Sustainability of the freshwater pearl mussel *M. margaritifera* and water quality in Haukås River

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Abstract

Haukås River, located in Western Norway and close to the city of Bergen, is one of the remaining places where the endangered European river mussel (*Margaritifera margaritifera*) is found and there is an ongoing conservational effort to increase the population, by reintroducing juveniles. The present study examines the water quality of the river and investigates different parameters, such as oxygen, temperature, pH, nitrite and biodiversity, which might influence the recruitment of the juveniles. The results showed that E.coli and phosphorous did not surpass the required critical level for reintroduction of the mussels; however the water of the river was not drinkable. Haukås river could be classified as oligotrophic to moderately eutrophic due to the phosphorous levels. However, further studies especially about oxygen levels, should be done in this area.

Haukåselv som ligger utenfor Bergen, er en av de gjenværende plasser hvor den uttryddningstruede elvemuslingen (*Margaritifera margaritifera*) befinner seg og det foregår arbeid med å øke populasjonen ved å reintrodusere juveniler. Denne studien/rapporten undersøker vannkvaliteten i elven og ser på forskjellige parametre, slik som oksygen, temperatur, pH, nitritt og biodiversitet, som kan påvirke rekrutteringen av juvenile muslinger. Resultatene viste at *E.coli* og fosfor ikke oversteg de kritiske nivåene nødvendig for reintrodusering av muslingene, men vannet i elven var ikke drikkbart. Haukåselven kan bli klassifisert som oligotrofisk til moderat eutrofisk pågrunn av fosfor nivåene. Men flere studier, spesielt om oksygennivå, bør bli utført i området.

Introduction

Global decline in freshwater pearl mussel *Margaritifera margaritifera* stocks has been recorded since the 1950s (Buddensiek, 1995; Geist, 2010). Although the primary cause is identified as juvenile recruitment, most conservation measures have seen only limited effects in large-scale recovery (Bolland et al., 2010; Geist, 2010). A wide range of factors influences the stock-dynamics in rivers with *M. margaritifera.* Anthropogenic effects are thought to contribute to the global decline. These include loss of native fish populations, siltation, pollution, acidification and habitat loss (Bolland et al., 2010). Norway hosts the largest remaining populations of *M. margaritifera* in the world and therefore has a special conservational responsibility (Larsen, 2005). In Norway the estimated 150 populations consist mostly of adult mussels as a results of the poor recruitment (Jakobsen et al., 2013). One possible solution to solve this problem is to culture juveniles in hatcheries and keep them there until they are big enough to survive reintroduction in the original habitat. The long term goal is to improve these habitats so reproduction can occur naturally in the river (Jakobsen et al., 2013).

The freshwater pearl mussel *Margaritifera margaritifera*

The freshwater pearl mussel provides vital ecosystem services in lotic ecosystems and is therefore an important species to conserve. The mussel can be classified as an indicator, flagship, umbrella and keystone species. Many species can fulfill some of these criteria, but the freshwater pearl mussel can be seen as an exception as it matches criteria involved in all of these concepts (Geist, 2010). In Norway the freshwater pearl mussel has been proposed as a priority species by the Norwegian Environmental Agency and it's on the red list of threatened species (Karlsson and Larsen, 2013) and it is on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, within the category "Endangered". In the European Red List it is categorized as "Critically Endangered" (Cuttelod et al., 2011).

As an indicator species the mussels can be used to assess the ecosystems health, as it has several stages which have different tolerance limits to river conditions for example sedimentation and oxygen (Geist, 2010; Young, 2005), as well as the co-occurrence of

specialized species, because the mussel only lives in a restricted range of habitats and is adapted to cool running waters, saturated with oxygen but low in nutrients (Geist, 2010).

As a keystone species the freshwater pearl mussel affect its habitat and ecosystem in many beneficial ways, especially by filtering and clarifying the water. With the capacity to filter 50L/day an adult freshwater pearl mussel provides vital ecosystem services in aquatic river systems (Ziuganov, 1994). Such as making filtered material available, i.e. nutrition and pseudofaeces (Larsen, 2005), and making it easier for light to penetrate and giving more potential for plant growth. This will increase biodiversity as the river or stream offers more food and hiding places (Geist, 2010). These effects are also why the mussels can be categorized as an umbrella species because conservation and protection of the mussels will benefit the entire ecosystem. The river mussel is an interesting pollution indicator because of its ability to store trace elements from the water and its high age potential (Dolmen and Kleiven, 1997). Additionally the freshwater pearl mussel has become a popular symbol and leading element of conservation campaigns, it can be called a flagship species (Geist, 2010).

Life cycle and habitat requirements

The freshwater pearl mussel is a bivalve (Order: Unionoida) that has a complex life cycle, which includes an obligate parasitic stage on host fish gills, a juvenile benthic stage mostly feeding on sediment and an adult filtering stage. The adult mussel is partly buried on the river bottom and filter-feeds by inhaling water through their siphons (Skinner et al., 2003) (Figure 1). The mussel matures at 10-15 years, and stays reproductive for about 75 years (Bauer, 1987). Fertilized eggs are developed in a pouch in the females gills for several weeks before the larva called glochidia is released in mid- to late summer (Hastie, 1999 cited in: Hastie & Young, 2003). Each female releases between one and four million glochidia in one or two days (Hastie, 2001b cited in: Skinner et al., 2003). Ross (1988, cited in: Moorkens, 1999) suggested that each female can produce 9.8 million eggs under favorable conditions. The high production is explained due to high mortality of glochidia, where only a few are inhaled by a host fish. Known host fish in Europe are brown

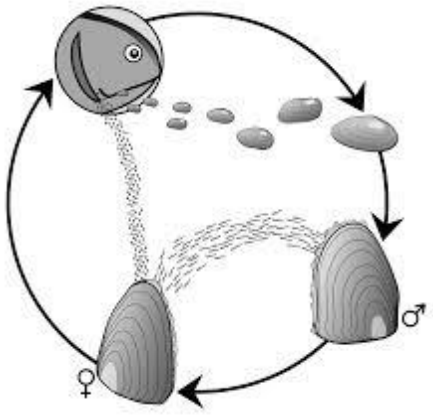

Figure 1: Life cycle of freshwater pearl mussel. The male mussel releases sperm to the female, glochidia are released into the water, glochidia attached to a host's (fish) gills, glochidia become juveniles, juveniles drop back to the sediment and the cycle begins again. *(Source: wikipedia.org)*

trout (*Salmo trutta)* and Atlantic salmon (*Salmo salar) (Larsen, 2005).* They attach to the hyper-oxygenated environment in the gills of the host and remains there for about 8 to 9 months (Mitchell, 2011). They use microvilli to get nourishment from the gill tissue during this time until they reach a suitable size to survive without the host (Ziuganov, 1994; Neizlin et al., 1994 cited in: Moorkens, 1999). Then, the glochidia detach, bury in the sediment of the river bottom for around five years and come out when they are able to withstand the fast flow of water in the streams (Cranbrook, 1976; Wells et al., 1983, both cited in: Moorkens 1999). Several factors affect the success of the reproduction during the life cycle of the mussel. The glochidia can drop off the host fish due to immune response from the fish, in the parasitic stage (Mitchell, 2011). The next stage is considered to be the most critical and vulnerable during the recruitment of mussels. Survival for juvenile freshwater mussels depends on whether the stream bed is suitable for recruitment that is good aeration and stable substratum (Geist and Auerswald, 2007). If the juvenile mussel lands on unfavorable substrate like mud or silt, they can perish. They are also likely to die if they do not attain a sufficient size on the fish host (Mitchell, 2011).

The river bank should consist of enough sand for the juveniles to bury and with boulders or rocks that stabilizes the substratum. Hastie et al. (2000) found that in a river in Scotland the optimum water depth was 0.3-0.4 m, and optimum water velocity was 0.25-0.75 m/s. Several studies have recorded similar results, in Upper Austria the stretches with flow velocities of 0.2-0.6 m/s and water depths of 0.25-0.5 m were most densely colonized (Jung et al., 2013). In a study by Bauer (1988) it was found that freshwater pearl mussel populations do not reproduce successfully at phosphorus levels higher than 30 µg/L. The study also indicates that the Freshwater Pearl Mussel preferred a pH lower than 7.5. The recruitment and establishment of juvenile mussels decreased with increasing levels of phosphate, calcium and BOD (biochemical oxygen demand). Maximum phosphates levels of < 30 µg/L is also supported by Oliver (2000) and Skinner et al. (2003) who also indicates that nitrate levels should be less than 1.0 mg/L. In a cage experiment by Buddensiek (1995) conductivity, ammonia, nitrate, phosphate, calcium, sodium, potassium and magnesium were found to be negatively related to survival and growth of juveniles. As many other studies, this shows that eutrophication affects survival and growth negatively.

Juvenile mussels are especially sensitive to pollutants between parasitic and burrowing life stages. Nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) are potentially harmful and can lead to a low survival rate for juvenile freshwater mussels (Skinner et al., 2003). Adult mussels can grow to 10 to 13 cm although size is variable in different healthy populations and are sexually dimorphic and it may take up to 20 years for a juvenile mussel to turn into an adult (Mitchell, 2011).

