Ecological Effects of Road Salt

The effects of road salt on the composition of macroinvertebrate fauna in three different streams receiving highway runoff.

CLAIRE BANT

NORWEGIAN UNIVERSITY OF LIFE SCIENCES
DEPARTMENT OF ECOLOGY AND NATURAL RESOURCE MANAGEMENT (INA)
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ABSTRACT

The present study focuses on the effects of road salt from highway runoff on macroinvertebrate communities. It was hypothesised that changes in salt concentrations will alter the composition of the macroinvertebrate fauna downstream of the reference sites. It is assumed that water quality will decrease as salt concentrations increase therefore resulting in a decrease in macroinvertebrate diversity and an absence of pollution sensitive species (EPT species). Three different streams situated in the south eastern part of Norway were investigated (Bolvikelva, Ljanselva and Gjersrudbekken).

There was very little difference in the macroinvertebrate communities recorded upstream and downstream. The majority of the variation in macroinvertebrate data found using RDA (Redundancy Analysis) was between streams. This variation between streams is probably due to a number of factors such as, differences in stream size, the urbanisation of the watershed and the variation in riparian zones.

There were no significant differences between the biological indices obtained at upstream and downstream sites or between seasons. This indicated no significant decrease in water quality between sites or seasons. Although the indices used in this study did demonstrate varying sensitivity. For example, ASPT often indicated a much higher water quality than the Shannon diversity index. No singular index is considered a suitable ”all round index” and a mixture of biological indices and tools would probably be a more effective monitoring technique.

Based on the macroinvertebrate data sampled in this study, macroinvertebrate communities showed no acute negative responses to road salt However some species displayed a higher sensitvity to chloride than others. Community studies alone may be insensitive to any negative effects road salt may have on macroinvertebrates. Many processes often occur at the individual level before a whole community reacts to any negative impacts. Further investigation using possible biomarkers at the individual level may provide earlier warning signs to possible negative effects of road salt.

CONTENTS

1. INTRODUCTION

The use of road deicing salt (NaCl) is at present the most popular aid in removing snow and ice from roads in Norway, and the total use of road deicing salt in Norway has increased significantly over the past few years (see Figure 1). The detrimental environmental effects of road salt are becoming of increased public concern and can vary from increased salt concentrations in groundwater (Kelly and Wilson, 2002), damage to roadside vegetation (Kayama et al., 2003, Viskari and Karenlampi, 2000) and negative effects on the water quality in rivers and streams. Studies have demonstrated that the high percentage of deicing salts that are removed by surface runoff can cause a significant increase in chloride concentrations in the receiving streams and rivers. Higher chloride concentrations in lakes (Thunqvist, 2003, Godwin et al., 2003, Ostendorf et al., 2001), is especially the case during spring season floods (Ruth, 2003a). Bækken and Haugen (2006) showed that one third of lakes studied had developed an obvious saltgradient as a result of road salt application. The study also showed that 95% of the lakes with a saltgradient also had an obvious oxygen gradient. A saltgradient can prevent full circulation in spring/autumn months and cause a decrease in oxygen towards the bottom of a lake (Torleif and Thrond, 2006).

Results from other Scandinavian studies have shown that road salt can have a significant effect on the water quality. Studies in both Sweden and Finland have demonstrated that the use of road salt can increase the mobilisation of heavy metals in roadside soils thereby negatively affecting stream water quality (Lofgren, 2001, Ruth, 2003b, Backstrom et al., 2004).

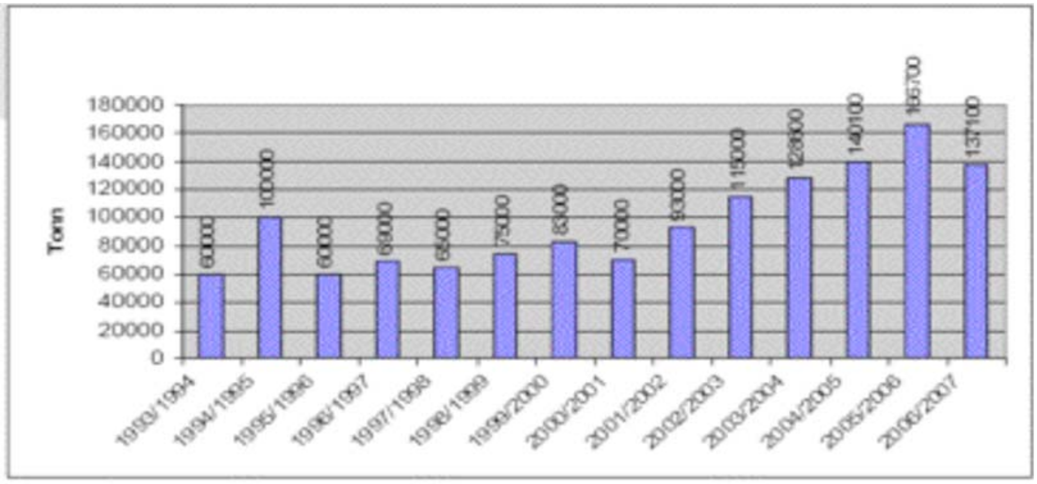


Figure 1 - Total salt (including sand) used in Norway from winter 1993 – 2007

Measuring water quality using macroinvertebrate species as biological indicators is a well-practiced technique and a number of different indices have been developed. The Trent biotic index (TBI) is based on two main effects of organic pollution (1) a reduction in community diversity, and (2) the loss of key indicator organisms from the clean water fauna (Gray, 2005). The index measures the amount of organic pollution in a stream depending on the presence or absence of six groups of indicator organisms and the number of other taxa present. Biological Monitoring Working Party (BMWP) and Average Score Per Taxon (ASPT) are two alternative indices often used to assess water quality. These indices are both based on the principle that different species have different pollution tolerances. These three pollution indices were originally developed for the assessment of streams in Britain. However, modifications have been made to the TBI and BMWP in order to make them suitable for the assessment of Norwegian streams. (Borgstrøm & Saltveit 1978; Aanes & Bækken 1989)

Observing macroinvertebrate community structure, indicator organisms e.g. %EPT species and the taxonomic composition of communities are all popular methods used when monitoring the water quality of rivers and streams (Charvet et al., 2000, Blausius and Merritt, 2002, Cao et al., 1997). The community structure of macroinvertebrates can alter with any change in the physical and/or chemical properties of their environment, therefore indicating changes in water quality. There are several advantages to using macroinvertebrates when monitoring water quality (Hellawell, 1986, Rosenberg and Resh, 1993); (1) they are omnipresent, (2) there are a large number of taxa therefore increasing the probability that some will react to a change in their environment, (3) they are relatively sedentary and have life spans that are long enough to provide an indication of changes in the environment over a period of time, (4) sampling is simple, cost efficient and (5) methods are well-established.

Several studies outside of Norway have focused on the effect road salt may have on macroinvertebrates (Benbow and Merritt, 2004, Crowther and Hynes, 1977). Blausius and Merrit (2002) conducted both field and laboratory experiments to test the effects of road salts on stream macroinvertebrates. They found that stream macroinvertebrate communities were not significantly affected to short term exposure to road salt (Blausius and Merritt, 2002). Benbow and Merrit (2004) studied the toxicity of road salt under different testing conditions. For most study species the LC50 chloride estimates were significantly higher than the 43 studied wetlands. However, due to differences in fauna, and the influence of factors such as altitude, geology and climate (Aanes and Bækken, 1989a) the same results may not be found in Norway.

As of yet there have been no previous studies in Norway on effects road salt may have on the community structure of macroinvertebrates in rivers and/or streams situated alongside roads. The present study will focus on any effects road salt may have on the composition of macroinvertebrate communities. It is hypothesised changes in salt concentrations will alter the composition of the macroinvertebrate fauna downstream of the reference sites. It is assumed that water quality will decrease as salt concentrations increase therefore resulting in a decrease in macroinvertebrate diversity and an absence of pollution sensitive species (EPT species).

2. Materials and Methods

2.1. Study streams

Three different streams situated in the south eastern part of Norway were investigated. All are in close vicinity to roads where salt is applied as part of winter maintenance.

Bolvikelva is situated in the northern part of the Herre catchment and starts at Kilevann in Skien, Telemark. The catchment consists of Precambrian metamorphic rocks, mainly augen gneiss, granite and foliated granite (http://www.ngu.no/kart/bg250/). The stream is about 2,5km long and flows predominantly through luxuriant and little disturbed natural areas from Siljandammen to River Herre. However, a main road, RV356, runs adjacent to part of the stream and road salting has recently (winter 2006/2007) been started as part of winter maintenance. All sampling sites were in relatively close vicinity to the RV356. In addition Statens Vegvesen have placed several measurement stations in this stream to continuously measure pH, conductivity, temperature and water level as part of their SaltSMART project (Kitterød, 2006).

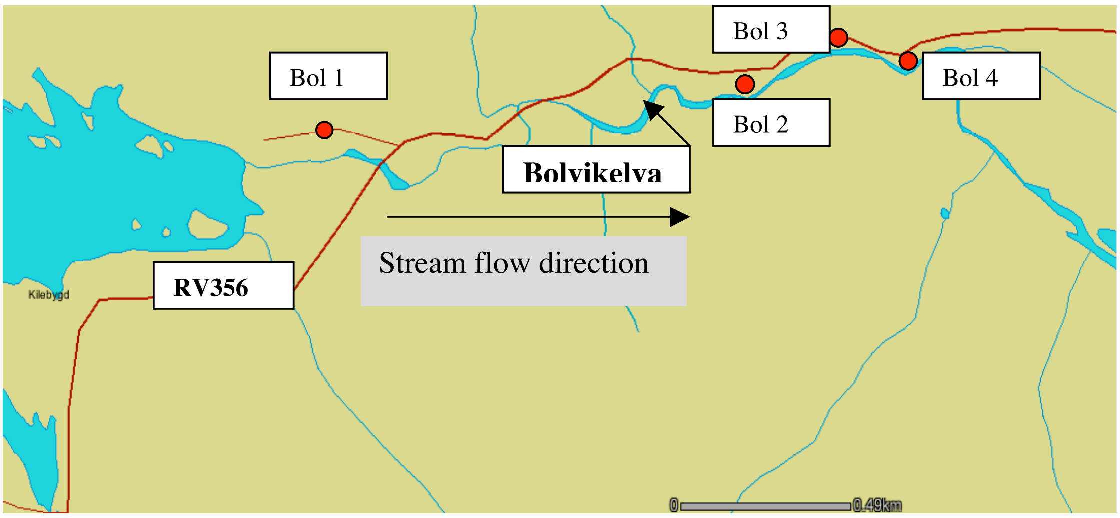


Figure 2 - Map of Bolvikelva and sampling sites (http://arcus.nve.no/website/nve/viewer.htm). The main road, RV356 is shown in red.

Ljanselva is situated in southeast Oslo and has a catchment area of 39m^2 that reaches the borders to Ski (Bremnes et al., 2006). The catchment is made up of igneous (quartz diorite, tonalite and trondhjemite), metamorphic (augen gneiss, granite and foliated granite) and some unspecified volcanic rocks (http://www.ngu.no/kart/bg250/). The main sources of Ljanselva are the lakes, Lutvann in the north and Stensrudtjern in the south. Woodlands dominate the Ljanselva catchment, although in the lower reaches it flows through a major urban area. The average water flow is approximately 5m^2/s and the river is known to carry a substantial amount of phosphorous bonded to particles carried from the surrounding roads and agriculture[1]. Ljanselva was chosen for this study due to its close proximity to a main road (E6) which is subject to heavy traffic and is salted heavily during the winter months.

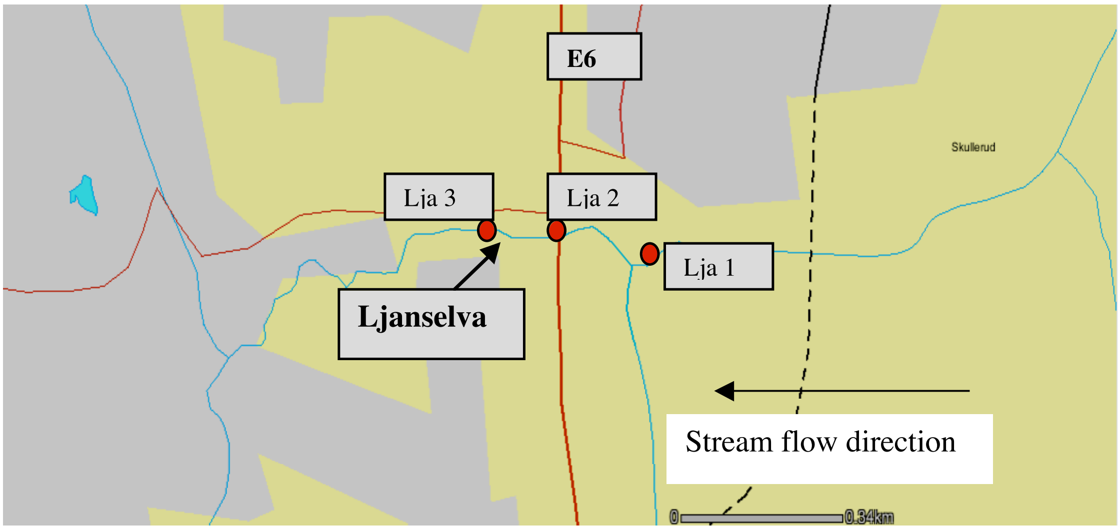


Figure 3 - Map of Ljanselva and sampling sites (http://arcus.nve.no/website/nve/viewer.htm). The main road, E6, is shown in red.

Gjersrudbekken is the most significant tributary of Ljanselva. It rises from Gjersrudtjern and flows northwest alongside Enebakkveien until it meets Ljanselva at Hauketo. The catchment is made up of rocks similar to Ljanselva, including both igneous (quartz diorite, tonalite and trondhjemite), metamorphic rocks (augen gneiss, granite and foliated granite) and some unspecified volcanic rocks (http://www.ngu.no/kart/bg250/). Pollution sources in the area include the motorway E18/E6, local roads, a water deposal site

[1] Fakta om Ljanselva (25.07.2005). http://www.vann-og-avlopsetaten.oslo.kommune.no/vassdrag_og_fjord/vassdragene_i_oslo/ljanselva/article49485-16066.html (accessed 23.04.2008)

(Grønmodeponiet), an incineration plant (Klemetsrud forbrenningsanlegg), a stone-crushing plant (Åsland pukkverk), a snow disposal site (Åsland snow deponi) and some light industry. A reference point was not included from this stream due to overgrown vegetation making it impossible to reach a suitable site.

