



## Article

# Comparative Study of Fatty Acid Composition of Muscles of Atlantic Cod (*Gadus morhua* Linnaeus, 1758) with Natural Diet and Feeding near Salmon Farms

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**Abstract:** Coastal aquaculture and local fisheries interact in shared marine environments, influencing each other synergistically and/or antagonistically. Salmon farming, notably with open-net sea cages along the Norwegian coast, attracts wild fish due to increased food availability from uneaten feed, but it also exposes wild fish to farm emissions like waste and toxic chemicals (de-lice treatments, antifouling and medical agents). The attraction behaviour of wild fish can impact fatty acid composition in fish tissues, influenced by the high terrestrial fat content in salmon aquafeed. We study how the Atlantic cod, aggregating around salmon farms in a subarctic fjord in Northern Norway, can be affected, potentially altering their natural diet and fatty acid profiles. Our study compares the muscle-tissue fatty acid compositions of cod caught near aquaculture facilities (impact) versus fish caught in neighbouring fjords (control), and we hypothesise decreased omega-3 fatty acids near farms. The analysis revealed no significant differences in the fatty acid concentrations or categories between the impacted and control fish, challenging our initial expectations. However, differences were found for C18:1(n9)t (elaidic acid), with a higher value in the impacted fish. These findings suggest that salmon farming's influence on cod's fatty acid profiles in the flesh (i.e., relevant for the nutritional quality of the fillets that consumers eat) may be limited or minimal despite their aggregative behaviours around farms. The threshold levels of salmon feed consumed by wild cod before it affects the quality and survival of, e.g., sperm or other life stages, are not known and require new investigations. This study underscores the complexity of interactions between aquaculture and wild fisheries, impacting both ecological dynamics and consumer perspectives on seafood quality and health benefits.

**Keywords:** aquaculture and wild fish interactions; spillover feed; salmon farming; fatty acids; cod population; environmental impact



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## 1. Introduction

Coastal aquaculture and local fisheries often coexist in shared marine environments, potentially leading to both synergistic and antagonistic interactions. Understanding these interactions is crucial for sustainable management and policy-making to ensure the coexistence of both sectors [1]. For example, Atlantic salmon (*Salmo salar*) farming along the Norwegian coast, using open-net sea cages, produces significant attraction to and aggregation of wild fish communities around farm cages [2,3]. This could be due to salmon farms

creating an artificial environment with an increased availability of food resources resulting from uneaten feed and waste generated by the farmed salmon. Fish farms essentially function as large Fish Aggregating Devices (FADs), offering structure within the pelagic environment but with greater food availability compared to conventional FADs. The uneaten portion of food pellets that escape through the cages likely increases their attractiveness. As a result, fish farms can influence the presence, abundance, residency and diets of fish in a specific area, potentially having significant impacts on local fisheries. Many fish species aggregate around the farms, taking advantage of the concentrated trophic resources. This change in feeding behaviour can affect the fatty acid composition of several tissues due to the composition of salmon aquafeed, which has a high proportion of terrestrial fats, as has been demonstrated in several studies for various species [4].

One of the most relevant species that aggregates around salmon farms is the Atlantic cod (*Gadus morhua*), with potential effects due to the large amount, i.e., 60,000–100,000 tonnes yearly, of waste feed that causes cod to gather around farms, changing their natural diet [5]. Cod is extensively consumed, with a large market share across Europe [6] and in local markets. Hence, people could be consuming cod that has been fished around salmon farms. The modification of fatty acid composition in fish affected by salmon farming has been detected in several tissues, such as the liver, gonads and muscle [7]. Changes in fatty acids in flesh/fillet could be relevant from the consumer's point of view because the intake of essential fatty acids could be reduced or altered. The recommendation for fish consumption, particularly those rich in omega-3 fatty acids (often referred to as  $\omega$ 3), is based on the numerous health benefits associated with these essential fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These omega-3 fatty acids have been linked to positive cardiovascular health, helping to lower blood pressure, reduce triglyceride levels and decrease the risk of heart disease, as well as affecting brain function, anti-inflammatory processes, vision and pregnancy, among other benefits [8].

