



Faculty of Applied Ecology and Agricultural Sciences

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Master thesis

## Effects of reduced liming on brown trout (*Salmo trutta* L.) in four small tributaries in eastern Norway.



Master in Applied Ecology

2020

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## Summary

Acidification of surface waters due to acidic precipitation has led to a reduction in biological diversity. Through international conventions, emissions causing the acidic precipitation has decreased. The challenges associated with acidification today is of a different character than it was earlier. Huge efforts have been done on a national scale in Norway to reduce acidification, mainly by adding lime to surface waters. Due to reduced acidic precipitation, lime treatment has been ceased in 800 locations, during the years 2004 - 2014. A monitoring of the water chemical and biological development in these locations adds knowledge about the consequences of ceased lime treatment.

In this study brown trout populations and water chemistry is monitored in four tributaries draining into Lake Råsjøen, Nannestad municipality, Norway. The four tributaries are currently under four different lime treatment regimes. Letter codes have been given to the tributaries Elsjøbekken (A), Storrsjøbekken (B), Botnetjernsbekken (C) and Trasletjernsbekken (D). Tributaries C and D have ceased lime treatment, tributary B is treated with lime in parts of its catchment area, and tributary A is fully treated with lime in its catchment area. The chemical parameters pH and conductivity were measured in field and water samples were collected and sent to a laboratory. Water temperature and relative barometric pressure were registered once an hour by mounted loggers. Brown trout and minnow have been captured in the tributaries using an electrical fishing apparatus. Brown trout were then marked using PIT-tags, to monitor growth and movement. Stationary antennas were mounted at the inlets to Lake Råsjøen, a portable antenna was used in the tributaries, in addition to registration of previously marked brown trout upon recapture.

The findings in this study indicates that the ceased lime treatment in tributary D were premature, based on the low abundance of brown trout compared to the other tributaries. Furthermore, tributary D has the lowest pH and ANC levels of the four tributaries. Tributary C does not show a low abundance of brown trout, but there are indications of a negative development as no female spawning brown trout are recaptured there and periodically low pH and ANC levels. Tributaries A and B are still treated with lime and show relatively high densities of brown trout and returning female spawning brown trout. The water chemistry of tributaries A and B are also more stable, compared to tributaries C and D.

## Sammendrag

Forsuringen av vann og vassdrag grunnet sur nedbør har ført til en reduksjon i biologisk mangfold. Gjennom internasjonale konvensjoner, har utslippene som førte til sur nedbør blitt redusert. Utfordringene tilknyttet forsuring i dag er av en annen karakter enn tidligere. En stor innsats har blitt gjort på et nasjonalt plan i Norge for å redusere forsuringen, hovedsakelig ved å tilføre kalk til vann. Som en følge av de reduserte sure nedbøren har også bruken av kalk blitt avsluttet i 800 lokaliteter i årene 2004 – 2014. En overvåkning av vannkjemisk og biologisk utvikling i disse lokalitetene øker kunnskapen rundt konsekvensene av avsluttet kalking.

I denne studien overvåkes brunørret populasjoner og vannkjemisk i fire bekker som drener inn i Råsjøen, Nannestad kommune, i Norge. De fire bekkene er under forskjellige kalkningsregimer Bokstavkoder er gitt til bekkene Elsjøbekken (A), Storrsjøbekken (B), Botnetjernsbekken (C) og Trasletjernsbekken (D). Bekk C og D har avsluttet kalking, bekk B er delvis kalket i nedbørsfeltet og bekk A er fullkalket i nedbørsfeltet. De kjemiske parameterne pH og konduktivitet ble målt i felt og vannprøver ble samlet og sent til et eksternt laboratorium. Vanntemperatur og relativt barometrisk trykk ble registrert en gang i timen av utplasserte loggere. Brunørret og ørekyte ble fanget i bekkene ved hjelp av el-fiskeapparat. Brunørret ble merket med PIT-tags, for å overvåke vekst og bevegelse. Stasjonære antenner ble montert i innløpene til Råsjøen, en bærbar antenne ble benyttet i bekkene, samt at tidligere merket brunørret ble registrert ved gjenfangst.

Funnene i denne studien indikerer at den avslutningen av kalkningen i bekk D var for tidlig, basert på lite antall brunørret, sammenliknet med de andre bekkene. Bekk D hadde og de laveste nivåene av pH og ANC av de fire bekkene. Bekk C hadde ikke det samme lave antallet brunørret, men det er indikasjoner på en negativ utvikling at ingen gyttende hunner av brunørret ble gjenfanget, samt periodisk lave pH og ANC verdier. Bekkene A og B blir fortsatt tilført kalk og viser relativt høye tettheter av brunørret og gjenfangster av gyttende hunner året etter. Vannkjemien er også mer stabil i bekkene A og B, sammenliknet med bekkene C og D.

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# 1. Introduction

## 1.1 Freshwater acidification.

Acidic rain is mainly caused by sulphur and nitrogen emissions from industrial activity. Mainly the chemical compounds ( $\text{SO}_2$ ) and ( $\text{NO}_x$ ) are responsible for the creation of acidic rain (Lydersen, et al., 2002). Historically, acidic rain caused decreased pH levels in waters, through chemical events in the watersheds (Gensemer & Playle, 1999). In general, the bedrock, and vegetation make most of Norway's soil naturally acidic. Through acidic rain, however, the soil in the watersheds became more acidic and the Acid Neutralizing Capacity (ANC) of the soil water was reduced. The reason for this is that increased acidity causes base cations to leak out from the soil. After this, aluminium starts to leak out as well, causing elevated concentrations of aqueous aluminium in acidified waters.

When aluminium is mobilized from the soil and into freshwater systems, a variety of aluminium compounds are formed (Barnes, 1975). In general, elevated concentrations of aqueous aluminium is toxic to freshwater biota, including fish (Gensemer & Playle, 1999). Dependent on physio-chemical conditions of the water, the toxicity of aluminium can vary substantially (Poléo, 1995), (Gensemer & Playle, 1999). In general, monomeric inorganic aluminium ( $\text{Al}_i$ ) is considered to be the most toxic Al-fraction (Poléo, 1995).

The chemically unstable, positively charged ions of aluminium ( $\text{Al}_i$ ), deposits on negatively charged ions forming complex polymers. Studies show that temperature and pH levels are crucial catalysts in the degree of ongoing polymerization (Poléo, Lydersen, & Muniz, 1991). These chemical processes are potentially toxic to fish.  $\text{Al}_i$  polymerize on chemically negatively charged compounds, both organic and inorganic (Poléo et al., 1991). Potentially, it may do so on fish gills as the mucus covering the gill is rich in negatively charged sites (Poléo, 1995; Nilsen et al., 2010). Depending on duration of exposure, the concentration of  $\text{Al}_i$  ions in the water, pH, temperature and concentration of total organic carbon (TOC), water might become toxic to fish (Lydersen, Salbu, & Poléo, 1992; Poléo & Muniz, 1993). When the fish gills are subject to clogging by  $\text{Al}_i$ , the gills produce more mucus as a defensive mechanism to rid itself of the irritation on the gills. This leads to more mucus available for complexation with aluminium leading to hypoxia and subsequent suffocation (Poléo, 1995). Laboratory experiments has shown that different fish species have different tolerance levels to the above-mentioned chemical and physical factors (Poléo et al., 1997). If the water has high ANC and TOC values, the overall Al-toxicity of the water is decreased, partly by increased competition by base cations for the binding sites on the fish gill, and partly by complexation of aluminium in the water by organic (Poléo, 1995; E. Lydersen, T. Larssen, & E. Fjeld, 2004).

In Norway, the first reports on fish deaths in hatcheries, lakes, and rivers in the southernmost part of the country date back to 1920 (Henriksen et al., 1989). However, it was not until the late 1950's that it was discovered that the increased acidity was linked to the acidity of the precipitation – and the term “acid rain” was brought to the public's attention (Gorham, 1958).

Acidic precipitation effected many fish populations within large areas. In the southern half, including east and west of Norway, fish populations were at best reduced, and at worst extinct (Henriksen et al., 1989). Since the 1970's, large efforts have been done both nationally and internationally, to reduce the emissions of acidifying compounds, such as the Gothenburg Protocol from 1999. This has resulted in a notable decrease in emissions of sulfuric and

nitrogen compounds (Klimont, Smith, & Cofala, 2013; Garmo et al., 2014). In freshwater systems pH has increased, and aqueous aluminium concentrations has decreased. Along with the improved water quality, aquatic biota, including fish populations have started to recover (Hesthagen, Fiske, & Saksgård, 2016). On the other hand, a general long-term damage might be the depletion of soil base cation levels (reduced ANC), caused by the long period with acidic precipitation (Watmough & Dillon, 2001). A water chemical recovery from acidification in Europe has accrued, and indications are primarily due to the decreased sulphur emissions (Garmo et al., 2014). With the chemical recovery of surface waters, several studies have shown that ANC levels, adjusted for TOC are a key factor in biological recovery (Hesthagen, Fiske, & Skjelkvåle, 2008; Hesthagen et al., 2016).

As atmospheric sulphur and nitrate levels has decreased, acidic precipitation is no longer the main issue (Garmo et al., 2014). Based on this, the lime treatment of surface waters in Norway has been reduced by 800 (pr. 2014) locations (Miljødirektoratet, 2016). However, some catchment areas may still have deposits of sulphur and nitrogen compounds in the catchment areas that exceed the natural tolerance limit (Lydersen et al., 2002; Westling & Sahlén Zetterberg, 2007). In those areas water chemistry will deteriorate when the anions are mobilized from the catchment area and continued lime treatment may be the only viable option to preserve aquatic biota (Clair & Hindar, 2011).

## 1.2 Study species

Brown trout is widely distributed in Norway, because of natural migration and by human introductions in the entire country. Although regarded as one species, variations in genetics and life history traits results in huge variation within the species such as habitat selection, lifecycles and feeding patterns (Jonsson, 1989; Klemetsen et al., 2003b). The brown trout is iteroparous, however, females may not survive more than one spawning, if conditions are too tough (Jonsson, 1989; Klemetsen et al. 2003). Brown trout has high variability in its life history traits and can adapt to several different environments (Jonsson & Jonsson, 2011). Brown trout have a wide variation in food and habitat selection, both within and between habitats. This results in a corresponding variation in size, growth rate and age at mature stage (Jonsson & Jonsson, 2011). Spawning usually occurs in tributaries or rivers during autumn, between September to December (Fleming & Reynolds, 2004). Water temperature, chemistry and oxygen saturation are all key factors for the embryo development (Einum, Hendry, & Fleming, 2002). The brown trout lays its eggs in the riverbed in a hole dug up by the female (Fleming & Reynolds, 2004). After spawning she covers the eggs with gravel and small stones. Hatching occurs in the following spring, and during the first weeks after hatching the small alevins feed on their yolk sac (Jonsson & Jonsson, 2011). The alevins are particularly sensitive to acidic aluminium rich water (Carrick, 1979b). When the yolk sac is digested, they emerge from the gravel and start feeding on their own. Young brown trout's display a somewhat aggressive and territorial behaviour, in their intense competition for nutrition (Jonsson & Jonsson, 2011). Their preference of habitat is determined by factors such as shelter, water flow velocity, depth and food abundance (Jonsson & Jonsson, 2011). After some time, it is common for brown trout to migrate from the tributaries where they hatched to a larger body of water, such as a larger river or lake. This is most often followed by a change in its feeding habitats and behaviour, and usually increased its growth rate (Jonsson, 1989).

In this study, brown trout (*Salmo trutta L.*) and minnow (*Phoxinus phoxinus L.*) were the only fish species found. The abundance of brown trout and minnow is used as biological indicator

for recovery after acidification, as studies have shown that these species are display different sensitive to acidic water (Poléo et al., 1997).

### **1.3 Main goal of this study**

The aim of this study is to see if the lime treatment influences brown trout and minnow in four tributaries draining into Lake Råsjøen.

- Is there a link between the water chemical parameters and the abundance of brown trout?
- Does the chemical parameters effect life history traits of brown trout in the four tributaries?

## 2. Material and Methods

### 2.1 Study Area

#### 2.1.1 Lake Råsjøen and its catchment area

The study area consists of four tributaries, all draining into Lake Råsjøen, in Nannestad municipality, Viken County, Norway (figure 1). Each tributary was given a letter code (A-D), divided into five stations of 100 m<sup>2</sup> with a number code (1-5). The lowest number was always the lower station closest to the outlet into Lake Råsjøen, with increasing number upstream along the tributary.

All four tributaries give the same possibility for brown trout migrating up the tributaries to spawn. The catchment areas of the four tributaries vary in size and properties in term of number of lakes and ponds from where the four tributaries originate (table 1). Amount of snow and time of spring floods vary between years (figure 2). Since the tributaries are relatively small, their water flow regime is sensitive to the amounts of precipitation, causing water levels to rapidly increase and decrease.



Figure 1: Map of the study area around Lake Råsjøen in Viken County, Norway, with the four tributaries marked: Elsjøbekken (A), Storrsjøbekken (B), Botnetjernsbekken (C), and Trasetjernsbekken (D).

(C), Trasletjernsbekken (D). The red lines indicate where stationary antennas for PIT-tag registration were placed. The other tributaries draining to Lake Råsjøen, was excluded from the study as they are prone to drought.

Table 1: Size of Lakes in study area and catchment areas to Tributaries Elsjøbekken (A), Storrsjøbekken (B), Botnetjernsbekken (C), Trasletjernsbekken (D) and Lake Råsjøen. X and Y are coordinates in UTM WGS 84.

| Site                 | Catchment area | Order | Lake area km <sup>2</sup> | MASL (m) | X      | Y       |
|----------------------|----------------|-------|---------------------------|----------|--------|---------|
| Søndre Bakkeholtjern | A              | 1     | 0.0266                    | 518      | 269458 | 6678742 |
| Nordre Bakkeholtjern | A              | 2     | 0.01                      | 515      | 269312 | 6678940 |
| Svartvann            | A              | 3     | 0.063                     | 507      | 268818 | 6679091 |
| Store Elsjø          | A              | 4     | 0.1527                    | 509      | 269238 | 6679432 |
| Lille Elsjø          | A              | 5     | 0.0548                    | 498      | 268929 | 6679938 |
| Kolsjøen             | B              | 1     | 0.0867                    | 538      | 266485 | 6681341 |
| Stårsjøen            | B              | 2     | 0.0256                    | 476      | 267534 | 6680908 |
| Botnetjern           | C              | 1     | 0.0126                    | 460      | 265962 | 6682624 |
| Kroktjern            | D              | 1     | 0.0113                    | 517      | 268288 | 6684333 |
| Torestjern           | D              | 2     | 0.0265                    | 508      | 268004 | 6683880 |
| Trasletjern          | D              | 3     | 0.0317                    | 473      | 268704 | 6683446 |
| Råsjøen              | All            |       | 0.885                     | 436      | 267242 | 6682233 |

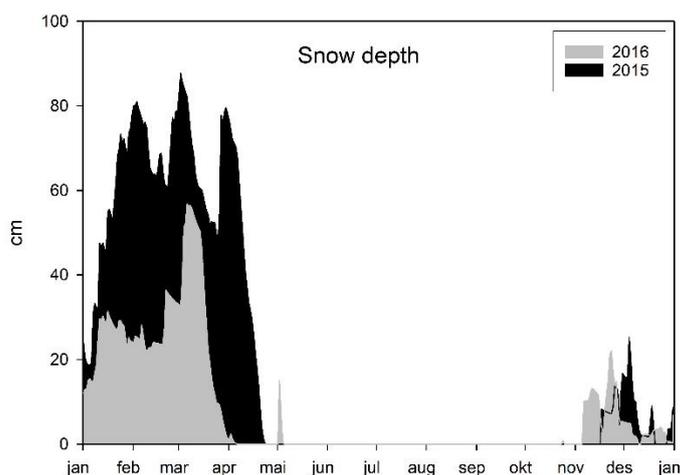
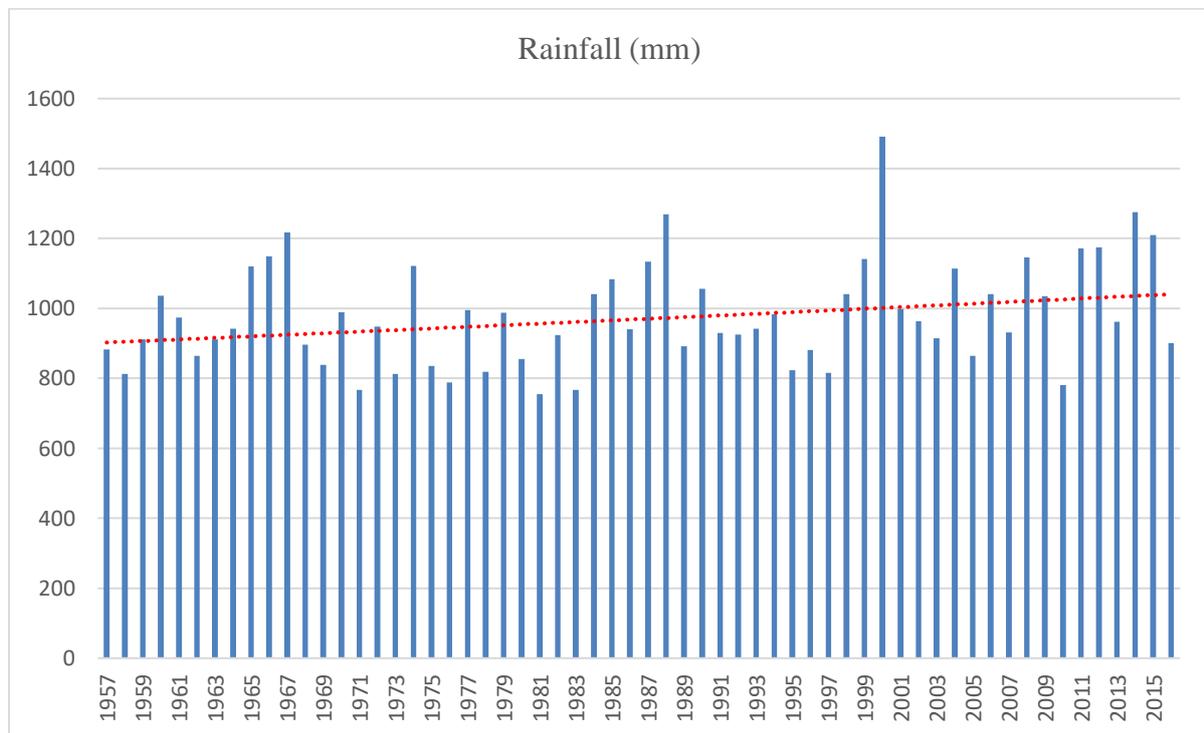


Figure 2: Presented is the amount of snow (cm) in the catchment area of Lake Råsjøen in 2015 (black segment) and 2016 (grey segment) by month. (Data form The Norwegian Meteorological Institute).

