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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A preliminary study into the influence of filtration on phytoplankton dynamics in an oligotrophic marine fish farm environment

Filtrasyonun oligotrofik deniz balık çiftliği ortamındaki fitoplankton dinamikleri üzerindeki etkisine dair ön çalışma

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Abstract: Fish farms play a crucial role in meeting the escalating demand for fish in human diets, yet their nutrient releases pose potential environmental risks. This study explores the influence of a fish farm in the eastern Aegean Sea on local phytoplankton dynamics, serving as an indicator of nutrient abundance. Designing a phytoplankton bioassay near the fish farm, natural phytoplankton communities were incubated within dialysis membrane bags, creating a confined environment for accessing farm-released nutrients before dispersing into surrounding seawater. Consequently, higher growth rates within the bags were anticipated compared to the ambient seawater. However, natural interactions within phytoplankton communities involve predator-prey dynamics, influencing the net growth rates of phytoplankton. To investigate different grazing pressures on the incubated phytoplankton, five experimental groups were established. Four of these groups involved filtering seawater through various mesh sizes (40 µm, 56 µm, 100 µm, and 150 µm) and then filling the dialysis membrane bags with the filtered water. The fifth group contained seawater without any filtration. Despite the oligotrophic nature of the ambient seawater, a remarkable increase in phytoplankton growth was observed inside the bags. Variable growth rates were observed among the groups, with unfiltered and 150 µm mesh-filtered bags exhibiting the highest growth rates, suggesting copepod absence may contribute. Although the species composition within the bags differed from that of the ambient seawater, the overall species diversity remained limited. A total of 33 phytoplankton taxa were identified in the seawater samples taken from the study site, comprising 17 diatom and 16 dinoflagellate species. *Pronoctiluca spinifera* (Lohmann) Schiller 1932 was documented for the first time along the Aegean Sea coast of Türkiye. This study enhances our understanding of how fish farming can impact phytoplankton communities and underscores the necessity for further investigations into the complex interactions between aquaculture and marine ecosystems in oligotrophic environments.

Keywords: Aquaculture interactions, bioassay, growth rate variations, species composition shifts, nutrient discharges

Öz: Balık çiftlikleri, beslenmede artan balık talebini karşılamada kritik bir rol oynamakta, ancak çiftliklerden salınan nütrientler çevre için potansiyel riskler oluşturmaktadır. Bu çalışma, doğu Ege Denizi'ndeki bir balık çiftliğinin yerel fitoplankton dinamikleri üzerindeki etkisini, fitoplanktonu, nütrient varlığının bir göstergesi olarak kullanarak incelemektedir. Doğal fitoplankton toplulukları, balık çiftliğine yakın bir konumda yerleştirilen bir fitoplankton biyoanalizi tasarlanarak diyaliz membran torbalarında inkübe edilmiştir. Bu sayede fitoplanktonun salınan besinlere denizel ortamda dağılmadan önce erişebileceği içerisinde bulundukları sınırlı bir ortam oluşturulmuştur. Bu nedenle, torbaların içindeki büyüme oranlarının dışarıdaki deniz suyuna kıyasla daha yüksek olması beklenmektedir. Ancak, fitoplankton kommüniteleri av-avcı dinamiklerini içerir ve bu da fitoplanktonun net büyüme oranlarını etkilemektedir. İnkübe edilen fitoplankton üzerinde farklı otlama (grazing) baskılarını incelemek için beş deneme grubu oluşturulmuştur. Bu grupların dördü, deniz suyunu çeşitli göz açıklığına sahip ağlardan (40 µm, 56 µm, 100 µm ve 150 µm) geçirerek, diyaliz membran torbalarının bu süzülmüş deniz suyu ile doldurulması ile oluşturulmuştur. Beşinci grup ise deniz suyu filtrasyon aşamasından geçirilmeden kullanılarak hazırlanmıştır. Ortam deniz suyunun oligotrofik doğasına rağmen, çiftliğe yakın konumlandırılan diyaliz membran torbalarının içindeki fitoplankton büyüme oranlarında belirgin bir artış gözlemlenmiştir. Özellikle gruplar arasında farklı büyüme oranları gözlemlenmiş, filtresiz deniz suyu ve 150 µm göz açıklığına sahip ağ ile filtrelenmiş deniz suyu ile hazırlanan torbalarda en yüksek büyüme oranları tespit edilmiştir. Bunun nedeninin, torbaların içinde kopepodların bulunmaması olabileceği düşünülmektedir. Torba içerisindeki tür kompozisyonu ortam deniz suyundan farklılıklar gösterirken, genel tür çeşitliliği sınırlı olarak kalmıştır. Çalışma bölgesinden alınan deniz suyu örneklerinde, 17'si diatom ve 16'sı dinoflagellat türü olmak üzere toplam 33 fitoplankton taksonu belirlenmiştir. *Pronoctiluca spinifera* (Lohmann) Schiller 1932 türü, Türkiye'nin Ege Denizi kıyısında ilk kez bu araştırmada kaydedilmiştir. Bu çalışma, balık çiftliklerinin fitoplankton komünitelerine nasıl etki edebileceğini anlama konusuna katkı sağlamakta ve oligotrofik ortamlardaki akuakültür ve deniz ekosistemleri arasındaki karmaşık etkileşimlerin daha fazla incelenmesi gerekliliğini vurgulamaktadır.

