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Bioaccumulation of Poly- and Perfluoroalkyl Substances (PFASs) and Mercury in European Perch (*Perca fluviatilis*)

Spatial distribution and forest clear-cut (CC) effects in Swedish lakes

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Abstract

Recently poly- and perfluoroalkyl substances (PFASs) have gained increasing attention due to their ubiquitous distribution in the environment and adverse effects to human and wildlife. PFASs have unique chemical and physical properties such as surfactant characteristics and are used for many industrial applications (e.g. firefighting, textile processing etc.). PFASs are not easily replaceable thus there have been efforts to use rather short chain PFASs than long chained ones (chain length of six or more perfluorinated carbons). Short chained PFASs are less likely to be toxic and bioaccumulative but still complex mixtures of PFASs can be found in animals and the environment.

Little is known about the environmental dynamics of PFASs and how Hg and specific PFASs correlate in biota. This study aims to assess interactions and accumulation patterns of PFASs and Hg in freshwater fish and if forest clear-cut (CC) and other factors (e.g. age, $\delta^{13}C$ etc.) can influence bioaccumulation. In this study samples of European perch (*Perca fluviatilis*) were taken after CC from five different lakes throughout Sweden (Björntjärn, Brobo-Kroktjärn, Gårdsjön, Långtjärn and *Umeå 4*) and from lake Kroktjärn European perch samples were collected before and after forest CC. In addition, the correlation between Hg and PFASs was investigated using Hg data from a previous master thesis study.

The results showed that long chained PFCAs (PFUnDA, PFTriDA, PFDoDA, PFDA and PFTeDA) and PFOS were most present in all samples with detection frequencies between 68 and 99%. Also PFAS levels in fish were not elevated after CC with ∑PFASs of 1.23±0.622 ng/g ww (mean±standard deviation) before CC compared to 1.13±0.146 ng/g ww after CC and showed no significant difference (ANOVA, R^2=0.0012, p>0.05). Furthermore, one conclusion in this study is that PFAS levels in fish were impacted by atmospheric deposition with a decreasing gradient from south (urban/industrial areas) to north (remote areas) (ANOVA, R^2=0.72, p<0.0001). Fish from lakes located in more populated areas (lake Gårdsjön) showed elevated levels of PFASs with a maximum of ∑PFASs=4.2 ng/g ww. Moreover, the PFASs-latitude correlations showed significant correlations for all PFASs (except 6:2 FTSA) (p<0.0001), indicating that the location of the lake is important for the accumulation of PFASs in fish. All PFASs correlated significantly with each other (except 6:2 FTSA) (p<0.0001). Four significant correlations were found between PFASs (PFDA (p<0.001), PFUnDA, PFDoDA and ∑PFCAs (p<0.05)) and Hg, thus they might have similar accumulation and transport pattern.

Keywords: PFAS, perfluoroalkyl substances, polyfluoroalkyl substances, European perch, forest clear-cut, bioaccumulation, lakes, mercury

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Abbreviations

µg	microgram
6:2 FTSA	6:2 Fluorotelomersulfonate
ANOVA	analysis of variances
bw	bodyweight
C	carbon
CC	clear-cut
DF	detection frequency
dw	dry weight
EFSA	European Food Safety Authority
EU	European Union
F	fluorine
FAO/WHO	Food and Agriculture Organisation/World Health Org.
FOSA	perfluorooctanesulfonamide
FOSAA	perfluorooctanesulfonamidoacetic acid
FOSE	perfluorooctanesulfonamidoethanol
g	gram
H	hydrogen
Hg	mercury
IS	internal standard
kg	kilogram
lat.	latitude
LC-MSMS	liquid chromatography–tandem mass spectrometry
Max.	maximum
MDL	method detection limit
MeHg	methylmercury
Min.	minimum
mL	milliliter
n	sample size
n.a.	not analyzed
N-EtFOSA	N-ethylperfluorooctanesulfonamide
N-EtFOSAA	N-ethylperfluorooctanesulfonamidoacetic acid
N-EtFOSE	N-ethylperfluorooctanesulfonamido-ethanol
N-MeFOSA	N-methylperfluorooctansulfonamide
N-MeFOSAA	N-methylperfluorooctanesulfonamidoacetic acid
N-MeFOSE	N-methylperfluorooctanesulfonamido-ethanol
Nr.	number
PerFASs	perfluoroalkyl substances
PFAA	perfluoroalkyl acid
PFASs	poly- and perfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoate
PFDoDA	perfluorododecanoate
PFDS	perfluorodecane sulfonate

PFHpA	perfluoroheptanoate
PFHxA	perfluorohexanoate
PFHxDA	perfluorohexadecanoate
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoate
PFOA	perfluorooctanoate
PFOcDA	perfluorooctadecanoate
PFOS	perfluorooctane sulfonate
PFPeA	perfluoropentanoate
PFSA	perfluoroalkyl sulfonic acid
PFTeDA	perfluorotetradecanoate
PFTriDA	perfluorotridecanoate
PFUnDA	perfluoroundecanoate
pg	pictogram
PolyFASs	polyfluoroalkyl substances
POPs	persistent organic pollutants
PP	polypropylene
p-value	probability value
Rpm	rounds per minute
R-value	pearson correlation coefficient
SD	standard deviation
TDI	tolerable daily intake
U.S. EPA	U.S. Environmental Protection Agency
ww	wet weight

1 Introduction

Poly- and perfluoroalkyl substances (PFASs) have been proven to be harmful to human and environment (Lau et al., 2007) due to their structure and consequently their bioaccumulation and persistence potential (Giesy & Kannan, 2001; J. W. Martin et al. 2003a). They can be transported over long distances in both water and atmosphere (Shoeib et al., 2006; Yamashita et al., 2005) and can be found in surface water, sediment, air and are traced in environmental specimen in remote regions (Lau et al., 2007; Shoeib et al., 2006; Stiehl et al., 2008).

Beside perfluorooctane sulfonate (PFOS), one of the most spread PFAS, mercury (Hg) is also an environmental pollutant that accumulates in water, sediment and air and has a long range dispersal. It poses a risk to human and wildlife because of its toxic potential and high prevalence and availability in the environment (Evers et al., 2008). Hg can enter water bodies due to surface runoff, and this transport can be enhanced by forest clear-cuts (CC) (Bishop et al., 2009; Sørensem et al., 2009). It has been shown that forest CC can increase surface runoff by up to over 100% (Rosén et al. 1996) and can cause elevation of Hg levels in fish in nearby lakes. Up to one quarter of Hg in fish can derive from forest harvesting due to mobilization of stored Hg in the soil (Bishop et al., 2009), which poses a threat to aquatic ecosystems. It is thus of great importance to evaluate the effect of forest CC on the occurrence of Hg and PFASs in fish and how these substances relate to each other.

1.1 PFASs

1.1.1 Background and Use

There is a broad industrial use of PFASs because of their specific chemical and physical properties. They are chemically and thermally inert, non-stick, hydrophilic and lipophobic and water resistant (Fielding, 1979; Herzke et al., 2012). Due to their special surface-active properties, they are very effective as surfactants at low concentrations and thus are used for firefighting and in textile processing to make carpets and clothes resistant to water and oil. In addition, it is used for the treatment of metal surfaces, for pesticides and for photography (Hekster & de Voogt, 2002; Stiehl et al., 2008).

Due to their many useful properties and their broad industrial application, they are economically very valuable. There has been a steady increase in production and use since 1970, even if the first PFASs were found in human serum already in 1968 (Giesy & Kannan, 2001). After the start of usage of PFASs in commercial products, wildlife samples were tested which also showed to contain PFASs (J. W. Martin et al., 2003a). In 2002, PFOS was voluntary phased-out by the main manufacturer 3M, which resulted in a global decrease of emissions of it. Nevertheless, it still can be found in animals and the environment (Ahrens et al., 2014; Bach et al., 2015). After 2002, the production of perfluorooctanoate (PFOA) increased dramatically and also elevated levels of perfluorobutanoate (PFBA) were found in

the environment as a result of the change to shorter chained substances (Möller et al., 2010; Weinberg et al., 2011). As a reaction to that and because PFOA was classified to be likely carcinogen to humans (Renner, 2006), the U.S. Environmental Protection Agency (U.S.EPA) created the PFOA Stewardship Program in 2006 to reduce PFOA and similar chemicals by 95% on a global basis until 2010 (U.S. EPA, 2006). In 2008, the use of PFOS and its derivatives were forbidden by the EU (European Parliament Council, 2006). The current goal is to reach an elimination of these chemicals at the end of 2015, whereas it is to mention that 3M already reached the Program´s goal at the end of 2008 (U.S. EPA, 2006; Lau et al. 2007).

1.1.2 Properties

In general, PFASs are man-made chemicals (Lehmler, 2005) but they can also occur naturally (two carbon (C) atoms or less). They are produced by two main processes, electrochemical fluorination and telomerization (Buck et al., 2011) and can be divided into three groups (Ahrens & Bundschuh, 2014):

- Perfluoroalkyl substances (PerFASs),
- Polyfluoroalkyl substances (PolyFASs) and
- Fluorinated polymers.

When a substance is called “fluorinated”, it can either be organic or inorganic and has at least one fluorine (F) atom. In the case of PFASs, the hydrogen (H) atoms from the original non-fluorinated compound are substituted by F atoms. The first group is characterized by a fully fluorinated alkyl chain and the second one by a partly fluorinated alkyl chain. PerFASs have in general an inflexible structure, which protects the molecule thus can only be attacked at non-fluorinated side chains (Key et al., 1997). The alkyl chain represents a chain of connected H and C atoms with the formula C_nH_{2n+1} and when the substituion takes place the formula changes to C_nF_{2n+1}-R, with n as the number of C atoms and R as the functional group (Buck et al., 2011).

C-F bonds have a large bond energy and are one of the strongest in organic chemistry; thus PFASs are very persistent (Key et al., 1997; Stiehl et al., 2008). Due to their low surface tension, the functional group is hydrophilic and the fluorinated chain is hydrophobic. This contributes to a lower degradability, even by strong acids, alkalis, or oxidizing agents and also it enhances the low solubility in water and organic solvent (Ahrens, 2011; Lau et al., 2007; Stiehl et al., 2008).

The degradability and toxicity of PFASs is mostly influenced by the chain length and the functional group (Ahrens, 2011). It can either be long chained or short chained, whereas among perfluoroalkyl acids (PFAAs) it can be distinguished between the long chained perfluoroalkyl sulfonates (PFSAs) with a chain length of six or more perfluorinated carbon atoms and perfluoroalkyl carboxylates (PFCAs) with a perfluorinated chain of seven carbon atoms or more (Buck et al., 2011). In general, it was found that the longer the chain, the more likely the substance is to be toxic and bioaccumulative, mostly due to their higher hydrophobicity (J. W. Martin et al., 2003a). When the chain consists of four C atoms or less, it was shown that there is no harm to the environment (Renner, 2006) or according to Olsen et al. (2009), substances with less than 6 perfluorinated Cs can easier be eliminated by various organisms than long chained PFASs. Also PFASs with short chains are more hydrophilic and have thus a larger mobility in water bodies whereas long chained rather bind

to sediment (Ahrens et al., 2010). Furthermore, it was shown that longer chained PFASs have a higher bioaccumulation potential in human and animals compared to shorter chained PFASs (Martin et al. 2003a; Kudo et al. 2001). Also with increasing chain length, a decreasing depuration rate occurs (Kudo et al., 2001). Thus the production shifted towards shorter chained PFASs (Möller et al., 2010). The main problem hereby is that long chained PFASs serve as the best surfactants in industrial products, and even if they are prohibited in some countries, the production might be moved to countries without regulations or where economic increase is more important than environmental issues (Lindstrom et al., 2011).

