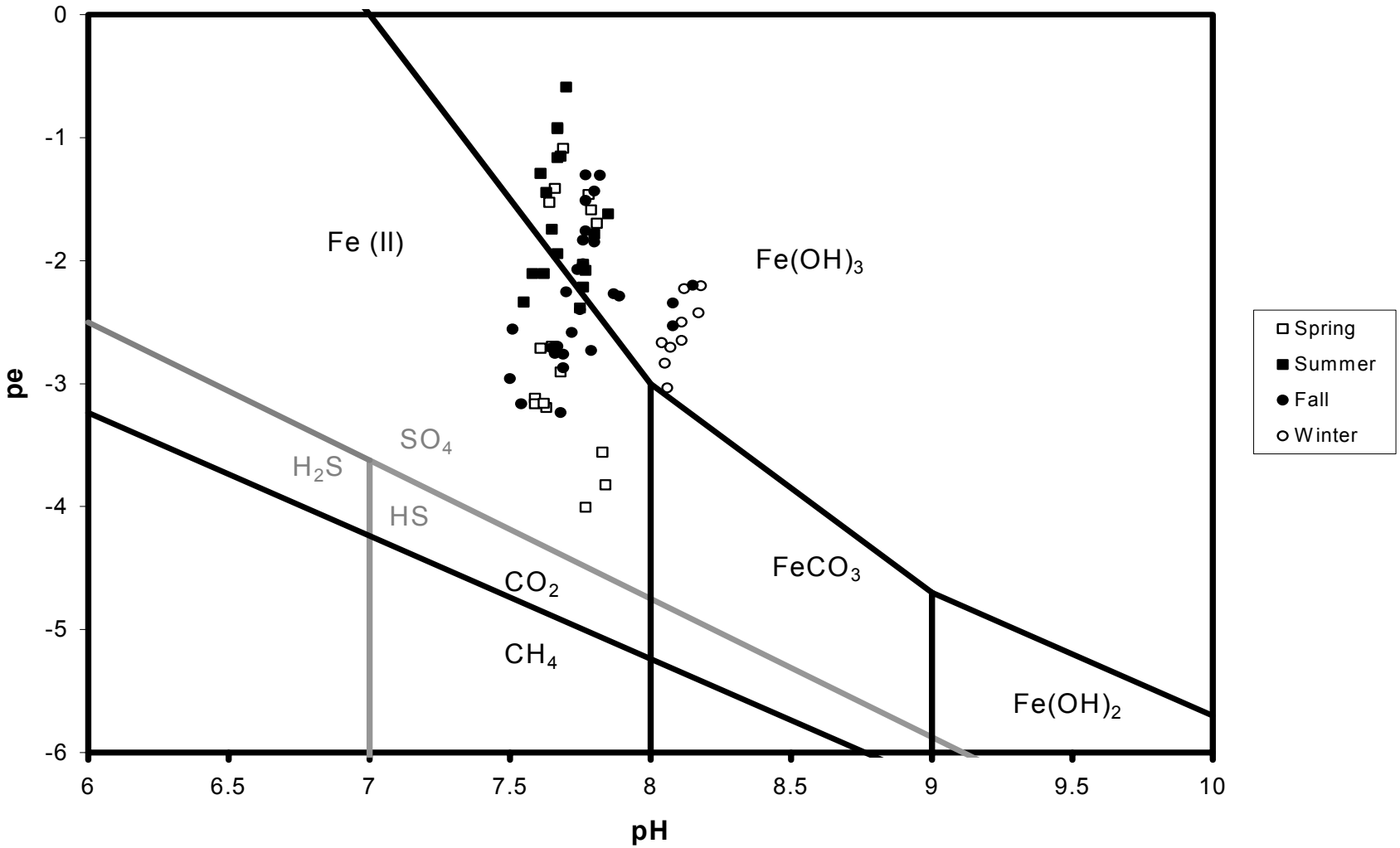
Winter Solstice = December 21, 2000

Figure 4-5 indicates an apparent seasonal shift in redox conditions. This shift follows the temporal trend of pH measurements, with pH increasing into the winter months and decreasing into the summer months.

Chemical Speciation Software

Given the pH of an environment and the activity of both species of a given redox couple an equilibrium redox potential can be calculated based on thermodynamic data for that couple. Chemical analysis of lagoon fluid was conducted in order to calculate expected Eh values and compare calculated potentials to measured potentials (Taken from Chapter 3, Table 3-9).

Figure 4-5: pe/pH phase diagram for Farm 10 data grouped by season collected.



The focus of this exercise was to input analytical data from the lagoons studied, then calculate an equilibrium Eh based on the redox couple selected for calculation. Recalculation of equilibrium Eh using a different couple was then undertaken reusing the same input parameters. Agreement between calculated and measured potential would indicate which couple is the potential determining reaction, and this couple would be used for further calculations.

Calculations were made using software developed by the United States Environmental Protection Agency (U.S. EPA), the chemical speciation program MinteqA2 (**6**). This program allows for the input of concentrations for selected chemical species, the selection of active redox couples, and the calculation of an expected equilibrium redox potential. This is all accomplished using thermodynamic data compiled by the U.S. EPA and stored on an internal database included with the MinteqA2 software.

Literature for the MinteqA2 software states that an extensive literature review was conducted in the culling of the thermodynamic constants used for the MinteqA2 database. Since these constants are the most important part of equilibrium calculations a specific number of key reaction constants were evaluated to ensure confidence in the calculated redox potentials.

In order to evaluate the MinteqA2 database, constants thermodynamic values used for select reactions were compared to values cited in other sources. Table 4-3 contains the values found in the MinteqA2 database and values cited in other sources. All MinteqA2 database values for the reactions considered compared favorably to values cited in the literature referenced, and the database was used as given.

Table 4-3: MinteqA2 database constants as compared to cited values.

Minteq Input	Minteq Constant	Cited from	Literature Value
$Fe^{3+} + 3OH^- = Fe(OH)_3$	log K = -3.191	NIST Database 46	log K = -3.2 [1]
Fe^{2+}/Fe^{3+}	E^0 = 0.771 V	Bard, Allen 1985	E^0 = 0.771 V [2]
HS^-/SO_4^{2-}	E^0 = 0.249 V	MINTEQ version 3.11	E^0 = 0.25 V [1]
Fe^{2+} + Acetate = Fe[Acetate]	log K = 1.4	NIST Database 46	log K = 1.4 [3] log K = 1.1 +/- .3 for Fe[Acetate] at 50 ^{0}C [3] log K = 2.1 +/- .6 for $Fe[Acetate]_2$ at 50 ^{0}C [3]
$Fe^{2+} + HPO_4^{2+} = FeHPO_4$ (aq)	log K = 15.975	NIST Database 46	log K = 16.0 [4]

[1] Pankow, J. 1991.Aquatic Chemistry Concepts. Lewis Publisher, Chelsea, Michigan.
[2] CRC Handbook of Chemistry and Physics, 81st edition
[3] Palmer, D., and Drummond, S. 1988. Potentiometric determination of the molal formation constants of ferrous acetate complexes in aqueous solutions to high temperatures. J. Phys. Chem. 92(23), 6795-6800.
[4] Stumm,W., and Morgan,J.1996. Aquatic Chemistry. Wiley-Interscience, New York

Table 4-4 lists the lagoon parameters used as inputs for the MinteqA2 software. Concentration values for the parameters listed were taken from the Grand Mean data set (Chapter 3, Table 3-9). Three redox couples were considered; the ferrous/ferric iron couple, the sulfate/sulfide sulfur couple, and the methane/carbon dioxide carbon couple. To complete the calculations, several assumptions needed to be made for important parameters where analytical data was not available. The parameters of interest and the estimates made are given in Table 4-5.

Table 4-4 : Lagoon data used for calculation of redox potential. Data reported for ammonium and sulfate in Chapter 3, Table 9 was modified to express concentration as a function of species, not as concentration of nitrogen or sulfur in species.

Minteq input parameters	
Parameter	**Parameter Estimate**
pH	measured pH
Mn^{2+}	total dissolved Mn
Cu^{2+}	total dissolved Cu
Fe^{2+}	total dissolved Fe
Zn^{2+}	total dissolved Zn
NH_4^+	(NH_4-N)* mgNH_4/mgN
PO_4^{3-}	dissolved OP-P
SO_4^{2-}	(SO_4-S)* mgSO_4/mgS
Ca^{2+}	total dissolved Ca
Mg^{2+}	total dissolved Mg
Na^+	total dissolved Na
K^+	total dissolved K
Cl^-	total dissolved Cl

Table 4-5: Assumptions for unknown parameters used in calculation of Eh using MinteqA2.

Parameter	Parameter Estimate
HS^-	Dissolution of FeS (ppt)
Fe^{3+}	Dissolution of $Fe(OH)_3$
CO_3^{2-}	1400 mg/L
Acetate	Remainder of ion imbalance after input of above parameters

As discussed earlier, at the pH levels found in the lagoons studied ferric iron would precipitate out of solution to form a ferric oxide compound. Poorly crystalline ferrihydrite ($Fe(OH)_3$) has the highest solubility product for ferric oxide minerals at the pH levels encountered, and as such would be the first compound to precipitate out of solution. Assuming that the iron redox cycle was rapid enough to prevent the formation of more stable crystalline solids, the concentration of free ferric iron was considered to be controlled by dissolution of ferrihydrite.

Carbonate concentration is an important species that was not analytically determined. An estimate for carbonate concentration was made using values for mean alkalinity for swine lagoons supplied through personal correspondence with Dr. John Classen, Department of Biological and Agricultural Engineering, North Carolina State University. The estimated value for carbonate concentration used was 1400 mg CO_3^{2-}/L.

Another parameter estimate of interest was that of the dissolved organic carbon concentration. Two assumptions needed to be made concerning this estimate: one as to the nature of the dissolved organic carbon, and two the concentration of the dissolved organic carbon species. In reality the dissolved carbon fraction of lagoon fluid likely is represented by a large group of diverse organic compounds. Identification of the true nature of the carbon fraction is beyond the scope of this study. However, complexation reactions between dissolved carbon compounds and free metals could be an important mechanism for metal removal from solution, so an educated estimate is necessary.

For our calculations we used acetate to represent the dissolved organic carbon fraction. This decision was based on the fact that acetate is the most common by-product of the anaerobic fermentation of complex organic compounds, the predominant biological

reaction in anaerobic swine lagoons (**2**). An estimate of the concentration was made using calculations of the ion balance in lagoon fluid using the Generalized Electroneutrality Equation (ENE):

$$\Sigma(z_i c_i) = 0$$

Where z_i are the charges of the various ions and c_i are the concentrations of those ions. Acetate concentration was assumed to satisfy the remainder of the ion imbalance after insertion of analytical data into the ENE equation.

Calculation of Equilibrium Redox Potentials

Data for the calculation of redox potential was taken from the Grand Mean data set, Chapter 3 Table 3-9. Initially three sampling dates were chosen arbitrarily in order to limit the calculation overhead. The sampling dates used were the first sampling date (Day 215), the last sampling date (321), and the middle of the sampling period (Day 279). Calculations were first conducted using the sulfur couples as the potential determining reaction.

