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Potential Impacts of Mediterranean *Mytilus Galloprovincialis* Mussel Farming in a Specific Area of Aegean Sea”

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Abstract

Intensive mussel cultures have an negative impact on the quality of the aquatic environment, mainly by depositing faeces, pseudo faeces and dead mussels in the sediments. Biodeposition can alter the characteristics of the sediment below culture systems. The organic enrichment of sediment under the mussel culture results in a reduction of dissolved oxygen in the water, with undesirable effects in mussel production and in water quality of the aquatic environment.

The aim of this research is to present the impact of long line Mediterranean mussel culture of *Mytilus Galloprovincialis* in aquatic ecosystem. Our results demonstrate the organic enrichment of mussel culture in relation to the reference area and to the coast, resulting in the lower concentrations of dissolved oxygen in the water. The release of P-PO4 under the mussel culture and the lower concentrations of chlorophyll-a due to the consumption of phytoplankton organisms by mussels were also confirmed. Finally, the concentrations of nitrogen nitrite and nitrate didn’t show statistically a remarkable change.

Introduction

In various countries, the contribution of aquaculture to the economic development has been an undeniable fact. In Greece indeed there has been a tremendous growth in aquaculture, including the cultivation of mussels, since the mid-1980s. The significant growth of mussel cultures in our country is mainly due to its simplicity of applying longline mussel culture, secondly due to the relatively low plant and production costs, because mussels do not require external feed input, and finally due to the favorable environmental conditions.

Mussel cultures help coastal waters against excessive phytoplankton blooms in response to anthropogenic actions resulting in eutrophication. Furthermore, an enhancement of water clarity due to filtration allows deeper light penetration and increase the growth of seagrasses [1].

Despite the growing interest in mussel farms, there is an increasing concern about the effects that shellfish farming may be having on the environment. A concentration of mussel culture activity makes the area where applied most susceptible to environmental problems in a variety of ways [2].

The impact of mussel culture is due to the increased nutrient loads, particularly organic phosphorus and nitrogen and inorganic nitrogen (ammonia) that might easily induce eutrophication [3]. Due to the high biodeposition, mussel farms create sediments that are characterised by suboxic to anoxic conditions [4]. Biodeposition processes affect environmental conditions in a wider area [5]. Mussel farms also result in a concentration and redistribution of nutrients. Conditions of localised enrichment can arise through excretion of dissolved inorganic nutrients into the water column and increased sedimentation of organic material below the farms in the form of faecal and pseudofaecal materials, dead mussels and associated epibiota. Sedimentation rates have been reported to be two to three times higher underneath the mussel farms compared to ambient rates outside the cultures [6].

The aim of this study was to evaluate the impact and the extent of influence of mussel culture on the quality of the aquatic ecosystem in a specific area of Aegean Sea (Prefecture of Pieria). Physico-chemical parameters: temperature, dissolved oxygen, salinity, pH, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate phosphate, particulate organic carbon and organic sediments and phytoplankton biomass (as chlorophyll-a) were investigated in order to evaluate water quality.

Materials and Methods

Mussel culture

In our study the Mediterranean mussel *Mytilus Galloprovincialis* was used, which is the cultivated species in Greece and more generally in the Mediterranean area. This species is ideally developed in the climatic and physico-chemical conditions that exist in the marine environment of our country. Specifically, it grows in waters with a salinity of 32-37‰ while it survives without problems and in sea waters which salinity ranges from 22-42‰. In addition to salinity, the temperature also plays an important role to the growth of the mussel. The temperature ranges from 10 and 26 °C with an excellent of 15-19 °C and the pH value ranges from 7.0 to 8.3 [7-10].

The mussel culture was selected in a specific marine area of the Prefecture of Pieria (Makrygialos- latitude: 40° 24’ 51, 8688”N, 22° 36’ 39, 4848”E). The mussel culture was of the “long line” (floating) type, four years of age and productivity per row of 15 tons. The distance between the rows of the culture was 5-7 m. The distance of the aquaculture from the coast was 500 m and its depth water was 12-14 meters.

From March to May of the next year, twelve monthly samples were taken (one per month: March: M, April: A, May: M, June: J, July: J, August: A, September: S, October: O, November: N, December: D, January: J, February: F, March: M, April: A, May: M). Due to adverse weather conditions, no measurements were made for three months.