1.1.3 Sources, Transport and Transformation

PFASs with a chain length of two C atoms or less are in general considered to occur naturally, but e.g. volcano activities can be a natural source of long chain PFASs (Ahrens, 2011). On the other hand, they are released due to anthropogenic activities, which include among others manufacturing processes, chain of supply, use of products and disposal. They can enter the aquatic environment by point sources like a waste water treatment plant or nonpoint sources like surface runoff, as this is the case for forest CC areas (Ahrens, 2011). If PFASs are not directly emitted during its product life cycle, they might enter the environment in an indirect way, because their precursor substances can be degraded or metabolized and end up as persistent pollutants as well (Buck et al., 2011). They can also be re-emitted into the environment from e.g. ice and can circulate in the complex global cycle of PFASs, which consists of permanent transformation and transport processes. It depends also if the PFASs are ionic or neutral. If they are ionic, like PFCAs and PFSAs, they are easier soluble in water compared to neutral PFASs. Thus these are mostly found in water but also attached to particles of soil and sediment and accumulated in the food chain due to the low ion vapour pressure. Neutral PFASs are not as persistent as ionic ones, have a higher vapour pressure and can undergo transformations like degradation, photolysis and hydrolysis (Ahrens, 2011). For example perfluorooctanesulfonamido ethanols (FOSEs) and perfluorooctane sulfonamides (FOSAs), two of the investigated neutral volatile PFASs, can be degraded to the two final breakdown products PFCAs and PFSAs (Jahnke & Berger, 2009). In larger terms it can be stated that PolyFASs can be transformed to PerFASs (Buck et al., 2011). In addition, PFCAs and PFSAs can be transported in gas phase or via aerosols (Mc Murdo et al., 2008; Webster & Ellis, 2010) and all PFASs can be transported by the ocean or sea spray to distant areas (Ahrens et al., 2010; Mc Murdo et al., 2008).

1.1.4 Occurrence in Fish

PFASs can be released into water bodies either from former emissions of long-chained substances or from the degradation of their steadily increasing precursors (Ahrens & Bundschuh, 2014). PFOS is one of the most present PFASs in the environment (Houde et al., 2006) and has been shown to be highly toxic to fish (Yamashita et al., 2005). It seems likely that PFASs accumulate in fish, thus several studies were conducted, and it was found that PFASs accumulate in blood protein rather than in adipose tissue, as many other persistent organic pollutants (POPs) (Jones et al., 2003).

Fish often constitute an important fraction at human and animal diet and has been proposed to be the main food intake of PFOS (Berger et al., 2009). It is therefore an

important test organism for bioaccumulation tests (J. W. Martin et al., 2003a). The main exposure route of contaminants for fish comes directly from the water, not from its food, and is taken up mostly via the gills (J. W. Martin et al., 2003b; Streit, 1992). Interestingly, piscivorous animals have higher PFAS levels than their diet, which proves the biomagnification potential of PFASs (Giesy & Kannan, 2001; Stiehl et al., 2008).

1.1.5 Exposure Routes and Toxicity

The main exposure route of PFASs for humans are fish consumption, inhalation of indoor air and intake of house dust and drinking water (Fromme et al., 2009; Kannan, 2011). Results of that can be, among others, irritation of eyes, adverse effects on the reproductive organs and the liver (D. Borg & Håkansson, 2012) as well as an increased risk for prostate cancer (Lehmler, 2005). Children are furthermore subject to higher levels of PFASs and that might affect the growth of their skeletons and organs (Kannan, 2011). It is also shown, that PFASs can be taken up easily but hardly metabolized and poorly excreted (Kemper & Nabb, 2005; Kudo et al., 2001). Until now, there is no internationally accepted limit for PFAS levels in fish but several proposals have been made by agencies and institutions for a tolerable daily intake (TDI). For PFOS, they all range between 0.1-0.3 µg/kg bw/day and for PFOA between 1.5-3 µg/kg bw/day (D. Borg & Håkansson, 2012; Fromme et al., 2009; Schuetze et al., 2010).

1.2 Hg

Accumulation of Hg in freshwater fish has been well studied and emissions of Hg have been one component that is crucial for the high levels of Hg found in freshwater fish today (Chan et al., 2003). It is derived from both, natural and anthropogenic sources and is emitted globally (Pirrone et al., 2001). The largest input of Hg into the aquatic environment is via atmospheric deposition (Hammerschmidt & Fitzgerald, 2006). Even if there is an overall decrease of Hg-levels in fish, the concentration in fish remains high. A study of Åkerblom et al. (2014) about Hg in fish from Swedish lakes showed that all lakes had fish with Hg concentrations that exceed the European Union (EU) Environmental Quality Standard (0.02 mg/kg) and the maximum levels set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) for Hg in fish used for human consumption (0.5–1.0 mg/kg) were also exceeded in 52.5% of all lakes (Åkerblom et al., 2014). Fish represents an important source of nutrition to animals (e.g. wild mink and river otter) as well as to humans (Basu et al., 2005), where the main exposure route is predatory fish and other aquatic foods (National Food Agency (NFA), 2012).

Usually, total Hg is analyzed and monitored for different programs and reports, whereas the organic form of Hg –methyl mercury (MeHg)- also the most bioavailable and toxic form, is the one that can cause biomagnification and operate adversely in the nervous system of humans and animals (Chan et al., 2003; Westcott & Kalff, 1996). The bioaccumulation of MeHg can be influenced by many factors (i.e. precipitation, temperature, acid deposition, watershed characteristics etc.) whereas land-use can affect the Hg load to the aquatic ecosystem crucially (Hammerschmidt & Fitzgerald, 2006). Up to one quarter of Hg in fish can derive from forest harvesting (Bishop et al., 2009). This can be explained by the fact that Hg, originated from atmospheric deposition, is stored in forest soils and is then

mobilized by management activities in forests. The enhanced transport may increase Hg loads to soil, biota, fish and even forest productivity in the long run and poses a threat to aquatic ecosystems (Bishop et al., 2009; Bringmark & Bringmark, 2001).

1.3 Objectives

This study focus on the analysis of 26 individual PFASs in European perch from six lakes in Sweden (Kroktjärn, Björntjärn, Brobo-Kroktjärn, Gårdsjön, Långtjärn and *Umeå 4*). European perch (*Perca fluviatilis*) were sampled from lake Kroktjärn before (2010/2011) and after forest CC (2013/2014) to investigate the impact from forest CC on accumulation of PFASs in fish. Fish from the other lakes (and from lake Kroktjärn), taken in 2010 and 2011, were analyzed to compare the spatial distribution of PFASs, and to evaluate if PFASs are correlated with Hg. The following hypotheses were studied:

- PFAS levels in fish are elevated after clear cutting close to lakes due to higher surface runoff,
- PFAS levels in fish in lakes are impacted by atmospheric deposition with a decreasing gradient from south (urban/industrial areas) to north (remote areas).

Similar studies regarding the distribution of PFASs were conducted by Ahrens et al. in 2010 but with fish from high mountain lakes in France and with focus on fish liver, while in this study muscle tissue is used. Also, Martin (2014) carried out a study to investigate the effects of forest harvests in European perch but with focus on Hg. This study can be seen as an extension of the study of Martin (2014).

2 Materials and Methods

2.1 Sampling and Selection of Lakes

The lakes Kroktjärn, Björntjärn, Brobo-Kroktjärn, Gårdsjön, Långtjärn and *Umeå 4* were selected (Figure 1, Table 1, detailed maps: Appendix C) based on *i)* a similar size (1.8-31.3 ha), *ii)* isolation from direct human influence, *iii)* location in a forestry area, and *iv)* location at a gradient from north to south to investigate the spatial pattern. For all lakes, the forestry CC was carried out in autumn 2012, whereas this is only important for lake Kroktjärn because the samples from this lake were compared before and after CC. For the other lakes, the samples were only compared before the CC took place.

Samples of European perch were taken in 2010, 2011, 2013 and 2014 from the selected lakes. After dissection, muscle tissue samples were freeze-dried and then stored in a freezer at -20°C until analysis.

Figure 1. Sampled lakes in Sweden that have been studied for PFASs content in European perch and also changes in PFAS content before and after clear-cut (CC) (Table 1).

Table 1. Coordinates, lake size and clear-cut (CC) area of the studied lakes.

Lake	Latitude	Longitude	Lake Size (ha)	CC Area (ha)
Brobo-Kroktjärn	N 61° 22'	E 15° 20'	10.93	19.4
Kroktjärn	N 60° 07'	E 13° 58'	4.77	72.7
Långtjärn	N 60° 01'	E 15° 52'	6.46	93.8
Gårdsjön	N 58° 03'	E 12° 01'	31.3	11.9
Björntjärn	N 63° 54′	E 18° 49′	3.87	55.3
Umeå 4 [a]	N 64° 15′	E 18° 45′	1.8	no CC

[a] data used from a research project coordinated by Ann-Kristin Bergström (Umeå University)

2.2 Sample Selection

The European perch samples from the field sampling were selected based on the availability of the following data:

- fish age,
- fish length,
- sample size and
- $\delta^{13}C$ and $\delta^{15}N$ isotopes

Fish specimen with an age of 3-5 years and a length of 10-16 cm were selected to reduce the influence of size/age variations on the results. Also the sample size from before and after CC was very uneven so for lake Kroktjärn the number of samples was limited to the ones that had a length between 12.9 and 16 cm. Lastly, the selected samples should include isotope data, which was only the case for a few from the whole database. In the end, 80 fish samples were selected for PFAS analysis. An overview of all samples of each lake is given in table 2.

Table 2. Statistics of fish samples: Sample size (n), sampling time, length, weight, age, Hg concentration and water content.

Lake	n	Sampling Time (year)	Mean Length (mm)	Mean Weight (g)	Mean Age (years)	Mean Hg Conc (mg/kg ww)	Mean Water Content (%)
Brobo-Kroktjärn	5	2011	156	41.4	n.a.	0.16	80
Kroktjärn (before CC)	14	2010-2011	139	26.6	3.6	0.63	80
Kroktjärn (after CC)	14	2013-2014	139	26.8	3.8	0.83	80
Långtjärn	7	2010	153	37.6	4.6	0.22	80
Gårdsjön	14	2010	144	32.4	3.6	0.28	80
Björntjärn	13	2011	148	32.8	3.8	0.36	81
Umeå 4	13	2011	129	20.45	3.2	0.37	81
	∑80						

The Hg content in the fish was already been analyzed at SLU (J. C. Martin, 2014). Furthermore, Martin (2014) applied the catchment boundaries with an automatic Arc GIS program on the detailed maps (Appendix C).

2.3 Chemicals

In total 26 PFASs were investigated covering a perfluorocarbon chain length from 4-18. As mentioned in the introduction, PerFASs and PolyFASs can be divided into several sub-groups. The substances of interest for this study belonged all to the PerFASs apart from 6:2 FTSA. All investigated substances, their acronyms and formulas are given in Table 3.

Table 3. Class, acronym and formula of investigated substances.