For the calculation of equilibrium redox potential involving the bisulfide/sulfate half reaction two variables must be known, the activity of sulfate (SO_4^{2-}) and the activity of bisulfide (HS^-). Sulfate concentration was directly measured while bisulfide concentration was not determined. In order to calculate a redox potential an estimate had to be made concerning bisulfide concentration. As a first estimate, the difference between measured total dissolved (> 0.4 micron) sulfur and measured total dissolved sulfate-sulfur (SO_4^{2-}-S) was taken as bisulfide concentration. Alternatively an assumption concerning a possible solid species controlling bisulfide concentration was made.

According to our data dissolved iron is available and more abundant then all other transition metals examined. Sulfide reacts readily with transition metals such as ferrous iron to precipitate ferrous sulfide, as given by the low solubility of crystalline ferrous sulfide. Therefore the assumption was made that dissolution of ferrous sulfide controlled the concentration of hydrogen sulfide in solution. Sulfide also binds readily with zinc and copper, both of which had measureable concentrations in the dissolved fraction. Calculations were also conducted using zinc and copper sulfide as mineral species controlling the activity of bisulfide in solution. Table 4-6 contains the results of the calulation of equilibrium redox potential using the non-sulfate fraction (total dissolved sulfur – dissolved sulfate sulfur) of total dissolved sulfur as an estimate of the bisulfide concentration. Table 4-7 contains the results of the calculation of equilibrium redox potential using ferrous iron, zinc, and copper sulfide as the mineral species controlling the activity of bisulfide in solution.

Table 4-6: Calculated redox potential using non-sulfate fraction of total dissolved sulfur as estimate of bisulfide concentration.

Sulfur Couple Redox Potential (in mV)				
		[HS^-] = ([Total diss S] - [SO_4^{2-} -S])		
	Measured Eh (mV)	Calculated Eh (mV)	Difference	Ionic strength
Day 215	-161	-268	107	6.3×10^{-2} M
Day 279	-131	-269	138	6.0×10^{-2} M
Day 321	-118	-279	161	6.3×10^{-2} M

Table 4-7: Calculated redox potential using iron, zinc, and copper sulfide as mineral species controlling bisulfide concentration in solution.

Sulfur Couple Redox Potential (in mV)							
		FeS source of [HS⁻]		ZnS source of [HS⁻]		CuS source of [HS⁻]	
	Measured Eh (mV)	Calculated Eh (mV)	Difference	Calculated Eh (mV)	Difference	Calculated Eh (mV)	Difference
Day 215	-161	-255	94	-225	64	-134	-27
Day 279	-131	-257	126	-226	95	-136	5
Day 321	-118	-267	149	-236	118	-147	29

Observing the calculated results given in Tables 4-6 and 4-7 it was determined that the data generated was sufficient to make a confident conclusion as to whether the calculated redox potential using the sulfur couple best approximates the measured potential.

With the exception of the potential calculated using copper sulfide as the mineral species controlling bisulfide activity, the data suggests that using the sulfur (SO_4^{2-}/HS^-) couple as the potential determining redox couple results in a calculated potential sizably lower then the measured potential. As a result of these calculations potential calculations using the methane (CH_4/CO_2) couple were not attempted because the expected deviation is greater than for the sulfur couple (e.g., see Figure 4-1).

In regards to the potential calculations involving copper sulfide species, the existence of this species is doubtful if the resulting concentration of bisulfide in solution is calculated. Table 4-8 contains the calculated concentration of bisulfide in equilibrium with copper sulfide using measured Eh and measured dissolved copper concentrations.

Table 4-8: Calculated concentration of bisulfide in equilibrium with copper sulfide using measured Eh and measured dissolved copper concentrations.

	Measured Eh (mV)	$[HS^-]$ (M)	mg HS^--S/L
Day 215	-161	8.93E-19	2.86E-14
Day 279	-131	6.30E-23	2.02E-18
Day 321	-118	4.26E-25	1.37E-20

Considering that emission of hydrogen sulfide gas from swine lagoons has been documented, as well as the existence of sulfide oxidizing bacteria in swine lagoons, the resulting bisulfide concentration must be considered too low to be realistic.

For the calculation of equilibrium redox potential involving the ferrous/ferric iron half reaction two variables must be known, the activity of ferrous iron (Fe^{2+}) and the activity of ferric iron (Fe^{3+}). Neither iron species was directly measured, rather total iron and total dissolved iron (> 0.4 micron) were measured. In order to evaluate which fraction gives a better estimate of the activity of ferrous iron in solution the total ferrous iron concentration necessary to explain observed redox potentials was calculated assuming ferrihydrite as the source of ferric iron. Table 4-9 contains the results of these calculations.

Table 4-9: Calculated total ferrous iron concentration necessary to correspond with measured redox potentials assuming ferrihydrite as the source of ferric iron. Data used taken from Grand Mean data set, Chapter 3, Table 3-9.

DAY OF YEAR	Measured Eh (mV)	Calculated [Fe^{2+}] (mg/L)	Total [Fe] (mg/L)	Difference	Total dissolved [Fe] (mg/L)	Difference
215	-161	11.20	1.60	9.60	0.60	10.60
229	-139	6.68	1.19	5.50	0.39	6.29
237	-100	2.14	1.01	1.12	0.32	1.82
244	-114	1.73	1.23	0.50	0.33	1.40
251	-89	1.73	1.17	0.57	0.37	1.36
258	-120	7.09	0.97	6.11	0.30	6.78
266	-124	3.10	0.89	2.21	0.37	2.73
272	-126	2.71	1.16	1.55	0.42	2.29
279	-131	4.20	1.55	2.65	0.55	3.65
286	-86	0.40	1.02	-0.62	0.48	-0.08
292	-91	0.34	1.15	-0.81	0.38	-0.03
299	-165	8.09	1.35	6.74	0.45	7.64
307	-142	5.89	0.98	4.91	0.49	5.39
314	-155	6.81	0.99	5.82	0.49	6.33
321	-118	1.08	0.90	0.18	0.50	0.58
			mean	3.07	mean	3.78
			stdev	3.15	stdev	3.21

Based on the data in Table 4-9 it appears total iron concentrations better agree with calculated iron concentrations. However, it is apparent that more iron is necessary then exists in the system. Calculated potentials using both total dissolved iron concentration and total iron concentrations as estimates of total ferrous iron concentration and ferrihydrite as the source of ferric iron are given in Table 4-10.

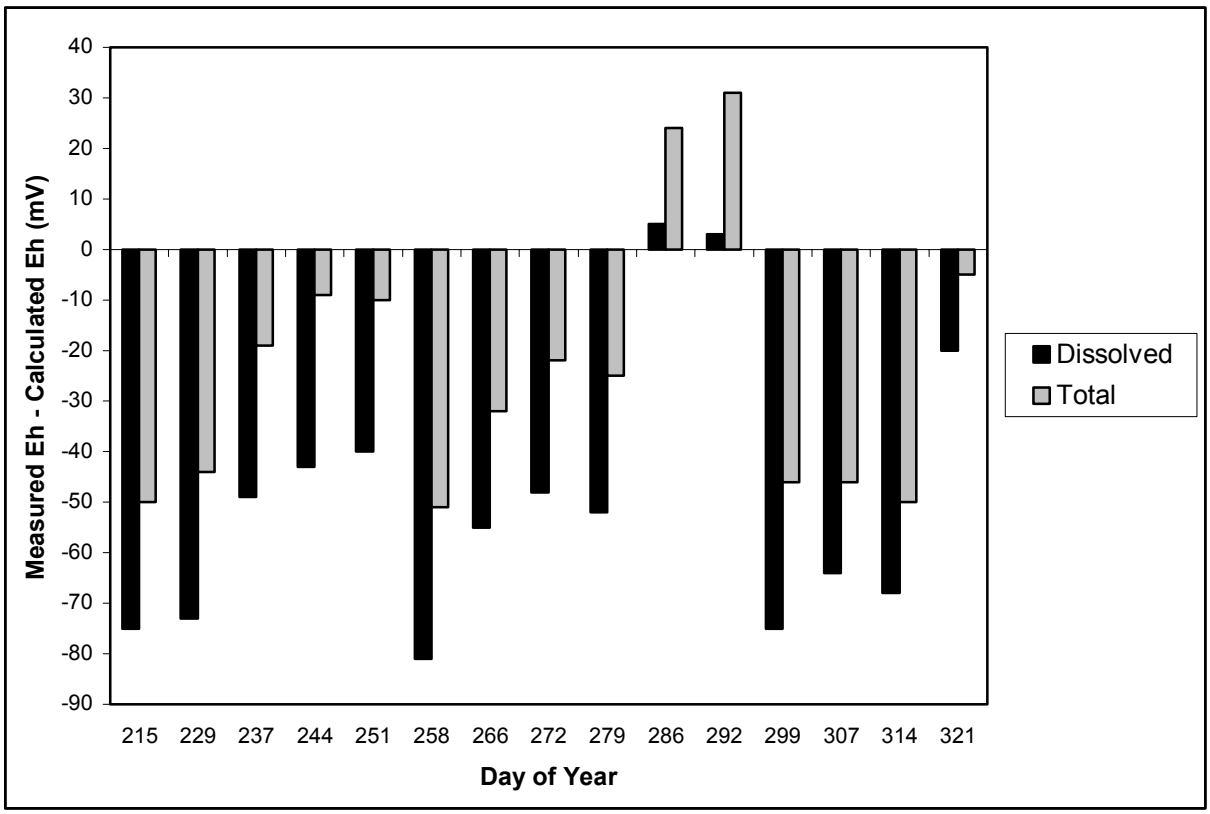
Table 4-10: Calculated potentials using total dissolved iron concentration and total iron concentration as the estimate of total ferrous iron concentration and ferrihydrite as the source of ferric iron. Data used taken from Grand Mean data set, Chapter 3, Table 3-9.

		Total dissolved Fe		Total Fe	
DAY OF YEAR	Measured Eh (mV)	Eh (mV)	difference	Eh (mV)	difference
215	-161	-86	-75	-111	-50
229	-139	-66	-73	-95	-44
237	-100	-51	-49	-81	-19
244	-114	-71	-43	-105	-9
251	-89	-49	-40	-79	-10
258	-120	-39	-81	-69	-51
266	-124	-69	-55	-92	-32
272	-126	-78	-48	-104	-22
279	-131	-79	-52	-106	-25
286	-86	-91	5	-110	24
292	-91	-94	3	-122	31
299	-165	-90	-75	-119	-46
307	-142	-78	-64	-96	-46
314	-155	-87	-68	-105	-50
321	-118	-98	-20	-113	-5

From the results of the chemical speciation calculations, dissolved ferrous iron (Fe^{2+}) is complexed primarily by dissolved orthophosphate. Approximately 25-40% of the total dissolved free ferrous iron concentration is complexed with orthophosphate. Carbonate and acetate also complex with ferrous iron to an appreciable extent, binding with approximately 6% and 12% of the total dissolved free ferrous iron, respectively. Acetate, as stated earlier, was used to represent all dissolved organic compounds.

Figure 4-6 contains a plot of the difference between measured Eh and calculated Eh (measured – calculated) over the sampling period (8/2/00-10/21/00).

Figure 4-6: Calculated difference between measured Eh and calculated Eh (measured – calculated) over the sampling period from 8/2/00-10/21/00. Data used taken from Grand Mean data set, Chapter 3, Table 3-9.

