DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

USE OF MEMBRANE DIALYSIS BAGS TO ESTIMATE IN SITU GROWTH RATE OF PHYTOPLANKTON IN A MARINE FISH CAGE SYSTEM

by

Betül BARDAKCI ŞENER

June, 2024

İZMİR

USE OF MEMBRANE DIALYSIS BAGS TO ESTIMATE IN SITU GROWTH RATE OF PHYTOPLANKTON IN A MARINE FISH CAGE SYSTEM

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by

Betül BARDAKCI ŞENER

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İZMİR

Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"USE OF MEMBRANE DIALYSIS BAGS TO ESTIMATE IN SITU GROWTH RATE OF PHYTOPLANKTON IN A MARINE FISH CAGE SYSTEM"** completed by **BETÜL BARDAKCI ŞENER** under supervision of **ASSOC. PROF. DR. GÜZEL YÜCEL GİER** and **ASSOC. PROF. DR. EYÜP MÜMTAZ TIRAŞIN** we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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USE OF MEMBRANE DIALYSIS BAGS TO ESTIMATE IN SITU GROWTH RATE OF PHYTOPLANKTON IN A MARINE FISH CAGE SYSTEM

ABSTRACT

Fish is considered a very healthy food for human consumption. There has however been concern that the fish farms themselves may pose a threat to human health in the sense that they may affect the environment adversely. The benthic region and the water column next to fish farms have been investigated in order to determine any such effect. There was no significant difference in nutrients was found between samples taken from fish farms or the water column next to the cages compared to samples taken from more distant reference points. However, because of irregular feeding regimes and currents, those measurements may be inaccurate. In addition to nutrient analysis, phytoplankton was used as a bio-indicator to determine the effects of fish farms on the environment. However, since phytoplankton moves with currents and also gets consumed by predators, the differences in the amounts of phytoplankton near and further away from farms cannot simply be explained as resulting only from nutrient levels released from farms. The aim of this thesis is to monitor nutrients released from fish farms into the environment by employing 4 bioassays within fish cages and at points positioned gradually away from the cages. The measured concentrations of nitrite+nitrate, orthophosphate and silicate in the ambient seawater compared with those inside the bags showed no significant temporal variation and were all within the range typically found in oligotrophic waters. Chlorophyll *a* concentrations measured in the ambient water were low as in the oligotrophic seas. The estimated growth rates of chlorophyll *a* concentrations and phytoplankton cell numbers inside the filtered and unfiltered bags were not statistically different. Species richness decreased dramatically after incubation period in all the bags. Three diatom species, *Leptocylindrus danicus*, *Nitzschia longissima* and *Cylindrotheca closterium* proliferated exponentially inside all bags, exceeding the overall average growth rate. According to results obtained from experiment 3, under continuous nutrient releases from fish farms, high population growth rates can be achieved in oligotrophic environments when phytoplankton losses due to grazing, advection and sinking are eliminated. According to experiments 2 and

4, growth rates decreased with increasing distance from the farm (up to 250 meters) due to a decrease in nutrient concentration. Growth was almost not observed upstream at a distance of 1000 meters, which served as the control station.

Keywords: Bioassay experiment, net growth rate, diatom, oligotrophic, nutrient enrichment

BİR DENİZ BALIĞI KAFES SİSTEMİNDE FİTOPLANKTONUN İN SİTU BÜYÜME ORANININ TAHMİNİNDE MEMBRAN DİYALİZ TORBALARININ KULLANIMI

ÖZ

Balık çiftliklerinde üretilen balıklar kaliteli protein ve yağ asidi bakımından insan beslenmesindeki açığı kapatmaya yardımcı olmaktadır. Ancak çiftlikler, faaliyetleri sonucunda bulundukları çevreyi etkileyebilmektedirler. Bu etkilerin ortaya konulabilmesi için ise bentik bölgede ve su kolonunda araştırmalar yapılmaktadır. Balık çiftliklerinde gerek kafeslerin bulunduğu su kolonunda gerekse etkilerden yeterince uzak olduğu düşünülen referans noktalardan alınan su örneklerindeki nütrientlere (besin elementi) ilişkin analizlerden elde edilen sonuçlar karşılaştırıldığında önemli bir fark görülememektedir. Çünkü yemleme rejimlerindeki düzensizlik ve akıntılar sebebiyle bu ölçümlerin hassasiyeti yeterli düzeyde değildir. Balık çiftliklerinin etkilerini su kolonu üzerinden belirleme çalışmalarında nütrient analizlerinin yanı sıra fitoplanktonun biyoindikatör olarak kullanıldığı araştırmalar da yapılmıştır. Bu projenin amacı; balık çiftliklerinden salınan nütrientin çevresel sulara geçişinin hızlı, kolay, etkili ve yenilikçi bir yöntemle gösterilmesidir. Bu amaçla balık çiftliklerinde kafesler içerisinde ve kafeslerden kademeli olarak uzaklaşan noktalarda 4 biyodeney tasarlanmıştır. Deniz suyundan ve torba içerisinden ölçülen nitrit+nitrat, ortofosfat ve silikat konsantrasyonları arasında anlamlı bir fark bulunmamıştır. Deniz suyundan alınan örneklerin tamamında klorofil *a* değerleri oligotrofik denizlerde görülen seviyededir. Torba içerisinden ölçülen klorofil *a* konsantrasyonları ile fitoplankton sayıları arasında anlamlı bir fark bulunmamıştır. Fitoplankton tür çeşitliliği her bir deney için inkübasyon sonunda ciddi şekilde düşmüş ve az sayıdaki dominant tür hakim olmuştur. Özellikle üç diatome türü *Leptocylindrus danicus*, *Nitzschia longissima* ve *Cylindrotheca Closterium* inkübasyon sonunda baskın hale gelen türlerdir ve popülasyon büyüme oranlarını geçmişlerdir. 3. Deney sonunda elde edilen sonuçlara göre oligotrofik koşullarda bile balık çiftliği gibi sürekli nutrient salınımının olduğu yerlerde fitoplankton akıntılar ve predatörlerin etkisinin ortadan kaldırıldığı diyaliz membran torbalarında in situ olarak oldukça fazla miktarlarda üreme gösterebiliyorlar. 2. ve 4. deney'e göre ise çiftlikten uzaklaştıkça (250m)

nutrient konsantrasyonundaki azalmaya bağlı olarak büyüme oranlarında da azalma görülmüş 1000 m'de ise büyüme hemen hemen hiç gözlenmemiştir.

Anahtar kelimeler: Biyodeney, net büyüme oranı, diyatome, oligotrofik, nutrientçe zenginleşme

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CHAPTER ONE
INTRODUCTION

The global demand for seafood continues to rise, driven by factors such as population growth, increased affluence, and shifting dietary preferences. Fish, renowned for its high-quality protein content, has been a cornerstone of human nutrition for centuries. However, the sustainability of wild fish stocks has become a pressing concern due to overfishing (Pauly, Christensen, Dalsgaard, Froese, & Torres, 1998), prompting the exploration of alternative solutions to meet the growing demand for fish. Among these solutions, aquaculture has emerged as a pivotal player in the seafood industry, offering a means to alleviate pressure on wild populations while providing a reliable source of fish for human consumption (Dimitriou et al., 2015).

Aquaculture, though offering promise as a sustainable food production method, is not without its environmental challenges. One of the key concerns is the potential impact of fish farming activities on the surrounding aquatic ecosystems, particularly in terms of nutrient pollution. Assessing nutrient concentrations in water columns has been instrumental in evaluating the environmental footprint of aquaculture operations (Dalsgaard & Krause-Jensen, 2006; Herbeck & Unger, 2013). However, traditional methods of nutrient assessment face inherent limitations, including the rapid diffusion of nutrients in marine waters and the sporadic nature of nutrient releases from fish farms. Consequently, accurately detecting and attributing increases in nutrient concentrations solely to aquaculture activities necessitates frequent and intensive sampling efforts, posing logistical and financial challenges (Dalsgaard & Krause-Jensen, 2006).

In response to these challenges, the utilization of bioindicators has emerged as a promising approach in assessing nutrient pollution in aquatic environments. Bioindicators, particularly primary producers such as phytoplankton, offer valuable insights into nutrient dynamics within marine ecosystems. Primary producers possess the ability to efficiently assimilate, and store nutrients present in the water, leading to observable changes in growth rates in response to fluctuations in nutrient availability. Moreover, the use of bioassays, such as incubating phytoplankton inside dialysis membrane bags, has proven highly effective in capturing the dynamic interactions

between phytoplankton populations and their surrounding environment (Dalsgaard & Krause-Jensen, 2006; Dimitriu et al., 2015; Pitta et al., 2009).

Despite advancements in our understanding of nutrient dynamics in aquaculture environments, significant knowledge gaps remain, particularly regarding the spatial extent and magnitude of nutrient influence emanating from fish farms. The influence of nutrient discharge from fish farms into the surrounding marine environment is known to diminish with distance from the point source, but the precise gradient and extent of this influence are not fully elucidated. By employing phytoplankton bioassays using dialysis membrane bags, previous studies have begun to shed light on the spatial patterns of nutrient pollution originating from fish farms (Dalsgaard & Krause-Jensen, 2006; Dimitriu et al., 2015; Pitta et al., 2009). However, further research is needed to comprehensively understand the dynamics of nutrient dispersal and its ecological implications.

Therefore, the overarching objective of this doctoral thesis is to contribute to the knowledge base surrounding the environmental impacts of aquaculture, with a specific focus on nutrient pollution and its spatial gradients in marine environments. This thesis aims to address key research questions, including the characterization of nutrient dynamics near fish farms, the development of innovative bioassay techniques for assessing nutrient pollution, and the exploration of ecological responses to nutrient enrichment. By combining field experiments, and laboratory analyses, and utilizing bioassays with phytoplankton as bioindicators, this thesis seeks to provide actionable insights for sustainable aquaculture management and marine conservation efforts.

CHAPTER TWO
LITERATURE REVIEW

2.1 Fish Farms and Their Potential Effects on Environment

Aquaculture plays a pivotal role in meeting the increasing demand for seafood, a trend expected to persist in the future (Gephart et al., 2021). One distinctive feature of aquaculture is its independence from freshwater sources, unlike other sectors of food production (Dimitriou 2015). This characteristic underscores the importance of aquaculture in sustainable food systems, particularly in regions where freshwater resources are limited. Despite its potential benefits, aquaculture also presents environmental challenges, notably through nutrient discharges from production processes. Marine finfish cage culture operates as an open system, where the introduction of juvenile fish and their feed is balanced by the output of harvested and escaped fish, as well as excretory products that contribute to the local pool of dissolved nutrients, and particulate organic matter in the form of waste feed and fecal material. Any inputs not recovered during harvest must be assimilated by the surrounding environment. Ensuring that the environment can effectively manage and absorb these waste materials in proportion to the scale of mariculture activity is crucial to prevent both local and broader disturbances to ecosystem function, such as bottom water oxygen depletion and eutrophication (Navarro, Leakey, & Black, 2008). It is well documented that fish farms contribute to the enrichment of the water column with organic and inorganic materials by releasing fish waste, excretions, uneaten feed, and scales, skin, mucus, vitamins, and therapeutic agents (Arzul, Clément & Pinier, 1996). Dissolved inorganic nutrients are introduced into the pelagic environment from fish farm cages through direct means, such as fish excretions containing substances like ammonium and urea, or indirectly through the breakdown of particulate organic waste. These inorganic nutrient inputs have the potential to stimulate phytoplankton growth, with aquaculture activities being associated with the formation of harmful phytoplankton blooms. They can also influence the composition of phytoplankton communities by altering nutrient ratios, such as an increased N:Si ratio favoring the growth of flagellates over diatoms. Moreover, due to the rich nutritional content of

waste food and feces, these dissolved organic nutrients likely serve as a highly labile nutrient source for heterotrophic bacteria (Navarro et al., 2008).

Traditionally, two primary methods have been employed to evaluate the impacts of nutrient discharge from fish farms into the marine environment. The first method involves regular sampling of the water column, followed by the measurement of nutrient levels. However, this approach has drawbacks. Nutrient release fluctuates daily based on farm feeding practices, requiring frequent sampling to accurately measure discharge. Additionally, nutrient analysis techniques have limited sensitivity to detect significant variations. Notably, nutrient concentrations may only significantly increase in regions with low current velocities, where water exchange is limited (Dalsgaard & Krause-Jensen, 2006). Another proposed method for assessing nutrient input is the use of phytoplankton as indicators. Research indicates that the initial response to increased eutrophication typically manifests in changes to phytoplankton abundance and species composition (Sidik, Rashed-Un-Nabi & Hoque, 2008). Despite this, the impact on primary production in fish farms due to nutrient enrichment varies considerably. While some studies suggest a notable increase in primary production, others report negligible effects (Price, Black, Hargrave & Morris, 2015). Challenges in detecting the impact of elevated nutrient levels on phytoplankton include rapid dilution in the water column due to strong currents and predation pressure from grazers (Dalsgaard & Krause-Jensen, 2006; Pitta et al., 2009). Furthermore, studies conducted at fish farms situated along the Mediterranean coast consistently indicate minimal to insignificant increases in chlorophyll a (chl a) concentrations in the water columns adjacent to the cages (La Rosa et al., 2002; Pitta et al., 2009). Price et al. (2015) have provided an overview of the current understanding of nutrient discharges from marine fish farms globally, as well as the direct consequences on water quality and subsequent impacts on primary production, including the potential formation of harmful algal blooms. Furthermore, Price et al. (2015) highlighted that the effects of individual fish farms on marine water quality have been mitigated under contemporary operational practices.

The impact of nutrient discharge from fish farms into the surrounding marine environment decreases as distance from the source increases, enabling the

identification of affected areas or ranges stemming from these farms. Utilizing phytoplankton bioassays with dialysis membrane bags proves to be an effective approach for monitoring nutrient pollution originating from fish farms across horizontal gradients or in relation to distance. Research conducted by Dalsgaard & Krause-Jensen (2006), Dimitrou et al. (2015) and Pitta et al. (2009) has utilized this method and observed increased primary productivity near Mediterranean fish farms, followed by declines as distance from the farms increased. Similarly, Herbeck & Unger (2013) in China reported similar outcomes using dialysis membrane bags.

Exploring how fish farms contribute to nutrient enrichment demands a thorough understanding of the many influencing factors involved. With fish farms dispersed across various geographical locations, each characterized by unique water dynamics and trophic statuses, the dynamics of nutrient release are inherently complex. Furthermore, the feeding patterns of farmed fish, influenced by seasonal variations and local environmental conditions, significantly impact nutrient outputs. Additionally, the species reared in these farms vary widely, introducing another layer of complexity to the analysis. Hence, attributing the findings of a single study to all fish farms proves challenging, as the intricate interplay of factors necessitates a site-specific approach. Thus, while nutrient concentration analyses serve as vital tools for detecting and understanding nutrient enrichment from fish farms, comprehensive assessments must account for the multitude of variables inherent in these diverse aquatic environments.

The monitoring and management of phytoplankton dynamics in marine environments, particularly in aquaculture settings such as fish cage systems, are essential for maintaining water quality, ecosystem health, and sustainable aquaculture production. Understanding the growth rates of phytoplankton, primary producers crucial to marine ecosystems, is fundamental in assessing nutrient availability, trophic dynamics, and potential environmental impacts of aquaculture operations.

2.2 The Importance of Phytoplankton

Marine phytoplankton play a significant role in global primary production, accounting for as much as half of it. They serve as a fundamental source of organic

matter for the majority of marine organisms and play a crucial role in regulating the global carbon cycle (Majewska et al., 2017)

Phytoplankton exhibit remarkable diversity and adaptability, necessitating a comprehensive understanding of their traits. These microorganisms are widely distributed across diverse taxonomic groups, characterized by microscopic sizes, buoyancy to resist sinking, and the capacity for photosynthesis, or in the case of mixotrophs, the ability to supplement photosynthesis with heterotrophic nutrition. They display notable morphological and functional diversity, shaped by selective adaptation to different habitats and environmental conditions (Salmaso, & Tolotti, 2021).

Phytoplankton are key producers in aquatic ecosystems and excellent bioindicator species for evaluating changes in water columns (Bauer, Conforti, Ruiz, & Gomez, 2012). This is attributed to their efficient uptake of biologically available nutrients, facilitated by their elevated cell surface area to volume ratio, as well as their rapid growth rates. Additionally, their adaptability to culture conditions enhances their utility for monitoring purposes. Phytoplankton have the ability to adjust their size and shape in response to various environmental factors such as light intensity, nutrient levels, water mixing, and predator presence. These adaptations serve to optimize their survival and growth. Cell size plays a significant role in algal growth rates. Generally, smaller cells have a higher surface-to-volume ratio, resulting in increased nutrient uptake rates relative to larger cells. Consequently, the availability of nutrients in an environment directly impacts phytoplankton composition and biomass. Changes in physical conditions, coupled with nutrient availability, can lead to shifts in species dominance, favoring those with higher surface-to-volume ratios under nutrient-rich conditions and vice versa (Reynolds, 2006).

In marine ecosystems, the availability of nutrients essential for microbial growth and energy is typically low, often reaching levels that restrict growth. Consequently, microbial organisms' ability to thrive depends heavily on their efficient utilization of resources, especially amidst fierce competition with other organisms. Additionally, the fluctuating stability of the water column influences nutrient distribution,

phytoplankton abundance, cell dimensions, and the broader configuration of the marine food web (Šolić et al., 2020).

Nutrient uptake and assimilation in phytoplankton represent intricate processes. Besides light, phytoplankton rely on inorganic nutrients for growth. However, the nutrients absorbed from the water by phytoplankton are often significantly diluted compared to their concentrations within the cell. Moreover, the presence of an element does not automatically ensure its availability. This indicates that for an element to be assimilated by the cells, it must be both soluble and diffusable (Reynolds, 2006).

2.3 Growth of Phytoplankton

The growth of phytoplankton involves an increase in cell numbers rather than cell size, achieved through cell divisions. The replication cycle begins with the birth of a cell and concludes with its division into two daughter cells. Throughout this period, intracellular processes are carried out, known as the generation time or doubling time (td).

The increase in cell numbers is the indicator of the rate of growth in biomass. This rate of increase encompasses not only cell division but also factors such as mortalities Hence, it's essential to elucidate concepts like growth rates (r), increase rate or specific rate of increase (r_n), and replication rate (r'). Replication rate (r') denotes the potential biomass increase before any cell losses (r_L) occur. Increase rate (r_n) refers to the accumulation of biomass, indicating changes in cell concentration (Reynolds, 2006). Therefore,

$$r' = r_n + r_L \tag{2.1}$$

Here now on we will call r_n as μ.

An equation can express the growth of cell numbers over time, defining cell number as a function of time, where time serves as the independent variable and cell number as the dependent variable. Assuming a scenario where a single cell of phytoplankton divides daily, corresponding to a generation time (td) of one day, after one day, two

daughter cells birth, denoted as N, representing the number of cells after each division (Figure 2.1).