Due to the relevance of these interactions between salmon aquaculture and wild cod consumption, a study was proposed with the objective of comparing the fatty acid composition of the muscle tissue of impacted cod, i.e., fish aggregated around aquaculture facilities, with that of control cod caught in neighbouring fjords without salmon farms. We hypothesised that the concentration of  $\omega$ 3 fatty acids should decrease in individuals affected by salmon farms.

## 2. Materials and Methods

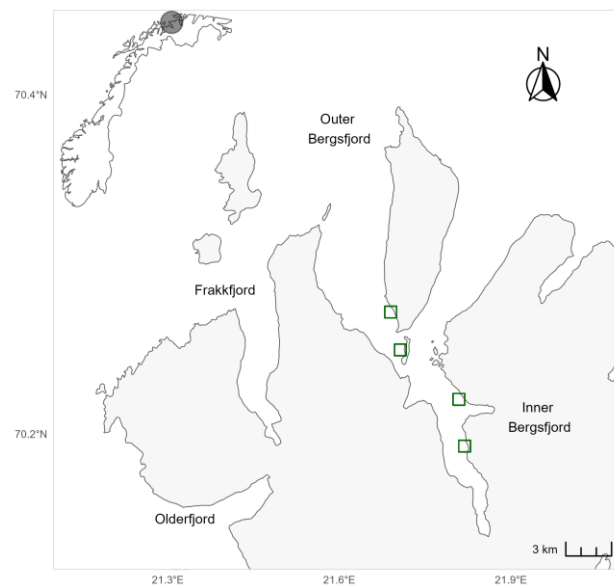
### 2.1. Study Area and Survey Design

This study involved collecting cod individuals at four locations: Inner Bergsfjord, the impacted fjord, where the four salmon farms are located, and Outer Bergsfjord, Frakkfjord and Olderfjord, which were considered control fjords (Figure 1). Fish that were considered impacted were sampled within less than 5 km from a salmon farm within Bergsfjord, while the control fish were sampled at larger distances. The sample period was between October 2019 and October 2020.

### 2.2. Sampling

Fish were captured using a jig with a single hook around the farm and, at some distance, gill nets and fish pots were introduced to improve capture efficiency. The muscle samples obtained for each location were as follows: Inner Bergsfjord = 53, Outer Bergsfjord = 77, Frakkfjord = 68 and Olderfjord = 59. Each fish was dissected, and samples of the homogenised flesh (approximately 5 g each) were obtained from individual fish from frontal and dorsal muscle, packed in aluminium foil, and frozen at  $-20^{\circ}\text{C}$  for later analysis. Due to the low fat concentration in the cod flesh, we pooled tissue samples from several individual fish (within the fjord) before analysing the fatty acids. The samples were grouped based on sex and maturity level. Before analysing the data based on control versus impact treatment, the data were examined, and no significant differences were found between the maturity levels or sexes. In

total, there were 4 groups per sampling location (mature–immature/male–female) with between 10 and 30 muscle samples each. The total number of fish used for the analysis was 112.



**Figure 1.** Geographical location of the study area. Inner Bergsfjord, the impacted fjord, where the salmon farms are located (green box), and Outer Bergsfjord, Frakkfjord and Olderfjord, which were considered control fjords. The gray circle indicates the geographical location of the study area on the Norwegian coast.

