Aquaculture and its effect on the wild fish resource in a Swedish lake

An evaluation of changes in fat content, protein content and trophic niche

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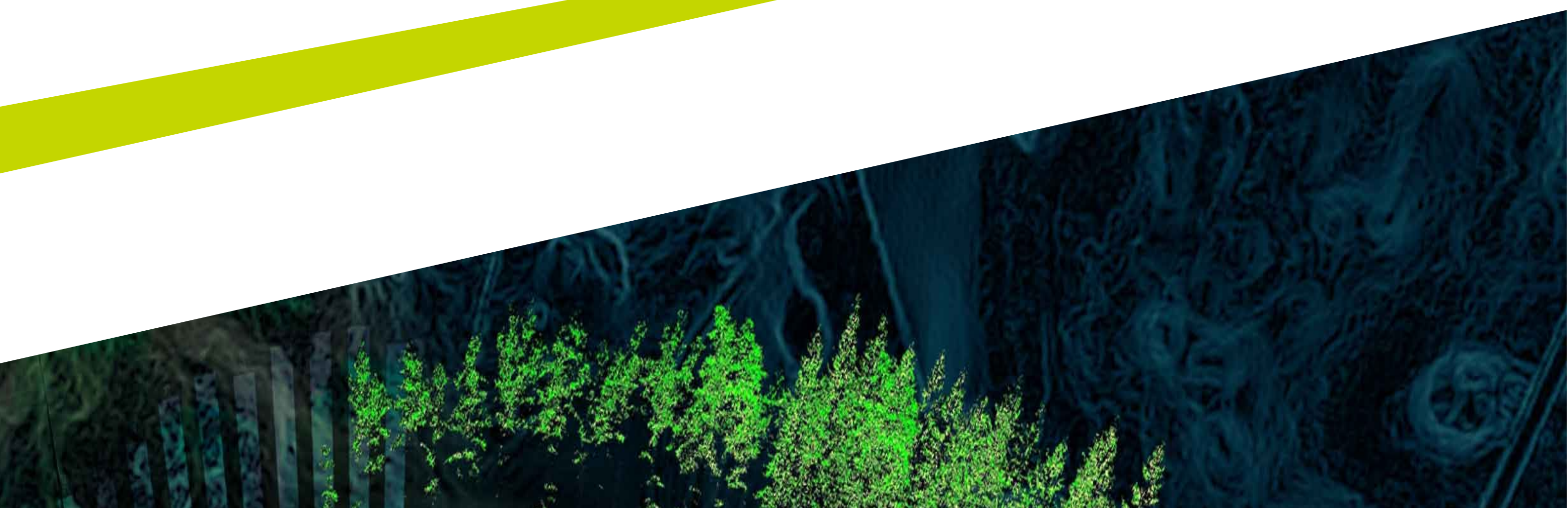

Aquaculture and its effect on the wild fish resource in a Swedish lake - an evaluation of changes in fat content, protein content and trophic niche

Effekten av matfiskodling på intilliggande ekosystem i en svensk insjö - en utvärdering av förändringar i fetthalt, proteinhalt och trofisk nisch

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Abstract

Fish is nutritious and important as a food source for many people. It contains high levels of protein, important omega-3 fatty acids as well as micronutrients. For a sustainable fish consumption, aquaculture is needed but it can affect nearby ecosystems in many ways. This study aimed to evaluate the effect of aquaculture on wild Arctic charr, burbot, perch and whitefish, in a lake with a newly established fish farm in northern Sweden. Fish were caught in three different locations (aquaculture, downstream, upstream) at three different occasions (year 2009, 2010, 2012). Fish caught in 2009 were sampled the first year after establishment of the aquaculture, with low impact from the farm and hence assumed to be "before" establishment, while 2012 and 2012 were assumed to be "after". Analyses of fat- and protein content were performed in all four species of the wild fish from the different capture locations and capture years. Additionally, stable isotopes of carbon and nitrogen were analysed in perch and whitefish from different capture years and capture locations. Fat content increased over time in burbot and whitefish whereas it both decreased and increased in perch, depending on capture location. Protein content did not change significantly over time in any of the species. Charr was not statistically evaluated due to low sample size. The stable isotope analysis indicated some changes after establishment of the aquaculture. Both perch and whitefish had an increase in range of $\delta^{15}N$. Perch did also have an increase in range of $\delta^{13}C$ for the same time period while whitefish had a lower range of $\delta^{13}C$. The changes in fat content in wild fish and the changes in trophic niche that were found, indicate leakage of nutrients from the fish farm. In some areas this might have a positive effect on the production of wild fish whereas in other areas this could be a problem. Additionally, there is a need for further studies of the effect on wild fish in order for farmed fish to be a part of a sustainable food supply.

Keywords: Aquaculture, wild fish, fat content, protein content, stable isotopes

Sammanfattning

Fisk är ett viktigt livsmedel för många människor då det är så näringsrik. Det innehåller mycket protein, viktiga omega-3-fettsyror samt vitaminer och mineraler. För att fiskkonsumtionen ska kunna vara hållbar är fiskodling viktigt och därmed något som ständigt utvecklas. Samtidigt kan fiskodlingar påverka närliggande ekosystem på många olika sätt. Den här studien hade som mål att undersöka vilken effekt en nyetablerad fiskodling hade på vild fisk i en sjö i norra Sverige. De vilda arter som inkluderades i denna studie var abborre, lake, röding och sik. Fisk fångades in för provtagning vid tre olika tillfällen (år 2009, 2010, 2012) och vid tre olika fångstlokaler (uppströms, nedströms, utanför odlingen). Fisk från 2009 fångades under det första året efter etableringen av fiskodlingen, med lite påverkan från odlingen och antogs därmed vara ”före” etableringen, medan fångster från 2010 och 2012 antogs vara ”efter”. Analys gjordes på mängden fett och protein för de fyra arterna utifrån år och fångstlokaler. Utöver fett och protein analyserades även stabila isotoper av kol och kväve i abborre och sik. Mängden fett ökade över tid i lake och sik samtidigt som den både ökade och minskade i abborre beroende på var den var fångad. Proteinmängden förändrades inte signifikant över tid i någon av fiskarterna. Statistiska värden för röding utvärderades inte på grund av bristfällig mängd prover. Analysen av stabila isotoper indikerade vissa förändringar efter etableringen av fiskodlingen. Både abborre och sik hade ökade värden av $\delta^{15}N$. Abborre hade också en ökning i $\delta^{13}C$ för samma tidsperiod, medan sik hade en minskning i $\delta^{13}C$. Förändringarna i mängden fett i den vilda fisken tillsammans med förändringar i trofisk nisch indikerade att näring läcker ut från fiskodlingen. I vissa områden kan näringsläckage ha en positiv effekt på vild fisk medan det på andra ställen kan innebära problem. Med det sagt behövs mer studier göras för att se vilka effekterna är på vild fisk och på så sätt kunna betrakta odlad fisk som en del av en hållbar livsmedelsförsörjning.

Nyckelord: Fiskodling, vild fisk, fetthalt, proteinhalt, stabila isotoper

Table of contents

List of tables

List of figures

Abbreviations

95% CI	95% confidence interval around the bivariate means
CD	mean distance to centroid
$\delta^{13}Cc$	stable isotope of C, the ratio of ^{13}C to ^{12}C, (c=corrected for variations in fat content)
$\delta^{15}N$	stable isotope of N, the ratio of ^{15}N to ^{14}N
DHA	docosahexaenoic acid, C22:6 n-3
dX_range	range between highest and the lowest values of $\delta^{13}Cc$
dY_range	range between highest and the lowest values of $\delta^{15}N$
EPA	eicosapentaenoic acid, C20:5 n-3
GLM	generalised linear models
MNND	mean nearest neighbour distance
PUFA	polyunsaturated fatty acids
SDNND	standard deviation of nearest neighbour distance
SEA	standard ellipse area
SEAc	corrected value for standard ellipse area
SIA	stable isotope analysis
TA	total area

1. Introduction

> "Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production. It is probably the fastest growing food-producing sector and now accounts for 50 percent of the world's fish that is used for food." (FAO 2022)

Fish is a great source of nutrition for many people. Fish is one of the most effective meat-producing animals and there is an estimation that two thirds of fish consumption year 2030 will be provided from aquaculture (The Swedish Board of Agriculture & Swedish Agency for Marine and Water Management 2021).

In 2018 the fish production worldwide was estimated to 179 million tonnes, with 82 million tonnes from aquaculture. Of the total production 156 million tonnes were used for human consumption leading to an overall estimation of annual fish consumption to 20.5 kg per capita. In 2018, 46% of the total production and 52% of the fish used as food came from aquaculture (FAO 2020). Fish not used for human consumption are normally used as feed, fishmeal or fish oil (FAO 2020). Asia in general and China particularly are the largest aquaculture producers globally. China is responsible for half of the global production and is also very diversified in regards of both organisms and production systems (Eriksson et al. 2017). In Europe, Norway is the biggest aquaculture producer (FAO 2020).

Wild fish stocks are becoming smaller due to overfishing in many places (Eriksson et al. 2017). At the same time the human population is becoming bigger. Aquaculture is one alternative to even out problems related to smaller wild stocks and a complement to counteract protein deficiency and other nutrient deficiencies connected to less available food to a growing population (Eriksson et al. 2017). However, with implementation and development of farms other problems arise. Aquacultures are known to be related to eutrophication by leakage of nutrients which can have impact on adjacent ecosystems and wild fish populations (Hixson 2014; Eriksson et al. 2017; Hansen et al. 2018).

1.1 Aquaculture production

Farming of fish can be performed in many different ways, by using different production systems. Fish farms can be situated in freshwater, seawater or in brackish water either along the coast, in lakes or in waterways. Farming can also be placed on land provided with either freshwater or seawater (Eriksson et al. 2017; FAO 2020; SWEMARC 2022). Aquacultures are often related to fish production, but can also be production of crustaceans (e.g. shrimp, crab, crayfish), molluscs (e.g. oysters, mussels) and aquatic plants (e.g. seaweed) (FAO 2020). Production can be performed in ponds, tanks, pens and cages. The systems can be closed or open, focusing on one or many species at the same time (SWEMARC 2022). The production can also be extensive or intensive. Extensive aquaculture is a way of farming where the fish (or other organisms) are not fed, but they rely on naturally occurring feed sources. Intensive aquaculture mean that the fish are fed (Eriksson et al. 2017).