Class and Substance	Acronym	Formula
Perfluoroalkyl sulfonic acid (PFSAs)		
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_3^-$
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_3^-$
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_3^-$
Perfluorodecane sulfonate	PFDS	$C_{10}F_{21}SO_3^-$
Perfluoroalkyl carboxylic acid (PFCAs)		
Perfluorobutanoate	PFBA	$C_3F_7CO_2^-$
Perfluoropentanoate	PFPeA	$C_4F_9CO_2^-$
Perfluorohexanoate	PFHxA	$C_5F_{11}CO_2^-$
Perfluoroheptanoate	PFHpA	$C_6F_{13}CO_2^-$
Perfluorooctanoate	PFOA	$C_7F_{15}CO_2^-$
Perfluorononanoate	PFNA	$C_8F_{17}CO_2^-$
Perfluorodecanoate	PFDA	$C_9F_{19}CO_2^-$
Perfluoroundecanoate	PFUnDA	$C_{10}F_{21}CO_2^-$
Perfluorododecanoate	PFDoDA	$C_{11}F_{23}CO_2^-$
Perfluorotridecanoate	PFTriDA	$C_{12}F_{25}CO_2^-$
Perfluorotetradecanoate	PFTeDA	$C_{13}F_{27}CO_2^-$
Perfluorohexadecanoate	PFHxDA	$C_{15}F_{31}CO_2^-$
Perfluorooctadecanoate	PFOcDA	$C_{17}F_{35}CO_2^-$
Perfluorooctanesulfonamides (FOSAs)		
Perfluorooctanesulfonamide	FOSA	$C_8F_{17}SO_2NH_2$
N-ethylperfluorooctanesulfonamide	N-EtFOSA	$C_8F_{17}SO_2N(C_2H_5)H$
N-methylperfluorooctansulfonamide	N-MeFOSA	$C_8F_{17}SO_2N(CH_3)H$
Perfluorooctanesulfonamidoethanols (FOSEs)		
N-ethylperfluorooctanesulfonamido-ethanol	N-EtFOSE	$C_8F_{17}SO_2N(C_2H_5)C_2H_4OH$
N-methylperfluorooctanesulfonamido-ethanol	N-MeFOSE	$C_8F_{17}SO_2N(CH_3)C_2H_4OH$

(Continued)

(Table 3 continued)

Class and Substance	Acronym	Formula
Perfluorooctanesulfonamidoacetic acids (FOSAAs)		
Perfluorooctanesulfonamidoacetic acid	FOSAA	$C_8F_{17}SO_2N(CH_2CO_2H)H$
N-ethylperfluorooctanesulfonamidoacetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2H_5)CH_2CO_2H$
N-methylperfluorooctanesulfonamidoacetic acid	N-MeFOSAA	$C_8F_{17}SO_2N(CH_3)CH_2CO_2H$
x:2 Fluorotelomersulfonates (x:2 FTSAs)		
6:2 Fluorotelomersulfonate	6:2 FTSA	$C_6F_{13}CH_2CH_2SO_3H$

The internal standard (IS) FXIS07 used for the analysis contained $^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, with a concentration of 20 pg/µL, and $^{13}C_8$-FOSA, d_3-N-MeFOSAA, d_5-N-EtFOSAA, d_3-N-MeFOSA, d_5-N-EtFOSA, d_7-N-MeFOSE and d_9-N-EtFOSE, with a concentration of 50 pg/µL. It was purchased from Wellington Laboratories from Canada and stored at -16°C.

Laboratory equipment was rinsed three times with methanol (LiChrosolv® 99.9%, Merck KGaA, Germany) or acetone (Suprasolv® 99.8%, Merck KGaA, Germany). Glassware were burnt over night at 400°C in an oven (Kendro, Laboratory Products). For the extraction also Superclean ENVI-Carb (Supelco®, 120/400, Bellefonte , USA) was used as well as glacial acetic acid (Suprapur®, 100%, Merck KGaA, Germany).

2.4 PFASs Analysis

The analytical method for analysis of PFAS in biota is described in detail by Ahrens et al. (2011).

2.4.1 Extraction

The extraction was carried out in batches of approximately 25 samples. Firstly, the muscle tissue was homogenized using a spatula. Aliquots of 1 g of the homogenized sample was weighted in a 50 mL polypropylene (PP)-tube and 8 mL methanol and 100 µL IS standard mixture (FXIS07) was added using plastic pipettes (VWR, Ultra High Performance). The tube was then placed on a wrist-action shaker (Gerhardt, Bonn) at 200 rpm for 30 minutes and then centrifuged (Eppendorf, Centrifuge 5810) at 3000 rpm for 15 minutes. The supernatant was decanted into a 15 mL PP-tube and the extraction was repeated but adding 4 mL methanol instead of 8 mL. After the second cycle of shaking and centrifuging, the supernatant was decanted into the 15 mL PP-tube and combined with the first fraction. Then the 15 mL PP-tubes were centrifuged at 3000 rpm for 5 minutes and the sample extract was concentrated to 1 mL using N_2 blow down (Organomation Associates Inc., N-EVAP™ 112). After concentration, the 1 mL extract was transferred into 1.7 mL centrifuge tubes (Eppendorf), which were prepared by adding 25 mg ENVI-Carb and 50 µL glacial acetic acid in each tube. The 1.7 mL tubes were vortex-mixed (Heidolph, REAX 2000) thoroughly for 30 seconds. Afterwards, the tubes were centrifuged at 4000 rpm for 15 minutes and 0.5 mL of the supernatant solution were transferred to autoinjector glass vials

(Eppendorf). The samples were vortex-mixed and stored in the freezer at -20°C until analysis by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-MSMS).

2.4.2 Instrumental Analysis

For the analysis of PFASs, the LC-MSMS analysis was also carried out according to Ahrens et al. (2011). Finally the data acquisition was performed using the software MassHunter 4.0 from Agilent Technologies.

2.4.3 Data Handling and Statistical Analysis

The statistical analysis was carried out using Microsoft Excel 2010 (Microsoft), JMP 10 (SAS Institute) and the box-whisker plots were generated in SigmaPlot (Systat Software GmbH).

To assure a good quality of laboratory work, mean of the blanks, method detection limits (MDL) and recovery were calculated (Table 4). For the blanks, five samples were taken and analyzed regarding their PFASs compounds (ng). The method detection limit was calculated using this formula: Mean blank+3*blank SD. For the compounds, where nothing was detected in the blanks, 0.05 ng was assumed (based on the lowest standard concentration in the calibration curve). To convert it to ng/g ww, the MDL was divided by two. The recovery was calculated by dividing the detected compound concentration by the average IS (100%) in percent for each sample. Out of this an average was calculated for each individual PFAS.

The PFASs concentration was calculated from dry weight (dw) into wet weight (ww) in ng/g, and values smaller than the MDL were set to zero. For each lake, the minimum, maximum, median, average, standard deviation, number detected, total number of samples and detection frequency (DF) was calculated. Only the ones with a DF>50% were used for further statistical analysis, which included 7 PFASs (i.e. 6:2 FTSA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA and PFOS). For statistical analysis, all zero values were replaced with 0.5 * MDL. Since the data was not normally distributed (statistically tested in JMP), they were transformed to common logarithmic data. By doing this it was possible to lay out not only non-parametric tests but also parametric tests (Field, 2009). This was done for the table of significances (Table 8) and for the Pearson Correlation matrix (Table 7) but not for the Whisker Plots (Figure 4 and 5) because there was no difference for the interpretation of the results when using logarithmic data.

Cluster analysis was used to visually describe which lakes were similar regarding the PFASs content in the samples (Ketchen Jr. & Shook, 1996). The data for the cluster analysis was calculated using the mean values of all samples of each lake. The resulting cluster diagram was used, to show which lakes were most similar to each other regarding the relative composition of individual PFAS species in European perch.

Pearson correlations between PFAS concentrations were used to investigate if the different substances have similar sources and correlate with each other showing R-value (Pearson correlation coefficient) and the *p*-value (probability value with a significance level of $\alpha = 0.05$). The closer R is to 1 or -1, the larger is the linear relationship, thus the substances are correlating stronger (Gasser et al., 2006).

Whisker plots were generated to visualize the trend of ∑PFASs before and after the forest CC. Furthermore, they were generated to visualize the trend of the spatial distribution

of ∑PFASs. Note that for the Whisker plots, the same data was used with a DF>50% but containing zeros. Moreover, ANOVA was used to emphasize the results of the spatial distribution and the CC effect. Before the ANOVA, the Levene´s test was conducted to assess the equality of variances.

To evaluate which factor had an influence on the accumulation of PFASs in fish, multivariate correlations were laid out and showed how several continuous variables for fish (age, length, weight, $\delta^{13}C$ and $\delta^{15}N$), latitude and all PFASs relate to each other. By counting the number of significant *p*-values and summarizing them, possible trends from factors were determined.

3 Results

3.1 Quality Assurance

The mean blanks ranged from a minimum of 0 ng/g ww (most compounds) to a maximum of 0.59 ng/g ww (PFPeA). The MDL ranged from a minimum of 0.0031 ng/g ww (FOSA) to 0.34 ng/g ww (PFPeA). The mean recovery±SD for all compounds except N-EtFOSA, N-EtFOSE and N-MeFOSE was 81±17% and for N-EtFOSA, N-EtFOSE and N-MeFOSE 12±8% but these substances were not detected in the samples thus the low value is not of concern.

Table 4. Mean blanks, method detection limit (MDL) and mean recovery for all compounds.

Compund	**Mean Blanks ±SD (ng) n=5**	**MDL (ng/g ww)**	**Mean Recovery ±SD (%) n=80**
PFBS	0	0.025	80±14
PFHxS	0	0.025	73±13
PFOS	0	0.025	80±14
PFDS	0	0.025	80±14
PFBA	0	0.025	64±12
PFPeA	0.59±0.026	0.34	73±13
PFHxA	0.21±0.015	0.13	73±13
PFHpA	0	0.025	81±16
PFOA	0	0.025	81±16
PFNA	0	0.025	86±16
PFDA	0.034±0.023	0.052	92±16
PFUnDA	0.0014±0.0031	0.0054	94±18
PFDoDA	0.00704±0.0066	0.014	83±17
PFTriDA	0	0.025	83±17
PFTeDA	0.0051±0.00701	0.013	83±17
PFHxDA	0	0.025	83±17
PFOcDA	0	0.025	83±17
FOSA	0.0034±0.00097	0.0031	54±13
N-EtFOSA	0	0.025	11±3
N-MeFOSA	0	0.025	47±37
N-EtFOSE	0	0.025	9±3
N-MeFOSE	0	0.025	17±18
FOSAA	0	0.025	96±23
N-EtFOSAA	0	0.025	117±25
N-MeFOSAA	0	0.025	96±23
6:2 FTSA	0	0.025	73±13
Mean Recovery all compounds ±SD (%) (Except N-EtFOSA, N-EtFOSE and N-MeFOSE)			81±17
Mean Recovery ±SD (%) for N-EtFOSA, N-EtFOSE and N-MeFOSE			12±8

3.2 Detection Frequency and Concentration Levels

For all substances that had a detection frequency (DF)>0, for the sums of the PFAS groups and the ∑PFASs, the minimum, maximum, median, mean and standard deviation (SD) was calculated (Table 5, Appendix A Table 9). The most frequently detected substances were PFUnDA and PFTriDA with a DF of 99% followed by PFOS with a DF of 80% and by PFDoDA and PFDA with a DF of 76% and 74%, respectively. Lastly PFTeDA had a DF of 68% and all other substances had a DF below 25%. This shows that the group of PFCAs was most commonly present in all samples. The maximum concentration level of 2.4 ng/g ww was for PFHxS and was only detected in one sample at lake Gårdsjön (resulting in the high ∑PFASs of 4.2 ng/g ww). 6:2 FTSA was also outstanding with 2.2 ng/g ww followed by much lower maximum concentration levels between 0.044 ng/g ww for PFNA and 0.93 ng/g ww for PFOS.

Table 5. Minimum, maximum, median, mean, standard deviation (SD) and detection frequency (DF) of all PFASs, which were detected in the samples.

