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Executive Summary

This internship report provides a comprehensive overview of a study focused on marine phytoplankton indigenous to the Bay of Bengal. The research encompassed a series of critical steps: the culture, growth observation, isolation, and identification of these microscopic organisms.

The methodology employed in this study was multifaceted, involving several key techniques. These included the systematic collection of samples from the marine environment, their subsequent cultivation under controlled laboratory conditions, detailed microscopic observation to study their characteristics, and various identification methods to accurately classify the phytoplankton species.

A core objective of this research was to deepen the understanding of phytoplankton diversity within the Bay of Bengal, to quantify their growth rates under experimental conditions, and to assess their crucial ecological significance within the broader marine ecosystem. Phytoplankton are fundamental to marine food webs and play a vital role in ecosystem productivity.

Ultimately, the findings generated from this study are intended to contribute meaningfully to two important areas: enhancing marine biodiversity assessments in the region and supporting more effective management strategies for fisheries resources. This research, therefore, bridges fundamental ecological understanding with practical applications for marine conservation and resource sustainability.

1. Introduction

1.1 Background of the Study

Algae, a group of photosynthetic autotrophs, thrive across a wide range of aquatic environments, including lakes, rivers, and oceans. By means of photosynthesis, they play a vital role in producing atmospheric oxygen, transforming water and carbon dioxide into carbohydrates through the utilization of solar energy. This group encompasses a broad spectrum of organisms, ranging from prokaryotic unicellular cyanobacteria to complex multicellular eukaryotic algae, each displaying unique structural and functional attributes (M.A. Ashraf, 2017).

Microalgae, a subset of the algal family, consist of highly diverse unicellular and multicellular organisms exhibiting both autotrophic and heterotrophic traits (Roque et al., 2018). They harness sunlight and convert it into chemical energy via metabolic pathways (Ozkurt, 2009), and can prosper in both saline and freshwater ecosystems (M.A. Ashraf, 2017).

In marine ecosystems, microalgae exist as microscopic, pelagic, single-celled organisms suspended throughout seawater. Typically ranging in size from 2 to 20 μm, they are capable of withstanding variations in salinity, temperature, pH, light intensity, and other environmental factors (Barsanti et al., 2008). The major groups of microalgae—such as diatoms, dinoflagellates, green algae, blue-green algae, and coccolithophores—are notable for their ecological importance and biochemical diversity. These organisms serve as rich sources of essential fatty acids, pigments, amino acids, and vitamins (P. Perumal, 2012).

Microalgae play a pivotal role in the success of coastal and marine aquaculture. They are indispensable in supporting the growth and development of fish, mollusks, shrimps, and oysters, especially in larval nutrition and bioencapsulation techniques (P. Perumal, 2012). Despite the identification of over 30,000 microalgae species, only a small fraction has been commercially exploited for high-value applications (Mata et al., 2010; Saha and Murray, 2018; Khan et al., 2018). The practice of cultivating microalgae for aquaculture purposes dates back to 1910, as first reported by Allen and Nelson (1910), and has since experienced substantial expansion alongside the growth of the aquaculture industry.

Currently, microalgae are not only recognized as a direct food source for humans and terrestrial livestock but also as a crucial component in the diets of farmed aquatic species, particularly mollusks and early larval stages of fish and crustaceans (Barnabe and Gilbert, 1994). Furthermore, microalgae have been identified as a promising candidate for biofuel production (Flynn KJ, 2010).

With the continued expansion of the aquaculture sector, the cultivation of microalgae has become increasingly essential, particularly for feeding organisms such as rotifers and fish. The success of microalgal cultivation often depends on selecting species that are well-adapted to local environmental conditions (Mata et al., 2010). Beyond aquaculture, microalgae are central to aquatic ecosystems, accounting for approximately 40% of global photosynthetic activity

(Moreno-Garrido I, 2008). They are responsible for producing roughly half of the atmospheric oxygen at any given time, underscoring their irreplaceable ecological function.

Microalgae cultivation is relatively simple and economical due to their capacity for photoautotrophic growth. Within the framework of a biorefinery approach, microalgae present vast potential for the production of numerous value-added products, with applications spanning food, medicine, nutraceuticals, pharmaceuticals, and biofuels (Báñez E and Cifuentes A, 2013).