The most common production system for fish used for food production in Sweden is open intensive systems (The Swedish Board of Agriculture 2021). The open systems are often cages, placed in the water with an open top just above the surface. They are normally round in shape and can have a diameter of approximately 10-60 metres and 7-15 metres deep. Feeding within large systems is often well adjusted to the production in terms of size of the farm, species and environmental factors. The feeding is often maintained with an even distribution, to limit waste. Additionally, the right environmental conditions for the fish are well monitored, since everything affecting health, appetite and growth of the fish will in the end affect the production. Therefore, oxygen, nutrients, diseases, temperature etc. are monitored and maintained. Open cages are normally used both in freshwater and along the coast, but when needed (due to more harsh weather conditions) the cages can be submerged and/or in enclosed net cages (Eriksson et al. 2017).

One negative aspect of open systems is that the impacts by environmental effects are greater than in the closed systems due to a constant interaction with the surrounding environment (Eriksson et al. 2017). A meta-study by Barrett et al. (2019) evaluated the impact of aquacultures on wild species. Their study shows that in many cases, wild fish are more prone to reside close to farms and the diversity of species are often higher within the same location. The effects on wild fish seen within the same study was that wild fish from an environment close to an aquaculture generally were bigger in size and weight. The increase in body mass of wild fish related to an aquaculture can in the same way be an indication of some lower qualities in fatty acids related with the feed from the aquaculture (Barrett et al. 2019).

Aquaculture systems can besides being open also be closed or semi-closed. Semi-closed systems often consist of closed tanks with a water distribution from a natural source and they can be placed on land. Closed systems have a circulation system of water and are not depending on water exchange. They are placed on land and the circulation of water are often controlled with filters. Advantages with systems that are closed or semi-closed are their lower impact on the adjacent environment due to none or less direct contact with natural water resources. Additionally, the closed systems are good for controlling the environment for the fish. Disadvantages are the high energy needed for controlling the environment in the closed systems, and high costs related to these (Eriksson et al. 2017).

1.2 Aquaculture in Sweden

Worldwide, aquaculture production has developed and increased during the last years. At the same time aquaculture production in Sweden is very low, only 1% of the total global production (Eriksson et al. 2017; SWEMARC 2022). In Sweden we import 90% of farmed fish consumed as food (SWEMARC 2022). The aim has been to expand aquaculture production in Sweden, but the number of aquaculture sites have decreased the last decades (The Swedish Board of Agriculture 2021). The reason for this is believed to be related to complicated regulations and legislations as well as administration time for these. Another reason believed to be related to the decrease in Swedish aquaculture sites are difficulties in where to locate aquacultures to limit negative impact on the environment (The Swedish Board of Agriculture & Swedish Agency for Marine and Water Management 2021). There is a demand and interest in developing the Swedish aquaculture production (Eriksson et al. 2017).

In Sweden in year 2020, there were 154 active production sites within aquaculture and 55 of them were production of fish for food consumption while 59 of them were producing fish for stocking and 40 of them were producing crayfish or mussels. Of the fish farmed for food production, 33 of the sites were located in the north of Sweden[1], 19 in the south of Sweden[2] and three in the east of Sweden[3] (The Swedish Board of Agriculture 2021). The most common fish species within food production is rainbow trout (*Oncorhynchus mykiss*) followed by Arctic charr (*Salvelinus alpinus*) (hereafter referred to as charr). Other species farmed in Sweden are eel, crayfish, mussels and oysters. Aquaculture production employed about 543 people in 2020 (The Swedish Board of Agriculture 2021). The total production of fish for

[1] Värmland, Dalarna, Gävleborg, Västernorrland, Jämtland, Västerbotten, Norrbotten

[2] Jönköping, Kronoberg, Kalmar, Blekinge, Skåne, Halland, Västra Götaland

[3] Stockholm, Uppsala, Södermanland, Östergötland, Örebro, Västmanland

food purposes 2020 was estimated to 9900 tonnes (fresh weight) (The Swedish Board of Agriculture 2021).

Aquaculture in Sweden is a part of the food supply despite the low production in relation to imported fish. The production contributes to work opportunities and is also an important factor for cultural and social reasons. It is hence a valuable production for rural- and coastal areas (The Swedish Board of Agriculture & Swedish Agency for Marine and Water Management 2021).

1.3 Sustainability

One problem with aquaculture is emissions of nutrients, especially nitrogen and phosphorus to adjacent ecosystems due to leakage of feed and faeces. From the feed used in the aquaculture, 3% ends up outside the system and can either be eaten by wild fish or affecting the environment by sedimentation (Hansen et al. 2018). Too high levels of nitrogen and phosphorus contributes to eutrophication which can affect the surrounding environment with an increase in production of phytoplankton, macrophytes, algae as well as zooplankton. This increased production can therefore benefit the environment as long as the balance between producers and consumers is not disturbed (Eriksson et al. 2017). An unbalanced nutrient distribution can affect the oxygen supply in the ecosystem. With this said, an establishment of an aquaculture could lead to many negative effects on the environment but could also contribute to an increase in biomass in the same area (Eriksson et al. 2017).

To decrease the environmental impact, experiments and modelling have been done to optimize the amount of feed that is used (Eriksson et al. 2017). Another step in making aquaculture more environmentally sustainable is to change the composition of the feed. The change in feed could lead to less usage of wild fish as a main ingredient. It could also be a way to optimise the nutrient efficiency and health aspects of fish. Studies have for example been done where plant material has been replacing fish oil and fishmeal, and the development of fish feed is an ongoing process (Hixson 2014).

Other environmental related issues connected to aquacultures, besides leakage of nutrients are usage of chemicals, escapes of fish and spread of diseases. Some of these issues can be avoided by considering type of production system. Closed systems, placed on land are in need of more resources to be able to supply the systems with the right conditions in terms of water, temperature etc. At the same time, the closed systems are more controllable in terms of leakage of feed, chemicals, escapes etc. The closed systems might be more energy consuming and more expensive, but at the same time maybe more environmentally friendly (Eriksson et al. 2017).

Aquaculture is a part of the food production. For a sustainable food production, different aspects within food security are important to consider. The fish as a food product needs to be safe for human health and non-hazardous. At the same time the production systems need to be safe for workers. The fish also have to meet consumer's qualitative and quantitative needs in terms of flavour, appearance but also nutritionally. Additionally, the production needs to be environmentally sustainable, making sure that effects on surrounding ecosystems are minimal. The aquaculture production also needs to fill legal and ethical criteria as well as be stable against changes in world politics and economics (Jennings et al. 2016).

1.4 Fish as a food source

Fish and seafood are one of the most nutritional foods, all nutrients considered (Tacon & Metian 2013). However, the amount and composition of nutrients can vary depending on species and production system, as well as on different processing- and cooking methods (FAO 2020). The Swedish Food Agency's recommendation is to eat fish 2-3 times per week to contribute to a healthier lifestyle and to reduce the risk of cardiovascular disease and other public health diseases (Swedish Food Agency 2021a). Fish contains essential amino acids and high-quality proteins. Fat fish is also rich in essential omega-3 fatty acids and polyunsaturated fatty acids (PUFA) (Nordic Council of Ministers 2014; FAO 2020). Fish can also contribute to the intake of vitamin A, B and D as well as iron, calcium, iodine, selenium and zinc. All these compounds together make fish a great healthy combination of nutrients even for a low amount (Nordic Council of Ministers 2014; FAO 2020). Fish could hence be an important food- and protein source in countries where the general animal protein intake is low (FAO 2020).

As fish may vary in composition due to species and origin it is difficult with general numbers regarding properties and content in fish as a food source. Fish are often categorised depending on fat content. Common classifications of fish according to fat content per 100 g of meat are <2 g fat for lean fish, 2-8 g fat for medium-fat fish and >8 g fat for fatty fish. The medium-fat and fatty fish are good sources of omega-3 fatty acids (Nordic Council of Ministers 2014).

Fish generally have a higher protein content compared to terrestrial animals in terms of edible meat per body weight and do also have a great amino acid content in comparison with other types of meat. Generally, fish contains more of the important omega-3 fatty acids compared to other types of meat (Tacon & Metian 2013).

1.4.1 Fat

One important macronutrient and energy source for the human body is fat. Amongst providing energy, fat is important for temperature regulation, protection of organs and for metabolic matters. Fat fish contain a high amount of the omega-3 unsaturated fatty acids eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA). EPA and DHA are important fatty acids responsible for many functions and structures within the human body, for example the growth of membranes, tissues and development of the brain (Becker 2013; Nordic Council of Ministers 2014).

Some previous studies performed on changes in nutrition between wild fish and farmed fish show that fat content is generally higher in farmed fish compared to wild fish, regardless of species and production system (Flick, Jr 2002; González et al. 2006; Nettleton & Exler 1992; Olsson et al. 2003; Suomela et al. 2016). Some of the studies also evaluated the content of omega-3 fatty acids in wild and farmed fish, as well as ratios of omega-3:omega-6. According to some measurements the content of omega-3 fatty acids is higher in farmed fish (Flick, Jr 2002; Tacon & Metian 2013). The higher content of omega-3 is directly linked to the composition of the feed. The feed is in these cases most probably containing higher levels of fish meal or fish oil which increases the omega-3 levels in the fish (Flick, Jr 2002; Tacon & Metian 2013).