Substance	DF (%)	Minimum (ng/g ww)	Maximum (ng/g ww)	Median (ng/g ww)	Mean (ng/g ww)	SD (ng/g ww)
PFBA	3.8	<0.025	0.36	n.a.	0.01	0.052
PFPeA	2.5	<0.34	0.53	n.a.	0.011	0.070
PFOA	8.8	<0.025	0.064	n.a.	0.003	0.012
PFNA	14	<0.025	0.044	n.a.	0.004	0.011
PFDA	74	<0.005	0.18	0.065	0.067	0.049
PFUnDA	99	0.038	0.64	0.24	0.24	0.13
PFDoDA	76	0.014	0.51	0.11	0.12	0.093
PFTriDA	99	0.048	0.85	0.23	0.24	0.14
PFTeDA	68	<0.013	0.11	0.022	0.023	0.021
PFHxS	1.3	<0.025	2.4	n.a.	0.030	0.26
PFOS	80	<0.025	0.93	0.13	0.19	0.19
6:2 FTSA	25	<0.025	2.2	n.a.	0.077	0.27
∑PFCAs	100	0.12	2.3	0.69	0.73	0.42
∑PFSAs	85	<0.025	2.9	0.13	0.22	0.35
∑PFASs	100	0.12	4.2	0.86	1.03	0.73

<x, below the respective method detection limit (MDL)
n.a. not analyzed

3.3 Composition Profile

The cluster analysis shows similar patterns of PFAS composition between the studied lakes (Figure 2). It can be seen that the samples from Björntjärn and Långtjärn had the most similar composition as well as the samples from Brobo-Kroktjärn and *Umeå 4*, whereas all four were also similar to Kroktjärn. The samples from Gårdsjön had a composition of PFASs that differs the most from the other lakes.

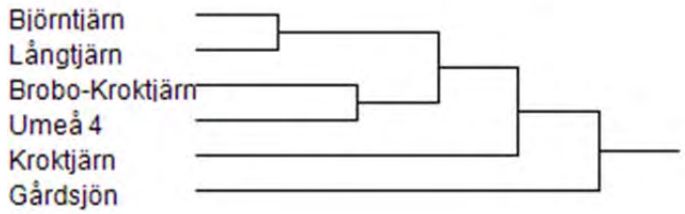

Figure 2. Cluster diagram of sampled lakes.

The relative composition of the detected PFASs (DF>50%) in the samples of the studied lakes showed that the PFASs with the highest contribution were PFTriDA and PFUnDA ranging from 18% to 34% and 20% to 31%, respectively (Figure 3, complete data in Appendix B, Table 10). A large portion was also PFOS ranging from 3% (*Umeå 4*) to 30% (Långtjärn). *Umeå 4* had an outstanding composition profile due to the occurrence of PFPeA (7%), which was not detected in any other lake and its low contribution of PFOS (3%). 11% of 6:2 FTSA were found in the compostion of lake Långtjärn and Kroktjärn (before CC) but only accumulating in very specific samples with a rather high level. In general, 6:2 FTSA was found to have different patterns than all other PFASs (Table 6 and 6).

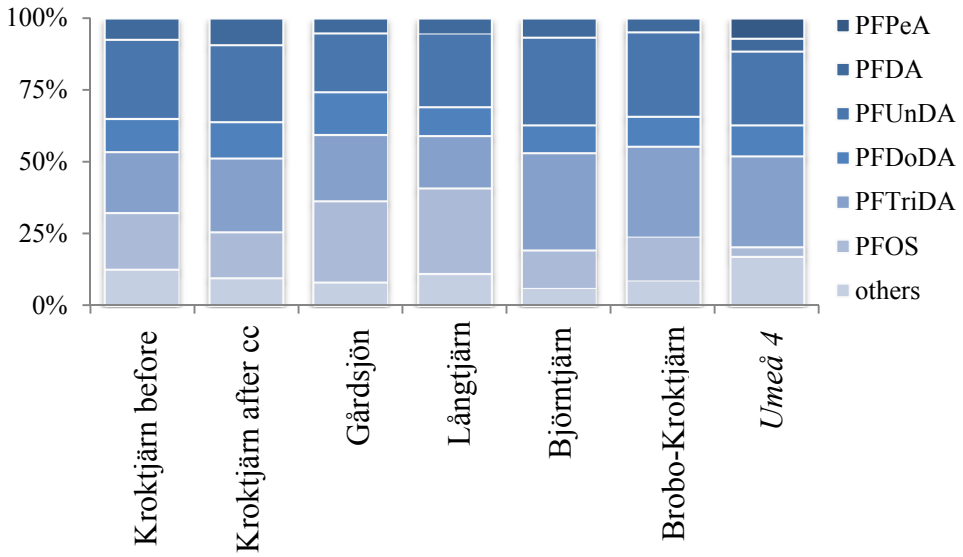

Figure 3. Composition profile of analyzed PFASs.

3.4 PFASs Concentrations Before and After Forest CC

Samples of European perch were taken from lake Kroktjärn before the forest CC in 2010 and 2011 as well as after the forest CC in 2013 and 2014 to show the accumulation of PFASs in European perch (Figure 4). Against our hypothesis, there was no observable effect of the forest CC on the PFAS levels in fish (ANOVA, R^2=0.0012, p>0.05), even if the CC area of 36% at lake Kroktjärn was one of the largest (Table 1) compared to the other lakes.

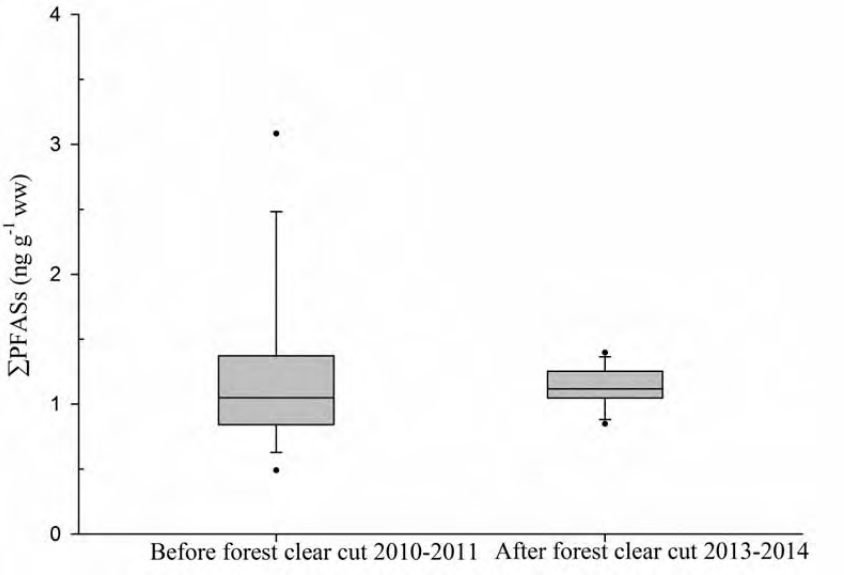

Figure 4. ∑PFASs before and after forest CC event.

The ∑PFASs before the CC event ranged between 0.49 ng/g ww and 2.5 ng/g ww (outlier: 3.08 ng/g ww, with 6:2 FTSA = 2.2 ng/g ww) and after the CC event between 0.85 and 1.4 ng/g ww. The median of the ∑PFASs changed from 1.05 ng/g ww to 1.1 ng/g ww (Appendix A, Table 9).

When looking at the results of the individual PFASs before and after forest CC, it was found that most PFASs showed just small variations or even a decreased level after the CC (Appendix A, Table 9).

3.5 Spatial Distribution

The ∑PFASs showed a slight and steady increase in concentrations from north to south (Figure 5, Table 6, Figure 6) and that there was a significant difference between the ∑PFASs of all the lakes (ANOVA, R^2=0.72, p<0.0001).

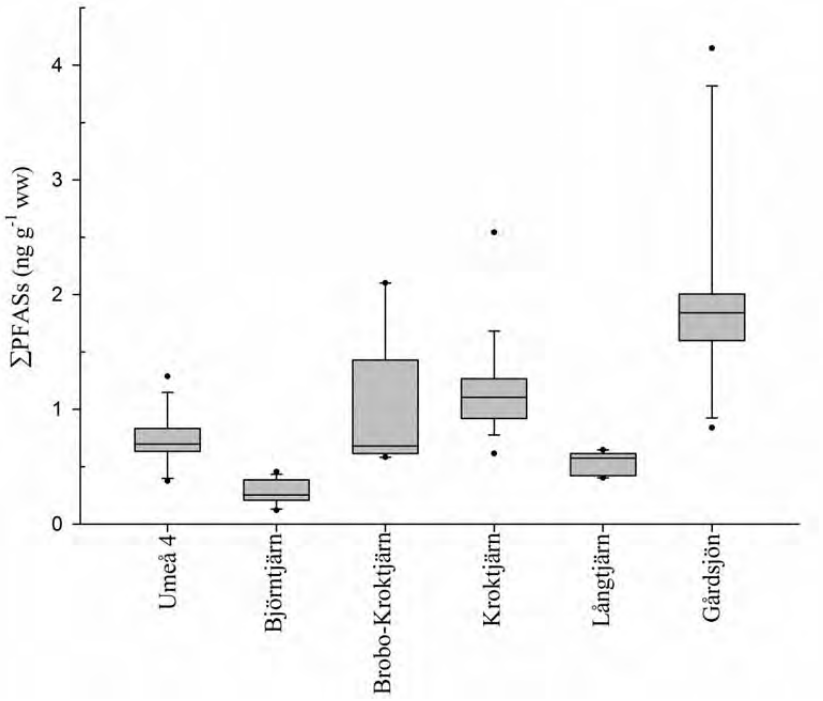

Figure 5. Spatial distribution of PFASs in different lakes over Sweden, arranged from north to south.

Values of the ∑PFASs ranged from a minimum of 0.12 ng/g ww for lake Björntjärn, a rather northerly located lake (latitude: N 63° 54′), to a maximum of 4.2 ng/g ww for lake Gårdsjön, the most southerly located lake of the studies lakes (latitude: N 58° 03'). The medians ranged between 0.25 and 1.8 ng/g ww for Björntjärn and Gårdsjön respectively. Correlations between all PFASs and the corresponding latitude were done to further emphasize the importance of the location of the lake (Table 6).

Table 6. Latitude-PFASs concentration correlations.

Substance	**R^2-value**	**R-value**	***p*-value**
PFDA	0.305	-0.55	<0.0001
PFUnDA	0.42	-0.65	<0.0001
PFDoDA	0.501	-0.71	<0.0001
PFTriDA	0.31	-0.56	<0.0001
PFTeDA	0.309	-0.56	<0.0001
PFOS	0.604	-0.78	<0.0001
6:2 FTSA	0.00028	-0.017	0.88
∑PFCAs	0.41	-0.64	<0.0001
∑PFSAs	0.29	-0.54	<0.0001
∑PFASs	0.403	-0.63	<0.0001

All PFASs, except of 6:2 FTSA, showed significant negative regression slopes (p<0.05) as well as low to moderate R^2-values, indicating that the latitude can predict the large scale spatial distribution of PFASs.

For each substance and latitudes, linear regressions were laid out with a confidence interval of 95% (Figure 6). All PFASs showed a negative correlation with latitude (Table 6, Figure 6). It implies that the further north the lake was situated (higher latitude), the less PFAS were detected in European perch. This is in agreement with the trend that is shown in Figure 5.