Anahtar Kelime: Akuakültür etkileşimleri, biyodeney, büyüme hızı değişimleri, tür kompozisyonu değişiklikleri, nutrient salınımları

INTRODUCTION

Aquaculture plays a critical role in filling the demand gap for seafood and will continue to do so in the future (Gephart et al., 2021). However, the activities of fish farms inevitably result in various inputs into the marine environment (Navarro et al., 2008). It is well-documented that fish farms enrich the water column with organic and inorganic substances by releasing fish feces, excretion, unconsumed feed, scale and skin shedding, mucus, vitamins, and therapeutic agents (Arzul et al., 1996). Two main methods have traditionally been used to assess the effects of nutrient input from fish farms into the marine environment. The first method involves regular sampling of the water column, with subsequent measurement of nutrient values. This method has two disadvantages. Firstly, the release of nutrients varies daily depending on the feeding regime at the farms, necessitating hourly sampling to measure the nutrient discharge accurately. The second issue

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with this approach lies in the limited sensitivity of nutrient analyses to detect significant differences. Farms are often situated in regions with high water exchange to maximize the influx of fresh seawater into the cages and minimize their environmental impact. Significant increases in nutrient concentrations due to nutrient release are only achievable in cases where the current velocity is low (Dalsgaard and Krause-Jensen, 2006). Another proposed method for measuring nutrient input into the water column is the use of phytoplankton as an indicator. The literature suggests that the initial impact on aquatic communities due to increased eutrophication begins with changes in the abundance and species composition of phytoplankton (Sidik et al., 2008).

The impact on primary production in fish farms as a result of nutrient enrichment varies widely, ranging from significant alterations to negligible changes. Price et al. (2015) conducted a comprehensive review, revealing that numerous studies suggest a significant increase in primary production in fish farms. However, within the same work, it is also noted that certain studies found no substantial effects on primary production in fish farms. The inability to detect the impact of increased nutrient levels in the water column on phytoplankton has been attributed to factors such as rapid dilution in the water column due to strong currents and water exchange (Dalsgaard and Krause-Jensen, 2006; Pitta et al., 2009), as well as predation pressure (grazing) by organisms that feed on phytoplankton (Pitta et al., 2009).

One of the most reliable methods used to estimate the *in situ* growth rates of marine phytoplankton is incubation inside dialysis bags. The effectiveness of dialysis bag experiments is based on their ability to maintain physicochemical contact between the enclosed phytoplankton population and the surrounding environment (Furnas, 1990).

