Master's Thesis

Effects of the endangered freshwater pearl mussel *Margaritifera margaritifera* on river ecosystem and water quality

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ABSTRACT

Freshwater mussels (Unionoida) can provide valuable ecosystem services through their filtration function, consequently improving or affecting water quality. The endangered freshwater pearl mussel *Margaritifera margaritifera* used to occur and can still occasionally occur in high densities. However, no study has been conducted regarding how *M. margaritifera* influences river ecosystem and water quality. The aim of this study was to investigate the impact of *M. margaritifera* on the boreal river through analysing water quality parameters, phytoplankton and zooplankton. Thus, two closely located rivers— *M. margaritifera* river and control river (no mussel)—in northern Finland were compared using water samples and *in situ* measurements performed at 50 m intervals from the first 500 m section downstream from the headwater lake of both rivers. Variables studied included water temperature, conductivity, pH, total suspended solids, optical dissolved oxygen and fluorometric estimates of blue-green algae and chlorophyll-a, as well as density and species composition of phytoplankton and zooplankton, in addition to *M. margaritifera* count. The estimated number of *M. margaritifera* in 500 m study section was about 32000. The response of measured variables in relation to the distance from the lake to the rivers were studied by regression analysis. The results point to the likelihood that *M. margaritifera* reduce blue-green algae, chlorophyll-a and dissolved oxygen as well as phytoplankton and zooplankton density, suggesting potentially remarkable ecosystem

effect and influence on water quality by *M. margaritifera* when occurring in high density.

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Bio- ja ympäristötieteiden laitos
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TIIVISTELMÄ

Makeanveden helmen simpukka *Margaritifera margaritifera* on uhanalainen laji. Makean veden simpukat (Unionoida) voivat tarjota arvokkaita ekosysteemipalveluja ja parantaa veden laatua suodatustoimintansa ansiosta. Mitään tutkimusta ei kuitenkaan ole tehty siitä, miten uhanalainen *Margaritifera margaritifera*—joka on aikaisemmin esiintynyt (ja voi esiintyä vielä nykyäänkin paikoittain) hyvin suurina tiheyksinä—vaikuttaa jokiekosysteemiin ja veden laatuun. Tämän tutkimuksen tarkoituksena oli tutkia jokihelmisimpukoiden vaikutuksia jokiin analysoimalla veden laatuparametreja sekä kasviplanktonin ja zooplanktonin tiheyttä kahdessa pohjois-suomalaisessa, lähekkäin sijaitsevassa joessa, joista toisessa esiintyy tiheä jokihelmisimpukkakanta, mutta toisessa simpukoita ei ole. Tutkimus tehtiin keräämällä vesinäytteitä ja suorittamalla mittauksia paikan päällä 500 metrin matkalla kymmenestä pisteestä 50 metrin välein jokien yläpuolella sijaitsevasta järvestä alkaen. Tutkitut muuttujat olivat veden lämpötila, sähkönjohtavuus, pH, suspendoituneet kiintoaineet, liuennut happi, fluorimetriset estimaatit sinilevälle ja klorofylli-a:lle, sekä kasviplanktoninja zooplanktonin tiheys ja lajisto. Arvioitu jokihelmisimpukkamäärä tutkitulla 500 metrin matkalla oli 32000 yksilöä. Jokien välisiä eroja mitattujen muuttujien käyttäytymisessä suhteessa etäisyyteen järvestä tutkittiin regressioanalyysillä. Tulokset viittasivat siihen, että *M. margaritifera* alentaa siniviherlevien, klorofylli-a:n ja liuenneen hapen pitoisuuksia sekä kasvuplanktonin ja

zooplanktonin tiheyttä. Tuloksista voidaan päätellä, että ainakin tiheä *M. margaritifera* -esiintymä saattaa pystyä vaikuttamaan veden laatuun ja jokiekosysteemin toimintaan.

TABLE OF CONTENTS

ABBREVIATIONS

Dist.	Distance
DO	Dissolved Oxygen
IRR	Incident Rate Ratio
FPM	Freshwater Pearl Mussel
MussCum	Cumulative mussel
TSS	Total Suspended Solids

1 INTRODUCTION

Freshwater mussels belong to the order Unionoida. The endangered freshwater pearl mussel (FPM), *Margaritifera margaritifera* is one of the longest living invertebrates usually live over 100 years (Bauer 1992, Helama and Valovirta 2008). Usually, they attain their sexual maturity at 10-15 years when the length generally exceeds 65mm (Young and Williams 1984). Freshwater mussels are one of the important components of the aquatic ecosystem and considered as an ecosystem engineer because of its various activities including burrowing and filtering capacities (Chowdhury et al. 2016, Vaughn 2018). Freshwater mussels perform different functions in aquatic systems. Vaughn (2018) categorized these functions as 1) regulatory services which include biofiltration and has direct influence on water purification; 2) supporting services that include nutrient recycling and storage (impact on water quality), structural habitat (impact on fish habitat), and substrate and food web modification (impact on biodiversity); and 3) provisioning and cultural services including use as a food source, as tools, jewellery and art, and for spiritual enhancement. Moreover, *M. Margaritifera* is considered as an indicator species to reflect ecosystem health of the water body since it satisfies all the criteria of indicator species (Geist 2010). Freshwater mussels can play a significant role to recover the biodiversity of a polluted lake (Chowdhury et al. 2016).

1.1 Habitats, distribution and present status of freshwater pearl mussels *M. margaritifera*

The mussels live buried or partly buried in coarse sand and fine gravel in clean, oligotrophic, fast-flowing and unpolluted rivers and streams (Young & Williams 1984). *M. margaritifera* is mostly found in cool, oxygen saturated running water. The most suitable habitat for *M. margaritifera* is the streams with bedrock, cobble, and gravel substratum, moderate flow velocities, low nutrient concentrations and low carbonate

content as well as the presence of adequate salmonid hosts (Geist 2010). Neutral to a slightly acidic condition of the water is the most preferable condition for *M. margaritifera* (Young & Williams 1984) and they also have the preference for the habitats that have more than 80% tree cover (Outeiro et al. 2007). In broad sense adults and juvenile *M. margaritifera* prefer almost similar habitats but adults can condone silty and muddy conditions for a long time conversely juveniles never found in this type habitat (Hastie et al. 2000).

Freshwater pearl mussel (FPM) is widely distributed from the arctic and temperate regions of western Russia, westwards through Europe to the northeastern seaboard of North America (Young et al. 2001). However, distribution of *M. margaritifera* is widespread and discontinuous in rivers and streams of Western and Central Europe (Saano et al. 2010). It has also found in oligotrophic streams of northern and central Europe (Lopes-Lima et al. 2017). The largest known population of *M. margaritifera* can be found in Norway and north-western Russia at present time (Oulasvirta et al. 2014). The species is about to elimination from streams of Belarus, Denmark, Lithuania, and Poland and the most alarming is that because of lack of recent recruitment more than 95% of the residual population is highly fragmented and functionally extinct in southern and central Europe (Geist 2010, Young et al. 2001). Because of valuable pearl, *M. margaritifera* has been exploited in Europe since pre-Roman times. Unfortunately, this species has classified as a critically endangered species in the 2011 IUCN European assessment on non-marine molluscs (Cuttelod et al. 2011) as well as one of the highly threatened freshwater bivalves worldwide (Giest 2010). In Finland, FPM is protected by the Nature Conservation Act since 1955 (Oulasvirta et al. 2014). There are 117 *M. margaritifera* rivers recognized in Finland while 106 *M. margaritifera* rivers located in northern part and only 11 *M. margaritifera* rivers situated in southern part of Finland (Oulasvirta et al. 2017).

1.2 Probable reasons for declining *M. margaritifera*

Several reasons are responsible for the extinction of this species for example habitat loss and fragmentation, overexploitation, pollution, loss of host fishes, introduction of non-native species, water abstraction and climate change (Lopes-Lima et al. 2017). Another important reason for the loss of mussels is the alteration of physicochemical characteristics of streambed which cause the problem for finding a suitable place for juvenile mussels (Geist and Auerswald 2007). Furthermore, mortality of adult mussels is largely influenced by higher nitrate concentration as well as increased level of phosphate and calcium. However, survival and establishment of juvenile influenced by biological oxygen demand (BOD) level of water (Bauer 1988). On the other hand, Taskinen et al. (2011) have investigated that persistence of glochidia may negatively be affected by low pH and high concentration of heavy metal which eventually leads local reduction of *M. margaritifera*. In Finland acidification and releasing of chemicals by humans to the water body are the main reasons for declination of *M. margaritifera* (Valovirta 1998).

1.3 Roles of mussels to improve water quality

Freshwater is the habitat for many aquatic organisms, but this important habitat is getting polluted and quality degraded because of natural and mainly anthropogenic activities. Process and application of chemicals to reduce the pollution level from freshwater are some extent tough and expensive compared to a biological process. Freshwater mussels can be an effective biological control tool to improve the water quality because of their filter feeding characteristics. Initially, mussels were categorized as suspension feeders, but it is now proved that mussels can feed different food particles like benthic as well as planktonic such as phytoplankton, rotifers, and detritus (Nichols et al. 2005). *M. margaritifera* is called as the keystone taxon since they are one of the major filter feeders in many lakes and rivers of the world (Mamun & Khan 2011, Geist 2010). A 61 mm-long mussel can filter maximum 0.5 to 1.0 L/h, but the volume

of filtered water depends on water temperature, species, animal size, population density as well as the testing procedure of water volume measurement (Vaughn et al. 2008). Mussels can clear 35% of suspended particles from the water column (Lummer et al. 2016).

