

A Metabolomic Approach To Detect Effects of Salmon Farming on Wild Saithe (*Pollachius virens*) Populations

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S Supporting Information

ABSTRACT: A metabolomics approach was used to analyze effects of salmon farming on wild saithe (*Pollachius virens*) populations. Saithe fish were captured at two salmon farms and at two control locations around the island of Hitra, Norway. Changes in diet seem to drive changes in metabolic status of fishes. The liver and muscle tissues, from the fishes captured around the farm, showed higher levels of lactate and certain amino acids (glutamine, glutamate, and alanine) and lower levels of glucose and choline than the fishes captured in the control locations, far from the farm locations. The higher levels of lactate and amino acids could be related to the facility of obtaining food around the farm and the deficit in choline to the deficit of this nutrient in the salmon feed. At each location the fish were captured with either benthic gill nets and automatic jigging machines, and this feature showed also variations in different metabolites.

KEYWORDS: aquafeed, fish populations, metabolites, aquaculture, NMR, chemometric

INTRODUCTION

Marine aquaculture and fisheries share space and resources, which may involve both potential synergies and unwanted interactions between these two important industries.^{1,2} In a context where worldwide aquaculture production is expected to grow, the development of tools to detect aquaculture–fisheries interactions is of particular relevance.³ Increased understanding of those interactions is required to manage them properly, avoiding conflicts among users.

One well-known consequence of salmon culture in coastal areas is the aggregation of wild fish in the vicinity of the farms, which feed on the nonconsumed pellets from the fish cage.⁴ Previous studies have detected compositional side effects in the fatty acids profile due to this trophic subsidy⁵ that may lead to alterations in the physiology and even the quality of wild fish targeted by artisanal fisheries.⁶

The set of techniques used to assess the influence of a pellet diet in wild fish is usually costly and time-consuming (e.g., fatty acid profile, trace elements analysis⁷). Alternatively, small molecules (i.e., metabolites) identifiable by nuclear magnetic resonance (NMR) can discriminate fish origin in different situations⁸ and may be a useful and cost-effective tool to trail effects of aquaculture on wild fish. NMR spectroscopy is a multicomponent detection technique that offers the opportunity to detect most of the mentioned molecules and study biological tissue.^{9–11} Most NMR analyses are based on signals from proton (¹H) nuclei, which is the most sensitive NMR nucleus. Protons in different local chemical environments produce signals at slightly different NMR frequencies and can therefore be observed at different positions in the spectrum. This position, termed chemical shift, allows the identification of individual components

in a sample. For a given signal, the area under the signal curve is proportional to the concentration of the compound that gives rise to the peak, allowing quantification of compounds in the samples. The spectra obtained from tissue extracts are better resolved and therefore allow a more precise assignment of peak identities. On the basis of the detailed information from extracts it is possible to obtain an optimal classification of the metabolic status of the fish in certain environment.

These techniques may have practical applications for the selection of specimens according to their qualitative and quantitative content of small molecules, which is of relevance for the nutritional value of fish.^{12–19} Therefore, analyses of the small molecules, such as leucine, valine, carnitine, creatine, glucose, or glycogen, may be used to discriminate individuals.¹² The small molecules have a potential to serve as markers to trace the history of the fish because the type and amount of metabolites are affected by physiological factors or stress prior to death. The latter would permit the possibility to examine the effect of the diet and classify the fish according to their biochemical composition.

Salmon farming is the largest aquaculture industry in Europe, with a production in 2014 of almost 1.3 million tons, which consumed >1.6 million tons of pelleted fish feed in Norway alone.¹³ Saithe (*Pollachius virens*) is one of the most important species for Norwegian local fisheries and is commonly attracted in large numbers to fish farms due to the abundance of lost

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salmon feed.¹⁴ Consequently, the food quality of the saithe may be modified in farming-intensive areas due to a switch from natural prey to a diet consisting of salmon pellets.² However, recent research indicates that the negative quality influence depends on the fishing gear used.^{2,15,22}

The present study aims to define the liability of metabolites, determined by NMR, for detecting the influence of salmon farming on wild fish physiology, by analyzing muscle and liver composition of wild saithe using NMR spectroscopy. Fish were captured around fish farms and control areas, using two alternative fishing gear (gill nets and angling), to define the suitability of NMR for environmental management of marine aquaculture.

MATERIALS AND METHODS

Fish Sample Preparation. Saithe were captured between the September 19 and 21, 2012, at two salmon farms and at two control locations (>5 km from the nearest farm) around the island of Hitra, Norway (63.603658° N/8.645661° E) (Supporting Information, Figure S1). At each location the fish were captured with either benthic gill nets or automatic jigging machines ($n = 8$). Fish were randomly chosen, but gut content for each individual was analyzed and hepatosomatic index calculated for avoiding incorrect treatment assignment. Average length (\pm standard error, SE) and weight (\pm SE) of farm-aggregated saithe ("farm") were 65.9 ± 1.6 cm and 3115.8 ± 211.1 g, respectively, whereas average length (\pm SE) and weight (\pm SE) of saithe captured far from salmon farming activity ("control") were 66.4 ± 2.6 cm and 2475.6 ± 261.8 g, respectively. Muscle and liver tissue samples (around 6 g) were collected from the captured fish and kept at -80 °C for further analysis. To obtain the polar metabolites for ^1H NMR experiments, the frozen stored samples were extracted using perchloric acid method.⁹

Chemicals. D_2O (99.9% purity) and sodium 3-trimethylsilyl-propionate-2,2,3,3- d_4 (TSP, 99% purity) were obtained from Aldrich (Steinheim, Germany); perchloric acid 70% (puriss p.a. ACS) was from Fluka Chemicals BioChemika (Buchs, Switzerland); and potassium carbonate (puriss p.a. ACS) was from Panreac (Barcelona, Spain).