1.4.2 Protein

Protein is an important macronutrient for the human body. It is important for cell structure and production of enzymes and hormones. Proteins are also involved in the immunological system as well as in transportation of nutrients within the body. Proteins do have more important functions besides the already mentioned (Abrahamsson & Hambræus 2013). Food from animal origin, for example fish, is a good source of all essential amino acids and hence protein of good quality (Swedish Food Agency 2022). Protein content in fish can be different depending on many factors, for example what species is consumed. A general estimation of protein content in fish is 20-35% protein (Nordic Council of Ministers 2014).

It is difficult to distinguish a general pattern regarding differences in protein content between farmed and wild fish. Studies show both higher and lower protein content in wild fish, as well as no detected differences (Nettleton & Exler 1992; Olsson et al. 2003; González et al. 2006; Jensen et al. 2013).

1.5 Stable isotopes and trophic niche

Analysing stable isotopes is a method used in many different biological studies. Stable isotope analysis (SIA) can be used to analyse many important ecological matters, for example physiology of plants and animals, diet composition, niche shifts and trophic structure. An isotope composition of an individual can reflect its diet (Post et al. 2007).

The isotopic niche can be used as a proxy for trophic niche. A trophic niche is a description of the ecology of a species or a community. The niche describes relations between the species (or community) and its environment, including how they live and what they eat and their adaption to different climates (Cohen 1977; Pocheville 2015; Nationalencyklopedin 2022). The niche also indicates hierarchies within an ecosystem as well as threats and competition of resources (Pocheville 2015; Nationalencyklopedin 2022; Linköping University 2022). When main focus within the niche is competition of food and how nutrition is consumed for different species, "food web" is often used (Cohen 1977). There are several components that have more than one isotope, but the stable isotopes of carbon (C) and nitrogen (N) are often used to analyse organisms and their role within a food web (Peterson & Fry 1987; Bearhop et al. 2004).

Stable isotopes are expressed as ratio of the heavy isotope to the light, compared to an international standard. The $\delta^{15}N$ is converted from the ratio of ^{15}N to ^{14}N and $\delta^{13}C$ from the ratio of ^{13}C to ^{12}C, both expressed in per mil (‰) (Newsome et al. 2007).

SIA of $\delta^{13}C$ and $\delta^{15}N$ in combination can give indications of different patterns for the species (or community). Higher $\delta^{15}N$ levels can for example indicate higher trophic level but can also be an indication of higher pollution level in regards of eutrophication (Newsome et al. 2007). SIA of nitrogen generally gives more information on trophic level. SIA of carbon gives more information on carbon source consumed. For example SIA of carbon can indicate feed sources for fish (Bearhop et al. 2004; Michener & Kaufman 2008) By combining $\delta^{13}C$ and $\delta^{15}N$ the isotopic niche of a species (or community) can be given as a proxy for trophic niche. This is a good measure to evaluate potential effects of aquaculture on wild fish communities.

1.6 Aim

The establishment of a fish farm is affecting the wild fish and the ecosystem in the lake, by leakage of nutrients. The aim of this study is to evaluate the effect of aquaculture on the wild fish resource by analyses of fat- and protein content as well as stable isotopes measured in wild fish in a lake at the time of establishment of a new site and a few years following establishment.In this study following hypotheses will be tested:

1. Fat content in wild fish increases after establishment of an aquaculture as a consequence of increased amounts of nutrients in the system.
2. Protein content in wild fish increases after establishment of an aquaculture as a consequence of increased amounts of nutrients in the system.
3. Fish downstream are more affected by the newly established aquaculture than fish upstream as a consequence to distribution of nutrients via the stream.
4. The isotopic niche of the wild fish changes in accordance with the isotope signal for the feed used in the fish farm

2. Method

2.1 Study area

Malgomaj is a lake located in Vilhelmina, a Swedish municipality in the county Västerbotten in the northern part of Sweden (64.75682°N, 16.20637°E). According to the Water Information System Sweden[4] (VISS 2022), the surface of the lake is 103 km^2. The lake is 70 km long and has a maximum depth of 100 metres (Umlax AB 2022).The ecological status of the lake is classified as “poor”, and its chemical status is classified as “heavily modified” (VISS 2022). The status of the fish fauna in Malgomaj 2019 was classified as “moderate”. Since the negative impact on the lake is considered very big there is no conditions for a long-term diverse and sustainable fish community (VISS 2022).

The fish farm in Malgomaj uses open cages and was established during 2008. The company Umlax AB have since then farmed Arctic charr and they employ 5-10 persons. The cages are located 1.5 km out in the lake (Umlax AB 2022).

2.2 Study species

For this study, four species were selected from a total of eight different study species. The selection was based on available data and their relevance as a human food source and the four species selected were: burbot, charr, perch and whitefish.

2.2.1 Arctic charr (*Salvelinus alpinus*)

Charr in Sweden can normally be found in lakes and waterways in the northern part of Sweden, often in the mountain areas (Swedish Agency for Marine and Water Management 2020a). They could also be found in lakes in the south of Sweden, but

[4] The Water Information System (VISS) is a database that has been developed by the Water authorities, the County Administrative Boards and the Swedish Agency for Marine and Water Management. The database is a tool to aid the improvement of our waters to comply with the EU Water Framework Directive. The database contains a comprehensive assessment of the ecological, quantitative and chemical status of the water as well as maps and geographic location.

due to acidification and stocking of other species most of the southern populations have disappeared (SLU Artdatabanken 2022a). Arctic charr, or other similar types of charr, are also found in Norway, Iceland, northern Russia and North America (SLU Artdatabanken 2022a). They prefer cold and clear water with high oxygen levels and do also prefer deeper parts of the lakes (Swedish Agency for Marine and Water Management 2020a). They can be up to 90 cm long. Charrs are carnivores, but their feed varies depending on their size and competition with other populations of feed supply. Small charrs normally eat invertebrates and plankton while bigger charrs eat fish (SLU Artdatabanken 2022a).

Charr is well-known and appreciated as food. In the stores it is most common to find farmed charr. The meat is often red, and charr can be cooked in many ways as well as be cured. Sweden is one of the biggest producers of farmed charr as food (Sjömatsfrämjandet 2022a). Wild charr is considered to be a medium fat fish since it contains between 2-8 g of fat per 100 g (Nordic Council of Ministers 2014; Swedish Food Agency 2021a). Raw charr (farmed) contains (per 100 g) 19.9 g protein and 7.94 g of fat, with an energy amount of 152 kcal/634 kJ (Swedish Food Agency 2021b).

2.2.2 Burbot (*Lota lota*)

Burbot can be found in most freshwaters in Sweden, as well as in brackish water (SLU Artdatabanken 2022b). In Swedish freshwater, burbot is the only species within the cod-family (Swedish Agency for Marine and Water Management 2020b). Burbot can be found in northern Europe, north-eastern Asia and parts of North America (SLU Artdatabanken 2022c). Burbot normally lives close to the bottom of lakes. They prefer cold and clear water and are most active during night-time in the summer (SLU Artdatabanken 2022c). Burbots are carnivores. Large individuals can eat smaller fish, crustaceans and fish roe while the smaller individuals eat small invertebrates. They can become 15-25 years of age and can be up to 120 cm long but are most commonly around 65 cm (SLU Artdatabanken 2022c).

Both the meat and the roe of burbot can be eaten and it is found in stores and fish counters in Sweden during winter (Sjömatsfrämjandet 2022b). Burbot is considered to be a lean fish since it contains less than 2 g of fat per 100 g (Nordic Council of Ministers 2014; Swedish Food Agency 2021a). Raw burbot contains (per 100 g) 16.5 g protein and 0.45 g of fat, with an energy amount of 71 kcal/297 kJ (Swedish Food Agency 2021b).

2.2.3 Perch (*Perca fluviatilis*)

European perch (hereafter referred to as perch) is one of the most common fish species in Sweden. Perch can be found in almost all Swedish freshwaters and also along the coast in brackish water but avoids too cold or fast flowing water. Besides Sweden, it can be found in northern Europe as a wild species and has also been introduced in Australia, South Africa and some southern parts of Europe (SLU Artdatabanken 2022d). During summer it prefers water with vegetation and shallow water and during winter it is more common to find perch in deep water (Swedish Agency for Marine and Water Management 2020c). Perch can be up to 61 cm long but with a normal length of 35 cm, and also commonly much smaller. Perch are carnivores. Small individuals eat zooplankton while bigger individuals eat insect larvae, crustaceans and smaller fish (SLU Artdatabanken 2022d).

Perch is a common fish to eat and can be a great alternative to many common food fishes seen in recipes and in restaurants (Sjömatsfrämjandet 2022c). Perch is considered to be a lean fish since it contains less than 2 g of fat per 100 g (Nordic Council of Ministers 2014; Swedish Food Agency 2021a). Raw perch contains (per 100 g) 19.8 g protein and 0.61 g of fat, with an energy amount of 86 kcal/359 kJ (Swedish Food Agency 2021b).

2.2.4 Whitefish (*Coregonus maraena*)

Whitefish can be found in lakes and rivers around most parts of Sweden. It is also common in the Baltic Sea and along the Swedish west coast (SLU Artdatabanken 2022e). They require cold and oxygen rich water (Swedish Agency for Marine and Water Management 2021). There are many different types of whitefish, and they are hence divided into different whitefish-species. Whitefish, similar to the Swedish species can be found in northern Europe, Asia and North America. In freshwater they are generally found in large, deep lakes (SLU Artdatabanken 2022e). They are carnivores and depending on type of whitefish the feed can be plankton, insects, crustaceans and molluscs and other fish (SLU Artdatabanken 2022e). Whitefish can be up to 60 cm long, but normally 15-40 cm (SLU Artdatabanken 2022e). Their weight can reach up to 5-6 kg (Swedish Agency for Marine and Water Management 2021).

Whitefish can be cooked and eaten in several ways e.g., fried, boiled, pickled, smoked. The roe is often used for Swedish caviar (Sjömatsfrämjandet 2022d). Whitefish is considered to be a lean fish since it contains less than 2 g of fat per 100 g (Nordic Council of Ministers 2014; Swedish Food Agency 2021a). Raw whitefish contains (per 100 g) 20.9 g protein and 0.64 g of fat, with an energy amount of 90 kcal/379 kJ (Swedish Food Agency 2021b).