Water Quality

One of the most important parameters for water quality is dissolved oxygen, because it affects the health of the aquatic ecosystem, fish mortality, odors and other qualities of surface water (Chin, 2012). Levels of thermo-tolerant coliform bacteria is the most commonly used indicator for fecal pollution, and analyzes can be used to determine if the fecal pollution is recent (Folkehelseinstituttet, 2004). Phosphorus has an important role of eutrophication of streams, and human activities often results in large fluxes of phosphorus, especially by runoffs from agricultural fields. Increased levels of phosphorus lead to higher primary production, which results in high bacteria populations, high rates of decomposition and depletion of dissolved oxygen in poorly mixed bottom waters (Correll, 1998). Temperature and pH is usually measured when monitoring water quality. In natural water bodies, pH affects biological and chemical reactions, control solubility of metal ions and affect natural flora and fauna (Chin, 2012).

Nitrogen occurs in freshwater systems in a variety of forms including: dissolved molecular N_2, ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and organic compounds (e.g. amino acids, amines, proteins). The input dynamics of nitrogen in lotic ecosystems are dependent on human activities, atmospheric fixation, surface and groundwater drainage, while the output is mostly effluent outflow, but also includes sedimentation and volatilization at the water surface. Nitrite in river ecosystems is primarily generated as an intermediate product of nitrification and denitrification (Bril, 2012).

Biodiversity Monitoring Work Package (BMWP) is a system which can be used to determine the level of pollution in a river and present the biological condition of a water body by a biological perspective (Armitage et al., 1983). Walley and Hawkes (1996) developed and improved this score system introducing computer based analysis. Each taxon corresponds to a specific revised BMWP score, with a range between 1 and >12; for instance, Peridae

(stoneflies) have revised BMWP score equal to 12.5. Taxa with a score close to 1 are considered tolerant to high water pollution levels, while taxa with a score close to 10 are considered highly sensitive to pollution. Revised BMWP scores are considered to represent more realistic the tolerance of families to pollution comparing to BMWP scores. A total BMWP score of a water body more than 100 indicates that the biological quality of the river is high and BMWP score less than 10 indicates that the biological quality of the river is poor.

Average score per taxon (ASPT) can also be used to assist the understanding of the biodiversity data, indicating the overall pollution tolerance of the different taxonomic groups found at the sampled area (Armitage et al., 1983; Balloch et al., 1976 cited in: Walley and Hawkes, 1996). The ASPT has a range between 1 and 10 and values over 7.0 indicate very good water quality, while values less than 3.9 indicate very poor water quality (Walley and Hawkes, 1997; Walley and Hawkes, 1996).

The Shannon index (H´) is a statistical index used to measure the heterogeneity of an area. It estimates the abundance and evenness of species present at a site; greater H´ value implies greater species diversity. In real ecosystems can range between 1.5 and 3.5, but rarely can have values more than four (Margalef, 1972 cited in: Magurran, 2009).

Haukås River

The Haukås River is located 20 km north of Bergen in an urban developing area with many possible runoff sources. The river is 4.5 km long and passes a large construction area, a camping area, a horse racing track and a heavily trafficked road (the E39) before reaching the sea. The river is quite small, varies from about 2 to 4 meters in wide and is about 0.5 meter in depth. Today the Haukås River is the only remaining habitat for the Freshwater Pearl Mussel (*Margaritifera margaritifera*) in Bergen municipality, and maintaining this population of mussels is therefore important. The freshwater pearl mussel was rediscovered in the Haukås River in 2002, and when examining the river several empty shells were found and most of the live mussels were old individuals and this indicates that the recruitment the last years is poor (Bjordal, 2014)(Bjordal, 2014)(Bjordal, 2014)(Bjordal, 2014)(Bjordal, 2014). After the rediscovery of the mussel in Haukås River, action has been taken from Bergen municipality to maintain a healthy population in the river. Several restrictions and environmental regulations were set when the planning of a nearby industrial area was conducted. During the construction work, several of the restrictions has not been followed, and is most likely the

reason for the drastic decrease of old, mature mussels, in this particular zone of the river. Despite this, it has been some recruitment in this zone (number II in Figure 2) the last years, and the area might therefore support a viable population. The survey of 2013 counted 738 live mussels in the river, and the population is estimated to be around 900 freshwater pearl mussels in the Haukås River (Bjordal, 2013b).

Figure 2: An overview of mussel abundance in different zones in the Haukås River in the survey in 2013. Red dots marks occurrence of the freshwater pearl mussel, *from (Bjordal, 2014)*.

The European LIFE programme

Since 1992, the European Union has co-financed more than 4000 projects with a focus on environment, nature conservation and climate action (LIFE programme, 1992). Many of these projects have been about the freshwater pearl mussel and its conservation. In countries like Sweden, Czech Republic and Germany as well as Great Britain, to mention a few, projects with a focus on improving the habitat and reproduction of the mussels have been financed, in part, by LIFE programme (1992), and the knowledge gathered from these efforts has been plenty. The main problems these projects have had to deal with, are similar to the

ones in this study, namely achieving successful recruitment and reproduction of juvenile mussels, and sediment issues has been a recurring reason to these problems (Degerman et al., 2009; LIFE-Nature project, 2006; PIP project, 2012).

Culturing project in Austevoll

In 2011 a cultivation project started in Austevoll, initiated by the Norwegian Directorate for Nature Management. This is a part of a national action programme for the conservation of the freshwater pearl mussel, which began in 2005. Cultivation trials in Austevoll started in 2011 and the production cultivation started in 2012. Two different methods were used to collect mussels for cultivation from Haukås River. First method was to collect host fish that is naturally infected by glochidia larva in their natural habitat. The host fish were then sent to the cultivation site where glochidia larva were harvested; in Haukås River, 351 mussels were collected in this way in 2012. From the Haukås River over 5000 mussels was estimated for harvesting in the spring 2013. The last method used in Haukås River was to collect glochidia larvae when they were released, bring them to the cultivation farm and let them infect the host fish in the cultivation farm. The mussels are kept in the cultivation farm until they are big enough to be reintroduced into their original habitat (Jakobsen et al., 2013). Ideally reintroduction in rivers with oxygen depleted sediments should bypass the burrowing juvenile stage, so that the reintroduced mussels avoid low oxygen levels, and are not dependent on burrowing in the sediment, but it is practically challenging as mussels may be as old as 5 years before they reach 2mm, and begin filter-feeding on the river bottom. Moreover partial burrowing in anoxic sediment might be harmful, even for juvenile mussles. Filter feeding is most likely dependent not on age, but size. Filter-feeding apparatus has been shown to be developed at 2 mm (Schartum, 2014). Growth indicates that the mussels should be ready for reintroduction when they are three to four years (Jakobsen et al., 2013). The oldest group of mussel collected from Haukås River is ready for reintroduction, and is to be reintroduced to the Haukås River in spring 2015. The need for finding suitable sites for the reintroduction is then urgent (Bjordal, pers. comm., September 2014).

Freshwater pearl mussel meander

A suggestion has been made in cooperation between the University of Bergen and Bergen municipality to build a freshwater pearl mussel meander in Haukås Gård, it will function as a mussel refuge, and as a source population for subsequent natural re-introduction of the

remaining river. The aim is to provide a refuge for the mussels from the Austevoll cultivation project which then infects the natural trout population in the river. The trout then hopefully spreads the mussels both upstream and downstream of the refuge, and if the habitat requirements are met, there will be a natural re-introduction. The site of the planned facilities is near the main stream of Haukås River in Haukås Gård, which is owned by Bergen municipality. The plan is to build a new meandering riverbed in area 2 in Figure 3, coming from the tributary small river in site 1 in Figure 3. To maintain stable water flow a hose could be put in the main river with water supply. The intake of water in the hose is suggested to be upstream of the tributary river from Bergen Travpark. 1 cost estimated was made at January 14th 2014, and set to be 466.000 NOK. The meander project will be in accordance to the municipalities zoning plan for Haukås (Bjordal, pers. comm., September 2014).

Figure 3: The area of Haukås Gård. 1 shows the tributary river. The area marked by 2 shows the planned meander, and C locates the extra supply of water in a hose to the meander.

Aim of study

Our study is part of a cooperation between Bergen municipality and the University of Bergen, aiming to reintroduce juvenile freshwater river mussels *Margaritifera margaritifera* in the Haukås River, and to achieve a sustainable population in the river. Therefore, the main focus of our study is to determine the water and habitat quality of the river and investigate different parameters such as oxygen and nitrite levels that might influence the recruitment of reintroduced juveniles.

Materials and methods

Eight sites were chosen for the sampling in cooperation with Bergen Municipality. The choice of these sites was made taking into account previous studies (Anders et al., 2013) in order to monitor the changes in the river through time as well as the potential sources that might affect the water quality of the area (Figure 4). The sites were sampled for *E.coli*, phosphorus and biodiversity.