Collection of water and sediment samples was made in three

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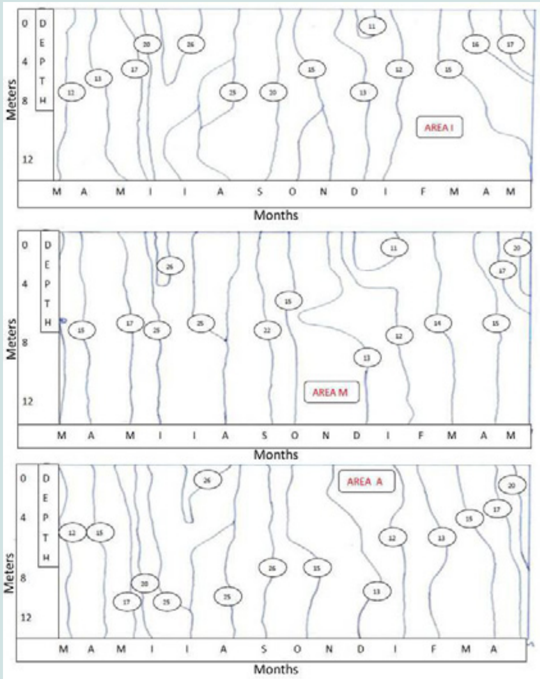

Figure 1: Temperature variation with depth at different months in sea water: coast (area A), mussel culture (area M) and reference area (area I).

different regions

A (coast): Area, 300 meters from the coast.

M (mussel culture): Area of mussel cultivation, which was 500 meters from the coast.

I (reference area): An area that was about 1,000 meters from the coast.

Samples of water were collected from 5 different points from both the sea surface and the bottom (10 to 20 cm above the sediment) in each area (A, M, I). A Niskin (General oceanics, inc.) water sampling probe was used for water sampling, with a capacity of two liters, and an Ekman type sampler was used in order to collect sediment.

Physico - chemical variables

The variables that were examined to determine the water and sediment quality were: temperature, dissolved oxygen, salinity, pH, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate phosphate, chlorophyll- a, particulate organic carbon and organic sediments.

On-site dissolved oxygen and water temperature measurements were made by means of a portable oxygen meter (WTW type OXI96). The dissolved oxygen and temperature measurements were carried out every two meters depth in order to draw their vertical distribution into the water column. The pH measurement was determined by WTW's portable pH meter, while salinity was determined by ATAGO S/Mill Salinity Portable Salinity Meter 0-100. The water and sediment samples were transferred to 2.5 liter glass containers at the Laboratory of Ecology and Environmental Protection of the Department Veterinary University of Thessaloniki.

The water sample bottles were immediately returned to the laboratory in insulated containers. These samples were filtered within 3h of collection through Whatman GF/C glass-fiber filters. Samples were kept frozen until analysis of chlorophyll-a, N-NO_3, N-NH_3, P-PO_4, POC, N-NO_2. Chlorophyll-a was determined by filtration of the water sample and its concentration in the extract was determined spectrophotometrically 665 nm. Determination of P-PO_4 was based on the reaction of the ions with an acidified molybdate reagent to yield a phosphomolybdate complex, the extinction of which was measured at a wavelength of 880 nm. Determination of N-NH_4 was based on the reaction of phenol and hypochlorite to form indophenols. At a wavelength of 630 nm the absorbance against a blank sample and a series of solutions of known concentration in ammoniacal nitrogen was used to determine the standard reference curve. Determination of N-NO_3 and N-NO_2 was based using the appropriate method. The nitrate was reduced to nitrite when the water sample ran through a column containing amalgamated cadmium filings. The produced nitrite was determined by diazotizing with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine to form a coloured azo dye, the extinction of which was measured at 543 nm. Determination of particulate organic carbon was done by wet ashing with a mixture of potassium dichromate and concentrated sulphuric acid. The absorbance of the sample was measured at a wavelength of 440 nm. The Standard Methods technique was applied to calculate sediment organic (% dry matter). According to technique the sample was initially dried at 103 °C to constant weight and then the analysis is continued with the combustion of the sample at 550±50 °C [11-14].