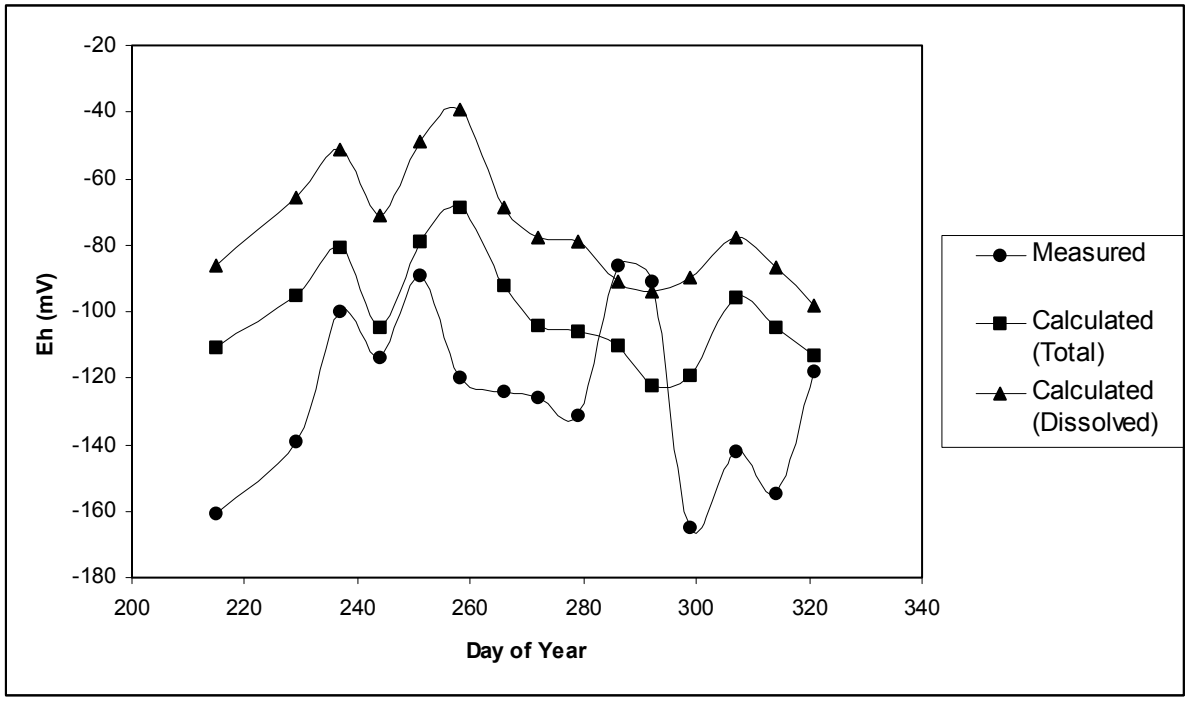


It is apparent from Figure 4-6 that measured potentials, with the exception of potentials measured on day 286 and 292, are consistently lower then calculated potentials. It is significant to note that calculated potentials using total iron as the

estimate of total ferrous iron concentration are within the +/- 50 mV garaunteed accuracy established by Braodley & James, the field redox probe manufacturer (Chapter 2).

Figure 4-7 is a plot of calculated potential versus measured potential. This plot was constructed using data averaged over depth and all six lagoons (Grand Mean) for the sampling period from 8/2/00-10/21/00. This data is found in Chapter 3, Table 3-9.

Figure 4-7: Measured redox potential and potential calculated using data averaged over depth and all six lagoons (Grand Mean) for the sampling period from 8/2/00-10/21/00.



As noted earlier, the measured redox potential appears to increase and "peak" on several sampling dates. Of those fluctuations, the calculated potentials seem to mirror this trend in measured potential for days 237, 251, and 307. The other possible fluctuations,

one occuring over days 286 and 292 and the other the increase on day 321, are not represented in the calculated potential.

Sources of Error in Redox Potential Calculations

It is apparent that for a significant number of sampling dates the calculated potential is greater then the measured potential. Furthermore, this phenomenon appears to occur consistently over the sampling period. This indicates the possibility of a systematic error in the calculation of equilibrium redox potential.

In order to explain the observed discrepancy between calculated and measured potentials there are two potential sources of error to consider. One involves experimental error in sample collection and the other involves theoretical errors in the assumptions made in the potential calculations.

One possible reason for the deviation between measured and calculated Eh could be the result of sample contamination. Air contamination of collected lagoon liquid could have resulted in chemical oxidation of aqueous ferrous iron. This would reduce the activity of free ferrous iron and result in an underestimation of the dissolved ferrous iron concentration. Calculated potentials would then appear higher then the actual potential of the system as measured in the field. Air contamination could have occurred during transport to the lab before freezing, or during filtration and centrifugation. Every attempt was made to limit the samples to atmospheric exposure, but contact was inevitable during filtration and centrifugation. In addition, storage in HDPE plastic containers could have resulted in air contamination, as plastic containers are considered to be porous with respect to gaseous contamination. It should be noted that air contamination would only

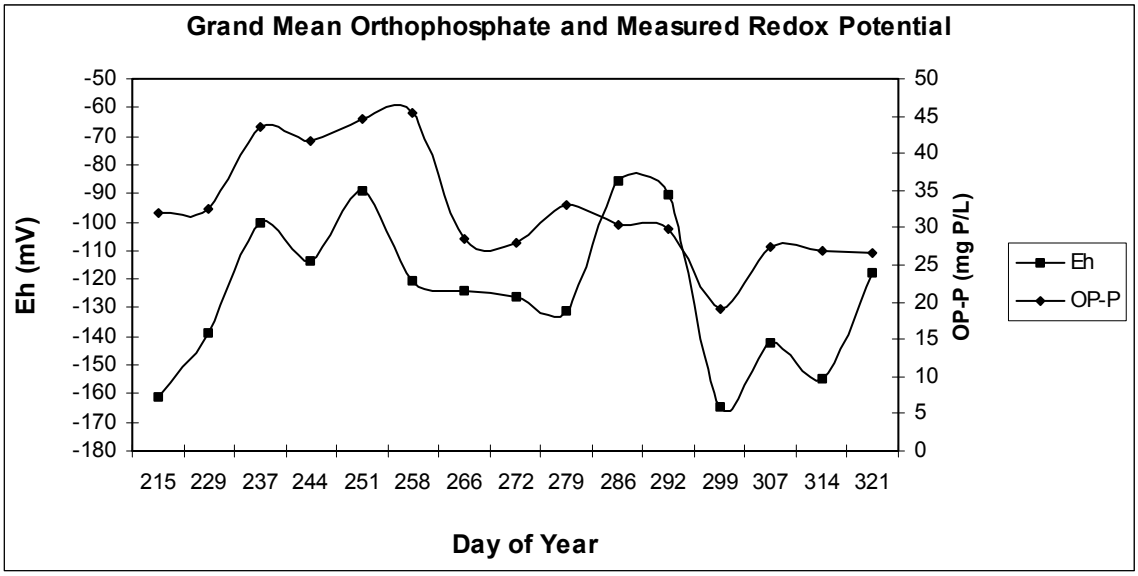
affect dissolved iron concentrations, and this phenomenon would have no effect on the total iron concentration.

The other possible explanation for the deviations observed involves error in the underlying assumptions made in the calculation of redox potential. This discussion can be in turn divided into two possibilities: error in the fundamental interpretation of the data, and error in the estimation of parameters involved in the potential calculations.

In terms of error in the interpretation of the data, the underlying concept of equilibrium redox potential could be the failing point. Calculated potentials represent redox potentials that would exist in the lagoon under equilibrium conditions. It is possible that redox potentials in lagoons undergo dynamic changes, and that the field measured Eh on some days represent conditions far from equilibrium. The error would then be in the comparison of calculated equilibrium potentials to measured non-equilibrium potentials, based on the assumption that both potentials should be equal.

Observation of the temporal plots of measured redox potential suggests that periodically there are rather large fluctuations in potential which seem to occur across all six lagoons. Evidence collected from the chemical analysis of lagoon fluid from all six lagoons seems to suggest that these fluctuations are real events. Considering that free ferrous iron concentration is controlled significantly through complexation with orthophosphate, plots of orthophosphate and Eh averaged over all six lagoons and all three depths (Grand Mean data, Chapter 3 Table 3-9) show similar trends (Figure 4-8).

Figure 4-8: Grand Mean Eh and orthophosphate values plotted vs. day of the year. Day 215 represents 8/2/00, and day 391 represents 1/25/01.



For the first observed fluctuations on days 237 and 251 both orthophosphate and Eh appear to follow similar trends. This is consistent with the idea that as the orthophosphate concentration increases, ferrous iron complexation would increase, with a concomitant increase in the measured Eh of a ferrous/ferric iron system. It is apparent from Figure 4-9 that the large fluctuation in measured redox potential around day 286 and 292 is not mirrored in the plot of dissolved orthophosphate. However, there is other evidence that this fluctuation might be a real event. Grand Mean (Chapter 3 Table 3-9) plots of dissolved sulfate and dissolved iron, both redox sensitive species, demonstrate large fluctuations around these sampling dates (Figures 4-9 and 4-10).

Figure 4-9: Grand Mean Eh and dissolved iron values plotted vs. day of the year. Day 215 represents 8/2/00, and day 391 represents 1/25/01.

