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AQUATIC FIELD AND LAB METHODS I

NORTHWEST CENTER FOR SUSTAINABLE RESOURCES (NCSR)
CHEMEKETA COMMUNITY COLLEGE, SALEM, OREGON
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Aquatic Field and Lab Methods I

The Northwest Center for Sustainable Resources is an Advanced Technological Education project funded by the National Science Foundation.

Aquatic Field and Laboratory Methods I (FISH 220) was developed at Grays Harbor College, Aberdeen, Washington, and was tested and revised at Mount Hood Community College, Gresham, Oregon. Materials were prepared by Don Samuelson, Lead Program Developer for NCSR. Samuelson holds a M.S. in General Science/Fisheries from Oregon State University, a B.A. in Chemistry/Biology from Pacific Lutheran University, and an A.S. in Marine Biology from Grays Harbor College.

Technology education programs in which this course is incorporated are described fully in the Center's report entitled, "Visions for Natural Resource Education and Ecosystem Science for the 21st Century." Copies are available free of charge.

Course materials are also posted on our website:
www.ncsr.org

Please feel free to comment or provide input.

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Table of Contents
Aquatic Field And Lab Methods I - FISH 220

Student Assessments

FISH 220 Course Outline

Aquatic Field & Lab Methods I

This course was developed to provide students with the analytical skills frequently used in fisheries, natural resources and environmental technologies. Through field and laboratory instruction in modern physical and chemical techniques and instrumentation, students will be able to collect, analyze and report water quality data with a high degree of precision and accuracy. Responsibility for maintaining high standards of analytical performance, quality control, and safety are major learning outcomes. A 6-7 week, team-driven, "real world" research project, conducted at a nearby watershed, allows students to apply all techniques, methods and skills learned in the first 5-6 weeks of the term.

Aquatic Field and Lab Methods I emphasizes chemical field and laboratory methods, while the follow-on course, *Aquatic Field and Lab Methods II* emphasizes biological field and laboratory methods.

TEXTS

Brassard, M. and D. Ritter. 1994. *The Memory Jogger II: A Pocket Guide of Tools for Continuous Improvement and Effective Planning*. First Edition. GOAL/QPC. 13 Branch St. Methuen, MA.

Cauros, M. 1994. *Environmental Sampling and Analysis for Technicians (ESAT).* CTC Press, Inc. Boca Raton, FL. 320 pp.

Mitchell, M.K., and W.B. Stapp. 1996. *Field Manual for Water Quality Monitoring: An Environmental Education Program for Schools*. Kendell/Hunt Publishing Co., Dubuque, Iowa. 77 pp.

Roberts, W. 1990. *Leadership Secrets of Atilla the Hun*. Warner Books, New York, N.Y.

Hermann, S. 1992. *Scientific Journal Keeping: The Grinnell System for Journal Writing*. The Evergreen State College Press, Olympia, WA.

COURSE DESCRIPTION

The primary goal of this field and laboratory course is to have students learn the techniques of collection, analysis and reporting of water quality data while gaining essential skills in the use of water quality instrumentation. Special emphasis is placed on:

- Precision and accuracy of analytical performance
- Adherence to the basic principles of the scientific method
- Proper use of glassware, instrument calibration, quality control and laboratory safety
- Incorporating a learning community model in this "capstone" course
- Encouraging the integration of ecological, economic, political and social disciplines essential to the resolution of critical water quality issues facing our watersheds
- Promoting self-confidence, responsibility, leadership, teamwork, and continuous quality improvement

COURSE OBJECTIVES

Upon completion of this course, students should be able to:

1. Use the tools of project planning, project management, teamwork, leadership and continuous quality improvement to plan and execute a "real world" water quality monitoring project, including: setting goals and objectives, scope of work, and timelines – using contemporary project planning software.

2. Demonstrate understanding and use of the scientific method in a field research study.

3. Demonstrate a high degree of skill, precision, and accuracy while performing the following water quality tests: barometric pressure, dissolved oxygen, fecal coliform, pH, B.O.D., temperature, total phosphate, nitrates, turbidity, total solids, conductivity, salinity, ammonia, hardness, alkalinity and discharge of water.

4. Work safely, both individually and in teams, in a supervised lab and field setting.

5. Express creativity through field notebooks and journals, seminars, and written and oral presentations of research reports.

6. Improve esteem and self-confidence while working in teams as leaders and followers.

STUDENT ASSESSMENT

Grades are based on a points system, with an approximate breakdown as follows:

SF-171 and State Forms	20 points
Resume and Letter of Application	40 points
Quiz I – Strong Inference	25 points
Quiz II – Lab Safety	25 points
Quiz III – Concentration of Solutions	25 points
MID-TERM Exam	200 points
Draft of Research Report	25 points
Final Research Report	75 points
Oral Presentations	50 points
Field Notebook/Journal	75 points
Lab/Field Participation	50 points
Peer Evaluation	50 points
Seminar Participation	100 points
Lab Field Skills	50 points
Instructor Evaluation	50 points
Total	840 points

TOPICS

I. Introduction to Chemical Field and Lab Methods
   A. Field Notebooks and Journals
   B. Biometrics
   C. Ecological Sampling Methods
   D. Ecological Research
   E. Seminars
   F. Learning Community Outcomes
   G. Research Reports and Oral Presentations
   H. Self Evaluations
   I. Portfolios

II. General Considerations in Sampling
   A. Objectives of a Sampling Program
   B. Preparation of Sampling Equipment
   C. Sample Preservation
   D. Field Quality Control

III. Field Tests and Sample Collection
   A. Calibration of Field Instruments
   B. Maintenance of Field Instruments
   C. The Most Common Field Tests, Instrumentation, and Calibration Procedures
   D. Surface Water Sampling

IV. Introduction to Quality Assurance and Quality Control (QA/QC); Sample Custody
   A. Quality Assurance Program
   B. Responsibilities of the QA/QC Technician
   C. Sample Custody
      1. Field custody
      2. Laboratory custody

V. Analytical Methods and References, Analytical System Calibration, and Performance Checks
   A. EPA Approved Methods and References for Analyzing Water Samples
   B. Calibration of Instruments
      1. Field instruments
      2. Laboratory instruments
   C. Standardization of Titrating Solutions
   D. Performance Checks of Laboratory Instruments

VI. Laboratory Safety

VII. Quality Management of Laboratory Instruments and Supplies; Quality Control Requirements
- A. Maintenance of Laboratory Instruments
- B. Quality Requirements for Laboratory Supplies
- C. Lab and Field Quality Control Checks
- D. Detection Limits
- E. Precision, Accuracy and Bias
- F. Quality Control Charts

VIII. Raw Data Conversion into Reportable Results; Approval of Analytical Data
- A. Responsibilities of the Analyst
- B. Calculations for Final Results
- C. Significant Figures
- D. Validation of the Analytical QC Checks

IX. Reporting Analytical Data; Introduction to Physical Properties
- A. Confidence Intervals
- B. Units Used to Express Analytical Results
- C. Report Format
- D. Solids, Salinity, Temperature and Turbidity

X. Introduction to Metals; Inorganic Non-Metallic Constituents
- A. Acidity, pH, Alkalinity and Hardness
- B. Nitrogen Compounds
- C. Dissolved Oxygen
- D. Phosphorous

XI. Organic Pollutants; Microbiological Parameters
- A. Biochemical Oxygen Demand (BOD)
- B. Coliform Bacteria Groups
    1. Total Coliform
    2. Fecal Coliform

XII. Regulations and Standards
- A. Introduction to Environmental Laws
- B. Drinking Water Standards
- C. Surface Waters
- D. Industrial Waters

XIII. Case Study: *The Rogue River Education Program*

XIV. Project Planning
- A. Team Building I
- B. Defining and Developing Scope-of-Work
  1. Mission
  2. Goals, Objectives, Tasks and Time Lines
  3. Prioritization
  4. Team Selection
- C. Continuous Quality Improvement
  1. Brainstorming
  2. Affinity/Tree Diagrams
  3. Flow/Fish Bone Diagrams
  4. Nominal Group Technique

XV. Project Management
- A. Team Building II
- B. Leadership and Supervision
- C. How to Conduct Meetings

XVI. Project Outcomes
- A. Research Report
- B. Oral Presentations
  1. General Audience
  2. Professional Audience
- C. Field Notebooks and Journals
- D. Student and Self-Evaluations
- E. Peer Evaluation
- F. Portfolios

Detailed Schedule

Week 1
Lecture: Introduction to Chemical Field and Laboratory Methods
Reading: Chapter 1 (pp 3-28) *Environmental Sampling and Analysis for Technicians* (ESAT)
Handouts: Federal Employment forms (SF-171), State Employment forms, Resume and Letter of Application Format
Assignment**:** SF171, State forms, 1st Draft Resumes and Letters of Application
Lecture: General Considerations In Sampling
Reading: Chapters 2 and 3 ESAT (pp 29-42)
LAB 1: Field Notebooks and Journals
LAB 2: Precision and Accuracy of Laboratory Balances
Lab Reading: The Grinnell System for Journal Keeping

Week 2
Lecture: Field Tests and Sample Collection
Seminar: Strong Inference and MWH
Reading: Chapter 4 and 5 ESAT (pp 79-90)
Lecture: Intro to Quality Assurance and Control; Sample Custody
Reading: Chapter 6 and 7 ESAT (pp 91-128)
Assignment: *Know location of Safety Equipment!*
Quiz I: Strong Inference and MWH
LAB 3: Reagent Preparation: Potassium Chloride for Portable Salinity, Conductivity, Temperature Meters; Calibration
LAB 4: Reagent Preparation; Winkler D.O. Test
Lab Reading: Chapter 1 and 2 WQM (pp 1-14)

Week 3
Lecture: Analytical Methods and References; Analytical System Calibration and Performance Checks
Reading: Chapters 8 and 9 ESAT (pp 139-162)
Seminar: Laboratory Safety
Assignment: Concentrations of Solutions
Lecture: Quality Management of Lab Instruments; Analytical System Calibration and performance Checks
Reading: Chapters 10 and 11 ESAT (pp 162-190)
Problem Solving: Concentrations of Solutions
LAB 5: Calibration and Use of the Handheld Thermometer and Mercurial Barometer; the Winkler Titration for Dissolved Oxygen; Precision and Accuracy
LAB 6: Calibration and Use of the YSI and Orion Portable Dissolved Oxygen Meters; Precision and Accuracy; Maintenance and Troubleshooting

Week 4
Lecture: Raw Data Conversion into Reportable Results; Evaluation and Approval of Analytical Data
Reading: Chapters 12 and 13 ESAT (pp 191-204)
Assignment Due: Concentration of Solutions Problem
Quiz II: Laboratory Safety (25 pts)
Lecture: Reporting Analytical Data; Introduction to Physical Properties
Reading: Chapters 14 and 15 ESAT (pp 205-232)
LAB 7: Biochemical Oxygen Demand (BOD) and Fecal Coliform Tests
LAB 8: pH, Alkalinity and Hardness

Week 5
Lecture: Introduction to Physical Properties; Inorganic Chemical Properties
Reading: Chapters 16 and 17 ESAT (pp 233-262)
Quiz III: Concentrations of Solutions
Lecture: Organic and Microbiological Pollutants
Reading: Chapter 18 ESAT (pp 263-300)
MID-TERM
LAB 9: Ammonia, Nitrates and Phosphates
LAB 10: Turbidity; Suspended and Dissolved Solids

Week 6
Lecture: Regulations and Standards
Reading: WQM pp 155-174; Read 1st half of *Leadership Secrets of Atilla the Hun*
Handouts: Project Planning; Project Management, Team Building I
LAB 11: Low ROPES/Challenge Field Exercise @ the Soccer Field
LAB 12: Ecosystem Study

Week 7
Lecture: Team Building I
Class Activities: Project Planning; Mission Statement Goals and Objectives; Tasks and Time Lines; Team Selection
Lecture: What are Skill Standards?
Lecture and Software Demonstration: Project Planning (PERT and Gannt)
LAB 13: Project Planning; Water Quality Research Study: Brainstorming and Tree Diagram; Developing Tasks and Time Lines; Prioritization; Assignment of Individual and Team Responsibilities
LAB 14: Project Planning; Prioritization of Field Study Activities (Lake Swano Watershed – this is Grays Harbor's model watershed that students use)

Week 8
Lecture: Continuous Quality Improvement Tools; Team Building II
Class Activity: Flow Diagrams, Fish Bone Diagram, Run Charts
Reading: 2nd half of *Leadership Secrets of Atilla the Hun*
Lecture: Continuous Quality Improvement
Lecture and Class Activity: Use CQI and Project Planning Tool to Plan Research Report and Oral Presentation to General and Professional Audiences
Assignment: Plans for Specific Projects
LAB 15: Project Management; Leadership and Supervision (Seminar); *Leadership Secrets of Atilla the Hun* (1st half)
LAB 16: Field Study Activities (Lake Swano Watershed, local watershed)

Week 9
Lecture: CQI Plan
Demonstration: AV Equipment and Materials Available for Presentations
Lecture and Demonstration: Format for Final Research Report
LAB 17: Teamwork and Continuous Quality Improvement; Seminar: *Leadership Secrets of Atilla the Hun* (2nd half); Work on Draft of Research Report
LAB 18: Field Study Activities (Lake Swano Watershed)

Week 10
Class Activity: Rehearsal for Student Presentation to General Audience
LAB 19: Field Study, Work on Final Research Report; Prepare for Oral Presentations
LAB 20: Field Study; Work on Final Research Report; Prepare for Oral Presentation; Student Demonstration of Water Quality Testing Skills

Week 11
Class Activity: Rehearsal for Student Presentation to Professional Audience
All Assignments Due
LAB 21: Complete Final Research Report; Oral Presentation to General Audience
LAB 22: Final Research Report Due; Oral Presentation to Professional Audience

LABORATORIES AND ACTIVITIES

Field Notebooks and Journals
Precision and Accuracy of Laboratory Balances
Reagent Preparation for Calibration and Use of Meters
Dissolved Oxygen
Gas Supersaturation
Fecal Coliform Testing
Biological Oxygen Demand
pH, Alkalinity and Hardness
Nitrogen and Phosphorus: Ammonia, Nitrates, Phosphates
Turbidity and Total Solids
ROPES Challenge Course

NOTES FOR INSTRUCTORS

Field Sites

Field sites include local sites on campus (Grays Harbor College's local site is the *Lake Swano Watershed*). Instructors should be resourceful in selecting their field locations.

Methods of Evaluation

I. Traditional

- < Lecture Quizzes
- < Mid-Term Exam
- < Practical Written and Oral Laboratory Exams
- < Individual Research Report
- < Individual Oral Presentations to Class
- < Final Exam

II. Non-Traditional (Learning Community Outcomes)

- < Field Notebooks and Journals
- < Written Self Evaluations
- < Written Evaluation of Student by Instructor
- < Resume and Letter of Application
- < Peer Review (teamwork, leadership, seminaring skills, levels of competency, performing water quality tests, written research report and oral presentation)
- < Team Research Reports
- < Team Oral Presentations (to general and professional audiences)
- < Five-minute videos, posters, or slide/talks of assigned water quality tests (includes a written script)
- < Project Plan (goals, objectives, scope-of-work, tasks and time lines)
- < A Portfolio (includes the above outcomes)

Additional Instructional Materials, Equipment, Software, and References

I. AV Equipment (available to students)

- < Overhead projectors, overhead projections and copy machines
- < 35 mm camera; color print and slide film
- < Slide projectors; power point projectors
- < Camcorders and tapes, poster boards

II. Hardware and Software

- < Connection to the Internet
- < Statistical Software (Statistix)
- < Project Planning Software (Microsoft)
- < Spreadsheet and Desktop Publishing Software (Excel, Corel Draw)
- < Computer Assisted Library Search (BIOSIS)

III. Additional References

- < American Public Health Association (APHA), American Water Works Association (AWWA), and the Water Pollution Control Federation (WPCF), 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th Ed.
- < Scham's Outline Series, *Theory and Problems of College Chemistry* (latest edition) Scham Publishing Co., New York.
- < U.S. Environmental Protection Agency, 1974. *Methods for Chemical Analysis of Water and Wastewater.*

Field Notebooks and Journals

INTRODUCTION

As natural resources, fisheries and/or GIS technicians, one of the most important tools you will need to possess will be to observe and record what goes on around you in the natural world. **Field notes** and **journals** are an important part of developing and improving your observation and data-collection skills over time, and you will be required to keep both a field notebook and journal this entire quarter. Observations, insights, and key information you learn in this class will not only help you in this class, but will provide an excellent record for you to refer back to whenever needed. You will develop record-keeping skills by keeping field notes and journals on a daily basis. Periodically, both your field notebooks and journals will be turned in to be reviewed by the instructor or his/her assistant.

Use your text, "*The Naturalist's Field Journal,*" as your guide for both format and content for your field notebooks and journals (see course outline—text and references).

FIELD NOTEBOOKS

The field notebook is usually a *Rite-In-The-Rain* type (either the 3.5" x 5" size, or the spiral bound). Both are designed to fit in your shirt or back pocket. Both types can be purchased for about $3-4 at the college bookstore. You may fill up several notebooks during one quarter! Always write in your notebooks using a #2 pencil or black waterproof ink. Most inks will "run-in-the-rain." Remember too, that field notebooks **always** go with you into the field.

Use your "Rite-in-the-Rain" field notebooks to record your observations and measurements (when field data forms are not provided). Also record questions and insights and make diagrams and sketches whenever possible. At the end of the day, you should always transcribe your field notes into your journal.

Items in your field notebooks should describe: *who?, what?, when?, where?, why?* and *how?*

1. Front Covers:

Include the following information on the front cover of your field notebooks:

- course number and course name
- a note that says, "If found, please return to: (your name, address and phone #)"

2. Inside the Front Cover:

Include the following checklist inside the front cover to serve as a reminder during the quarter:

- date/time
- weather observations
- locations, landmarks
- routes to and from your destinations
- field notes from instructors or guest speakers (in the field)
- mileage to and from destinations
- plants, animals and habitats observed
- questions and/or insights
- *qualitative observations* based upon your senses of sight, smell, hearing, touch and taste
- *quantitative measurements* (when field data sheets are not provided)
- animal signs (tracks, scat, calls, etc.)
- maps, drawings and sketches
- who was with you on your data-collection trip?

NATURALIST'S JOURNAL

You will transcribe notes taken in the field into your journal. Unlike your field notebooks, your journal *does not* go into the field.

Your naturalist's journal consists of three major sections: The Journal, Species Account and Diary.

1. The Journal

This first section is much like your field notebook. Much of the information you recorded that same day in the field can be transcribed to the Journal section of your journal. *Avoid* personal feelings, emotions, and comments of a non-scientific nature in this section (save these for your Diary). Journal entries are a bit more formal than field notebook entries (pay more attention to complete sentences, grammar and punctuation). Journal entries can serve as *legal evidence* and are often bound and placed in library collections.

Follow the examples in Herman's text for both format and content, and you can't go wrong. It goes into great detail as to the specific format and style of the field journal. You will be required to learn and use this style. Consult Herman's text whenever you are in doubt. His book is based upon the *Grinnell System of Journal Writing*, which is a very disciplined type of technical writing.

2. *Species Account*

The first time you see a particular species of a plant or animal, it should be noted in your field notebook. *Sketch it right on the spot!* Describe the habitat you found it living in. What other species of plant and animals were also living in this habitat? Describe what life stage or season of the year you observed the plant or animal.

As you transcribe this new species to your Species Account section of your journal, describe in full detail what you observed the first time you noted it. This may take more than one page. You may have to look up some scientific information or even key out the species the first time you record it. At a minimum, you should include:

- common name
- scientific name (genus and species)
- other taxonomic classifications (phylum, class, order, and family)
- date/time of observation
- location
- habitat
- season of the year
- other plants and animals nearby
- ecological relationships (interactions with other members of the observed ecosystem)
- physical, chemical, and biological characteristics (size, color, smell, sex, life stage, texture, etc.)
- sketch of what you observed

With the second and subsequent sightings, you only have to make general observations such as date, time, location—place these entries directly below your previous entries for this species. Draw additional sketches if the appearance is different than previous observations (i.e., perhaps this eagle is an adult compared to the immature one you saw the first time; or you now observe a salmonberry [*Rubus spectabilis*] in winter—without leaves—as compared to the first observation in the spring of the year).