Statistical analysis

ANOVA method was used in order to evaluate the possible interaction between the factors of "area", "depth" and "time" factors in the formulation of the physicochemical and biological parameters that was studied. At the same time, one-way analysis (ANOVA) based

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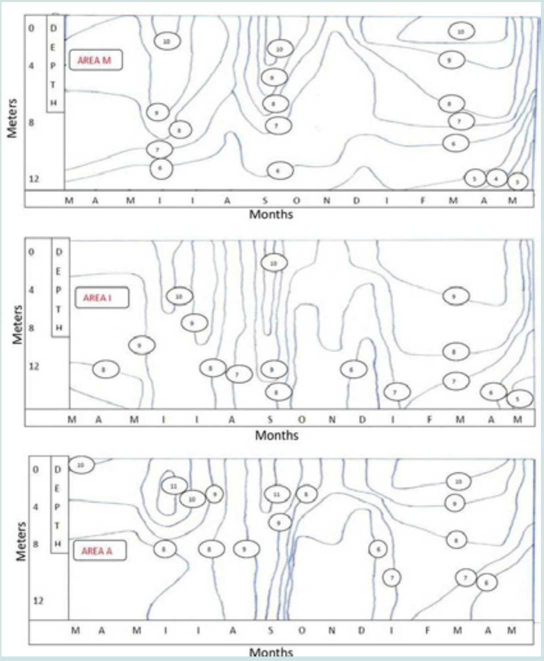

Figure 2: Dissolved Oxygen variation with depth at different months in sea water: coast (area A), mussel culture (area M) and reference area (area I).

on either time or region was used with or without the transformation of primary data. Duncan's multiple ranging control was used for the cases of homogeneity, unlike the Kruskal - Wallis parametric control method that was used for the cases of heterogeneity. Then the Mann - Whitney control method followed. Finally for the factor depth the t distribution method was used to statistically evaluate its fluctuation differences. All significance checks were made at a significance level of α = 5% and for the analysis the SPSS 8.0 statistical program was used. A three-dimensional charting program, Surfer Version 7.00, Surface Mapping System (Golden Software Inc. 1999), presents the seasonal and spatial distribution of parameter values that were measured at the surface and at the bottom of the research area of coast, mussel and sea. The horizontal x-axis represents the parallel offshore of 500 meters, while the y-axis represents the vertical distance from the shore of 1.100 meters. In particular, the sampling points for the coastline are defined for the y-axis in the 200 to 300 meter zone, for the mussel cultivation in the zone between 450 and 550 meters, while for the sea in the zone between 900 and 1,000 meters. The coordinates of the sampling points for the x axis are those of 0 meters, 250 meters and 500 meters. Strongly colored red areas show the highest seasonal concentrations of the variables [15-17].

Results and Discussion

Temperature

The temperature of the water which ranged from 10-26 °C is considered satisfactory for the survival of the mussels. According to the isothermal curve diagrams referring to time, no changes are observed between the three sampling areas. Also no thermal stratification was observed, since the vertical lines in the isothermal curve diagrams indicate the mixing of the water due to the current flow. High temperatures (24 to 25 °C) at the bottom during the summer months help aerobic decomposition of organic matter resulting in oxygen consumption (5.5-6.5 mg O_2/L) and the increase in concentrations of ammonium nitrogen (Figure 1).

Soluble oxygen (mg O_2/L)

The concentration of dissolved oxygen in water was found to be higher in the surface of the three areas (9-13 mg/L), which is attributed mainly to the high photosynthetic activity of phytoplankton organizations. On the other hand, lower oxygen values were found at the bottom of mussel cultivation (3 mg/L). The decomposition of organic substances that were accumulated at the bottom by metabolic excretions of mussels (faeces and pseudo-faeces) and the deposition of dead mussels explain low concentrations of dissolved oxygen. Despite the consumption of dissolved oxygen from mussels, there were no significant differences found on the surface of the water up to five meters depth in all three sampling areas. This may be attributed to high photosynthetic activity and to oxygen production, as well as to the continuous flow of water from the currents. Other researchers found an increased consumption of oxygen due to the degradation of the organic substance that was accumulated in the sediment in the form of faecal and pseudo-faecal matter [5,18-20].

The elevated temperatures that were observed in July and August (25 °C) in combination with the presence of organic (8.5% dry matter) confirm the low oxygen concentrations during this period in mussel culture (5 mg/L). Also, from February to May, the lowest concentration in dissolved oxygen (3-4 mg/L) is found at the bottom of mussel cultivation, which is attributed, despite the relatively low temperature values, to the large quantities of organic material that was found at the bottom (9.5% dry matter) (Figure 2).