The Mediterranean Sea is typically characterized as oligotrophic, as its waters naturally contain very low nutrient concentrations (Krom et al., 1991; Mura et al., 1996). Notably, the oligotrophic nature of the marine environment where this study took place, which hosts a fish farm, theoretically provides an advantageous position for observing the effects of nutrient input. The activities of fish farms result in nutrient input into the water column, making these areas unique research sites for investigators.

In addressing the challenge of detecting the impact of increased nutrient levels from fish farming in oligotrophic environments, a bioassay was conducted at a fish farm located in Çandarlı, Denizköy (İzmir, Türkiye), where natural phytoplankton assemblages responded to nutrient releases resulting from fish farming. Utilizing dialysis bags in the bioassay provided a confined environment for phytoplankton, minimizing potential losses due to factors such as daily migrations, grazing, or drifting caused by open sea currents. As a result, higher growth rates within the bags compared to ambient seawater were anticipated, excluding factors causing losses in open water and aiming to reveal the effect of the fish farm on the water column. Inevitably, since natural phytoplankton assemblages were used as inoculum, interspecies competition and predator-prey interactions persisted within the dialysis bags. By applying various filtration treatments to the inoculum, the goal was to measure the highest growth rates inside the bags. The technique of using dialysis membrane bags for the *in situ* incubation of natural phytoplankton is applied for the first time in Türkiye in this study.

MATERIAL AND METHODS

Study area

The study site was a fish farm located in Çandarlı, Denizköy, situated in the northeastern Aegean Sea of Türkiye (38°58'33"N, 26°47'22"E; Figure 1). The farm is situated at a distance of approximately 1 km from the shore, and the water depth in the farm area ranges from 50 to 70 meters. The fish farm was established and began production 4 years before the experiment commenced. The annual production capacity of the farm is approximately 1000 tonnes of sea bream and sea bass. Fish were automatically fed once daily. The highest recorded current speed in the area was 20 cm/s. The experiment was conducted from the 20th to the 23rd of July 2020 and was based on a protocol established in a previous study by Mura et al. (1996).

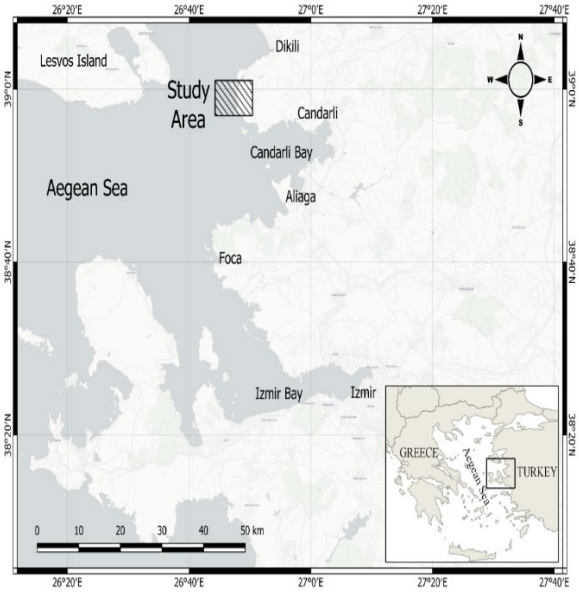


Figure 1. Location of the study area in Çandarlı (the eastern Aegean Sea)

Experimental design

The experiment was designed for the *in situ* incubation of natural phytoplankton communities within dialysis membrane bags for a duration of three days. Dialysis membrane bags facilitate the exchange of molecules smaller than proteins with the surrounding environment. The bags utilized in the experiment were constructed from Spectra/Por® 1 dialysis

membrane tubing. Each bag was sealed at both ends using 110 mm Spectra/Por® closures (nylon). Each bag had a total volume of 600 mL. Seawater containing natural phytoplankton communities was collected from the 50 cm surface layer within the fish farm using a Nansen bottle. Five experimental groups were established to examine varying grazing pressures on the incubated phytoplankton. 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.

