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MARINE ANALYTICAL PARAMETERS FOR SEA FARMING

A Comprehensive Technical Manual

Edited and Compiled by

Divu D., Suresh Kumar Mojjada, Swathi Lekshmi PS., Prachi Siddharth Bagde, Ramshad TS., Muktha Menon, V V R Suresh, Grinson George

Indian Council of Agricultural Research

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FOREWORD

As India sets ambitious targets to expand its mariculture industry, aiming to achieve 4 million tonnes of fish production by 2050, the path ahead is both promising and challenging. The growth of this sector is crucial for meeting the increasing demand for fish and seafood while ensuring sustainable practices. However, managing mariculture operations, particularly in open sea environments, presents significant challenges in holistic management. The vast and dynamic nature of the environment underscores the importance of monitoring and managing the ecosystem sustainably.

The comprehensive analytical guide for assessing the environmental parameters could be instrumental in addressing these challenges. Effective management of water quality and environmental conditions is critical for the success and sustainability of mariculture operations. The need for a detailed, accessible resource has become increasingly evident.

Recognizing this need, a comprehensive compilation of resource developed to support practitioners in overcoming these challenges. The Marine Analytical Parameters for Sea farming: A Comprehensive Technical Manual, authored by Divu et al., 2025, fills this void by providing a thorough and practical guide to assessing and managing water quality parameters. This manual is designed to address the complexities of mariculture environments with detailed methodologies and practical applications. Offering valuable insights and step-by-step protocols, this guide equips stakeholders, farmers, and research students with essential tools for effective water quality management.

ICAR-CMFRI's commitment to extended its technical support to the mariculture sector is exemplified through developing valuable techno-scientific resources. I commend Divu et al. for their dedication in creating this essential resource. It promises to be a cornerstone reference for enhancing water quality management practices and supporting the growth of India's mariculture industry.

Dr. Grinson George
Director
ICAR-Central Marine Fisheries Research Institute

PREFACE

Maintaining a balanced environment is essential for sustaining life on Earth. As human activities increasingly disrupt this balance, the need for accurate and effective environmental monitoring becomes more critical. Understanding the complex interactions between chemicals and living organisms requires precise analysis and sophisticated techniques. The field of environmental analysis has significantly evolved from its origins in ancient practices to the advanced methods we use today. Analytical chemistry, which focuses on identifying and measuring substances, plays a crucial role in this progress. This evolution underscores the importance of using modern tools and methodologies to address contemporary environmental challenges.

The **Marine Analytical Parameters for Sea farming: A Comprehensive Technical Manual** has been developed to address these critical needs, providing a comprehensive guide to the techniques and methods used in environmental analysis with a specific focus on mariculture. Designed for mariculturists, students, researchers, stakeholders, and entrepreneurs, this manual offers practical, detailed guidance for managing water quality and environmental conditions effectively.

To ensure this manual is both practical and user-friendly, extensive research and refinements have been undertaken. The development process involved a thorough review of existing literature, consultations with experts, and iterative testing of methodologies. This rigorous approach ensures that the manual is accurate, accessible, and applicable in real-world settings.

Divided into seven chapters focusing on different types of parameters, the content is crafted to be clear, accurate, and practical. This manual is a valuable tool for anyone dedicated to studying, understanding, and protecting our environment.

By equipping readers with the knowledge and tools necessary for effective environmental and biological assessment, this manual aims to support the ongoing efforts to enhance mariculture practices and promote sustainable development. It is our hope that this resource will contribute to a deeper understanding of environmental management and inspire continued innovation and dedication in the field.

Dr. Divu D
Senior Scientist, Mariculture,
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ABOUT THE MANUAL

The Marine Analytical Parameters for Sea farming: A Comprehensive Technical Manual, serves as a vital resource for anyone involved in the analysis and management of environmental and biological parameters within mariculture. This comprehensive guide is meticulously designed to address the complexities of aquatic environmental monitoring, providing practical and detailed methodologies essential for ensuring optimal conditions in mariculture systems. Divided into seven well-structured chapters, the manual covers all major parameters critical to effective water quality management and biological assessment. Each chapter delivers in-depth explanations and step-by-step instructions, reflecting extensive research and expert input to offer the most current and accurate information. While the primary focus is on mariculture, the methodologies presented are adaptable to freshwater and brackish water aquaculture, making the manual a versatile tool for various aquatic farming applications. The user-friendly format, with clear instructions and practical tips, ensures that both seasoned professionals and newcomers can easily apply the techniques and enhance their operational practices. By addressing contemporary environmental challenges such as pollution and climate change, this manual not only supports effective management strategies but also contributes to the sustainable development of the aquaculture industry, making it an invaluable asset for mariculturists, researchers, students, and stakeholders alike.

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CHAPTER 1

Laboratory Safety and Standard Operating Procedures (SOPs)

Toms C. Joseph, Divu D., Swathi Lekshmi P.S., Muktha Menon

Introduction

Ensuring safety and efficiency in the laboratory is paramount for successful scientific research and operations, particularly in fields involving intricate analyses and delicate procedures like mariculture. This chapter provides comprehensive guidelines on standard operating procedures (SOPs) in the lab, understanding potential hazards, routine laboratory activities, and emergency procedures. Adhering to these guidelines ensures not only the integrity of the research but also the safety and well-being of all laboratory personnel.

1.1 Standard Operating Procedures in the Lab

Standard Operating Procedures (SOPs) are detailed, written instructions designed to achieve uniformity in the performance of specific functions. They are essential for maintaining quality control, ensuring consistency, and fostering a safe working environment.

Preparation and Documentation

Ensure all SOPs are documented, easily accessible, and well-understood by all personnel involved.

Regularly review and update SOPs to incorporate new techniques, equipment, and safety protocols.

Conduct training sessions and refresher courses to keep staff informed about the latest SOPs.

Use of Equipment

Follow the manufacturer's instructions and SOPs for the correct use and maintenance of laboratory equipment.

Schedule regular calibration and maintenance for all equipment to ensure accuracy and reliability.

Keep detailed records of equipment usage, maintenance, and calibration.

Chemical Handling

Handle, store, and dispose of chemicals properly, and ensure proper ventilation to reduce exposure to hazardous fumes.

Ensure Material Safety Data Sheets (MSDS) are available for all chemicals used in the laboratory.

Use appropriate personal protective equipment (PPE) such as gloves, lab coats, and safety goggles.

Standard Operating Procedures

Maintain good personal hygiene.

Use face shields, respirators, and other personal protective equipment (PPE).

Label all chemical containers with the correct names and hazard information.

Store chemicals in accordance with their hazard category and ease of use.

Maintain accurate records of time-sensitive chemicals and proper disposal on expiry.

1.2 Understanding the Hazard

Awareness of potential hazards in the laboratory is crucial for preventing accidents and ensuring a safe working environment.

Chemical Hazard

- Identify and understand the risks associated with the chemicals used, including toxicity, flammability, and reactivity.
- Use chemical fume hoods when handling volatile or hazardous substances.
- Ensure proper storage of chemicals to prevent reactions and spills.

Biological Hazard

- Be aware of the risks posed by biological agents such as bacteria, viruses, and other pathogens.
- Implement biosafety measures, including the use of biosafety cabinets and proper sterilization techniques.
- Follow decontamination protocols for waste disposal and equipment cleaning.
- Eating, drinking, applying cosmetics, and hair grooming are prohibited in the working area.
- Access to the laboratory shall be limited to authorized personnel.
- Wash hands before and after laboratory work.
- When transporting infectious biological samples, proper precautions shall be taken to ensure safety and containment.

Physical Hazard

- Recognize hazards related to equipment, such as centrifuges, autoclaves, and other machinery.
- Ensure proper training for equipment use and enforce the use of safety guards and interlocks.
- Conduct regular safety audits and inspections to identify and mitigate physical hazards.

Ergonomic Hazards

- Address potential ergonomic risks, such as repetitive strain injuries and improper workstation setup.
- Promote proper posture and provide ergonomic equipment to reduce strain.
- Encourage regular breaks and stretching exercises to prevent musculoskeletal disorders.

Understand the hazards

Familiarize yourself with the hazards associated with laboratory analysis.

Review & understand the Safety Data Sheets for all chemicals involved.

Use suitable containment measures when using hazardous & volatile chemicals.

Work under direct supervision at all times & Never work alone in the laboratory.

Be alert and proceed with caution at all times in the laboratory.

1.3 Routine Laboratory Activities

Routine laboratory activities are essential for maintaining a safe and efficient working environment because they ensure the consistent application of safety protocols, the proper functioning of equipment, and the early detection of potential hazards.

Daily Inspections

Conduct daily checks on equipment, safety showers, eyewash stations, and emergency exits to ensure proper functionality.

Check for any signs of wear and tear or malfunction in equipment and address issues promptly.

Verify that all safety signage and labels are visible and up-to-date to maintain a clear and safe working environment.

Housekeeping

Keep the laboratory clean and organized. Properly label and store chemicals, samples, and equipment.

Dispose of waste materials promptly and correctly, following proper segregation and disposal procedures.

Ensure workbenches and equipment are cleaned and disinfected regularly.

Inventory Management

Maintain an up-to-date inventory of chemicals, reagents, and consumables.

Regularly check expiration dates and restock supplies as needed.

Implement an inventory tracking system to monitor usage and prevent shortages.

Training and Refresher Courses

Ensure all laboratory personnel receive adequate training on SOPs, safety protocols, and emergency procedures.

Conduct regular refresher courses to keep everyone informed of the latest safety practices.

Encourage continuous learning and professional development through workshops and seminars.

Routine Round up

Maintain clean workspaces to avoid contamination by extraneous substances.

Inspect equipment prior to use to ensure functionality and adhere to the manufacturer's operational guidelines.

Dispose of chemical and biological waste in compliance with institutional regulations.

Conduct regular safety drills and refresher training sessions

1.4 Emergency Procedures

Having clear, well-documented emergency procedures is vital for effectively managing incidents and minimizing harm.

Emergency Contact Information

- Display emergency contact numbers prominently in the laboratory.
- Ensure all personnel are familiar with whom to contact in case of an emergency.
- Establish a communication plan for notifying relevant authorities and stakeholders.

Spill Response

- Develop and implement a spill response plan for handling chemical, biological, and other hazardous spills.
- Equip the lab with spill kits and ensure all personnel are trained in their use.
- Follow proper containment and cleanup procedures to minimize exposure and environmental impact.

Fire Safety

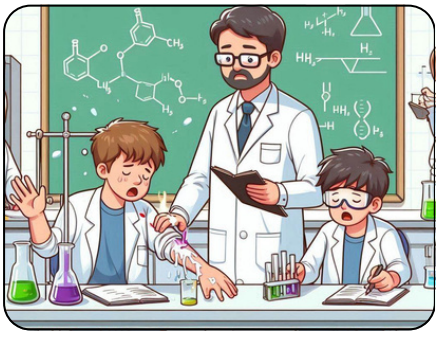
- Install and maintain fire extinguishers, smoke detectors, and fire alarms.
- Conduct regular fire drills and ensure all personnel know how to use fire safety equipment.
- Implement fire prevention measures, such as proper storage of flammable materials and electrical safety protocols.

Medical Emergencies

- Have first aid kits readily available and ensure personnel are trained in basic first aid and CPR.
- Establish procedures for reporting and responding to medical emergencies.
- Maintain a list of medical facilities and emergency services near the laboratory.

Emergency Procedures

1. Know the proper emergency response procedures for accidents or injuries in the laboratory.
2. Know the locations of emergency equipment such as eyewash stations, safety showers, fire extinguishers, and first aid kits.
3. Know the nearest fire alarm locations and at least two exit routes, ensuring they are prominently displayed for easy visibility.
4. Report accidents, injuries, and unsafe conditions to the supervisor immediately.



Conclusion

Adhering to standard operating procedures, understanding potential hazards, maintaining routine laboratory practices, and having well-defined emergency procedures are essential components of a safe and productive laboratory environment. By following the guidelines outlined in this chapter, laboratory personnel can ensure the safety, efficiency, and reliability of their work, ultimately contributing to the advancement of scientific research and mariculture operations. Ensuring laboratory safety and compliance with SOPs not only protects personnel but also enhances the quality and integrity of scientific research.

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CHAPTER 2

Analytical Procedures for Estimation of Physical Parameters

Chinnadurai S., Swathi Lekshmi P S, Suresh Kumar Mojjada, Prachi Siddharth Bagde

Introduction

Physical parameters refer to the measurable properties of water that describe its physical characteristics and conditions. These parameters are crucial in determining the quality and suitability of water for various aquatic environments, including mariculture. Monitoring physical parameters helps in understanding the dynamics of water bodies and their impact on the organisms living within them. Understanding and monitoring the physical parameters of water is crucial for effectively managing aquatic environments, particularly in mariculture. Physical parameters such as temperature and transparency play a vital role in determining water quality and the overall health of aquatic ecosystems. These parameters influence various biological, chemical, and physical processes, impacting the growth and well-being of marine organisms.

Temperature, Light intensity and transparency are among the most significant physical parameters to assess. Temperature affects metabolic rates, reproductive cycles, and the solubility of gases like oxygen, directly influencing the health and growth of aquatic species. Transparency, measured using methods like the Secchi disc, indicates water clarity and light penetration, which are essential for photosynthesis and primary production in the aquatic food web. Proper light intensity is a critical factor in promoting healthy plant growth in which light serves as a vital energy source through the process of photosynthesis. Accurate measurement and regular monitoring of these physical parameters help in maintaining optimal conditions for mariculture operations. By ensuring the right temperature and clarity levels, mariculturists can enhance the productivity and sustainability of their practices, ensuring the well-being of fish and shellfish in their care

2.1 Estimation of Water Temperature

Temperature is a fundamental physical parameter in aquatic environments, playing a critical role in influencing the metabolic rates, growth, reproduction, and overall health of marine organisms. It affects various biochemical processes, dissolved oxygen levels, and the breeding cycles of many species. Monitoring and maintaining optimal temperature conditions are crucial for the successful management of mariculture systems.

In mariculture, temperature impacts several key aspects:

- **Metabolic Rates:** Higher temperatures generally increase the metabolic rates of aquatic organisms, influencing their growth and development.
- **Dissolved Oxygen Levels:** Temperature affects the solubility of oxygen in water, which is crucial for the respiration of marine life.
- **Reproductive Cycles**: Many aquatic species have temperature-dependent breeding cycles.
- **Disease Susceptibility**: Temperature fluctuations can stress organisms, making them more susceptible to diseases.
- **Feeding Efficiency:** Optimal temperature ranges can enhance feeding efficiency and nutrient absorption.

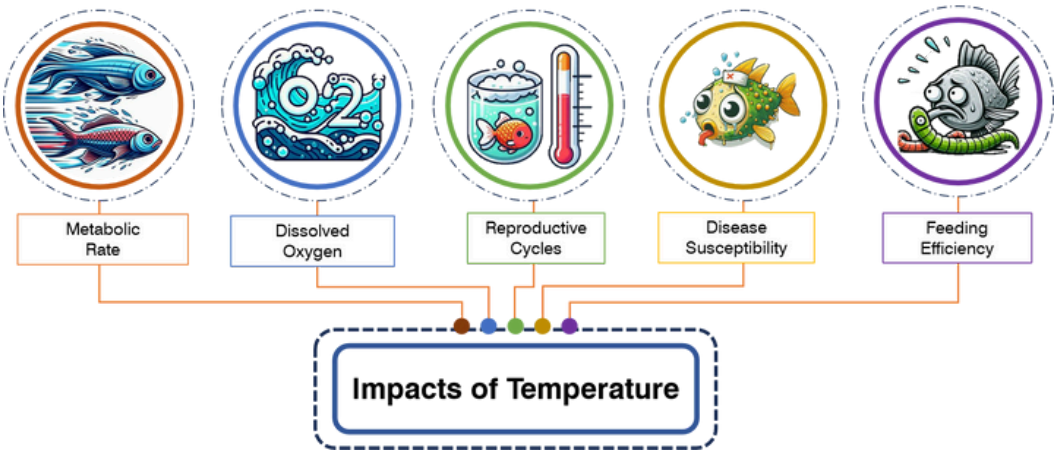

Fig 2.1 Impacts of Temperature Fluctuation (An Infographic)

Principle

The measurement of water temperature is based on the principle that the temperature of the surrounding water will equilibrate with the temperature-sensing device. This device, typically a thermometer or temperature probe, measures the kinetic energy of the water molecules, which is interpreted as temperature. Accurate measurement requires the device to be in thermal equilibrium with the water and free from external influences that might skew the reading.

Equipment Required

- Thermometer : Digital thermometer, mercury thermometer, or alcohol thermometer. For precision, a temperature probe with a data logger can be used.
- Field Notebook and Pen: To record readings along with relevant details.

Procedure

01 Calibration

- Ensure the thermometer or temperature probe is calibrated according to the manufacturer's instructions.

02 Preparation of the Thermometer

- Turn on digital thermometers or temperature probes to ensure they are functioning.
- For mercury or alcohol thermometers, check for air bubbles and remove them by gently tapping.

03 Submerging the Thermometer

- Submerge the thermometer or probe in water at the desired depth. For surface temperature, immerse just below the surface.
- For measurements at different depths, use a weighted line or a depth-specific thermometer.

04 Equilibration

- Allow the thermometer or probe to equilibrate to the water temperature. This may take a few minutes.

05 Reading the Temperature

- Read the temperature from the scale on the thermometer or the digital display.
- For data loggers, record the temperature from the connected device.

06 **Recording Data**

- Record the temperature in the field notebook with the date, time, and location of measurement. Note any relevant conditions such as weather, time of day, and water depth.

07 **Repeating Measurements**

- Take multiple readings at different times and locations for comprehensive monitoring. This helps understand temperature variations in the water body.

2.2 Estimation of Water Transparency

Transparency, also referred to as turbidity, is a key indicator of water quality in mariculture systems. It measures the clarity of water by determining how well light can pass through it, which is crucial for assessing the health and productivity of aquatic environments. In mariculture, transparency is vital because it affects various biological and chemical processes. High turbidity, caused by suspended particles, algae, or organic matter, can limit light penetration, impair photosynthesis, and disrupt the growth of aquatic plants and phytoplankton. This limitation can reduce oxygen levels in the water, impacting the overall health of fish and shellfish. Furthermore, poor transparency can affect the efficiency of feeding in filter-feeding organisms and may contribute to increased disease risks. By maintaining appropriate transparency levels, mariculture practitioners can enhance production efficiency, prevent algal blooms, and ensure a balanced aquatic ecosystem, which is essential for the optimal growth and health of cultured species. Regular monitoring helps in managing water quality effectively and promoting a sustainable mariculture environment.

Principle

The Secchi disk method measures water transparency by determining the depth at which a white or black-and-white disk becomes invisible and reappears. The disk, when lowered into the water, reflects the amount of light penetrating the water column. As the disk is lowered, it eventually becomes obscured by the water's turbidity or suspended particles. The depth at which this occurs and where it reappears provides a measure of the water's clarity. The average of these two depths is used to calculate the Secchi depth, which can then be used to estimate the euphotic zone, indicating the depth at which sufficient light penetrates for photosynthesis.

Materials Required

- Rope
- Measuring tape

Instruments required

- The Secchi disk (or Secchi disc), as created in 1865 by Angelo Secchi, is a plain white, circular disk 30 cm used to measure water transparency or turbidity in bodies of water. The upper surface of the Sechi disk is divided into four quadrants, each alternately painted black and white. A stable structure is positioned at the centre of the upper surface, around which a graduated rope is affixed. At the centre of the lower surface, a small weight is attached to aid in sinking the Secchi disc to the desired depth.

Fig 2.2 Sechi Disc

Procedure

01 Preparation

- Ensure the Secchi disk is clean and in good condition. Attach the disk to a graduated rope or measuring tape, marked in meters and centimeters for accurate depth readings.

02 Deployment

- Slowly lower the Secchi disk into the water on the shaded side of the boat or platform to avoid glare and reflections.
- Maintain a steady and slow descent to avoid creating waves or disturbing the sediment.

03 Observation

- Continue lowering the disk until it becomes invisible from the surface. Note this depth as the “disappearance depth”. Then, slowly raise the disk until it reappears and becomes visible again. Record this depth as the “reappearance depth”.

04 Measurement

- The Secchi depth is the average of the depths at which the disk disappears and reappears.
- Use the formula: Secchi Depth = (Depth at disappearance + Depth at reappearance) / 2.

05 Recording Data

- Record the Secchi depth, date, time, weather conditions, and any observations about the water conditions (e.g., colour, presence of algae, clarity).

06 Clean-up

- Rinse the Secchi disk with clean water after use and store it properly to maintain its condition.

Note

- Lower the Secchi disk vertically into the water to avoid disturbing the water column and achieve accurate readings.
- Choose a time of day or position where sunlight glare on the water surface is minimized to prevent wrong readings.
- If possible, have multiple observers take measurements to account for individual variations in eyesight and perception.

2.3 Estimation of Light Intensity

Proper light intensity is a critical factor in promoting healthy plant growth and development. For plants, light serves as a vital energy source through the process of photosynthesis. Inadequate or excessive light can lead to a range of issues, from stunted growth to poor flowering. Understanding light levels in different habitats is crucial for determining the optimal conditions for plant growth and survival. By accurately assessing light availability using a lux meter, ecologists can study how variations in natural light impact plant health, productivity, and species distribution. This information is essential for implementing strategies related to habitat management, conservation, and agricultural practices.

Principle

The measurement of light intensity using a lux meter is based on photometric detection. The lux meter employs a photodetector, usually a photodiode, that generates an electrical current when exposed to light. The magnitude of this current is directly proportional to the intensity of the light. This current is then converted into a voltage, which the meter processes and displays as a lux value

Measurement of Light Intensity

Illuminance is the metric used to measure light intensity within a space. It is expressed in footcandles or lux, which represent the amount of light (lumens) falling on a surface per unit area.

Specifically, light intensity is measured in lumens per square foot (footcandles) or lumens per square meter (lux). Lumens (lm) are the unit of measurement we use to quantify the amount of visible light the human eye can see.

Table 2.1 - Various Light Levels

Category	Lux	Footcandles
Bright Summer Day	100000	10000
Full Day light	10000	1000
Overcast day	1000	100
Twilight	10	1
Full Moon	<1	<0.1

Table 2.2 - Various Light Level Ranges

Type of Plants	Lux
Low Light plants	500-2500
Medium Light plants	2500-10000
Bright Light plants	10000-20000
Very Bright plants	20000-50000

Table 2.3 - Various Light Levels for Various Plants

Type of Plants	Lux
Aechmea fasciata (Bromeliad)	807 to 1614
Anthurium sp	807 to 1614
Aspidistra elatior (Cast - Iron Plant)	207 to 807
Begonia sp (other than metallica and rex basket)	>10764
Calathea sp	807 to 1614
Chlorophytum elatum (Spider plant)	1614 to 10764
Cordyline fruticose	807 to 1614
Eucheuma sp	6000
Kappaphycus alvarezii	333 - 1000
Nephrolepsis cordifolia	807 to 1614
Sansevieria sp (Snake plant or Mother-in-Laws Tongue)	270 to 807

Lighting experiments use a light meter (also known as an illuminance meter or lux meter) to measure the amount of light in a sampling area. They are widely used for their ease of use and portability. The light meter contains a sensor that detects the light falling on it and provides the user with a measurable illuminance reading. A key advantage of using a lux meter is that it can be calibrated. Lux meters are typically configured to the CIE standard illuminate A for accurate measurements.

Materials Required

- Lux Meter
- Data Recording too
- Measuring Tape
- Power source

Procedure

01 Calibration

- Ensure lux meter is properly calibrated according to the manufacturer's instructions.

02 Measure ambient Light in the chamber

- Turn off any lights in the room that contain the incubation chamber to be measured.
- Turn on the light meter and allow the reading to stabilize. Once it has stabilized, record the lux value displayed on the meter. This value will serve as the baseline measurement, representing the ambient light level.
- Once this baseline is recorded, proceed with the experiment, ensuring that the lights remain off for the entire duration of the experiment.

03 Turn on the Lights and take measurement

- From the incubation chamber, ensure that light meter is properly set up to record the new reading. Be patient and allow the lighting to reach its full brightness before taking the measurement.
- Turn on the light meter and allow the reading to stabilize. Once it has stabilized, record the lux value displayed on the meter.

04 Note differential reading

- Subtract the ambient light level from the illuminated level to obtain the differential measurement, which represents the amount of light produced by the existing luminaires..
- If the room lights are on during the measurement, subtract this correction from the light measured in the incubation chamber.

Fig 2.3 Lux Meter

Note

- Ensure the lux meter is properly calibrated before use.
- Position the sensor at the exact location and angle where the measurement is needed.
- Maintain a consistent distance between the light source and the sensor for all measurements.
- Be aware of reflective surfaces that might alter the light reaching the sensor.
- Take multiple readings to ensure accuracy and consistency.

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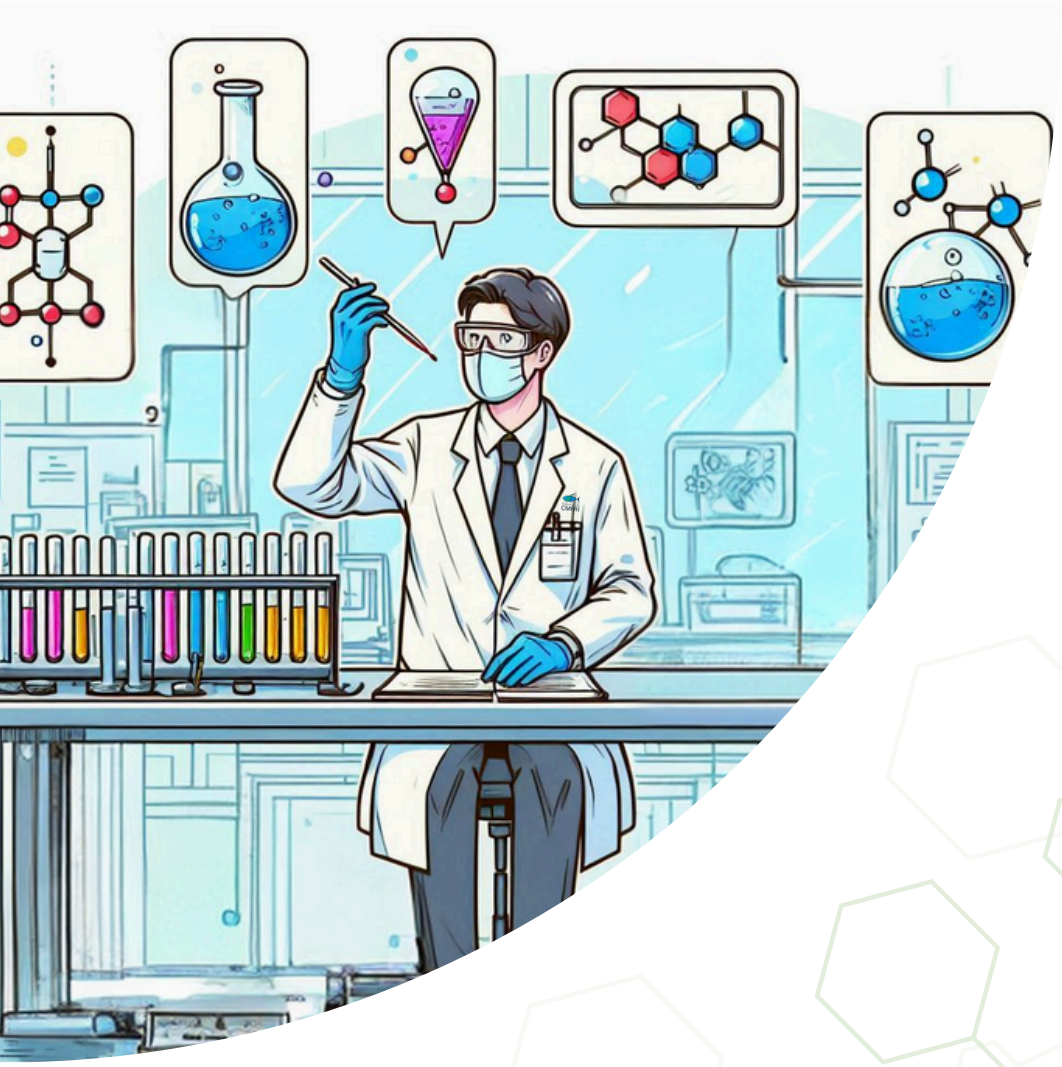
CHAPTER 3



Analytical Procedures for Estimation of Chemical Parameters

Suresh Kumar Mojjada, Chinnadurai S., Prachi Siddharth Bagde, Aarsha Subramanian

Introduction

Chemical parameters are essential metrics that provide critical insights into the quality and composition of water in aquatic environments. In mariculture, analyzing chemical parameters is fundamental to maintaining optimal conditions for the health and growth of marine species. This chapter delves into the various critical chemical parameters in assessing and managing water quality in mariculture systems.