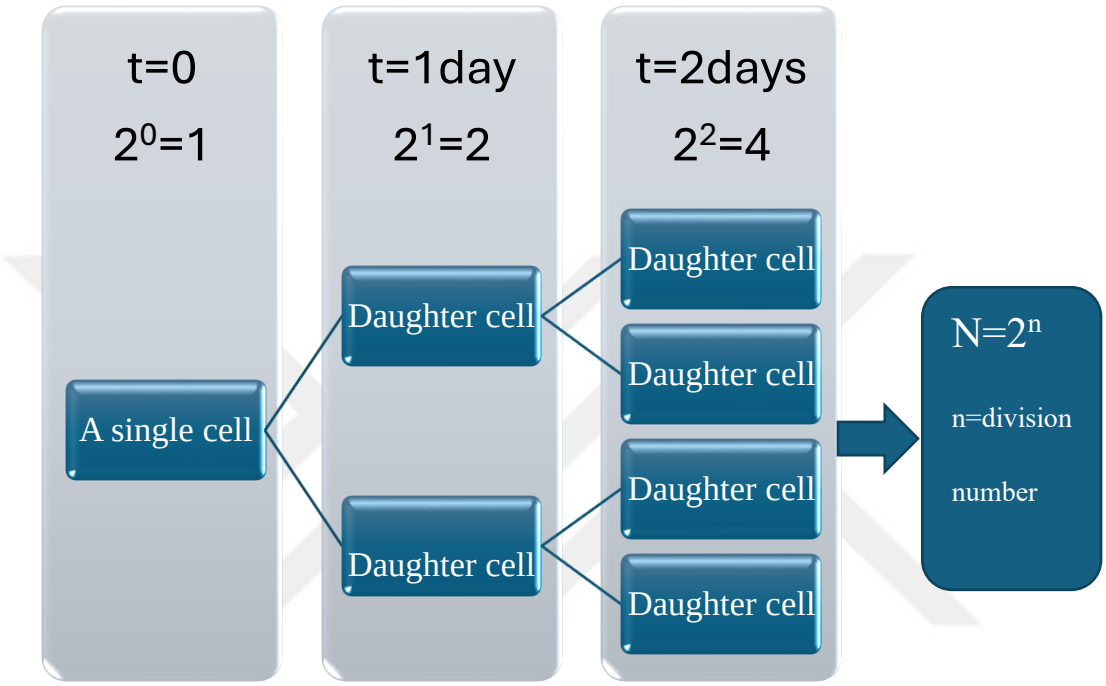


Figure 2.1 Diagram of calculating cell numbers for each cell division

$$n = \frac{t}{td} \tag{2.2}$$

$$N = 2^{t/td} \tag{2.3}$$

In a phytoplankton population, growth begin with any cell number which is N_0, N_t is the population at time t

$$N_t = N_0 . 2^{t/td} \tag{2.4}$$

Doubling time or generation time is td, doubling rate is 1/td which is denotes with *v*.

$$N_t = N_0 . 2^{v.t}, \quad 2 = e^{ln2} \tag{2.5}$$

$$N_t = N_0.(e^{ln2})^{t/td} = N_0.e^{\frac{ln2}{td}.t} \quad (2.6)$$

Doubling of a biomass expressed by the natural logarithm of 2 (ln2=0.693) and the change in biomass with time provides the rate. Therefore, a doubling per day corresponds to a populations specific growth rate of 0.693 d^{-1}(Reynolds, 2006). The doubling time, *td*, is a species-specific characteristic. The doubling time, td, is inherent to each species. Each phytoplankton species possesses a genetically predetermined doubling time under ideal conditions. Thus, we can establish μ*:*

$$\frac{ln2}{td} = \frac{0.693}{td} = \mu\text{=specific growth rate} \quad (2.7)$$

$$N_t = N_0.e^{\mu.t} \quad (2.8)$$

$$\frac{N_t}{N_0} = e^{\frac{ln2.t}{td}} \quad (2.9)$$

$$\frac{N_t}{N_0} = e^{\mu.t} \quad (2.10)$$

$$\frac{ln(N_t/N_0)}{t} = \mu \quad (2.11)$$

To estimate the specific growth rate of a population, a series of growth measurements needed. Essentially, the slope of the regression line derived from natural log-transformed measurements against time (experimental period) was employed to estimate growth rates (μ d^{-1}) (Lürling, 2006).

2.4 Estimating Specific Growth Rates of Phytoplankton

To investigate the potential rates of cell replication in phytoplankton involves studying the dynamics of isolates under carefully controlled laboratory conditions. Initially, the performance of cells from a particular species is assessed under highly idealized conditions and the optimal performance is determined (Reynolds, 2006). Under optimal laboratory conditions, phytoplankton species exhibit their fastest rates

of species-specific growth when cultivated in prepared standard media. These media are specifically formulated to saturate the resource requirements of the organisms. In such environments, constant and continuous light exposure, at an intensity adequate to saturate photosynthesis, and steady, optimal temperatures facilitate maximal growth rates. However, even under these ideal conditions, there is typically a notable 'lag phase' during which the inoculated cells adjust to their new environment. Within a day or two, the isolated population begins to rapidly increase, often doubling its mass at regular intervals. It's during this exponential phase that the maximal rate of replication is achieved, when the observed net rate of increase is believed to be equal to the intrinsic rate of increase (Reynolds, 2006).

In natural ecosystems, phytoplankton may frequently encounter suboptimal conditions regarding light intensity, temperature, and nutrient availability, resulting in growth rates that fall below their maximum potential. However, the influence of each of these factors can be quantified by manipulating their levels under controlled laboratory conditions. Through such experimentation, one can elucidate the specific impact of light, temperature, and nutrient levels on phytoplankton growth rates.

Determining the rates of cell replication and population growth in natural habitats has long been considered challenging. This difficulty stems from the fact that what typically observed is a fluctuating population density, expressed as the species' rate of increase, which does not fully capture the rate of cell replication due to unquantified dynamic losses of entire cells occurring simultaneously. Consequently, the net rate of change can be negative without necessarily indicating a failure in true growth; rather, it may imply that the rate of loss surpasses that of replication. Additionally, the issues of patchiness and advection introduce further complexities, such as compounded sampling errors, where even the observed rate of population change may prove insufficient as a basis for analysis (Reynolds, 2006).

Laws (2013) conducted a comprehensive review of methodologies used to assess in situ growth rates of phytoplankton wherein he delineated three primary methods. These include ^{14}C labeling of chlorophyll-a and carotenoids, the dilution method, and cell cycle analysis, with the latter being specifically applicable to single phytoplankton species. There are also several methodological challenges in estimating phytoplankton

growth rates. Firstly, there's the issue of incubation vessel size, where earlier studies suggested that larger bottles were necessary to prevent artifacts associated with photochemical pigment destruction. However, subsequent research found conflicting results regarding the impact of bottle size on growth rate estimates. Despite this, it has become standard practice to use larger bottles to minimize potential artifacts. Secondly, the cleanliness of sampling techniques is crucial, as any introduction of toxic substances during sample collection could stress plankton, affecting metabolic measurements. This recognition emerged around 1980. Finally, the duration of incubations has evolved over time. Older methods required longer incubation periods, leading to potential artifacts due to bacterial attachment and sunlight effects. With the advancement of techniques like the ^{14}C method, shorter incubations have become standard, allowing for more precise estimates of carbon uptake (Laws, 2013).

The study conducted by Broise & Palenik (2007) introduced in situ phytoplankton microcosms in 1 l bottles with water renewal as a novel approach for assessing pollution impact on marine ecosystems. Results indicated that partial water renewal every two days helped maintain phytoplankton cell counts comparable to ambient seawater levels over a two-week period. Finding underscores the importance of mimicking natural conditions in microcosm experiments and suggests that water renewal could mitigate bloom-like behavior observed in closed systems.

2.5 Dialysis Membrane Bags Method for in situ Estimation of Growth Rates

The use of membrane dialysis bags for estimating phytoplankton growth rates in marine environments involves enclosing natural phytoplankton assemblages or cultured phytoplankton in semi-permeable bags immersed in situ within the water column. In situ incubation of natural phytoplankton communities in dialysis membrane bags is considered as a reliable method for measuring marine phytoplankton growth rates (Furnas, 1990). These bags allow for the exchange of nutrients, gases, and other dissolved substances between the enclosed phytoplankton and the surrounding water while preventing the ingress of grazers and large particles. By measuring changes in phytoplankton abundance, biomass, or physiological parameters within the dialysis bags over time, researchers can estimate the net growth rate of phytoplankton under ambient environmental conditions. As a result, incubation in dialysis membranes

provides the cells with an environment that is quite similar to natural conditions (Broise & Palenik, 2007).

A membrane acts as a selective barrier, separating two phases while aiding the separation process. Pressure, concentration, and temperature are some of the factors that drive this process. In many membrane processes, the feed is separated into two parts: retentate (which the membrane retains or rejects) and permeate. The rate at which a particular solute flows over the membrane is principally determined by its shape, charge, and size. Furthermore, the temperature, viscosity, and mixing rate of the solution all influence the transport process (Bilad, Arafat, & Vankelecom, 2014).

In membrane dialysis, solutes or nutrients diffuse across the membrane from the feed side (high concentration) to the permeate side (low concentration), propelled by a concentration gradient. Phytoplankton contained in a dialysis membrane bag are suspended in the saltwater column. Nutrients from saltwater naturally move into the dialysis bags, shifting from high to low concentrations. This technique retains phytoplankton inside the bag, while the semipermeable barrier keeps other species out. As long as the phytoplankton continue to consume nutrients, a concentration gradient exists between the bag's inner and outer surfaces, promoting continued nutrient diffusion from the seawater. One of the most difficult challenges is dealing with biofouling, which can reduce membrane permeability (Bilad et al. 2014).

In this study 6-8 kDa molecular weight dialysis membrane was used. This pore size described with the notation of kDa. Also, conversion to nm to Da is approximately 1:1 according to the simulations for calculation of proteins. This conversation factor is dependent to the proteins folding position and denaturation phase. Therefore, to understand if a molecule can pass through the membrane dialysis bag, it is necessary to know the molecular weight of this molecule. For instance, molecular weight of NO_3- is 62, which is equal to 62 Da, therefore 6000-8000 Da pore size dialysis membrane is considered as transparent to NO3- . This simple exercise is enough to explain if nutrients capable of transferring through the inside and outside the membrane. However, some enzymes and proteins cannot pass through the membrane pores since their molecular weights bigger than the membranes pores.

Dialysis bag cultures have been extensively employed in scientific research, especially in the examination of microorganisms (Schultz & Gerhardt, 1969).

Trainor (1965) conducted one of the earliest documented experiments involving the incubation of algae within dialysis bags. Following this, Jensen, Rystad & Skoglund (1972) showcased successful algae incubation in dialysis bags, achieving remarkable levels of density. Jensen et al. (1972) elucidated that the dialysis culture technique was appropriate for studying marine algal growth, specifically with marine phytoplankton such as *Thalassiosira pseudonana* and *Phaeodactylum tricornatum*.

A study conducted by Vargo, Hargraves, & Johnson (1975) showed regenerated cellulose dialysis membranes are an ideal surface for epiphytic bacteria when they incubated in the marine environment. In this study interior surface of the membrane is covered with 10^{7} cells cm^{-2} after 3 days of incubation in summer, while in winter after 17 days.

In the study conducted by Dodson & Thomas (1977), the dialysis method permits the gradual alteration of conditions from simulated upwelling to oligotrophy over an extended duration in laboratory settings, without causing significant disruption to the algal culture. The upwelling conditions persisted for 8 days for *Chaetoceros affinis* and 14 days for *Gymnodinium splendens,* followed by extended periods of oligotrophy lasting 80 days and 65 days, respectively. After prolonged oligotrophic conditions, algal cultures demonstrated survival and exhibited the capacity to endure the nutrient concentrations often falling below the detection limits of standard analytical techniques. Thus, the implementation of dialysis cultures facilitated the emulation of oceanic conditions within laboratory settings.

Toth (1980) employed dialysis bags to mitigate the influence of bottle or enclosure effects and evaluate the production of bacterioplankton and phytoplankton. By comparing the growth rates of phytoplankton and bacteria in bottle and dialysis bag incubations, dialysis bags were determined to outperform closed bottles. Specifically, dialysis bags demonstrated significantly higher algal production, showing a 40% increase compared to bottles.

Watanabe (1987) developed a dialysis culture chamber to analyze the nitrogen-to-carbon (N/C) and phosphorus-to-carbon (P/C) ratios and in situ growth rates of six distinct phytoplankton species. These species were individually incubated in a hypereutrophic pond for four days.

The practice of incubating single phytoplankton species or natural phytoplankton communities in dialysis bags at sea has been widely adopted (Furnas, 1982; Jensen et al., 1972; Mura & Agusti, 1996). Researchers have also explored the impacts of fish farms by conducting in situ incubations of natural phytoplankton communities within these environments (Dalsgaard & Krause-Jensen, 2006; Dimitriu et al., 2015; Pitta et al., 2009). Furthermore, dialysis bags have been utilized to investigate predator-prey interactions (Grattepanche & Katz, 2020; Martin, Pinedo & Sardá, 1996) and examine the effects of toxins on individual species (Rodriguez, Alfonso, Turrell, Lacaze & Botana, 2011).

2.6 Diversity and disturbances

Although marine water masses are connected across the planet, the composition and abundance of the phytoplankton exhibit drastic variations both spatially and temporally. Differences in nutrient scarcity and light availability present distinct challenges for algal growth, while variations in tolerance levels and adaptations to extreme conditions play a crucial role in selecting species.

Phytoplankton communities thrive as long as they have enough nutrients and adequate light for growth. However, if either resource becomes limited, the rate of new recruits decreases. Species with traits that make them better adapted to handle these limitations or more flexible in overcoming them become crucial. Those species that can cope with the scarcity survive, while others that are less specialized may struggle or decline. Competition for these limited resources reduces species diversity over time, ultimately leaving only the species best suited to dominate.

Following a disturbance event, succession takes place in an ecosystem, causing changes over time in factors like species diversity and composition (Connell, 1978). These changes significantly affect phytoplankton communities, which are sensitive to

environmental fluctuations such as light, temperature, nutrient levels, and grazing pressure. Therefore, fish farms can significantly alter the composition of phytoplankton communities by releasing nutrients as a result of their production activities.

The theory of competitive exclusion, (Gause, 1934), explains succession by suggesting that only a few species can coexist in a stable state. Hardin (1960) encapsulates this idea succinctly in his famous assertion "Complete competitors cannot coexist". However, recent studies, reveal that fish farms often harbor a higher diversity of species, challenging this principle. This contradicts the notion of limited species coexistence. This discrepancy is often attributed to the paradox of phytoplankton identified by Hutchinson (1961), which is further explained by the intermediate disturbance hypothesis proposed by Connell (1978), emphasizing non-equilibrium interpretations.

The most commonly referenced work is by Connell (1978), where he discussed the limitations on species diversity in rainforests and coral reefs, leading him to propose the well-known intermediate disturbance hypothesis (IDH). In summary the essence of the theory:

- Without interference, competitive exclusion will gradually decrease the number of species to a minimum.
- With frequent and severe disturbances, only pioneer species are expected to return after each event.
- When disturbances occur at moderate frequencies or intensities, there are more chances for diverse species to establish themselves after each event, but they often don't mature into stable, competitive states before the next disturbance.

The Intermediate Disturbance Hypothesis (IDH) was later applied to phytoplankton succession by Reynolds (1988).

Tsanagaki et al. (2013) noted a rise in diversity around fish farms in the oligotrophic eastern Mediterranean, linking it to the IDH. Reynolds (1988) highlighted the

importance of the interaction between increasingly smaller intermediate scales, ranging from 20 to 200 hours, and the growth rates of phytoplankton species in sustaining high species diversity. Furthermore, Gaedeke & Sommer (1986) showed that species coexistence could be greater than under steady-state conditions when the intervals between disturbances surpassed the average generation times.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

The study site was a fish farm located in Çandarlı, Denizköy, in the northeastern Aegean Sea of Türkiye (38°58'33"N, 26°47'22"E) (Figure 3.1). The farm has a distance of approximately 1 km from the shore and water depth beneath range in 40-70 m. The fish production started four years prior to the experiment date, and about 1000 tonnes of sea bass and sea bream are produced there per year. Fish were mechanically fed once a day. The maximum measured current speed in the region was 20 cm/s. Four bioassays were conducted following a procedure adapted from Dalsgaard & Krause-Jensen (2006) and Mura et al. (1996). Three stations were selected for this study: Station 1 inside the fish farm, Station 2 located 250 meters away from the farm and positioned downstream in the primary current direction, and another station 1000 meters upstream from the farm, which served as the control. (Figure 3.2 and Figure 3.3).

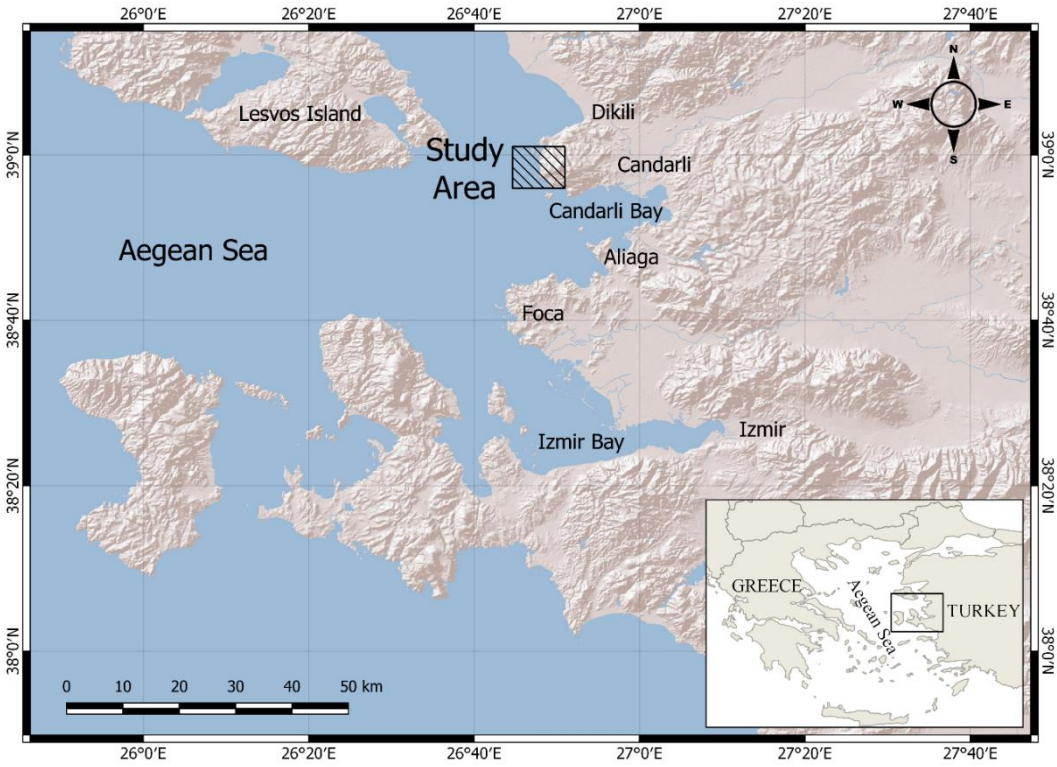


Figure 3.1 Location of the study area in Çandarlı, Izmir, Türkiye (the eastern Aegean Sea)

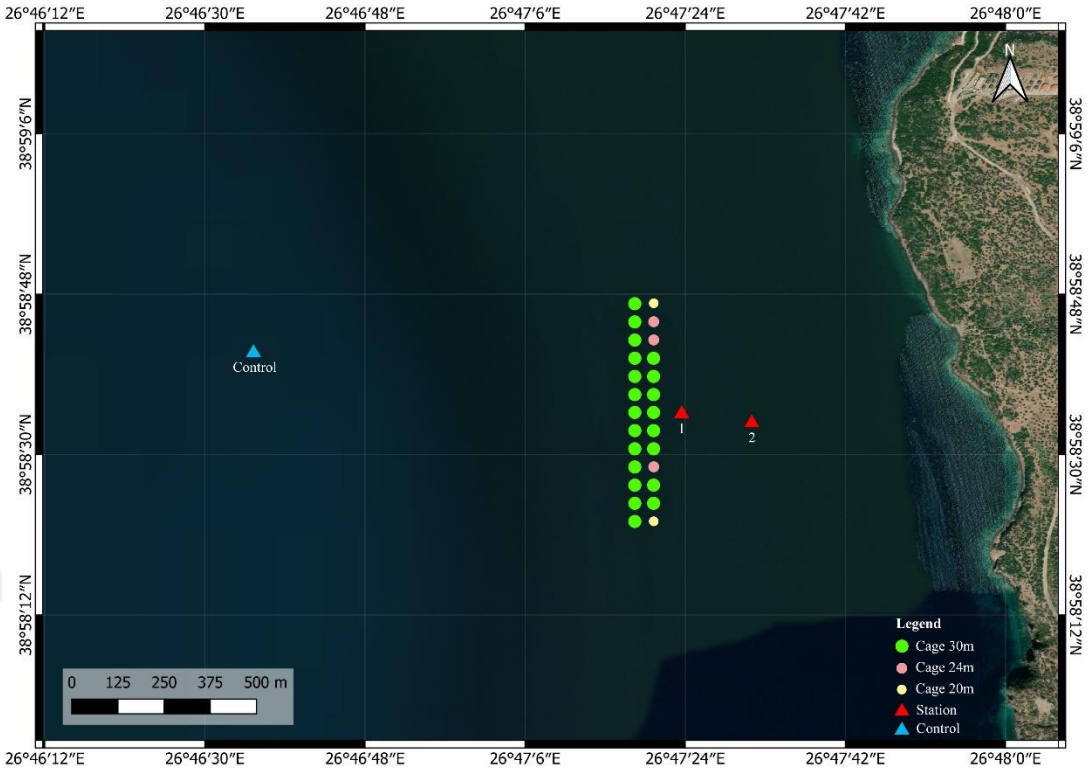


Figure 3.2 Stations of the bioassays

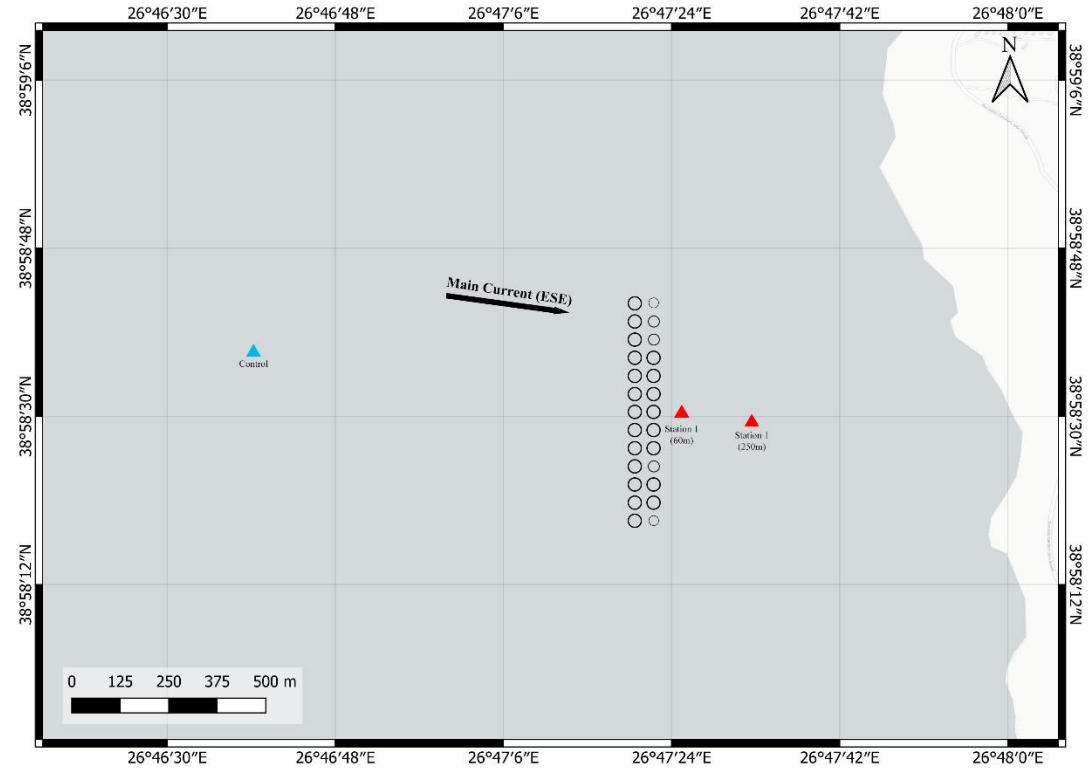


Figure 3.3 Bioassay stations with main current direction

3.2 Bioassay set-up and sampling

The bioassay was essentially *in situ* incubation of natural phytoplankton communities inside dialysis membrane bags, and it was designed to monitor the change of the growth rate of enclosed phytoplankton. Dialysis membrane bags enable the exchange of molecules smaller than proteins with the surrounding environment. The dialysis membrane bags used in the experiment were made of Spectra/Por® 1 dialysis membrane tubing with a molecular weight cut off 6-8 kD and a flat width of 100 mm. Each bag was closed on both ends with 110 mm Spectra/Por® closures (nylon) produced for membrane bags. The membrane tube was cut into 30-cm pieces. Each bag was 6.4 cm in diameter, 20 cm in length, and had a volume of 600 mL. The membranes equilibrate with ambient water in less than 4 hours (Mura et al., 1996) (Figure 3.4). The bags were soaked in deionized water overnight to allow for glycerin purification.