3. *Diary*

The Diary section of your journal is where you can be the most creative. There are almost no restrictions on what you can write about in this section. Many students like to express their emotions and feelings about what they saw in the field that day, or how the class itself is going for them (good or bad). It provides a chance to "vent some steam" if necessary or "go nuts over something that really turned you on" that day. Remember that your diary entries will *be kept strictly confidential*. No one but your instructor and assistant will ever see what you write here. We often make comments in this section that either compliment or disagree with your comments and/or thoughts. Please keep our comments confidential too!

NOTE: *Try to write **equally** in all three (3) sections every day you write in your journal.*

PROCEDURE

For this first exercise, you will have a chance to use all of your senses to observe a "bit of nature" around you.

A. Field Scenario

You will choose a small portion (a few square meters) of the natural environment around you to observe for this exercise. This involves sitting quietly in your chosen spot and recording in your field notebooks all the required information (see the checklist you've put inside the front cover of your notebook), plus *everything* else you see, hear, smell, touch, or taste within your "private space." Pay special attention to the living things you observe: Where are they?, what are they doing?, what sounds do they make?, what do they look like? (provide a sketch), how big are they? (measure them), what color are they? (NOTE: *You should be equipped with a metric ruler and a hand lens*). Also note the physical and chemical environment: air temperature, weather, clouds, soil type, water conditions, elevation, etc.

Be sure to observe, both close in and far away from your chosen location, for at least 90 minutes. Record as much as you can during this time. (NOTE: *This field notebook scenario was the "brainchild" of Ms. Judy Moore, Biology Instructor, Yakima Valley Community College, Yakima, WA. It is included with her permission*).

B. Naturalist's Field Journal Exercise

The same day (or night) after you make your initial field observations in your field notebooks, take some time to transcribe your records *equally* in the three (3) sections of your journals. There should be plenty of information to fill a number of pages in each section. Be sure to consult Steve Herman's text for *format* and *content*. You may also have to consult taxonomic keys for animals and plants when you do your species accounts. (NOTE: *Sketches should be your own, not a photocopy of a picture from a book.)*

Remember that in the Diary section of your journal, it's your chance to reflect on how this nature observation exercise related to you as a person. *How did you feel as you were sitting quietly observing and writing? Does this bring up any memories of your past? How do you, or humans in general, fit into or relate to nature today?* This section of your journal will feel very much like you are writing an English paper—you have the freedom to explore and expound upon your feelings, emotions, opinions and put them into writing, very unlike most scientific or technical writing you will do. And it's OK to take a few notes in your field notebook to jog your memory later when you write them in more detail in your diary.

LAB PRODUCTS

Turn in your field notebooks and journals.

Precision and Accuracy of Laboratory Balances

INTRODUCTION

The term **balance** refers to any instrumentation or apparatus that is used to weigh substances in a laboratory. **Gravimetric analysis** is the process of weighing various substances or items with **precision** and **accuracy**, sometimes at an accuracy level of 0.0001 grams.

Many measurements and tests performed in a laboratory utilize the concept of **weight** in their calculation. Every chemical has a specific **atomic weight**, listed on the label of the container, which is utilized in making solutions.

The metric system is used to weigh substances or items in a laboratory. Generally, measurements are recorded in grams (g) or milligrams (mg), and must be converted if they are different. The degree of accuracy is usually one unit less than it actually reads on the balance.

PROCEDURE

Materials & Equipment

Balances
Triple beam balance
Sartorius electronic pan balance (top loading)
Mettler analytical balance

Materials
Weighing paper
Safety goggles
Weighing boats
Metal pans
A metric weight

Objects to weigh
125 ml. glass beaker
Paper clip
Rubber stopper

Getting Started

The weight of the container used to weigh something (i.e., weigh paper, weighing boat, or pan) must be removed from the final measurement by **taring** the balance (tare = container's weight). It is possible to "zero" a balance with the weight of a container already on it; thus, whatever is added for purposes of weighing is the actual reading taken off the instrument. Often it is recommended to perform several weighings and average the results. Any readings that are drastically different from the others are discarded, if the reason for the difference cannot be explained.

When you begin your exercise, the balance should be clean, with all weights at zero and knobs in the "lock" or "off" position. This protects the sharpness of the knife edge used in precise weight measurements. If it is not clean or "arrested to zero," note the condition in your lab notes, and proceed to clean the balance, and re-check the zero setting.

NOTE: *Never blow on the pan of an analytical balance to remove chemicals spilled or left in the pan. Moisture is added, changing the overall weight, and moisture can be introduced into the balance itself. A camel-hair paint brush should be used instead.*

Although no particular preparation is necessary for weighing objects (a paper clip, stopper, and beaker), it should be noted that there is moisture clinging to the surfaces of the items (and most others), which changes the true weight. The true weight can be obtained by placing the items in a drying oven at 200°F for several hours, and then moving items to a dessicator, which allows temperature equilibration within the container. Weighing at that time provides true (dry) weight.

NOTE: *Do not handle any of the items with fingers, as the oils present also change the weight. Tongs should be used instead.*

Taking Weight Measurements

Working in groups of three or four, for each item, weigh the item 3 times on each type of balance, and average the weights obtained (thus, with 3 objects to weigh, and 3 different balances; 3 X 3 X 3 = 27 separate measurements will be recorded; 3 average weights will be recorded for 3 items per each balance). You will then determine the standard deviation of your sample weights to examine the precision of that method, and determine the accuracy of each method by calculating standard weights.

Remember:

- Be sure to set all weights back to zero after each weighing.
- Never weigh a warm object.
- Always use weigh paper or the boat – never place chemicals directly on the bare pan.
- Always handle the balance gently and carefully.
- Try to place the object to be weighed in the center of the pan.
- Weighing your item is not difficult, but requires attention to detail.
- It is important to read and record to the *correct decimal point!*
- Make sure the balance is level, free of vibration and located in a draft-free location.

USE OF BALANCES

A. Triple beam balance

A common laboratory balance used by beginning chemistry students, this balance is used to weigh objects to the nearest 0.01g. When using this balance, always use weighing paper or a weigh boat – never put anything directly in the pan. Clean up any spills, and always leave the balance on "arrest" or "set" so that it is not swinging freely on the knife-edge (this can dull the edge and ruin the accuracy).

Using the triple beam balance:

1. Before placing an object on the balance, using the leveling screws, level it and check that the position of the bubble is in the middle of the circle. Adjust as necessary.

2. Determine the "rest" or "zero" point of the balance by setting all the weights of the unloaded balance to zero while allowing the beam to swing freely. Make sure the weights are firmly seated.

3. Release the beam by turning the beam arrest knob ½ turn to the right. As the beam swings, the pointer will move up or down on the pointer scale. As it swings, count the number of divisions (above the zero) that it moves up and the number of divisions (below the zero mark) that it moves down. The sum of the two counts will give the position at which the pointer would come to rest (the rest point). Make two or three measures of the rest point to be sure. The rest point is used for reference in the weighing operation.

4. Clean the balance if necessary with a soft brush or clean wipes.

5. If the balance is not zeroed, add or subtract the adjustment weights and repeat step 3 until balanced.

6. Place the object to be weighed on the pan of the balance (remember to use a weigh boat if it is a chemical). The beam will move down to the left. Move the 10 g weight until the beam moves down to the right. Then move the 10 g weight backwards one notch. Repeat with the 1 gram weight. Once the proper settings of these weights have been found, the sliding 0.01 g weight is moved to the right to a position in which the beam swings freely. At this time, the motion of the pointer on the pointer scale is noted to see if it corresponds to the rest point. If it does not, the sliding mass is moved slightly to the right or left until the motion of the pointer is such that the original rest point is obtained upon addition of the pointer scale divisions.

7. At this point, the mass should be carefully read and recorded. It is always a good idea to double-check the reading of the mass. When a sample of a substance is weighed on a piece of paper or in a container, the mass of the paper or empty container is first determined. Then the substance is placed on the paper or in the container and the total mass is determined. The mass of the substance is then found by subtracting the mass of the paper or container from the total mass.

B. Pan balance

This balance is often used in the lab to weigh heavier or bulkier objects/amounts. Although it reads to the nearest hundredth (0.01g) it may only be accurate to the nearest tenth (0.1g). It is very easy to tare.

Using the pan balance:

1. Turn the balance on and release the pan with the "arrest" knob. Make sure it reads zero on the digital readout. Adjust with zero knob, if necessary.

2. Place the object to be weighed on the center of the pan.

3. If you are weighing something in a container, the weight of the container can be easily tared by using the zero adjust knob. You are now only going to read the weight of whatever is added to the container.

4. Note and record the digital reading.

5. Repeat the procedure to complete the lab requirements.

C. Analytical balance

This balance is one of the most precise and accurate, and it's perhaps the trickiest to use. *It will measure down to 0.0001g!* On many models, the most you can weigh using this type of balance is 100 grams. It is used for measuring minute amounts of chemicals and generally is reserved for dry weights (not liquids). It requires a steady hand when adding chemical to a weigh paper to avoid spills or adding too much of whatever you are trying to measure out – *and small amounts can add up fast!* You must work in a confined space where the pan of the balance is protected by glass. In our lab, there are doors on the right and left for access by your hand and the spatula or spoon.

Using the analytical balance:

1. Make certain the arrestment knob on the left of the balance is in position I. (Refer to the arrestment knob positions diagram on the balance). The knob will be in the vertical position and there will be no light in the scale window. The beam is arrested.

2. Turn all the weight knob controls (knobs 2-6) to zero. All index lines on these knobs will be in the vertical position and the scale reading will be "00."

3. Making use of the leveling feet on the front and back of the balance, adjust until the balance is level (by noting the position of the bubble in the circle).

4. Clean the pan and the balance if necessary, using a camel hair brush or Kimwipe. Close the windows to prevent air currents.

5. Release the beam completely by gently turning the arrestment knob counterclockwise about one-third turn. A brake will stop the knob at this position.

IMPORTANT NOTE: *Do not force the knob at any time!*

6. Using the zero adjustment knob on the right side of the balance, adjust the "0" line on the vernier to coincide exactly with the "00" line on the main scale in the scale window. Be certain to look directly in front of the scale to prevent parallax error.

7. The balance is now zeroed. Gently return the arrestment knob back one-third turn to the vertical position. The light will go out on the main scale.

8. Open the side window, placing a weigh paper or other type of weighing receptacle in the center of the pan.

9. Close the window and turn the arrestment knob one-third turn clock-wise to partial beam release. A brake will stop the knob. The balance is now semi-released and the weight knobs can be adjusted. Turn the 10g weight knob clockwise one position at a time until the main scale goes up. Then turn the knob back one position.

10. Repeat with the 1g knob until the main scale moves up, then turn back one position. Repeat with 0.1g knob.

11. Finally, completely release the beam by *gently* turning the arrestment knob 1 clockwise turn. Allow about ten seconds for the scale to reach equilibrium and record the mass to the nearest tenth of a milligram.

12. Arrest the beam by *gently* returning the arrestment knob to the vertical, arrested position. The light in the main scale will go out.

13. Open the side window, place the chemical on the weighing paper or in the receptacle, and repeat procedure.

14. Open the side window, remove the materials from the pan, close the window, adjust the weight knobs to zero, and clean the area.

PRODUCTS & CLEANUP

- Turn in data sheets and definitions (one set per team).
- Clean up all equipment and lab space.
- Turn off instrumentation.
- Put all equipment and supplies away in the proper place.
- Write a short team report and turn it in at the beginning of the next lab period; be sure to include the following in your write-up:
    - Difference between precision and accuracy
    - Advantages/disadvantages of each weighing method

LABORATORY BALANCES
DATA SHEET

Date	Name	Item Weighed	Trial #	Tr. Beam balance(g)	Pan balance(g)	Analytical balance(g)	Comments
		125 ml Beaker	#1				
			#2				
			#3				
			Avg. Wt.				
			Std. Dev.				
		Paper Clip	#1				
			#2				
			#3				
			Avg. Wt.				
			Std. Dev.				
		Rubber Stopper	#1				
			#2				
			#3				
			Avg. Wt.				
			Std. Dev.				

DEFINITIONS

Define the following terms:

Tare

Gravimetric Analysis

Precision

Accuracy

Equilibrium

Balance

Dessicator

NOTES FOR INSTRUCTORS

Learning Objectives: The student understands the terms precision and accuracy and how these terms apply to the use of laboratory balances.

Learner Outcomes: The student will demonstrate the ability to correctly use three (3) types of laboratory balances: the triple beam, pan and analytical balances, and be able to choose the appropriate balance for each of a variety of weighing applications.

More on taring the balance

Methods:

- If you have not tared the container on the balance, the weight of the container must be taken first and then subtracted from the total weight to get the weight of the item.

- If you are weighing out potassium chloride (KCl) in a weigh boat, and you need 1.537 grams of it, you have *two options:*

Weigh the container, record your answer, and add 1.537. The number you get is the total grams you should see on the digital read out after you *carefully* add the 1.537g. KCl to your container with a spatula or spoon.

-or-

Use the zero knob on the balance (and procedure for zeroing that balance) to re-zero the balance with the container already on the pan. Then when you add the 1.537 grams, your digital readout should read exactly 1.537 grams.

Reagent Preparation for Calibration and Use of Meters

INTRODUCTION

Salinity, **conductivity** and **temperature measurements** are made in sea water, brackish waters (estuaries), and fresh water as part of routine water quality sampling. Conductivity is especially important to measure in fresh waters, as part of the electroshocking process, to choose the correct current and voltage settings on the electroshocker (different current and voltage intensities are required for different sizes and species of fish). This laboratory exercise details reagent preparation (potassium chloride) for calibration and use of salinity, conductivity and temperature (S-C-T) meters (Yellow Springs Instruments, Model 33, S-C-T Meter).

Conductivity (in µmhos/cm) is the measurement of electrical conductance a water sample would exhibit if measured between opposite faces of a 1.0-cm cube. **Salinity** is the number of grams of salt/kilogram of a sample ($^{o}/_{oo}$ = parts per thousand). This measurement assumes the sample contains a "standard" sea water salt mixture. The sample **temperature** is measured in degrees Celsius.

PROCEDURE

Setup

A. Adjust meter to zero (if necessary) by turning the bakelite screw on the meter face so that the meter needle coincides with the zero on the conductivity (µmhos/cm) scale.

B. Calibrate the meter by turning the switch to the redline by adjusting the meter needle with the redline control to the red line on the scale. If this cannot be accomplished, replace the batteries.

C. Plug the probe into the probe jack on the side of the instrument.

D. Put the probe in the solution to be measured.

I. Temperature

A. Set the switch to TEMPERATURE.

B. Place the probe in solution, and after allowing time for the probe temperature to come to equilibrium, read the temperature on the bottom scale of the meter in degrees Celsius.

II. Salinity

A. Switch the instrument to the SALINITY position and read salinity on the red 0-40 $^{o}/_{oo}$ meter range.

B. Depress the CELL TEST button. The meter reading should fall less than 2%. If greater, the probe is fouled and the measurement is in error. Clean the probe and re-measure.

III. Conductance

A. Switch the meter to the X 100 µmhos/cm range. If the reading is below 50 on the 0-5000 meter scale, switch to the next lower range (X 10 µmhos/cm). If the reading is still below 50, switch to the next lower range (X 1 µmhos/cm). Read the meter scale and multiply that reading by the range (X 100, etc.). The answer is the reading in µmhos/cm (measurements are not temperature compensated).

NOTE: Remember to always start on the ***least sensitive scale*** *(X 100), working progressively down to the most sensitive scale (X 1). This prevents damage to the instrument.*

B. When measuring in the X 100 and X 10 ranges, depress the CELL TEST button. The meter reading should fall less than 2%. If greater, the probe is fouled and the measurement is in error. Clean the probe and re-measure.

NOTE: *The cell test does not function on the X 1 range.*

Calibrating/Sampling Protocol

Students should read the instruction manual for the YSI - Model 33 S-C-T Meter, and practice using the meter at four sampling sites which represent fresh, brackish, and sea water samples. Students should work in teams of 2-3.

1. Each team will prepare the 0.01 Normal solution of KCl using the analytical grade reagents and deionized water provided. Obtain a temperature and conductivity reading for your standard solutions and deionized water used to make the standard solution. Using the Temperature/Conductivity Table and Cell Constant formula (from the Cell Calibration/ Standard Solution section above), calculate the cell constant (K) for your instrument and probe. Once you start to collect conductivity readings in the field, you will need to multiply your values by K (the cell constant). Be sure to use the same meter and probe each time you go out in the field (write the serial numbers of your probe and meter in your field notebooks and field data sheets).

2. Each team of students will take triplicate samples for conductivity, salinity and temperature at each sampling site.

3. Record your data on your field data sheet. Make qualitative observations both in your field notebook and in the comments section of your data sheets.

4. When you have completed your measurements, be sure to turn off your instrument, rinse the S-C-T probe in distilled water, dry off the instrument and probe, and stow it away in the designated storage cabinet.

PRODUCTS

Write up a one-page report that discusses the protocol and precautions for measuring conductivity in fresh, brackish and sea water. Include your data sheet as an enclosure to this report. Be ready to discuss the results you obtained.

Salinity-Conductivity-Temperature
Laboratory/Field Data Sheet

Samplers Names: _____

Conductivity from 0.01 N KCl Standard Solution = _____ µ mhos/cm
Conductivity of deionized water used to make standard KCl solution = _____ µmhos/cm
Temperature of Standard Solution = _____ °C
Cell Constant _____ on _____ (date) Serial # (instrument) _____; Serial # (probe) _____

Date/Time	Location	Temperature (°C)	Conductivity (µmhos/cm)	Salinity (0/00)
	1. Location #1	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____
	2. Location #2	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____
	3. Location #3	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____
	4. Location #4	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____	1. _____ 2. _____ 3. _____
Comments:				

NOTES FOR INSTRUCTORS

The Probe

Description:

The YSI 3300 Series Conductivity Probes are designed for field use, embodying construction and design for rugged, accurate service. Each probe features a built-in cell constant of 5.00 ± 0.1, a precision YSI temperature sensor of ± 0.1°C accuracy at 0°C, and ± 0.3°C at 40°C; and a low capacitance cable assembly terminating in a three terminal 0.25" diameter phone type connector. The Model 3310 probe has a 10 ft. cable and the Model 3311 has a 50 ft. version. Other lengths are available on special order. The probe has a rigid PVC body with platinized, pure nickel electrodes and a cable providing resistance to a wide range of water-borne substances.

Refer to S-C-T Meters instruction booklet, *Yellow Springs Instruments*, Model 33 S-C-T Meter.

Maintenance:

The only maintenance required is battery replacement, using two "D" size alkaline flashlight cells. Accuracy will not be maintained if zinc-carbon "D" cells are used. Battery replacement is indicated when the redline adjustment cannot be accomplished. Replace batteries every six months to reduce the danger of corrosion due to leaky batteries. To replace batteries, remove the six screws from the rear plate. The battery holders are color coded. The Positive (+ button) end must be on red.

Calibration:

It is possible for the temperature knob to become loose or slip from its normal position. In an emergency, the dial can be re-positioned. *To emphasize* – this is an emergency procedure only, and when this occurs, the instrument should be returned to the factory for proper re-calibration at the earliest opportunity.

Cleaning:

When the cell test indicates low readings, the probable cause is dirty electrodes – from hard water deposits, oils, organic matter, etc. For normal cleaning, soak the electrodes for 5 minutes in a bathroom tile cleaning preparation. For stronger cleaning, soak for 5 minutes in a solution made of 10 parts distilled water, 10 parts isopropyl alcohol and 1 part HCl.

NOTE: *Always rinse the probe after cleaning and before storage.* CAUTION!: *Do not touch electrodes inside the probe. Platinum black is soft and can be scraped off.*

Probe use

Obstructions near the probe can disturb readings. At least two inches of clearance must be allowed from non-metallic underwater objects. Metallic objects such as weights should be kept at least 6 inches from the probe.