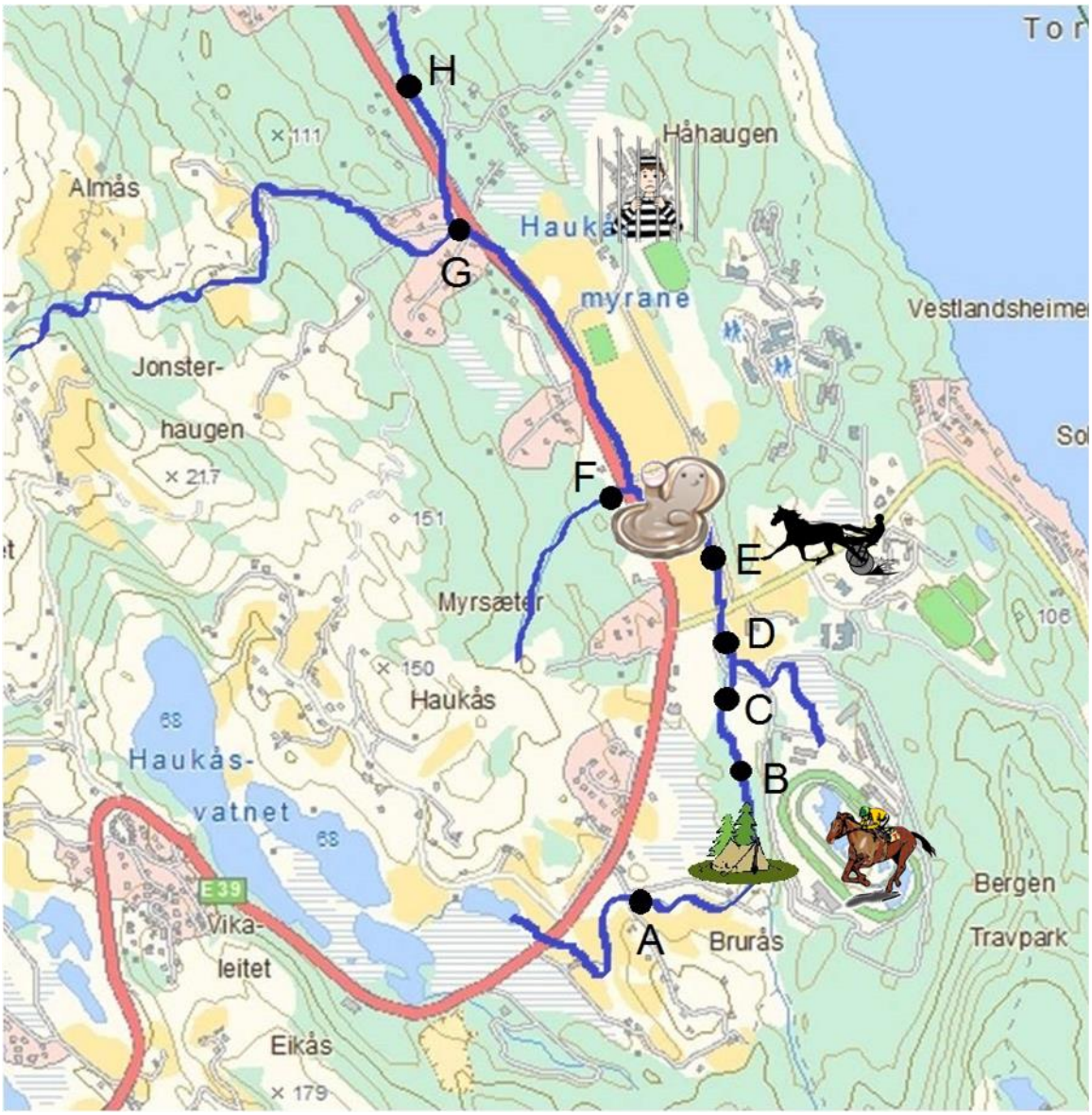

Figure 4: Map presenting the sampling area in Haukås River as well as the potential sources that might affect the water quality of the river (prison of Bergen, Horse track, camping, all of them are presented to the map with relevant pictures).

The sampling took place the 9th of September, 2014. The weather was cloudy; there was 0.1 mm of rainfall between 09.00 h and 11.00h, and the temperature varied from 10.8 to 17.1 º C. The previous week the precipitation in the area was moderate, with 11.2 mm on 02.09.2014 and 8.7 mm the day prior to sampling (08.09.2014). The temperature differed between 14.3 and 16.4 ºC during the same period (Meteorologisk institutt, 2014).

Description of sites

Site A

(60°29'2.6"N 5°22'41.5"E)

Site A is located close to "Bergen Camping Park" and the river runs underneath the camping through a large plastic tube, making an artificial barrier. High vegetation and overhanging trees characterizes the site. The bottom of the river consists sand and gravel. At least 5 brown river trouts (*Salmo trutta fario)* were observed, they appeared to be juveniles (Figure 5). Some garbage and an old bicycle were found on the riverside.

Site B

(60°29'16.2"N 5°22'50.9"E)

This site is located in a conifer forest and characterized by flat land profile on both sides of the river. The vegetation is dense and moss beds cover many trees (Figure 5). In this site, four iron un-galvanized rods with a distance of 3-4 m upstream and downstream from each other were collected. These rods were placed to measure the oxygen level in the sediment, since the oxidation of iron can show presence of oxygen.

Site C

(60°29'20.4"N 5°22'47.9"E)

This site is located at the beginning of the conifer forest and quite close to site B. Only water samples were taken, and nitrate measurement was recorded on this site. This was decided taking into account the close distance to site B.

Site D

(60°29'24.6"N 5°22'47.0"E)

Site D is located in a dense forest area. Vegetation grows on both sides of the stream. West of the river the land is flat while on the east side the landscape is steeper. The sediment is sandy and muddy which made it difficult to walk with waders. Branches of the trees stretch out across the water and there was is a lot of vegetation in the stream (Figure 5).

Site E

(60°29'32.6"N 5°22'41.0"E)

This site is located close to a known population of adult river mussels (Bjordal, 2013 in Anders et al., 2013). West of the river is an agricultural field and on the east side a horse race track runs parallel to the river. The vegetation of the surrounding area consists dense and tall grass. There was also grass found in the stream. A few meters upstream trees branched out

across the river (Figure 5). The sediment was sandy and rocky. In this site 2 iron rods were collected, one in the middle of the river and one close to the riverbank, on the agriculture field side. These rods were placed, together with the iron rods in site B, to measure the oxygen level of the sediment.

Site F

(60°29'36.0"N 5°22'25.0"E)

Located close to the E39 main road, Steinestøvvegen, which is heavy trafficked, this site is characterized by dense vegetation on both sides of the river, as well as in the stream. The land is steep west of the road (Figure 5). 30 meters upstream there is a large tree with many of its branches growing across the stream. The sediment was very muddy and it was difficult to walk.

Site G

(60°29'54.7"N 5°21'56.8"E)

Site G is located under the small bridge leading to Almåshaugane. The site is close to the E39 main road, which is heavily trafficked. On each side of the site, there is a lot of vegetation, moss, grass and ferns. Trees on each side have branches that stretched over the stream, and some branches had fallen into the water (Figure 5). The sediment was sandy and rocky with much vegetation. There was also spotted two trouts at this location.

Site H

(60°30'7.5"N 5°21'39.8"E)

Located near a house in Almåsdalen where there was some construction work. Some building materials were observed close to the west bank. There is much vegetation on this site, on both sides of the stream. East of the river a large tree and its branches stretched out across the sampling site (Figure 5). The sediment is mostly sandy but also rocky and it is some vegetation in the stream.