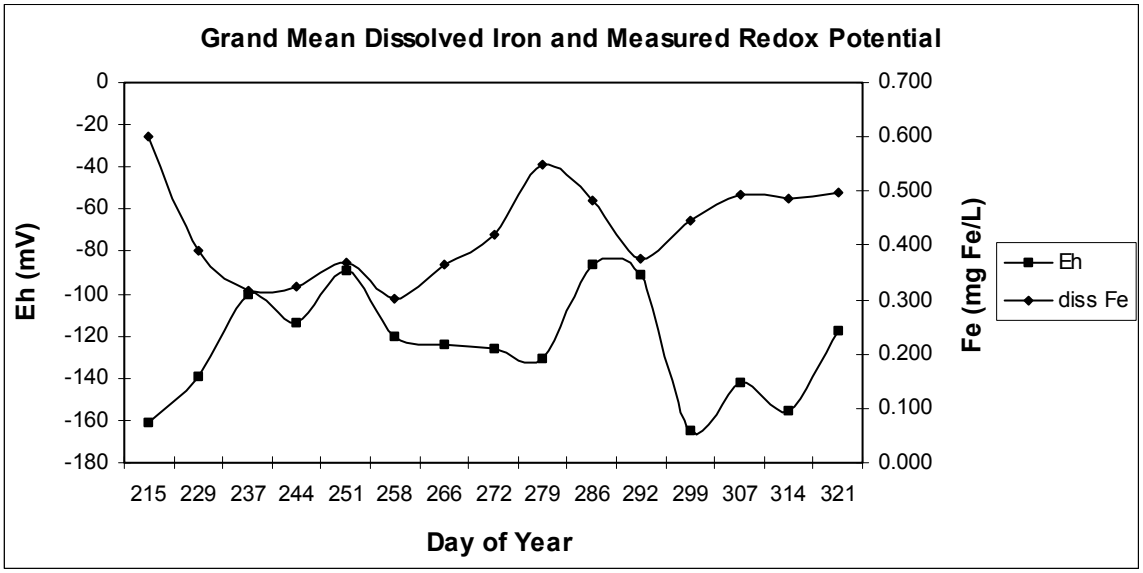
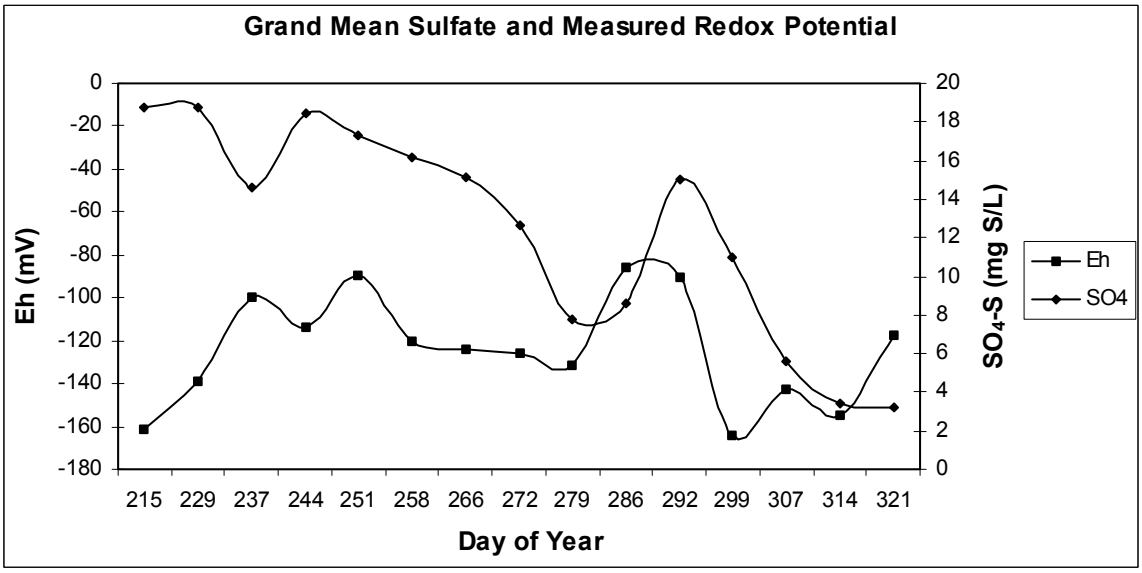


Figure 4-10: Grand Mean Eh and dissolved sulfate values plotted vs. day of the year. Day 215 represents 8/2/00, and day 391 represents 1/25/01.



At the beginning of the sampling period, dissolved iron concentration decreases while measured redox potential increases. This occurs as expected given the assumption that the majority of dissolved iron in this system would be found as ferrous iron. Dissolved iron concentration then increases and peaks at approximately 550 ppb on day 279. Redox potential remains relatively stable during this period. Following this increase dissolved iron concentration decreases while measured potential increases to a maximum of –86 mV on day 286.

Observing Figure 4-10, the increase in redox potential on days 286 and 292 appears to coincide with a large increase in sulfate concentration. Sulfate concentration shows a steady decline until day 279, after which it increases almost two fold from approximately 8 mg SO_4-S/L to 15 mg SO_4-S/L on day 292.

Considering all the data discussed above it seems likely that fluctuations in measured redox potential represent real events. Noting that all six lagoons seem to fluctuate synchronously suggests that these fluctuations are influenced by local climate changes. Figure 4-11 is a plot of Eh and lagoon temperature averaged over all six farms and over all three depths (Grand Mean data, Chapter 3 Table 3-9) as well as a plot of precipitation measured at the Clinton Horticultural Crops Research Station in Clinton, NC.

Observing Figure 4-11, it appears that the increase in potential on day 251 coincides with both a drop in lagoon temperature and a rainfall event. The increase in potential noted on day 321 also appears to occur with both a drop in lagoon temperature and a rainfall event. The large increase in redox potential noted on days 286 and 292 appears to coincide with a drop in lagoon temperature, but does not appear to coincide

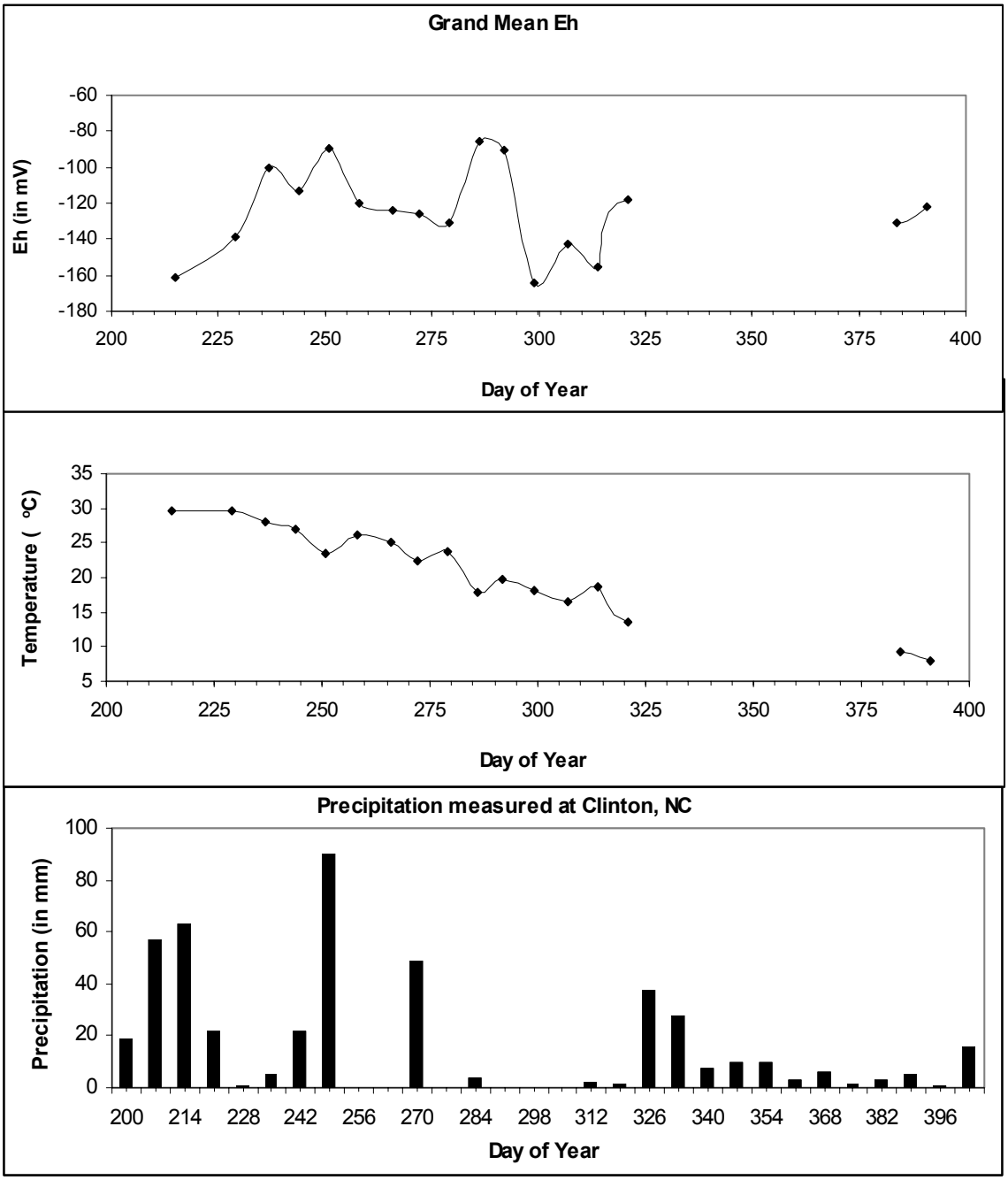
with a rainfall event. A rainfall event does occur during the period from day 263 through 270, at least 16 days before the increase in potential was measured.

It is possible that these drops in lagoon temperature effect the microbial communities active in cycling redox sensitive species. Another possibility is that oxygen, nitrate, sulfate, and other oxidized species introduced into the lagoon during rainfall events have a measurable effect on lagoon redox potential. In conclusion, it appears that measured redox potentials fluctuated widely around a mean potential. This could be interpreted as the lagoons having a mean potential representative of near equilibrium conditions for the iron redox couple, where certain events occur that affect this balance. The response to these events can be seen in the fluctuations in measured redox potential.

Alternatively the error could lie in the assumptions made concerning estimated parameters used in the MinteqA2 calculations. One possible area of error could be in the selection of the iron oxide solid phase. Solubility products for solid phases differ based on whether the solid phase is poorly crystaline or crystalline. Our assumption was that in the lagoons studied the solid phase was poorly crystaline ferrihydrite. Amorphous solids have a higher solubility and would result in a higher ferric iron concentration, which would result in a calculated potential higher then expected.

It is also possible that the error lies in the selection of the solid phase species. With the large concentration of both orthophosphate and bicarbonate in lagoon environments it is possible that the iron oxide solid phase could be an amalgam of iron phosphate, iron carbonate, and iron oxide mineral facies. As an example, ‘green rust’ compounds are partially oxidized ferrous oxide mineral species interlayered with

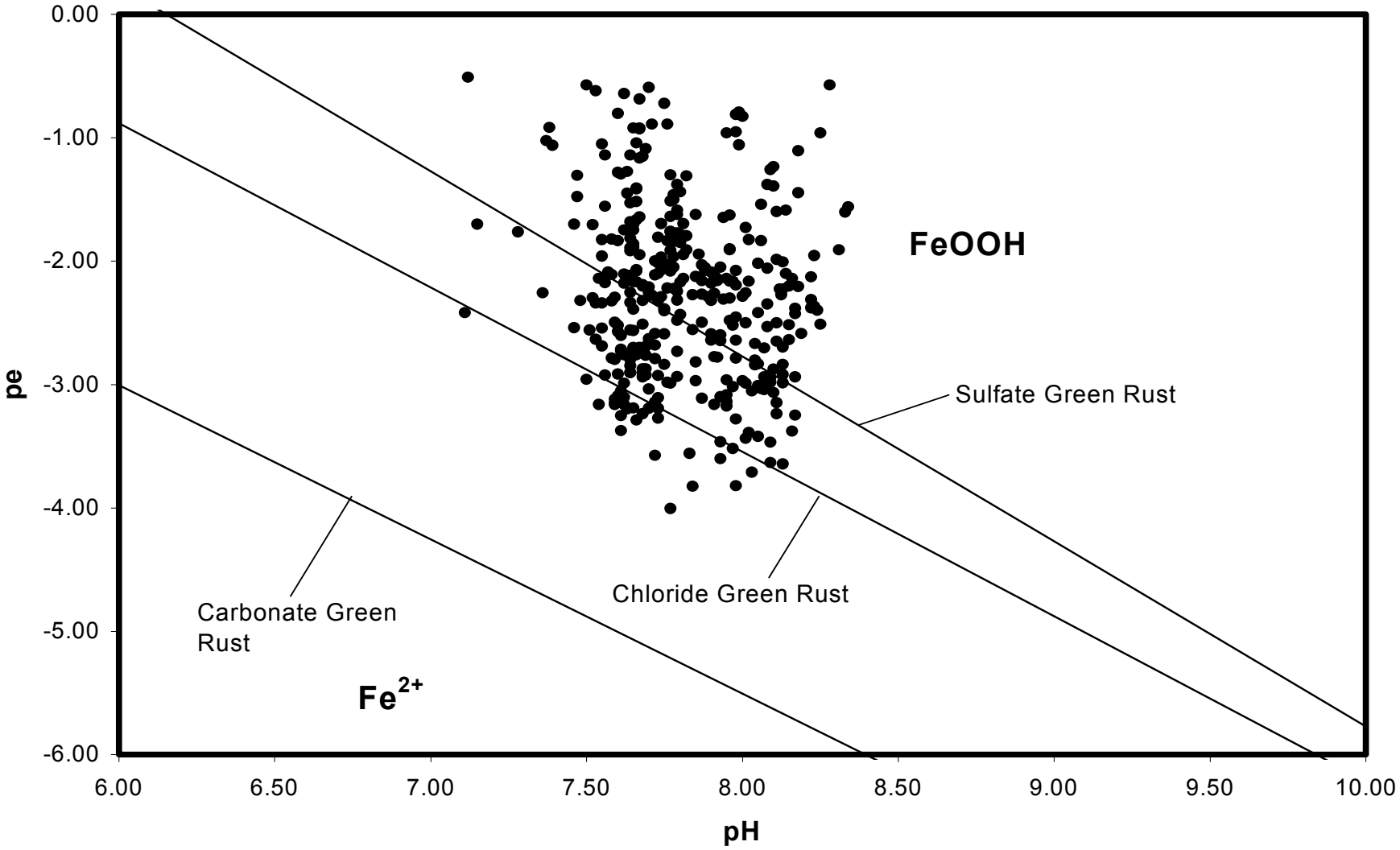
Figure 4-11: Grand Mean Eh, Grand Mean lagoon temperature and precipitation measured at Clinton, NC. Day 215 represents 8/2/00, and day 391 represents 1/25/01. Each rainfall data point represents total rainfall (in mm) for the seven day period ending on the day plotted.



