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The Effects of Marine Mucilage Aggregates on the Mediterranean Mussel *Mytilus galloprovincialis* Aquaculture in the Sea of Marmara (Türkiye)

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ABSTRACT

There is an international pressure to increase the worldwide expanse of marine mussel farms. However, the mussel farms are challenged with environmental problems such as mucilage aggregate events. Thus, it is essential to assess the effects of mucilage on the mussel aquaculture and to eradicate the harm and socioeconomic effects they confer on society. Our study addressed this issue by establishing a demographic baseline for commercially important mussel species prior to the proposed aquaculture in the area. This baseline was established at a recently set up commercial mussel farm located approximately 4km north of Erdek port and around 2km from Ocaklar port in the Marmara Sea region of Türkiye. Monthly samples of spats attached to the collectors and mussel meat yields were addressed, and seawater temperature and chlorophyll *a* contents were measured throughout this study (36 months) conducted at a commercial mussel farm. Year-round biological data from 2019 to 2022 were recorded before, during, and after the mucilage event on the Mediterranean mussel, *Mytilus galloprovincialis*. Moreover, samples were taken from an area inside the mussel aquafarm. As a result of this study, differences before, during, and after the mucilage aggregate event were detected in the number of the Mediterranean mussel spat attached to the collectors and the meat yields of the farmed mussels. This demographic baseline is the only data available, at a sufficient spatial and temporal resolution, for evaluating the effects of a mucilage aggregate event in the mussel aquafarm, and it fills an important data gap for risk assessment.

INTRODUCTION

Around the world, urbanization and industrialization are causing various environmental problems due to population growth. In coastal societies, rapid industrialization and population growth have put pressure on the oceans creating various problems that affect the natural life (**Holon *et al.*, 2015**).

Regardless of the city's waterfront location, all cities' liquid waste ends up in the ocean to a greater or lesser extent. Over time, this pollution accumulates and reaches a level that cannot be eliminated naturally (**Hanna, 1992; Fukue *et al.*, 1999; Font *et al.*, 2019**). About 25% of Türkiye's population lives along the coast of the sea of Marmara, which seems like an inland sea. The total population of the provinces of İstanbul, Kocaeli, Yalova, Bursa, Balıkesir, Çanakkale and Tekirdağ surrounding the sea of Marmara is approximately 24,437,500 **(TÜİK, 2022)**.

The sea of Marmara is connected to the Black Sea through the Bosporus and to the Aegean Sea through the Dardanelles Strait. While surface currents are flowing from the Black Sea to the Aegean Sea, there are also under surface currents flowing from the Aegean Sea to the Black Sea. The total volume of the Marmara Sea is approximately 3,377 cubic kilometers **(Altan, 2014)**. In other words, the per capita seawater volume in provinces coastal to the sea of Marmara is about 160,000 cubic meters. It is known that the main problem in the sea of Marmara stems from the fact that a large part of the Turkish industrial companies are located along the coast of the sea of Marmara. In recent years, there has been an increase in the formation of mucilage, or "sea saliva," or "sea snot," which is an organic substance resulting from the growth of floating and benthic algae in the ocean. Slime can also be defined as organic material secreted into water by phytoplankton as a result of the overgrowth of phytoplankton due to environmental factors **(Misic *et al*., 2011)**. Mucilage, in terms of its structure and properties, is generally a collection of gelatinous aggregates. Mucilage is an important stress factor that affects numerous variables such as light, temperature, oxygen, and pH in surface, coastal, and deep-sea environments, with profound effects on benthic organisms **(Claudet & Fraschetti, 2010)**.

Phytoplankton are the main producers, providing food and oxygen to all aquatic organisms, including zooplankton, through photosynthesis **(Jouenne *et al*., 2007)**. Phytoplankton require a carbon source, illumination, and essential nutrients like nitrogen, phosphorus and sulfur to facilitate primary production **(Gligora *et al*., 2007)**. The concentrations of nitrogen and phosphorus compounds in the aquatic environment are elevated due to unregulated release from agricultural and industrial practices, as well as inadequate treatment of urban wastewater **(Van Drecht *et al*., 2009; Frost *et al*., 2019; Li *et al*., 2021)**. The sudden and rapid growth of phytoplankton populations occurs when nitrogen and phosphorus loads are elevated, alongside favorable conditions for growth such as heat, light and trace elements. This unregulated proliferation leads to the formation of "algal blooms" **(Jassby 2005; Pei *et al*., 2019)**. In 2021, the Marmara Sea witnessed the emergence of mucilage, which can be induced by extensive algal blooms **(Tüfekçi *et al*., 2010)**.