### 2.3. Laboratory Fatty Acid Analyses

Fatty acid composition was analysed following the methodology described below. Briefly, total lipids were extracted from 0.5 to 3 g of muscle sample by homogenising it in a chloroform/methanol mixture (2:1 *v/v*) using a tissue disruptor. The total lipid extraction followed the method of [9], with non-lipid impurities being removed by washing with a 0.88% (*w/v*) KCl solution. Lipid weight was determined gravimetrically after solvent evaporation and overnight vacuum desiccation. Fatty acid methyl esters (FAMES) were prepared via acid-catalysed transesterification of the total lipids according to Christie's method (1982). The total lipid samples underwent overnight trans-methylation at 50 °C in a 2 mL mixture of 1% H<sub>2</sub>SO<sub>4</sub> in methanol (with 1 mL of toluene to dissolve neutral lipids). The resulting methyl esters were extracted twice with a 5 mL mixture of hexane–diethyl ether (1:1, *v/v*) after neutralisation with 2 mL of 2% KHCO<sub>3</sub>. After drying under nitrogen, they were dissolved in 0.5 mL of iso-hexane. FAME analysis was performed using gas–liquid chromatography mass spectrometry with a flexible fused silica capillary column and Sigma-Aldrich FAME Mix as standards. Individual fatty acid concentrations were expressed as percentages of the total content.

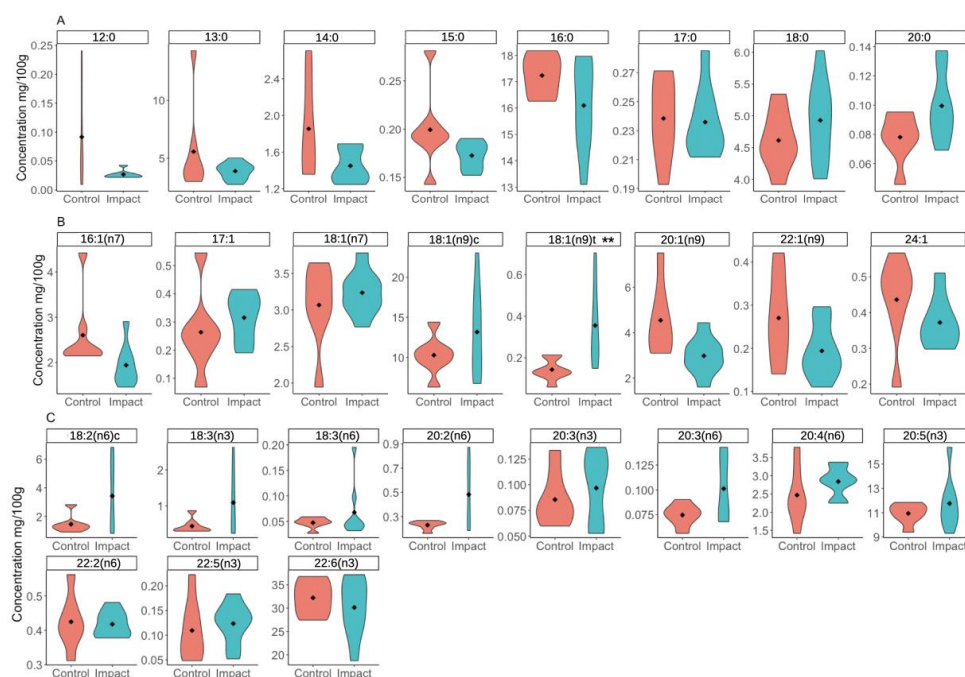
### 2.4. Data Analysis

Fatty acid composition was compared between treatment groups (impact and control) in terms of percentage, calculated from the concentration data (mg/100 g). We conducted a thorough statistical analysis to examine potential differences in cod sex and gonad maturity (Figure S1). After ensuring that there were no significant variations attributable to these factors, we proceeded to analyse the data, focusing solely on the control versus impact factor. This approach allowed us to simplify our analysis and concentrate on the primary experimental conditions, ensuring the robustness and clarity of our findings (Figure S1). Violin plots were used to compactly display the continuous distribution of fatty acids between the impact and control groups because they are a versatile data visualisation tool that can be particularly useful for understanding the distribution of a dataset. Violin plots are effective for comparing the distribution of a continuous variable

across multiple groups, allowing one to identify skewness and outliers. Fatty acids were grouped into the following categories: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Graphical representation was carried out with R software using *ggplot2* and *ggord* [10]. Statistical differences in fatty acids and category concentrations were tested using the Wilcoxon–Mann–Whitney test using the library *gtsummary* [11]. This method serves as a non-parametric analogue to the independent samples *t*-test, suitable when the response variable is not normally distributed. Treatment was considered a fixed factor with two levels: impact versus control. The overall fatty acid composition data were visualised using a PCA after eliminating the less concentrated fatty acids (6 fatty acids < 1 mg/100 g).