A further reduction of dissolved oxygen in sediments is likely to create anaerobic conditions for the degradation of organic matter and for the formation of toxic gases such as methane (CH_4), hydrogen

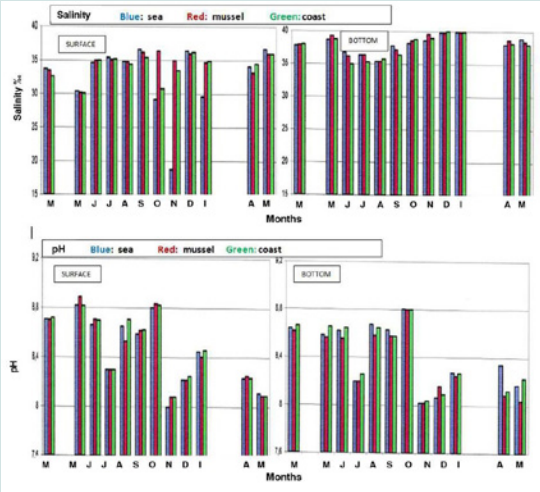

Figure 3: Monthly variation of pH and salinity (‰).

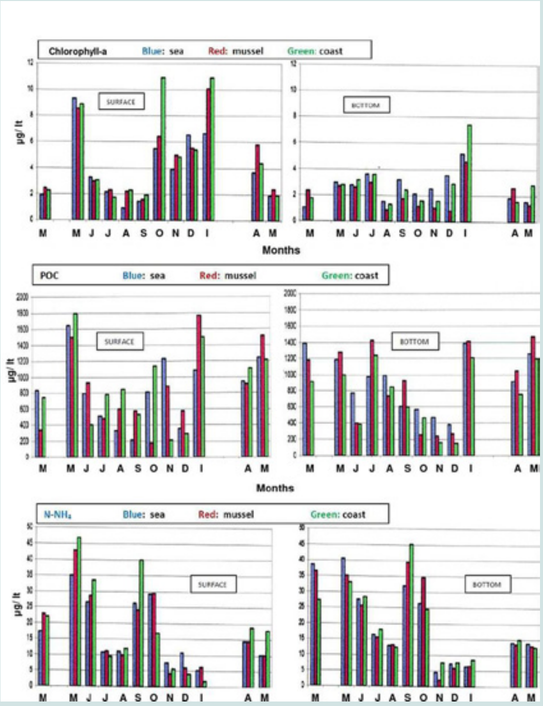

Figure 4: Monthly variation of a, POC, N-NH4 (μg/lt).

sulfide (H_2S), creating problems to the environment as well as to the mussel culture itself. The fact that no anaerobic conditions were found at the bottom is due to the young age of culture and possibly to the streams that partially disperse its organic bottoms.

The results are similar to the findings of other researchers which indicate that the oxygen is used by mussels for the basic functions (breathing and filtering). In contrast, another research did not show a significant change in the concentration of dissolved oxygen in water despite the presence of mussels (D.p.), which was attributed in the continuous flow of water currents [21-26].

pH

The highest values (8.6 to 8.8) that were observed on the surface of the three areas in relation to the bottom may be attributed to the photosynthetic activity of the autotrophic phytoplankton organisms. On the contrary, the decomposition of organic material that was accumulated at the bottom in the form of faeces and dead mussels contributes to lower pH values at the bottom [22,25]. However, pH values were appropriate for the growth of mussels (Figure 3).

Salinity

Salinity values ranged within the limits of mussel growth, with an

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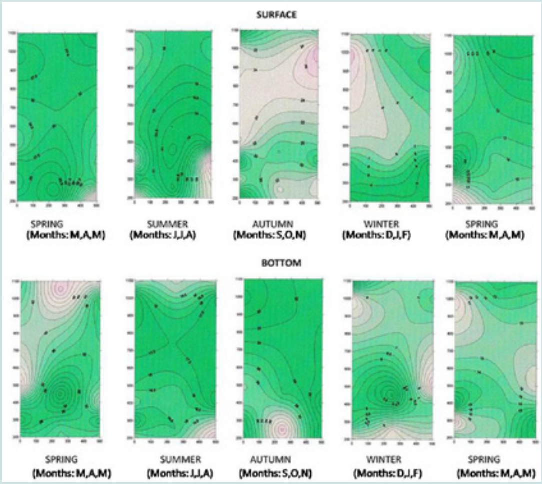

Figure 5: Seasonal variation of NH4 (μg/lt).