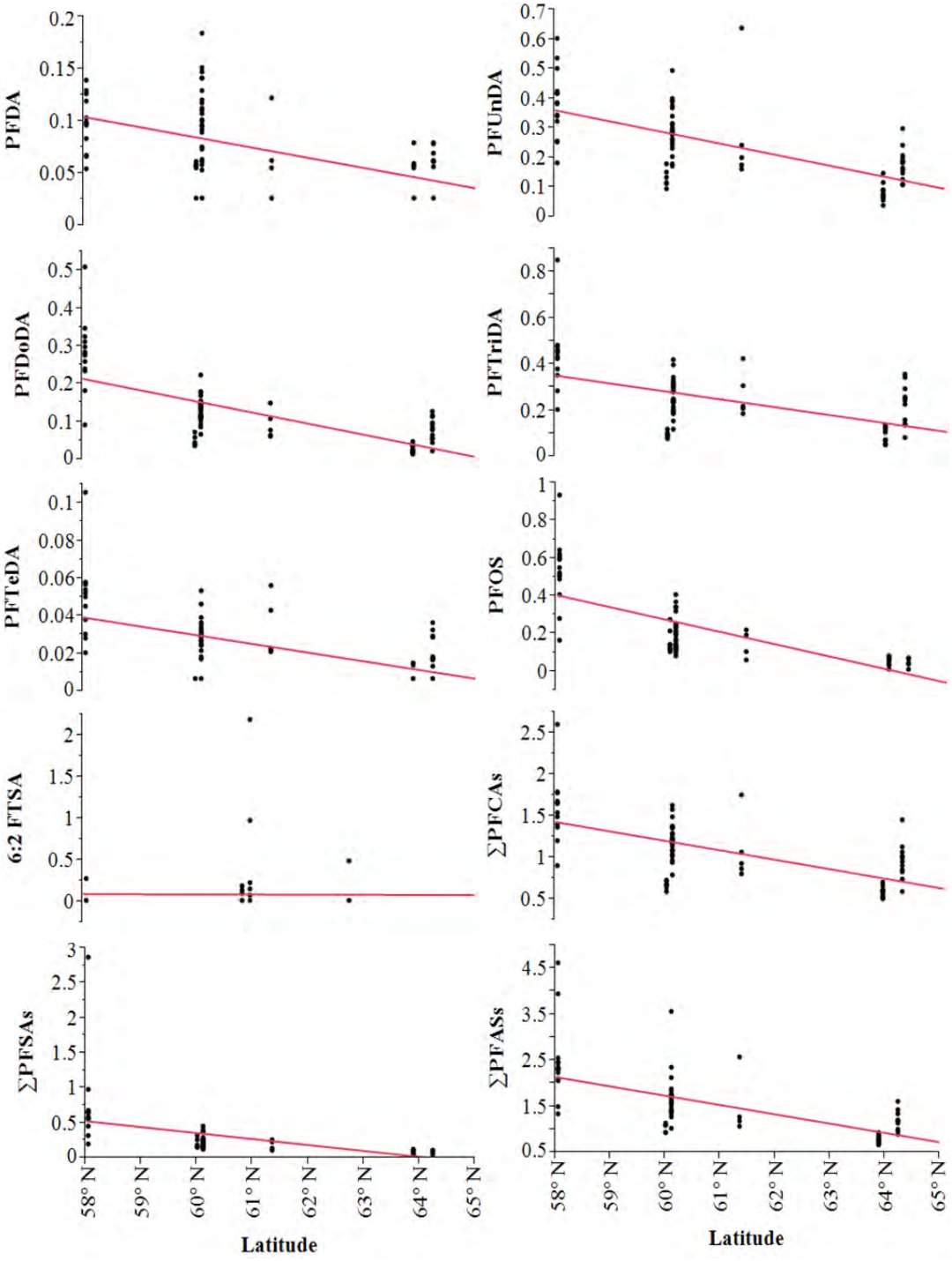

Figure 6. Linear regressions of individual PFASs, ∑PFCAs, ∑PFSAs and ∑PFASs (ng/g ww) over latitude.

3.6 Correlations of Hg and PFASs

Pearson correlations were laid out to see how each PFAS and Hg correlated across all lakes. It was found that almost all PFASs were correlated with each other highly significantly ($p<0.0001$) except from 6:2 FTSA (Pearson Correlation matrix, Table 7). It could also be demonstrated that Hg was correlating with PFDA ($p<0.001$), PFUnDA, PFDoDA and ∑PFCAs ($p<0.05$).

Table 7. Correlations and probability value of all investigated PFASs and Hg (n=80) using log data.

Substance		6:2 FTSA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFOS	∑PFCAs	∑PFSAs	∑[a]	∑PFASs
6:2 FTSA	R											
	p-value											
PFDA	R	-0.13										
	p-value	0.25										
PFUnDA	R	-0.015	0.82									
	p-value	0.90	1.3E-20****									
PFDoDA	R	-0.044	0.72	0.93								
	p-value	0.67	5.01E-14****	3.8E-36****								
PFTriDA	R	-0.0013	0.62	0.84	0.91							
	p-value	0.99	9.2E-10****	2.0E-22****	8.2E-32****							
PFTeDA	R	-0.069	0.55	0.72	0.81	0.89						
	p-value	0.54	1.4E-07****	4.0E-14****	2.4E-19****	5.4E-29****						
PFOS	R	-0.20	0.66	0.73	0.69	0.48	0.46					
	p-value	0.071	2.7E-11****	2.9E-14****	2.1E-12****	5.5E-06****	2.05E-05****					
∑PFCAs	R	-0.052	0.78	0.94	0.95	0.93	0.86	0.65				
	p-value	0.65	9.7E-18****	2.2E-37****	2.3E-40****	3.8E-36****	1.3E-24****	9.07E-11****				
∑PFSAs	R	-0.202	0.65	0.73	0.71	0.54	0.52	0.95	0.69			
	p-value	0.073	6.08E-11***	2.4E-14****	9.8E-14****	1.9E-07****	8.9E-07****	6.4E-43****	1.5E-12****			
∑FTSAs/FOSAs/	R	0.96	-0.071	0.042	0.008	0.040	0.010	-0.12	0.005	-0.13		
FOSEs/FOSAAs	*p*-value	5.2E-46****	0.53	0.71	0.94	0.73	0.93	0.28	0.97	0.26		
∑PFASs	R	0.15	0.72	0.89	0.89	0.84	0.79	0.71	0.92	0.79	0.25	
	p-value	0.17	5.2E-14****	1.3E-27****	1.8E-28****	1.2E-22****	5.0E-18****	1.9E-13****	3.9E-33****	4.6E-18****	0.027*	
Hg (mg/kg ww)	R	0.039	0.37	0.25	0.23	0.22	0.19	0.005	0.26	-0.061	0.087	0.16
	p-value	0.73	8.6E-04***	0.024*	0.037*	0.050	0.099	0.96	0.019*	0.59	0.44	0.15

*p<0.05;**p<0.01;***p<0.001;****p<0.0001;
[a] FTSAs/FOSAs/FOSEs/FOSAAs

3.7 Other Significant Factors

More correlations were tested between the PFAS concentrations and a variety of factors (Table 8). For this, the significant *p*-values were counted for each correlation and summed up.

Table 8. Individual PFASs and ∑PFASs that correlated significantly ($p<0.05$) with different factors (length, weight, age, $\delta^{13}C$, $\delta^{15}N$ and latitude) and number of significant *p*-values.

	Kroktjärn	**Gårdsjön**	**Långtjärn**	**Björntjärn**	**Brobo-Kroktjärn**	***Umeå 4***	**Total (Nr. of Significances)**
Length	-	-	∑PFASs	PFDA, PFTeDA	-	∑PFSAs	**4**
Weight	-	-	PFOS, ∑PFSAs, ∑PFASs,	PFDA	-	∑PFASs	**5**
Age	-	-	-	PFTeDA	-	PFDoDA, PFTeDA, ∑PFCAs	**4**
$\delta^{13}C$	PFDoDA, PFTriDA, PFTeDA, ∑PFCAs, ∑PFASs	-	PFDoDA, PFTriDA	-	-	PFDoDA, PFTriDA, PFTeDA, ∑PFCAs	**11**
$\delta^{15}N$	-	-	PFDA, ∑PFASs	-	-	PFTriDA	**3**
Total (Nr. of Significances)	**5**	**0**	**8**	**4**	**0**	**10**	**27**

It was found that one key factor was $\delta^{13}C$ with a number of significant *p*-values of 11. This was shown for lake Kroktjärn, Långtjärn and *Umeå 4* for PFDoDA, PFTriDA, PFTeDA, ∑PFCAs and ∑PFASs.

4 Discussion

4.1 PFASs

4.1.1 Comparison of PFAS Concentrations to Literature Data

In this study, the ∑PFAS ranged between 0.12 and 4.2 ng/g ww (Table 5), which is similar to the study of Sjöholm (2015) investigating the fish species *L. megastoma, L. intermedius, L. gorguari, C. gariepinus* and *O. niloticus* from lake Tana in Ethiopia (between 0.2 and 2.1 ng/g ww). In contrast, ∑PFAS concentrations were higher in lake trout, arctic char, brown trout and rainbow trout from several high mountain lakes in France (∑PFCAs ranged between 20.7 and 36.1 ng/g ww) compared to this study (∑PFCAs = 0.12 ng/g ww and 2.3 ng/g ww) (Ahrens et al., 2010). The high ∑PFCA concentrations in France were explained by atmospheric deposition of PFCAs or their precursors, originated from long-range transport or the nearby city Grenoble. In addition, Giesy & Kannan (2001) found that PFOS concentrations in biota were higher in industrialized and more populated areas than in remote regions. This was also found in this study with PFOS levels ranging between 0.16 and 0.93 ng/g ww for lake Gårdsjön (lat: N 58° 03') and between <0.025 (<MDL) and 0.068 ng/g ww for lake *Umeå 4* (lat: N 64° 15′) (Appendix A, Table 9).

4.1.2 Spatial Distribution

∑PFASs showed highest concentration at lake Gårdsjön, which is located in southern Sweden approximately 90 km from the city Gothenburg (∑PFASs = 4.2 ng/g ww) (Appendix A, Table 9). The cluster analysis (Figure 2) showed that lake Gårdsjön differed from its composition of PFASs from the other lakes the most. Whereas the composition profile (Figure 3) showed the same results as the concentration levels (PFTriDA, PFUnDA and PFOS were the main contributing PFASs), except from lake *Umeå 4*, which had besides 32% PFTriDA and 25% PFUnDA, as much as 7% PFPeA and only 3% PFOS (Appendix B, Table 10). Also 6:2 FTSA (belonging to the category “others” in Figure 3) contributed with 11% to the composition of lake *Umeå 4*, making it the most outstanding lake. However, when looking at latitude-PFAS correlations, almost all had a significant decreasing north-south gradient (p-value <0.0001) apart from 6:2 FTSA, indicating that the location of the lake plays an important role in the accumulation of PFASs. All individual PFASs, except from 6:2 FTSA, followed the same pattern as ∑PFASs and concentrations decreased from south to north (Figure 6).

Ahrens et al. (2010) showed that the transport and bioaccumulation of PFCAs and PFSAs were compound specific and that long chained PFASs have a higher

potential to accumulate in the food chain. This could also be observed in this study with PFUnDA ($C_{10}F_{21}CO_2^-$) and PFTriDA ($C_{12}F_{25}CO_2^-$) with a DF of 99% followed by PFOS ($C_8F_{17}SO_3^-$) with a DF of 80%, as the dominant PFASs (Table 5). The finding of mostly long chained PFASs, on the other hand, disagrees with the fact that the main source of contaminants comes directly from the water (as mentioned in the introduction) since short chained PFSAs and PFCAs are more water soluble according to Ahrens et al. (2010), thus they should be found more.

When it comes to PFOS though, the same study of Ahrens et al. (2010) showed that PFOS distribute in a different way than the other detected PFASs and its source is not the closest industrial region but rather due to the consistent atmospheric deposition. This is in contrary to what was found in this study where PFOS levels decreased from southern Sweden (lake Gårdsjön) to northern Sweden (lake *Umeå 4*). One explanation for it could be the difference in altitude of the studied lakes, which influences the distribution of emissions.