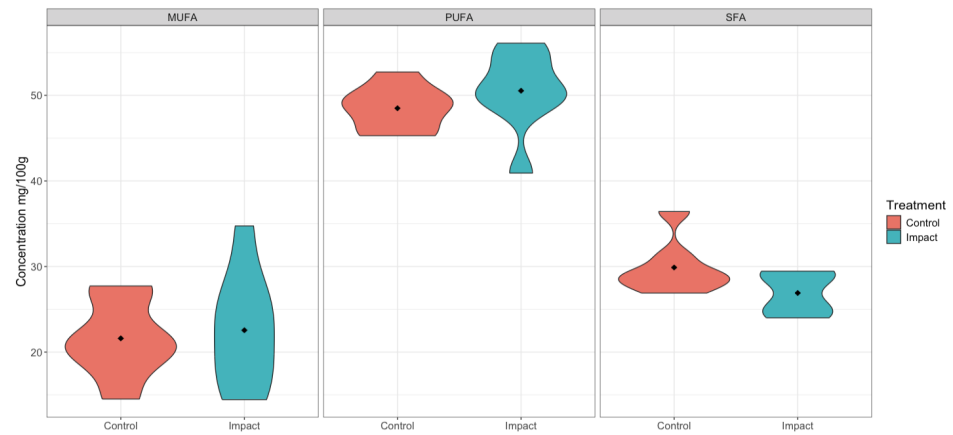
### 3. Results

The most relevant fatty acids were C22:6(n3) (docosahexaenoic acid or DHA), with 0.94 ( $\pm 0.13$  S.E) and 0.96 ( $\pm 0.06$  S.E.) mg/100 g in the control and impact groups, respectively, followed by C16:0 (palmitic acid) with 0.5 mg in both the control ( $\pm 0.06$  S.E.) and impact ( $\pm 0.08$  S.E.) groups (Table A1, Figure S2). C20:5(n3) (eicosapentaenoic acid or EPA) was also quite relevant, with 0.32 and 0.36 mg/100 g in the control and impact groups, respectively ( $\pm 0.05$  S.E.), followed by C18:1(n9)c (oleic acid) with 0.3 ( $\pm 0.09$  S.E.) and 0.45 ( $\pm 0.32$  S.E.) mg/100 g in the control and impact groups, respectively (Figure S1, Table A1). When analysing the significant differences in the concentration of fatty acids between the two treatments, significant differences were found only for C18:1(n9)t (elaidic acid) with low concentrations but a higher value in impact (Figure 2, Table A1).



**Figure 2.** Violin plot representation of concentration (mg/100 g) of fatty acids for cod flesh (control and impact). (A) = SFAs (saturated fatty acids); (B) = MUFAs (monounsaturated fatty acids); and (C) = PUFAs (polyunsaturated fatty acids). The black rhombus indicates the mean of the two treatments. Asterisks indicate the significant differences from the Wilcoxon–Mann–Whitney test (\*\*  $p < 0.001$ ; Table A1).

Regarding the different categories of fatty acids (Figure 3), PUFAs showed the highest concentration, averaging around 1.5 mg/100 g in both treatments. MUFAs showed values of 0.64 ( $\pm 0.2$  S.E.) mg/100 g in the control group and 0.76 ( $\pm 0.44$  S.E.) mg/100 g in the impact group, without statistical differences between the treatments, and SFAs showed values averaging around 0.8 mg/100 g (Table 1).