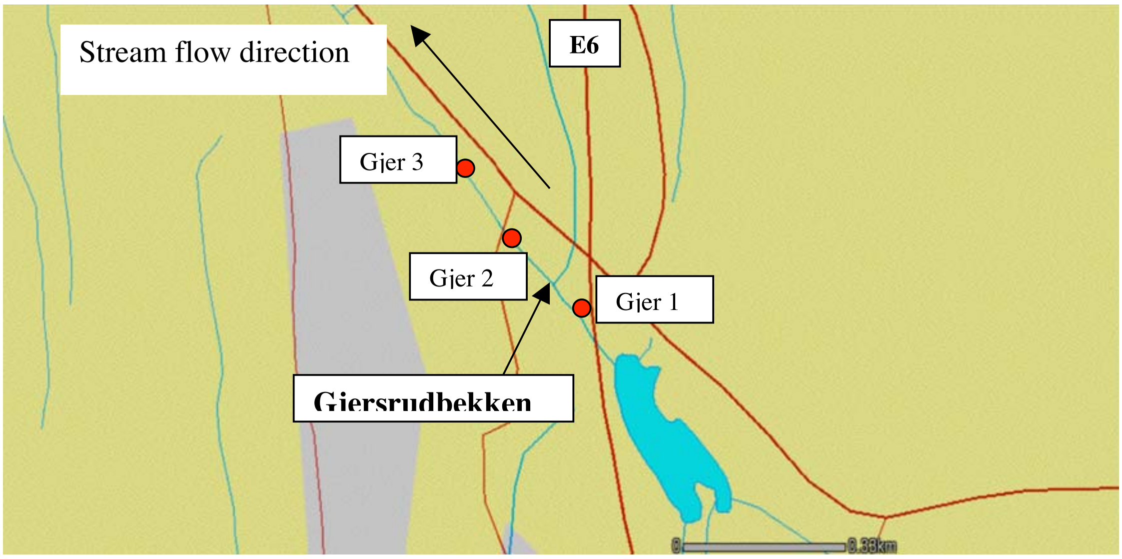


Figure 4 - Map of Gjersrudbekken and sampling sites (http://arcus.nve.no/website/nve/viewer.htm).

2.2. Sampling

Both chemical and biological samples were collected at three/four sites on each stream; where possible one reference site upstream and two/three sites downstream were selected. Characteristics such as stream width (m), stream depth (m) and substrate type (*phi*) (Wentworth, 1922, Allan, 1995) are based on field observations.

Table 2.1. Physical characteristics of the study sites. References sites are marked with an asterisk. Phi values are presented in bold characters.

Site abbreviation	Location	Altitude (m.a.s.l)	Stream width (m)	Stream depth (m)	Substrate (mm) ***Phi* value**
LJA 1*	E 26683.81 N 6643350.06	104	0.5	2	2- 8 mm *Fine-very fine gravel* **-1**
LJA 2	E 266566.03 N 6643376.82	97	0.5	1.5	64-256mm *small cobble* **-6**
LJA 3	E 266495.79 N 6643373.47	96	1	2	3.9–62.5 μm *medium silt* **-4**
GJE 1*	E 366898.83 N 6640329.76	124	2	2	64-256mm *small cobble* **-6**
GJE 2	E 266663.03 N 6640562.22	152	1.5	3	64-256mm *small cobble* **-6**
GJE 3	E 266586.1 N 6640637.47	131	1.5	2	64-256mm *small cobble* **-6**
BOL 1*	E 182275.52 N 6566701.47	69	0.5	4.5	64-256mm *large cobble* **-6**
BOL 2	E 183387.49 N 6566854.42	54	0.5	10	64-256mm *small cobble* **-6**
BOL 3	E 183699.59 N 6566963.96	64	1	4.5	32–64mm *very course gravel* **-6**
BOL 4	E 183854.6 N 6566950.53	55	1.5	3	1–2 mm *very course sand* **0**

Macroinvertebrates were collected in two separate periods, in the winter (during salting) and in late summer/early autumn (before salting). The standard kick sample method

(Hynes, 1961a) was used to collect the samples. Three, 30 second kick samples were taken at each site. During the sampling at Bolvikelva site 3 (Bol 3), three, one-minute kick samples were taken due to the large substrate size. The hand net frame measured 30cm x 30cm with a mesh size 250μm. The net is placed on the riverbed and the area just upstream of the net is disturbed (with the foot using a kicking motion), the invertebrates are then carried into the net by the current of the stream (Hynes, 1961b). Samples were taken mainly from riffles since they generally contain the greatest diversity of macroinvertebrates due to plentiful oxygen and food levels. However riffle dwelling organisms generally require high oxygen levels and low levels of pollution (Osmond, 1995). Taking this in to consideration and in an attempt to identify as many different habitats and collect as many individuals as possible the net was moved around the site. The samples were preserved in 70% ethanol and later identified in the laboratory.

In the laboratory, samples were subsampled using a method based on the subsampling method described in the United States Environmental Protection Agency Wadeable Streams Assessment Benthic Laboratory Methods Manual (Barbour, 1999). The method was based on the random selection of 500 organisms used to represent the whole assemblage. However, several of the samples had less than 500 individuals present in which case all of the individuals present were counted and identified. The individuals belonging to the Plecoptera, Ephemeroptera and Trichoptera families were later identified to species. Other individuals were identified to class or family. Subsampling is often used to reduce the specimen counts to a reasonable number which in turn reduces the cost and time of processing macroinvertebrate samples (Resh and Rosenberg, 1993, Vinson and Hawkins, 1996). The validity of subsampling methods to assess the biological condition of a site has often been discussed and it is generally agreed that the chosen method of subsampling may determine the quality of the results (Barbour and Gerritsen, 1996, Vinson and Hawkins, 1996, Courtemanch, 1996).

The following taxonomic handbooks were used;

Ephemeroptera: Elliot *et al*. 1988; Brittain *et al*. 1996; Engblom, 1996, Merritt & Cummins, 1984
Plecoptera: Solem, 1996; Merritt & Cummins, 1984.
Trichoptera: Wallace *et al*. 1990; Edington & Hildrew, 1995; Solem & Andersen, 1996.

2.3. Environmental variables

Water samples were taken at all sites in both seasons and were analyzed for pH, TOC, conductivity, salinity, chloride, nitrate, sulphate and fluoride. In-situ water samples were analysed using a W-21 SDI-12 Water Quality Multi-probe measured pH, conductivity (mS m^{-1}) temperature, dissolved oxygen (mg/L), total organic carbon (TOC mg L^{-1}) and salinity. Multi-probe in-situ samples were not taken at Bolvikelva in January 2008, and as a result of this both temperature and dissolved oxygen were disregarded in the multivariate statistical analysis (since the CANOCO program is unable to handle missing variables for further details see pg47 In Leps, 2009). Additional water samples were also taken and analyzed in the accredited laboratory at UMB and tested for chloride, sulphate, fluoride and nitrate (NO_3-N μg L^{-1}).

Table 2.2 Chemical data for the study sites. Reference sites are marked with an asterisk. Temperature and dissolved oxygen data was removed due to them being absent from Bolvikelva January 2008 data.

Site (abbr)	**Date**	**pH**	**TOC mg L^{-1}**	**Chloride mg L^{-1}**	**Sulphate mg L^{-1}**	**Nitrate mg L^{-1}**	**Fluoride mg L^{-1}**
Lja 1*	March 08	6.99	5.12	3.29	4.97	014	0.062
Lja 2	March 08	7.27	6.25	7.03	6.13	<0.03	0.068
Lja 3	March 08	7.33	4.81	7.3	6.45	0.22	0.07
Lja 1*	October 08	7.74	5.84	3.11	5.35	0.047	0.063
Lja 2	October 08	7.59	5.96	4.76	6.86	0.103	0.072
Lja 3	October 08	7.67	5.99	4.66	6.97	0.115	0.073
Gje 1	March 08	7.31	6.08	39.0	18.4	0.74	0.102
Gje 2	March 08	7.26	136	38.6	18.0	<0.03	0.096
Gje 3	March 08	7.27	364	39.1	18.7	<0.03	0.098
Gje 1	October 08	7.82	11.17	23.06	22.26	0.355	0.119
Gje 2	October 08	7.75	10.96	23.83	25.28	0.393	0.123
Gje 3	October 08	7.95	10.96	23.26	25.62	0.387	0.124
Bol 1*	January 08	6.92	5.74	4.11	2.96	0.260	0.093
Bol 2	January 08	7.01	5.55	3.43	2.98	0.260	0.089
Bol 3	January 08	6.96	5.51	3.07	2.88	0.240	0.090
Bol 4	January 08	6.98	5.63	3.14	3.00	0.250	0.091
Bol 1*	October 08	7.23	5.58	2.44	2.41	0.073	0.094
Bol 2	October 08	7.46	5.57	2.96	2.45	0.080	0.097
Bol 3	October 08	8.19	5.54	3.02	2.49	0.085	0.098
Bol 4	October 08	7.54	5.57	3.12	2.56	0.085	0.097

2.4. Biological Indices

Various macroinvertebrate species often have different tolerance levels to pollution and other disturbances. As a result, these tolerance differences in community structure are often used as an indicator of water quality (Armitage et al., 1983, Resh and Rosenberg, 1993). The development of biological indices has enabled us to assess the merit of water quality in a simple but reliable manner. Biotic indices are based on the general theory that downstream from a pollution source there is a change in biota (Friedrich, 1996). Over the years numerous indices have been developed and today there are over 50 are present (Depauw and Vanhooren, 1983). However, Lydy et al. (2000) point out that not all indices are as effective or as accurate as others (Lydy et al., 2000, Hughes, 1978, Boyle et al., 1990) and they may be insensitive to different levels of pollution. The accidental presence of “rare species” may also cause some inaccuracy of results if not handled carefully (Vinson and Hawkins, 1996). Lydy et al (2000) concluded that the most effective method in detecting changes in water quality was to use a mixture of indices and other analytical tools. Taking this into consideration several indices were used in the present study; the Trent Biotic Index (Woodiwiss, 1964, Borgstrøm, 1978),, Chandler Score (Armitage et al., 1983, Chandler, 1970)., BMWP score (BMWP, Biological Monitoring Working Party. Final report: assessment and presentation of the biological quality of rivers in Great Britain., 1978, Aanes and Bækken, 1989b), ASPT (Armitage et al., 1983), and finally the % EPT species index.

TBI scores are based on the presence or absence of six groups of indicator organisms each with different oxygen requirements which indicates pollution tolerance. Each site is assigned a score between 0 and 10. Better water quality is indicated by higher scores. The Trent Biotic Index (TBI) used in this study was an adaptation for Norwegian conditions (Woodiwiss, 1964, Borgstrøm, 1978). This modification was made due to some slight differences in species present in Norwegian streams. For example, *Gammarus* does not occur in Norwegian streams, leading to a gap in the index (Borgstrøm, 1978). Two main criticisms of the TBI are its insensitivity to mild pollution, and the fact that it is only applicable in riffle areas of the stream. However, a number of the main elements of the TBI have been adapted and used to develop other indices, such as the Chandler score index. The Chandler score index (Chandler, 1970) shows obvious differences in diversity and abundance between clean and polluted parts of a river and has a continuous gradation from polluted to clean conditions. The Chandler score index used in this study was adapted to the study due to the absence of key organisms or in the case where not all organisms were identified down to species as required in the original index.

Regardless of the popularity of biotic indices when assessing water quality the fact that tolerance levels are location and pollution specific must also be taken in to consideration., For example, the BMWP index was developed in Great Britain and cannot be used in all rivers worldwide (Lydy et al., 2000). Biotic indices must therefore be chosen and handled with caution.

Further drawbacks of the above indices are that they require identification of organisms to species level demanding more time and effort. Therefore alternative indices have been developed where only identification to the family level is required to use them. The Biological Monitoring Working Party (BMWP) is such an index. Each family is allocated a score between 1 and 10 reflecting their pollution tolerance levels. Those families that are least pollution tolerant have higher scores. The final BMWP score is calculated by adding together the individual scores of all indicator organisms present (family level).

The Average Score Per Taxon (ASPT) is an alternative index which is calculated by dividing the BMWP score with the number of indicator families present in the sample (Friedrich, 1996). As with the TBI and Chandler Score index the overall conclusion of the BMWP and ASPT is the same, the higher the score the better the water quality. ASPT is at present recommended for the European Water Framework Directive (European Water Framework Directive. Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy., 2000).

Other indices often used to assess water quality are diversity indices. These focus on the species diversity of a community by combining the species richness and community balance (evenness). There are a number of different diversity indices nevertheless the Shannon-Wiener diversity index (Weber, 1973) was chosen for this study since it is most frequently used when dealing with aquatic systems (Washington, 1984). It is generally assumed that high species diversity values indicate a well-balanced and undisturbed community, while low values indicate stress or impact (Metcalfe, 1989).

The major drawback of diversity indices is their apparent lack of accuracy. Lydy et al. (2000) demonstrates that diversity indices can be misleading. When comparing diversity, similarity and biotic indices for changes in macroinvertebrate community structure and stream quality they found that diversity indices could indicate opposite results to that which had occurred. They found that diversity indices should not be used alone to assess water quality, as often changes did not occur in diversity but instead in community structure (Lydy et al., 2000).

The final index used in this study was the percentage of EPT (*Ephemeroptera*, *Plecoptera* and *Trichoptera*) species. Mayflies (*Ephemeroptera*), stoneflies (*Plecoptera*) and caddisflies (*Trichoptera*) are often considered intolerant of pollution (Lydy et al., 2000, Patty, 2005) therefore their presence indicates good water quality. A shift in benthic invertebrate community would be indicated by a change in the percentage of EPT present. A study in USA showed that EPT richness was found to be the most useful when compared with family richness or total richness (Lenat and Resh, 2001).

When considering the use of indices to assess water quality one must always take into consideration their drawbacks. No index can best reflect the changes in water quality and community structure alone, therefore the most effective and accurate investigations of water quality are those with a mixture of indices and biological tools (Lydy et al., 2000).

2.5. Community Analyses

In this thesis species composition was analysed using the canonical ordination techniques; Detrended Correspondence Analysis (DCA), Principal Component Analysis (PCA) and Reduncy Analysis (RDA) (ter Braak, 1998). Ordination is used mainly in exploratory data analysis rather than in hypothesis testing. It summarizes and interprets species data using environmental variables. The reaction a species may have towards certain environmental variables is often difficult to determine and measure, the use of species composition can therefore provide more information on the environmental situation than many environmental variables. The overall aim of canonical ordination is to detect the pattern that displays any relations between the species and the observed environment (Jongman et al., 1987).

Detrended Correspondance Analysis (DCA)

Detrended Correspondance Analysis (Hill, 1980) is based on Correspondance Analysis (CA) and was designed to aid in the interpretation of ecological data. It was developed to remove the arch effect produced when using a CA. This removal is achieved in two main steps; (1) detrending and (2) rescaling. DCA is a form of indirect gradient analysis where the sites and environmental variables are plotted along axes on the basis of data on species composition (Jongman et al., 1987). Sites situated closely together are said to be similar in species composition, and also sites located close to a species is assumed to have a high abundance of that particular species.

Principle Components Analysis (PCA)

PCA is an unconstrained linear ordination method (also known as a indirect gradient analysis) and are used to identify patterns within data. The unconstrained methods attempt to find a variable that best explains the variation in species data this variable is then taken as the ordination axes. The PCA shows the distribution of species along ”ideal” ordination axes and patterns are displayed in a way where similarities and differences between species are highlighted.

Redundancy Analysis (RDA)

RDA is the canonical form of PCA and although it is often neglected by ecologists it can be useful when used in combination with PCA. Redundancy expresses how much of the variance in one set of variables can be explained by the other. In an RDA ordination diagram the correlation between the species and environmental variables are displayed.

Options used in the Canoco statistical program.

In this thesis the DCA was used to determine whether a unimodal or linear ordination method should be used. This analysis identifies whether there are any structures among the macroinvertebrates (Meland, 2001). This is determined by the longest length of gradient. If the longest length of gradient exceeds 4.0, unimodal methods are most suitable. If it is less than 3.0 then linear methods of ordination are ideal (Leps, 2009) Linear methods of ordination were determined to be the most suitable in this case and a PCA was used to identify any patterns within the macroinvertebrate data. These results were then used together with the measured environmental variables in an RDA to identify if any environmental variables could describe the macroinvertebrate structure. Manual forward selections by Monte-Carlo permutation tests were used to identify and select which variables were most influential in the analysis. In order to examine how well the environmental variables explained the distribution of species the % species-enviroment correlation taken from the RDA was compared with the % species-environment correlation from the PCA. The samples from both seasons were analysed together to identify any variation between seasons (salting Vs pre-salting). An RDA was also run to investigate how much of the macroinvertebrate data was determined by chloride (using altitude as a covariable).

