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A metabolomic approach to detect effects of salmon farming on wild saithe (*Pollachius virens*) populations

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ABSTRACT

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 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 of the farm locations. The higher levels of lactate and amino acids could be related with the facility to obtain food around the farm and the deficit in choline with the deficit of this nutrient in the salmon feed. At each location the fish were captured with either benthic gillnets and automatic jigging machines, and this feature showed also variations in different metabolites.

1. 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 on those interactions are 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 non-consumed pellets from the fish cage [4]. Previous studies have detected compositional side-effects in the fatty acids profile due to this trophic subsidy[5] 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 analysis[7]). Alternatively, small molecules (i.e. metabolites) identifiable by Nuclear Magnetic Resonance (NMR) can discriminate fish origin in different situations[8], and may be a useful and cost effective tool to trail effects of aquaculture on wild fish. NMR spectroscopy is a multi-component detection technique that offers the opportunity to detect most of the mentioned molecules and study biological tissue[9-11]. Most NMR analysis are based on signals from proton (^{1}H) 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. Based on 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 since the type and amount of metabolites is 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 more than 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, are commonly attracted in large amounts to fish farms due to the abundance of lost 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, 1522].

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 analysing muscle and liver composition of wild saithe using NMR spectroscopy. Fish were captured around fish farms and control areas, using two alternative fishing gear (gillnets and angling), in order to define the suitability of NMR for environmental management of marine aquaculture.

2. MATERIALS AND METHODS

2.1. Fish sample preparation

Saithe were captured between the 19th and 21st of September of 2012 at two salmon farms and at two control locations (> 5 km from the nearest farm) around the island Hitra, Norway (63.603658ºN / 8.645661ºE) (Supporting Information, Figure S1). At each location the fish were captured with either benthic gillnets and automatic jigging machines (n=8). Fish were randomly chosen, but gut content for each individual was analysed and hepatosomatic index calculated for avoiding incorrect treatment assignment. Average length (±standard error; SE) and weight (±SE) of farm-aggregated saithe ("Farm") were 65.9 ±1.6 cm and 3115.8 ±211.1 g respectively; whereas average length (±SE) and weight (±SE) of saithe captured far from salmon farming activity ("Control") was 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 ºC for further analysis. In order to obtain the polar metabolites for ^{1}H NMR experiments, the frozen stored samples were extracted using perchloric acid method[9].

2.2. Chemicals

D_2O (99.9% purity) from Aldrich (Steinheim, Germany); sodium 3-trimethylsilyl-propionate-2,2,3,3,-d4 (TSP, 99% purity) from Aldrich (Steinheim, Germany); perchloric acid 70% (puriss p.a. ACS) from Fluka Chemicals BioChemika (Buchs, Switzerland); and potasium carbonate (puriss p.a. ACS) from Panreac (Spain).

2.3 In vitro ^{1}H NMR spectroscopy

All NMR experiments were performed on a Bruker Avance 400 MHz equipped with a 5 mm ^{1}H-BB-^{13}C TBI probe with an actively shielded Z-gradient. ^{1}D solution state ^{1}H NMR experiments were acquired with a recycle delay of 2 s, 32.768 time domain points and with 2.556 s of acquisition time. The number of scans was 2253. Spectra were apodized by multiplication with an

97 exponential decay producing a 0.3 Hz line broadening in the transformed spectrum. Direct ^{1}H NMR
98 was performed using SPR-W5-WATERGATE[16]. Twelve ppm and -2 ppm and were outside the
99 spectral window. The ^{1}H NMR spectra were reduced to ASCII files using custom-written *ProMetab*
100 software (version 2.1)[17] and peak alignment using *i*coshift (version 1.0; available at
101 www.models.kvl.dk)[18]. All ^{1}H NMR spectra processing have been performed in MATLAB (The
102 MathWorks, Natick, MA) using a AMD Turion X2, 2.20GHz processor with 4GB of RAM. High-
103 resolution MR spectra of perchloric acid extracts from liver and muscle were first examined to
104 provide detailed information about water soluble components[9]. Identification of individual
105 components for muscle and liver was done by comparison to published values of chemical shifts,
106 knowledge of the biochemical composition of fish skeletal muscle and liver and the identification of
107 signals was obtained from 2D NMR spectra[9, 10, 12]. The assignment of the different resonances was
108 listed in Table 1. Hypoxanthine, a molecule which is a good indicator for tissue freshness, was not
109 detected in the ^{1}H NMR spectra.

110 **2.4 Chemometric analysis and experimental design.**

111 For the statistical analysis of spectroscopy data we performed a peak alignment[18]. When the peaks
112 were aligned, robust principal components analysis (robust PCA)[19] and partial least square with
113 linear discriminant analysis (PLS-LDA)[20] were performed. MATLAB version 6.5 from MathWorks
114 was used for the calculations. Robust PCA was carried out using the LIBRA toolbox[19] and PLS-
115 LDA was carried out using the *plslda* toolbox[20]. Two fixed factors were considered for statistical
116 analysis: influence of aquaculture, with two treatments (Farm and Control) and fishing gear, also
117 with two treatments (gillnet and jigging).

118 In a supervised method, such as PLS-LDA, the most common approach is to select a number of the
119 data for to make a mathematical model. This model can be used for the prediction of new
120 independent samples. The independent samples used for to validate the model are samples excluded
121 in the construction of the mathematical model. With our ^{1}H 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 so many models as samples, but in every model was excluded one sample. This approach had two advantages: we can detect quickly samples wrong classified and all models are very similar. We our data, 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 (gillnet and jigging)).

3. RESULTS

The ^{1}H 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 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.

In order to analyze the ^{1}H NMR metabolic profile spectra, we used an unsupervised chemometric method such as robust PCA[19]. The scores plots from liver tissues samples displayed a good separation between the Farm and Control fishes (Figures 1.A and 1.B). The separation between the samples was determined by the loadings from PC2 (Figure 1.D). The loadings were not real data, but they can be interpreted as such in order to evaluate the importance of the different metabolites in the distribution of the samples in the scores plots. The loadings from PC2 (Figure 1.D) 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 (gillnet or jigging) (Figure 1.C). The loadings from PC1 indicated that the fish captured with gillnet had higher concentrations of alanine and lactate, and lower concentrations of glucose, glycerol, carnitine and choline (Figure 1.C).With muscle tissue, the situation was very similar when the ^{1}H NMR spectra were analysed 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 concentration of

lactate and alanine, and lower concentration of choline than the Control fishes (Figure 2.C). The influence of the fishing method on the metabolomic profile was less clear in the ^{1}H 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-LDA[20] showed that ^{1}H NMR data was able to discriminate powerfully between Farm and Control fishes (Figure 3), using the approach described in Materials and Methods section. The liver tissue from Farm fish had a higher concentration of lactate, amino acids (alanine, glutamine and glutamate) and carnitine (loadings from C1) and lower concentration of taurine than the liver tissues from Control fishes. When the capture fish method was considered as the metabolomic variable (gillnet or jigging), the fish captured with gillnet had higher concentration of lactate and alanine, and lower concentration of glucose and glycerol than the fishes captured with jigging (Figure 4).