Weights are attached to the cable of the YSI 3310 and 3311 Probes. The YSI 3327 weights are supplied in pairs with a total weight of 4 oz per pair. Should it become necessary to add more weight to overcome water currents, we suggest limiting the total weight to two pounds (8 pairs). For weights in excess of two pounds, use an independent suspension cable. In either case, weights must be kept at least 6 inches away from the probe.

Gentle agitation by raising and lowering the probe several times during a measurement ensures flow of sample solution through the probe and improves the time response of the temperature sensor.

Cell Calibration and Standard Solutions

The YSI 3300 Series Cells are calibrated to absolute accuracy of 1.5% based on a standard solution. Since the literature on conductivity does not indicate a consistently accepted standardization method, we have chosen the 0.01 normal KCl solution method as our standard (as determined by Jonas and Bradshaw in 1937). Recent textbooks, as well as the ASTM standards, concur with this choice.

The solution is prepared by diluting 0.745 grams of pure dry KCl with distilled water until the solution fills to 1.0 liter. The table below shows the values of conductivity this solution would have if the distilled water were non-conductive.

NOTE: *Since even high purity distilled water is slightly conductive, the measured conductivity will be higher by an amount equal to the water's conductivity.*

Temperature (°C)	Conductivity (Absolute Micromhos/cm^3)
15	1141.5
16	1167.5
17	1193.6
18	1219.9
19	1246.4
20	1273.0
21	1299.7
22	1326.6
23	1352.6
24	1380.8
25	1408.1
26	1436.5
27	1463.2
28	1490.9
29	1518.7
30	1548.7

The operator may use the standard solution and the table to check accuracy of a cell's constant or to determine an unknown constant. The formula is shown below:

$$K = \frac{R(C_1 + C_2)}{10^6}$$

where: K = Cell constant
R = Measured resistance in ohms
C_1 = Conductivity in absolute micro-ohms
C_2 = Conductivity in absolute micro-ohms of the distilled water used in making solution

R, C_1 and C_2 must either be determined at the same temperature or corrected to the same temperature to make the equation valid.

NOTE: For further information on conductivity and the above standard information, refer to ASTM Standards Part 23 (*Standard Methods of Tests for Electrical Conductivity*), or *Water and Industrial Waste Water* ASTM Designation D1125-64.

Dissolved Oxygen

INTRODUCTION

Dissolved oxygen (DO) is the amount of oxygen present in a natural water body or wastewater stream. The level of DO will depend on physical, chemical and biological activity present in the water. It is indicative of the overall health of the water body and influences the potential for living organisms, including plants and animals, to exist and thrive. Water that is high in oxygen supports numerous aerobic species, while water that is low in oxygen limits the number and quality of species that can survive. Anaerobic species can survive with little or no oxygen. DO is important, obviously, because aerobic organisms need oxygen in specific quantities to conduct cellular respiration.

Sunlight powers the ultimate source of oxygen in our atmosphere and water by driving photosynthetic processes of green plants and other chlorophyll-bearing organisms. Water contains dissolved oxygen, which comes from both the atmosphere (particularly through wave and current action), and from plants within the water (aquatic plants).

NOTE: *The chemical reaction of photosynthesis takes place during daylight (sunlight) hours, with oxygen given off as an end product. Consequently, the level of dissolved oxygen in water is highest at the end of the day and lowest just before dawn. This is an important point to remember, particularly when setting up sampling plans.*

This lab demonstrates three methods of calculating dissolved oxygen:

1. *Titration method:* the **Winkler Titration** method.

2. *Meter method:* Using a meter with a **membrane electrode.**

3. *Kit titration method:* The **LaMotte** or **HACH kit**, which is a "modularized" titration method often used in the field.

While the Winkler, or iodometric, method provides a higher degree of precision and accuracy than the field kits, one should obtain similar results for any given sample using any of these three methods.

APPLICATION

DO is a standard water quality test to determine the relative level of health (or level of pollution) in a given water body. Water quality is defined by standards for specific uses or criteria. For example, the standard for water for swimming is different than that for drinking water, which is different for that needed to grow oysters. Additionally, cold-water fish need more oxygen than warm water fish. For local information and applications, research your state's Water Quality Standards.

Low dissolved oxygen usually is caused by an excess of organic waste, such as sewage components. During organic breakdown of this waste, bacteria and other organisms undergo respiration, which utilizes oxygen, resulting in decreased dissolved oxygen levels. Thus, untreated or raw sewage in streams, lakes and estuaries results in imbalanced ecosystems, stressing aquatic life adapted to certain water conditions.

Testing for DO is an important factor in overall water quality assessment. Dissolved oxygen is measured in milligrams O_2 per liter of water sampled (mg/L). For a body of water to be considered healthy, the percent (%) saturation of DO should be above 90%. DO is calculated by measuring the mg/L of oxygen in the sample, and then correcting it, if necessary, for atmospheric pressure and temperature.

PROCEDURE

Materials and Equipment

LaMotte Dissolved Oxygen Test Kit
HACH Dissolved Oxygen Test Kit
YSI Dissolved Oxygen Meter, Model # 57
YSI Dissolved Oxygen Meter, Model # 54
YSI Dissolved Oxygen Meter, Model # 58
YSI Dissolved Oxygen Monitor, Model # 56
YSI Submersible Stirrer, Model #5696
YSI Dissolved Oxygen Probe, 5700 series
Sample bottles, labeled
Ice chest/ice (if storing samples)
Rubber boots
Thermometer
1 ml pipettes (at least 3)
Gloves, latex or rubber
Safety goggles
Waste disposal bottle
Water sampler (if needed)

Preparation

Teams of three to four students will prepare to go to the sampling site. Preparing to take water samples means careful planning for collecting, cataloging and analyzing the sample so that the data obtained is reliable or of high confidence. Taking good field notes is mandatory, including drawing maps of the sample area.

NOTE: *Packing adequate supplies and materials will ensure a successful sampling venture!*

Bring the following to the field site:

(12) 300-ml BOD bottles
(1) HACH or LeMotte kit
(1) DO meter with 10-50 ft. probe
(1) Kemmerer sampler

Each team will sample four stations or locations around the lake. Three depths will be taken at each station: surface, mid-depth and 1.0 foot from the bottom. Tests will be conducted on site – *with the exception of the Winkler method* – in which samples will be collected and preserved on site, but processed in our lab.

To get familiar with each method, each student should sample one depth with all three (3) sampling methods.

Sampling

Several factors should be considered prior to sampling, including method of analysis, site accessbility, and what components are being sampled. Planning is necessary – questions should be addressed, including: *How much sample should be collected? What influence will diurnal fluctuations in photosynthesis and temperature have on DO readings? Will you access your site by boat, on foot or from a bridge?*

Your instructor will provide details.

Analysis

NOTE: *See handouts (following lab) for procedures and tables noted.*

Kit Method: Follow the kit's directions for collecting and fixing the sample. After the sample is stabilized, continue by following titration directions.

Winkler Titration Method: Prepare necessary reagents in sufficient quantities according to procedure. Place enough reagents in labeled bottles – these will be taken to the field to fix samples as they are collected. Follow collection and sampling procedures. Return to lab and proceed with titration.

Meter Method: Prepare and calibrate the meter and probe according to the operation manual for the particular instrument used (i.e., YSI Model 54, 56, 57, or 58 DO meters; and 5700 series DO probe). Record 3 separate meter readings at the sampling site, following the correct sampling procedure. Average the results by adding all the readings together and dividing by the number of readings taken. Follow the measurement table and examples to determine the correct percent (%) saturation.

Calculations/Results

Correcting for Atmospheric Pressure or Altitude:
When you have obtained a reading in mg/L, check whether you need a correction factor for atmospheric pressure (using the Measurement Table provided on page 38). If so, take a reading from a barometer (atmospheric pressure expressed in mm Hg), or find your altitude on the chart. Follow across the line to determine your correction factor and multiply your mg/L answer by that number (the Measurement Table for Winkler Titration is found after the Preparation/Solutions section).

Examples:

1) If you don't have a barometer, but know your altitude is 542 feet, and your reading for dissolved oxygen was 9 mg/L:

$$9 \text{ mg/L} \times .98 = 8.55 \text{ mg/L}$$

2) Your barometer reading was 654 mm Hg, and your reading for dissolved oxygen was 11 mg/L.

$$11 \text{ mg/L} \times .86 = 9.46 \text{ mg/L}$$

Determining Percent Saturation (at Various Water Temperatures) Using a Nomogram:

Look at the nomogram (chart) that correlates temperature with DO (listed below the atmospheric pressure/altitude measurement table). Find your dissolved oxygen reading in mg/L and lay a ruler from that point on the chart up to the temperature reading above. You can then read the % saturation on the diagonal line of the chart.

Examples:

1. If your adjusted reading is 8.55 mg/L, and the temperature is 20 degrees celsius:

   % saturation = ~91%.

2. If your adjusted reading is 9.46 mg/L and the temperature is 12 degrees celsius:

   % saturation = ~85%

NOTE: *It should be noted that some DO meters automatically correct for atmospheric pressure altitude and temperature. Check the operating manual for your specific meter for these options.*

PRODUCTS

1. Turn in data sheets and field notes.

2. Clean up all equipment and lab space; turn off instrumentation; put away all equipment and supplies in their proper place.

3. Write a short team report and turn it in at the beginning of the next lab period; include the following in your write-up:

   a. The difference between precision and accuracy of the different methods.
   b. The advantages/disadvantages of each method.

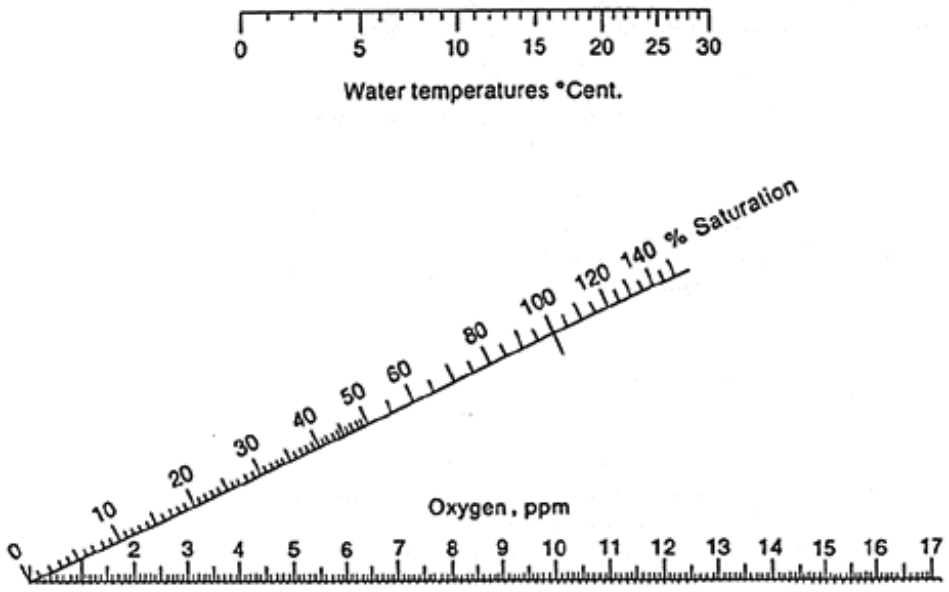
Measurement Table

To determine correction factor for atmospheric pressure/altitude

When you have completed all the steps for the method of analysis and have obtained a reading in mg/L, use the Measurement Table below to check whether you need a correction factor for atmospheric pressure (see Calculations/Results for details).

Atmospheric Pressure (mm Hg)	Equivalent Altitude (feet)	Correction Factor
775	-540	1.02
760	0	1.00
745	542	.98
730	1,094	.96
714	1,688	.94
699	2,274	.92
684	2,864	.90
669	3,466	.88
654	4,082	.86
638	4,756	.84
623	5,403	.82
608	6,065	.80
593	6,744	.78
578	7,440	.76
562	8,204	.74
547	8,939	.72
532	9,694	.70
517	10,472	.68

Nomogram (To determine % saturation based on correlation between DO and water temperatures)



Rawson's nomagram of oxygen saturation values at different temperatures and altitudes. Hold ruler or dark-colored thread to join an observed temperature on the upper scale with the observed dissolved-oxygen value on the lower scale. The values or units desired are read at points where the thread or ruler crosses the other scale. The associated table supplies correction values for oxygen saturation at various altitudes. For example, if 6.4 ppm of oxygen is observed in a sample having an altitude of approximately 500 m (1,640 feet), the amount of oxygen that would be present at sea level under the same circumstances is found by multiplying 6.4 by the factor 1.06, giving the product 6.8; then the percentage saturation is determined by connecting 76.8 on the lower scale with the observed temperature on top scale and noting point of intersection on the middle (diagonal) scale.

LaMotte DO Kit Method

Collecting and Fixing the Sample:

1. To avoid contamination, thoroughly rinse the sampling bottle with sample water.

2. Cap the bottle and submerge it to the desired depth. Remove the cap to allow the bottle to fill.

3. Tap the sides of the submerged bottle to release air bubbles. Replace cap while bottle is still submerged.

4. Retrieve bottle and examine carefully to make sure no air bubbles are inside.

5. Fix the sample by adding 8 drops of Manganese Sulfate Solution and 8 drops of Alkaline Potassium Iodide Azide Solution. Cap and mix by inverting the bottle several times. A precipitate will form. Allow precipitate to settle below the shoulder of the bottle before proceeding.

6. Use 1g spoon to add one level measure of Sulfamic Acid Powder. Cap and gently shake until reagent and precipitate have dissolved. A clear-yellow to brown-orange color will develop, depending on the oxygen content of the sample.

NOTE: *At this point, contact between the sample and the air will not affect the test results. It is not necessary to continue the analysis at that time; numerous samples can be collected and fixed and taken back to the lab for titration.*

Titrating the Sample:

1. Fill the titrator tube to the 20 ml line with the fixed sample and cap it. If the color of the fixed sample is already a very faint yellow, skip to step 3 immediately below.

2. Fill the *Direct Reading Titrator* with Sodium Thiosulfate Solution. Insert the titrator into the center hole of the titration tube cap. While gently shaking the tube, slowly press the plunger to titrate until the yellow-brown color is reduced to a very faint yellow.

3. Remove the titrator cap and be careful not to disturb the Titrator plunger, as you add 8 drops of Starch Indicator Solution. Sample should turn blue.

4. Replace the cap and Titrator. Continue titrating until the blue color just disappears. Read the test result where the plunger tip meets the scale. Record as ppm (mg/L) dissolved oxygen.

YSI Oxygen Meter Method (Model 54)

To Prepare Meter for Use:

1. To prepare oxygen probe for operation, connect the two probe plugs to the jacks on the side of the instrument.

2. With the instrument turned off, check the mechanical zero of the meter (pointer should indicate zero). Adjust with the screw on the meter case. Recheck when the position of the instrument is changed.

3. Switch to the RED LINE position and adjust the meter to the red line with the front panel control.

4. Place the probe in the calibration medium.

5. Switch to the TEMP position and read the temperature when the meter is ready.

6. Switch to the ZERO position and adjust the meter to zero with the ZERO control.

7. Switch to the 0.10 or 2.0 ppm position and calibrate the instrument with the CAL control.

To Prepare Probe for Use:

1. Add distilled water to the KCl crystals and dissolve completely. Transfer a part of the KCl solution to an eyedropper bottle.

2. Remove the sensor guard from the probe (where applicable).

3. Remove the protective membrane and "O" ring. Select a membrane from the vial and lay it on a clean sheet of paper – be careful to handle only by the ends.

4. Support the probe in the vertical position. Using your thumb, secure the membrane to the side on the probe.

5. With the eyedropper, fill the central hole — avoiding air bubbles. Wet the gold electrode and the lucite around it. The surface tension of the KCl will cause a large drop or meniscus to form above the electrode. This will ensure complete contact between the membrane and the KCl.

6. Stretch the membrane over the top of the electrode. Stretch the "O" ring into place – inspect for a wrinkle-free membrane. A taut, smooth membrane surface is required. A lax membrane will result in erratic performance and slow speed of response. Remove the excess membrane about 1/8" beyond the "O" ring with scissors. NOTE: *A small air bubble may appear under the membrane. This is normal.*

7. Remove excess KCl solution from outside the probe.

8. Replace the sensor guard. The probe is ready for operation.

To Calibrate:

Place the probe in a water sample of known oxygen concentration. Agitate the sample (or probe) to prevent oxygen depletion around the cathode of the probe. Adjust the CAL control to give the reading of the oxygen concentration sample. For more information, consult the instruction manual.

YSI Dissolved Oxygen Meter Method (Models 56, 57, 58)

To Prepare Meter for Use:

NOTE: *Model 56 has a chart recorder. All references to chart, pen, paper, etc. are for Model 56 only.*

1. Connect the YSI Model 5739 Oxygen to the receptacle on the side of the case. Tighten the connector finger enough to ensure a proper waterproof seal. If the YSI Model 5695 submersible stirrer is used, connect it to the probe at the same time.

2. Open the case lid. Pull out the chart paper drawer by grasping the handles on either side of the chart paper and pulling up. Check for sufficient paper supply. If additional paper is needed, see the section on chart paper in the operator's manual for proper paper loading.

3. Check dessicant condition (if the instrument is to be used in an environment of 70% relative humidity or above). The dessicant should be blue in color; if it appears white, see the section in the manual on *Dessicant Maintenance* for drying the dessicant.

4. Check battery charge. Switch POWER to On and PEN INPUT to ZERO. Adjust PEN ZERO to read O on the 0-10 red scale on the scale plate.

5. Turn PEN INPUT switch to BAT CHK. Recharge batteries if the pen will not move or if the pen indicates low battery charge. NOTE: "10" on the 0-10 red scale on the scale plate is full charge; "0" indicates lack of charge. See the section in the manual on *Instrument Battery* for battery charging.

To Prepare Probe for Use:

The theory of operating a probe sensor revolves around two electrodes wetted with potassium chloride (KCl) electrolyte, which are sealed from the environment by an oxygen permeable Teflon™ membrane. There is a gold cathode (negative) directly under the membrane and a silver anode (positive) that is in the center of the probe. The cathode consumes any oxygen that is dissolved in the thin layer of electrolyte between the cathode and the membrane. This frees four electrons from the cathode for each molecule of oxygen consumed and produces an electric current passing between anode and cathode proportional to the quantity of oxygen consumed. The silver anode reacts with chloride ions, accepting electrons from the electrolyte to complete the probe's conduction path.

Whenever oxygen pressure outside the membrane is greater than zero, oxygen diffuses through the membrane into the probe. The current created by the entering oxygen indicates the environmental oxygen partial pressure. The probe current can be calibrated at the instrument into required measurements like milligrams per liter dissolved oxygen (by weight).

Follow these directions:

1. Prepare the electrolyte provided in the service kit by dissolving the KCl crystals in the dropper bottle with distilled water. Fill the bottle to the top.

2. Unscrew the sensor guard from the probe and remove the "O" ring and membrane. Thoroughly rinse the sensor with distilled water.

3. Prepare a new probe for use with the half-sensitivity membrane.

4. Lightly pencil an "X" across the probe's gold-sensing surface.

5. Wet the special contoured sanding tool from the service kit with distilled water. Hold the tool's abrasive face uniformly against the probe's sensing surface and slowly twist the tool in a circular fashion until all traces of the pencil mark are erased.

6. Use the tool to slightly radius the outer edge of the probe face. Rinse the surface with distilled water to remove all particles. NOTE: *If the probe has been in use for some time, and the gold cathode appears tarnished or shows a slight silver ring on the inner edge; it should be restored by sanding.*

Fill the probe with electrolyte as follows:

1. Grasp the probe in your left hand. When preparing the probe, the pressure compensating vent should be to the right. Successively fill the sensor body with electrolyte while pumping the diaphragm with the eraser end of a pencil or similar soft, blunt tool. (With practice you can hold the probe and pump with one hand while filling with the other.)

2. Secure a membrane under your left thumb. Add more electrolyte to the probe until a large meniscus completely covers the gold cathode. NOTE: *Handle membrane material with care; keep it clean and dust free; touch it only at the ends.*

3. With the thumb and forefinger of your other hand, grasp the free end of the membrane. Using a continuous motion, stretch the membrane UP, OVER, and DOWN the other side of the sensor. Stretch DOWN until the membrane "cap " forms smoothly over the "O" ring groove.