In Vitro ^1H NMR Spectroscopy. All NMR experiments were performed on a Bruker Avance 400 MHz equipped with a 5 mm ^1H -BB- ^{13}C TBI probe with an actively shielded Z-gradient. ^1D solution state ^1H NMR experiments were acquired with a recycle delay of 2 s, 32.768 time domain points, and 2.556 s of acquisition time. The number of scans was 2253. Spectra were apodized by multiplication with an exponential decay producing a 0.3 Hz line broadening in the transformed spectrum. Direct ^1H NMR was performed using SPR-W5-WATERGATE.¹⁶ Twelve ppm and -2 ppm and were outside the spectral window. The ^1H NMR spectra were reduced to ASCII files using custom-written *ProMetab* software (version 2.1)¹⁷ and peak alignment using *icoshift* (version 1.0; available at www.models.kvl.dk).¹⁸ All ^1H NMR spectra processing have been performed in MATLAB (The MathWorks, Natick, MA, USA) using a AMD Turion X2, 2.20 GHz processor with 4GB of RAM. High-resolution MR spectra of perchloric acid extracts from liver and muscle were first examined to provide detailed information about water-soluble components.⁹ Identification of individual components for muscle and liver was done by comparison to published values of chemical shifts and knowledge of the biochemical composition of fish skeletal muscle and liver, and the identification of signals was obtained from 2D NMR spectra.^{9,10,12} The assignments of the different resonances are listed in Table 1. Hypoxanthine, a molecule that is a good indicator for tissue freshness, was not detected in the ^1H NMR spectra.

Chemometric Analysis and Experimental Design. For the statistical analysis of spectroscopy data we performed a peak alignment.¹⁸ When the peaks were aligned, robust principal components analysis (robust PCA)¹⁹ and partial least-squares with linear discriminant analysis (PLS-LDA)²⁰ were performed. MATLAB version 6.5 from MathWorks was used for the calculations. Robust PCA was carried out using the LIBRA toolbox,¹⁹ and PLS-LDA was carried out

Table 1. Resonance Assignments with ^1H Chemical Shifts of Metabolites Identified in NMR Spectra of Perchloric Acid Extract from Tissues of Wild Saithe (*Pollachius virens*)

compound	proton	multiplicity	δ ^1H
leucine/isoleucine	$-\text{CH}_3$	d	0.97
valine	$-\text{CH}_3$	d	1.19
lactate	$-\text{CH}_3$	d	1.34
alanine	$-\text{CH}_3$	d	1.49
lysine	$-\text{CH}_2$	m	1.73
acetate	$-\text{CH}_3$	s	1.92
glutamine/glutamate	$-\text{CH}_3$	m	2.14
glutamate	$-\text{CH}_2$	m	2.35
glutamine	$-\text{CH}_2$	m	2.43
anserine	$-\text{CH}_3$		2.73
creatine	$-\text{NCH}_3$	s	3.04
choline	$-\text{NCH}_3$	s	3.13
phosphocholine	$-\text{N}(\text{CH}_3)_3$	s	3.21
β -glucose	$-\text{C}2\text{H}$, ring	dd	3.22
carnitine	$-\text{N}(\text{CH}_3)_3$	s	3.26
taurine	$-\text{S}-\text{CH}_2$	t	3.42
β -glucose	$-\text{C}5\text{H}$, ring	ddd	3.47
β -glucose	$-\text{C}3\text{H}$, ring	t	3.49
choline	βH	m	3.51
glycine	αH	s	3.58
glycerol	1,3H β	dd	3.64
glycerolphosphocholine	βH	dd	3.68
anserine	$-\text{NCH}_3$		3.69
α -glucose	$-\text{C}3\text{H}$, ring	t	3.70
β -glucose	$-\text{C}6\text{H}$, ring	dd	3.70
aspartic acid	αH	dd	3.78
α -glucose	$-\text{C}5\text{H}$, ring	m	3.84
β -glucose	$-\text{C}6\text{H}$, ring	dd	3.89
creatine	$-\text{CH}_2$	s	3.93
lactate	$-\text{CH}$	q	4.11
adenosine	H1		6.09
histidine (in anserine)	$-\text{C}4\text{H}$, ring	S	6.88
tyrosine	$-\text{C}3,5\text{H}$ ring	m	6.91
tyrosine	$-\text{C}2,6\text{H}$ ring	m	7.19
histidine (in anserine)	$-\text{C}2\text{H}$, ring	s	8.23
formate	$-\text{CH}$	s	8.52

using the *plslda* toolbox.²⁰ Two fixed factors were considered for statistical analysis: influence of aquaculture, with two treatments (farm and control), and fishing gear, also with two treatments (gill net and jigging).

In a supervised method, such as PLS-LDA, the most common approach is to select a number of the data to make a mathematical model. This model can be used for the prediction of new independent samples. The independent samples used to validate the model are samples excluded in the construction of the mathematical model. With our ^1H NMR spectra for the different samples, we made PLS-LDA models. Every model was made with all samples less one. In every case, the model was validated with the sample excluded. In other words, we made many models as samples, but in each model was excluded one sample. This approach had two advantages: we can detect quickly samples wrongly classified and all models are very similar. All samples were classified in the correct group when any of the two factors were considered (influence of aquaculture, with two treatments (farm and control) or fishing gear, also with two treatments (gill net and jigging)).