1.2 Purpose of the Internship

The goal of this internship was to address the issues presented by Bangladesh in feed management for its expanding inland fish production sector. Bangladesh maintains the third position globally in inland fish production, following China and India. Despite enormous prospects in fisheries and aquaculture, feed management poses hurdles in Bangladesh because to worldwide rivalry in culture techniques. The biggest difficulties in addressing the demand for high-quality feed originate from the high costs of feed ingredients and final products. To meet the current and future demand, growing "live feed" or microalgae, which is commonly called "green gold," is a good choice for local farmers who want to feed their fish in an environmentally beneficial way. However, the lack of awareness and acknowledgment of commercial microalgae culture among rural fisherman communities poses a challenge. The internship also intends to provide insights into the many phases of microalgae growth, allowing commercial fish farms estimate phytoplankton community blooming behaviour in pond ecosystems. Modern technical support and understanding are necessary for building effective culture and management methods. A full understanding of the cultural behaviour and kinetics of microalgae growth is vital for the sector's future contribution and development.

1.3 Objectives of the Internship

The objectives of this internship are:

1. To gain practical knowledge of marine phytoplankton culture techniques, including media preparation and growth maintenance under laboratory conditions.
2. To monitor and interpret phytoplankton growth patterns through direct observation and measurement tools.
3. To isolate and identify key phytoplankton species using microscopy and taxonomic methods.
4. To assess the ecological significance of phytoplankton diversity in the Bay of Bengal, focusing on their role in marine food webs and ecosystem productivity.

2. Institutional Overview

Bangladesh Fisheries Research Institute (BFRI)

Established in 1984 and commencing operations in 1986, the Bangladesh Fisheries Research Institute (BFRI) is an autonomous government research institution dedicated to advancing

knowledge in aquaculture, fisheries management, and marine biology. Headquartered in Mymensingh, BFRI operates several specialized research stations across the country, focusing on various aquatic ecosystems, including freshwater, brackish water, and marine environments.

BFRI's primary objectives encompass:

- Conducting scientific research to enhance fish production and ensure sustainable fisheries management.
- Developing and disseminating innovative technologies for aquaculture and fisheries.
- Collaborating with national and international organizations to address challenges in the fisheries sector.
- Providing training and capacity-building programs for stakeholders involved in fisheries and aquaculture.

Figure 1: Marine Fisheries and Technology Station (MFTS), Cox’s Bazar

Marine Fisheries and Technology Station (MFTS), Cox’s Bazar

The Marine Fisheries and Technology Station (MFTS), established in 1991, is a pivotal branch of BFRI located in Cox’s Bazar. Spanning approximately 4 hectares, MFTS is strategically positioned to facilitate research on marine fisheries and coastal ecosystems.

Key features and research activities at MFTS include:

- **Specialized Laboratories**: The station houses five specialized laboratories equipped for advanced oceanographic and ecological studies, enabling comprehensive research on marine biodiversity and environmental parameters.
- **Hatchery and Cistern Complexes**: MFTS operates both indoor and outdoor cistern complexes and a hatchery, supporting the breeding and cultivation of various marine species, including shrimp and finfish.

- **Research Vessel**: The station possesses a research vessel equipped for conducting riverine surveys and experimental fishing, facilitating in-depth studies of marine resources and ecosystems.
- **Research Focus Areas**:
    - Assessment of economically important marine fishery resources.
    - Development of breeding and farming techniques for marine species, including shrimp.
    - Exploration of crop cycle-based fish and shrimp farming methods.
    - Advancement of processing, product development, and conservation techniques for marine products.

2.1 Research Facilities and Activities at BFRI, MFTS

Laboratory Infrastructure

The Marine Fisheries and Technology Station (MFTS) is equipped with specialized laboratories designed to support advanced research in marine biology and fisheries science. These facilities include:

- **Marine Biological Laboratories**: Dedicated to the study of marine organisms, including phytoplankton, zooplankton, and various fish species.
- **Water Quality Assessment Labs**: Equipped to analyze critical parameters such as salinity, pH, dissolved oxygen, and nutrient concentrations, essential for monitoring marine and aquaculture environments.
- **Seaweed Processing Laboratory Complex**: Established under the "Seaweed Culture & Seaweed Product Development in Bangladesh Coast" research project, this facility focuses on the cultivation, processing, and product development of seaweeds.