Understanding and monitoring these parameters help ensure that the aquatic environment remains conducive to the physiological needs of cultured organisms. Variations in chemical parameters can significantly affect metabolic processes, nutrient availability, and overall ecosystem stability. Therefore, regular and precise analysis of these parameters is crucial for the effective management and sustainability of mariculture operations.

The primary chemical parameters discussed in this chapter include pH, salinity, alkalinity, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), and organic carbon. Each of these parameters plays a vital role in determining the suitability of water for supporting marine life. Accurate measurement and control of these parameters are imperative for preventing stress, disease, and mortality among cultured species.

By systematically evaluating these chemical parameters, mariculturists can implement informed management practices to enhance water quality, optimize growth conditions, and promote the overall productivity of mariculture systems.

3.1 Estimation of pH

pH is a fundamental parameter that quantifies the acidity or alkalinity of water, reflecting the hydrogen ion concentration. In mariculture, monitoring pH is crucial as it directly affects the physiological processes of aquatic organisms, including growth, reproduction, and health. Optimal pH levels ensure balanced nutrient availability and biochemical processes, which are vital for maintaining the stability and productivity of aquaculture systems. The ideal pH range for most mariculture environments is typically between 7.5 and 8.5. Deviations from this range can lead to stress, reduced growth rates, and increased susceptibility to diseases in cultured species. Thus, regular pH monitoring is essential for managing water quality and ensuring a thriving mariculture environment.

Principle

The pH of a water sample is determined using a pH meter, which measures the voltage difference between a pH-sensitive electrode and a reference electrode immersed in the solution. This voltage difference correlates with the hydrogen ion concentration, which is converted into pH units. The pH meter is calibrated with standard buffer solutions of known pH values to ensure accuracy. The pH of the sample is then read directly from the meter's display.

Sample collection

For accurate pH measurement, water samples should be collected in clean, acid-washed containers to prevent contamination. Use a container with minimal headspace to avoid introducing air, which can alter the pH. Submerge the container below the water surface to collect a representative sample and fill it. Label each sample with essential details such as the sampling location, date, and depth. Transport the samples to the laboratory promptly and avoid exposing them to extreme temperatures to ensure reliable pH readings.

Materials Required

- Beaker
- Wash bottle with Distilled Water
- Tissue paper

Instruments Required

- pH meter: A pH meter is an essential scientific instrument used to measure the acidity or alkalinity of a solution. It provides precise and reliable pH readings, which are crucial in various fields, including chemistry, biology, environmental science, and mariculture. The pH meter consists of a probe that senses hydrogen ion activity in the solution and a meter that displays the pH value. Accurate pH measurements are vital for maintaining optimal conditions for biological processes, ensuring the health and productivity of aquatic organisms in mariculture, and monitoring water quality in environmental studies. Regular calibration and proper maintenance of the pH meter are essential for obtaining consistent and accurate results, making it an indispensable tool in both laboratory and field settings.

Fig 3.1 Digital pH Meter

Procedure

01 Calibration:

- Turn on the pH meter and allow it to warm up if required by the manufacturer.
- Rinse the pH probe with distilled water and gently blot it dry with tissue paper or a lint-free cloth to avoid contamination.
- Immerse the probe into the pH 7.0 buffer solution. Wait for the reading to stabilize, then adjust the meter to read exactly 7.0 pH using the calibration knob or buttons.
- Rinse the probe again with distilled water and dry it.
- Repeat the calibration process with pH 4.0 and pH 10.0 buffer solutions if a two- or three-point calibration is required. Always rinse and dry the probe between solutions.

02 Sample Measurement

- Rinse the probe with distilled water and gently blot it dry.
- Immerse the probe into the sample water. Ensure the sample is well mixed and at a uniform temperature for accurate measurement.
- Wait for the reading to stabilize. This may take a few seconds to a minute, depending on the meter.
- Record the pH value displayed on the meter.

03 Post-Measurement Care:

- Rinse the probe with distilled water and gently blot it dry. Store the probe according to the manufacturer's instructions, typically in a storage solution or with a protective cap to keep the electrode moist.

Note

- It is important to note that hydrogen ion activity (proton) can be averaged and convert back it into pH.
- Calibrate the pH meter using standard buffer solutions before each use to ensure accuracy.
- Properly clean and condition the pH electrode according to the manufacturer's instructions before use. Ensure that the electrode is hydrated if it is stored dry.
- pH readings are temperature-dependent. Use a temperature probe to ensure the sample and buffer solutions are at the same temperature.
- Allow sufficient time for the pH meter reading to stabilize after immersing the electrode in the sample.

Alternative Methods

Titration

Titration is a classical quantitative technique used to determine the concentration of an acidic or basic solution. In an acid-base titration, a solution of known concentration (the titrant) is gradually added to the solution of unknown concentration (the analyte) until the reaction reaches the equivalence point, typically indicated by a colour change using an appropriate indicator. The volume of titrant required to reach this point is used to calculate the concentration of the analyte. The calculation is performed using the formula:

$$C_{analyte} = \frac{C_{titrant} \times V_{titrant}}{V_{analyte}}$$

where C represents concentration and V represents volume. This method provides precise and reliable pH measurements, but it is more time-consuming and requires careful technique.

pH Strips

pH strips are an accessible and straightforward method for measuring pH. These strips are impregnated with pH-sensitive dyes that change colour in response to the acidity or alkalinity of the solution. The colour change is compared against a standard pH colour chart to estimate the pH value. While pH strips offer rapid results and ease of use, they lack precision compared to electronic methods and do not provide information about the strength of acids or bases.

Multiparameter kits and pH probe

A multiparameter probe is an advanced tool that measures pH using a combination of electrodes. The glass electrode interacts with hydrogen ions in the sample, while the reference electrode maintains a stable potential. The difference in electrical potential between these electrodes, due to the pH difference across the glass membrane, is measured and converted into a pH value. This method offers high precision and can be used for continuous monitoring, making it suitable for detailed and real-time pH analysis in various aquatic environments. Similarly, a pH probe operates on the same principle, utilizing a glass electrode and reference electrode to determine the hydrogen ion concentration in a sample. pH probes are often used for spot-check measurements and are highly portable, making them ideal for fieldwork where quick and accurate pH readings are required. Both multiparameter probes and pH probes provide reliable and efficient means of assessing pH levels, essential for maintaining the health and stability of aquatic systems.

Fig 3.2 a. A multiparameter Kit for pH estimation, b. A pH probe

3.2 Estimation of Salinity

Salinity, defined as the "total amount of solid materials in grams dissolved in one kilogram of seawater when all the carbonate has been converted to oxide, bromine and iodine replaced by chlorine, and all organic matter completely oxidized," is a fundamental parameter in marine science. It quantifies the concentration of dissolved salts, primarily sodium chloride, in seawater. Accurate salinity measurement is essential for understanding marine water properties, as it affects water density, stratification, and the distribution of marine organisms. In mariculture, maintaining optimal salinity levels is crucial for the health, growth, and productivity of cultured species. Variations in salinity can induce osmotic stress, disrupt metabolic functions, and impact survival rates, highlighting the need for precise salinity monitoring and management in aquaculture systems

Principle

In seawater analysis, halogen compounds such as chloride, bromide, and iodide are titrated with silver nitrate, using potassium chromate as an indicator. When silver nitrate is added to the seawater sample, it reacts with halogen ions to form insoluble silver halides, except for fluoride. The reaction proceeds until all halide ions are precipitated as silver halides. After the precipitation of halide ions, excess silver ions begin to react with potassium chromate, forming a red precipitate of silver chromate. The appearance of this faint red colour signals the endpoint of the titration. The volume of silver nitrate required to reach this endpoint reflects the total halogen content, thus providing an accurate measure of the seawater's chlorinity. The chlorinity is then used to calculate salinity, based on the standard conversion factor.

Sample collection

For accurate salinity measurements, collect seawater samples using clean, acid-washed containers. Submerge the container below the water surface to avoid contamination and fill it completely. Properly label each sample with relevant details such as sampling location, date, and depth. Store the samples in a cool, dark place and transport them to the laboratory promptly to ensure their integrity and the precision of the salinity analysis.

Materials Required

- Burette with stand
- Conical flask (250 mL)– 1 No
- Measuring cylinder (50 mL) – 1 No

- Micropipette with tip
- Standard flask (1 L) -1 No
- Standard flask (100 mL) – 1 No
- Wash bottle with Distilled water

Reagents Required

- Silver nitrate solution: 37.11 g of GR/reagent grade silver nitrate is dissolved in 1 L distilled water
- Potassium chromate solution: 8g of the salt is dissolved in 100 mL of distilled water.

Procedure

01 Sample Preparation

- Measure a known volume (e.g., 50 mL) of the seawater sample using a pipette and transfer it to the titration flask.

02 Indicator Addition

- Add a few drops of potassium chromate (K_2CrO_4) indicator to the water sample in the flask. The sample will turn yellow due to the indicator.

03 Titration

- Fill the burette with a standard solution of silver nitrate ($AgNO_3$).
- Slowly add the silver nitrate solution from the burette to the water sample while constantly swirling the flask.
- Continue the titration until a persistent reddish-brown precipitate (silver chromate, Ag_2CrO_4) forms. This indicates the endpoint of the titration.

04 Calculation

- Record the volume of silver nitrate solution used to reach the endpoint.
- The concentration of chloride ions (Cl^-) in the sample can be calculated using the formula:

$$\text{Salinity } (Cl^- \text{ in g/L}) = \frac{\text{Volume of } AgNO_3 \times Molarity\ of\ AgNO_3 \times 35.45}{Volume\ of\ sample\ in\ L}$$

- Multiply the chloride concentration by a factor (usually 1.805) to convert it to salinity (total dissolved salts).

Note

- Uniform sample usage is suggested, preferably 50mL, so the ionic concentration to achieve the endpoint remains constant.
- The pH of the solution must be between 7–8 since at lower pH, the, chromate ion is protonated and the chromic acid form dominates in the solution and the chromate ion concentration becomes too low to produce the precipitate at the equivalence point.
- A good amount of indicator solution must be used. Otherwise, the endpoint might be achieved too soon, disrupting the chances of getting accurate results.
- Caution should be taken to notice the colour change of the indicator solution from yellow to brick red, and the tube must be shaken to get accurate results.
- Take a minimum of three results to obtain accurate readings for the experiment.

Alternative Methods

Refractometer

A refractometer is an optical instrument used to measure the refractive index of a liquid, which can be correlated to various properties such as concentration or salinity. The principle behind a refractometer is based on Snell's Law, which states that the angle of light passing from one medium to another changes due to the differing speeds of light in those media. In a refractometer, a beam of light is directed through the liquid sample, and the extent of light bending (refraction) is measured. This bending is related to the concentration of dissolved substances in the liquid.

Fig 3.3 Refractometer

How to use a Refractometer?

01 Periodically check and adjust the refractometer using a calibration solution (usually distilled water) to ensure accuracy, setting it to 0.

02 Place a few drops of the liquid sample onto the prism of the refractometer and close the cover plate to spread it evenly.

03 Look through the eyepiece to observe the light pattern and read the refractive index or concentration on the scale.

04 Note the reading indicated by the scale.

05 Wipe the prism and cover plate with a soft, lint-free cloth to remove any residue.

Conductivity Meter and multiparameter Kit

A conductivity meter is a precise instrument used to measure the electrical conductivity of a solution, which is directly related to its salinity. It operates by passing an electric current through the solution and measuring the resulting conductivity, which increases with the concentration of dissolved salts. This method allows for accurate and rapid assessment of salinity levels, making it essential for monitoring aquatic environments. A multiparameter kit designed specifically for salinity measurement combines conductivity and salinity sensors in one device, offering the convenience of obtaining salinity data along with other relevant measurements if needed. These kits are particularly useful for providing a detailed assessment of water conditions, ensuring accurate and consistent monitoring of salinity levels critical for the health and productivity of aquatic organisms.

Fig 3.4 Conductivity Meter

3.3 Estimation of Alkalinity

The alkalinity present in water predominantly arises from salts, including carbonates, bicarbonates, phosphates, nitrates, borates, silicates, and the presence of hydroxyl ions in a free state. However, the prevalent composition of alkalinity-inducing ions in most bodies of water primarily consists of carbonates and bicarbonates, with minimal concentrations of other contributing ions. In mariculture, maintaining appropriate alkalinity levels is essential for the health and growth of cultured species. Alkalinity influences the stability of pH in the water, which in turn affects the metabolic processes of aquatic organisms. High or low alkalinity can lead to stress, impaired growth, and increased mortality in cultured species. Therefore, regular monitoring of alkalinity is crucial for the effective management of mariculture systems.

Principle

The determination of total alkalinity, carbonates, and bicarbonates can be conducted through titration of the water sample using a strong acid such as HCl or H_2SO_4. Initially, the sample is titrated to a pH of 8.3 employing phenolphthalein as an indicator, which turns the solution colorless when all the hydroxyl and carbonate ions are neutralized. This first titration yields the phenolphthalein alkalinity (P). Next, the titration continues to a pH range of 4.2 to 5.4 using methyl orange or a mixed indicator, which changes the solution colour to light pink or red. This second endpoint provides the total alkalinity (T). The values obtained from these titrations allow for the computation of carbonates, bicarbonates, and hydroxyl ions present in the water sample. The total alkalinity is calculated based on the volume of acid required to reach the endpoints and is usually expressed in milligrams per liter (mg/L) of calcium carbonate ($CaCO_3$).

Sample collection

Collect samples using clean, acid-washed bottles to avoid contamination. Rinse the bottle with the source water three times, then submerge it below the surface to fill, ensuring no air bubbles are trapped. Seal the bottle tightly and label it with relevant information, such as the sampling location, date, and time. Store the samples in cool, dark conditions and transport them to the laboratory as soon as possible. Proper handling and timely analysis are essential to obtain accurate alkalinity measurements.

Materials Required

- Measuring cylinder (250 mL) – 1 No
- Beaker – 2 Nos
- Burette with stand – 1 No
- Conical Flask – 1 No
- Pipette – 1 No
- Standard flask (1 L) – 4 Nos
- Standard flask (100 mL) – 1 No
- Wash bottle with Distilled Water

Reagents Required

- **Hydrochloric Acid (0.1 N).**
  1. Prepare 1 N HCl: Dilute concentrated 12 N HCl (specific gravity 1.18) 12 times. For example, mix 8.34 mL of 12 N HCl with distilled water to make up to 100 mL.
  2. Prepare 0.1 N HCl: Further dilute the 1 N HCl by mixing 100 mL of it with distilled water to make up to 1000 mL.
  3. Standardize: Standardize the 0.1 N HCl solution using standard sodium carbonate solution.
- **Methyl Orange Indicator**
  1. Dissolve 0.5 g of methyl orange powder in distilled water.
  2. Dilute the solution to a final volume of 100 mL.
  3. Store the solution in a dark place.
- **Phenolphthalein Indicator**
  1. Dissolve 5 g of phenolphthalein in 500 mL of ethanol.
  2. Add 500 mL of distilled water to the solution.
  3. Add 0.02 N sodium hydroxide drop by drop until the solution turns a faint pink colour.
- **Sodium Thiosulphate (0.1 N)**
  1. Dissolve 25 g of sodium thiosulphate in 1 liter of distilled water.
- **Sodium Carbonate (0.1 N)**
  1. Dissolve 5.3 g of sodium carbonate (previously dried at 250 °C for about 4 hours) in distilled water.
  2. Make up the solution to a final volume of 1 liter.

Procedure

01 Preparation

- Pipette 100 mL of the sample into a clean conical flask.

02 Residual Chlorine Removal

- To remove any residual chlorine that could interfere with the titration, add 1 drop of sodium thiosulphate to the sample. This step ensures accurate measurement of alkalinity by preventing chlorine from reacting with the titrant.

03 Add Phenolphthalein Indicator

- Add 2 drops of phenolphthalein indicator. If the pH is above 8.3, the solution will turn pink, indicating the presence of hydroxide and carbonate alkalinity.

04 First Titration

- Titrate the sample with standard hydrochloric acid from the burette until the pink colour disappears. This measures the alkalinity associated with hydroxide and carbonate ions. Record the volume of hydrochloric acid used (V_1).

05 Add Methyl Orange Indicator

- After the pink colour has disappeared, add 2 drops of methyl orange indicator.

06 Second Titration

- Continue titrating with hydrochloric acid until the colour changes from yellow to orange. This indicates the end point for bicarbonate alkalinity. Record the total volume of hydrochloric acid used (V_2).

06 Calculation

1) Phenolphthalein alkalinity (P) as mg/L CaCO3 =

$$Phenolphthalein\ alkalinity(P)\ as \frac{mg}{L} CaCO_3 = \frac{(V_1 * Normality\ of\ HCl) * 1000 * 50}{mL\ of\ Sample}$$

2)Total alkalinity T as mg/L CaCO3 =

$$Total\ alkalinity(T)\ as \frac{mg}{L} CaCO_3 = \frac{(V_2 * Normality\ of\ HCl) * 1000 * 50}{mL\ of\ Sample}$$

Where, V1 = ml of HCl used with only phenolphthalein
V2 = ml of total HCl used with only phenolphthalein and methyl orange.
P = Phenolphthalein alkalinity
T = Total alkalinity

Fig 3.5 a. Sample after adding phenolphthalein indicator b. Sample after first titration, c. Sample after adding Methyl orange indicator, d. Final titration

Note

- Do not leave the indicator solution uncovered as it contains alcohol that can evaporate.
- Be careful not to spill the mixed indicator solution containing dye onto your skin.
- If it spills in your skin mark will remain for 2-3 days.

3.4 Estimation of Dissolved Oxygen

Dissolved Oxygen (DO) refers to the amount of oxygen that is present in water and available for biological use. It is a critical parameter in aquatic environments, playing a key role in supporting the respiration of fish, invertebrates, and microorganisms. DO is essential for various physiological and metabolic processes, including respiration, nutrient cycling, and waste decomposition. In mariculture, maintaining adequate DO levels is crucial for the health and growth of cultured species. Low DO levels can lead to hypoxia, resulting in stress and mortality among aquatic organisms, while optimal DO concentrations are necessary for maximizing growth rates and ensuring the overall productivity and sustainability of aquaculture systems. The Winkler method is a classic titrimetric technique used to determine dissolved oxygen (DO) concentrations in water.

Sample collection

For accurate measurement of dissolved oxygen using the Winkler method, sample collection must be performed with care to preserve the integrity of the dissolved oxygen content. Collect samples using clean, air-tight bottles to prevent exposure to the atmosphere, which could alter oxygen levels. Fill the bottles, avoiding any air bubbles, and immediately add Winkler's Reagents A and B to fix the oxygen content in the sample. This fixation ensures that the dissolved oxygen is preserved until the sample can be analyzed in the laboratory. Proper handling and prompt fixation are crucial for reliable results.

Principle

The Winkler method, also known as the Winkler titration, operates on the principle of oxidizing manganous dioxide (bivalent manganese) with dissolved oxygen in a sample, leading to the creation of a tetravalent compound. Upon acidification of the solution containing this tetravalent compound, free iodine is released through the oxidation of potassium iodide. The quantity of liberated iodine corresponds directly to the dissolved oxygen content in the sample, which is subsequently quantified through titration using a standardized solution of sodium thiosulphate.

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + 2K_2SO_4$$

A white precipitate suggests minimal dissolved oxygen within the sample. Conversely, a brown precipitate signifies the presence of dissolved oxygen, which has interacted with manganous hydroxide to generate manganic oxide.

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$

On addition of acid the precipitate is dissolved forming manganic sulphate.

$$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$$

As a result of the immediate interaction between this compound and the previously introduced potassium iodide, iodine is promptly released, leading to the characteristic iodine colouring of the sample.

$$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2$$

The quantity of iodine molecules liberated during the reaction is equivalent to the number of oxygen molecules dissolved in the sample. This can be accurately assessed through titration against a standard sodium thiosulphate solution, employing starch as an indicator.

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$

Materials Required

- Beaker – 1 No
- Burette with stand – 1 No
- Conical flask (250 mL)– 1 No
- D.O Bottle – 4 Nos
- Measuring cylinder (10 mL, 50 mL) – 1 No
- Micropipette with tip
- Standard flask (1 L) – 1 No
- Standard flask (100 mL) – 2 Nos
- Wash bottle with distilled water

Reagents Required

- **Winkler solution A**

1. Dissolve 36 grams of analytical reagent grade manganous sulphate monohydrate, $MnSO_4.H_2O$, in distilled water, and adjust the volume to 100 mL.

- **Winkler solution B**

1. Dissolve 100 g of sodium hydroxide in 100 mL of distilled water.
2. Dissolve 27 g of potassium iodide in 100 mL of distilled water and mix the two solutions. Large amount of heat is liberated during the process. Both the sodium hydroxide and potassium iodide used in this method should be of analytical reagent grade.

- **Standard thiosulphate solution (0.025N):**

1. Dissolve 6.205 g sodium thiosulphate in 1 litre distilled water.

- **Starch Indicator**

1. Dissolve 1 g of starch powder in a small amount of cold distilled water to make a smooth paste.
2. Heat the paste gently in boiling distilled water while stirring continuously until it becomes clear and thickens.
3. Allow the solution to cool to room temperature.
4. Store the starch solution in a dark bottle, as it can degrade with light.

Procedure

01 Sample Collection

- Collect the water sample in a BOD bottle, ensuring no air bubbles are trapped. Fill the bottle completely and seal it with a stopper.

02 Add Winkler's Reagents

- Add 1-2 mL of Winkler's A (manganous sulfate solution) and 1-2 mL of Winkler's B (alkaline iodide reagent) to the sample. Invert gently to mix. A brownish or yellowish precipitate may form. (Preparation procedure in reagents required section).

03 Add Sulfuric Acid

- Add 1-2 mL of concentrated sulfuric acid (H_2SO_4) to the sample to dissolve the precipitate and release iodine.

04 Titrate with Sodium Thiosulphate

- Titrate the sample with standardized sodium thiosulphate solution until the iodine colour fades (Preparation procedure in reagents required section).

05 Add Starch Indicator

- Add a few drops of starch indicator solution to the sample. The solution will turn blue if iodine is present.

06 Continue Titration

- Continue titrating until the blue colour disappears, indicating that all iodine has reacted.

07 Calculate DO

- Record the volume of sodium thiosulphate used and calculate the DO concentration based on this volume and the sample volume.

Calculation Formula

$$DO = \frac{(V_{std} - V_{sample}) \times N_{std} \times 8000}{V_{sample}}$$

V (std) = Volume of sodium thiosulphate solution used in the blank titration (mL)

V (sample) = Volume of sodium thiosulphate solution used for the sample (mL)

N (std) = Normality of the sodium thiosulphate solution (N)

8000 = Constant for converting the result to mg/L

Fig. 3.6 Precipitation formation after adding Conc. Sulfuric Acid to the water sample

Fig 3.7 Gradual Color Change during titration

Note

- Use high-quality reagents, especially manganous sulphate and potassium iodide, to ensure accurate results.
- Employ precise titration techniques, including standardized solutions and appropriate indicators, to accurately determine the concentration of dissolved oxygen.
- Maintain consistent temperatures throughout the procedure, particularly during the iodometric titration, to minimize errors.
- Prevent contamination of reagents and samples by using clean glassware and avoiding contact with atmospheric oxygen during the procedure.

Alternative Method

D.O using Multiparameter probe

A multiparameter probe measures dissolved oxygen (DO) using the principle of either electrochemical or optical sensing. In the electrochemical method, an oxygen-permeable membrane covers a cathode and anode submerged in an electrolyte. Oxygen diffuses through the membrane and is reduced at the cathode, producing a current proportional to the oxygen concentration. In the optical method, the probe uses a luminescent dye that emits light when excited. Oxygen molecules reduce this luminescence, and the decrease in emitted light is measured and correlated to the DO concentration.

3.5 Estimation of Biological Oxygen Demand

Biological Oxygen Demand (BOD) is a critical water quality parameter that quantifies the amount of oxygen consumed by microorganisms during the decomposition of organic matter in water over a specified period. It serves as an important indicator of organic pollution, with higher BOD values reflecting greater levels of organic matter and potential stress on aquatic ecosystems. Elevated BOD levels can lead to reduced dissolved oxygen in the water, adversely affecting aquatic life. In mariculture, where maintaining optimal water quality is vital for the health and growth of cultured species, BOD measurements are essential. High BOD values, often due to organic waste from feed and metabolic by-products, can cause oxygen depletion, leading to stress or harm to aquatic organisms. Effective management of BOD levels ensures that dissolved oxygen remains sufficient, supporting a healthy environment for marine life and enhancing the sustainability and productivity of aquaculture systems. By monitoring and controlling BOD, mariculture operations can mitigate the risks associated with organic pollution and promote a balanced and thriving aquatic ecosystem..

Principle

The principle of the Biological Oxygen Demand (BOD) test is based on the measurement of the amount of oxygen consumed by microorganisms during the breakdown of organic matter in a water sample over a specified period. This process occurs naturally as microorganisms decompose organic pollutants, leading to a reduction in the dissolved oxygen levels. By measuring the decrease in oxygen concentration before and after the incubation period, the BOD indicates the extent of organic pollution. A higher BOD value signifies a greater amount of organic material and potential for increased microbial activity

The subsequent equations will simplify the biodegradation process:

Organic matter + O_2 + microorganisms = CO_2 + H_2O + new microbial cells

Ammonia + O_2 + microorganisms = NO_3 + H_2O + new microbial cells.