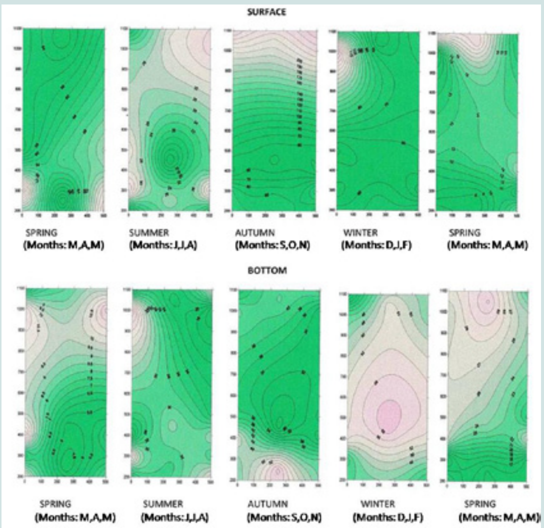

Figure 6: Seasonal variation of N-NO3 (μg/lt).

exception in November where low values (18‰) were found in the sea. This decrease in salinity is probably due to the large rainfall of the previous month and to the possible inflow of fresh waters from neighboring rivers. On the contrary, the gradual increase in salinity in June (from 30‰ in May to 35‰) may be due to the low rainfall in May and τo the low inflow of fresh water from neighboring rivers. The heavy rainfall that was observed in February may have contributed to a decrease in salinity in the coming months (30‰ to 33‰). The lowest values that were observed on the surface in all three regions compared to corresponding of bottom were expected, since fresh water as lighter remained on the surface (Figure 3).

Ammonium nitrogen (mg N-NH_4/L)

Higher concentrations of N-NH_4 were found at the bottom compared with the concentrations found at the surface at the three sampling sites (Figure 4). Particularly in the summer (July and August), the concentrations of ammonium nitrogen in the bottom (15.52±1.02 mg/L and 13.12±1.14 mg/L, respectively) were higher than those in mussel culture on the surface (11.23±1.51 mg/L and 9.97±1.25 mg/L respectively).

This fact may be attributed to the degradation of organic matter in the sediment. The seasonal and spatial distribution of the ammonia nitrogen concentration is shown in the three-dimensional graph of ammonium nitrogen (Figure 5). This figure shows the highest concentrations of ammonium nitrogen in the bottom of the three areas in the summer months, as well as the highest concentrations at the bottom of mussel cultivation in September (39.4±5.6 mg/L and in October (34,67±3,88 mg/L).

The highest concentrations of ammonium nitrogen, although not statistically significant, that were found on the mussel area relative to the reference area in March, May, June and July, may be attributed to the release of ammonium nitrate by mussels. Ammonia release from mussels due to degradation of dead mussels is also noted by other researches [5,22-24,27-31].

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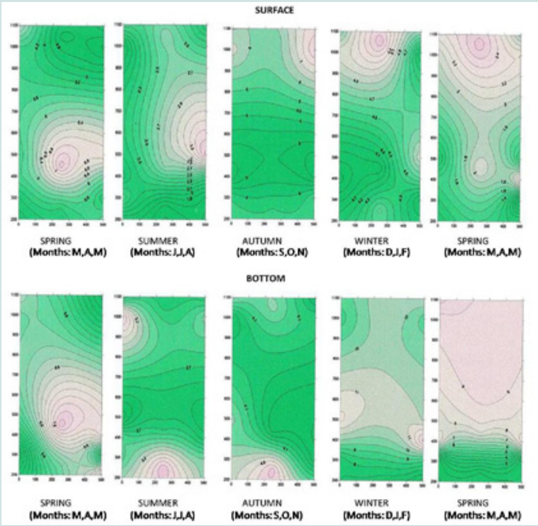

Figure 7: Seasonal variation of N-NO2 (µg/lt).