A circular PVC tubing frame with a 2-meter diameter was securely affixed to the ropes that anchored the automatic fish feeding system to the seabed of the fish farm. Using cable ties, the 11 dialysis bags were attached to a horizontal rope running along the PVC frame. Lead weights (2 kg) were strategically positioned at both ends of the rope to submerge it at a depth approximately 50 cm below the surface (Figure 2).

At the commencement and conclusion of the experiment, 1 L samples were collected from unfiltered seawater and seawater filtered through different mesh sizes (as mentioned above) to study the nutrient contents of the ambient seawater, environmental variables, and phytoplankton community in the study area.

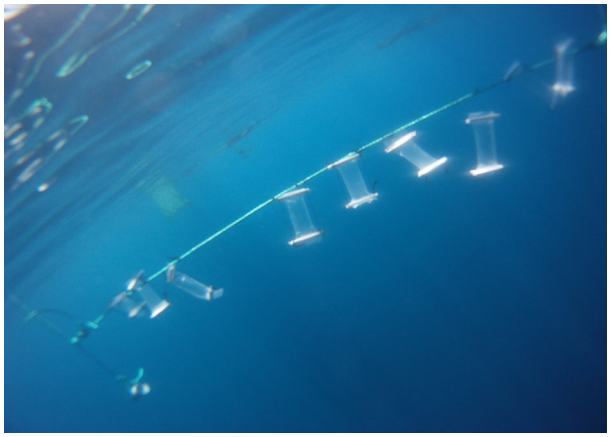


Figure 2. Photograph of the deployed experimental arrangement, 50 cm below the sea surface

Taxonomic identification and enumeration of phytoplankton species

At the end of the incubation period, water samples of approximately 200-300 mL were obtained from the bag contents. These samples, along with the seawater samples collected at the study site both at the onset and conclusion of the experiment, were used for the examination of phytoplankton. All samples underwent fixation with a 0.4% acidic Lugol's solution for this purpose. Subsequently, following sedimentation, taxonomic identification and enumeration of phytoplankton were carried out in the laboratory using an Olympus BX50 fluorescence microscope at a magnification of 100x. In order to determine the taxonomic composition of phytoplankton species, the following resources were consulted: Tomas (1997) and Gomez et al. (2010).

Nutrients and environmental variables

Samples designated for dissolved inorganic nutrients and chl *a* were prescreened through 210 µm nylon mesh to remove larger particles. After prescreening, these samples were further filtered through Whatman GF/F glass microfiber filters. The resulting filtrates were then stored at -20°C and analyzed subsequently for nitrite (NO_2), nitrate (NO_3), orthophosphate (PO_4), and reactive silica (SiO_2) using a SKALAR autoanalyzer (Skalar, De Breda, Netherlands) employing colorimetric methods adapted from the standard seawater analyses as outlined by Grasshoff et al. (1999). For analysis of chl *a*, the filters were folded and placed inside glass tubes, immediately frozen for subsequent laboratory analysis. Extraction of chl *a* was carried out using 90% acetone for a duration of 24 hours, followed by quantification using the fluorometric method described by Strickland and Parsons (1972). Seawater temperature and salinity measurements were conducted using the portable Sea-Bird 37 SM instrument (Sea-Bird Electronics Inc., Bellevue, WA, USA). Dissolved oxygen was measured using the Winkler method (Grasshoff et al., 1999). Secchi disk depth and pH were measured *in situ*.