If the ^{1}H NMR spectra from muscle tissue were analysed 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) concentration 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 concentration in choline and taurine than Farm fishes. With PLS-LDA analysis of ^{1}H NMR spectra, there was a very good classification of the muscle tissues samples when the fishing method was considered as metabolomic variable (Figure 6).

4. DISCUSSION

Salmon farming affected metabolic composition of main tissues, such as muscle and liver, of wild fish aggregated to fish farms, most likely because of the fish feed eaten by the wild fish. ^{1}H 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. Additionally 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 ^{1}H 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 faeces. It has been shown that up to 45% of the diet originates from pellets and/or faeces[22]. Conversely, in a natural sea 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 consume of high fat content feed, as it has been demonstrate for gadoids associated with salmon farms[14]. This increase in fat content could be driving the observed intergroup differences in the metabolites profile, especially in liver tissue as most of the lipid metabolism takes place in the liver[24]. Saithe is a gadoid, and this family accumulate fat in the liver as energy reservoir, and high lipid content salmon feed may contribute accumulation of fat in the liver. The liver is the centre 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, both in 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 to 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 in order 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 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 was found in Farm fish. However, the Control fish had higher levels of choline than the Farm fish. Choline has several important metabolic roles. The neurotransmiter acetylcholine is a derivate to the choline, such as the phosphatidylcholine (lecithin). Phosphatidylcholine has structural functions in membranes and in the 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 necessity for the 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 are eating mainly fish, crustaceans, echinoderms, and polychaetes[14, 23], and this natural diet should reduce diet deficient.

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, since 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 that the Control fish. A subsequent decrease in the pH of the muscle could have modified the physical properties of the tissue, since certain muscle proteins may have lost their water-holding capacity by a 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 presumable 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 because a slow death (e.g. trawl, trammel and gill nets) provide lower quality fish when compared to

255 techniques with a quick sacrifice and fish bleeding (e.g. longline, jigging). Quality is intimately
256 related to the metabolism exhibited by fish when it is captured by a certain fishing method, but
257 other factors as handling and storage are important driving forces of fish quality[31]. The fishing gear
258 will influence the levels of pre-capture stress, and the direct relation between this stress and the
259 lactic acid production in the muscle is known for saithe[29]. Other key factor altering final
260 metabolomic profile and flesh quality could be the bleeding of the fish, because the post-mortem
261 lactic acid accumulation is significantly reduced when the fish is properly bled[32]. The excess of fat
262 due to a diet consisting of salmon pellets could also affect flesh quality depending on fish capture
263 technique and handling[33].

264 Salmon farming aggregate large numbers of gadoids most likely due to the abundance of lost
265 salmon feed[4, 14]. Therefore, salmon farming seem to influence metabolic profiles of wild fish but
266 other factors as capture method should also be considered when explaining metabolomics profile
267 changes. Further studies are needed to ascertain physiological and ecological consequences of a
268 pellet diet for wild fish assemblages and the interaction with other factors such as fish migrations,
269 physiological seasonal changes as reproduction, fishing gear and fish handling.

270 **5. CONCLUSIONS**

271 Salmon farming interact with wild fish populations in a complex way[2]. Changes in diet seem to
272 drive changes in metabolic status of important tissues such as liver and muscle in wild fish
273 aggregated at fish farms. These changes could also be affected by fishing techniques. Using a
274 metabolomic approach by ^{1}H NMR, it is possible to classify the individual depending on farming
275 influence and fishing gear, hence this technique could be useful for monitoring influence of fish
276 farming on local fisheries and also, the metabolomic results could explain potential variations in the
277 fillet quality.

278

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Supporting Information. Figures with the study area around Hitra Island, Norway, 1H NMR spectrum of perchloric acid extract from liver and muscle of wild saithe (*Pollachius virens*). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Mikkelsen, O.; van den Berg, C.; Schroder, K., Determination of labile iron at low nmol L-1 levels in estuarine and coastal waters by anodic stripping voltammetry. *Electroanalysis* **2006,** *18* (1), 35-43.

2. Uglem, I.; Karlsen, O.; Sanchez-Jerez, P.; Saether, B., Impacts of wild fishes attracted to open-cage salmonid farms in Norway. *Aquaculture Environment Interactions* **2014,** *6* (1), 91-103.

3. Cataudella, S.; Massa, F.; Crosetti, D., *Interaction Between Aquaculture and Capture Fisheries: a methodological perspective*. FAO: Rome, 2005; Vol. N. 78, p 229.

4. Dempster, T.; Uglem, I.; Sanchez-Jerez, P.; Fernandez-Jover, D.; Bayle-Sempere, J.; Nilsen, R.; Bjorn, P., Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. *Marine Ecology Progress Series* **2009,** *385*, 1-14.

5. Fernandez-Jover, D.; Martinez-Rubio, L.; Sanchez-Jerez, P.; Bayle-Sempere, J.; Jimenez, J.; Lopez, F.; Bjorn, P.; Uglem, I.; Dempster, T., Waste feed from coastal fish farms: A trophic subsidy with compositional side-effects for wild gadoids. *Estuarine Coastal and Shelf Science* **2011,** *91* (4), 559-568.

6. Ottera, H.; Karlsen, O.; Slinde, E.; Olsen, R., Quality of wild-captured saithe (Pollachius virens L.) fed formulated diets for 8 months. *Aquaculture Research* **2009,** *40* (11), 1310-1319.

7. Arechavala-Lopez, P.; Fernandez-Jover, D.; Black, K.; Ladoukakis, E.; Bayle-Sempere, J.; Sanchez-Jerez, P.; Dempster, T., Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and sea bass. *Reviews in Aquaculture* **2013,** *5* (3), 137-157.