RESULTS

The ^1H NMR metabolic profile spectra aqueous liver extract (Supporting Information, Figure S2) showed that the profile was dominated by different signals assigned to metabolites such as

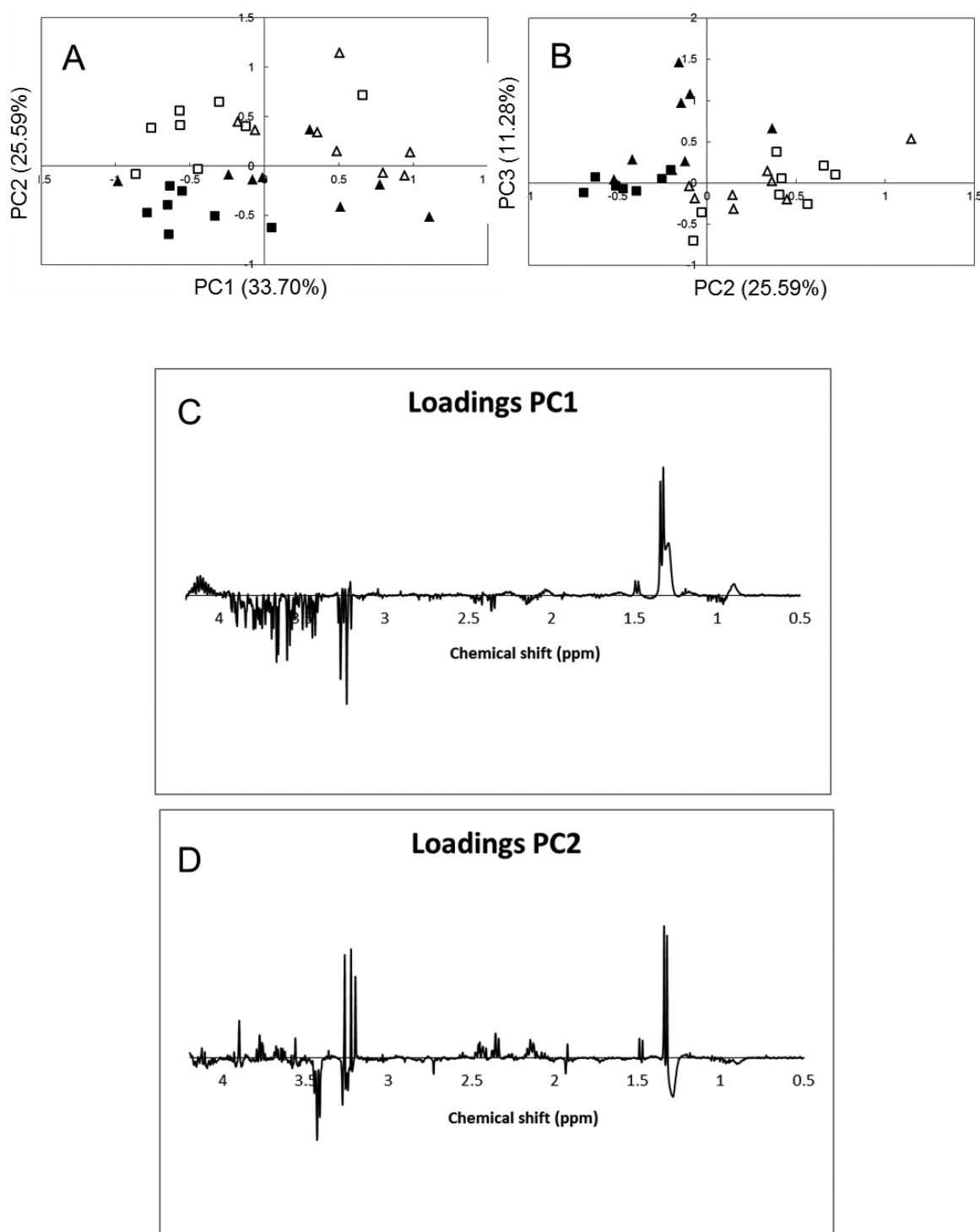


Figure 1. Robust PCA performed on ¹H NMR spectra from liver tissues of wild saithe (*Pollachius virens*): (A, B) scores plots from PC (■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net; (C, D) loadings plots from PCs. The first principal component (PC1) was described by 33.70%, the second principal component (PC2) by 25.59%, and the third principal component (PC3) by 11.28% of the variations.

glucose, glycerol, lactate, alanine, choline, and taurine. Other metabolites, such as acetate and several amino acids, were also assigned (glutamine, glutamate, leucine, valine, and isoleucine). Signals in the aromatic region (below 6 ppm) were assigned to the nucleosides/nucleobases, adenosine, inosine, uridine, uracil, and aromatic amino acid. In the case of muscle, the ¹H NMR spectra were dominated by signals from lactate, anserine, choline, creatine/phosphocreatine (Supporting Information, Figure S3). Signals from taurine, amino acids (alanine, glycine, glutamine,

glutamate, histidine, leucine, isoleucine, lysine, and valine), carbohydrates, and nucleosides or nucleotides (adenosine, ATP) were also observed.

To analyze the ¹H NMR metabolic profile spectra, we used an unsupervised chemometric method such as robust PCA.¹⁹ The scores plots from liver tissue samples displayed a good separation between the farm and control fishes (Figure 1A,B). The separation between the samples was determined by the loadings from PC2 (Figure 1D). The loadings were not real data, but they