Furthermore, pearl mussels can help to accumulate metals and nutrients from water for example, each ton of pearl oyster material removed approximately 703 g metals, 7452 g nitrogen and 545 g phosphorus from the water of Port Stephens (Gifford et al. 2005). High mussel densities can lead to biological oligotrophication through decreasing nutrients and phytoplankton biomass which ultimately result in increased water clarity (Chowdhury et al. 2016). Filtration activities by unionids were the reason of biological oligotrophication in the River Spree, Germany through reducing phytoplankton biomass and total phosphorus, thereby increased water clarity (Welker & Walz 1998). Real time changes of water quality can detect through analyzing different physiological responses of mussel such as gaps that is shell opening and closing, variations in heart rate, and changes in filtration and behavior (Goodchild et al. 2016, Hauser 2015, Hartmann et al. 2016). Hartmann et al. (2016) used *Anodonta anatina* to observe changing behavioral response because of de-icing salt pollution and found large variation in filtration behavior. Moreover, a sudden high mortality rate of mussels can be an indication of a high level of pollution of that water body (Mamun & Khan 2011).

1.4 Ecosystem services provided by freshwater mussels

The filtering activity of mussels not only improve the water quality but also has significant impacts on the aquatic ecosystems such as transferring nutrients and energy from water column to sediments which leads increased production across the trophic level (Vaughn et al. 2008). Howard and Cuffey (2006) studied that freshwater mussels are important to maintain the local food web of a water system and stimulate benthic production through removing large volume of particulate matter from the water

column and converting those as faeces and pseudofaeces. These nutrient-rich biodeposit are transferred from the water column to the bottom and act as available sources of food to the benthic community. Moreover, mussels' shells act as habitat for many benthic biota (Vaughn et al. 2008). Therefore, mussels' presence can increase benthic invertebrate production which is an important food source for young salmon and trout (Oulasvirta et al. 2014). However, mussels excrete can be readily taken up by algae and heterotrophic bacteria since these are soluble nutrients (Vaughn 2018). Mussels can improve oxygenating of stream bed through bioturbation (Boeker et al. 2016) which may enhance production of macroinvertebrates and can provide better quality salmonid spawning grounds (Lummer et al. 2016) by the same way of effects of burrowing lamprey larvae (Boeker and Geist 2016).

Mussels can purify and clear the water through filtering suspended particles. This can facilitate light penetration, eventually stimulating primary production and making a better habitat for clear-water dependent species. This condition may favour abundance and growth of higher trophic levels such as fish and availability of increased fish hosts can bring positive effects on reproduction success of freshwater mussels (Lummer et al. 2016). Mussels can also alter the algae community by influencing nutrients in streams which consequently affect water quality (Atkinson et al. 2013a). Consequently, reduction of mussels can affect different services given by mussels such as Vaughn et al. (2015) found that biofiltration, nitrogen and phosphorus cycling and nitrogen, phosphorus and carbon storage provided by mussels declined almost 60% over 20 years in the Kiamichi River in southeastern Oklahoma, U.S. Recently it was also shown that freshwater mussels can filter larvae of fish parasites from water (Mironova et al. 2018) and thus, affect transmission of parasites in the aquatic ecosystems.

However, no study has been conducted to measure impacts of freshwater pearl mussel (FPM) to the water body to realize its ability to improve water quality and ecosystem. This knowledge is particularly important to enhance the conservation efforts of endangered freshwater pearl mussel. This is the first attempt to evaluate the function

of *M. margaritifera* in a river ecosystem and will give an idea how the very large FPM populations that prevailed in our rivers in the past may have affected the rivers. The main objectives of this study were:

- to study the impacts of *M. margaritifera* on the quality of boreal river water through analysing different water quality parameters,
- to identify the effects of *M. margaritifera* in river ecosystem by studying its impact on phytoplankton and zooplankton density.

1.5 Hypothesis

Filter-feeding of freshwater mussels has many consequences in the aquatic ecosystem. Mussels remove algae, bacteria and organic particles (McMahon 1991). Mussels can clear a remarkable proportion of suspended particles (Lummer et al. 2016). Mussels have been observed to reduce phytoplankton biomass and phosphorus content and increase water clarity of river, leading to biological oligotrophication in high mussel densities (Chowdhury et al. 2016, Welker and Walz 1988). The decrease of chlorophyll-a concentration due to mussel filtering activity has been observed in a number of field and laboratory studies (Bunt et al. 1993, Caraco et al. 1997, Pace et al. 1998, Reeders et al. 1989, Soto & Mena 1999). Freshwater mussels remove large amount of particulate matter from water column (Howard and Cuffey 2006). Smith et al. (2012) found in an experimental aquaria that mussel can significantly reduce turbidity. Mussels have been found to reduce N-fixing blue-green algae (Atkinson et al. 2013b). Caraco et al. (2000) found that inclusion of zebra mussel in the Seneca River caused declining of dissolved oxygen concentration.

In accordance with these findings, my hypotheses were as follows. As compared to the control river, *M. margaritifera* river is characterized by a decrease, or steeper decrease, in phytoplankton density, chlorophyll-a, blue-green algae density, suspended solids and turbidity. The large biomass of mussels could also result in reduced oxygen content of water. If phytoplankton density will decrease, this could also lead to a

reduced density of zooplankton. No specific prediction could be made with respect to the effect of *M. margaritifera* on water colour, conductivity, pH, phytoplankton species richness and zooplankton species richness (Table 2).

2 MATERIALS AND METHODS

2.1 Study Area

Two closely located northern Finnish rivers from the river Tornionjoki catchment were selected for this study (Figure 1); control river (Table 1) where no freshwater pearl mussels (FPM) were present and the *M. margaritifera* river (Table 1) where FPM were present (Oulasvirta et al. 2014). Both rivers were studied for the first 500 m section downstream from the headwater. The section was selected because of the high abundance of mussels in the upper part of the *M. margaritifera* river. The total *M. margaritifera* population of this 6.3 km long river is 131 000 individuals and *M. margaritifera* is the only mussel species in the river (Oulasvirta et al. 2014).

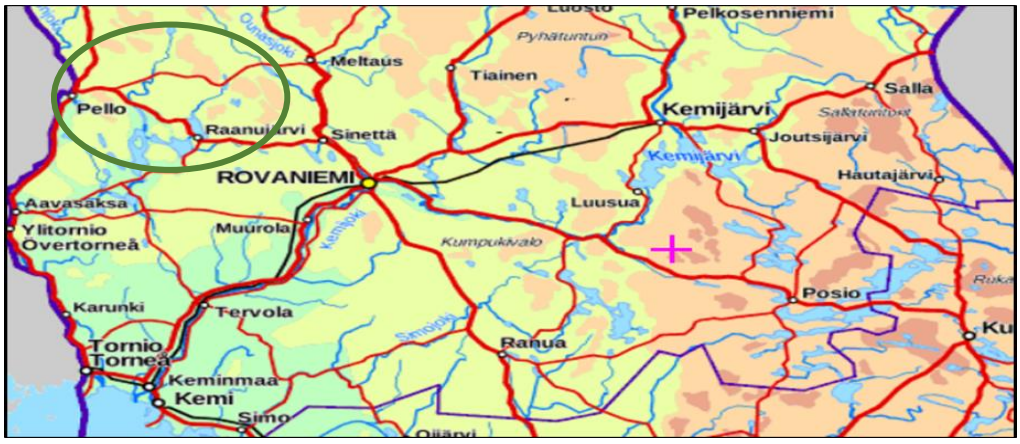

Figure 1. Location of study rivers near Pello, Finland (within the circle on the map).

Table 1. Characteristics of the control river and *M. margaritifera* river.

Parameter	**Control river**	***M. margaritifera* river**
Catchment area number[1]	67.932	67.933
Elevation[1], m	86.9	169.8
Mean runoff[2], m^3/s	0.7	0.3
River width[3], m	4 - 5	1.5 – 2
Mussel number estimate (in 500m section)[3]	0	32000

[1]jarviwiki.fi (read October 10th, 2018); [2] The Finnish drainage basin register, VALUE tool; [3] measured in this study.

2.2 Sampling

The sampling was conducted on 18th July 2017. Both rivers were sampled from ten points along the 500 m study section (50 m apart) on the same day. The first point was 50 m downstream from the lake and consequently, the tenth point was 500 m downstream from the lake. All ten points were sampled for water quality with a handheld YSI6600V2-4 multiparameter sonde (YSI Inc., Yellow Springs, OH, USA) measuring conductivity, pH, total suspended solids, optical dissolved oxygen and fluorometric estimates of blue-green algae and chlorophyll, and by collecting water samples in 5 L prerinsed plastic containers. The sample size for each parameter was 10. The readings obtained from YSI sonde for dissolved oxygen and blue green algae cells were used for statistical analysis. Rest of the parameters (conductivity, pH, water colour, turbidity, total suspended solids, chlorophyll-a, phytoplankton density and species richness, zooplankton density and species richness) analysed in laboratory to get more reliable results and readings obtained from laboratory analysis used for statistical analysis. Response variables of the study are given in Table 2.

Table 2. Mean, standard deviation and predicted changes due to effect of *M. margaritifera* of all water quality parameters (n=10) measured *in situ* sonde and in laboratory.