4.1.3 Forest Clear-Cut Effect

No significant change of the PFAS concentration were found before and after CC (p>0.05). This could be explained by the fact that the CC has no observable effect on the PFASs level in fish from this particular lake. Also that there might have been less precipitation before sampling thus less surface runoff or that the runoff after CC was not as high as found by Rosén et al. (1996) (up to 100% and more). It is also possible that there is, despite a higher runoff after the CC, a strong sorption of PFASs onto soil particles so they are not released with the runoff. Moreover, PFASs could bind onto the sediment in the lake so the equilibrium state will be reached quickly. Lastly there might be an observable effect only after several years.

However, in a study by Martins (2014) a significant increase in Hg concentration of 10% in perch after the CC was found. Furthermore, it was found that the lakes with higher CC areas had also higher mean Hg concentration after the CC than the lakes with the smaller CC areas.

4.1.4 Other Significant Factors

The accumulation of PFASs in fish was strongly explained by $\delta^{13}C$ (Table 8). The fractionation of C and N isotopes in fish muscle is determined by the diet of the fish, i.e. feeding habits as well as excretion (Wada, 2009). This means that the nutrition and its depuration plays also an important role when it comes to PFASs but to which extent is not clear and it is out of the scope of this study.

Moreover, long time exposure has to be considered due to the fact that PFASs have been continuously released into the environment, they have been accumulating for several years thus influencing many generations of fish. Even if research recently made achievements regarding the effects of PFASs on the environment, due to the continuous changes and/or phase out of chemicals and the following change in mixture of substances, it is still a very challenging field that has to be investigated.

4.1.5 TDI and Type of PFASs

When comparing the results of the concentration levels to the TDI levels (only existing for PFOS and PFOA) suggested by the European Food Safety Authority (EFSA), it was shown that the maximum detected PFOS and PFOA level of 0.93 ng/g ww and 0.064 ng/g ww respectively (Appendix A Table 9) were far below the suggested dose of 150 ng/kg bw per day and 1500 ng/kg bw per day respectively (D. Borg & Håkansson, 2012). This can be calculated when assuming a consumption of 195 g of fish (one meal portion according to Lindstrom et al. (2011)) and a body weight of 70 kg:

$$\mathrm{TDI_{PFOS}} = \frac{0.93\frac{ng}{g\ ww} \cdot 195\ \mathrm{g}}{70\ kg} = 2.6\ \frac{ng}{kg\ bw} < 150\ \frac{ng}{kg\ bw}$$

$$\mathrm{TDI_{PFOA}} = \frac{0.064\frac{ng}{g\ ww} \cdot 195\ \mathrm{g}}{70\ kg} = 0.18\ \frac{ng}{kg\ bw} < 1500\ \frac{ng}{kg\ bw}$$

The results of the detected concentration levels also correspond with what was found by Ahrens et al. (2010), as described in the following sentence. PFOA was not detected (compared to a DF of 9% in this study) because of its low bioaccumulation potential (J. W. Martin et al., 2003a) and PFOS was detected in all samples (compared to a DF of 80% in this study) due to the fact that it was found to be the most present PFAS in biota (Ahrens & Bundschuh, 2014). In general, the detection of the type of PFASs, mostly long-chained PFASs, was the same but total levels in this study were much lower than in the study by Ahrens et al. (2010). In this study, 12 different PFASs (PFBA, PFPeA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxS, PFOS and 6:2 FTSA) were detected, whereas the study conducted in France a total of 7 different PFASs were detected (PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA and PFPeDA) (Ahrens et al., 2010). Also the study done by Sjöholm (2015) showed a lower number of PFASs (7 in total- PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS and PFOS) than the results from this study. The substances that were detected in all three studies (PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA and PFOS) belong to the group of PFCAs, apart from PFOS (belongs to PFSAs). However, all had a chain length of 8 C atoms or more, which proves again that long chained PFASs have a higher bioaccumulation potential than shorter chained PFASs, even if different species were investigated and mixtures of PFASs can have unpredictable species related effects (Ahrens & Bundschuh, 2014).

4.2 PFASs and Hg

While all PFASs with a DF>50%, except of 6:2 FTSA, correlated in a highly significant way (p<0.0001, Table 7) there were only four significant correlations between PFASs and Hg (PFDA p<0.001, PFUnDA, PFDoDA and ∑PFCAs p<0.05). Similar results were found by Ahrens et al. (2010), where almost no significant

correlations were found apart from Hg and PFCAs (p<0.05). All substances in fish muscle that correlate significantly indicate similar accumulation pattern and transport. Thus, it can be stated that all PFASs, except of 6:2 FTSA, and Hg correlating with three PFCAs are subject to this statement. This, however, disagrees with the study of Sjöholm (2015), where only one significant correlation occurred between Hg and PFOS. Note that these studies were done in Ethiopia at a lake that was close to populated areas as well as to agricultural land and thus might influence the results.

5 Conclusions

The aim of this study was to assess if *i)* PFAS levels in fish were elevated after clear cutting close to lakes and *ii)* that PFAS levels in fish were impacted by atmospheric deposition with a decreasing gradient from south (urban/industrial areas) to north (remote areas). The results showed that hypothesis *i*) could be rejected (PFAS levels were not elevated after CC) and that hypothesis *ii)* was accepted (PFAS levels were impacted according to the gradient).

Other than Hg, PFASs did not show a clear impact of forest management practices (ANOVA, R^2=0.0012, p>0.05), even if the CC area of 72.7 ha was quite big compared to the size of lake Kroktjärn (4.77 ha). The surface runoff of the prone area would thus be large enough to show an effect as for Hg, where the mean Hg ww±mean SD increased from 0.45±0.25 mg/kg to 0.84±0.15 mg/kg (J. C. Martin, 2014) compared to mean ∑PFASs±mean SD, which even decreased from 1.2±0.62 ng/g ww to 1.1±0.15 ng/g ww (Appendix A, Table 9). In general, the group of PFCAs was most present in all samples, more precisely, long chained PFCAs. It proves again that they have a higher bioaccumulation potential than shorter chained PFASs.

When investigating the impact of the spatial distribution of the lakes over Sweden, it showed that the lakes in the more populated, southerly part of the studied area (Gårdsjön, Kroktjärn, Brobo-Kroktjärn) were more prone to PFASs accumulation since fish from the southern lakes (especially Gårdsjön) showed elevated levels of PFASs (ANOVA, R^2=0.72, p<0.0001). Furthermore, the correlations of latitude and PFASs were all significant (p<0.0001) except of 6:2 FTSA, indicating that the location of the lake plays an important role. Lake Gårdsjön and *Umeå 4* were the lakes that differed most regarding their composition compared to the other lakes.

Four significant correlations were found between PFASs and Hg (PFDA (p<0.001), PFUnDA, PFDoDA and ∑PFCAs (p<0.05)), which might be explained by the fact that PFASs mostly come from anthropogenic sources and Hg is an element occurring in nature. Nevertheless PFDA, PFUnDA, PFDoDA and Hg seem to have similar accumulation and transport pattern as well as all PFASs, except of 6:2 FTSA.

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8 Appendix

Appendix A. Lake specific statistical data of all detected PFASs

Table 9. Minimum, maximum, median, mean, standard deviation (SD) and detection frequency (DF) of all detected PFASs sorted after lakes.

Lake	Year		6:2 FTSA	PFBA	PFPeA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFHxS	PFOS	∑PFCAs	∑PFSAs	∑[a]	∑PFASs
Kroktjärn	2010/2011	Min	<0.025	<0.025	<0.336	<0.025	<0.025	<0.005	0.17	0.066	0.12	<0.013	<0.025	0.079	0.41	0.079	<0.003	0.49
		Max	2.2	0	0	0	0.037	0.18	0.49	0.22	0.33	0.036	0	0.406	1.3	0.406	2.2	3.1
		Median	0	0	0	0	0	0.092	0.27	0.11	0.23	0.018	0	0.21	0.73	0.21	0	1.05
		Mean	0.24	0	0	0	0.009	0.090	0.30	0.12	0.23	0.016	0	0.22	0.76	0.22	0.24	1.2
		SD	0.60	0	0	0	0.014	0.052	0.087	0.037	0.057	0.013	0	0.11	0.21	0.11	0.60	0.62
		DF (%)	29	0	0	0	29	86	100	100	100	64	0	100	100	100	29	100
Kroktjärn	2013/2014	Min	<0.025	<0.025	<0.336	<0.025	<0.025	0.061	0.18	0.090	0.20	0.025	<0.025	0.112	0.73	0.11	<0.003	0.85
		Max	0.228	0.36	0	0	0.035	0.15	0.40	0.22	0.42	0.053	0	0.25	1.2	0.25	0.23	1.4
		Median	0	0	0	0	0	0.104	0.30	0.15	0.30	0.031	0	0.18	0.88	0.18	0	1.1
		Mean	0.016	0.055	0	0	0.004	0.107	0.30	0.14	0.29	0.033	0	0.18	0.93	0.18	0.016	1.1
		SD	0.059	0.11	0	0	0.011	0.023	0.051	0.031	0.064	0.008	0	0.047	0.13	0.047	0.059	0.15
		DF (%)	7.1	21	0	0	14	100	100	100	100	100	0	100	100	100	7.1	100
Gårdsjön	2010	Min	<0.025	<0.025	<0.336	<0.025	<0.025	0.054	0.18	0.091	0.20	0.020	<0.025	0.16	0.56	0.16	<0.003	0.84
		Max	0.28	0	0	0.054	0.037	0.14	0.602	0.51	0.85	0.105	2.4	0.93	2.3	2.9	0.28	4.2
		Median	0	0	0	0	0	0.098	0.38	0.28	0.45	0.053	0	0.54	1.3	0.57	0	1.8
		Mean	0.020	0	0	0.013	0.005	0.098	0.38	0.28	0.44	0.051	0.17	0.53	1.3	0.70	0.020	2.0
		SD	0.071	0	0	0.018	0.012	0.024	0.11	0.090	0.14	0.019	0.608	0.17	0.37	0.62	0.071	0.83
		DF (%)	7.1	0	0	36	14	100	100	100	100	100	7.1	100	100	100	7.1	100
Långtjärn	2010	Min	<0.025	<0.025	<0.336	<0.025	<0.025	<0.005	0.095	0.037	0.074	<0.013	<0.025	0.106	0.22	0.106	<0.003	0.402
		Max	0.19	0	0	0	0	0.061	0.18	0.071	0.12	0	0	0.27	0.37	0.27	0.19	0.65

(Continued)

(Table 9 continued)