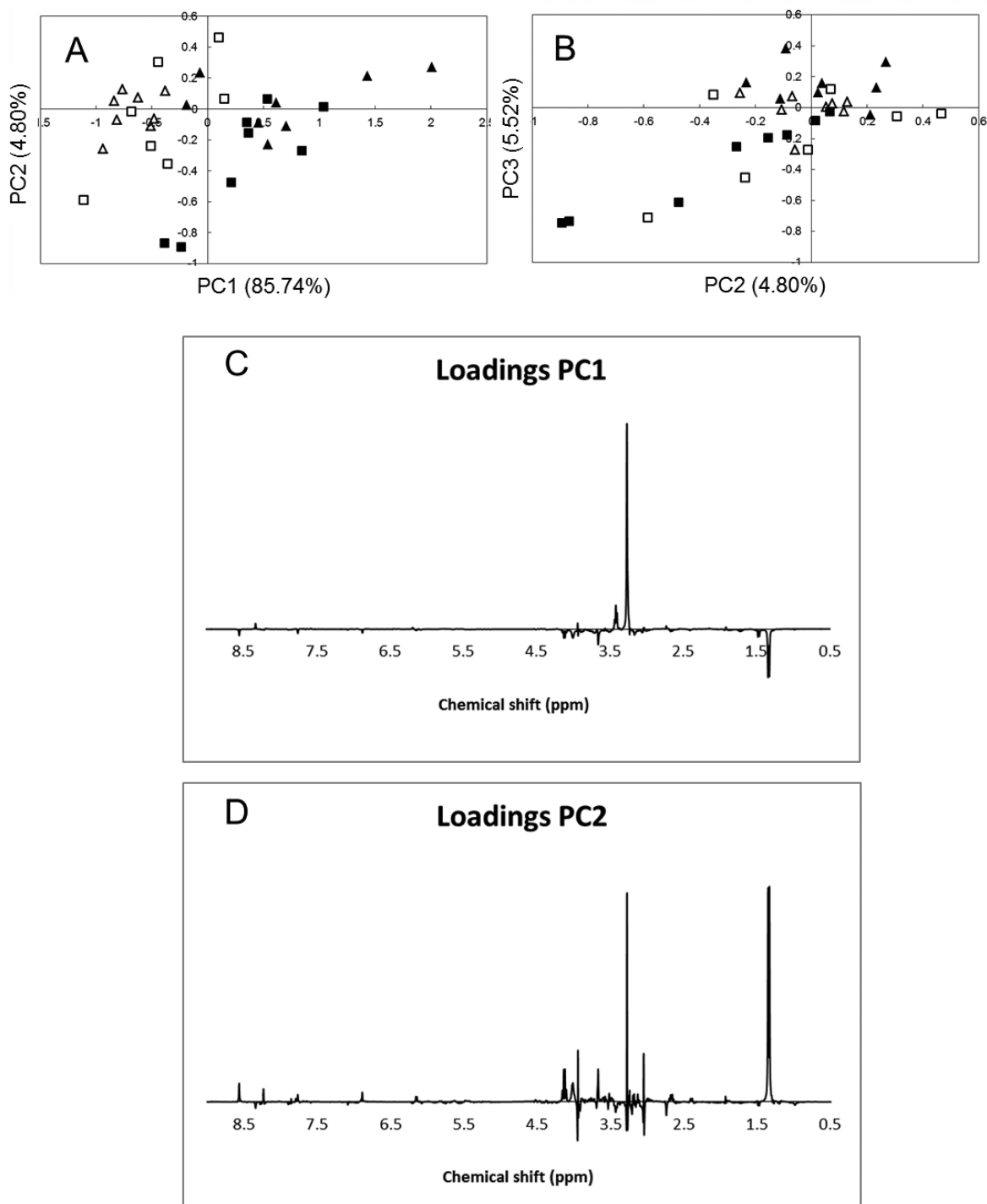


Figure 2. Robust PCA performed on ¹H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*): (A, B) scores plots from PC (■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net); (C, D) loadings plots from PCs. The first principal component (PC1) was described by 85.74%, the second principal component (PC2) by 4.80%, and the third principal component (PC3) by 3.52% of the variations.

can be interpreted as such to evaluate the importance of the different metabolites in the distribution of the samples in the scores plots. The loadings from PC2 (Figure 1D) showed that in the liver tissue, the farm fishes had higher lactate, amino acids (glutamine, glutamate, and lysine) and carnitine concentrations and lower taurine concentrations than the control fishes. The loadings from PC1 could be more related to the fish capture method (gill net or jigging) (Figure 1C). The loadings from PC1 indicated that the fish captured with gill nets had higher

concentrations of alanine and lactate and lower concentrations of glucose, glycerol, carnitine, and choline (Figure 1C). With muscle tissue, the situation was very similar when the ¹H NMR spectra were analyzed by robust PCA. The loadings from PC1 determined the distribution of the samples in the scores plots (Figure 2). The loadings from PC1 displayed that the farm fishes had higher concentrations of lactate and alanine and a lower concentration of choline than the control fishes (Figure 2C). The influence of the fishing method on the metabolomic profile was