3. RESULTS

During the two subsampling periods January/March (salting season) and October 2008 (pre-salting season) approximately 10 000 specimens were identified to either family or species. The total number of EPT species accounted for 20% of all the material, where in this case the Ephemeroptera family was the most dominant, accounting for approximately 19.6% of the total subsampled material. The families Plecoptera and Trichoptera accounted for 8.2% and 4.1% of the subsampled material, respectively. 15 Ephemeroptera species, 12 Plecoptera species, 24 Trichoptera species were recorded. The most abundant taxon found was the family Chironomidae accounting for approximately 31% of the subsampled material.

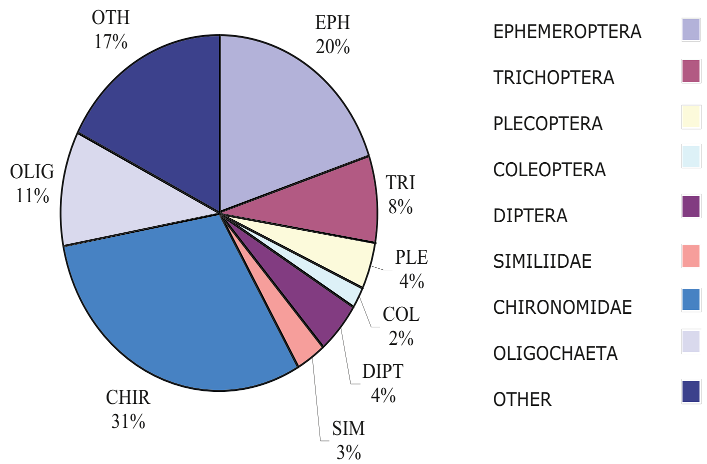


Figure 3.1. The total subsampled macroinvertebrate data. Percentage individuals of each taxon.

3.1. Bolvikelva

In comparison with the reference site the occurrence of the taxon Chironomidae decreased downstream, possibly indicating an improvement in water quality. At the downstream site 3 in both seasons this decrease was more dramatic than at other downstream sites, their occurrence also began to increase downstream from site 3. This decrease in dominance downstream resulted in an increased dominance of the ephemeropteran community.

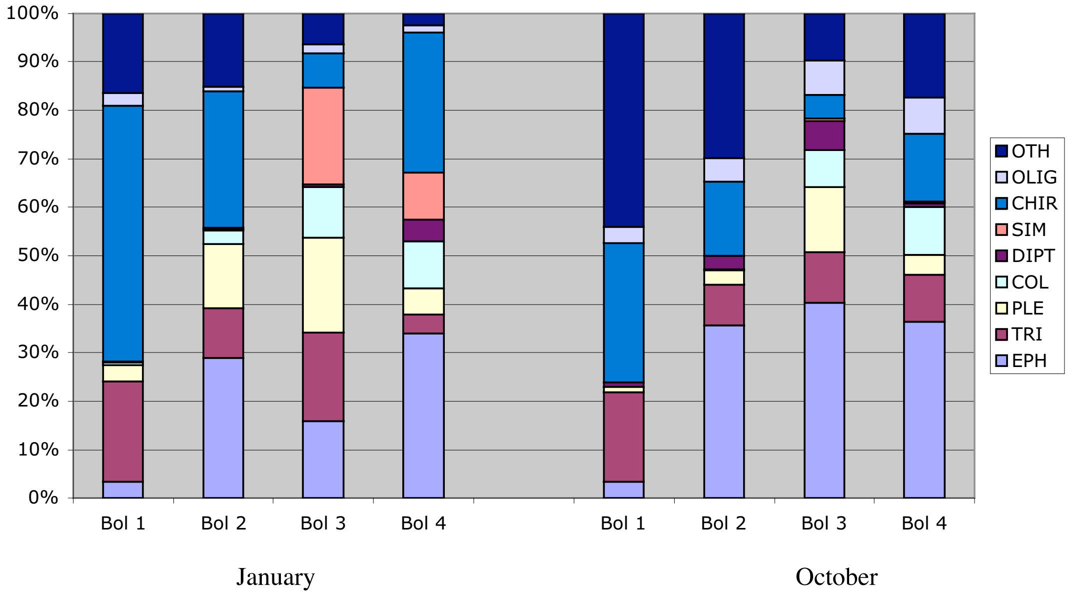


Figure 3.1.1. Percentage composition between the different taxa collected from Bolvikelva. Reference sites are indicated by asteriks.

A change within the EPT community was also clearly noticeable in both seasons from the reference site to the downstream sites. A switch in dominance from the trichopteran community to the ephemeropteran community downstream was visible. One exception to this pattern downstream was at site 3 in january where the plecopteran community are most dominant.

During the salting season (January), 19 trichopteran, 8 plecopteran, and 9 ephemeropteran species were recorded. The most dominant species overall was the plecopteran *Amphinemura borealis*. This dominance however was not completely evident until the third site (Bol 3) downstream from the reference point. Upstream at the reference site (Bol 1) Trichoptera were the dominant, with the major species being *Neureclipsis bimaculata*. This species almost disappeared completely downstream, with only two individuals found at the final downstream site (Bol 4). Immediately downstream (Bol 2) from the reference site the Ephemeroptera species *Baetis rhodani* was most abundant, the only site where *B. rhodani* was recorded. A final shift in species dominance was evident at the final downstream site (Bol 4) where the ephemeropteran species *Centroptilum luteolum* became the dominant species. However as with *Baetis rhodani* this was the only site where this species was present.

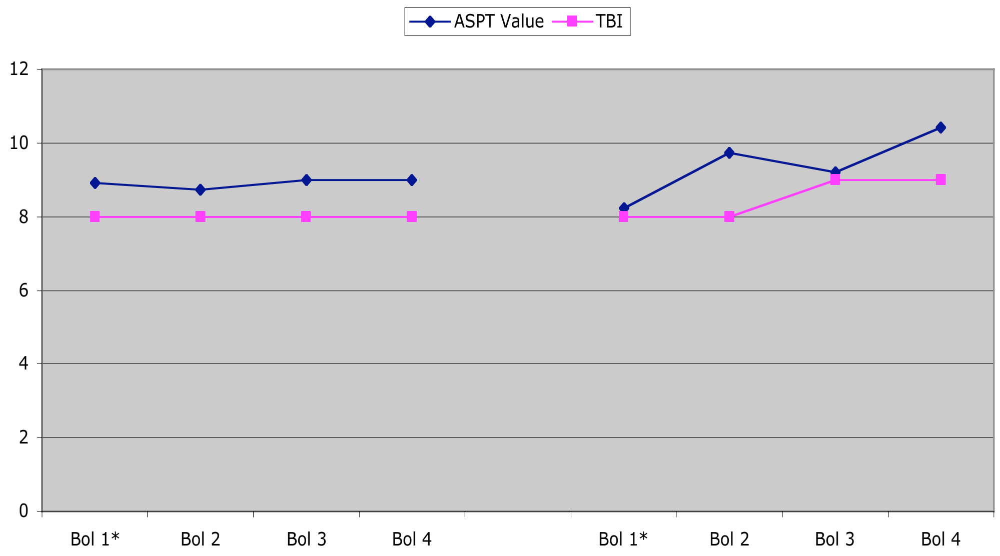
In the pre-salting season (October), 15 trichopteran species, 7 plecopteran species and 11 ephemeropteran species were present. The trichopteran species *Brachycentrus subnubilus* was the most dominant species overall, however, this dominance altered from the reference site (Bol 4) to the downstream sites to species from the Ephemeroptera family. At the downstream sites (Bol 5,6 and 7) *Baetis fuscatus, Baetis rhodani* and *Caenis horaria* became the most dominant species, respectively. These species are almost only recorded at the sites where they are most dominant and much fewer specimens are found at all other sites. Unlike the ephemeropteran species the dominant trichopteran *B. subnubilus* was present in relatively similar numbers throughout the stream system.

Biotic Indices

In January there was very little difference between the ASPT values and TBI scores upstream and those downstream. The Shannon diversity index increased very slightly downstream from the reference site indicating a slight improvement in water quality downstream. However, this was not the case at the final downstream site (Bol 4) where the index decreases marginally. There were some dramatic differences in the water quality indicated by the Shannon diversity index, TBI score and ASPT value. Both the TBI Score and the ASPT value indicated a very good water quality, whereas the Shannon Diversity Index indicated poor water quality.

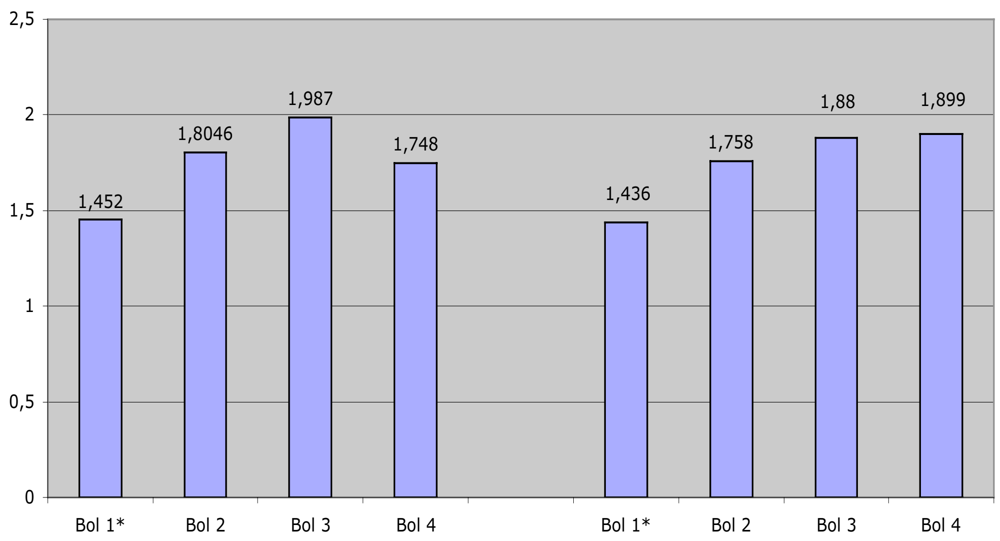
There was a slight decrease downstream in the obtained Chandler score except at the downstream site Bol 3, where there is a significance increase in the score. This indicates that the water quality was better than the other sites at Bol 3 in January. Overall the

a)



January October

b)

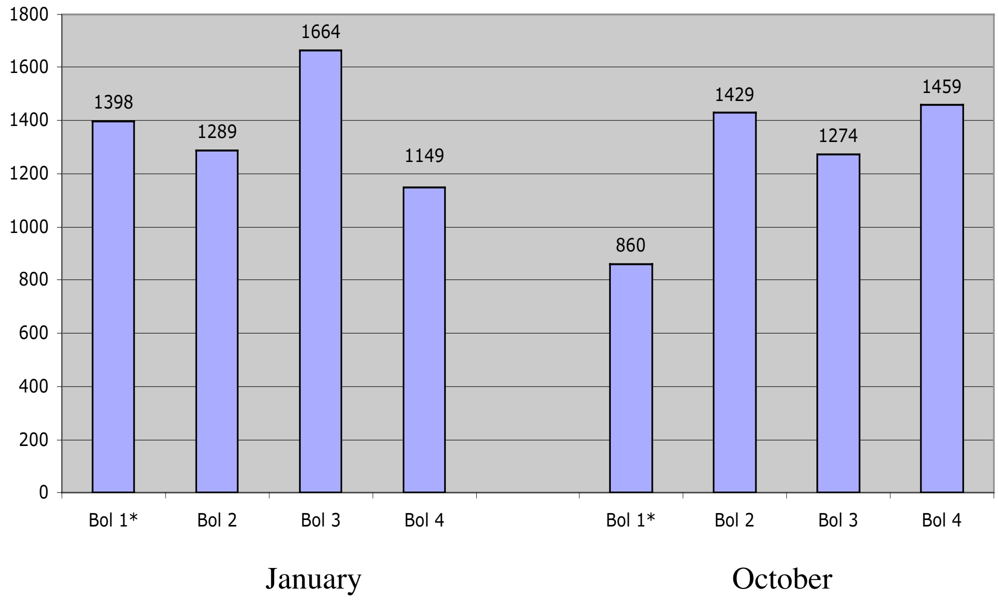


January October

Figure 3.1.2. a) TBI score and ASPT values; b) Shannon diversity index. All indices are calculated from the sites sampled at Bolvikelva in January and October 2008. Reference sites are indicated by asteriks.

% EPT species recorded in january increased downstream with a minor decrease at the final site (Bol 4). This increase in abundance of EPT species indicates improving water quality downstream from the reference point.

a)



b)

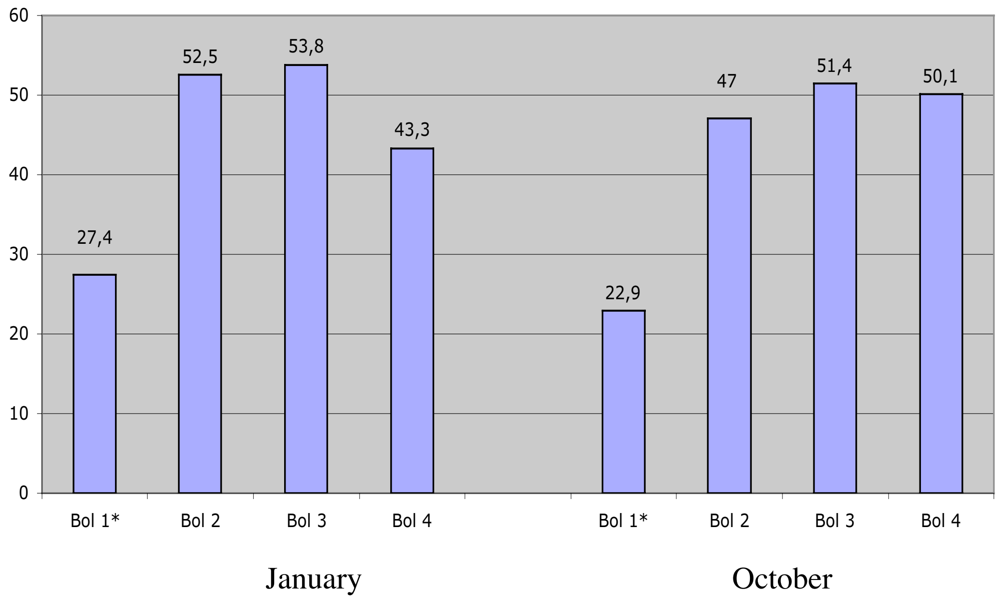


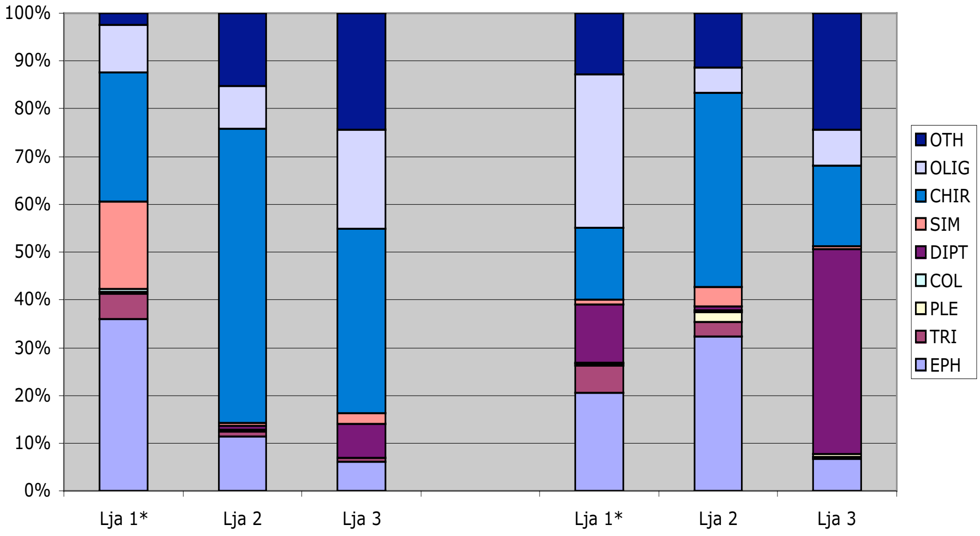
Figure 3.1.3. a) Chandler score; b) % EPT species. All indices are calculated from the sites sampled at Bolvikelva in January and October 2008. Reference sites are indicated by asteriks.