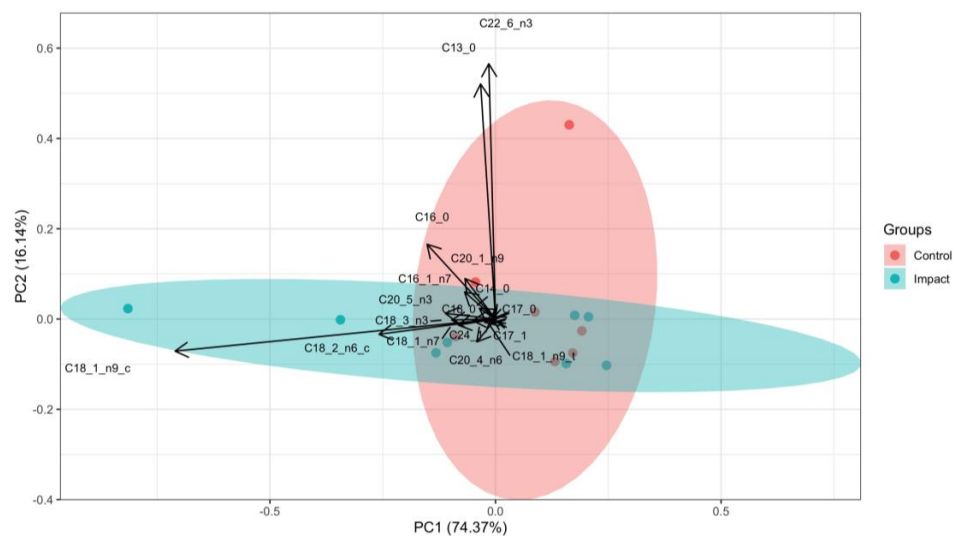


**Figure 3.** Violin plot representation of percentage of categories of fatty acids for cod flesh (control and impact). MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; and SFAs = saturated fatty acids. The black rhombus indicates the mean of the two treatments. The Wilcoxon–Mann–Whitney tests did not indicate significant differences at the  $p < 0.05$  level (Table 1).

**Table 1.** Mean ( $\pm$ S.E.) concentration (mg/100 g) of fatty acid categories (MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; and SFAs = saturated fatty acids), with  $p$ -value from the Wilcoxon–Mann–Whitney test.

$p$ -Value	Impact	Control	Fatty Acid Categories
0.96	0.76 (0.44)	0.64 (0.20)	MUFA
0.46	1.56 (0.27)	1.42 (0.16)	PUFA
0.46	0.83 (0.16)	0.88 (0.16)	SFA

Regarding the multivariate analysis, the fatty acid composition of cod muscle revealed an overlap among samples from the impacted cod and control fish (Figure 4). However, the impacted samples showed an arrangement along the x-axis (PC1 = 74.37%), with C18:1(n9)c (oleic acid), C18:3(n3) (linolenic acid) and C18:2(n6)c (linoleic acid) represented separately in the ordination plot. Conversely, the control samples exhibited a closer arrangement along the y-axis (PC2: 16.14%), primarily influenced by a single sample, with C22:6(n3) (docosahexaenoic acid or DHA), C20:1(n9) (gondoic acid) and C16:0 (palmitic acid) having the strongest correlation with this axis.



**Figure 4.** Bidimensional representation of Principal Component Analysis (PCA) of fatty acid concentrations for control and impacted muscle cod samples.

#### 4. Discussion

Coastal aquaculture is expected to increase markedly in Northern Norway [5], potentially affecting the diet and fatty acid composition of wild fish in these regions. Therefore, Atlantic cod, especially the coastal cod ecotype [12], could be affected by salmon aquaculture, as fish aggregate around salmon farms [2,3] and may feed there the whole year round. In 2022, around 1000 registered fish farm localities produced 1.62 million tonnes of Atlantic salmon along the Norwegian coast [13]. However, the main findings of the present work show minor differences in the concentration of the individual and main groups of fatty acids when comparing cod muscle from localities near aquaculture facilities to those from remote areas. Contrary to our expectations, the EPA and DHA levels were consistently high and similar between the treatment and control groups. Therefore, from a fatty acid perspective, there appear to be no major nutritional changes in consuming cod grown near salmon facilities compared to fish caught elsewhere. Both groups of fish had relatively high polyunsaturated fatty acid (PUFA) concentrations in their flesh.