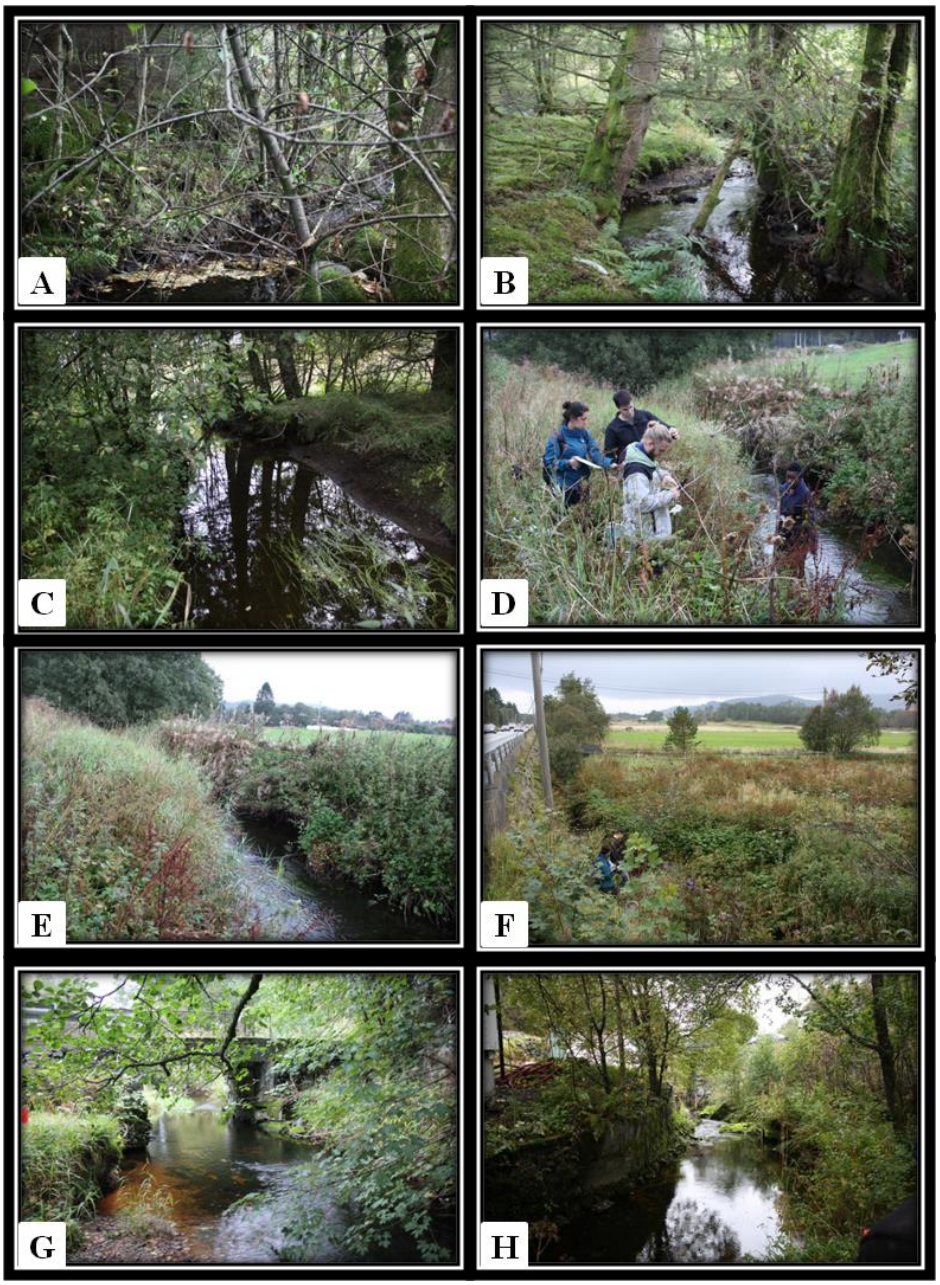

Figure 5: Sampling sites all along the study area of Haukås River.

Sampling Procedure

Biodiversity

Kick sampling was done using a bucket, which was open at one end and had a fine rectangular mesh (mesh size 1 mm) at the other end. The bucket was held by one person at the bottom of the river with the open end facing the current. Another person standing 30-50 cm away from the bucket, kicked the sediment into the bucket. The sediment and organisms

would flow with the water into the bucket. The sediment was kicked for one minute after which the sample in the bucket was washed into a 200 mL bottle for analysis later in the laboratory. Water from the same site was used in order to avoid contamination from another site.

Identification of the animals was carried out later in the afternoon in the UiB laboratory. Samples from sites with rocky bottom were transferred directly to rectangular aluminium plates and fresh water added to them. Organisms easily seen with naked eye were removed from the plate using forceps and sorted in a plate with small wells. Some samples were stained with rose bengal to make organisms more visible. Samples taken from sites with soft bottom were poured into the aluminium plates in smaller quantities and organisms easily seen were sorted with forceps and transferred to well plates. Later on the sediment was sieved with a finer mesh to get a filtrate, which was put on an aluminium plate. Staining was done to make the organisms visible. All organisms were identified by microscopy. Organisms were placed under a light microscope (different magnifications) and identified as close to family level as possible. Revised BMWP and ASPT scores were used to categorize biological and water quality per each site. It is important to mention that in order to calculate the BMWP score of site E, *Pediciidae* was considered to be a taxonomic group with score equal to 5.5, since this family according to Paisley et al. (2007) contributes to the BMWP score of *Tipulidae*.

Thermo-tolerant coliform bacteria

For the sampling of thermo-tolerant coliform bacteria were used sterile 250 mL bottles. The bottles were attached to a 2 meter long sampling rod in order to be lowered down without getting contaminated. The opening was facing downwards reaching approximately half the water column depth and then turned so it filled with water. The person taking the sample stood either on the riverbank or downstream in the river so that the sample did not become contaminated. The sterile bottles were opened right before the sample was taken and closed right after. We had a limitation of 20 samples at which we distributed throughout the sites. From site C, D, F and H we took two samples and from sites A, B, E and G we took three samples. The samples were kept cool in a cooler box until they were delivered to the Bergen Vann Laboratories. The analysis in the laboratory followed the membrane filter method NS ISO 8199, January 2002 (Bergen Vann KF 2014, pers. comm., 10 September 2014). The agar plates were incubated for 21 (± 3) hours at 44.5 (± 0.2) °C, the colonies were counted and

multiplied in relation to the dilution. The method followed the Norwegian standards NS 4792 1st edition May 1990 (Bergen Vann KF 2014, pers. comm., 10 September 2014). Difference in Thermo-tolerant coliform bacteria levels, between the sites was tested using a one-way ANOVA**.** Significant differences between the sites were found using a Tukey HSD test.

Phosphorus

The phosphorus samples were taken in non-sterile 50 mL Falcon tubes. The tubes were rinsed in the river 3 times before the sample was taken. The sampling method was the same as for the thermo-tolerant coliform bacteria, and the phosphorus samples were also taken in half of the water column depth. It was taken one sample in each site, except site A, where two samples were taken.

The samples were kept cool in a cooler box until they were delivered at Eurofins Laboratories. They were then analysed after Norwegian Standard NS-EN ISO 15681-2 by means of NS-EN ISO 6878 procedure (Eurofins 2008, pers. comm., 17 September).

Temperature and pH

A total of eight readings for pH and eight for temperature were taken, one at each site by immersing a portable pH meter (pH 3110, 32362 Weilheim) just below the water surface and taking the readings when the meter stabilised. The pH-metre was calibrated prior to use.

Nitrite

Nitrite samples were analysed on site using a Hanna Instrument 764 Checker® Ultra Low Range spectrophotometer (@525 nm), using an adapted EPA Diazotization method 354.1 (EPA, 1971) to yield the concentration of nitrogen-nitrite (NO_2^--N) in ppb (parts per billion). The results were converted from the NO_2^--N concentration to the nitrite ion concentration (NO_2^-), by multiplying the reading with a factor of 3.29. Two 10 mL samples were filled at each sample site in the middle of the water column; one for calibration and a second for the reaction. The resolution of the analysis is 1 ppb (NO_2^--N) with a range between 0-200 ppb. The accuracy is ± 10 ppb ± 4 % of reading at 25 ºC (Hanna-Instruments, 2014).

Oxygen

Oxygen was measured from two pre-determined sites, which were characterized as an interesting site to reintroduce juvenile freshwater mussels. Two iron rods were inserted into the riverbed at site E and four at site B. A plastic marker indicated the border between

sediment and water. All six un-galvanized rods placed in the riverbed in July 2014 were used as a qualitative test to show the anoxic zones in the sediment below the bottom of the river. In presence of oxygen the rods were expected to be rusted, because aerobic bacteria use oxygen as an electron donor; absence of oxygen would be indicated with lack of rust. As the rods only indicate whether there is oxygen or not, we cannot see changes during periods. Additionally all the sampled sites were investigated for sulphur smells in the sediment, which is a strong indicator of sulphur-reducing bacteria, thus revealing anoxic zones.

Measuring width, depth and flow rate

Width, depth and current were all measured. The measurement of the current was executed by two people extending a rope for a known distance up to 10 m. By dropping a plastic bottle into the stream and recording the needed time for the bottle to travel the known distance, we calculated the velocity. The flow rate was calculated by multiplying the velocity with the cross-sectional area.

Results

Biodiversity

Biodiversity was grouped according to the revised BMWP score (Walley and Hawkes, 1996) of the organisms. Families which had revised BMWP scores of 1-3.9 were grouped as those indicating poor, 4-6.9 fair, 7-9.9 good and 10-12.9 excellent water quality respectively. This means that the taxa Annelidae, Planorbidae, Ceratopogonidae, Sphaeriidae, Thaumaleidae and Chironomidae were graded as indicators of poor, Baetidae, Elmidae, Hydropteridae, Pedicidae and Tipulidae were grouped those indicating fair, Leuctridae and Polycentropodidae as indcators of good, Taeniopterygidae and Chloroperlidae as indicators of excellent water quality. Percentages of organisms according to site are presented in Figure 6.

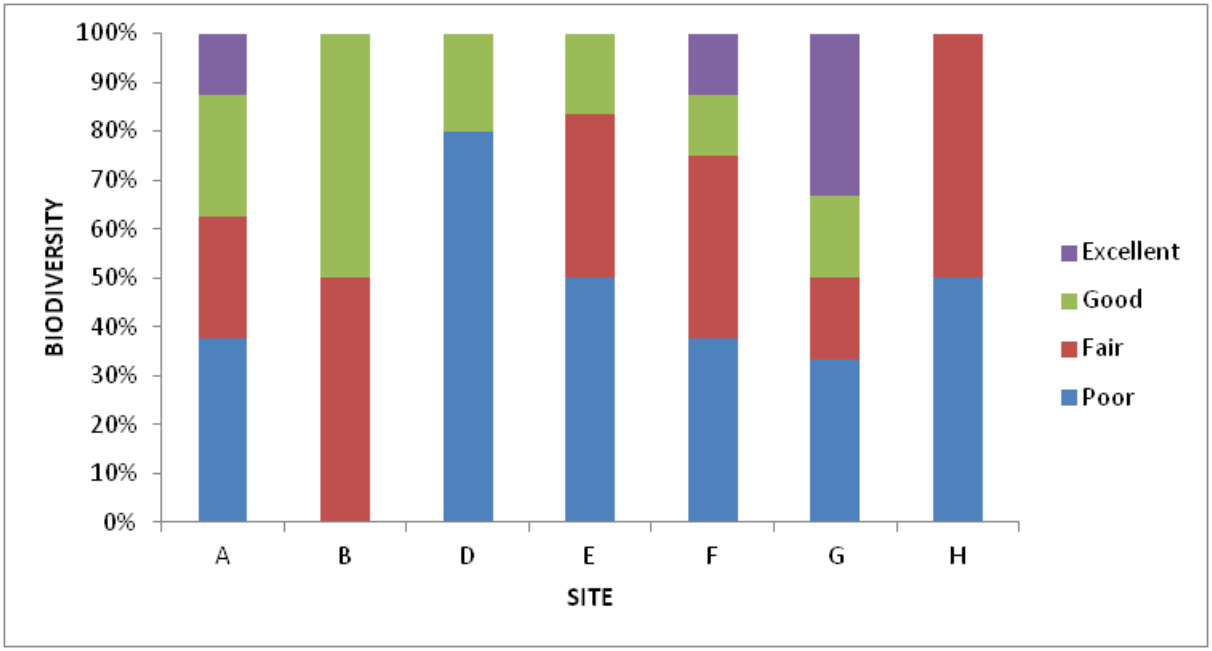

Figure 6: Taxonomic families categorized according the water quality status of each sampling site. Groups "Excellent", "Good", "Fair" and "Poor" are groups resulted by using revised BMWP scores. Haukås River, 2014.