The TBI scores and ASPT values from the october sampling both indicate a more significant improvement in water quality than those obtained in january. A significant improvement in water quality downstream from the reference site is indicated by the increase in the TBI score and the ASPT value. This improvement in water quality is also demonstrated by increases downstream in the Shannon diversity index, the Chandler Score and the % EPT species. However, once again the TBI score and ASPT value paint a different picture to that of the Shannon diversity index. As demonstrated in the january results they both indicate a much higher water quality than the one indicated by the Shannon diversity index.

3.2. Ljanselva

There were three dominant taxon recorded in the Ljanselva samples. During the salting season (March) Chironomidae was the dominant taxon. This dominance altered in the pre-salting season when Oligochaeta and Diptera were present in higher numbers, especially downstream from the reference site (Lja 4). The numbers of EPT species decreased dramatically downstream indicating a negative change in water quality. Despite this occurrence being more pronounced in the samples from March it was also evident to a certain degree in the October samples.

Between seasons there is very little difference in the abundance and presence of certain EPT species. Ephemeroptera species *Baetis niger* (219 specimens) was overall the most dominant, extremely closely followed by *Baetis rhodani* (211 specimens). All the Ephemeroptera species decreased downstream. Once again *Baetis niger* was the dominant species at the first two sites (Lja 4 and Lja 5). There was a significant decrease in numbers of individuals recorded at downstream site Lja 6.



March October

Figure 3.2.1. Percentage composition between the different taxa collected from Ljanselva. Reference sites are indicated by asteriks.

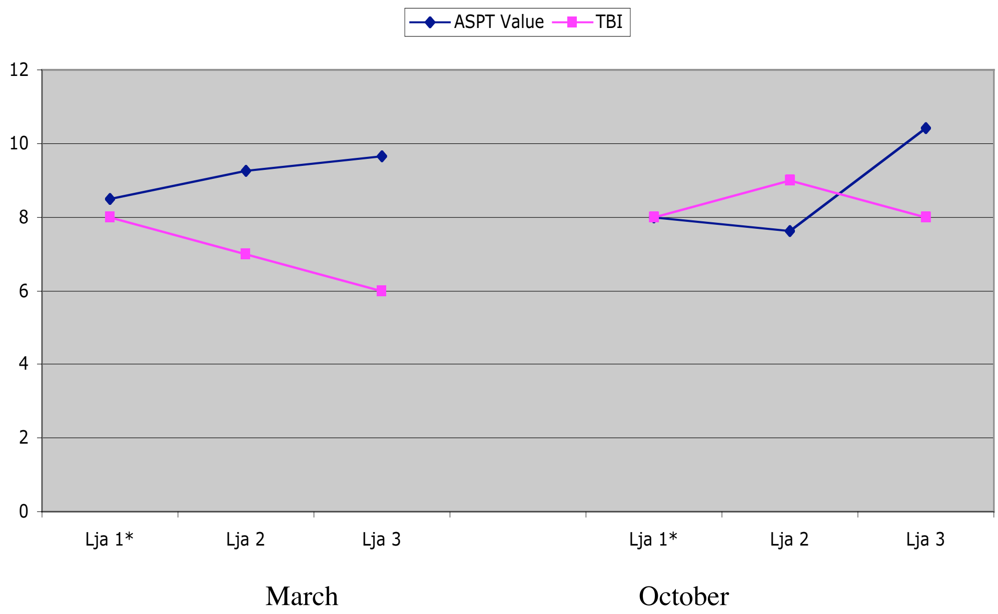
Biotic Indices

The ASPT value and TBI indices generally disagreed with each other in both seasons. The ASPT slightly increased downstream in March, whereas the TBI score decreased quite dramatically. This pattern is reversed in October when both indices fluctuate. The TBI score obtained moderatley increases immediately downstream (Lja 5) and then decreases at the final site (Lja 6). As for the ASPT values obtained, they also flucuate slightly however they demonstrate a dramatic improvement in water quality downstream.

The Shannon diversity index fluctuates in both seasons, with the lowest diversity being found at the second site (Lja 2/Lja 5). This decrease in diversity is nevertheless more pronounced in the salting season. Once again the Shannon diversity index and ASPT value indicate different situations, the Shannon diversity index implies a lower water quality than that of which is indicated by the ASPT value. The Chandler score obtained in March also disagreed with the Shannon diversity index and implied a dramatic decrease in diversity rather than the fluctuation in diversity shown by the Shannon

Shannon diversity index. The % EPT species index in March showed a greater decrease downstream than that found in October. There was an increase in % EPT species recorded immediately downstream followed by a dramatic decrease at the final site.

a)



b)

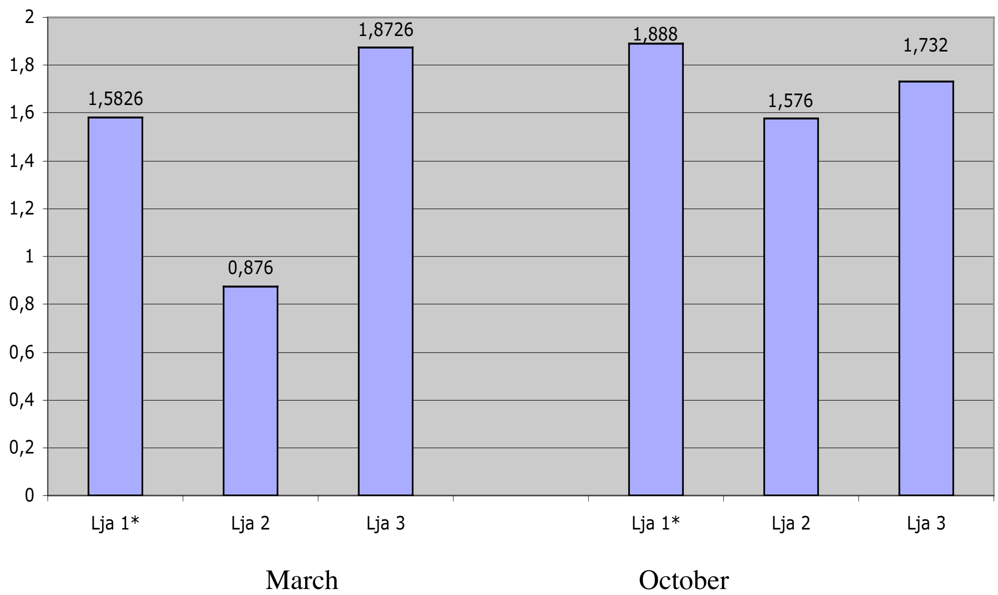
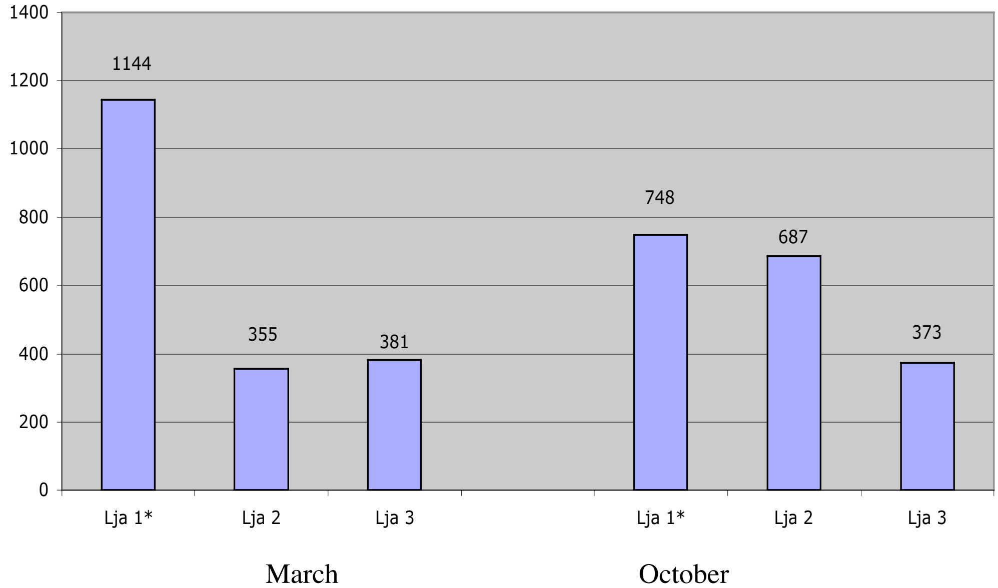


Figure 3.2.2. a) TBI score and ASPT values; b) Shannon diversity index. All indices are calculated from the sites sampled at Ljanselva in March and October 2008. Reference sites are indicated by asteriks.

a)



b)

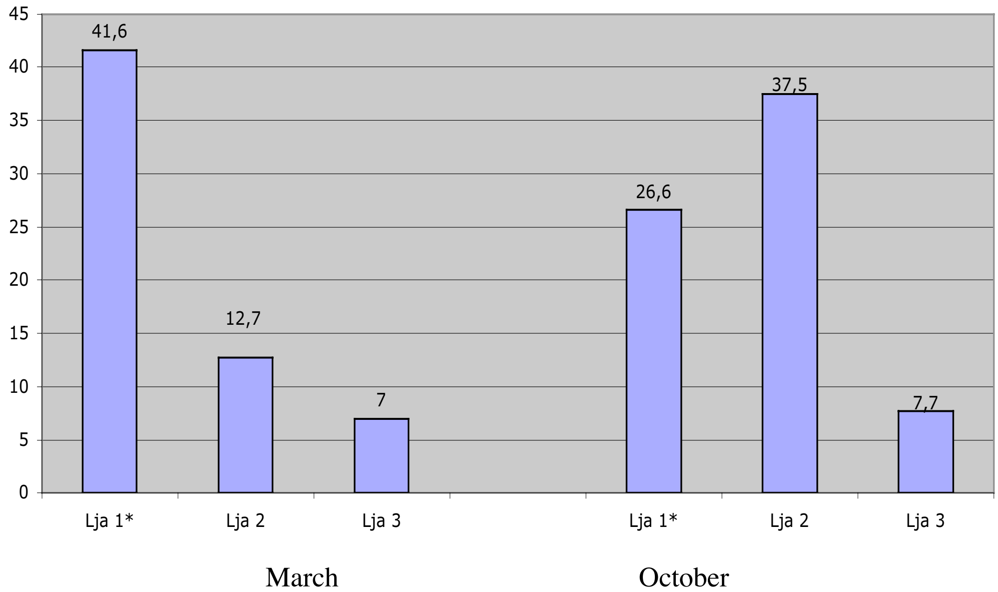


Figure 3.2.3. a) Chandler score; b) % EPT species. All indices are calculated from the sites sampled at Ljanselva in March and October 2008. Reference sites are indicated by asteriks.

3.3. Gjersrudbekken

There was an obvious overall dominance by the taxon Chironomidae. An increase in the abundance of Ephemeroptera individuals did not effect this dominance. The increase

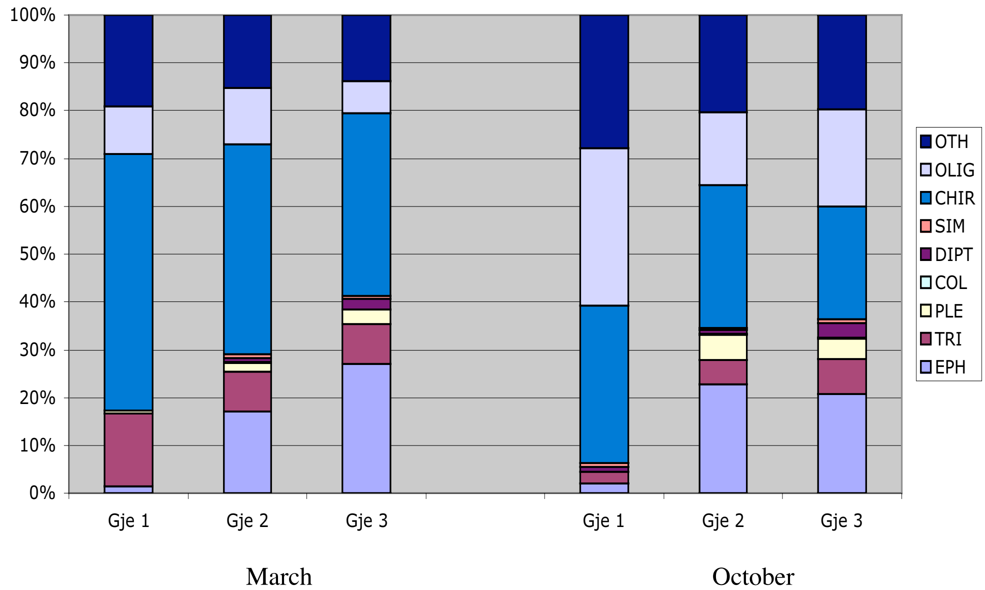


Figure 3.3.1. Percentage composition between the different taxa collected from Gjersrudbekken. Note that Gjersrudbekken has no upstream reference sites.

in Ephemeroptera abundance is more likely to have effected the occurrence of other species. A dominance shift within the EPT community is visible downstream, changing between the trichopteran community (more dominant upstream) and the ephemeropteran community (more dominant downstream). This shift is not very clear in the pre-salting season at upstream site Gje 1, possibly due to the small number of EPT specimens recorded. The plecopteran community is also completely absent from Gje 1.

Overall the most abundant species was *Ameletus inopinatus*. This abundance was evident in both seasons. The presence of this species declines downstream during the salting season and is completely absent from the upstream site (Gje 1) during the pre-salting season. During the salting season (March), 11 trichopteran, 5 plecopteran, and 7 ephemeropteran species were recorded. Ephemeroptera species *Ameletus inopinatus* was overall the most dominant (396 specimens). The Trichoptera species with the highest occurrence was *Neureclipsis bimaculata,* and the majority of tricopteran species showed a decrease downstream except a limited number. The most abundant Plecoptera was *Nemoura cinerea*. However the Plecoptera family was present in extremely low numbers and many species were absent in the upstream samples (Gje 1).