Water quality Parameters	**Control river**		***M. margaritifera* river**		
	Mean±S.D.	Measuring method	Mean±S.D.	Measuring method	Predicted changes
Conductivity, mS/m (25°C)	1.79±0.03	Laboratory	1.91±0.05	Laboratory	-
pH	6.80±0.06	Laboratory	7.00±0.07	Laboratory	-
Dissolved oxygen, mg/L	9.85±0.09	*In situ* sonde	9.77±0.17	*In situ* sonde	Decrease
Water color, mg Pt L^{-1}	63.77±1.62	Laboratory	44.94±2.70	Laboratory	-
Turbidity, NTU	1.54±0.57	Laboratory	1.36±0.27	Laboratory	Decrease
Total Suspended solids, mg/L	1.20±0.37	Laboratory	1.02±0.16	Laboratory	Decrease
Chlorophyll-a, µg/L	4.96±0.42	Laboratory	2.67±0.58	Laboratory	Decrease
Blue-green algae, cells/mL	356.36±89.22	*In situ* sonde	296.99±123.46	*In situ* sonde	Decrease
Phytoplankton density, cells/50mL	5852.66±2003.10	Laboratory	9772.38±8563.06	Laboratory	Decrease
Phytoplankton species richness	14.50±2.12	Laboratory	14.50±2.32	Laboratory	-
Zooplankton density, ind./50mL	2.82±0.99	Laboratory	1.54±0.70	Laboratory	Decrease
Zooplankton species richness	19.10±2.88	Laboratory	14.50±1.96	Laboratory	-

Water quality measures were obtained by holding the multiparameter sonde submerged at the sampling point for one minute after the optics were cleaned automatically or by submerging the 5 L sample container upstream in the water by

hand, at a depth of 10-15 centimetres. Water samples were held in cool and dark prior to the analysis conducted at the water laboratory of the Department of Biological and Environmental Science, University of Jyväskylä.

Samples for phytoplankton and zooplankton were collected from the same depth range as the water quality samples. Zooplankton sample was collected by submerging 10 L water bucket in the river for 3 times as quickly as possible and filtered 30 L of collected water through 100 µm plankton net. The concentrated sample was then emptied in a 500 mL prerinsed plastic container and the net was rinsed twice bringing a total sample volume of 100 mL from the 30 L of raw river water and preserved by adding 100 mL of ethanol (ETAX A12, ALTIA Oyj, Finland) directly on site. Phytoplankton was collected directly from the sampling depth to 100 mL amber glass bottles and 5 drops of Lugol solution was added to the samples directly on site. Consequently, plankton samples were held in cool and dark prior to the analysis (until September 20, 2017). From each sampling point, numbers of mussels were counted using an Aqauscope (underwater viewing device) from a 2 m wide transect across the river.

2.3 Laboratory analysis

Water quality analysis for pH, turbidity, conductivity, water colour and chlorophyll-a were conducted in the laboratory on the next day and analysis for total suspended solids (TSS), organic and inorganic suspended solids started (and finished on the following days). Eventually, phytoplankton and zooplankton density as well as species richness were calculated in the laboratory.

2.3.1 pH measurement

pH of all the water samples was measured in the laboratory by using pH meter (Metrohm 744 pH meter, CH-9101 Herisau, Switzerland) which is consist of combination electrode- glass and calomel electrodes. Before measuring, the meter was

calibrated with standard solutions of pH 4 and pH 7. About 50 mL sample was taken into the beaker and electrodes were submerged into the sample until pH reading become stable. The stable reading was recorded from the screen of pH meter (SFS 3021).

2.3.2 Turbidity

Turbidity was measured by using a turbidity meter (Merck Turbiquant 1500T, Merck KgaA, Darmstadt, Germany) which is based on nephelometric determination of water turbidity (scattering of light from suspended particles in the water). The method is based on comparison with reference solutions with known scattering properties. To conduct this analysis cuvette was rinsed with 20 mL water for 3 times. The sample was placed in a 30 mL cuvette and placed it into the meter and reading (nephelometric turbidity units, NTU) was recorded immediately.

2.3.3 Conductivity

Conductivity (mS/m, at 25 °C) was measured in the laboratory by using conductivity meter (CDM2e, Radiometer Copenhagen, Denmark). Conductivity is quotient of electrolyte current density and strength of electric field that grows when the electrolyte concentration of the solution increases. The more the ions, the higher the conductivity. Conductivity meter contains a flask where current electricity travels between platinum electrodes. Conductivity of the sample is a product of electrical conductivity and flask media and needs to be corrected for temperature at 25 °C. Meter gives the correct value immediately after submerging the electrode into the sample (SFS-EN 27888).

2.3.4 Water colour

Water colour was determined with a spectrophotometer (Shimatzu UV1800 UV-spectrophotometer, Kyoto, Japan). Spectrophotometric method (ISO 7887:2011) compares the sample to the known concentration of platinum cobalt chloride solution. Water colour was measured at wavelength 420 nm. Prior to this, the device was zero

adjusted with distilled water and the sample was filtered (GF/C, Whatman, info on filter). A calibration curve was made with the concentration on x-axis and absorbance on y-axis producing the eventual water colour in mg Pt L^{-1}.

2.3.5 Suspended Solids

Suspended solids were measured by using the Finnish standard (SFS 3037). Shortly described, a clean filter (GF/C, Whatman) was placed on the filtration sinter and wet with a drop of distilled water. A couple of seconds suction was applied; the filter was carefully removed (not to change the weight) and dried in an oven for one hour in +105 ±5 ° C. The dried filter was stored in desiccator and weight with a precision of ± 0.1 mg. After that weighed filter was placed on the filtration stone sinter. The volumes of well shaken filtered sample were varied from 750 mL to 1500 mL depending on accumulation of suspended solids on filter. Suction was continued until the filter was dry. Distilled water (40 mL) was used for rinsing particles from the sides of the chamber. The filter was then carefully removed and placed in the oven to be dried for at least 2 hours in+105 ±5 ° C. Weight of dried filter paper was stabilized then again in the desiccator and measured. TSS of the samples were calculated by using equation 1.

TSS of the sample mg/L= 1000 (a-b)/V……. eq (1)

Where, a=combined weight of the filter and the filtrate (mg), b= weight of the filter (mg), V= volume of sample filtrated (ml).

Inorganic suspended solids (TSS ash weight) were gained by annealing (burn in high temperature) the filter after TSS measurement for 2 hours in +550 °C in porcelain crucible. Weight was then stabilized in the desiccator and measured. Amount of organic suspended solids (burned away in the oven) was also determined, amount of organic suspended solids, mg/L = TSS- ash weight.

2.3.6 Chlorophyll-a

Chlorophyll-a of water sample has assessed in the laboratory according to ISO 10260:1992. To measure Chlorophyll-a (µg/L) first the sample water needed to filter by using GF/C. For filtering, we used 1000 mL of well shaken sample for each sample. Then algae with the pigment left on the filter were extracted in 20 mL of ethanol A16. After that, all the bottles were closed with a cap and wrapped with Al foil. Then these bottles were placed in the fridge (+4°C) for overnight (cold ethanol extraction). In the next day, the extracts were filtered through a large filter by folding the filter in a small plastic funnel and placed it on a 100 mL conical glass. The extract was then measured optically. Calibration of blank sample with A16 ethanol was done at wavelengths 665 and 750 nm. Finally, the concentration of chlorophyll-a of the samples were measured by using equation 02.

$$Cc=A*Ve*1000/(Vs*d*Kc) \quad \text{eq (02)}$$

where Cc = Chlorophyll-a concentration µg/L; A= A665-A750; A665= sample absorbance at wavelength 665 nm and A750= sample absorbance at wavelength 750 nm; Ve = volume of ethanol (mL); Vs= volume of sample filtered (L); d= length of the cuvette usually used in the measurement (5 cm); Kc = 83.4. Chlorophyll-a absorption coefficient (in 94% ethanol).

2.3.7 Identification and counting of Phytoplankton

To count and identify phytoplankton species in the sample, 50 mL of the well shaken sample was taken into a 50 mL tube with a counting chamber and left to settle for 24 hours (2 replications for each sample). For calculation of phytoplankton cells per 50 mL sample, an Uthermol counting chamber was used (Utermöhl 1958). After completion of settlement process, the counting chamber was moved to microscope gently without any disturbance. An inverted microscope with 20X objective was used to identify and count phytoplankton. Phytoplankton taxa or species were identified based on

morphological characteristics like colour, shape and colony structure. During the counting process, imaginary transects along chamber were followed to prevent counting same field again. For counting, from each sample, 10 views were observed. Views were selected randomly by following imaginary transects. The total number of individual phytoplankton species were calculated according to equation 03.

*Total number of individual species in 50 mL sample= (Area of the chamber / Area of the view) * Average number of species……eq (03)*

2.3.8 Identification and counting of zooplankton

For identification and counting of zooplankton, an inverted microscope was used with 20X objective. Since the original sample was concentrated, sub-samples were diluted by taking a 25 mL sample and 25 mL water in the tube with the chamber. The sample was covered with coverslip and samples were kept for 12 hours to settle. After settling, the process counting chamber was moved carefully to the microscope for counting. For zooplankton counting, imaginary transects along chamber was also followed and all the transects were observed to identify and count all the zooplankton. The total number of zooplanktons per litre was counted by using equation 04.

Total number of zooplankton per litre sample = Number of counted specimen(Volume of the concentrated sample / volume of subsamples counted) / Sample volume……eq (04)*

2.4 Data Analysis

2.4.1 Data generation and handling

For statistical use, *in situ* multiparameter sonde data was observed for any disturbances in the readings and an average of 3 to 8 blind recordings was counted for each parameter measured at each sampling point. Water quality data was presented as singular values apart from Chlorophyll-a and Suspended Solids which were averages of 3 replicates. Zooplankton sample was also calculated from a single value, but phytoplankton sample was calculated as an average of 2 replicates. Phytoplankton and

zooplankton density were expressed as per 50 mL volume of sample. For statistical analysis to see mussels' effect in the *M. margaritifera* river, mussels were estimated as cumulative mussel (MussCum) which indicates the total number of mussels from 1st to last sampling point of *M. margaritifera* river. The data sets of conductivity, pH, dissolved oxygen (DO), turbidity, total suspended solids, chlorophyll and zooplankton density were close to average value, but data sets of water colour, blue-green algae and phytoplankton density were spread out (Table 2).