8. Arechavala-Lopez, P.; Uglem, I.; Fernandez-Jover, D.; Bayle-Sempere, J.; Sanchez-Jerez, P., Immediate post-escape behaviour of farmed seabass (Dicentrarchus labrax L.) in the Mediterranean Sea. *Journal of Applied Ichthyology* **2011,** *27* (6), 1375-1378.

9. Gribbestad, I.; Aursand, M.; Martinez, I., High-resolution 1H magnetic resonance

spectroscopy of whole fish, fillets and extracts of farmed Atlantic salmon (Salmo salar) for quality assessment and compositional analyses. *Aquaculture* **2005,** *250* (1-2), 445-457.

10. Martinez, I.; Bathen, T.; Standal, I.; Halvorsen, J.; Aursand, M.; Gribbestad, I.; Axelson, D., Bioactive compounds in cod (Gadus morhua) products and suitability of H-1 NMR metabolite profiling for classification of the products using multivariate data analyses. *Journal of Agricultural and Food Chemistry* **2005,** *53* (17), 6889-6895.

11. Viant, M.; Bundy, J.; Pincetich, C.; de Ropp, J.; Tjeerdema, R., NMR-derived developmental metabolic trajectories: an approach for visualizing the toxic actions of trichloroethylene during embryogenesis. *Metabolomics* **2005,** *1* (2), 149-158.

12. Wagner, L.; Trattner, S.; Pickova, J.; Gomez-Requeni, P.; Moazzami, A., H-1 NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (Salmo salar). *Food Chemistry* **2014,** *147*, 98-105.

13. Norwegian Directorate of Fisheries. 2015.

14. Dempster, T.; Sanchez-Jerez, P.; Fernandez-Jover, D.; Bayle-Sempere, J.; Nilsen, R.; Bjorn, P.; Uglem, I., Proxy Measures of Fitness Suggest Coastal Fish Farms Can Act as Population Sources and Not Ecological Traps for Wild Gadoid Fish. *Plos One* **2011,** *6* (1).

15. Rotabakk, B.; Skipnes, D.; Akse, L.; Birkeland, S., Quality assessment of Atlantic cod (Gadus morhua) caught by longlining and trawling at the same time and location. *Fisheries Research* **2011,** *112* (1-2), 44-51.

16. Lam, B.; Simpson, A., Direct H-1 NMR spectroscopy of dissolved organic matter in natural waters. *Analyst* **2008,** *133* (2), 263-269.

17. Viant, M., Improved methods for the acquisition and interpretation of NMR metabolomic data. *Biochemical and Biophysical Research Communications* **2003,** *310* (3), 943-948.

18. Savorani, F.; Tomasi, G.; Engelsen, S., icoshift: A versatile tool for the rapid alignment of 1D

NMR spectra. *Journal of Magnetic Resonance* **2010,** *202* (2), 190-202.

19. Verboven, S.; Hubert, M., LIBRA: a MATLAB library for robust analysis. *Chemometrics and Intelligent Laboratory Systems* **2005,** *75* (2), 127-136.

20. Li, H.; Liang, Y.; Xu, Q.; Cao, D., Key wavelengths screening using competitive adaptive reweighted sampling method for multivariate calibration. *Anal Chim Acta* **2009,** *648* (1), 77-84.

21. Sanchez-Jerez, P.; Fernandez-Jover, D.; Bayle-Sempere, J.; Valle, C.; Dempster, T.; Tuya, F.; Juanes, F., Interactions between bluefish Pomatomus saltatrix (L.) and coastal sea-cage farms in the Mediterranean Sea. *Aquaculture* **2008,** *282* (1-4), 61-67.

22. Skog, T.; Hylland, K.; Torstensen, B.; Berntssen, M., Salmon farming affects the fatty acid composition and taste of wild saithe Pollachius virens L. *Aquaculture Research* **2003,** *34* (12), 999-1007.

23. Homrum, E.; Hansen, B.; Steingrund, P.; Hatun, H., Growth, maturation, diet and distribution of saithe (Pollachius virens) in Faroese waters (NE Atlantic). *Marine Biology Research* **2012,** *8* (3), 246-254.

24. Tocher, D.; Castell, J.; Dick, J.; Sargent, J., Effects of Salinity on the Fatty-Acid Compositions of Total Lipid and Individual Glycerophospholipid Classes of Atlantic Salmon (Salmo-Salar) and Turbot (Scophthalmus-Maximus) Cells In Culture. *Fish Physiology and Biochemistry* **1995,** *14* (2), 125-137.

25. Moyes, C. D.; West, T. G., Exercise metabolism of fish. In *Biochemistry and Molecular Biology of Fishes*, W., H. P.; P., M. T., Eds. Elsevier Science Amsterdam, 1995; Vol. 4, pp 367–392.

26. Uglem, I.; Dempster, T.; Bjorn, P.; Sanchez-Jerez, P.; Okland, F., High connectivity of salmon farms revealed by aggregation, residence and repeated movements of wild fish among farms. *Marine Ecology Progress Series* **2009,** *384*, 251-260.

27. Nelson, D.; Michael, C., *Lehninger Principles of Biochemitry*. 2014; p 1198.

373 28. Lovell, T., *Nutrition and feeding of fish*. 2. ed.; Kluwer Academic Publishers: Boston, Mass.,
374 1998; p ix, 267 s.

375 29. Roth, B.; Rotabakk, B., Stress associated with commercial longlining and recreational
376 fishing of saithe (Pollachius virens) and the subsequent effect on blood gases and chemistry.
377 *Fisheries Research* **2012,** *115*, 110-114.

378 30. Love, R., Variability in Atlantic Cod (Gadus-Morhua) From Northeast Atlantic - Review Of
379 Seasonal and Environmental Influences on Various Attributes of Flesh. *Journal of the Fisheries*
380 *Research Board of Canada* **1975,** *32* (12), 2333-2342.

381 31. Esaiassen, M.; Nilsen, H.; Joensen, S.; Skjerdal, T.; Carlehog, M.; Eilertsen, G.; Gundersen,
382 B.; Elvevoll, E., Effects of catching methods on quality changes during storage of cod (Gadus
383 morhua). *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology* **2004,** *37* (6),
384 643-648.

385 32. Chiba, A.; Hamaguchi, M.; Kosaka, M.; Tokuno, T.; Asai, T.; Chichibu, S., Quality
386 Evaluation of Fish Meat by Phosphorus-31-Nuclear Magnetic-Resonance. *Journal of Food Science*
387 **1991,** *56* (3), 660-664.