Figure 3.4 Dialysis membrane bag (Personal archive, 2020)

Seawater samples containing natural phytoplankton communities were collected from the 50 cm surface layer using a Nansen bottle (Figure 3.5). Each bioassay was filled with seawater collected from its respective station where the bioassays were to be placed. In each experiment, not only were the contents of the bags analyzed, but ambient seawater samples were also tested both at the beginning and at the end of the experiment. These ambient seawater samples were collected and filtered according to the same experimental groups used to prepare the bags and used as control.

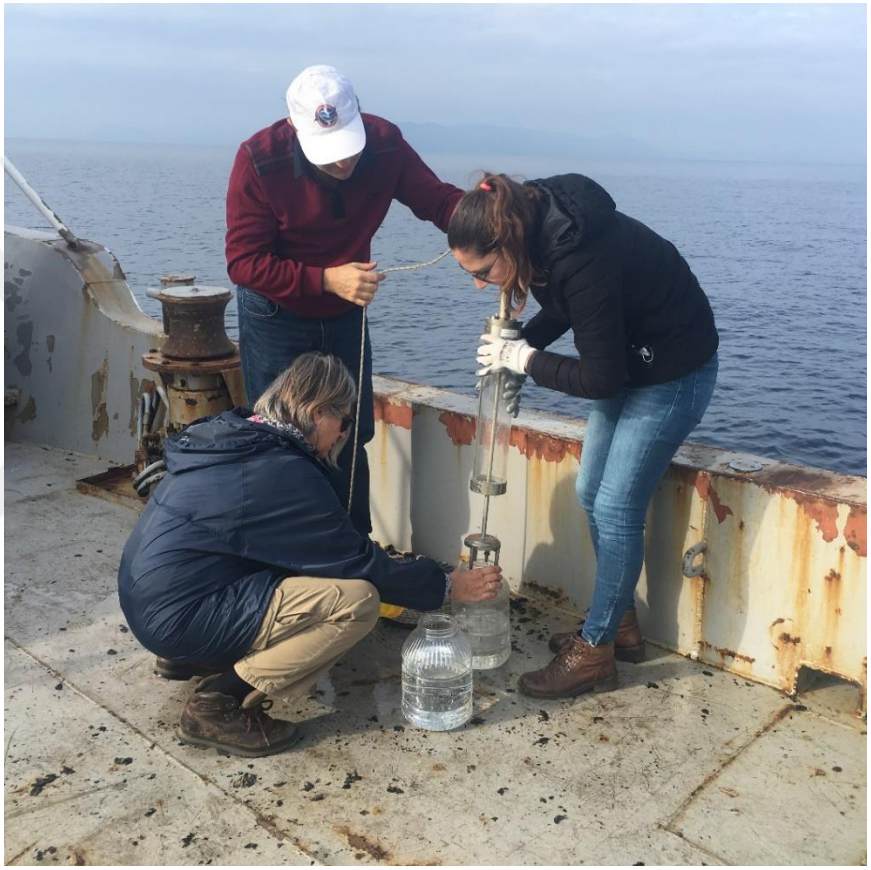


Figure 3.5 Water samples collection with Nansen bottle (Personal archive, 2020)

A circular PVC tubing frame with a diameter of 2 meters was firmly attached to the ropes that anchored the mechanical fish feeding system to the fish farm's seafloor. The dialysis bags were secured to a horizontal rope running along the PVC frame using cable ties. Weights were carefully placed at both ends of the rope to submerge it to around 50 cm below the surface The frame was, in turn, anchored to the sea floor with ropes attached to a 500 kg concrete block to prevent it from dragging and to keep it in place on the surface (Figure 3.6).

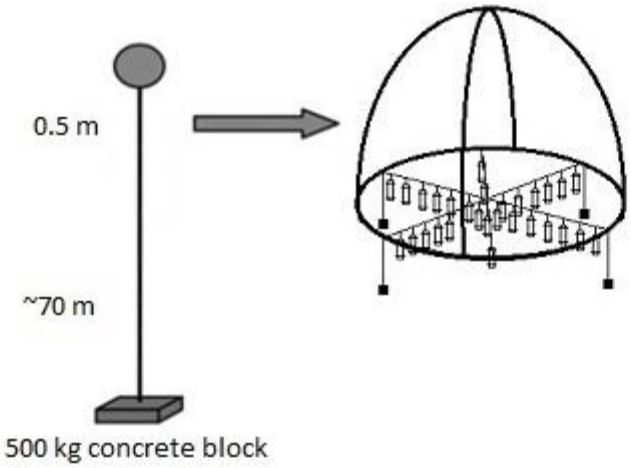


Figure 3.6 Diagram of the bioassays

Four experiments were carried out successfully.

The first experiment was initiated on July 20, 2020. In the first experiment five experimental groups were established in Station 1 to examine varying grazing pressures on the incubated phytoplankton. This experiment was the initial experiment and to test our experimental setup. Therefore, only one station was used. Four experimental groups were generated by filtering the seawater through meshes with different mesh sizes (40 µm, 56 µm, 100 µm, 150 µm) and subsequently filling the filtered water into the bags. The fifth group was formed using seawater that underwent no filtration. The experimental group prepared with unfiltered seawater comprised 3 bags with identical characteristics, while the remaining groups were tested using 2 bags each, resulting in a total of 11 dialysis membrane bags and was retrieved after a duration of three days. This timeframe was chosen based on a study conducted by Furnas (1992), which indicated that a minimum of two days is required to reliably observe growth responses. Additionally, the permeability of dialysis membrane bags decreases over time due to biofouling affecting pore integrity. Two bags from this bioassay were lost, each with different treatments, likely due to strong currents (Figure 3.7).

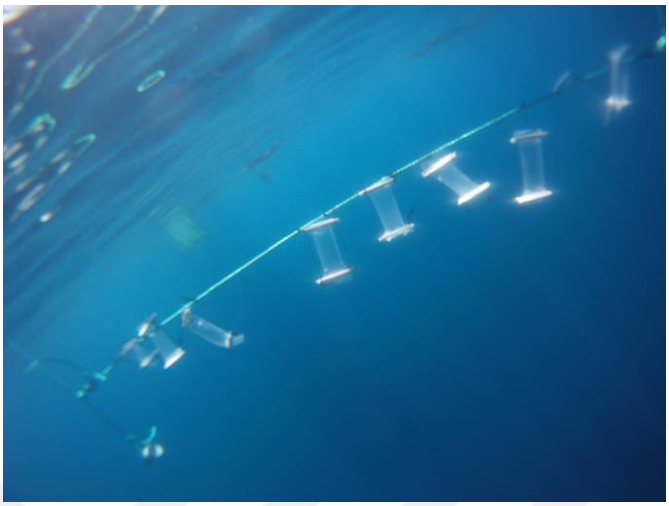


Figure 3.7 Deployment of experimental setup 50 cm below sea surface (Personal archive, 2020)

The second experiment was conducted on August 11, 2020 (Figure 3.8). In this bioassay, the first experiment was repeated with one modification: the treatment involving 56 µm mesh filtered sea water was omitted, as its results were similar to those obtained with bags filled with 40 µm mesh filtered sea water. Additionally, a new station was introduced to detect nutrient gradients near the fish farm. This station was positioned approximately 250 meters downstream from Station 1. Consequently, two stations were deployed downstream in the main current direction. Within these stations, four different treatments were implemented within the bags to assess varying predation pressures: unfiltered sea water, 40 µm filtered sea water, 100 µm filtered sea water, and 150 µm filtered sea water. Triplicate bags were filled with 40 µm filtered sea water across all stations, while other treatments were prepared as duplicates.

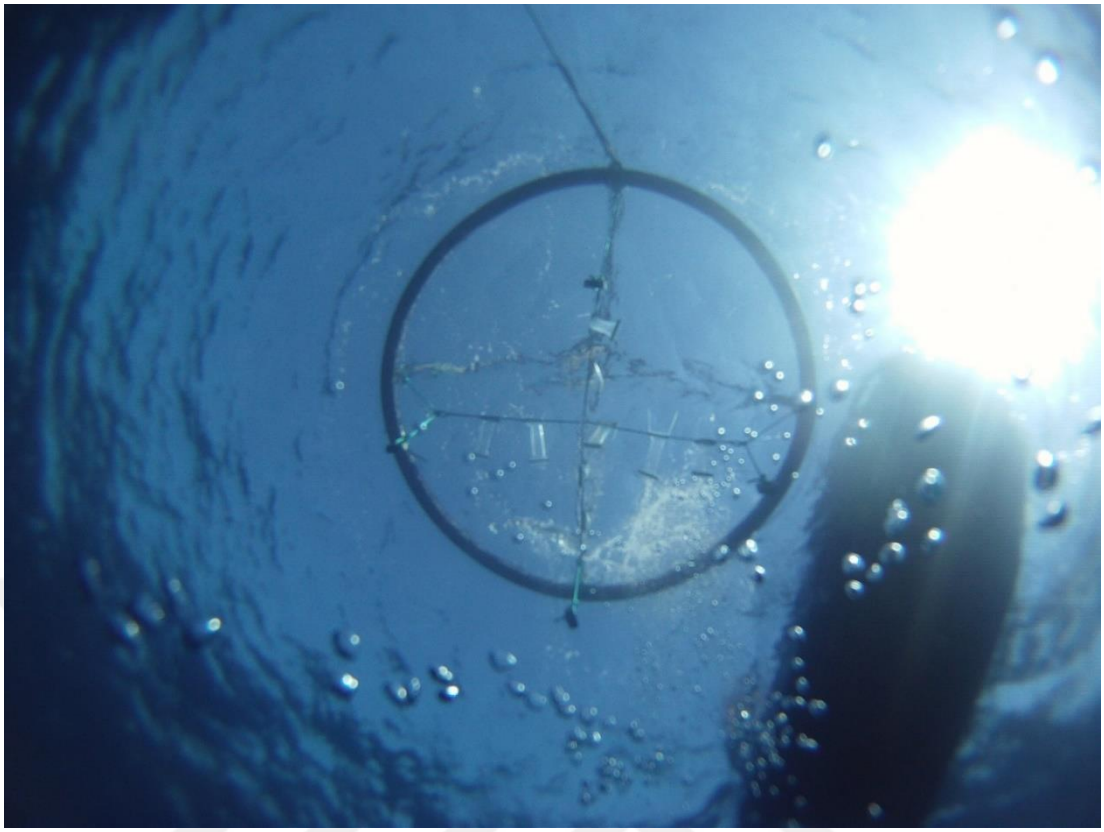


Figure 3.8 Second experiments underwater photography (Personal archive, 2020)

In the third experiment, the aim is different from the previous experiments. The third experiment, conducted to determine the growth rate of phytoplankton assemblages over six days (corresponds to a minimum of two generations for the majority of phytoplankton (Pomati et al., 2013)), involved daily retreatment and analysis of bags. This experiment commenced on September 1, 2020. The installation of the bioassay is shown in Figures 3.9, 3.10, and 3.11. Underwater view of the bioassay are shown in Figure 3.12.

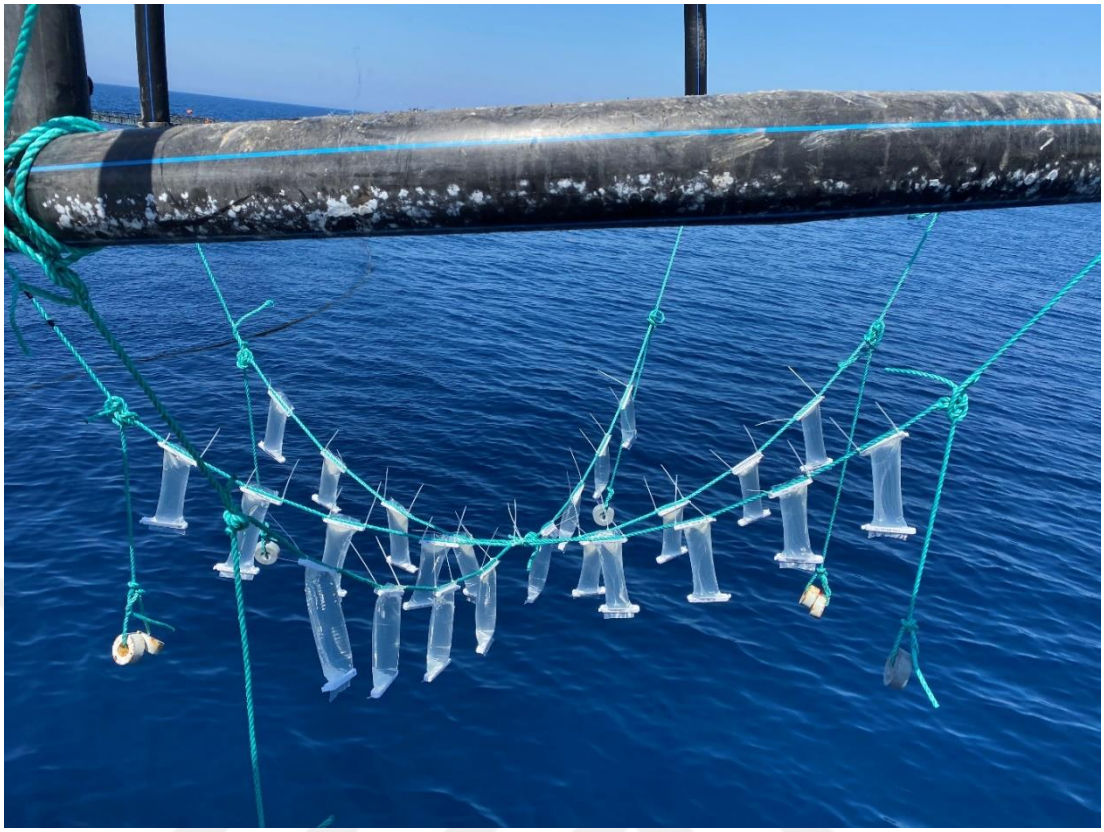


Figure 3.9 Installation of the bioassay (Personal archive, 2020)

In this experiment, 24 bags were prepared: half with unfiltered sea water and half with 150 μm filtered sea water. Ambient seawater from the incubation depth, along with two dialysis bags for each treatment (four bags in total), were sampled daily. The ambient seawater samples were divided into two groups: one filtered through a 150-μm mesh and one unfiltered. These ambient seawater samples served as controls. By the end of the sixth day, all the bags used in the experiment had been successfully retrieved. The contents of the bags were immediately divided into aliquots for further analysis after sampling.



Figure 3.10 Installation of the dialysis membrane bags (Personal archive, 2020)



Figure 3.11 Dialysis membrane bags attached to the PVC tubing frame (Personal archive, 2020)

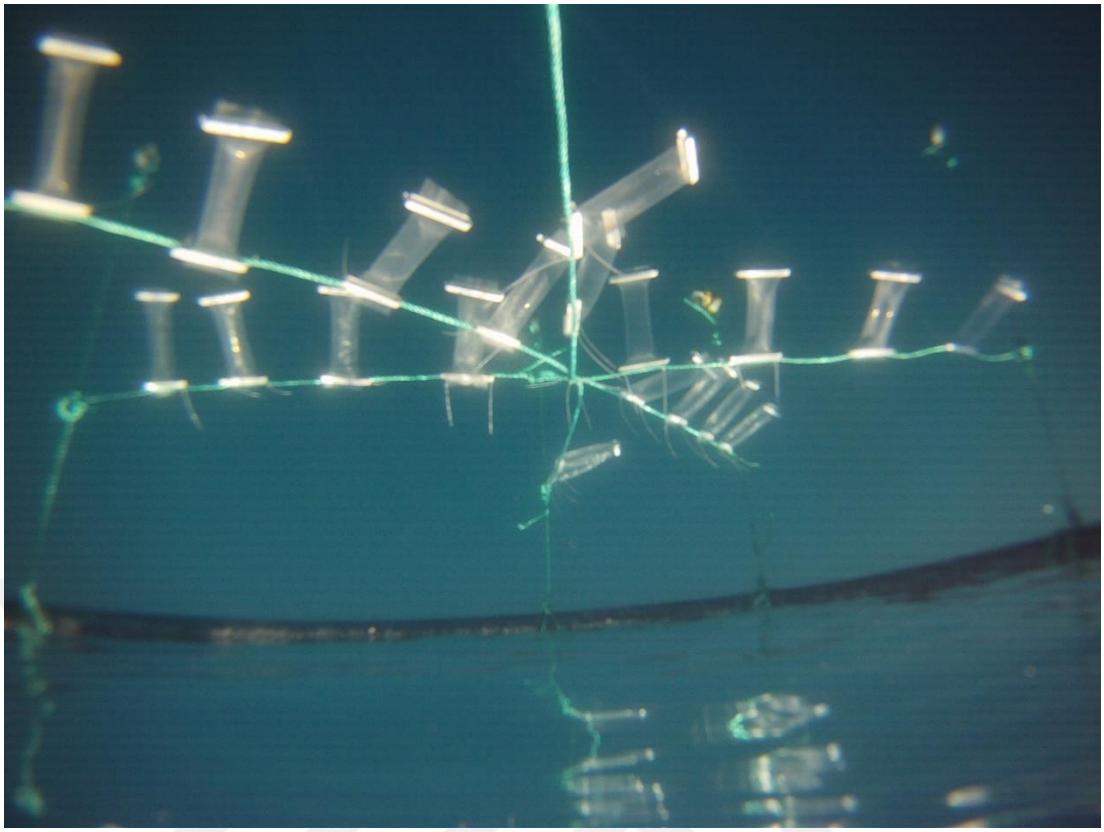


Figure 3.12 Dialysis membrane bags incubated 50 cm below the seasurface (Personal archive, 2020)

The fourth experiment, which began on October 27, 2020, originally planned for retrieval after three days of incubation. However, due to an earthquake striking Izmir on October 30, 2020, the incubation period was extended, and bag retrieval occurred on day 5 instead.