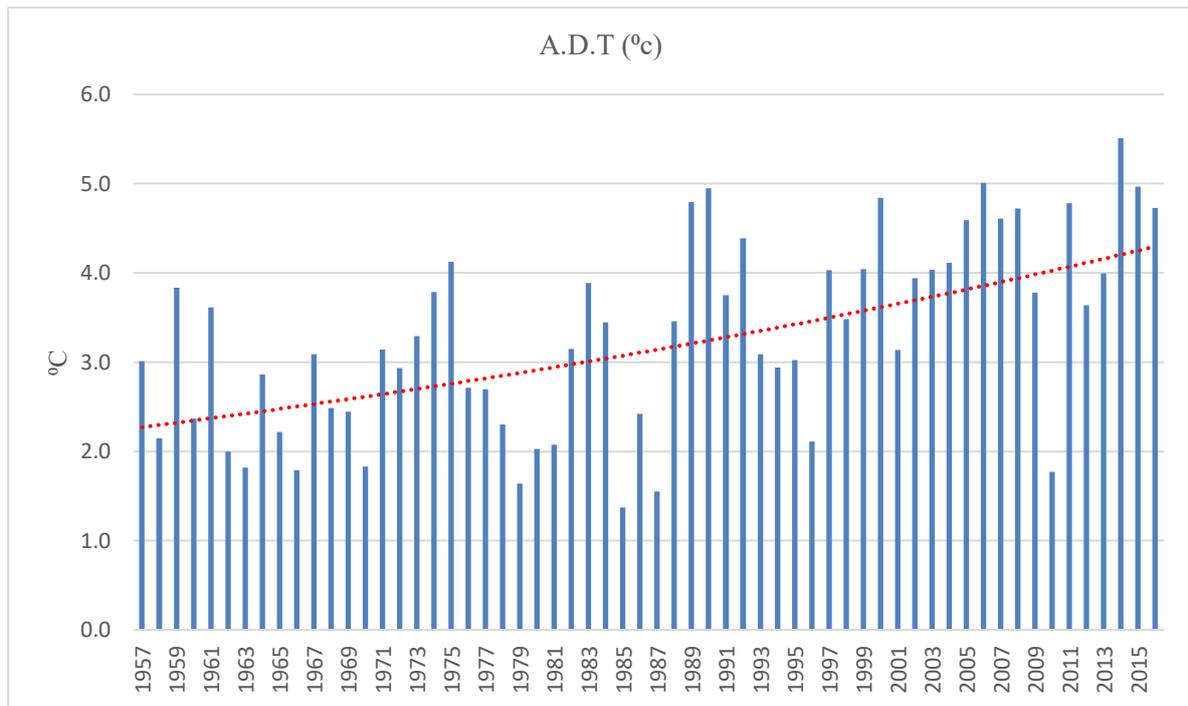
The amount of snow and the time of the spring flood varies between the years (figure 2). In both years there is a repaid decline in the amount of snow in the catchment area of Lake

Råsjøen. In 2015, all the snow has melted in the last week of April. In 2016 the snow melted in late March. The occurrence of the spring flood varied with one month between the two years.



*Figure 3: The total amount of rainfall in mm/year in the catchment area of Lake Råsjøen in the years 1957 – 2016. (Data from The Norwegian Meteorological Institute).*

During the last 61 years, there has been a small general increase in the amount of rainfall in the catchment area.



*Figure 4: Shows average day air temperature (A.D.T) development in the catchment area of Lake Råsjøen, in the years 1957 – 2016. The model shows that the average day temperature has increased from 1957 to 2016. (Data form The Norwegian Meteorological Institute).*

The average day-temperature/year presented in figure 4 is overall increasing in the catchment area of Lake Råsjøen during the last 61 years. It is reasonable to assume that the average water temperature of tributaries is also increased during this period, but this study lacks historical water temperature data to support this assumption.

## 2.2 The tributaries to Lake Råsjøen

A total of eleven tributaries is draining into Lake Råsjøen (figure 1). Of the eleven, only four tributaries have a stable water flow through the year. As a result, these four were selected for this study. To get compatible results, the same methods for data collection was applied to the selected tributaries.

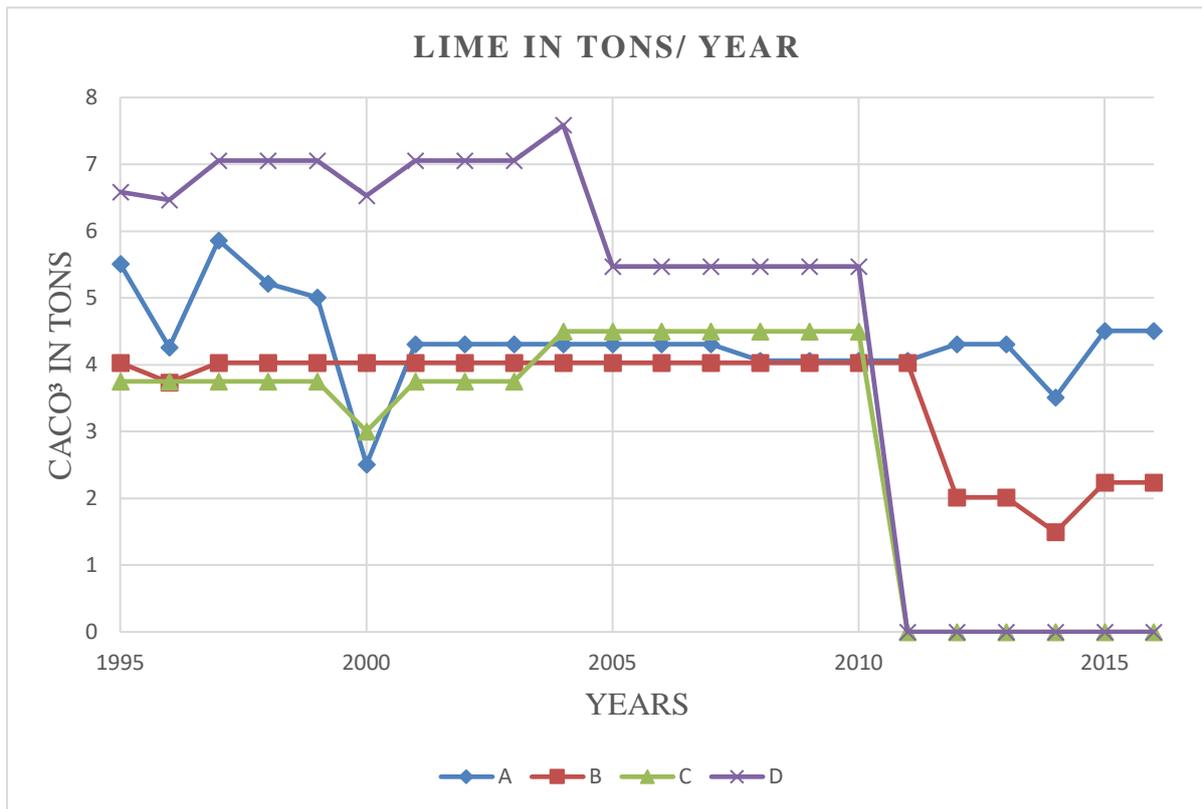


Figure 5: Amount of lime ( $\text{CaCO}_3$ ) in tons added for each of the four tributaries catchment areas in the years 1995 - 2015. Tributary A: Elsjøbekken (Blue line), tributary B: Storrsjøbekken (red line), tributary C: Botnetjersbekken (Green line) and tributary D: Trasletjersbekken (purple line). (Data provided by County Governor in Oslo and Viken).

The history of supply of lime and the current lime treatment in the four tributaries are different. Presented in figure 5 is a visual representation of the lime treatment regimes in the four tributaries of this study. Waterbodies in the catchment area of tributary A has been limed continuously since 1995 until the present study was undertaken, with approximately the same amount of lime each year ( $\pm 0.67$  tons) since 2001. Tributary B has been treated with lime during the same period as tributary A, but the amount of lime was reduced by 50 % in 2012. Tributary A and tributary B are still being treated with lime during this study. Tributary C received roughly the same amount of lime each year ( $\pm 1.5$  tons) until lime treatment was ceased in 2011. Tributary D was supplied with averagely 6.95 tons ( $\pm 0.58$  tons) lime from 1995 to 2004. From 2005 to 2010 tributary D yearly received 5.5 tons lime. Lime treatment was ceased in tributary D in 2011.

The stream morphology is roughly similar in the four tributaries included in this study. The four tributaries have areas with suitable spawning substrate for brown trout, high variance in pools, riffles, and bottom substrate size within tributaries. Suitable habitat for juvenile brown trout, such as bigger rocks and undercut banks are also present in all four tributaries.

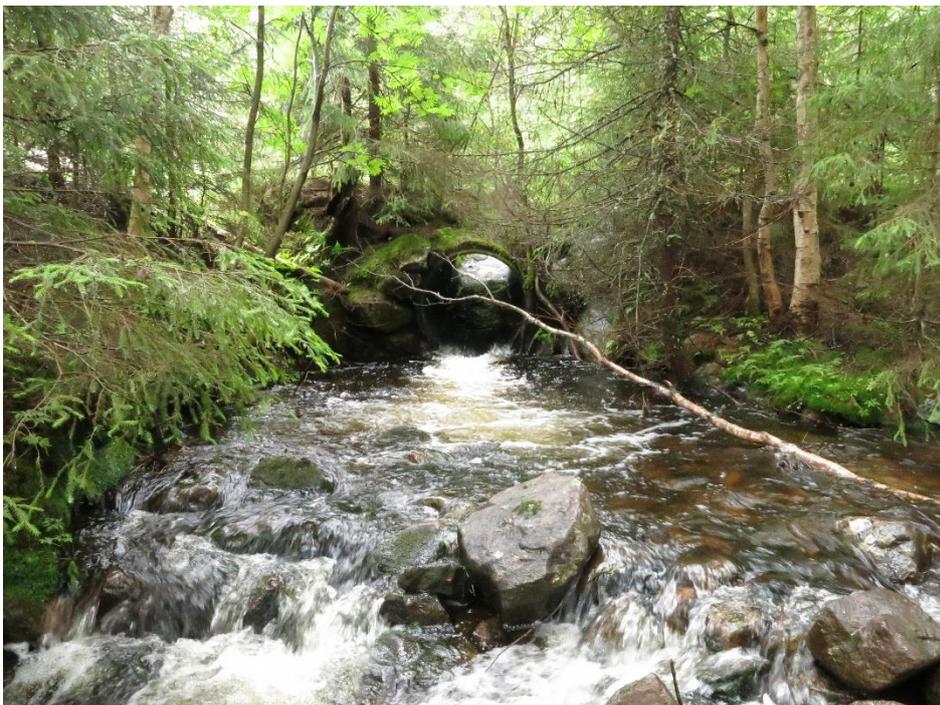
### 2.2.1 Tributary A: Elsjøbekken.

Tributary A (figure 1) is the only tributary in this study where the entire catchment area is presently treated with lime. Closest to Lake Råsjøen, the first 50 m of the tributary is

dominated by sand, with a mosaic of gravel and pebbles. The substrate of the next 100 m is dominated by rocks and gravel, considered to be optimal spawning substrate for the brown trout (figure 6). Further upstream the topography is slightly steeper, and larger rocks and boulders dominate. There are, however, parts with gravel and thereby spawning habitat for brown trout. The tributary has natural variation in vegetation along its banks, undercut banks occur and its natural course is intact. There are two manmade objects in the tributary, a culvert (figure 7), and a small dam. The small dam is further upstream than the study area. Spawning brown trout from Lake Råsjøen have been observed upstream of both installations, so they are likely not preventing fish to pass, at least not during normal water flow.



*Figure 6: Dominant substrate in the first 150 m of Elsjøbekken (tributary A).*



*Figure 7: The pipe in tributary A.*

## 2.2.2 Tributary B: Storrsjøbekken

Tributary B (figure 1) exhibits a bit different stream morphology compared to tributary A. Stations B1 – B4 are quite like stations A4 and A5 in tributary A. Station B5 is dominated by sand and mud in the tributary. The road and the pipe between B4 and B5 act like a small dam so stream velocity is very slow in B5, compared to the rest of tributary B. The pipe under the road could represent a possible obstacle for small fish and adult fish during low water flow (figure 8). However, brown trout captured and marked downstream the pipe have been recaptured upstream of the pipe. There are relatively steep, natural waterfalls between station B3 and B4 but there is no indication that this is an obstacle, as marked brown trout have been registered here by the backpack reader. The rest of the tributary runs its natural course, has in general suitable substrate for spawning and natural vegetation along its banks.



*Figure 8: Tributary B, Storrsjøbekken runs through a small pipe under a road. During normal water flow the pipe is possibly an obstacle for brown trout.*

## 2.2.3 Tributary C: Botnetjernsbekken

Tributary C (figure 1) has a similar stream morphology to tributary B, but it is smaller and is more sensitive to drought. Beyond station C5, approximately 300 meters upstream from Lake Råsjøen it runs through marshes, the water flow is slow, and the bottom substrate is dominated by mud, unfit substrate for spawning brown trout. However, during draughts and low water-levels in the tributary, this portion may serve as a refuge for brown trout. Stations C1 – C4 runs seemingly naturally, with natural vegetation along its banks. Station C5, might have been made more straight than natural, as it partially runs along a forest road. Between station C3 and C4 the road crosses the tributary by means of a concrete bridge. There is no indication that this bridge prevents movement of fish. In general, the entire tributary houses suitable substrate for spawning brown trout (figure 9).



*Figure 9: Tributary C, Botnetjernsbekken is dominated by larger rocks and gravel at the tributary floor.*

#### **2.2.4 Tributary D: Trasletjernsbekken**

The first station of tributary D (D1, figure 1) is defined by large boulders and steep slopes with high discharge. There are small pockets of substrate present, that can be used by brown trout to spawn, but the amount is limited, compared to control tributary A. However, at the inlet to Lake Råsjøen, it runs in a wide fan with plenty of suitable substrate for brown trout to spawn. Stations D2 - D3 exhibit similar stream morphology to tributaries B and C, with suitable substrate for brown trout to spawn. Tributary D originates from the small pond Trasletjern, which have been used as a reference location for ceased lime-treatment by NIVA since 2005 (Hindar, 2008). A waterfall on the border between stations D1 and D2, might serve as an obstacle for fish, during low water flow (figure 10).



*Figure 10: Tributary D, Trasletjernsbekken, the border between station D1 and D2 is beyond this waterfall. Station D1 is dominated by boulders and large rocks in station 1, with a few pockets of substrate suited for brown trout spawning.*

### **2.3 Mounted installations.**

To monitor the temperature and relative barometric pressure in the four tributaries, HOBO U20L data logger was deployed (figure 12). Temperature (°C) and relative barometric pressure (kps) was automatically recorded once each hour. This was done to account for potential differences in temperature between the tributaries which may affect the findings. Fish are exothermic animals, so potential different temperatures in the four tributaries could impact growth rate (Elliott, 1976). The temperature and pressure loggers were mounted inside protective plastic cylinders, to ensure they were not damaged by debris during floods. Loggers were then placed in the tributaries on the bottom of poles with a relative water gauge mounted (figure 11). All loggers were activated and placed in the four tributaries in July of 2015 and retrieved in November of 2016. To collect the data, the logger was placed in a shuttle connected to USB interface HOBO base station U-4 and analysed by HOBOWare Pro software.



*Figure 11: A water level gauge was mounted in a small pool to have a reference point on water levels. The gauge was bolted into rock, to make it withstand floods and debris. The same equipment was mounted in each of the four tributaries.*



*Figure 12: HOBOT U20L, barometric pressure and water temperature data logger. This device was set to record water temperature and barometric pressure once per hour. This yielded an expected battery life of > 2 years. The device was placed under water and mounted to the water level gauge.*

### 2.3.1 Antenna systems

All antenna systems, verification boxes and PIT-tags were supplied by Oregon Rfid<sup>®</sup>.

Single antenna HDX readers were mounted at the outlet to Lake Råsjøen in all the four tributaries (figure 13). This was done to monitor the marked brown trout's movement in and out of the tributaries. Brown trout was marked using Passive Integrated Transponder tags (PIT-tags). The antennas were powered through solar panels. As expected, there were not enough power to maintain operation of the antennas during winter when sunlight is scarce in Norway. To adjust for this, verification boxes were placed within the reading field of the antenna. It was set up to make a PIT-tag visible to the antenna every 30 minutes. When the tag was visible in the dataset upon collection, it verified operational time and down time of the antenna system. The antenna loop of the stationary antennas was suspended to the bottom of the tributaries to be as little exposed to debris (that could potentially damage or reduce the read efficiency) as possible. Dehumidify boxes were placed along with the electronic compartments to reduce humidity, which could damage the equipment. All electronics connected to the stationary antennas were placed in an aluminium box (figure 14).



*Figure 13: Stationary PIT-tag antenna mounted at the inlet of a tributary to Lake Råsjøen. In the tree in the upper left corner of the image, is the solar panel, powering the station inside the aluminium box. Inside the box is the electronic components which register passing brown trout marked with PIT-tags.*



*Figure 14: The inside of the aluminium box containing the PIT-tag antenna registration station with the following components 1: PIT-tag registration box, 2: batteries, 3: Solar panel regulator box, 4: Dehumidifier to reduce humidity inside the box.*



*Figure 15: Backpackreader operator in action. The portable antenna system is used to account for marked brown trout still residing in the tributary. The extended plastic tube, with a loop in the end is the antenna, while the registration system and batteries are carried in a small backpack.*

A portable antenna, hereafter referred to as the backpack reader (BPR) (figure 15), was used in the four tributaries as a secondary registration system. The BPR supply registrations of brown trout marked with PIT-tag in the tributaries that has not left the tributary. This portable antenna was used within the limits of the five stations, in addition to an extra 100 m<sup>2</sup> upstream. A test of the detection probability was done to ensure that this was a credible method. First an operator went through the area with the BPR. Then the same area was fished using an electrical

fishing apparatus. The fish caught was then scanned with the handheld easy tracker. When the data from this small experiment was analysed, we found an estimated detection probability of 80 %. In part, the BPR could now account for marked brown trout, still residing in the tributary, not possible to register by the stationary antenna.

### **2.3.2 Water chemical parameters**

The water chemical parameters pH and conductivity were registered in field when the equipment was available. The apparatus used was WTW 3420 multimeter with a pH electrode: WTW Sensolyt 900-P and a conductivity electrode WTW TetraCon 925. The pH electrode was calibrated with buffers of pH 4.01, pH 7.0 and 10.0 before use, to ensure precise measurements.

Water samples from each of the four tributaries were collected on eight different occasions from the 25<sup>th</sup> of October 2015 to the 28<sup>th</sup> of March 2017. The samples were sent to Eurofins laboratory in Norway for further analysis. The complete list of parameters analysed, with International Organization for Standardization (ISO) are listed in the appendix section of this thesis. (*Appendix Table 1*).

## **2.4 Study species**

Data collection on study species was performed in 2015 and 2016. Brown trout and minnow were captured using an electric fishing apparatus. This was done in mid-August of both years. In October – November 2015 and 2016, spawning brown trout were captured with an electric fishing apparatus. Length and weight of all brown trout and minnow were registered. Brown trout > 80 mm, including spawning brown trout in autumn, were marked using PIT-tags to monitor body growth and movement patterns upon recapture.

### **2.4.1 Capture and marking of brown trout**

All five stations in each tributary was fished with an electrical fishing apparatus (*geomega FA-3*) three times with  $\geq$  half an hour between each removal, in accordance to Norwegian standard methods of Electrical fishing. (Bohlin et al., 1989). Captured brown trout and minnow body length was recorded down to nearest millimetre, and body weight to the nearest point zero gram using an electronic scale. The scale used was “My weight iBalance i5500<sup>®</sup>” with an accuracy of 0.1g. Scales were collected from brown trout for age determination and tissue was collected from each brown trout, by removing a piece of the adipose fin. By removing the adipose fin, marked brown trout would have a visual marker to prevent a double marking by PIT-tag upon recapture.

Brown trout with a body length of > 80 mm were sedated using Benzocaine interspersed to 5ml /10l water concentration. The brown trout were one at a time put in a bucket with the sedative. When the trout no longer responded to handling, it was marked by making a small incision in the abdomen (figure 16) using an ethanol (96 %) sterilized scalpel. An ethanol sterilized PIT-tag was placed inside the abdomen. Individual number of PIT-tags were recorded for each brown trout during the tagging process using Oregon Rfid’s Easy tracker for full-duplex (FDX) and half-duplex (HDX) tags (figure 17). The brown trout were then given an ID-number corresponding with the tributary and station it was captured. The incision wound was then sterilized with a drop of ethanol and the brown trout put into a container with

water and added oxygen for surveillance. When the brown trout awoke and displayed natural behaviour, it was released in the same station and tributary as it was captured.

In 2016 all captured previously marked brown trout were registered with body length, body weight and capture station. This made it possible to register actual growth from 2015 to 2016 and potential movement. Both years, brown trout  $\geq 80$  mm were tagged using a 12 mm HDX-B (read only) PIT-tag and brown trout  $\geq 120$  mm were tagged with a 23 mm HDX-B (read only) PIT-tag. The treatment was done with the license from Norwegian Food Safety Authority (FOTS, ID 6700).