Sample collection

To ensure accurate Biological Oxygen Demand (BOD) measurements, water samples must be collected and preserved carefully. Use clean, non-reactive bottles, such as glass or plastic, to avoid contamination. Collect samples from representative points in the water body, ensuring they are well-mixed and not affected by surface scum or sediments. Fill the bottles to the brim to minimize air contact, which could alter oxygen levels. Immediately seal the containers to prevent any change in oxygen levels and store them in a cool, dark place. Transport samples to the laboratory within a few hours of collection to maintain their integrity and ensure reliable BOD results.

Materials Required

- Beaker – 1 No
- BOD bottles (300 mL) – 6 Nos
- Burette with stand – 1 No
- Conical flask (250 mL) – 1 No
- D.O Bottle – 4 Nos
- Measuring cylinder (10 mL, 50 mL) – 1 No
- Micropipette with tip
- Standard flask (1 L) – 1 No
- Standard flask (100 mL)– 2 Nos
- Wash bottle with distilled water

Instruments Required

- BOD Incubator

Reagents Required

- **Winkler solution A**
  1. Dissolve 36 grams of analytical reagent grade manganous sulphate monohydrate, $MnSO_4$. H_2O, in distilled water, and adjust the volume to 100 mL.
- **Winkler solution B**
  1. Dissolve 100 g of sodium hydroxide in 100 mL of distilled water.
  2. Dissolve 27 g of potassium iodide in 100 mL of distilled water and mix the two solutions. Large amount of heat is liberated during the process. Both the sodium hydroxide and potassium iodide used in this method should be of analytical reagent grade.
- **Standard thiosulphate solution (0.025N):**
  1. Dissolve 6.205 g sodium thiosulphate in 1litre distilled water

- **Starch Indicator**

1. Dissolve 1 g of starch powder in a small amount of cold distilled water to make a smooth paste.
2. Heat the paste gently in boiling distilled water while stirring continuously until it becomes clear and thickens.
3. Allow the solution to cool to room temperature.
4. Store the starch solution in a dark bottle, as it can degrade with light.

Procedure

01 Prepare BOD Dilutions

- Prepare three BOD bottles by transferring the following sample volumes and filling them up with dilution water to the 300 mL mark:
- 5 mL sample
- 15 mL sample
- 20 mL sample

02 Create Two Sets of Samples

- Prepare one set of each of the above dilutions to measure the initial dissolved oxygen (DO) on day 0.
- Prepare a second set of each of the above dilutions to be placed in the BOD incubator.

03 Measure Initial DO

- For the Day 0 DO analysis set, measure the initial DO using the Winkler method as outlined previously.

04 Incubate the Samples

- Place the BOD bottles from the incubation set in a BOD incubator.
- Incubate the samples at 20°C for 5 days.

05 Measure DO After 5 Days

- After the 5-day incubation period, measure the DO in the incubated samples using the Winkler method.

05 Record the Data

- Record the initial and final DO concentrations for each sample dilution.
- Calculate the BOD using the difference between the initial and final DO concentrations.

Table 3.1 - Data Recording Format

Sample Volume (mL)	Initial DO (mg/L)	Final DO (mg/L)	Dilution Factor	BOD (mg/L) = (Initial DO - Final DO) × Dilution Factor
5			60	
15			20	
20			15	

Sample Calculation

- Assume:
  Initial DO for 5 mL sample = 8.0 mg/L
  Final DO for 5 mL sample = 4.0 mg/L
  Dilution Factor for 5 mL sample = 300/5 = 60

$$BOD = (8.0\ mg/L - 4.0\ mg/L) \times 60 = 240\ mg/L$$

- Repeat the calculation for the 15 mL and 20 mL samples accordingly using their respective dilution factors:
  Dilution Factor for 15 mL sample = 300/15= 20
  Dilution Factor for 20 mL sample = 300/20=15

3.6 Estimation of Chemical Oxygen Demand

Chemical Oxygen Demand (COD) measures the amount of oxygen required to chemically oxidize both organic and inorganic substances in water, serving as a key indicator of water pollution. It provides a comprehensive assessment of water quality by quantifying the total oxygen demand exerted by all chemical oxidants, including those that are not readily biodegradable. In mariculture, monitoring COD is essential for maintaining optimal water quality. Elevated COD levels can signal significant organic pollution, leading to oxygen depletion that negatively impacts the health and growth of cultured species. By regularly measuring COD, aquaculture managers can effectively manage waste and ensure a sustainable and healthy environment for aquatic life, thus supporting the overall success and productivity of mariculture operations.

Principle

The Chemical Oxygen Demand (COD) test is based on the principle that a strong oxidizing agent, such as potassium dichromate, can completely oxidize almost all organic compounds in a sample to carbon dioxide (CO_2) under acidic conditions. During the test, the sample is treated with potassium dichromate in concentrated sulfuric acid at reflux temperature, with silver sulfate acting as a catalyst and mercuric sulfate added to remove chloride interference. After the oxidation process, any remaining potassium dichromate is quantified by titration with a standard solution of ferrous ammonium sulfate (FAS), using Ferroin as an indicator to detect the end point. This method measures the total oxygen demand of the sample, reflecting the concentration of both biodegradable and non-biodegradable substances.

Sample collection

For accurate Chemical Oxygen Demand (COD) analysis, water samples should be collected in clean, airtight containers to prevent contamination and preserve the sample's integrity. Submerge the container below the water surface to avoid trapping air bubbles and fill it to minimize air space. Label the containers with details such as sampling location, date, and time. Store and transport the samples in a cool, dark environment and deliver them to the laboratory as soon as possible, preferably within 24 hours, to ensure reliable results.

Materials Required

- Burette with stand – 1 No
- Wash bottle with distilled water – 1 No
- Measuring Cylinder (50 mL) – 1 No
- COD tubes
- Standard flask (1 L) – 1 No
- Beaker – 1 No
- Glass bottle (500 mL) – 1 No
- Conical flask – 1 No

Instruments Required

- COD apparatus
- Water distillation unit
- pH meter
- Weighing balance
- Magnetic Stirrer
- Hot air oven

Reagents Required

- **Potassium Dichromate Solution (0.25 N)**
  1. Dissolve 12.25 g of potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1 liter using a magnetic stirrer to ensure complete dissolution.
- **Sulfuric Acid Reagent**
  1. Slowly add 5.5 g of silver sulfate (Ag_2SO_4) to 1 liter of concentrated sulfuric acid (H_2SO_4). Allow the mixture to stand for at least one day before use.
- **Ferroin Indicator**
  1. Dissolve 1.485 g of 1,10-phenanthroline monohydrate and 695 mg of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) in distilled water and dilute to 100 mL, using a magnetic stirrer.
- **Standard Ferrous Ammonium Sulfate (FAS) Solution (0.1 N):**
  1. Dissolve 39.2 g of ferrous ammonium sulfate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) in distilled water. Add 20 mL of concentrated sulfuric acid (H_2SO_4), cool, and dilute to 1 liter with the help of a magnetic stirrer to ensure complete dissolution.

Procedure

01 Prepare COD Tubes:

- Add 0.4 gm of mercuric sulfate ($HgSO_4$) and 20 ml of the sample (or 20 ml of KHP solution diluted with 20 ml of distilled water) to each COD tube. Include 1 or 2 crushed stones to aid mixing.

02 Add Reagents

- Introduce 10 ml of 0.25 N potassium dichromate ($K_2Cr_2O_7$) and 30 ml of concentrated sulfuric acid (H_2SO_4) to each tube. Swirl gently and check the solution colour. If green, add more of the standard $K_2Cr_2O_7$ (known volumes) until the colour remains orange.

03 Digest the Sample

- Place the tubes in a COD apparatus, attach a condenser, set the temperature to 150°C, and digest for 2 hours. Allow the samples to cool to room temperature after digestion.

04 Prepare for Titration:

- Transfer the cooled sample to a conical flask, dilute with 150 ml of distilled water, and titrate with 0.1 N ferrous ammonium sulfate (FAS). Add 3-4 drops of Ferroin indicator.

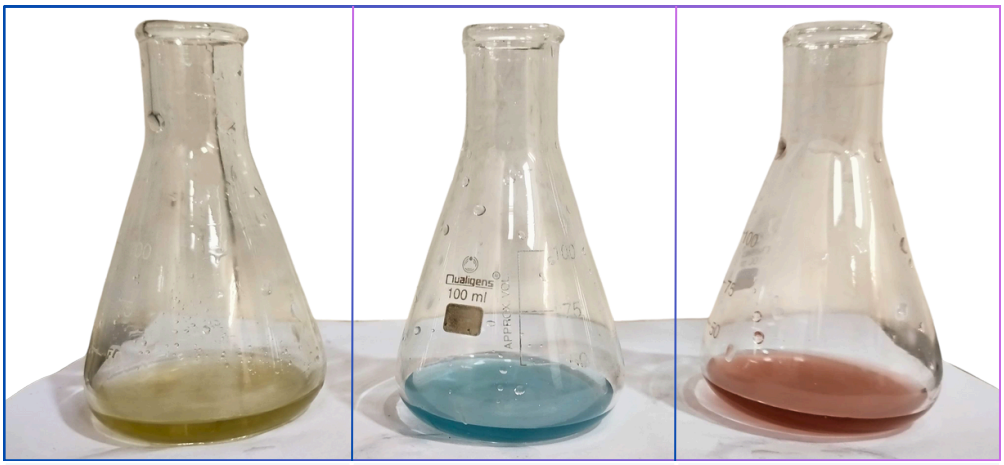
05 Perform Titration

- Determine the end point by observing a color change from blue-green to reddish brown.

06 Calculation

- **COD = a-b X N X 8000/ml of sample solution**

Where, a-Reading blank solution, b-Reading with sample solutions and N-Normality of FAS Solution.



a. After Digestion | b. Before end point | c. After End point

Fig. 3.8 Chemical Oxygen Demand Estimation

Note

- Use high-quality reagents to ensure accurate results.
- Employ precise titration techniques, including standardized solutions and appropriate indicators, to accurately determine the concentration of dissolved oxygen.
- Maintain consistent temperatures throughout the procedure, particularly during the iodometric titration, to minimize errors.
- Prevent contamination of reagents and samples by using clean glassware and avoiding contact with atmospheric oxygen during the procedure.

3.7 Estimation of Organic Carbon

Organic carbon in soil, originating from both natural processes and anthropogenic activities, plays a crucial role in aquatic ecosystems. It serves as a fundamental energy source for microbial communities, fueling the base of the food web and contributing to the overall carbon cycle. In mariculture, the management of organic carbon levels in sediments is essential to maintain optimal water quality and prevent detrimental conditions such as hypoxia, which can adversely affect the health and growth of cultured species. Elevated levels of organic carbon can lead to increased biological oxygen demand (BOD), potentially causing oxygen depletion and stressing aquatic organisms. Additionally, excessive organic carbon can promote the growth of harmful algal blooms, further compromising the aquatic environment and the productivity of mariculture operations. Therefore, regular monitoring and accurate estimation of organic carbon are vital for sustainable aquaculture practices and the preservation of aquatic health. It can be quantified by the titrimetric procedure which involves the gradual addition of a solution with an accurately known concentration (a standard solution) to a solution of unknown concentration (the sample to be analysed) until the reaction between the two solutions is complete. The completion of the reaction is monitored using an indicator, which induces a colour change at the endpoint.

Principle

Organic carbon estimation in soil involves digesting the sample with potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). The heat released during the dilution of sulfuric acid facilitates the oxidation of organic matter in the soil. After the digestion, the remaining, unreacted potassium dichromate is quantified by titrating with a standard solution of ferrous sulfate ($FeSO_4$) or ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$). The amount of potassium dichromate reduced by the organic carbon in the sample is determined based on the difference between the initial and final concentrations of potassium dichromate, providing a measure of the organic carbon content.

Sample collection

For accurate organic carbon estimation, it is crucial to collect soil samples properly to ensure representativeness and minimize contamination. Collect samples using clean, dry tools to avoid contamination from other sources. Choose sampling sites that are representative of the area being studied. Avoid collecting samples from disturbed or atypical areas unless those are specifically of interest.

- Sampling Tools: Use stainless steel or plastic tools to prevent contamination. Avoid using metal tools that may react with the soil.
- Sample Size: Collect sufficient soil from several locations within the sampling area to create a composite sample. Typically, 500 grams to 1 kilogram of soil is recommended.
- Handling: Place the collected soil in clean, labeled containers or bags. Ensure the containers are sealed tightly to prevent moisture loss or contamination.
- Storage and Transport: Store samples in a cool, dry place if they cannot be analyzed immediately. Transport them to the laboratory as soon as possible, maintaining their integrity by keeping them cool and dry.

Materials Required

- Conical Flask
- Measuring Cylinder (50 Ml,100 mL)– 1 No
- Dropper
- Petri dish
- Burette with stand – 1 No
- 0.5 mm Sieve

Reagents Required

- **Potassium Dichromate Solution (1 N)**
  1. Dissolve 49 grams of potassium dichromate in distilled water and dilute to 1 liter.
- **Sulfuric Acid + Silver Sulfate**
  1. Dissolve 25 grams of silver sulfate in 1 liter of concentrated sulfuric acid.
- **Diphenylamine Indicator Solution**
  1. Dissolve 0.5 grams of diphenylamine in a mixture of 100 mL concentrated sulfuric acid and 20 mL distilled water. Store in a colored bottle to protect from light.
- **Ferrous Ammonium Sulfate (0.5 N)**
  1. Dissolve 196 grams of analytical reagent (A.R.) grade ferrous ammonium sulfate ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$) in distilled water. Add 20 mL of concentrated sulfuric acid and dilute to 1 liter.

Procedure

01 Sample Preparation

- Weigh 1 g of air-dried, finely ground soil sample passed through 0.5mm sieve and transfer it into a 500 mL conical flask.

02 Oxidation

- Add 10 mL of 1 N potassium dichromate solution to the soil in the flask.
- Add 20 mL of concentrated sulfuric acid with silver sulfate to the flask, ensuring it is added rapidly to initiate the oxidation process. Swirl the flask gently to mix the contents thoroughly.
- Allow the mixture to stand for 30 minutes for complete oxidation of the organic carbon.

03 Addition of Indicator and Titration

- Add 200 mL of distilled water to the flask.
- Add 10 mL of 85% phosphoric acid and 1 mL of sodium fluoride (NaF) solution.
- Add 2-3 drops of diphenylamine indicator solution to the mixture.
- Titrate the solution with 0.5 N ferrous ammonium sulfate (FAS) solution, stirring continuously.
- The endpoint of the titration is indicated by a colour change from blue-violet to a greenish hue.

04 Blank Determination

- Run a blank determination by following the same procedure without the soil sample to account for any potential reagent impurities or background interferences.

04 Calculation

- Calculate the organic carbon content using the formula:

$$Organic\ Carbon\ (\%) = \frac{(B - S) \times N \times 0.003 \times 100}{Weight\ of\ soil\ Sample\ (g)}$$

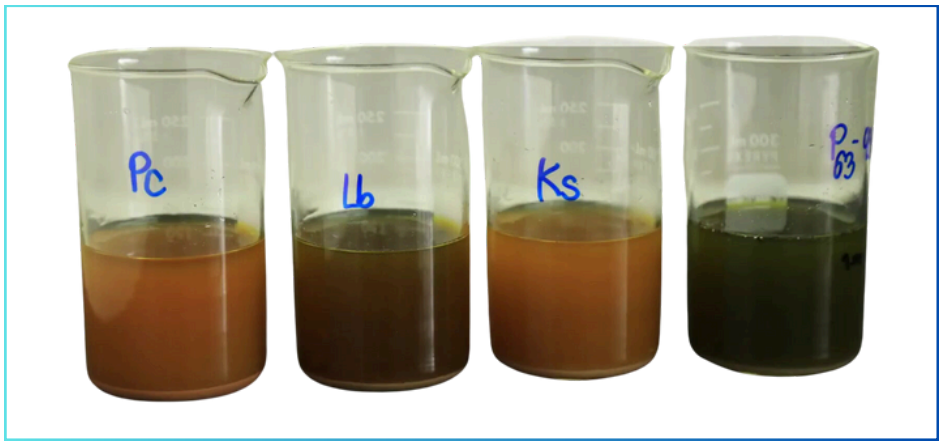


Fig. 3.9 Soil Samples Kept for Digestion

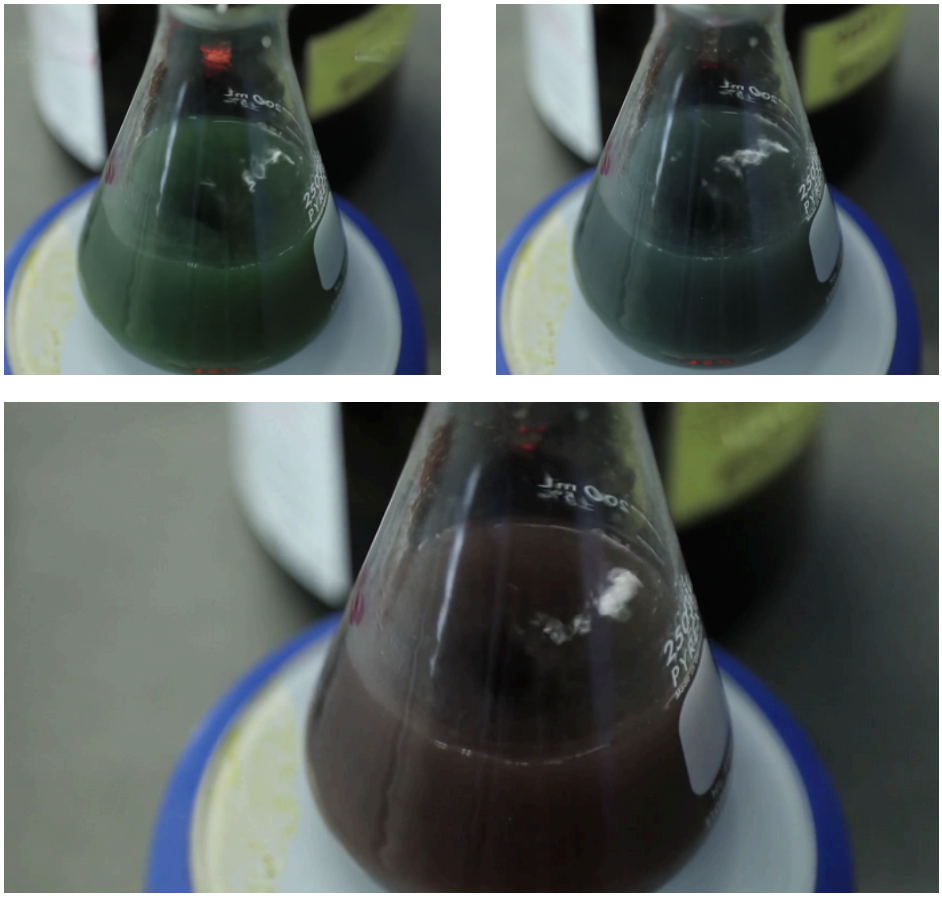


Fig. 3.10 Gradual Change in color during titration

Note

- Maintain a consistent temperature throughout the titration process to minimize errors caused by temperature-dependent reaction rates.
- Perform blank titrations using distilled water or a blank solution containing all reagents except the sample to correct for any background colour or interference.
- Perform acid digestion of the sample to convert organic carbon into a form suitable for oxidation by potassium dichromate.

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CHAPTER 4

Nutrient Analysis

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4 Contents

Introduction

Nutrients are fundamental components of mariculture, which plays an important role in the growth, health, and productivity of aquatic organisms. In mariculture systems, the availability and balance of essential nutrients such as ammonia, nitrite, nitrate, phosphate, and silicate are critical to maintaining optimal water quality and biological productivity. Ammonia, nitrite, and nitrate plays a key role in the nitrogen cycle, which is involved in the process of protein synthesis in fish and other marine species. Ammonia is excreted by fish as a waste product and, if this nutrient bioaccumulates in the fish tissue, it can affect the health and growth of the fish. Through nitrification, ammonia is converted to nitrite and then to nitrate, while this form is less toxic, it still can pose risks if concentrations become too high. Phosphate, another vital nutrient, plays a significant role in cellular energy transfer, DNA synthesis, and skeletal formation in marine organisms. It is essential for the growth of phytoplankton, which forms the base of the aquatic food web, providing nourishment for higher trophic levels, including fish and shellfish.

Silicate is particularly important in marine ecosystems as it is a necessary nutrient for the growth of diatoms, a group of algae that are primary producers and a key food source for many marine organisms. Diatoms contribute significantly to the primary productivity of aquatic systems, and their presence is indicative of a healthy and balanced ecosystem.

Monitoring and estimating the levels of these nutrients in mariculture systems is crucial for several reasons. Firstly, it helps in preventing nutrient imbalances that can lead to water quality deterioration. Excessive levels of nutrients, particularly nitrogen and phosphorus compounds, can result in eutrophication, which can trigger harmful algal blooms (HABs).

Secondly, maintaining appropriate nutrient levels is essential for promoting optimal growth conditions for cultured species. Nutrient deficiencies can stunt growth and reduce the overall health and productivity of the farmed organisms.

Furthermore, understanding nutrient dynamics is essential for the sustainable management of mariculture systems. Sustainable aquaculture practices aim to minimize environmental impacts while maximizing production efficiency. By closely monitoring nutrient levels, aqua culturists can implement strategies to reduce waste, optimize feed usage, and enhance the overall sustainability of their operations.

4.1 Estimation of Ammonia (NH_3)

Ammonia (NH_3) is a compound commonly found in groundwater, surface water, and wastewater, originating from the breakdown of nitrogen-containing organic matter. It plays a crucial role in many biological processes and is a precursor for amino acid and nucleotide synthesis. In mariculture, ammonia is produced as a metabolic waste product by fish and other aquatic organisms and from the decomposition of uneaten feed and organic matter. Although ammonia is an essential part of the nitrogen cycle, high levels in mariculture systems can be toxic, causing stress, reduced growth rates, and increased mortality in cultured species. Therefore, accurate monitoring and management of ammonia levels are essential for the overall health and productivity of mariculture ecosystems. It can be quantified with the help of a spectrophotometer, which works on Beer Lambert's law.

Principle

Ammonia concentration is measured using a colorimetric method. In this process, ammonia reacts with hypochlorite and phenol in the presence of sodium nitroprusside to produce a blue-coloured compound, indophenol blue. This blue colour's intensity, which correlates with ammonia concentration, is quantified by measuring absorbance at 640 nm with a spectrophotometer. Beer-Lambert's law states that absorbance is directly proportional to the concentration of the analyte, allowing accurate determination of ammonia levels.

Sample collection

Representative samples should be collected using clean glassware. Rinse the container with the source water, then submerge it below the surface to avoid scum and debris and collect the representative water sample. Label the sample with the date, time, and location of collection. Store the sample in a cool environment and transport it to the laboratory promptly to maintain integrity.

Analytical Procedures

Stock Preparation for Standardization

- **Mother Stock Solution:** Dissolve precisely 1.321 grams of Ammonium Sulphate in a 1000 mL standard flask using distilled water, ensuring complete dissolution. Adjust the volume to exactly 1000 mL with distilled water. This solution is referred to as the “Mother Stock”.

- **Working Standard Solution:** From the Mother Stock solution, carefully pipette 1mL and transfer it into a separate 100 mL standard flask. Dilute the solution to volume with distilled water, maintaining precision in measurement. This diluted solution is designated as the "Working Standard".
- **Calibration Standards:** Utilizing the Working Standard solution, sequentially pipette volumes of 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL into separate 100 mL standard flasks. Adjust each solution to volume with distilled water, ensuring accurate dilution.
- **Absorbance Measurement:** Extract precisely 10 mL from each prepared standard solution and transfer it into suitable containers. Add the requisite reagents according to the protocol mentioned below. Measure the absorbance of each solution at 640 nm using a spectrophotometer.
- **Standard Curve Construction:** Construct a standard curve by plotting the absorbance readings against the corresponding concentrations of the standard solutions. Ensure that the regression coefficient (R^2) of the standard curve exceeds 0.98, indicating a high degree of linearity and reliability in the calibration.

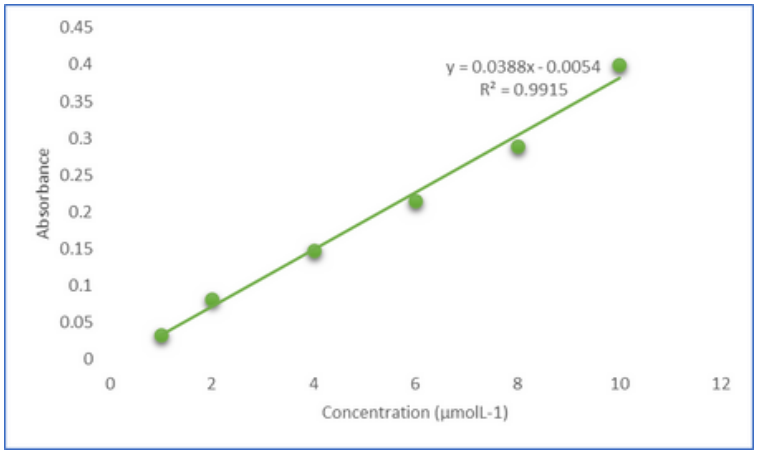

Fig. 4.1 Standardization Curve for Ammonia

Materials Required

- Beaker – 3 Nos
- Funnel – 2 Nos
- Measuring Cylinder (25 mL,10 mL) – 1 No
- Micropipette and Tip
- Pipette – 1 No
- Standard Flask 100 mL - 8 Nos

- Standard Flask (50 mL,250mL & 1L)– 1 No
- Test tube holder – 1 No
- Test tubes - 7 Nos
- Tissue Paper
- Wash bottle with Distilled Water

Instrument Required

- UV Visible Spectrophotometer

Reagents Required

- **Phenol Solution:** Prepare the phenol solution by combining 5.5 mL of liquefied phenol (≥89%) with 95% ethyl alcohol and adjusting the final volume to 50 mL. It is recommended to prepare this solution every week to ensure freshness. Adequate ventilation should be ensured during the preparation process.
- **Sodium Nitroprusside Solution:** Dissolve 1 gram of Sodium nitroprusside in 200 mL of distilled water. Store the solution in amber bottles to protect it from light degradation. The solution remains stable for one month.
- **Alkaline Citrate Solution:** Prepare the alkaline citrate solution by dissolving 50 grams of sodium citrate and 2.5 grams of sodium hydroxide in distilled water. Adjust the volume to 250 mL.
- **Oxidizing Solution:** The oxidizing solution is formulated by combining 25 mL of alkaline citrate solution with 6.25 mL of sodium hypochlorite. It is essential to prepare this solution freshly as needed.

Procedure

01 Sample and Standard Preparation

- Take 10 mL of each standard solution or sample in clean test tubes.

02 Addition of Reagents

- Add 0.4 mL of Phenol solution to each test tube.
- Add 0.4 mL of Sodium Nitroprusside solution to each test tube.
- Add 1 mL of oxidizing solution to each test tube.

03 Incubation

- Incubate the mixture for at least 1 hour, allowing blue colour development. The incubation can be extended up to 24 hours if necessary.

04 Measurement

- Measure the absorbance of each solution at 640 nm using a spectrophotometer.