Research Equipment

To facilitate comprehensive research activities, MFTS is furnished with a range of scientific instruments, including:

- **Microscopes**: For detailed examination and identification of microscopic marine organisms.
- **Spectrophotometers**: Used in the analysis of water samples to determine various chemical properties.
- **Incubators**: Maintain controlled environmental conditions for the cultivation and study of marine species.
- **Research Vessel**: A specialized boat equipped for conducting riverine surveys and experimental fishing, enabling in-situ data collection and sampling.

Ongoing Research Projects

MFTS undertakes a variety of research initiatives aimed at enhancing the understanding and management of marine resources:

- **Marine Fisheries Assessment**: Evaluating the stock and diversity of economically important marine fishery resources to inform sustainable exploitation strategies.
- **Plankton Biodiversity Studies**: Investigating the diversity and ecological roles of plankton communities, which are fundamental to marine food webs.
- **Seaweed Research**: Developing cultivation techniques, assessing nutritional values, and exploring value-added products derived from seaweeds, contributing to alternative livelihoods and industries.
- **Breeding and Farming Techniques**: Innovating methods for the breeding and cultivation of marine species, including shrimp and finfish, to support aquaculture development.
- **Fish and Shrimp Farming Methods**: Exploring crop cycle-based farming systems to optimize production and sustainability in coastal aquaculture.

Through these facilities and research endeavors, MFTS plays a pivotal role in advancing marine science and supporting the sustainable development of Bangladesh's fisheries sector.

3. Internship Activities

3.1 Overview of Daily Work Schedule

1. **Briefing:** Summary of the ongoing projects and activities conducted by MFTS.
2. **Fieldwork**: Water sampling from the Bay of Bengal using phytoplankton net with 20-micron pore size.
3. **Mix Culture**: A primary culture setup was initiated using the collected phytoplankton samples.
4. **Plankton Isolation**: After a few days, dominated plankton species are isolated using 24-well plate.
5. **Pure Culture**: Isolated *Skeletonema Sp.* were cultured using both Artificial Sea Water and Natural Sea Water for a comparative analysis.
6. **Laboratory work**: Glassware preparation and disinfection process, F/2 nutrient media preparation, Seawater preparation, aeration and foiling.

4. Methodology

4.1 Sample Collection

To investigate the diversity and growth of indigenous marine phytoplankton, seawater samples were collected from the coastal waters of the Bay of Bengal, particularly near Laboni Beach of the Cox's Bazar. The sampling procedure was carefully designed to ensure the effective collection and preservation of phytoplankton for subsequent laboratory analysis and culture.

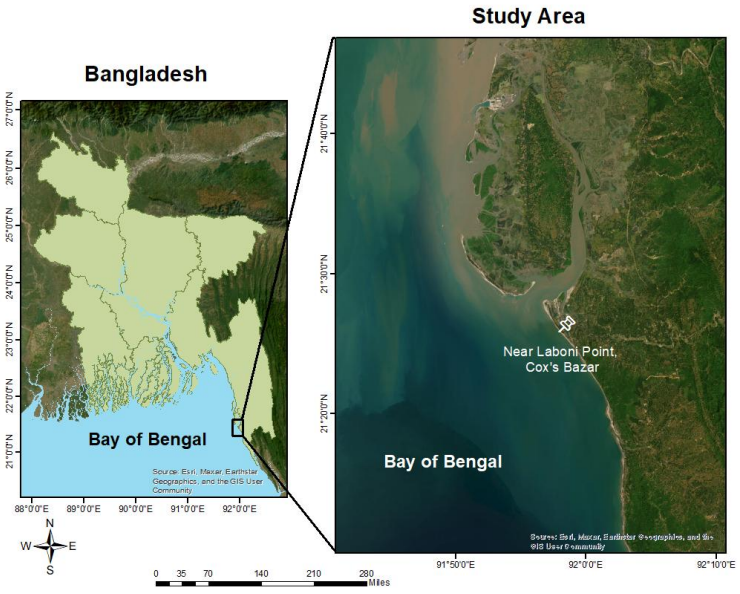


Figure 2: Sample Collection Site Map

Sampling Methodology

- **Use of Plankton Nets**:

  A plankton net with a mesh size of 20 micrometers (μm) was used to collect phytoplankton-rich seawater. This mesh size is standard for capturing a wide range of nano- and microphytoplankton while allowing finer particles and smaller organisms to pass through. The net was towed horizontally and vertically through the water column at different coastal sites to ensure a representative collection of phytoplankton species from the surface and subsurface layers.