2.3 Fish sample collection

Wild burbot, charr, perch and whitefish were sampled via netting in Malgomaj during the end of August and beginning of September in 2009, 2010 and 2012. The fish were sampled at three locations: one 1760-2760 meters upstream of the fish farm (upstream location), one 270-900 meters from the cages (aquaculture location), and one 2390-4360 meters downstream of the fish farm (downstream location). All fish were caught between 1-28 metres depth, regardless of capture location. Fish were frozen and stored after capture pending further analyses for fat content, protein content and stable isotopes.

Some fish were also measured and weighed. Mean values for weights and lengths were calculated together with standard error of mean (SEM) for each species, capture location and capture year.

2.4 Analyses of fat content, protein content and stable isotopes

Frozen fish were thawed, and after thawing the fillets were separated from skin and bones. Two cross sections (3-5 g each) from each fillet were cut out to perform analyses on fat- and protein content as well as stable isotope analysis, according to standard methods.

For analyses of fat- and protein content, pieces of the fillets were homogenised (Losmixer, Miris AB, Uppsala, Sweden) and filtered. The homogenised and filtered samples were then analysed using mid-infrared transmission (MIT) (Miris AB, Uppsala, Sweden) spectroscopy according to Elvingson & Sjaunja (1992). Standard methods for fat- and protein were used for calibration (Elvingson & Sjaunja 1992; Byström et al. 2006; Quinton et al. 2007; Larsson et al. 2012).

The pieces of fillets used for stable isotope analysis (SIA) were freeze-dried, milled and homogenised using a mixer mill (Retsch GmbH, Haan, Germany). Thereafter the samples were dried for 16 hours at 70°C and stored in exsiccator pending analyses. Analysis was after that performed on an Elemental Analyzer-Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) and allowed for simultaneous measurements of $\delta^{15}N$ and $\delta^{13}C$ along with mass fractions of N and C (Ohlsson & Wallmark 1999; Werner et al. 1999). Feed samples, containing feed used in the farm 2008 (old) and 2009 (new), where also analysed for stable isotopes.

2.5 Data analyses and statistical analyses of fat content and protein content

To evaluate effects of fish farm establishment on wild fish, generalised linear models (GLM) were used to examine the response variables fat- and protein content with the explanatory variables: time for capture (year) and capture location (place: aquaculture, downstream, upstream). Aquaculture was used as a reference site. For all models, the interaction between time (year) and location (place) were initially included. When non-significant ($p>0.05$), the interaction term was removed. All analyses were done in R version 4.1.3 (2022-03-10) and significance set to $\alpha < 0.05$.

Normal distribution of data for each species was tested prior to statistical analyses using Shapiro test for normality and histograms. When normality was not met, data were log transformed. Results based on transformed data are indicated by an asterisk in the result tables.

The residuals for all models were also analysed using Shapiro test for normality. Additionally, the residuals were visually evaluated using Q-Q plot by examining data points looking for any major deviation from the distribution line.

Burbot caught in the aquaculture location were excluded from the statistical analysis for both fat- and protein content due to inadequate number of samples from 2009 (n=0) and 2012 (n=1). All samples were included in plotted data, and seen in figures, but not included in the statistical analysis. Results from GLMs for burbot hence only include downstream- and upstream location.

For charr, the number of samples was low (n=17), compared to the sample size for the other species. It was therefore not possible to perform relevant statistical analyses for neither fat- nor protein content and only visual assessments were performed.

2.6 Data analysis and statistical analysis of stable isotopes

Only species with enough data available were used to analyse the isotope niche and therefore only perch and whitefish were considered.

Stable isotopes are affected by the fat content in the animal due to fractionation during the lipid synthesis. Since fat content in fish can vary between species and individuals, the effect of fat on the stable isotopes is relevant in this study. According to a study of Post et al. (2007) the effect of fat content on the stable isotope ratio of carbon should be corrected when comparing species with varying fat content. All values for $\delta^{13}C$ were hence corrected according to Post et al. (2007) to normalize for variations in fat content. The following equation for aquatic organisms was used to calculate $\delta^{13}C_{corrected}$ ($\delta^{13}Cc$):

$$\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$$

Isotope niche metrics (Layman et al. 2007) were calculated for each species, sampling location and year using the SIBER (Stable Isotope Bayesian Ellipses in R) package for R version 2.1.6.

To evaluate differences in the isotope niche between different capture locations and capture times the following niche metrics were used: total area (TA) of the convex hull and a corrected value for standard ellipse area (SEAc). The correction in SEAc was used to adjust for low sample size in the standard ellipse area (SEA) (Layman et al. 2007). The 95% confidence interval around the bivariate means (95% CI) was visually studied in figures containing plotted data.

In addition, changes in the total community of perch and total community of whitefish, represented by fish from the three different sampling locations were evaluated. The communities were also categorised according to time period. Data from year 2009 was categorised as “before” and data from 2010 and 2012 was categorised as “after”. In this way changes in stable isotopes could be compared and the years 2010 and 2012 could indicate changes within the food web and trophic niche, with time.

The evaluation of each community was using the following metrics: range between highest and the lowest values of $\delta^{15}N$ (dY_range) and $\delta^{13}Cc$ (dX_range), total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND) and standard deviation of nearest neighbour distance (SDNND) in the bi-plot space. The dY_range and dX_range represent changes within a food web where changes in dY_range indicates changes in trophic diversity and dX_range indicates patterns in consumption of carbon sources. Both TA and CD gives indications on trophic variety within the food web. The TA gives information on how big niche space that is used by the group studied and CD indicates how the group is located in relation to the mean values of $\delta^{15}N$ and $\delta^{13}Cc$. The density of the studied group is indicated by MNND and SDNND (Layman et al. 2007).

3. Results

A summary of analyses of fat- and protein content as well as stable isotopes of carbon and nitrogen for all four fish species (burbot, charr, perch, whitefish), categorised by capture location (aquaculture, downstream, upstream) and capture year (2009, 2010, 2012) are presented in table 1. Additionally, values from feed samples, containing feed used in the farm 2008 and 2009, analysed for stable isotopes are also presented in table 1. Total number of fish analysed were 82 burbot, 17 charr, 130 perch and 228 whitefish.

A summary of mean values of measured weights and lengths for the four fish species are presented in table 2. The weights ranged between 110-230 g for burbot, 14-709 g for charr, 75-128 g for perch and 142-349 g for whitefish. The lengths ranged between 27-33 cm for burbot, 12-38 cm for charr, 19-23 cm for perch and 24-29 cm for whitefish.

Table 1. Results overview – Values for all samples analysed for each species (burbot, charr, perch, whitefish), grouped by capture year (2009, 2010, 2012) and capture location (aquaculture, downstream, upstream). Values presented are mean values together with standard error of mean and number of samples analysed (n), for the variables: fat content (%), protein content (%) and stable isotope analysis of carbon ($\delta^{13}Cc$) and nitrogen ($\delta^{13}N$). Values from stable isotope analysis of carbon ($\delta^{13}Cc$) and nitrogen ($\delta^{13}N$) for feed used in the farm 2008 and 2009 are also presented.

Variable	Species	Location	2009	2010	2012
Fat content (%)	**Burbot**	Aquaculture	N/A (n=0)	0.79 ± 0.06 (n=12)	2.20 ± 0.00 (n=1)
		Downstream	0.51 ± 0.12 (n=4)	1.01 ± 0.16 (n=5)	0.97 ± 0.04 (n=19)
		Upstream	0.75 ± 0.17 (n=6)	0.58 ± 0.04 (n=7)	1.54 ± 0.08 (n=27)
	Charr	Aquaculture	N/A (n=0)	2.03 ± 0.00 (n=1)	19.29 ± 3.23 (n=6)
		Downstream	3.94 ± 0.85 (n=3)	N/A (n=0)	2.08 ± 0.72 (n=2)
		Upstream	5.51 ± 1.38 (n=4)	N/A (n=0)	3.35 ± 0.00 (n=1)
	Perch	Aquaculture	1.80 ± 0.12 (n=13)	1.07 ± 0.05 (n=15)	0.84 ± 0.05 (n=13)
		Downstream	1.38 ± 0.09 (n=16)	0.89 ± 0.04 (n=20)	1.10 ± 0.05 (n=11)
		Upstream	1.22 ± 0.17 (n=17)	0.54 ± 0.03 (n=15)	1.25 ± (0.09 (n=10)
	Whitefish	Aquaculture	3.37 ± 0.25 (n=30)	1.92 ± 0.08 (n=75)	3.48 ± 0.23 (n=41)
		Downstream	2.62 ± 0.36 (n=13)	1.61 ± 0.24 (n=17)	2.15 ± 0.24 (n=21)
		Upstream	2.13 ± 0.60 (n=7)	1.81 ± 0.58 (n=10)	4.18 ± 0.49 (n=14)
Protein content (%)	**Burbot**	Aquaculture	N/A (n=0)	18.99 ± 0.68 (n=12)	16.65 ± 0.00 (n=1)
		Downstream	15.40 ± 0.31 (n=4)	15.46 ± 0.71 (n=5)	15.78 ± 0.19 (n=19)
		Upstream	15.71 ± .0.28 (n=6)	15.28 ± 0.96 (n=7)	16.03 ± 0.33 (n=27)
	Charr	Aquaculture	N/A (n=0)	20.43 ± 0.00 (n=1)	16.55 ± 0.37 (n=6)
		Downstream	16.13 ± 0.21 (n= 3)	N/A (n=0)	16.50 ± 0.21 (n=2)
		Upstream	17.02 ± 0.22 (n=4)	N/A (n=0)	16.60 ± 0.00 (n=1)
	Perch	Aquaculture	18.34 ± 0.15 (n=13)	19.61 ± 0.26 (n=15)	18.86 ± 0.17 (n=13)
		Downstream	18.01 ± 0.12 (n=16)	18.34 ± 0.28 (n=20)	18.19 ± 0.22 (n=11)
		Upstream	17.56 ± 0.17 (n=17)	17.76 ± 0.38 (n=15)	18.90 ± 0.20 (n=10)
	Whitefish	Aquaculture	17.81 ± 0.13 (n=30)	20.07 ± 0.18 (n=75)	18.89 ± 0.21 (n=41)
		Downstream	18.32 ± 0.23 (n=13)	19.27 ± 0.33 (n=17)	18.63 ± 0.25 (n=21)
		Upstream	18.19 ± 0.12 (n=7)	19.88 ± 0.63 (n=10)	18.58 ± 0.28 (n=14)
$\delta^{13}Cc$ (‰)	**Perch**	Aquaculture	-26.25 ± 0.41 (n=10)	-26.52 ± 0.26 (n=9)	-26.45 ± 0.30 (n=13)
		Downstream	-26.09 ± 0.40 (n=11)	-26.09 ± 0.43 (n=9)	-26.49 ± 0.25 (n=11)
		Upstream	-25.47 ± 0.43 (n=11)	-25.04 ± 0.27 (n=10)	-25.80 ± 0.25 (n=10)
	Whitefish	Aquaculture	-29.09 ± 0.42 (n=13)	-29.12 ± 0.40 (n=19)	-29.57 ± 0.28 (n=41)
		Downstream	-27.60 ± 0.79 (n=10)	-28.39 ± 0.70 (n=10)	-28.39 ± 0.42 (n=21)
		Upstream	-27.93 ± 0.61 (n=7)	-27.82 ± 0.49 (n=10)	-29.78 ± 0.29 (n=14)
$\delta^{15}N$ (‰)	**Perch**	Aquaculture	6.30 ± 0.19 (n=10)	7.42 ± 0.18 (n=9)	8.33 ± 0.29 (n=13)
		Downstream	6.32 ± 0.23 (n=11)	7.63 ± 0.16 (n=9)	8.24 ± 0.16 (n=11)
		Upstream	6.69 ± 0.30 (n=11)	7.64 ± 0.13 (n=10)	8.36 ± 0.13 (n=10)
	Whitefish	Aquaculture	7.29 ± 0.15 (n=13)	7.72 ± 0.08 (n=19)	8.40 ± 0.08 (n=41)
		Downstream	7.21 ± 0.22 (n=10)	7.79 ± 0.17 (n=10)	8.61 ± 0.14 (n=21)
		Upstream	7.42 ± 0.37 (n=7)	8.04 ± 0.15 (n=10)	8.50 ± 0.19 (n=14)
			2008	**2009**	
$\delta^{13}Cc$ (‰)	**Feed**		-21.56 ± 0.06 (n=6)	-24.66 ± 0.73 (n=4)	
$\delta^{15}N$ (‰)			11.03 ± 0.05 (n=6)	14.06 ± 0.41 (n=4)	