Consumers generally prefer wild fish from extractive fisheries over fish from produced from aquaculture, citing reasons such as taste, safety, and nutritional value [6]. However, studies show only slight reductions in quality for fish near salmon farms and no significant nutritional concerns for cod in these environments. For example, comparisons of the fillet quality of saithe (*Pollachius virens*) captured near or more than 5 km away from salmon farms in Norway generally showed good quality, though impacted fish averaged a slightly lower quality [14].

In our study, the results indicated that there were no significant differences in the composition of most fatty acids between the treatments, with the exception of elaidic acid. Elaidic acid exhibited a very low concentration variance, but it was higher in the impact treatment group. Elaidic acid is a trans-fatty acid and, specifically, the trans-isomer of oleic acid. It is a predominant industrial trans fatty acid, commonly found in partially hydrogenated vegetable oils, which are used in a variety of processed foods to increase shelf life and stability. This finding suggests that while the overall fatty acid profiles remain consistent, specific fatty acids like elaidic acid may serve as key indicators of metabolic or dietary variations. Contrary to expectations, EPA and DHA levels were consistently high and similar between the treatment and control groups. Therefore, from a fatty acid perspective, there appear to be no major nutritional differences in consuming cod grown near salmon facilities compared to fish caught elsewhere. Both groups of fish had relatively high polyunsaturated fatty acid (PUFA) concentrations in their flesh. However, the excess weight that C20:1-n9 (gondoic acid) and C16:0 (palmitic acid) had over the controls is remarkable, especially as these saturated fatty acids are a typical component of aquaculture feeds formulated with terrestrial vegetables [15].

Young cod primarily feed on crustaceans, and as they grow, their diet shifts to include a larger proportion of fish. Across all age groups, cod also consume some annelid worms. The key crustaceans in their diet include Caridae, Astacidea, Anomura and Brachyrrhyncha species. The most significant fish species consumed are herring, Norway pout, haddock, whiting, sand eel (*Ammodytes* spp., Ammodytidae) and dab (*Limanda limanda*, Pleuronectidae) [16]. On the other hand, fatty acid analysis has determined that cod feed on both benthic species and zooplankton, as indicated by the dominance of fatty acids such as 20:4(n6) and 20:1(n9), respectively. In the present study, 20:4(n6) showed a significant concentration in both treatments. Meanwhile, 20:1(n9) was important in the ordination of the control individuals, which may suggest a greater utilisation of pelagic resources by this group [7].

Plant material in the feed has reduced the importance of fish oil for salmon feeding. The feed now mainly includes cereal grains and vegetable oils (e.g., corn, canola, soybean, or palm oil), which can be incorporated into wild fish tissue, particularly when there is an excess of spilled feed in the marine environment [14]. Elevated concentrations of fatty acids from terrestrial sources, such as 18:1(n9) or 18:2(n6), in fish tissues already serve as valuable biomarkers, indicating the consumption of waste feed by wild fish associated with

cages [4,7]. In the present study, these fatty acids were the primary drivers of the ordination results concerning cod muscle samples from areas near salmon farm installations, which is consistent with other studies that use these fatty acids as indicators of the impact of salmon farms.

Cod are quite prevalent in fjords, as demonstrated by combining individual tracking data from acoustic telemetry with genetic analyses, particularly the more sedentary Norwegian coastal cod, which is currently in a depleted state and shows a higher level of fjord residency compared to migratory Northeast Arctic cod [17]. However, coastal cod can also exhibit high mobility within the fjord, similarly to saithe [18]. This suggests that cod, even when near salmon farms, consume a significant amount of marine trophic resources combined with lost aquafeed, at least when the density of salmon farms is relatively low, as in our study system. In our dataset, the cod seem to maintain the availability of PUFAs, as detected in the muscle fatty acid analysis. Additionally, changes in specific fatty acids, such as docosahexaenoic acid, eicosapentaenoic acid and arachidonic acid, can reveal the dietary influence on fish growth and development. However, the threshold levels of salmon feed (amount, duration and timing) consumed by wild cod before it affects the quality of eggs, sperm or other life stages are not known and require new investigations to be identified [5].