- **Sedimentation and Concentration**:

  After collection, the seawater samples were left to settle (sedimentation) for several hours in dark containers. This process allowed suspended particles and plankton cells to gradually concentrate at the bottom, improving the efficiency of subsequent filtration and culturing.

- **Preservation for Microscopy and Analysis**:

  For species identification and further microscopic observation, a portion of the concentrated samples was preserved using formaldehyde at a final concentration of 4%. This fixative is commonly used to prevent biological degradation and maintain the structural integrity of phytoplankton cells, making them suitable for long-term storage and analysis.

- **Filtration for Culture Preparation**:

  Another portion of the concentrated plankton suspension was filtered and prepared for inoculation into culture media. This involved removing excess debris and separating viable phytoplankton cells for growth under controlled laboratory conditions.

4.2 Culture Techniques

Glassware Preparation

All glassware underwent a day-long immersion in bleaching water followed by a thorough detergent wash. Subsequently, the test tubes and flasks utilized as culture vessels underwent sterilization through autoclaving at 121ºC under 29.4 psi (2 atm) pressure. The sterilization duration was set at 10-30 minutes for test tubes and small flasks, while larger 10-liter liquid volumes underwent a sterilization period of 1-4 hour. Then dried in the oven.

Figure 3: Glassware preparation for culture

Media Preparation

Cultivating microalgae is all about maximizing cell numbers in the quickest period feasible, as natural nutrients in freshwater and ocean aren't sufficient for vigorous algal growth. There are different formulas for the culture medium, each optimized for distinct types of microalgae and cyanobacteria, displaying the diversity in these cultivation methods.

For experimental culture, Guillard F/2 media is used to culture the marine microalgae.

Table 1: Guillard's F/2 Media used to Culture Marine Microalgae (Guillard, 1975)

Solution	Component	Chemical Formula	Concentration
A	**Nitrate and phosphate stock solution**		
	Sodium nitrate	$NaNO_3$	75 g/L
	Sodium Phosphate	NaH2PO4	5 g/L
	Silicate	Na2SiO3.9H2O	30 g/L
B	**Trace Metals**		
	Ferric chloride	FeCl3.6H2O	3.5g/L
	Disodium EDTA	Na2EDTA	4.3g/L
Dissolved in 900ml distilled water. Added 1ml of each of the following trace metal solutions.			
C	**Trace metal stock solution**		
	Copper sulfate	$CuSO_4 \cdot 5H_2O$	1.96 g
	Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	4.40 g
	Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	1.26 g
	Manganese chloride	$MnCl_2 \cdot 4H_2O$	36.0 g
	Cobalt chloride	$CoCl_2 \cdot 6H_2O$	2.0 g
Make up the volume to 1 liter with distilled H_2O Add 1ml per liter of seawater of the above solutions (A&B)			
D	**Vitamin stock solution**		
	Thiamine (Vitamin B_1)		20 mg
	Cyanocobalamin (Vitamin B_{12})		1 mg
	Biotin		1 mg

Figure 4: Media preparation for live feed culture

4.3 Calculation Method

Number of viable cells/ml = Average number of cells/square × Dilution factor $\times 10^6$

Where,

$$\text{Average number of cells/square} = \frac{\text{Total number of cells in square}}{\text{Number of squares}}$$

$$\text{Dilution factor} = \frac{\text{Final volume}}{\text{Volume of cells}} = \frac{\text{Volume of cells} + \text{Volume of Trypan Blue}}{\text{Volume of cells}} = \frac{100+80}{100} = 0.8$$