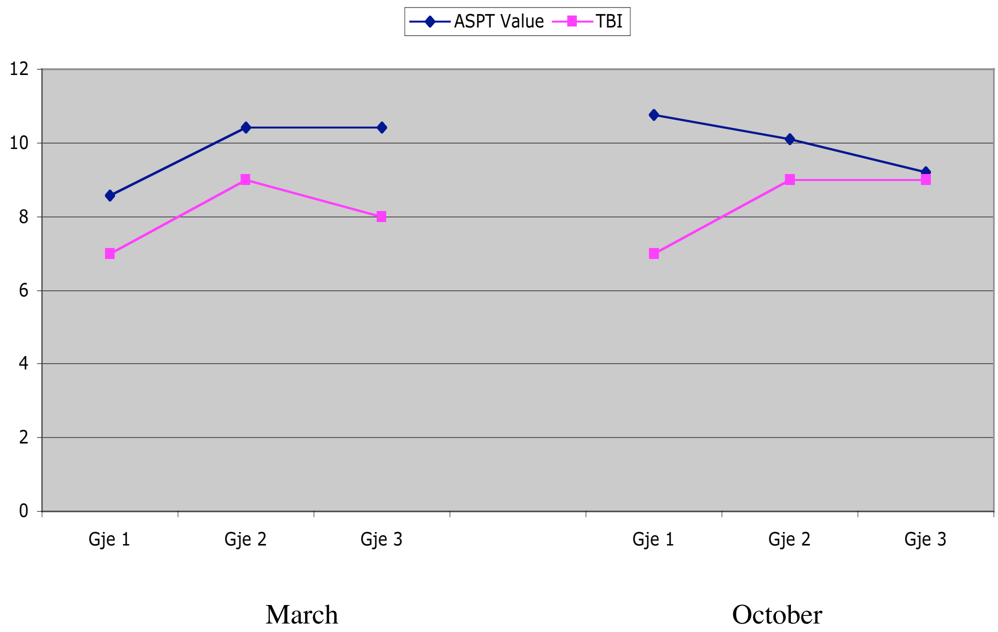
In the pre-salting season (October), 14 trichopteran, 4 plecopteran, and 9 ephemeropteran species were recorded. As recorded in March the same species in each family was the most abundant, Trichoptera *Neureclipsis bimaculata*, Plecoptera *Nemoura cinerea*, and Ephemeroptera *Ameletus inopinatus*.

Biotic Indices

The TBI score and ASPT value generally increases downstream in the March samples at Gjersrudbekken. However as the TBI score indicates a minor decrease in water quality at the final sampling site downstream (Gje 3) this decrease is not indicated by the ASPT value which stays more or less the same. Unlike the four other biological indices (TBI, ASPT, Chandler Score, and % EPT species) the Shannon diversity index implies an overall decrease in water quality downstream. There was some major disagreement between the indices ASPT and Shannon diversity index. Overall ASPT indicated a very high water quality, whereas the Shannon diversity index implied a low water quality.

In October the obtained ASPT values tended to disagree with the indications of the other indices. A gentle downstream decrease in water quality was concluded from the ASPT values, whereas the other indices (TBI, Shannon diversity index, Chandler score and % EPT species) displayed a dramatic increase in water quality downstream. The dramatic increase in the Chandler score and the % EPT species present could be explained by the difference in obtained values at the upstream site Gje 1 between seasons.

a)



b)

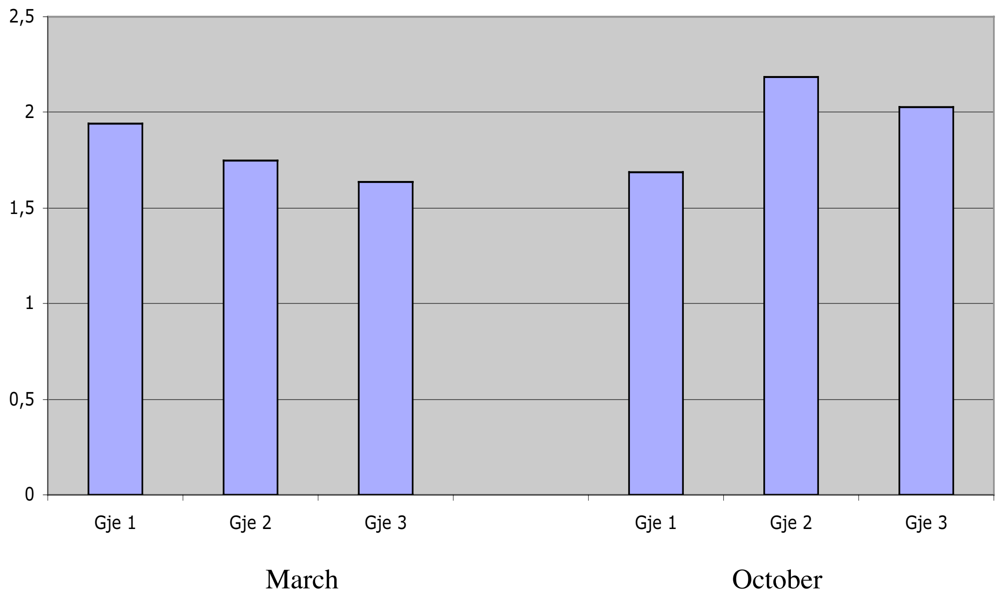
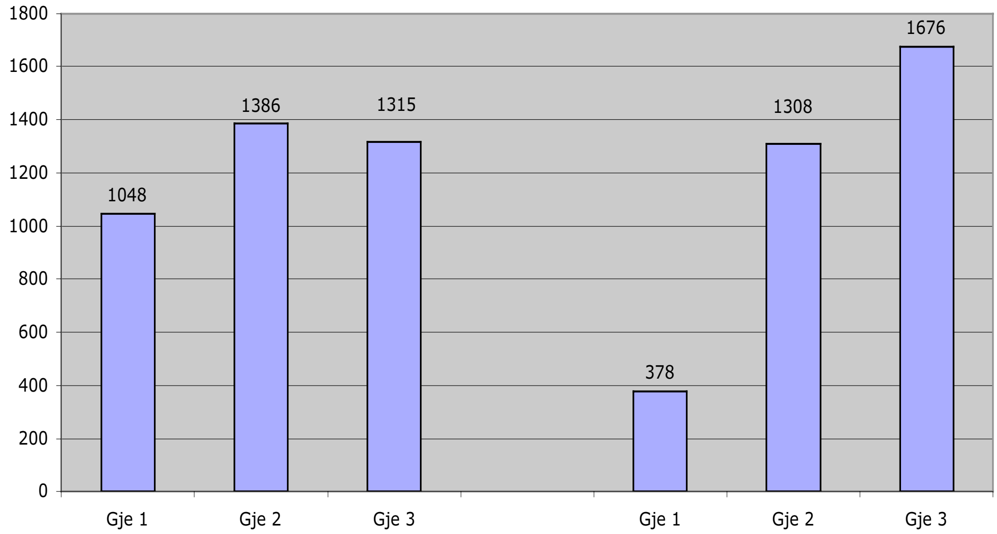


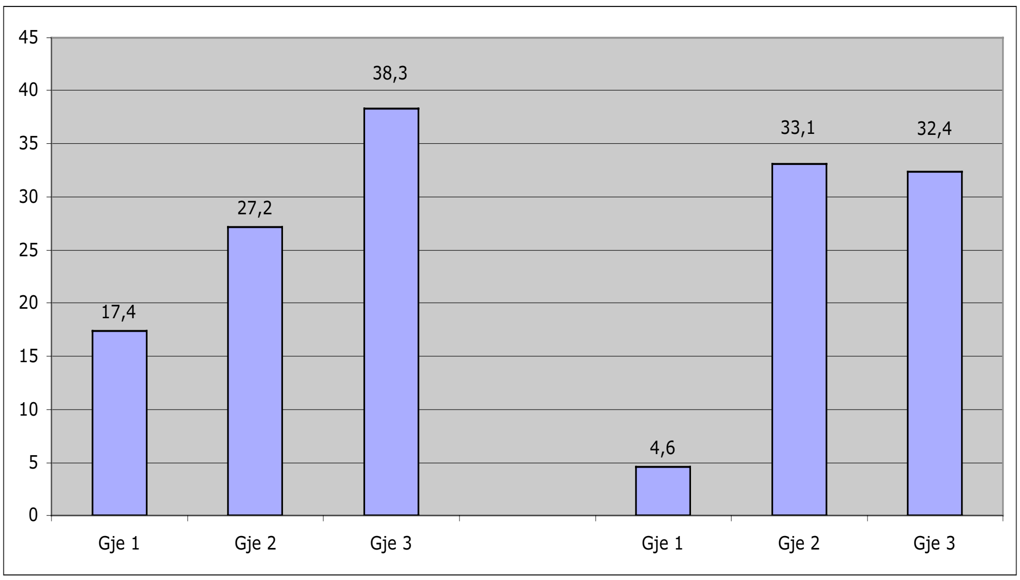
Figure 3.3.2. a) TBI score and ASPT values; b) Shannon diversity index. All indices are calculated from the sites sampled at Gjersrudbekken in March and October 2008. No reference sites were recorded at this stream.

a)



March October

b)



March October

Figure 3.3.3. a) Chandler score; b) % EPT species. All indices are calculated from the sites sampled at Gjersrudbekken in March and October 2008. No reference sites were recorded at this stream.

3.4. Community Analyses using PCA and RDA

PCA Analysis

The importance of the first ordination axis using a PCA and the EPT species was 0.254 (eigenvalue) and the first two axes explained 42.7 percent of the variation among species (Table 3.4.1.). The largest seperation in data is between Gjersrudbekken/Ljanselva and Bolvikelva, indicated by their seperation on the first principal axis. There is very little variation amongst seasons and the majority of the variation appears between streams. The upstream sites at Gjersrudbekken (Gje 1 and Gje 4) were indicated as species poor sites as they lay close to zero along the first axis. The Ephemeroptera and Plecoptera species are relatively evenly distributed among the sites, whereas Trichoptera species are distributed mainly on the right side of the diagram related to Bolvikelva. This demonstrates that Trichoptera are present in greater abundance at Bolvikelva.

Axis	1	2	3	4
Eigenvalue	0.254	0.172	0.134	0.099
Length of gradient	0.988	0.832	0.876	0.938
Cumulative % variance of species data	25.4	42.7	56.1	66.0
Cumulative % variance of species – environment relation	32.1	47.6	60.9	72.2

Table 3.4.1. Summary of samples analysed by PCA. Eigenvalue, length of gradient, cumulative percentage variance of species data and cumulative percentage of species-environment relation of each PCA axis are listed.

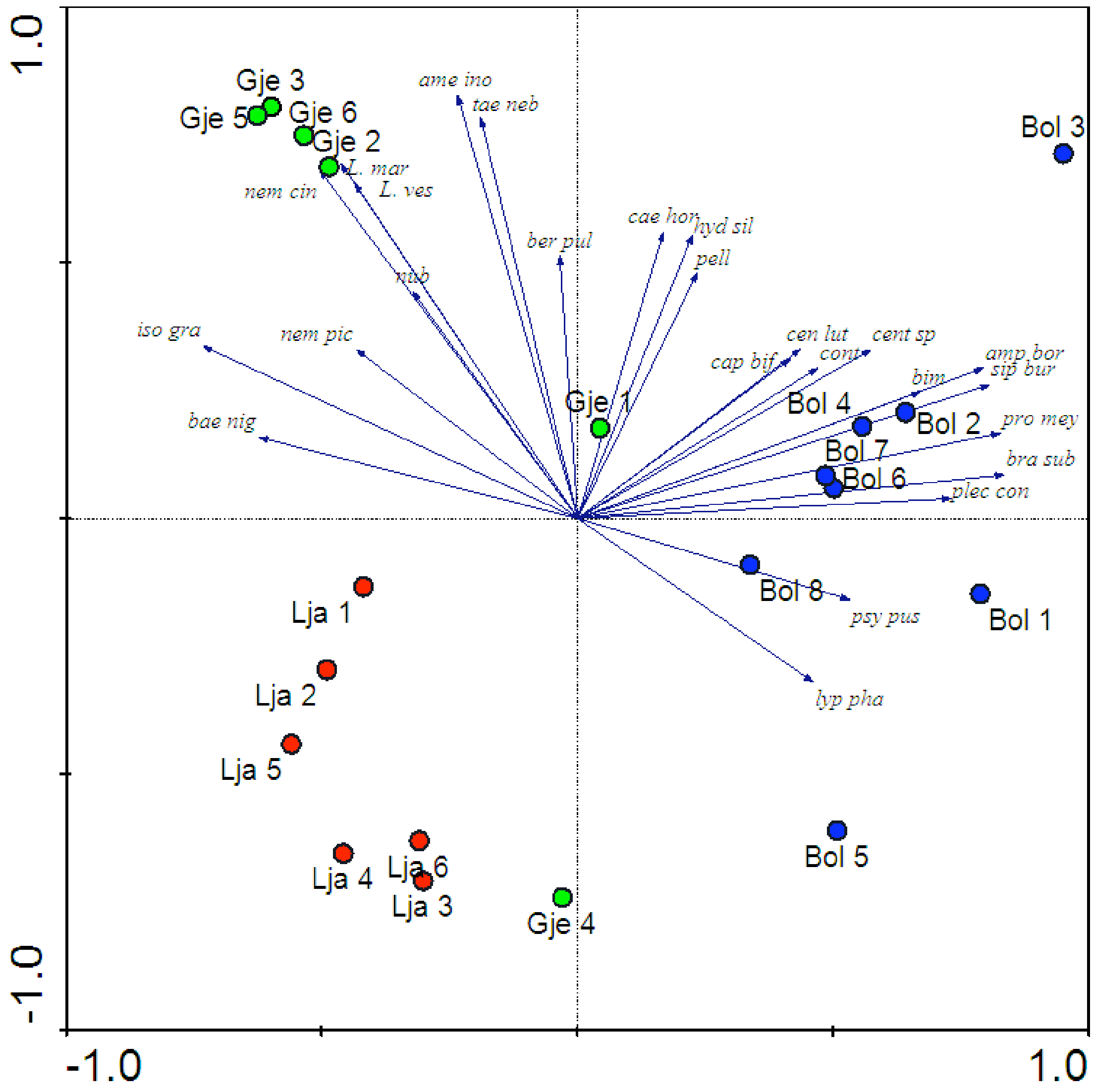


Figure 3.4.1. PCA based on macroinvertebrate samples from both salt season and pre-salt season. For clarity only 50% of the species that explained the variation the most are visualized. Descriptions of the site and species abbreviations see Appendix 1.

RDA Analysis

According to the manual forward selection Monte-Carlo permutation test five environmental variables; altitude (m.a.s.l.), season and chloride were the most significant variables ($p < 0.05$, Monte-Carlo permutation test) when explaining the variation in species data. The other variables were therefore dismissed. When comparing the % species data variation explained by the RDA (0.250) there was a relatively low difference compared to the % species data variation explained by the PCA (0.254), indicating that the above environmental variables explained a large part of the variation. The importance of the first RDA axis was 0.250 (eigenvalue) and explained 25 percent of the variation in species data (Table 3.4.3).

Axis	1	2	3	4
Eigenvalue	0.250	0.131	0.109	0.085
Length of gradient	0.992	0.905	0.923	0.917
Cumulative % variance of species data	25.0	38.0	48.9	57.5
Cumulative % variance of species – environment relation	32.3	49.2	63.3	74.4

Table 3.4.3. Summary of samples analysed by RDA. Eigenvalue, length of gradient, cumulative percentage variance of species data and cumulative percentage of species-environment relation of each RDA axis are listed.