The aim of this bioassay was to examine the spatial gradient of nutrient influence in the vicinity of a fish farm, with the goal of observing a gradual decrease in nutrient levels with distance and ultimately, the fading of this influence. For this experiment, three stations were utilized, with two stations being identical to those used in the second experiment, and a third station positioned upstream from the cages, serving as a control situated 1000 meters from the farm. Within these stations, three different treatments were employed within the bags to assess varying predation pressures: unfiltered sea water, 40 μm filtered sea water, and 150 μm filtered sea water. Notably, the decision was made to exclude the 100 μm filtered sea water bags in this bioassay, as observed growth outcomes in the second experiment showed similarities

between the 100 μm and 150 μm bags. Bioassays were established with the help of scuba divers (Figure 3.13). Bioassay lifted and established to the sea with crane (Figure 3.14). Bioassays drifted to the stations with boats (Figure 3.15). In Figure 3.16, bioassay setup in Station 1 was shown and an Figure 3.17 fish farm was seen.

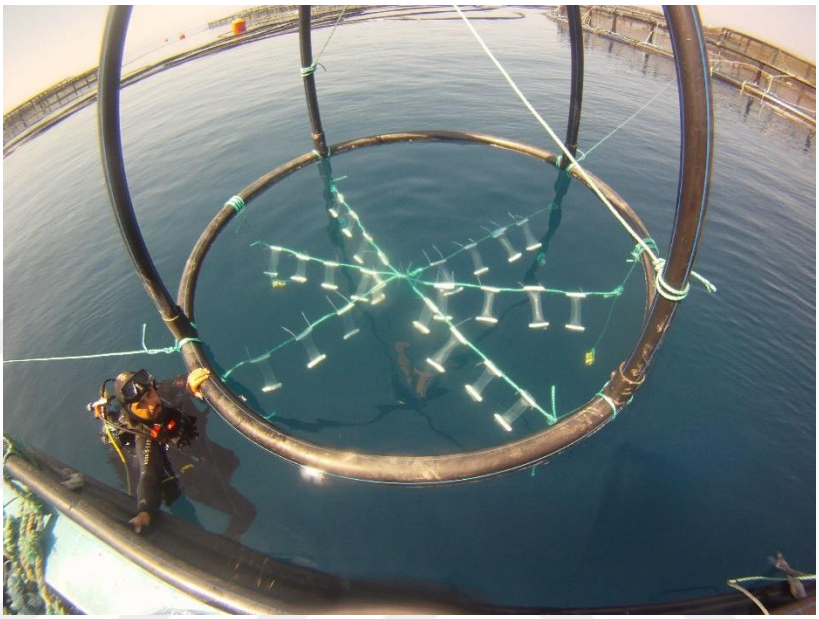


Figure 3.13 Establishment with scuba divers (Personal archive, 2020)



Figure 3.14 Experimental setup while establishing with crane (Personal archive, 2020)



Figure 3.15 Experimental setup while drifting to the stations (Personal archive, 2020)



Figure 3.16 Experiment setup in fish farm (Personal archive, 2020)

Details of each experiment were given in Table 3.1, Table 3.2 and Table 3.3.

Table 3.1 Experiment dates and incubation times

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Date	20.07.2020	11.08.2020	01.09.2020	27.10.2020
Incubation time	3 days	3 days	6 days	5 days

Table 3.2 Stations of experiments

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Stations	Fish farm (Barge)	Fish farm (Barge)	Fish farm (Barge)	Fish farm (Barge)
		250m		250m
				Control (1000m)

Table 3.3 Stations, bag types and bag numbers in each experiment

		Stations						Bag numbers
		Barge		250 m		Control		
		Established	Retrieved	Established	Retrieved	Established	Retrieved	
First Experiment (3 days)	40µm	2	1					11
	56µm	2	1					
	100µm	2	2					
	150µm	2	2					
	Unfiltered Sea water	3	3					
	Total	11	9					
Second Experiment (3 days)	40µm	2	2	2	2	2	2	27
	56µm	2	2					
	100µm	2	2	2	2	2	2	
	150µm	2	2	2	2	2	2	
	Unfiltered Sea water	2	2	3	3	2	2	
	Total	10	10	9	9	8	8	
Third Experiment (6 days)	40µm							24
	56µm							
	100µm							
	150µm	12	12					
	Unfiltered Sea water	12	11					
	Total	24	23					

Table 3.3 continued

		Stations						Bag numbers
		Barge		250 m		Control		
		Established	Retrieved	Established	Retrieved	Established	Retrieved	
Fourth Experiment (5 days)	40µm	3	3	3	3	2	2	22
	56µm							
	100µm							
	150µm	2	2	3	3	3	3	
	Unfiltered Sea water	2	2	2	2	2	1	
	Total	7	7	8	8	7	6	



Figure 3.17 Fish farm (Personal archive, 2020)

3.3 Taxonomic identification and quantification of phytoplankton species

Following the incubation period, the contents of the bags were divided into aliquots for subsequent analysis. Specifically, 200 ml from each bag was preserved in a 0.4% Lugol's solution. Ambient seawater samples collected for 5 L samples both filtered and unfiltered, were also subjected to the same preservation procedure. Preserved samples

were transferred into sedimentation chambers using graduated cylinders of various sizes (1000 ml, 250 ml, 100 ml, 10 ml), where they were left undisturbed for one week. This sedimentation period was optimized based on the size and density characteristics of the phytoplankton species present in the samples. Following the sedimentation period, clear supernatant water was carefully removed from each sedimentation chamber using a pipette or gentle siphoning method. Special care was taken during this step to avoid disturbing the settled phytoplankton at the bottom of the chambers. The preserved samples were utilized for the identification and enumeration of phytoplankton ranging in size from 20 to 200 µm, through sedimentation (Figure 3.18) followed by examination under a microscope (Olympus BX50 Fluorescence Microscope) at 100x magnification. The taxonomic references utilized included Tomas (1997), Koray (2001), and Gomez et al. (2010).



Figure 3.18 Sedimentation steps (Personal archive, 2020)

3.4 Nutrients and environmental variables

Samples for dissolved inorganic nutrients and chl *a* were prescreened using 210 µm nylon mesh to eliminate bigger particles. Following prescreening, these samples were further filtered using Whatman GF/F glass microfiber filters. The resulting filtrates

were then stored at -20°C before being analyzed for nitrite (NO_2), nitrate (NO_3), orthophosphate (PO_4), and reactive silica (SiO_2) with an SKALAR autoanalyzer (Skalar, De Breda, Netherlands) using colorimetric methods adapted from the standard seawater analyses as described by Grasshoff et al. (1999). For chl *a* examination, the filters were folded and insert inside glass tubes, which were then frozen for later laboratory analysis. Chl *a* was extracted with 90% acetone for 24 hours, then measured using the fluorometric technique published by Strickland & Parsons (1972). Seawater temperature and salinity were measured with the portable Sea-Bird 37 SM equipment (Sea-Bird Electronics Inc., Bellevue, WA, USA) (Figure 3.19). The Winkler technique was used to measure the dissolved oxygen concentration. The Secchi disk depth and pH were determined *in situ* (Figure 3.20). In Figure 3.20 preparing membrane dialysis bags were shown.

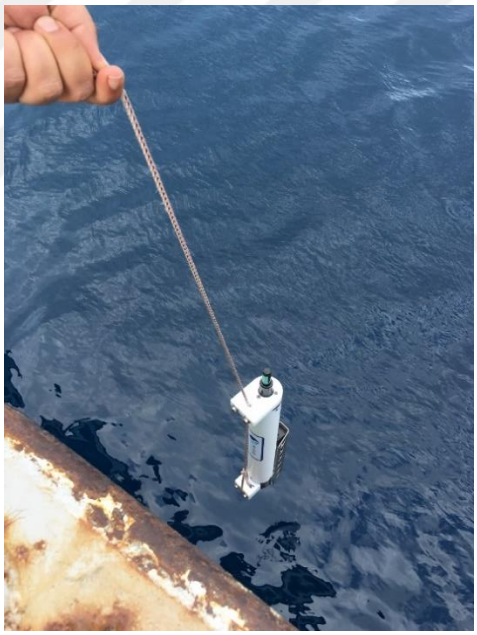


Figure 3.19 CTD measuring with Sea-Bird 37 SM (Personal archive, 2020)



Figure 3.20 Sechi disc measuring (Personal archive, 2020)



Figure 3.21 Filling the bags with sea water (Personal archive, 2020)

3.5 Statistical analyses

The average values of concentrations of each nutrient measured from the ambient water and the water contained inside the dialysis bags were compared using independent t-tests. Simple linear regression analyses treating the observed concentrations of each nutrient in the seawater separately as the dependent variable against time (day) as predictor were used to detect any significant temporal variation in the nutrient concentrations during the experiment period.

The population growth rates of phytoplankton inside dialysis bags and phytoplankton in ambient water were determined using chlorophyll concentrations and cell counts. Throughout the experiment, both were measured and recorded daily, and the calculations were based on the exponential growth model (Reynolds, 2006; Snedecor & Cochran, 1989;):

$$Y_t = Y_0 e^{rt} \tag{3.1}$$

Yt is the dependent variable that specifies the chl *a* concentrations or cell counts at time *t* (day). Y_0 is the starting concentration of chl *a* or cell number at time t=0 (i.e., at the begining of the experiment), and *r* is the population's specific growth rate, given in terms of chl *a* concentration or cell count. The growth model was linearized using the logarithmic transformation, and the specific growth rate *r* was calculated as the slope of a linear regression of the natural logarithm of *Y* vs *t* (Reynolds, 2006; Snedecor & Cochran, 1989). An analysis of covariance (ANCOVA) was also performed to see if the estimated Y_0 and *r* values differed across dialysis bags containing filtered and unfiltered seawater. The growth rates of various dominant species in the ambient water and inside the dialysis bags were also measured in terms of cell count. Changes in concentrations (either chl *a* or cell numbers) within the dialysis bags after incubation were used to calculate the gross rates (r_{gross}). Net growth rates (r_{net}) were estimated using the changes in concentrations in the ambient seawater during the experiment. The subtraction between the gross and net rates were the loss rates (r_{loss}) (Mura et al., 1996; Sommer et al., 2005; Toth, 1980).

Various indices were employed to evaluate the species diversity and evenness of phytoplankton communities. Diversity was assessed by using Shannon-Weiner index (H') and Simpson's index (D) (Magurran, 1988; Krebs, 1999). The ratio of observed diversity (H') to maximal diversity (H_{max}), may occur in a situation if all species were equally abundant, was used to measure evenness (E) (Magurran, 1988).

$$H' = -\sum_{i=1}^{S}(p_i) \times log_2(p_i) \tag{3.2}$$

$$D = 1 - \sum_{i=1}^{S} p_i^2 \tag{3.3}$$

$$E = \frac{H'}{H_{max}} = \frac{H'}{log_2(S)} \tag{3.4}$$

In the preceding formulations p_i represents the fraction of the ith species in terms of cell numbers, and S represents the total number of phytoplankton species or species richness in the community. The Shannon-Wiener index may be written in terms of species numbers using exponentiation with 2 as the base and H' as the exponent. This is also known as Hill's number *N1* (Krebs, 1999). An additional form of Simpson's index, known as Simpson's reciprocal index (D^{-1}), was also employed to represent diversity estimates in terms of species counts. Simpson's reciprocal index is equivalent to Hill's number *N2* (Krebs, 1999).

$$2^{H'} = N_1 \tag{3.5}$$

$$D^{-1} = \frac{1}{\sum_1^S p_i^2} = N_2 \tag{3.6}$$

For each day of the experiment, all indices were calculated for each bag and its ambient seawater. All statistical tests were performed using R software version 4.0.3 (R Core Team, 2020), with a significance level of 5%.

CHAPTER FOUR
RESULTS

4.1 First Experiment

The physical properties remained consistent during experiment period (Table 4.1). During the experiment, average wave heights were continuously modest (<1 m), as observed and recorded by fish farm staff. Table 4.2 shows the nutrient concentrations recorded in both ambient seawater and the dialysis membrane bags. The concentrations of nitrite + nitrate, orthophosphate, and reactive silica measured in the ambient seawater varied from 0.08 to 0.23, 0.03 to 0.07, and 0.21 to 0.45, respectively. The nutrient concentrations detected in the bags were slightly greater than those in ambient seawater (Table 4.2).

Table 4.1 Physical properties of the ambient seawater

Physical Properties					
Days	**Temperature (°C)**	**Salinity (ppt)**	**Dissolved Oxygen (mg L^{-1})**	**pH**	**Secchi Depth (m)**
0	23.4	39.4	7.9	8.1	24.3
3	23.3	39.5	7.8	8.1	24.6

Table 4.2 The nutrient values measured in both the ambient seawater and dialysis membrane bags at the beginning and end of the experiment. Note that nutrient values inside the dialysis membrane bags represent averages. UF: unfiltered treatment

Medium	**Nutrients**	**Days**									
		0					**3**				
		40µm	**56µm**	**100µm**	**150µm**	**UF**	**40µm**	**56µm**	**100µm**	**150µm**	**UF**
Ambient Seawater	Nitrite+ Nitrate (µM)	0.18	0.14	0.09	0.06	0.11	0.18	0.23	0.19	0.22	0.18
	Orthophosphate (µM)	0.09	0.01	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08
	Silicate (µM)	0.26	0.21	0.32	0.26	0.39	0.35	0.45	0.37	0.33	0.34
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	-	-	0.22	0.4	0.43	0.21	0.28
	Orthophosphate (µM)	-	-	-	-	-	0.03	0.05	0.07	0.07	0.05
	Silicate (µM)	-	-	-	-	-	0.52	0.58	0.35	0.42	0.46

The concentrations of chl *a* and phytoplankton cell numbers, determined from both unfiltered and filtered ambient seawater, as well as from unfiltered and filtered dialysis bags, at the beginning and end of the experiment, are all presented in Table 4.3. Gross (r_{gross}), net (r_{net}), and loss (r_{loss}) growth rates, derived from the chl a values and phytoplankton cell numbers in both the ambient seawater and dialysis bags (Table 4.3), are displayed in Table 4.4. As evident from Table 4.3, the chl a concentrations and phytoplankton cell numbers in the samples collected from the ambient seawater, regardless of the filtering treatment, were very similar at the start and end of the experiment. Consequently, no significant phytoplankton growth was observed (t-test), and the net growth rate was zero (Table 4.4). However, after three days of incubation, a substantial increase in cell numbers and chl a concentrations within the dialysis bags became evident (Table 4.3). At the conclusion of the experiment, there was an over 18-fold increase in chl a values observed in the bags incubated with unfiltered seawater in comparison to the ambient seawater. Likewise, for the dialysis bags incubated with filtered seawater, the observed increases were approximately 11-fold at 40 µm, 12-fold at 56 µm, 11-fold at 100 µm, and 17-fold at 150 µm filtration (Table 4.3). Similarly, the increases in phytoplankton cell numbers in the bags subjected to filtration were more than 9-fold at 40 µm, nearly 12-fold at 56 µm, 10-fold at 100 µm, and 16-fold at 150 µm filtration, with close to 17-fold increase in the bags incubated with unfiltered seawater (Table 4.3).

Table 4.3 . Estimates of chl a concentrations and phytoplankton cell numbers from the unfiltered and filtered ambient seawater samples, as well as from the incubated bags. Reported results for the bags represent the average values of the sampled bags for each treatment, UF representing the unfiltered treatment

Medium	**Growth Variable**	**Days**									
		0					**3**				
		40µm	**56µm**	**100µm**	**150µm**	**UF**	**40µm**	**56µm**	**100µm**	**150µm**	**UF**
Ambient Seawater	chl *a* µg L^{-1}	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	Cell L^{-1}	72860	72356	71346	79823	76439	76435	78923	73265	74391	75693
Dialysis Membrane Bags	chl *a* µg L^{-1}	-	-	-	-	-	0.65	0.7	0.68	1.01	1.09
	Cell L^{-1}	-	-	-	-	-	689797	883409	703460	1221128	1292972

The ANCOVA analysis indicated a classification of the bags into two discernible groups based on growth rates estimated from chl a concentrations. The initial group comprised unfiltered bags and those filtered through a 150 µm mesh size, demonstrating notably higher growth rates compared to the second group, which included bags filtered through 40 µm, 56 µm, and 100 µm mesh sizes. When growth rates were assessed based on cell counts, the ANCOVA results delineated three separate groups of bags. The bags in the first group, with higher growth rates, were the same as those identified in the analysis using chl *a* contents, namely the bags filtered with a 150 µm mesh size and unfiltered bags. The second group included only the bags filtered through a 56 µm mesh size. The gross growth rate of this group was lower than that of the first group but higher than that of the third one, which consisted of the bags filtered with 40 µm and 100 µm mesh sizes (Table 4.4). In each of the five experimental groups, the two growth rates estimated from two different dependent variables, i.e., chl *a* concentrations and cell numbers, were comparable. The Pearson's product-moment correlation coefficient between these two measurements was 0.99.

Table 4.4 Gross growth rates, net growth rates, and loss growth rates estimated from chl a values and phytoplankton cell numbers observed both in the ambient seawater and inside the dialysis membrane bags (*UF representing the unfiltered treatment)

Medium	**Treatment**	**Growth Rates**		
		r_{gross} (d^{-1})	r_{net} (d^{-1})	r_{loss} (d^{-1})
Estimated from chl *a* values	40µm	0.80	0.00	0.80
	56µm	0.82	0.00	0.82
	100µm	0.81	0.00	0.81
	150µm	0.95	0.00	0.95
	UF*	0.97	0.00	0.97
Estimated from phytoplakton cell numbers	40µm	0.75	0.02	0.73
	56µm	0.83	0.03	0.81
	100µm	0.76	0.01	0.75
	150µm	0.91	-0.02	0.93
	UF*	0.94	0.00	0.95

A total of 33 phytoplankton taxa were identified in the seawater samples taken from the study site during the experiment. Of these, 17 were diatom species, while the remaining 16 belonged to dinoflagellate species (Table 4.6). *Pronoctiluca spinifera* (Lohmann) Schiller, 1932 (Family: Protodiniferaceae, Class: Dinophyceae) was

detected in the unfiltered seawater sample collected at the first day of the experiment (Figure 4.1), (Table 4.6). This species had previously been reported only in the Black Sea waters of Türkiye by Öztürk (1998), and this study provides the first record of its presence on the Aegean Sea coast of Türkiye. Upon examination of the phytoplankton community composition, it became evident that diatoms dominated, especially within two prominent families: Leptocylindraceae and Bacillariaceae. In contrast, when assessing dinoflagellate abundance, the Ceratiaceae family stood out as having the highest number of individuals.



Figure 4.1 *Pronoctiluca spinifera* (scale bar 25 µm)

Table 4.5 shows the number of species, diversity indices, and evenness values for both filtered and unfiltered ambient seawater samples collected on the first and last

days of the experiment, as well as similar estimates for all dialysis bags collected on the final day of the experiment. In general, it was observed that with an increase in the mesh size of filtration, there was a corresponding rise in the number of species, aligning with expectations. Unfiltered samples exhibited the highest species count. This pattern was similarly reflected in the species diversity indices and evenness values. The filtration stage prevented the entry of certain taxa, notably chain-forming diatoms such as Chaetoceros spp., which have relatively larger cell sizes, into the dialysis bags (Table 4.6 and Table 4.7).

The trend of rising species richness with increasing mesh size of filtration was also observed in the dialysis bags at the end of the experiment. While many species found in the ambient seawater were not encountered inside the bags, certain species were observed in much higher numbers than those recorded in the ambient seawater. In particular, the diatom species *Leptocylindrus danicus* Cleve, 1889 was present in exceptionally high quantities in all bags (Figure 4.2), contributing to the lower evenness values in Table 4.5 compared to the ambient seawater. Additionally, this species was seen to form chains consisting of 8-10 cells inside the bags. Overall, species diversity was notably low in all incubated bags, as evidenced by the N_1 and N_2 values presented in Table 4.5. The contents of all bags at the end of the incubation period comprised a total of 11 diatom species and 8 dinoflagellate species (see Table 4.7).

Samples collected from the ambient seawater also contained microzooplankton, including ciliate protozoans and copepod nauplii, although their concentrations were very low. No adult copepod was found inside any bag, but ciliates were present in all bags (Some of the identified genera include Favella sp., Eutintinnus sp., Strobilidium sp., and Mesodinium sp.).