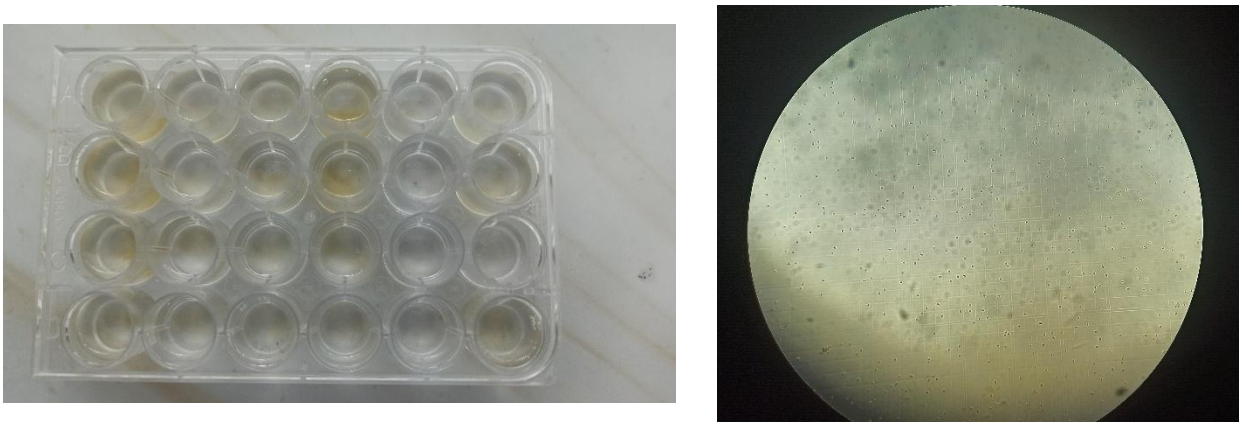


Figure 5: Serial dilution and Cell count using Sedgewick Rafter counting

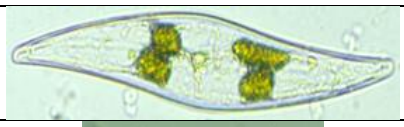
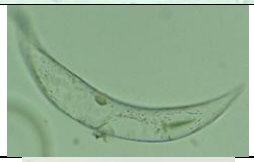
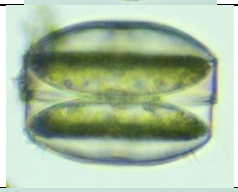
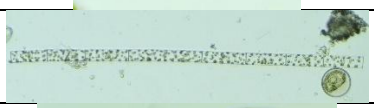
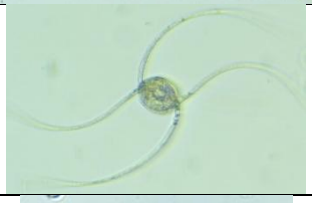
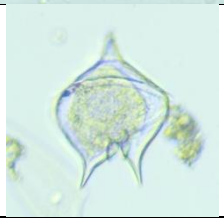
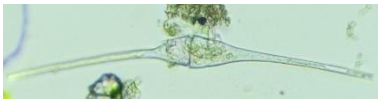
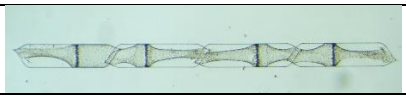
4.4 Identification of Phytoplankton

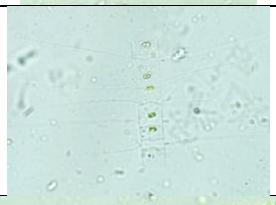
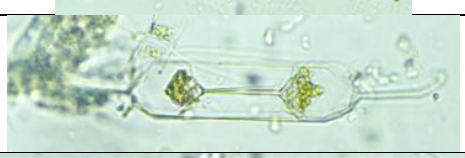
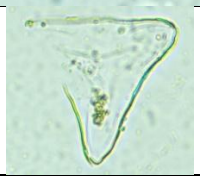
Morphological identification of marine microalgae was conducted using both light and fluorescence microscopy at magnifications of 10x and 40x. These microscopic techniques allowed for detailed visualization of cell structures, sizes, shapes, and pigmentation which are the key features necessary for accurate identification of phytoplankton species.