negatively charged ions of the general formula $[Fe_4^{(II)} Fe_2^{(III)} (OH)_{12}][Anion * 2H_2O]$. Experimentally determined redox potentials for the equilibrium between goethite and green rust compounds with sulfate, chloride, and carbonate have been reported (**3**).

Figure 4-12 is a pe/pH phase diagram constructed using the reported pe/pH relationship for the green rust compounds and a plot of all measured data. Measured redox potentials fall close to the equilibria between goethite and both the sulfate green rust and the chloride green rust. It is apparent that further study into the nature of the ferric iron solid phase would be necessary to better define the nature of the iron redox couple in swine lagoon environments.

Figure 4-12 : pe/pH phase diagram constructed using redox couple between green rust compound and goethite.



Alternatively this discrepancy could be the result of an overestimation of dissolved ferric iron concentration due to dissolution of ferrihydrite. Considering that lagoons contain a significant amount of dissolved organic compounds, dissolved phosphate, and bacterial mass it is possible that adhesion of such components to the surface of a suspended ferrihydrite crystal could reduce its solubility. As a result the dissolution of suspended iron oxide minerals in lagoon environments would maintain a lower dissolved ferric iron concentration then would be calculated using constants for chemically pure iron oxide minerals.

Measured vs. Calculated Potential

It is apparent from Figure 4-7 that measured and calculated potentials appear to follow similar trends, but do not have similar magnitudes. In order to have better agreement between calculated and measured potential, the assumptions concerning the activity of ferric iron (Fe^{3+}) could be refined. As mentioned before, both ferrous and ferric iron activity must be known in order to calculate an equilibrium redox potential. In order to estimate ferric iron activity the assumption was made that the dissolution of ferrihydrite is responsible for ferric iron concentration in solution. The possibility exists that ferric iron concentration is controlled by a different mineral phase. In order to estimate the solubility product of a ferric solid phase that would result in the necessary ferric iron activity to correlate calculated potentials to measured potentials, the ferric solid phase solubility product was varied within the speciation program until agreement between measured and calculated potential was within 5 mV. Table 4-11 tabulates the result of this exercise.

Table 4-11: Calculated ferric solid phase solubility product when calculated potential agrees with measured potential within 5mV. Data used taken from Grand Mean data set, Chapter 3, Table 3-9.

		Total Dissolved Fe		Total Fe	
DAY OF YEAR	Measured Eh (mV)	Eh (mV)	log K	Eh (mV)	Log K
215	-161	-162	-1.90	-163	-2.30
229	-139	-136	-2.00	-141	-2.40
237	-100	-104	-2.30	-104	-2.80
244	-114	-118	-2.40	-117	-3.00
251	-89	-90	-2.50	-90	-3.00
258	-120	-115	-1.90	-122	-2.30
266	-124	-130	-2.15	-127	-2.60
272	-126	-130	-2.30	-127	-2.80
279	-131	-131	-2.30	-135	-2.70
286	-86	-90	-3.20	-89	-3.55
292	-91	-94	-3.20	-95	-3.65
299	-165	-161	-2.00	-166	-2.40
307	-142	-143	-2.10	-143	-2.40
314	-155	-158	-2.00	-152	-2.40
321	-118	-121	-2.80	-119	-3.10
		mean	-2.34	mean	-2.76
		stdev	0.43	stdev	0.43

Table 4-12 illustrates the solubilty product of ferric oxide species available in the MINTEQA2 database.

Table 4-12: Solubility product of ferric oxide mineral species available within the MINTEQA2 database.

Iron Oxide Mineral	log K_{SP}
$Fe(OH)_3$ (Ferrihydrite)	-3.191
FeOOH (Lepidocrocite)	-1.371
FeOOH (Goethite)	-0.491
Fe_2O_3 (Hematite)	1.418
Fe_2O_3 (Maghemite)	-6.386

Observing Table 4-12, it appears that calculated solubility products fall in between the solubility products for ferrihydrite and lepidocrocite.

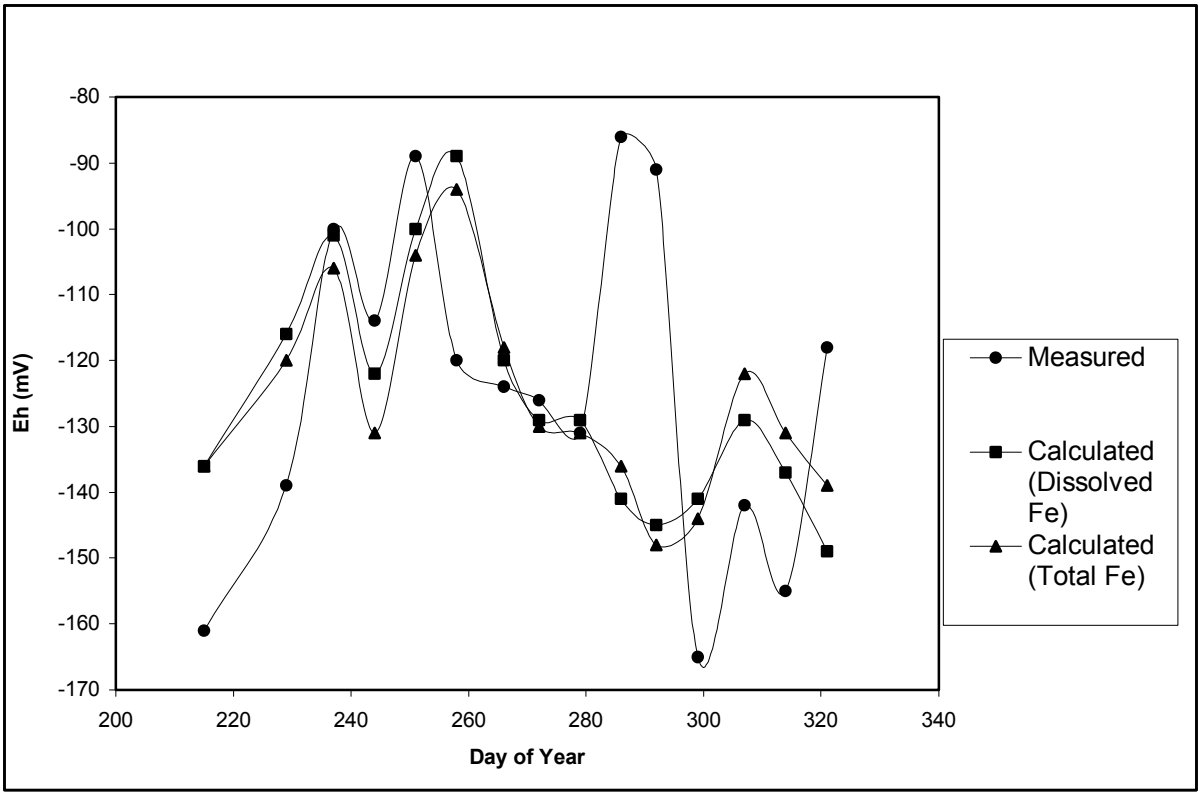
Using the mean calculated solubility product to calculate equilibrium Eh values for the iron couple results in closer approximation of measured Eh values. Table 4-13 contains calculated redox potentials for both total and total dissolved iron using the respective calculated mean solubility product.

Table 4-13: Calculated equilibrium redox potentials for total and total dissolved iron using calculated mean solubility product values. Data used taken from Grand Mean data set, Chapter 3, Table 3-9.

DAY OF YEAR	Measured Eh (mV)	Total Dissolved Fe (log K = -2.34)	Difference	Total Fe (log K = -2.76)	Difference
215	-161	-136	-25	-136	-25
229	-139	-116	-23	-120	-19
237	-100	-101	1	-106	6
244	-114	-122	8	-131	17
251	-89	-100	11	-104	15
258	-120	-89	-31	-94	-26
266	-124	-120	-4	-118	-6
272	-126	-129	3	-130	4
279	-131	-129	-2	-131	0
286	-86	-141	55	-136	50
292	-91	-145	54	-148	57
299	-165	-141	-24	-144	-21
307	-142	-129	-13	-122	-20
314	-155	-137	-18	-131	-24
321	-118	-149	31	-139	21

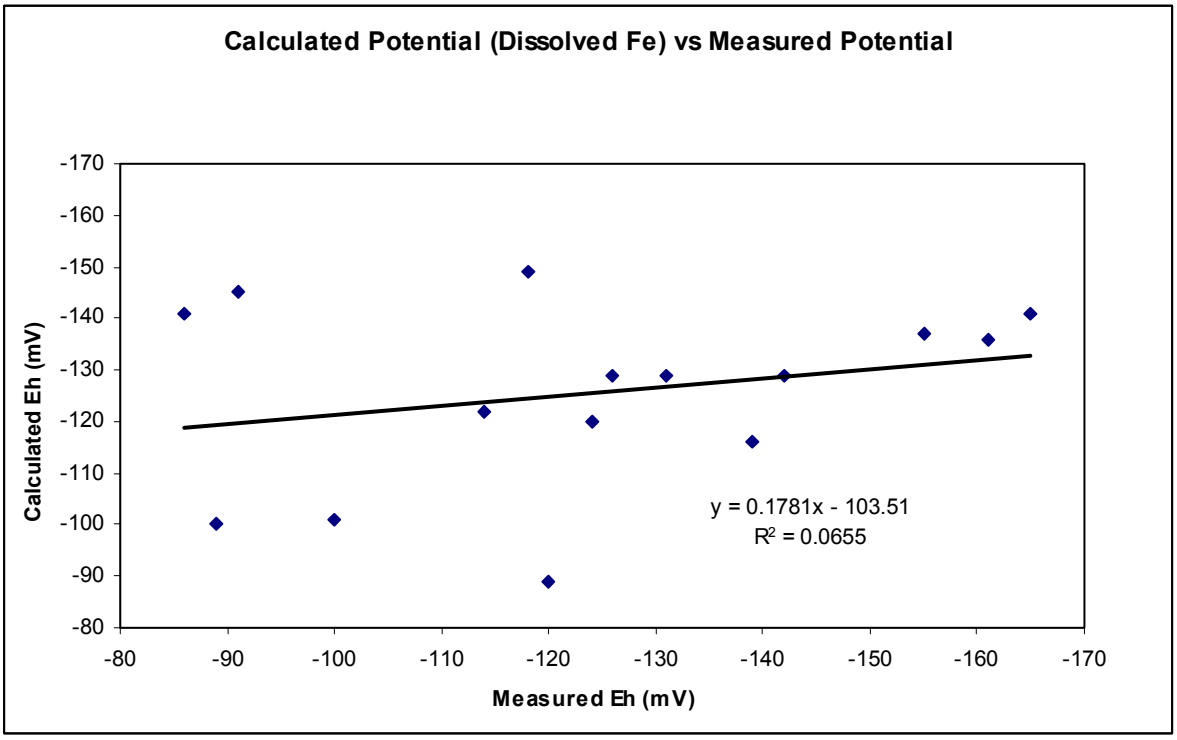
Figure 4-13 contains a plot of measured and calculated potential for both total and total dissolved iron concentrations as a function of sampling day using calculated ferric solid phase solibility product values.