Baetis sp and *Agapetus ochripes* displayed a preference for higher altitudes, whereas the Plecoptera species *Siphonoperla burmeisteri*, *Amphinemura borealis*, and *Protonemura meyeri* preferred much lower altitudes. Other species that also preferred relatively high altitudes included *Isoperla grammatica*, *Brachyptera risi*, and *Nemoura pictetii* in spite of displaying a greater association to high levels of chloride. The majority of Trichoptera species were negatively correlated with chloride and altitude except *Hydropsyche augustipennis*. Trichoptera species preferred lower levels of both chloride and altitude (see figure 3.4.3). However the species *Hydropsyche augustipennis* was positively correlated with high levels of chloride and showed no significant correlation to altitude. Overall the majority of EPT species were recorded during the salt season (winter).

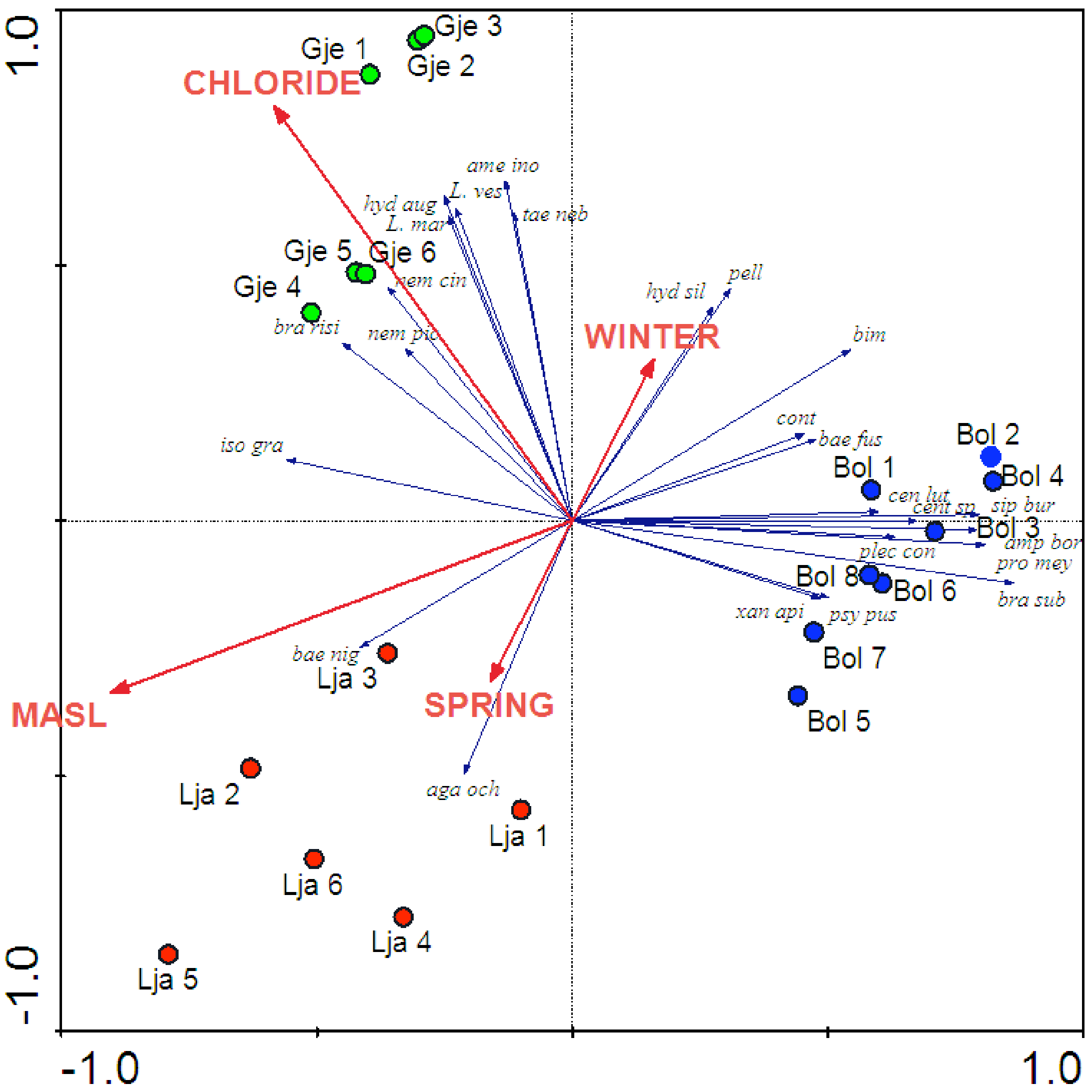


Figure 3.4.2. RDA based on the macroinvertebrates and the most relevant environmental parameters. For clarity only 50% of the species that explained the variation the most are visualized. For descriptions of the site and species abbreviations see Appendix 1.

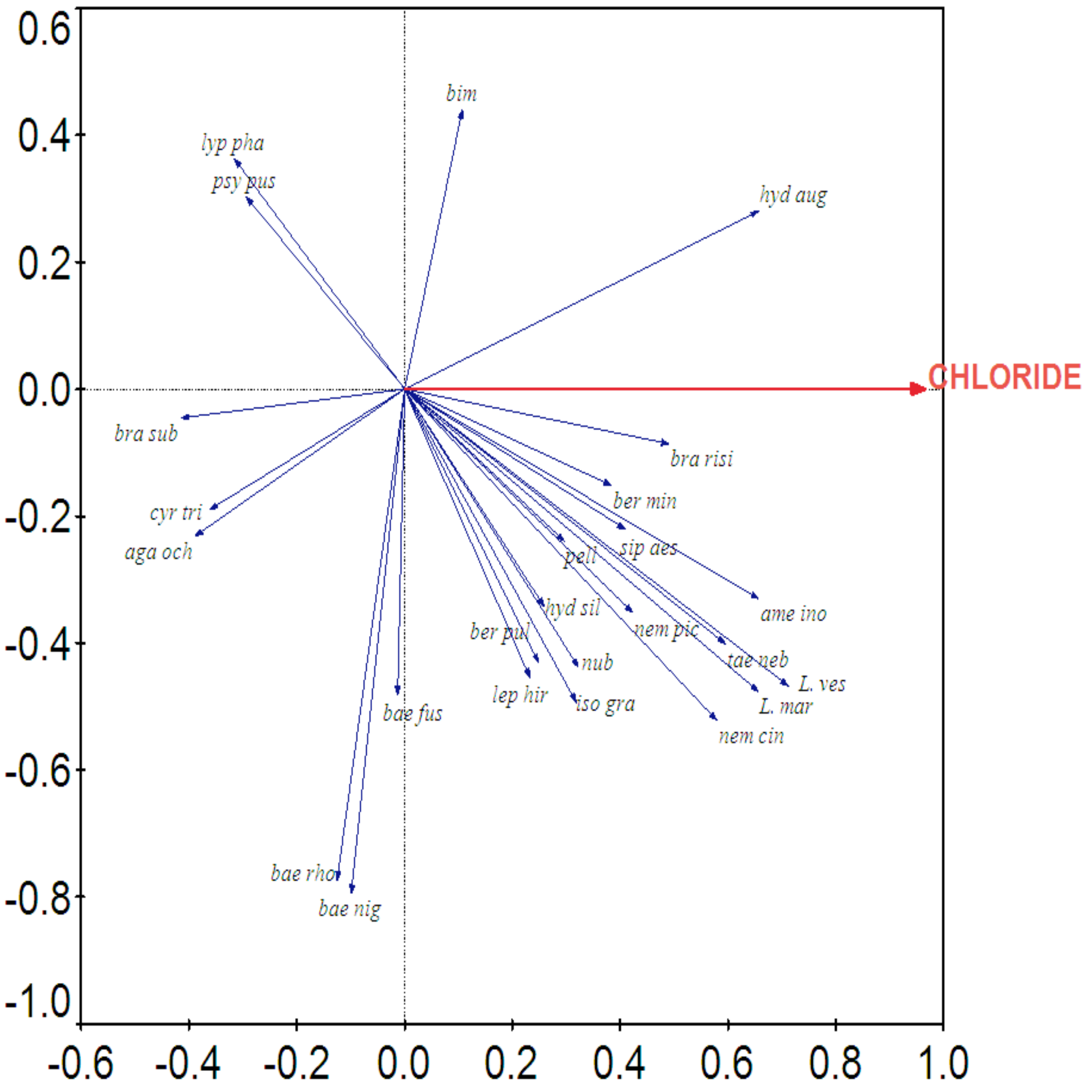


Figure 3.4.3. RDA based on the macroinvertebrates and chloride with altitude as a covariable. For clarity only 50% of the species that explained the variation the most are visualized. For descriptions of the site and species abbreviations see Appendix 1.

The variation between upstream and downstream sites was tested using a general linear ANOVA (Minitab 15. Statistical Software). Water variables and biotic indices were tested for normality (Minitab 15. Statistical Software), and those that did not pass the normality test were $\log_{10}$ transformed. Biotic indices were not significantly different upstream and downstream in either season (see figures 3.4.4 & 3.4.5). This was also the case when reference sites and downstream sites were tested alone. Shannon diversity, TBI and % EPT demonstrated more variation downstream than upstream. During the salting season TBI was much lower and Shannon diversity showed slightly more variation that during the pre-salting season.

Water variables also showed no significant differences when the upstream sites were compared to downstream sites (figure 3.4.6). However, when the downstream sites were tested alone pH demonstrated significant differences between seasons (p-value = 0.000) (figure 3.4.7). The pH levels were much lower during the salting season. TOC and Nitrate showed more variation during the salting season, whereas Fluoride showed less. Chloride demonstrated slight variation during the salting season however there was no significant differences between sites or seasons.

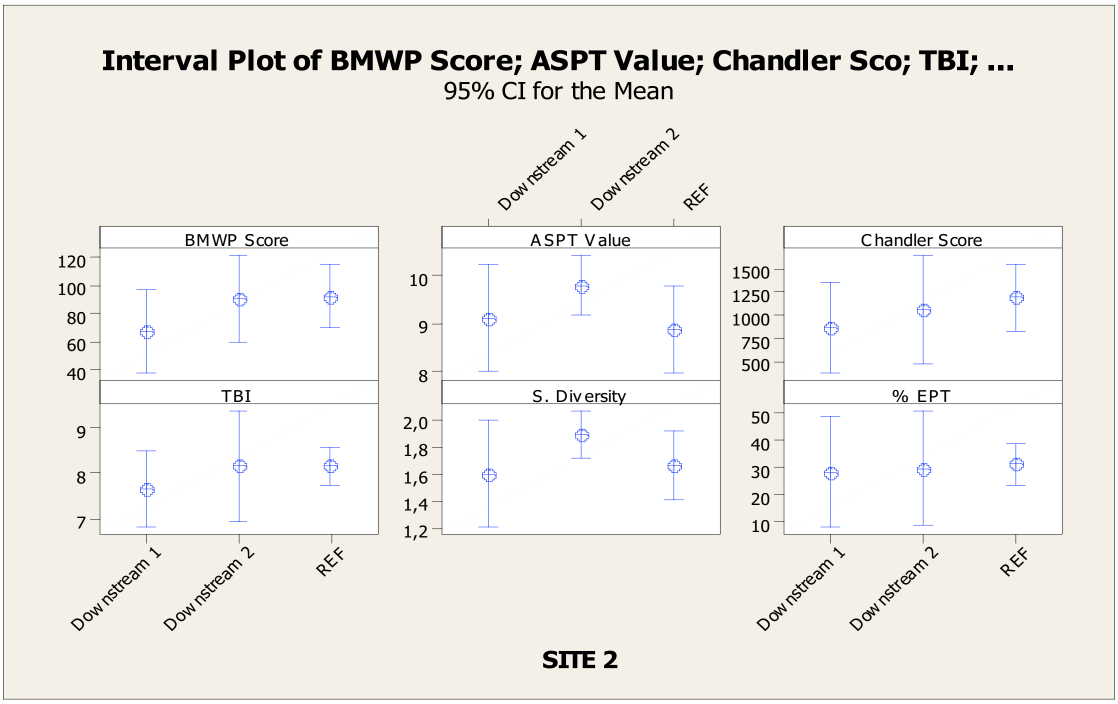


Figure 3.4.4. Interval plots showing differences in biological indices at reference (upstream) and downstream sites.

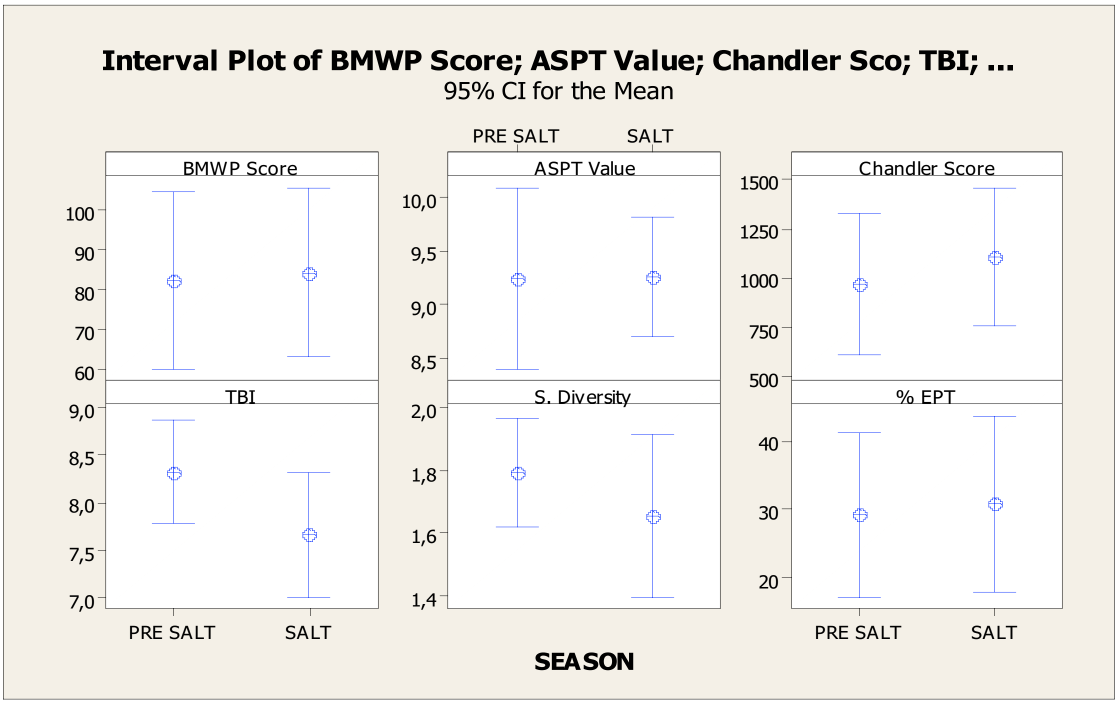


Figure 3.4.5. Interval plots showing differences in biological indices between seasons.