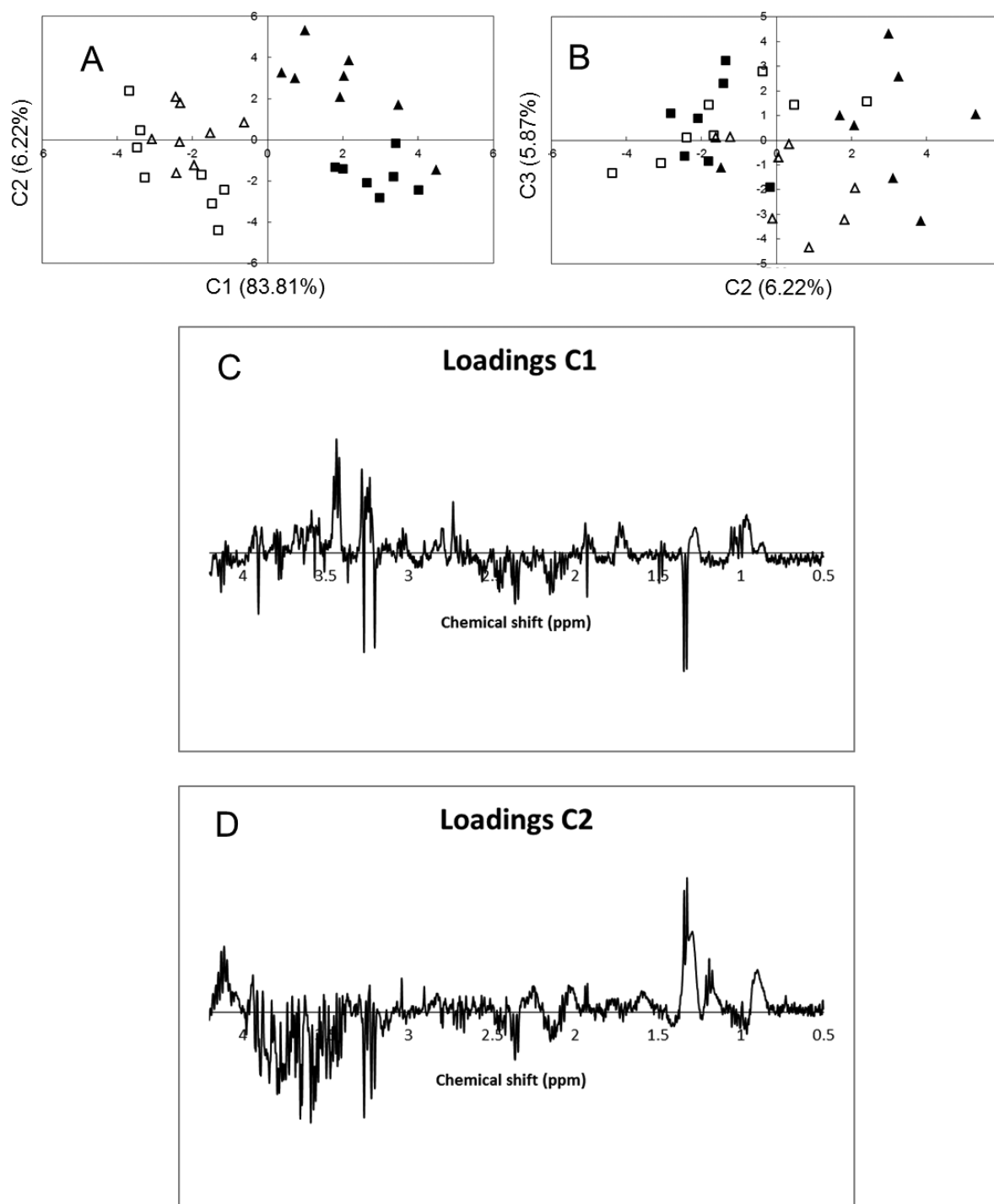


Figure 3. PLS-LDA performed on ^1H NMR spectra from liver tissues of wild saithe (*Pollachius virens*) using the proximity to the farm as classification criteria: (A, B) scores plots from PLS-LDA (■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net; (C, D) loadings plots from PLS-DA. The first component (C1) was described by 83.81%, the second component (C2) by 6.22%, and the third component (C3) by 5.87% of the variations.

less clear in the ^1H NMR data from muscle tissue than in liver tissue. The liver is the central tissue in the energetic metabolism, and it is an organ that quickly adapts to situations of stress, as it would be the catch of fish. However, the metabolic changes in the muscle were lower because this tissue would need more time to adapt to situations of stress.

The results from a supervised multivariate method such as PLS-LDA²⁰ showed that ^1H NMR data were able to discriminate powerfully between farm and control fishes (Figure 3), using the

approach described under [Materials and Methods](#). The liver tissue from farm fish had higher concentrations of lactate, amino acids (alanine, glutamine, and glutamate), and carnitine (loadings from C1) and a lower concentration of taurine than the liver tissues from control fishes. When the capture fish method was considered as the metabolomic variable (gill net or jigging), the fish captured with gill net had higher concentrations of lactate and alanine and lower concentrations of glucose and glycerol than the fishes captured with jigging (Figure 4).