4. Secure the end of the membrane under the forefinger of the hand holding the probe.

5. Roll the "O" ring over the end of the probe. There should be no wrinkles in the membrane or trapped air bubbles. Some wrinkles may be removed by lightly tugging on the edges of the membrane beyond the "O" ring.

6. Trim off the excess membrane with scissors or a sharp knife. Check that the stainless steel temperature sensor is not covered by excess membrane.

7. Shake off the excess KCl and reinstall the sensor guard. A bottomless plastic calibration bottle is provided with the probe for convenient calibration. Place a small piece of moist towel or sponge in the bottle and insert the probe into the open end. NOTE: *This ensures 100% humidity for accurate calibration and helps protect the probe against drying out in storage.* Membranes will last indefinitely, depending on usage. Average replacement is 2 to 4 weeks. However, should the electrode be allowed to evaporate and an excessive volume of air form under the membrane, or the membrane become damaged, thoroughly flush the reservoir with KCl and install a new membrane. Also replace the membrane if erratic readings are observed or calibration is not stable. User-prepared electrolyte can be made by making a saturated solution of reagent grade KCl and distilled water and then diluting the solution to half strength with distilled water. Adding two drops of Kodak Photoflo® per 100 ml of solution assures good wetting of the sensor. Hydrogen sulfide, sulfur dioxide, halogens, neon, nitrous oxide and carbon monoxide are interfering gases. If you suspect erroneous readings, it may be necessary to determine if these are the cause.

To Calibrate:

Calibration consists of exposing the probe to a known oxygen concentration, such as air or water of a known oxygen content, and appropriately adjusting the Model 56 reading to match the known sample. This calibration is based on the *air calibration technique:*

1. Place a moist sponge or a piece of cloth in a plastic calibration bottle. Loosen the bottle lid about ½ turn and slip the bottle over the probe guard up to the body. Place the probe in a protected location (where temperature is not changing) or wrap it in a cloth or other insulator.

2. Setting the instrument: POWER to ON

3. CHART SPEED to 1 CM/HR

4. PEN INPUT to 02 & °C

5. 02 RANGE to 0-100%

6. 02 FILTER to OFF

7. After one minute, adjust the 02 CALIB control to give a full scale dissolved oxygen reading near 10. Switch PEN INPUT to 02 & °C

8. Wait for the pen to draw straight lines on the chart.

NOTE: *The accuracy of your data depends on the accuracy of your calibration. Following a* membrane change, probe stabilization may require several hours. For best calibration accuracy allow the probe to stabilize with polarization voltage on overnight.

Then:

1. Set 02 RANGE to 0-100%
2. Switch PEN INPUT to ZERO
3. Set CHART SPEED to RAPID
4. Turn 02 filter to OFF
5. Adjust PEN ZERO control to indicate 0 on the 0-10 chart scale
6. Switch PEN INPUT to 02 CALIB control until the pen traces at the calibration value on the 0-10 chart scale
7. Switch CHART SPEED control to 1 CM/HR

Dissolved Oxygen Data Sheet

Enter Dissolved Oxygen measurements below:

Type of Instrument/Kit	Date/ Time	mg/L	% Sat.	Notes

Definitions

Aerobic – pertaining to organisms that require air or free oxygen to survive.

Anaerobic – pertaining to microorganisms that do not need air or free oxygen to survive.

mg/L – milligrams per liter; also known as ppm or parts per million. It is a typical unit of scientific measurement. 1 mg/L is equal to one part per million.

Water quality – a standard with specific criteria for tests such as dissolved oxygen, pH or turbidity.

Dissolved oxygen – oxygen that is dissolved in water and available for organisms.

Titration – in chemistry, the process of finding how much of a substance is contained in a solution by measuring how much of another substance is necessary to add to the solution in order to produce a specific reaction. (In this case, it is a color change that is used as the indicator.)

Precipitate – a substance that is separated out from a solution as a solid by a chemical reaction.

Photosynthesis – the chemical reaction or process by which water and carbon dioxide with the addition of light are converted into sugars and oxygen.

Meniscus – the curved upper surface of a column of liquid. Resulting from capillarity, it is convex when the walls of a container are dry, concave when they are wet. All aqueous (watery) solutions have a concave meniscus.

NOTES FOR INSTRUCTORS

Learning Objective: The student understands the rationale for performing dissolved oxygen testing.

Learner Outcomes: The student demonstrates the ability to determine the level of dissolved oxygen in a water sample by kit, meter, and laboratory titration methods, within acceptable levels of precision, accuracy and time limits.

The instructor or instructional assistant will normally prepare the Winkler dissolved oxygen reagents. Refer to instructions for the preparation of reagents, collection, and preservation of samples, titration, and data analysis for the iodometric (Winkler) method. See Standard Methods for the Examination of Water and Wastewater, 19th Edition, 1995.

The azide modification of this method works well for most sewage, effluents, and streams in the Pacific Northwest. It is especially useful if water samples contain more than 50 mg NO_2^- N/L and not more than 1 mg ferrous iron/L

The most important thing to remember when taking a sample is that it should be representative of the water body you are trying to assess.

Sample bottles should be narrow-mouthed glass bottles with the underside of the cap or stopper being pointed to help eliminate trapped air. When filling the sample bottle, it should be immersed in the water a sufficient length of time for it to fill several times. While still holding the container under water, tap the side to dislodge any air bubbles that might be sticking to the side of the container. Samples need to be fixed on site to prevent changes in DO from agitation in transport back to the lab. Avoid shaking or agitating the containers in any way.

Several factors should be considered prior to sampling, including method of analysis, site accessibility, and what components are being sampled. Planning is necessary – questions should be addressed, including: *How much sample should be collected? What influence will diurnal fluctuations on photosynthesis and temperature have on DO readings? Will you access your site by boat, on foot or from a bridge?*

If sampling from a boat, it is important to take your sample at known, multiple depths. Be sure the boat is balanced as you lean over to obtain the sample.

Boat sampling is used mostly for lakes, bays or impounded water bodies where water is deep.

If sampling on foot, be sure you are far enough from shore in the water body to get a representative sample – ideally, your distance from shore should be at a depth taken about midway between the top and bottom of the water body (obviously, the water body must be shallow; i.e., a stream). The speed of currents, riffles, etc., must be considered when selecting your sampling site, and always determine if the site is safe. Alternatively, sometimes samples can be taken from shore with the aid of an extended reach sampler.

Sampling is done on foot for streams, lower velocity and/or shallower water bodies.
Sampling from a bridge takes extra cable to reach the approximate halfway point to the bottom of the water body. A weight may also be necessary to keep the sampler in the appropriate place. Occasionally there is need to stir the water, if it is impounded (e.g., dammed), in an attempt to homogenize the water prior to sampling (creating at least one cubic foot per second [cfs] of flow). This is done to maintain a fresh oxygen sample to the probe.

Sampling from a bridge is used to accommodate sampling in difficult locations, like steep banks, fast water, or unstable substrates.

Gas Supersaturation

NOTE: *Information has been used with permission from the* ***Field Manual for the ES-2 and ES-3 Weiss Saturometers.*** *ECO Enterprises, 2821 N.E. 55th, Seattle, WA 98105.*

INTRODUCTION

What Is Supersaturation?

Dissolved gasses are present in all fish-bearing waters, usually entering the water directly from the air. Normally, the dissolved gas pressure is equal to the atmospheric (barometric) pressure just above the air-water interface (14.7 pounds per square inch [psi] or 760 mm of mercury at sea level). 100% saturation is considered normal and ideal.

Occasionally, natural or man-made conditions occur that upset this balance. Supersaturation can be caused by dam spillways, waterfalls, power plant outflows, algae blooms and other sources. If the percent saturation goes higher than 110%, aquatic life is threatened and fish kills can occur. Conversely, if the percent saturation goes below 80%, not enough dissolved oxygen is present to support aquatic life. Undersaturation (de-aerated waters, below 100% saturation) can occur in some well or spring waters, and in still or stagnant ponds.

Supersaturation causes a disease in fish called the "gas-bubble" disease, where air bubbles come out of solution in the blood vessels of fish – causing blood vessel blockage, rupture and death. This disease is similar to the bends experienced by human divers coming to the surface too quickly from a deep dive.

The Saturometer

The *Weiss Saturometer* is a field-portable instrument which monitors dissolved gas parameters. In this activity, you will learn about using a saturometer to calculate percent saturation.

NOTE: *For many applications, this number (% Saturation) is the only number needed to assure safety of the aquatic environment and to assure legal requirements are within proper limits. Check with state, federal and tribal fisheries in your particular geographic area to confirm safe levels of % saturation for the fish species endemic to your region.*

PROCEDURE

Work in teams of 2-4 students. Do the following:

1. Determine the barometric pressure using a mercurial barometer. Verify your reading by calling a local airport/airfield.

2. Using the *ES-2 Weiss Saturometer* (long-handled meter), determine the percent saturation for a local lake and creek. Record your measurements on the field data sheet provided.

3. Using the *ES-3 Weiss Saturometer* (short-handled meter), determine the percent saturation in the headbox of a local fish hatchery. Also measure the percent saturation in at least one of the fish troughs containing fish fry. Record your measurements on the field data sheet provided.

4. Calculate the percent saturation for all measurements you have taken as described below. Record percent saturation for each sample on the data sheet provided.

USING THE SATUROMETER AND MAKING CALCULATIONS

Before immersing the saturometer sensor in water, the pressure gauge should indicate a zero reading. This is the point at which the pressure inside the instrument is equal to the local atmospheric (barometric) pressure. After a reading is taken, the gauge pointer will indicate a positive or negative dissolved gas pressure reading in millimeters of mercury (mm Hg). To convert this number into percent saturation, the atmospheric pressure should be measured with an aneroid or mercurial barometer, or calculated from altitude charts and local weather bureau or airport data. The following formula is then used:

$$\text{Percent (\%) Sat.} = \frac{\text{P atm} + \text{DP}}{\text{P atm}} \times 100$$

.... where % Sat. = the total dissolved gas saturation (indicates supersaturation or undersaturation)

Patm = the local barometric pressure in mm Hg (760 mm Hg is the nominal value at sea level)

D P = saturometer reading in mm Hg

Example: Barometric pressure is 765 mm Hg, DP is + 100 mm Hg

$$\text{\% Sat.} = \frac{765 + 100}{765} \times 100 = 113\% \text{ Saturation}$$

The actual dissolved gas pressure present in this sample = 765 X 1.13 (113% Sat.) = 865 mm Hg.

PRODUCTS

At the end of this lab you will be responsible for:

- Rinsing both the ES-2 and ES-3 saturometers and stowing them away.
- Completing your data sheets.
- Turning in one (1) data sheet for your team at the beginning of the next lab/field period.

MATERIALS AND SUPPLIES

Field notebook
Lab exercise and data sheets
Mercurial barometer
ES-2 and ES-3 Weiss Saturometer
Hand-held calculator

Gas Saturation Data Sheet

Calculations			
Lake _____	_____Creek	Hatchery Head Box	Hatchery Trough

Calculations: Percent (%) Saturation = $\frac{\text{P at}}{\text{-}}$

Calculations			
Lake _____	_____Creek	Hatchery Head Box	Hatchery Trough

Barometric Pressure (from mercurial barometer) = _____ mm Hg Barometric Pressure (from local air field) = _____ mm Hg

Comments: _____

_____

NOTES FOR INSTRUCTORS

Field sites for this activity include a lake, lake outfall to a stream, and a fish hatchery.

How Was The Saturometer Invented?

Large fish kills in the early 1970s on the Columbia and Snake River systems in the Pacific Northwest prompted the Environmental Protection Agency (EPA) to take action to solve the problem. An inexpensive, field portable, accurate instrument to measure dissolved gas pressures at various locations was needed. Dr. Ray F. Weiss, with the Scripps Institute of Oceanography, in conjunction with people from the Region 10 EPA in Seattle, Washington, designed the first operational *saturometer*. ECO Enterprises redesigned and started manufacturing these instruments in 1972.

Principles of Operation

The saturometer operates basically as an artificial fish gill. Dimethyl silicone rubber tubing is used for the sensing membrane. This small, hollow tubing will allow the passage of dissolved gasses from the outside (water) to the interior (pressure gauge, referenced to local atmospheric pressure) for readout. Over 300 square inches (200 ft.) of this tubing is used in the ES-2 instrument.

The tubing is wound on frames into a compact sensor. A perforated outer shell protects the membrane tubing from damage and allows rapid water transfer. A manually operated water-jet pump emits high velocity streams of water across the tubing, accelerating water transfer and removing air bubbles from the tubing that interfere with dissolved gas diffusion. The water-jet pump is not needed in undersaturated waters, as no bubbles can form.

A specially calibrated low-volume pressure gauge is used to measure the dissolved gas pressure. This gauge measures from -100 to +400 mm of mercury with a full-scale accuracy of better than ± 1%. A pressure-relief valve is located just behind the gauge to quickly vent internal pressure after a reading is taken, for testing, leak detection and calibration purposes.

Saturometer Use in Rivers, Lakes & Streams

Before industrial man started utilizing our waters for power generation, irrigation and flood control, the only source of supersaturation was waterfalls. They trapped atmospheric gasses and carried them into deep pools where the gasses went into solution. Nature's way of dissipating the resulting supersaturated condition was to take these waters over turbulent, shallow sections of the river that agitated the water, bringing the dissolved gasses in contact with the air-water interface where they could dissipate and return to the atmosphere.

The advent of large dams on many fish-bearing rivers and streams eliminated these turbulent sections by creating large, deep reservoirs that prevented effective dissipation of excess dissolved gas pressures. Dam spillways, when in operation and not properly designed, contributed more to

the supersaturation levels. Hot water from power generating facilities (nuclear or other) entering these waters further contributed to this problem.

Unlike the air we breathe, dissolved gas pressure and composition in water are highly variable and can be adversely affected by many different factors and conditions. The location, control, regulation or elimination of these undesirable factors falls under the jurisdiction of regulatory agencies such as the EPA, state, federal and private fisheries agencies, power companies and utility districts, the Departments of the Interior and Commerce, and private industry. All are all involved in controlling and maintaining a healthy aquatic environment for the fisheries industry.

The *Weiss Saturometer* is a field portable, accurate instrument which monitors dissolved gas parameters. Both rugged and inexpensive, skilled personnel are not necessary to obtain fast, accurate readings.

After the monitoring site is reached, the sensor membrane section is immersed in the water and the valve is closed. A few minutes of agitation with the water pump or swishing back and forth will indicate whether the water is:

1. supersaturated (pointer on gauge will go clockwise, +)

-or-

2. undersaturated (pointer on gauge will go counterclockwise, –)

-or-

3. If no change in the reading occurs, equilibration has been reached, and that reading should be noted.

NOTES: *If water is undersaturated, no agitation or pumping is needed as air bubbles will not form on the tubing. If supersaturated conditions are present, vigorous agitation and pumping every 30 seconds of agitation should be done.*

Other information should be recorded, preferably on a data sheet, and should include date, time, location, barometric pressure (either measured or calculated from weather service data and altitude charts), water and air temperature. NOTE: *If percent nitrogen is required, a dissolved oxygen determination should be made with either a dissolved oxygen probe or the Winkler method.*

If tests at difficult sites or from a boat are required, it may be wise to use the wrist-straps on the ES-2 or tie a line around the handle to prevent loss of either type of instrument.

CAUTION: *Do not dry the instrument or expose it to open flame or fire as the tubing may be destroyed. Care should be taken to keep the gauge dry and free from corrosion. In taking field readings, do not allow foreign objects such as sticks, sand, etc., to enter the sensor area.*

Alternatives to the Saturometer

An alternative method to the saturometer (or used in conjunction for averaging readings) is using a pump or syringe. While immersed in water, positive pressure should be applied (or by sucking on the valve opening to provide a vacuum), then the valve should be closed so a reading of 50 - 100 mm Hg above the expected reading is obtained. The pump or syringe is then allowed to bleed down to the equilibration point. (Agitation may still be needed in supersaturated waters.) As mentioned, both methods can be employed and the average value computed for very accurate readings. Either method takes generally 4-15 minutes to obtain a reading.

Saturometer Use In Hatcheries and Research Facilities

An active saturometer-monitoring program in government or private hatcheries can prove of great value in preventing fish kills due to supersaturation or oxygen depletion, and in regulating the aquatic atmosphere through dynamic control of aerators and source water selection, metering and composition control. Many hatcheries now employ almost totally artificial (man-made) control of the amount and type of dissolved gases present in their fish-bearing waters. The advent of intensive fish-farming programs and modern research facilities intensifies the necessity to accurately measure the composition and amount of dissolved gasses present.

It has been proven that fish kills due to dissolved gas supersaturation are not necessarily due to nitrogen alone, but the sum total of all dissolved gas partial pressures. It is necessary to maintain a proper nitrogen/oxygen for fish. Many hatcheries, fish farm facilities and research institutes are now altering this nitrogen/oxygen balance to obtain optimum growth rates of fingerlings and fry, often with great success. The necessity of maintaining these de-aerated and then oxygen enriched waters at the optimal percent saturation pressure dictates an active monitor and control program due to the complexity of these systems. One failure or mistake gone unnoticed for a given length of time can cause the loss of millions of young fish.

A sudden change in source water composition and percent saturation occasionally occurs. The first warning signs of trouble can be a lot of belly-up fish. But with a monitoring program, such conditions can be detected and corrected before fatalities occur. The same is true if an aerator and circulation pump systems should spring a leak (usually at a fitting) in a high pressure or high velocity section of piping, or in a suction section on the inlet side of a pump – allowing atmospheric air to be injected into that point via venturi action – causing supersaturation to be present past that leakage point. If the condition persists, fatalities can occur. With a spot-check saturometer-monitoring program, the leak can be located and fixed before fatal conditions occur.

Algae blooms often occur in larger holding ponds. They produce prodigious amounts of dissolved oxygen that can reach harmful or fatal levels. By taking readings on a grid pattern using triangulation measurements, a thermal map can be constructed – pinpointing the algae bloom source. Localized herbicide can then be selectively applied to control this condition without poisoning the entire pond. Also, stagnant ponds or lakes, or the presence of too many fish in a given body of

water, can lead to oxygen depletion. Again, this condition can be detected and corrected with an active saturometer-monitoring program before fatalities occur.

ES-3 "mini" versus long-handled ES-2 Saturometers

The ES-3 "mini" or lab saturometer is better suited for use in smaller size fish tanks, as it occupies less space and removes less dissolved gas from the water than the larger ES-2 instrument. The longer handle on the ES-2, however, allows hatchery personnel better access to outdoor holding ponds which may have water levels a few feet below the ground surface.

NOTE: *Saturometers can be sterilized with acetone, boiling water, dilute chlorine or other anti-bacterial agents to prevent the possible spread of diseases from tank to tank or pond to pond.*

CONVERSION FACTORS

Atomic Values: O_2 = 22.392 liters/mole
N_2 = 22.403 liters/mole
Ar = 22.390 liters/mole

Atomic Weights: O_2 = 31.9988 grams/mole
N_2 = 28.0134 grams/mole
Ar = 39.948 grams/mole

Composition of air: O_2 = 20.946%, N_2 = 78.084%, Ar = .934%

Concentration: 1 ppm = 1 mg/liter (approx./fresh water)
.001 gm/L = 1 oz./7400 gallons (approx./fresh water)
1 gm/liter = 1000 parts per million (approx./fresh water)

Conversion of ml/L to mg/L: 1 ml/L O_2 = 1.42903 mg/L
1 ml/L N_2 = 1.25043 mg/L
1 ml/L Ar = 1.78419 mg/L

Distance: 1 ft. = .3048 meters
1 meter = 3.281 feet

Hydrostatic Pressure: 1 meter of fresh water @ 20° C = 73.43 mm Hg
1 foot of fresh water @ 20° C = 22.89 mm Hg
1 meter of seawater @ 35 ppt, 20° C = 74.88 mm Hg
1 foot of seawater @ 35 ppt, 20° C = 23.34 mm Hg

Pressure: 1 Std. atmosphere = 760 mmHg = 1.013 Bar = 10.35 meter (32.2 ft.) of fresh water at 20° C = 10.15 meter (32.56 ft.) of seawater at 35 ppt and 20° C = 14.22 lbs./sq. in. = 101.325 kilopascals = 760 torr @ 0° C = 1 Kgf/cm^2

Temperature: $^{o}C = 5/9 (F^{o} - 32)$ $^{o}F = (9/5\ ^{o}C) + 32$ $^{o}K = ^{o}C + 273.15$

Volume: 1 cc = 6.1 X 10^{-3} cu. in. 1 cu. ft. = 28.32 liters
1 cu. in. = 16.387 cc 1 liter = .0353 cu. ft. = 67.91 cu. in. = 1.06 quarts

REFERENCES

ECO Enterprises, 1985. *Weiss saturometer field manual for the ES-2 & ES-3 instruments*. ECO Enterprises, 2821 N.E. 55th, Seattle, WA 98105. (206) 525-4784 or (206) 523-9300; also, 1-800-426-6937 (except WA).