*Figure 16: Placement of a 12 mm PIT-tag in the abdomen of a brown trout. The placement of the PIT-tag in the abdomen meant that as little muscular functions of the brown trout were affected as possible. Furthermore, the PIT-tag would be closest to the bottom mounted antenna loop, increasing the likelihood of a successful registration upon passing the antenna.*



*Figure 17: Handheld PIT-tag reader. With the handheld reader, the number of the PIT-tag placed inside the abdomen of brown trout could be read. The long number on the display is the number of the PIT-tag.*

In 2015, 344 brown trout recruits  $\geq 80$ mm (12mm PIT-tag) and 178 migratory spawning brown trout (23 mm PIT-tag) were marked. The larger PIT-tag inserted in brown trout  $> 120$  mm, was done to increase detection probability (Burnett et al., 2013). In 2016, 463 brown trout recruits  $\geq 80$  mm (12 mm PIT-tags) and 185 migratory spawning brown trout (23 mm PIT-tag) were tagged. In sum, a total of 1170 brown trout were marked. In both years, an additional 1240 brown trout  $< 80$  mm and 82 minnows were registered with length and weight. Brown trout with a body length of  $< 80$  mm were not marked with PIT-Tag to prevent high mortality rates (Larsen et al., 2013). The consequence of this is that all brown trout  $< 80$  mm are statistically treated as 0+.

## 2.5 Statistical analysis

When using an electrical fishing apparatus, quantity of fish captured during three efforts within a defined area can be used to calculate estimated population size, catchability, and densities of fish within the defined area. In this study, all defined areas (stations) were 100 m<sup>2</sup>, to ensure compatible results. Populations size, catchability and densities were calculated using methods compiled in (Bohlin et al., 1989). Estimates were conducted for each of the five stations in each tributary and results were divided into brown trout body length  $\geq 80$  mm and  $< 80$  mm. It was then possible to compare results both within and between the four tributaries. Estimates were not conducted on minnow, as the observations were too few. Historical density results (# brown trout /m<sup>2</sup>) is provided by Norges Jeger- og Fiskerforbund, Akershus, collected by Bjørn Otto Dønnum (1998), unpublished data.

To analyse and present data, Microsoft Excel and JMP Pro 14.0, SAS institute were used. All field data was written into Excel and the PIVOT table function was used to sort the data. Generalized linear models were used to test if there were differences between tributaries and years on brown trout length and weight. Generalized linear models were made through JMP Pro 14.0.

Data from the stationary antennas were sorted in Excel using the filter function and pivot tables function. A lot of brown trout had been staying close to the antenna loop and been registered multiple times, creating vast amounts of data. This data was reduced to one registration pr.  $> 2$  hours pr. day pr. PIT-tag number, to make it more manageable.

### 3. Results

#### 3.1 Water chemistry

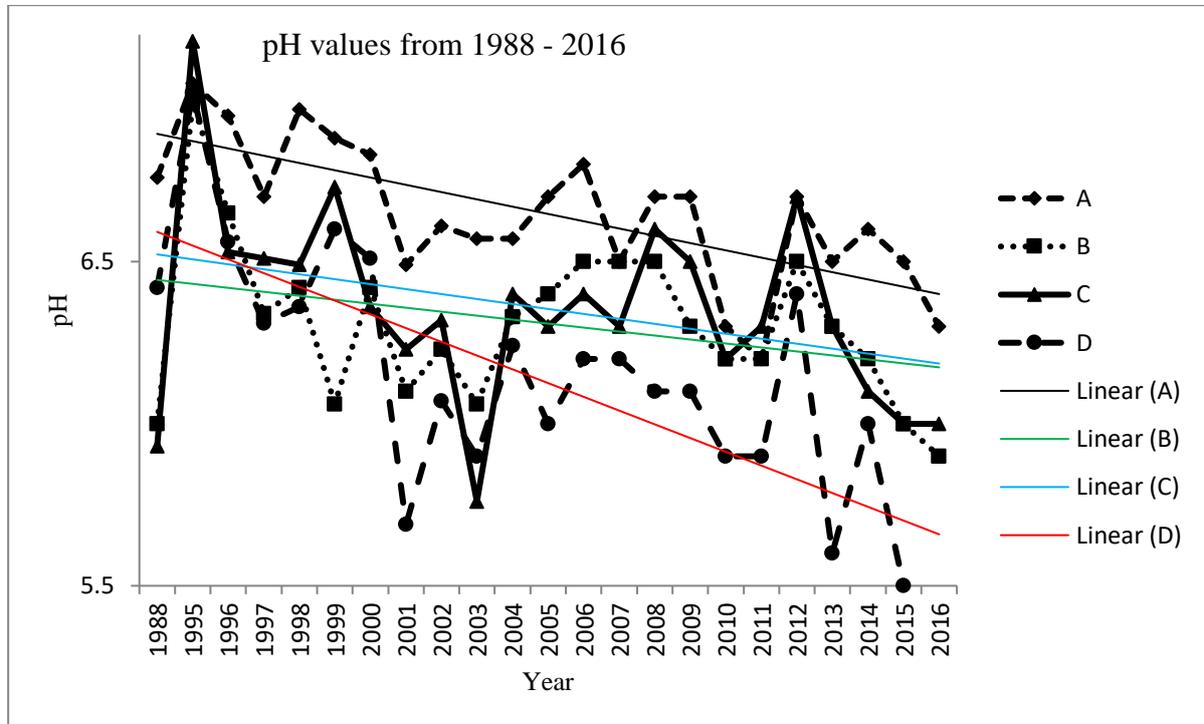


Figure 18: Development of pH values in the four tributaries from 1988 – 2016. A: Elsjøbekken. B: Stårnsjøbekken. C: Botnetjernsbekken and D: Trasletjernsbekken. Linear models show the average decline of pH values. Data provided by Fylkesmannen i Oslo og Viken.

The development of pH levels is decreasing in all four tributaries in the period from 1988-2016 (figure 18). Tributary D (red line) has the steepest level of decrease in pH levels, compared to tributaries A, B and C. Tributary A (black line) has had a steeper decline in pH levels than tributaries B and C, but has in general higher values. Tributaries B (green line) and C (blue line) has the most stable pH values, of the four tributaries.

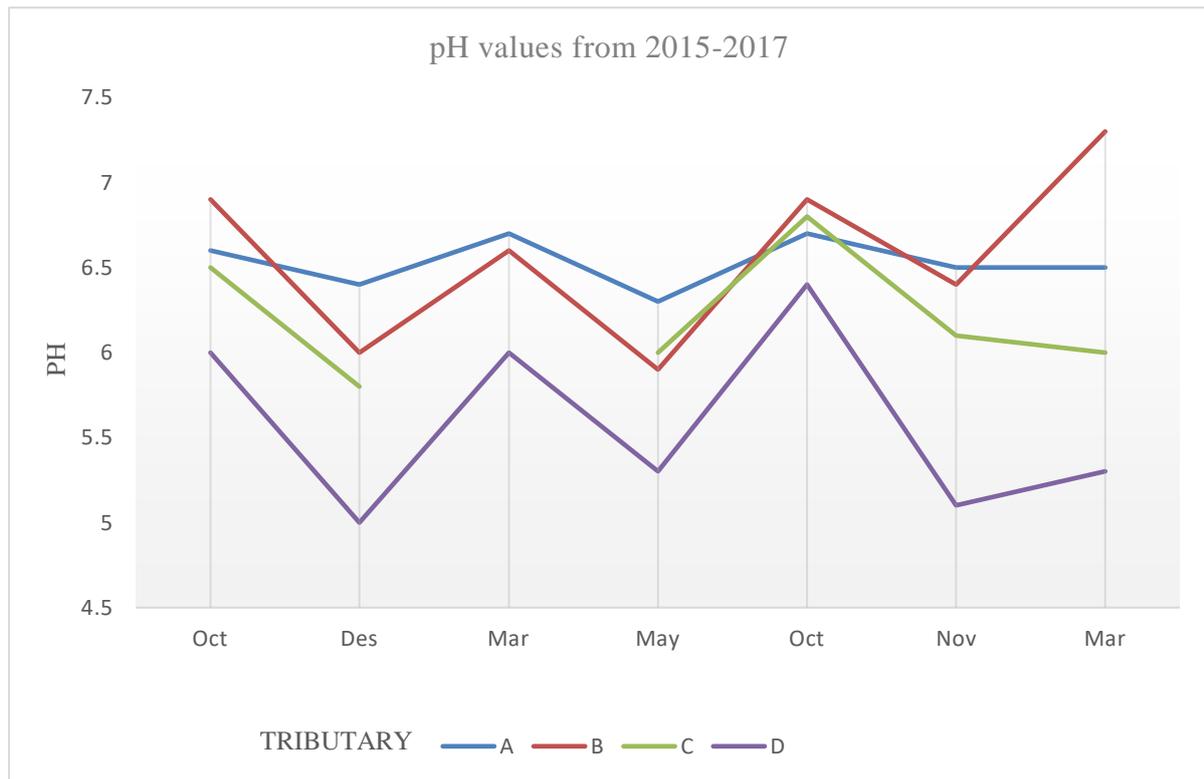


Figure 19: pH values from water samples collected and sent to an external lab. Values fluctuate with each sample. Tributary C lacks one sample in March 2016.

The fluctuations in pH levels roughly correspond between tributaries and samples (figure 19). The findings of this study show that the average pH levels from tributary A (pH average  $6.5 \pm 0.4$ ) and B (pH average  $6.5 \pm 1.4$ ), are similar, but with higher fluctuations in tributary B. Tributary C (pH average  $6.2 \pm 1.0$ ) has a smaller average than tributaries A and B. Tributary D (pH average  $5.5 \pm 1.4$ ) has the lowest average pH level of the four tributaries.

Table 2: Shows field measurements of pH and conductivity ( $\mu\text{S}/\text{cm}$ ) along with the relative water level from the water level gauge placed in each of the four tributaries.

| Date       | Tributary | Relative water level (cm) | pH   | $\mu\text{S}/\text{cm}$ |
|------------|-----------|---------------------------|------|-------------------------|
| 04.08.2015 | A         | 42                        | 6.66 | 16.5                    |
| 13.10.2016 | A         | 36                        | 7.32 | 23                      |
| 05.08.2015 | B         | 50                        | 5.90 | 14.4                    |
| 13.10.2016 | B         | 27                        | 7.30 | 30.6                    |
| 05.08.2015 | C         | 55                        | 5.94 | 13.4                    |
| 13.10.2016 | C         | 26                        | 6.80 | 22.9                    |
| 05.08.2015 | D         | 50                        | 5.11 | 14.4                    |
| 13.10.2016 | D         | 30                        | 6.80 | 18.5                    |

Field measurements of water quality in 2015 data was done during a declining water level after a small flood. 2016 represent relatively low water levels (table 2). The relative water

level is values from the water level gauge mounted in each tributary. The results indicate that pH levels and conductivity decrease when water levels in the tributaries increase.

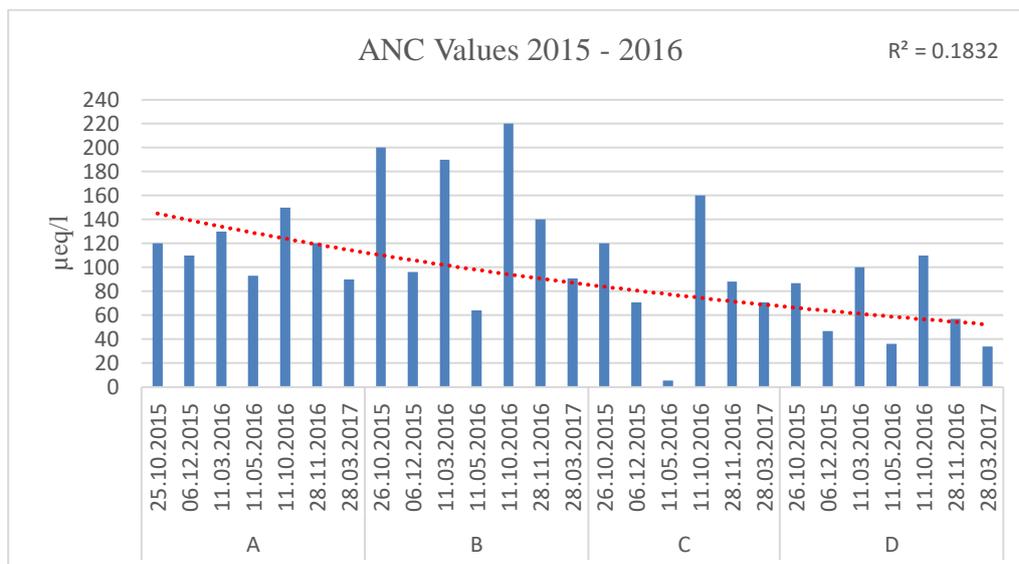
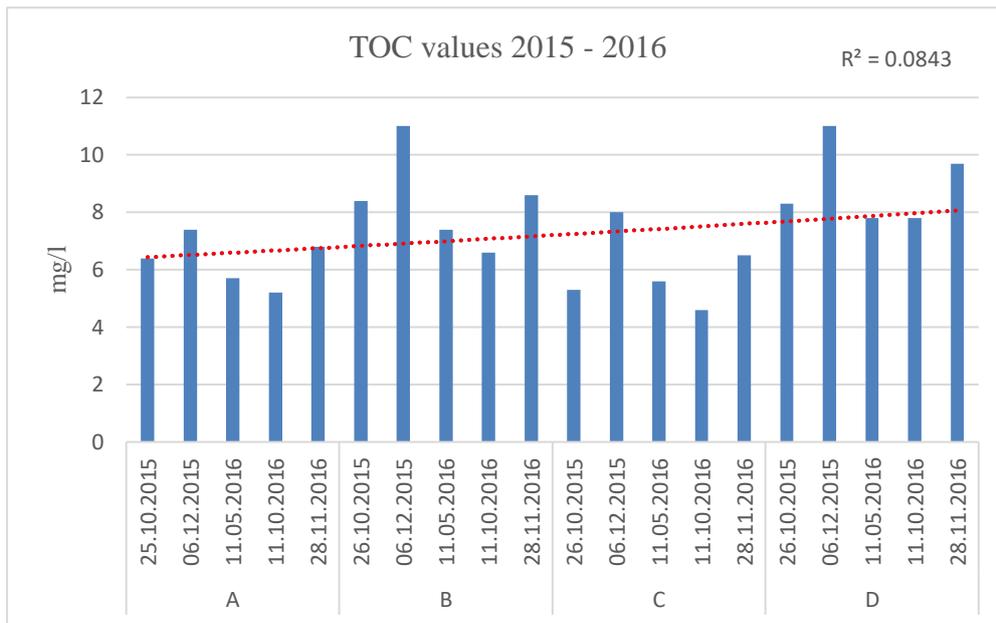


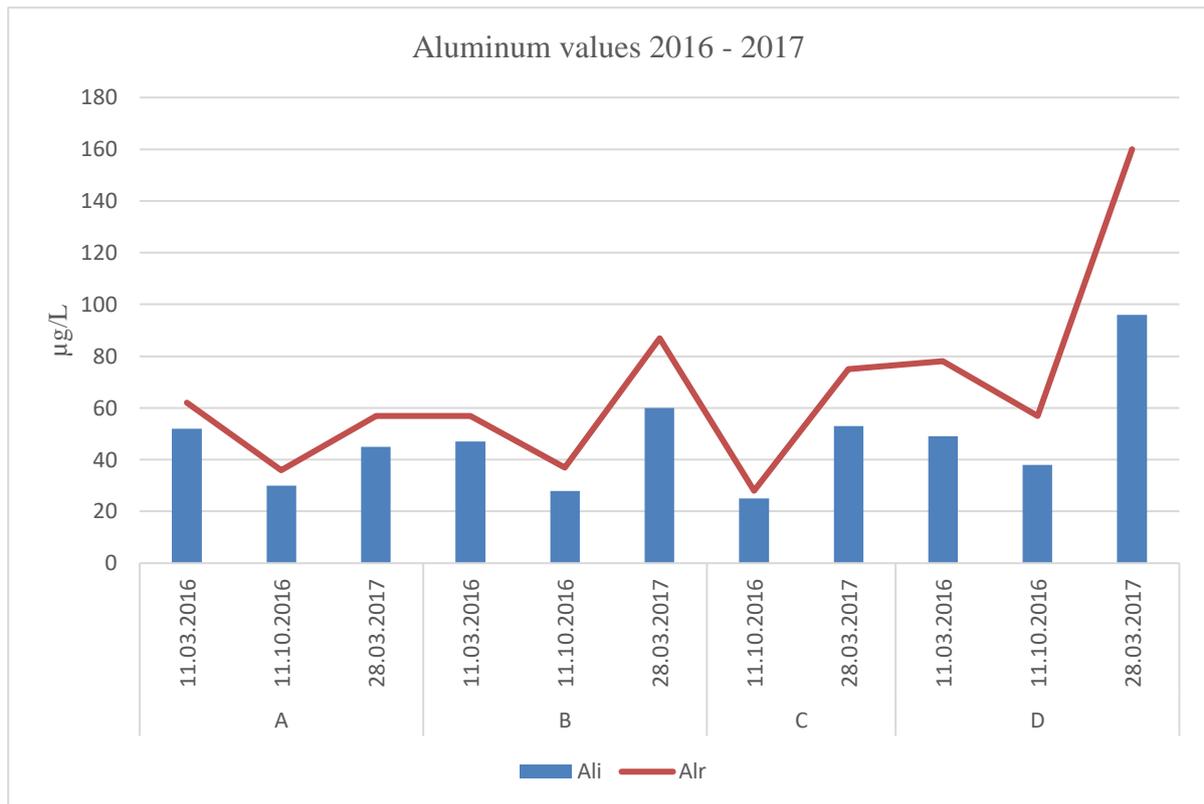
Figure 20: ANC (Acid Neutralizing Capacity) values from tributaries A (Elsjøbekken), B (Storrsjøbekken), C (Botnetjernsbekken) and D (Trasletjernsbekken) from 2015 to 2017. The linear model is the mean (red line) represent average ANC levels within and between tributaries.

The variation in ANC values are relatively stable ( $\pm 60 \mu\text{eq/l}$ ) in control tributary A, compared to tributary B ( $\pm 156 \mu\text{eq/l}$ ), tributary C ( $\pm 154.5 \mu\text{eq/l}$ ) and tributary D ( $\pm 76 \mu\text{eq/l}$ ) (Figure 20). The declining trend by tributary in ANC values corresponds with the lime treatment regimes in the tributaries (figure 5). The very low value in tributary C from the sample collected on the 11<sup>th</sup> of May 2016, is likely an error from the external laboratory, and could explain the low fit of the regression line ( $R^2 = 0.1832$ ).



*Figure 21: TOC (Total Organic Carbon) values from tributaries A (Elsjøbekken). B (Storrsjøbekken). C (Botnetjernsbekken) and D (Trasletjernsbekken). The linear model is the mean and indicates a small increase in TOC levels from tributary A to D.*

There are relatively even values of TOC in the four tributaries, with only a small degree of variation between samples (figure 21). The results show small fluctuations in TOC values between samples. Samples from tributary A average TOC  $6.3 \text{ mg/l} \pm 1.0$ . Tributary B, average TOC  $8.4 \text{ mg/l} \pm 5.6$ . Tributary C average TOC  $6.0 \text{ mg/l} \pm 3.4$ . Tributary D average TOC  $8.92 \text{ mg/l} \pm 6.4$ .



*Figure 22: The concentration of aqueous aluminium, divided into inorganic monomeric aluminium ( $Al_i$ ) and total aluminium ( $Al_r$ ) in three water samples collected between March 2016 to March 2017.*

The levels of aluminium fluctuate to some extent between water samples within tributaries (figure 22). Tributary C lacks one registration due to an error at the external laboratory. The values of both  $Al_i$  and  $Al_r$  are relatively stable between tributaries, except from the sample collected on the 28<sup>th</sup> of March 2017, where tributary D shows elevated aluminium levels.