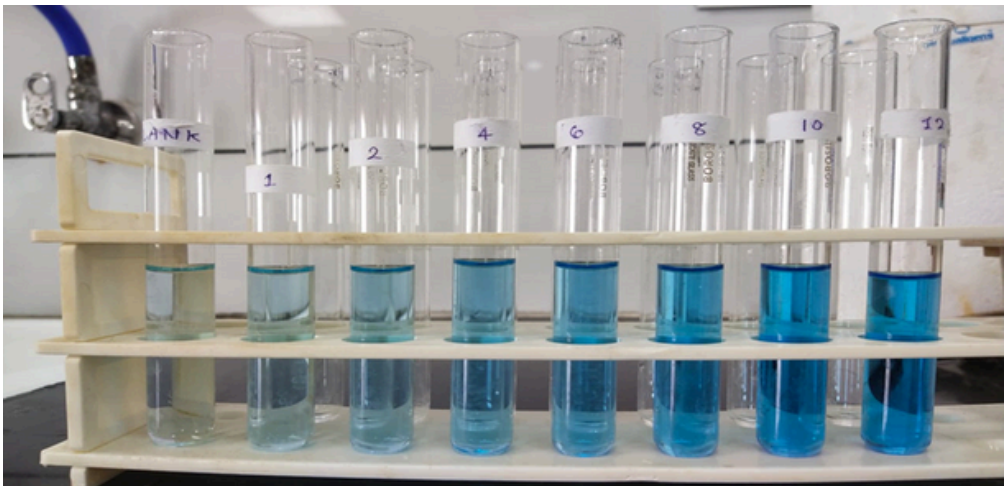

Fig. 4.2 Preparation of Ammonia Standard Solutions at Different Concentrations for Spectrophotometric Analysis

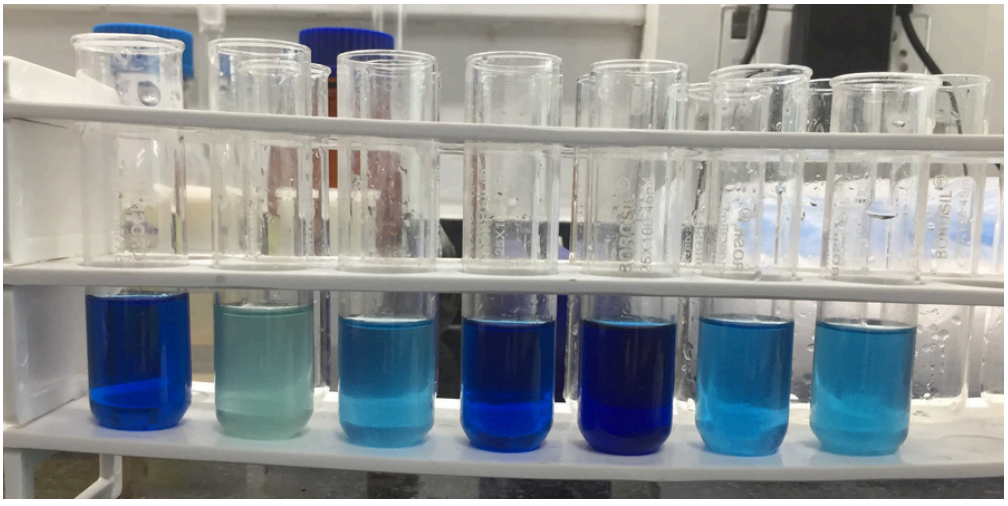

Fig. 4.3 Varying Blue Intensities Reflect Different Ammonia Concentrations in Samples

Note

- Cover samples with plastic wrap.
- Let colour develop at room temperature.
- Colour will be stable for at least 24 hours.

Alternative Method

Ammonia Testing Kit

Ammonia concentration can be quantified using a saltwater test kit through a precise procedure. Begin by adding 5 mL of the sample to a test tube, followed by 8 drops of Test Solution 1. Then, add 8 drops of Test Solution 2 to the mixture. Shake well. Allow the test tube to sit undisturbed for 10 minutes, during which a colour change will occur. The ammonia concentration, measured in parts per million (ppm), can be determined by comparing the resulting colour with the provided colour chart.

Fig. 4.4 Ammonia Testing Kit

4.2 Estimation of Nitrate(NO_3^-)

Nitrate (NO_3^-) is the principal form of reactive inorganic nitrogen in oceanic waters, serving as a crucial nutrient for primary productivity in mariculture systems. It is essential for the synthesis of biomolecules, protein production, energy metabolism, and various physiological processes in aquatic organisms. While nitrates are vital for plant nutrients, excessive levels can lead to water quality issues, causing stress and health problems in cultured species. High nitrate concentrations can accelerate eutrophication, resulting in increased aquatic plant growth and altering the ecosystem balance in mariculture environments.

Principle

Nitrate in seawater is quantitatively reduced to nitrite by passing the sample through a column containing cadmium filings coated with metallic copper. The resulting nitrite is diazotized with sulfanilamide and then coupled with N-(1-naphthyl)-ethylenediamine to form a highly colored azo dye, whose absorbance is measured spectrophotometrically. A correction is applied for any nitrite initially present in the sample.

Sample collection

Representative samples should be collected using clean glassware. Rinse the container with the source water, then submerge it below the surface to avoid scum and debris and collect the representative water sample. Label the sample with the date, time, and location of collection. Store the sample in a cool environment and transport it to the laboratory promptly to maintain integrity. Precise documentation of collection details is essential to ensure the accuracy and reliability of the subsequent analysis.

Analytical Procedures

Stock Preparation for Standardization

- **Mother Stock Solution:** Dissolve precisely 0.345 grams of sodium nitrite in a 1000 mL standard flask, ensuring complete dissolution. Adjust the volume to exactly 1000 mL with distilled water. This solution is referred to as the “Mother Stock”.
- **Working Standard Solution:** From the Mother Stock solution, accurately measure and transfer 10 ml into a separate 100 mL standard flask. Dilute the solution to volume with distilled water, maintaining precision in measurement. This diluted solution is designated as the “Working Standard”.

- **Calibration Standards:** Utilizing the Working Standard solution, sequentially pipette volumes of 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL into separate 100 mL standard flasks. Adjust each solution to volume with distilled water, ensuring accurate dilution.
- **Absorbance Measurement:** Extract exactly 5 mL from each prepared standard solution and transfer it into suitable containers. Add the necessary reagents according to the protocol for nitrate analysis which is mentioned below. Measure the absorbance of each solution at 505 nm using a spectrophotometer.
- **Standard Curve Construction:** Construct a standard curve by plotting the absorbance readings against the corresponding concentrations of the standard solutions. Ensure that the regression coefficient (R^2) of the standard curve exceeds 0.98, indicating a high degree of linearity and reliability in the calibration.

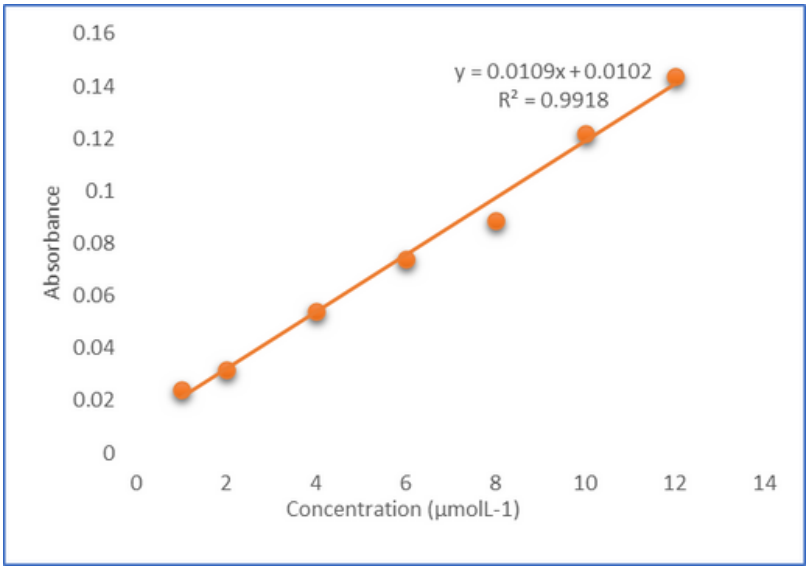

Fig. 4.5 Standardization Curve for Nitrate

Materials Required

- Beaker – 2 Nos
- Funnel – 2 Nos
- Measuring Cylinder (25 mL,10 mL) – 1 No
- Micropipette and Tip
- Pipette – 1 No

- Standard Flask 100 ml - 8 Nos
- Standard Flask 1L – 1 No
- Tissue Paper
- Volumetric Flask (25mL) – 7 Nos
- Wash bottle with Distilled water

Instrument Required

- UV Visible Spectrophotometer

Reagents Required

- **Concentrated Ammonium Chloride Solution:** Dissolve 125 g of analytical reagent grade ammonium chloride (NH_4Cl) in 500 mL of distilled water.
- **Dilute Ammonium Chloride Solution:** Dilute 50 mL of the concentrated ammonium chloride solution to 2000 mL with distilled water.
- **Cadmium-Copper Filings (99.9% purity):**
  1. Stir 100 g of cadmium filings (particle size >0.5 mm) with 500 mL of a 2% w/v copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution until the solution turns clear and copper particles appear.
  2. Fill the column with dilute ammonium chloride solution and gradually add the cadmium-copper mixture to form a column approximately 30 cm in length.
  3. Tap the column gently to ensure proper settling, and wash thoroughly with dilute ammonium chloride solution.
  4. Adjust the flow rate to ensure that 100 mL of solution passes through in 8 to 12 minutes.
  5. When not in use, ensure the column is completely covered with dilute ammonium chloride solution.
- **Sulfanilamide Solution:** Dissolve 5 g of sulfanilamide in a mixture of 50 mL of concentrated HCl and approximately 300 mL of distilled water.
- **N-(1-naphthyl)-ethylenediamine (NNED) Dihydrochloride Solution:** Dissolve 0.5 g of NNED dihydrochloride in 500 mL of distilled water. Store the solution in a dark bottle and renew it monthly.
- **Synthetic Seawater:** Dissolve 310 g of sodium chloride (NaCl), 100 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), and 0.5 g of sodium bicarbonate ($NaHCO_3 \cdot H_2O$) in 10 L of distilled water.
- **Standard Nitrate Solution:**
  1. Dissolve 1.02 g of potassium nitrate (KNO_3) in 1000 mL of distilled water.
  2. Take 2 mL of this solution and dilute to 1000 mL with synthetic seawater to prepare a standard nitrate solution with a final concentration of 20 µg-at N/L.
  3. Store the standard solution in a dark bottle and prepare it fresh before use.

Procedure

01 Sample and Standard Preparation

- Add 2 mL of concentrated ammonium chloride to the sample.
- Transfer 100 + 2 mL of the sample into an Erlenmeyer flask and mix the solution thoroughly.
- Pour approximately 5 mL of the mixture onto the top of the column and allow it to pass through.
- Add the remaining sample to the column and position the drained Erlenmeyer flask beneath the collection tube.
- Collect approximately 40 mL of the eluate and discard it.
- Collect about 50 mL in a graduated cylinder and transfer this portion back into the Erlenmeyer flask containing the original sample.

02 Addition of Reagents

- Add 1 mL of sulfanilamide solution to the 50 mL sample using a pipette.
- Mix thoroughly and allow the reaction to proceed for more than 2 minutes but no longer than 8 minutes.
- Add 1 mL of naphthylethylene diamine (NNED) solution and mix immediately.

03 Incubation & Measurement

- Within 2 hours, measure the absorbance of the solution in a 1 cm cuvette against distilled water at a wavelength of 543 nm using a spectrophotometer.
- Adjust the measured absorbance by subtracting the absorbance of the reagent blank.

04 Substitution

- Repeat the procedure using 100 mL of dilute ammonium chloride solution instead of the seawater sample as a blank.
- Measure the absorbance using the same 1 cm cuvette.
- Subtract the blank absorbance from each sample's absorbance value.

05 Dilution

- Carry out the procedure with 100 mL of dilute nitrate solution and measure the absorbance (extinction) for each column.
- Calculate the factor F using the formula:

  F = 20 / Es,

  where Es is the corrected extinction of the standard.

06 Calculation

- µg- at N/l = (Corrected extinction x F) - 0.95 C

Where C = the concentration of nitrite in the sample in µg at N/L

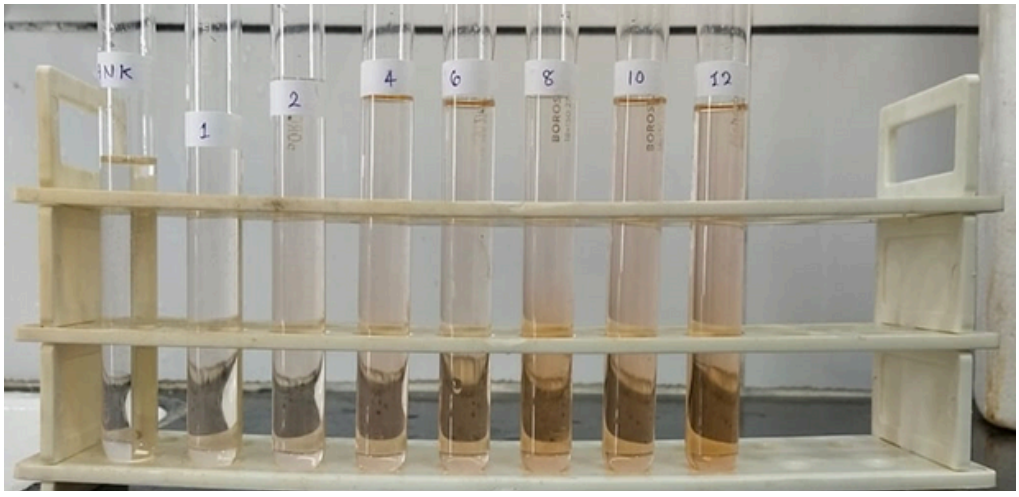

Fig. 4.6 Preparation of Nitrate Standard Solutions at Different Concentrations for Spectrophotometric Analysis

Note

- Use clean containers, avoid contamination, and store samples in the dark at low temperatures.
- Monitor the colour development during the reaction. Ensure that the colour development is proportional to the nitrate concentration.
- Control the reaction conditions, including pH and temperature, as they can significantly impact the accuracy of the method.

Alternative Method

Nitrate Testing Kit

Nitrate concentration can be quantified using a saltwater test kit through a precise procedure. Begin by adding 5 mL of the sample to a test tube, followed by 10 drops of Test Solution 1. Then, add 10 drops of Test Solution 2 to the mixture. Shake well. Allow the test tube to sit undisturbed for 10 minutes, during which a colour change will occur. The Nitrate concentration, measured in parts per million (ppm), can be determined by comparing the resulting colour with the provided colour chart.

Fig. 4.7 Nitrate Testing Kit

4.3 Estimation of Nitrite (NO_2^-)

Nitrite (NO_2^-) is an intermediate in the nitrogen cycle and is present in various water bodies, including mariculture systems. It is a product of both the microbial reduction of nitrate and the oxidation of ammonia. Nitrite plays a crucial role in the nitrogen cycle by acting as a bridge between the oxidation states of nitrogen, facilitating the conversion of ammonia to nitrate through nitrification and the reduction of nitrate back to nitrogen gas via denitrification. In mariculture systems, the balance of these processes is critical for maintaining water quality and ensuring the health of cultured species. Elevated nitrite levels can impair the ability of fish and other marine organisms to transport oxygen, leading to methemoglobinemia, also known as "brown blood disease." Therefore, regular monitoring and control of nitrite concentrations are vital to prevent toxic buildup and ensure optimal growth and survival rates in mariculture operations.

Principle

Nitrite concentration is determined using a colorimetric method in which nitrite reacts with sulfanilamide in an acidic solution to form a diazonium salt. This salt then couples with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to produce a pink-coloured azo dye. The intensity of the colour, measured at 543 nm using a spectrophotometer, is directly proportional to the nitrite concentration in the sample. This measurement is based on Beer Lambert's law, which states that absorbance is directly proportional to the concentration of the solution, the path length, and the molar absorptivity.

Sample collection

Representative samples should be collected using clean glassware. Rinse the container with the source water, then submerge it below the surface to avoid scum and debris and collect the representative water sample. Label the sample with the date, time, and location of collection. Store the sample in a cool environment and transport it to the laboratory promptly to maintain integrity. Precise documentation of collection details is essential to ensure the accuracy and reliability of the subsequent analysis.

Analytical Procedures

Stock Preparation for Standardization

- **Mother Stock Solution:** Dissolve precisely 0.689 grams of sodium nitrite in a 1000 mL standard flask, ensuring complete dissolution. Adjust the volume to exactly 1000 mL with distilled water. This solution is referred to as the "Mother Stock".
- **Working Standard Solution:** ·From the Mother Stock solution, accurately measure and transfer 10 ml into a separate 100 mL standard flask. Dilute the solution to volume with distilled water, maintaining precision in measurement. This diluted solution is designated as the "Working Standard".
- **Calibration Standards:** Utilizing the Working Standard solution, sequentially pipette volumes of 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL into separate 100 mL standard flasks. Adjust each solution to volume with distilled water, ensuring accurate dilution.
- **Absorbance Measurement:** Extract precisely 10 mL from each prepared standard flask and transfer it into appropriate containers. Add the requisite reagents according to the protocol mentioned below. Measure the absorbance of each solution at 540 nm using a spectrophotometer.
- **Standard Curve Construction:** Construct a standard curve by plotting the absorbance readings against the corresponding concentrations of the standard solutions. Ensure that the regression coefficient (R^2) of the standard curve exceeds 0.98, indicating high linearity and reliability in the calibration process.

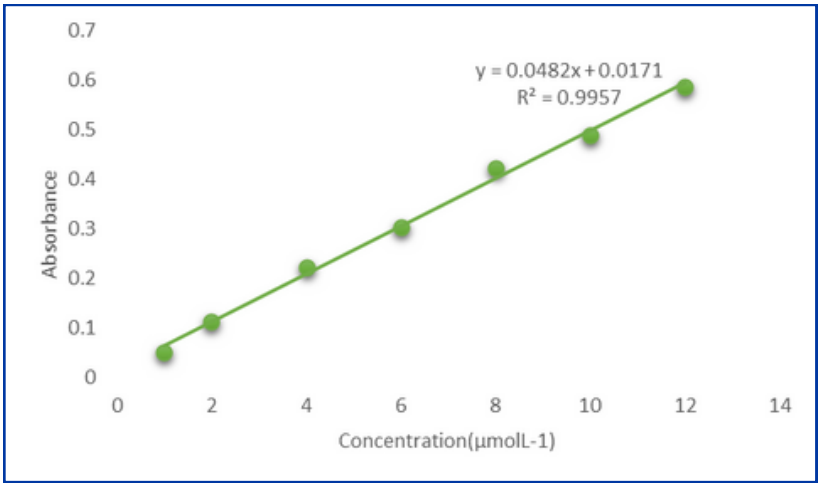

Fig. 4.8 Standardization Curve for Nitrite

Materials Required

- Beaker – 2 Nos
- Funnel – 2 Nos
- Measuring Cylinder (25 mL,10 mL) – 1 No
- Micropipette and Tip
- Pipette – 1 No
- Standard Flask (100 mL) - 8 Nos
- Standard Flask (500 mL) – 1 No
- Standard Flask (1 L) – 2 Nos
- Test tube holder – 1 No
- Test tubes - 7 Nos
- Tissue Paper
- Wash bottle with Distilled Water

Instrument Required

- UV Visible Spectrophotometer

Reagents Required

- Prepare the **Sulphanilamide solution** by adding 100 mL of concentrated Hydrochloric Acid (Con HCl) to 600 mL of distilled water. Dissolve 10 grams of crystalline sulphanilamide in the solution and adjust the volume to 1 L. Store the solution in a dark environment, and it will remain stable for one month.
- Dissolve 0.5 grams of N-(1-Naphthyl) ethylenediamine (NED) in 500 mL of distilled water to prepare the **NED solution.** Store the solution in a dark location, and it will maintain stability for a period of one month.

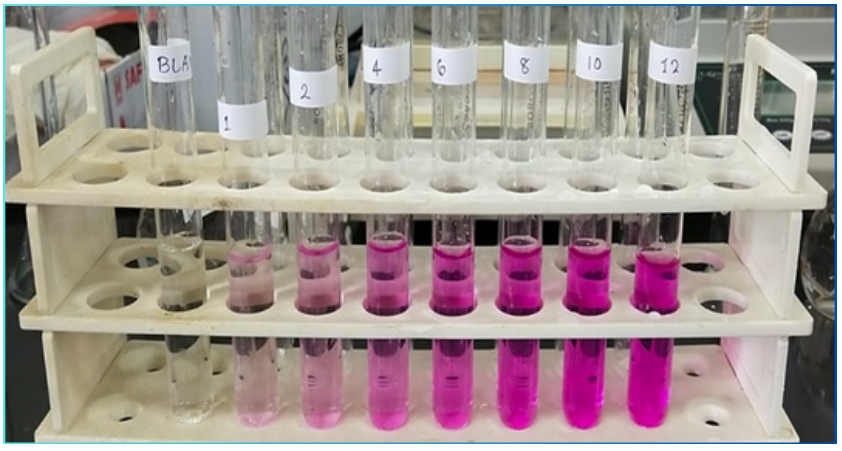

Fig. 4.9 Preparation of Nitrite Standard Solutions at Different Concentrations for Spectrophotometric Analysis

Procedure

01 Sample Preparation

- Take 10 mL of the sample in a clean test tube.

02 Addition of Reagents

- Add 0.2 mL of Sulphanilamide to the sample.
- Allow the mixture to react for 1-4 minutes.
- Add 0.2 mL of N-(1-Naphthyl)ethylenediamine (NED) to the test tube.

03 Incubation

- Incubate the mixture for 10-15 minutes to allow the colour to develop.
- A pink-coloured azo dye complex will be formed, with increasing intensity in proportion to the concentration of nitrite.

04 Measurement

- Measure the absorbance of the solution at 540 nm using a spectrophotometer
- Correct the measured extinction by subtracting both turbidity and reagent blank. Calculate the nitrite-nitrogen concentration in microgram-atoms of nitrogen per liter (pg-at N/liter) from the expression:

µg-at N/liter = corrected extinction x F

Where F is a factor obtained as described in section Nitrate above.

Note

- Preserve samples immediately after collection to prevent the conversion of nitrate to nitrite, as nitrate interference can affect the accuracy of nitrite estimation.
- Acidify the samples with a suitable acid (usually hydrochloric acid) to ensure the conversion of nitrate to nitrite before analysis.
- Allow sufficient time for colour development after the addition of reagents. This step is critical for accurate measurement.
- Be aware of potential interferences, such as substances that may react with the reagents or produce a colour similar to the nitrite complex.

Alternative Method

Nitrite Testing Kit

Nitrite concentration can be quantified using a saltwater test kit through a precise procedure. Begin by adding 5 mL of the sample to a test tube, followed by 5 drops of Test Solution 1. Then, add 5 drops of Test Solution 2 to the mixture. Shake well. Allow the test tube to sit undisturbed for 10 minutes, during which a colour change will occur. The Nitrite concentration, measured in parts per million (ppm), can be determined by comparing the resulting colour with the provided colour chart.

4.4 Estimation of Phosphate

Phosphate is a crucial nutrient for aquatic ecosystems, playing a significant role in aquatic plants and microorganisms growth and metabolism. In mariculture systems, phosphate originates from feed and metabolic waste. While essential at low concentrations, excessive phosphate can lead to eutrophication, causing algal blooms and degrading water quality. Managing phosphate levels is vital for maintaining a balanced ecosystem and promoting the health and productivity of cultured species.

Principle

Phosphate concentration is determined using a colorimetric method where ammonium molybdate and antimony potassium tartrate react in an acid medium with orthophosphate to form a heteropoly acid, known as phosphomolybdate acid. This complex is then reduced by ascorbic acid to form an intensely blue-coloured complex. The intensity of the blue colour, measured at 880 nm using a spectrophotometer, is directly proportional to the phosphate concentration in the sample, adhering to Beer Lambert's law, which states that absorbance is directly proportional to the concentration of the solution, the path length, and the molar absorptivity.

Sample collection

Representative samples should be collected using clean glassware. Rinse the container with the source water, then submerge it below the surface to avoid scum and debris and collect the representative water sample. Label the sample with the date, time, and location of collection. Store the sample in a cool environment and transport it to the laboratory promptly to maintain integrity. Precise documentation of collection details is essential to ensure the accuracy and reliability of the subsequent analysis.

Analytical Procedures

Stock Preparation for Standardization

- **Mother Stock Solution:** Dissolve precisely 0.186 grams of Potassium Dihydrogen Phosphate in a 1000 mL standard flask, ensuring complete dissolution. Adjust the volume to exactly 1000 mL with distilled water. This solution is referred to as the "Mother Stock".
- **Working Standard Solution:** From the Mother Stock solution, carefully extract 1 mL and transfer it into a separate 100 mL standard flask. Dilute the solution to volume with distilled water, ensuring accuracy in measurement. This diluted solution is termed the "Working Standard".
- **Calibration Standards:** Utilizing the Working Standard solution, sequentially pipette volumes of 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, 10 mL, and 12 mL into separate 100 mL standard flasks. Adjust each solution to volume with distilled water, maintaining precision in dilution.
- **Absorbance Measurement:** Extract precisely 10 mL from each prepared standard flask and transfer it into appropriate containers. Add the requisite reagents according to the protocol mentioned below. Measure the absorbance of each solution at 880 nm using a spectrophotometer.
- **Standard Curve Construction:** Construct a standard curve by plotting the absorbance readings against the corresponding concentrations of the standard solutions. Ensure that the regression coefficient (R^2) of the standard curve exceeds 0.98, indicating high linearity and reliability in the calibration process.

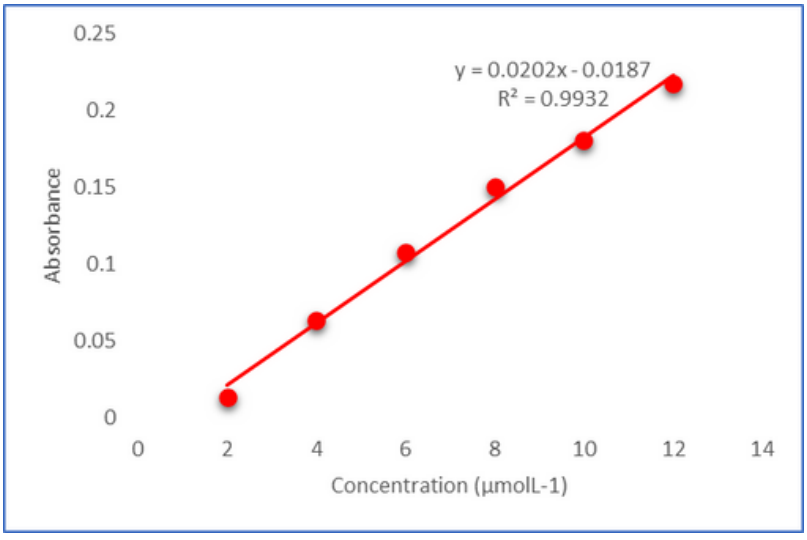

Fig. 4.10 Standardization Curve for Phosphate

Materials Required

- Beaker – 2 Nos
- Funnel – 2 Nos
- Measuring Cylinder (25 mL,10 mL) – 1 No
- Micropipette and Tip
- Pipette – 1 No
- Standard Flask (100 mL) - 8 Nos
- Standard Flask (500 mL) – 1 No
- Standard Flask (1 L) – 2 Nos
- Test tube holder – 1 No
- Test tubes - 7 Nos
- Tissue Paper
- Wash bottle with Distilled Water

Instrument Required

- UV Visible Spectrophotometer

Reagents Required

- Sulphuric Acid Solution 5N : Prepare a 5 Normal (5N) sulfuric acid solution by diluting 70 mL of concentrated sulphuric acid with distilled water to a final volume of 500 mL, or by diluting 14 mL of concentrated sulfuric acid in 100 mL of distilled water.
- Potassium Antimony Tartrate Solution: Dissolve 1.37 grams of Potassium Antimony Tartrate in 400 mL of distilled water. Store in a glass stopper bottle.
- Ammonium Molybdate Solution: Dissolve 4 grams of Ammonium Molybdate in 100 mL of distilled water. Store the solution in a glass storage bottle.
- Ascorbic Acid Solution: Dissolve 1.76 grams of ascorbic acid in 100 mL of distilled water. The solution remains stable for one week when stored at 4°C.
- Mixed Reagent: Combine the reagents mentioned above in the following proportions to prepare 100 mL of the combined reagents:

1. Normal Sulfuric Acid (H_2SO_4): 50 mL
2. Potassium Antimony Tartrate Solution: 5 mL
3. Ammonium Molybdate Solution: 15 mL
4. Ascorbic Acid Solution: 30 mL (Mix each reagent sequentially, one by one. The resulting mixed reagent remains stable for duration of 4 hours.)