Statistical analyses

The average concentrations of each nutrient, measured in both the ambient water and the water enclosed within the dialysis bags, were compared using independent *t*-tests (Snedecor and Cochran, 1989). The population growth rates of phytoplankton within the dialysis bags and those present in the ambient water were estimated using chl *a* concentrations and cell numbers. Calculations were based on the exponential growth model (Snedecor and Cochran, 1989; Reynolds, 2006):

$$Y_t = Y_0 e^{rt}$$

Here, Y_t is the dependent variable that describes the cell numbers or chl *a* concentrations at time *t* (day). Y_0 is the initial number of cells or amount of chl *a* concentration at time *t*=0 (i.e., at the start of the experiment), and *r* is the specific growth rate of the phytoplankton community, either in terms of cell counts or chl *a* concentration. The growth model was linearized with logarithmic transformation, and the specific growth rate *r* was then estimated as the slope of the linear regression of the natural logarithm of *Y* versus *t* (Snedecor and Cochran, 1989; Reynolds, 2006). Moreover, an analysis of covariance (ANCOVA) was carried out to examine potential variations in the estimated *r* values among the five

experimental groups, namely, the dialysis bags containing seawater subjected to four different filtration treatments and one unfiltered seawater. Changes in concentrations (chl *a* or cell numbers) within the dialysis bags after incubation were utilized to represent the gross rates (r_{gross}). Net growth rates (r_{net}) were calculated based on changes in concentrations in the ambient seawater between the start and end of the experiment and served as the control (Mura et al., 1996). The disparity between the gross and net rates was designated as the loss rates (r_{loss}).

Various indices were employed to evaluate species diversity and evenness within the phytoplankton communities. Diversity was quantified using both the Shannon-Weiner index ($H´$) and Simpson's index (D) (Magurran, 1988; Krebs, 1999). The ratio of observed diversity ($H´$) to maximum diversity (H_{max}), which represents the diversity achievable under conditions where all species are equally abundant, served as a measure of evenness (E) (Magurran, 1988).

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

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

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

In the above formulations, the quantity p_i represents the proportion of the ith species in terms of cell numbers, and S represents the total number of phytoplankton species or species richness within each community. For a more explicit interpretation of diversity, the Shannon-Wiener index can be expressed in an alternative form using exponentiation with 2 as the base and $H´$ as the exponent, commonly referred to as Hill's number N_1 (Krebs, 1999). Additionally, an alternative form of Simpson's index, known as Simpson's reciprocal index (D^{-1}), is used to express diversity estimates in terms of the number of species. Simpson's reciprocal index corresponds to Hill's number N_2 (Krebs, 1999). All the indices were calculated for each dialysis bag and the ambient seawater for both the start and end of the experiment. Prior to the tests, the necessary assumptions of normality and homoscedasticity were assessed using an F-test, the Shapiro–Wilk normality test, and normal quantile-quantile plots (Snedecor and Cochran, 1989). All statistical tests were conducted using R software version 4.1.3 (R Core Team, 2022), with a significance level set at 5%.

RESULTS

The physical properties remained unaltered throughout the duration of the experiment (Table 1). According to observations and records from the fish farm personnel, the average wave heights during the experiment were consistently low (<1 m). The nutrient concentrations, measured both in the ambient seawater and within the dialysis membrane bags, are shown in Table 2. The concentrations of nitrite + nitrate, orthophosphate, and reactive silica determined in the ambient seawater ranged from 0.08 to 0.23, 0.03 to 0.07, and 0.21 to 0.45, respectively. The nutrient concentrations measured in the bags were slightly higher than those from the ambient seawater (Table 2).

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 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 3), are displayed in Table 4. As evident from Table 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).

However, after three days of incubation, a substantial increase in cell numbers and chl *a* concentrations within the dialysis bags became evident (Table 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 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 3).

Table 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 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	0.03	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

Table 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 results revealed that the bags could be categorized into two distinct groups in terms of growth rates estimated from chl *a* concentrations. The first group consisted of unfiltered bags and those filtered through a 150 µm mesh size, and they exhibited significantly higher growth rates than the second group, which comprised bags prepared by filtering through 40 µm, 56 µm, and 100 µm mesh sizes (Table 4). When growth rates were estimated from cell counts, The ANCOVA results indicated 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). 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. 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)

Source	Treatment	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 5). In the unfiltered seawater sample collected on the initial day of the experiment, *Pronoctiluca spinifera* (Lohmann) Schiller, 1932 (Family: Protodiniferaceae, Class: Dinophyceae) was found (Figure 3), (Table 5). 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.