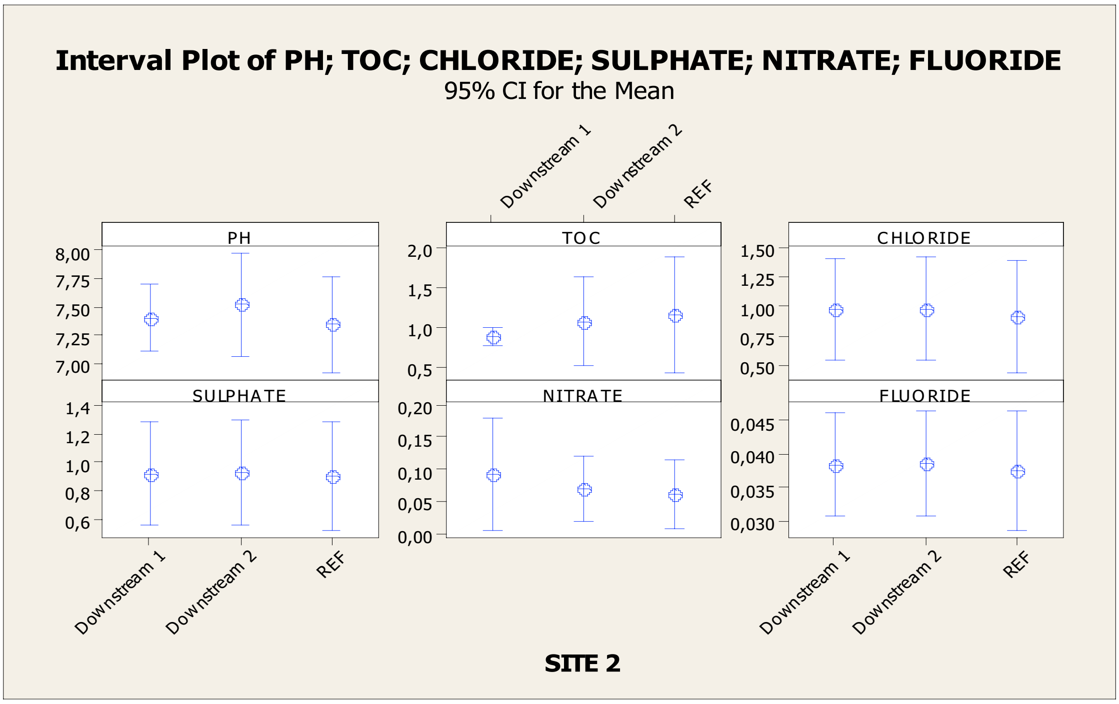


Figure 3.4.6. Interval plots showing differences in water variables at reference (upstream) and downstream sites.

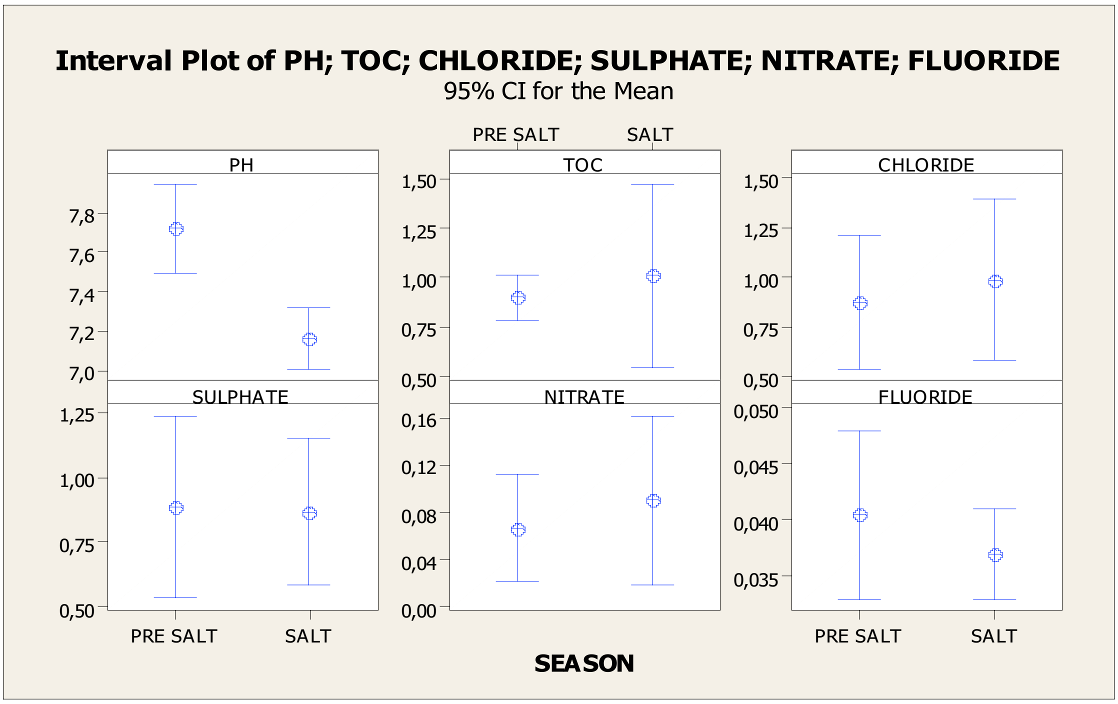


Figure 3.4.7. Interval plots showing differences in water variables between seasons (downstream sites only).

4. DISCUSSION

4.1. EVALUATION OF MACROINVERTEBRATES AND INDICES

4.1.1. Bolvikelva

January

A change in macroinvertebrate community downstream is present at Bolvikelva however a stressed community is not indicated. Although there is an obvious heavy reduction in the presence of Trichoptera downstream there is also a significant increase in Ephemeroptera and a slight increase in Plecoptera. The increase in numbers of the Plecoptera larvae (especially *Amphinemura borealis*) at downstream site Bol 3, could be caused by possible road salt dilution effects of the tributary inlet present. In this case dilution effects of the tributary need further investigation and there may be other factors causing the abundance of *Ampinemura borealis*. A decrease in the number of Chironomidae individuals recorded is also noticeable downstream, especially at site Bol 3. The decrease in abundance of Chironomidae downstream may imply an improvement in water quality, and the presence of Plecoptera would support this theory.

The Shannon diversity index displayed a greater diversity present downstream at site Bol 3. The diversity experienced after this site showed an significant decrease. However, the Shannon diversity index recorded failed to suggest a high water quality whereas the indications of the TBI Scores and ASPT values suggested a very high water quality. This could be due to their different approaches, the Shannon diversity index score decreases when one or two species dominate and is higher when the abundance is equally distributed between all species (Meland, 2001). The dominance of even a pollution sensitive taxon (For example; Plecoptera) would decrease the score and imply a possible stressed environment even though this may not be the case at all. A look at the Shannon diversity index and the dominant taxa (in this case the taxon *Simuliidae*) alone may have suggested stressed conditions at site Bol 3, however the fact that there was a high % EPT species present (epecially Plecoptera) would suggest that in this case that stress at this site was not at all severe.

October

The samples taken during the pre-salting season showed similar patterns in the macroinvertebrate community to those taken during the salting season. Once again the abundance of Chironomidae was greatest at the reference site and the % EPT species present increased gradually upstream. One main difference between the seasons is the dominance of Ephemeroptera at site Bol 3. The sensitive taxon Plecoptera are still present however they are recorded in smaller numbers than those experienced in January. This may indicate a slightly lower water quality at this site in October or could be due to natural variation. Hatching is often induced by certain temperatures and some species hatch later in the year.

The biological indices failed to indicate an overall reduction in water quality downstream, however the Chandler score does imply a slight reduction between site Bol 2 and site Bol 3. The abundance of a particular species would not decrease the Chandler score, due to the fact that it is based on a scoring system. Each species is assigned a particular score based on not only their abundance but also their pollution tolerance (Spellerberg, 1991). A greater abundance in pollution tolerant species recorded would therefore increase the Chandler score. Biotic indices however tell us very little about the community structure and diversity.

4.1.2. Ljanselva

March

Pollutant tolerant Chironomidae are the dominant taxon at all sites, suggesting a relatively high level of pollution. The number of Chironomidae individuals present were at their highest directly downstream (Lja 2) from the Skullerud highway treatment pond and directly situated under the main road (E6). Both these factors would have a notable negative impact on the water quality downstream.

The obvious decline in % EPT species present downstream indicates a negative change in water quality. The Shannon diversity index only indicates such changes at the site immediately downstream from the wastewater treatment plant and shows an increase in diversity further downstream. The increase in diversity further downstream may be due to the fact that macroinvertebrate communities often recover from pollution effects naturally when distance increases from the pollution source (Storey et al., 1991, Williams and Hynes, 1976). TBI indicated some a poorer water quality downstream than upstream. The TBI is based on the fact that certain species disappear and species diversity decreases as organic pollution increases, it fails however to take into consideration the differences in pollution tolerant species. The ASPT showed the opposite pattern downstream, probably due to the fact that the presence of pollution sensitive species are taken into consideration when calculating the ASPT and it is therefore more accurate in detecting mild pollution.

October

Although some downstream pollution was indicated by the Chandler score, % EPT species and TBI it was not as pronounced as the indices calculated in March. This may be due to greater amounts of runoff reaching the stream during the salting season than the pre-salting season. One cannot assume that the decrease in diversity downstream is associated to road salting alone. Studies have shown that road salt may release heavy metals accumulated in the sediments or roadside soils (Lofgren, 2001, Backstrom et al., 2004, Shanley, 1994).

Once again the TBI and ASPT values disagree with each other, especially at the final downstream site Lja 3. Here the ASPT displays a dramatic increase in water quality whereas the TBI displays a steady decrease. The ASPT is probably incorrect as the Shannon diversity index, Chandler score and %EPT species all indicate a diminished water quality downstream. This may be due to the rare occurrence of sensitive families including Siphlonuridae, Heptageniidae, and Perlolidae.

4.1.3. Gjersrudbekken

March

The overall dominance of Chironomidae in this stream indicates possible poor water quality. Kefford *et al* (2003) found that the taxon Chironomidae are however one of the most salt-sensitvie groups with the salinity LC_{50} value approximately 10 $mScm^{-1}$. This demonstrates that organic pollution probably has more of an effect on macroinvertebrate communities than salinity levels. Nevertheless salinity is one variable which often complements organic pollution (Laws, 2000). Despite the fact that pollution sensitive EPT species are present, they are only recorded in relatively low numbers.

The species *Ameletus inopinatus* occur in high numbers at site Gje 1 (upstream site) during the salting season and declines downstream. This species is particularly sensitive to organic pollution (Malmqvist and Hoffsten, 1999, Falkirk Council Area Biodiversity Action Plan, 2009) and its decline downstream implies a possible increase in organic pollution. The abundance of Ephemeroptera species *Baetis rhodani* increased slightly downstream whereas numbers of *Ameletus inopinatus* decreased drastically downstream. *Baetis rhodani* is known to be tolerant of organic pollution (Elliott et al., 1988), in contrast to *Ameletus inopinatus* which is negatively affected by pollution, metal pollution in particular (Wiseman et al., 2004). The drastic decreases in *Ameletus inopinatus* together with high numbers of the taxon Chironomidae downstream may indicate a negative change in water quality downstream. Trichoptera numbers recorded almost halved downstream. This is unlikely to be caused by their sensitivity to NaCl since no significant differences in chloride levels were recorded. However, some macroinvertebrates display larger drift due to high exponering to salt (Crowther and Hynes, 1977, Blausius and Merritt, 2002). Although Crowther and Hynes (1977) found that the drift of no specific specimen was affected there was an increase in drift present among all species. Since no samples were taken during or immediately after heavy rainfall episodes it is possible that any effects on drift may have been missed. Trichoptera are also more tolerant (LC_{50} values range 9->26 $mScm^{-1}$) than some salt-sensitive *Baetidae* (LC_{50} values range 5.5-6.2 $mScm^{-1}$) which were recorded downstream (Kefford et al., 2003).

Of the four biological indices used, only the Shannon diversity index indicated a possible decrease in water quality downstream. The increase in TOC levels downstream also indicate a slight water quality reduction. The total organic carbon (TOC) levels recorded increased downstream from 0.85 to 2.56 (mg/L). TOC levels demonstrate an increase in organic contaminants present, which in turn may cause a depletion in oxygen and a presence of toxic substances. This is unlikely to be the case in this situation since the TOC levels were relatively low. The decrease in water quality downstream implied by the dominance of pollution tolelerant taxon is supported by the lack of pollution sensitive taxon found downstream (especially EPT species). Both *Baetis niger* and *Baetis rhodani* only appear downstream in both seasons. *Baetis rhodani* is known to be somewhat tolerant to mild levels of pollution which could explain its presence downstream (Hellawell, 1986).

October

The dominance of taxon such Chironomidae, aquatic Oligochaeta and the limited numbers of EPT species present at all sites illustrate the poor water quality of Gjersrudbekken. Whereas the increase in percent of pollution sensitive Ephemeroptera, Trichoptera and Plecoptera found downstream may indicate an improvement in water quality.

Both the situations mentioned above are implied using the biological indices. TBI, Shannon diversity index and % EPT species present all indicate an improvement in water quality downstream, whereas ASPT indicates a decrease in water quality. The Shannon diversity index does not however suggest the same high water quality which is suggested by the other indices. The low diversity score experienced may be due to the high abundance of Chironomidae.

4.2 CHLORIDE

Despite the fact that upstream and downstream chloride levels were not tested as being statistically significant there were still some slight differences. The fact that neither of the samples were statistically significant could be due to the chosen time of sampling. Sampling after heavy rainfall would have more likely displayed some significant differences. Storms and heavy rainfall would increase the time it takes chemicals such as road salt to reach the stream. Sharp fluctuations in heavy rainfall could also cause other ecological effects, such as those associated with heavy metals. Some studies have observed positive correlations between NaCl and heavy metals especially after heavy rainfall (Ruth, 2003b, Mason et al., 1999).

The highest chloride levels recorded at Bolvikelva in January was at the site Bol 1 (reference) with a slight decrease in levels downstream. This was not the case during the pre-salting season samples where levels increased steadily downstream. A change in migration patterns of road salt may be a possible explanation for this situation.

At Ljanselva the chloride levels also increased downstream during the salting season and there was also an increase in chloride levels downstream during the pre-salting season although not as evident. Demers and Sage (1990) found that elevated chloride levels were not always a short term event and recorded elevated chloride levels up to six months after salt application had stopped. This could also be the case during the pre-salting season at Ljanselva. Scott (1981) also found that both urbanization of the watershed and discharge affects the level and duration of road salt concentrations in a watercourse. Yet it must also be taken in to consideration that chloride levels in streams are related to several other factors; drainage pattern of the roadway, length of the road drained, topography, geology, and groundwater concentrations (Scott, 1981, Williams et al., 2000, Williams et al., 1997).

Chloride levels in Gjersrudbekken were the highest found throughout the whole study. There were discrepencies between salting season (March) and pre-salting season (October) with the highest chloride levels being found surprisingly during the pre-salting season. The higher chloride levels in this case may be due to the migration pattern of the road salts. It is possible that during periods of little rainfall the stream receives more of its water from groundwater sources rather than surface runoff. Groundwater concentrations of chloride may have accumulated and therefore release higher levels of chloride in to the stream during the pre-salting season. Measurement of groundwater concentrations was beyond the scope of this study and further research may provide more information.

4.3. MACROINVERTEBRATE COMMUNITY

As shown in the ordination diagram the greatest variation in macroinvertebrate data found was between streams and not within the streams. Both Ljanselva and Gjersrudbekken displayed similar macroinvetebrate communities and this could be due to the size and location of the streams. Ljanselva and Gjersrudbekken are similar in size, whereas Bolvikelva is somewhat larger. Bolvikelva is also a more isolated stream with a more varied riparian zone and more vegetation present along the banks of the stream compared to the other two streams. Ljanselva and Gjersrudbekken are located in more built up areas. The urbanization of the watershed and the riparian zone would have a number of effects on both the stream flow and the macroinvertebrate community present (Sandin, 2009, Townsend et al., 2003). Corkum (1992) found that macroinvertebrate communities were strongly associated with site-specific factors such as riparian vegetation and land use. The regional location of a stream may also affect macroinvertebrate communities present since many species are adapted and colonised according to a streams physcial and chemical characteristics.

Effects on macroinvertebrates present in ponds and lakes would most likely differ to those found in streams. It is often the case that different chloride levels are recorded at different water levels in ponds and lakes with highest chloride levels recorded at the lower levels (Novotny et al., 2008, Mayer et al., 1999). An increase in salinity at the lake bottom may prevent lake overturn and release of heavy metals from porewater in to the overlaying water (Bækken and Haugen, 2006).