388 33. Regost, C.; Arzel, J.; Cardinal, M.; Laroche, M.; Kaushik, S., Fat deposition and flesh
389 quality in seawater reared, triploid brown trout (Salmo trutta) as affected by dietary fat levels and
390 starvation. *Aquaculture* **2001,** *193* (3-4), 325-345.

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394 **Table 1**. Resonance assignments with ^{1}H chemical shifts of metabolites identified in NMR spectra of

395 perchloric acid extract from tissues of wild saithe (*Pollachius virens*).

Compound	Proton	Multiplicity	δ ^{1}H
Leucine/Iseleucine	$-CH_3$	d	0.97
Valine	$-CH_3$	d	1.19
Lactate	$-CH_3$	d	1.34
Alanine	$-CH_3$	d	1.49
Lysine	$-CH_2$	m	1.73
Acetate	$-CH_3$	s	1.92
Glutamine/glutamate	$-CH_3$	m	2.14
Glutamate	$-CH_2$	m	2.35
Glutamine	$-CH_2$	m	2.43
Anserine	$-CH_3$		2.73
Creatine	$-NCH_3$	s	3.04
Choline	$-NCH_3$	s	3.13
Phosphocholine	$-N(CH_3)_3$	s	3.21
β-Glucose	-C2H, ring	dd	3.22
Carnitine	$-N(CH_3)_3$	s	3.26
Taurine	$-S-CH_2$	t	3.42
β-Glucose	-C5H, ring	ddd	3.47
β-Glucose	-C3H, ring	t	3.49
Choline	βH	m	3.51
Glycine	αH	s	3.58
Glycerol	1,3Hβ	dd	3.64
Glycerolphosphocholine	βH	dd	3.68
Anserine	$-NCH_3$		3.69
α-Glucose	-C3H, ring	t	3.70
β-Glucose	-C6H, ring	dd	3.70
Aspartic Acid	αH	dd	3.78
α-Glucose	-C5H, ring	m	3.84
β-Glucose	-C6H, ring	dd	3.89
Creatine	$-CH_2$	s	3.93
Lactate	-CH	q	4.11
Adenosine	H1		6.09
Histidine (in anserine)	-C4H, ring	S	6.88
Tyrosine	-C3,5H ring	m	6.91
Tyrosine	-C2,6H ring	m	7.19
Histidine (in anserine)	-C2H, ring	s	8.23
Formate	-CH	s	8.52

396

Figure Legends

Figure 1. Robust PCA analysis performed on ^{1}H NMR spectra from liver tissues of wild saithe (*Pollachius virens)*. A) and B). Scores plots from PC. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and 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 .

Figure 2. Robust PCA analysis performed on ^{1}H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*). A and B) Scores plots from PC. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and 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.

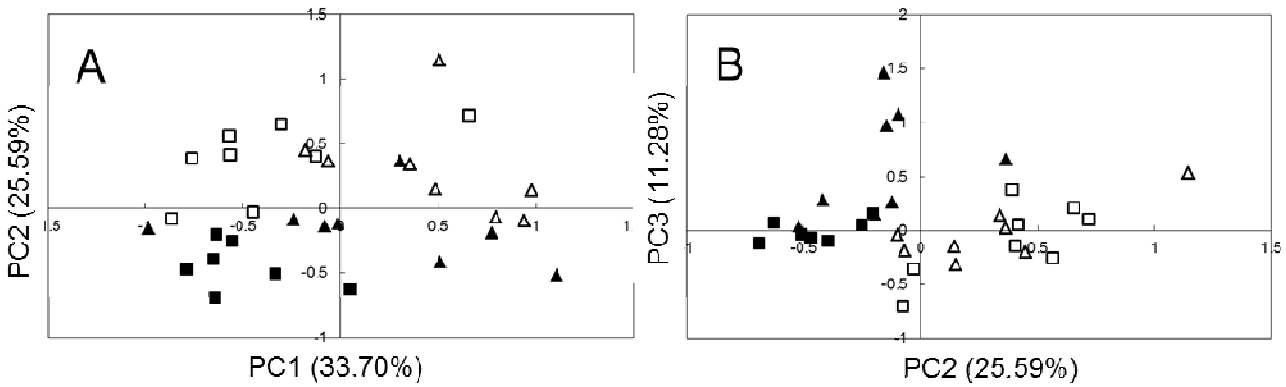
Figure 3. PLS-LDA performed on ^{1}H NMR spectra from liver tissues of wild saithe (*Pollachius virens*) using the proximity to the farm as classification criteria. A and B) Scores plots from PLS-LDA. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and 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.

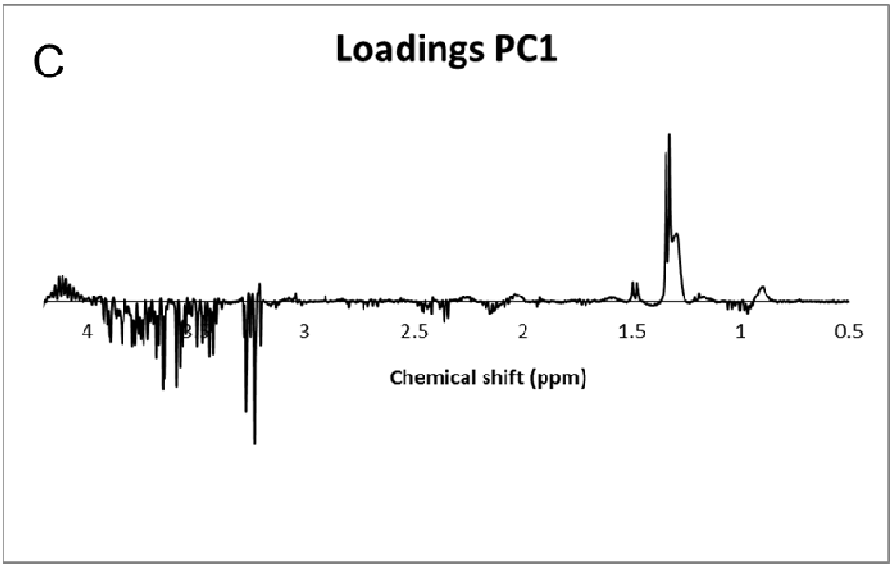
Figure 4. PLS-LDA performed on ^{1}H NMR spectra from liver tissues of wild saithe (*Pollachius virens*) using the fishing method as classification criteria. A and B) Scores plots from PLS-LDA. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and D) 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.