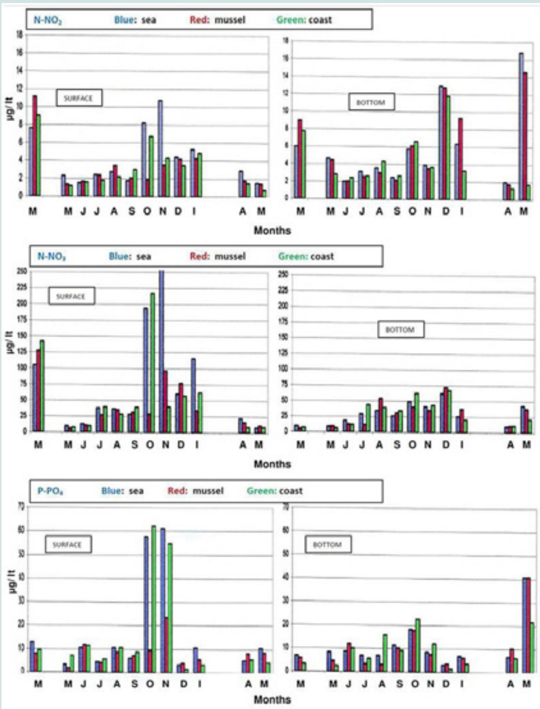

Figure 8: Monthly variation of N-NO2, P-PO4, N-NO3 (µg/lt).

Nitrite and nitrate nitrogen (mg N-NO_3/L, mg N-NO_2/L)

Nitrate nitrogen was found to be at higher concentrations in area I (from 8.19 to 469.96 mg/L) and in the coast (from 8.12 to 217.4 mg/L) compared to the concentrations in the mussel culture (from 6.51 to 127.44 mg/L).

This is probably due to increased inflows of nitrate water from the rivers firstly due to heavy rainfall and secondly due to the reduction of the speed of sea currents in mussel farming. At the bottom, nitrate nitrogen concentrations were lower than those on the surface, while significant differences were observed between the three regions. The above is illustrated in the three-dimensional graph of seasonal and spatial distribution of the nitrate nitrogen concentration (Figure 6 and 7).

The highest concentrations were observed on the surface of the sea due to the possible inflow of nitrate waters from adjacent rivers. However, the increase in nitrogen nitrate concentration that was found mainly in the bottom of mussel culture in December (72.45±1.86 mg/L) and January (37.71±3.02 mg/ L) can to be attributed to nitrification

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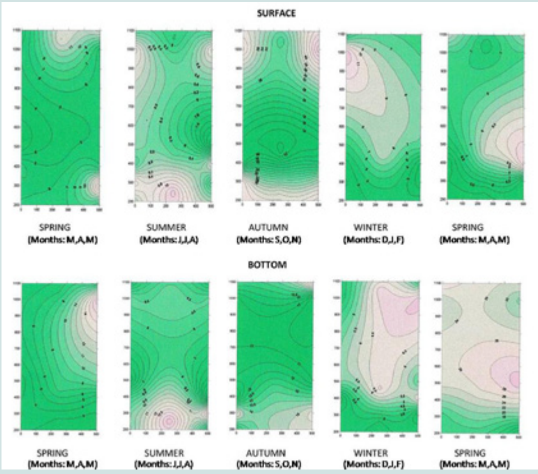

Figure 9: Seasonal variation of P –PO4 (μg/lt).

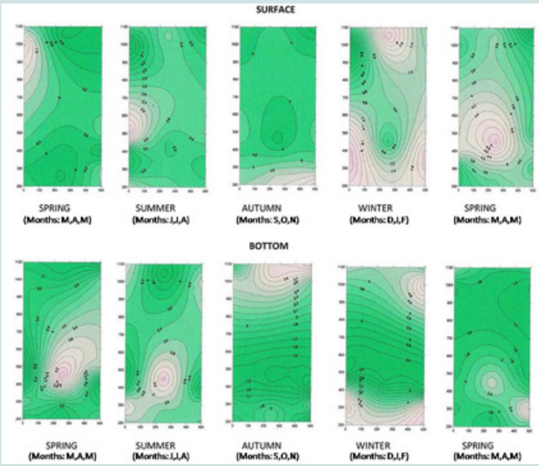

Figure 10: Seasonal variation of a (μg/lt).

of its nitrogen ammonium as a result of the decomposition of organic substances.

Nitrogen nitrate concentrations were at higher values particularly in December (12.81±0.88 mg/ L), January (9.28±0.11 mg/ L) and May (14.6±0.35 mg/ L) at the bottom compared to the surface (Figure 8).