Figure 4-13: Measured and calculated potential for both total and total dissolved iron concentrations averaged over depth and all six lagoons (Grand Mean) for the sampling period from 8/2/00-10/21/00.



Correlation plots of calculated versus measured potential (Figure 4-14) suggest poor correlation between measured and calculated potential. Observing Figure 4-13, calculated potentials appear to closely follow the trend of measured potential, with the exception of the measured potential on days 286 and 292. If the data from days 286 and 292 are discarded the correlation between measured and calculated potential improves. Figure 4-15 contains correlation plots of calculated potentials using total iron concentration and total dissolved iron concentration versus measured potential with data from days 286 and 292 excluded.

Figure 4-14: Correlation plot of measured vs. calculated potential for total and total dissoved iron concentrations.



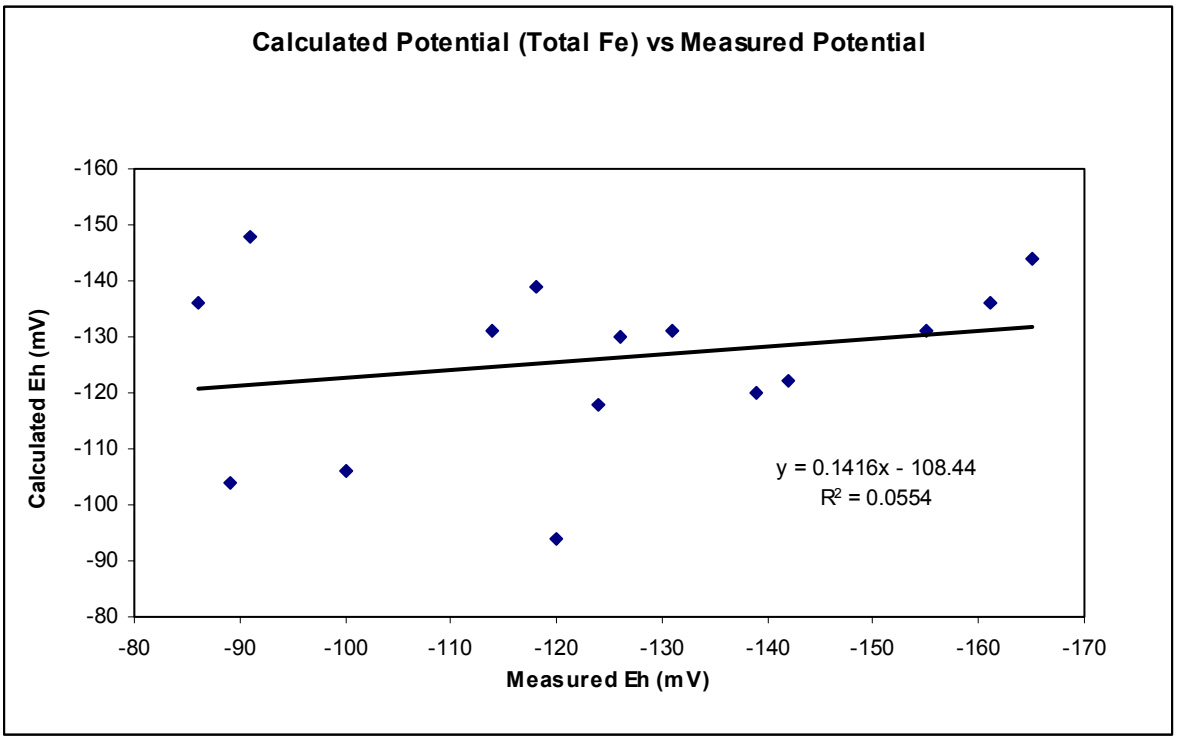
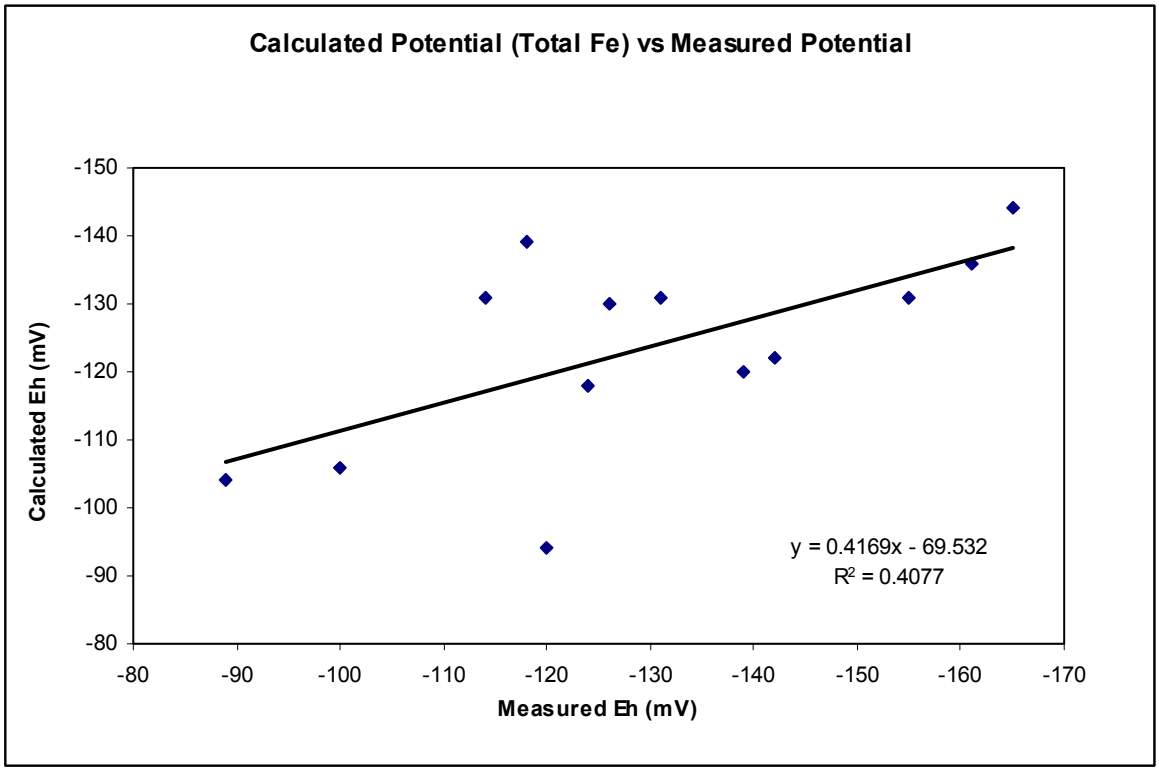
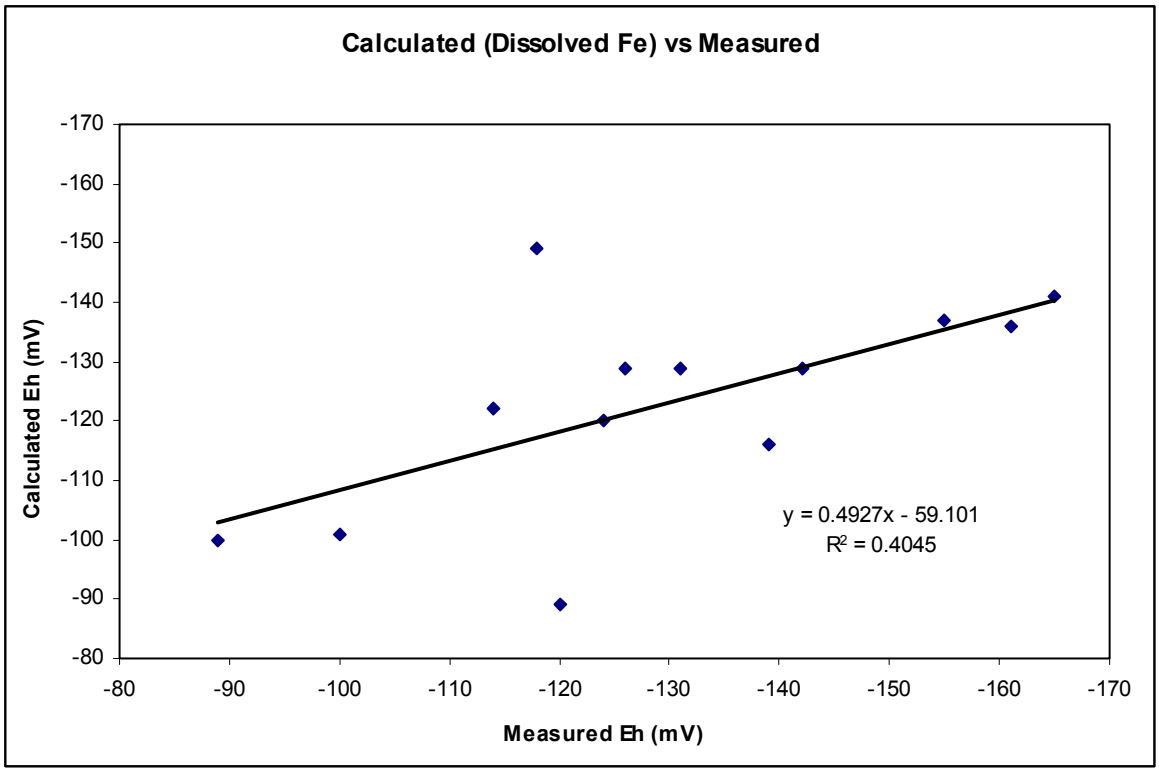


Figure 4-15: Correlation plot of measured vs. calculated potential for total and total dissolved iron concentrations with data from days 286 and 292 discarded.