Procedure

01 Sample Preparation

- Take 10 mL of the sample in a clean test tube.

02 Addition of Reagents

- Add 1.6 mL of the mixed reagent to the sample.

03 Incubation

- Incubate the mixture for 10-15 minutes to allow the colour to develop.

04 Measurement

- Measure the absorbance of the solution at 880 nm using a spectrophotometer within 30 minutes of the incubation period.

Note

- Let all reagents reach room temperature before they are mixed.
- Mix in the order given.
- If turbidity occurs mix & stand for a while.

Alternative Method

Phosphate Testing Kit

Phosphate concentration can be quantified through a precise procedure using a saltwater test kit. Begin by adding 5 mL of the sample to a test tube, followed by 6 drops of Test Solution 1. Then, add 6 drops of Test Solution 2 to the mixture. Shake well. Allow the test tube to sit undisturbed for 10 minutes, during which a colour change will occur. The Phosphate concentration, measured in parts per million (ppm), can be determined by comparing the resulting colour with the provided colour chart.

4.5 Estimation of Silicate

Silicate is an essential nutrient in marine environments, contributing to the growth of diatoms, which are a key component of the marine food web. In mariculture, silicate levels must be monitored to ensure optimal conditions for the growth of these and other siliceous organisms. An imbalance in silicate concentration can affect the productivity and health of cultured species. High silicate concentrations can lead to excessive diatom growth, which may outcompete other phytoplankton, disrupting the balance of the ecosystem. Therefore, maintaining appropriate silicate levels is crucial for the sustainability of mariculture operations.

Principle

Ammonium molybdate at acidic pH reacts with silicate to form a yellow complex. Oxalic acid is then added to destroy any molybdo phosphoric acid that may form, which is essential even if phosphate is known to be absent. This yellow complex is then reduced by ascorbic acid to form a blue-coloured complex. The intensity of the blue colour, which is measured at a specific wavelength using a spectrophotometer, is directly proportional to the silicate concentration in the sample. This method follows Beer Lambert's law, stating that absorbance is directly proportional to the concentration of the solution, the path length, and the molar absorptivity.

Sample collection

Representative samples should be collected using clean glassware. Rinse the container with the source water, then submerge it below the surface to avoid scum and debris and collect the representative water sample. Label the sample with the date, time, and location of collection. Store the sample in a cool environment and transport it to the laboratory promptly to maintain integrity. Precise documentation of collection details is essential to ensure the accuracy and reliability of the subsequent analysis.

Analytical Procedures

Stock Preparation for Standardization

- **Mother Stock Solution:** Dissolve precisely 0.960 grams of Sodium fluorosilicate in a 1000 mL standard flask, ensuring complete dissolution. Adjust the volume to exactly 1000 mL with distilled water. This solution is referred to as the "Mother Stock".

- **Working Standard Solution:** ·From the Mother Stock solution, accurately measure and transfer 10 ml into a separate 100 mL standard flask. Dilute the solution to volume with distilled water, maintaining precision in measurement. This diluted solution is designated as the “Working Standard”.
- **Calibration Standards:** Utilizing the Working Standard solution, sequentially pipette volumes of 1 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL into separate 100 mL standard flasks. Adjust each solution to volume with distilled water, ensuring accurate dilution.
- **Absorbance Measurement:** Extract precisely 50 mL from each prepared standard flask and transfer it into appropriate containers. Add the requisite reagents according to the protocol mentioned below. Measure the absorbance of each solution at 810 nm using a spectrophotometer.
- **Standard Curve Construction:** Construct a standard curve by plotting the absorbance readings against the corresponding concentrations of the standard solutions. Ensure that the regression coefficient (R^2) of the standard curve exceeds 0.98, indicating high linearity and reliability in the calibration process.

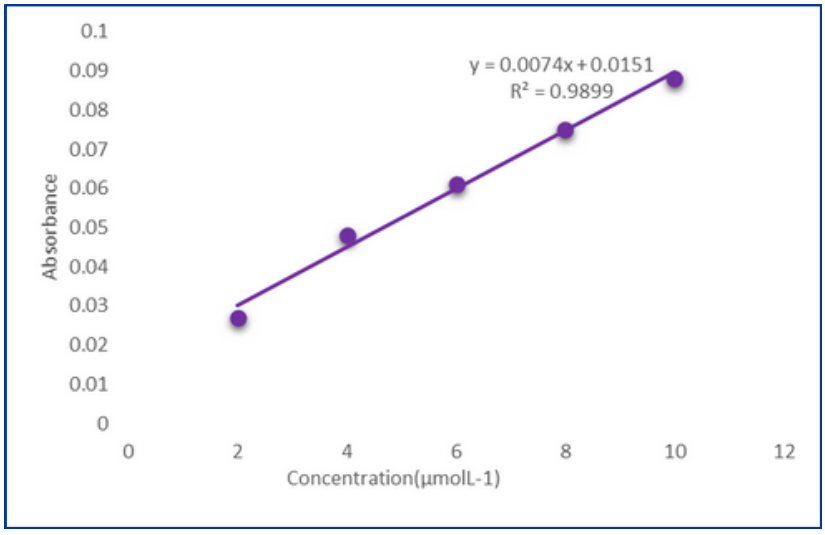

Fig. 4.11 Standardization Curve for Silicate

Materials Required

- Beaker – 2 Nos
- Funnel – 2 Nos
- Measuring Cylinder (25 mL,10 mL) – 1 No
- Micropipette and Tip
- Pipette – 1 No
- Plastic Standard Flask (100 mL)- 10 Nos
- Standard Flask (1L) – 1 No
- Test tube holder – 1 No
- Test tubes - 7 Nos
- Tissue Paper
- Wash bottle with Distilled Water

Instrument Required

- UV Visible Spectrophotometer

Reagents Required

- Sulphuric Acid : Prepare by mixing 25 mL of concentrated sulfuric acid with 75 mL of distilled water.
- Acid Molybdate Reagent: Dissolve 2 g of ammonium molybdate in 70 mL of water, add 6 mL of concentrated HCl, and dilute to 100 mL.
- Oxalic Acid Solution: Prepare a saturated solution of oxalic acid by agitating 10 grams of oxalic acid dihydrate with 100 mL of distilled water. Store the solution at room temperature in a plastic bottle.
- Metol-Sulphite solution: Dissolve 5 g of metol and 3 g of anhydrous sodium sulfite in 240 mL of water, then dilute to 250 mL and filter. Filter the solution using Whatman No. 1 filter paper, and store the filtered solution in a dark glass bottle.
- Reducing agents: Mix 100 mL of the metol-sulfite solution with 60 mL of a 10% oxalic acid solution. While maintaining cooling conditions, gradually add 120 mL of a 25% sulfuric acid solution. Dilute the final mixture to a total volume of 300 mL

Treatment of apparatus

- Graduated flasks should be soaked overnight in a 1:1 mixture of concentrated nitric acid and sulfuric acid to enhance their resistance to solubility. After soaking, rinse the flasks thoroughly with tap water, then with distilled water. Drain the flasks but avoid drying them completely, as this may reduce their resistance to solubility.

Procedure

01 Sample Preparation

- Take 20 mL of sample into 50 mL graduated flask with 3 mL of acid molybdate reagent and mix thoroughly.

02 Addition of Reagents

- After 10 minutes, add 15 mL of the reducing agent and dilute to 50 mL with distilled water

03 Incubation

- Allow the mixture to stand for 3 hours.

04 Measurement

- Measure the absorbance of the solution at 810 nm using a spectrophotometer.

Note

- Silicate analysis should be performed using plastic containers because silicate can adsorb onto glass surfaces, leading to inaccurate results.
- Optimize the reaction conditions such as pH and temperature to ensure complete complex formation.
- Be aware of potential interferences that may affect the accuracy of the method. Common interferences include other anions that can form complexes with molybdate or absorb light at the same wavelength.

Fig. 4.12 Preparation of Silicate Standard Solutions at Different Concentrations for Spectrophotometric Analysis

Alternative Method

Silicate Testing Kit

Silicate concentration can be quantified using a saltwater test kit through a precise procedure. Begin by adding 5 mL of the sample to a test tube, followed by adding test solution to the sample. Shake well. Allow the test tube to sit undisturbed for 10 minutes, during which a colour change will occur. The Silicate concentration, measured in parts per million (ppm), can be determined by comparing the resulting colour with the provided colour chart.

Fig. 4.13 Silicate Testing Kit

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CHAPTER 5

Major Ions

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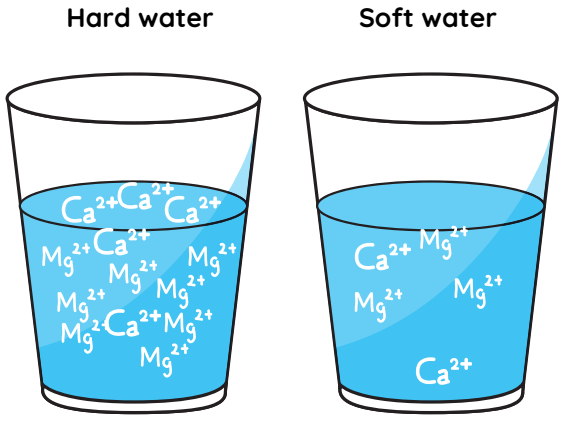
Introduction

Water hardness is a fundamental physical parameter defined by the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water. This characteristic of water significantly influences several biological and chemical processes within aquatic environments. The presence of these ions plays a crucial role in various physiological functions and ecological interactions. For example, calcium and magnesium are essential for the formation of shells and exoskeletons in invertebrates, contributing to their structural integrity and survival. Similarly, these minerals are vital for bone health in fish, supporting their growth and development.

In mariculture, managing water hardness is paramount for the health and productivity of cultured species. Optimal hardness levels support the physiological needs of aquatic organisms, including maintaining proper osmoregulation and enzyme function. The stability of the aquatic environment, which is influenced by hardness, directly impacts the overall well-being of cultured species. For instance, the availability of calcium in water is crucial for the development of strong shells in shellfish and for the skeletal health of fish.

Deviation from the ideal hardness range can lead to several issues. Low hardness levels can result in inadequate mineral availability, causing stress and hindering growth in aquatic organisms. On the other hand, excessively high hardness can lead to mineral imbalances, potentially resulting in detrimental effects on aquatic life. Both scenarios can affect the productivity of mariculture operations, leading to reduced growth rates, increased susceptibility to diseases, and overall lower survival rates of cultured species.

Therefore, effective management and regular monitoring of water hardness are essential for ensuring the success and sustainability of mariculture operations. By maintaining appropriate hardness levels, mariculturists can support the optimal growth and health of cultured species, contributing to the overall stability and productivity of the aquatic system.



Water hardness can be classified into two types:

1. Temporary Hardness
2. Permanent Hardness

Temporary Hardness: This type of hardness is primarily caused by dissolved bicarbonates of calcium and magnesium, such as calcium bicarbonate ($Ca(HCO_3)_2$) and magnesium bicarbonate ($Mg(HCO_3)_2$). It can be significantly reduced by boiling the water. When heated, calcium bicarbonate converts to calcium carbonate, water, and carbon dioxide:

$$Ca(HCO_3)_2 \xrightarrow{\text{heating}} CaCO_3 \downarrow + H_2O + CO_2$$

Similarly, magnesium bicarbonate converts to magnesium hydroxide and carbon dioxide:

$$Mg(HCO_3)_2 \xrightarrow{\text{heating}} Mg(OH)_2 \downarrow + 2\ CO_2$$

Permanent Hardness: This type of hardness is due to the presence of dissolved chlorides, nitrates, and sulphates of calcium, magnesium, iron, and other metals, including compounds like calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), calcium sulfate ($CaSO_4$), magnesium sulfate ($MgSO_4$), iron sulfate ($FeSO_4$), and aluminum sulfate ($Al_2(SO_4)_3$). Unlike temporary hardness, permanent hardness cannot be removed by boiling; it requires chemical treatment for removal.

Principle

Hardness in water is determined using a complexometric titration method with ethylenediaminetetraacetic acid (EDTA). EDTA forms colourless, stable complexes with calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions present in the water. To ensure the pH of the solution remains optimal (pH 9-10), a buffer solution of ammonium chloride (NH_4Cl) and ammonium hydroxide (NH_4OH) is used. Eriochrome Black-T (EBT) is employed as an indicator. In the presence of EBT, the calcium and magnesium ions form an unstable, wine-red complex. As EDTA is added, it reacts with the Ca^{2+} and Mg^{2+} ions, displacing the EBT from its complex and causing a colour change to blue, which indicates the endpoint of the titration. The volume of EDTA used to reach this endpoint is used to calculate the hardness of the water sample.

5.1 Estimation of Permanent and Temporary Hardness

Sample collection

To ensure accurate hardness measurement, collect water samples using clean, acid-washed containers to avoid contamination. Submerge the container below the water surface and fill it completely to eliminate air bubbles, which can affect the analysis. Clearly label each sample with details such as sampling location, date, and depth to maintain proper documentation. Store the samples in a cool, dark place and transport them to the laboratory promptly. Adhering to these procedures ensures the integrity of the samples and the reliability of calcium and magnesium concentration estimates.

Materials Required

- Burette with stand – 1 No
- Conical flask – 1 No
- Pipette – 1 No
- Dropper – 1 No
- Measuring Cylinder (100 mL) – 1 No
- Standard Flask (250 mL) – 1 No
- Wash bottle with Distilled water

Instrument Required

- Weighing balance

Reagents Required

- **Preparation of standard hard water solution**
  1. Dissolve 1 gram of pure, dry calcium carbonate ($CaCO_3$) in a minimal amount of dilute hydrochloric acid (HCl).
  2. Evaporate the resulting solution to dryness using a water bath.
  3. Dissolve the dry residue in distilled water to make a final volume of 1 liter.
- **Ethylenediaminetetraacetic acid (EDTA) 0.01M**
  1. Add precisely 0.931 grams of ethylenediaminetetraacetic acid (EDTA) to a 250 mL standard flask, then fill the flask to the 250 mL mark.
- **Buffer solution**
  1. Add 67.5 grams of ammonium chloride (NH_4Cl) to 570 milliliters of concentrated ammonia solution.
  2. Dilute the mixture with distilled water until the total volume reaches 1 liter.
- **Preparation of Indicator (EBT)**
  1. Dissolve 0.5 g of Eriochrome Black–T in 100mL alcohol.

Procedure

01 Standardization of EDTA Solution:

- Rinse and fill a burette with the EDTA solution.
- Pipette 20 ml of standard hard water (M_1) into a conical flask.
- Add 4 ml of buffer solution and 2 drops of Eriochrome Black T (EBT) indicator.
- Titrate with the EDTA solution until the wine-red colour changes to clear blue.
- Let the volume of EDTA used be 'X' ml.
- Use the formula M1V1=M2V2 to determine the molarity of the EDTA solution, where

  M 1 = Molarity of standard hard water (0.01 M)
  V 1 = Volume of standard hard water (20 ml)
  M 2 = Molarity of EDTA
  V 2 = Volume of EDTA used (X ml)

02 Determination of Total Hardness

- Rinse and fill the burette with the EDTA solution.
- Pipette 20 ml of sample water (V_3) into a conical flask.
- Add 4 ml of buffer solution and 2 drops of indicator.
- Titrate with the EDTA solution until the wine-red colour changes to clear blue.
- Let the volume of EDTA used be 'Y' ml.
- Use the formula M2V2=M3V3 to determine the molarity of the sample water, where

  M2= Molarity of EDTA
  V2= Volume of EDTA used (Y ml)
  M3= Molarity of sample water
  V3= Volume of sample water (20 ml)

- Calculate total hardness using

$$Total\ Hardness = M_3 \times \frac{Molecular\ weight\ of\ CaCO_3(100) \times 1000}{Volume\ in\ ml} = M_3 \times 105\ ppm$$

03 Determination of Permanent Hardness

- Take 100 ml of sample water in a 250 ml beaker.
- Boil to remove temporary hardness until the volume is reduced by half, then cool to room temperature.
- Filter through filter paper to remove insoluble salts.
- Adjust the volume to 100 ml with distilled water.
- Pipette 20 ml of this solution (V_4) into a conical flask.
- Add 4 ml of buffer solution and 2 drops of indicator.
- Titrate with the EDTA solution until the wine-red colour changes to clear blue.
- Let the volume of EDTA used be 'Z' ml.
- Use the formula M2V2=M4V4 to determine the molarity of the permanent hardness, where:

  M2= Molarity of EDTA
  V2= Volume of EDTA used (Z ml)
  M4= Molarity of permanent hard water
  V4= Volume of permanent hard water (20 ml)
- Calculate permanent hardness using:

$$Permanent\ \ Hardness = M_4 \times \frac{Molecular\ weight\ of\ CaCO_3(100) \times 1000}{Volume\ in\ ml} = M_4 \times 105\ ppm$$

04 Determination of Temporary Hardness

- Calculate temporary hardness by subtracting permanent hardness from total hardness:

Temporary Hardness=Total Hardness−Permanent Hardness

The hardness of the given water sample is as follows:
Total hardness = ---------- ppm
Permanent hardness = ---------- ppm
Temporary hardness = ---------- ppm

Fig. 5.1 Gradual change in colour during titration for hardness analysis

Note

- The factor 105 ppm accounts for the molecular weight of $CaCO_3$ and practical rounding.
- Maintain a stable pH throughout the titration process, typically around pH 10, to optimize the formation of metal-EDTA complexes.
- Allow sufficient time for the formation of stable metal-EDTA complexes during the titration to ensure complete and accurate chelation of calcium and magnesium ions.
- Perform blank corrections by titrating a blank solution containing all reagents except the sample to account for any background colour or interference.
- Employ precise and consistent titration techniques, including slow and controlled addition of the EDTA solution while continuously monitoring the colour change, to accurately determine the endpoint.

5.2 Estimation of Calcium and Magnesium based Hardness

The estimation of water hardness is based on complexometric titration. The hardness is determined by titrating the water sample with a standard solution of ethylenediaminetetraacetic acid (EDTA), a complexing agent. Since EDTA is insoluble in water, its disodium salt is used in this experiment. EDTA can form four or six coordination bonds with metal ions. A blue dye, Eriochrome Black T (ErioT), is used as the indicator in this titration. ErioT also forms a complex with calcium and magnesium ions, causing a colour change from blue to pink during the process.

A back titration is performed using a solution of magnesium chloride. This solution forms a complex with the excess EDTA molecules until the end-point, where all the excess EDTA has been complexed. Once all the excess EDTA is bound, the remaining magnesium ions from the magnesium chloride solution begin to complex with the Eriochrome Black T (ErioT) indicator, causing an immediate color change from blue to pink.
The main reaction is $Ca^{2+} + EDTA^{4-} \rightarrow [Ca\text{-}EDTA]^{2-}$

Back titration $EDTA^{4-} + Mg^{2+} \rightarrow [Mg\text{-}EDTA]^{2-}$

Indicator reaction: note, ErioT is blue and ErioT-Mg is pink

$ErioT + Mg^{2+} \rightarrow ErioT\text{-}Mg$

Materials Required

- Burette
- Pipette 20 mL
- Conical flasks 250 mL
- Volumetric cylinder 100 mL

Reagent Preparation

- EDTA: (ethylenediamine tetraacetic acid) - 500 mL of a 0.05 $molL^{-1}$ solution. Weigh 9.31 g of the EDTA salt and dissolve it in 500 mL of distilled water in a volumetric flask.
- Buffer: Dissolve 7.0 g of ammonium chloride in 57 mL concentrated ammonia. Dilute to 100mL with distilled water in a volumetric flask. The pH should be 10.5.
- $MgCl_2.6H_2O$: 0.025 $molL^{-1}$ solution. Weigh 2.54 g of magnesium chloride hexahydrate and dilute to 500 mL with distilled water in a volumetric flask.
- ErioT indicator: Dissolve 0.2 g of Eriochrome Black T indicator in 15 mL of concentrated ammonia solution and 5mL absolute ethanol.

Procedure

01 Standardisation of EDTA Solution

- Follow the steps for standardisation as mentioned in the previous section.

02 Titration Method for Seawater Samples

- Pipette 10 mL of the sample solution into a conical flask.
- Add 20 mL of 0.05 mol L^{-1} EDTA solution.
- Add 10 mL of ammonia buffer, 50 mL of distilled water and 1 mL of Eriochrome Black T indicator solution.
- Titrate the sample with the standard 0.025 molL^{-1} magnesium chloride solution until a permanent pink colour appears.

03 Titration Method for Freshwater Samples

- Add a 100 mL of the sample solution into a 250 mL conical flask.
- Prepare a 0.005 mol L^{-1} EDTA solution by diluting the 0.05 mol L^{-1} EDTA solution by a factor of 1/10. Add 20mL of this diluted EDTA to the sample solution.
- Add 10 mL of the ammonia buffer and 1 mL of Eriochrome Black T indicator solution.
- Prepare a 0.0025 mol L^{-1} magnesium chloride solution by diluting the 0.025 mol L^{-1} magnesium chloride solution by a factor of 1/10.
- Titrate the sample solution with this 0.0025 mol L^{-1} magnesium chloride solution until a permanent pink colour appears.

Calculation

- Calculate the total moles of EDTA added to the sample solution.
- Calculate the moles of magnesium chloride solution used in the back titration. According to the titration equation given below, the moles of Mg^{2+} will be equivalent to the moles of excess EDTA.

$$EDTA^{4-} + Mg^{2+} \rightarrow [Mg\text{-}EDTA]^{2-}$$

- Given the ratio of Ca^{2+} + Mg^{2+}: EDTA = 1 : 1, Calculate the moles of Ca^{2+} and Mg^{2+} that were complexed with EDTA by subtracting the moles of excess EDTA from the total moles of EDTA added to the sample. The resulting value represents the moles of Ca^{2+} and Mg^{2+} present in the sample solution.

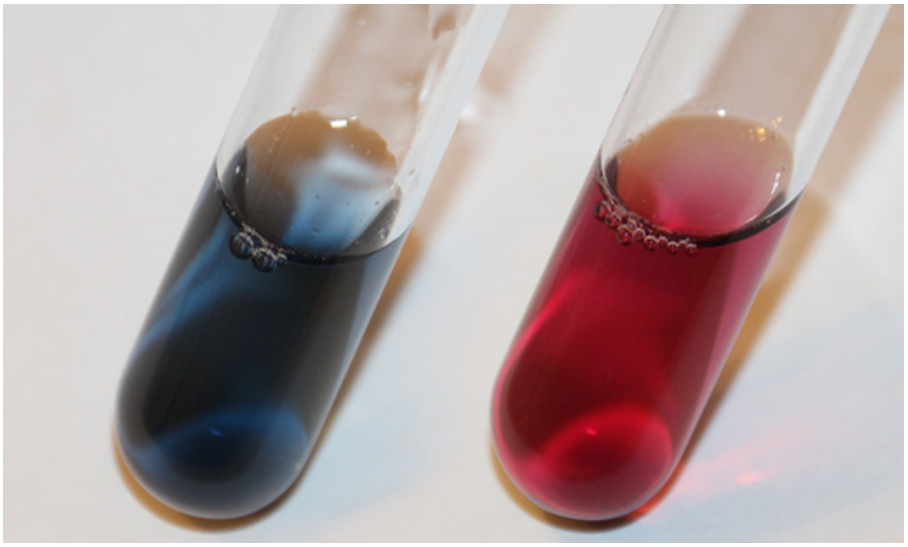


Fig. 5.2 Gradual change in colour during analysis

Note

- Lab coats, safety glasses and enclosed footwear must be worn at all times in the laboratory.
- Concentrated ammonia solution used in preparing buffer and indicator solutions is highly corrosive. Rubber gloves must be worn, and caution exercised when handling.
- Both the buffer and indicator solutions may release ammonia gas, which can be harmful if inhaled in large quantities. Work in a fume hood or a well-ventilated area.
- The ammonia buffer (pH ~ 10.5) used here is needed as Eriochrome Black T only changes colour in the pH range 7.