2.4.2 Statistical analysis

Obtained data, i.e. values of each studied variable, were first plotted against distance from lake to find patterns of change. If a contrasting trend was observed in a given measured water quality parameter or variable, it could signal the effect of *M. margaritifera*. For example, if the chlorophyll-a value would decrease by distance downstream from the starting point (below the lake) in the *M. margaritifera* river but not in the control river, that would be interpreted as a possible effect of mussels—since mussels' filter and remove phytoplankton which would be seen as reduced chlorophyll-a values.

We assumed the trends—if they are found—to be linear. If patterns (trends) were observed, regression analysis was used to investigate a) the possible contrasting trends between *M. margaritifera* river and control river and b) the relationship between a given parameter and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point). In the regression analyses, the cumulative number of mussels (MussCum) was considered as an explanatory variable. The river (*M. margaritifera* river and control river) and distance from the lake (0-500 m) were considered as indicator variables so that the *M. margaritifera* river received a value of 1 and control river a value of 0.

The response variables for regression analyses, selected by visual inspection for possible trends, were dissolved oxygen (DO), conductivity, turbidity, total suspended solids (TSS), chlorophyll-a, blue-green algae, phytoplankton and zooplankton density, and phytoplankton and zooplankton species richness. The linear regression model was used for DO, turbidity, TSS and blue-green algae and log-linear regression model was used for phytoplankton density, zooplankton density, phytoplankton species richness and zooplankton species richness since their values were an integral value. All the models were done by using R program (R Core Team. R, 2018). For the standard linear regression model, the response variable was considered as continuous and normally distributed. For example, in case of DO the mathematical form of the model was

$$\mathrm{E}(DO) = \beta_o + \beta_1 M.margaritiferariver + \beta_2 dist + \beta_3 M.margaritiferariver.dist.$$

However, log linear regression model was applied for numerical occurrences where the model assumed Poisson distribution for responsible variable. The mathematical form of this model for example in case of phytoplankton density is given below.

$$\log(phyto) = \beta_o + \beta_1\, M.margaritiferariver + \beta_2 dist + \beta_3\, M.margaritiferariver.dist.$$

3 RESULTS

3.1 Distribution and number of *M. margaritifera* in *M. margaritifera* river

The abundance of *M. margaritifera* was not uniform in different observed points of *M. margaritifera* river. The highest number of *M. margaritifera* (about 300) was found in the point observed around 350 m distance of *M. margaritifera* river and lowest number (about 35) was found around 50 m distance (Figure 2). The cumulative number of *M. margaritifera* over the 500 m section was 1285 (Figure 3). As 2 m wide cross-transects of the river were searched for mussels in each sampling point, it can be estimated that a total of 32000 *M. margaritifera* individuals occured in the 500 m study section of the *M. margaritifera* river.

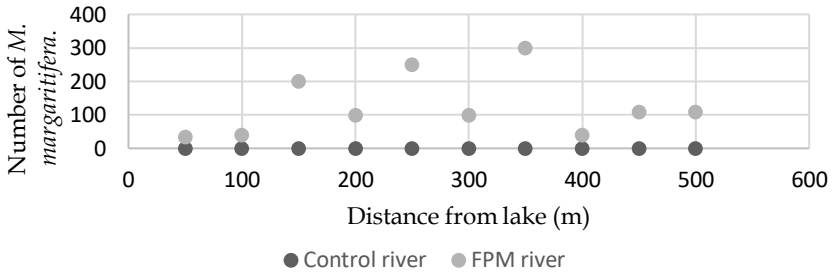

Figure 2. Distribution of *M. margaritifera* in 500 m study section of *M. margaritifera* river and control river, as counted from a 2 m wide transect across the river.

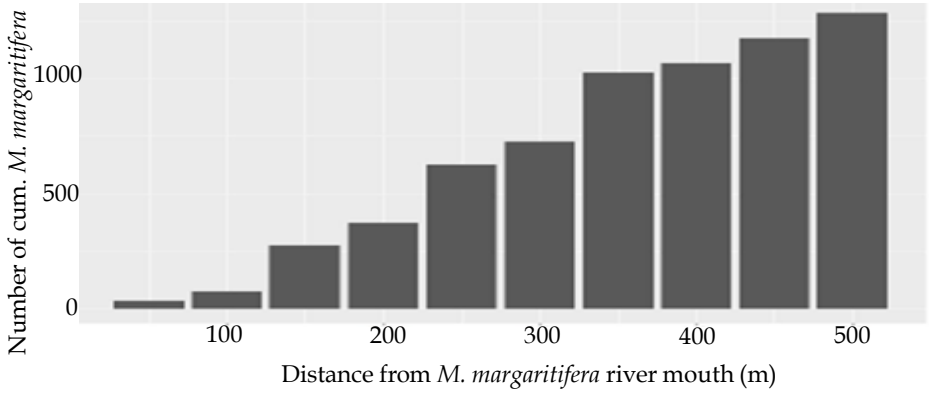

Figure 3. Cumulative *M. margaritifera* count with distance in *M. margaritifera* river.

3.2 Water quality parameters

Visual inspection indicated that the change with respect to distance from the lake was parallel in the *M. margaritifera* and control river in the case of conductivity, pH and water color (Figure 4) and they were, thus, not further studied using regression analysis. On the opposite, there appeared to be more or less contrasting trend with respect to distance in dissolved oxygen, turbidity, total suspended solids, blue-green algae, chlorophyll-a, phytoplankton density and zooplankton density as well as in phytoplankton species richness and zooplankton species richness (Figure 4). Therefore, regression analyses were performed for these variables/parameters (Tables 3-18, Figures 5-18).

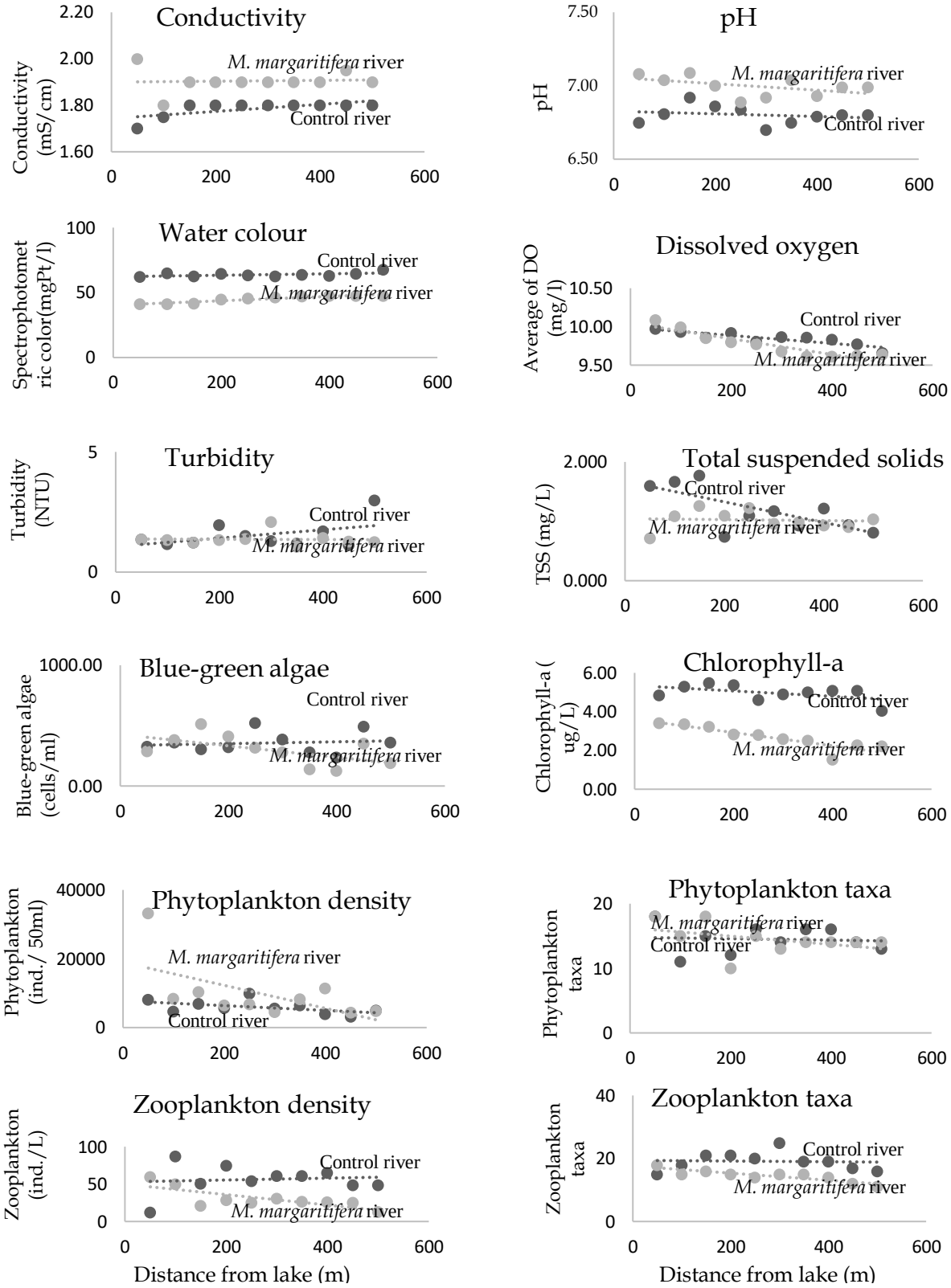

Figure 4. Trends with respect to distance from below the lake for measured parameters and variables.