Conclusions Regarding the Identification of Active Redox Couples

According to the calculated potentials it appears that the iron (Fe^{2+}/Fe^{3+}) couple results in a calculated potential closest to the field measured redox potential. As such it was assumed that the Fe^{2+}/Fe^{3+} redox couple exists in the lagoons sampled and was detected with the redox probe. The existence of a HS^{-}/SO_4^{2-} couple in lagoon effluent is also probable, although the data collected suggests that the redox probe did not detect it. It has been stated that due to the necessity for microbial catalysis this reaction does not produce an exchange current that can be measured by conventional redox measuring techniques (**5**). Further evaluation of the redox chemistry occuring in the swine lagoons studied will included the Fe^{2+}/Fe^{3+} redox couple as a possible reaction involved in the production of di-nitrogen gas.

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Chapter 5

N_2 Production in Swine Lagoon Systems

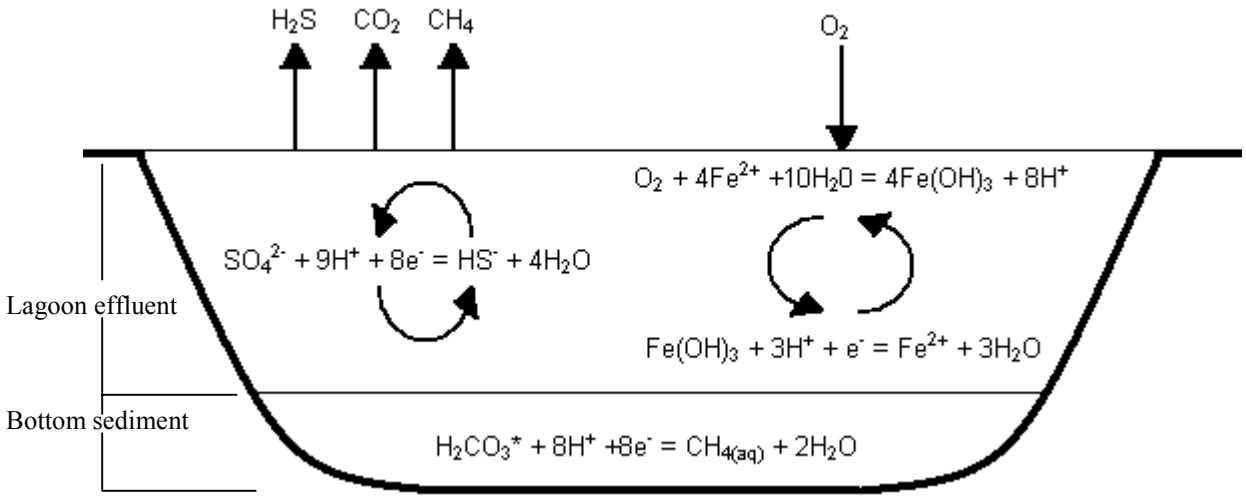
Recent research concerning gas emissions from swine waste lagoons documented the emission of di-nitrogen (N_2) gas from these systems (**9**). Emissions from two lagoons included in our study, Farm 10 and Farm 20, were monitored during the period from July to November 1998. During this period emission rates of N_2 gas averaged 120 and 9 kg N_2-N ha^{-1} day^{-1} for Farms 10 and 20 respectively. To identify possible mechanisms for emission of N_2 in swine lagoons the redox processes active in these systems were evaluated.

Based on our investigation of 6 anaerobic swine lagoons in eastern North Carolina, several conclusions were made concerning the nature of the redox conditions in these environments. Redox potentials measured in the field and potentials calculated using analytical data are consistent with the existence and measurement of an iron redox couple. It is also probable that there is a sulfur couple active in these environments. Evidence for its existence consists of the documented emission of hydrogen sulfide gas (**1,2,14**), our identification of sulfate in lagoon effluent, and the observation of purple sulfur bacteria (which convert bisulfide to sulfate) on the surface of the lagoons studied. There is also reason to believe that there is the existence of an active carbon redox couple, given that methane emission is documented in swine lagoon environments (**14**). A nitrogen redox couple was not considered to be active based on the fact that nitrate and/or nitrite were not detectable in lagoon effluent.

Figure 5-1 is a schematic diagram of the redox processes we believe are active in the swine lagoons studied. It is my assertion that Fe^{3+} is the electron acceptor with the

highest oxidation potential found in the swine lagoons studied. Ferric iron (Fe^{3+}) is generated through the reaction of ferrous iron (Fe^{2+}) with oxygen (O_2). Although dissolved oxygen is undetectable in lagoon effluent the surface of the lagoon is in contact with the atmosphere. Assuming that oxygen is continuously diffusing through the lagoon surface its consumption must be rapid and thorough. Oxidation of ammonium using oxygen as the terminal electron acceptor, known as nitrification, is not assumed to be active considering that in low oxygen environments nitrification is inhibited in the presence of significant dissolved carbon (**15**). Ferrous iron reacts rapidly with oxygen and can occur independently of microbial catalysis (**15**). It is therefore my assertion that oxygen diffusing through the surface of the lagoon environment would react rapidly and readily with dissolved ferrous iron.

Figure 5-1 : Schematic diagram of the redox reactions considered active in anaerobic swine lagoons.



Mechanisms of N_2 Formation

Ammonium is the predominant nitrogen species in swine lagoon fluid, accounting for approximately 80% of the total nitrogen (TKN) according to data supplied by the North Carolina Cooperative Extension Service, North Carolina State University (available at www.soil.ncsu.edu/publications/soilfacts/AG-439-04). Furthermore, nitrate/nitrite nitrogen was undetectable in the lagoons studied. Three mechanisms are known that could result in the transformation of ammonium nitrogen to di-nitrogen gas: anaerobic ammonium oxidation (Anammox), oxygen limited autotrophic nitrification/denitrification (OLAND), and traditional nitrification/denitrification.

One common thread between these three mechanisms is a need for an initial input of nitrate/nitrite nitrogen. Anammox occurs with the oxidation of ammonium using nitrite/nitrate as the terminal electron acceptor according to the following reaction (**13,16**):

$$NH_4^+ + NO_2^- = N_2 + 2H_2O \quad (1)$$

OLAND involves a two-step process (**10**). The first step is the oxidation of ammonium using oxygen as the terminal electron acceptor under conditions of low oxygen concentration (< 3mg/L):

$$NH_4^+ + 1.5O_2 = NO_2^- + H_2O + 2H^+ \quad (2)$$

The second step involves the oxidation of ammonium with nitrite/nitrate as the terminal electron acceptor with the production of di-nitrogen gas according to the following reaction:

$$NH_4^+ + NO_2^- = N_2 + 2H_2O \quad (3)$$

Traditional nitrification and denitrification occurs via the following two reactions respectively (**3**):

$$NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O \quad (4)$$

$$C_6H_{12}O_6 + 4NO_3^- = 6CO_2 + 6H_2O + 2N_2 \quad (5)$$

For the denitrification reaction (equation 5), in which nitrate is used as a terminal electron acceptor for the oxidation of organic matter, glucose ($C_6H_{12}O_6$) was used to represent a complex organic molecule. In order for all three processes to be feasible, a mechanism must exist for the introduction or production of nitrate/nitrite in swine lagoon systems.

Ferric Iron (Fe^{3+}) as an Electron Acceptor

Based on the results of our investigation into the redox processes active in swine lagoons, the possibility exists that ferric iron could act as an electron acceptor for ammonium oxidation. In order to evaluate the feasability of this idea one can estimate the extent of iron-induced ammonium oxidation (and resultant N_2 production) in the lagoon effluent and compare that to the reported N_2 flux data. For Farm 10, where we have an estimate for the lagoon area of 2.67 hectares (**9**), we can estimate the total volume using a formula taken from (**7**):

$$\text{Volume} = (L*W+ (L-d*s)*(W-d*s)+4*(L-d*s)*(W-d*s))*d/6 \quad (6)$$

Where L refers to the length of the lagoon, W refers to the width of the lagoon, d represents the depth of the lagoon, and s refers to the slope of the lagoon (3=3:1). The parameters used for this calulation are given in Table 5-1.

Table 5-1: Parameters used for the calulation of total lagoon volume for Farm 10.

Parameter	Estimate
L	231m
W	116 m
d	2.6 m
s	3

The volume calculated for Farm 10 using this approach is approximately 64,000 m^3 ($6.4x10^7$ L). The mean total iron concentration (Chapter 3, Table 3-2) in the lagoon effluent for Farm 10 is approximately 1.4 mg/L (=1.4 g/m^3). Therefore the total mass of iron contained within the lagoon fluid is approximately 90 kg Fe, or 1612 moles of iron molecules. For the oxidation of one molecule of ammonium, six electrons must be transferred to a terminal electron acceptor (Table 5-2). Hypothetically, if this total pool of iron cycled completely from ferric iron to ferrous iron once it would result in the oxidation of 269 moles of ammonium molecules per cycle, which if denitrified would result in the production of approximately 3.75 kg of di-nitrogen (7.5 kg N_2-N). Dividing this value by the surface area of the lagoon (2.67 ha) a flux estimate of 2.8 kg N_2-N ha^{-1} $cycle^{-1}$ can be calculated. This value is significantly less then the reported average flux of 120 kg N_2-N ha^{-1} day^{-1} (**9**). It is observed that in order for this mechanism to be plausible the iron cycle described would have to occur approximately 43 times a day. In contrast if nitrate is used as the oxidant for ammonium oxidation, such as occurs in the Anammox or OLAND processes, this would result in a flux twice that of what was calculated.

In order to estimate the amount of iron oxidation possible through contact with atmospheric oxygen the flux of oxygen through the surface layer of the lagoon can be estimated using the following equation (**5**):

$$F_C = k_L(C_S - C) \quad (7)$$

Where F_C equals the flux of dissolved oxygen across the water surface, k_L represents the surface transfer coefficient, C_S represents the saturation dissolved oxygen concentration, and C represents the actual dissolved oxygen concentration. The value k_L is calculated using formulas empirically developed to calculate reaeration in lakes. The only variable in k_L calculation is wind speed over the lake surface. Wind speed values reported for Farm 10 measured over an 11 day period and averaged over 24 hour intervals ranged from 123 to 352 cm sec^{-1} (**9**). Using these values for windspeed and averaging the k_L values produced using several of the reported formulas (**5**) estimates for the surface transfer coefficient are 0.326 and 1.068 m day^{-1} for the minimum and maximum windspeeds respectively. The value for the saturation concentration of dissolved oxygen for seawater with a comparable chlorinity to Farm 10 at 25 ^{0}C is approximately 8.2 g m^{-3} (**5**).

Using a C value of 0 and the values cited above, equation 7 predicts an oxygen flux of 26.7 and 87.6 kg O_2 ha^{-1} day^{-1} for the minimum and maximum windspeeds respectively. For a one day period at Farm 10 (Surface area = 2.67 ha) this would result in the flux of 71.3 and 233.9 kg O_2 day^{-1}. Considering that each molecule of oxygen reacts to oxidize four molecules of iron, this would result in the oxidation of 499 and 1637 kg Fe day^{-1} for the minimum and maximum windspeeds respectively. For the maximum windspeed value this represents the possibility of oxidizing the total amount of iron approximately 18 times over a one day period.