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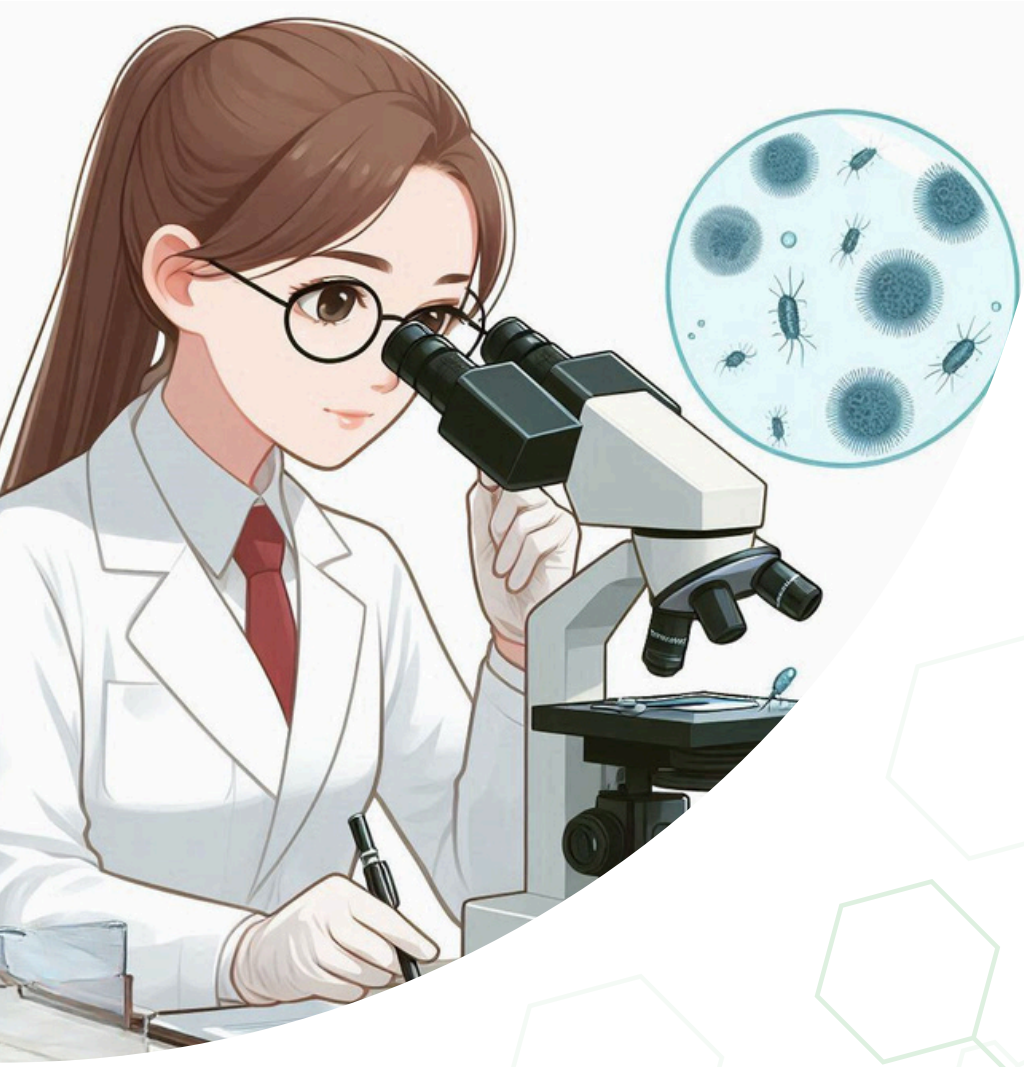
CHAPTER 6



Estimation Of Biological Parameters

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6 Contents

Introduction

Biological parameters are vital indicators of the health and functioning of aquatic ecosystems. They encompass various aspects of living organisms and their interactions within the environment. Understanding these parameters is crucial for managing and optimizing aquatic systems, especially in mariculture, where the health and productivity of cultured species are closely linked to the overall ecosystem dynamics.

These biological parameters include

- Macrophyte vegetation,
- Benthos,
- Phytoplankton,
- Zooplankton,
- Primary productivity, and
- Chlorophyll estimation.

Macrophyte vegetation consist of aquatic plants which plays a crucial role in primary production through photosynthesis. Benthos consists of organisms living on or in the sediments of water bodies, playing significant roles in nutrient cycling and habitat structuring. Phytoplankton are microscopic plants that drive primary production through photosynthesis, forming the foundational basis of aquatic food webs. Zooplankton, small drifting animals, feed on phytoplankton and are essential in transferring energy through the food chain. Primary productivity measures the rate at which primary producers convert sunlight into organic matter, reflecting the overall energy available in the ecosystem. Chlorophyll estimation provides insights into phytoplankton biomass and primary productivity by measuring the concentration of chlorophyll pigments.

Monitoring these biological parameters offers valuable insights into the health, productivity, and sustainability of aquatic systems. In mariculture, understanding these factors is crucial for optimizing growth conditions, managing nutrient inputs, and ensuring the overall balance of the ecosystem. Effective management of biological parameters helps maintain a productive and sustainable environment for cultured species, supporting the success and longevity of mariculture operations.

6.1 Estimation of Macrophyte Vegetation

Macrophyte vegetation sampling is the systematic process of collecting and analysing data on aquatic plants, including their abundance, distribution, and diversity, within a specified study area. This sampling helps to understand plant communities, monitor ecological changes, and assess environmental conditions in aquatic ecosystems.it provides a systematic approach to assess plant abundance, distribution, and diversity.

This method allows for the monitoring of changes over time, characterization of different habitat types, and evaluation of the impact of mariculture activities on local vegetation. Additionally, transect sampling aids in collecting baseline data, inventorying plant species, and managing resources effectively. Overall, it supports informed decision-making and contributes to the sustainability and health of mariculture systems.

Standardized Methods for Sampling Analysis

- **Sampling period** - Sampling and surveys of aquatic macrophyte communities are conducted on the basis of investigation requirements.
- **Selecting Macrophyte Sampling Locations** - Sampling areas should be representative of the overall habitat at the site. Sampling locations may include similar areas within or adjacent to other habitat types. Locations selected for all replicate samples collected at a site should be as similar to each other.
- **Recording Site Characterization, Habitat and Land Use Data** – Record the following details: Station ID, Date, Town, Waterbody Name, and Water Level (categorized as High, Medium, or Low based on specific criteria). Capture one or more representative digital photos of the monitoring site, and assign a photo number to each. If not previously recorded during earlier sampling, document the GPS details.
- **Recording Physical/Chemical Measurements in the Field** - physical/chemical water quality measurements are taken (temperature, dissolved oxygen, pH, specific conductance) of the study area.
- **Methodologies for Sampling Analysis** - Sampling analysis at representative locations should be conducted using standardized methodologies such as the line transect or belt transect methods. The values obtained from the sampled areas can be extrapolated to estimate the values for the entire study area.
- **Line transect** – It is string laid along the ground in a straight line between the two poles. This method involves the observer moving along a line through a study area looking to left and right sides for the target animal or plant. It is a specialized technique used by ecologists to estimate the density or total number of plants in a study area where counting all individuals is not possible.

Points to note

1. All objects on the transect line are detected.
2. Objects do not move in response to the observer before the detection is noted.
3. Objects are only counted once.
4. Objects are recorded at the point of initial detection.
5. Distances are measured without errors.
6. Transect lines are randomly located in the study area.

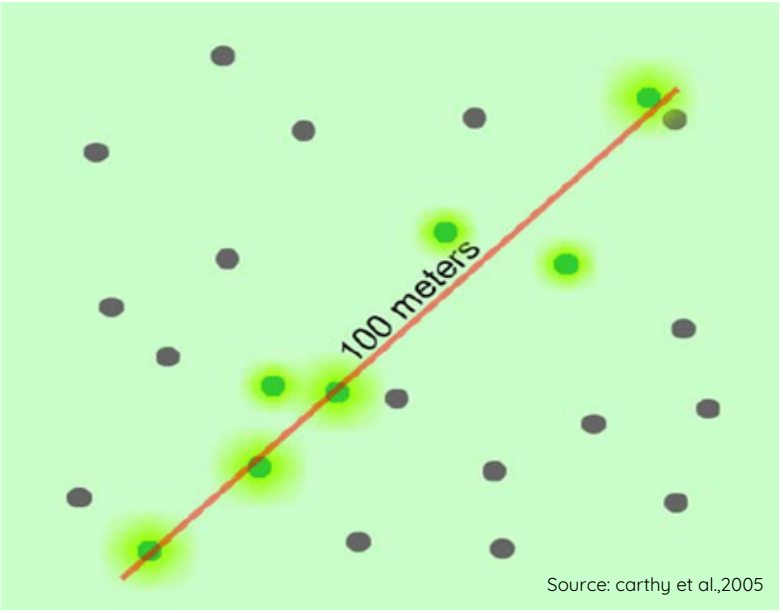

Fig. 6.1 Line transect

- **Belt transect** - This method provide data useful for making management decisions and was reasonably robust to observer skill level, but was inefficient due to time required to locate and place quadrats. The belt transect method involves laying out a strip (belt) of a specified width across the study area. Observers record the presence of target plants or animals within this belt.

Materials required

- GPS unit to record site location
- Clip board and pencils
- Cooler with ice
- Zip lock cover
- Permanent marker
- Wetland Aquatic Vegetation Sampling Forms
- Map/imagery of lake/ pond/ wetland (with bathymetry or depth information where available).
- Waterproof camera

- Measuring tape
- List of Rare/Threatened/Endangered species in freshwater aquatic environments
- ID information for invasive plant species

Fig. 6.2 Belt transect

Procedure

01 Establishing a Transect for Detailed Observation

- A measuring tape or rope is stretched across the area of interest to create a linear path, known as a transect.
- The length of transect depend on study area. Along the transect, specific intervals or segments which are of defined area are selected for detailed observation.

02 Systematic Observation Along the Transect

- Researchers systematically move along the transect, examining each segment for the presence, abundance, and types of plant species or other organisms.
- Observations on dominant species and changes in species composition along the transect needed to be documented.

03 Species Identification and Data Recording

- Investigator identifies and classifies the species while walking along the transect, and another person records the data.

- If the genus is known but the species is unknown, assign a number to the genus and include a brief description of identifying characteristics. Check for the presence of invasive species, as well as rare, threatened, or endangered species. Collect a voucher specimen of any potential invasive species for confirmation with the appropriate authority.
- Estimate and record the relative abundance of each species.
  Dominant > 60% Common 21-60% Sparse 2-20% Present < 2%
- Record the observed growth form for each species as: Free-floating, Floating, Leaved, Submergent, Emergent.
- Examine the material retrieved for the presence of animals such as freshwater sponges, crustaceans, snails, small fish, or other vertebrates.

Advantages

- Provides a structured approach to studying species distribution and environmental gradients.
- Enables the observation of large areas within a defined path.
- Facilitates collection of measurable data on species abundance and diversity.
- Useful for analyzing changes in species composition across environmental gradients.

Limitations

- It only captures data along a linear path, which does not include species outside the transect.
- Requires time, planning, and often multiple personnel to execute effectively.
- The path may not represent the entire study area's diversity..
- Results may vary with seasons, requiring repeated surveys for comprehensive data.

Note

- Use standardized methods to clean and disinfect sampling equipment.
- Place any unidentified plants in a plastic zip-lock, labelled with the station number, date, and corresponding pseudonym using a permanent marker.
- Photograph unknown plants to document features and growth habits by noting photo numbers for reference.
- Minimize damage to the habitat and avoid disrupting wildlife during the survey..
- Assign roles (e.g., identifier, recorder) to ensure efficiency and accuracy..

6.2 Estimation of Phytoplankton

Phytoplankton, derived from the Greek "phyton," meaning plant, are autotrophic microorganisms comprising both prokaryotic and eukaryotic algae. They occupy the upper layers of water bodies, particularly where there is sufficient light to facilitate photosynthesis. Phytoplankton's photosynthetic activity contributes significantly to half of the total global primary production. Additionally, they serve as the primary nutritional source for zooplankton, collectively establishing the foundation of the marine food chain. The growth of phytoplankton populations depends upon the levels of light exposure and nutrient availability within their environment.

Though normally found in solitary form, phytoplankton can aggregate into extensive chains or colonies with spherical shapes, some of which are large enough to be visible by the naked eye. Phytoplankton exhibit responsiveness to both the physical and chemical conditions present in the aquatic environment. On occasion, their rapid reproductive rate leads to the occurrence of blooms. Algal blooms can have significant impacts on the chemistry of water, particularly on parameters such as pH and dissolved oxygen (DO). During photosynthesis, when algae assimilate carbon dioxide, the pH of the water increases due to the elevated levels of hydroxide ions. Conversely, during respiration, when carbon dioxide is released, the hydroxide ion concentration decreases, consequently lowering the pH of the water.

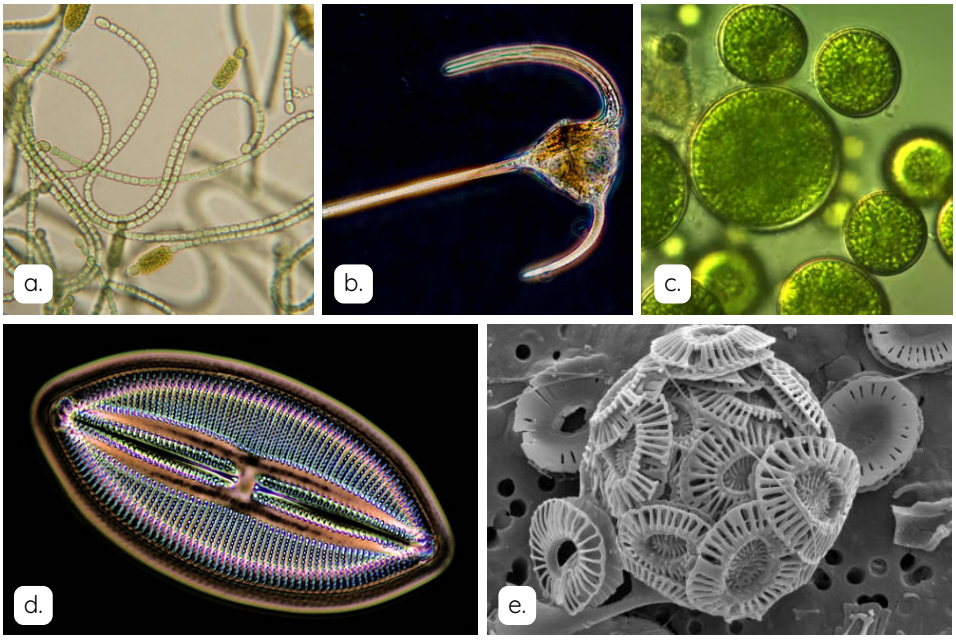

Fig. 6.3 Types of Phytoplankton , a. Cyanobacteria, b. Dinoflagellate, c. Green Algae, d. Diatoms, e. Coccolithophores

Sample collection

Surface Sampling

A plastic bucket tied to a rope suffices adequately for surface sampling. The most used method for acquiring a concentrated sample of phytoplankton involves towing a cone-shaped net made of bolting silk through the water. The broader end of the net remains open due to a metal ring, which is connected to the tow rope via a rope bridle. The narrower end is sealed with either a metal or plastic bucket. A net with 200 meshes per inch (with a mesh aperture size of 0.054 mm) is best for diatom collection. It should be towed at a speed ensuring full extension just beneath the water surface. For deeper water sampling, attaching heavy weights to the net bridle and towing at a similar low speed is advisable. As plankton accumulates during the haul, the net's filtering efficacy diminishes progressively. Thus, the duration of the haul depends on phytoplankton density. In dense phytoplankton areas, a haul lasting 5 or 10 minutes may suffice, while in other conditions, 20 or 30 minutes may be more suitable.

Sampling from various depths

Samples can be collected from various depths by using Niskin sampler. The Niskin bottle is a development of the Nansen bottle patented by Shale Niskin in March 1966. Instead of a metal bottle sealed at one end, the 'bottle' is a tube, usually plastic to minimize contamination of the sample, and open to the water at both ends. Each end is equipped with a cap which is either spring-loaded or tensioned by an elastic rope. The action of the messenger weight is to trip both caps shut and seal the tube.

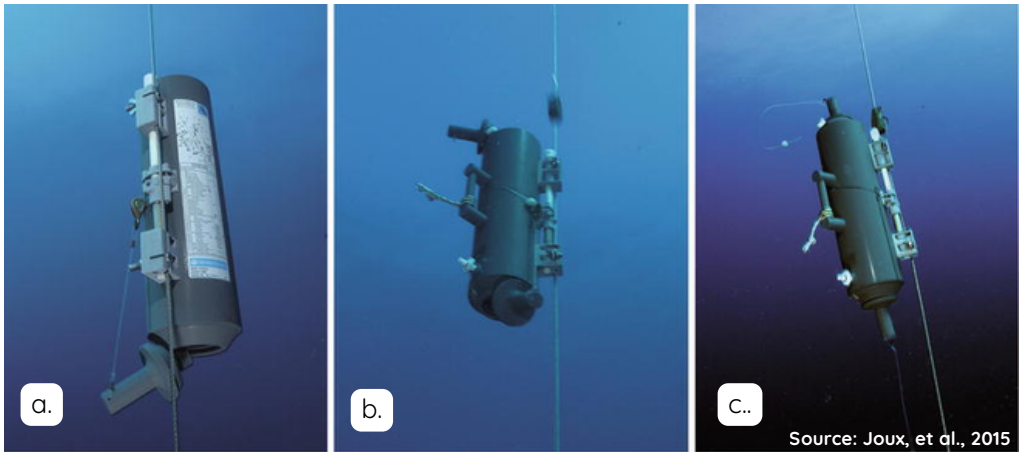

Fig. 6.4 Niskin bottle at sea. (a) Bottle positioned at the sampling depth. Valves are open. (b) The “messenger” triggers the closure. (c) The valves are closed. The water contained in the cylinder is isolated from the outside. The bottle can then be brought on board

Preservation

Whenever possible, samples ought to be examined in their fresh, living state. Allowing samples to stand after collection can lead to two notable consequences. Firstly, the organisms within the sample may consume numerous diatom cells, thereby damaging many others. Secondly, certain plant species may undergo rapid division, resulting in a false perception of abundance within the sample. Cylindrotheca closterium, a common diatom, frequently exhibits this behavior.

For short-term storage, these effects can be reduced by maintaining samples in a cool environment, such as a cold room or refrigerator. However, for long-term preservation, the addition of preservatives like Lugol's iodine is necessary. Lugol's iodine solution, comprising 10 grams of iodine, and 20 grams of potassium iodide in 200 mL of distilled water, with the inclusion of 20 grams of glacial acetic acid added 2-3 days before use, ensures superior preservation of flagellates. Lugol's iodine should be added to water samples at a ratio of 1:100 volume of water. Add 3 millilitres of Lugol's Iodine to one litre of the seawater sample. Allow this mixture to stand undisturbed for 24 hours within a measuring cylinder. Once all particulate matter has settled to the bottom, cautiously siphon off the supernatant water, ensuring that the settled sediment remains undisturbed. This process typically results in a reduction of the original one-litre sample to approximately 60 millilitres. Subsequently, employ solely clear glass bottles for storing these reduced samples, as plastic containers are prone to absorbing iodine from the solution.

Advantages of Lugol's Solution

- Lugol's solution is comparatively less harmful in comparison to aldehyde-based or other more toxic fixatives.
- Iodine enhances the sinking of cells in settling chambers.
- Lugol's stains cells a dark brown colour which makes counting easier.

Counting

A conventional microscope with ×10 and ×40 objective lenses is suitable for the examination of sub samples mounted in seawater under a cover slip.

Sedgewick rafter cells

The volume of planktonic microalgae filtered from 1 litre of water was adjusted to a 60 mL concentrate. After thoroughly shaking the settled sample, transfer 1 mL of this concentrated sample to the Sedgwick-Rafter counting cell, which has a volume of 1 mL. Calculate the number of microalgae present across all one thousand grids. Repeat this counting process three times and calculate the average. Utilize the formula to determine the total number of planktonic algal species present in one litre of water sample.

$$N = \frac{n \times v}{V}$$

Where,

N = no. of planktonic algae per litre of water filtered

n = average no. of planktonic algae in one ml. of sample

v = volume of plankton concentrates in mL.

V = total volume of water filtered in litre

In the case of chain forming species, the number of chains has to be counted.

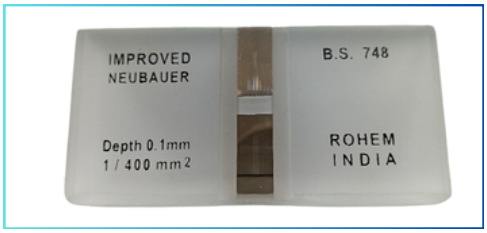

Fig. 6.5 Sedgewick Rafter

Haemocytometer

The haemocytometer with an improved Neubauer ruling requires thorough rinsing and drying. Its counting chamber comprises two surfaces with grids, separated by canals. A drop of uniformly mixed algal suspension, derived from the 60 mL reduced sample described earlier, is dispensed from a pipette. Both chambers must be filled to ensure the coverslip is properly seated. If the algal suspension spills over either side, the chamber should be cleaned and refilled. Each half of the haemocytometer surface consists of nine large grids, with only the algal cells within the four large corner grids (numbered 1 to 4) to be counted. Furthermore, each larger corner grid is subdivided into 16 smaller quarters.

Number of cells per litre = n/g× 10000 ×v/V

Where n is the number of algal cells counted, g is the number of corner grids covered, 'V' is the volume of sample, and 'v' is the volume to which the sample is reduced after settlement.

Note

- Sampling should be conducted during periods of minimal disturbance to avoid disrupting phytoplankton populations.
- Proper training in microscopy and taxonomic identification is essential for reliable species identification.
- Cells positioned on the border are included in the count if at least half of the cell lies within the square, ensuring accuracy and avoiding duplication of counts.

6.3 Estimation of Zooplankton

Zooplankton holds a pivotal role within the pelagic food web, as it is responsible for the transfer of organic energy generated by unicellular algae through photosynthesis to higher trophic levels, including pelagic fish stocks that are exploited by humanity. The term "plankton" was introduced by Victor Hensen, a pioneer in quantitative plankton and fishery research, in 1887. It originates from the Greek word "Planktos," meaning "to wander," encompassing all organisms drifting in water whose locomotive capabilities are insufficient to withstand currents.

Zooplankton primarily obtains carbon by consuming phytoplankton, subsequently utilizing it either for metabolic energy through respiration or upon death, contributing to the planktic food web as biomass or detritus. Their sizes vary greatly, ranging from tiny flagellates measuring mere micro meters to massive jellyfish spanning up to 2 meters in length. Zooplankton is categorized into five size classes, spanning from nanoplankton to mega plankton. Due to denser nature of organic material compared to seawater, it tends to sink into open ocean ecosystems, moving away from coastlines and transporting carbon in the process. This phenomenon, referred to as the biological pump, plays a significant role in oceans serving as the largest carbon sink on Earth

Table 6.1 - Classification of Zooplankton based on Size

Category	Size Range	Description
Femtoplankton	0.02-0.2 µm	Marine viruses
Picoplankton	0.2-2 µm	Small eukaryotic protists; bacteria; Chrysophyta
Nanoplankton	2-20 µm	Heterotrophic nanoflagellates feeding on bacteria
Microplankton	20-200 µm	Protozoans like ciliates
Mesoplankton	0.2-20 mm	Metazoans (e.g., Copepod, Medusa etc.)
Macroplankton	20-200 mm	Members of hydrozoidae, mysids, siphonophores, scyphozoans, ctenophores.
Megaplankton	> 200 mm (almost 8 inches)	Metazoans (e.g., Jellyfish)

Sample collection

Zooplankton collection typically involves the utilization of nets. These nets come in various sizes and designs to meet different sampling needs. The different nets can broadly be put into two categories:

- **Open nets** primarily employed for horizontal and oblique hauls, and
- **Closed nets** equipped with messengers to retrieve vertical samples from specific depths.

The mesh size of the netting material plays a crucial role in determining the type of zooplankton captured. Finer mesh nets tend to capture smaller organisms, including larval stages and eggs of planktonic species and fish, whereas nets with coarser mesh are better suited for capturing larger plankton and fish larvae. For optimal quantitative and qualitative zooplankton sampling, it's advisable to conduct horizontal zooplankton sampling either before dawn, after dusk, or during the night.

Horizontal hauls employ several types of nets, including Bongo nets, Horizontal ring nets, and Horizontal Multi Net Trawls. These nets are specifically designed for capturing zooplankton in horizontal water columns. On the other hand, vertical hauls utilize different nets which include the Nansen Vertical Closing Net, the Indian Ocean Standard Net (IOSN), and the Clark Bumpus Sampler. These nets are good at collecting zooplankton samples from specific vertical layers of the water column. Additionally, a specialized high-speed sampler known as Hardy's Continuous Plankton Recorder is often towed behind ships or vessels to obtain continuous plankton samples over extended distances.

The standard plankton net, commonly used for plankton collection (such as the IOE-Standard net with a 1 square meter mouth and 300µm mesh size), consists of a conical bolting silk bag affixed to a ring or hoop. Three thin rope bridles are attached to the ring, spliced to a small ring for connection to a towing rope or warp. A weight is affixed to the warp to aid in sinking the net to the desired depth and maintaining horizontal mouth positioning. For qualitative sampling, the standard net is towed at speeds of 1-1.5 knots for a duration of 10 minutes. Bongo net can used to obtain multiple samples at a time.

For quantitative assessment, it is essential to determine the volume of water filtered through the net to estimate the quantity of plankton per unit volume accurately. This requires attaching a calibrated flow meter at the mouth of the net. The quantitative process starts with rinsing the plankton net using a suitable flow of seawater, effectively washing plankton from the net material and concentrating it in the cod end. Subsequently, the cod end is unscrewed to extract the specimen for further analysis. Filtered seawater can serve for concentration and material transfer purposes.

Total water filtered can be obtained by the formula

$$V = A \times R k$$

Where,
K = Calibration constant
A = Mouth area of the net
R = Flow meter reading
V = Volume of water filtered

Fig. 6.6 A flow meter attached to plankton net

Fixation

For the fixation of Zooplankton seawater formalin solution comprising approximately 4% formaldehyde can be used. This solution is prepared by adding 20 ml of 40% formaldehyde to 200 ml of seawater containing plankton. The solution is further buffered with sodium borate and strontium chloride for stability. It is important to use analytical grade formalin for fixation, as commercial formalin often contains iron compounds that can lead to the formation of a brown precipitate of iron hydroxide which complicates zooplankton identification. For the preservation of calcareous shelled zooplankton, maintaining the pH of the preservation fluid at 8.2 is essential.

Preservation

Following the fixation process, the zooplankton specimens are transferred and securely stored in air tight containers, ensuring sufficient quantity of preservative is added. Predominantly, buffered formalin solution, typically ranging from 4 to 5% concentration, serves as both a fixative and preservative agent. Other preservatives used include 70% ethanol or 40% isopropanol.

Biomass Estimation

The term biomass denotes the live weight or the amount of living matter present in the zooplankton sample. Volumetric Methods like Displacement volume and Settling volume can be done to estimate biomass.

Settling Volume

Transfer preserved samples into either a 50 mL or 100 mL measuring cylinder, ensuring thorough mixing. The residual portion of the sample is rinsed into the cylinder using filtered seawater from a wash bottle. Subsequently, the mixture is left undisturbed for a period of 24 hours, after which the settled volume is measured. It is important to note that the morphology of distinct organisms can influence the measurement of settling volume.

Displacement Volume

The limitations associated with settling volume can be overcome by displacement volume. In this method, the plankton sample, from which interstitial water has been removed, is introduced into a predetermined volume of filtered zooplankton. Subsequently, the filtered zooplankton, devoid of interstitial water, is carefully transferred to a measuring cylinder containing a known volume of 4% buffered formalin, using a spatula. The observed increase in volume indicates plankton volume.

Sub Sample

The Folsom plankton is used to subsample a zooplankton specimen. Specimen is introduced into the drum of the splitter, followed by a slow, oscillating rotation of the drum. Internally partitioned, the drum segregates the sample into equal fractions. Subsequently, a fraction may be reintroduced into the drum for additional splitting iterations. This process continues until an appropriate subsample is obtained for subsequent counting. Following subsampling, it is necessary to rinse the splitter to recover any organisms attaching to the walls of the drum.

Fig. 6.7 Folsom Plankton Splitter

Operation of Folsom Plankton Splitter

01 The specimen is introduced into the drum of the splitter, which is then rotated slowly in an oscillating manner.

02 The drum segregates the sample into equal fractions, each containing 50% of the component.

03 One of the 50% fractions is reintroduced into the drum of the splitter, and the drum is again slowly rotated in an oscillating manner.

04 The drum further segregates this sample into equal fractions, each now containing 25% of the component.

05 This process is repeated until an appropriate subsample is obtained.

Counting

Following the splitting process, the sorting and enumeration of specimens is carried out. For counting Sedgewick rafter cells are used. This task is undertaken in two distinct stages: primary sorting and secondary sorting. In primary sorting, the sample is initially segregated into taxonomic groups. Where as in secondary sorting involves a more detailed examination, where significant groups of organisms or specimens are further sorted into their respective families and genera, allowing for a finer resolution of taxonomic classification. Extremely large specimens such as jelly fish have to be separated before taking measurements in order to avoid unusual values.