3.3 Results of regression analyses

In the regression model below, regressions model coefficients (B) are presented with 95% confidence intervals (CI), and p-values for indicator and explaining variables are given. Below, "Dist." refers the effects of distance in the control river and "*M.margaritifera* river: Dist." refers comparison of *M. margaritifera* river to the control river, and "*M.margaritifera* river" indicates level difference in the distance between two rivers.

3.3.1 Dissolved oxygen

Regression model showed that in the *M. margaritifera* river the dissolved oxygen (DO) level reduced 0.00103 mg/L (-0.00052 + (-0.00051)) per 1 m distance (dist), reduction is higher than the decreased DO level for same distance in control river (0.00052 mg/L), the difference being statistically significant (p=0.014) (Table 3). Thus, there was a statistically significant difference in regression slopes for the *M. margaritifera* river and control river for dissolved oxygen, so that oxygen decreased more quickly by distance in the *M. margaritifera* river than in the control river. Consequently for 100 m distance predicted value for the *M. margaritifera* river was around 0.1 mg/L DO and for control river was around 0.05 mg/L (Figure 5). The second model is only related to the *M. margaritifera* river to see the effects of mussels on measured water quality parameters. It has found that single mussel reduced 0.00034 mg/L DO (Table 4) from the *M. margaritifera* river which was statistically significant (p<.001) and 500 cumulative mussels reduced about 0.175 mg/L DO (Figure 6).

Table 3. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for dissolved oxygen (DO). The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	DO (mg/L)		
	B	*CI*	*p*
(Intercept)	9.98913	9.90-10.07	<.001
M.margaritifera river	0.05779	-0.06—0.18	.324
Dist.	-0.00052	-0.00— -0.00	.001
M.margaritifera river: Dist.	-0.00051	-0.00— -0.00	.014
Observations		20	
R2/ adj. R2		.846 / .817	

Table 4. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for dissolved oxygen (DO). The regression model analysed relationship between DO and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	DO (mg/L)		
	B	*CI*	*p*
(Intercept)	9.99123	9.91-10.08	<.001
MussCum	-0.00034	-0.00— -0.00	<.001
Observations		10	
R2/ adj. R2		.872 / .856	

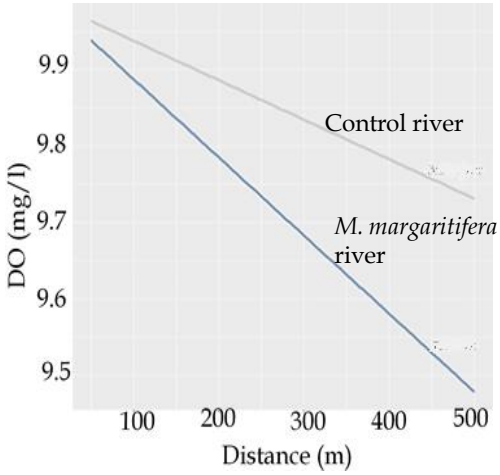

Figure 5. Predicted values of dissolved oxygen (DO) for both rivers, as given by the regression model. The level difference between the rivers has been removed.

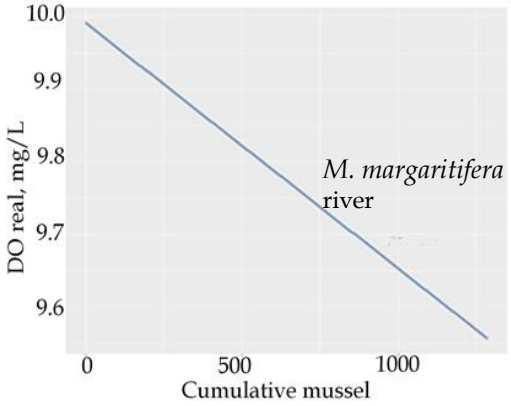

Figure 6. Effect of cumulative mussel count on dissolved oxygen (DO), as indicated by the regression model.

3.3.2 Turbidity

Regression model showed that in the *M. margaritifera* river very small amount of turbidity has decreased i.e. -0.0001 NTU (0.0017+(-0.0018)) per 1 m distance which was not statistically significant (p=.198) but in the control river turbidity increased 0.0017 NTU by 1 m distance (Table 5). Therefore, predicted values for turbidity in control river was increasing with increased distance but in the *M. margaritifera* river predicted value was almost the same from 50 m to 500 m distance. Consequently, for 100 m distance around 0.17NTU turbidity was increased in control river and in *M. margaritifera* river for 100 m distance there was almost no change of turbidity (Figure 7). The second model (only related to *M. margaritifera* river) showed that mussel has not changed the turbidity of *M. margaritifera* river (MussCum B=0.0000) (Table 6).

Table 5. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for turbidity. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Turbidity (NTU)		
	B	*CI*	*p*
(Intercept)	1.0647	0.44-1.69	0.002
M.margaritifera river	0.3127	-0.57—1.19	0.462
Dist.	0.0017	-0.00— -0.00	0.085
M.margaritifera river: Dist.	-0.0018	-0.00— -0.01	0.198
Observations		20	
R2/ adj. R2		.210 /.062	

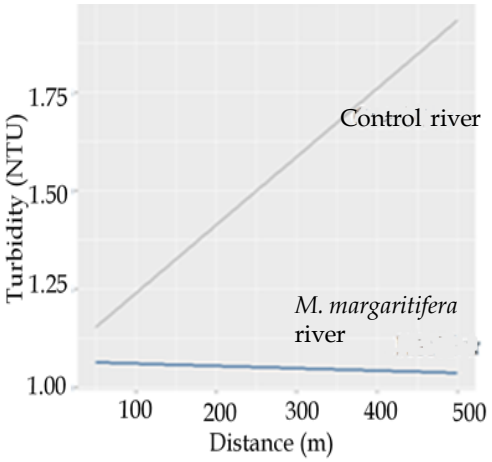

Figure 7. Predicted values of turbidity for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 6. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for turbidity. The regression model analysed relationship between turbidity and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	Turbidity (NTU)		
	B	*CI*	*p*
(Intercept)	1.3873	1.00 - 1.77	<.001
MussCum	-0.0000	-0.00 — -0.00	0.855
Observations		10	
R2/ adj. R2		.004 /-.120	

3.3.3 Total Suspended Solids (TSS)

The first regression model showed that there was no change of TSS concentration per 1 m distance in *M. margaritifera* river (-0.0017+0.0017=0 mg/L) however, in control river 0.0017 mg/L TSS has reduced for 1 m distance which is statistically being significant (p=0.033) (Table 7). Therefore, in the control river TSS concentration decreased at a higher rate than the *M. margaritifera* river by distance. Moreover, predicted value showed that for 100 m distance 0.18 mg/L TSS has reduced in control river but in *M. margaritifera* river there was almost no change of TSS with 100 m distance (Figure 8). The second model showed that mussel did not influence for changing TSS concentration in *M. margaritifera* river (MussCum B=0) (Table 8).

Table 7. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for total suspended solids (TSS). The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	TSS (mg/L)		
	B	*CI*	*p*
(Intercept)	1.675	1.35 – 2.00	<.001
M.margaritifera river	-0.63	-1.10 — 0.16	0.011
Dist.	-0.0017	-0.00 — -0.00	0.003
M.margaritifera river: Dist.	0.0017	0.00 — 0.00	0.033
Observations		20	
R2/ adj. R2		.484 /.387	

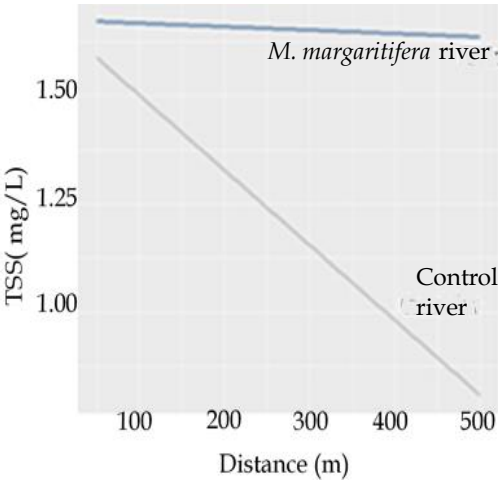

Figure 8. Predicted values of total suspended solids (TSS) for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 8. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for turbidity. The regression model analysed relationship between turbidity and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	TSS (mg/L))		
	B	*CI*	*p*
(Intercept)	1.0482	0.82 - 1.27	<.001
MussCum	-0.0000	-0.00 — 0.00	.760
Observations		10	
R2/ adj. R2		.012 /-.111	

3.3.4 Blue-green algae

The density of blue-green algae represents the presence of cyanobacteria in the water body. From the regression model, it has been found that in the *M. margaritifera* river 0.4723 cells/mL (0.0773+(-0.5496)) blue-green algae has reduced per 1 m distance (statistically not significant, p=.098) but in control river for 1 m distance 0.0773 cells/mL blue-green algae has increased (Table 9). Thus, the regression lines of blue-green algae were in opposite direction between two rivers. Predicted value for *M. margaritifera* river showed that around 50 cells/mL blue-green algae declined per 100 m distance and in control river around 12 cells/mL blue-green algae increased for 100 m distance (Figure 9). Furthermore, single mussel reduced 0.00034 cells/mL blue-green algae from *M. margaritifera* river which was statistically significant (p=.046) (Table 10). Thus, reduction of blue-green algae from the *M. margaritifera* river can be considered as effect of *M. margaritifera,* consequently 500 cumulative mussels reduced around 85 cells/mL of blue-green algae from the same river (Figure 10).