Numerous factors contribute to the formation of mucilage, with the initial one being the discharge of surplus carbohydrate compounds into the oceanic surroundings caused by the overgrowth of phytoplanktonic species through photosynthesis **(Berto *et al*., 2005)**. In instances where climatic changes, eutrophication, and thermal stratification affect the water, there are alterations in oxygen, light, pH and temperature parameters. These fluctuations can lead to the rapid formation of mucilage, resulting in anoxic conditions and the subsequent demise of marine flora and fauna on the seabed **(Karlson *et al*., 2021)**.

A report published by the Intergovernmental Oceanographic Commission of UNESCO revealed that the northeastern part of the Marmara Sea experienced the first occurrence of mucilage in the autumn of 2007 **(Aktan *et al*., 2008)**. The study found that diatoms, with a concentration of more than 107 cells per liter, were present in the samples collected from the mucilage event formation. Furthermore, the report noted the simultaneous occurrence of the dinoflagellates and mucilage (36 x 103 per liter) and a significant rise in the coccolithophores species off the coast of İstanbul. Large quantities of mucilage aggregates have been found to have an impact on fishing and recreational diving activities. Additionally, sediment and mussels have been observed to contain common benthic mucilage aggregates. The negative effects of heavy precipitation on the benthic ecosystem have also been documented **(Aktan *et al*., 2008)**. In 2021, **Yurga (2022)** observed the distribution of phytoplanktonic species in the marine saliva of the sea of Marmara. The findings revealed the presence of 1 dinoflagellate species and 5 diatom species from 5-liter seawater samples collected from 6 different experimental stations along the Marmara Sea **(Yurga, 2022)**.

Mussels can contribute significantly to the biological regulation of mucilage by consuming phytoplankton through their water filtration feeding mechanism. A mussel of average size, measuring 7- 8cm in length, possesses the capacity to filter a volume of 10 to 15 liters of water per hour, effectively removing various organic and inorganic particles **(Steeves *et al*., 2022)**. Mussels possess such attributes that enable them to hinder the development of sea saliva through the consumption of phytoplankton, thereby assuming a significant role in the biological combat against mucilage **(Asmus & Asmus 1991; Heath *et al*., 1995; Menge *et al*., 2009)**. To eradicate the adverse effects of marine saliva, it is imperative to enhance the cultivation of mussels and augment the quantity of infrastructure.

MATERIALS AND METHODS

Study site

The study took place at a private mussel farm situated approximately 4km north of the Erdek port and around 2km from the Ocaklar port of Erdek district in Balıkesir in the Marmara Sea region. The satellite image in Fig. (1) highlights the marked area of the mussel farm in red. The sea surface geographic coordinates of this farm area are: 40º 27' 56.51'' N – 27º 42' 20.97'' E, 40º 27' 43.75'' N – 27º 42' 37.66'' E, 40º 27' 39.73'' N – 27º 42' 32.87'' E, and 40º 27' 53.26'' N – 27º 42' 16.47'' E.

Fig. 1. Map of the mussel farm located in the study site (Highlighted in red)

The research was conducted for over a period of 36 months, from September 2019 to September 2022. Mucilage of the mussel farm where our study was conducted can be easily seen by eye (Figs. 2, 3).