Table 2. A summary of all weights and lengths measured for each species (burbot, charr, perch, whitefish), grouped by capture year (2009, 2010, 2012) and capture location (aquaculture, downstream, upstream). Values presented are mean values together with standard error of mean and number of samples analysed (n).

	Species	Location	2009	2010	2012
Weight (g)	**Burbot**	Aquaculture	N/A (n=0)	177 ± 24 (n=12)	110 ± 0 (n=1)
		Downstream	230 ± 89 (n=4)	176 ± 28 (n=5)	220 ± 19 (n=19)
		Upstream	151 ± 29 (n=6)	164 ± 20 (n=7)	180 ± 12 (n=28)
	Charr	Aquaculture	N/A (n=0)	139 ± 0 (n=1)	709 ± 60 (n=6)
		Downstream	14 ± 3 (n=3)	N/A (n=0)	77 ± 0 (n=1)
		Upstream	16 ± 1 (n=4)	N/A (n=0)	16 ± 0 (n=1)
	Perch	Aquaculture	86 ± 5 (n=13)	115 ± 15 (n=15)	123 ± 10 (n=13)
		Downstream	75 ± 5 (n=16)	120 ± 7 (n=20)	109 ± 13 (n=11)
		Upstream	83 ± 11 (n=17)	128 ± 10 (n=15)	128 ± 14 (n=10)
	Whitefish	Aquaculture	142 ± 16 (n=30)	177 ± 9 (n=74)	155 ± 17 (n=41)
		Downstream	183 ± 59 (n=13)	225 ± 31 (n=17)	184 ± 31 (n=21)
		Upstream	213 ± 44 (n=7)	349 ± 89 (n=10)	158 ± 18 (n=13)
Length (cm)	**Burbot**	Aquaculture	N/A (n=0)	N/A (n=0)	27 ± 0 (n=1)
		Downstream	32 ± 3 (n=4)	N/A (n=0)	33 ± 1 (n=19)
		Upstream	29 ± 2 (n=6)	N/A (n=0)	31 ± 1 (n=28)
	Charr	Aquaculture	N/A (n=0)	N/A (n=0)	38 ± 1 (n=6)
		Downstream	12 ± 1 (n=3)	N/A (n=0)	17 ± 3 (n=2)
		Upstream	12 ± 0 (n=4)	N/A (n=0)	13 ± 0 (n=1)
	Perch	Aquaculture	20 ± 0 (n=13)	N/A (n=0)	22 ± 1 (n=13)
		Downstream	19 ± 0 (n=16)	N/A (n=0)	21 ± 1 (n=11)
		Upstream	19 ± 1 (n=17)	N/A (n=0)	23 ± 1 (n=81)
	Whitefish	Aquaculture	25 ± 1 (n=30)	N/A (n=0)	25 ± 1 (n=41)
		Downstream	25 ± 2 (n=13)	N/A (n=0)	27 ± 1 (n=21)
		Upstream	29 ± 2 (n=7)	N/A (n=0)	24 ± 1 (n=14)

3.1 Fat content

In burbot the fat content changed significantly over time for fish caught upstream, Changes in fat content in burbot from the aquaculture location was only visually presented (figure 1) due to inadequate sample size and any interpretation should hence be done with great care. Since aquaculture was excluded, downstream was the reference for the results (table 3) and an interaction between year and the upstream location could be seen (figure 1, table 3).

The fat content in perch changed significantly over time. In the aquaculture location the fat content in perch decreased over time. In the downstream- and upstream location the fat content decreased from 2009 to 2010 to be higher again in 2012. There was an interaction effect between time (year) and location, both downstream and upstream (table 3). The interaction effect indicated that changes in fat content over time differed between different locations.

Fat content in whitefish changed significantly over time and there was a significant interaction between year and the upstream location (figure 1, table 3). In the upstream location the fat content increased over time while in the aquaculture- and downstream location the fat content in 2010 was lower compared to 2009 and 2012. However, there was no significant change in fat content in whitefish from the downstream location compared to whitefish from aquaculture location (table 3).

For charr, the fat content was only visually presented due to a low sample size (n=17) and any interpretation should be done with great care. The fat content ranged between 2-31% (figure 1). Based on the available data (figure 1) no differences in fat content appear to exist between the downstream- and upstream location and no meaningful comparison could be made for charr in the aquaculture location. It appeared as if fat content of charr decreased in the downstream- and upstream location (figure 1).

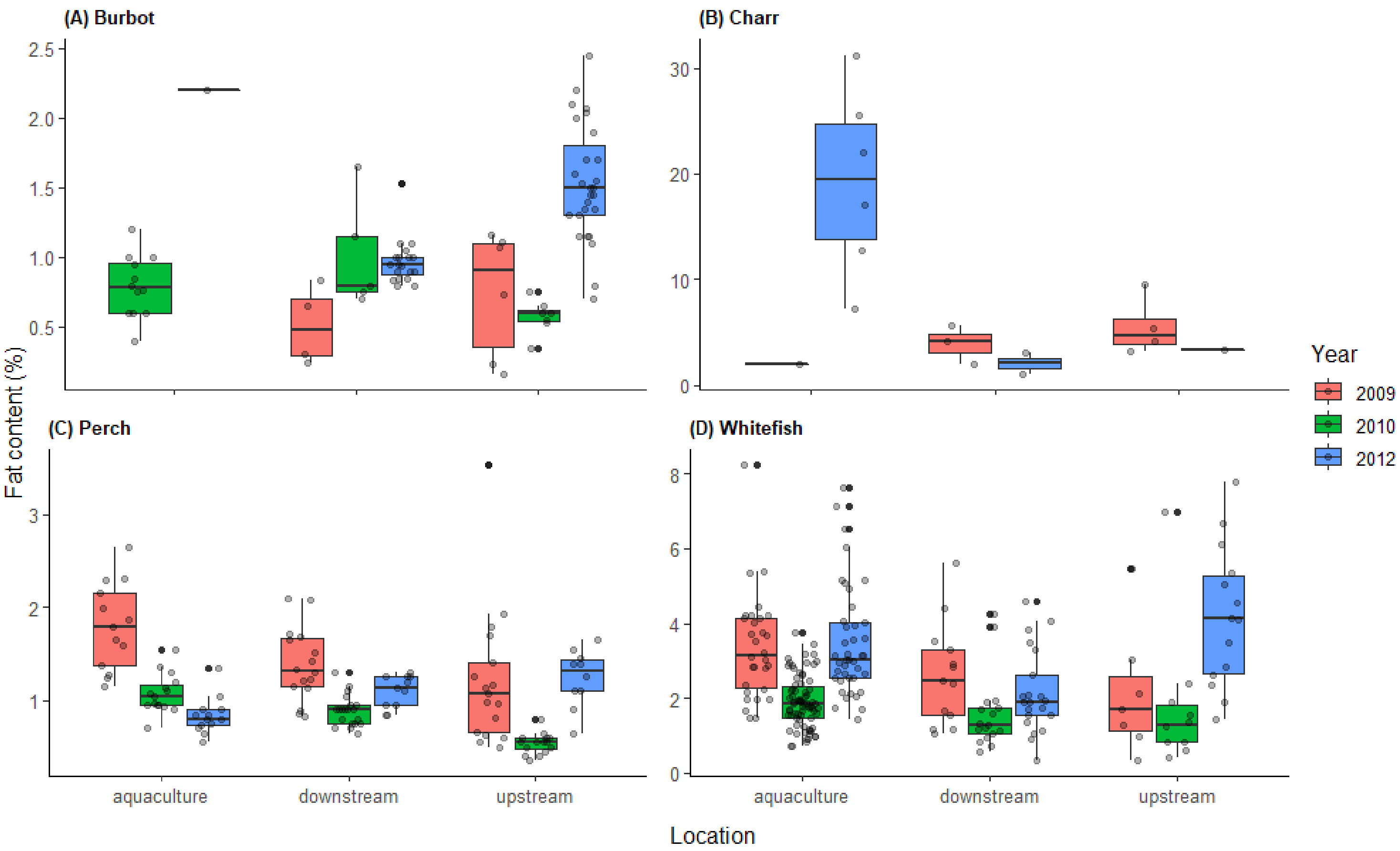

Figure 1. Boxplots showing fat content (%) in burbot (A), charr (B), perch (C) and whitefish (D) caught in three different locations (aquaculture, downstream, upstream) in different years (2009, 2010, 2012). In the boxplot, the box shows the first quantile, median (black line) and third quantile. The whiskers show minimum and maximum values. All data points are plotted with circles (not filled). Outliers are filled, black circles next to its corresponding data point. Note that the values of the fat content (y-axis) are not equal for the four species.