A total number of 18 taxonomic groups were found in the river. Some river sites had macroinvertebrates belonging to the orders Emphemeroptera, Plecoptera and Tricoptera (i.e. Baetidae and Hydropteridae). All sites except A and B have Diptera (Chironomidae). Sites A and F had the highest number of families (Appendix I, Table 1). The most common taxonomic groups were Annelida, which was found in all sites except B and H, and Elmidae, which was found in all sites except in D. In site E there were found individuals belonging to the family of Pediciidae (order: Diptera).

The total BMWP of the river was 31.8 and the average ASPT per site was 6. This classifies the river as one with poor biological and good water quality respectively. The individual classification of the sites is presented in Appendix I, Table 1. ASPT scores of each site are presented in Figure 7.

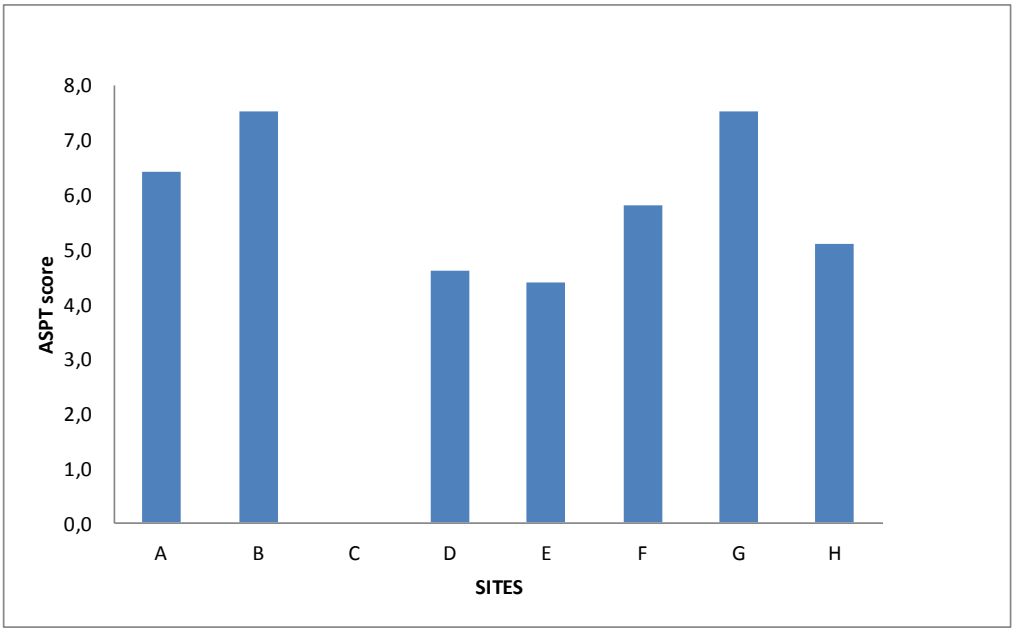

Figure 7: ASPT score for each site, Haukås River, 2014.

The Shannon index was calculated for all sites as follows:

$$H_n = -\sum(p_i \, ln \, p_i)$$

where $p_i = \frac{n_i}{N}$ is the relative abundance of each species; n_i is the number of individuals in each taxonomic group and N is the total number of all individuals. According to the values given by this index, sites B and H have most poor environmental conditions than all the other sites (Appendix I, Table 1).

Temperature and pH

Water temperature varied from 11 °C on site G to 15 °C on site A. The mean temperature was 13.14 °C (± 1.48) °C. The pH ranged from 7.08 to 7.72 and the average pH value was 7.27 (± 0.21) (Appendix I, Table 2).

Thermo-tolerant coliform bacteria

Thermo-tolerant coliform bacteria (TTCB) levels ranged from 20 col/100 mL, to 420 col/100 mL. The mean was 155.5 (± 100.71) col/100 mL (Figure 8). TTCB levels varied significantly

between sites (p=0.0019, F=6.89. TTCB levels in site E was significantly different from site A, B and C (p=0.00158, p=0.0027, p=0.012 respectively). There was an increase in TTCB levels from site A, downstream to site H (p=0.0036) (Figure 8).

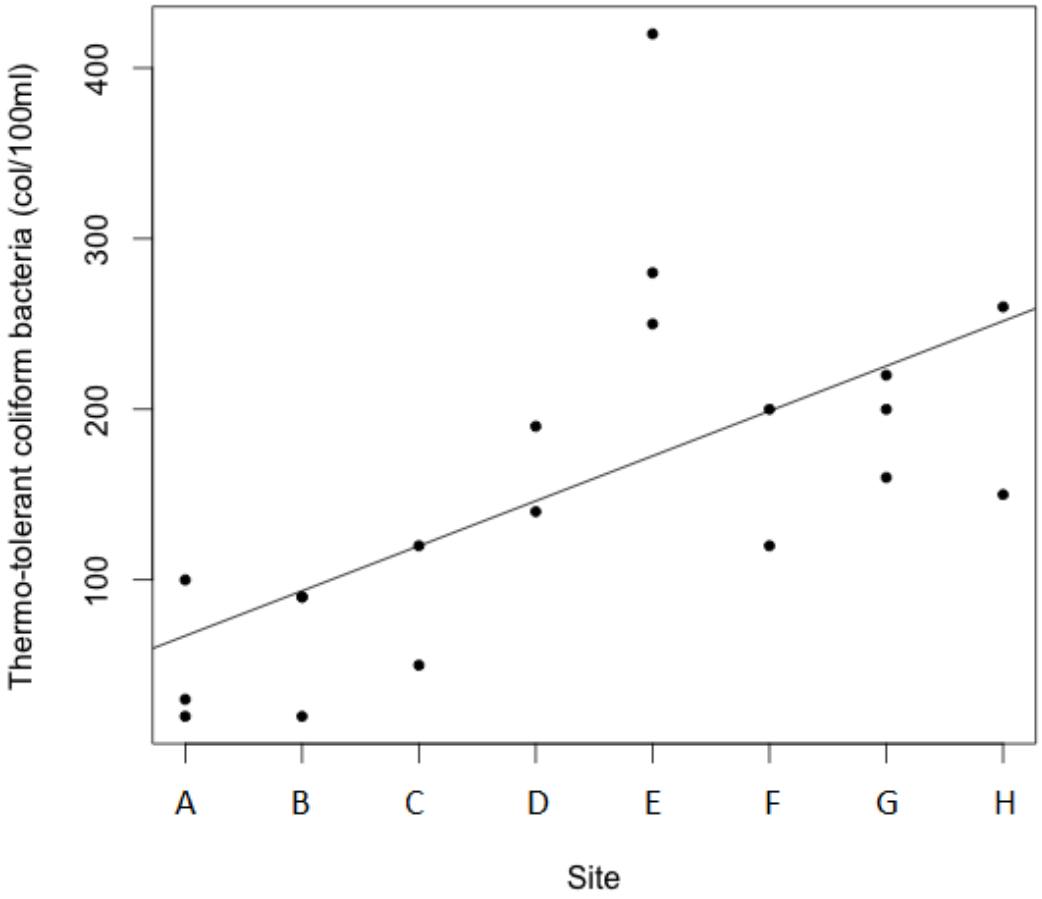

Figure 8: The scatter plot shows levels of thermo-tolerant coliform bacteria (col/100 mL) at sampling sites. The line shows an increase in levels downstream the river.

Phosphorus

The phosphorus level ranged from 7.8 µg/L to 22 µg/L (Appendix I, Table 3). The mean value was 16.59 (± 5.86) µg/L.

Oxygen

Depth of oxygen reduction revealed an overall shallow zone for juvenile settlement (Appendix I, Table 4). Results varied both between and at same sites. Largest variation was at site E, Sulphur smell was detected at sites E and G.

Nitrite

Concentration of Nitrite ($NO2^-$) in (µg/L), showed variation spatially between sites, with factors up to 21 (Site E (69.09 µg/L) compared with site H (3.29 µg/L) (Figure 9). No apparent variation trend from upstream to downstream was evident. Lack of replication prevented comparisons of differences between sites.