Calculation

After estimation of zooplankton Biomass the standing stock values are converted into per cubic meter and is calculated as follows:

Volume of zooplankton (mL/m3) = Total volume of zooplankton/Volume of water filtered(V)

Wet weight of zooplankton (g/m3) = Total wet weight of zooplankton/Volume of water filtered(V)

Faunal Composition

Larger organisms, categorized as macroplankton and typically found in low abundance, can be identified and enumerated through direct observation with the naked eye. Conversely, members of microplankton necessitate identification and enumeration under a dissection microscope. Information regarding faunal composition and the relative abundance of various zooplankton taxa and species are obtained through the counting of plankton present in the samples. For zooplankton enumeration, it is customary to examine a subsample or aliquot ranging from 10 to 25%.

Any individual items posing identification challenges should be separately preserved in separate specimen tubes and labelled for subsequent identification by experts. Organisms of particular interest can be segregated for further measurement, identification, and enumeration. When inputting data into a spreadsheet, designate sample stations along the rows and parameters, including the number of different species, along the columns. This would help easy statistical analysis of the data.

Total number of zooplankton specimens/ individuals of all groups =Total counts of the specimens (say x). (g/m3) /Volume of water filtered (V) = No/m3

Total number of specimens of a particular zooplankton taxon = Total counts (x) / Volume of water filtered(y) = No/m3

Mounting

Mounting in microscopy entails placing samples on a glass slide for microscopic observation, securely holding the specimen in place. Permanent glass slides are made using natural or synthetic resins, with Canada balsam and lactophenol commonly employed as mounting agents.

Note

- The initial responses of zooplankters to fixatives and preservatives typically involve rapid and jerky movements, as well as the contraction of body and appendages. These reactions can hinder species identification. Narcotizing solutions are used to overcome this.
- Fixed specimens must undergo cleaning of any attached material, such as detritus or precipitate. This process involves delicately removing extraneous substances with fine forceps or needles, ensuring specimens remain undamaged.
- Special care must be taken to ensure that seawater used for receiving samples is at ambient temperature, salinity, and free of contaminants. Sampling procedures are designed to minimize physiological stress and physical damage to organisms.

6.4 Estimation of Benthos

Benthos are Invertebrate organisms that are visible to the naked eye and can be retained by a U.S. Standard No. 30 sieve (595 µm mesh). They inhabit or attach to substrates (e.g., cobble, gravel, snags) in aquatic environments for at least part of their life cycle. The benthic fauna, commonly referred to as the bottom fauna, holds significant importance within the marine ecosystem's food web. Fish and crustaceans dwelling near the seabed primarily consume these benthic organisms, thus considering the abundance of benthic fauna a crucial determinant of the demersal fishery potential within a specific marine region. These organisms have an important role in aquatic environments' food chains, actively participating in nutrient processing and cycling while serving as primary food resources for numerous aquatic animals.

In recent years, there has been a growing emphasis on long-term research related to marine benthic communities, particularly within the context of pollution studies and ecosystem health assessment. Macroinvertebrates have emerged as valuable biological indicators due to their presence in aquatic environments, relatively limited mobility compared to other organisms, and ease of collection owing to their size. Additionally, conducting comprehensive chemical and physical analyses for a diverse array of pollutants is not feasible. Nevertheless, aquatic biota exhibit wide responses to various potential pollutants, including both synergistic and antagonistic effects.

It is generally accepted that animals retained within a mesh sieve ranging from 0.5 to 2.0 mm are classified as macrofauna, whereas those that pass through this sieve but are retained by a sieve with a mesh size of approximately 60µm are designated as meiofauna.

Table 6.2 - Classification of Benthos based on Size

Size Range	Classification
200-2000 µm	Macro invertebrates
< 595 µm	Micro invertebrates
40-200 µm	Meiofauna
< 40 µm	Microfauna

Table 6.3 - Various Groups of Benthos

Category	Size Range
Insecta	Mayflies, Caddisflies, Stoneflies, Beetles etc
Turbellaria	Flatworms
Annelida	Oligochaeta (aquatic earthworms), Hirudinea (leeches)
Crustacea	Amphipoda (Scuds), Isopoda (aquatic sowbugs), Decapoda (crayfish, shrimp)
Hydra carina	Water mites
Gastropoda	Snails
Pelecypoda	Freshwater mussels

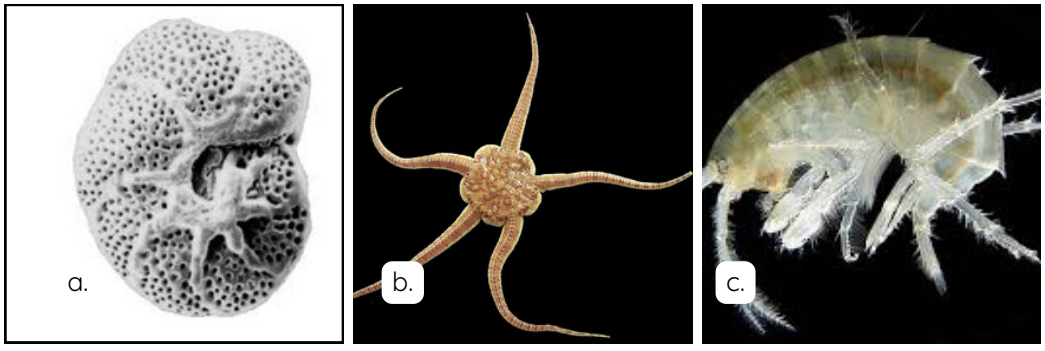

Fig. 6.8 - Examples of Benthos a) Foraminifera b) Brittle Star c) Amphipod

Sample collection

Various instruments can be utilized for the collection of sediment samples which includes Peterson grab or Van Veen grab, Kick net, Dredger, Sediment corer,

1) A Petersen grab or Van Veen grab is used for gathering benthic samples from near-shore waters. It is advisable to deploy the grab gently at each designated site, ensuring the winch wire maintains a vertical position. In instances of considerable depth or swift currents, supplementary weights need to be attached to the grab, with positional stability achieved by navigating against the current or utilizing anchorage. Arrangements for the reception and processing of samples should be readily available on the vessel's deck. Immediate recording of sediment temperature is required when the grab is brought down to the deck. Duplicate samples should be obtained parallel, noting the precise date and time of collection. Particular attention is to ensure that the second duplicate is not taken from an area that may have been depleted by the first duplicate.

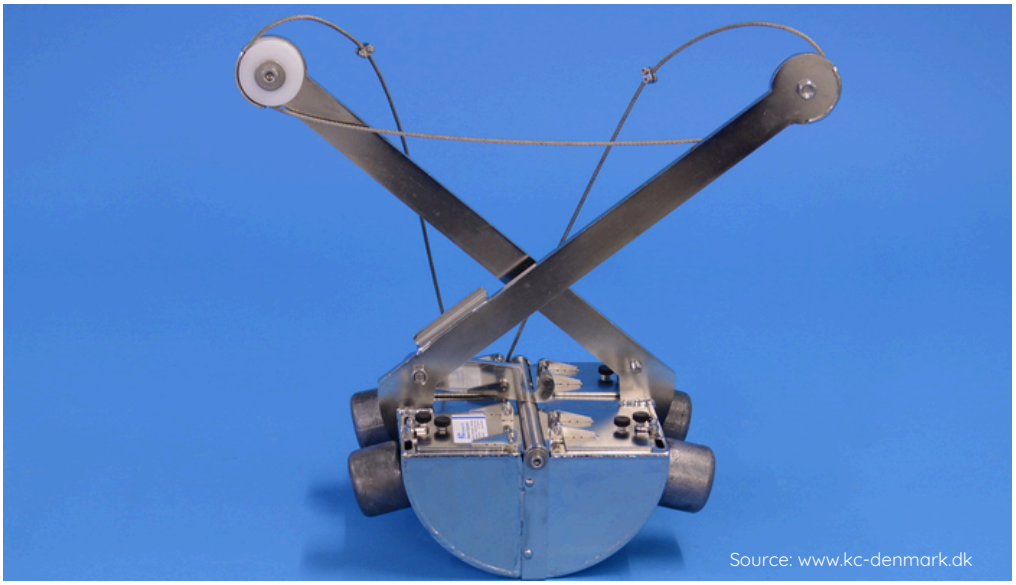

Fig. 6.9 Van-Veen Grab

2) Kick net is a 1 x 1-meter square mesh net used in aquatic environments, particularly for collecting benthic macroinvertebrates from stream and river beds. It consists of a mesh net attached to a frame, which is placed on the streambed.

Operation

Select sampling sites that accurately represent the area to be sampled, and determine the number of replicates needed. This method is particularly effective in riffle areas of streams, where the water flows relatively quickly over cobble,

gravel, and sand substrates. It is important to note that this sampling technique requires at least two people to perform effectively.

The first team member enters the stream from the downstream side and positions the net in the water with its opening facing upstream, in an undisturbed area free of large rocks or debris. The second team member enters the stream 1 to 2 meters upstream from the net, lifting and scrubbing rocks underwater to dislodge organisms. They then disturb the stream bed by kicking it while moving downstream toward the net. Once the area has been thoroughly disturbed, the person holding the net lifts the net out of the water with a gentle scooping motion. After a sample has been collected, empty the contents of the net into a white pan, and remove any debris after inspection for clinging organisms. When completed, transfer the contents of the white pan into a wide-mouthed jar. using forceps, pick off any organisms that have attached to the net fabric and place them into the jar.

After sampling is complete, add required amount of fixative or preservative to fully cover the substrate and organisms in the container. Label each container with the date, site name, sample location, and replicate number. Transfer the samples to the designated laboratory for further processing.

Source:forestry-suppliers.com

Fig. 6.10 Kick net

3) Dredger is an instrument for collecting bottom sediment samples by dragging empty container along the floor of water body. It typically consists of a scoop or bucket attached to a handle or cable, which is lowered to the seabed or riverbed to collect material. It is used for the collection of large quantity of samples.

Operation

Select sampling sites that are representative of the area to be sampled and determine the number of replicates needed. Dredges are particularly suitable for use in deep water or areas with soft bottom sediments. Attach the dredge to a heavy rope and attach a weighted messenger to length of rope. Lower the dredge slowly, particularly during the final 0.5 meters of descent above the substrate surface. If the water depth is not known, lower the dredge to the bottom and then raise it to ensure it reaches the desired depth. Trigger the dredge by either dropping the weighted messenger or letting the rope loose.

Lift the filled dredge to the surface using a smooth, even motion to prevent disturbing the contents. Care must be taken while retrieving and handling the dredge, as it can be very heavy when loaded. Empty the contents of the dredge into a container. Gently pour all the contents of the container into a soil sieve. Use a gentle spray of water to break up compacted particles and to facilitate passing of sediment through the sieve. The material remaining on the sieve is the collected sample. Place the sieve in a container of water to concentrate materials and organisms to one side. Remove rocks and large debris that do not have clinging organisms, and scrape them into a wide-mouthed jar. After sampling is complete, add required amount of fixative or preservative to fully cover the substrate and organisms in the container. Label each container with the date, site name, sample location, and replicate number. Transfer the samples to the designated laboratory for further processing.

Fig. 6.11 Dredger

4) Sediment Corers are used for collecting sediment from the ocean floor; they work by pushing or grabbing sediment into containers. A core sample is usually a cylindrical section of a naturally-occurring substance. Most core samples are obtained by drilling with special drills into the substance, such as sediment or rock, with a hollow steel tube, called a core drill. The hole made for the core sample is called the core hole.

Operation

Select sampling sites that are representative of the area to be sampled and determine the number of replicates needed. Sediment coring is particularly useful in estuarine environments, such as tidal flats or salt marshes, and is often conducted during low tide. It is recommended that the cores have a minimum diameter of 8.8 centimetres (cm) and a length of 10 cm. Insert the core into the sediment to the desired depth based on the study objectives. To remove the core from the sediment, cap the top of the core and gently rotate it while slowly pulling it out of the sediment. Once the core is removed, cap the bottom end to prepare it for later processing.

Empty the contents of core by pouring into a U.S standard No soil sieve for benthos. Use a gentle spray of water to break up compacted particles and to facilitate passing of sediment through the sieve. The material remaining on the sieve is the collected sample. Place the sieve in a container of water to concentrate materials and organisms to one side. Remove rocks and large debris that do not have clinging organisms, and scrape them into a wide-mouthed jar. After sampling is complete, add required amount of fixative or preservative to fully cover the substrate and organisms in the container. Label each container with the date, site name, sample location, and replicate number. Transfer the samples to the designated laboratory for further processing.

Fig. 6.12 Multiple Sediment corer

Processing Sample onboard

Transfer the sediment sample carefully into a container without spillage. Rinse the sediment through a sieve using a continuous flow of water, ensuring neither spillage nor mesh clogging occurs. Multiple sieves can be utilized if fauna size composition is of interest. For cases where size information is not required, a single sieve with a 500 µm mesh can be used for segregating macrofauna. Regularly inspect sieve mesh sizes for damage and wear. To reduce damage to delicate organisms, sieving may be conducted by submerging the sieve in a sufficiently deepwater bath to fully cover the mesh screen. Subsequently, the sediments should be gently agitated until they are washed out.

Benthic organisms retained within each sieve should be collected into separate bottles and preserved with 4% formalin onboard. To prepare the preservation solution, collect the sample in approximately 100 mL of seawater and add 10 ml of 40% formalin. In instances of sample contamination with debris, tube-dwelling polychaetes, large animals, or substantial residual sediment, particularly in compact clay sediments, increase the formalin concentration to 10% or even 20%.This material is a suspected carcinogen (U.S. Environmental Protection Agency, 1981) and should only be handled in well-ventilated areas while wearing gloves and eye protection. Any skin contact with formalin should be promptly flushed with large amounts of water. Staining enhances the sorting process and improves efficiency. Nevertheless, excessive staining can affect species identification. The addition of a few drops of Rose Bengal stain (at a dilution of 1:500) to the sample will fasten sorting, as organisms acquire deep purple coloration.

Processing Sample in Laboratory

Transfer the contents of the sample container into a U.S standard No sieve for benthos and rinse with water to remove the fixative or preservative. Collect the rinsate and dispose it properly. Gently shake the tray to ensure the contents are evenly distributed. Transfer the contents of the sieve onto a white plastic tray. Visually inspect for large debris. Separate the organisms from the unwanted material and group them into similar taxonomic categories (e.g., Order, Family) for identification and enumeration.

A benthos sample may sometimes contain high density of organisms, requiring significant time for processing. In such cases, a sub-sample may be taken from the original sample. The method for sub-sampling can vary depending on the investigator's convenience and the type of sample. For example, the bottom of the white tray can be divided into a grid with 10 numbered squares. Gently shake the tray to ensure the contents are evenly distributed. A random number generator is used to select one square, and the organisms within that square are removed. This sub-sample represents the sample from that specific location or replicate.

Larger animals are to be individually collected, counted, and weighed before proceeding with the sorting and enumeration of smaller specimens. The sorting and enumeration of smaller organisms requires the use of a dissection microscope along with fine brushes, needles, and forceps. However, specimens such as Oligochaeta, Chironomidae, and certain mayfly structures require mounting on glass slides and can be identified under a compound microscope. Initial sorting should involve categorizing specimens into four primary taxonomic groups: segmented worms (Annelida), mollusks (Mollusca), arthropods (Arthropoda), and other marine invertebrates. These sorted specimens are then placed into separate sample vials, each appropriately labelled for identification.

The determination of displacement volume and wet weight of sorted animals is noted prior to their identification and enumeration. Wet weight analyses are preferred for routine and monitoring surveys. While analyses of dry weight and ash-free dry weight may be conducted under specific circumstances, but they are generally not recommended for faunal studies due to material destruction during the process. To estimate dry weight, the formalin-preserved material should be dried at 60°C until a constant weight is achieved (typically 12-24 hours, or longer if necessary depending on material thickness). Ash-free dry weight estimation should follow the measurement of dry weight. This is accomplished by incinerating the sample at 500°C in an oven until weight constancy is attained (approximately 6 hours, depending on sample and object size).

The determination of wet weight involves weighing the specimens after removal of external fluid using filter paper. The animals are carefully positioned on filter paper and gently moved until no residual moisture remains, ensuring that excessive pressure is avoided. In the case of animals with shells, their weight is typically measured inclusive of their shells; however, for bivalves, any excess water should be drained before weighing. Tube-dwelling species (polychaetes) must be extracted from their tubes before weighing. Echinoids and ascidians should be punctured to release excess water prior to blotting on filter paper.

Broken animals shell only be counted by their intact anatomical features, such as their heads in the case of polychaetes, or the hinges of bivalves with attached pieces of tissue. Subsequent to identification, all samples are correctly labelled and stored in the laboratory. Once the sample is identified and laboratory documentation is finalized, all taxonomic information is entered into a Microsoft Access database.Various indices may be calculated using the final count of organisms. The specific indices employed will depend on the objectives of the investigation which includes total number of organisms, total number of taxa, ratio of pollution sensitive organisms to pollution tolerant organisms (e.g., Ephemeroptera, Plecoptera, Trichoptera [EPT}: chironomid ratio), percent dominant taxa, diversity indices, and statistical analysis.

Note

- Using formalin for the preservation or fixation of sponges and ctenophores is strongly discouraged, as it may lead to rapid disintegration or complete destruction of the animals.
- Inspect the debris for any unusual clusters of twigs, leaves, or sand, as these may serve as protective enclosures for certain organisms. During such cases, both the protective casing and the organism within should be carefully collected.
- If there is any doubt regarding the identity of an object, it is advisable to collect it without including it in the count. Notification to a senior biologist is recommended in such cases.
- Training individuals in the proper operation of samplers can help reduce injuries resulting from equipment misuse.
- Spillage during the transfer of samples need to be noted.

6.5 Estimation of Chlorophyll

Chlorophyll pigments, including types a, b, and c, are vital components within marine phytoplankton, with chlorophyll a being the most significant as it directly indicates the biomass of phytoplankton in marine ecosystems. Chlorophyll is essential for photosynthesis, a process that produces oxygen, sequesters carbon, provides food, and maintains ecological balance. This process underpins the marine food web, supporting the growth of organisms at higher trophic levels, such as fish and shellfish in mariculture systems. Monitoring chlorophyll levels is crucial, as abnormal concentrations can signal nutrient imbalances, excessive algal growth (eutrophication), or environmental stressors that could affect mariculture productivity and sustainability. Regular assessment of chlorophyll concentrations enables mariculturists to optimize feeding practices, manage nutrient inputs, and ensure the overall health of the aquatic ecosystem.

Principle

The estimation of chlorophyll involves extracting the pigment from phytoplankton cells using a solvent such as acetone, methanol, or ethanol. The extracted solution is then subjected to spectrophotometric analysis, where the absorbance of light at specific wavelengths is measured. Chlorophyll a absorbs light primarily at wavelengths of 430 nm (blue) and 662 nm (red). By measuring the absorbance at these wavelengths, the concentration of chlorophyll a can be determined. The absorbance values are used in specific equations to calculate the concentration of chlorophyll in the sample, providing an estimate of the phytoplankton biomass in the marine environment. This method allows for accurate monitoring of chlorophyll levels, aiding in the assessment of primary productivity and the overall health of the aquatic ecosystem.

Sample Collcetion

To collect samples for chlorophyll estimation, use clean, opaque containers to prevent light exposure, which can degrade chlorophyll. Rinse the containers with the sample water multiple times to remove any potential residues. Submerge the container below the water surface to collect a representative sample, ensuring it is filled completely and without trapping air bubbles. Seal the container tightly to prevent any external contamination during transport. Label the container clearly with essential details such as sampling location, date, and time. After collecting the samples, filter the water through a glass fiber filter to concentrate the phytoplankton. Store the filters in a dark, cool environment, ideally below -20°C, until analysis. Analyze the samples promptly to ensure accurate chlorophyll estimation.

Materials Required

- Centrifuge tube (15 mL) – 3 No for triplicate analysis
- 47 mm microfilter paper or 4.5cm Whatman GF/F glass filter paper.
- Dropper – 1 No
- Measuring cylinder (1L, 50 mL)– 1 No
- Tarson bottle (1 L) – 3 Nos
- Tissue paper
- Wash bottle with distilled water
- Zooplankton net – 1 No
- Black cover

Instrument Required

- UV Visible spectrophotometer
- Cooling Centrifuge

Reagents Required

- **Acetone**
  1. Use analytical grade acetone
- **Magnesium carbonate suspension**
  1. Add 1g of finely powdered magnesium carbonate to 100mL of distilled water in a stoppered flask, then vigorously shake to suspend the powder.

Procedure

01 Sample Filtering

- Filter 500 mL to 5 L samples through a 0.3 mm mesh nylon net to remove larger organisms.

02 Addition of Magnesium Carbonate

- Add 2-3 drops of magnesium carbonate suspension and mix well.

03 Volume Filtering

- Filter the measured volume through a 47 mm Millipore filter or a 4.5 cm Whatman GF/C glass filter paper.

04 **Preparation for Pigment Extraction**

- Place the filter in a 15 mL stoppered centrifuge tube.

05 **Addition of Acetone**

- Add 8 mL of 90% acetone to the Millipore filter or 10 mL to the glass filter, then shake well.

06 **Pigment Extraction**

- Extract the pigments by refrigerating the tube in complete darkness for 20 hours. If possible cover it with black cover.

07 **Warming the Extract**

- Remove the tubes and allow them to warm up in the dark until they reach room temperature.

08 **Adjusting Extract Volume**

- Add 90% acetone to make the extracts up to 10 mL for the Millipore filter and 12 mL for the glass filter.

09 **Centrifugation**

- Centrifuge the extract at 4000 rpm for 10 minutes and decant the supernatant into a 10 mm path cuvette.

10 **Absorbance Measurement**

- Measure the absorbance at 750, 665, 645, and 630 nm against a cell containing 90% acetone.

11 Calculations

- The amounts of pigments in the sample are calculated using the formula of Strickland and Parsons (1972).

C (chlorophyll a) = 11.6 E665 – 1.31 E645 –0.14 E630
C (chlorophyll b) = 20.7 E645 – 4.34 E665 – 4.42 E630
C (chlorophyll c) = 55 E630 – 4.64 E665 –16.3 E645

Where,
"E" represents the absorbance values obtained at various wavelengths as mentioned earlier, which have been corrected based on the reading obtained at 750 nm.
Chlorophyll a, b and c are the amounts of chlorophyll.

- Calculate the concentration of pigments in sea water from the equation:
  mg pigment/m^3 = C * 10 / V

Where C is a value obtained from the Strickland and Parsons equations and V is the volume of seawater filtered in litre

Note

- The addition of magnesium carbonate is necessary as it serves the purpose of safeguarding the phytoplankton chlorophyll against acidification, thereby preventing its decomposition into phaeophytin pigments.
- Using of Millipore filters is advantages as they dissolve entirely in acetone, pose no complications during the centrifugation process, and requires no specific precautions during filtration.
- Glass filters need to be shaken vigorously for an extended period until complete dissolution. This process grinds the cells through physical shaking, leading to enhanced extraction of cell pigments.
- Thoroughly drain the filter under suction before removing it from the filtration equipment.

6.6 Estimation of Primary Productivity

The term "primary productivity" refers to the rate at which radiant energy is absorbed through photosynthetic processes conducted by green plants and algae, subsequently stored as organic substances. This rate is expressed in such terms as kilocalories per square per year (kcal/m /yr).

Gross primary production (GPP)

The complete amount of sunlight captured and converted into organic matter through photosynthesis is known as gross primary production (GPP). This includes the organic material used up during respiration within the measured timeframe. It's also known as total assimilation.

Net Primary Production (NPP)

The energy that's remaining after respiration and stored as organic material during the measurement period is called net primary production. It's also known as apparent photosynthesis. Net production represents the energy accessible to the heterotrophic components of the ecosystem.

The flow of energy during primary production can be expressed by the formula.

Gross primary production (GPP) =Net primary production (NPP) + Respiration (R)

Primary productivity is essential for ecosystems as it forms the base of the food chain, thereby supplying energy for all organisms. It influences biodiversity, and oxygen production and also maintains life through the conversion of inorganic compounds into organic substances, thereby facilitating the flow of energy throughout ecosystems. Monitoring primary productivity helps mariculturists assess the availability of food resources for cultured species and understand ecosystem dynamics. Adequate primary productivity indicates a healthy and nutrient-rich environment, supporting growth and reproduction among mariculture organisms. Conversely, low productivity levels may signal nutrient deficiencies or environmental stressors that could impact the health and productivity of cultivated species. So proper monitoring is essential to enhance the overall efficiency and sustainability of aquaculture practices.

Factor that promotes Primary Productivity

Environmental factors that promote net productivity includes warm temperatures, ample rainfall, flowing water carrying nutrients in natural ecosystems, as well as irrigation, fertilizer application, and pest control in agricultural settings. Terrestrial ecosystem productivity is influenced by various factors including carbon dioxide levels, light availability, temperature, moisture, nutrient availability, and soil texture. Ecosystems with optimal conditions of these factors tend to exhibit higher productivity, aiming to maximize photosynthesis.

Principle

This method is based on the assessment of dissolved oxygen levels in water samples (measured in ml/L) using Winkler's method. Six BOD bottles are employed for this procedure. The dissolved oxygen content in the initial (I), Dark (D), and Light (L) bottles, each with a capacity of either 125 mL or 250 mL, after a designated incubation period, is used to measure primary productivity. The duration of the incubation period varies depending on the nature of the water sample such as it spans 3.0 hours for seawater, whereas it extends to 2.5 hours for shallow estuarine water.

Sample Collection

Use a clean water sampling bottle for taking water sample. Submerge the container to the desired depth and collect water samples without introducing air bubbles. Measure light intensity at the same depth using a light meter. Securely seal the container to prevent light exposure during transport. Label the sample with details such as location, date, depth, and time. Store it in a cool, dark place and quickly transport it to the laboratory.

Materials Required

- Dissolved oxygen bottles (125 mL) - 4 numbers clear, 2 numbers dark with black cloth
- Dropper
- Burette with stand
- Wash bottle with distilled water

Reagents Required

- **Winkler solution A**
  1. Dissolve 36 grams of analytical reagent grade manganous sulphate monohydrate, $MnSO_4 . H_2O$, in distilled water, and adjust the volume to 100 mL.
- **Winkler solution B**
  1. Dissolve 100 g of sodium hydroxide in 100 mL of distilled water.
  2. Dissolve 27 g of potassium iodide in 100 mL of distilled water and mix the two solutions. Large amount of heat is liberated during the process. Both the sodium hydroxide and potassium iodide used in this method should be of analytical reagent grade.
- **Standard thiosulphate solution (0.025N):**
  1. Dissolve 6.205 g sodium thiosulphate in 1 litre distilled water

- **Starch Indicator**

1. Dissolve 1 g of starch powder in a small amount of cold distilled water to make a smooth paste.
2. Heat the paste gently in boiling distilled water while stirring continuously until it becomes clear and thickens.
3. Allow the solution to cool to room temperature.
4. Store the starch solution in a dark bottle, as it can degrade with light.