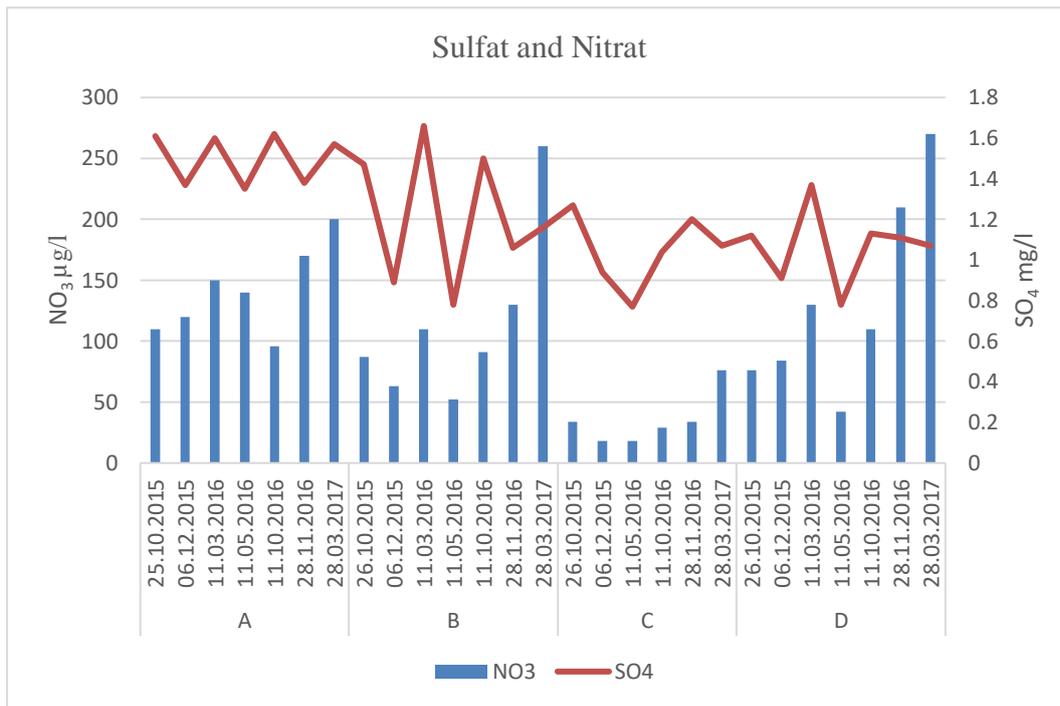
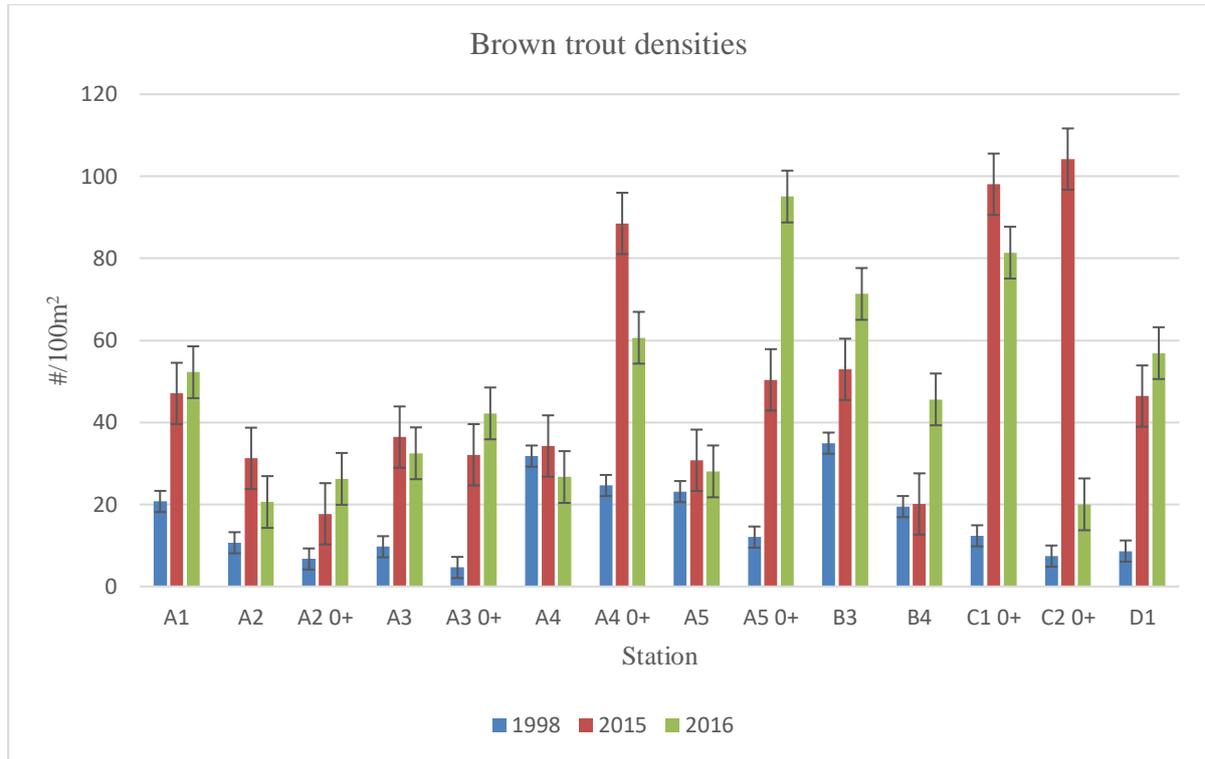


Figure 23: Concentrations of sulphate (SO<sub>4</sub>) and nitrate (NO<sub>3</sub>) from the seven water samples collected between 2015 and 2017 in tributaries A (Elsjøbekken), B (Storrsjøbekken), C (Botnetjernsbekken) and D (Trasletjernsbekken).

The concentrations of sulphate and nitrate varies between samples (figure 23). Sulphate concentrations in the samples have a decreasing trend form A-D but the sulphate fluctuates between samples. Tributary A generally has the highest values of sulphate (SO<sub>4</sub> average 1.5 mg/l, ±0.27), followed by tributary B (SO<sub>4</sub> average mg/l, ±0.88), tributary D (SO<sub>4</sub> average 1.07 mg/l, ±0.59) and tributary C (SO<sub>4</sub> average 1.04 mg/l, ±0.5).

The average concentration of nitrate was highest in tributary A (NO<sub>3</sub> average 140.9 µg/l, ±104), followed by tributary D (NO<sub>3</sub> average 131.7 µg/l, ±228), tributary B (NO<sub>3</sub> average 113.2 µg/l, ±208) and tributary C (NO<sub>3</sub> average 34.8 µg/l, ±58).

### 3.2 Brown trout



*Figure 24: Comparison of historical electro-fishing data on brown trout densities/100 m<sup>2</sup> ±SE (whiskers), between 1998 (blue columns) 2015 (red columns) and 2016 (green columns). 1998; unpublished data, Bjørn Otto Dønnum. Letter representing tributary A: Elsjøbekken, B: Storrsjøbekken, C: Botnetjernsbekken and D: Trasletjernsbekken and number is station. 0+ indicates data on brown trout <1 year old.*

The comparison of data from 2015 and 2016 (red and green columns), and historical data from 1998 (blue columns), suggest that there has been a general increase in the densities of brown trout (#/100 m<sup>2</sup>) from 1998 to 2015 and 2016 (figure 24). In general, values on brown trout < 80 mm (0+) fluctuates more between the years 2015 and 2016, then values on brown trout > 80 mm pr. station.

### 3.2.1 Brown trout (<80 mm)

*Table 3: Estimated population sizes of brown trout <80mm in the years 2015 and 2016 of each station in the four tributaries  $\pm$  SE and number of brown trout <80mm body length captured (n).*

| Station | 2015     |              |    | 2016     |             |    |
|---------|----------|--------------|----|----------|-------------|----|
|         | Pop. Est | $\pm$ SE     | n  | Pop. Est | $\pm$ SE    | n  |
| A1      | 79.57    | $\pm$ 23.10  | 52 | 83.33    | $\pm$ 13.23 | 64 |
| A2      | 23.02    | $\pm$ 1.49   | 22 | 34.10    | $\pm$ 8.73  | 26 |
| A3      | 41.73    | $\pm$ 21.66  | 25 | 54.85    | $\pm$ 6.22  | 47 |
| A4      | 115.06   | $\pm$ 120.76 | 41 | 78.83    | $\pm$ 17.40 | 56 |
| A5      | 65.50    | $\pm$ 20.66  | 43 | 123.58   | $\pm$ 92.16 | 51 |
| B1      | 92.82    | $\pm$ 12.60  | 73 | 82.01    | $\pm$ 24.28 | 53 |
| B2      | 18.72    | $\pm$ 1.20   | 18 | 79.59    | $\pm$ 19.26 | 55 |
| B3      | 52.53    | $\pm$ 2.38   | 50 | 44.52    | $\pm$ 14.03 | 31 |
| B4      | 6.54     | $\pm$ 1.28   | 6  | 13.38    | $\pm$ 3.88  | 11 |
| B5      | 4.04     | $\pm$ 0.24   | 4  | 9.54     | $\pm$ 1.16  | 9  |
| C1      | 100.03   | $\pm$ 5.98   | 90 | 83.01    | $\pm$ 4.12  | 77 |
| C2      | 100.03   | $\pm$ 5.98   | 90 | 19.22    | $\pm$ 4.35  | 16 |
| C3      | 66.88    | $\pm$ 31.70  | 38 | 15.34    | $\pm$ 7.41  | 11 |
| C4      | 93.74    | $\pm$ 8.32   | 80 | 8.01     | $\pm$ 0.12  | 8  |
| C5      | 38.11    | $\pm$ 10.68  | 28 | 23.39    | $\pm$ 10.80 | 16 |
| D1      | 14.68    | $\pm$ 22.98  | 7  | 21.85    | $\pm$ 3.57  | 19 |

The estimated population size of brown trout < 80 mm pr. station shows a high degree of variation within tributaries and between years (table 3). The higher the standard error value (SE), the higher statistical uncertainty of the observation, hence many stations have a high degree of uncertainty in the population estimates. In general, the standard error of the population estimates is high in all stations, with a few exceptions. Tributary D has only one station presented (D1), as this was the only station brown trout < 80 mm were captured.

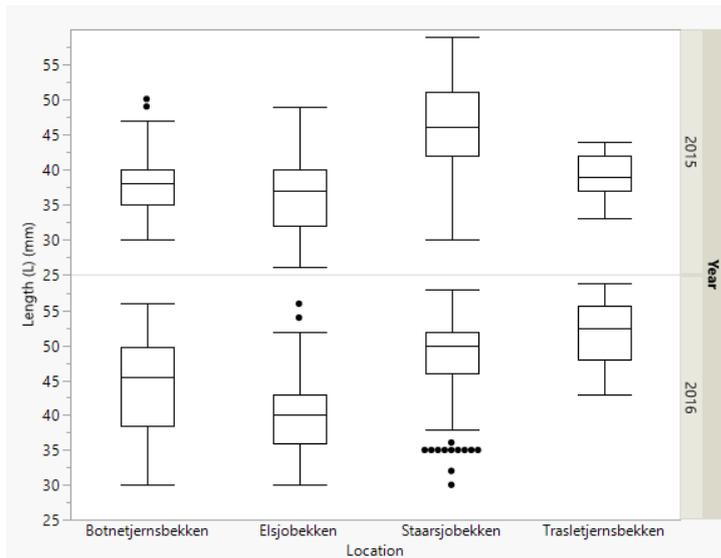


Figure 25: Boxplot showing body length distribution in tributary of brown trout < 80 mm between the tributaries and years. Tributary C (Botnetjernsbekken), A (Elsjøbekken), tributary B (Storrsjøbekken), and tributary D (Trasletjernsbekken) Boxes entail 50% of the observations with horizontal lines displaying medians. Black dots represent values outside the 25 percentiles (Outside normal distribution).

The findings on body length distribution of brown trout < 80 mm between locations and years shows that tributary D has even results between years (figure 25). In 2015 tributary A had the lowest variation in body length, compared to the other tributaries. In 2016 tributary D had the biggest body lengths of brown trout < 80 mm. Generally, no big differences in body length distribution between tributaries and years were found, when comparing results between tributaries.

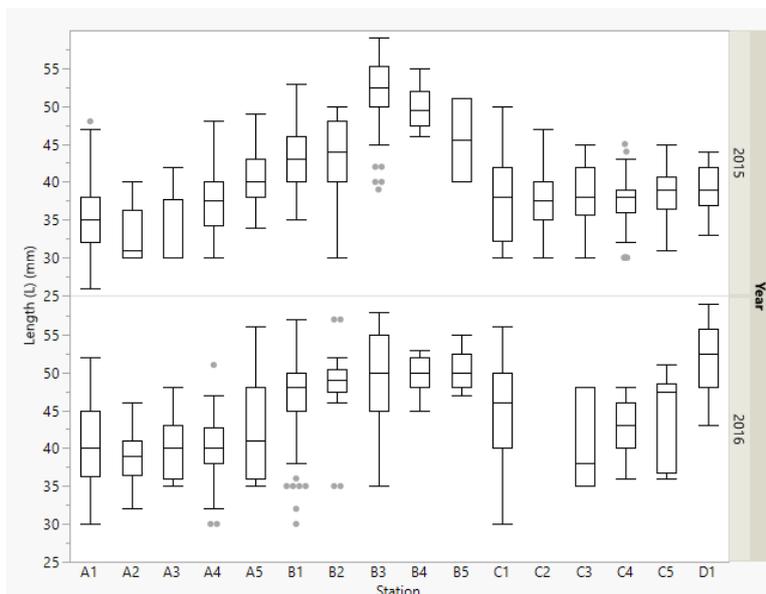
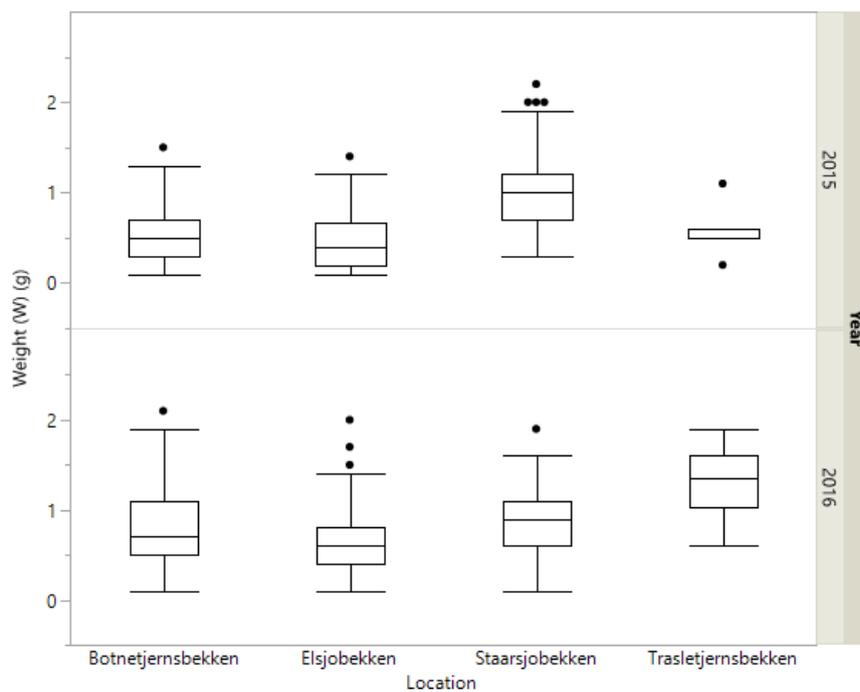


Figure 26: Body length distribution of brown trout < 80 mm between stations and years. Letter representing location and number representing station. Boxes entail 50% of the observations with horizontal lines displaying medians. Grey dots represent values outside the 25 percentiles (Outside normal distribution).

When comparing the body length of brown trout  $< 80$  mm between all stations and years the results show that there are higher differences in body length, then what is visible comparing locations and years (figure 26). Body length variation is most affected by station (*logworth 101.447*) and secondly by year (*logworth 29.853*). Both station and year are statistically significant ( $p < 0.001$ ) (*Appendix: table 5*). In 2016 station D1 had the biggest brown trout  $< 80$  mm, however, tributary D is only represented with one station, compared to the other three tributaries who is represented with five stations each. This is because station D1 was the only station in which brown trout  $< 80$  mm were captured.



*Figure 27: Body weight variation of brown trout  $< 80$  mm between tributaries and years. Boxes entail 50% of the observations with horizontal lines displaying medians. Grey dots represent values outside the 25 percentiles (Outside normal distribution).*

The variation in body weight of brown trout  $< 80$  mm between tributary are lesser in 2016 than in 2015 (figure 27). In 2015 the variation in body weight is very small in tributary D, compared to the other tributaries, likely as a result of the low number of brown trout  $< 80$  mm captured in tributary D (table 3, D1). Variation in body weight are mostly affected by tributary (*Logworth 27.538*) but also by year (*Logworth 6.479*). Both station and year are statistically significant ( $p < 0.001$ ). (*Appendix table 6*).

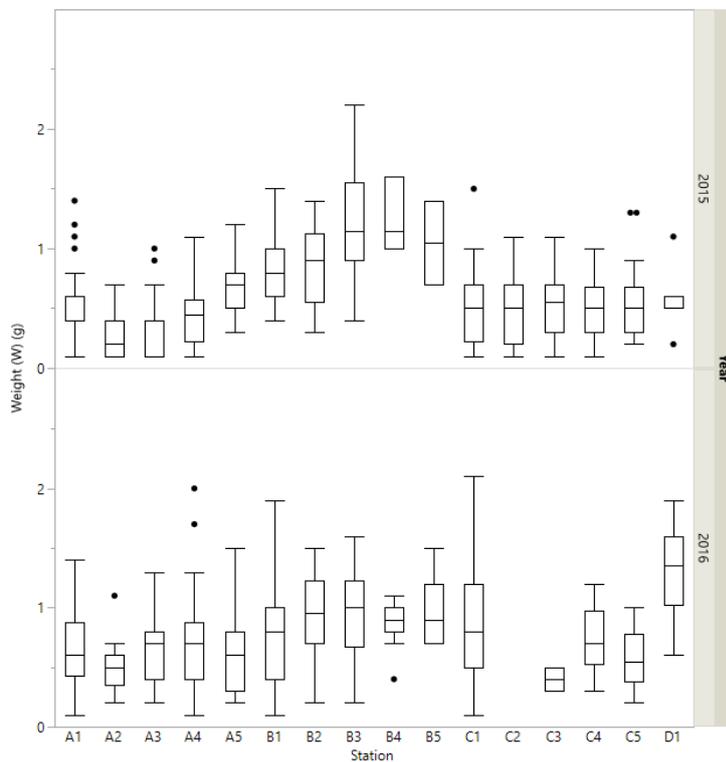


Figure 28: Weight distribution of brown trout < 80 mm between stations and years. Boxes entail 50 % of the observations with horizontal lines displaying medians. Grey dots represent values outside the 25 percentiles (Outside normal distribution).

The variation of weight is statistically significant between both station (Logworth 27.538) and year (Logworth 6.479) (figure 28). There are however a few exceptions, as station A2, B5 and C3, did not show statistical significance between weight and station due to too high variation. ( $p > 0.05$ ) (Appendix table 7).