Figure 5. PLS-LDA performed on ^{1}H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*) using the proximity to the farm as classification criteria. A and B) Scores plots from PLS-

LDA. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and 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.

Figure 6. PLS-LDA performed on ^{1}H NMR spectra from muscle tissues of wild saithe (*Pollachius virens*) using the fishing method as classification criteria. A and B) Scores plots from PLS-LDA. (■) Control with angling; (▲) Control with gillnet, (□) Farm with angling; (Δ) Farm with gillnet. C and 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.


A
PC2 (25.59%)
PC1 (33.70%)
B
PC3 (11.28%)
PC2 (25.59%)


C
Loadings PC1
Chemical shift (ppm)

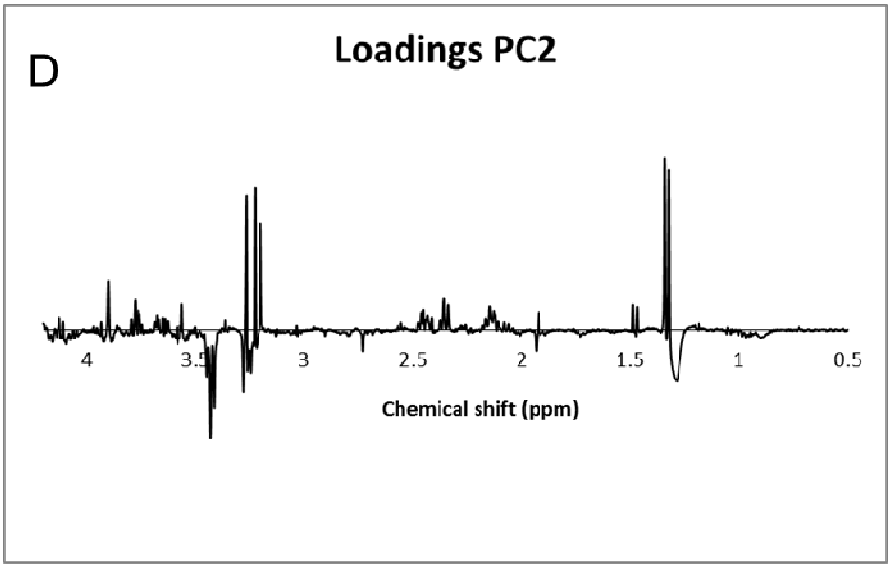

D
Loadings PC2
Chemical shift (ppm)

Figure 1.

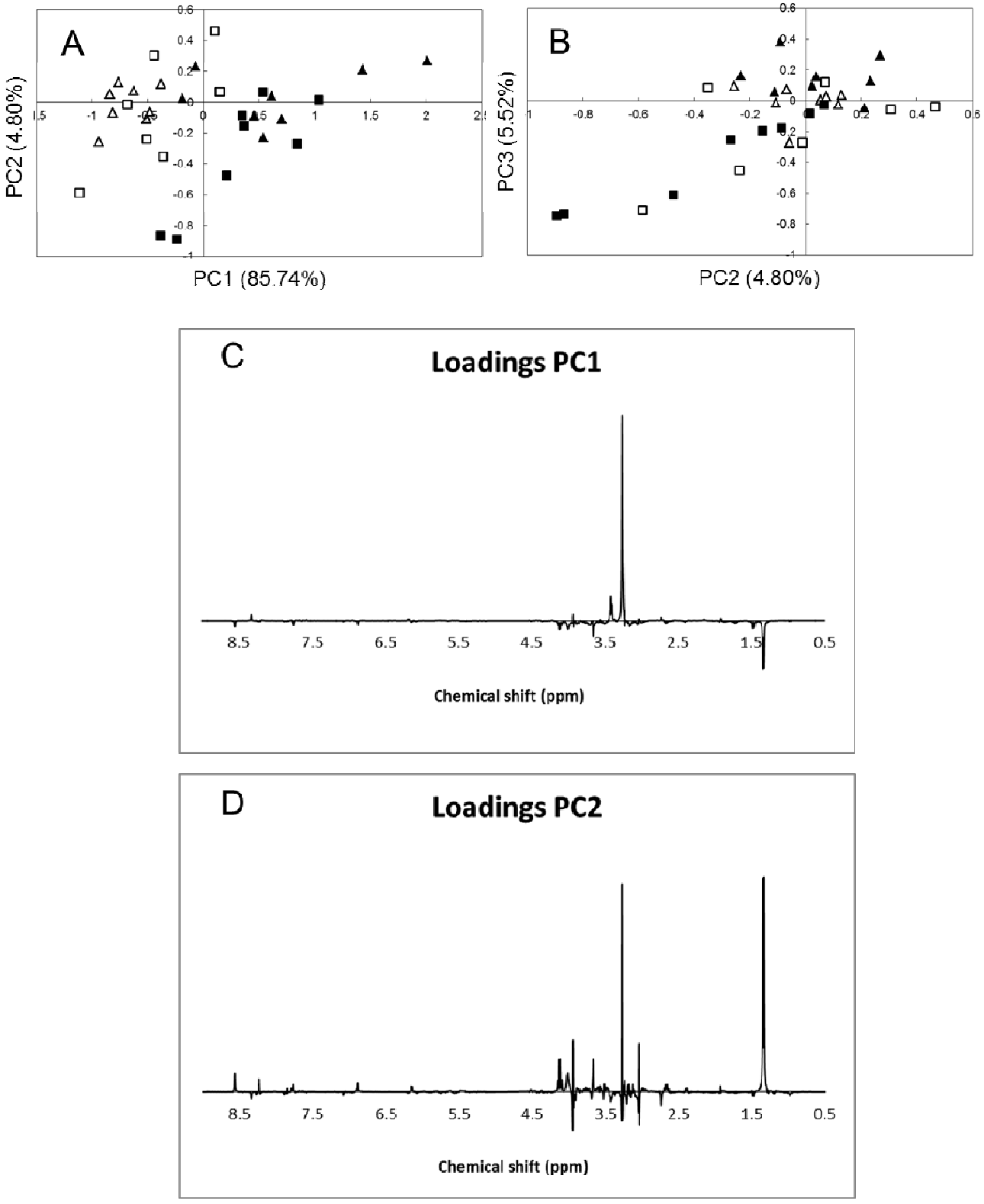

A
PC2 (4.80%)
PC1 (85.74%)
B
PC3 (5.52%)
PC2 (4.80%)
C
Loadings PC1
8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
Chemical shift (ppm)
D
Loadings PC2
8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
Chemical shift (ppm)

Figure 2.