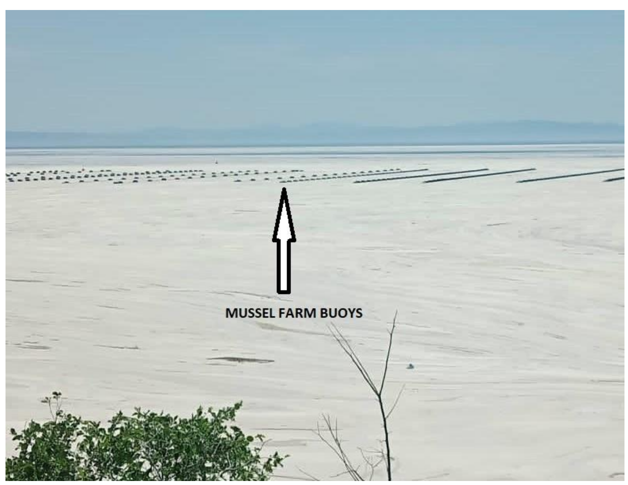

Fig. 2. General view of the Mussel farm and the Musilage formation

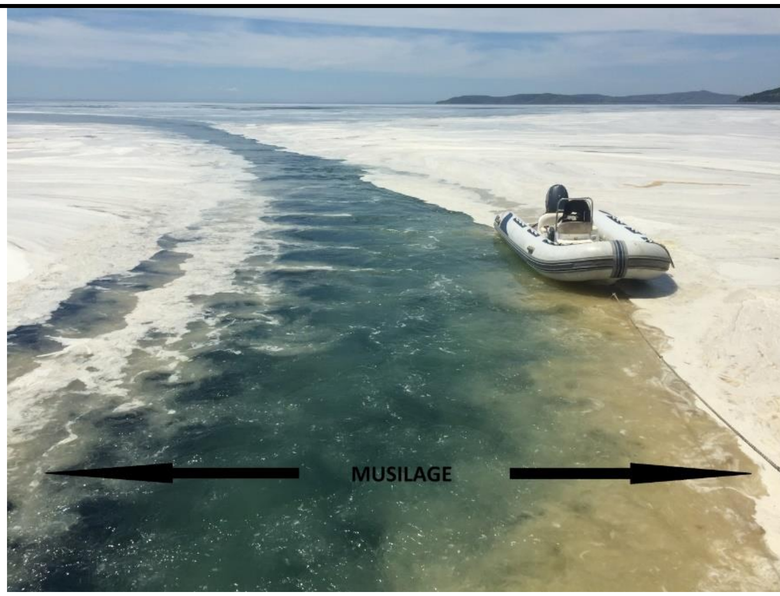

Fig. 3. Mucilage formation at the mussel farm

Starting in September 2019, the mussel collectors were placed on the existing long rope system at the farm monthly. After a period of 3 months, each collector was retrieved from the water, and the number of juvenile mussels (or, Spats) attached to them were examined and recorded. Additionally, the conditions of the mussels, specifically their meat yield ratios, were assessed by sampling those that had reached the market size of 5- 7cm within the same date range every 3 months. To identify the month with the highest meat yield, the adductor muscles of the mussels were carefully removed, and the meat and shell parts were separated and weighed precisely using a precision scale (Weightlab Instruments, WH-503T). Samples were obtained from fully grown mussels in both systems, and various biometric measurements such as length, width, thickness, fresh meat weight, and total meat weight were taken using a caliper (MITUTOYO®) and the precision scale (Weightlab Instruments®, WH-503T). These measurements were essential in calculating the meat yield.

The meat yield in mussels was calculated using the following equation:

Meat Yield (%) = (Fresh Meat Weight / Total Live Weight) x 100 **(Okumuş & Stirling, 1998)**.

Water temperature measurements were monthly conducted during the entire duration of the study. The collected samples were stored in an icebox and subsequently transferred to the laboratory. To analyze chlorophyll *a* (Chl *a*) content as $\mu g.L^{-1}$, water samples underwent filtration using GF/C filter paper and a vacuum pump, followed by an immersion in acetone (90%) for 24 hours. Following centrifugation, the samples were subjected to measurement using a UV-visible spectrophotometer (OPTIMA® SP- 300) at specific wavelengths, namely 630, 650, 665, and 750nm **(Strickland & Parsons, 1972)**.

Data analysis

The Python Statistical Program was utilized to conduct a Tukey's honestly significant difference (HSD) test to analyze the statistical differences in attachment rates and conditions among groups (i.e. before, during, and after mucilage event) for each month. To compare the attachment rates or conditions for each month across the "before", "during" and "after" groups, a paired t-test was performed.

RESULTS

The average annual mussel spat attachment numbers per collector exhibited mean values of 204.30± 170.60, 29.20± 58.00, and 681.80± 571.90 for the data sets corresponding to the periods "before," "during," and "after" the occurrence of mucilage, respectively (Table 1 & Fig. 4).

Table 1. Monthly and annual average mussel spat attachment numbers per collector, chlorophyll *a*, temperature and mussel meat yield (%).