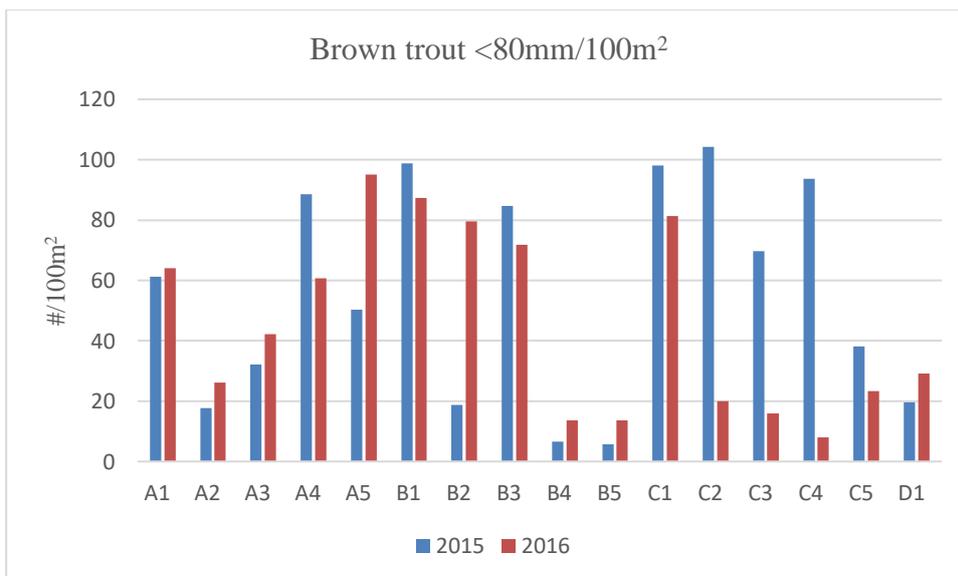


Figure 29: Brown trout < 80 mm/100 m<sup>2</sup>, distributed by stations and years A: Elsjøbekken. B: Storrsjøbekken. C: Botnetjernsbekken and D: Trasletjernsbekken.

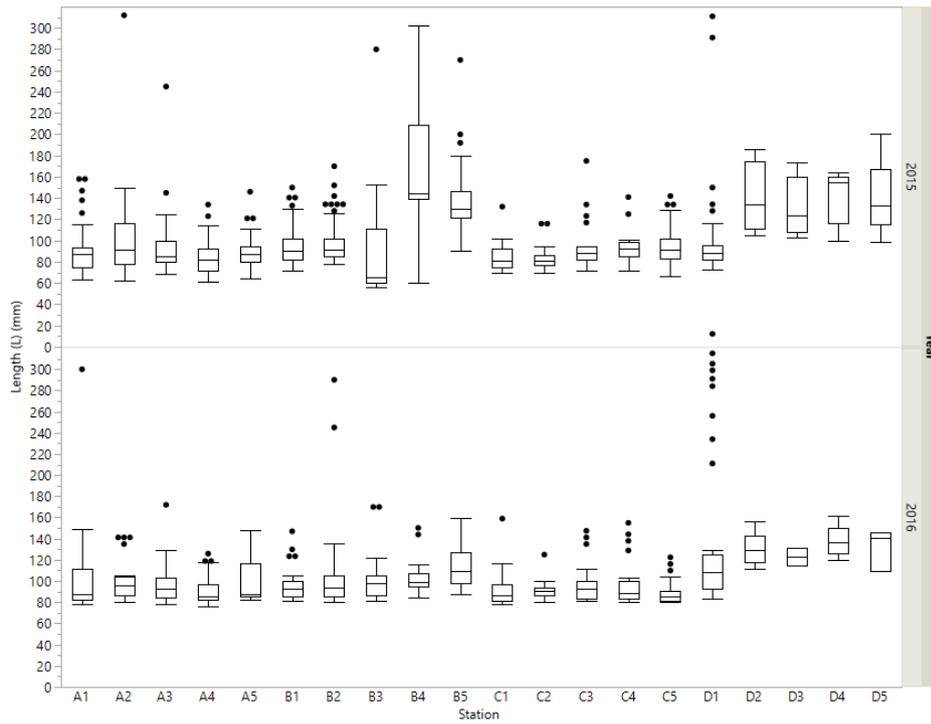
The density of brown trout  $< 80$  mm/100m<sup>2</sup> in tributary A and B shows the most stable densities between the years 2015 and 2016 (figure 29). Results from tributary C fluctuates more between the years, compared to A and B. Tributary D has only one station presented as this was the only station where brown trout  $< 80$  mm was captured, but show relatively even results between years.

### 3.2.2 Brown trout $> 80$ mm

Table 4: Estimated population sizes of brown trout  $> 80$  mm in the years 2015 and 2016 of each station,  $\pm$  SE and number of brown trout  $> 80$  mm captured ( $n$ ).

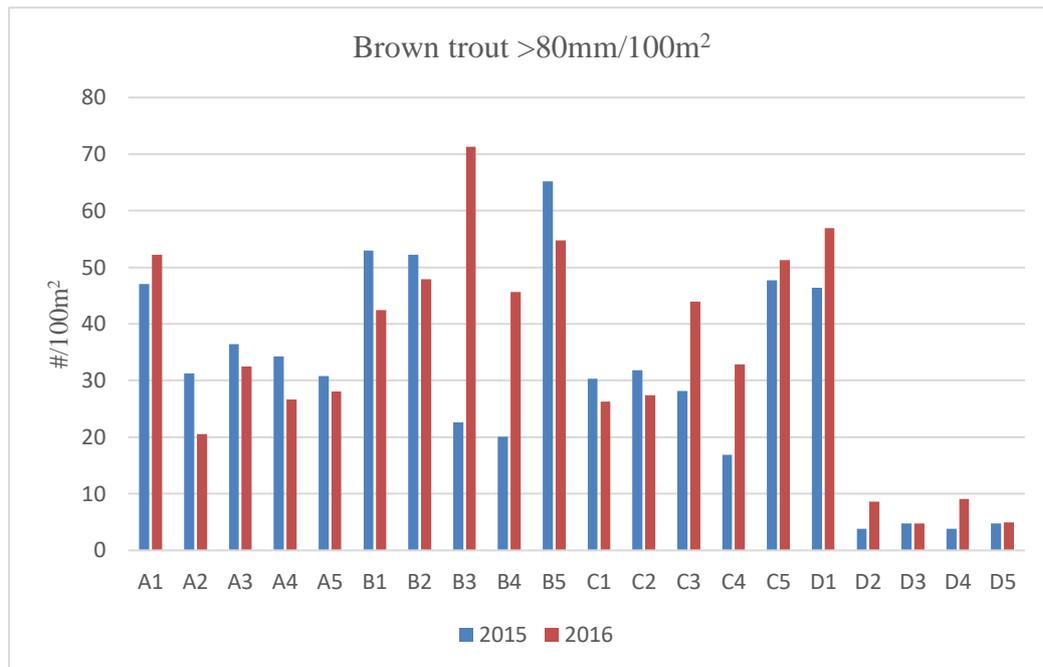
| Station | 2015     |             |     | 2016     |             |     |
|---------|----------|-------------|-----|----------|-------------|-----|
|         | Pop. Est | $\pm$ SE    | $n$ | Pop. Est | $\pm$ SE    | $n$ |
| A1      | 61.18    | $\pm$ 2.73  | 58  | 67.90    | $\pm$ 17.68 | 47  |
| A2      | 40.61    | $\pm$ 3.43  | 37  | 26.77    | $\pm$ 5.48  | 22  |
| A3      | 47.34    | $\pm$ 9.25  | 37  | 42.22    | $\pm$ 6.58  | 35  |
| A4      | 44.52    | $\pm$ 14.03 | 31  | 34.69    | $\pm$ 5.69  | 29  |
| A5      | 40.00    | $\pm$ 4.64  | 35  | 36.48    | $\pm$ 2.59  | 34  |
| B1      | 49.76    | $\pm$ 7.38  | 41  | 39.87    | $\pm$ 3.66  | 36  |
| B2      | 52.24    | $\pm$ 6.71  | 44  | 47.87    | $\pm$ 1.95  | 46  |
| B3      | 14.03    | $\pm$ 0.19  | 14  | 44.22    | $\pm$ 3.80  | 40  |
| B4      | 19.71    | $\pm$ 3.57  | 17  | 44.69    | $\pm$ 2.61  | 42  |
| B5      | 45.67    | $\pm$ 4.11  | 41  | 38.35    | $\pm$ 1.63  | 37  |
| C1      | 30.93    | $\pm$ 5.22  | 26  | 26.83    | $\pm$ 3.24  | 24  |
| C2      | 30.55    | $\pm$ 5.98  | 25  | 26.35    | $\pm$ 0.71  | 26  |
| C3      | 27.00    | $\pm$ 10.53 | 19  | 42.22    | $\pm$ 6.58  | 35  |
| C4      | 16.92    | $\pm$ 1.48  | 16  | 32.88    | $\pm$ 1.24  | 32  |
| C5      | 47.72    | $\pm$ 2.58  | 45  | 51.32    | $\pm$ 1.52  | 50  |
| D1      | 34.81    | $\pm$ 3.82  | 31  | 42.67    | $\pm$ 1.00  | 42  |
| D2      | 4.04     | $\pm$ 0.24  | 4   | 9.07     | $\pm$ 0.30  | 9   |
| D3      | 5.03     | $\pm$ 0.19  | 5   | 5.03     | $\pm$ 0.19  | 5   |
| D4      | 4.04     | $\pm$ 0.24  | 4   | 9.54     | $\pm$ 1.16  | 9   |
| D5      | 5.03     | $\pm$ 0.19  | 5   | 5.22     | $\pm$ 0.67  | 5   |

Presented in table 4 is the estimated population size of brown trout  $> 80$  mm  $\pm$  SE pr. station in the four tributaries in this study. The standard error (SE) is generally low, and so the results are likely credible. Tributaries A, B and C shows high population size compared to tributary D. The exception is station D1, which has an estimated population size on the same level as the other stations. A significantly lower population estimate is found in stations D2 - D5.



*Figure 30: Distribution of Brown trout > 80 mm body length between station and year. All brown trout marked with PIT-tag. Black dots represent values outside normal distribution.*

Tributary A displays stable body lengths between year and station. B and C display a bit more variation, while D have generally bigger body length (figure 30). In general, the four tributaries are significantly different in body length between stations, except form tributary B (appendix table 9).



*Figure 31: Brown trout > 80 mm/100 m<sup>2</sup>, distributed by capture station in tributaries A: Elsjøbekken. B: Storrsjøbekken. C: Botnetjernsbekken and D: Trasletjernsbekken.*

The densities of brown trout > 80 mm/100 m<sup>2</sup> fluctuates between years and station (figure 31). Tributary A and B shows the most stable results between years and stations, while the fluctuations are highest in tributary C. Tributary D has very low densities of brown trout and fluctuations between years in stations D2 - D5. There is no apparent relationship with the different lime treatments (figure 5) in tributaries A, B and C, but a negative effect on stations D2 – D5 cannot be excluded.

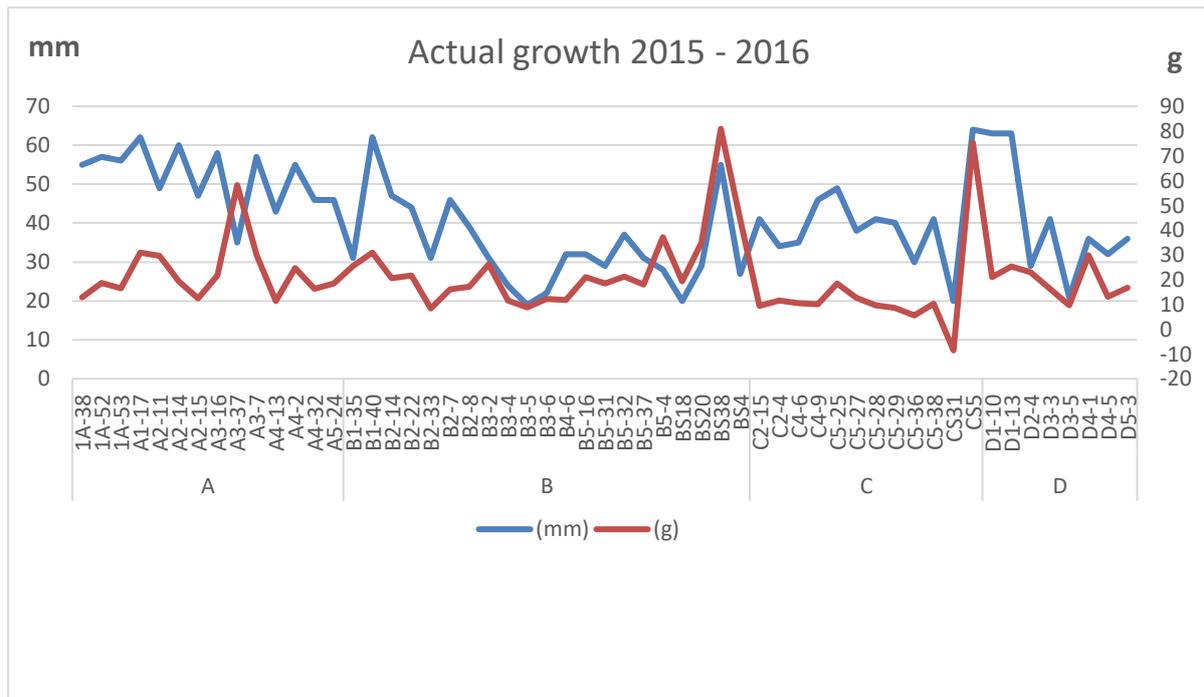


Figure 32: Presented is measured actual growth difference of recaptured brown trout > 80 mm marked in 2015 and recaptured in 2016. Blue line is growth in body length (mm) and red line is growth in weight (g). Y-axis are categorized into tributaries and ID numbers representing individual brown trout. (n = 55).

The difference in body length and body weight of 55 brown trout > 80 mm between the years 2015 and 2016, is based on measurements in the field on recaptured brown trout, with the same equipment each year (figure 32). Captures was made in the middle of August both years to get as compatible data as possible. In tributary C, there was a higher increase in length then weight, with the brown trout, ID number CS31 showing an increase in body length and decrease in body weight. The recaptures from station D2 - D5 showed very low levels of body growth.

Table 5: Shows age distribution of brown trout > 80 mm in the four tributaries, in august 2015. A (Elsjøbekken). B (Storrsjøbekken). C (Botnetjernsbekken) and D (Trasletjernsbekken). Values are number of brown trout, based on subset sample of available data.

| Age | A   | B  | C  | D  |
|-----|-----|----|----|----|
| 1+  | 174 | 97 | 24 | 26 |
| 2+  | 24  | 51 | 1  | 11 |
| 3+  | 1   | 19 |    | 7  |
| 4+  | 2   | 2  |    | 5  |
| 5+  |     | 3  |    | 1  |
| 6+  |     | 1  |    |    |

Tributary C has only 1- and 2-year olds present, while the other three tributaries have a higher degree of age distribution. The higher age distribution in tributary B and D, are because of stationary stream dwelling brown trout.

### 3.2.3 Spawning brown trout

Presented below are the results from the spawning brown trout, captured in the autumn of 2015 and 2016.

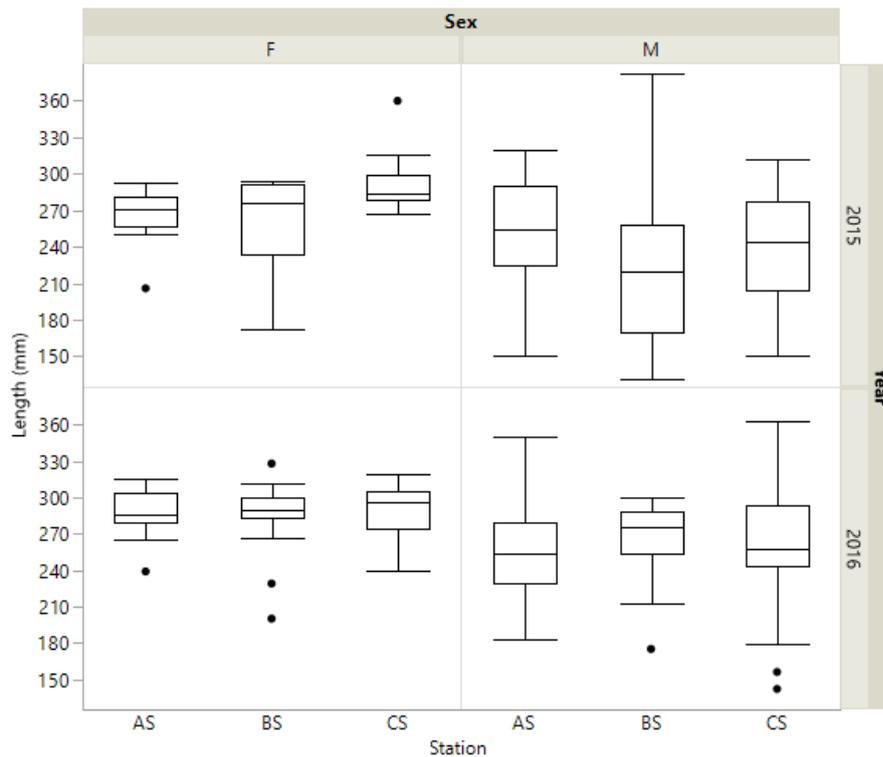


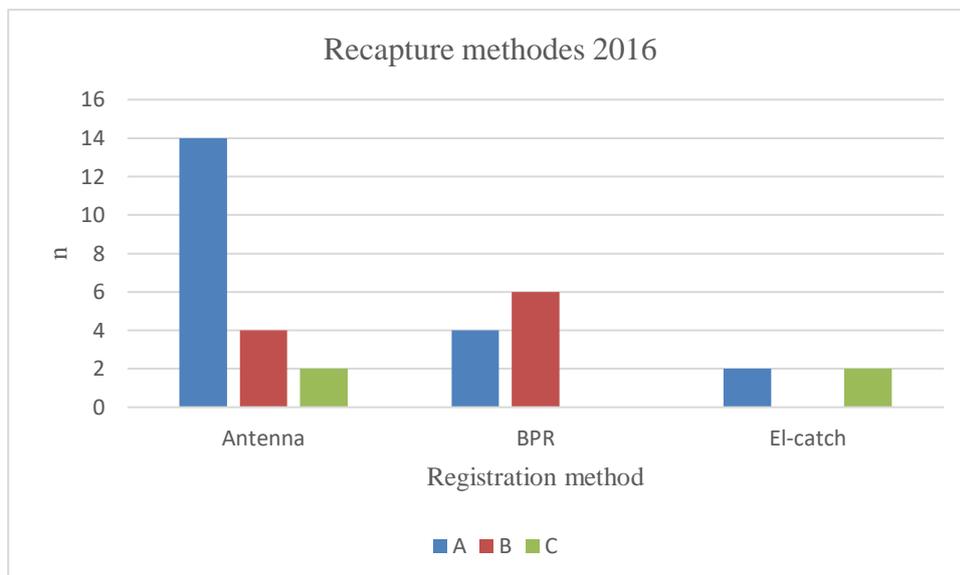
Figure 33: Distribution of body length (mm) of migratory spawning brown trout between tributaries and years. Black dots represent values outside the 25 percentiles (Outside normal distribution). AS (Elsjøbekken). BS (Storrsjøbekken) and CS (Botnetjernsbekken).

Tributary A (AS) displays a relatively stable body length between years, in both males and females, with a bigger variation in male body length (figure 33). Tributary B (BS) and C (CS) display more variation between body length in years.

*Table 6: Number of spawning Brown Trout captured (n) and sex-ratio Males (M)/Females(F) divided into each tributary and year. Numbers include recaptures of brown trout marked in 2015 and recaptured in 2016.*

| <b>Tributary</b> | <b>n</b><br><b>2015</b> | <b>n</b><br><b>2016</b> | <b>M</b><br><b>2015</b> | <b>F</b><br><b>2015</b> | <b>Sexration</b><br><b>2015</b> | <b>M</b><br><b>2016</b> | <b>F</b><br><b>2016</b> | <b>Sexratio</b><br><b>2016</b> |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------------------------------|-------------------------|-------------------------|--------------------------------|
| <b>A</b>         | 67                      | 73                      | 33                      | 21                      | 1.57                            | 49                      | 30                      | 1.63                           |
| <b>B</b>         | 52                      | 49                      | 35                      | 6                       | 5.83                            | 21                      | 32                      | 0.66                           |
| <b>C</b>         | 53                      | 64                      | 30                      | 22                      | 1.36                            | 37                      | 28                      | 1.32                           |
| <b>D</b>         | 0                       | 0                       | 0                       | 0                       | 0                               | 0                       | 0                       | 0                              |

The number of migratory spawning brown trout males divided by females yields the sex ratio (table 6). Tributary A and C displays stable sex-ratios in both years, while B have 5.83 males/female in 2015 and 0.66 males/female in 2016. No spawning brown trout were captured in tributary D.