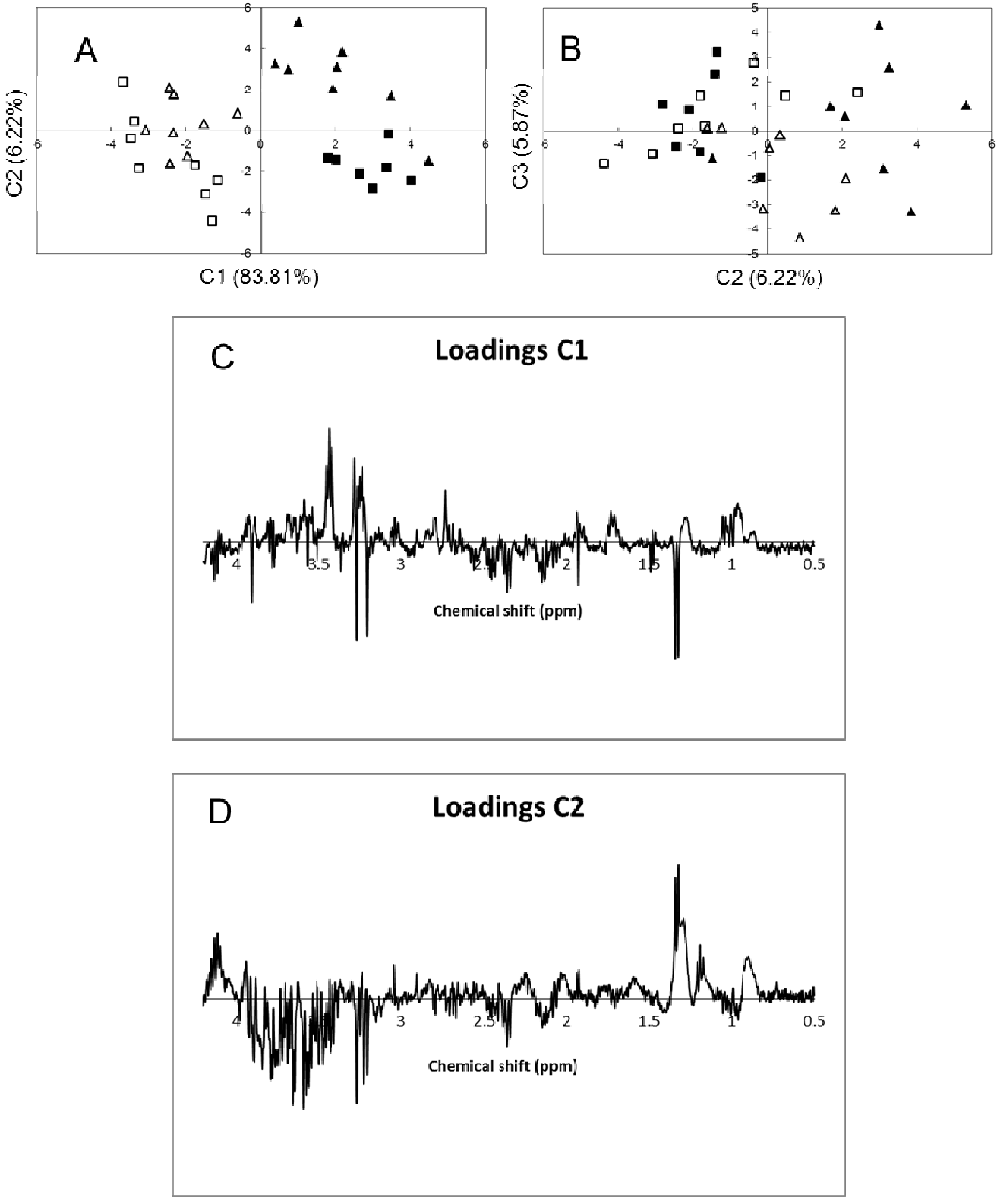

A
C2 (6.22%)
C1 (83.81%)
B
C3 (5.87%)
C2 (6.22%)
C
Loadings C1
Chemical shift (ppm)
D
Loadings C2
Chemical shift (ppm)

Figure 3.