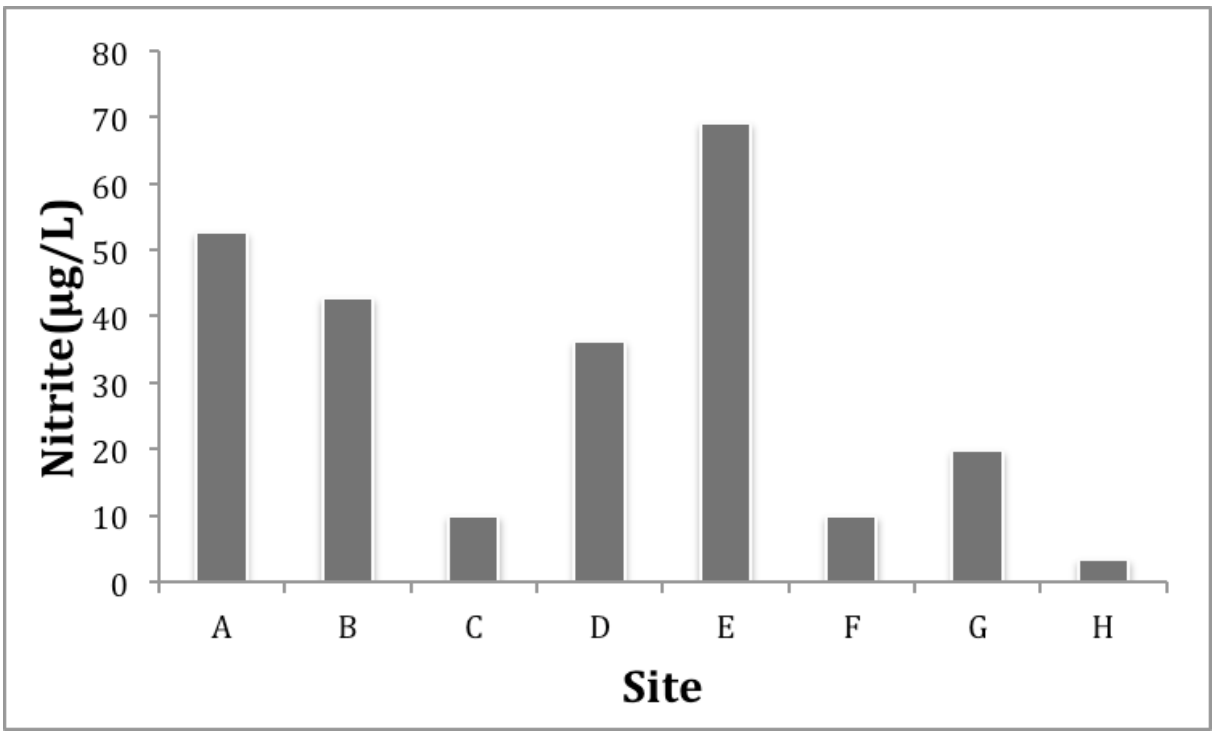

Figure 9: Concentration of Nitrite (NO_2^-) in (µg/L), with an accuracy of ±10 (µg/L) at 25 °C. Results varied spatially between sites with factors up to 21 (Site E compared with site H). No apparent variation trend from upstream to downstream is evident. Lack of replication prevented comparisons of difference between sites.

Measuring width, depth and flow rate

Flow rate was calculated for each sampling site, except for site C. The results are showed in Table 5 (Appendix I, Table 5).

Discussion

Summary of results

According to the present outcomes, the examined physical and chemical parameters in this study indicate that the water quality requirements for the juvenile freshwater pearl mussel in the Haukås River are met. The oxygen assessment in the sediment showed alarming anoxic zones relatively shallow in the sediment. Phosphorus and nitrite levels (nitrite is more toxic than nitrate) in the water were probably not sufficient enough to negatively affect the mussels substantially, and the pH levels are, with the exception of site D, under the recommended limit of 7.5. The biodiversity and E.coli levels indicate that the biological quality of the river is poor, but the effect this has on the mussels is uncertain.

Methodical issues

All measured parameters have collected data on the water running through the river and therefore on the environment in which the adult mussels are exposed. The bottleneck for recruitment of the freshwater mussel in Haukås River has been recognized as juvenile settlement and survival after the glochdic-parasitic stage in the interstitial zone; however data on the actual physical and chemical environment where the juveniles are exposed are lacking. Nevertheless, oxygen data from the iron rods, the parameter which is suspected to be the primary determinant of successful juvenile recruitment (Bjørn and Reiser, 1991; Chapman, 1988; Geist and Auerswald, 2007), are referring to the interstitial zone. The two sites examined showed absence of oxygen at different, but shallow depths, based upon the lack of rust on the un-galvanised metal rods. The juveniles also need fine sediment for successful growth (Degerman et al., 2009). The sediment of some of the sampling sites consisted of mud, which could hinder the survival of the juveniles (Degerman et al., 2009). To improve the statistical testing, several phosphorus samples at each site is needed. We had only one sample taken from site B-H, and two samples taken from site A. Samples of thermo-tolerant coliform bacteria were taken in multiples of two or three on each site. Contamination occurred in three samples. In site F both samples were contaminated when the bottle was lowered into the river and touched the bottom substrate. In one of three samples in site B, the bottle detached from the rod and had to be picked up, and lowered once more. This probably did not affect the result, as the number of colonies was the same as for another sample in this site.

Discussion of results

The tributary river from "Bergen Travpark" and horse racing track has previously been detected as a pollution source of the Haukås River (Anders et al., 2013; Bjordal, 2013a). The proposed area for the mussel refuge, and the reintroduction of *M. margaritifera* are immediately downstream of this confluence point. Thus, the present study will focus on these areas.

In the period after the "rediscovery" of the freshwater pearl mussel in 2002, it has been known that the tributary river coming from "Bergen Travpark" has intermittently contaminated some areas of Haukås River, with fine particles, e-coli and nutrients (Anders et al., 2013). This is also reflected in the missing appearance of mussels in this part of the river. In the first 300 m downstream of the confluence point, no live mussels were found in the surveys conducted in 2002 and 2013 (Bjordal, 2013b). Attempts to mitigate the environmental impact of "Bergen Travpark" by sand capturing, proved to be successful; however additional measures are needed (Bjordal, 2013a). Furthermore a capture and filter-ditch has been constructed along the horse training track, which is situated parallel to the river (in the area around site E). Although the measure is effective, it is dependent on Horse-Track personnel to ensure that maintenance of the training track does not disrupt the filter-ditch (Bjordal, 2013a). Additionally it is also clear that the measure is not sufficient to avoid runoff from the training track, as it clogs easily and loses its functions during heavy rainfall.

Even though these measures have been in place since summer 2013, pollution and runoff have previously been detected from "Bergen Travpark", highlighting the need to extended and improve the water quality further (Anders et al., 2013; Bjordal, 2013a). Last year's report (Anders et al., 2013) shows increased values of phosphorus between our sites D and E,which coincides with the confluence point from the tributary river related to "Bergen Travpark", and the horse racing track running parallel to the river at site E. The specificity of the different sites which detected the pollution seems to exclude the road, nearby industrial development and direct agricultural runoff from the crop field situated west of site E. If the pollution source was the road, the pollution would have been detected at multiple measuring sites, which were along the road, including many upstream. Similarly, if the industrial development was the pollution source, measurements from the upstream sites would have detected it. Although the road and the industrial development most likely are not the source for the specific pollution in

site D and E, the river system might be affected by that, as parts of the river is located close to the main road E39, which is heavily trafficked. Sewage and drainage pipes goes out to the river, and that contributes to contamination, Bjordal (2013b) states that this could also be an acute problem if traffic accidents occur.

However, in regard to runoff from agricultural activities there are many possible entries to the river system, it is possible that runoff originating from the vicinity of "Bergen Travpark" might flow into the same tributary. Another pathway could also be the small patch of cropland situated west of site E, which might use manure as natural fertilizer. Indeed our results seem to support this, as there was no increased P levels in the immediate vicinity of the cropland (which might indicate artificial fertilizer use), but high e.coli counts which could originat from natural fertilizer of the cropland or the horse training track and manure use. Ultimately it is assumed that the pollution contribution from the small cropland is negligible compared to "Bergen Travpark", and the horse training track.

The outcomes of the present study are in accordance with the hypothesis about these contamination sources. As thermo-tolerant coliform bacteria (TTCB) can be used to assess faecal contamination in the environment and thus identify pollution sources (Paruch and Mæhlum, 2012), the increased level of TTCB in site E supports that the pollution is likely from horse manure from the horse training track or natural fertilizing manure from the agricultural field. The levels of TTCB measured in the Haukås River shows that it is contaminated by faecal matter in such an amount that makes it unsuitable for human to drink and bathe in it (Andersen et al., 1997). However, it is difficult to assess whether the relation of TTCB is of harm or benefit for the mussels.

Biodiversity indices found that Site D, had the worst biological quality of our sampled sites, and presented 80 % of poor quality indicators. Abundance of species can be used to indicate the quality of water (Sharma and Chowdhary, 2011) and the low biodiversity results are consistent with the tributary pollution from "Bergen Travpark". Most organisms that were found in this area of the river were different from upstream biodiversity, and are indicators of pollution, with high tolerance for low oxygen concentrations, such as annelids (Chadde-Schumaker, 2014). In fact our oxygen results showed a high variability in site E (downstream from site D and the confluence point), but the rod placed near the bank and not in the middle, had anoxic values 0.5cm under river bed; which indicates that heavy sedimentation occurs in

the low flow areas. As fine particles require less turbulence to sediment than larger particles, areas with slower flow, such as river beds, accumulate fine particles.