Figure 4.2 Diatom bloom (*Leptocylindrus danicus*) after the incubation period

Table 4.5 Phytoplankton species richness and species diversity observed in the unfiltered (UF) and filtered (40 µm, 56 µm, 100 µm, and 150 µm) ambient seawater as well as in the unfiltered and filtered dialysis membrane bags

		Days									
		0					**3**				
		40µm	**56µm**	**100µm**	**150µm**	**UF**	**40µm**	**56µm**	**100µm**	**150µm**	**UF**
Ambient Seawater	Species richness (*S*)	9	16	18	24	29	13	15	21	21	25
	Shannon Index (*H'*)	2.64	3.07	3.30	3.65	3.90	2.92	3.10	3.48	3.51	3.63
	Simpson's Index (*D*)	0.78	0.79	0.82	0.85	0.86	0.78	0.82	0.83	0.84	0.83
	N_1 ($2^{H'}$)	6.24	8.39	9.88	12.59	14.92	7.58	8.57	11.16	11.36	12.39
	N_2 (D^{-1})	4.61	4.82	5.59	6.73	6.91	4.63	5.53	5.73	6.14	6.18
	Evenness (*E*)	0.83	0.77	0.79	0.80	0.80	0.79	0.79	0.79	0.80	0.78
Dialysis Membrane Bags	Species richness (*S*)	-	-	-	-	-	5	7	12	16	19
	Shannon Index (*H'*)	-	-	-	-	-	0.30	0.34	0.67	0.61	0.66
	Simpson's Index (*D*)	-	-	-	-	-	0.08	0.08	0.16	0.13	0.14
	N_1 ($2^{H'}$)	-	-	-	-	-	1.23	1.27	1.59	1.52	1.58
	N_2 (D^{-1})	-	-	-	-	-	1.08	1.09	1.19	1.15	1.16
	Evenness (*E*)	-	-	-	-	-	0.13	0.12	0.19	0.15	0.16

Table 4.6 Catalog of phytoplankton species identified in samples from ambient seawater

Taxa	Days									
	0					3				
	40µm	56µm	100µm	150µm	UF	40µm	56µm	100µm	150µm	UF
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin, 1964	+	+	+	+	+	+	+	+	+	+
Nitzschia longissima (Brébisson) Ralfs, 1861	+	+	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+			+	+	+
Chaetoceros affinis Lauder, 1864		+	+	+	+	+	+	+	+	+
Chaetoceros decipiens Cleve, 1873							+	+	+	+
Grammatophora marina (Lyngbye) Kützing, 1844				+	+					
Hemiaulus hauckii Grunow ex Van Heurck, 1882	+	+	+	+	+	+	+	+	+	+
Leptocylindrus danicus Cleve, 1889	+	+	+	+	+	+	+	+	+	+
Licmophora sp.		+	+	+	+			+	+	+
Navicula sp.	+	+	+	+	+	+		+	+	+
Dactyliosolen fragilissimus (Bergon) Hasle, 1996	+	+	+	+	+		+	+	+	+
Guinardia flaccida (Castracane) H.Peragallo, 1892					+		+	+	+	+
Guinardia striata (Stolterfoth) Hasle, 1996	+	+	+	+	+		+	+	+	+
Pseudosolenia calcar-avis (Schultze) B.G.Sundström, 1986				+	+					
Rhizosolenia sp.				+	+					
Pleurosigma sp.			+	+	+	+	+	+	+	+
Thalassionema nitzschioides (Grunow) Mereschkowsky, 1902					+					

Table 4.6 continued

Taxa	Days									
	0					3				
	40µm	56µm	100µm	150µm	UF	40µm	56µm	100µm	150µm	UF
Tripos fusus (Ehrenberg) F.Gómez, 2013		+	+	+	+	+	+	+	+	+
Tripos furca (Ehrenberg) F.Gómez, 2013						+	+	+	+	+
Tripos lineatus (Ehrenberg) F.Gómez, 2021					+					
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman, 2020		+	+	+	+					
Tripos trichoceros (Ehrenberg) Gómez, 2013				+	+					
Dinophysis acuminata Claparède & Lachmann, 1859		+	+	+	+	+		+	+	+
Oxyphysis oxytoxoides (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011		+	+	+	+			+	+	+
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III, 1966					+					
Oxytoxum scolopax F. Stein, 1883		+	+	+	+	+	+	+	+	+
Podolampas elegans F.Schütt, 1895				+	+					
Podolampas palmipes F. Stein, 1883						+	+	+	+	+
Prorocentrum gracile F.Schütt, 1895			+	+	+					+
Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+	+	+	+	+	+
Pronoctiluca spinifera (Lohmann) Schiller, 1932					+					
Protoperidinium depressum (Bailey) Balech, 1974				+	+					
Protoperidinium steinii (Jørgensen) Balech, 1974								+	+	+

Table 4.7 Catalog of phytoplankton species detected within dialysis membrane bags

Taxa	Day 3				
	40μm	**56μm**	**100μm**	**150μm**	**UF**
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin, 1964			+	+	+
Nitzschia longissima (Brébisson) Ralfs, 1861		+	+	+	+
Pseudo-nitzschia sp.			+	+	+
Chaetoceros affinis Lauder, 1864		+	+	+	+
Chaetoceros decipiens Cleve, 1873					
Grammatophora marina (Lyngbye) Kützing, 1844			+	+	+
Hemiaulus hauckii Grunow ex Van Heurck, 1882	+	+	+	+	+
Leptocylindrus danicus Cleve, 1889	+	+	+	+	+
Licmophora sp.					+
Navicula sp.					
Dactyliosolen fragilissimus (Bergon) Hasle, 1996					+
Guinardia flaccida (Castracane) H.Peragallo, 1892					
Guinardia striata (Stolterfoth) Hasle, 1996					
Pseudosolenia calcar-avis (Schultze) B.G.Sundström, 1986					
Rhizosolenia sp.				+	+
Pleurosigma sp.					
Thalassionema nitzschioides (Grunow) Mereschkowsky, 1902			+	+	+

Table 4.7 continued

Taxa	Day 3				
	40µm	**56µm**	**100µm**	**150µm**	**UF**
Tripos fusus (Ehrenberg) F.Gómez, 2013				+	+
Tripos furca (Ehrenberg) F.Gómez, 2013		+	+	+	+
Tripos lineatus (Ehrenberg) F.Gómez, 2021	+	+	+	+	+
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman, 2020					+
Tripos trichoceros (Ehrenberg) Gómez, 2013					
Dinophysis acuminata Claparède & Lachmann, 1859					
Oxyphysis oxytoxoides (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011				+	+
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III, 1966					
Oxytoxum scolopax F. Stein, 1883				+	+
Podolampas elegans F.Schütt, 1895					
Podolampas palmipes F. Stein, 1883					
Prorocentrum gracile F.Schütt, 1895			+	+	+
Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+
Pronoctiluca spinifera (Lohmann) Schiller, 1932					
Protoperidinium depressum (Bailey) Balech, 1974					
Protoperidinium steinii (Jørgensen) Balech, 1974					

4.2 Second Experiment

The physical characteristics remained unaltered at both stations for the entire duration of the experiment (Table 4.8 and Table 4.9). Based on observations and records provided by the fish farm personnel, the average wave heights remained consistently below 1 m throughout the experiment.

Table 4.8 Physical properties of the ambient seawater at Station 1

Station 1					
	Physical Properties				
Days	**Temperature (℃)**	**.pH**	**Dissolved Oxygen (mg/l)**	**Salinity (ppt)**	**Secchi Depth (m)**
0	23.7	8.2	7.9	39.4	24.3
3	23.9	8.2	7.8	39.3	24.5

Table 4.9 Physical properties of the ambient seawater at Station 2

Station 2					
	Physical Properties				
Days	**Temperature (℃)**	**.pH**	**Dissolved Oxygen (mg/l)**	**Salinity (ppt)**	**Secchi Depth (m)**
0	23.8	8.1	7.8	39.4	25.2
3	23.9	8.1	7.7	39.3	25.3

The nutrient concentrations, assessed in both the ambient seawater and within the dialysis membrane bags, are displayed in Tables 4.10 and Table 4.11. Nutrient levels in the ambient seawater and within the bags exhibited remarkable similarity, with no notable temporal variation observed in any of the nutrients.

Table 4.10 Nutrient concentrations at Station 1 from both ambient seawater and dialysis membrane bags

Station 1									
Medium	Nutrients	Days							
		0				3			
		40µm	100µm	150µm	UF	40µm	100µm	150µm	UF
Ambient Seawater	Nitrite+ Nitrate (µM)	0.28	0.20	0.29	0.33	0.43	0.37	0.42	0.38
	Orthophosphate (µM)	0.03	0.02	0.03	0.03	0.02	0.03	0.03	0.03
	Silicate (µM)	0.38	0.44	0.38	0.31	0.56	0.48	0.43	0.51
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	-	0.45	0.85	0.84	0.92
	Orthophosphate (µM)	-	-	-	-	0.03	0.05	0.04	0.06
	Silicate (µM)	-	-	-	-	0.88	0.39	0.72	0.92

Table 4.11 Nutrient concentrations at Station 2 from both ambient seawater and dialysis membrane bags

Station 2									
Medium	Nutrients	Days							
		0				3			
		40µm	100µm	150µm	UF	40µm	100µm	150µm	UF
Ambient Seawater	Nitrite+ Nitrate (µM)	0.26	0.27	0.29	0.31	0.44	0.49	0.37	0.48
	Orthophosphate (µM)	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.02
	Silicate (µM)	0.32	0.31	0.33	0.41	0.63	0.57	0.59	0.61
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	-	0.86	0.82	0.54	0.79
	Orthophosphate (µM)	-	-	-	-	0.05	0.04	0.04	0.04
	Silicate (µM)	-	-	-	-	0.64	0.47	0.32	0.9

Table 4.12 presents the gross (r_{gross}), net (r_{net}), and loss (r_{loss}) growth rates, calculated from both chl *a* values and phytoplankton cell numbers, observed in both the ambient seawater and dialysis bags at Station 1. Similarly, Table 4.13 displays the corresponding data for Station 2. The ch *a* concentrations and phytoplankton cell numbers in the samples obtained from the ambient seawater, irrespective of the filtering treatment or stations, exhibited close resemblance at both the beginning and end of the experiment. As a result, no noteworthy phytoplankton growth was detected according to the *t*-test, and the net growth rate was determined to be zero (Table 4.12 and Table 4.13). Nevertheless, following three days of incubation, a notable rise in cell numbers and chl *a* concentrations within the dialysis bags was observed at both stations (Table 4.12 and Table 4.13). In station 1, from chl *a* values estimation, across all treatment groups (40µm, 100µm, 150µm, and UF), the gross growth rates (r_{gross}) gradually increased, indicating higher productivity with increasing filter pore size.

Notably, the unfiltered treatment (UF) exhibited the highest growth rates. From phytoplankton cell numbers estimation**,** the gross growth rates varied among treatments, with the UF treatment again showing the highest growth rates, albeit with some variability across filter pore sizes (Table 4.12).

Table 4.12 Gross, net, and loss growth rates calculated from chl *a* values and phytoplankton cell numbers recorded in both ambient seawater and within dialysis membrane bags at Station 1 (UF indicating the unfiltered treatment).

Station 1				
Medium	**Treatment**	**Growth Rates**		
		r_{gross} **(d^{-1})**	r_{net} **(d^{-1})**	r_{loss} **(d^{-1})**
Estimated from chl *a* values	40µm	0.49	0.01	0.49
	100µm	0.69	0.01	0.69
	150µm	0.90	0.01	0.90
	UF	1.05	0.13	0.92
Estimated from phytoplakton cell numbers	40µm	0.27	-0.05	0.32
	100µm	0.46	0.01	0.45
	150µm	0.52	-0.04	0.56
	UF	0.64	-0.01	0.65

Unlike Station 1, the gross growth rates estimated from chl a in Station 2 showed mixed trends across different treatments. Notably, the 40µm treatment exhibited negative growth rates. Similarly, the growth rates estimated from phytoplankton cell numbers varied across treatments, with some treatments showing negative net growth rates (r_{net}) and higher loss growth rates (r_{loss}) compared to Station 1. Station 2, located further away from the fish farm, exhibited lower growth rates compared to Station 1 across both chl *a* values and phytoplankton cell numbers of estimations.

Table 4.13 Gross, net, and loss growth rates calculated from chl a values and phytoplankton cell numbers recorded in both ambient seawater and within dialysis membrane bags at Station 2 (UF indicating the unfiltered treatment).

Station 2				
Medium	**Treatment**	**Growth Rates**		
		r_{gross} (d^{-1})	r_{net} (d^{-1})	r_{loss} (d^{-1})
Estimated from chl *a* values	40µm	-0.09	-0.04	-0.06
	100µm	0.50	0.01	0.50
	150µm	0.44	-0.23	0.66
	UF	0.67	0.01	0.66
Estimated from phytoplakton cell numbers	40µm	0.13	0.02	0.11
	100µm	0.24	-0.02	0.26
	150µm	0.35	0.03	0.32
	UF	0.32	0.01	0.32

Table 4.14 Diversity indices

Medium	**Indices**	**Treatments**	**Days**			
			0		**3**	
			Barge	**250**	**Barge**	**250**
Ambient Sea Water	**Species Richness (S)**	40µm	27	31	13	6
		100µm	32	37	17	8
		150µm	45	38	17	8
		UF	47	38	17	8
	Shannon Index (H')	40µm	4.30	4.69	2.94	2.36
		100µm	4.55	4.73	3.27	2.65
		150µm	5.08	4.97	3.07	2.61
		UF	5.29	4.89	3.08	2.64
	Simpson's Index (D)	40µm	0.94	0.95	0.82	0.78
		100µm	0.94	0.94	0.85	0.82
		150µm	0.96	0.96	0.83	0.81
		UF	0.97	0.95	0.84	0.82
	N_1 ($2^{H'}$)	40µm	19.64	25.73	7.66	5.13
		100µm	23.41	26.57	9.64	6.29
		150µm	33.84	31.41	8.42	6.11
		UF	39.25	29.69	8.46	6.24
	N_2 (D^{-1})	40µm	15.55	20.86	5.54	4.57
		100µm	17.95	18.22	6.81	5.42
		150µm	24.60	24.35	5.89	5.36
		UF	31.33	21.17	6.08	5.51
	Evenness (E)	40µm	0.90	0.95	0.79	0.91
		100µm	0.91	0.91	0.80	0.88
		150µm	0.92	0.95	0.75	0.87
		UF	0.95	0.93	0.75	0.88

Table 4.14 continued

Medium	Indices	Treatments	Days			
			0		3	
			Barge	250	Barge	250
Dialysis Membrane Bags	Species Richness (S)	40µm	-	-	33	28
		100µm	-	-	36	30
		150µm	-	-	38	37
		UF	-	-	38	37
	Shannon Index (H')	40µm	-	-	4.72	4.49
		100µm	-	-	4.84	4.52
		150µm	-	-	4.97	4.82
		UF	-	-	5.05	4.85
	Simpson's Index (D)	40µm	-	-	0.95	0.94
		100µm	-	-	0.95	0.94
		150µm	-	-	0.96	0.95
		UF	-	-	0.96	0.95
	N_1 ($2^{H'}$)	40µm	-	-	26.42	22.40
		100µm	-	-	28.70	22.87
		150µm	-	-	31.36	28.33
		UF	-	-	33.18	28.82
	N_2 (D^{-1})	40µm	-	-	20.05	17.21
		100µm	-	-	21.54	17.06
		150µm	-	-	24.34	20.58
		UF	-	-	28.46	21.39
	Evenness (E)	40µm	-	-	0.95	0.93
		100µm	-	-	0.94	0.92
		150µm	-	-	0.95	0.93
		UF	-	-	0.96	0.93

Table 4.14 displays the number of species, diversity indices, and evenness values for both filtered and unfiltered ambient seawater samples obtained on the first and last days of the experiment, as well as equivalent estimates for all dialysis bags collected on the last day of bioassay. In general, increasing the filter mesh size resulted in a corresponding increase in the number of species, as expected. Unfiltered samples had the greatest species count. This trend was also seen in the species diversity indexes and evenness scores. The filtering stage prohibited some taxa from entering the dialysis bags, particularly chain-forming diatoms like *Chaetoceros* spp., which have higher cell sizes (Table 4.15 and Table 4.16). After the incubation period, the dialysis bags showed a tendency of increasing species richness with increasing filtration mesh size. While many species identified in ambient seawater were not detected within the bags, many species were observed in greater quantities than those found in ambient seawater. Overall, species diversity was significantly low in all incubated bags, as demonstrated by the *N1* and *N2* values shown in Table 4.14.

Table 4.15 Catalog of phytoplankton species detected within ambient seawater

Taxa	0. Day								3. Day							
	Station 1				Station 2				Station 1				Station 2			
	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF
Cylindrotheca closterium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitzschia longissima	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacteriastrum sp.	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Chaetoceros affine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chaetoceros decipiens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Climacosphenia elongata	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Climacosphenia moniligera		+	+	+		+	+	+	+	+	+	+			+	+
Coscinodiscus sp.	+	+	+	+	+	+	+	+		+	+	+			+	+
Hemiaulus hauckii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leptocylindrus danicus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Licmophora sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pinnularia sp.		+	+	+			+	+								
Dactyliosolen phuketensis			+	+	+	+	+	+	+	+	+	+			+	+
Guinardia flaccida	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Guinardia striata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhizosolenia sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Striatella unipunctata	+	+	+	+	+	+	+	+			+	+	+	+	+	+
Thalassionema nitzschioides			+	+	+	+	+	+	+	+	+	+			+	+
Neoceratium contrarium		+	+	+		+	+	+			+	+			+	+
Proboscia alata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoceratium tricoceros		+	+	+		+	+	+		+	+	+	+	+	+	+
Neoceratium kofoidi			+	+			+	+								
Dinophysis caudata		+	+	+	+	+	+	+		+	+	+	+	+	+	+
Gyrodinium spirale			+	+												

Table 4.15 continued

Taxa	0. Day								3. Day							
	Station 1				Station 2				Station 1				Station 2			
	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF
Gonyaulax			+	+												
Lithodesmium undulatum				+		+	+	+	+	+	+	+	+	+	+	+
Oxyphysis oxytoxoides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ditylium brightwellii	+	+	+	+		+	+	+	+	+	+	+			+	+
Lioloma	+	+	+	+					+	+	+	+			+	+
Neoceratium fusus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoceratium lineatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoceratium macroceros	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Dinophysis acuminata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corythodinium tessellatum																
Oxytoxum scolopax	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Podolampas elegans			+	+												
Podolampas palmipes																
Prorocentrum micans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Protoperidinium depressum			+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyrophacus sp.			+	+												
Grammatophora marina																
Navicula sp.			+	+	+	+	+	+	+	+	+	+				
Dactyliosolen fragilissimus			+	+					+	+	+	+	+	+	+	+
Pseudosolenia calcaravis			+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plerosigma sp				+												
Tripos furca	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tripos tricoceros			+	+	+	+	+	+								
Prorocentrum glacile	+	+	+	+	+	+			+	+	+	+	+	+	+	+
Protoperidinium steinii			+	+												

Table 4.16 Catalog of phytoplankton species detected within membrane dialysis bags

Taxa	3. Day							
	Station 1				Station 2			
	40µ	100µ	150µ	UF	40µ	100µ	150µ	UF
Cylindrotheca closterium	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+	+	+	+
Nitzschia longissima	+	+	+	+	+	+	+	+
Bacteriastrum sp.								
Chaetoceros affine	+	+	+	+				
Chaetoceros decipiens		+	+	+				
Climacosphenia elongata								
Climacosphenia moniligera								
Coscinodiscus sp.								
Hemiaulus hauckii	+	+	+	+	+	+	+	+
Leptocylindrus danicus	+	+	+	+	+	+	+	+
Licmophora sp.	+	+	+	+				
Pinnularia sp.								
Dactyliosolen phuketensis						+	+	+
Guinardia flaccida								
Guinardia striata								
Rhizosolenia sp.	+	+	+	+				
Striatella unipunctata		+	+	+				
Thalassionema nitzschioides								
Neoceratium contrarium								
Proboscia alata	+	+	+	+				
Neoceratium tricoceros								
Neoceratium kofoidi								
Dinophysis caudata								
Gyrodinium spirale								
Gonyaulax sp.								
Lithodesmium undulatum								
Oxyphysis oxytoxoides	+	+	+	+				
Ditylium brightwellii								
Lioloma								
Neoceratium fusus	+	+	+	+		+	+	+
Neoceratium lineatum								
Neoceratium macroceros								
Dinophysis acuminata								
Corythodinium tessellatum								
Oxytoxum scolopax								
Podolampas elegans								
Podolampas palmipes								
Prorocentrum micans		+	+	+				
Protoperidinium depressum								
Pyrophacus sp.								
Grammatophora marina								
Navicula sp.								
Dactyliosolen fragilissimus								
Pseudosolenia calcaravis								
Plerosigma sp		+	+	+				
Tripos furca	+	+	+	+	+	+	+	+
Tripos tricoceros								
Prorocentrum gracile	+	+	+	+				
Protoperidinium steinii								

4.3 Third Experiment

Table 4.17 displays the physical characteristics and nutrient concentrations of both the ambient seawater and the water contained within the dialysis membrane bags. Throughout the study period, the seawater temperature consistently ranged between 24.3 and 24.9 ℃. Dissolved oxygen, pH, and salinity levels remained consistent. Secchi depth exhibited minor fluctuations, ranging from 24.3 to 25 meters. According to observations and records provided by the fish farm staff, average wave heights remained below 1 meter throughout the experiment period. The nutrient concentrations in both the ambient seawater and within the bags were highly similar (Independent *t*-test, for nitrite+nitrate, $t = 0.68$, df = 12, $P = 0.51$; for orthophosphate, $t = 1.08$, df = 12, $P = 0.30$; for silicate, $t = 0.09$, df = 12, $P = 0.93$) and there were no notable temporal fluctuations observed in any of the nutrients (Linear regression, for nitrite+nitrate, $r^2 = 0.08$, $F = 2.26$, df =1, 12, P = 0.16; for orthophosphate, $r^2 = 0.05$, $F = 0.27$, df = 1, 12, $P = 0.60$; for silicate, $r^2 = 0.04$, $F = 0.48$, df = 1, 12, $P = 0.50$).