Fecal Coliform Testing

INTRODUCTION

Fecal coliform is the common name of a type of bacteria commonly found in the intestinal tract of warm-blooded animals – including humans. Although there are numerous strains of coliform bacteria, many are considered non-pathogenic and are present in the intestinal tracts of healthy individuals. Obviously, they are released into the environment when they are expelled from the gut. Although not necessarily harmful, their presence in a water sample is indicative of fecal contamination and may also indicate the presence of other, more harmful microbes. Examples of harmful water-borne bacteria include those causing hepatitis, dysentery, or cholera.

Sources of **fecal contamination** include failing septic systems, animal waste that runs off farmland, and untreated domestic sewage. For example, the city of Victoria on Vancouver Island, British Columbia, Canada, dumps its sewage directly into the waters of the nearby bay and the Straits of Juan de Fuca.

Two methods are commonly used for determining fecal coliform:

1. Membrane Filter Technique
2. Most Probable Number Method

PROCEDURE

For this exercise, we will use samples collected from effluent outfalls at a local waste treatment plant and a pulp mill. You will work in teams of 2-3 students, collecting samples at each location.

To determine fecal coliform content of your samples, handouts provided will guide you through each method (Membrane Filter and Most Probable Number).

IMPORTANT NOTES:

- *The most important aspect of this assay is maintaining a sterile environment. All bottles and other glassware must be sterilized prior to use. This can be accomplished with either an autoclave (121° C for 15 minutes), using a pressure cooker (15 lbs. psi), or by heating in a conventional oven (170° F for 60 minutes). And remember, plastics cannot be put into the drying oven. If using a plastic filtration system, it should be placed in boiling water for 5 minutes.*

- *It is mandatory that all materials in contact with the sample are sterile, even at the sampling site. Always employ and maintain sterile technique.*

SAMPLING

Preparing to take water samples means having a plan for collecting, cataloging and analyzing the sample so that the data obtained is reliable. For example, having an idea of a range for fecal coliform contamination that is expected (based on knowledge of the site), or how accessible your sample site is, all influence your preparations. Taking good field notes is mandatory.

Collecting the sample depends on several factors including method of analysis, site accessibility, and what is being sampled. This also influences how much sample should be collected. Will you access your site by boat, on foot or from a bridge?

NOTE: *Like other water testing, the most important thing to remember when taking a sample is that it should be representative of the water body you are trying to assess, and, that the sample is uncontaminated.*

Collecting the sample.

1) Grasp a sterilized sample container (normally a 250-ml nalgene bottle) near its base in one hand. A sterile plastic bag, whirl pak style, may be used.

2) Remove the cap with the opposite hand and retain the cap without contaminating the cap or the interior surface. For hard to access areas, place the bottle in the custom sample holder prior to removing the lid.

3) Gently plunge the bottle, mouth down, six to twelve inches into the water, avoiding introduction of any surface scum or any other material that is not representative. Do not scrape or scour any submerged materials into the sample.

4) Point the bottle slightly upward and into the current away from the hand, leg, side of boat, or any other possible source of contamination.

5) If there is no current, create an artificial current by pushing the bottle horizontally forward in a direction away with the hand. Tip the top of the bottle upward as the bottle is moved.
6) Withdraw the full sample bottle carefully and pour off excess sample until the bottle is

approximately ¾ full. There must be air space of one to two inches above the top of the sample.

7) Cap the container immediately.

8) Mark the container with sample location, date and time name of sample collector, and any other pertinent observations.

NOTE: *Samples must be "read" 24 hours after they are placed in the incubator, even if that is not a scheduled lab time.*

MEASUREMENT

Fecal coliform is measured in number (#) of colonies per 100 mL of water sample. This is obtained by collecting the sample, preparing it for analysis, incubating it for 24 hours at 44.5°C, and then counting the number of fecal colonies that have grown on the membrane grid under a microscope.

The calculation:

# of fecal colonies counted X 100/mL of sample = # colonies/100 mL

NOTE: *If the sample has been diluted in order to get a reading between 20 and 60 colonies per 100 mL of water, you must multiply your # of counted colonies back by the same factor that was used to dilute (1:10 or 1:100 for example).*

PRODUCTS

- After the lab, clean up and wash all glassware
- Calculate sample results
- Turn in lab notes, definitions worksheet, lab report, data sheets (provided)

- *Lab report:* Write up a one-page summary of this exercise. Include in your discussion:

  - An introduction about the importance of the fecal coliform test

  - A comparison of results you obtained from the waste treatment plant (municipal waste) and the pulp mill (industrial waste)

  - Links this test may have with local health hazards

Definitions Worksheet

Write out the definitions of these words:

Fecal Coliform

Non-pathogenic

Hepatitis

Dysentery

Cholera

Sanitary sewer

Combined sewer overflows

Water quality

Storm sewer

HANDOUT
MEMBRANE FILTER (MF) TECHNIQUE FOR FECAL COLIFORM

REFERENCE

USAPA. 1985. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, 1992.

EQUIPMENT

Filtration unit
Manifold
Autoclave
Microscope
Electric vacuum pump
Bunsen burner
Incubator or water bath

MATERIALS

Sample bottles, sterilized and labeled
Dilution bottles w/screw tops, 99 mL capacity
Test tubes, glass screw tops, 20-25 mL capacity
Containers for buffer solutions, glass w/screw tops, 1000 mL capacity
Culture dishes – petri-type, disposable and presterilized w/absorbent pads thick enough to hold 1.8-2.2 mL. of medium
Plastic bags - sterile, waterproof whirl-pak type for immersing samples (in petri dishes) into water-bath
Filter membranes, presterilized, individually wrapped membranes w/gridlines for counting colonies
Forceps or tweezers – with a rounded tip and without corrugations on inner sides of tips (sterilize by dipping in 95% alcohol and flaming)
Test tube rack, plastic coated and sterilized
Gloves, latex or rubber
Sampler, if needed
Safety goggles
Rubber boots
Waste disposal bottle
Thermometer
Pipettes, disposable and sterilized (1 mL and 10 mL).

REAGENTS

Culture media:
Use 2 mL packets provided. Keep media refrigerated. Check expiration date.

Buffer Solution:

a. Dissolve 3.4 g potassium phosphate monobasic (KH_2PO_4) in 50 mL distilled water.

b. Use pH meter and adjust pH to 7.2 by adding 1N NaOH and dilute to 100 mL with distilled water.

c. Dissolve 8.1 g magnesium chloride ($MgCl_2 \cdot 6H_20$) in ~100 mL water.

d. Add 4.0 mL of the magnesium sulfate solution and 1.0 mL of the potassium phosphate monobasic solution to 800 mL distilled water and autoclave for 30 minutes. This will be used as the buffered dilution water. Autoclave with the caps screwed on loosely. When cooled, screw caps tightly before storing.

ANALYSIS

1) Begin sample analysis promptly after collection. It should be processed within one hour if not kept cool, or up to 6 hours if it has been kept cool.

2) Before starting your analysis, pour some of the sample into a separate beaker to determine the pH (never put probes directly into the sample bottle). Based on background information on bacteria density in that sample at that particular pH, estimate the volume expected to yield a countable membrane and select two additional quantities representing one-tenth and ten times this volume respectively. Use sample volumes that will yield counts between 20 and 60 fecal coliform colonies per membrane. If the count is < 20, increase the sample size until a minimum of < 100 mL sample has been used. If 100 mL will not filter, use enough filters until enough sample has been used to add up to 100 mL. If the bacterial density of a sample is not known, filter several decimal volumes to establish the fecal coliform density.

3) Open the disposable petri dish and add the M-FC broth to the petri dish. Put cover of dish back on.

4) Start to filter the sample by unwrapping the base of the sterile filtration unit and putting it into the manifold (or filtration flask). Be careful not to touch the porous plate on the top.

5) Using sterile forceps, place a sterile filter over the porous plate of the base, grid side up. Be sure not to include the paper separator.

6) Carefully place the funnel unit over base; it is held in place magnetically. If less than 20 mL of sample is to be filtered, pour in about 50 mL of buffer solution.

7) Pipette amount of sample (minimum of 1 mL) with a sterile pipette directly into the funnel. Be careful not to touch the buffer solution with the pipette (use a bulb to pipette or a gun). Put the pipette back into the sample. Add enough buffer solution to make about 100 mL.

8) Start the vacuum pump and turn manifold to "ON." Filter sample. With filter still in place, rinse funnel by filtering three 20 to 30-mL portions of sterile buffer solution. Swish the buffer solution around in the funnel to make sure that sides are rinsed.

9) Remove the funnel and immediately remove filter with sterile forceps. Place it on the sterile pad in the petri dish with a rolling motion to avoid entrapment of air. Use the forceps to go around the edges of the membrane to make sure it will stick to the pad. Do not touch the center portion of the membrane. Put the cover back on.

10) Make sure that the petri dishes are marked with sample name, date, sample size and dilution. Place the petri dishes upside down on the counter. Run a duplicate sample using the same procedure as just described.

When doing a dilution series on a sample, filter the highest dilution first, then go to the next highest dilution using the same filtration unit, and so on, without washing or sterilizing the unit in between. When going on to the next sample, start with a sterile filtration unit. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtration.

If this happens, treat any further filtrations as a new filtration series starting with a sterile filtration unit. Remember also that each new sample and each new dilution within a dilution series requires a new sterile pipette. If large volumes, such as 50 and 100 mL, are to be filtered (i.e., river samples), use the graduation markings on the side of the filter funnel.

11) Place the petri dishes in Whirl-Pak waterproof bags, squeeze air out and close securely by rolling up the top of the bag. Place them upside down, and submerge them in water bath, incubating for 24 hours at 44.5°C (±0.2°C). Make sure that the dishes are completely submerged, and check the temperature of the water — *making sure it is within the range*. Note the time when the dishes are put into the water bath. Place all prepared cultures in the water bath within 30 minutes after filtra tion.

12) After 24 hours it is time to read the results of the test. Samples are removed from the water bath and allowed to sit for 5-10 minutes. Non-fecal colonies that have also grown during incubation will fade in color during this time. Fecal colonies will appear blue or blue-green and have a stippled, metallic-sheen surface.

13) Note background bacterial counts on the plate by counting colonies in a representative grouping of 4 grid squares on the filter pad. Colony number should be interpreted as follows:

Five (5) colonies or less = Low density
6-15 colonies = Medium density
16 or more = Heavy density

14) Compute density of fecal coliforms in a sample (NOTE: use only coliforms from membrane filters that produced colony counts within the desired range of 20-60 colonies). Report the density as fecal coliforms/100 mL, using the following equation:

$$\text{Fecal coliform colonies/100 mL} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample filtered}}$$

QUESTIONS

If you did not obtain appropriate numbers of colonies on your sample plates, what might be the reason? What would you do to correct this?

HANDOUT
MOST PROBABLE NUMBER (MPN) METHOD FOR FECAL COLIFORM

REFERENCE

USPHS, 1985. 18th Edition Section. *Standard Methods for the Examination of Water and Wastewater*. Pages 9-66 to 9-80.

EQUIPMENT

Autoclave – set for 121°C and 15 lbs. PSI – follow manual for loading and use
Air incubator – temperature set @ 35° (±0.5°)C. (monitor temp. on 24-hr. chart)
Water incubator – set at 44.5° (±0.2°)C. (monitor temp. on 24 hr. chart)

MATERIALS

Sample bottles, sterilized and labeled – wide mouth with screw tops w/screw tops, (99-mL capacity)
Test tubes, glass screw tops (20 to 25-mL capacity)
Containers for buffer solutions, glass w/screw tops, (1000-mL capacity)
Test tube rack, plastic coated and sterilized
Gloves (latex or rubber)
Sampler (if needed)
Safety goggles
Rubber boots
Waste disposal bottle
Thermometer
Pipettes, disposable and sterilized (1 mL and 10 mL)

NOTE TO INSTRUCTORS: *Reagent preparation should be done well in advance of this lab (at least one week). It would be advantageous to practice the analysis prior to having students doing it.*

REAGENTS

Culture Media:

Lauryl Tryptose Media, commercially made by HACH

- Single strength with MUG, in individual screw cap tubes with inverted Durham tubes for gas collection (#22175-15). This media strength is acceptable for dilution series beginning at 1 mL or less and using 1 mL of solution per dilution tube.

- Concentrated media without MUG, in individual screw cap vials with inverted Durham tubes for gas collection (#21014-15). This media strength is acceptable for dilution series beginning at 10 mL of solution per dilution tube.

E.C. Media, commercially made by HACH

- Single strength, with MUG, in individual screw cap tubes with inverted Durham tubes for gas collection, (#22824-15). This media can be used in conjunction with a florescent light for determining the presence of *E. coli*.

Buffer Solution:

a) Dissolve 34 g potassium phosphate monobasic (KH_2PO_4) in 500 mL distilled water.

b) Use pH meter and adjust pH to 7.2 ($\pm$0.2) by adding 1 g NaOH and dilute to 1L with distilled water.

c) Dissolve 81.1 g magnesium chloride ($MgCl_2 \cdot 6H_20$) in ~ 1 L distilled water.

d) Add 1.25 mL stock of the stock phosphate buffer solution and 5.0 mL of the magnesium chloride solution to 1 L distilled water.

e) Dispense in amounts that will provide 99 ($\pm$2.0) mL or 9 ($\pm$0.2) mL after autoclaving for 15 minutes. Autoclave with the caps screwed on loosely. When cooled, screw on tightly before storing.

ANALYSIS

1) Samples should be processed within one hour if not kept cool, within 6 hours if they have been in a cooler with ice.

2) Pour some of the sample into a separate beaker to determine pH (never put probe directly into sample bottle). Based on previous information on bacteria density in the sample at that particular pH, estimate the dilution series expected to yield 5 positive vials at the highest concentration, and lesser positive reactions in the following two dilution series. If the density is not known, run several decimal volume dilution series to establish the fecal coliform density.

3) Arrange the Lauryl Tryptose fermentation tubes in rows of five tubes in each rack. Use a minimum of three rows of five tubes each.

4) Prepare dilution series as outlined in the following section. Shake each sample and dilution vigorously at least 25 times.

5) Inoculate each of the five tubes in the highest concentration with 1 mL of the sample. (If you are using the single strength media, to use 1 mL of inoculum. If the concentrated media is used, inoculate with 10 mL of sample).

6) Proceed to the next series of five tubes and add 1 or 10 mL of the 1:10 dilution. Proceed in this fashion until there are at least three series of five tubes with descending concentrations of the original sample.

7) Incubate at 35°C (± 0.5°C). After 24 (±2) hours, shake each tube gently and examine it for gas and shades of yellow color (which indicates an acidic reaction). If there is no gas, growth, or acidic reaction, re-incubate. Check at the end of 48 (±3) hours. Record the presence or absence of gas or growth.

CONFIRMATION TEST

1) Submit all presumptive fermentation tubes showing any amount of gas or growth or acidic reaction within 48 hours to the confirmation test.

2) Rotate the positive presumptive tube gently. Using a 3 mm diameter metal or disposable sterile loop, transfer growth from the presumptive tube to the EC broth tube. Place the tubes into the bath within 30 minutes of being inoculated.

3) Incubate the inoculated EC broth tubes in a water bath at 44.5°C (±0.5°C) for 24 (±2) hours. The tubes in the water bath are to be immersed to the upper end of the media.

4) Within 24 hours, interpret your results as follow:

POSITIVE REACTION = Gas production indicates with growth of fecal coliforms.

NEGATIVE REACTION = No growth, or growth without gas production (indicating a source other than the digestive tract of warm-blooded animals).

5) Calculate the MPN from the number of positive EC broth tubes.

- Select 3 dilution tubes that yield positive results in all five tubes in the least dilute concentration and the two next succeeding higher dilutions. Use the results from the three volume set in computing the MPN/100 mL

- If the least dilute sample series does not yield all 5 positive reactions, use the next highest positive three-volume series.

MPN Method

Data sheet

Date	Team Members	Sample Location	Sample # and Rep #	Counted Colonies/100 mL H_20	Dilution Factor	Total # Fecal Colonies

MPN Method

Data Sheet

Date	Team Members	Sample Location	Sample # and Rep #	Counted Colonies/100 mL H_20	Dilution Factor	Total # Fecal Colonies

NOTES FOR INSTRUCTORS

Learning Objectives:
The student understands the relationship between fecal coliform testing as it pertains to water quality.

Learner Outcomes:
The student demonstrates fecal coliform testing using membrane filter (MF) and most probable number (MPN) assays.

Field samples:
Samples for this field exercise were obtained at a local pulp mill and treatment plant. These have been found to be effective field sites for this exercise.

Background information:
There are different types of sewer systems that might carry fecal contamination into a water body. Sanitary sewer piping contains raw sewage and leads to a sewage treatment plant where there is mechanical filtration (primary), biological respiration/decomposition (secondary), and possibly another treatment (tertiary) before the sewage is discharged into a water body. Storm sewers carry surface runoff from rainfall only. However, surface runoff carries whatever is on the land along with the water into the storm drains and into a local water body. This is known as stormwater runoff and may contain fecal contamination. Combined sewer overflows (CSO) are sewer systems where storm and sanitary sewers are connected and which allow raw sewage to overflow into storm sewers during times of high runoff due to rain and/or flooding. These systems are being eliminated for obvious reasons and are no longer being built.

State water quality regulations restrict the number of bacteria that are legally acceptable in a water body for a given use. For instance, in water with an "AA" rating, drinking water is allowed no detectable fecal bacteria in samples. In bays where shellfish are grown, the number of fecal colonies allowable in a sample (100 mL of water) is 50. In waters where swimming is likely, the level is 200 colonies/100 mL of water (sample).

It is possible for organisms found in pulp and paper effluents to give a positive test for fecal coliforms. These bacteria originate and thrive, given a specific environment, from organic sources other than animals. Four kinds of coliform bacteria are: *Escherichia coli, Klebsiella, Enterbacter,* and *Citrobacter*.

Application:
One reason that fecal coliform is tested routinely is because it is a relatively easy and inexpensive test that gives reliable results. If a particular problem requires it, further testing can be implemented. For instance, one can determine the source of the fecal bacteria, whether it is of human origin or comes from a specific animal.

When sampling bacteria, it is a good idea to collect numerous samples to minimize variability. Sometimes it is advisable to compare wet and dry weather samples in order to identify the source.

Biological Oxygen Demand

INTRODUCTION

Biological oxygen demand, or BOD, is a measure of how much organic material is in a water sample utilizing oxygen in the process of decomposition. **Organic material** includes detritus (dead, decaying plants and other organisms), as well as organic chemicals from industrial sources, and wastewater.

This organic material can be increased in water by adding nutrients (such as fertilizer runoff from farms and lawns) and by direct input of organic material from wastewater and industrial waste. Sources of nutrients arise from either point or nonpoint source pollution. Human and animal wastes are prime sources of nutrients when they enter nearby water bodies. Water that contains high levels of nutrients accelerates plant growth, and when plants die, this eventually leads to accelerated decomposition processes (aerobic respiration of organic material). **Aerobic bacteria** feed on the organic material, using oxygen in the process, thus depleting the supply of oxygen.

BOD is the quantity of oxygen needed to satisfy microbial requirements in the breakdown of organic materials.