Effect of fine-particle sediment on Dissolved Oxygen

Also fine sediment both organic and inorganic, with high and low Biochemical Oxygen Demand (BOD) respectively, are both suspected to be principal reason for the shallow hyporheic anoxic zones discovered in this study. This result is in agreement with other studies (Geist and Auerswald, 2007; Österling et al., 2010). The sedimentation of fine particles affects the permeability of Dissolved Oxygen (DO) through the river substratum. Oxygen permeability is reduced as the fine particles impedes diffusion, thus the BOD does not penetrate deep into the hyporheic zone; simultaneously respiration of Oxygen occurs in the hyporheic zone, leading to DO levels insufficient for juveniles (Bjørn and Reiser, 1991; Chapman, 1988; Geist and Auerswald, 2007). Additionally metabolites diffuse less into the river, which can create micro-toxic areas for juveniles. Furthermore increased organic sediment loads can increases respiration rates, and thus the BOD (Österling et al., 2010). The hyporheic zone is defined between the free-flowing surface water~~,~~ and the groundwater. The exchange~~s~~ rates of water quality is influenced by the substratum permeability (Boulton et al., 1998; Malcolm et al., 2008). Groundwater typically has low DO concentrations, while surface water has high concentrations (Packman et al., 2004), meaning the juveniles may experience very different DO levels according to local differences and depth of settlement.

Oxygen levels found during this study were not quantified, but displayed anoxic (non-reducing Fe) in sediment depths not consistent with juvenile mussel requirements. Glochidia burrow and settle in the hyporheic zone after its parasitic stage on the fish. Juvenile burial depths have not been pinpointed accurately, but there is consensus that they do not bury deeper than 20 cm (Degerman et al., 2009; Quinlan et al., 2014). Geist and Auerswald (2007) set a maximum of 10-15 cm, Buddensiek et al. (1993) investigated 10 cm, while (Tarr, 2008) looked at 2 cm. In the present study, the measurements only contain depth of anoxic and oxic zones, and not DO concentrations from 08.07.14-09.09.14 (70 days). Consequently the habitable zone must lie above the observed anoxic limit. DO near the anoxic limit is likely much lower than near the substratum surface; indeed Buddensiek et al. (1993) found significant differences in 1 cm intervals. It is possible that juveniles must trade-off between high DO and possibly more food (bigger particles), but more prone to the current flow and predation with subsequent mortality during floods or heavy rain; while deeper settling in the

hyporheic zone has lower DO inflow, but is more protected from floods (Quinlan et al., 2014). Adult mussel have the ability to survive periods of relatively low DO periods, but are far less likely to experience as low DO levels as their hyporheic juveniles (Geist and Auerswald, 2007; Skinner et al., 2003). In a study by Oliver (2000) DO saturation requirements for *M. margaritifera* was found to be between 90-110 %, but these levels lack empirical (*in situ)* data, and are probably too high to explain the presence of mussels in many rivers, especially considering temporal variability of DO. Summers with high productivity generally have low DO (as a result of high BOD), while winter generally have high DO (Malcolm et al., 2011; Oliver, 2000).

Indications of nutrient runoff

The most common cause of eutrophication in streams are excessive concentrations of phosphorus and nitrogen, and a concentration of 20 µg/L of phosphorus will often cause a eutrophication problem in streams (Correll, 1998). Low nitrogen and phosphorus levels are the primary nutrients limiting plant/algae growth, and excessive concentrations often lead to a regime shift; from higher macroscopic plants and organisms, to microscopic and algal communities, that in turn outcompete higher plants in nutrient rich environments (Folke et al., 2004).

The measurements of phosphorus support the hypothesis of the tributary river from "Bergen Travpark" as a significant contamination source of Haukås River as the levels of phosphorus in Haukås river increased in site D. Phosphorus level continued to stay on this level further downstream in the river and could be a result of nonpoint source runoff from the agricultural field (Carpenter et al., 1998), and from equine manure through drainage water from the horse race track located beside the river (Parvage et al., 2011). Compared to the survey performed by Anders et al. (2013), the phosphorus level is lower than the year before in this area. All the sites had phosphorus levels below the critical value of 30 µg/L for successful reproduction of freshwater pearl mussel (Bauer, 1988) and levels of phosphorus is then not likely to be a limiting factor for successful juvenile recruitment. Still monitoring of phosphorus levels should be conducted over a period of time to check for variations during seasons, especially because of the agricultural field and periods of more heavily rainfall which influence runoff from the field and the horse race track area.

Nitrogen also have a direct effect on the mussel population, although there are not many studies regarding NO_2^- requirements for mussel, but instead nitrite and nitrate (oxidized nitrogen) have been assessed in a number of studies (Moorkens, 2000). Total Oxidized Nitrogen (TON) or nitrate are mostly used in studies regarding mussel requirements (Geist and Auerswald, 2007; Quinlan et al., 2014). In a recent study, Geist and Auerswald (2007) found significant positive correlation between nitrite and growth, while a negative correlation between growth and nitrate was also presented. A study by Soucek and Dickinson (2012) about a different bivalve, *Megalonaias nervosa*, showed nitrite concentrations of 177 mg /L lead to 50 % mortality after 96 hours; nitrite, nitrate and ammonium are potentially harmful for juveniles, with nitrite and ammonium more toxic than nitrate (Eybe et al., 2013). Detritus is known to reduce balance and facilitate the nitrification from nitrite and ammonium to nitrate, rendering it less harmful (Eybe et al., 2013). Additionally juveniles immersed in the hyporheic zone would be exposed to different nitrite levels than our samples (from the river), especially detritus and nitrification effects may lead to different levels. Water quality could have essential importance to the survival of juvenile mussels. Up to 100 % mortality could be caused by low pollution levels recorded during the time the mussels attempt to establish in the sediment (Skinner et al., 2003), where this specific time period is crucial, because juveniles are less tolerant to pollution comparing to the adults (Eybe et al., 2013).

Successful reintroductions

So far the only successful example of reintroduction is from the river Lutter in Germany, where they have been able to stimulate recruitment of juvenile mussels. This was done by infecting brown trout with glochidia and return them to the river as well as reduce flow of sediment into the river by creating sediment traps (Altmüller and Dettmer, 2006). There are however many projects in the European Union LIFE programme that have been conducted with a focus on the conservation of mussels. The improvement of sediment in the streams have had a big focus in all of these projects, and the lack of oxygen is recognized as a one of the main threat to the mussels (Degerman et al., 2009; LIFE-Nature project, 2006; LIFE Nature Program, 2007; LIFE Náyade, 2007). The substrate that the mussels find suitable must be stable, consisting of sand and gravel, preferably close to large rocks and stones, and it must be well oxygenated (Degerman et al., 2009).

Solutions and conclusions

Stream-bed interstitial habitats are considered to have an important effect on the ecological function of rivers (Mueller et al., 2013a). Although juvenile mussel burial depths have not been pinpointed accurately, there is consensus that they do not bury deeper than 20 cm (Degerman et al., 2009; Quinlan et al., 2014). It is also known that during the time the mussels attempt to establish in the sediment, up to 100 % mortality could be caused by low pollution levels (Skinner et al., 2003) and this time period is crucial, because juveniles are less tolerant to pollution comparing to the adults (Eybe et al., 2013). Therefore, to restore a stream, such as Haukås River, intended to be used for the reintroduction of juvenile mussels, one of the priorities should be the improvement of the sediment (Mueller et al., 2014). This is in accordance with LIFE programme (1992) conclusions, where it is mentioned that the substrate is essential when it comes to successful recruitment. The improvement of the sediments must be a part of conservation projects for the freshwater pearl mussel. A potential solution to this problem might be the dredging of the river; however such a drastic action is a costly and only temporary solution in case that the source of mud remains uncontrolled The mussels may fail to increase in number within the following years because the juveniles can't recruit to the river. A suggestion to this could be the planting of trees in the river catchment, especially in areas where there is likely runoff (i.e. horse track, agricultural fields). This may work as a permanent solution to the accumulation problem of mud in the sediment due to soil erosion. Also, it should be mentioned that the planted trees should preferably be part of existing flora close to the river banks and the number should not be in excess. Another method, used successfully in the case of Germany (Altmüller & Dettmer, 2006) and which could be applied to Haukås River, would be the introduction of fish infected with glochidia.

Taking into account the life cycle of the freshwater mussel and the years that are needed until an adult is ready for spawning (Bauer, 1987), any attempts for restoration of freshwater mussel habitat should be made in long term perspectives. The successful restoration of stream substrata is costly and time intense but is probably the most essential aspect of ecosystem health in rivers (Geist and Auerswald, 2007).

Success is most likely to come from various projects which take an integral approach to the four underlying aspects; legal protection and policing, public awareness, habitat restoration and artificial breeding (Thomas et al., 2010).

Appendix I - Tables

Table 1: Biodiversity, BWMP and ASPT scores at sampling sites, Haukås river, 2014. Source of score evaluations (Walley and Hawkes, 1996).