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 5, Table 6). Unfiltered samples exhibited the highest species count. This pattern was similarly reflected in the species diversity indices and evenness values. Table 7 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.



Figure 3. *Pronoctiluca spinifera*

Table 5. List of phytoplankton species observed in the ambient seawater samples

Classis	Family	Taxa	Days									
			0					3				
			40 µm	56 µm	100 µm	150 µm	UF	40 µm	56 µm	100 µm	150 µm	UF
Bacillariophyceae	Bacillariaceae	*Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, 1964	+	+	+	+	+	+	+	+	+	+
		Nitzschia longissima (Brébisson) Ralfs, 1861	+	+	+	+	+	+	+	+	+	+
		Pseudo-nitzschia sp.	+	+	+	+	+			+	+	+
	Chaetocerotaceae	*Chaetoceros affinis* Lauder, 1864		+	+	+	+	+	+	+	+	+
		Chaetoceros decipiens Cleve, 1873							+	+	+	+
	Grammatophoraceae	*Grammatophora marina* (Lyngbye) Kützing, 1844				+	+					
	Hemiaulaceae	*Hemiaulus hauckii* Grunow ex Van Heurck, 1882	+	+	+	+	+	+	+	+	+	+
	Leptocylindraceae	*Leptocylindrus danicus* Cleve, 1889	+	+	+	+	+	+	+	+	+	+
	Licmophoraceae	*Licmophora* sp.		+	+	+	+			+	+	+
	Naviculaceae	*Navicula* sp.	+	+	+	+	+	+		+	+	+
	Rhizosoleniaceae	*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.				+	+					
	Pleurosigmataceae	*Pleurosigma* sp.			+	+	+	+	+	+	+	+
	Thalassionemataceae	*Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902					+					
Dinophyceae	Ceratiaceae	*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				+	+					
	Dinophysaceae	*Dinophysis acuminata* Claparède & Lachmann, 1859		+	+	+	+	+		+	+	+
	Oxyphysaceae	*Oxyphysis oxytoxoides* (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011		+	+	+	+			+	+	+
	Oxytoxaceae	*Corythodinium tesselatum* (F.Stein) Loeblich Jr. & Loeblich III, 1966					+					
		Oxytoxum scolopax F. Stein, 1883		+	+	+	+	+	+	+	+	+
	Podolampadaceae	*Podolampas elegans* F.Schütt, 1895				+	+					
		Podolampas palmipes F. Stein, 1883						+	+	+	+	+
	Prorocentraceae	*Prorocentrum gracile* F.Schütt, 1895			+	+	+					+
		Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+	+	+	+	+	+
	Protodiniferaceae	*Pronoctiluca spinifera* (Lohmann) Schiller, 1932					+					
	Protoperidiniaceae	*Protoperidinium depressum* (Bailey) Balech, 1974				+	+					
		Protoperidinium steinii (Jørgensen) Balech, 1974								+	+	+

Table 6. List of phytoplankton species observed inside the dialysis membrane bags