Table 9. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for blue-green algae. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Blue-green algae (cells/mL)		
	B	*CI*	*p*
(Intercept)	335.098	189.56-480.63	<.001
M. margaritifera river	91.7754	-114.04 – 297.59	0.359
Dist.	0.0773	-0.39 – -0.55	0.731
M. margaritifera river: Dist.	-0.5496	-1.21 – -0.11	0.098
Observations		20	
R2/ adj. R2		.286 /.153	

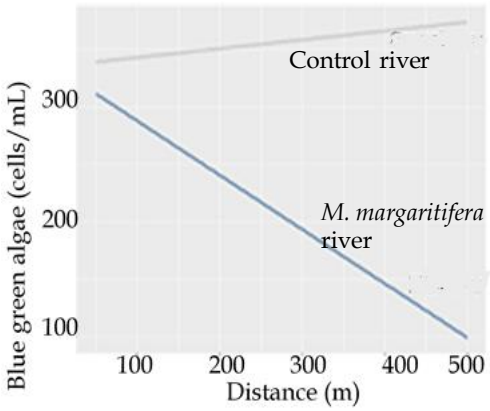

Figure 9. Predicted values blue-green algae for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 10. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for blue-green algae. The regression model analysed relationship between blue-green algae and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	Blue-green algae (cells/mL)		
	B	*CI*	*p*
(Intercept)	411.011	277.61-544.41	<.001
MussCum	-0.0003	-0.34 — -0.00	0.046
Observations		10	
R2/ adj. R2		.410 /.337	

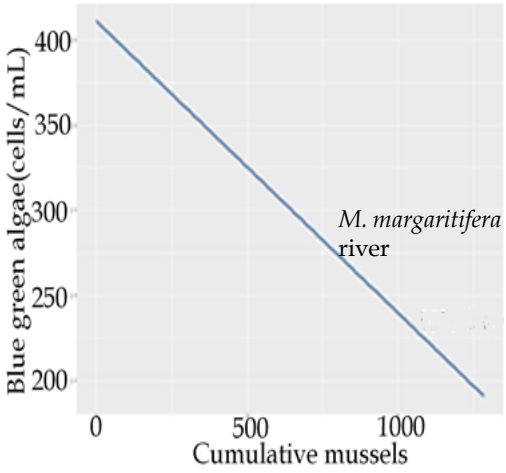

Figure 10: Effect of cumulative mussel count on blue-green algae, as indicated by the regression model.

3.3.5 Chlorophyll-a

Regression model for chlorophyll-a showed that in the *M. margaritifera* (M.m.) river the concentration of chlorophyll-a declined 0.0034 µg/L (-0.0014 + (-0.0020)) for 1 m distance, that is higher than the reduced chlorophyll-a concentration for 1 m distance in control river (0.0014 µg/L) but the difference was not statistically significant (p=0.073) (Table 11). Consequently for 100 m distance predicted value for the *M. margaritifera* river was around 0.375 µg /L and for the control river was around 0.25 µg/L (Figure 11). From the second model, it has been found that single mussel reduced 0.0011 µg/L chlorophyll-a (Table 12) from *M. margaritifera* river which was statistically significant (p<.001). Thus, it can be considered that *M. margaritifera* significantly contributed to reduce chlorophyll-a concentration from the *M. margaritifera* river and eventually 500 cumulative mussels removed about 0.5 µg/L of Chlorophyll-a from the same river (Figure 12).

Table 11. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for chlorophyll-a. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Chlorophyll-a (µg/L)		
	B	*CI*	*p*
(Intercept)	5.3461	4.85-5.84	<.001
M. margaritifera river	-1.7408	-2.44 —-1.05	<.001
Dist.	-0.0014	-0.00— 0.00	0.082
M. margaritifera river: Dist.	-0.002	-0.00— 0.00	0.073
Observations		20	
R2/ adj. R2		.941 /.929	

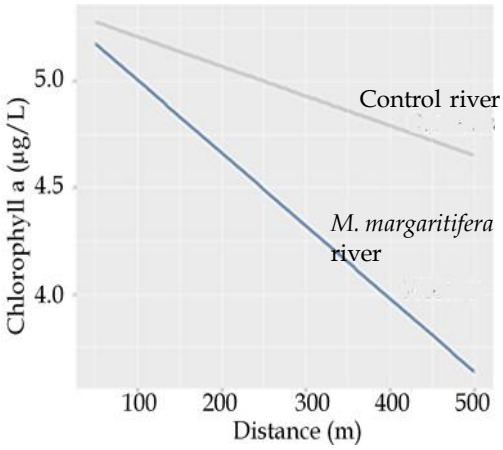

Figure 11: Predicted values chlorophyll-a for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 12. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for chlorophyll-a. The regression model analysed relationship between chlorophyll-a and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	Chlorophyll-a (µg/L)		
	B	*CI*	*p*
(Intercept)	3.4149	3.04—3.79	<.001
MussCum	-0.0011	-0.00—-0.00	<.001
Observations		10	
R2/ adj. R2		.789 /.762	

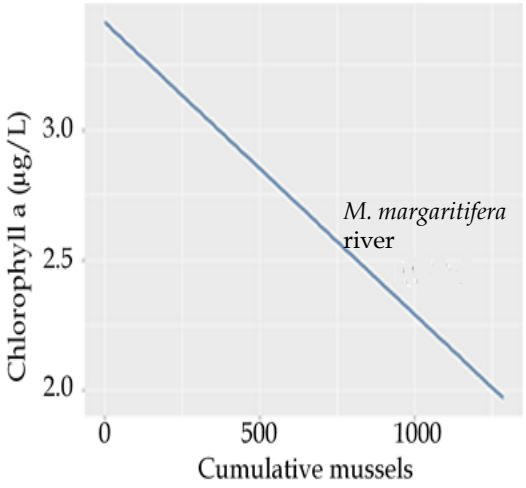

Figure 12. Effect of cumulative mussel count on chlorophyll-a, as indicated by the regression model.

3.3.6 Phytoplankton density

The obtained IRR (incident rate ratio) coefficients from the Poisson regression model are interpreted as percent changes and when IRR value is less than 1 it expresses

negative relationship between two factors. From this model it has been found that in *M. margaritifera* river 0.36% ((0.9988 X 0.9976)-1) X100) phytoplankton density has reduced for 1 m distance which is higher than the phytoplankton reduction in the control river per 1 m distance ((0.9988-1) X100) = 0.12%) but the higher percentage of phytoplankton reduction in the *M. margaritifera* river was not statistically significant (p=.161) (Table 13). Because of the difference in regression slopes of phytoplankton density for the *M. margaritifera* river and control river, phytoplankton density decreased at a higher rate in the *M. margaritifera* river by distance than in the control river (Figure 13). However, form the second model it can be considered that presence of *M. margaritifera* in *M. margaritifera* river could be responsible factor for higher phytoplankton reduction than control river since one *M. margaritifera* reduced 0.9989 ind./50 mL phytoplankton from *M. margaritifera* river (Table 14). Effect of cumulative mussels count on phytoplankton density, as indicated by the regression model, has given in Figure 14.

Table 13. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for phytoplankton density. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Phytoplankton (ind./50mL)		
	IRR	*CI*	*p*
(Intercept)	7998.61	3726.43-15644.13	<.001
M. margaritifera river	2.887	1.23 — 7.11	0.03
Dist.	0.9988	1.00 — 1.00	0.361
M. margaritifera river: Dist.	0.9976	0.99 — 1.00	0.161
Observations		20	

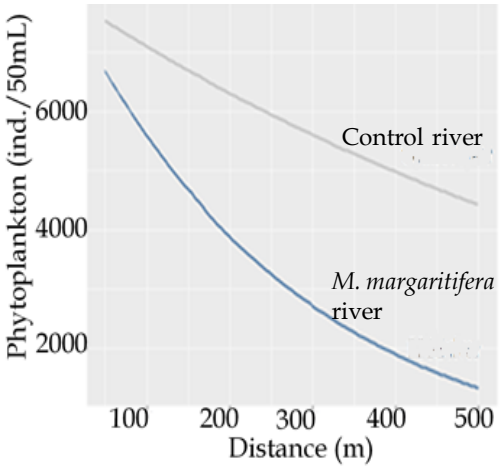

Figure 13. Predicted values phytoplankton density for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 14. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for phytoplankton density. The regression model analysed relationship between phytoplankton density and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

	Phytoplankton (ind./50mL)		
	IRR	*CI*	*p*
(Intercept)	17761.2	9369.60-30802.80	<.001
MussCum	0.9989	1.00 — 1.00	0.06
Observations		10	

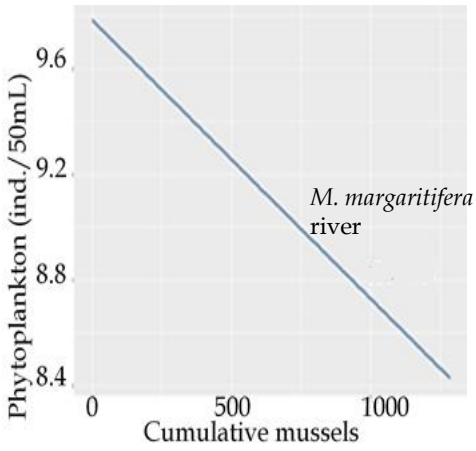

Figure 14. Effect of cumulative mussel count on phytoplankton density, as indicated by the regression model.

3.3.7 Phytoplankton Species richness

In the *M. margaritifera* river 26 different phytoplankton species has been identified and in the control river 32 phytoplankton species has been identified (Appendix 1). *Dinobryon*, *Synedra*, *Tabellaria* and *Urosolenia* were the dominant species for both rivers. In the *M. margaritifera* river number of phytoplankton taxa decreased 0.05% ((0.9999X0.9996)-1) X100) for 1 m distance which was not statistically significant (p=0.449) and in control river phytoplankton taxa number decreased 0.01 % ((0.9999 - 1) X 100) for same distance (Table 15). Thus, in the control river for 100 m distance, the predicted value for the phytoplankton taxa was almost 0 but, in the *M. margaritifera* river the predicted value for the same distance was around 1 which indicates higher decreasing rate compared to the control river (Figure 15).