Table 3. Summary of generalised linear models (GLM) for change in fat content in burbot, perch and whitefish. For each species the intercept, capture time (year) and capture location (place) are presented. Results for perch and whitefish include all locations (downstream and upstream compared to aquaculture), while results for burbot only include downstream- and upstream location (upstream compared to downstream). Year:Place shows interaction effects.

	Estimate	Std. Error	t value	Pr (>|t|)
Burbot*				
(Intercept)	-347.809	125.369	-2.774	0.007
Year	0.173	0.062	2.773	0.007
Placeupstream	-380.907	162.807	-2.340	0.022
Year:Placeupstream	0.190	0.081	2.341	0.022
Perch*				
(Intercept)	466.760	95.565	4.884	<0.001
Year	-0.232	0.048	-4.883	<0.001
Placedownstream	-360.819	136.078	-2.652	0.009
Placeupstream	-579.271	137.554	-4.211	<0.001
Year:Placedownstream	0.179	0.068	2.651	0.009
Year:Placeupstream	0.288	0.068	4.209	<0.001
Whitefish*				
(Intercept)	-194.646	81.631	-2.384	0.018
Year	0.097	0.041	2.395	0.017
Placedownstream	232.433	145.571	1.597	0.112
Placeupstream	-520.872	175.060	-2.975	0.003
Year:Placedownstream	-0.116	0.072	-1.599	0.111
Year:Placeupstream	0.259	0.087	2.975	0.003

* = log-transformed values

3.2 Protein content

Protein content (%) did not change significantly over time for any of the species: burbot (p-value: 0.342), charr (p-value: 0.861), perch (p-value: 0.498) or whitefish (p-value: 0.711). Time (year) was therefore removed from the GLM (table 4).

The protein content in burbot caught in the downstream location was not significantly different than in burbot caught in the upstream location (figure 2, table 4). However, data for protein content in burbot was not normally distributed. Additionally, data from burbot caught in the aquaculture location was only visually presented due to inadequate sample size. Protein content appeared to be higher in samples from the aquaculture location compared to from the downstream- and upstream locations (figure 2), but any interpretation should be done with great care.

In general, the protein content in perch did not change over time. However, there was an interaction with time in the upstream location, which indicated an increase in protein content over time in perch in the upstream location, compared to the aquaculture location where no change over time was seen (figure 2, table 4). Protein content in perch was not significantly higher or lower in the downstream location compared to the aquaculture location (table 4).

Protein content in whitefish was lower in samples from the downstream location compared to the aquaculture location. The data was however not normally distributed, and more samples were collected from the aquaculture location compared to the downstream and upstream location. More samples in the aquaculture location resulted in a larger variation in values compared to samples from the other capture locations (figure 2, table 4).

Protein content in charr appeared to be generally lower downstream and upstream, compared to the aquaculture location (figure 2). However, due to the low number of samples (as described within the fat content result), charr protein data were only visually assessed. Similar to fat content, there was a large variation in protein content in charr caught in the aquaculture location (figure 2). The protein content in charr caught in the upstream location appeared to be slightly higher than in charr in the downstream location.

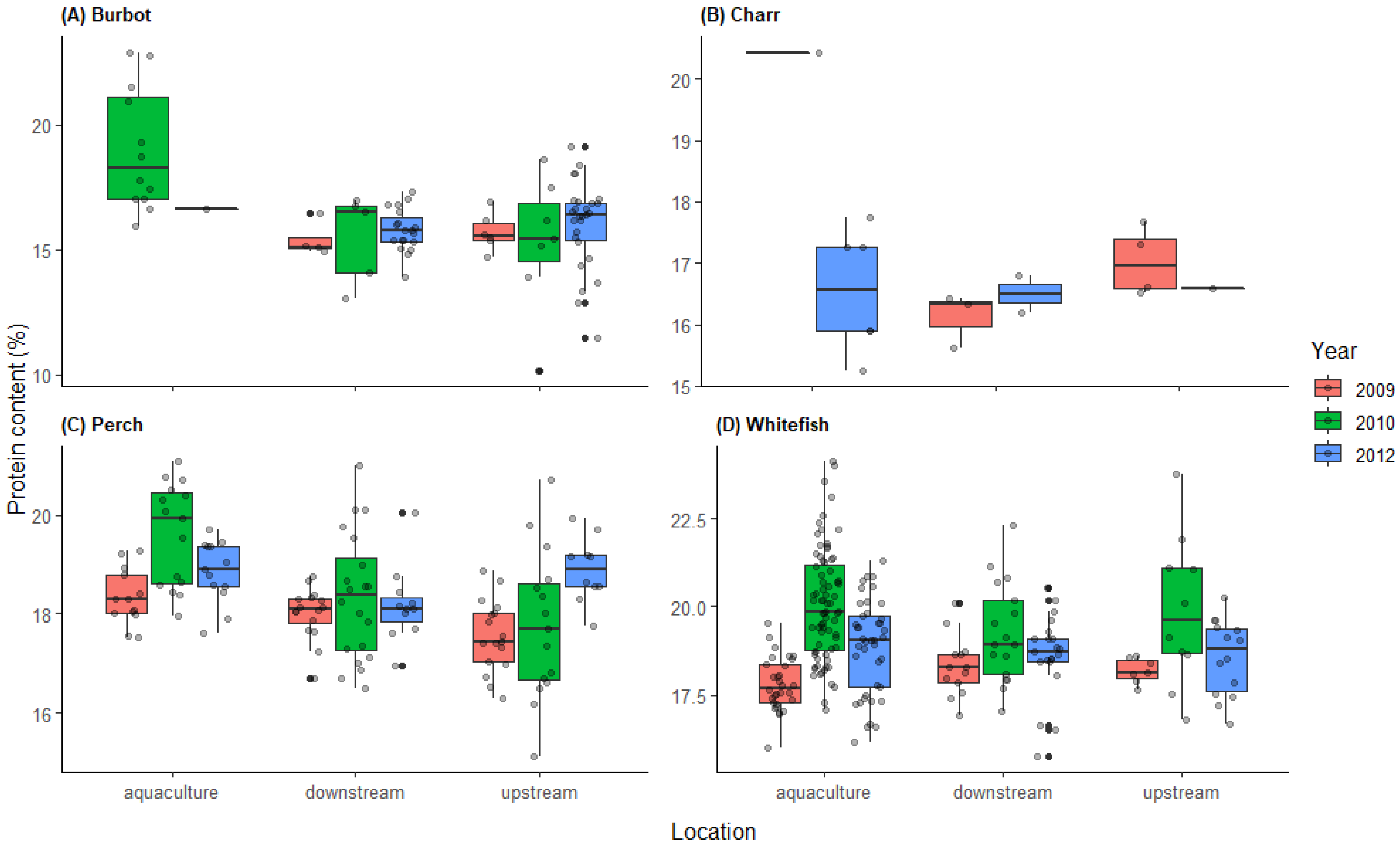

Figure 2. Boxplots showing protein content (%) in burbot (A), charr (B), perch (C) and whitefish (D) caught in three different locations (aquaculture, downstream, upstream) in different years (2009, 2010, 2012). In the boxplot, the box shows the first quantile, median (black line) and third quantile. The whiskers show minimum and maximum values. All data points are plotted with circles (not filled). Outliers are filled, black circles next to its corresponding data point. Note that the values of the fat content (y-axis) are not equal for the four species.

Table 4. Summary of generalised linear models (GLM) for change in protein content in burbot, perch and whitefish. For each species the intercept, capture time (year) and capture location (place) are presented. Results for perch and whitefish include all locations (downstream and upstream compared to aquaculture), while results for burbot only include downstream- and upstream location (upstream compared to downstream). Year:Place shows interaction effects.

	Estimate	Std. Error	t value	Pr (>|t|)
Burbot				
(Intercept)	15.668	0.292	53.645	<0.001
Placeupstream	0.180	0.381	0.474	0.637
Perch				
(Intercept)	-154.356	255.269	-0.605	0.547
Year	0.086	0.127	0.679	0.498
Placedownstream	79.491	363.484	0.219	0.827
Placeupstream	-733.809	367.428	-1.997	0.048
Year:Placedownstream	-0.040	0.181	-0.221	0.826
Year:Placeupstream	0.365	0.183	1.995	0.048
Whitefish*				
(Intercept)	2.955	0.007	450.633	<0.001
Placedownstream	-0.025	0.013	-1.975	0.050
Placeupstream	-0.019	0.016	-1.190	0.235

* = log-transformed values

3.3 Stable isotope analysis

3.3.1 Perch

Changes in values of $\delta^{15}N$ in perch was observed three years after establishment of the aquaculture compared to after one year, in the 95% confidence interval around the bivariate means (95% CI) for all locations (aquaculture, downstream, upstream) (bold lines, figure 3). By studying the 95% CI it appeared to be a clear overlap between the downstream- and upstream location during first year of establishment of aquaculture as well as within three years after (bold lines, figure 3). The 95% CI for the aquaculture location appeared to overlap a bit more one year after establishment and did also appear to have a more positive $\delta^{13}C$ value compared to the other locations.