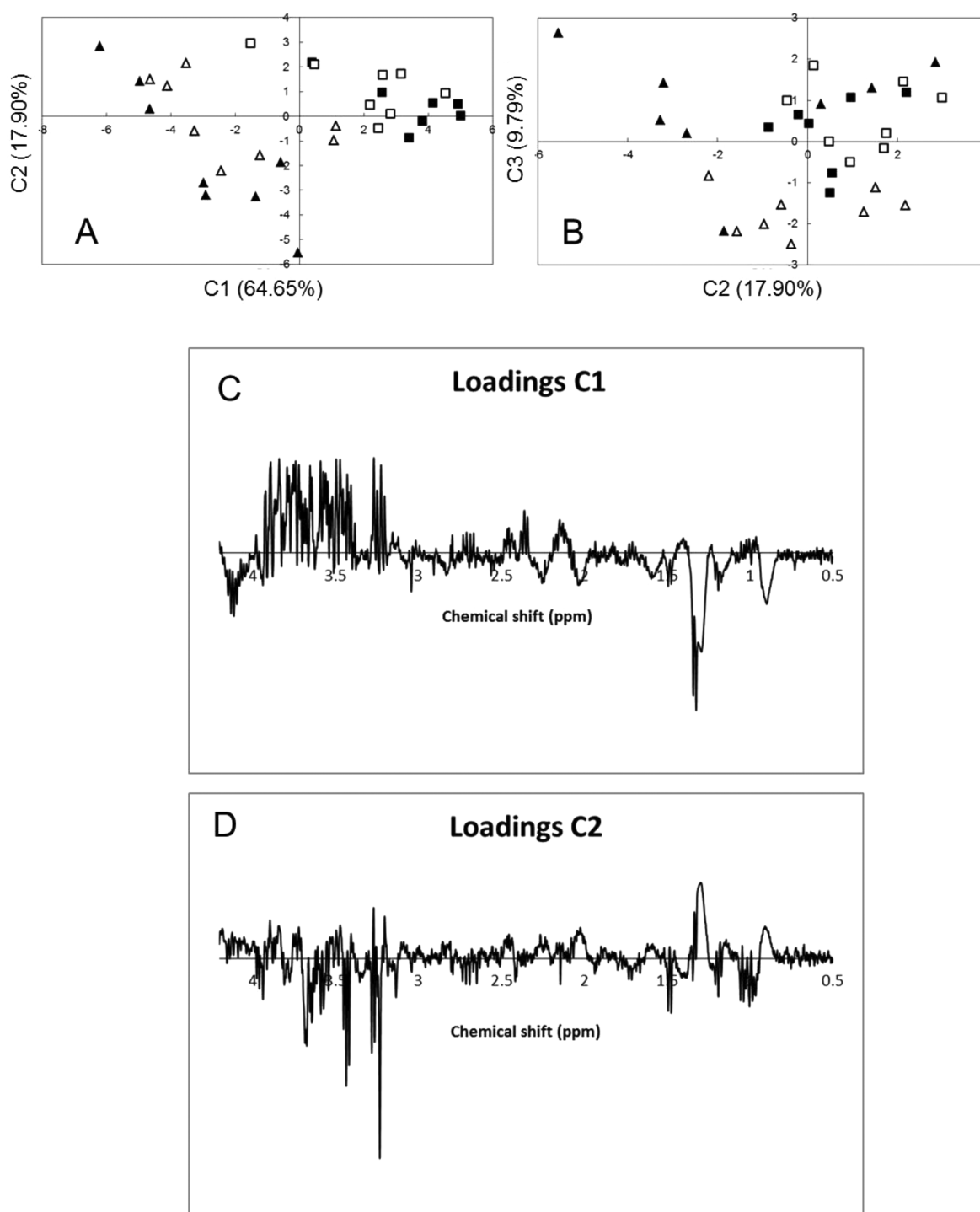


Figure 4. PLS-LDA performed on ^1H NMR spectra from liver tissues of wild saithe (*Pollachius virens*) using the fishing method as classification criteria: (A, B) scores plots from PLS-LDA (■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net; (C, D) loadings plots from PLS-LDA. The first component (C1) was described by 64.65%, the second component (C2) by 17.90%, and the third component (C3) by 9.79% of the variations.

If the ^1H NMR spectra from muscle tissue were analyzed by PLS-LDA, between farm and control fishes the discrimination power was also very high (Figure 5). When the proximity of the farms was considered in the classification, the lactate and amino acids (glutamine, glutamate, and alanine) concentrations were higher in muscle tissues from farm fishes than in muscle tissues from control fishes (Figure 6). However, the muscle tissues from control fishes displayed higher concentrations of choline and

taurine than farm fishes. With PLS-LDA analysis of ^1H NMR spectra, there was a very good classification of the muscle tissues samples when the fishing method was considered as metabolomic variable (Figure 6).

DISCUSSION

Salmon farming affected metabolic composition of main tissues, such as muscle and liver, of wild fish aggregated to fish farms,

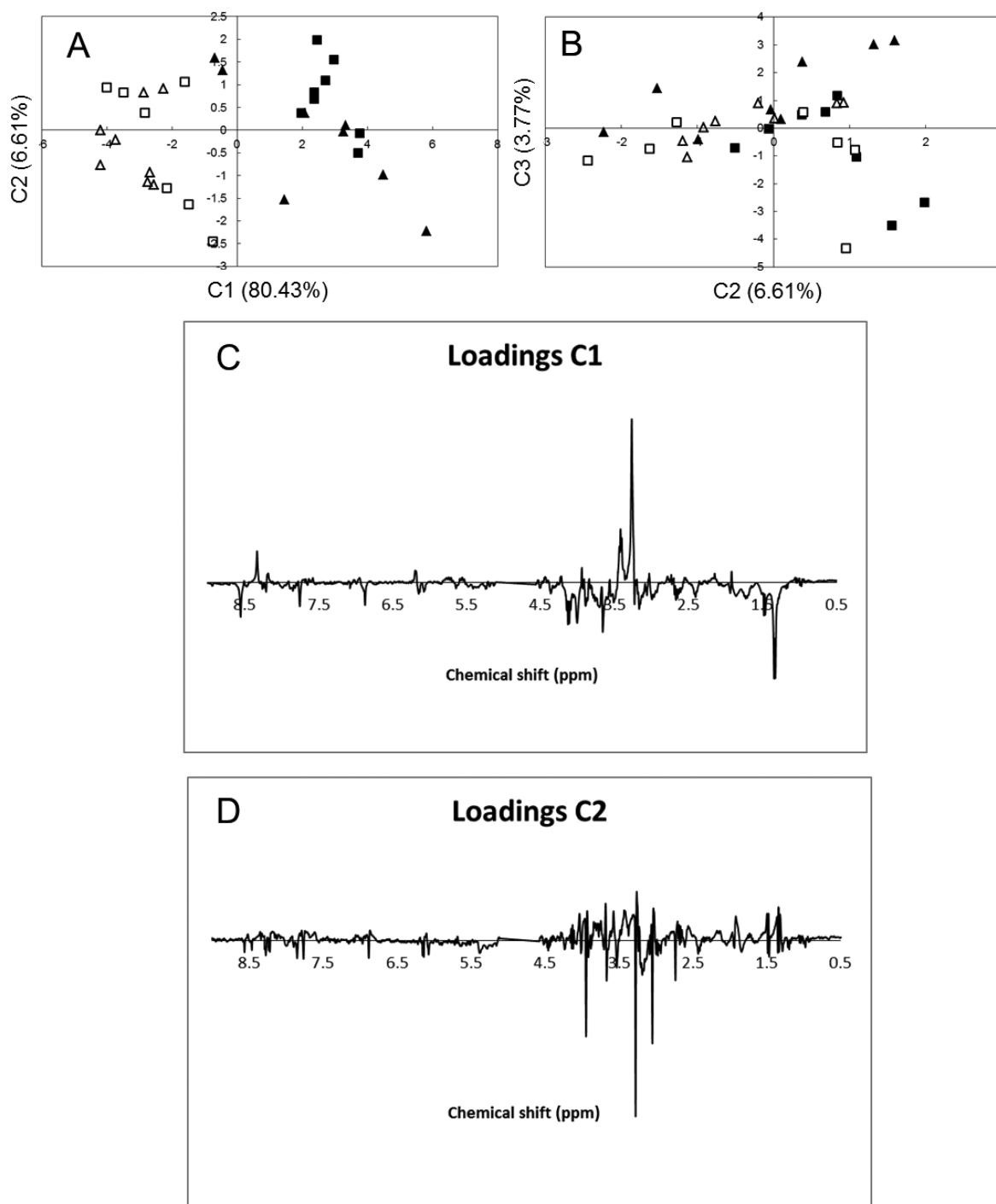


Figure 5. PLS-LDA performed on ^1H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*) using the proximity to the farm as classification criteria: (A, B) scores plots from PLS-LDA ((■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net); (C, D) loadings plots from PLS-LDA. The first component (C1) was described by 80.43%, the second component (C2) by 6.61%, and the third component (C3) by 3.77% of the variations.

most likely because of the fish feed eaten by the wild fish. ^1H NMR proved to be a valuable and cost-effective tool for monitoring aquaculture–wild fish interactions by metabolomic changes. Saithe, in the same way as other species that use fish farms as an artificial trophic niche,²¹ experience metabolic changes, which could have negative or even positive physiological effects. In addition to fish farming influence, the fishing technique also affected the physiology of the fish due to the differential stress caused by the different fishing gears.