Table 15. Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for phytoplankton taxa. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Phytoplankton Taxa		
	IRR	*CI*	*p*
(Intercept)	14.8023	12.04 — 18.07	<.001
M. margaritifera river	1.1044	0.83 — 1.47	0.503
Dist.	0.9999	1.00 — 1.00	0.825
M. margaritifera river: Dist.	0.9996	1.00 — 1.00	0.449
Observations		20	

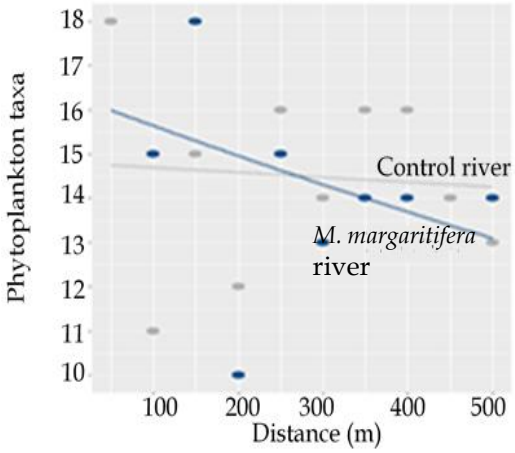

Figure 15. Predicted values phytoplankton taxa for both rivers, as given by the regression model. The level difference between the rivers has been removed.

3.3.8 Zooplankton density

Poisson regression model showed that zooplankton density increased 0.02% (1.0002-1) X 100)) by 1 m distance in control river and decreased 0.24% (1.0002X0.9974)-1) X100)) in the *M. margaritifera* (M.m.) river for 1 m distance and this reduced amount in the *M. margaritifera* river statistically being significant (p=0.040) (Table 16). Therefore, the difference between regression slopes for the *M. margaritifera* river and control river for zooplankton density was statistically significant, thus zooplankton decreased by distance in the *M. margaritifera* river and increased with distance in control river (Figure 16). Furthermore, the second model showed that because of one mussel, 0.9993 ind./50 mL zooplankton has reduced from the *M. margaritifera* river which was also statistically significant (p=.008) (Table 17). Consequently, effect of cumulative mussels count on zooplankton density as indicated by Figure 17.

Table 16: Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for zooplankton density. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

Zooplankton (ind./50mL)			
	IRR	*CI*	*p*
(Intercept)	798.294	522.17—1183.48	<.001
M. margaritifera river	1.0361	0.54—1.95	0.914
Dist.	1.0002	1.00— 1.00	0.754
M. margaritifera river: Dist.	0.9974	1.00— 1.00	0.04
Observations		20	

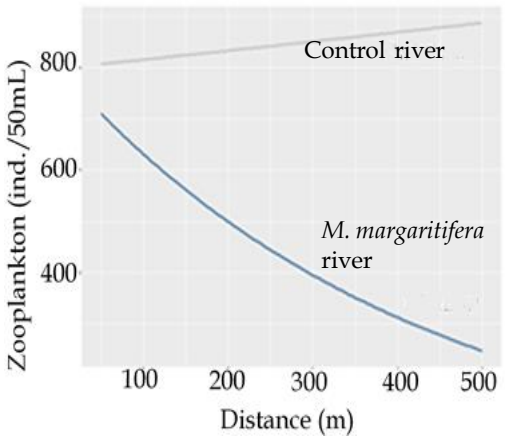

Figure 16: Predicted values zooplankton density for both rivers, as given by the regression model. The level difference between the rivers has been removed.

Table 17: Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for zooplankton density. The regression model analysed relationship between zooplankton density and cumulative number of mussels (MussCum, accumulated number of mussels when proceeded from the 1st sampling point to the last sampling point).

Zooplankton (ind./50mL)			
	IRR	*CI*	*p*
(Intercept)	712.759	534.59-932.09	<.001
MussCum	0.9993	1.00— 1.00	0.008
Observations		10	

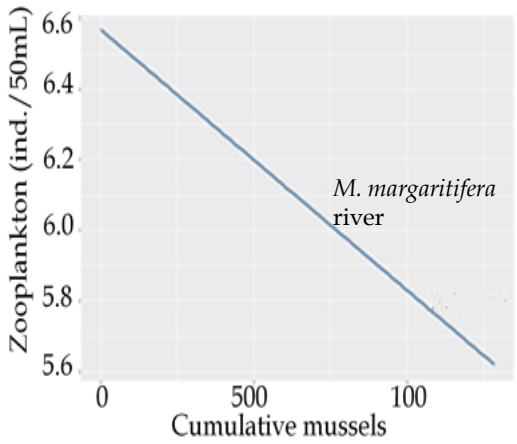

Figure 17: Effect of cumulative mussel count on zooplankton density, as indicated by the regression model.

3.3.9 Zooplankton Species richness

In this study 34 zooplankton species/taxa have found in control river and 28 species /taxa has found in *M. margaritifera* river (Appendix 2). Poisson regression model showed that in the *M. margaritifera* river 0.08% ((0.9999X0.9993)-1) X 100) zooplankton taxa number decreased by 1 m distance which was higher than the reduced amount (0.01% = ((0.9999-1) X 100))) of zooplankton taxa number in the control river by 1 m distance. The difference between reduced amount of zooplankton taxa number in *M. margaritifera* river and control river was not statistically significant (p=0.096) (Table 18). However, the predicted value for 100 m distance in the *M. margaritifera* river was around 2 but in the control river this value was around 0 per 100 m distance (Figure 18).

Table 18: Estimates of regression model coefficients (B), 95% confidence intervals of coefficients (CI) and p-values for zooplankton taxa. The regression model analysed the possible contrasting trends between *M. margaritifera* and control river.

	Zooplankton Taxa		
	IRR	*CI*	*p*
(Intercept)	19.4017	16.46—22.76	<.001
M. margaritifera river	0.9202	0.72—1.17	0.509
Dist.	0.9999	1.00—1.00	0.834
M. margaritifera river: Dist.	0.9993	1.00—1.00	0.096
Observations		20	

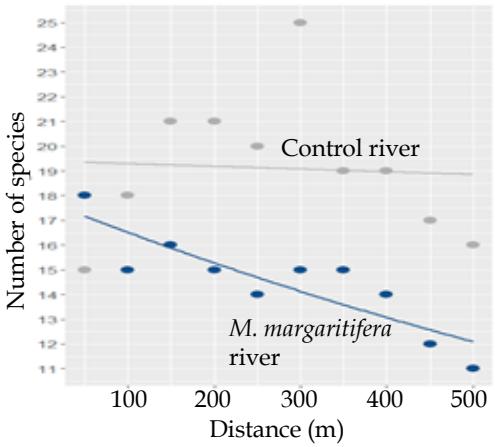

Figure 18: Predicted values zooplankton taxa for both rivers, as given by the regression model. The level difference between the rivers has been removed.

4 DISCUSSION

Some studies have been conducted to investigate the relationship of different variables for example dissolved oxygen, chlorophyll-a, phytoplankton grazing, zooplankton communities with freshwater mussels such as zebra mussel (Caraco et al. 1997, Caraco et al. 2000, Osterling et al. 2007, Pace et al. 1998, Soto & Mena 1999). This is the first study to investigate the effects of *M. margaritifera* on river ecosystem and water quality parameters but there were only 10 sampling points per river which were relatively a small sample size to get the accurate result. However, the bigger sample size is better to get more accurate and reliable results, but the sample size is often determined by logistic considerations. In this case, selecting another pair of rivers in the similar environmental condition was difficult and time-consuming to make bigger sample size as well as the irregular number of mussels in selected rivers was another constraint. In other words, it will be extremely demanding to have more river pairs, and given the present resources, it was completely impossible to increase the number of river pairs in this study. Nevertheless, obtained trends from regression analysis demonstrated relationships among different water quality variables and *M. margaritifera*, as well as helped to realize how the dense population of *M. margaritifera* could affect river ecosystem. Obviously repetition of the study like this would be useful to get more confident and certain results.

Dissolved oxygen is one of the important water quality parameters for the survival of aquatic life. A certain amount of dissolved oxygen level is the combined result of primary production and respiration of all components of an aquatic ecosystem. Maintenance of dissolved oxygen level also depends on various physical aspects which influences stratification and gas exchange of the water body, as well as eutrophication. From the current study, it has been found that a dense *M. Margaritifera* bed may significantly decrease dissolved oxygen level of boreal river water. This decreased

dissolved oxygen level may be due to respiration effects of *M. Margaritifera*. Caraco et al. (2000) identified lowering of dissolved oxygen in the Seneca River as a result of direct respiration by zebra mussel or due to indirect effects of declining of other benthic animals and increasing of water clarity. Decreased dissolved oxygen concentration may have significant impacts on food webs, biogeochemical cycles, and aesthetics of aquatic systems.

Turbidity refers water clarity due to the presence of suspended particles which can be influenced by the presence of mussels. In the laboratory experiment, mussel can reduce up to 32% turbidity from water (Smith et al. 2012). Osterling et al. (2007) found that freshwater mussel (zebra mussel) can reduce turbidity rapidly at high mussel density but zebra mussel cannot decline turbidity or decline at a lower rate when mussel density is lower. However, mussels can enhance addition of materials in water through deposition of faeces and pseudofaeces (MacIsaac & Rocha 1995). This study has been found that *M. Margaritifera* does not have any statistically significant influence on turbidity and total suspended solids in the *M. Margaritifera* river. Although in the control river turbidity showed a positive trend and total suspended solids showed a negative trend with distance but the level of turbidity and total suspended solids were almost same throughout the whole study section in the *M. Margaritifera* river. This may indicate that *M. Margaritifera* may have some indirect influence in maintaining a certain level of turbidity and total suspended solids in boreal river water.