Months	BEFORE THE MUCILAGE				DURING THE MUCILAGE				AFTER THE MUCILAGE			
	2019-2020 SEPTEMBER				2020-2021 SEPTEMBER				2021-2022 SEPTEMBER			
	Attachment	Chl-*a**	Temperature**	Meat Yield***	Attachment	Chl-a	Temperature	Meat Yield	Attachment	Chl-a	Temperature	Meat Yield*
September	32	0.58	23.4	18.5±2.8	32	0.71	24.0	21.5±1.7	187	0.68	23.0	11.5±1.4
October	36	0.54	19.6	19.5±2.5	15	0.84	20.8	17,6±2.8	230	0.56	17.8	15,4±1.9
November	67	0.69	17.4	19,0±1.9	9	0.82	16.8	15,4±3.1	204	0.81	15.2	23.8±2.0
December	128	0.61	13.2	17.5±2.9	0	0.77	13.3	14,3±1.8	255	0.44	12.3	26.9±1
January	255	1.18	10.3	17.5±1.7	0	1.42	11.0	13,7±2.4	609	0.93	9.8	26,1±2.5
February	398	1.63	9.2	20.5±2.0	0	1.99	10.0	15.6±2.5	721	1.27	8.5	27,0±1.7
March	147	2.18	10.8	22,2±1.9	0	1.84	10.3	22±4,8	907	2.51	7.7	29.5±3.8
April	329	0.59	11.2	24,0±1.6	0	0.26	12.6	21,4±3.1	1113	0.92	11.3	31.4±2.9
May	522	0.47	17.0	26,0±2.9	0	0.26	17.3	21.8±2.0	2220	0.69	17.6	32,5±3.9
June	470	1.03	22.3	29,0±2,1	0	1.25	23.3	18.8±2.3	1196	0.80	22.3	30,1±1.3
July	223	2.14	25.1	28,0±4,8	0	1.58	26.7	15.8±2.0	841	2.71	24.0	24.7±2.8
August	17	1.90	25.8	25,0±2.2	136	1.25	27.0	12.3±2.3	264	2.54	26.0	32.6±3.4
September	32	0.71	24.0	21.5±1.7	187	0.68	23.0	11.5±1.4	117	2.62	22.5	30.01±4.7
Mean	204.3	1.1	17.6		29.2	1.1	18.2		681.8	1.3	16.8	
Standard Deviation	170.6	0.6	5.9		58.0	0.5	6.1		571.9	0.9	6.2	

*): Water chlorophyll *a* amount as µg. L^{-1}

**): Water temperature as °C

***): Meat yield as % (N=15)

The values for water chlorophyll *a* amounted: 1.1± 0.6, 1.1± 0.5, and 1.3± 0.9 µg.L^{-1}, and for water temperature, the values were 17.6± 5.9, 18.2± 6.1, and 16.8± 6.2°C, "before," "during," and "after" the mucilage, respectively (Table 1).