This increase is probably due to the decomposition of the organic material that was deposited at the bottom. Other researchers support that the increased nitrate and ammonia concentrations are attributed firstly to the reduced number of phytoplankton organisms (due to their consumption of mussels), secondly to the excretion of organic material from the *Dreissena polymorpha* mussels and finally to the degradation of dead mussels [31-33].

Phosphorus of orthophosphate ions (mg P-PO$_4$/ L)

Phosphorus of orthophosphate ions was detected on the surface at higher concentrations in the sea (from 3.23 to 61.28 mg/ L) and in coast (from 1.65 to 62.44 mg/ L) than in the mussel culture (from 1.88 to 23.68 mg/L), particularly in October and November. This is mainly attributed to the influx of nutrients components from neighboring sources, in combination with the movement of the sea currents (B - NW winds).

In contrast, the bottom compared to the surface shows an increase in the concentration of phosphates in mussel culture, particularly in June (12.03±1.99 mg/ L), April (10.23±4.25 mg/ L) and May (40.81±2.5 pg/ L) (Figure 8). These results support the general findings of other researchers about the rich mussel excretions in nutrients. The above is also illustrated in the three-dimensional graph of seasonal and spatial distribution of phosphorus concentration of orthophosphate ions (Figure 9).

The increased concentrations of phosphate in October and November are combined both with increased concentrations of nitrate during the same months and with a gradual increase in chlorophyll from October to December. The decrease that was observed in June is attributed to nitrogen intake of diatoms and macrophytes and supports the general findings of other researchers [22,25,26,30,31,34-37].

Chlorophyll-a

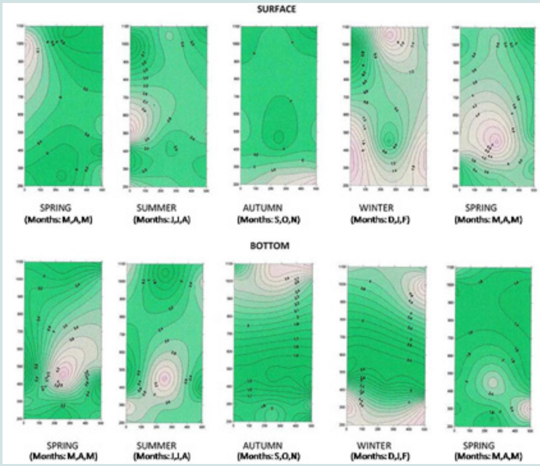

Figure 11: Seasonal variation of POC (μg/lt).

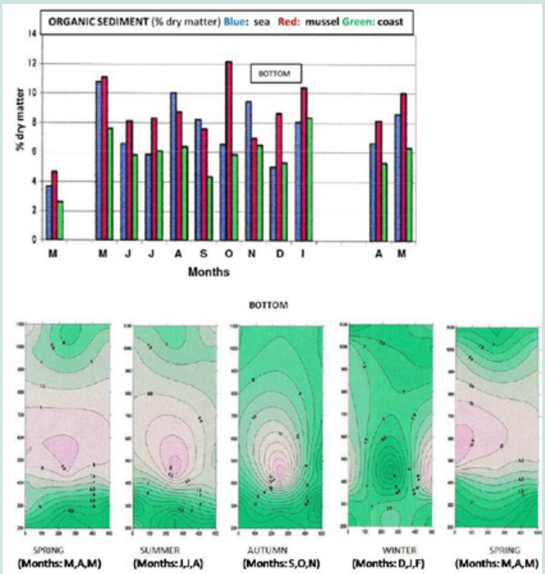

Figure 12: Monthly and seasonal variation of organic sediment (μg/lt).

Higher concentration of chlorophyll a was found on the surface of mussel culture compared to the reference area and to the coast for five out of the total twelve of months that the sampling lasted. No differences were found between mussel culture and reference area, apparently due to overexploitation of phytoplankton organisms from phosphate and nitrate inflows from neighboring rivers and due to surface currents. The consumption of phytoplankton from mussels is evident from the findings at the bottom where in most months (except March and April) there is a decreased concentration of chlorophyll a in mussel culture compared to the coast and the sea (Figure 4). The decrease in chlorophyll a is in an agreement with other researchers [30,38-41].