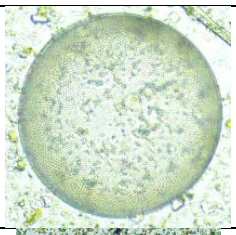
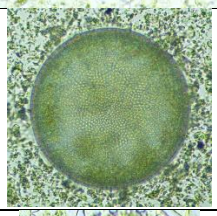
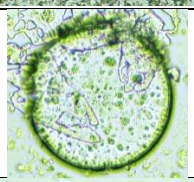
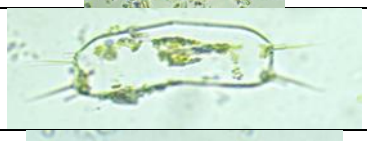
To ensure taxonomic accuracy, the identification process was supported by referencing the comprehensive species catalogue presented in the book *Plankton of Bangladesh* authored by Dr. Sk. Ahmad Al Nahid. This resource provided a region-specific guide to phytoplankton diversity in Bangladesh, which was particularly useful in cross-verifying observed morphological traits with known species descriptions found in local coastal waters.

Table 2: Identified phytoplankton species with their scientific name

Serial No.	Species	Species Name
1		*Skeletonema costatum*

2		*Pleurosigma normanii*
3		*Rhizosolenia robusta*
4		*Ceratium hirundinella*
5		*Ceratium trichoceros*
6		*Thalassiosira aestivalis*
7		*Aulacoseira granulata*
8		*Chaetoceros sp*
9		*Dinophysis caudata*
10		*Protoperidenium pallidum*
11		*Cylindrotheca Closterium*
12		*Rizhosolenia imbricata*

13		*Tripos trichoceros*
14		*Ceratium furca*
15		*Tintinnopsis radix*
16		*Xystonella treforti*
17		*Chaetoceros sp.*
18		*codonella amphorella*
19		*Ditylum brightwellii*
20		*Chaetoceros radican*
21		*Licmophora C.Agardh*

22		*Coscinodiscus traducens*
23		*Coscinodiscus argus*
24		*Coscinodiscus luctuosus*
25		*Thalassiosira sp.*
26		*Coscinodiscus radiosus*
27		*Coscinodiscus centralis*
28		*Thalassiosira sp.*
29		*Thalassiosira punctigera*

5. Results and Discussion

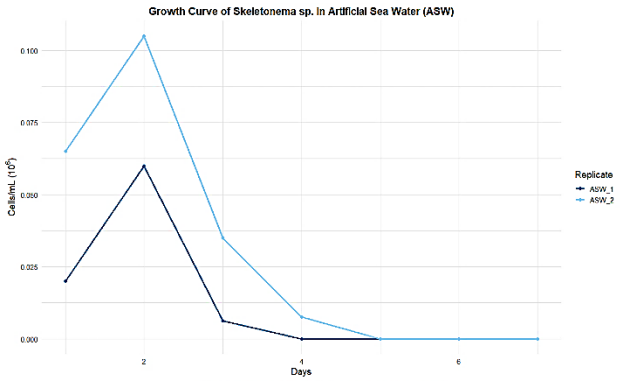
5.1 Culture and Growth Observations

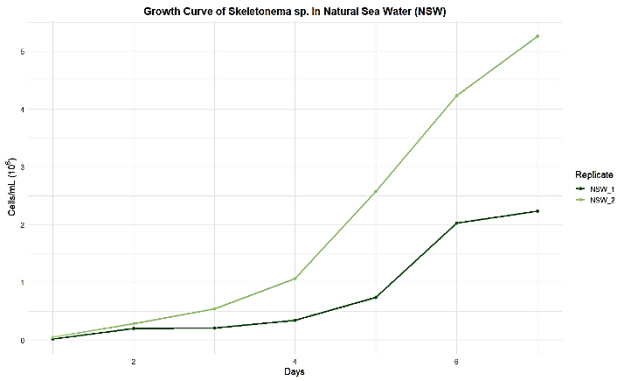
Cell density (in millions of cells per milliliter) of *Skeletonema sp.*, a genus of marine diatoms, cultured over a 7-day period in two different types of seawater:

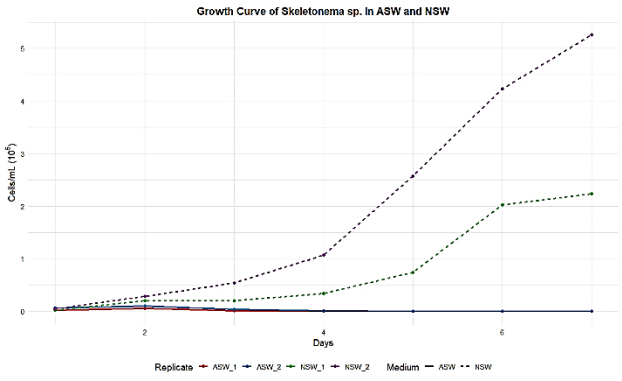
- ASW = Artificial Seawater (with two replicates: ASW_1 and ASW_2)
- NSW = Natural Seawater (with two replicates: NSW_1 and NSW_2)

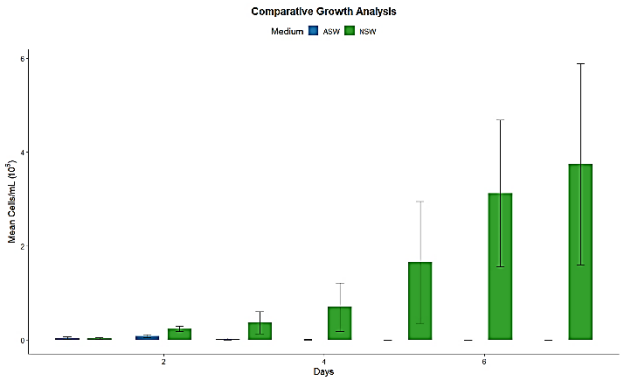
Table 3: Cell density of *Skeletonema sp.* (in millions of cells per milliliter) in artificial seawater (ASW) and natural seawater (NSW)

Days	**ASW_1 (10^6 cells/ml)**	**ASW_2 (10^6 cells/ml)**	**NSW_1 (10^6 cells/ml)**	**NSW_2 (10^6 cells/ml)**
1	0.02	0.065	0.02	0.05
2	0.06	0.105	0.2	0.28
3	0.00625	0.035	0.205	0.535
4	0	0.0075	0.34	1.065
5	0	0	0.735	2.567
6	0	0	2.02	4.23
7	0	0	2.23	5.26









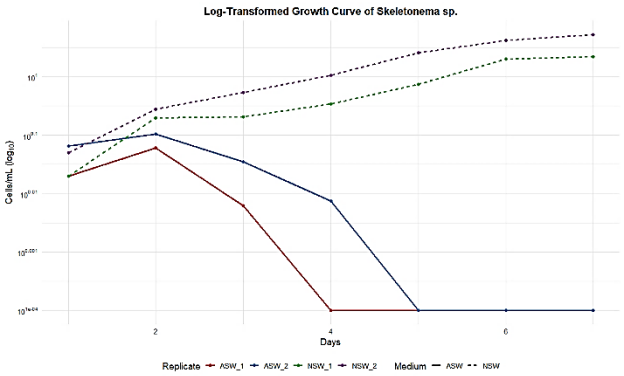


Figure 6: Growth Characteristics of *Skeletonema sp.* in Artificial and Natural Seawater

Key Findings and Interpretation:

- **Growth in Natural Seawater (NSW):**
    - The table clearly shows a sustained and significant increase in cell density (106 cells/ml) in both NSW replicates (NSW_1 and NSW_2) over the 7-day experimental period.
    - The "Growth Curve of *Skeletonema sp.* in Natural Sea Water (NSW)" graph visually confirms this, displaying characteristic S-shaped or exponential growth curves, indicating a healthy and proliferating population.
    - The "Log-Transformed Growth Curve of *Skeletonema sp.*" for NSW further emphasizes the exponential nature of this growth, as the data points tend to align linearly on the logarithmic scale, particularly after an initial lag phase which indicates consistent cell division and population expansion.
- **Lack of Growth/Decline in Artificial Seawater (ASW):**
    - The table reveals that cell density in ASW (ASW_1 and ASW_2) initially shows a slight increase (peaking around Day 2) but then rapidly declines to zero by Day 4 or Day 5.
    - The "Growth Curve of *Skeletonema sp.* in Artificial Sea Water (ASW)" graph vividly depicts this trend: an initial, minor peak followed by a precipitous drop, indicating that the organism not only fails to grow but also experiences a rapid die-off in ASW.
    - The "Log-Transformed Growth Curve of *Skeletonema sp.*" for ASW corroborates this decline, showing a steep linear decrease on the logarithmic scale after the initial transient increase, characteristic of exponential death or degradation of the population.