A particular effect of fish farming is that the ^1H NMR profiles from liver and muscle tissues of farm fishes showed less dispersion in the score plots compared to the controls. The latter is likely due to the prevalence of salmon feed as trophic resource, which is quite homogeneous with respect to nutritional content compared to natural prey. Saithe aggregated to salmon farms normally obtain a considerable proportion of their food from lost pellets or perhaps also salmon feces. It has been shown that up to 45% of the diet originates from pellets and/or feces.²² Conversely, in a natural sea

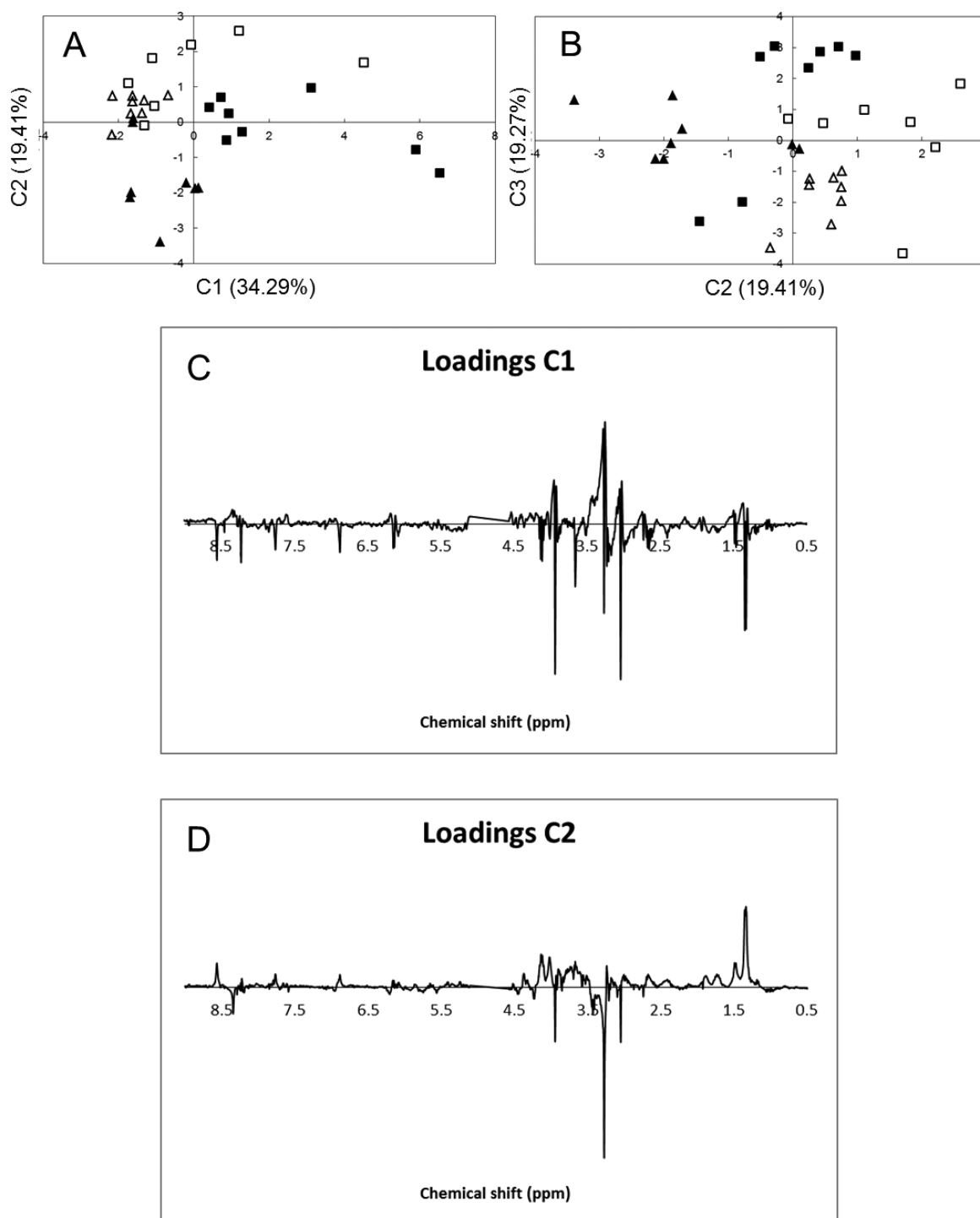


Figure 6. PLS-LDA performed on ^1H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*) using the fishing method as classification criteria: (A, B) scores plots from PLS-LDA ((■) control with angling; (▲) control with gill net; (□) farm with angling; (△) farm with gill net); (C, D) loadings plots from PLS-LDA. The first component (C1) was described by 34.29%, the second component (C2) by 19.41%, and the third component (C3) by 19.27% of the variations.

environment, the diet is expected to be more diverse,²³ which is reflected in a more variable metabolite profile.

It is noticeable that, although total length of control and farm individuals was similar, the fish weight was larger in the latter group, which results in a higher condition index for aggregated fishes. This effect is directly linked to the consumption of high-fat content feed, as has been demonstrated for gadoids associated with salmon farms.¹⁴ This increase in fat content could be driving

the observed intergroup differences in the metabolite profile, especially in liver tissue as most of the lipid metabolism takes place in the liver.²⁴ Saithe is a gadoid, and this family accumulates fat in the liver as an energy reservoir, and high lipid content salmon feed may contribute accumulation of fat in the liver. The liver is the center of the energetic metabolism in the organism, and it controls and buffers the variation in the food intake, affecting metabolite composition.