Lake	Year		6:2 FTSA	PFBA	PFPeA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFTeDA	PFHxS	PFOS	∑PFCAs	∑PFSAs	∑[a]	∑PFASs
		Median	0	0	0	0	0	0.055	0.13	0.045	0.093	0	0	0.14	0.302	0.14	0	0.57
		Mean	0.062	0	0	0	0	0.033	0.13	0.050	0.091	0	0	0.16	0.305	0.16	0.062	0.53
		SD	0.075	0	0	0	0	0.028	0.026	0.011	0.014	0	0	0.058	0.049	0.058	0.075	0.097
		DF (%)	43	0	0	0	0	57	100	100	100	0	0	100	100	100	43	100
Björntjärn	2011	Min	<0.025	<0.025	<0.336	<0.025	<0.025	<0.005	0.038	0.014	0.048	<0.013	<0.025	<0.025	0.12	<0.025	<0.003	0.12
		Max	0.10	0	0	0.064	0.026	0.079	0.15	0.047	0.13	0.015	0	0.079	0.34	0.079	0.101	0.46
		Median	0	0	0	0	0	0	0.077	0.024	0.072	0	0	0.055	0.22	0.055	0	0.25
		Mean	0.013	0	0	0.005	0.002	0.023	0.083	0.026	0.087	0.002	0	0.040	0.23	0.040	0.013	0.28
		SD	0.032	0	0	0.017	0.007	0.030	0.027	0.010	0.029	0.005	0	0.029	0.070	0.029	0.032	0.099
		DF (%)	15	0	0	7.7	7.7	38	100	100	100	15	0	69	100	69	15	100
Brobo-Kroktjarn	2011	Min	<0.025	<0.025	<0.336	<0.025	<0.025	<0.005	0.163	0.059	0.19	0.021	<0.025	0.059	0.43	0.059	<0.003	0.58
		Max	0.49	0	0	0.034	0	0.122	0.64	0.15	0.42	0.056	0	0.22	1.4	0.22	0.49	2.1
		Median	0	0	0	0	0	0.055	0.20	0.078	0.22	0.023	0	0.101	0.58	0.101	0	0.68
		Mean	0.097	0	0	0.007	0	0.048	0.28	0.092	0.27	0.033	0	0.13	0.73	0.13	0.097	0.95
		SD	0.20	0	0	0.013	0	0.046	0.18	0.034	0.087	0.014	0	0.067	0.36	0.067	0.20	0.58
		DF (%)	20	0	0	20	0	60	100	100	100	100	0	100	100	100	20	100
Umeå 4	2011	Min	<0.025	<0.025	<0.336	<0.025	<0.025	<0.005	0.107	0.023	0.081	<0.013	<0.025	<0.025	0.21	<0.025	<0.003	0.37
		Max	0.29	0	0.53	0	0.044	0.079	0.30	0.13	0.36	0.036	0	0.068	1.29	0.068	0.29	1.3
		Median	0.095	0	0	0	0	0.056	0.18	0.076	0.25	0.017	0	0	0.605	0	0.095	0.70
		Mean	0.091	0	0.067	0	0.005	0.036	0.18	0.078	0.23	0.017	0	0.021	0.61	0.021	0.091	0.72
		SD	0.087	0	0.16	0	0.013	0.034	0.051	0.029	0.078	0.012	0	0.028	0.24	0.028	0.087	0.22
		DF (%)	62	0	15	0	15	54	100	100	100	77	0	38	100	38	62	100

[a] FTSAs/FOSAs/FOSEs/FOSAAs

<x, below the respective method detection limit (MDL)

Appendix B. Data for composition profile

Table 10. Composition of samples from all studied lakes.

	PFPeA	**PFDA**	**PFUnDA**	**PFDoDA**	**PFTriDA**	**PFOS**	**others**
Kroktjärn before CC	0	8	28	11	21	20	13
Kroktjärn after CC	0	10	26	13	26	16	10
Gårdsjön	0	5	20	15	23	28	8
Långtjärn	0	5	25	10	18	30	11
Björntjärn	0	7	31	10	34	13	6
Brobo-Kroktjärn	0	5	29	11	31	15	9
Umeå 4	7	4	25	11	32	3	17
Unit for all: %							

Appendix C. Topographic maps of the catchment areas of the studied lakes (after CC)

Legend for topographic maps:

Water


Clear Cut Area

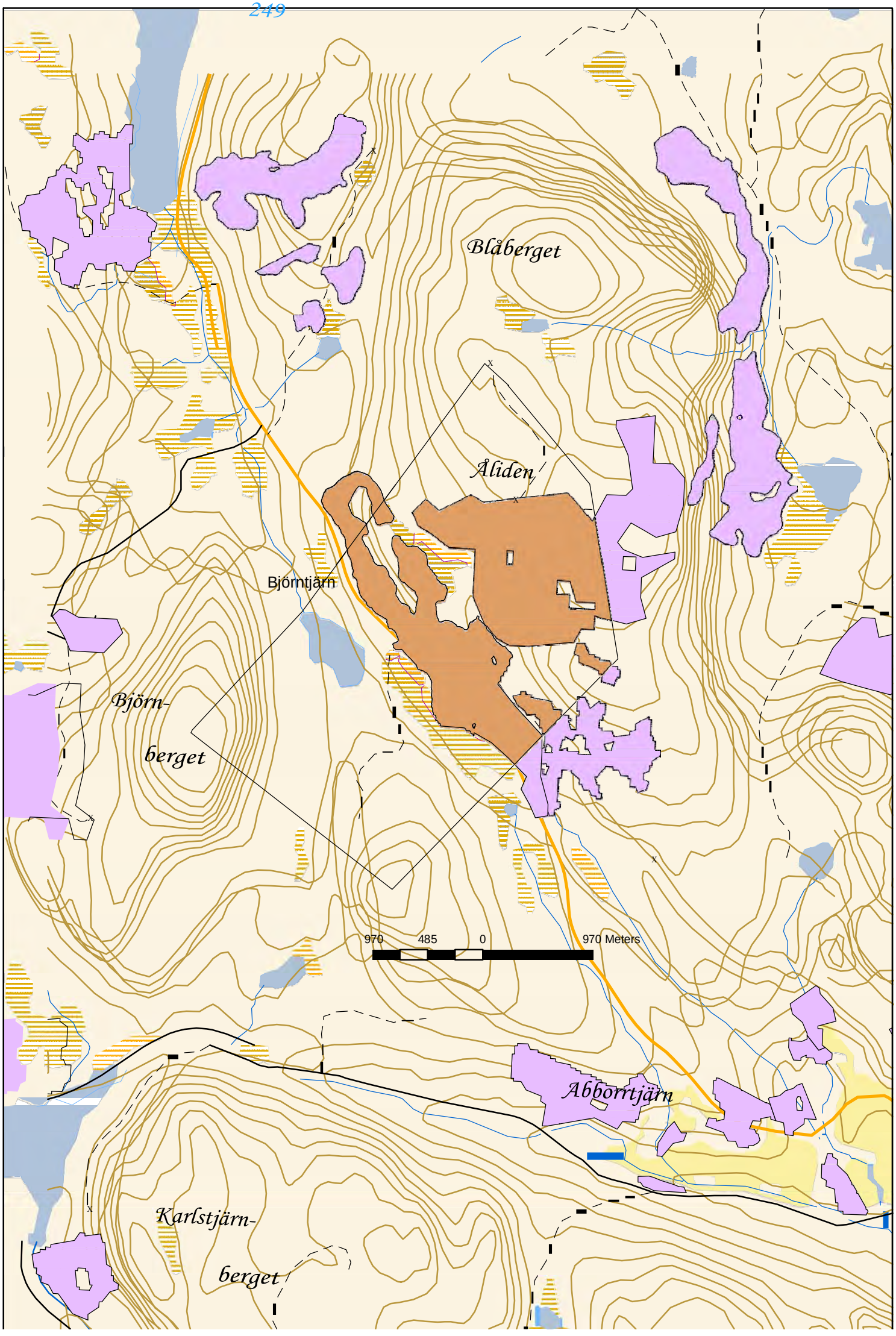

Clear Cut Area

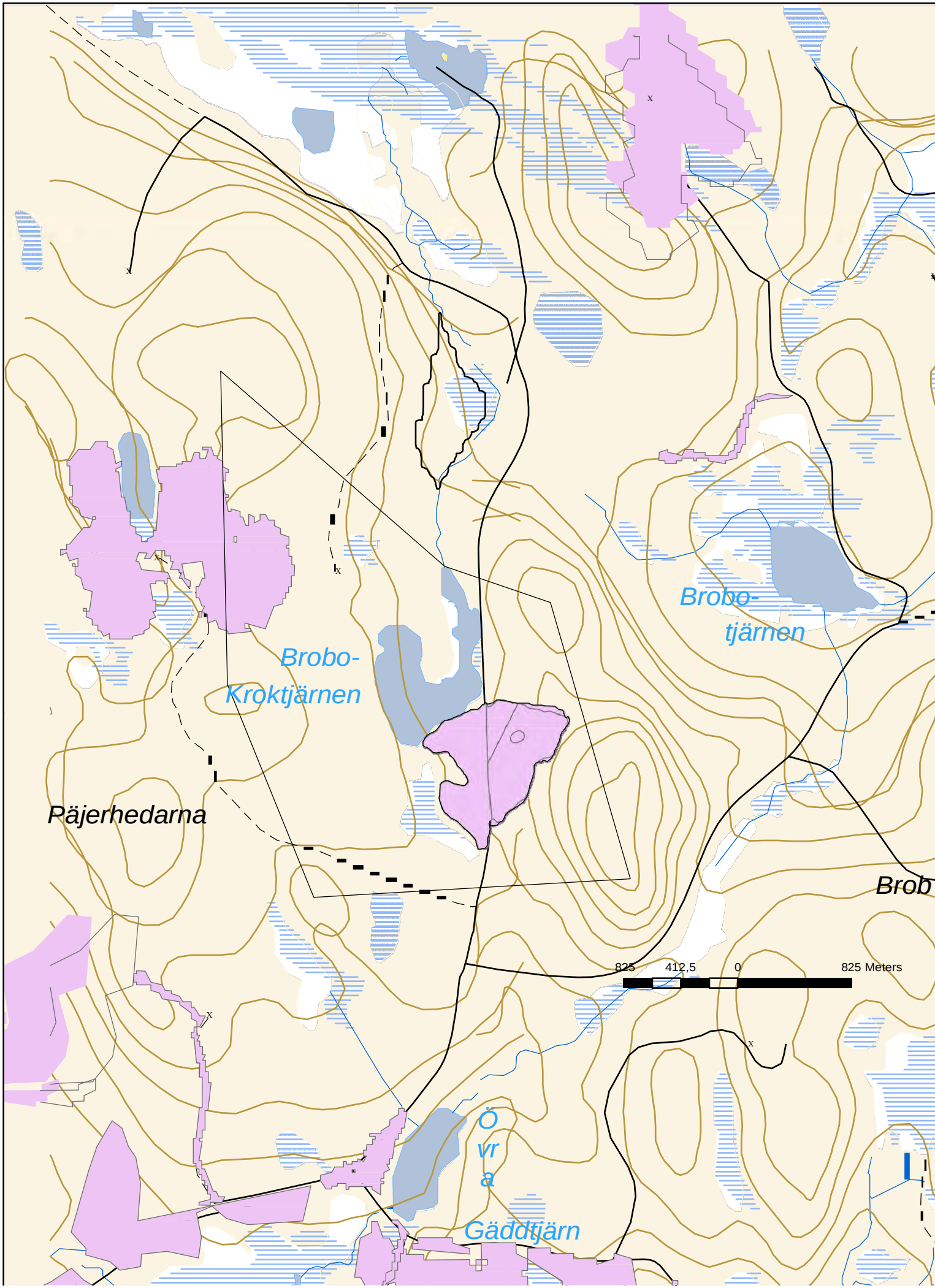

Wetland

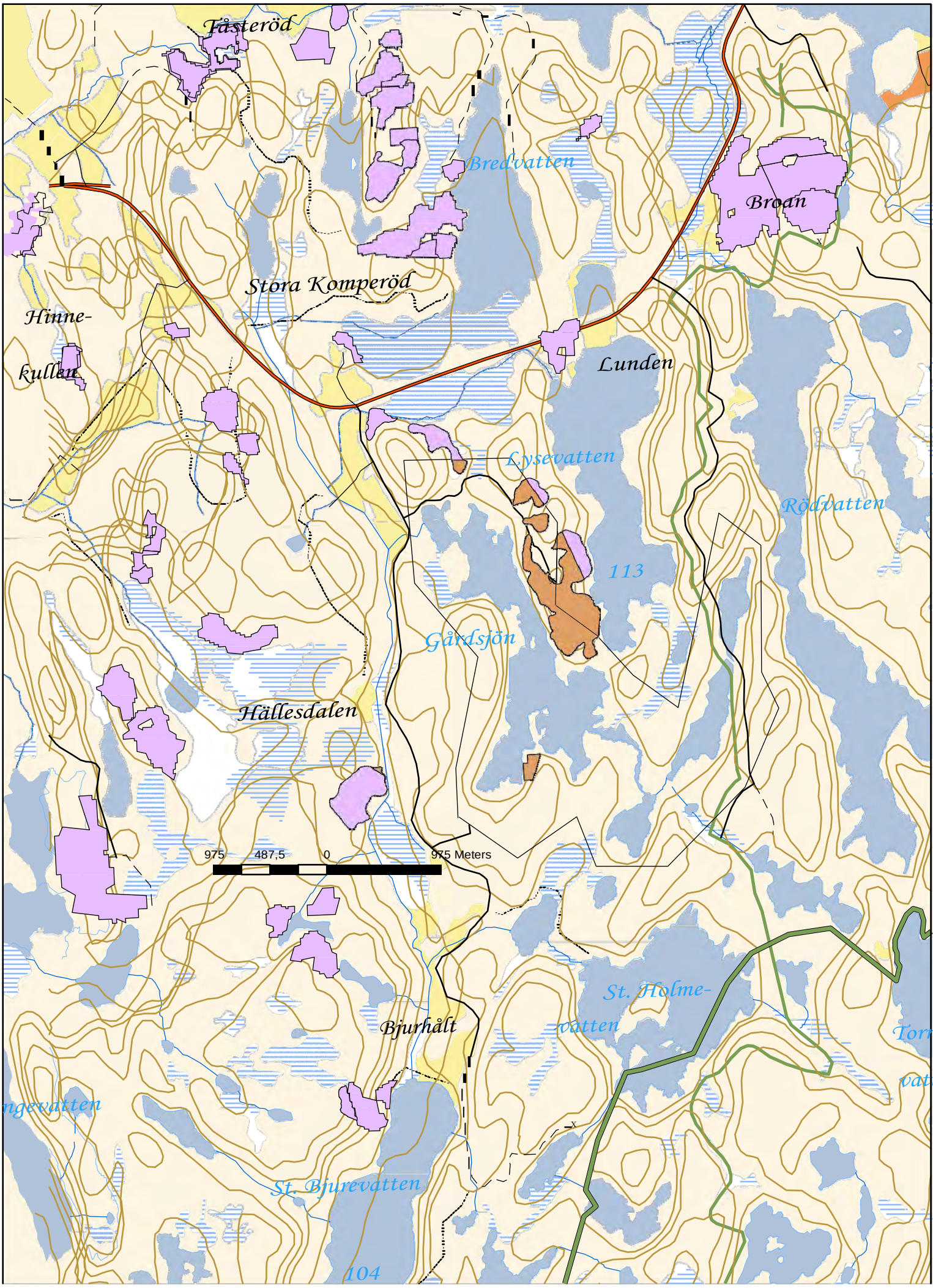
Contour Line

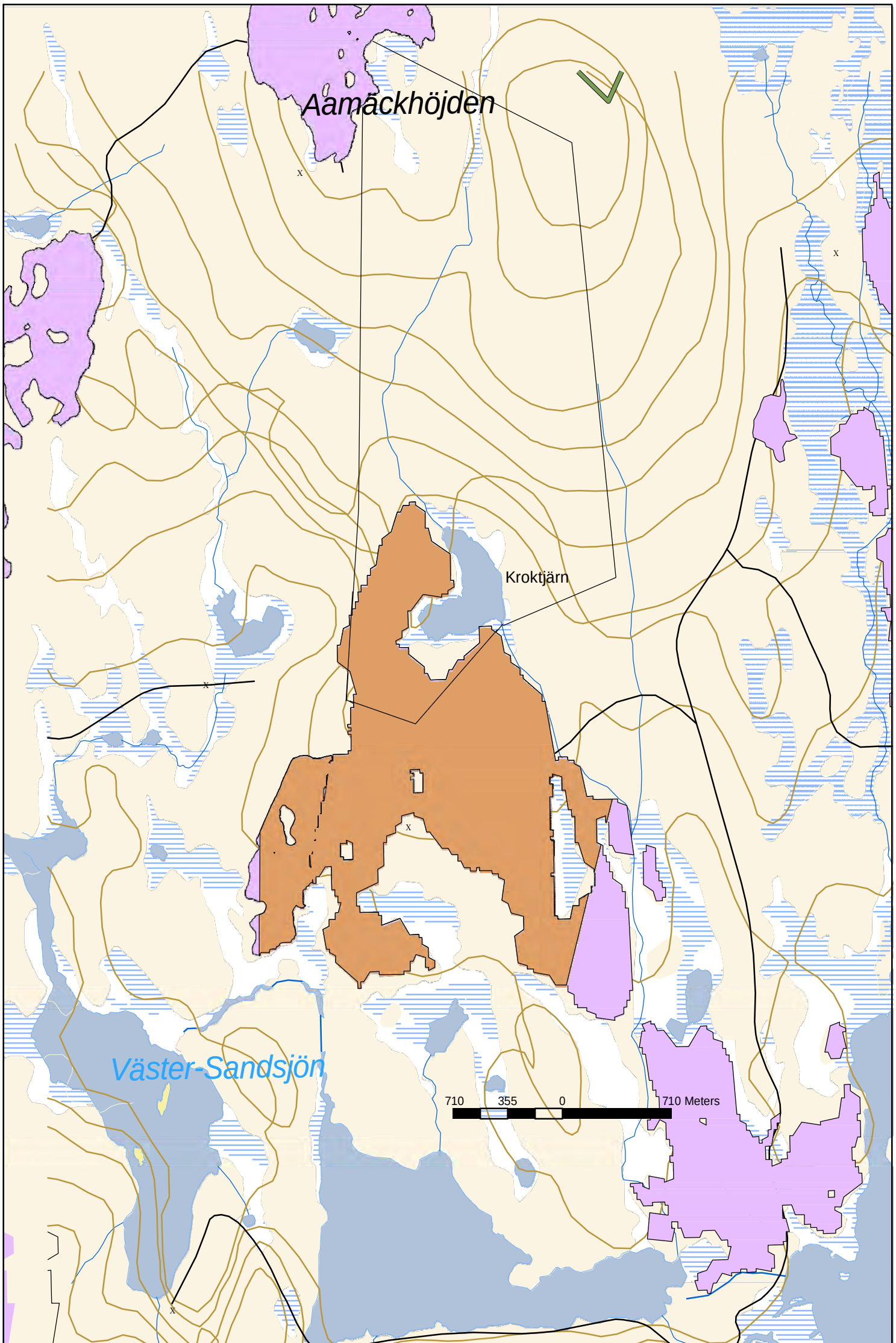
Catchment Boundaries

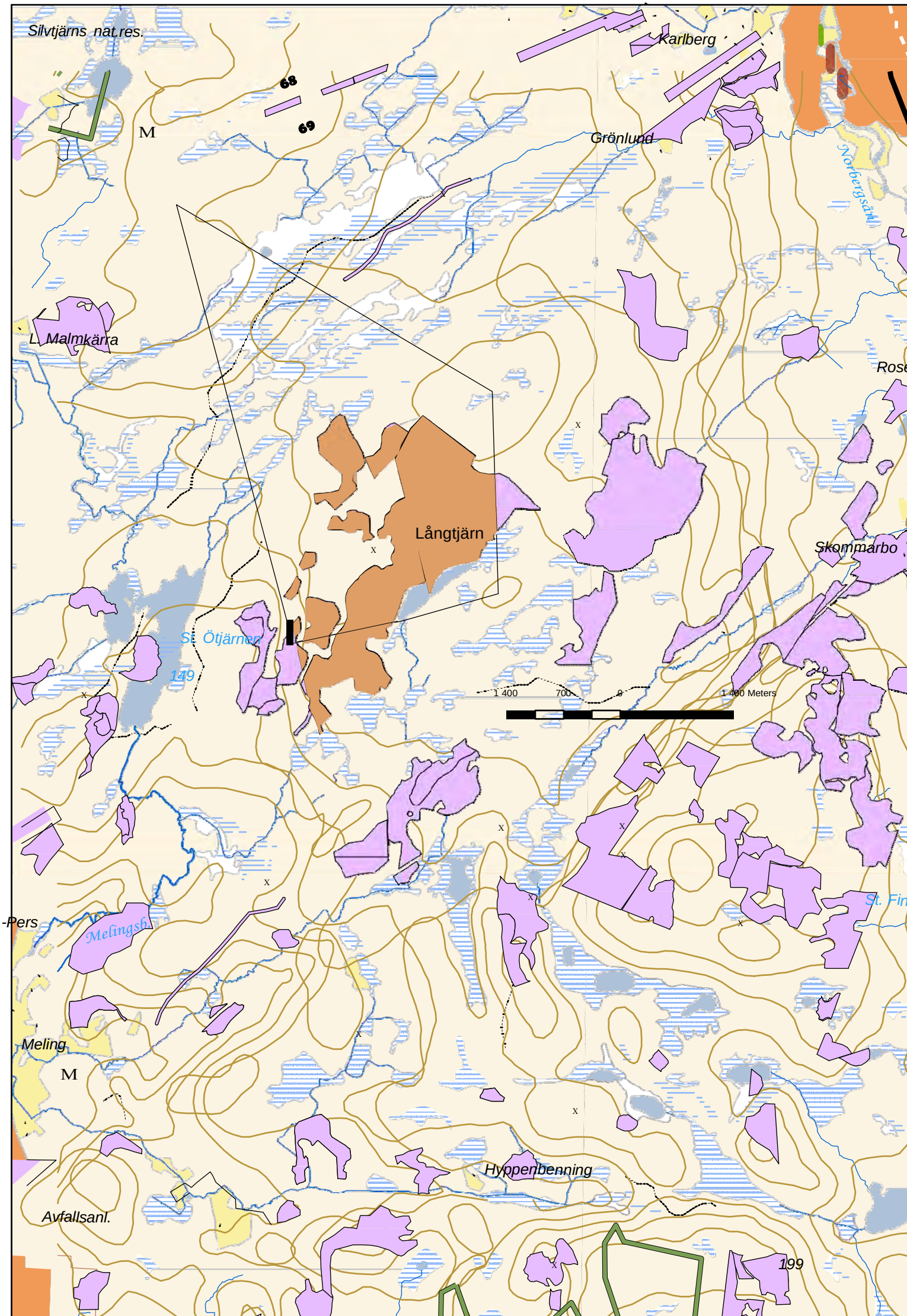
Topographic map of the catchment area of lake Björntjärn……....47
Topographic map of the catchment area of lake Brobo-Kroktjärn…….....48
Topographic map of the catchment area of lake Gårdsjön……..…..49
Topographic map of the catchment area of lake Kroktjärn……..…..50
Topographic map of the catchment area of lake Långtjärn……..…..51


Blåberget
Åliden
Björntjärn
Björn-
berget
970
485
0
970 Meters
Abborrtjärn
Karlstjärn-
berget


Brobo-
Kroktjärnen
Brobo-
tjärnen
Päjerhedarna
Brob
825
412,5
0
825 Meters
Övra
Gäddtjärn


Tåsteröd
Bredvatten
Broan
Stora Komperöd
Hinne-
kullen
Lunden
Lysevatten
Rödvatten
113
Gårdsjön
Hällesdalen
975
487,5
0
975 Meters
St. Holme-
vatten
Bjurhålt
St. Bjurevatten
104


Aamäckhöjden
Kroktjärn
Väster-Sandsjön
710
355
0
710 Meters


Silvtjärns nat.res
Karlberg
68
69
M
Grönlund
Norbergsån
L. Malmkärra
Rose
Långtjärn
Skommarbo
St. Ötjärnen
149
1 400
700
0
1 400 Meters
St. Fin
-Pers
Melingsh.
Meling
M
Hyppenbenning
Avfallsanl.
199