*Figure 34: Amount of returning spawning brown trout males and females captured and marked in 2015, recaptured in 2016 by Stationary antenna. (Antenna) Backpack reader (BPR) and electrical fishing (El-catch) in tributaries A (blue columns), B (red columns) and C (green columns).*

The total amount of recaptured spawning brown trout males and females in the tributaries A, B and C and which registration method yielded the highest number of registrations (figure 34). The stationary antenna was the most effective way to register PIT-tag marked brown trout in tributary A (blue columns), with the portable antenna as second and last the electrical fishing apparatus. In tributary B (red columns) the backpack reader (BPR) was the method who yielded most recaptures of PIT-tag marked brown trout, with the antenna as second and no recaptures using electronic fishing apparatus. In tributary C (green columns) the stationary antenna (antenna) and electrical fishing apparatus (El-catch) was the only methods that registered PIT-tag marked brown trout.

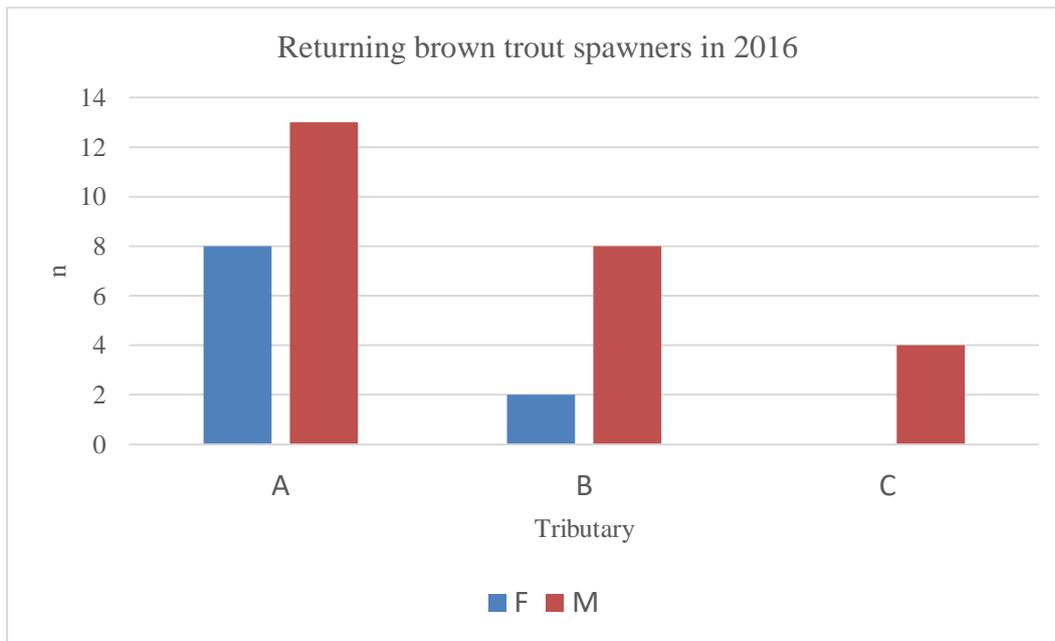


Figure 35: Sex distribution of recaptured spawning brown trout females (F) (blue columns) and males (M) (red columns) in the tributaries A, B and C. No spawning brown trout were captured in tributary D in 2015 and 2016.

The amount of returning spawning brown trout registered in 2016 and marked in 2015, was highest in tributary A, with tributary B as second and tributary C with the fewest returning spawning brown trout (figure 35). The amount of returning females shares the same pattern, except for tributary C, where no female was registered as recaptures.

### 3.2.4 Stationary antenna registrations.

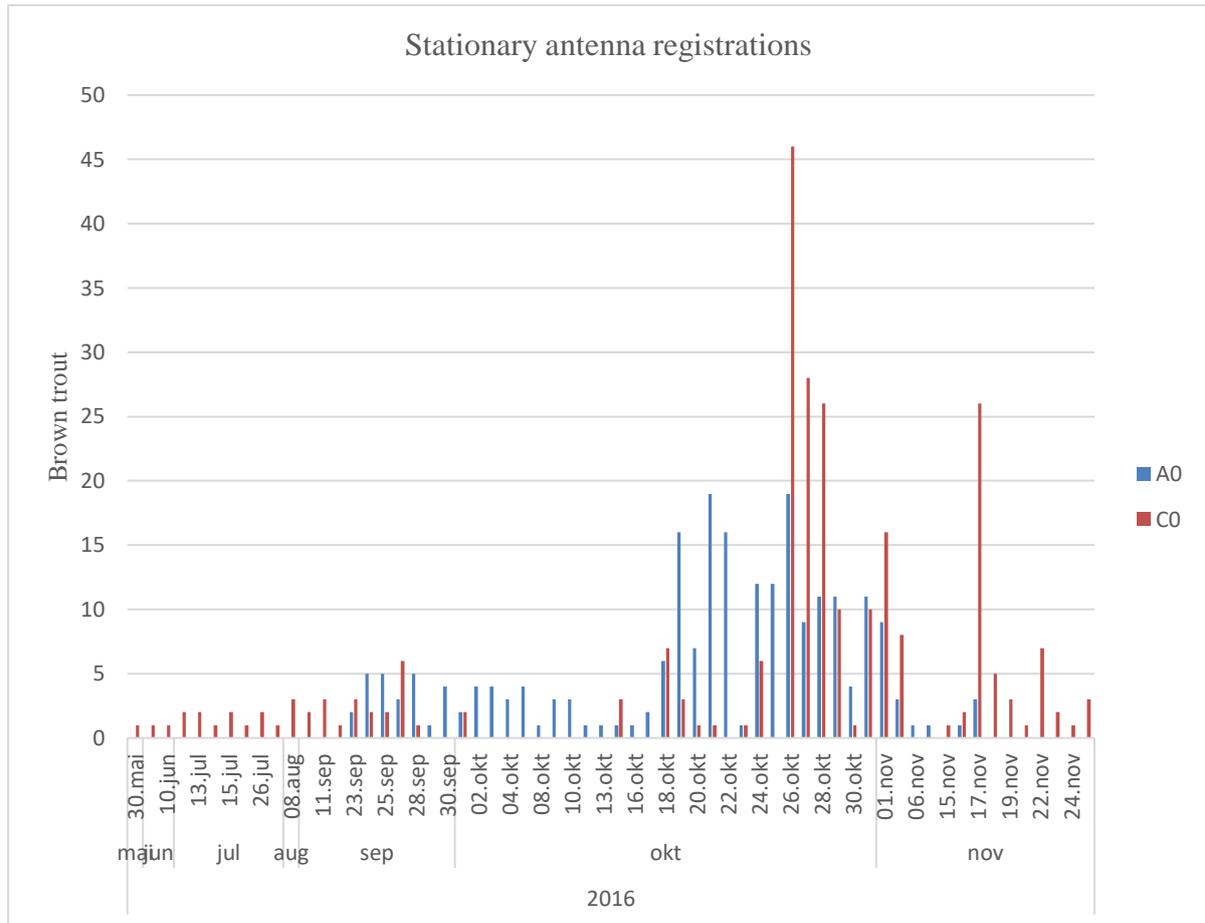
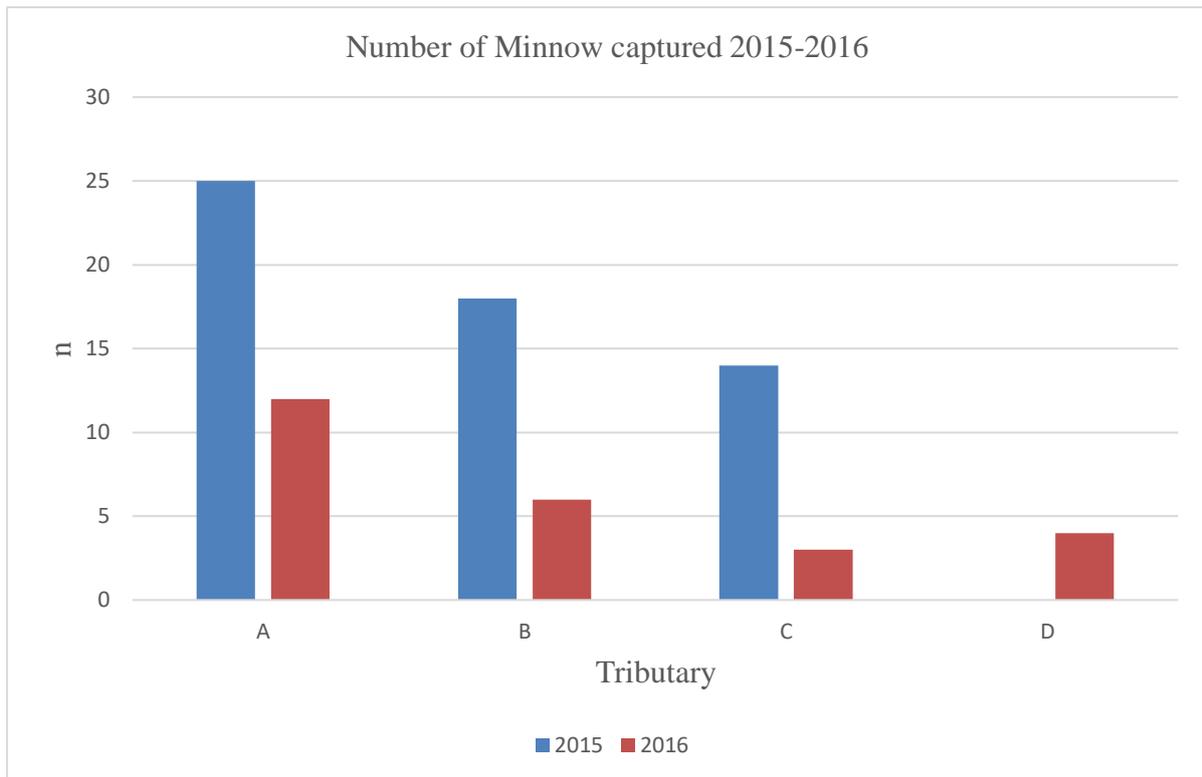


Figure 36: Marked brown trout passing the stationary PIT-tag antenna mounted in the tributaries A0 (Elsjøbekken) (blue columns) and C0 (Botnetjernsbekken) (red columns) from May to November, 2016. Figure including juvenile and spawning brown trout marked in 2015.

The activity of PIT-tag marked brown trout in and out of the tributaries A and C varies with month and tributary (figure 36). There is some activity in all the months. However, there is a peak in brown trout passing the stationary antenna in the from the 18<sup>th</sup> of October to the 1<sup>st</sup> of November in both tributaries. On the 17<sup>th</sup> of November there was also high activity in tributary C (C0).

### 3.2.5 Minnow



*Figure 37: Number of minnows captured in 2015 and 2016, in the four tributaries A (Elsjøbekken). B (Storrsjøbekken). C (Botnetjernsbekken) and D (Trasletjernsbekken).*

The number of captured minnows is decreasing in all tributaries from 2015 to 2016 (figure 37). The number of captured minnows also decrease by tributary from A to D, except for tributary D, where no minnows were captured in 2015, and four in 2016.

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## 4. Discussion

Acidification of surface waters has been and still are a great challenge in the northern hemisphere (Garmo et al., 2014). Originally, acidic precipitation due to emissions from the industry was the main challenge (Clair & Hindar, 2011). After several international conventions have successfully reduced emissions in Europe, the main challenge today is the long-term effect to the catchment areas. Acid precipitation has depleted the base cations and thereby reduced soil ANC levels in many regions both in Europe and North America (Lawrence et al., 1999). The severity of the long term depletion of base cations are dependent on geological composition (Mosher et al., 2010).

The chemical and the biological recovery after acidification has been the topic of many studies (Henriksen et al., 1989; Kroglund et al., 2001; Westling & Sahlén Zetterberg, 2007). The criteria of full recovery are debated in scientific communities. Aqueous ANC levels has been the most used criteria to determine chemical recovery. However, TOC levels have an effect on the ANC of surface waters, and today a ANC level adjusted for TOC concentration and charge densities is most commonly used (Lydersen, Larssen, & Fjeld, 2004). The level of mobilized aluminium ions ( $Al_i$ ) from the soil, is the most determinative factor for toxicity to fish in fresh water. Furthermore, acidic episodes are usually connected to water flow and may reach toxic levels for biota during floods, especially during spring (Laudon & Bishop, 1999). Toxic levels of  $Al_i$  may endure longer than the flood itself, depending on the deposition of base cations, such as calcium in the catchment area (Laudon & Bishop, 1999; Lawrence et al., 1999; Andersen, 2006).

To counteract acidification, lime has been added to waters, both still and running in Norway by The Norwegian Environment Agency since 1983 (Miljødirektoratet, 2016). As acidic precipitation has receded, so has the extensive use of lime (Miljødirektoratet, 2016). The decision to reduce or cease lime treatment in an area, must be based on that chemical and biological recovery after acidification is fulfilled.

### 4.1 Water chemical parameters.

#### pH and aluminium

The pH values presented in this study are from different sources. The historical values (figure 30) are based on water samples collected once a year, mainly conducted by volunteers from local hunting and fish associations. The results (figure 30) might yield higher pH values compared to an annual average of pH levels. Even so, the pH level is generally decreasing from 1988 to 2016. Most of the water-samples of which figure 30 is based on, are collected in summer each year, because the forest roads are often not accessible by car in spring. Table 8 shows that in general, that pH decreases with increased water levels. In figure 31 the fluctuations of pH are displayed from seven water samples. Tributaries A, B and C are generally high regarding pH values, compared to tributary D which in general have lower pH levels, with bigger fluctuations between samples. Studies have shown that the potential highest toxicity of aluminium polymerization is between pH 5.0 and 6.0 (Poléo, 1995). Tributary D is within pH levels between 5.0 and 6.0 in six out of seven samples. Figure 34 shows that tributary D has relatively similar  $Al_i$  values as tributaries A, B and C. However, in March of 2017 tributary D displays a relatively high concentration of 96  $\mu\text{g/L}$   $Al_i$ . Water levels in the tributaries seem to influence negatively on water quality in tributaries. During floods,  $Al_i$

concentrations may be higher than recorded in this study. Laboratory studies have shown that the mortality rate of brown trout are at its highest after 700 hours, within the same pH range, but with three times the  $Al_i$  concentrations in the water then registered in this study (Poléo et al., 1997). The tributaries in this study rapidly increase and decrease in water levels, determined by the amount and length in time of precipitation, which in turn may reduce the exposure time of toxic water to biota. However, studies have shown that the mobilization of high concentrations of  $Al_i$  might have effect long after the flood has receded (Andersen, 2006). The spring and autumn floods seem to be the longest periods of elevated water levels in the tributaries. The two seasons are key factors in the life history of a brown trout, spawning during autumn floods and the emerging brown trout fry during spring (Jonsson, 1989). These coinciding phenomena could explain the low population estimates and densities of brown trout (table 4) (figure 31) in tributary D, stations D2 - D5. Either the spawning brown trout in these stations are too fatigued by the water quality to spawn, or the survival rate of the fry are close to zero.

Water temperature must be considered, as the aluminium polymerization ratio is a more important factor to acute toxicity than the concentrations of  $Al_i$  itself (Poléo, 1995). With temperature being known to be a catalyst in chemical reactions in general, studies have shown that pH between 5.5 and 6.5 and temperatures between 10-20°C have the most acute toxicity to fish, due to the rate that aluminium polymerase on fish gills, causing hypoxia (Poléo, 1995). Temperature loggers placed in the four tributaries show that the water temperature is roughly similar in the four tributaries each month of the year (Appendix figure 1). During March 2016 water temperature was on average 0°C in tributary D. It is likely that the temperature was approximately the same in March 2017. The low water temperature during spring floods, with the highest registered concentration of  $Al_i$  may account for the survival of some brown trout in tributary D, due to a relatively slow rate of aluminium polymerization in low temperatures. The water chemical development of Trasletjern, from which tributary D directly originates are well documented. In 2008 NIVA published a rapport on water chemical development in lakes, after lime treatment had ended. In 2004 lime treatment was ceased in Trasletjern and water chemical parameters were registered in 2005, 2006 and 2007. They found that  $Al_i$  concentrations increased from 10 µg/L in 2005 to 31-47 µg/L in 2007. Ten years later, the findings in this study is that the concentration of  $Al_i$  has doubled in tributary D, with the highest concentration measured at 96 µg/L. pH has also shown a negative development from 5.9-6.8 in the period 2005-2007, while this study found a pH range of 5.1-6.8 (Hindar, 2008).

Since the catchment area of all four tributaries in this study are relatively close geographically, they receive roughly the same amount of water during the rainfall. Figure 3 shows that the total amount of precipitation/year has increased in the last 61 years. Even this small increase in the amount of precipitation/year could potentially influence water chemistry and biota in the four tributaries, as they are relatively small and water levels are sensitive to the amount of precipitation. It is during spring and autumn that the water levels generally are high in the four tributaries in this study. Figure 2 shows that the amount of snow and time of spring floods in the catchment area varies between years. It also shows that by May, most of the snow is gone from the catchment area. This inclines that the water samples collected each year in June, is likely collected during normal- to low water flow in surface waters and might not show a representative pH value. Furthermore, pH is not measured in field, but sent to an external laboratory. This in turn prolongs the time between the sample was taken and when the water chemistry is analysed and might affect the chemical results. pH alone does not account for water toxicity to biota, but studies have found correlations between mortality rates of juvenile brown trout and low pH (Carrick, 1979a) (Hesthagen et al., 2008). The crucial component in

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water toxicity is aluminium ions ( $\text{Al}_i$ ) (Poléo et al., 1991; Poléo, 1995). However, the level of toxicity to fish is determined by several physio-chemical factors. Among them are temperature (Poléo et al., 1991), pH (Poléo, 1995), conductivity (Alstad et al., 2005), TOC and ANC (Lydersen et al., 2004). ANC levels and TOC levels may countereffect or reduce the toxicity of waters with mobilized aluminium ions, while pH and temperature acts as catalysts in the rate of aluminium polymerization (Westling & Sahlén Zetterberg, 2007).

### **ANC and TOC**

The Acid neutralizing capacity (ANC) is the difference between base cations and strong acid anions (Lydersen et al., 2004). In this study the average ANC values across the study period are higher in tributaries A and B, compared to tributaries C and D (figure 32). Tributary D shows the lowest ANC values. This is likely a consequence of the still undergoing lime treatment in tributaries A and B, as the added lime contributes to the neutralization of acidic components, i.e. the added lime becomes a part of the total ANC. Tributary C has one sample with very low ANC levels, likely caused by an error of the external lab (figure 20). If this sample is removed from the data set, the  $R^2$  value increases from 0.1832 to 0.325. The trendline would explain more of the variation between samples without the possible error.

In general, the TOC levels and thereby the dissolved organic carbon (DOC) levels in Norway's surface waters have increased in the last decade (Garmo et al., 2014). As a result ANC levels must be higher in order to sustain brown trout populations in humic lakes (Hesthagen et al., 2008). The TOC levels in the four tributaries of this study are relatively even (figure 21). However, there is a slight increase of TOC values from tributary A through D. Tributary C and D has a higher amount of marshes in their catchment area, than tributaries A and B. This could account for the slightly higher TOC values.

## **4.2 Brown trout**

In Norway, brown trout is the fish species documented to be the most affected by acidification of surface waters (Trygve Hesthagen, Sevaldrud, & Berger, 1999). Studies have shown that brown trout is not the most acidification sensitive fish species in Norwegian surface waters (Poléo et al., 1997). The reason for claiming that brown trout is the most affected species by acidification might be that this fish species is one of the most abundant in Norway, or it could be the relatively low number of studies on acidification effects on other fish species compared to brown trout.