Comparative Analysis:

- The "Growth Curve of *Skeletonema sp.* in ASW and NSW" graph provides a powerful side-by-side comparison, making the contrasting growth patterns

immediately evident. The ASW replicates show a downward trajectory, while the NSW replicates exhibit a strong upward trend.

- The "Comparative Growth Analysis" likely summarizes the mean growth over time, predominantly reflecting the successful growth in NSW due to the negligible densities in ASW.

These graphs collectively demonstrate that natural seawater is a highly conducive medium for the growth and proliferation of *Skeletonema sp.*, supporting robust exponential population increase. Conversely, artificial seawater, under the experimental conditions tested, is detrimental to the survival of *Skeletonema sp.* leading to a rapid decline and eventual eradication of the population. This implies that natural seawater contains essential components or conditions (specific trace elements, organic compounds, appropriate pH buffering, microbial communities, or nutrient availability) that are either absent, insufficient, or unfavorably balanced in the artificial seawater formulation, which are critical for the sustained growth and viability of *Skeletonema sp.*

5.2 Challenges and Limitations

Phytoplankton identification, isolation, and culturing is a delicate and complex procedure that demands meticulous attention and strict aseptic techniques throughout the entire workflow. One of the primary challenges encountered during the internship was the high risk of contamination, which can easily compromise the validity and reliability of experimental results. Even slight contamination, especially during pure culture maintenance, can lead to overgrowth of unwanted species, masking the targeted phytoplankton and rendering the culture unusable for further study.

Maintaining a pure culture stock is particularly challenging. Even minor contamination from external sources—whether during seawater preparation, glassware handling, or nutrient media formulation—can lead to the proliferation of unwanted microorganisms, masking or outcompeting the target species.

Each stage of the workflow requires rigorous attention:

- **Seawater Preparation:** Natural or artificial seawater must be filtered and sterilized properly to avoid introducing foreign organisms.
- **Glassware Washing and Disinfection:** All equipment must be thoroughly cleaned and autoclaved to ensure a sterile working environment.
- **F/2 Nutrient Media Preparation:** The media must be prepared under sterile conditions, as it is a potential medium for rapid microbial growth if contaminated.
- **Inoculation and Subculturing:** These steps demand careful handling, as they are most prone to cross-contamination

Another major limitation faced during the internship was the morphological identification of the phytoplankton species. Many phytoplankton genres, especially diatoms, exhibit subtle structural differences that are difficult to distinguish under basic compound microscopes. This often

restricts identification to the genus level rather than species-level precision. Lack of access to advanced microscopic techniques such as Scanning Electron Microscopy (SEM) or molecular tools like DNA barcoding posed further limitations on the taxonomic resolution of the samples.

Overall, success in phytoplankton culture and identification hinges on maintaining sterile protocols, employing precise laboratory techniques, and overcoming limitations in observational tools and resources.

6. Conclusion

The 15-day internship undertaken at the Bangladesh Fisheries Research Institute's (BFRI) Marine Fisheries Technology Station proved to be highly productive, yielding significant insights into the marine phytoplankton of the Bay of Bengal. The internship provided valuable data regarding the diversity of these organisms, their distribution patterns within the waters, and their specific growth characteristics under experimental conditions.

A particularly noteworthy accomplishment of this internship was the successful isolation and subsequent culture of 29 distinct strains of phytoplankton. This achievement is a substantial contribution to the scientific documentation of marine biodiversity specifically within Bangladeshi waters. By identifying and culturing these diverse strains, the research enhances the foundational knowledge of the local marine ecosystem. Such documentation is vital for future ecological studies, conservation efforts, and the potential application of these indigenous phytoplankton in aquaculture or other biotechnological fields.

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