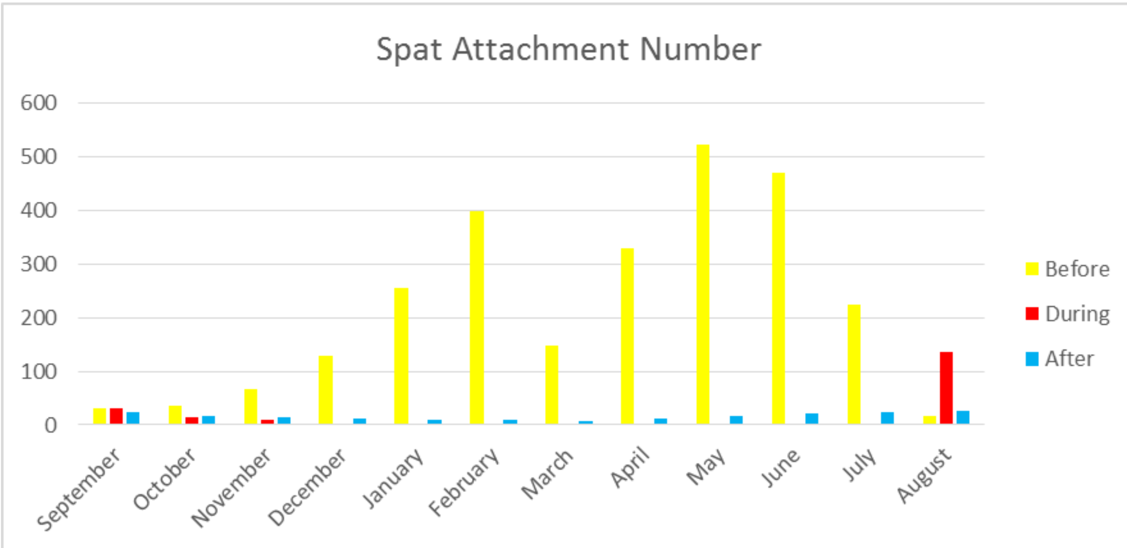

Fig. 4. Monthly average mussel spat attachment numbers per collector

The meat yield rates changed between 17.5 & 25.8, 11.5 & 22, and 11.5 and 32% for the data set corresponding to the periods "before," "during," and "after" the mucilage aggregates, respectively (Table 1 & Fig. 5).

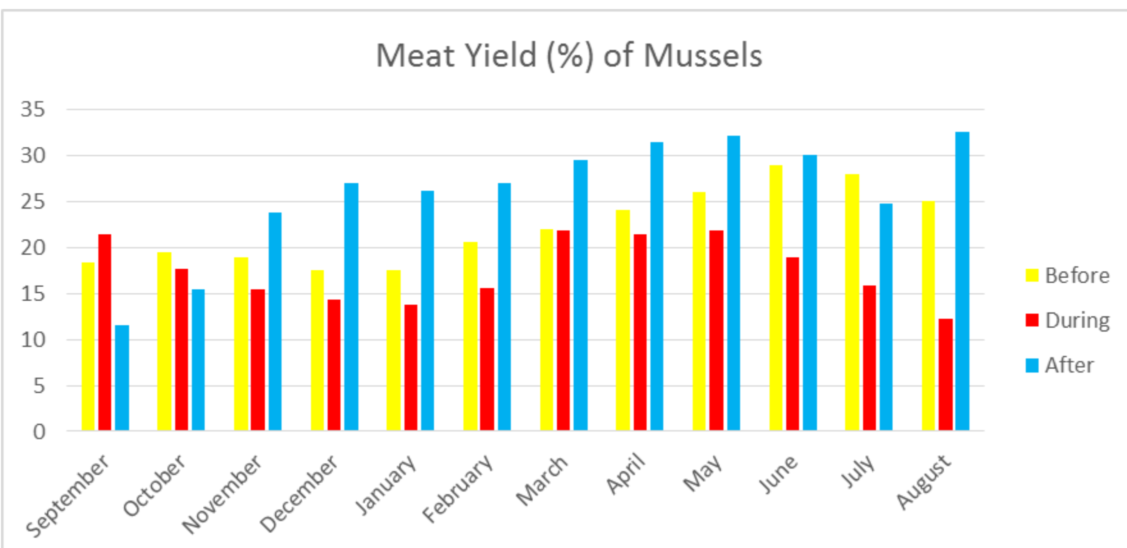

Fig. 5. Monthly average meat yield (%) of mussel

The presence of mucilage had a severe impact on mussel attachment (Table 1 & Fig. 4). Throughout the period from December 2020 to July 2021, when mucilage was present in the Marmara Sea's water column, mussel attachment rates were recorded as zero. The one-way ANOVA analysis indicated significant variations in attachment rates among the three periods ($P \leq 0.05$, f= 34.41). Furthermore, Tukey's HSD test revealed significant differences in attachment rates between the "after" and "before" periods, the "after" and "during" periods, as well as the "before" and "during" periods ($P \leq 0.05$). Paired t-test results also confirmed significant differences between the "before," "during," and "after" groups for the months spanning December to August.

The attachment rates experienced a decline during the periods of intense mucilage events. Nevertheless, the underlying cause may not be apparent. The reduction in attachment rates could be attributed to the decrease in the population of adult mussels that serve as a source of mussel larvae. The excessive mucous material can result in benthic hypoxia, which may escalate to an anoxic state. Consequently, benthic organisms, particularly those that are immobile, are unable to flee from the affected regions **(Rinaldi *et al*., 1995; Dzierżyńska-Białończyk *et al*., 2019)**. The exchange of gases with the overlying water or direct mechanical suffocation can lead to mortality **(Cornello *et al*., 2005)**. Investigating the neighboring natural populations will offer a solution to this problem in a comparable scenario. In the current research, a thorough examination of the mortality rates was not conducted; nevertheless, the decline in the adult mussel population in the region is likely one of the dependable factors.