The RDA triplot showed the most determining variables affecting macroinvertebrate communities in this study were chloride and altitude (m.a.s.l.). The majority of the trichopteran species present preferred lower levels of chloride and lower altitudes. This was confirmed by conducting a RDA using chloride as the only environmental variable and altitude as a co-variable. There is little detailed ecotoxicological information on the species which were found to be sensitive to high chloride levels in this study (*Agapetus ochripes, Cyrnus trimaculatus, Brachycentrus subnubilus, Psychomyia pusilla* and *Lype phaeopa*), therefore a laboratory investigation including these species would be justified. Although the recorded chloride levels in this study may not be acute and lead to mortality within the trichopteran community other physiological processes may have been affected. Claus et al (2005) demonstrated that the increase in salt levels affected the leaf shredding activity and abundance of caddisfly (Trichoptera). Several laboratory studies have also demonstrated that different levels of NaCl may effect the osmoregulatory and physiological processes of some invertebrates (Blausius and Merritt, 2002; Crowther and Hynes, 1977). Increased salinity levels do not effect all freshwater macroinvertebrate species in the same way, and studies have demonstrated that salinity tolerance levels vary greatly (Kefford et al., 2005, Dunlop et al., 2008, Kefford et al., 2007). This is also demonstrated in the ordination diagram where different species are related to different levels of chloride. This may give some indication of a salt gradient, but the data here is limited. More samples from more sites should be taken to establish a salt index.

The lack of statistically significant differences found between biotic indices at sites and between seasons also indicate the low impact road salt had on macroinvertebrate communities. It is generally agreed that a mixture of all indices and tools are more effective when analysing biological data (Lydy et al,. 2000). Such field studies on macroinvertebrate communities are insensitive and earlier warning signs may detected by studying individuals and any biomarkers (Barata et al., 2005, Damasio et al., 2007, Mutwakil et al., 1997). Many processes often occur at the individual level before a whole community shows any signs of negative environmental effects. Macroinvertebrate communities are also affected by many other factors than pollution such as stream flow, substrate, predation and competition (to name just a few). Communities can take longer to recover from pollution effects than individuals. Overall both biomarkers and community studies have their limitations and therefore a combination of both methods would be more reliable and effective in detecting effects of pollution (Damasio et al., 2008, Faria et al., 2006, Picado et al., 2007).

4.4. CONCLUSION

Based on the macroinvertebrate data sampled in this study, macroinvertebrate communities showed no acute negative responses to road salt. This agrees with Blausius and Merritt (2002) when they demonstrated a lack of macroinvertebrate community changes in relation to the NaCl levels experienced today. They concluded that road salt runoff would have little negative impact due to snow melt dilution (Blausius and Merritt, 2002). Nonetheless, community studies alone may be insensitive to any negative effects road salt may have on macroinvertebrates and individual studies may be more suitable. Many processes often occur at the individual level before a whole community reacts to any negative impacts. Further investigation using possible biomarkers at the individual level may provide earlier warning signs to possible negative effects of road salt .

It is important to remember when studying the effects of road salt on macroinvertebrate communities in the field it is particularly difficult to determine the effects of salinity and those of other underlying factors. Factors such as the presence of heavy metals and interactions within the macroinvertebrate assemblage will also effect the community structure and diversity. It should also be taken in to consideration that although no acute negative effects have been observed in this case other chronic effects could be taking place. In both these cases an additional laboratory study may help determine the any other factors influencing the macroinvertebrate community and any chronic effects of road salt.

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APPENDIX I: MACROINVERTEBRATE DATA

Table 1. Macroinvertebrate data sampled in January/March and October 2008. The abbreviations are used in PCA and RDA diagrams. Reference sites are marked with asteriks.

Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1*	Bol 2	Bol 3	Bol 4	Bol 5*	Bol 6	Bol 7	Bol 8	Lja 1*	Lja 2	Lja 3	Lja 4*	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
EPHEMEROPTERA																					
BAETIDAE																					
Baetis (LEACH, 1815)																					
Baetis sp.											4		5	17	5	3				9	12
rhodani (PICTET, 1843-45)	bae rho		85	1		5	14	31	4	117	7	12	39	27	9		38	56		11	10
fuscatus (LINNAEUS, 1761)	bae fus	2	7		14		38	9	8	9							6	4		3	5
niger (LINNAEUS, 1761)	bae nig		36	5	2			6	3	47	47	14	48	59	4		17	15		24	23
Procloeon (BENGTSSON, 1915)																					
bifidum (BENGTSSON, 1912)	pro bif									4	1										
Centroptilum sp. (EATON, 1869)			3	19						1										1	3
Centroptilum luteolum (MÜLLER, 1776)	cen lut		2	8						4								1			1
HEPTAGENIIDAE																					
Heptagenia																					
fuscogrisea (RETZIUS, 1783)	hep fus								1												
rithrogena germanica (EATON, 1870)	rit ger									1					1						
SIPHLONURIDAE																					
Siphlonuridae (ULMER, 1920)																					
siphlonurus aestivalis (EATON, 1903)	sip aes																1				
ameletus inopinatus (EATON, 1887)	ame ino	15	7	39	78		76	89	114			1				114	89	76		78	39
LEPTHOPHLEBIIDAE																					
Leptophlebia (WESTWOOD, 1840)																					

Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1	Bol 2	Bol 3	Bol 4	Bol 5	Bol 6	Bol 7	Bol 8	Lja 1	Lja 2	Lja 3	Lja 4	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
vespertina (LINNEAUS, 1758)	L. ves		1				1	1	5					2		1	9	11	1	19	5
marginata (LINNEAUS, 1767)	L. mar				1												1	13		7	7
Caenis																					
horaria (LINNEAUS, 1758)	cae hor		1	6	1		5		20							2		1		8	8
Caeniidae																					
luctuosa (BURMEISTER, 1917)	cae luc						32	3	3								1		8		
PLECOPTERA																					
TAENIOPTERYGIDAE																					
Taeniopteryx (PICTET, 1841)																					
nebulosa (LINNÈ, 1758)	tae neb		1	2						1						2	1	1		3	2
Bracyptera (NEWPORT, 1849)																					
risi (MORTON, 1896)	bra ris						1							2	2	2	3	2			1
NEMOURIDAE																					
Amphinemura (RIS, 1902)																					
borealis (MORTON, 1894)	amp bor	4	26	58	15		2	25	1												
Nemoura (Latreille, 1796)																					
cinerea (RETZIUS, 1783)	nem cin			1										2			2	5		18	16
Nemurella (KEMPNY, 1898)																					
pictetii (KLAPALEK, 1900)	nem pic													1			1	2			

Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1	Bol 2	Bol 3	Bol 4	Bol 5	Bol 6	Bol 7	Bol 8	Lja 1	Lja 2	Lja 3	Lja 4	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
Protonemura (KEMPNY, 1898)																					
meyeri (PICTET, 1841)	pro mey	5	15	10	2	2		8	2												
PERLOLIDAE																					
Isoperla (BANKS, 1903)																					
grammatica (PODA, 1761)	iso gra									1	2		1		1		1	1		2	3
CHLOROPERLIDAE																					
Siphonoperla (ZWICK, 1867)																					
burmeisteri (PICTET, 1841)	sip bur	8	22	17	6		6	2													
Xanthoperla (ZWICK, 1967)																					
apicallis (NEWMAN, 1836)	xan api	1				2	5	19	14												
CAPNIIDAE																					
Capnia (PICTET, 1841)																					
bifrons (NEWMAN, 1839)	cap bif			4																	
Capnopsis (MORTON, 1896)																					
schilleri (ROSTOCK, 1892)	cap sch			1		1															
PERLIDAE																					
dinocras cephalotes (CURTIS, 1827)	din cep								1												

Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1	Bol 2	Bol 3	Bol 4	Bol 5	Bol 6	Bol 7	Bol 8	Lja 1	Lja 2	Lja 3	Lja 4	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
TRICHOPTERA																					
RHYACOPHILIDAE (PICTET, 1834)																					
nubila (ZETTERSTEDT, 1840)	rhy nub	4	3	1		1					4		1	4			5	4		3	5
HYDROPSYCHIDAE (CURTIS, 1834)																					
Hydropsyche (PICTET, 1834)																					
contubernalis (McLACHLAN, 1865)	cont	15	9	8		7		2	1	5	1					2	6			1	2
pellucidula (CURTIS, 1834)	pell	11	10	2		1	2										16	2			6
siltalai (DÖHLER, 1963)	hyd sil	12	21	9	1		1	3		1		1	1	3		3	9	11		5	
augustipennis (CURTIS, 1834)	hyd aug	14					1	1		2			1			17	1	7	6	3	7
POLYCENTROPODIDAE (ULMER, 1903)																					
Plectrocnemia (STEPHENS, 1836)																					
conspersa (CURTIS, 1834)	plec cons	14	4	8	2	5	1	1		1			2			1			1		2
Neureclipsis (McLACHLAN, 1864)																					
bimaculata (LINNEAUS, 1758)	bim	31	1	15	2	11	6	3	6							45	1	3		1	
Cyrnus																					
trimaculatus (CURTIS, 1834)	cyr tri								1	1			1								
Polycentropus																					
flavomaculatus (PICTET, 1834)	pol fla	4	3	6		2		3	5				2	1	2				2	4	5

Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1	Bol 2	Bol 3	Bol 4	Bol 5	Bol 6	Bol 7	Bol 8	Lja 1	Lja 2	Lja 3	Lja 4	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
BERAEIDAE																					
Beraea (STEPHENS, 1833)																					
pullata (CURTIS, 1834)	ber pul			3			1	3	14				2				4	4		1	4
Beraeodes (EATON, 1867)																					
minutus (LINNEAUS, 1761)	ber min			1															1	1	5
PSYCHOMYIIDAE																					
Pyschomyia																					
pusilla (FABRICIUS, 1781)	psy pus	2				3	3	2													
Lype (McLACHLAN, 1878)																					
phaeopa (STEPHENS, 1836)	lyp pha	4	2			7	4	1		1		1	2			2					
Tinodes (CURTIS, 1834)																					
waeneri (LINNEAUS, 1758)	tin wae			1		4	4			2						3					1
PHRYGANEIDAE																					
trichostegia minor (CURTIS, 1834)	tri min						1														2
GLOSSOSOMATIDAE																					
agapetus ochripes (CURTIS, 1834)	aga och									7			13	1							
MOLANNIDAE																					
molanna albicans (ZETTERSTEDT, 1840)	mol alb	1																			
molanna angustata (CURTIS, 1834)	mol ang																				1

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Taxa and species	Abbreviation.	Bolvikelva								Ljanselva						Gjersrudbekken					
		Bol 1	Bol 2	Bol 3	Bol 4	Bol 5	Bol 6	Bol 7	Bol 8	Lja 1	Lja 2	Lja 3	Lja 4	Lja 5	Lja 6	Gje 1	Gje 2	Gje 3	Gje 4	Gje 5	Gje 6
LEPTOCERIDAE																					
oecetis testacea (CURTIS, 1834)	oec tes									2		1									1
LEPIDOSTOMATIDAE																					
lepidostoma hirtum (FABRICIUS, 1775)	lep hir			1	1					5		1					2	3		1	
PHILOPOTAMIDAE																					
wormaldia occiptalis (PICTET, 1834)	wor occ												1								
BRACHYCENTRIDAE																					
brachycentrus subnubilus (CURTIS, 1834)	bra sub	2	35	18	2	19	15	21	15												
Hydroptila tineoides (DALMAN, 1819)	hyd tin	1			1				4												

APPENDIX II: BIOTIC INDICES

Trent Biotic Index (Woodiwiss, 1964, in Metcalfe, 1989)

Key Groups	Diversity	Total number of groups present				
		0-1	2-5	6-10	11-15	16+
		Biotic Index				
Plecoptera present	> 1 species	-	VII	VIII	IX	X
	1 species	-	VI	VII	VIII	IX
Ephemeroptera present Excl. Baetis rhodani	> 1 species	-	VI	VII	VIII	IX
	1 species	-	V	VI	VII	VIII
Trichoptera and/or B. rhodani present	> 1 species	-	V	VI	VII	VIII
	1 species	IV	IV	V	VI	VII
Gammarus present	All above absent	III	IV	V	VI	VII
Asellus present	All above absent	III	IV	V	VI	VII
Tubificidae and/or red Chironomidae	All above absent	I	II	III	IV	-
All above absent		-	I	II		

Groups in the modified version (Saltveit & Borgstrøm 1978)

1. Turbellaria
2. Oligochaeta
3. Hydracarina
4. Plecoptera
5. Ephemeroptera
6. Trichoptera
7. Coleoptera
8. Chironomidae
9. Simuliidae
10. Other Diptera
11. Hirudinea
12. Lamellibrachiata
13. Gastropoda
14. Asellus
15. Other groups

Families and scores in the Biological Monitoring Working Party (Armitage 1983)

Families	Score
Siphlonuridae Heptageniidae Leptophlebiidae Ephmerellidae Potamanthidae Ephemeridae Taeniopterygidae Leuctridae Capniidae Perlodidae Perlidae Chloroperlidae Phrygganeidae Molanidae Beraeidae Odontoceridae Leptoceridae Goeridae Lepidostomatidae Brachycentridae Sericostomatidae	10
Astacidae Lestidae Agriidae Gomphidae Cordulegasteridae Aeshnidae Corduliidae Libelluidae Psychomyiidae Philopotamidae	8
Caenidae Nemouridae Rhyacophilidae Polycentropodidae Limnephilidae	7
Neritidae Viviparidae Ancylidae Hydroptilitdae Unionidae Corophiidae Gammaridae Platycnemidiae Coenagriidae	6
Mesoveliidae Hydrometridae Gerridae Nepidae Naucoridae Notonectidae Pleidae Corixidae Hapliplidae Hygrobiidae Dystischidae Gyrinidae Hydrophilidae Clambidae Helodidae Dryopidae Elminthidae Chrysomelidae Curculionidae Hydropsychidae Tipulidae Simuliidae Planariidae Dendrocoelidae	5
Baetidae Sialidae Piscicolidae	4
Valvatidae Hydrobiidae Lymnaeidae Physidae Planorbidae Sphaeriidae Glossiphoniidae Hirundidae Erphobdellidae Asellidae	3
Chrionomidae	2
Oligochaeta (whole class)	1

Chandler Biotic Index (adapted from Mason, 1981)

Row		Groups present in sample	Abundance in sample				
			Present 1-2	Few 3-10	Common 11-50	Abundadant 51-100	Very abundant 100 +
			Points scored				
1	Each species of	*Crenobia alpina* Taenopterygidae, Perlidae, Perlolidae, Isoperlidae, Chloroperlidae	90	94	98	99	100
2	Each species of	Leuctridae, Capniidae, Nemouridae (excluding *Amphinemura*)	84	89	94	97	98
3	Each species of	Ephemeroptera (excluding *Baetis*)	79	84	90	94	97
4	Each species of	Cases caddis, Megaloptera	75	80	86	91	94
5	Each species of	Rhyachophila (Trichoptera)	65	70	77	83	88
6	Genera of	Coleoptera, Nematoda	51	55	61	66	72
7		*Amphinemoura* (Plecoptera)	47	50	54	58	63
8		*Baetis* (Ephemeroptera)	44	46	48	50	52
9	Each species of	Uncases caddis (excl. *Rhyacophila*)	38	36	35	33	31
10	Genera of	Hydracarina	32	30	28	25	21