Ferric oxide minerals in lagoon effluent represent a possible mechanism for transport of an oxygenated iron species to the bottom of the lagoon. Settling of oxygenated iron could possibly result in bacterial utilization of oxygen for oxidative

processes, such as ammonium oxidation. Alternatively, iron oxide crystals bound to bacterial surfaces could be used for oxidative processes within the lagoon fluid column. Dissimilatory iron reduction is considered to be a mechanism for the use of ferric oxide as a terminal electron acceptor for the biological oxidation and breakdown of complex carbon compounds (**6**). Considering that ammonium is abundant in lagoon environments, it is possible that some microorganism would develop the ability to use ferric oxide minerals to oxidize ammonium for energy production.

Nitrate/Nitrite as Intermediaries in the Production of N_2

Thermodynamically, the oxidation of ammonium by ferric iron (e.g. as controlled by ferrihydrite dissolution) is a reaction that results in the production of energy that could be used for microbial growth. Table 5-2 contains reaction data for the oxidation of ammonium by ferrihydrite.

Table 5-2 : Thermodynamic equilibrium data for the overall reaction of ammonium and ferrihydrite. Values taken from (4).

Redox Couple Half Reactions	**log K**
$Fe(OH)_3 + e^- + 3H^+ = Fe^{2+} + 3H_2O$	15.8
$1/6NO_2^- + e^- + 4/3H^+ = 1/6NH_4^+ + 1/3H_2O$	15.1
$1/8NO_3^- + e^- + 5/4H^+ = 1/8NH_4^+ + 3/8H_2O$	14.9
For the oxidation of ammonium to nitrite by ferrihydrite:	**log K**
$Fe(OH)_3 + 1/6\ NH_4^+ + 5/3\ H^+ = Fe^{2+} + 1/6\ NO_2^- + 8/3\ H_2O$	0.7

Data pertinent to the calculation of the free energy change for the oxidation of ammonium by ferrihydrite is included in Table 5-3.

Table 5-3: Thermodynamic data for the calculation of the Gibbs free energy of formation for the reaction of ammonium and ferrihydrite. Values taken from (15).

Species	G_f (in kcal/mol) at 298.15 ^{0}K
$Fe(OH)_3$	-166.5
H^+	0
Fe^{2+}	-18.85
H_20	-56.687
NH_4^+	-18.97
NO_2^-	-7.7

The Gibbs free energy of reaction for the oxidation of ammonium by ferrihydrite was calculated to be –1.6 kcal/mol. The negative sign of the free energy of reaction indicates that energy is released during this reaction. Although this reaction does not result in a significant amount of energy production, it is important to note that the denitrification of the nitrate produced by this reaction results in the production of energy. Denitrification is a process by which microorganisms use nitrate/nitrite as the terminal electron acceptor in the oxidation of organic matter. Coupling the ferric oxidation of ammonium to denitrification of nitrate would result in an overall production of energy which could be used for microbial growth. As an example for the general denitrification reaction (equation 3) cited above, where glucose is oxidized using nitrate as the terminal electron acceptor, the Gibbs free energy of the reaction can be calculated using the data given in Table 5-4.

Table 5-4 : Thermodynamic data for the calculation of the Gibbs free energy of formation for the denitrification with glucose. Values taken from (8).

Species	G_f (in kcal/mol) at 298.15 ^{0}K
$C_6H_{12}0_6$	-217.6
NO_3^-	-26.43
CO_2	-94.25
H_20	-56.69
N_2	0

The calculated Gibbs free energy of this reaction is –582.32 kcal/mole.The energy released from this reaction could then be used for microbial growth.

Hydroxylamine as an Intermediary in the Production of N_2

Another possible mechanism for ferric oxidation of ammonium involves the production of hydroxylamine as an intermediate step. Table 5-5 lists the equilibrium constants for the oxidation of ammonium to nitrate using ferrihydrite as the oxidant.

Table 5-5 : Thermodynamic equilibrium data for the oxidation of ammonium to hydroxylaamine and the subsequent oxidation of hydroxylamine to nitrate. Values taken from (4) and (11).

Reaction 1:	log K
$NH_4^+ + H_2O = NH_3OH^+ + 2H^+ + 2e^-$	-45
$2[Fe(OH)_3 + e^- + 3H^+ = Fe^{2+} + 3H_2O]$	2(15.8)
$NH_3OH^+ + OH^- = NH_2OH + H_2O$	8.18
$NH_4^+ + 2Fe(OH)_3 + 3H^+ = 2Fe^{2+} + NH_2OH + 5H_2O$	-5.22

Table 5-5 continued: Thermodynamic equilibrium data for the oxidation of ammonium to hydroxylaamine and the subsequent oxidation of hydroxylamine to nitrate. Values taken from (4) and (11).

Reaction 2:	log K
$1/6\ NH_2OH + 1/3\ H_2O = 1/6\ NO_3^- + e^- + 7/6H^+$	-11.3
$Fe(OH)_3 + e^- + 3H^+ = Fe^{2+} + 3H_2O$	15.8
$1/6NH_2OH + Fe(OH)_3 + 11/6H^+ = 1/6NO_3^- + Fe^{2+} + 8/3H_2O$	4.5

From the information stated in Table 5-5 it is observed that for every molecule of hydroxylamine produced six electrons are available during hydroxylamine oxidation. Therefore although the initial oxidation of ammonium to hydroxylamine appears to be thermodynamically infeasible, when coupled with the oxidation of hydroxylamine the overall reaction would result in a positive equilibrium constant. This reaction would then be considered spontaneous and energy yielding.

Influence of Sulfur Redox Couple on Formation of N_2

Di-nitrogen gas can also be produced through the oxidation of hydrogen sulfide using nitrate as the terminal electron acceptor (**12**). The organsisms responsible for this conversion use this process to liberate energy for growth when oxygen is not available for the oxidation of sulfide. Reduction of nitrate results in the formation of di-nitrogen gas according to the reaction as given in Table 5-6. Again an initial input of nitrate is needed in order for this reaction to come to completion.

Table 5-6 :Thermodynamic equilibrium data for the oxidation of hydrogen sulfide by nitrate. Values taken from (4).

For the oxidation of hydrogen sulfide by nitrate:	log K
$1/5NO_3^- + e^- + 6/3H^+ = 1/10N_2 + 3/5H_2O$	21.1
$1/8H_2S + 1/2H_2O = 1/8SO_4^{2-} + e^- + 5/4H^+$	-5.2
$1/5NO_3^- + 1/8H_2S + 3/4H^+ = 1/10N_2 + 1/8SO_4^{2-} + 1/10H_2O$	15.9

Deposition of Oxidizing Agents into Lagoon Environments

Another possible input of nitrate or nitrite into lagoon environments is rainfall. Temporal trends seen across all six lagoons suggest that lagoon chemistry fluctuates with climate changes and weather events. It is possible that rain events could be significant sources of nitrate and oxygen. According to data from the Clinton Horticultural Research Station (available at http://www.ncdc.noaa.gov), rainfall for the period from July through Novemeber 2000, a 154 day period beginning on June 27th and ending on November 28th, totalled 45.52 cm with an average nitrate concentration of 1.9 mg NO_3^- L^{-1}. For Farm 10, one of the lagoons observed, a good estimate of lagoon surface area is approximately 26,700 m^2 (**9**). That would result in a total rain volume of 12,154 m^3, and a nitrate input of approximately 5.3-kg NO_3-N. This would suggest a significant input of nitrate, and considering that nitrate is undetectable in lagoon effluent its removal must occur. Assuming that denitrification results in the conversion of all this nitrate to di-nitrogen gas would result in the production of 5.3 kg N_2-N. Dividing this value by the surface area of the lagoon and the days constituting this rain period we can come to a flux estimate of 0.013 kg N_2-N ha^{-1} day^{-1}. This value seems hardly significant when compared to the reported average daily flux of 120 kg N_2-N ha^{-1} day^{-1} for Farm 10 during this same time period in 1998.

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Chapter 6

Conclusions

Swine lagoons are commonly used as an integral part of large-scale swine production in North Carolina. Recent research has documented the measurement of significant amounts of di-nitrogen gas (N_2) being emitted from the surface of swine lagoons in North Carolina (**2**). Nitrogen in lagoon environments is largely comprised of organic nitrogen and ammonium. The conversion of ammonium to di-nitrogen gas in anaerobic swine lagoons will require a change in the oxidation state of nitrogen [NH_4^+ with N(-III) to N_2 with N(0)] and thus must be coupled with other redox reactions which may be present.

Of the published mechanisms for the potential conversion of ammonium to di-nitrogen gas, all require the formation of either nitrite or nitrate before final conversion to di-nitrogen gas (**1,3,4,5**). Swine lagoons are known to emit methane and can be sources of hydrogen sulfide emissions as well. Thus there are at least two redox reactions already known to be active in swine lagoons. Our hypothesis was that there exists in swine lagoons at least one additional redox couple that can interact with ammonium to lead to the formation of nitrite or nitrate, and that the chemical species that compose this redox couple exist in sufficient amounts within anaerobic swine lagoons to result in the formation of significant (measurable) amounts of di-nitrogen gas.

In order to evaluate the redox processes active in the swine lagoons studied Eh, pH, and lagoon temperature measurements were taken in the field, and lagoon liquid samples were collected and analyzed for a variety of chemical constituents. These measurements were taken both as a function of depth and as a function of day of the year.

Statistical analysis of the collected data suggests that there could be some slight stratification within lagoon environments. However, it was decided that the magnitude of the differences observed do not necessitate treatment of the data according to depth within the lagoon. Observation of the data as a function of time suggests that there are temporal trends to the data which seem to occur across all the lagoons studied, suggesting that climate changes effect the chemical processes occuring within the lagoons.

Based on this attempt to identify the redox couple or couples responsible for the field measured redox potential, measured and calculated redox potentials suggest the existence of an iron (Fe^{3+}/Fe^{2+}) redox couple. Within the lagoon ferric iron (Fe^{3+}) represents an electron acceptor that could possibly drive the oxidation of ammonium to nitrate/nitrite, ultimately resulting in the production of di-nitrogen gas.

Thermodynamically, the oxidation of ammonium by ferric iron (e.g. as controlled by ferrihydrite dissolution) is a reaction that results in the production of energy that could be used for microbial growth. The oxidation of ammonium by ferric iron is proposed to occur according to the following reaction :

$$Fe(OH)_3 + 1/6\ NH_4^+ + 5/3\ H^+ = Fe^{2+} + 1/6\ NO_2^- + 8/3\ H_2O \qquad (1)$$

The calculated equilibrium constant for this reaction is $10^{0.7}$. The Gibbs free energy of reaction for this reaction was calculated to be –1.6 kcal/mol. The negative sign for the free energy of reaction indicates that energy is released during this reaction. Although this reaction does not result in a significant amount of energy production, it is important to note that the denitrification of the nitrite produced by this reaction results in the production of energy. Coupling the ferric oxidation of ammonium to denitrification of nitrate/nitrite would result in an overall production of energy which could be used for

microbial growth. Although there are no known organisms who use ferric iron for the oxidation of ammonium to nitrate/nitrite, the large number and biodiversity of species found in swine lagoons suggest that such a reaction is not implausible.

In order to definitively ascertain whether the iron redox couple is active and involved with the production of di-nitrogen gas in swine lagoons, further research would have to center around identifying and quantifying the ferrous and ferric iron species present in swine lagoon environments. In addition it would be necessary to identify an organism or organisms found in swine lagoons that use ferric iron as a terminal electron acceptor.

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