The potential significance of the second reason, which may be even more important, lies in the direct adverse impacts of the mucilage on the larvae. During the early stages of their life, mussel larvae possess cilia on a velum that enables them to adjust their position in the water and feed **(Sprung, 1984)**. The survival of these larvae is heavily influenced by environmental factors such as oxygen levels, food availability, and pollutants **(Widdows, 1991)**. If an adult, population manages to produce a certain number of larvae, these offspring could potentially be affected by a range of factors induced by the presence of mucous. These factors include impaired swimming ability, potential anoxic conditions, particularly at greater depths, and limited food availability, among others. Furthermore, it is crucial to gain a better understanding of the impact of mucilage on the period preceding embryo formation and how it affects fertilization. Table (2) shows that the mussels were less affected during the mucilage event in comparison. The mean values of meat yield ratios for "before", "during", and "after" the event was significantly different according to the one-way ANOVA ($P \leq 0.05$, f= 12.67). The Tukey's HSD test revealed significant differences in "condition" between the "after" vs "during" groups ($P = 0.0039$), and the "after" vs "before" groups ($P = 0.0074$), but not between the "during" vs "before" groups ($P = 0.9388$). Mucus material on the mussels can cause their valves to remain closed for too long, reducing their filtration capacity and meat condition **(Cornello *et al*., 2005)**. Even a weak appearance of mucilage can affect mussel's filtration rates and meat condition, as observed during the massive mucilage event that affected large parts of the Marmara Sea until August 2020 **(Cornello *et al*., 2005)**.

The visual observation of the mucilage event on mussel buoys and musilage on mussels under the water during September 2020 was a significant outcome of this study (Figs. 6- 7). The mucilage reached its peak during the spring months of 2021, gradually narrowing down in the early summer of the same year, and eventually subsiding by summer 2021.