To further illustrate:
Increased Nutrients Increased Plant Growth—— Plants Die (Increased

Organic Material)* —— Aerobic Bacteria/Microbes feed on organic material

(Increased Decomposition)** —— Depletes Dissolved Oxygen (DO)

* Source of Biological Oxygen Demand
** Creates Biological Oxygen Demand

Thus, water pollution, in the form or organic waste or nutrients, affects DO. Overall, a certain population of microbes is necessary to decompose organic material; however, if the population becomes excessive, low DO will result. This not only affects water quality, but also fish habitat and viability.

BOD is measured by an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of a water sample. The test measures oxygen consumption for five days at 20°C.

PROCEDURE

Students will work in teams of 2-3. You will be given water samples that have been obtained from either the local sewage treatment plant or pulp mill bio-ponds. Each team will be responsible for creating their own data sheet for recording BOD data.

BOD is measured in mg/L or parts per million (ppm) (as is DO). Calculations must include any dilution factor utilized in the test. The BOD test is performed similarly to that of DO – but note that the sample must be incubated for five-days in a controlled temperature water bath or dark, room temperature environment.

Follow instructions on the *BOD Analysis Handout* included.

As you do your analysis, note the following:

- Care must be taken at all times to ensure that no air is entrapped in the sample bottles. Prior to incubation, place a water seal of some sort on the bottle.

- An excess of acidity or alkalinity will interfere with the determination. All samples must be neutralized to pH of 6.5 to 7.5 using 1.0N sulfuric acid or 1.0N sodium hydroxide prior to testing. Care must also be taken to ensure that all equipment is clean and proper laboratory procedures are used. All glassware, stills and carboys should be thoroughly cleaned at regular intervals.

- Samples for BOD analysis may degrade significantly during storage between collection and testing, resulting in low BOD values. Minimize reduction by analyzing promptly or by cooling to near freezing during storage. Even at low temperatures, storage time should be kept at a minimum. Analysis should be initiated within two hours of collection, if not cooled. If cooled to 4° C or below, testing should start within six hours of collection.

DEFINITIONS

Define the following terms:

Biological oxygen demand

Seed culture

Organic

Inorganic

Point pollution

Non-point pollution

PRODUCTS

During the lab.

- Design a data sheet for recording BOD data.
- Maintain sterile technique.
- Clean up spills and wash hands immediately if there is skin contact.

Following the lab.

- Clean up all equipment, benches; wash utensils, bottles etc.
- Turn in both a blank and completed worksheet with BOD analysis for your samples.

EQUIPMENT

Air incubator, capable of maintaining samples at 200° (± 1°) C, with all light excluded
Wheaton 306-mL BOD bottle with flared top and ground glass stoppers
Carboy, clear glass, 9 L, calibrated in .5 L intervals
ORION 250A pH meter
Siphon tube. Construct so that water in the .5 L calibrated carboy can be siphoned into individual BOD bottles without introduction of air bubbles
Dissolved oxygen analyzer, electrode membrane type for DO measurement (YSI Model 50B with a 5905 probe with auto-stirrer)
Magnetic stirrer and stir bars
10-mL graduated pipets, calibrated in 1/10 mL increments
25 mL graduated pipettes, calibrated in 1/4 mL increments
200 mL, 300 mL, and 600 mL glass beakers
100 mL and 250 mL graduated cylinders
Water distiller and holding tank
Water seal caps for BOD bottles

REAGENTS

Phosphate buffer (LabChem Inc., LC18500-1, pH 7.2; APHA for BOD)
Magnesium sulfate (LabChem Inc., LCI6500-1, 2.25%; APHA for BOD)
Calcium chloride (LabChem Inc., LC12730-1, 2.75%; APHA for BOD)
Ferric chloride (LabChem Inc., LC14300-1, 0.025%; APHA for BOD)
1N sulfuric acid
1N sodium hydroxide
Glucose-glutamic acid solution (HACH BOD Standard Solution Ampules, #14865-10)
Dilution water
Seed material (unchlorinated, municipal sewage)

REFERENCE

Standard Methods, 19th Edition. 1995. Method 5210 B. 5-Day BOD Test, page 5-3.

HANDOUT
BIOLOGICAL OXYGEN DEMAND (BOD) ANALYSIS

1. Prepare a BOD data sheet.

2. Prior to each test series, a sufficient quantity of unchlorinated sewage should be obtained from the sewage treatment plant. Prior to running seed control and seeding sample bottles, recap and homogenize (shake well).

   NOTE: *When excess seed is discarded and the labware is prepared for dishwashing, dilute excess and liberally disinfect labware with disinfectant.*

!CAUTION!: *Domestic sewage may contain harmful organisms. Familiarize yourself with handling biohazards.*

3. Pour 75-100 mL or appropriate aliquot of a well-shaken BOD sample into 200-mL beaker. Add Teflon/poly-ethylene coated stir bar.

4. Let samples suspected of containing low chlorine residuals stand for one to two hours to dissipate chlorine. Samples containing high amounts of chlorine residual should be treated with sodium sulphite (see *Standard Methods*, 19th Edition, page 5-4).

5. Neutralize the sample to between 6.5 and 7.5 pH with NaOH and H_2SO_4 as required. Use care to not dilute the sample by more than 0.5%. Samples must be 20° (+ 1°) C prior to neutralization and any adjustment of the pH recorded. Use approximately five drops/100 mL = 0.5% dilution.

6. Set up clean, distilled water-rinsed BOD bottles in an appropriate order for testing. Include three bottles for dilution water blank; two bottles for seed correction; three bottles for sugar standard; and bottles as necessary depending on sample dilution rates.

   NOTE: *Increase all tests to four bottles if the Winkler Method is used.*

7. Pipette correct aliquot of samples into appropriate BOD bottles. Aliquot may vary according to sample and seed strength. Sample volume should be enough to deplete the DO by at least 2.0 mg/L and still leave at least 1.0 mg/L residual after 5 days of incubation.

8. Pipette sugar standard. (Use only if there is no HACH Sugar Standard.) Standard HACH aliquot is three mL per bottle.

9. Pipette Alpha-Trol BOD standard. Four (4) mL per bottle is the appropriate aliquot.

10. Pipette the appropriate quantity of seed to achieve a 0.2 mg/L to 0.6 mg/L uptake of oxygen in the dilution water into the "seed" bottles. This quantity should be enough to cause 40% to 70% oxygen depletion in five days. Use two bottles (same set on and off) for the Meter Method and four bottles (one set on and one set off) for the Winkler Method.

11. Pipette appropriate aliquot of seed into all BOD bottles except the dilution (BLANK) water bottles, as determined by using Table 1 (see *Standard Methods* text, *Method 5210B*; 5-Day BOD Test for the appropriate aliquots of seed to deliver).

12. Obtain dilution water and add the four buffers – based on 1 mL of buffer per liter of dilution water in 9 L carboy. Shake vigorously for one minute.

13. Siphon dilution water into BOD bottles. Use two (2) bottles for initial DO measurement. Stopper the other two uncontaminated samples for checking quality of dilution water check at the end of five days. For QA purposes, if a depletion of 0.2 mg/L or more occurs, look for a cause. Flag data on your report if DO depletion is elevated above 0.2 mg.

14. Measurement of the initial DO is run on all samples. They are then stoppered, water sealed, and placed in an incubator at 200° C ± l °C, for five days.

15. On a daily basis, three BLANK and three SUGAR bottles should be prepared. Measure DO immediately in one of the BLANKS and one of the SUGAR bottles. After measuring the DO in the BLANK, use that bottle to get a pH and temperature value for the dilution water. Stopper and water seal the other two, then incubate. This is the BLANK and SUGAR BOD. This practice enables an initial DO calibration reading to be acquired without contaminating the second set of bottles.

16. After incubation for five days at 20°C in the dark, remove the bottles from the incubator and run the appropriate test for DO. Record the incubator temperature daily.

17. Calculate BOD-5 values.

18. Acid-wash BOD bottles as necessary to minimize contamination of samples.

19. Once a week, run a DO meter versus Winkler comparison to verify accuracy of the meter. Also test the dilution water for dissolved solids content to check water quality.

CALCULATIONS

A. Dissolved Oxygen (DO)

1. *Winkler Titration*

   Each mL of 0.025 N sodium thiosulfate is equivalent to 0.2 mg DO. Therefore, when the 200-mL of sample is taken, each mL of titrant represents 0.2 mg DO/ 200 mL of sample, or 1 mg DO/L of sample.

2. *DO Probe*

Read directly from meter in accordance with manufacturer's instructions.

B. Seed Correction (SC)

SC mg/L = DO Depletion X Correction Factor (CF)

NOTE: *Look up CF on Table 1, Seed Correction Factor Table (see Standard Methods text, Method 5210B; 5-Day BOD Test).*

C. BOD-5 of Seeded Sample

1. BOD-5 represents the net loss of DO, in five days, expressed in mg/L

2. BOD = ([DOI – DOF] - SC) / (SV/3)

where:

DOI = DO (mg/L) before incubation
DOF = DO (mg/L) after incubation
SC = sewage correction
SV = volume (in mL) of sample tested
SC = 0 (for unseeded samples)

NOTE: *Multiply mg/L by the dilution ratio if sample is diluted.*

Example for a 1:15 dilution:

100 mg/L X 15 = 1500 mg/L

REPORTING RESULTS

- Report results to the nearest mg/L.
- Do not report more than three significant figures.
- If a nitrification inhibitor is used, state so on your worksheet. This is not the normal practice.
- Report any unusual testing conditions, such as frozen samples and BOD's, done in time periods other than five days.

NOTES FOR INSTRUCTORS

Included here are descriptions for the preparation of dilution water and an alternative test for BOD. Instructors can involve students in these activities as they apply to the BOD lab.

I. Preparation of BOD Dilution Water

Because most wastewaters contain more oxygen-demanding materials than there is dissolved oxygen (DO) available in air-saturated water, it is necessary to dilute the BOD samples prior to incubation. This ensures proper and accurate measurements of DO depletion all the way to the end-point. (NOTE: *If you ran the same type of samples all the time you would know this already*.) However, since this is a learning lab, you do not already know into what range your samples will fall. Thus, adequate amounts of fresh, distilled dilution water should be kept on hand for running BOD tests with multiple dilutions. The water is conditioned or buffered immediately prior to use by the addition of a nutrient/buffer. This provides a suitable environment for bacterial growth. This "seed" helps the decomposition process take place, but this added bacterial nutrient necessitates proper maintenance and cleansing of the dilution water carboys and associated equipment.

Equipment

Carboys for distilled water
Still for preparing fresh water

Reagents

A. Reagent grade sulfuric acid

B. Nutrients/Buffers

1. Phosphate Buffer: Dissolve (8.5 g KH_2PO_4, 33.4 g $Na_2HP0_4 \cdot 7H_20$ and 1.7 g NH_4Cl) in water and dilute to 1 L.

2. Magnesium Sulfate Buffer: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in 1 L distilled water.

3. Calcium Chloride Buffer: Dissolve 27.5 g $CaCl_2$ in 1 L distilled water.

4. Ferric Chloride Buffer: Dissolve 0.25 g $FeCl_3 \cdot 6H_20$ in 1 L distilled water.

Points to consider:

1. Maintain carboys clean and free of any bacterial growth.

2. As necessary, clean carboys with concentrated sulfuric acid and rinse well.

3. The carboy is then stored in a cabinet with a cotton plug and allowed to naturally condition for seven days when it is again placed in service to run BOD series. Do not fill the carboy into the neck area or initial DO levels will be reduced.

4. Maintaining the cabinet temperature in the 20°C range will provide sufficient initial DO to run an accurate test. Lower cabinet temperatures will raise initial DO, while higher temperatures will reduce DO levels.

5. Water quality can be seriously affected by major gas leaks within the area of a lab. To minimize this affect and maximize natural oxygen conditioning, the cotton plug is used. However, a technician must always be aware of the possibility of unwanted gases being absorbed.

6. Once a week the quality of the water contained in the carboys, prior to the addition of the buffers/nutrients, is checked for levels of dissolved solids by running a conductivity measurement. This value is logged on the BOD sheet. If the conductivity of the water is greater than 2.0 mhos, check the distilling unit, source water, and pre- and post-filtering units.

Safety Precautions

- Use extreme care and caution when performing the acid cleaning.

- The nutrients are labeled as POISON and should be handled accordingly.

- *Carboys are heavy and bulky and should be handled carefully!*

II. Glucose-Glutamic Acid Test (BOD Check)

NOTE: *To be used only if there is no HACH BOD Standard available.*

The BOD test can be influenced greatly by numerous toxicants, poor seed material, or poor technique. Distilled waters are relatively inactive, causing low results in BOD analysis. Therefore, the technician should check water quality, seed effectiveness, and analytical technique by making BOD measurements on pure organic compounds. The recognized measurement is the *Glucose-Glutamic Acid check.*

Reagents and Equipment

D-Glucose Anhydrous ($C_6H_{12}0_6$) granular preferred
L-(+) Glutamic Acid
2000-mL volumetric flask
Hot plate
Mettler scale
Desiccant
Oven
Thermometer (20-100°C)
Glass stir rod
50-mL beaker
Magnetic stirrer and stir bar

Points to consider:

1. Rinse all glassware with demineralized water and maintain cleanliness throughout the process.

2. Place sufficient aliquots of glucose and glutamic acid into the oven for one hour to dry at 103 degrees Celsius prior to measurement.

3. Clean, rinse and fill a 2000-mL volumetric flask with 1000-1500 mL demineralized water and place in refrigerator to bring temperature down below 20°C.

4. After one hour, desiccate the glucose and glutamic acid, then measure .30 g of each into a clean and dry 50-mL beaker using the Mettler scale and analytical measuring techniques.

5. Add demineralized water to the glucose-glutamic mix and dissolve with the addition of heat.

6. Add the sugar mixture to the volumetric and place on the hot plate to bring temperature up to 20°C, constantly mixing.

7. Technician runs a five-day BOD on a 2% dilution of the glucose-glutamic acid standard.

8. Use 7 mL and 9 mL samples added to a standard 300 mL BOD bottle and dilute to volume with "seeded" dilution water.

9. If the five-day 20°C BOD value of the sugar standard is outside the range of 198 +/- 30.5 mg/L (167.5 to 228.5), reject any BOD determinations made with the seed and dilution water and seek the cause of the problem.

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pH, Alkalinity and Hardness

INTRODUCTION

pH is defined as the concentration of hydrogen ions in water expressed as moles per liter (Moles/L = Moles · L^{-1}). It is mathematically defined as the negative log of the hydronium ion (H_3O^+). It is a measurement of the concentration of hydrogen (H^+) ions in a solution, in this case, water. Actually, it is the chemical activity of those ions that is of more interest than just the concentration.

Although *activity* and *concentration* of ions in a sample may be equitable in very weak solutions, they become less so as other ions, such as minerals, are introduced. Although pH is relatively easy to measure, it is an important water quality test because it is indicative of other conditions that can influence water quality and the ability to support fish or other organisms.

Alkalinity is defined as the ability of water to neutralize acid; it is the sum of titratable bases. The alkalinity of a substance may be quite different from the pH. The presence/activity of hydroxyl ions (OH^-) influences alkalinity. Alkalinity is an important indicator of the need for water treatment. Hydroxyl ion concentration is measured as pOH and is expressed as mg/L of equivalent calcium carbonate. The test for alkalinity is a titration with sulfuric acid to a pH of 8.3 (phenolphthalein end point), or 5.1, 4.8, 4.5, or 3.7 (total alkalinity), depending on the alkalinities of water samples.

Hardness is presence/absence of calcium and magnesium ions in the water solution (or sample). Hardness is measured as water's ability to precipitate soap (in mg/L). Minerals are suspended in solution at lower pH; at higher pH they are insoluble and precipitate out of solution.

pH, alkalinity, and hardness will be measured for water samples using methods described in this laboratory.

APPLICATION

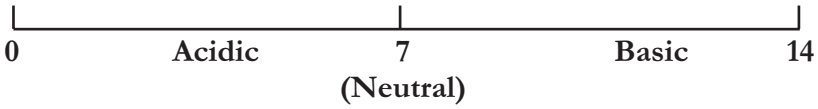
Most organisms have specific pH requirements. Some organisms, such as bacteria, can exist in a wide range of pH measurements, while other species, such as young trout, require a very specific pH and will die if the pH is altered out of that narrow range. Acid rain originates when chemical compounds such as SO_2 and NO_2, primarily from auto emissions and power plants fueled by coal, are released into the atmosphere. Alkalinity is of concern when determining if water is suitable for irrigation. Hardness is also a concern to pipe integrity as the build-up of mineral deposits on

metal piping can eventually restrict the flow of liquid through it. Washing clothes requires more soap in hard water and there is often a residue that remains on the clothing which dulls brightness. Sometimes you can even taste the difference in drinking water when the water is hard. Often iron accompanies the calcium and magnesium in hard water, which gives water a "metallic" flavor.

MEASUREMENT

The pH scale ranges from one (1) to fourteen (14) with the value of seven (7) as neutral. You've probably heard of shampoo being "pH balanced" – this means little in actuality, but it is meant to indicate "pH balanced for your hair." Body pHs range from extremely acid (stomach acid) to near neutral blood pH. Among household products, examples of those with high pH (considered "basic"), include products like baking soda, ammonia and bleach. Examples of low pH "acidic," include vinegar, cola and battery acid.

pH scale:



Since pH is actually an exponent of the concentration of the hydrogen ion concentration, it is important to note that a unit change of pH from 7 to 6, for example, is really a ten-fold change in how acidic or basic the substance is. Thus, a lake with a pH of 6 is 100 times (10x10) more acidic than one with a pH of 8.

PROCEDURE

Using clean, deionized water-rinsed, 1.0-L Nalgene containers, collect triplicate water samples for pH, alkalinity and hardness tests at three locations (one lake surface, two creek locations).

In teams of two-three students, read the instructions thoroughly for each test, and make preparations for collecting field samples. Each team will do readings on three replicate samples ("reps") at each of three sites. NOTE: *Each team member should complete each test.*

Record your collective results on the data sheet provided.

A. pH Measurement

Equipment

HACH Portable pH Meter, Model, 43800-00
HACH Combination Electrode, Model 44300
HACH Combination Electrode, w/temperature sensor, Model 44200
CORNING pH/Temperature Meter 4
ORION pH/Millivolt Meter, Model 811

Materials

Safety goggles
Rubber boots
Meter/probe
Buffers
Squirt bottle of deionized water
Paper towels or Kimwipes

NOTES: *Before using the pH meter it must first be calibrated using fresh buffers with known pH. For calibration/standardization purposes, commercially-available buffers are available. They will be used to standardize the meter.*

If you expect your sample to fall in the acidic range, use buffers of 4.01 and 7.0. If you expect more alkaline results, use buffers of 7.0 and 10.0 for calibration purposes.

Also:

- Make sure that all probes/electrodes are plugged into the correct meter and properly seated
- Check the solution in the reference electrode to see that it is full
- Make sure the probe is clean and the electrode is not cracked or broken

The Portable HACH pH Meter: Model 43800-00

Calibration

1) Press the POWER key. The display will light.

2) Press the pH key.

3) Press the AUTO/MANUAL key. The AUTO indicator will light. The S1 and pH indicators will flash. Zeros will appear in the display.

4) Place the electrode into a pH 4.01 buffer solution and press the DISPENSER button. For the best accuracy, allow 30 seconds to elapse before performing step 5.

5) Press the STANDARD key and wait until the pH indicator stops flashing. The S2 indicator will begin flashing. The actual pH value will appear in the display. If the solution temperature deviates from 25°C, the display will show the actual pH (and not 4.01). (NOTE: *The temperature display will show the actual solution temperature if the temperature probe is connected.*)

6) Rinse the electrode with deionized water and blot dry with a paper towel. Place the electrode into a pH 7.00 buffer solution and press the DISPENSER button. For best accuracy, allow 30 seconds to elapse before performing step 7.

7) Press the STANDARD key. S2 will stop flashing. Wait until the pH indicator stops flashing. The actual pH value will appear in the display. If the solution temperature deviates from 25°C, the display will show the actual pH and not 7.00.

8) Press the pH key. Rinse the electrode with deionized water (or a portion of the sample to be measured) and blot dry with a paper wipe. Place the electrode into the sample and press the Dispenser Button. The meter now measures pH.