The densities of brown trout collected in 1998, compared to the findings of this study, shows an increase in the abundance of brown trout in all the four tributaries (figure 24). An explanation for this could be natural variation in production of brown trout from year to year, or it could be a result of the lime treatment regime. It is possible that in 1998 the brown trout population in the four tributaries were still recovering from acidification, even though all tributaries were treated with lime since 1995 (figure 5). Studies have shown that chemical recovery of surface waters was mainly driven by a reduction in sulphur deposits in the catchment areas (a key compound in acidic rain) in the period up to 1999 (Garmo et al., 2014). After 1999 the chemical recovery rate seemed to slow down (Garmo et al., 2014).

The process of less acidic precipitation on water chemistry is delayed by the processes in the catchment area (Garmo et al., 2014). In addition to the delayed chemical recovery, studies have shown that the biological recovery may be even more delayed than the chemical recovery

(Hesthagen et al., 2011). Furthermore, the rate of recovery of aquatic biota may differ between bodies of water (Gray & Arnott, 2009). These findings support the assumption that the four tributaries were still under chemical and biological recovery during the late 1990s.

The estimated population size of brown trout < 80 mm (table 3) fluctuates between years, and the standard error (SE) is generally higher than the standard error on estimated population size of brown trout  $\geq 80$  mm (table 4). The estimated catch probability is higher for brown trout  $\geq 80$  mm than for brown trout < 80 mm but varies little between years. (Appendix table 2). This indicates that brown trout  $\geq 80$  mm are easier to catch than smaller brown trout during electro fishing and in turn explains why the estimated population size is more stable between years for brown trout  $\geq 80$  mm, than brown trout < 80 mm. Furthermore, the estimated population size on brown trout  $\geq 80$  mm is relatively similar between years and stations. Tributary D shows a very small population in all stations, except station D1 in both years. It is possible that the brown trout residing in station D1, escapes into Lake Råsjøen when the water quality becomes poor and returns when the water chemical state is better. There are very low densities of brown trout in stations D2 – D5 compared to the other stations in this study (figure 31). This is likely a result of episodic water toxicity, as pH and Al<sub>i</sub> show the highest fluctuations in tributary D compared to the other tributaries (figure 19, 22). This study finds no other explanation for the high fluctuations, than the different lime regimes in the four tributaries. The brown trout captured in stations D2 – D5 are small in body size and display a very little growth between years (figure 32). This could be the cause of scarce food abundance or genetical properties. Studies have found genetical determination of resilience to acidification within strains of brown trout (Gjedrem & Rosseland, 2012). The small body size may also be beneficial to surviving periodic aluminium polymerization (Alstad et al., 2005).

The lack of brown trout < 80 mm body length (apart from the first meters of station D1), the high fluctuations in crucial chemical parameters, including the drop in ANC levels, suggest that tributary D was not chemically nor biologically recovered from acidification when the lime treatment was ceased in 2011 (figure 5). A repeated lime treatment will increase pH and ANC levels, insuring first chemical recovery, followed by biological recovery after acidification (Westling & Sahlén Zetterberg, 2007).

### **4.3 Spawning brown trout**

No spawning brown trout were captured in tributary D during late autumn in 2015 and 2016. But there is likely spawning to some extent as brown trout < 80 mm were captured in the first meters of station D1. Based on this, it is likely that some extent of brown trout spawning occurs in tributary D, if not the brown trout < 80 mm have migrated there from other tributaries. In 2015 brown trout ID number A4-4 were marked on the 7<sup>th</sup> of august and recaptured on the 13<sup>th</sup> of august in station D1. Brown trout A4-4 was 90mm in body length at the time, migrating down 200 m of tributary A, across Lake Råsjøen and up into tributary D in 6 days. The change of tributary has not been commonly found among brown trout recruits in this study but raises the question if the brown trout recruits found in station D1 are hatched there or has migrated there. A future genetical analysis might provide an answer.

Among spawning brown trout, body length shows higher variation males than in females (figure 33). A higher variation in brown trout males, than females are likely due to life history traits such as habitat use, diet selection, inherited genetic differences or sexual maturation (Jonsson, 1989). The backpack reader antenna and physical recaptures has accounted for some

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male brown trout that has sexually matured in the tributaries, without any registration of migrating to Lake Råsjøen (Appendix table 8). These findings could explain the higher body size variation in males than in females, and that this is a natural occurring phenomenon and not a result of the different lime treatments.

Many brown trout die after their first spawning season (Jonsson, 1978). However, repeated spawning in both male and female brown trout's have been documented (Klemetsen et al., 2003a). The mortality rate of spawning brown trout is dependent on the amount of energy used during spawning (Berg, Thronæs, & Bremset, 1997). In this study the amount of repeating spawning brown trout varies between tributaries (figure 36). Tributary A has the highest number of repeating spawners, with a decreasing number in tributary B and no females and only a few males in tributary C. Brown trout males use less energy on the development of gonads than females (Berg et al., 1997). It is thereby possible that the mortality rate of spawning brown trout is limited by water chemistry in the three tributaries, explaining the declining trend of recaptured repeating spawners is parallel to the lime treatment regime (figure 5). It is thereby plausible that water chemistry is the reason for the reduction in recaptured brown trout from tributary A to C, and the lack of spawning brown trout from Lake Råsjøen in tributary D.

#### **4.4 Minnow**

The total number of minnows caught in the four tributaries decrease with lime treatment regime (figure 29). The declining number of minnows from Tributary A to tributary D, indicates that the water quality is limiting the abundance of minnow. Minnow has been observed to have lower tolerance levels to pH and  $Al_i$ , than brown trout (Poléo et al., 1997) (Norrgren, Gl, & Malmberg, 1991). It is thereby likely that the different lime treatment in the four tributaries is the explanation for the decreasing abundance of minnow from tributary A-D, due to reduced water quality.

Minnow is known to be a competitor to juvenile brown trout through feeding niches (Museth et al., 2007). A study has shown that in some lakes the brown trout abundance is decreased by 35 % (Museth et al., 2007). It is thereby possible that the abundance of brown trout recruits in the tributaries is affected by the abundance of minnow, rather than water quality. It is also likely that the minnow's effect on brown trout increase by number of minnows. The number of minnows present between years varies from 2015 to 2016 (figure 29). In the amount of minnow captured in these years are representative for the common abundance of minnow, then the low minnow abundance is likely not affecting brown trout populations in the four tributaries.

#### **4.5 Stochastic events**

The tributaries in this study are relatively small and are prone to alterations on small and large scale due to stochastic events. In the years 2015-2016, several stochastic events have occurred in each of the four tributaries that may have influenced our findings. A flood filled the antenna station in tributary D with water, completely destroying the electronic devices inside. Potential antenna registrations were lost, as the memory card was destroyed (figure 38). In 2016 during heavy rains, the riverbank in tributary C slid out resulting in huge boulders altering one of the pools in station four. Floods or droughts may displace fry less than one year (0+) further down

the tributaries, resulting in elevated densities of this group closer to Lake Råsjøen, than higher in the tributary. It may also cause early departures of brown trout fleeing from the tributary and into Lake Råsjøen. All four tributaries have some form of possible migratory obstacle for fish, dependent on water discharge. It is thereby plausible that high or low water discharge may affect population estimates pr. station. The capture of brown trout has not been attempted during high discharge in any of the four tributaries, due to personnel security risk and low visibility in the water. There has been made captures during lower than normal discharge. The effects of high discharge on estimated population size of brown trout has not been evaluated in this study, due to lack of comparison data during high discharge. During an autumn flood in 2015, the relative water gauge along with the temperature and relative pressure logger was washed out from the bank and transported downstream (figure 39). This led to a small gap in the registered temperature and relative pressure data. Furthermore, the relative water level is not compatible before and after the displacement of the water gauge, as the pool it was mounted in, was changed by the flood.



*Figure 38: A flood has filled the antenna station with water in tributary D in summer 2016.*



*Figure 39: Displacement of water level gauge and U20L logger after a flood in 2015, causing faulty temperature and barometric pressure data.*

#### **4.6 Management Implications.**

It would be an impossible task to survey the chemical and biological recovery of all surface waters, both due to economical and the share work load reasons. However, a small scale surveillance of the degree of recovery should be possible. One could argue that a precautionary principle is the continued use of lime, but that would not be sustainable for ever. The bed rock composition in the catchment area of this study should be taken into account, when transferring the findings in this study to other tributaries, as the geological composition in a catchment area will affect water chemical parameters (Mosher et al., 2010).

#### **4.7 Conclusion**

The chemical and biological factors of tributary D show no sign of recovery after acidification. It was thereby too early to stop lime treatment of tributary D's catchment area. Tributary C has satisfactory chemical and biological parameters, but the no returning female spawning brown trout are a cause for concern. Tributary C may still be affected by earlier lime treatments. Tributaries B and A are still treated with lime and it is thereby impossible to conclude if they are recovered from acidification.

#### **4.8 Further studies**

- Continued surveillance of chemical and biological parameters in the four tributaries to account for natural variation.
- Genetic studies on brown trout in the tributaries may disclose genetical differences in brown trout populations, between and within tributaries.

- The relationship between TOC and  $Al_i$  bindings; what are the chemical thresholds for breaking these bindings, causing a re-mobilization of  $Al_i$ .
- A thorough analysis of the bedrock in the catchment areas of the four tributaries, to provide transferable experiences from this study to other areas with similar geology.

## 5. Acknowledgements

Dr. Ruben Alexander Pettersen, thank you. I would not be where I am today in my life, without the ideas support, and motivation you offer. To my supervisors at Høgskolen i Innlandet, Antonio Bjørn Stefano Poléo and Kjartan Østbye, thank you for your support in all manners. I would also like to express my gratitude to my employer Norges Jeger- og Fiskerforbund, Akershus, thank you Per Rune Stav and Hjalmar Eide. The voluntary workers from Nannestad JFF, Rune Johnsen, Mats Larsson, and Aasmund Torgeir Brustad, for their invaluable assistance in the field. Terje Wivestad from Fylkesmannn i Oslo og Viken for believing in the project, founding and attendance at the lime-seminar in Trondheim, were the project was presented through a poster. A big thanks to the family landowners Young Fearnley Aas Gaard and their staff, for always being helpful and for the free use of the forest roads. My family and friends for their patience and support and to everyone who has contributed to this project.

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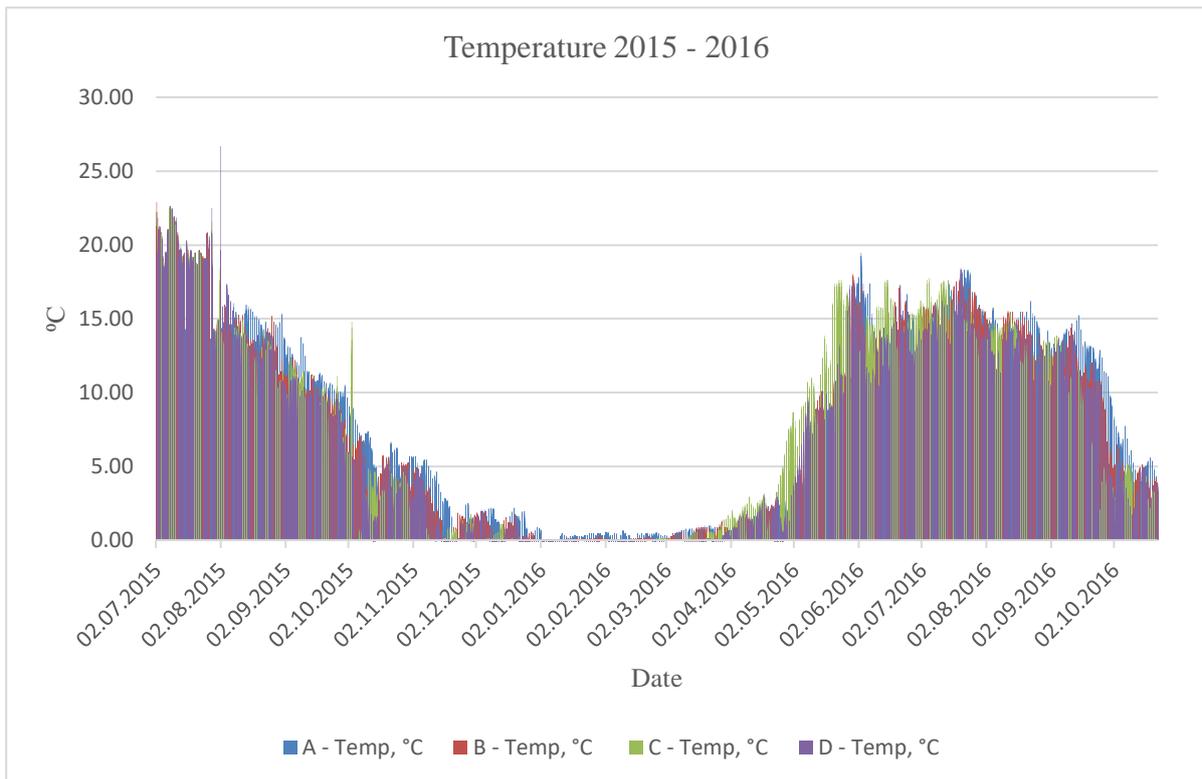
## 6. Appendix

*Appendix table 1: Parameters analysed. Standards used on water samples in Eurofhins laboratory.*

| <b>Parameter</b>                   | <b>Analysis standard</b> |
|------------------------------------|--------------------------|
| ALK Alkalinity                     | (ISO 9963-1)             |
| Calcium (CA)                       | (ISO 11885:2009)         |
| Acid Neutralization Capacity (ANC) | (ISO not stated)         |
| Chloro (Cl)                        | (EPA Method 325.2)       |
| Color                              | (NS-EN ISO 7887)         |
| Potassium (K)                      | (NS-EN ISO 11885:2009)   |
| Total organic carbon (TOC)         | (NS-EN 1484 IR)          |
| Magnesium (Mg)                     | (NS-EN ISO 11885:2009)   |
| Sodium (Na)                        | (NS-EN ISO 11885:2009)   |
| Nitrate (N-NO <sub>3</sub> )       | (NS EN ISO 13395)        |
| pH                                 | (NS-EN ISO 10523:2012)   |
| Total phosphorus (P-tot)           | (NS-EN ISO 15681-2)      |
| Sulfur (SO <sub>4</sub> )          | (NS-EN ISO 10304-1:2009) |

*Appendix table 2: The estimated catch probability during electro fishing in all stations. both years. The estimated catch probability is in general lower for brown trout < 80 mm than for brown trout > 80 mm.*

| Station        | Brown trout > 80 mm |        |           |        | Station        | Brown trout < 80 mm |        |           |        |
|----------------|---------------------|--------|-----------|--------|----------------|---------------------|--------|-----------|--------|
|                | 2015                |        | 2016      |        |                | 2015                |        | 2016      |        |
|                | Est catch           | SE     | Est catch | SE     |                | Est catch           | SE     | Est catch | SE     |
| <b>A1</b>      | 0.63                | ± 0.07 | 0.32      | ± 0.13 | <b>A1</b>      | 0.30                | ± 0.12 | 0.39      | ± 0.10 |
| <b>A2</b>      | 0.55                | ± 0.10 | 0.44      | ± 0.16 | <b>A2</b>      | 0.65                | ± 0.12 | 0.38      | ± 0.16 |
| <b>A3</b>      | 0.40                | ± 0.13 | 0.45      | ± 0.12 | <b>A3</b>      | 0.26                | ± 0.18 | 0.48      | ± 0.10 |
| <b>A4</b>      | 0.33                | ± 0.15 | 0.45      | ± 0.14 | <b>A4</b>      | 0.14                | ± 0.17 | 0.34      | ± 0.11 |
| <b>A5</b>      | 0.50                | ± 0.12 | 0.59      | ± 0.10 | <b>A5</b>      | 0.30                | ± 0.13 | 0.16      | ± 0.14 |
| <b>B1</b>      | 0.44                | ± 0.12 | 0.54      | ± 0.11 | <b>B1</b>      | 0.40                | ± 0.09 | 0.29      | ± 0.12 |
| <b>B2</b>      | 0.46                | ± 0.11 | 0.66      | ± 0.08 | <b>B2</b>      | 0.66                | ± 0.13 | 0.32      | ± 0.12 |
| <b>B3</b>      | 0.87                | ± 0.09 | 0.54      | ± 0.10 | <b>B3</b>      | 0.64                | ± 0.08 | 0.33      | ± 0.15 |
| <b>B4</b>      | 0.48                | ± 0.17 | 0.61      | ± 0.09 | <b>B4</b>      | 0.57                | ± 0.26 | 0.44      | ± 0.23 |
| <b>B5</b>      | 0.53                | ± 0.10 | 0.67      | ± 0.09 | <b>B5</b>      | 0.78                | ± 0.21 | 0.62      | ± 0.19 |
| <b>C1</b>      | 0.46                | ± 0.14 | 0.53      | ± 0.13 | <b>C1</b>      | 0.54                | ± 0.07 | 0.58      | ± 0.07 |
| <b>C2</b>      | 0.43                | ± 0.15 | 0.76      | ± 0.09 | <b>C2</b>      | 0.54                | ± 0.07 | 0.45      | ± 0.18 |
| <b>C3</b>      | 0.33                | ± 0.20 | 0.45      | ± 0.12 | <b>C3</b>      | 0.24                | ± 0.15 | 0.34      | ± 0.25 |
| <b>C4</b>      | 0.62                | ± 0.14 | 0.70      | ± 0.09 | <b>C4</b>      | 0.47                | ± 0.08 | 0.89      | ± 0.11 |
| <b>C5</b>      | 0.62                | ± 0.09 | 0.70      | ± 0.07 | <b>C5</b>      | 0.36                | ± 0.16 | 0.32      | ± 0.22 |
| <b>D1</b>      | 0.52                | ± 0.12 | 0.75      | ± 0.07 | <b>D1</b>      | 0.19                | ± 0.38 | 0.49      | ± 0.16 |
| <b>D2</b>      | 0.78                | ± 0.21 | 0.80      | ± 0.14 |                |                     |        |           |        |
| <b>D3</b>      | 0.82                | ± 0.17 | 0.82      | ± 0.17 |                |                     |        |           |        |
| <b>D4</b>      | 0.78                | ± 0.21 | 0.62      | ± 0.19 |                |                     |        |           |        |
| <b>D5</b>      | 0.82                | ± 0.17 | 0.65      | ± 0.24 |                |                     |        |           |        |
| <b>Average</b> | 0.57                |        | 0.60      |        | <b>Average</b> | 0.44                |        | 0.43      |        |



*Appendix figure 1: Measured water temperature in the four tributaries from 2015 to 2016.*

Appendix table 3: Generalized linear model. Variation of length between station and year. brown trout < 80 mm.