	Site A		Site B		Site D		Site E		Site F		Site G		Site H	
BMWP scoring taxa	**Score**	**NR of individuals**	**Score**	**NR of individuals**	**Score**	**NR of individuals**	**Score**	**NR of individuals**	**Score**	**NR of individuals**	**Score**	**NR of individuals**	**Score**	**NR of individuals**
Annelida	3.5	2			3.5	5	3.5	6	3.5	3	3.5	6		
Baetidae									5.3	4				
Ceratopogonidae					3.7	1								
Chironomidae							3.7	9	3.7	7	3.7	3	3.7	10
Chloroperlidae											12.4	10		
Elmidae	5	1	6.4	1			6.2	2	6.2	2	6.4	2	6.4	3
Gomphomidae											8	4		
Hydroptilidae	6.7	2												
Leuctridae	9.9	2					9.9	3						
Pediciidae							*5.5**	1						
Planorbidae	2.9	10					2.9	2	2.9	12				
Polycentropodidae	8.6	9	8.6	1	8.6	7			8.6	3				
Sphaeriidae	3.6	11			3.6	29								
Taeniopterygidae	10.8	2							10.8	1	10.8	4		
Thaumaleidae					3.7	1								

Tipulidae					5.5 1		
Non - BMWP scoring taxa							
Acari			2	2	5		5
Nematoda		1	1	25			
Richness	8	3	7	8	9	6	3
total BMWP	51	15	23	32	47	45	10
Biological quality	**Fair**	**Poor**	**Poor**	**Poor**	**Fair**	**Fair**	**Very poor**
total ASPT	6.4	7.5	4.6	5.3	5.8	7.5	5.1
Water quality	**Good**	**Very good**	**Poor**	**Fair**	**Fair**	**Very good**	**Fair**
Shannon Index (H)	1.75	1.10	0.96	1.54	1.93	1.66	0.98

Table 2: Temperature and pH in surface water at the sampling sites. Site C was not measured because it is located close to site B.

Site	Temperature (°C)	pH
A	15.0	7.20
B	13.8	7.21
C	-	-
D	13.7	7.72
E	13.7	7.27
F	13.6	7.08
G	11.0	7.20
H	11.2	7.22

Table 3: Phosphorus levels (µg/L) in midwater at the sampling sites.

Site	Phosphorus level (µg/L)
A	11.0
A	9.5
B	7.8
C	15.0
D	21.0
E	22.0
F	22.0
G	22.0
H	19.0

Table 4: Depth of oxygen reduction in bottom sediment layer. Duplicates of iron rods were placed only on sites E and B.

Oxygen depth (cm)	Site
3.0	B-Near bank (upstream)
1.3	B-Middle of river (upstream)
4.0	B – Near bank (downstream)
4.5	B – Middle of river (downstream)
4.7	E-Middle of river
0.5	E-Near bank

Table 5: Measurements of Width, depth and calculation of flow rate, Haukås River, 2014.

Site	Width (m)	Depth (m)	Flow rate (m3/s)
A	2.63	0.50	0.12
B	2.70	0.40	0.14
D	2.35	0.60	0.07
E	3.70	0.50	0.59
F	2.50	0.70	0.28
G	3.60	0.78	0.66
H	3.80	0.40	0.36

Appendix II – R script

```
#Temperature
temp.df<-read.table(pipe('pbpaste'), header=T, dec=',')
> head(temp.df)
Temperature
1        15.0
2        13.8
3        13.7
4        13.7
5        13.6
6        11.0
> attach(temp.df)
mean(Temperature)
[1] 13.14286
> sd(Temperature)
[1] 1.476321

#pH
> ph.df<-read.table(pipe('pbpaste'), header=T, dec=',')
> head(ph.df)
pH
1 7.20
2 7.21
3 7.72
4 7.27
5 7.08
6 7.20
> attach(ph.df)
mean(pH)
[1] 7.271429
> sd(pH)
[1] 0.2059473

#Thermo-tolerant coliform bacteria
e.coli<-read.table(pipe('pbpaste'), header=T, dec=',')
> head(e.coli)
Site col measurement
1    A 100          1
2    A  30          2
3    A  20          3
4    B  90          1
```

```
5    B  90         2
6    B  20         3
> attach(e.coli)

> mean(col)
[1] 155.5
> sd(col)
[1] 100.7067

> oneway.lm <- lm(col~Site)
> anova(oneway.lm)
Analysis of Variance Table

Response: col
Df Sum Sq Mean Sq F value   Pr(>F)
Site       7 154345 22049.3  6.8994 0.001944 **
 Residuals 12  38350  3195.8
---
 Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

a1<-aov(col~Site)
> TukeyHSD(a1)
Tukey multiple comparisons of means
95% family-wise confidence level

Fit: aov(formula = col ~ Site)

$Site
diff       lwr       upr     p adj
B-A   16.66667 -150.39915 183.73248 0.9999333
C-A   35.00000 -151.78526 221.78526 0.9961448
D-A  115.00000  -71.78526 301.78526 0.3990321
E-A  266.66667   99.60085 433.73248 0.0015759
F-A  110.00000  -76.78526 296.78526 0.4478740
G-A  143.33333  -23.73248 310.39915 0.1142654
H-A  155.00000  -31.78526 341.78526 0.1338859
C-B   18.33333 -168.45192 205.11859 0.9999402
D-B   98.33333  -88.45192 285.11859 0.5714441
E-B  250.00000   82.93419 417.06581 0.0027385
F-B   93.33333  -93.45192 280.11859 0.6265651
G-B  126.66667  -40.39915 293.73248 0.1980550
H-B  138.33333  -48.45192 325.11859 0.2173168
D-C   80.00000 -124.61300 284.61300 0.8345594
```

```
E-C  231.66667   44.88141 418.45192 0.0119977
F-C   75.00000 -129.61300 279.61300 0.8721694
G-C  108.33333  -78.45192 295.11859 0.4648013
H-C  120.00000  -84.61300 324.61300 0.4524702
E-D  151.66667  -35.11859 338.45192 0.1478651
F-D   -5.00000 -209.61300 199.61300 1.0000000
G-D   28.33333 -158.45192 215.11859 0.9989610
H-D   40.00000 -164.61300 244.61300 0.9950279
F-E -156.66667 -343.45192  30.11859 0.1273512
G-E -123.33333 -290.39915  43.73248 0.2200261
H-E -111.66667 -298.45192  75.11859 0.4312570
G-F   33.33333 -153.45192 220.11859 0.9971348
H-F   45.00000 -159.61300 249.61300 0.9900965
H-G   11.66667 -175.11859 198.45192 0.9999972

> fit.lme<-lme(col~as.numeric(Site), random=~+1|measurement)

anova(fit.lme)
numDF denDF  F-value p-value
(Intercept)          1    16 74.23413  <.0001
as.numeric(Site)     1    16 11.57899  0.0036

> summary(fit.lme)
Linear mixed-effects model fit by REML
Data: NULL
AIC      BIC    logLik
224.8371 228.3986 -108.4186

Random effects:
 Formula: ~+1 | measurement
(Intercept) Residual
StdDev: 0.003290754 80.71302

Fixed effects: col ~ as.numeric(Site)
Value Std.Error DF  t-value p-value
(Intercept)      40.82911  38.22770 16 1.068050  0.3013
as.numeric(Site) 26.36112   7.74691 16 3.402791  0.0036
Correlation:
 (Intr)
as.numeric(Site) -0.882

Standardized Within-Group Residuals:
 Min         Q1         Med         Q3         Max
```

-1.26024407 -0.64093651 -0.05518526 0.17858166 3.06475058

Number of Observations: 20
Number of Groups: 3

> plot(col~as.numeric(Site), xlab='Site', ylab='Thermo-tolerant coliform bacteria (col/100ml)', pch=20)
> abline(a=40.82911, b=26.36112)

#Phosphorus
phos<-read.table(pipe('pbpaste'), header=T, dec=',')
attach(phos)
mean(Level)
[1] 16.58889
> sd(Level)
[1] 5.856288

Appendix III – Row data

Date	Site	Coodrinates	Width (m)	Depth (m)	Area (m^2)	Velocity (m/s)	Flow rate (m^3/s)	Temperature	pH	Nitrite	NR of E.coli samples taken	NR of phosphorus samples taken
9/9/2014	A	60°29'2.6"N 5°22'41.5"E	2.63	0,50	1.32	0,09	0,12	15	7,2	16	3	2
9/9/2014	B	60°29'16.2"N 5°22'50.9"E	2.70	0.40	1,08	0,13	0,14	13,8	7,21	13	3	1
9/9/2014	C	60°29'20.4"N 5°22'47.9"E	-	-			0,00	-	-	3	2	1
9/9/2014	D	60°29'24.6"N 5°22'47.0"E	2.35	0.60	1,41	0,05	0,07	13,7	7,72	11	2	1
9/9/2014	E	60°29'32.6"N 5°22'41.0"E	3.70	0.50	1,85	0,32	0,59	13,7	7,27	21	3	1
9/9/2014	F	60°29'36.0"N 5°22'25.0"E	2.50	0.70	1,75	0,16	0,28	13,6	7,08	3	2	1
9/9/2014	G	60°29'54.7"N 5°21'56.8"E	3.60	0.78	2,81	0,23	0,66	11	7,2	6	3	1
9/9/2014	H	60°30'7.5"N 5°21'39.8"E	3.80	0.40	1,52	0,24	0,36	11,2	7,22	1	2	1

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