9) To review, press the REVIEW key. Press the REVIEW key through its increments to return to measuring pH. In the automatic buffer recognition mode, buffer solutions pH 4.01, 7.00 and 10.00 may be used in any sequence.

Using the portable HACH pH meter

1) Press the POWER key to turn on the meter.

2) Press the pH key. The pH indicator will light.

3) Rinse the electrode thoroughly with deionized water (or a portion of the sample to be measured) and blot dry with a paper towel.

Press the DISPENSER button once to dispense electrolyte. When the PROBE INDICATOR stops flashing (indicating a stable condition), read the sample pH from the upper display.

Analysis

After the meter is calibrated with the proper buffers, take a reading by inserting the probe attached to the meter into the water. Take enough time with your reading that the needle stops moving or the digital read-out stops changing or flashing (depending on which meter you have used). Record the results. If you are using the lab pH meter, a more accurate reading is obtained if the sample is being stirred. The sample should be placed on a magnetic stir plate with a stirring bar in the beaker holding the sample.

Record the readings you've obtained from your meter on the data sheet provided.

B. Alkalinity Measurement

Materials

Alkalinity kit
250-mL Erlenmeyer flask
100-mL graduated cylinder

Methods

1) Attach a clean straight-stem delivery tube to a 1.6N Sulfuric Acid Titration Cartridge. Twist the cartridge onto the titrator body.

2) Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3) Take a water sample by filling a clean 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask.

4) Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix. If a pink color does not develop, proceed to Step 7.

5) If the sample turns pink, titrate the sample with 1.6N sulfuric acid while swirling the flask. The end point is a change from pink to colorless.

6) Read and record the concentration of phenolphtholein alkalinity (as mg/L $CaCO_3$) from the digital counter window.

7) Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the same sample and swirl to mix.

8) Continue to titrate with the 1.6N sulfuric acid solution until the color changes to a light greenish blue-gray (pH 5.1), a light bluish pink-gray (pH 4.8) or a light pink (pH 4.5). Read and record the total alkalinity (as mg/L $CaCO_3$) from the digital counter window.

C. Hardness Determination

Equipment

Hardness kit

Materials

250-mL Erlenmeyer flask
100-mL graduated cylinder

Methods

1) Attach a clean straight-stem delivery tube to a 0.800M EDTA Titration Cartridge. Twist the cartridge onto the titrator body.

2) Flush out the delivery tube by turning the knob until titrant begins flowing from the end of the tube. Wipe the tip and reset the counter to zero.

3) Take a water sample by filling a clean 100-mL graduated cylinder to the 100-mL mark. Pour the sample into a clean 250-mL Erlenmeyer flask.

4) Using the 1-mL calibrated dropper, add 1-mL of Buffer Solution Hardness 1, and swirl to mix.

5) Add the contents of one ManVer® 2 Hardness Indicator Powder Pillow and swirl to mix.

6) Titrate the sample with the 0.800M EDTA solution while swirling the flask until the color changes from red to pure blue. Titrate slowly toward the end point to allow time for the reaction and color change to take place, especially for water samples below 20°C (68°F).

Read and record the total hardness (as mg/L $CaCO_3$) from the digital counter window.

PRODUCTS

1. Obtain pH, alkalinity and hardness readings at various sample locations or on samples provided
2. Use proper lab technique
3. Turn in your data and definitions sheets
4. Clean up all equipment and lab space, turn off instrumentation, and put all equipment and supplies away in their proper place
5. Write a short team report and turn it in at the beginning of the next lab period. The report should include the following:

    - The difference between precision and accuracy
    - Advantages/disadvantages of each method

Definitions Worksheet

Define the following terms:

Ion

Buffer

Calibrate

pH

Alkalinity

Hardness

pH, Alkalinity and Hardness
Data Sheet

Samplers' Names_____

Date/Time	Location	pH	Alkalinity (mg/L)	Hardness (Mg/L)	Comments/Observations
	Lake_____				
	Rep #1				
	Rep #2				
	Rep #3				
	Creek_____				
	Rep #1				
	Rep #2				
	Rep #3				
	Creek _____				
	Rep #1				
	Rep #2				
	Rep #3				

NOTES FOR INSTRUCTORS

Learning Objective: Students will understand the concepts of pH, alkalinity and hardness as they relate to water quality and healthy fish habitat.

Learner Outcomes: Students will demonstrate the ability to conduct tests for pH, alkalinity and hardness using colorimetry and meter methods.

End Points in Alkalinity Measurements

The following end points are recommended for determination of the total alkalinity in water samples of various compositions and alkalinities:

Sample Type	End Point
Alkalinity about 30 mg/L	pH 5.1
Alkalinity about 150 mg/L	pH 4.8
Alkalinity about 500 mg/L	pH 4.5
Silicates or phosphates	pH 4.5
Industrial wastes or complex mixtures	pH 3.7

To determine the indicator color at the total alkalinity end point, mix the contents of one pH buffer powder pillow (optional item) of the desired pH with 50-mL of demineralized water in a 250-mL Edenmeyer flask and add one Bromcresol Green-Methyl Red Indicator Powder Pillow. Use the color of the buffer/indicator solution as a reference when titrating samples.

When titrating to pH 3.7, use a Bromphenol Blue Indicator Powder Pillow in place of a Bromcresol Green Methyl Red Indicator, both in the reference solution and in the sample. The end point is a color change from purple through blue to green.

Nitrogen & Phosphorous: Ammonia, Nitrates, Phosphates

INTRODUCTION

As we learned in the Biological Oxygen Demand Lab, adding nutrients to water can affect ecosystems significantly. Nutrients in water bodies provide growth material (food) for all life forms dependent on that water body. A water body devoid of nutrients is unable to support fish, amphibians and other life forms. Conversely, an excess amount of nutrients can become a detriment to the health of the water body and its inhabitants. Two common nutrients found in excess in water bodies are nitrogen and phosphorous (N, P), which you might use to fertilize your lawn.

Nitrogen is found in several organic or inorganic chemical forms. Review "The Nitrogen Cycle." Ammonium (NH_3), ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are all components of the cycle. Each of these components can have a different influence on water quality.

Phosphorous, in the form of phosphate (PO_3), is a limiting factor for plant growth. An excess of this nutrient encourages so much plant growth that this vegetation, as it reaches the point in its growth cycle where it begins to decay, utilizes oxygen in the water, with resulting dissolved oxygen (DO) levels that are too low (or even nonexistent). This process is called **eutrophication**, and it ultimately destroys life forms in that water body.

For this exercise, we will be evaluating both nitrogen (ammonia, nitrites and nitrates) and phosphorous levels in water samples.

Application

Nitrogen and phosphorous are primary nutrients required for growth and development of life forms, both plant and animal. Nitrates and phosphates become a potential problem when they run off the land into a water body, leach down through the soil into the water table, or somehow affect plants or animals utilizing a water supply.

In the case of nitrates, humans can be affected by a disease called *methamaglobanemia* from drinking water high in nitrates. Mostly, this condition affects infants (up to 6 months of age) that drink baby formula made with water high in nitrates. The condition can be corrected by improving the water source.

Common sources of nitrogen and phosphorous include fertilizers, failing septic systems, and animal waste.

PROCEDURE

Measurement

Nutrient levels are usually measured in mg/L (in parts per million). They are determined using a colorimetric comparison.

Teams of 2-3 students will make necessary preparations to take field samples at 3 sites. The instructor will provide specifics regarding exact sampling locations on the day of your field exercise.

Methods

See exercises for phosphorous and nitrogen component analysis.

PRODUCTS

- Clean up equipment.
- Calculate sample results.
- Turn in data sheets.

Definitions Worksheet

Define the following terms:

Nitrogen cycle

Eutrophication

Denitrification

Mineralization

Colorimetry

NOTES FOR INSTRUCTORS

Learning Objective: Students will understand the influence of nutrients on water quality and fish health.

Learner Outcomes: Students will demonstrate use of a comparator and/or test kits to determine ammonia, nitrate and phosphate levels in water samples.

Measuring total phosphorous, ammonia nitrogen, and nitrate nitrogen:

We use handouts from the following source:

Blair, Jane. Ed. 1996. *Student Watershed Research Project (3rd Ed.).* A Saturday Academy Publication. Oregon Graduate Institute of Science and Technology. P.O. Box 91000. Portland, OR 97291-1000

Other sources include:

- Cauros, M. 1994. *Environmental Sampling and Analysis for Technicians (ESAT).* CTC Press, Inc. Boca Raton, FL. 320 pp.

- Mitchell, M.K., and W.B. Stapp. 1996. *Field Manual for Water Quality Monitoring: An Environmental Education Program for Schools*. Kendell/Hunt Publishing Co., Dubuque, Iowa. 77 pp.

Turbidity & Total Solids

INTRODUCTION

Turbidity is an expression of how clear a water sample is. When you look at different water samples, some may appear totally clear, while others are cloudy, or even opaque under some circumstances. This is because they have differing capabilities to transmit light due to particulates that are suspended in the water. These particulates are called **total solids**. Suspended solids may eventually settle out (to the bottom of the water body, or in our case, a sample) – these *settleable solids* will settle out depending on their weight and size.

Application

The concern for turbidity is widespread. Food products that may ultimately be consumed need to be prepared with clear, clean water. Numerous manufacturing processes also require a high degree of clarity. Turbidity can also affect photosynthesis in natural waters that, of course, contributes to overall water quality. It is a large concern of hatcheries, which use filtering processes, because too much particulate can smother incubating eggs or clog gills of emergent fry.

Turbidity-treatment processes include those that coagulate, settle particulate, or filter surface waters.

Measurement

A **turbidimeter** operates on the principle of **nephelometry** – where the meter measures the amount of light that is scattered from a directed beam by particles suspended in the sample. The units of measurement are *nephelometric turbidity units* (NTU) or *Jackson turbidity units* (JTU). Particulate parameters, such as color, particle size, and particle shape in a water sample will affect turbidity measurement.

Total solids are measured in water samples by determining their weight differential. The sample wet weight (in a tared container) is recorded, as is the dry weight, after all moisture is driven off, by placing the sample in a drying oven for an adequate amount of time. The dry weight divided by the wet weight gives a percentage of total solids by weight.

PROCEDURE

Equipment & Materials

Turbidimeter	Nalgene sample bottles
Secchi Disk	Drying oven
Suction flask	Pan balance
Vacuum pump	250-mL beaker
Imhoff cone	Gelman glass filter paper (.048 microns)
Dessicator	Ring stand
Funnel	

Teams of 2-3 students will collect field samples at 3 local sites. Samples will be analyzed for all parameters in the lab except Secchi disc measurements which will be taken in the field.

Sample preparation is minimal when performing a turbidity measurement. Sufficient sample, at least 1.5 L, must be obtained to perform both turbidity and total solids exercises. At a minimum, replicate samples should be taken at each station.

Formazin is used to standardize or calibrate the turbidimeter. When properly mixed, it is uniform in the number, size, and shape of its particles, thus making it an ideal turbidity standard. The unit of measure, and thus the calibration of this instrument, is Nephelemetric Turbidity Units (NTU) based on Formazin. Calibration samples may be obtained by diluting Formazin stock suspension using "turbidity-free" water. For our exercise, we will use already prepared Formazin standards purchased from a supplier.

NOTE: *Detailed procedures are provided as handouts at the end of this lab.*

Methods

Turbidity. The meter itself must be calibrated prior to each day's use and periodically checked to obtain correct readings.

Settleable solids. Set up the Imhoff cone on a ring stand.

Suspended solids. Set up a vacuum assembly by attaching a piece of rubber tubing between a suction flask and the side spigot on the water tap. Place a funnel in the top of the flask and line it with filter paper.

Dissolved solids. Evaporate a known amount of the filtrate from the suspended solids test to get dissolved solids.

Total solids. Add settleable, suspended and dissolved solids together to get total solids.

Analysis

Turbidity. Measurements are read directly from the meter. Samples should be shaken vigorously before taking a reading. Samples should be read within 24 hours of being taken. If this is not possible, keep them in a dark place to avoid bacterial or algal growth that can affect the reading.

When using the Secchi disk, lower it off the downwind side of the boat. The disk is lowered into the water on a graduated line. Take your reading from directly above the disk. The reading is taken off the graduated line at the point where the disk just barely disappears from view.

Settleable Solids. Pour a shaken sample of known volume (1 liter) into the cone and allow settling for 45 minutes. Stir the edges to keep residual sample from clinging to the sides of the cone. Take readings every few minutes and record the graduated unit to which the solids have settled. This will be evident by the "layered" look in the cone. Units are ml/L. Follow the procedures in the handout provided for detailed instructions on this method.

Suspended Solids. Place enough filter paper (one for each sample or replicate) in a drying oven for several hours prior to doing this part of the lab. When removed from the oven, the paper should be placed in the dessicator and allowed to cool. Weigh and record the weight of each filter paper; then place them in the funnel on top of the suction flask.

Take a known sub-sample (1.0 L, 250 mL, or 500 mL) used in settleable solids and pour it through the filter paper that you weighed and placed in the funnel. Turn the tap water on to create a vacuum which will help pull the sample through the filter paper. Place the filter (with residue) back in the drying oven for several hours (160°F). Weigh periodically during the drying process until no further weight loss is noted. (This weight loss is due to the release of moisture from the filter disc.)

Dissolved solids. Take a known amount of the filtrate from the suspended solids (after filtering) and place it in a tared beaker. The beaker should have been dried in the oven and allowed to cool in a dessicator prior to weighing. Pour in the liquid and place it on a hot plate to evaporate the liquid. Reweigh the beaker on a pan balance that measures to 0.001g when all moisture is gone.

PRODUCTS

1. Calculate sample results and turn in completed data sheets.
2. Clean up lab area and equipment.
3. As a group, prepare a report that addresses the following:

    - Why do we perform each of these tests? How do they relate to high water quality and healthy fish?

    - When might we use one test opposed to another (i.e., the Secchi disc rather than the turbidity meter)?

    - How might we improve precision and accuracy in each test?

Turbidity Data Sheet

Team members: _____

Date& Time	Specific Location	Secchi Disc (ft/m)	Turbidity (NTUs)	Comments
	_____ Creek			
	Rep #1			
	Rep #2			
	Lake _____			
	Rep #1			
	Rep #2			
	_____ Creek			
	Rep #1			
	Rep #2			
Observations: (i.e., weather, water color, and point or non-point sources of turbidity)				

Turbidity Data Sheet

Team members: _____

Date& Time	Specific Location	Setteable Solids (mg/L)	Suspended Solids (mg/L)	Dissolved Solids (mg/L)	Comments
	_____ Creek				
	Rep #1				
	Rep #2				
	Lake _____				
	Rep #1				
	Rep #2				
	_____ Creek				
	Rep #1				
	Rep #2				
Observations: (i.e., weather conditions, water color, point and/or non-point sources of sediments):					

Definitions Worksheet
Define the following terms:

Turbidity

Total solids

Settleable solids

Suspended solids

Dissolved solids

Nephelometry

Equilibrate

Imhoff cone

Dessicant

Turbidimeter

Cuvette

HANDOUT #1
The T2100A Laboratory Turbidimeter

NOTE: *For further details, refer to the instruction manual for the instrument.*

Standardizing the meter

Instrument standardization is achieved with the aid of four liquid turbidity standards, one for each range (except the lowest range). The turbidities of all four standards are based on Formazin dilutions. The standards (furnished with the instrument) are rated at 0.61, 10, 100 and 1000 NTU and are contained in sealed glass tubes. The first (0.61) is a liquid chlorobenzene solution and the last three are liquid latex solutions. When placed in the instrument, the standards scatter all amounts of light proportional to the NTU ratings specified on the tubes. Standardization is accomplished by selecting the desired range, placing the appropriate standard in the instrument and, with the light shield in place, adjusting the STANDARDIZE control to obtain a meter reading equal to the NTU value of the standard. When standardizing at the 100 and 1000-NTU ranges, the cell riser must be used.

NOTE: *These four standards are secondary standards and are not stable indefinitely. When flocculation begins, they should be replaced.*

!CAUTION!: *Chlorobenzene is flammable and may be a skin irritant!*

In the design of optical instruments, the matter of minimizing stray light in the optical system is important. In the 2100A Turbidimeter, the amount of stray light has been determined to be 0.035 to 0.040 NTU on the 0-0.2, 0-1 and 0-10 ranges. This is significant only on the 0-0.2 range and it is recommended that 0.04 be subtracted from the reading when using this range.

Using the turbidimeter

1. Before turning the instrument on, note if the meter needle is at the zero point. If it is not, zero by turning the small screw located on the meter.

2. Turn the power switch on. The amplifier and power supplies in the instrument are stable with line voltage and temperature changes. However, due to the inherent characteristics of photomultiplier tubes, maximum accuracy will be achieved if the instrument is allowed to operate for approximately 12 hours before the instrument is standardized and readings are taken.

3. Check the instrument for proper focusing. Vibrations during shipment may cause lamp movement.

4. Place sample in the optical well and place the light shield in position.

IMPORTANT NOTES

- The sample size for all turbidity measurements should be 25 (± 1 ml). Variations in sample volume can affect the accuracy of the determinations.

- When measuring the lower ranges (0-10, 0-1 and especially 0-0.2 NTU), air bubbles in the sample will cause false high readings. Before covering the cell with the light shield, observe the sample in its cell. If finely divided air bubbles are present, five minutes may be required before the bubbles can rise past the photomultiplier tube so that a valid reading can be taken. Bubbles may be eliminated rapidly and completely by dipping the end of the filled sample cell into an ultrasonic cleaning bath. When measuring the turbidity of viscous or thick solutions, it may be necessary to use a centrifuge to remove entrapped air from the sample. If a centrifuge is used, place rubber cushions in the centrifuge cups and fill the cups with water prior to inserting the turbidimeter sample cells. This will reduce the possibility of damage to the sample cells.

- If a water sample being tested is supersaturated with oxygen, air bubbles may appear on the sides of the sample cell in sufficient numbers to prevent turbidity measurement. This problem can be corrected by placing a drop of membrane-filtered Triton X-1000 Solution in the cell before filling with the water sample.

- When measuring high amounts of turbidity, it may be necessary to dilute the sample in order to bring it within the range of the instrument. If the sample is extremely turbid or highly colored, the meter may read less than the actual amount of turbidity present. When a sample appears to contain more turbidity than the meter reads, the sample should be diluted with another portion of sample that has been filtered. Diluting with distilled or deionized water may dissolve some of the turbidity. The remeasured turbidity of the diluted sample should then be multiplied by the dilution factor to obtain the turbidity of the original sample. If the accuracy of the reading is still questionable, further dilutions should be conducted.

- When the measurements are complete, best performance will be gained from the photomultiplier tube by: 1) removing the sample cell from the cell holder; 2) closing the sample compartment door; and 3) leaving the range switch in the 1000 or 100 position. The instrument is designed to run continually and leaving it on will improve the performance of the lamp and multiplier tube.

- The liquid standards supplied with the instrument should be stored in their case and kept at room temperature. Excess light and/or heat will affect their stability. Use care when handling the standards to avoid scratching or otherwise marring the glass surfaces. When in use, they must be clean and free of finger-prints.

Range Selection:

The sensing range of the turbidimeter is changed by turning the range selection switch on the front panel. Each range has been factory calibrated and readjustment should not be necessary except in some cases when either the photomultiplier tube or the high voltage circuit board is replaced.

Cleaning the Lenses:

Unplug the power cord and open the back panel of the turbidimeter. The lens assembly is held in place by a brass retaining screw on the side of the cell holder assembly. See Figure 3 of the instruction manual. Before removing the lens assembly, be sure to mark its original position in the cell holder so that the factory focusing of the instrument is not changed when the unit is reassembled. Wipe each lens with a tissue or towel that will not leave an oil film.

Lens and focus:

Each cell holder assembly is focused at the factory with the aid of the focusing template furnished with the instrument. Inside the template is a translucent plastic disc engraved with a circle. When the template is placed in the cell holder assembly, the instrument is properly focused if the image of the lamp completely fills the circle but does not extend beyond it. If the image is off-center, or too small or large, readjustment should be made.