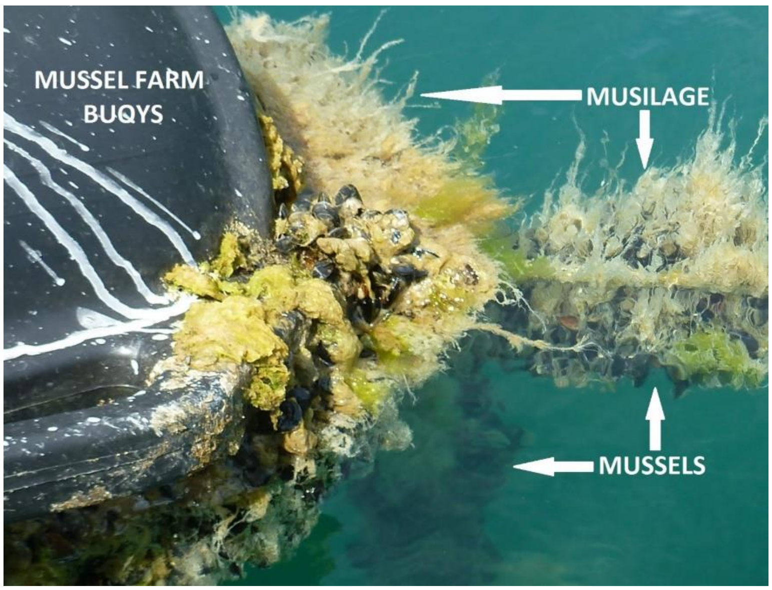

Fig. 6. The Mussel farm's buoy and the mucilage formation

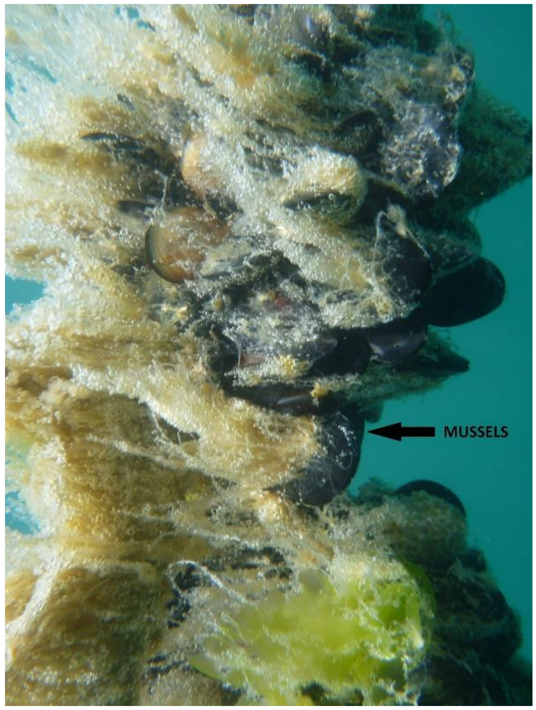

Fig. 7. Underwater image of the mussel and the mucilage

Table (1) reveals that there was no presence of the juvenile mussels, or spats, on the mussel collectors for a duration of 8 months, commencing from December 2020 and concluding in June 2020. Furthermore, the attachment of spats subsequent to the mucilage period (September 2021- 2022) was roughly three-fold greater compared to the period prior to mucilage (September 2019-2020).

During the mucilage period (September 2020- 2021), the average meat yield stands at approximately 18%. Following the mucilage period, there was an observed increase of around 11% in meat yield over the course of one year (September 2021-2022).

This study represents a pioneering effort in the region as it explores the correlation between mucilage formation and mussel cultivation. Furthermore, the escalating intensity of mucilage formation has adverse implications for aquaculture operations in mussel farms.

DISCUSSION AND CONCLUSION

The impact of mussel cultivation on the reduction of phytoplankton levels in the environment and its subsequent effect on eutrophication has been a subject of research for several decades **(Stigebrandt *et al*., 2015; Kotta *et al*., 2020)**. Recent studies have focused on the role of mussels in mitigating nutrient accumulation and improving water quality **(Kotta *et al*., 2020)**. Mussels possess the ability to filter water, sustaining themselves by continuously extracting and utilizing particles, particularly phytoplankton, as a food source. Since seawater remains in constant motion, nutrients are consistently introduced into the environment, providing a favorable condition for sedentary mussels to thrive **(Carlsson *et al*., 2012)**. A study conducted in the eastern Skagerrak aimed to address the serious environmental problem of eutrophication, which incurs significant costs for society on a global scale. The proposed solution involved reducing nitrogen inputs and exploring the potential of mussel farming. To assess the impact of mussel farming on the nitrogen cycle, a study was carried out in the Gullmar Fjord on the Swedish west coast. The findings revealed a 20% reduction in net nitrogen transport (both dissolved and particulate total) at the fjord's mouth **(Lindahl *et al*., 2005)**. While existing commercial mussel farms already provide this service without charge, the societal benefits can be significantly enhanced through the proper utilization of these farms. **Lindahl *et al*. (2005)** conducted a study to determine the most economical ways to enhance water quality. Their findings indicate that mussel farming is a viable and cost-effective solution to mitigate excess nitrogen in fjords and coastal regions. The study suggests that mussel farming can be an effective nutrient reduction measure in watersheds with favorable environmental conditions. Consequently, incorporating mussel farming into incentive schemes aimed at reducing eutrophication in fjords and coastal waters can potentially enhance their cost-effectiveness.

The extensive occurrence of marine mucilage that impacted vast areas of the Marmara Sea persisted for several months until September 2021 **(Özalp, 2021)**. Fishing activities experienced negative consequences as a result of mucilage accumulation, leading to the obstruction of fishing nets **(Kavzoğlu *et al*., 2021)**, consequently causing a halt in fishing operations. Furthermore, the public expressed apprehension regarding the potential detrimental impact of consuming mucilage-contaminated fish on human health. Moreover, the devastating effects of mucilage extended to benthic marine life, as it enveloped sediments and suffocated aquatic organisms **(Eren, 2021)**.

Another study suggested that longline mussel farming could be used as a method to mitigate the impacts of eutrophication and remove excess nutrients from the environment **(Timmerman *et al*., 2019)**. Additionally, the study found that the concentration of chlorophyll *a* decreased and the Secchi depth increased, particularly in the vicinity of the mussel farms. Consequently, the implementation of mussel farming has the potential to enhance the cost-effectiveness of incentive programs targeting the reduction of eutrophication in coastal waters.

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