Classis	Family	Taxa	Day 3				
			40 µm	56 µm	100 µm	150 µm	UF
Bacillariophyceae	Bacillariaceae	*Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin, 1964			+	+	+
		Nitzschia longissima (Brébisson) Ralfs, 1861		+	+	+	+
		Pseudo-nitzschia sp.			+	+	+
	Chaetocerotaceae	*Chaetoceros affinis* Lauder, 1864		+	+	+	+
		Chaetoceros decipiens Cleve, 1873					
	Grammatophoraceae	*Grammatophora marina* (Lyngbye) Kützing, 1844			+	+	+
	Hemiaulaceae	*Hemiaulus hauckii* Grunow ex Van Heurck, 1882	+	+	+	+	+
	Leptocylindraceae	*Leptocylindrus danicus* Cleve, 1889	+	+	+	+	+
	Licmophoraceae	*Licmophora* sp.					+
	Naviculaceae	*Navicula* sp.					
	Rhizosoleniaceae	*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.				+	+
	Pleurosigmataceae	*Pleurosigma* sp.					
	Thalassionemataceae	*Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902			+	+	+
Dinophyceae	Ceratiaceae	*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					
	Dinophysaceae	*Dinophysis acuminata* Claparède & Lachmann, 1859					
	Oxyphysaceae	*Oxyphysis oxytoxoides* (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira, 2011				+	+
	Oxytoxaceae	*Corythodinium tesselatum* (F.Stein) Loeblich Jr. & Loeblich III, 1966					
		Oxytoxum scolopax F. Stein, 1883				+	+
	Podolampadaceae	*Podolampas elegans* F.Schütt, 1895					
		Podolampas palmipes F. Stein, 1883					
	Prorocentraceae	*Prorocentrum gracile* F.Schütt, 1895			+	+	+
		Prorocentrum micans Ehrenberg, 1834	+	+	+	+	+
	Protodiniferaceae	Pronoctiluca spinifera (Lohmann) Schiller, 1932					
	Protoperidiniaceae	*Protoperidinium depressum* (Bailey) Balech, 1974					
		Protoperidinium steinii (Jørgensen) Balech, 1974					

Table 7. 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

Medium	Indices	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

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),

contributing to the lower evenness values in Table 7 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 7. The contents of all bags at the end of the incubation period comprised a total of 11 diatom species and 8 dinoflagellate species (Table 6).

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. *Leptocylindrus danicus* bloom

DISCUSSION

An increase in primary production due to additional nutrient input is readily detectable in waters with oligotrophic characteristics (Pitta et al., 1999). Fish farms established in oligotrophic marine environments, such as the Aegean Sea, should serve as examples of places where such a nutrient increase can be observed. Nevertheless, in the present study, both in the ambient seawater and inside the bags, nitrite+nitrate and orthophosphate concentrations measured at the beginning and end of the experiment (Table 2) fall within the range of values defined by Ignatiades et al. (1992) as characteristic of the oligotrophic Aegean Sea. Furthermore, chl *a* concentrations of the study area were notably low, measuring approximately 0.06 µg L^{-1} (Table 3) and was consistent with the range reported for other oligotrophic regions in the eastern Mediterranean (e.g., Pitta et al., 1999). The current findings concerning the physical, chemical, and biological attributes of the water column align with a previous study investigating three fish farms in the western Aegean Sea (Pitta et al., 1999). La Rosa et al. (2002), in their study on the impact of fish farm activities on the water column in the Tyrrhenian Sea in the northwestern Mediterranean, similarly reported no significant increase in nutrient or chl *a* contents in the water column adjacent to the cages throughout the year. According to Gowen and Bradburry (1987), the dispersion of wastes released from fish farms is influenced by factors such as the farm's surface area, the settling velocity of uneaten feed, and the depth of the water beneath the cages. Moreover, strong currents can disperse phytoplankton far from the farm area (Navarro et al., 2008). Another critical factor contributing to the difficulty in detecting phytoplankton response to nutrient enrichment may be the grazing effect, as highlighted by Pitta et al. (2009).

The present study, conducted during the summer season between 20 to 23 July 2020, holds particular significance due to its timing. This period aligns with the natural seasonal patterns of the Mediterranean Sea, characterized by low production rates during the summer months (López-Sandoval et al., 2011). What makes the findings particularly noteworthy is the significant contrast observed within this oligotrophic environment. Despite the prevailing oligotrophic conditions in the study area and the absence of a discernible net phytoplankton growth rate in the ambient seawater (Table 3), the gross growth rates based on both chl *a* values and cell counts exhibited a significant increase inside the dialysis membrane bags at the end of the 3-day incubation period. This unexpected and substantial growth was a consistent observation across all treatments. Similar observations of phytoplankton blooms under low nutrient conditions were made in an *in situ* diffusion culture system by Furnas (1982).