Pace et al. (1998) found that after the successful invasion of zebra mussel in the Hudson River chlorophyll concentration declined from 30 µg/L to <5 µg/L. In 1992, chlorophyll concentration was lowest when zebra mussel concentration was highest in Hudson river (Caraco et al. 1997). *Dreissena polymorpha* was an effective filter feeder to reduce chlorophyll concentration in European lakes (Reeders et al. 1989) as well as in North American lakes (Bunt et al. 1993). Moreover, Soto & Mena (1999) found in their laboratory experiment that *Diplodon chilensis* has reduced a significant amount of chlorophyll concentration within 18 days. In this study, a statistically significant

concentration of chlorophyll-a also reduced by distance in the *M. margiritifera* river compared to the control river, indicating the decreasing effect of *M. margaritifera* on chlorophyll-a.

Mussels are powerful filter feeder, in large productive river mussels principally feed phytoplankton (Thorp et al. 1998). Enough densities of mussels can decline phytoplankton population and change nutrient cycle (Phillips 2007). Pigneur et al. (2014) estimated a 70% annual reduction of phytoplankton biomass and 61% annual decrease of primary production in situation of highest concentration of invasive *Corbicula* in the River Meuse. Wilson (2003) also found that zebra mussel (*Dreissena polymorpha*) has decreased by 53% phytoplankton biovolume within one week into the experimental unit. In this study, it has also been found that in the *M. margiritifera* river phytoplankton reduction was almost three times higher compared to the control river and 1285 cumulative *M. margiritifera* reduced about 10 phytoplankton cells from 50 mL *M. margiritifera* river water. This reduction of phytoplankton population may be a result of two effects - I) direct effects of filter feeding and II) indirect effects of modification of nutrient cycle. These two effects combinedly can also change phytoplankton community in the river (Arnott & Vanni 1996). Although the obtained result from this study was only marginally statistically significant (p=.060) but combined with reduced chlorophyll-a it is quite safe to that *M. margaritifera* probably reduces phytoplankton density in the river. In both studied rivers phytoplankton community was dominated by diatom species. However, it has to be noted that the decreasing trend of phytoplankton in *Margaritifera* river was affected by the very high phytoplankton density value of the first sampling point (Fig. 4).

On the other hand, alteration of the nutrient cycle can also influence abundance of blue-green algae. Atkinson et al. (2013b) found in rivers of southern Oklahoma about 26% higher blue-green algae in the sites of without mussel with nitrogen limited than the sites with high mussel densities with co-limited (N and P). They recognized that modification of nutrient cycle was the reason for this difference of blue-green algae

abundance. Freshwater mussels also can collect blue-green algae cells from the water column which is another reason for reduction of blue-green algae density (Wood et al. 2006). This study also supports these previous studies, as it has found that *M. margiritifera* can remove statistically significant amount of blue-green algae cells from *M. margiritifera* river. However, Arnott & Vanni (1996) reported that freshwater mussel may enhance the growth of blue-green algae by providing favorable condition through changing the nutrient regime.

In the current study, phytoplankton taxa number decreased by the distance in the *M. margiritifera* river, but the decreased amount was not statistically significant. On opposite, in control river there was almost no change of phytoplankton taxa number with the distance (from first sampling point to last sampling point).

Zooplankton community in the *M. margiritifera* river and control river was dominated by rotifers and abundance of zooplankton was clearly distinct between two rivers. In the *M. margiritifera* river, zooplankton concentration decreased at a statistically significant rate by the distance but in the control river zooplankton density increased with the distance. Therefore, it is safe to consider that the presence of *M. margiritifera* in *M. margiritifera* river was probably the main reason for the reduction of zooplankton density. The reduction of phytoplankton and blue-green algae density as well as chlorophyll-a concentration by *M. margiritifera* may modify the food web of the *M. margiritifera* river which consequently affected availability of zooplankton in this river. Since this is the first study to observe the impact of *M. margiritifera* on river water, there is no evidence of direct predation of zooplankton by *M. margiritifera.* One probable reason for declined zooplankton concentration in *M. margiritifera* river may be the reduction of phytoplankton concentration. Thus, this result indicates that *M. margaritifera* may compete for food with zooplankton as they both are grazing on phytoplankton and thereby maybe decrease zooplankton density. Food quality and quantity is another important factor for zooplankton reproduction such as after mid-1992 zooplankton reproduction declined significantly in the Hudson River because of

limited abundance of phytoplankton. Timing of zooplankton decline was consistent with timing of phytoplankton decline in Hudson river (Caraco et al. 1997). Pace et al. (1998) found that in Hudson river total zooplankton biomass decreased more than 70% after the invasion of zebra mussels and they recognized that lowering of zooplankton biomass was associated with decreasing chlorophyll concentration in Hudson river. In this study, the number of zooplankton taxa was not significantly changed in the *M. margiritifera* river.

In summary from this study, it has been found that

- *M. margiritifera* may act as an effective filter feeder for reduction of chlorophyll-a, blue-green algae and phytoplankton concentration.
- *M. margiritifera* may have significant influence to decrease dissolved oxygen of boreal river water.
- Turbidity and total suspended solids may not be influenced by the presence of *M. margiritifera*.
- *M. margiritifera* may have significant effect for the reduction of zooplankton abundance from boreal river water.

The results indicate that ecosystem and water quality impacts of dense *M. margaritifera* bed can be pronounced throughout the river ecosystem. Thus, the results highlight the potentially lost ecosystem functions with extirpation of the endangered and extinct freshwater mussels, such as *M. margaritifera*.

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APPENDIX 1. List of identified phytoplankton species/taxa and total number of phytoplankton cells in per milliliter volume of sample.

Control river		***M. margaritifera* river**	
Species/taxa name	Number of total cells per mL sample	Species/taxa name	Number of total cells per mL sample
Aulacoseria	19	*Aulacoseria*	26
Asterionella	52	*Asterionella*	15
Aphanocapsa	4	*Anabaena*	9
Anabaena	3	*Bitrichia*	5
Bitrichia	3	*Cyclotella*	13
Bacillariales	2	*Crucigeniella*	216
Cyclotella	18	*Cosmarium*	4
Crucigeniella	7	*Dinobryon*	191
Cosmarium	6	*Desmodesmus*	1
Cryptophyte	1	*Diatoma*	24
CF. *Chlorophyceae*	1	Diatomophyceae	135
Dinobryon	97	*Euglena*	6
Desmodesmus	1	*Flagilaria*	2
Diatoma	45	*Limnothrix*	11
Diatomophyceae	43	*Mallomonas*	34
Euglena	4	*Oscillatoriales*	4
Euastrum	2	*Peridinium*	64
Eudorina	1	*Pediastrum*	1
Flagilaria	1	*Planktothix*	3
Mallomonas	6	*Planktolyngbya*	3
Monoraphidium	1	*Pseudanabaena*	1
Microcystis	1	*Staurastrum*	1
Peridinium	57	*Synedra*	106
Pediastrum	2	*Scenedesmus*	13

Pseudanabaena	1	*Tabellaria*	140
Staurastrum	3	*Trachelomonas*	1
Synedra	139	*Urosolenia*	874
Scenedesmus	1	Unidentified	3
Staurodesmus	1	-	-
Tabellaria	182	-	-
Tetrastrum	1	-	-
Urosolenia	371	-	-
Unidentified	106	-	-
Woronichinia	2	-	-

APPENDIX 2. List of identified zooplankton species/taxa and total number of individuals per liter volume of sample.

Control river		***M. margaritifera* river**	
Species/Taxa name	Number of total individuals per liter sample	Species/Taxa name	Number of total individuals per liter sample
Asphalanchna sp.	2020	*Asphalanchna sp.*	160
Bosmina sp.	2220	*Ascomorpha sp.*	920
Calanoida nauplis	540	*Bosmina sp.*	600
Calanoida copepodite	80	*Brachionus sp.*	40
Cyclopoida Naupilus	3960	*Cyclopoida Naupilus*	440
Cyclopoida copepodite	780	*Cyclopoida copepodite*	40
Copepoda nauplis	1280	*Copepoda sp.*	40
Cladocera	120	*Conochilus Unicornis*	2360
Conochilus Unicornis	2980	*Conochilus hippocrepis*	1080
Conochilus hippocrepis	920	*Collotheca libera*	720
Chydorus sphaericus	220	*Daphnia sp.*	80
Chydorus sp.	40	*Difflugia sp.*	200

Collotheca libera	120	*Euchlanis dilatata*	80
Daphnia sp.	840	*Epistylis rotans*	80
Difflugia sp.	620	*Gastropus stylifer*	160
Eudiaptomu sp.	360	*Keratella sp.*	59400
Gastropus stylifer	80	*Kelicottia longispina*	1960
Heliozoa sp.	2720	*Lecane luna*	1280
Keratella sp.	40800	*Lecane lunaris*	1320
Kelicottia longispina	8640	*Ploesoma hudsoni*	680
Lecane luna	540	*Polyarthra sp.*	4720
Lecane lunaris	0	*Polyphemus pediculus*	40
Lecane ludwigii	40	*Pompholyx sulcata*	680
Lecane sp.	40	*Rotifera sp.*	1160
Limnosida frontosa	40	*Synchaeta sp.*	1920
Ploesoma hudsoni	600	*Trichocera sp.*	200
Polyarthra sp.	16540	*Vorticella Sp.*	11240
Rotifera sp.	560	Unidetified	200
Synchaeta sp.	1760	-	-
Trichocera sp.	80	-	-
Tintinidium fluviatile	240	-	-
Tintinnopsis locustris	120	-	-
Vorticella Sp.	78220	-	-
Unidentified	1180	-	-