By visually studying the ellipses for the trophic niches, they appeared to overlap to a large extent (dashed lines, figure 3). Both the total area (TA) of the convex hull and the corrected value for standard ellipse area (SEAc) were lower in the downstream- and upstream location one year after establishment while they were higher in the aquaculture location (table 5).

After three years of fish farming in the lake, the dY-range, related to $\delta^{15}N$, was lower while the dX-range, related to $\delta^{13}C$, was higher compared to values from the first year (table 6). The TA decreased for perch in all sampling locations sampled during the last years of the sampling period compared to during the first year of farming. Decreased TA indicated less variation within the sampling group, compared to earlier sampling period (table 6). The mean distance to centroid (CD), indicating how the group is located to the mean mean values of $\delta^{15}N$ and $\delta^{13}Cc$ was higher three years after establishment of the aquaculture. The mean nearest neighbour distance (MNND) together with the standard deviation of nearest neighbour distance (SDNND), indicating the density of the group, were also higher for the same period. These higher values together (CD, MNND, SDNND) showed a larger variation within the sampling locations in their distance between each other.

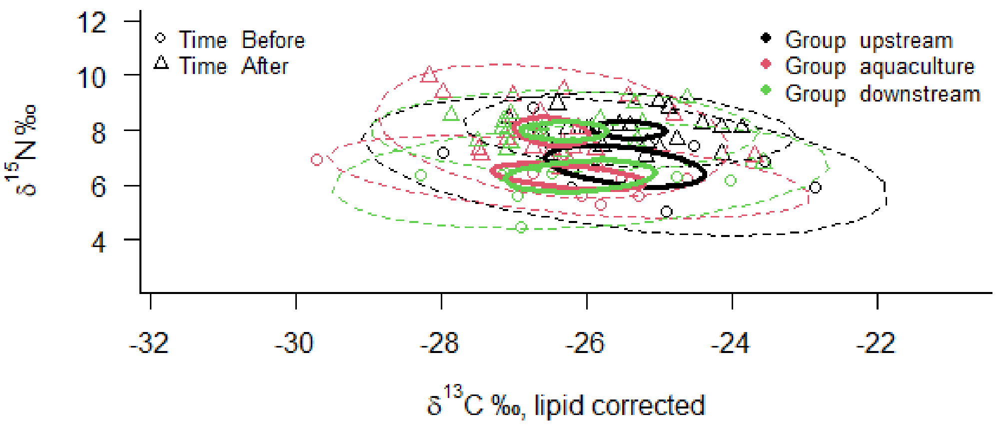

Figure 3. Isotopic niche of perch grouped by location and before and after establishment of aquaculture. The dashed lines indicate standard ellipse area (SEA) and the bold lines mark 95% confidence interval around the bivariate means (95% CI). Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after". The three smaller 95% CI, marked with bold lines, in the upper part of the figure show results from time period "after". The three in the bottom show results from time period "before".

Table 5. Stable isotope analysis of perch showing total area (TA) of the convex hull, standard ellipse area (SEA) and corrected value for standard ellipse area (SEAc) of fish caught in different locations before and after establishment of the aquaculture. Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after".

	TA	SEA	SEAc
Before			
Upstream	9.41	4.40	4.89
Aquaculture	4.44	2.33	2.62
Downstream	7.59	3.36	3.74
After			
Upstream	4.42	1.60	1.69
Aquaculture	8.75	3.00	3.15
Downstream	6.33	2.07	2.19

Table 6. Stable isotope analysis of perch showing range between the highest and the lowest values of $\delta^{15}N$ (dY_range) and $\delta^{13}Cc$ (dX_range), total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND) and standard deviation of nearest neighbour distance (SDNND) of fish before and after establishment of the aquaculture. Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after".

	dY_range	dX_range	TA	CD	MNND	SDNND
Before	0.39	0.77	0.02	0.35	0.34	0.32
After	0.04	1.06	0.002	0.43	0.41	0.42

3.3.2 Whitefish

Changes in values to higher δ^{15}N and lower δ^{13}C was observed in whitefish three years after establishment of the aquaculture compared to after one year, in 95% CI for all locations (aquaculture, downstream, upstream) (bold lines, figure 4). By studying the 95% CI it appeared to be an overlap between all locations and both time period studied (bold lines, figure 4). The 95% CI as for the downstream location appeared to have a more positive δ^{13}C value compared to the other locations.

By visually studying the ellipses for the isotopic niches, they appeared to overlap (dashed lines, figure 4). The ellipse for the downstream location was observed to have a higher δ^{13}C value during the first year of aquaculture, compared to later (dashed lines, figure 4). SEAc was lower in the downstream- and upstream location one year after establishment while it was higher in the aquaculture location (table 7). TA was lower in all locations one year after establishment of the aquaculture (table 7).

After three years of fish farming in the lake, both the dY-range (δ^{15}N) and dX-range (δ^{13}C) for whitefish were lower compared to values from the first year (table 8). TA, CD, MNND and SDNND were all smaller three years after establishment of the fish farm, compared to after one year (table 8). All these decreased values (dY_range, dXrange, TA, CD, MNND and SDNND indicated a more homogenic sampling group three years after establishment of the aquaculture, compared to before.

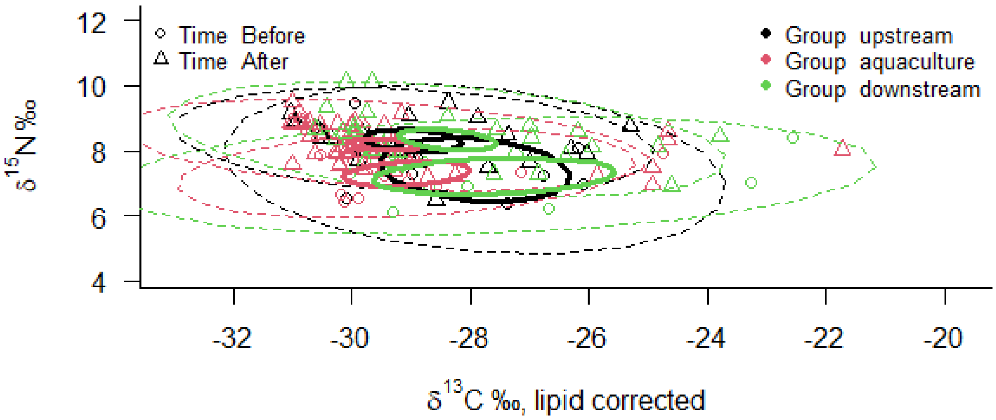

Figure 4. Isotopic niche of whitefish grouped by location and before and after establishment of aquaculture. The dashed lines indicate standard ellipse area (SEA) and the bold lines mark 95% confidence interval around the bivariate means (95% CI). Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after". The three smaller 95% CI, marked with bold lines, in the upper part of the figure show results from time period "after". The three in the bottom show results from time period "before".

Table 7. Stable isotope analysis of whitefish showing total area (TA) of the convex hull, standard ellipse area (SEA) and corrected value for standard ellipse area (SEAc) of fish caught in different locations before and after establishment of the aquaculture. Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after".

	TA	SEA	SEAc
Before			
Upstream	8.85	5.67	6.80
Aquaculture	4.34	2.70	2.95
Downstream	12.14	5.98	6.73
After			
Upstream	9.62	3.39	3.55
Aquaculture	14.75	3.06	3.12
Downstream	13.15	4.08	4.22

Table 8. Stable isotope analysis of whitefish range between the highest and the lowest values of $\delta^{15}N$ (dY_range) and $\delta^{13}Cc$ (dX_range), total area (TA), mean distance to centroid (CD), mean nearest neighbour distance (MNND) and standard deviation of nearest neighbour distance (SDNND) of fish before and after establishment of the aquaculture. Fish caught in 2009 are categorised as "before" and fish caught in 2010 and 2012 are categorised as "after".

	dY_range	dX_range	TA	CD	MNND	SDNND
Before	0.21	1.49	0.14	0.60	0.65	0.45
After	0.16	1.04	0.03	0.37	0.51	0.05

4. Discussion

The changes in fat content and stable isotopes that were observed in this study could indicate that wild fish were affected by leakage of nutrients from food producing aquaculture. The results suggested some changes in uptake and utilisation of nutrients after establishment of the aquaculture.

4.1 Nutrients

The fat content changed over time in burbot, perch and whitefish. Important to consider is that these three species are all considered as lean fishes (Nordic Council of Ministers 2014). The mean values for fat content in this study for these species were between 1.08-2.57%. Even though significant changes were seen after the establishment of the aquaculture, these changes might not be noticeably big in the total amount of fat in terms of fish as a food source. However, it is an important and interesting indication, that there were significant changes over time in the wild fish. The changes in fat content, regardless of how big or small, could however have significant biological impact on the wild fish.

In this study only total fat content was considered. When discussing health aspects of fish as a food source one of the main aspects, especially in fat fish, is the high levels of omega-3 fatty acids and high levels of PUFA compared to other animal food products (Tacon & Metian 2013; Nordic Council of Ministers 2014; FAO 2020). Further studies are needed to evaluate if changes in fat content in wild fish are relevant in terms of the wanted PUFA and omega-3 fatty acids. This study was looking at the feed source from a quantitative perspective, and not a qualitative perspective.