The higher levels of different metabolites such as lactate and amino acid (glutamine, glutamate, and alanine) found in farm fish, in both liver and muscle tissues, are residues from anaerobic lactic fermentation. This anaerobic lactic fermentation is conditioned by the low oxygen transport to the muscle tissue and can be related to fish mobility and fitness,²⁵ both being supposed to be higher in those fish not associated with farm facilities (i.e., control fish). This is supported by tagging studies, which show that saithe have long residence times around fish farms with repeated movements between nearby facilities.²⁶ Therefore, the farm fish could be having lower oxygen transport to the muscle and, as a consequence, higher lactate and alanine concentrations in its metabolism.²⁵ The lactate produced in the muscle tissue should be translated to the liver (Cori cycle).²⁷ In the liver, the lactate can be transformed into glucose by the gluconeogenesis pathway.²⁷ The Cori cycle could explain the increased lactate level in the liver and muscle tissues in farm fishes. In the same way, in the muscle tissue, different proteins would be degraded to obtain energy. The carbon skeleton of the amino acid would be used in the energy pathways, but the amino group should be translated to the liver as glutamine and alanine.²⁷ The glutamate is the more important intermediate in the deamination process of the amino acid.²⁷ In addition, the glutamine and glutamate are intermediate in the urea cycle in the liver.²⁷ In the liver tissue, the farm fish had a higher level of carnitine. This molecule is the acyl group's carrier to the mitochondrial matrix for the β -oxidation,²⁷ which is the source of energy for the gluconeogenesis.²⁷ The energy necessity was probably higher in the farm fish by the high lactate and alanine levels that should be transformed in glucose.

In muscle tissue, as explained above, the highest levels of lactate and alanine were found in farm fish. However, the control fish had higher levels of choline than the farm fish. Choline has several important metabolic roles. The neurotransmitter acetylcholine is a derivative of the choline, such as the phosphatidylcholine (lecithin). Phosphatidylcholine has structural functions in membranes and in lipid transport. Also, choline is an important methyl donor for methylation reactions.²⁷ Choline can be synthesized in the body from methionine or cysteine.²⁷ The synthesis in the body is not enough to reach the choline necessary for normal fish development, and deficit in choline can be produced by a methionine scarcity in the fish diets.²⁸ The fish feeds can be deficient in choline because the soybean seeds are rich in choline, but this is lost during the processing (i.e., the fat of the oilseed is removed before preparation of the feed, and the choline is also removed with the rest of lipids). For this reason the salmon feed is supplemented with choline chloride,²⁸ but this supplementation could be insufficient for the wild fish population around the farm. In the same way, it is important to consider that the proteins with animal origin are richer in methionine than the protein from plants. Gadoids are carnivorous, with a high trophic level, and eat mainly fish, crustaceans, echinoderms, and polychaetes,^{14,23} and this natural diet should reduce diet deficiency.

The lactic fermentation produces only two ATP molecules per glucose molecule. It is a very inefficient metabolic process, and it produces principally lactic acid, because it is an anaerobic process. The accumulation of lactic acid decreases the pH of the muscle tissue.²⁹ Moreover, lactic acid concentration is related to the glycogen stored in the living muscle, because the glucose of the glycogen is the substrate in the glycolysis. The glycolysis is the first metabolic pathway in the lactic fermentation. The level of the glycogen in the muscle is determined by the nutritional

status of the fish. Probably, farm fish can store more glycogen with a pellet diet, and therefore the lactic acid in the muscle was higher than in the control fish. A subsequent decrease in the pH of the muscle could have modified the physical properties of the tissue, because certain muscle proteins may have lost their water-holding capacity through partial denaturation.³⁰ This fact should have an effect on flesh quality for human consumers because a change in the surface charge of the muscle proteins, due to a presumably lower pH, enhances the water loss, and this feature determines the muscle toughness and a lower quality of the muscle.³⁰

The observed differences in tissue composition due to the fishing gear are in concordance with other studies, which have pointed, ultimately, to changes in the quality of flesh depending on the capture method.¹⁵ In extensive cases, those fishing methods involving exhaustion of fish leading to a slow death (e.g., trawl, trammel, and gill nets) provide lower quality fish when compared to techniques with a quick sacrifice and fish bleeding (e.g., longline, jigging). Quality is intimately related to the metabolism exhibited by a fish when it is captured by a certain fishing method, but other factors such as handling and storage are important driving forces of fish quality.³¹ The fishing gear will influence the levels of precapture stress, and the direct relationship between this stress and lactic acid production in the muscle is known for saithe.²⁹ Another key factor altering the final metabolomic profile and flesh quality could be the bleeding of the fish, because the post-mortem lactic acid accumulation is significantly reduced when the fish is properly bled.³² The excess of fat due to a diet consisting of salmon pellets could also affect flesh quality depending on fish capture technique and handling.³³

Salmon farming aggregates large numbers of gadoids, most likely due to the abundance of lost salmon feed.^{4,14} Therefore, salmon farming seems to influence metabolic profiles of wild fish, but other factors such as capture method should also be considered to explain metabolomics profile changes. Further studies are needed to ascertain physiological and ecological consequences of a pellet diet for wild fish assemblages and the interaction with other factors such as fish migrations, physiological seasonal changes such as reproduction, fishing gear, and fish handling.

In conclusion, salmon farming interacts with wild fish populations in a complex way.² Changes in diet seem to drive changes in metabolic status of important tissues such as liver and muscle in wild fish aggregated at fish farms. These changes could also be affected by fishing techniques. Using a metabolomic approach by ¹H NMR, it is possible to classify the individual depending on farming influence and fishing gear; hence, this technique could be useful for monitoring the influence of fish farming on local fisheries and, also, the metabolomic results could explain potential variations in fillet quality.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b04765.

Figures with the study area around Hitra Island, Norway, and ¹H NMR spectrum of perchloric acid extract from liver and muscle of wild saithe (*Pollachius virens*) (PDF)

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Notes

The authors declare no competing financial interest.

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