The findings confirmed that the filtration successfully eliminated all predator species larger than 150 µm. However, the filtration treatment could not completely prevent the entry of all ciliates into the bags. The inability to completely eliminate grazers from the bags may account for the unexpected result of significantly lower growth rates observed in the bags subjected to filtration using mesh sizes between 40 and 100 µm, as compared to those estimated for the remaining bags containing seawater filtered with a 150 µm mesh size and unfiltered seawater. Some portion of phytoplankton production in the bags filtered with 100 µm mesh size and below was likely consumed by the ciliates in the medium. Pitta et al. (1999) conducted a similar experiment at a fish farm in Sitia, Crete, employing a method where half of the dialysis membrane bags were filled with seawater filtered through a 25 µm mesh size while the other half contained unfiltered seawater. After a ten-day incubation period, during which the chl *a* content of the bags was measured, they observed the highest concentrations in the filtered bags. The explanation provided for this phenomenon was the complete exclusion of ciliates from the bags. Ciliates, with sizes exceeding 20 µm, are the primary grazers of nano-sized fractions of phytoplankton (Zöllner et al., 2009).

Another possible explanation for significantly higher growth rates observed inside the unfiltered bags and those filtered with a 150 µm mesh size may be as follows: Considering that a significant portion of the phytoplankton observed in the study were of a size smaller than 150 µm, it

is likely that using a 150 µm mesh size for filtration, aside from some chain-forming diatoms, did not result in a substantial difference in phytoplankton composition compared to the unfiltered bags. When designing the experiment, it was hypothesized that the presence of adult copepods in the unfiltered bags could potentially differentiate them from those subjected to the 150 µm filtration. However, no adult copepods were found inside either type of bag. This absence may account for the high phytoplankton growth rate inside the bags compared to that in the ambient seawater. Pitta et al. (1999) also suggested, that due to the limited volume of the bags, copepods were unlikely to graze on the microphytoplankton inside them. An experiment with bigger bags comprising larger volumes of seawater may be more suitable for detecting copepod grazing. Additionally, a Nansen bottle may not be the optimal tool for sampling adult copepods, and a more appropriate sampling device should be considered.

The number of phytoplankton species at the study site increased with the enlarging filtration mesh size, while unfiltered samples contained the highest species counts as expected (Table 7). A similar trend was reflected in the species diversity indices and evenness values. Likewise, species richness inside the incubated dialysis bags increased with larger filtering mesh sizes. However, species diversity inside all bags remained notably lower than those estimated for differently treated ambient water samples (Table 7). Many phytoplankton species present in the ambient water were conspicuously absent inside the bags (Table 5, Table 6). In contrast, certain species were observed in significantly higher densities than those in the ambient seawater. This resulted in generally very low N_1, N_2, and evenness values (Table 7). A similar substantial increase in cell densities for some phytoplankton species inside 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 an incubation period of just three days, as in the present study, is sufficient to observe these changes.

CONCLUSIONS

Overall, 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.

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AUTHORSHIP CONTRIBUTIONS

Betül Bardakçı Şener: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing -original draft, writing - review & editing. Eyüp Mümtaz Tıraşın: Conceptualization, formal analysis, funding acquisition, investigation, project administration, resources, supervision, writing - review & editing.

CONFLICT OF INTEREST STATEMENT

The authors affirm that they do not have any known financial interests or personal associations that might have given the impression of exerting influence over the study described in this manuscript.

ETHICS APPROVAL

This manuscript does not involve any animal studies conducted by the authors. The experimental research was carried out using natural phytoplankton communities.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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