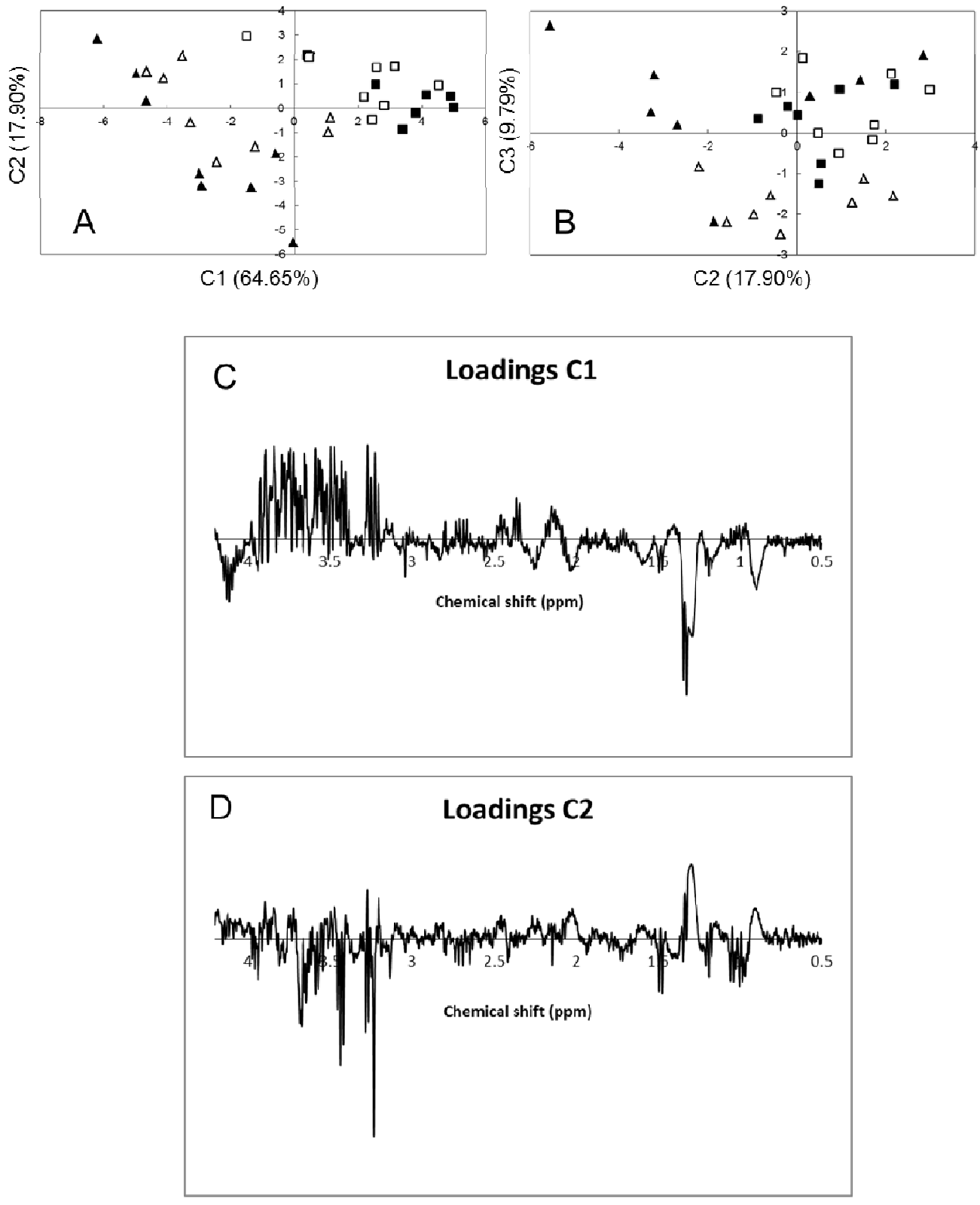

A
C2 (17.90%)
C1 (64.65%)
B
C3 (9.79%)
C2 (17.90%)
C
Loadings C1
Chemical shift (ppm)
D
Loadings C2
Chemical shift (ppm)

Figure 4.

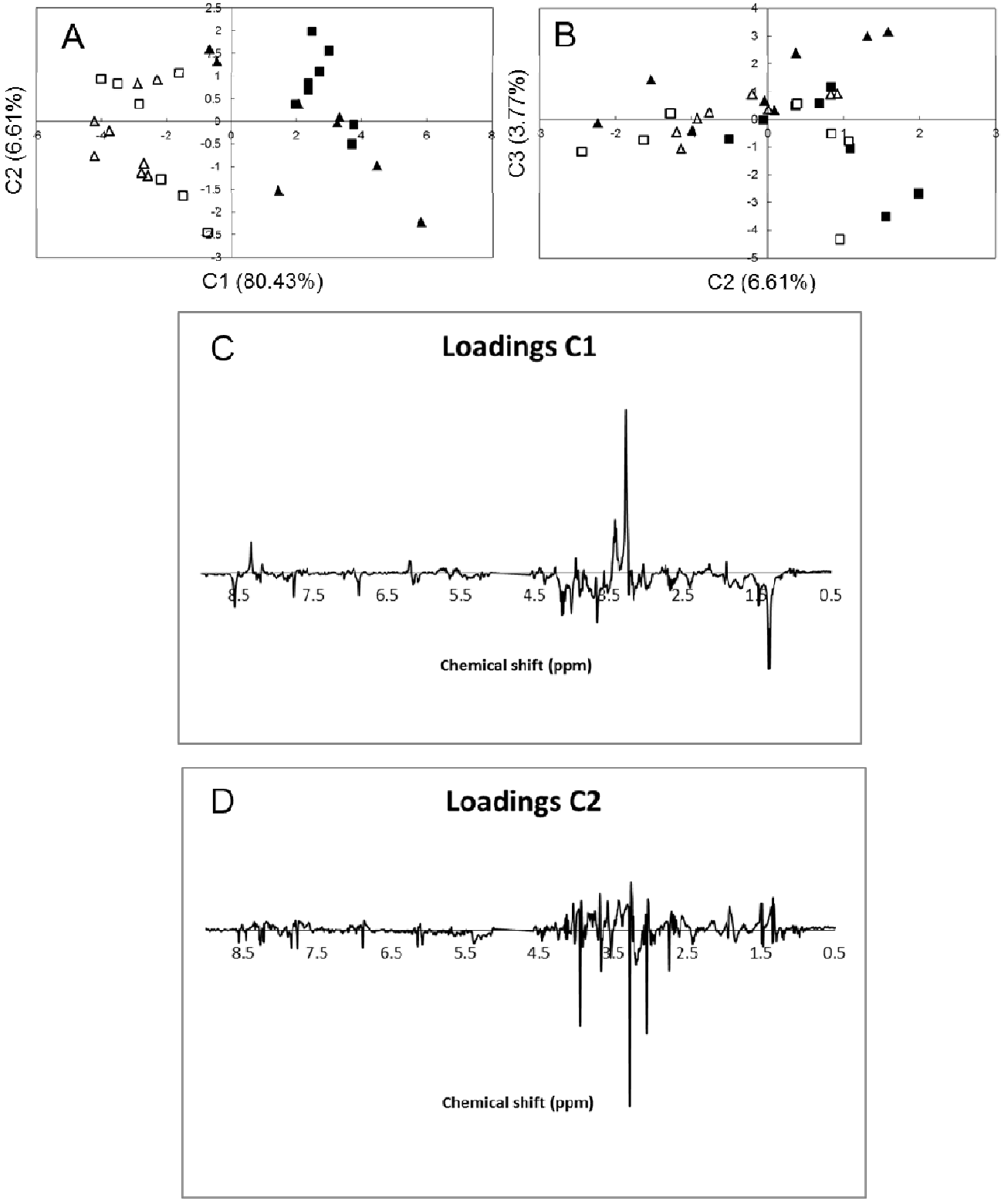

A
C2 (6.61%)
C1 (80.43%)
B
C3 (3.77%)
C2 (6.61%)
C
Loadings C1
Chemical shift (ppm)
D
Loadings C2
Chemical shift (ppm)

Figure 5.