Procedure

01 Sample Collection

- Collect water samples in 4 clear BOD bottles and 2 dark BOD bottles.

02 Initial Fixation

- Fix two of the clear bottles immediately with Winkler A and B reagents.

03 Incubation

- Incubate the remaining two clear bottles and two dark bottles for a duration of 3 hours at the location of sample collection.

04 Post-Incubation Fixation

- After incubation, fix the clear and dark bottles with equal volumes of Winkler A and B reagents.

05 Titration

- Titrate against sodium thiosulphate and calculate the O_2 concentration in mL.

06 Calculation

- Let O_2 of light bottle after incubation = x
- Let O_2 of dark bottle after incubation = y
- Let O_2 of light bottle initially fixed = z

- **Gross production = (x-y) * 0.536 / PQ * t mg C/1/hr**
- **Net production = (x-z) * 0.536 / PQ * t mg C/1/hr**

Where PQ is the photosynthetic quotient = 1.25
't' is the number of hours of incubation = 3 hrs.

Note: For expressing the productivity in m3 /day, multiply the above by 10000; assuming 10 hrs being the sunshine hours affecting photosynthesis in a day.

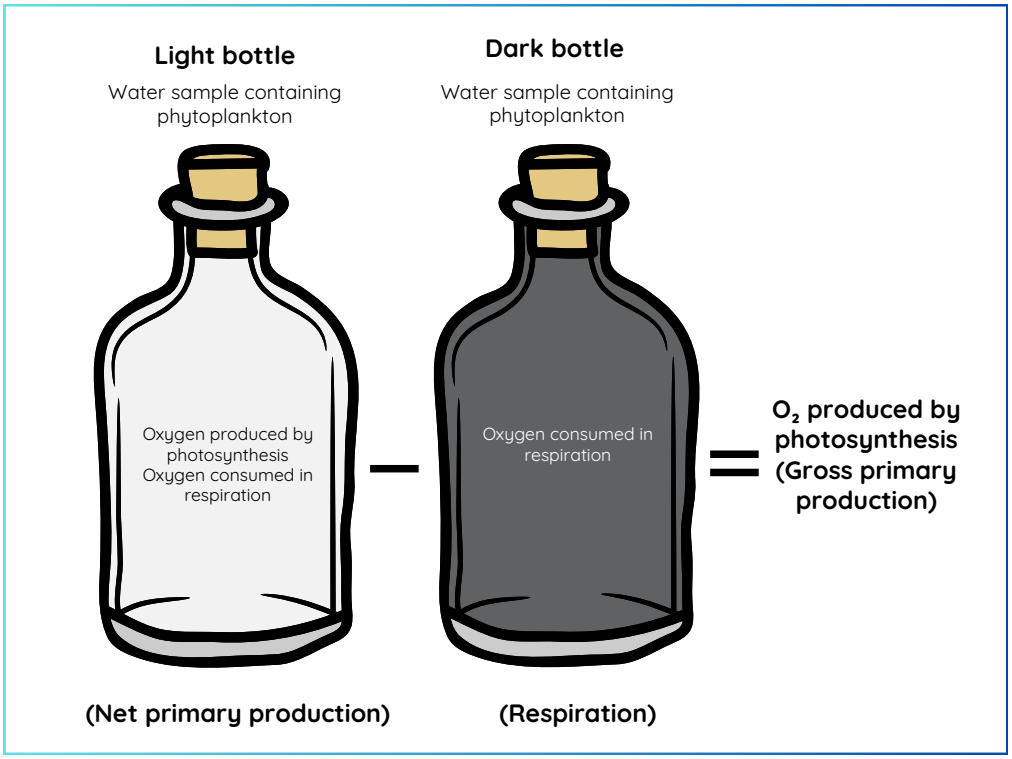

Fig. 6.13 Diagrammatic Representation of light and dark bottle reaction

Note

- Consider environmental variables, such as temperature, light availability, nutrient levels, and water chemistry, that may influence primary productivity.
- Handle samples carefully to avoid contamination and preserve the integrity of the collected data.
- Normality of Sodium thiosulphate solution should not exceed 0.005 (for more accuracy).

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CHAPTER 7

Proximate Composition analysis

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Introduction

Proximate composition analysis of fish feed is a fundamental process in evaluating the nutritional quality and ensuring the dietary requirements of aquaculture species are met. This analysis primarily focuses on determining key parameters such as moisture, crude protein, ash, crude fibre, carbohydrate, and crude fat. Each of these components plays a crucial role in the overall health, growth, and development of fish, making their assessment essential in mariculture practices.

Moisture: The moisture content in fish feed is a critical factor that influences its stability, shelf life, and nutritional quality. Moisture levels affect the feed's susceptibility to microbial growth, spoilage, and palatability. Maintaining optimal moisture levels is crucial to prevent feed degradation, ensure ease of digestion for fish, and maintain the overall quality and effectiveness of the feed. This is particularly important in mariculture, where feed stability can impact the success of aquaculture operations.

Crude Protein: Protein is a vital macronutrient required for the growth, repair, and maintenance of fish tissues. It plays a significant role in metabolic processes and overall health. Fish have specific protein requirements that vary by species and life stage. Assessing the crude protein content of fish feed is essential for formulating diets that meet these requirements, ensuring optimal growth rates, feed conversion efficiency, and overall fish performance. High-quality protein sources in fish feed contribute to the robust development of aquaculture species, making this parameter a cornerstone of nutritional assessment.

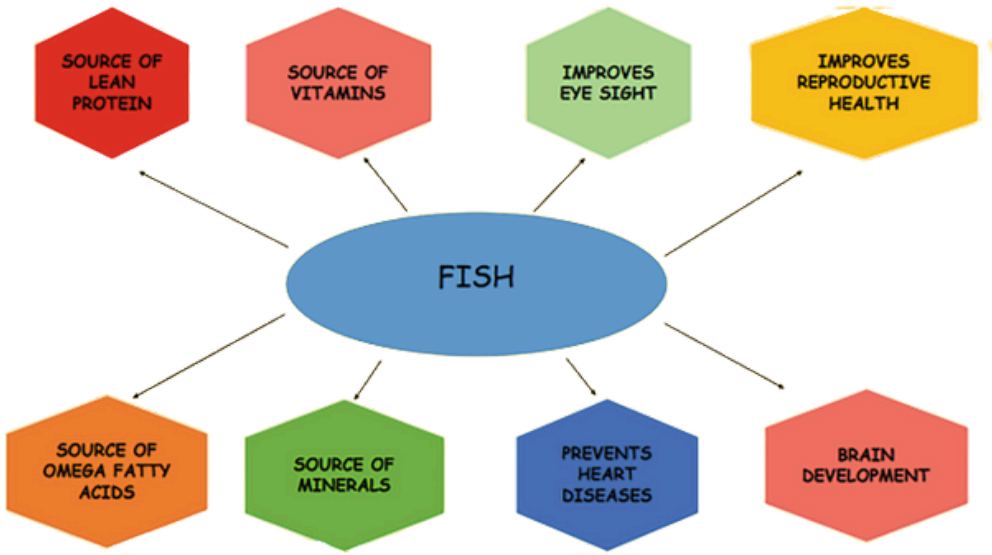
Crude Fat: Fat serves as a concentrated source of energy and provides essential fatty acids necessary for various physiological functions in fish. Adequate levels of crude fat in fish feed are crucial for maintaining energy balance, supporting growth, improving feed efficiency, and enhancing the overall health of fish. The right balance of fats ensures that fish receive the necessary energy for their activities and metabolic processes, promoting better growth performance and overall well-being.

Crude Fibre: Analyzing the crude fibre content in fish feed is essential for understanding the indigestible portion of the feed, which can influence digestive health and gut function in fish. Incorporating small amounts of fibre into a fish's diet can have benefits for their digestive health. Fibre helps to promote gut motility and ensures that nutrients pass through the digestive tract efficiently. Ensuring the appropriate level of fibre is thus important for maintaining digestive balance and avoiding gastrointestinal issues.

Carbohydrate: Carbohydrates are vital energy-yielding nutrients in commercial fish feed formulations, and their nutritional value varies among fish species, with warmwater fish capable of utilizing higher levels than coldwater and marine fish. While no specific dietary requirement for carbohydrates exists, their absence can lead to the catabolism of proteins and lipids for energy. Therefore, providing an appropriate level of carbohydrates tailored to the cultured fish species is crucial for promoting optimal growth and enhancing feed efficiency in aquaculture.

Ash: The ash content represents the total mineral composition in fish feed, including essential macro and trace minerals. Assessing ash content is crucial because minerals are vital for various physiological functions such as bone development, osmoregulation, and enzyme activation. Proper mineral balance supports the growth and health of aquaculture species, making ash analysis an important aspect of feed quality evaluation.

The importance of assessing the proximate composition of fish feed in mariculture cannot be overstated. Ensuring that fish receive a balanced and nutritious diet is fundamental to achieving optimal growth, health, and productivity. Regular analysis of these key parameters allows mariculturists to make informed decisions about feed formulation, ingredient selection, and dietary adjustments. This not only supports the efficient and sustainable production of aquaculture species but also contributes to the environmental sustainability of mariculture operations.



7.1 Estimation of Moisture Content

Understanding the moisture content in fish feed is crucial for various reasons. It directly impacts the nutritional quality and shelf life of the feed, guiding processing decisions to ensure product quality and stability. Optimizing moisture levels enhances feed efficiency and supports fish health and performance. By monitoring and controlling moisture content, aquaculture operations can ensure the provision of high-quality, nutritionally balanced feed for optimal fish growth and well-being.

Principle

The determination of the loss in mass during the drying process of a specified material under controlled conditions serves as a measure of the moisture content within the material.

Sample collection

Use clean, dry containers or sample bags to avoid contamination and moisture loss or gain. Collect representative samples from various locations within the batch, ensuring the sample is homogenized without compacting the feed. Immediately transfer the samples into the containers, seal them tightly, and label them clearly with batch details. Store the samples in a cool, dry place and transport them to the laboratory for analysis.

Instrument Required

- Moisture Dish - made of porcelain, silica, glass or aluminium.
- Hot air oven oven - maintained at 105°C.
- Desiccator

Fig. 7.1 Instruments Required for Moisture Analysis

Procedure

01 Sample Weighing

- Accurately weigh approximately 5 grams of the prepared sample into a moisture dish that has been previously air-dried in an oven and weighed.

02 Initial Drying

- Position the dish within an oven maintained at 105 ± 1°C for a duration of 4 hours.

03 Cooling and Weighing

- Allow the dish to cool in a desiccator, then weigh it.
- Record the weight obtained at this point.

04 Repeated Drying and Weighing

- Repeat the cycle of drying, cooling, and weighing at intervals of 30 minutes until the variance between two consecutive weighings is less than 1 milligram.

05 Final Drying

- Dry the dish again until the loss in mass between two successive weighing is less than 2 mg.
- Finally, record the final mass obtained.

06 Calculations

- **Moisture (%) by Mass = [(W1 - W2) / W] * 100**

Where,
W1 = weight in g of the dish with the material before drying,
W2 = weight in g of the dish with the material after drying
W = weight in g of the empty dish.

Note

- Ensure that the feed samples are representative and properly prepared according to standardized methods. Homogenize the sample to obtain an accurate representation of moisture distribution.
- Follow established protocols for temperature and duration specific to the type of feed being analysed.
- Perform moisture content measurements in replicates to assess precision and ensure the reliability of results. Analyse multiple samples from different parts of the feed batch to account for variability.
- Use dish having an effective surface area enabling the test portion to be distributed so as to give a mass per unit area of not more than 0.3 g/cm^2.
- Use grinding mill that is made of material which does not absorb moisture.

7.2 Estimation of Fat content

Measuring fat content in fish feed is vital for optimizing the nutritional balance and promoting the growth and health of aquatic species. Fat serves as a concentrated source of energy and essential fatty acids, influencing metabolic processes, immune function, and reproduction in fish. By accurately determining fat levels in feed, aquaculture practitioners can ensure that fish receive the necessary nutrients for optimal growth, development, and resistance to diseases. Additionally, monitoring fat content enables adjustments in feed formulations to meet the specific dietary requirements of different fish species at various life stages, contributing to sustainable and efficient aquaculture production.

The determination of crude fat content in food items can be effectively carried out through the utilization of petroleum ether or diethyl ether in a Soxhlet extraction apparatus. This method involves the extraction of dried and ground material, followed by the quantification of fat content with precision and convenience.

Principle

The extraction of crude fat is carried out with petroleum ether or diethyl ether in a Soxhlet apparatus. Subsequently, the solvent is volatilized after-extraction, and the mass of the residue is determined to measure the fat content accurately.

Sample collection

Use dry containers or sample bags to prevent contamination and moisture absorption. Collect representative samples from different locations within the feed batch, ensuring thorough mixing to achieve homogeneity. Transfer the sample into the container and seal the container tightly to prevent exposure to air and light, which can affect fat content. Label the sample clearly with details such as batch number, date, and location. Store the samples properly to prevent oxidation until they are transported to the laboratory.

Materials Required

- Water bath
- Weighing balance
- Soxhlet apparatus (With Extraction thimble)

Procedure

01 Ether Extraction

- Measure 2 grams of the feed and introduce it into a continuous extraction apparatus. Allow the material to extract with ether for a duration of 18 hours. Normally 6-8 hours for complete extraction and 18 hours is recommended for feeds with high fat content.
- Remove the ether through distillation.

02 Initial Drying

- Place the flask containing the residue on a boiling water bath and dry it in an oven set at 110 ± 1°C until the loss in mass between two successive weighing is below 2 mg.

03 Ether Washing

- Shake the residue with 2 to 3 milliliters of ether at room temperature, allowing it to settle, and then decant the ether.

04 Repeated Extraction

- Repeat the extraction process until no more residue dissolves.

05 Calculations

- **Fat, % by mass = (M1 – M2) * 100/ M**

Where,

M1 = mass in g of the Soxhlet flask with the extracted fat.

M2 = mass in g of the empty Soxhlet flask.

M = mass in g of the material taken for the test.

Source: www.velp.com

Fig. 7.2 Soxhlet Apparatus

Note

- Ensure that the fish feed samples are finely ground and homogenized to facilitate efficient extraction of fat.
- Determine the optimal extraction time and temperature based on the characteristics of the feed and solvent. Longer extraction times may be required for samples with higher fat content.
- Follow safety protocols when handling flammable solvents and operating the Soxhlet apparatus to prevent accidents and exposure to harmful chemicals.
- Allow sufficient time for solvent evaporation after extraction to ensure complete removal of the solvent from the fat residue.

7.3 Estimation of Protein Content

Estimating the protein content in fish feed is crucial for ensuring balanced nutrition, optimizing feed formulation, and promoting healthy growth in fish. By accurately determining protein levels, fish farmers can make diets to meet specific nutritional requirements, maximize growth potential, and maintain cost efficiency in aquaculture operations. Additionally, proper protein levels contribute to overall fish health and resilience against diseases, ultimately enhancing the sustainability and productivity of fish farming efforts.

The Kjeldahl method is widely used for determining the crude protein content in food products. The protein content of foods is calculated from total nitrogen measurements by multiplying a suitable conversion factor. This conversion factor is selected based on the nitrogen percentage present in a particular protein.

Principle

The sample undergoes oxidation in the presence of sulphuric acid, whereby nitrogenous compounds are transformed into ammonium sulphate. Mercury is added into the digestion mixture as a catalyst, while alkali sulphate serves as a boiling point enhancer. An excess of alkali is introduced to liberate ammonia, which is then quantitatively distilled into a measured volume of standard hydrochloric or sulphuric acid. The acid not neutralized by ammonia, is titrated back with standard alkali, providing a measure of the nitrogen content present in the sample.

Sample collection

Collect representative samples in clean dry containers using a clean scoop or spatula. Seal the container tightly to prevent exposure to air and moisture, which can affect protein content. Label the sample clearly with details such as batch number, date, and location. Store the samples in a cool, dry place until they are transported to the laboratory.

Materials Required

For Digestion

- Kjeldahl flasks (500 to 800 mL capacity) - 1 No
- A heating device (heater/burner) – 1 No

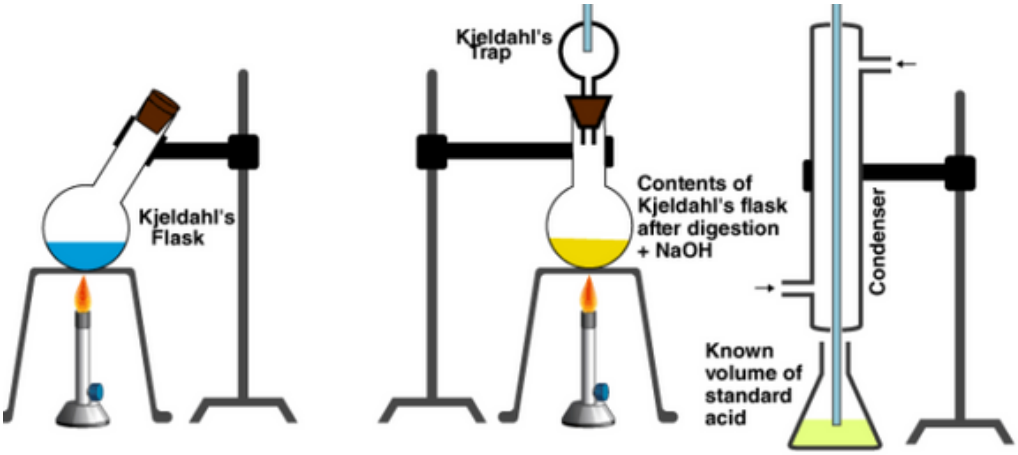
For Distillation

- Round bottom flask (1 litre capacity) – 1 No
- Splash head - 1 No
- Condenser – 1 No

- Trap – 1 No
- Beaker (500 mL capacity) – 1 No
- Receiving funnel – 1 No
- Standard Flask (1L) – 1 No
- Measuring cylinder (50 mL, 500 mL) – 1 No
- Burette with stand – 1 No
- Volumetric flask – 1 No

Reagents Required

- Concentrated Sulphuric Acid, AR Grade
- Potassium Sulphate or Anhydrous Sodium sulphate, AR Grade
- Sodium Hydroxide Solution - In a standard Flask with distilled water dissolve about 450 g solid sodium hydroxide, cool, and make up to 1 L.
- Hydrochloric or Sulphuric Acid, Standard Solution: (0.1N or 0.5N). Standardize against sodium hydroxide standard solution.
- Sodium Hydroxide Standard Solution - 0.1 N. Standardize against primary standard and against standard acid solution.
- Methyl Red Indicator - Dissolve 1 g methyl red in 200 mL alcohol.



Source: byjus.com

Fig. 7.3 Working of Kjeldahl's Distillation Unit

Procedure

01 Sample Weighing

- Accurately weigh between 0.7 to 2.2 grams of the fish feed sample.
- Ensure the ratio of salt to acid (m/v) is approximately 1:1 at the end of digestion for proper temperature control.

02 Addition of Reagents

- Add 0.7 grams of mercury oxide or 0.65 grams of mercury, 15 grams of powdered potassium sulphate or anhydrous sodium sulphate, and 25 mL of sulfuric acid to the flask. If available non-mercury catalyst options like (copper sulfate or selenium) can be used.
- Note: Each gram of fat consumes 10 mL and each gram of carbohydrate consumes 4 mL sulfuric acid during digestion.

03 Initial Heating

- Place the digestion flask in an inclined position on a heating apparatus and gently heat until foaming subsides.
- Add a small amount of paraffin or silicon antifoam to reduce foaming.

04 Boiling and Digestion

- Continue boiling the mixture vigorously until the solution becomes clear.
- Maintain the boiling for 1 to 2 hours and then allow it to cool.

05 Dilution and Prevention of Complex Formation

- Add approximately 200 mL of distilled water to the cooled solution.
- To prevent complex formation, add 25 mL of either sulphide or thiosulfate solution.
- Thiosulphate or sulphide solution may be mixed with the sodium hydroxide solution before addition to the flask, and stir the mixture to precipitate the mercury.

06 Making the Solution Alkaline

- Incline the flask and add, without agitation, 25 grams of sodium hydroxide to make the solution strongly alkaline.

07 Distillation Setup

- Immediately connect the flask to the distillation bulb or trap on the condenser, with the tip of the condenser immersed in a measured quantity of standard acid (usually 50 mL of 0.5 N acid).

08 Mixing and Heating

- Rotate the flask to thoroughly mix the contents, then begin heating until all ammonia has distilled over.
- (Lower the receiver before discontinuing distillation and rinse the tip of the condenser with distilled water.)

08 Titrate excess acid with alkali

- Titrate the excess acid in the receiver with standard 0.1 N sodium hydroxide, using methyl red as an indicator.

09 Calculations

- **Protein, % by mass = (B -S) * N * 1.4 * K / W**

where,
B = volume in ml of 0.1 N alkali used for titration for blank
S = volume in ml 0.1 N alkali used for titration for sample
N = normality of alkali used for titration,
K = Kjeldahl factor of Fish feed (5.7 to 6.25)
W = weight, in g, of sample taken for test

Note

- Sample to be analysed should be homogeneous.
- Non-mercury catalyst options like (copper sulfate or selenium) can be used. Mercury catalyst is now discouraged globally due to environmental hazards.
- Choose a suitable catalyst to optimize the digestion process and ensure complete conversion of nitrogen.
- Determine the strength of NaOH before use.
- Add an excess of alkali to liberate ammonia from the digested sample, enabling quantitative distillation.

7.4 Estimation of Ash

Measuring the ash content in fish feed is critical for evaluating the total mineral composition, which supports vital physiological functions such as bone development, osmoregulation, and enzyme activation in aquatic species. The ash fraction represents a mixture of all mineral elements present in the feed. While it does not indicate the quantity of individual minerals, ash analysis is useful for calculating parameters like Nitrogen-Free Extract (NFE) by difference from dry matter. Additionally, ash content can reveal potential contamination, such as soil or added salts.

The estimation of ash content involves oxidizing all organic matter in a weighed sample through high-temperature incineration in a muffle furnace, and subsequently weighing the residue. This method is particularly applicable to fish feeds and products with low carbohydrate content. However, it should be noted that the high temperatures used may volatilize some elements, such as potassium, sodium, chlorine, and phosphorus, and may cause fusion of the mineral matter, potentially affecting the precision of results.

Principle

The method is based on the complete combustion of organic material in a high-temperature muffle furnace, leaving behind inorganic mineral residues (ash), which are then weighed to determine the ash content.

Instrument Required

- Muffle Furnace
- Silica or Platinum Crucibles
- Analytical Balance:
- Desiccator

Procedure

01 Preparation of Crucibles:

- Heat a clean silica or platinum crucible at 600°C in a muffle furnace for one hour. Cool it in a desiccator and record its empty weight (W_1).

02 Sample Preparation

- Accurately weigh 2 grams of the dried feed sample and place into the pre-weighed crucible. Record the combined weight (W_2).

03 Initial Heating(charring)

- Place the crucible containing the sample on a clay triangle over a low flame to char the organic matter, avoiding loss of material.

04 Ashing in Muffle Furnace

- Transfer the charred sample to a muffle furnace maintained at 600°C. Incinerate for 6-8 hours or until a consistent white or grayish ash is obtained.

05 Cooling and Final Weighing

- Cool the crucible in a desiccator and weigh it (W_3). Reheat the crucible for an additional 30 minutes, cool, and reweigh to ensure that the ash weight is constant (variation less than 0.5mg between two weighing).

06 Calculation

- Use the formula below to calculate the ash content

$$\text{Ash content}\left(\frac{\text{g}}{100\text{g}}\right) = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

Where:

- W1 – Weight of empty crucible (g)
- W2 – Weight of crucible + sample before ashing (g)
- W3 – Weight of crucible + ash (g)

Note

- Ensure crucibles are completely dry before use to avoid moisture interference.
- Handle hot crucibles with tongs or heat-resistant gloves to prevent burns or contamination.
- The muffle furnace should be calibrated to maintain accurate and consistent temperature.
- Confirm complete combustion by achieving constant ash weight after reheating.
- Be aware of possible loss of volatile minerals (like Na, K, P and Cl during ashing.

7.5 Total Carbohydrate

Carbohydrates play a critical role in the formulation of fish feed, serving as a primary source of energy that supports various metabolic processes. Their inclusion in diets is particularly important for warmwater fish, which can efficiently utilize higher carbohydrate levels compared to coldwater and marine species. Although fish do not have a defined dietary requirement for carbohydrates, a lack of these nutrients can result in the breakdown of proteins and lipids for energy, potentially impairing growth and overall health. Moreover, providing an appropriate carbohydrate level tailored to the specific needs of the cultured fish species not only promotes optimal growth but also enhances feed efficiency and sustainability in aquaculture practices. By ensuring a balanced diet that includes carbohydrates, aquaculture producers can support the health and productivity of their fish, ultimately leading to more successful and sustainable farming operations.

The carbohydrate content can be calculated based on difference calculation.

% Carbohydrate =100 % - (% moisture + % ash + % crude fibre + % crude protein + % fat)

if sugars/starches are needed separately, specific methods like Anthrone method can be used

7.6 Crude Fibre

Crude fibre is an important component in fish feed formulations, contributing to the overall digestive health and gut function of fish. Although fish have limited ability to digest fibre, the presence of crude fibre can aid in promoting gut motility and ensuring the efficient passage of feed through the digestive tract. This can be particularly beneficial in preventing gastrointestinal issues and improving overall feed utilization. Additionally, crude fibre helps to maintain a balanced diet, allowing fish to assimilate nutrients more effectively. By incorporating appropriate levels of crude fibre into fish feed, aquaculture producers can support optimal growth, enhance feed efficiency, and contribute to the overall well-being of the fish, ensuring a more sustainable and productive aquaculture system.

The crude fibre content can be determined by sequential digestion of the sample with acid (1.25 % H_2SO_4) and alkali (1.25 % NaOH). The residue left over after digestion is washed with alcohol (ethanol) and ether (petroleum ether) and then transferred to a preweighed crucible, dried in an oven and then muffled. The difference in weight before and after muffling is noted. Soxhlet washing with (ethanol and ether) should be thorough to avoid overestimation of fibre due to retained fats.

Table 7.1 - Instruments and AOAC Methods for Proximate Composition Analysis

Parameter	AOAC Method Number	Reference	Instruments Required
Moisture	934.01	AOAC, 2023	Moisture Dish, Hot Air Oven, Weighing balance, Desiccator
Crude Protein	2001.11	AOAC, 2023	Kjeldahl Digestion Unit, Distillation Unit, Burette
Crude Lipid	991.36	AOAC, 2023	Soxhlet Apparatus, Weighing balance
Crude Fibre	930.10	AOAC, 2023	Muffle Furnace, Weighing balance
Ash	950.46	AOAC, 2023	Muffle Furnace, Desiccator, Weighing balance
Total Carbohydrate	Calculated based on difference calculation	-	-

Conclusion

In summary, proximate composition analysis of fish feed is a vital process in aquaculture that ensures nutritional adequacy and promotes optimal growth and health of fish. Understanding the roles of moisture, protein, fat, fibre, carbohydrates, and ash allows aquaculture practitioners to formulate diets tailored to the specific needs of different fish species. Regular monitoring and quality control measures are essential to maintain feed quality, contributing to sustainable and efficient aquaculture practices.

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