The protein content did not change significantly over time in any of the species. There were no interaction effects between year and location, except for perch in the upstream location, indicating an increase in protein content compared to the aquaculture location. In general, protein content in fish does not vary very much between species, in contrast to fat content (Nordic Council of Ministers 2014). Also, protein content in wild fish, compared to farmed fish showed no general differences (Nettleton & Exler 1992; Olsson et al. 2003; González et al. 2006;

Jensen et al. 2013). This could be an indication that measuring only protein content could be insufficient in terms of evaluating effects of fish farms on the wild species. By only measuring the protein content it is difficult to distinguish between species and production systems. To be able to find specific changes in protein, there is a need to study the quality of the protein and the amino acids. Again, this study is only looking at the food source from a quantitative perspective, and not a qualitative perspective and further studies are needed.

As already mentioned, only the total content of fat and protein are not enough indicators of the quality of the wild fish. Other aspects that would be needed to analyse further is size of the fish. For example, weight ranged between 14-709 g for fish used in this study. Since the observed weights and lengths measured were not further statistically analysed, no conclusions can be drawn related to size. From a human food perspective, it would be interesting to study possible changes in the size of the fish related to implementation of an aquaculture. Further analyses would be needed to be able to draw conclusions regarding the correlation between fat content and size, as well as general changes in size, and how these show effects on wild fish. Also, other parameters regarding the sampled fish would be important to consider in further studies such as age, gender and whether the size distribution of the fish differs in different parts of the lake. Additionally, the fish feed composition used in the fish farm would be needed to analyse to be able to draw conclusions regarding the fat quality and to be able to improve feeding, if needed.

4.2 Stable isotopes

The results from the SIA for perch indicated a higher dX-range (related to in $\delta^{13}C$) three years after the establishment of the aquaculture while the results for whitefish indicated a lower a dX-range for the same period of time. The higher dX-range in perch could be interpreted as bigger variation of carbon in the feed and possibly a new carbon source. A new source of carbon in the feed could suggest that perch adapt easy to changes in the environment and hence can use more nutrient resources to grow a bigger population. With the findings of perch possibly expanding their nutrition intake in terms of carbon source this could be a positive effect of farms leaking out nutrients for the resource of perch. Leakage of nutrients to the adjacent environment will happen in open systems to some extent, and these nutrients can be useful for the wild fish in the form of more available feed. For ecosystems with poor nutritional supply this could be positive. With that statement, it is however important to consider further studies on other types of effects on wild fish and how they could be related to quality of the fish and feed from farms.

There was a decrease in dY_range (related to $\delta^{15}N$) in both perch and whitefish three years after establishment of the aquaculture. The direction in the changes of $\delta^{15}N$ followed the same pattern and direction as the newer feed for the aquaculture. There was however a difference between perch and whitefish in the changes of $\delta^{13}C$ where perch did not follow the same pattern as the change in feed, when comparing the old feed against the new (table 2). The relationship between the changes in $\delta^{13}C$ and $\delta^{15}N$ could indicate that the fish used more of the same feed as before. They were on a more similar trophic level, taking up less of the trophic niche, especially the whitefish where the changes in $\delta^{13}C$ were the same, or lower in the time period "after". For perch, where the changes in $\delta^{13}C$ were higher in the "after" period. The trophic niche was higher due to larger separations between capture locations, especially upstream. In addition, all of the other metrics studied (TA, CD, MNND, SDNND) for whitefish appeared to indicate more homogeneity as a group, after three years of establishment of the fish farm. For perch the same values varied more, probably suggesting a wider separation within the group. Without knowing more, perch could be suggested to benefit from this in comparison with the whitefish, due to a higher trophic niche. On the other hand, the whitefish could be suggested to be more specialised and adapting to changes in the environment and have no need for a wider separation to find feed.

The stable isotope analysis was however only performed within two of the study species and could therefore only give a small indication of changes, not to be seen as representative for the whole ecosystem. Interaction between species could be affected by leakage of nutrients. The interaction between species could however also affect the overall effects of leakage. As observed in this study inter-species variations in response to the establishment of an aquaculture is likely and it is therefore important to further study with more species, to increase the understanding of aquaculture establishment and its effect on wild fish.

4.3 Risks and future perspectives

Even though the data for charr could not be statistically evaluated due to low sample size, another potential effect on the surrounding ecosystem from the aquaculture was found. The fat content in charr caught in the aquaculture location, considered as wild, had fat percentages within the range of 2-31% while values from the Swedish Food Agency estimates a fat content for wild charr up to 8% (Swedish Food Agency 2021b). These high values of charr from these samples suggest that they might not have been wild from the beginning, it could be charr that had escaped from the aquaculture or due to dominant behaviour between individuals. This could be a problem in many ways. There is an economical risk for the producers if fish escapes but there is also a problem for the ecosystem and surrounding area by higher risk of spreading diseases and competition within the ecosystem (Eriksson et al. 2017).

One of the hypotheses for this study was that fish downstream should be more affected by the establishment of an aquaculture than fish upstream. This hypothesis was based on a theory of water transporting nutrients from the aquaculture downstream. None of the results indicated a general change for fish caught specifically downstream or upstream and no general pattern was seen related to sampling location. This could mean that nutrients did not spread very much from the aquaculture. However, this is not excluding the fact that the fish can move towards the nutrient source. These findings could indicate that the nutrients from the aquaculture are not affecting any of the other locations, but there could also be a change in where the fish populations are located.

For this study, one has to keep in mind that the data should be looked at with caution. This is only one study performed for one represented system containing a few species with some varying in numbers of samples. It would have been interesting to do further studies containing bigger sample groups. It would also have been important to perform a similar study with a control site for the water, to be able to look for other environmental parameters affecting the ecosystem.

For fish as a food source, aquaculture has the opportunities to develop more. Globally the production is important and have been increasing the last years while it in Sweden have been decreasing (FAO 2020; SWEMARC 2022). Instead, we import almost all farmed fish from Norway and export a lot of fish for feed production (Eriksson et al. 2017). An important question is: why do we import and export a lot of the fish that could be produced locally? Instead of importing and exporting majority of the fish, a more sustainable alternative could be to better use already existing alternatives. The wild species used in this study, burbot, perch and whitefish, are not taking up a lot of space in the supermarkets in Sweden today. It would be of interest to investigate if these species could be used in a wider range and use of wild fish could be developed further. It would also be of interest to investigate if diversity could expand in number of species used for farming today. Maybe the native wild species are well suited for farming, and this would have been interesting to investigate further.

Fish farming in Sweden has decreased in comparison with the international production. One aspect of the decreasing aquaculture production in Sweden is related to bureaucracy (The Swedish Board of Agriculture & Swedish Agency for Marine and Water Management 2021). The goal is however to increase fish farming production (The Swedish Board of Agriculture 2021). If the findings in this study can be applied for more systems an increase of farming sites could lead to an increase in wild fish due to more available nutrients and energy. An increase in wild fish could either be a problem by competition within ecosystem, or an asset by helping to prevent the eutrophication to some extent and at the same time contribute to a bigger food supply of wild fish. Further studies are hence needed for more details in effects on wild fish, and the possibilities of taking advantages of a bigger wild fish supply.

4.4 Conclusion

The aim of this study was to evaluate the effect of aquaculture on the wild fish resource. Changes in fat content in wild fish and some changes in trophic niche were found, which could indicate that leakage of nutrients from the fish farm affected the wild fish in the lake. However, significant changes in protein content were not detected. At the same time, this study only covers a small part of the ecosystem and a fraction of the wild fish community and could therefore not be considered to represent the whole system. Indications could be seen, but further studies are needed: both with bigger sample sizes, more diverse samples and also newer data to be able to see consequences related to the environment in the lake today (14 years after establishment of the fish farm). Fish downstream were not particularly more affected than the fish upstream and from this study, this hypothesis could be rejected. However, also here more studies are needed.

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Popular science summary

You have probably been taught since you were kid that fish is good for you, and that it is important to eat fish every week. Fish is a common everyday food full of nutrients. Fish contains lots of proteins and fat, which help to build a strong and healthy body. Fish do also contain vitamins and minerals important to help your body to stay as healthy as possible.

To be able to eat all the healthy fish you need you can go fishing by yourself. If you are not able to provide for your own fish and have to buy it, you can either by wild fish or farmed fish. There are many sustainability related problems connected to both the wild fish and the farmed fish. Wild caught fish are often related to overfishing and disturbing ecosystems. An alternative to the wild fish is farmed fish. Fish farms can be performed in many ways, and one example of that are big net cages placed in a lake. By establishing fish farms, countries without a coast can provide for their own fish besides leaving the wild fish undisturbed. A problem with this type of production is that feed and faeces from the fish in the fish farm is leaking out in the lake. All this nutrition found in the feed and faeces can affect the ecosystem in the lake. Other fish and organisms can eat the feed while algae and other growing organisms can be fertilised by the nutrients.

In this study we analysed to see if a new fish farm in a Swedish lake was affecting wild burbot, charr, perch and whitefish. The measurements that were looked at were possible changes in fat content and protein content in the wild fish after establishment of an aquaculture. Also, stable isotopes were analysed in perch and whitefish. Stable isotopes of nitrogen and carbon are chemical markers, or signals, which can show traces of nutrients, or feed in the fish. By looking at these signals there is a possibility to see changes in how, and where the fish communities live. In this way it is possible to see if the feed given to the fish farm is leaking out and become food for the wild fish.

The results from the analyses of the wild fish indicated that fat content changed in wild fish after the establishment of a new fish farm. Burbot and whitefish had more fat, while perch had changes in fat content both going up and down. Protein content did not change in any of the fish. Charr was excluded from the analyses since the number of samples were too few to be representative. The analyses also showed changes in the stable isotopes, which could be a sign of the wild fish eating feed that were connected to the feed in the fish farm.

These findings of changes in fat content and changes in stable isotopes, gave signs that the wild fish was affected by the fish farm, and nutrients leaking out. This could both be a good and a bad thing. The bad thing is that if the farm is leaking out nutrients, the surrounding environment in the lake can be affected and changed in a way that is not favourable. The good thing is that wild fish populations can grow and become bigger, which can be a good alternative for many new food fishes.

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