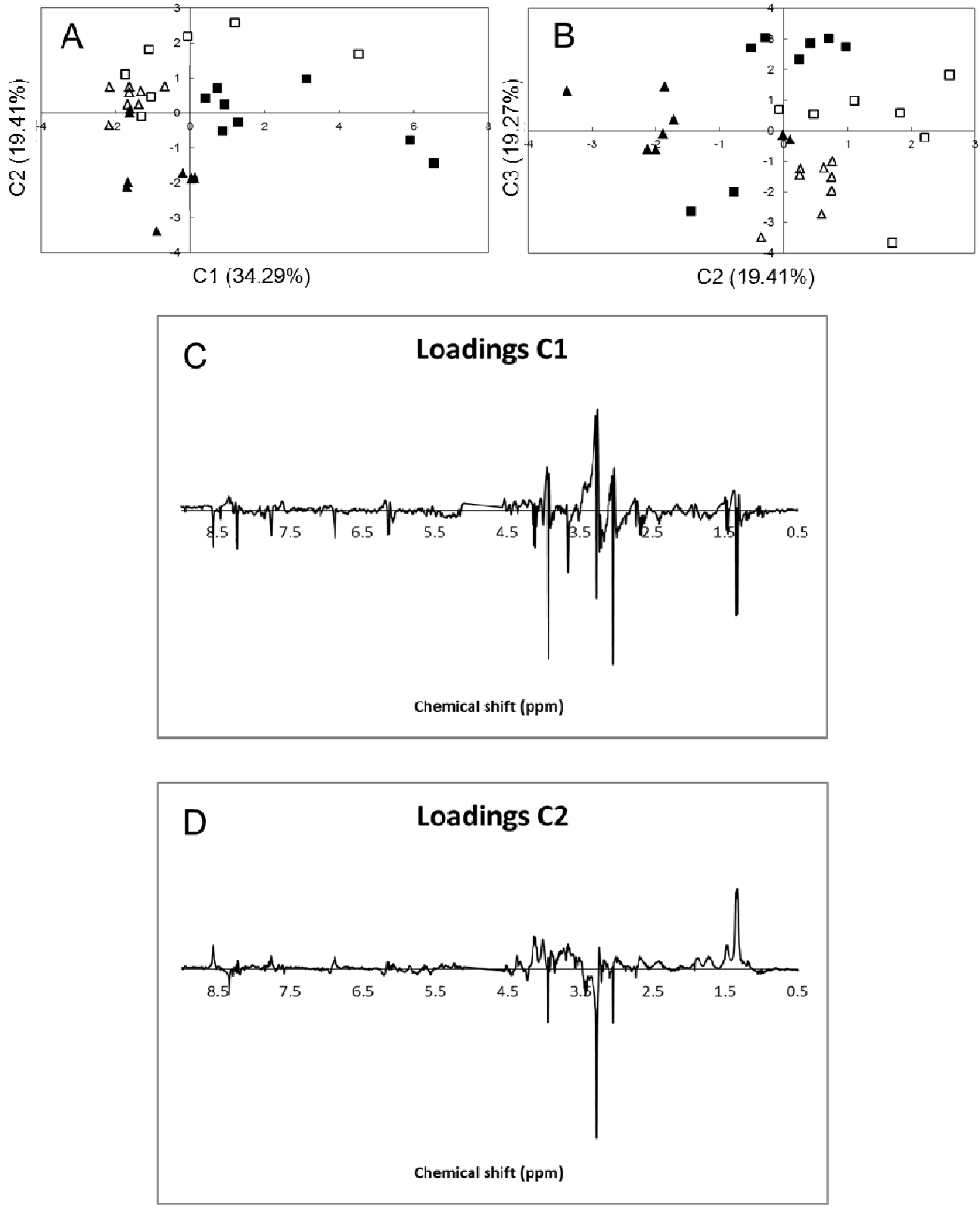
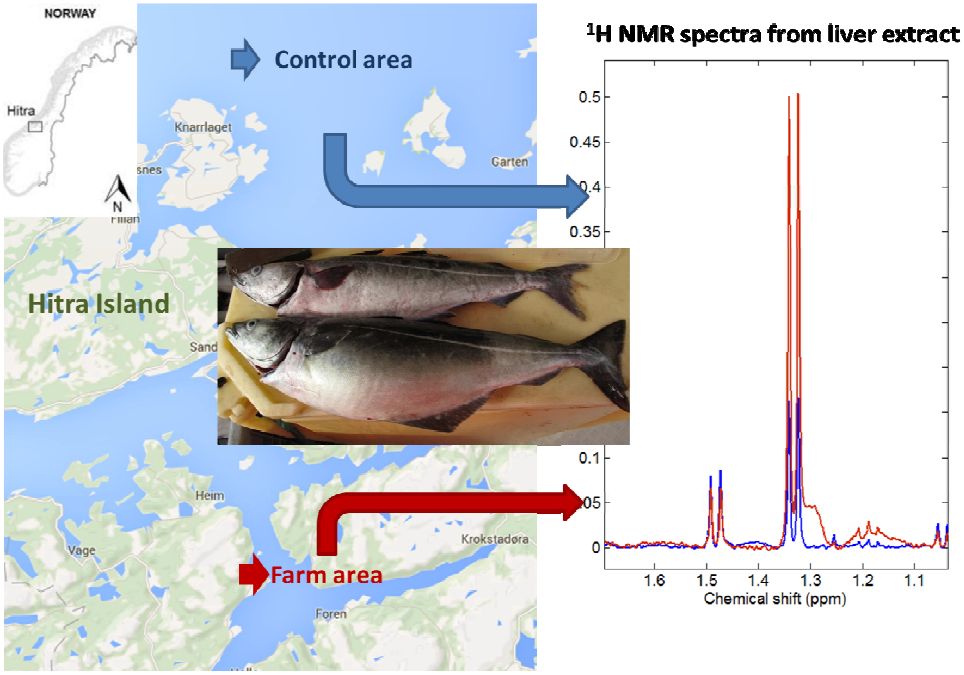

A
C2 (19.41%)
C1 (34.29%)
B
C3 (19.27%)
C2 (19.41%)
C
Loadings C1
8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
Chemical shift (ppm)
D
Loadings C2
8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
Chemical shift (ppm)

Figure 6.


NORWAY
Hitra
Control area
Knarrlaget
Garten
N
Hitra Island
Sand
Heim
Vage
Krokstadøra
Farm area
Foren
^{1}H NMR spectra from liver extract
0.5
0.45
0.4
0.35
0.1
0
1.6
1.5
1.4
1.3
1.2
1.1
Chemical shift (ppm)

TOC Graphic.