The increased concentrations of phosphate in October and November in the sea and near the coast are combined with increased concentrations of nitrate nitrogen over the same months and explain the gradual increase in chlorophyll from October 6.43±0.5 mg/ L) until January (10.07±0.88 mg/L). The above is also evident in the three-dimensional graph of seasonal and spatial distribution of the chlorophyll- a concentration (Figure 10).

Particulate Organic Carbon (μg C/ L)

Particulate Organic Carbon (POC) was measured on the surface at higher concentrations in mussel culture (from 178.2 to 1768.8±233.4 μg/ L) and near the coast (from 213.84 to 1797.8 μg/ L) (Figure 4). At the bottom, increased concentrations in mussel culture compared to the coast and in the sea were observed in May, July, September, January, April and May, but these increases were not statistically significant. High concentrations at the bottom should be mainly due to the sedimentation of organic material derived from mussel cultures in the form of faeces and dead mussels. The three-dimensional graph of seasonal and spatial distribution of the particulate organic carbon concentration the increased concentrations of particulate organic

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carbon at the bottom of mussel culture (Figure 11), due to the excretion of faecal matter as well as due to dead mussel deposition.

Also, the reduced concentration of particulate carbon on the surface of mussel cultivation for some months is attributed to phytoplankton consumption by mussels and is directly related to the reduced concentration of chlorophyll. In July and September, with high metabolic activity, there is an increase, not statistically significant, of particulate organic carbon at the bottom of mussel culture. The presence of high densities of the *Dreissena polymorpha* mussel population in other research resulted in a constant increase of the flow of particulate material from the water column to the sediment, especially in the summer period. The high value of particulate carbon that was observed in mussel culture and in the reference area in January coincides with the increased concentration of chlorophyll due to the low consumption of phytoplankton from mussels. From November to January, POC was measured at higher values in the surface than the bottom, which is attributed to the decline in phytoplankton consumption by mussels.

Organic sediments (% dry matter)

Organic sediments have been found at higher values for several months in mussel culture compared to the coast and the sea. The statistically significant differences between mussel culture and coast as well as between mussel and sea are apparently due to the organic material that was deposited at the bottom in the form of fecal and pseudo-faecal as well as dead mussels. The three-dimensional graph of seasonal and spatial distribution of the organic concentration illustrates the elevated concentrations of organics at the bottom of the mussel culture apparently due to the deposition of faecal matter and mussels (Figure 12).

Charging the bottom of mussel cultivation from organic material has led to reduced concentrations of dissolved oxygen in this area and to a higher concentration of ammonium nitrogen in the bottom rather than the surface. In July and in August, higher concentrations of ammonium nitrogen at the bottom that were observed over the surface, may be attributed to the degradation of sedimentary organic matter, which was trapped due to reduced currents from the coast to mussel culture. Thus, the concentration of ammonium nitrogen in culture in July from 11.2 μg/L on the surface increases to 15.5 μg/L, while in August it increases respectively from 9.9 μg/L to 13.1 μg/L. At the same time, the concentration of dissolved oxygen in water is maintained at low levels (5 μg/L). Other researchers have shown that the organic content of the sediment increases with the deposition of faeces and pseudofaeces, resulting in an increase in microbial activity and to the consumption of a higher amount of oxygen due to degradation of organic substance [40,42-47].

Conclusion

This study indicates that mussel culture greatly influenced the quality of the aquatic environment and the characteristics of the underlying sediments regarding the biological and physico-chemical parameters. The effects of mussels on nutrient cycling include marked changes in the nitrogen (form of $N\text{-}NO_3$, $N\text{-}NH_3$, $N\text{-}NO_2$) and phosphorous distribution (form of $P\text{-}PO_4$) both at surface and bottom. The ammonium excreted by mussels is immediately available for primary production; therefore mussels have a positive effect on primary production by increasing the nitrogen turnover in the water column. The release of $P\text{-}PO_4$ under the mussel culture and the lower concentrations of chlorophyll a due to the consumption of phytoplankton organisms by mussels were also confirmed. Higher concentrations of organic material and particulate organic carbon demonstrate the organic enrichment of mussel culture in relation to the reference area and to the coast, resulting in the lower concentrations of dissolved oxygen in the water. Further reduction of dissolved oxygen in sediments due to the accumulation of organic material over the years can create anaerobic conditions for the degradation of organic matter.

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