Table 4.17 Daily values of the physical properties and nutrients of the ambient seawater and values of the nutrients inside the bags

Medium	Variables		Days						
			0	1	2	3	4	5	6
Ambient Seawater	Physical Properties	Temperature (℃)	24.30	24.40	24.40	24.50	24.90	24.70	24.80
		Salinity (ppt)	39.50	39.40	39.50	39.40	39.50	39.50	39.40
		Dissolved Oxygen (mg/l)	7.80	7.80	7.90	7.80	7.80	7.90	8.00
		pH	8.20	8.20	8.20	8.10	8.20	8.10	8.10
		Secchi Depth (m)	24.30	24.50	23.90	24.70	25.00	24.60	24.40
	Nutrients	Nitrite+ Nitrate (μM)	0.23	0.24	0.28	0.25	0.33	0.25	0.26
		Orthophosphate (μM)	0.03	0.01	0.02	0.01	0.03	0.02	0.02
		Silicate (μM)	0.32	0.16	0.22	0.32	0.2	0.25	0.18
Dialysis Membrane Bags	Nutrients	Nitrite+ Nitrate (μM)	-	0.26	0.29	0.26	0.33	0.27	0.27
		Orthophosphate (μM)	-	0.02	0.02	0.01	0.04	0.02	0.03
		Silicate (μM)	-	0.18	0.19	0.33	0.25	0.23	0.19

Table 4.18 displays chl *a* concentrations and phytoplankton cell numbers obtained from both unfiltered and filtered ambient seawater, as well as from unfiltered and filtered bags. There was no noticeable growth observed in the phytoplankton

population, as indicated by chl *a* concentrations and phytoplankton cell numbers in both unfiltered and filtered ambient seawater. In other words, estimates of the net growth rate (r_{net}) did not significantly deviate from 0 (Linear regression, for chl *a* concentrations in the unfiltered seawater, r^2=0.07, F=1.45, df=1, 5, P=0.28; in the filtered seawater, r^2=0.09, F=1.57, df=1, 5, P=0.27; for cell numbers in the unfiltered seawater, r^2=0.19, F=2.43, df= 1, 5, P=0.18; in the filtered seawater, r^2=0.32, F=3.80, df=1, 5, P=0.11).

In the first two days of the incubation period, t the contents of both the unfiltered and filtered bags did not show any recognizable growth response in terms of cell numbers, even though the chl *a* concentrations increased (Table 4.18). Following two days of incubation, on the third day of the experiment, an elevation in cell numbers was noted, accompanied by a more noticeable increase in chl *a* concentrations (Table 4.18). After six days of incubation, the chl *a* concentrations surged by 82-fold in the unfiltered bags and by more than 100-fold in the filtered bags compared to levels observed in the ambient water. The phytoplankton community composition and growth underwent significant changes, with chl *a* concentrations reaching 4.92 µg L^{-1} for unfiltered bags and 5.07 L^{-1} for filtered bags, compared to initial concentrations of only 0.06 µg L^{-1} and 0.05 µg L^{-1}, respectively (Table 4.18). The filtration treatment (<150 µm) resulted in the removal of certain taxa, particularly chain-forming diatoms like *Chaetoceros* spp., due to their size. Nevertheless, despite the initial concentrations being different, the final chl *a* concentrations in the filtered and unfiltered bags showed comparable levels after the incubation period (Table 4.18). A similar growth pattern was also observed with cell numbers.

The overall population growth rates of phytoplankton, as measured by chl *a* concentrations, within the filtered and unfiltered bags were 0.653 ± 0.056 (rate ± standard error) and 0.686 ± 0.051 d^{-1}, respectively. However, there was no statistically significant difference between these two growth rate estimates. (ANCOVA, F=0.20, df=1, P=0.66). Therefore, the common growth (gross) rate (r_{gross}) calculated for the bags was 0.669 ± 0.037 d^{-1}. that the net growth rate (r_{net}) estimates of the phytoplankton communities based on chl *a* concentrations was 0, the loss growth rate (r_{loss}) was precisely equivalent to the gross rate (r_{gross}) mentioned previously.

Table 4.18 Daily estimates of chl a concentrations and phytoplankton cell numbers from both the unfiltered (UF) and filtered (F) ambient seawater, as well as from the unfiltered and filtered bags. Results reported for bags are the mean values of the two sampled bags for each treatment per day

Medium	Growth Indicator	Days													
		0		1		2		3		4		5		6	
		UF	F	UF	F	UF	F	UF	F	UF	F	UF	F	UF	F
Bags	chl *a* µg L^{-1}	0.06	0.05	0.34	0.31	0.34	0.68	0.73	1.15	2.11	2.14	4.24	3.39	4.92	5.07
	Cells L^{-1}	228000	144000	-	-	-	-	380117	437340	1545455	1548492	3692308	2422288	7963636	8529412
Ambient Seawater	chl *a* µg L^{-1}	0.06	0.05	0.06	0.05	0.06	0.05	0.06	0.04	0.05	0.03	0.05	0.03	0.06	0.05
	Cells L^{-1}	228000	144000	161497	114105	152510	133390	167220	119720	149700	105630	139460	100900	171040	118730

The growth rates estimated from the growth model based on the phytoplankton cell numbers for the filtered and unfiltered bags were 0.703 ± 0.090 and 0.669 ± 0.113 d^{-1}, respectively. Again, the ANCOVA test of interaction revealed no significant difference between the slopes of the filtered and unfiltered bag groups (ANCOVA, F=0.06, df=1, 14, P=0.82). The estimated common growth (gross) rate (r_{gross}) was, therefore, 0.686 ± 0.069 d^{-1} (Table 4.19), which was equivalent to a 98% daily increase in cell numbers in the untransformed scale. The two phytoplankton population growth rates estimated from two different dependent variables, i.e., chl *a* concentrations and cell numbers, were almost identical. Pearson's product-moment correlation coefficient between these two variables was 0.90 (t-test, t=8.23, df= 16, P<0.001).

Microscopic investigations of the phytoplankton composition in daily samples from the research location revealed the presence of 30 phytoplankton species, including 19 diatoms and 11 dinoflagellates (Tables 4.21 and 4.22). The natural phytoplankton population was dominated by diatoms, particularly taxa from the families Leptocylindraceae and Bacillariaceae. Dinoflagellates were predominantly comprised of the Neoceratium genus. Analysis of the dialysis membrane bags' contents indicated that while certain species present in the surrounding seawater vanished, others exhibited higher cell concentrations compared to those found in the ambient water. Following the incubation period, *Guinardia flaccida, G. striata, Dinophysis acuminata*, *Corythodinium tesselatum*, *Padolampas elegans*, *P. palmipes*, and *Protoperidinium depressum* were no longer present in the bags (Table 4.22). Nevertheless, *Leptocylindrus danicus, Nitzschia longissima*, *Cylindrotheca closterium* and *Hemiaulus hauckii* experienced exponential growth within all bags following the initial two days of the incubation period (Figure 4.3). Significant changes in the phytoplankton communities within the bags were noted in the subsequent days. From day three to six of the experiment, *L. danicus* emerged as the predominant contributor to the phytoplankton community. The proportion of this species steadily rose each day, reaching 53% of the total phytoplankton cell count in the filtered bags and 27% in the unfiltered bags by the end of the bioassay. The growth rates of the four prevalent phytoplankton species, namely *L. danicus*, *N. longissima*, *C. closterium,* and *H. hauckii*, were also computed using the cell numbers collected from both the filtered and unfiltered bags. As the ANCOVA tests showed no notable distinction in slopes

between the filtered and unfiltered bag groups for any species, overall rates (rgross) were determined from combined data (Table 4.19).

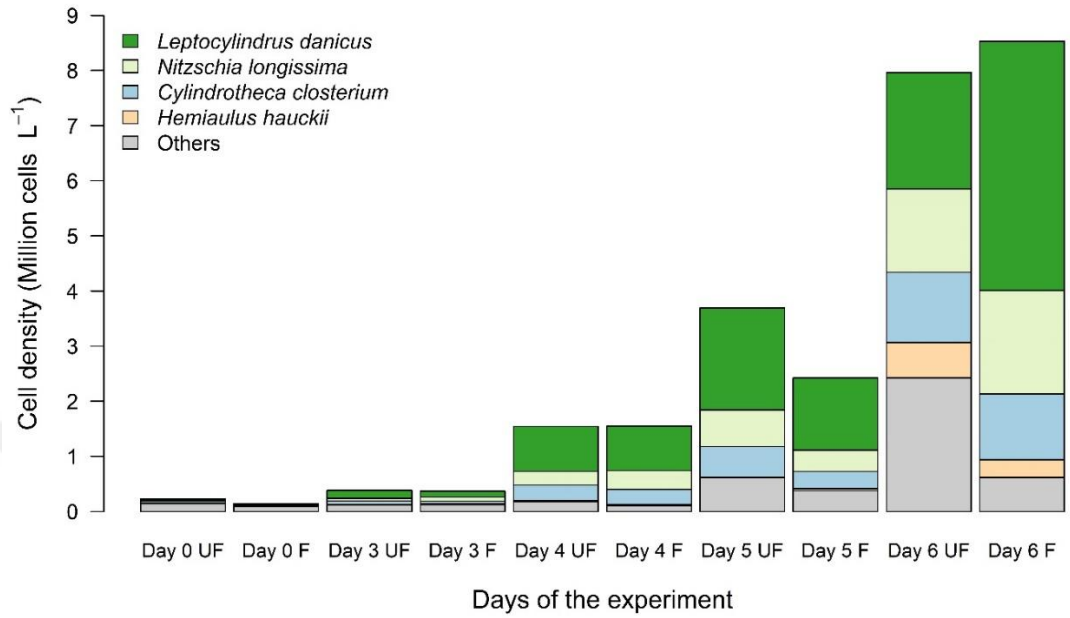


Figure 4.3 Variations in the cell densities (number of cells L-1 in millions) and relative contributions of the more abundant phytoplankton species over the experiment period inside the unfiltered (UF) and filtered (F) dialysis membrane bags. The cell densities are the mean values of the two replicate bags for each treatment per day.

The zooplankton community in the ambient seawater mainly consisted of the microzooplankton ciliate protozoans and copepod nauplii and the mesozooplankton adult copepods (Table 5). Copepods were scarce (< 12 individuals L^{-1}), whereas ciliates were more abundant (< 30 individuals L^{-1}). Adult copepods were absent in the bags, but copepod nauplii were present in both the unfiltered and filtered bags (< 4 individuals L^{-1}). As in the ambient seawater, ciliates (Table 6) were abundant inside the bags (< 10 individuals L^{-1}).

Table 4.19 Growth rates derived from phytoplankton cell numbers recorded in both the ambient seawater and within the dialysis membrane bags, encompassing all species as well as the four predominant species within the bags. The asterisk (*) indicates an estimate that is not significantly different from zero

Taxa	r_{gross} ± SD (d^{-1})	r_{net} ± SD (d^{-1})	r_{loss} (d^{-1})
All species	0.69 ± 0.07	-0.04* ± 0.03	0.69
L. danicus	0.85 ± 0.08	-0.08 ± 0.03	0.92
N. longissima	0.93 ± 0.05	0.02* ± 0.02	0.93
C. closterium	0.63 ± 0.09	-0.07 ± 0.03	0.70
H. hauckii	0.47 ± 0.17	-0.08* ± 0.04	0.47

Table 4.20 depicts the estimated values of the phytoplankton species richness, various diversity indices, and evenness observed in the unfiltered and filtered ambient seawater as well as inside the unfiltered and filtered dialysis membrane bags for each day during the experiment period. Species richness, species diversity, and evenness showed very minor daily fluctuations in the ambient seawater throughout the experiment. The differences between the daily estimated values for all indices for the unfiltered and filtered ambient seawater were all negligible (Table 4.20). Species richness and species diversity inside the bags, however, showed drastic variations and decreased substantially during the experiment. While a total of 30 and 29 phytoplankton species were present on the first day in the unfiltered and filtered bags, respectively, only 8 phytoplankton species were found in the bags on the final day. The same trend can be seen for species diversity, especially from Hill's numbers, N1 and N2, presented in Table 4.20.

Table 4.20 Daily observations of phytoplankton species richness and species diversity in both the unfiltered (UF) and filtered (F) ambient seawater, as well as in the unfiltered and filtered dialysis membrane bags, throughout the experiment duration

Medium	Indices	Treatments	Days						
			0	1	2	3	4	5	6
Ambient Sea Water	Species richness (*S*)	UF	30	27	28	28	28	27	29
		F	29	27	27	27	27	26	27
	Shannon Index (*H'*)	UF	4.21	4.03	4.20	4.39	4.47	4.37	4.41
		F	4.09	3.99	4.22	4.26	4.30	4.24	4.29
	Simpson's Index (*D*)	UF	0.93	0.91	0.92	0.94	0.94	0.94	0.94
		F	0.92	0.91	0.93	0.93	0.93	0.93	0.94
	N_1 ($2^{H'}$)	UF	18.46	16.31	18.33	21.02	22.09	20.61	21.27
		F	17.04	15.84	18.61	19.15	19.68	18.90	19.61
	N_2 (D^{-1})	UF	14.05	11.14	12.53	15.94	17.50	16.19	16.17
		F	12.27	11.12	14.68	13.69	14.88	14.71	15.60
	Evenness (*E*)	UF	0.86	0.85	0.87	0.91	0.93	0.92	0.91
		F	0.84	0.84	0.89	0.90	0.90	0.90	0.90
Dialysis Membrane Bags	Species richness (*S*)	UF	-	-	-	16	13	8	8
		F	-	-	-	18	12	8	8
	Shannon Index (*H'*)	UF	-	-	-	3.06	2.14	2.12	2.76
		F	-	-	-	3.33	1.95	2.04	1.90
	Simpson's Index (*D*)	UF	-	-	-	0.81	0.66	0.69	0.83
		F	-	-	-	0.85	0.65	0.66	0.65
	N_1 ($2^{H'}$)	UF	-	-	-	8.35	4.41	4.34	6.79
		F	-	-	-	10.04	3.85	4.11	3.74
	N_2 (D^{-1})	UF	-	-	-	5.20	2.93	3.19	6.04
		F	-	-	-	6.63	2.84	2.91	2.83
	Evenness (*E*)	UF	-	-	-	0.77	0.58	0.71	0.92
		F	-	-	-	0.80	0.54	0.68	0.63

Table 4.21 Catalog of phytoplankton species observed in the samples of seawater from the ambient seawater

Taxa	Days									
	0					3				
	40µm	56µm	100µm	150µm	UF	40µm	56µm	100µm	150µm	UF
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin, 1964	+	+	+	+	+	+	+	+	+	+
Nitzschia longissima (Brébisson) Ralfs, 1861	+	+	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+			+	+	+
Chaetoceros affinis Lauder, 1864		+	+	+	+	+	+	+	+	+
Chaetoceros decipiens Cleve, 1873							+	+	+	+
Grammatophora marina (Lyngbye) Kützing, 1844				+	+					
Hemiaulus hauckii Grunow ex Van Heurck, 1882	+	+	+	+	+	+	+	+	+	+
Leptocylindrus danicus Cleve, 1889	+	+	+	+	+	+	+	+	+	+
Licmophora sp.		+	+	+	+			+	+	+
Navicula sp.	+	+	+	+	+	+		+	+	+
Dactyliosolen fragilissimus (Bergon) Hasle, 1996	+	+	+	+	+		+	+	+	+
Guinardia flaccida (Castracane) H.Peragallo, 1892					+		+	+	+	+
Guinardia striata (Stolterfoth) Hasle, 1996	+	+	+	+	+		+	+	+	+
Pseudosolenia calcar-avis (Schultze) B.G.Sundström, 1986				+	+					
Rhizosolenia sp.				+	+					
Pleurosigma sp.			+	+	+	+	+	+	+	+
Thalassionema nitzschioides (Grunow) Mereschkowsky, 1902					+					

Table 4.21 continued

Taxa	Days									
	0					3				
	40µm	56µm	100µm	150µm	UF	40µm	56µm	100µm	150µm	UF
Tripos fusus (Ehrenberg) F.Gómez, 2013		+	+	+	+	+	+	+	+	+
Tripos furca (Ehrenberg) F.Gómez, 2013						+	+	+	+	+
Tripos lineatus (Ehrenberg) F.Gómez, 2021					+					
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman, 2020		+	+	+	+					
Tripos trichoceros (Ehrenberg) Gómez, 2013				+	+					
Dinophysis acuminata Claparède & Lachmann, 1859		+	+	+	+	+		+	+	+
Oxyphysis oxytoxoides (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011		+	+	+	+			+	+	+
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III, 1966					+					
Oxytoxum scolopax F. Stein, 1883		+	+	+	+	+	+	+	+	+
Podolampas elegans F.Schütt, 1895				+	+					
Podolampas palmipes F. Stein, 1883						+	+	+	+	+
Prorocentrum gracile F.Schütt, 1895			+	+	+					+
Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+	+	+	+	+	+
*Pronoctiluca spinifera (*Lohmann) Schiller, 1932					+					
Protoperidinium depressum (Bailey) Balech, 1974				+	+					
Protoperidinium steinii (Jørgensen) Balech, 1974								+	+	+

Table 4.22 Catalog of phytoplankton species observed in the samples of seawater from the dialysis membrane bags

Taxa	Day 3				
	40μm	**56μm**	**100μm**	**150μm**	**UF**
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin, 1964			+	+	+
Nitzschia longissima (Brébisson) Ralfs, 1861		+	+	+	+
Pseudo-nitzschia sp.			+	+	+
Chaetoceros affinis Lauder, 1864		+	+	+	+
Chaetoceros decipiens Cleve, 1873					
Grammatophora marina (Lyngbye) Kützing, 1844			+	+	+
Hemiaulus hauckii Grunow ex Van Heurck, 1882	+	+	+	+	+
Leptocylindrus danicus Cleve, 1889	+	+	+	+	+
Licmophora sp.					+
Navicula sp.					
Dactyliosolen fragilissimus (Bergon) Hasle, 1996					+
Guinardia flaccida (Castracane) H.Peragallo, 1892					
Guinardia striata (Stolterfoth) Hasle, 1996					
Pseudosolenia calcar-avis (Schultze) B.G.Sundström, 1986					
Rhizosolenia sp.				+	+
Pleurosigma sp.					
Thalassionema nitzschioides (Grunow) Mereschkowsky, 1902			+	+	+

Table 4.22 continued

Taxa	**Day 3**				
	40µm	**56µm**	**100µm**	**150µm**	**UF**
Tripos fusus (Ehrenberg) F.Gómez, 2013				+	+
Tripos furca (Ehrenberg) F.Gómez, 2013		+	+	+	+
Tripos lineatus (Ehrenberg) F.Gómez, 2021	+	+	+	+	+
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman, 2020					+
Tripos trichoceros (Ehrenberg) Gómez, 2013					
Dinophysis acuminata Claparède & Lachmann, 1859					
Oxyphysis oxytoxoides (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011				+	+
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III, 1966					
Oxytoxum scolopax F. Stein, 1883				+	+
Podolampas elegans F.Schütt, 1895					
Podolampas palmipes F. Stein, 1883					
Prorocentrum gracile F.Schütt, 1895			+	+	+
Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+
Pronoctiluca spinifera (Lohmann) Schiller, 1932					
Protoperidinium depressum (Bailey) Balech, 1974					
Protoperidinium steinii (Jørgensen) Balech, 1974					

4.4 Fourth Experiment

In the fourth experiment, an additional station located 1000 meters away from the farm, serving as a control, was introduced. Table 4.23, Table 4.24 and Table 4.25 display the physical characteristics of both the ambient seawater and the water inside the dialysis membrane bags collected from various stations. The seawater temperature maintained consistency within the range of 21.5-22.3 ℃ throughout the study period. Dissolved oxygen levels, pH, and salinity also remained stable. Secchi depth exhibited minor fluctuations ranging from 24.6 to 25.2 meters. Based on observations and reports provided by fish farm personnel, average wave heights remained low (less than 1 meter) throughout the experiment duration.