HANDOUT #2
DRT/100D Turbidimeter

Calibrating the meter

The reference standard supplied with this instrument is a pure liquid sealed in glass. It has a nominal value of 0.1 NTU and the actual value is marked on the top.

Extreme care should be taken to avoid surface scratches on the Reference Standard Cuvette. Scratches, together with dust or film, will cause analysis error. The Reference Standard optical surface should be wiped clean each time it is to be used and it is important to use a lint-free wiper such as a Kimwipe. Avoid touching the cuvette with your fingers as they leave a residual oil.

If cleanliness of the Reference Standard is in doubt, wash with detergent, rinse several times and polish with Kimwipes.

1. Place the Reference Standard (purchased commercially) in the optical well and place the light shield in position over the Reference Standard. The light shield should be used for all readings except when the flow-thru unit is in use.

2. Place the front panel range switch in lowest NTU range.

3. Adjust the reference adjust screw as necessary to cause the display to read the same value as the Reference Standard value. This value is marked on the top of the reference stan dard.

   The instrument is now referenced to the factory Formazin calibration and unknown samples may be read directly in NTUs. For the best accuracy, the instrument should be standardized at least once a day for in-line applications, and just before each grab sample measurement.

Using the turbidimeter

1. Place the unknown sample in the optical well and place the light shield in position.

2. Set the range switch located on the front panel to an appropriate range.

3. Read the value on the digital display and then record it. Settling particles or air in the sample may cause the digital reading to change rapidly.

HANDOUT #3

Settleable Solids

(from *Standards Methods*, 19th Edition, 1995)

Settleable solids may be reported on either a volume (ml/L) or weight (mg/L) basis.

Volume

This test requires only the Imhoff cone and Imhoff cone stand.

1. Fill the cone to the 1L mark with a well-mixed sample and allow to settle for 45 minutes. Gently spin the cone or stir and settle for 15 minutes more.

2. Record volume of settleable solids in the cone as ml/L. If settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from the volume of settled solids. Where there is a separation of settleable and floating material, do not estimate the floating material as settleable solids.

Gravimetric

This method requires:

Glass vessel at least 9cm in diameter
Drying oven
Siphon tubing or large pipette gun
65-mm planchet, aluminum or stainless steel
Dessicator
Analytical balance

1. Determine total suspended solids of well-mixed sample.

2. Pour a well-mixed sample into a glass vessel using at least 1 L and sufficient to give a depth of 20 cm. Alternatively you can use a larger diameter glass vessel and more sample. Let stand quietly for one hour and without disturbing the settled or floating material, siphon off 250 mL from the center of the container at a point halfway between the surface of the settled material and the liquid surface.

3. Determine total suspended solids (mg/L) of this supernatant liquor. These are non-settleable solids.

NOTES FOR INSTRUCTORS

Learning Objective: Students will understand the relationship between turbidity and total solids as they relate to water quality and fish health.

Learner Outcomes: Students will demonstrate use of a turbidimeter and the ability to calculate total, suspended, settleable and dissolved solids

ROPES Challenge Course

INTRODUCTION

As we begin our "capstone" field project we will focus on developing teamwork, project planning, self-esteem and self-confidence. We will also get a chance to practice our leadership, supervisory, and "following and listening" skills. One excellent way to begin enhancing all of the above behaviors, attitudes and skills is to participate in a ROPES/Challenge course.
While we cannot make attendance at a ROPES course mandatory, and we do realize you each have other commitments (family, work, school, etc.) we have found that there is no better way to begin a "capstone" field study (or learning community) than this. *ROPES is an incredibly effective process to develop teamwork.*

PROCEDURE

Wearing a sturdy pair of shoes with good arch and ankle support is essential. Bring layered clothing and raingear (we *won't* cancel if it rains or snows).

You don't have to be afraid of some of the more difficult challenges (i.e., the "pamper" pole and "hole-in-space"). You *can* ask your team's permission to excuse you from certain events. You'll be amazed just how much you will be able to accomplish, both individually, and as a team!

PRODUCTS

Following each event, teams will reflect on the significance of that event, either to us as individuals, or as a team. These periods of reflection are definitely as important as the physical challenges themselves.

There will be *no* write-ups due from this field exercise, *just be there, we really can't afford to have anyone miss this one.*

NOTES FOR INSTRUCTORS

This exercise is a demonstration of team-building in this course. Similar exercises to ROPES can be accomplished to build students' confidence and to encourage working together.

Ecosystem Study

INTRODUCTION

This "capstone" portion of this course will provide you the opportunity to put into practice all of the knowledge and skills learned in previous classes and specifically, those skills learned in the first five (5) weeks of this course.

SCHEDULE

Outline during week #6:

I. Project Planning—Research Project

A. Brainstorming—Affinity and Tree Diagrams
B. Defining the Scope-of-Work
C. Develop Tasks and Time Lines
D. Prioritization of Tasks
E. Assignment of Personnel

1. Individual assignments
2. Team assignments

Throughout the research study (Weeks 7-11) we will work on developing the following skills:

II. Project Management—Research Project

A. Leadership and Supervision
B. Teamwork
C. Continuous Quality Improvement (CQI)

Weeks 8 - 11 will be devoted to the research study itself. You will have the opportunity to participate in the design of your research project (i.e., when and where you meet as teams and as a class, and to some degree the "end products" you complete as individuals and as a class).

NOTES FOR INSTRUCTORS

Learning Community/Linked Courses

The overall delivery of this research study will take on the nature of a "learning community," either a formal one which is team taught by two or more faculty, or an informal one, where two or more faculty "link" their individual courses together (for example, FISH 220, Technical Writing classes, and Ecology classes) to accomplish learning outcomes that jointly satisfy the requirements of each of the individual courses. Students may be enrolled in just one (1) or perhaps all of the linked courses.

Marketing And Advertising

Learning communities, if they involve team teaching of multiple courses, often require campus-wide marketing and advertising. A poster or advertisement included in the college's course catalog, or run in the local newspaper, is an effective way of getting the message out about the course. It is important to inform and educate all divisions of the college (admissions, councilors, faculty, staff, administrators, etc.) about your learning community at least one quarter before it is taught. Planning by the faculty co-instructing a learning community should start at least two (2) quarters in advance. *One year is not too far out!*

Course Objectives/Time Lines

- During the first week of the learning community, faculty and students will:
- Develop course objectives (requirements)
- Develop a typical weekly schedule
- Develop a corporate mission statement. The mission statement will continue to be developed throughout the quarter.

Brainstorming is used extensively during the first two weeks to develop an affinity diagram, a tree diagram and a set of time lines for the research study. These are all continuous quality improvement (CQI) tools that allow student to practice management, leadership, teamwork, organizational, facilitation, listening and following skills. Other quality management tools used are flow diagrams, fish bone diagrams and multivoting (nominal group technique) for gaining consensus. The *Memory Jogger Plus +* is an inexpensive pocket guide for students to learn these management and leadership tools.

Seminars

Seminaring is a key ingredient of a learning community. One of the major goals of our learning communities is to explore ecological and technological issues from various (i.e., organizational, historical, economic, social and political) perspectives.

Oral Presentations

The following notes may be useful to present to students when embarking upon oral presentations.

Notes for a Successful Public/Professional Meeting:

Before the Event ~

Firm up the entire program well in advance; one to two months, if possible.

Be certain speakers know their topic and time requirements.

Outline program contents for publicity channels, including places and dates.

Complete planning for all physical facilities:

a. Meeting spaces; lunch and break areas.

b. Adequate exhibit spaces.

c. Registration supplies and personnel.

d. Audio-visual equipment and assistants.

e. Signs and bulletin boards for guidance of invited guests and speakers.

f. Invitations to guests, presenters, and the media.

Line up community support and participation.

Line up staff of hosts and hostesses.

Printing or copying of brochures, programs.

Arrange for photography and photographer.

During the Event ~

Make sure someone is in charge and that everyone knows their responsibilities.

Start sessions on time; announce at the beginning that this will be standard procedure.

Keep sessions and speakers on schedule.

Public address, recording and audio-visual equipment:

a. Have it ready and warmed up ahead of schedule.

b. Have qualified operators available.

c. Allow rehearsal time for all speakers to practice with A/V equipment.

d. Assign persons to turn lights off and on.

Keep program on schedule.

Make a good photographic record of the program.

Give credits to the planners and host/hostesses.

After the Event ~

Personalized thank-you letters to speakers and assistants.

Return all borrowed equipment and/or supplies.

Pay all bills promptly.

Assessment

Assessment is considerably different (and usually more comprehensive) in a capstone learning community than a traditional class. Some of the assessment tools used include: a written self-evaluation by each student, an instructor-written evaluation of the student, comprehensive peer and instructor evaluations, and a portfolio which includes all of the "end products" (learning outcomes) including a final research report. This portfolio has proven very useful to the student/graduate for application to a 4-year college or university, and applying for a job. It tends to provide a much more comprehensive assessment of the student than just a grade on a transcript.

List of End Products (Learning Outcomes)

A list of "end product" requirements is to be completed by each student—instructors may use the following list as a guide in developing their own assessment schedules. They may select the end-products they wish to utilize for this course based on the specific needs of the class.

Possible End-Products:

Federal Employment Forms/College Courses
WA State Employment Application
Resume/Cover Letter (1st Draft)
Resume/Cover Letter (Final Draft)
Seminar participation
Concentration of Solutions Problems
Participation in ROPES Challenge Exercise

Extra Credit Opportunities:
USF&W Service and/or DNR/DOE Grant Application
One (1) news article for local newspaper

Equipment Proficiency		
Methods/Instrument	**Date Learned**	**Date Evaluated**
Field and Laboratory balances		
YSI Model 54 DO meter		
YSI Model 57 DO meter		
Orion DO meter		
YSI Temperature, Cond., Salinity meter		
Winkler DO		
Hach Kits (all types)		
Turbidometer		
Spectrophotometer (all tests)		
Mercurial Barometer		
Saturometer		
pH meter (lab)		
pH meter (field)		
Sediment analysis		

Each student should demonstrate proficiency (at level 5 or higher). Levels of proficiency can be established by the instructor or the industry. For example, a Level 1 might indicate that the student has *observed* the procedure, but has not yet *performed* it; while a level 6 might indicate *total mastery* of the method, performed without assistance.

Evaluation Charts

Below are evaluation charts for:

- Self-evaluation by students
- Instructor evaluation
- Peer review

Instructors may use these as required for the assessment of student performance. Instructors are encouraged to add/delete columns as necessary.

Self Evaluation of Performance

Students	Seminaring	Lab/Field Participation	Project Planning	Teamwork	Leadership	Research Report (1st Draft)	Research Report (Final Draft)	Video Script	Oral Presentation

Please rate yourself and your teammates' performance in each of the areas above (zero (0) is lowest, five (5) is highest). Your ratings will be kept strictly confidential.

Instructor Evaluation of Student Performance

Name	Field Study	Seminar	FN/ Journal	Planning	Teamwork	Leadership	Video Script	Lab/Field Participation	Research Rpt./Oral Presentation

Students are evaluated by the instructor in the above areas (zero (0) is lowest; five (5) is highest). Ratings are kept strictly confidential.

Peer Review of Performance

Students	Seminaring	Lab/Field Participa-tion	Project Planning	Teamwork	Leadership	Research Report (1st Draft)	Research Report (Final Draft)	Video Script	Oral Presentation

Please rate yourself and your teammates' performance in each of the areas above (zero (0) is lowest, five (5) is highest). Your ratings will be kept strictly confidential

Summary

Since each capstone learning community is "student-driven," there is no single outline to use as an example. Students are allowed many choices during the quarter. Faculty must relinquish the "control" or "authority" normally held in a traditional course. This does not mean, however, that the students will not be productive. The opposite is most often true. Once the synergy of the "community" kicks in (this often happens in the 3rd-5th week of the quarter, when students finally accept the fact that they are 100% responsible for their own learning), "near miraculous learning" can occur and learning outcomes are accomplished within the prescribed time lines. All of this requires much "risk taking" on the parts of both the faculty and students making up the community.

Additional References

Brassard, M. 1989. *Memory Jogger Plus +*. First Edition. GOAL/QPC. 13 Branch Street, Methuen, MA 01844.

Department of the Navy. 1992. *Handbook for Basic Process Improvement*. Chief of Naval Operations Executive Steering Committee and the Department of the Navy's TQL Office, Washington, D.C.

Watershed Related Terms

The following is a list of watershed-related terms that could be used for definitions and description for the final oral exam:

Accuracy
Adaptive management
Alkalinity
Baseline survey
Bed load
Benthic
Biodiversity
Bioregion
Buffer strips
Canopy
Channel forming discharge
Community
Conductivity
Coniferous
Control
Cover
Cubic feet per second
Cumulative effect
Debris flow
Deciduous
Discharge
Dissolved load
Dissolved oxygen
DOM (Dissolved Organic Matter)
Ecosystem
Ecosystem management
Ecotone
Electroshocking
Ephemeral
Erosion
Experimental design
First order stream
Flood plain
Fluvial
Geomorphology
GIS (Geographic Information Systems)

Glacial flour
GPS (Global Positioning Systems)
Gradient
Gully erosion, gullying
Habitat units
Hardness
Hydraulic permit
Intermittent stream
Large woody debris
Limiting factor
Macroinvertebrates
Mass wasting
Meander
Non-point source pollution
Parameter
pH
Plunge pool
Point source pollution
Pool
Population
Precision
Reach
Replicate
Riffle
Riparian zone
Scoured
Side Channel
Stream segment
Stream typing
Surface erosion
Suspended sediments
Synergistic
Tailout
Thalweg
Time lines
Total solids
TQM (Total Quality Management)
Watershed
Wetlands

Example
Oral Exam - Study Guide (100 points)

Points

(25) 1. You will be asked to orally *define* and *discuss* five (5) of the terms from the attached list of watershed monitoring terms. The instructor will *randomly* select terms, so you'll have to know them all.

(25) 2. Describe (in detail) *one* or *more* of the following water quality monitoring procedures:

- Winkler Method for dissolved oxygen
- Settleable and suspended solids
- YSI Oxygen meter (include calibration)

(25) 3. Be prepared to discuss benefits (or disadvantages) of Continuous Quality Improvement (CQI) as a set of project planning and management tools to carry out our research study this quarter (i.e., did they help or hinder our goal to complete several *major* team "end products?"). You will be graded strictly on how well you defend your point of view (pro or con); *not* on what you think the instructor wants to hear.

(25) 4. Be prepared to discuss pros or cons of the "learning community" (teamwork, seminars, students teach other students, journals, oral presentations, portfolios) approach to learning verses more traditional approaches. Again, you will be graded on how well you defend your point of view.

Example
Mid-Term (164 points)

NAME: _____

Points

(15) 1. Define three (3) of the following terms as discussed in lecture.

a. water quality criterion
b. water pollution
c. accuracy
d. water quality standard
e. precision

(6) 2. List two (2) of the four (4) criteria used in selecting a physical or chemical method for laboratory use.

(3) 3. Why should we treat data collected by field methods differently than laboratory derived data?

(3) 4. How should the data obtained from field instruments be reported?

(6) 5. Before precision and accuracy data can be determined for a given analytical method, at least five (5) basic assumptions must be satisfied concerning the sample the method and the results obtained. (List three (3) of these)

(15) 6. Outline the procedure you would use in setting up a precision determination for an analytical method. Include information about the water to be tested, the concentrations involved, replicates, instrumental conditions and potential problems.

(8) 7. What are the key elements that should be included in the resulting precision statement? List four (4) of them.

(4) 8. Accuracy data can be used in two ways. Describe them.

Matching-letters may be used more than once (22 pts)

_____ $\frac{\text{number of moles of solute}}{\text{kilogram of solvent}}$

_____ using analytical grade reagents

_____ $\frac{\text{number of moles of solute}}{\text{number of liters of solution}}$

_____ $\frac{\text{number of milli-equivalents of solute}}{\text{number of milliliters of solution}}$

_____ reproducibility among replicates

_____ falsification of data

_____ $\frac{\text{number of gram-formula weights of solute}}{\text{number of liters of solution}}$

_____ reported as a range of standard deviations

_____ reported as percent recovery

_____ $\frac{\text{number of gram-equivalent weights of solute}}{\text{number of liters of solution}}$

a. molarity
b. precision
c. measurement of quality
d. professional pride
e. normality
f. control of quality

Fill In The Blank (6 pts)

1. One (1) gram of solute dissolved in one (1) liter of solution has a concentration of one (1) part per _____.

2. One (1) milligram solute dissolved in one (1) liter of solution has a concentration of one (1) part per _____.

3. One (1) microgram solute dissolved in one liter of solution has a concentration of one (1) part per _____.

Short Answer

(10) 1. In a solution consisting of NaCl dissolved in water:

a. Which substance is the solute?
b. Which is the solvent?
c. At what points do we have a saturated NaCl solution?
d. A supersaturated solution of NaCl exists when (complete this statement)_____.
e. A solution of NaCl in which the concentration is known is called a _____ solution.

(15) 2. Concentration Problems (Show work!)

a. What is the molarity of a solution containing 16.0 g CH_3OH per liter of solution? (Atomic weights: C = 12, H = 1, O = 16)

b. How many grams of solute are required to prepare 1 liter of 1FPb $(NO_3)_2$? (Atomic weights: Pb = 207, N = 14, O = 16)

c. To what extent must a given solution of concentrated 40 mg $AgNO_3$ per ml be diluted to yield one of concentration 16 mg $AgNO_3$ per ml?

Multiple Choice
Circle the most correct answer (12 pts)

1. In the laboratory we should:

a. pour acid into water
b. pour water into acid
c. add both water and acid in equal amounts
d. never mix acid and water

2. To make up a set of heavy metal standard solutions you should start with:

a. bacteria free tap water and analytical grade reagents
b. clean tap water and at least industrial grade reagents
c. deionized water and industrial grade reagents
d. deionized water and analytical grade reagents

3. Pipetting by mouth should:

a. only be done when you know what you are pipetting
b. be practiced on pure water samples before going on to strong acids and bases
c. never be done
d. be done only when the instructor is not in the room

4. Which of the following is *NOT* a recommended practice when using an analytical balance:

a. when not in use, the beam should be raised from the knife edge
b. weights should always be added in the full-release position
c. balance should always be mounted on a shock-proof table
d. operator should avoid spillage of corrosive chemicals on the pan or inside the balance case

5. A good field balance for weighing wet salmon eggs is:

a. a solution balance
b. electronic pan balance
c. analytical triple beam balance
d. a portion balance
e. both a and d
f. both a and c

6. Circle the *INCORRECT* response regarding food and chemicals in the laboratory:

a. food should never be stored in the laboratory refirigerator
b. chemicals should never be stored in the lunchroom refrigerator
c. small amounts of food can be stored in the laboratory refrigerator providing the space is well marked
d. glass laboratory beakers should be marked with the initial of the laboratory technician if used for drinking coffee
e. b and c
f. c and d

True or False (8 pts)

_____ Bases such as sodium and potassium hydroxide, either in solid or liquid form, can cause severe burns to the skin or eyes.

_____ Large amounts of acids and bases should be transported to the laboratory in a suitable polyethylene carrier.

_____ Contact lenses are an acceptable substitute for safety glasses in the laboratory.

_____ Broken glass should always be disposed of in a special marked container.

_____ All rules may be overlooked when the instructor is not present.

Short Essay (32 pts)

1. What should be done with unmarked bottles, beakers, or flasks containing unknown chemicals in the laboratory?

2. If you had to smell a chemical from a bottle in the laboratory, what would be the most acceptable procedure?

3. Explain how you would calibrate a buret for analytical use.

4. Calculate the cell constant of a conductivity meter given the following information (Show work):

$$L = \frac{K_1 + K_2}{K_x}$$

Where:

- L = Cell constant
- K_1 = Conductivity of KCl solution at temp. of measurement mmhos
- K_2 = Conductivity of deionized water used to prepare reference solutions (mmhos)
- K_x = Measured conductance (mmhos)

Given: K_1 = 7,138 mmhos
K_2 = 32 mmhos
K_x = 7,000 mmhos

5. What is meant by ruling theory?

6. What is meant by multiple working hypotheses?

7. Describe the meaning of strong inference.

8. How are multiple working hypotheses and strong inference related?