**Generalized Linear Model Fit**

Overdispersion parameter estimated by Maximum Likelihood  
 Response: Length (L) (mm)  
 Distribution: Normal  
 Link: Identity  
 Estimation Method: Maximum Likelihood  
 Observations (or Sum Wgts) = 1092

**Whole Model Test**

| Model      | -LogLikelihood | ChiSquare | DF | Prob>ChiSq |
|------------|----------------|-----------|----|------------|
| Difference | 319,632328     | 639,2647  | 16 | <,0001*    |
| Full       | 3321,34366     |           |    |            |
| Reduced    | 3640,97599     |           |    |            |

**Goodness Of Fit**

| Fit Statistic | ChiSquare | DF   | Prob>ChiSq | Overdispersion |
|---------------|-----------|------|------------|----------------|
| Pearson       | 28027,35  | 1075 | <,0001*    | 25,6661        |
| Deviance      | 28027,35  | 1075 | <,0001*    |                |

AICc  
6679,3248

**Effect Summary**

| Source  | LogWorth | PValue  |
|---------|----------|---------|
| Station | 101,447  | 0,00000 |
| Year    | 29,853   | 0,00000 |

[Remove](#) [Add](#) [Edit](#)  FDR

**Effect Tests**

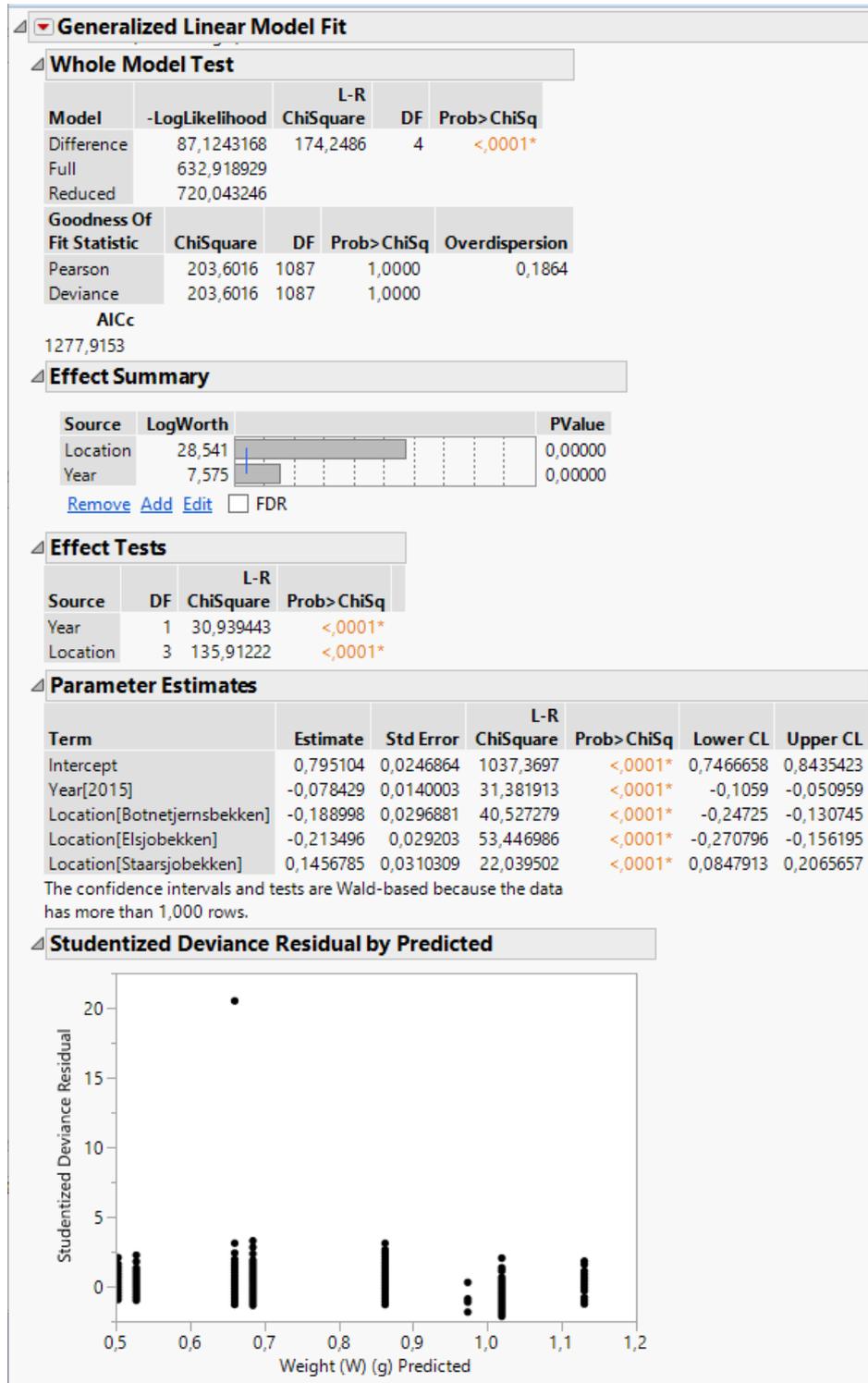
| Source  | DF | ChiSquare | Prob>ChiSq |
|---------|----|-----------|------------|
| Station | 15 | 524,56609 | <,0001*    |
| Year    | 1  | 132,1284  | <,0001*    |

**Parameter Estimates**

| Term        | Estimate  | Std Error | ChiSquare | Prob>ChiSq | Lower CL  | Upper CL  |
|-------------|-----------|-----------|-----------|------------|-----------|-----------|
| Intercept   | 42,324303 | 0,2010798 | 44303,975 | <,0001*    | 41,92975  | 42,718856 |
| Station[A1] | -4,158183 | 0,5271229 | 62,227638 | <,0001*    | -5,192488 | -3,123877 |
| Station[A2] | -6,654522 | 0,7846769 | 71,920377 | <,0001*    | -8,194192 | -5,114851 |
| Station[A3] | -5,282467 | 0,6328384 | 69,676763 | <,0001*    | -6,524204 | -4,04073  |
| Station[A4] | -4,075359 | 0,5338036 | 58,286598 | <,0001*    | -5,122773 | -3,027945 |
| Station[A5] | -1,457947 | 0,5195892 | 7,8734192 | 0,0050*    | -2,47747  | -0,438424 |
| Station[B1] | 2,663878  | 0,4701372 | 32,1055   | <,0001*    | 1,7413884 | 3,5863676 |
| Station[B2] | 3,2412337 | 0,8612116 | 14,164492 | 0,0002*    | 1,5513892 | 4,9310782 |
| Station[B3] | 8,8442701 | 0,5665322 | 243,7108  | <,0001*    | 7,7326369 | 9,9559032 |
| Station[B4] | 6,8450236 | 1,1689233 | 34,290821 | <,0001*    | 4,5513961 | 9,1386511 |
| Station[B5] | 5,6602396 | 1,448796  | 15,263535 | <,0001*    | 2,8174538 | 8,5030253 |
| Station[C1] | -1,112584 | 0,4214904 | 6,9677161 | 0,0083*    | -1,93962  | -0,285548 |
| Station[C2] | -3,243194 | 0,5627866 | 33,209188 | <,0001*    | -4,347477 | -2,13891  |
| Station[C3] | -2,181615 | 0,7762386 | 7,8988869 | 0,0049*    | -3,704728 | -0,658502 |
| Station[C4] | -2,906701 | 0,555476  | 27,382317 | <,0001*    | -3,99664  | -1,816762 |
| Station[C5] | -0,891744 | 0,7963329 | 1,2539807 | 0,2628     | -2,454285 | 0,6707977 |
| Year[2015]  | -2,024291 | 0,1708069 | 140,45434 | <,0001*    | -2,359443 | -1,689139 |

The confidence intervals and tests are Wald-based because the data has more than 1,000 rows.

Appendix table 4: Generalized linear model. Variation of weight between station and year. brown trout < 80 mm.



Appendix table 5: Generalized linear model. Variation of weight between station and year. brown trout < 80 mm.

**Generalized Linear Model Fit**

Overdispersion parameter estimated by Maximum Likelihood  
 Response: Weight (W) (g)  
 Distribution: Normal  
 Link: Identity  
 Estimation Method: Firth Adjusted Maximum Likelihood  
 Observations (or Sum Wgts) = 1092

**Whole Model Test**

| Model      | -LogLikelihood | ChiSquare | DF | Prob>ChiSq |
|------------|----------------|-----------|----|------------|
| Difference | 103,985244     | 207,9705  | 16 | <,0001*    |
| Full       | 616,058002     |           |    |            |
| Reduced    | 720,043246     |           |    |            |

**Goodness Of Fit Statistic**

| Fit Statistic | ChiSquare | DF   | Prob>ChiSq | Overdispersion |
|---------------|-----------|------|------------|----------------|
| Pearson       | 197,4103  | 1075 | 1,0000     | 0,1808         |
| Deviance      | 197,4103  | 1075 | 1,0000     |                |

AICc  
1268,7535

**Effect Summary**

| Source  | LogWorth | PValue  |
|---------|----------|---------|
| Station | 27,538   | 0,00000 |
| Year    | 6,479    | 0,00000 |

[Remove](#) [Add](#) [Edit](#)  FDR

**Effect Tests**

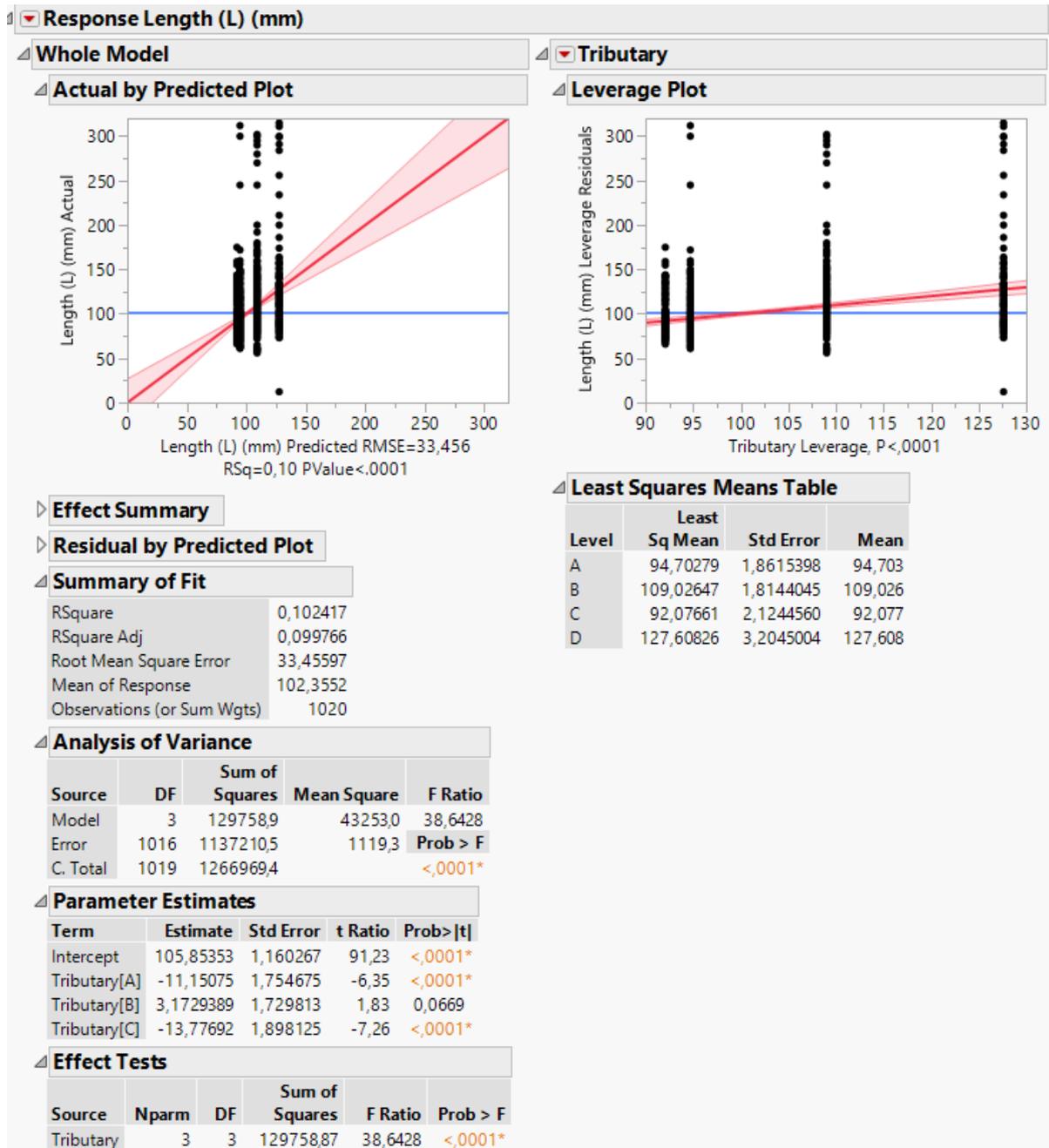
| Source  | DF | ChiSquare | Prob>ChiSq |
|---------|----|-----------|------------|
| Station | 15 | 169,63407 | <,0001*    |
| Year    | 1  | 26,055162 | <,0001*    |

**Parameter Estimates**

| Term        | Estimate  | Std Error | ChiSquare | Prob>ChiSq | Lower CL  | Upper CL  |
|-------------|-----------|-----------|-----------|------------|-----------|-----------|
| Intercept   | 0,7300482 | 0,0168757 | 1871,4505 | <,0001*    | 0,6969351 | 0,7631612 |
| Station[A1] | -0,138074 | 0,0442391 | 9,7412612 | 0,0018*    | -0,224879 | -0,05127  |
| Station[A2] | -0,128303 | 0,0658544 | 3,7958189 | 0,0514     | -0,257521 | 0,0009144 |
| Station[A3] | -0,228527 | 0,0531113 | 18,514051 | <,0001*    | -0,33274  | -0,124314 |
| Station[A4] | -0,16477  | 0,0447997 | 13,527191 | 0,0002*    | -0,252675 | -0,076866 |
| Station[A5] | -0,098359 | 0,0436068 | 5,0876679 | 0,0241*    | -0,183923 | -0,012795 |
| Station[B1] | 0,0926892 | 0,0394565 | 5,518506  | 0,0188*    | 0,0152688 | 0,1701096 |
| Station[B2] | 0,1697782 | 0,0722776 | 5,5176765 | 0,0188*    | 0,0279571 | 0,3115992 |
| Station[B3] | 0,3996045 | 0,0475465 | 70,63563  | <,0001*    | 0,3063102 | 0,4928989 |
| Station[B4] | 0,2483016 | 0,0981025 | 6,40618   | 0,0114*    | 0,0558078 | 0,4407954 |
| Station[B5] | 0,2140176 | 0,1215909 | 3,0981103 | 0,0784     | -0,024565 | 0,4525998 |
| Station[C1] | -0,075166 | 0,0353738 | 4,5152057 | 0,0336*    | -0,144575 | -0,005756 |
| Station[C2] | -0,190528 | 0,0472321 | 16,272095 | <,0001*    | -0,283206 | -0,097851 |
| Station[C3] | -0,125746 | 0,0651462 | 3,7257335 | 0,0536     | -0,253574 | 0,0020818 |
| Station[C4] | -0,14823  | 0,0466186 | 10,110094 | 0,0015*    | -0,239704 | -0,056757 |
| Station[C5] | -0,150443 | 0,0668326 | 5,067189  | 0,0244*    | -0,28158  | -0,019306 |
| Year[2015]  | -0,073611 | 0,014335  | 26,368488 | <,0001*    | -0,101739 | -0,045483 |

The confidence intervals and tests are Wald-based because the data has more than 1,000 rows.

Appendix table 6: Generalized linear model. Variation of length between station and year. brown trout >80mm.



Appendix table 7: Notes on brown trout movement and registration methods.

| ID    | 2015 |           |            | 2016        |        | Note                    |   |
|-------|------|-----------|------------|-------------|--------|-------------------------|---|
|       | Sex  | Station M | Date       | Station R.C | n Date |                         |   |
| BS28  | M    | BS        | 10.10.2015 | B0          | 9      | 21.05 - 03.06.2016      | Antenna only  |
| AS31  | M    | AS        | 07.10.2015 | A0          | 6      | 08.10 - 24.10.2016      | Antenna only  |
| BS32  | M    | BS        | 10.10.2015 | A0          | 1      | 25.10.2016              | Antenna only  |
| AS25  | M    | AS        | 07.10.2015 | A0          | 2      | 23.09 - 28.09.2016      | Antenna only  |
| A3-34 | M    | AS        | 07.10.2015 | (A0)A3-A5   | 5      | 11.08. - 12.11.2016     | A0, 12.11.2016, last record.  |
| AS35  | M    | AS        | 07.10.2015 | A0-A5       | 72     | 24.09 - 29.10.2016      | A0 10.10.2015, LAST RECORD OF 2015  |
| AS50  | F    | AS        | 11.10.2015 | A0          | 4      | 21.10 - 25.10.2016      | Antenna Only  |
| AS16  | F    | AS        | 07.10.2015 | A2-A5       | 9      | 25.10.2015-11.08.2016   | REMAIND IN TRIBUTARY  |
| AS60  | F    | AS        | 10.10.2015 | A0          | 3      | 21.10 - 25.10.2016      | Antenna Only  |
| BS18  | M    | BS        | 10.10.2015 | B3-B5       | 4      | 17.08. - 11.10.2016     | REMAIND IN TRIBUTARY, SNEAKY FUCKER? † 152MM, 2016 132MM, 2015                                |
| BS38  | M    | BS        | 10.10.2015 | BS          | 1      | 26.10.2016              | PHYSICAL RECAPTURE ONLY   |
| BS43  | F    | BS        | 10.10.2015 | A1-A5       | 6      | 13.04 - 06.12.2016      | BPR R.C ONLY, CHANGED TRIBUTARY!  |
| AS30  | M    | AS        | 07.10.2015 | A1-A2       | 7      | 25.10.2015 - 11.08.2016 | BPR ONLY. HAS NOT LOST TAG, HAS MOVED UPSTREAM FROM A1-A2 2015-2016.                          |
| AS52  | M    | AS        | 10.10.2015 | A0          | 3      | 27.09-29.10.2016        | 1 MONTH IN THE TRIBUATRY  |
| AS13  | M    | AS        | 07.10.2015 | A0          | 7      | 23.09 - 25.10.2016      | 1 MONTH IN THE TRIBUATRY  |
| BS19  | ND   | BS        | 10.10.2015 | B3-B5       | 3      | 26.10.2015 - 18.08.2016 | BPR ONLY. HAS NOT LOST TAG, HAS MOVED UPSTREAM FROM B3-B5 2015-2016.                          |
| AS19  | M    | AS        | 07.10.2015 | A0-AS       | 5      | 19.10 - 25.10.2016      | ANTENNA AND PHYSICAL R.C  |
| BS13  | M    | BS        | 10.10.2015 | B3-B5       | 5      | 13.04 - 18.08.2016      | BPR ONLY SNEAKY FUCKER?   |
| B5-26 | M    | B5        | 15.08.2015 | B3-BS       | 8      | 10.10.2015 - 18.08.2016 | BPR AND PHYSICAL R.C. READY TO SPAWN PHYSICAL R.C 10.10.2015 - SNEAKY FUCKER.L:150MM.         |
| AS1   | M    | AS        | 07.10.2015 | A0-AS       | 44     | 27.09 - 25.10.2016      | ANTENNA AND PHYSICAL R.C, ONE MONTH IN TRIBUTARY  |
| CS5   | M    | CS        | 08.10.2015 | C1-CS-C0-1  | 103    | 26.10.2015 - 17.11.2016 | R.C ALL METHODS. ANTENNA B0 16.05.2016 - 30.05.2016. CHANGED TRIBUTARY L:234MM 2016           |
| CS41  | M    | CS        | 08.10.2015 | CO          | 3      | 27.09.2016              | Antenna only  |
| BS8   | F    | BS        | 10.10.2015 | B4-B6       | 4      | 26.10.2015 - 18.08.2016 | BPR R.C ONLY, NOT REGISTERED OUT OF TRIBUTARY, BUT ABOVE STUDY AREA.                          |
| BS20  | M    | BS        | 10.10.2015 | B3-B4       | 3      | 26.10.2015 - 18.08.2016 | BPR R.C ONLY.   |
| BS50  | M    | BS        | 10.10.2015 | B0          | 18     | 15.09 - 30.09.2016      | ANTENNA R.C ONLY. 15 DAYS IN TRIBUTARY  |
| AS3   | ND   | AS        | 07.10.2015 | AS-B0-A0    | 11     | 15.09 - 25.10.2016      | ANTENNA AND PHYSICAL R.C, 2016: 15.09-29.09 TRIB B, 18.10 - 25.10: TRIB A. CHANGED TRIBUTARY! |
| B1-35 | ND   | B1        | 10.08.2015 | B1-B0       | 147    | 10.11.2015 - 30.09.2016 | ANTENNA AND PHYSICAL R.C, B1 2015 AND 2016. TRAVELS A LOT IN AND OUT OF TRIBUTARY             |
| B3-3  | ND   | B3        | 11.08.2015 | B2-B4       | 6      | 26.10.2015-11.10.2016   | BPR ONLY.   |
| A3-31 | M    | A3        | 06.08.2015 | A3-A4       | 3      | 25.10.2015-13.04.2016   | BPR ONLY.   |
| A3-37 | ND   | A3        | 06.08.2015 | A3-A5       | 11     | 07.10.2015-11.08.2016   | BPR AND PHYSICAL R.C, STATIONARY, 2016 L:280MM  |