Table 4.23 Physical properties of the ambient seawater at Station 1

	Physical Properties				
Days	**Temperature (℃)**	**Salinity (ppt)**	**Dissolved Oxygen (mg/l)**	**pH**	**Secchi Depth (m)**
0	22.3	39.2	7.9	8.1	25.2
5	21.6	39.2	7.8	8.2	25.3

Table 4.24 Physical properties of the ambient seawater at Station 2

	Physical Properties				
Days	**Temperature (℃)**	**Salinity (ppt)**	**Dissolved Oxygen (mg/l)**	**pH**	**Secchi Depth (m)**
0	22.2	39.3	7.9	8.2	24.6
5	21.6	39.2	8	8.1	24.8

Table 4.25 Physical properties of the ambient seawater at Control Station

	Physical Properties				
Days	**Temperature (℃)**	**Salinity (ppt)**	**Dissolved Oxygen (mg/l)**	**pH**	**Secchi Depth (m)**
0	22.3	39.3	8	8.2	25.6
5	21.5	39.3	8	8.2	25.8

Table 4.26, Table 4.27 and Table 4.28 showcases the nutrient compositions of both the ambient seawater and the water collected from the dialysis membrane bags across all stations. The nutrient compositions in the ambient seawater and the bags were

notably similar across all stations, and there were no notable temporal changes observed for any of the nutrients listed in the table.

The results showed significantly lower growth rates at this station compared to those observed at 250 meters, indicating a further reduction in nutrient concentration and negligible impact on phytoplankton growth. Additionally, the growth rates in bags filled with unfiltered seawater were lower at both stations compared to those at the Station 1, further supporting the role of farm-derived nutrients in promoting phytoplankton growth.

Table 4.26 Nutrient concentrations at Station 1 from both ambient seawater and dialysis membrane bags

Medium	Nutrients	Days					
		0			5		
		40µm	150µm	UF	40µm	150µm	UF
Ambient Seawater	Nitrite+ Nitrate (µM)	0.32	0.3	0.28	0.49	0.46	0.39
	Orthophosphate (µM)	0.02	0.03	0.04	0.04	0.03	0.02
	Silicate (µM)	0.33	0.37	0.32	0.62	0.53	0.61
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	0	42	0.37
	Orthophosphate (µM)	-	-	-	0.03	0.04	0.055
	Silicate (µM)	-	-	-	0.43	0.41	0.52

Table 4.27 Nutrient concentrations at Station 2 from both ambient seawater and dialysis membrane bags

Medium	Nutrients	Days					
		0			5		
		40µm	150µm	UF	40µm	150µm	UF
Ambient Seawater	Nitrite+ Nitrate (µM)	0.25	0.32	0.31	0.4	0.41	0.38
	Orthophosphate (µM)	0.02	0.03	0.04	0.03	0.03	0.02
	Silicate (µM)	0.37	0.34	0.4	0.57	0.52	0.6
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	0.58	0.55	0.47
	Orthophosphate (µM)	-	-	-	0.03	0.025	0.05
	Silicate (µM)	-	-	-	0.48	0.47	0.52

Table 4.28 Nutrient concentrations at Control Station from both ambient seawater and dialysis membrane bags

Medium	Nutrients	Days					
		0			5		
		40µm	150µm	UF	40µm	150µm	UF
Ambient Seawater	Nitrite+ Nitrate (µM)	0.28	0.27	0.3	0.43	0.41	0.4
	Orthophosphate (µM)	0.03	0.04	0.03	0.04	0.04	0.02
	Silicate (µM)	0.3	0.34	0.38	0.66	0.69	0.58
Dialysis Membrane Bags	Nitrite+ Nitrate (µM)	-	-	-	0.51	0.56	0.6
	Orthophosphate (µM)	-	-	-	0.03	0.03	0.04
	Silicate (µM)	-	-	-	0.53	0.55	0.6

Table 4.29 Diversity indices

Medium	Indices	Treatments	Days					
			0			5		
			Station 1	Station 2	Control Station	Station 1	Station 2	Control Station
Ambient Sea Water	Species richness (S)	40	29	28	26	27	28	25
		150	28	34	35	37	31	28
		UF	31	39	41	45	34	28
	Shannon Index (H')	40	4.54	4.55	4.44	4.42	4.40	4.27
		150	4.58	4.75	4.69	4.79	4.61	4.40
		UF	4.66	4.87	4.96	5.09	4.71	4.48
	Simpson's Index (D)	40	0.94	0.94	0.94	0.94	0.93	0.93
		150	0.94	0.95	0.94	0.95	0.95	0.94
		UF	0.95	0.95	0.95	0.96	0.95	0.94
	N_1 ($2^{H'}$)	40	23.21	23.38	21.78	21.41	21.10	19.29
		150	23.84	26.95	25.76	27.76	24.48	21.11
		UF	25.22	29.22	31.11	33.95	26.24	22.27
	N_2 (D^{-1})	40	17.41	18.24	17.10	16.23	15.17	14.64
		150	17.31	19.91	17.54	19.54	18.71	15.82
		UF	19.10	20.29	21.26	22.71	18.70	17.00
	Evenness (E)	40	0.93	0.95	0.95	0.93	0.91	0.92
		150	0.92	0.93	0.91	0.92	0.93	0.91
		UF	0.94	0.92	0.93	0.93	0.93	0.93

Table 4.29 contiuned

Medium	Indices	Treatments	Days					
			0			5		
			Station 1	Station 2	Control Station	Station 1	Station 2	Control Station
Dialysis Membrane Bags	Species richness (S)	40	-	-	-	7	5	7
		150	-	-	-	7	7	8
		UF	-	-	-	7	7	8
	Shannon Index (H')	40	-	-	-	2.61	2.14	2.75
		150	-	-	-	2.10	2.41	2.90
		UF	-	-	-	2.25	2.37	2.90
	Simpson's Index (D)	40	-	-	-	0.82	0.74	0.84
		150	-	-	-	0.66	0.77	0.86
		UF	-	-	-	0.71	0.76	0.85
	N_1 ($2^{H'}$)	40	-	-	-	6.10	4.41	6.73
		150	-	-	-	4.30	5.32	7.48
		UF	-	-	-	4.74	5.18	7.45
	N_2 (D^{-1})	40	-	-	-	5.55	3.91	6.45
		150	-	-	-	2.94	4.40	6.99
		UF	-	-	-	3.49	4.18	6.82
	Evenness (E)	40	-	-	-	0.93	0.92	0.98
		150	-	-	-	0.75	0.86	0.97
		UF	-	-	-	0.80	0.84	0.97

Table 4.29 displays the number of species, diversity indices, and evenness values for both filtered and unfiltered ambient seawater samples obtained on the first and last days of the experiment, as well as equivalent estimates for all dialysis bags collected on the last day of bioassay. In general, increasing the filter mesh size resulted in a corresponding increase in the number of species, as expected. Unfiltered samples had the greatest species count. This trend was also seen in the species diversity indexes and evenness scores. The filtering stage prohibited some taxa from entering the dialysis bags, particularly chain-forming diatoms like *Chaetoceros* spp., which have higher cell sizes (Table 4.30 and Table 31). After the incubation period, the dialysis bags showed a tendency of increasing species richness with increasing filtration mesh size.

Table 4.30 Catalog of phytoplankton species observed in the samples of ambient seawater

Taxa	0. Day									3. Day								
	Station 1			Station 2			Control Station			Station 1			Station 2			Control Station		
	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF
Cylindrotheca closterium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitzschia longissima	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacteriastrum sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chaetoceros affine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chaetoceros decipiens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Climacosphenia elongata		+	+		+	+			+		+	+						
Climacosphenia moniligera		+	+															
Coscinodiscus sp.								+	+		+	+				+	+	+
Hemiaulus hauckii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leptocylindrus danicus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Licmophora sp.									+		+	+		+	+			
Pinnularia sp.					+	+			+									
Dactyliosolen phuketensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Guinardia flaccida	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+
Guinardia striata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhizosolenia sp.	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Striatella unipunctata	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thalassionema nitzschioides	+	+	+		+	+		+	+		+	+	+	+	+	+	+	+
Neoceratium contrarium	+	+	+			+	+	+	+		+	+		+	+			
Proboscia alata				+	+	+		+	+	+	+	+	+	+	+	+	+	+
Neoceratium tricoceros	+	+	+				+	+	+	+	+	+	+	+	+	+	+	+
Neoceratium kofoidi					+	+		+	+		+	+						
Dinophysis caudata	+	+	+	+	+	+	+	+	+			+	+	+	+		+	+
Gyrodinium spirale									+			+						
Gonyaulax												+			+			

Table 4.30 continued

Taxa	0. Day									3. Day								
	Station 1			Station 2			Control Station			Station 1			Station 2			Control Station		
	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF
Lithodesmium undulatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxyphysis oxytoxoides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ditylium brightwellii	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+
Lioloma									+						+			
Neoceratium fusus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoceratium lineatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+
Neoceratium macroceros	+	+	+	+	+	+	+	+	+	+	+	+		+	+			
Dinophysis acuminata	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+
Corythodinium tessellatum						+		+	+			+						
Oxytoxum scolopax	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Podolampas elegans						+		+	+		+	+						
Podolampas palmipes					+	+					+	+						
Prorocentrum micans	+	+	+	+	+	+				+	+	+	+	+	+			
Protoperidinium depressum									+		+	+						
Pyrophacus sp.												+						
Grammatophora marina						+						+						
Navicula sp												+						
Dactyliosolen fragilissimus				+	+	+	+	+	+	+	+	+	+	+	+			
Pseudosolenia calcaravis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Plerosigma sp												+						
Tripos furca	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tripos tricoceros	+	+	+	+	+	+		+	+	+	+	+				+	+	+
Prorocentrum glacile					+	+	+	+	+				+	+	+	+	+	+
Protoperidinium steinii						+												

Table 4.31 Catalog of phytoplankton species observed in the samples of seawater from the dialysis membrane bags

Taxa	5. Day								
	Station 1			Station 2			Control Station		
	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF
Cylindrotheca closterium	+	+	+	+	+	+	+	+	+
Pseudo-nitzschia sp.	+	+	+	+	+	+	+	+	+
Nitzschia longissima	+	+	+	+	+	+	+	+	+
Bacteriastrum sp.									
Chaetoceros affine	+	+	+				+	+	+
Chaetoceros decipiens					+	+			
Climacosphenia elongata									
Climacosphenia moniligera									
Coscinodiscus sp.									
Hemiaulus hauckii	+	+	+	+	+	+	+	+	+
Leptocylindrus danicus	+	+	+	+	+	+	+	+	+
Licmophora sp.									
Pinnularia sp.									
Dactyliosolen phuketensis							+	+	+
Guinardia flaccida									
Guinardia striata									
Rhizosolenia sp.									
Striatella unipunctata									
Thalassionema nitzschioides									
Neoceratium contrarium									
Proboscia alata									
Neoceratium tricoceros									
Neoceratium kofoidi									
Dinophysis caudata									
Gyrodinium spirale									

Table 4.31 continued

Taxa	5. Day								
	Station 1			Station 2			Control Station		
	40µ	150µ	UF	40µ	150µ	UF	40µ	150µ	UF
Gonyaulax									
Lithodesmium undulatum									
Oxyphysis oxytoxoides									
Ditylium brightwellii									
Lioloma									
Neoceratium fusus								+	+
Neoceratium lineatum									
Neoceratium macroceros									
Dinophysis acuminata									
Corythodinium tessellatum									
Oxytoxum scolopax									
Podolampas elegans									
Podolampas palmipes									
Prorocentrum micans	+	+	+						
Protoperidinium depressum									
Pyrophacus sp.									
Grammatophora marina									
Navicula sp.									
Dactyliosolen fragilissimus									
Pseudosolenia calcaravis									
Plerosigma sp.									
Tripos furca									
Tripos tricoceros					+	+			
Prorocentrum glacile									
Protoperidinium steinii									

4.5 Light microscopy images of some phytoplankton species

Some examples of microscopy images captured during phytoplankton cell enumeration and identification (Figure 4.4, Figure 4.5, Figure 4.6, Figure 4.7, Figure 4.8, Figure 4.9, Figure 4.10, and Figure 4.11). The scale bars featured in the photographs denote a length of 25 µm.



Figure 4.4 *Corythodinium tessellatum* (Personal archive, 2020)



Figure 4.5 Binary fission of a *Coscinodiscus* spp. (Personal arhive, 2020)

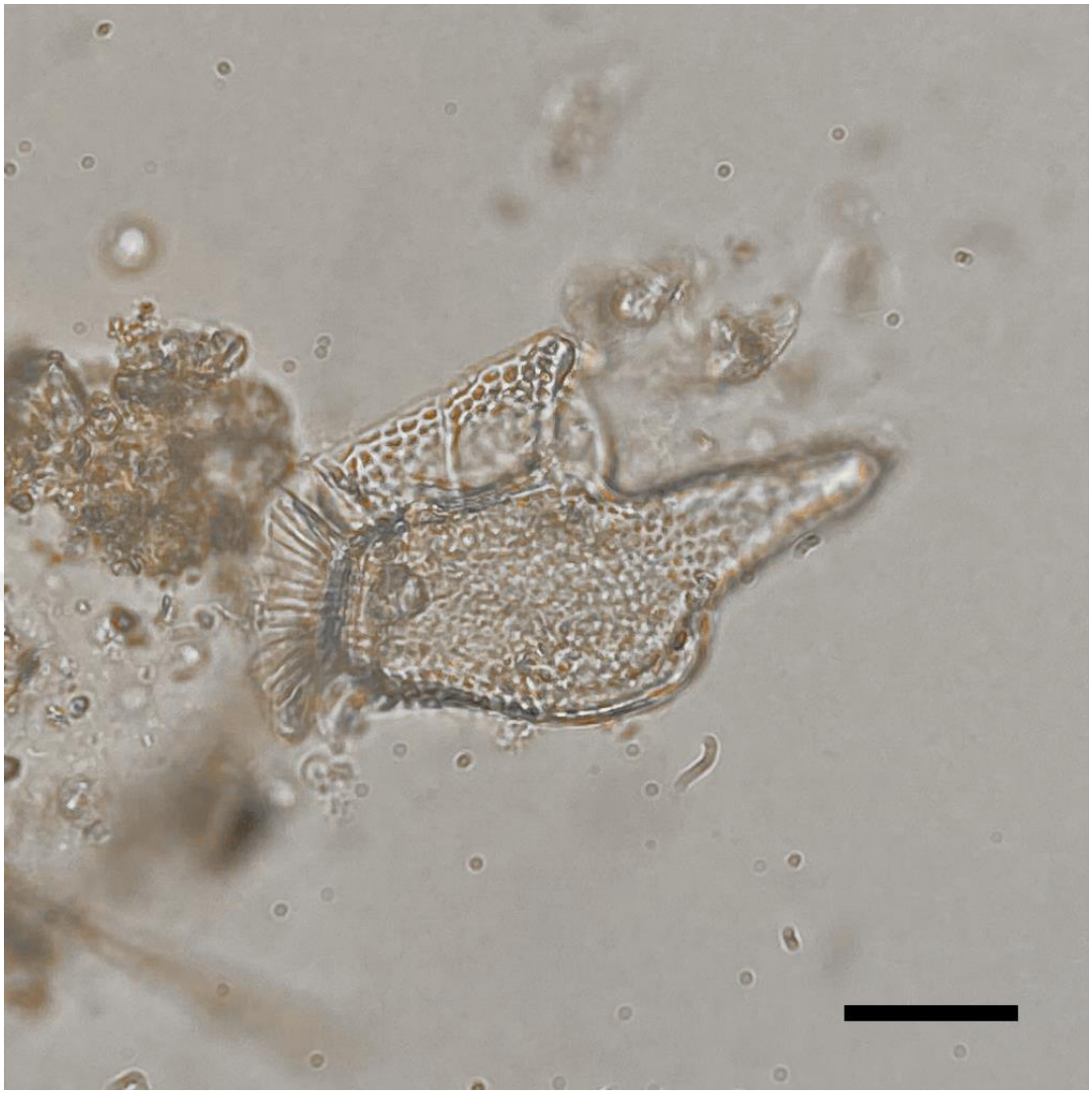


Figure 4.6 *Dinophysis caudata* (Personal archive, 2020)



Figure 4.7 *Hemiaulus hauckii* (Personal archive, 2020)



Figure 4.8 *Leptocylindrus danicus* (Chain forming) (Personal archive, 2020)



Figure 4.9 *Lichmophora* sp. on *Obelia geniculata* (Personal archive, 2020)



Figure 4.10 *Tripos furca* (Personal archive, 2020)

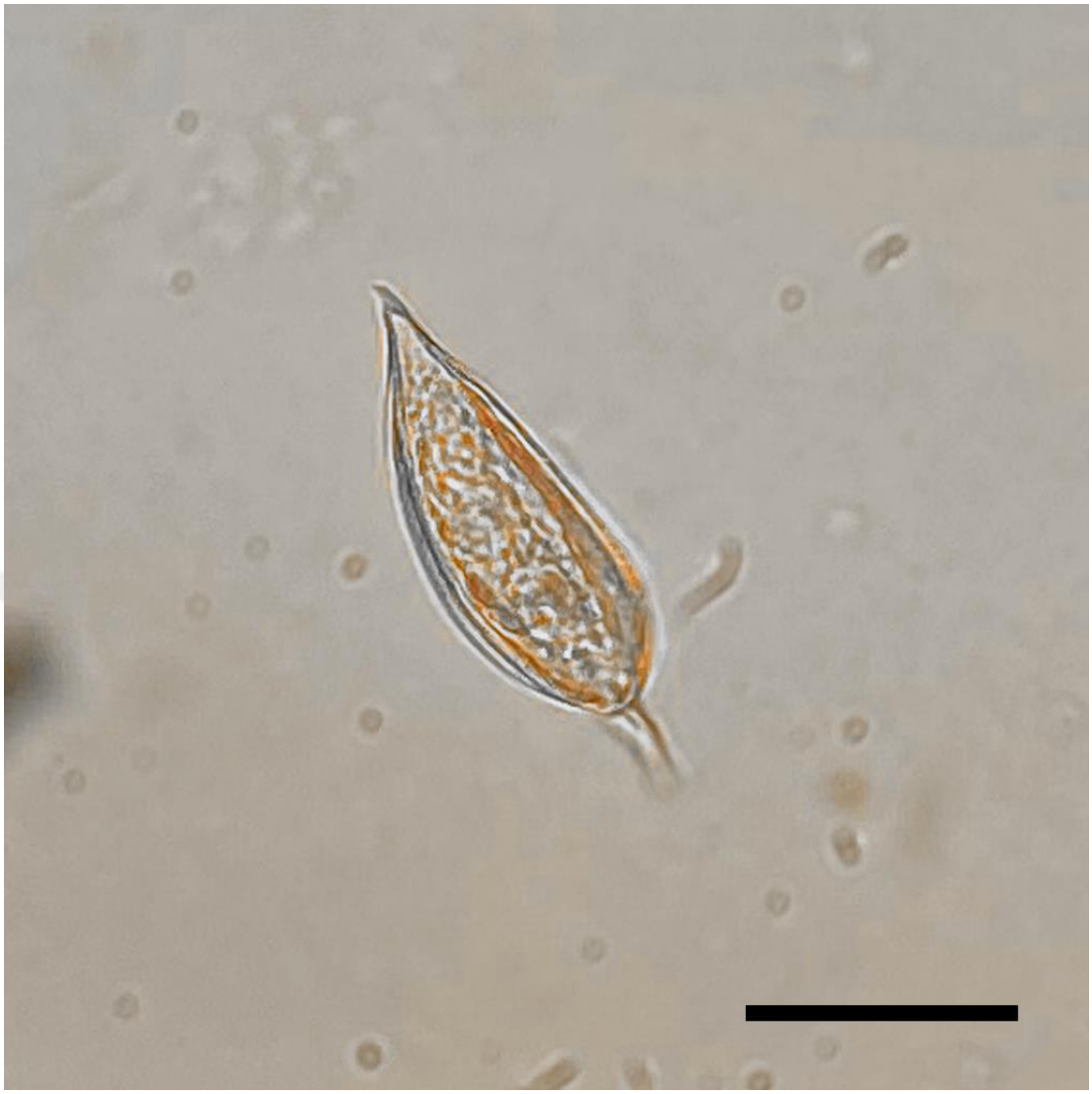


Figure 4.11 *Prorocentrum gracile* (Personal archive, 2020)

CHAPTER FIVE
DISCUSSION

Investigating the water quality of marine environments is of great importance in aquaculture. The degree of eutrophication in cultivation areas is determined by the chemical, biological, and physical characteristics of the water, as well as the aquaculture method and the scale of production (Gençay & Büyükışık, 2006). Many studies have been conducted to measure the environmental impacts of fish farms. However, most studies that rely solely on measuring nutrient levels have not been able to reach significant conclusions about the significant differences in the effects of fish farms on surrounding waters. In alternative studies based on the use of phytoplankton as bioindicator organisms, the composition and abundance of phytoplankton species in samples taken at regular intervals from the water column have been examined, but no significant differences have been found between samples taken from farm and reference points. In Turkey, studies on the effects of fish farm waste on the environment have generally focused on benthic communities, while studies on the water column have been limited. To address these issues, four experiments conducted between August and October 2020, aimed to assess the spatial distribution of nutrient gradients and their influence on biological productivity in coastal waters adjacent to the fish farm. Additionally, the study aimed to investigate natural interactions within phytoplankton communities, involving predator-prey dynamics that influence the net growth rates of phytoplankton. To explore different grazing pressures on the incubated phytoplankton, five experimental groups were established, comprising seawater filtered through various mesh sizes (40 µm, 56 µm, 100 µm, and 150 µm) and unfiltered seawater. Variable growth rates were noted among these groups as a result of species interactions, including prey-predator interactions

The second experiment, initiated on August 11, 2020, built upon the findings of the first experiment by replicating its design with slight modifications. Notably, the treatment involving 56 µm mesh filtered sea water was omitted due to its similarity to the results obtained with bags filled with 40 µm mesh filtered sea water. Furthermore, a new station positioned approximately 250 meters away from the original experiment site was introduced to detect nutrient gradients near the fish farm. This addition

enabled a more comprehensive assessment of nutrient dispersal patterns in the vicinity of the aquaculture facility. Within these stations, multiple treatments were implemented to evaluate varying predation pressures, providing valuable insights into the ecological responses of marine organisms to nutrient enrichment. The third experiment had a different objective, focusing on daily changes within the bags over a six-day period. The fourth experiment expanded the scope by adding a Control Station, situated 1000 meters away from Station One, to evaluate the extent of nutrient effects. These experiments collectively aimed to understand the influence of nutrient gradients on phytoplankton growth rates and to assess predator-prey dynamics within coastal waters adjacent to the fish farm.

In this discussion, the findings of these four experiments were analyzed, explored their implications for ecosystem management, and identified avenues for future research to advance our understanding of nutrient enrichment in marine environments.

The bioassays found that the daily concentrations of nitrite+nitrate and orthophosphate assessed in both the ambient seawater and the water within the dialysis membrane bags across all four experiments and stations, consistently fell within the range of values delineated by Ignatiades et al. (1992) for the typical oligotrophic offshore waters of the Aegean Sea. Furthermore, the chl *a* concentrations, serving as an indicator of phytoplankton biomass in the ambient water within the study area, remained low, fluctuating between 0.03 and 0.06 μg L^{-1} across all bioassays. Indeed, these concentrations fell within the range observed in other oligotrophic areas within the eastern Mediterranean, as documented by Pitta et al. (1999). According to Price et al. (2015), numerous studies have struggled to identify a phytoplankton response associated with nutrient loading from fish farm effluent, particularly in oligotrophic waters. The present findings are consistent with both this review and a prior investigation that explored the physical, chemical, and biological attributes of the water column in three fish farms situated in the western Aegean Sea, conducted by Pitta et al. (1999). In their study, the authors reported consistently low nutrient and chl *a* concentrations across three consecutive seasons. La Rosa et al. (2002), investigating the impacts of fish farm operations on the water column in the Tyrrhenian Sea within the northwestern Mediterranean, similarly documented no notable rise in nutrient or

chl *a* levels in the water column adjacent to the cages throughout any month within a year. As outlined by Gowen & Bradburry (1987), the dispersion of wastes discharged from fish farms is anticipated to be influenced by factors such as the surface area of the farm, the settling velocity of uneaten feed, and the depth of water beneath the farm. Hence, depth plays a crucial role in determining the distribution of nutrients discharged from the farm. In the present study, the bioassays were positioned at a water depth of approximately 40 meters. Dalsgaard & Krause-Jensen (2006) observed that fish farms are typically situated in regions with robust water currents to facilitate seawater renewal for the caged fish and minimize environmental impact. Consequently, nutrients released from the farms may swiftly dilute in large water volumes, potentially resulting in an increase in concentrations too slight to be detected by standard analytical methods. Moreover, strong currents could scatter and transport phytoplankton far from the farm area, as noted by Navarro et al. (2008). Another reason for the challenge in discerning the impacts of fish farms on phytoplankton is proposed to be the water body's residence time, which is shorter than the generation time of phytoplankton, as argued by Price et al. (2015). Additionally, the grazing effect may also be a critical factor in the inability to detect the phytoplankton response to nutrient enrichment, as emphasized by Pitta et al. (2009). A comparison of the measurements of nutrient concentrations in the water within the bags and those in the ambient water across all experiments did not reveal any significant differences. This outcome was anticipated, given that the dialysis membranes employed for the bioassay were semipermeable. This result suggests that the permeability of the membrane pores remained relatively constant throughout the experiment period, thereby exerting no discernible influence on the nutrient transfer into the bags from the surrounding environment.

Despite the oligotrophic conditions prevailing in the study area and the absence of detectable phytoplankton net growth rates (rnet) in terms of both chlorophyll a concentrations and phytoplankton cell numbers in either the unfiltered or filtered ambient seawater, the chlorophyll a contents of both the unfiltered and filtered dialysis membrane bags exhibited an increase after the incubation period in all of the experiments. In the thirs experiment daily measurements revealed that the increase in phytoplankton cell numbers within the bags only became evident on the third day of

the experiment following two days of minimal recognizable growth. According to Furnas (1991), enumerating phytoplankton assemblages in containment within low biomass oligotrophic waters might pose challenges, particularly when sample volumes are constrained, leading to potential imprecision. However, in the current study, initial phytoplankton cell concentrations were easily quantifiable because the sample volumes (1 L) obtained from the ambient seawater were adequately sized. Yet, this was not the scenario for the volumes available from the dialysis membrane bags. These volumes ranged between 200-300 mL, which proved relatively insufficient for precise and accurate enumeration during the first two days of the experiment. Another explanation for the initial two days of little or no growth is that even under optimal laboratory conditions, isolated natural phytoplankton typically require a certain lag phase before they can proliferate at their maximum growth rate. Inoculated cells typically require a day or two to acclimate to their new medium, as noted by Reynolds (2006). Hegseth & Sakshaug (1983), in a study on the in situ culture of three diatom species inside dialysis membrane bags in the Trondheimsfjord in northwestern Norway, began sampling only after two days of incubation to allow the cultures to adapt to the medium inside the bags. Similarly, in an experiment off Blanes Bay in the northwestern Mediterranean focusing on examining apparent growth rates of natural phytoplankton communities enclosed within dialysis bags, Mura et al. (1996) waited for 3 days before sampling the contents inside the bags. However, Furnas (1982) conducted a study in Narragansett Bay along the Atlantic coast of the northeastern USA, where only a two-day-long growth experiment in plexiglass chambers was deemed sufficient to observe not only smaller changes in numbers in slow-growing species but also high growth in other rapidly growing species. Furnas (1982) utilized a 10-µm mesh size for filtration, and phytoplankton species of such small size (< 10 µm) tend to reproduce more rapidly, as noted by Branco et al. (2020). This could explain why only a two-day incubation period was adequate to observe measurable changes. In the third experiment, the slight increase in chlorophyll a concentrations during the first two days, but the absence of a similar observation when enumerating cell concentrations using a light microscope, may simply be attributed to the limitations of the light microscope in distinguishing phytoplankton in the nano- and pico-size range.

The filtration step was implemented to prevent phytoplankton predators from infiltrating the bags. The objective was to create an environment as free from predators as possible while maintaining the inoculum assemblages closely resembling the natural populations in the ambient environment, thereby minimizing losses due to grazers. According to the findings, the filtration step effectively eliminated all predator species larger than 150 µm. However, because the size of most ciliate species in the ambient seawater was smaller than the pore size of the filter, filtration could not prevent all ciliates from entering the bags. Additionally, certain phytoplankton species larger than 150 µm (such as chain-forming diatoms and those with larger cell sizes) were also excluded from the bags. This could potentially explain why the initial chl *a* and cell concentrations in the unfiltered bags were slightly higher than those observed in the filtered bags. The findings presented here align with those obtained by Mura et al. (1996) in a similar experimental setup. In their study, the authors employed 40- and 150-µm pore sizes for filtration and unfiltered treatments, respectively. Despite initial differences in concentrations, they observed similar final abundances in differently treated bags. Mura et al. (1996) proposed that although the initial inoculum concentrations varied due to filtration, phytoplankton communities in each treatment still reached their maximum growth rates when the incubation period was sufficiently long. However, the results from an experiment conducted in a fish farm in Sitia, Crete (in the eastern Mediterranean) by Pitta et al. (1999) contrast with those of the present study. The methodology employed in the study was similar, where half of the dialysis membrane bags were filled with seawater filtered through 25-µm meshes, while the other half contained unfiltered seawater. However, after a ten-day incubation period, when the chlorophyll a content of the bags was measured, the researchers found the highest chlorophyll a concentrations in the filtered bags. They attributed this observation to the complete exclusion of ciliates from the bags, as ciliates, which typically exceed 20 µm in size, are the primary grazers of the nano fractions of phytoplankton, as noted by Sommer et al. (2005) and Zöllner et al. (2009).

In the current study, besides measuring the chlorophyll a content of the bags, the microphytoplankton (>200 µm) communities, whose primary predators are copepods as noted by Sommer et al. (2005), were also investigated. No adult copepods were detected inside either type of bag, which accounts for the higher growth rates of

microphytoplankton inside the bags compared to in the ambient seawater. Additionally, heterotrophic dinoflagellates, often a significant biomass component among phagotrophic protists within the microplankton size range, are believed to play a substantial role in consuming diatom blooms compared to copepods and other mesozooplankton (Sherr & Sherr, 2007). However, during the incubation period, a scarcity of dinoflagellates inside both filtered and unfiltered bags was observed. Larger dinoflagellate species were notably absent from the filtered bags due to their size, a pattern persisting across all consecutive days in the unfiltered bags. These findings are consistent with observations by Furnas (1990), who noted limitations in diffusion culture that may not fully support the growth of certain species or groups, including flagellates and dinoflagellates. The scarcity of dinoflagellate species within both types of bags during incubation suggests a reduction in grazing pressure exerted by dinoflagellates, likely contributing positively to the growth rate of diatoms within the bags.

Succession occurs within an ecosystem subsequent to a disturbance event, leading to temporal alterations in ecosystem attributes such as species diversity and composition (Connell, 1978) These dynamics profoundly impact phytoplankton communities, which are susceptible to environmental fluctuations, including variations in light, temperature, nutrient concentrations, and grazing pressure. Consequently, fish farms have the potential to substantially modify the community structure of phytoplankton, as they release nutrients as a byproduct of their production processes.

The competitive exclusion theory (Gause, 1934) illuminates how succession unfolds, suggesting that only 1-3 species can inhabit an equilibrium state. Later, Hardin (1960) succinctly captured the essence of this principle with his iconic statement: “Complete competitors cannot coexist.” However, the observed richness within fish farms contradicts this principle, as revealed by the present study. This phenomenon is often attributed to the paradox of phytoplankton (Hutchinson, 1961), which is explained by the intermediate disturbance hypothesis (Connell, 1978) through non-equilibrium interpretations. Originally aimed to explain the phenomenon of high species diversity in tropical rainforests and coral reefs, the Intermediate Disturbance Hypothesis (IDH) (Connell, 1978) was later applied to phytoplankton succession by

Reynolds (1988). The IDH proposes that intermediate frequency or intensity of disturbances increases diversity, and only a few species will dominate in the absence of disturbance. Ambient seawater samples collected from fish farms have revealed a notable difference in microphytoplankton diversity compared to incubated bags, despite prevailing oligotrophic conditions. This finding aligns with Tsanagaki et al. (2013), who similarly observed increased diversity near fish farms in the oligotrophic eastern Mediterranean, attributing it to the IDH. The interaction between increasingly smaller intermediate scales, ranging from 20 to 200 hours, and the growth rates of phytoplankton species plays a crucial role in maintaining high species diversity (Reynolds, 1988). Moreover, Gaedeke & Sommer (1986) demonstrated greater species coexistence than steady-state conditions when the intervals between disturbances exceeded the average generation times. Nutrients discharged from the farms primarily originate from daily feeding practices, falling within the range of intermediate disturbance due to the 24-hour feeding regime. The results indicate a community gross growth rate of 0.686 ± 0.069 in the third experiment suggesting a generation time of approximately 1. This implies that the generation time of natural phytoplankton communities did not exceed the disturbance intervals, as indicated by Gaedeke & Sommer (1986), given the daily feeding regime in fish farms. Nonetheless, the rich diversity of phytoplankton observed within fish farms reflects the dynamic nature of the environment influenced by variations in nutrient discharge intervals and intensities, as well as the role of water currents in nutrient dispersal within the water column. As a result, achieving an equilibrium state in the vicinity of the fish farm is challenging, fostering an environment of intermediate disturbance.

Compared to the persistently oligotrophic conditions in the ambient seawater, the bags achieved a relatively more balanced state. Inside, an environment enriched with nutrients from the fish farm was established, allowing phytoplankton to readily utilize these nutrients without losses from advection, accessing them before dilution in the vast water body. However, despite these favorable conditions, phytoplankton diversity within the bags was lower than in ambient seawater after the incubation. One possible explanation is that some species may have been unable to adapt to the new environment. For instance, dinoflagellates are not culturable species (Furnas, 1982). This could be attributed to factors such as the microcosm effect, as the bags may be

small enough to exhibit such effects (Padisák & Toth, 1981), or the lack of horizontal daily migrations that cannot be achieved inside the bags.

Prior to observing the experiment's results, the expectation was that small-sized species would proliferate within the bags, as oligotrophic waters are typically dominated by pico and nano-sized phytoplankton species (Tsanagaki et al., 2013). However, the present bioassay results deviated from this expectation. Diatoms exhibited high growth rates inside the bags, likely because ciliate grazing on diatoms was negligible. Ciliates prefer nanosized species as prey over diatoms (Sommer et al., 2005), and copepods and heterotrophic dinoflagellates were absent in the bags. The experiment reveals the dominance of diatom species within both types of bags, underscoring their unique ability, known as the "Winnie the Pooh strategist" (Thingstad et al., 2005), to exhibit a higher nutrient affinity despite their larger cell volume.

Field studies, laboratory bioassays, and enclosure experiments are limited in their capacity to depict environmental heterogeneities, often yielding outcomes that reflect community averages (Padisák & Toth, 1981). The six-day incubation period in the third experiment offers merely a snapshot of the environment, providing a narrow perspective that fails to capture the full spectrum of environmental changes and successions. Six days constitute a brief duration inadequate for observing the succession of dominant species.

The prevailing oligotrophic conditions, as evidenced by the available nutrient concentrations in both the ambient seawater and inside the bags were evidently adequate for phytoplankton reproduction. The occurrence of extreme growth, akin to a bloom, inside the bags, coupled with the observation of a net growth rate (rnet) not significantly different from 0 in the ambient water, underscores this point. A similar bloom-like growth, despite extremely low nutrient conditions, was also noted in in situ diffusion culture systems by Furnas (1982).

While variations in phytoplankton species richness, species diversity, and evenness in the ambient seawater were minor, significant changes were observed within the bags. Following a lag of two days, certain species experienced exponential growth, leading to a bloom-like phenomenon inside the bags. The species richness markedly

decreased with *L. danicus, N. longissimi, and C. closterium* surpassing the overall average growth rate observed inside the bags. A similar notable increase in cell densities of certain phytoplankton species within dialysis bags during an experiment was also documented by Mura et al. (1996). This observed phenomenon suggests that only a few taxa can tolerate experimental manipulations and that an incubation period of only six days, as in the present study, is adequate to detect these changes.

The second and fourth experiments were conducted to assess the impact of farm-derived nutrients on phytoplankton growth at different distances from the fish farm. In the second experiment, trials were set up at three stations: two stations similar to the first experiment and an additional station located 250 meters away, representing a farther distance from the farm. The results indicated that, consistent with previous observations, the highest growth was observed at the station closest to the farm, with a gradual decrease in growth observed as the distance from the farm increased. This suggests that the concentration of farm-derived nutrients decreases with distance, leading to a reduction in phytoplankton growth potential.

In the fourth experiment, bioassays were simultaneously set up at 3 stations to determine the effect of farm-derived nutrients. In addition to the second experiment, a reference station located 1000 meters away from the farm was added, where it was assumed that farm-derived nutrients did not reach. Three different types of bags were tested in this experiment. Dialysis membrane bags filled with seawater filtered through 40 µm and 150 µm, and unfiltered seawater were incubated with natural phytoplankton communities. As observed in other experiments, the highest growth was observed at the barge station (Station 1). Similarly, as in other experiments, growth was highest in bags filled with unfiltered seawater, while a decrease in growth was observed as the mesh size decreased. When measurements at the station 250 meters away were examined, again, the highest growth was observed in bags filled with unfiltered seawater, while growth decreased as the mesh size decreased. However, this time, the growth amounts in all bags were lower compared to the growth rates at the barge station. This indicates that the concentration of farm-derived nutrients is lower at 250 meters, and therefore, it is not sufficient to support growth in phytoplankton as much as in the barge station. When the bags at the reference point were examined, it was observed that the growth in each bag type was significantly low, and there was no

significant difference between bag types. This is because farm-derived nutrients cannot reach up to 1000 meters away, and oligotrophic seawater is not sufficient to support growth in phytoplankton. As a result, it can be said that the concentration of nutrients released from the fish farm decreases at 250 meters, and its effect was negligible at 1000 meters.

Overall, the results from these experiments suggest that farm-derived nutrients have a measurable impact on phytoplankton growth, with higher concentrations near the farm and diminishing effects as distance increases. The establishment of a control station at 1000 meters provided valuable insights into the extent of nutrient dispersal and its influence on phytoplankton dynamics. This is because farm-derived nutrients do not reach 1000 meters away, and oligotrophic seawater is not sufficient to support phytoplankton growth. Consequently, it can be concluded that the concentration of nutrients released from the studied fish farm decreases at 250 meters, but its effect is not diminished in 250 m away. These findings underscore the importance of understanding nutrient distribution patterns in coastal waters adjacent to fish farms and their implications for ecosystem health and management.

CHAPTER SIX
CONCLUSIONS

The findings of this study have demonstrated that the nutrient discharge from a fish farm established in an oligotrophic marine area did not alter the prevailing oligotrophic conditions in the environment. The unexpectedly high growth rates observed inside the bags can be attributed to the confinement of phytoplankton within the bags, preventing them from drifting away with currents or being subject to daily migration or grazing, factors that would typically limit their growth in the open sea. Additionally, the phytoplankton enclosed within the dialysis membrane bags positioned close proximity to the fish farms benefit from the unique advantage of accessing and utilizing the nutrients released from the farm before these nutrients disperse and dilute within the surrounding seawater. These combined factors likely contributed to the substantial increase in phytoplankton growth observed in the study. The present work represents the first bioassay and in situ phytoplankton incubation experiment using dialysis membrane bags in the Eastern Aegean Sea and all other Turkish seas. The results from this research may serve as a foundation for estimating the growth rates of natural phytoplankton communities in future investigations in Turkish waters.

However, this study discusses possible reasons why the effects of fish farms cannot be detected in the seawater column, whether in nutrient concentrations, chl *a* concentrations, or net phytoplankton growth rates. These reasons include:

- Nutrients are dispersed from the seawater immediately.
- Nutrients are consumed by phytoplankton and transferred up the food webs via grazing.
- The residence time of nutrient-receiving water bodies is shorter than the generation time of phytoplankton.
- Higher grazing pressure on primary producers resulted in nearly zero net growth rates.

- Losses of phytoplankton through advection (horizontal movement) or sinking (vertical movement).

The detection, however, was achieved using dialysis membrane bags. The reasons why higher growth rates were achieved inside the bags after the incubation period are listed above:

- Dialysis membrane bags provided an environment for the inoculum that was free from losses caused by advection and sinking.
- Dialysis bags created an advantage for the inoculum by being in close proximity to the nutrient resource originating from the fish farm.
- By excluding zooplankton via filtration inside the bags, a relatively grazer-free environment for microphytoplankton was achieved.

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