It has been demonstrated that a high consumption of fish rich in omega-3 ( $\omega$ 3) fatty acids significantly reduces the risk of cardiovascular diseases, inflammatory diseases and negative influences on brain development and function. The consumption of omega-3 fatty acids may even benefit mental health [19]. Therefore, from a human consumption standpoint, changes in fatty acid profiles could be important, even for lean fish such as cod, which contains a low quantity of fat in its flesh (<4g/100 g). However, the present study did not find major changes in the composition of fatty acids in cod muscle, suggesting that from a nutritional perspective for consumers, these changes do not appear to have significant effects.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/aquacj4040018/s1>: Figure S1. Violin plot representation of percentage of categories of fatty acids for cod flesh, considering the sex and gonad maturity (control and impact). MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; and SFAs = saturated fatty acids. The black rhombus indicates the mean of the three treatments. The Wilcoxon–Mann–Whitney tests did not indicate significant differences at the  $p < 0.05$  level. Figure S2. Ordered fatty acids for A = control group and B = impact group regarding the concentration (mg/100 g).

**Author Contributions:** Conceptualisation, P.A.B. and T.B.; methodology, I.M.S., B.-S.S., P.S.-J., N.K. and P.S.-J.; formal analysis, P.S.-J., T.J. and J.A.; data curation, P.S.-J.; writing—original draft preparation, P.S.-J.; writing—review and editing, T.B., T.v.d.M., P.A.B. and B.-S.S.; supervision, T.B.; project administration, P.A.B.; funding acquisition, P.A.B. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki. Since no experiments were conducted on these fish, approval from Mattilsynet/FOTS was not required. We confirm that the fish were handled in accordance with this regulation, "Forskrift om kvalitet på fisk og fiskevarer", as outlined in Norwegian legislation FOR-2013-06-28-844 (<https://lovdata.no/dokument/SF/forskrift/2013-06-28-844>), which governs the acceptable capture and handling of fish.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data can be made available upon request to the authors of the paper.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Appendix A

**Table A1.** Concentration (mg/100 g; mean and standard error) of fatty acids in cod flesh for control and impacted individuals. *p*-value of Wilcoxon rank sum exact test; Fisher's exact test.

Fatty Acid	Name(s)	<i>p</i> -Value	Impact	Control
12:00	Lauric_docecanoic	0.072	0.0009 (0.0005)	0.0025 (0.0023)
13:00	Tridecanoic_trideculic	0.34	0.12 (0.05)	0.17 (0.13)
14:00	Myristic_tetradecanoic	0.34	0.046 (0.017)	0.056 (0.023)
15:00	Pentadecanoic_Pentadecylic	0.46	0.0054 (0.0013)	0.0059 (0.0018)
16:00	Palmitic_hexadecanoic	0.87	0.50 (0.08)	0.50 (0.06)
16:1(n7)	Palmitoleic	0.15	0.06 (0.03)	0.08 (0.04)
17:00	Margaric_Heptadecanoic	0.87	0.0074 (0.0018)	0.0069 (0.0007)
17:01	cis_10_Heptadecenoic	0.15	0.010 (0.005)	0.007 (0.004)
18:00	Stearic	0.094	0.151 (0.022)	0.134 (0.012)
18:1(n9)t	Elaidic	0.009	0.011 (0.007)	0.004 (0.001)
18:1(n9)c	Oleic	0.61	0.45 (0.32)	0.30 (0.09)
18:1(n7)	Vaccenic	0.61	0.104 (0.038)	0.090 (0.021)
18:2(n6)c	Linoleic	0.69	0.13 (0.12)	0.04 (0.02)
20:00	Arachidic_icosanoic	0.072	0.0031 (0.0009)	0.0023 (0.0006)
18:3(n6)	gamma_Linolenic_Acid_GLA	0.54	0.0020 (0.0013)	0.0014 (0.0004)
20:1(n9)	cis_11_Eicosenoic_gondoic	0.15	0.09 (0.04)	0.14 (0.07)
18:3(n3)	Linolenic	0.61	0.041 (0.044)	0.013 (0.007)
20:2(n6)	Eicosadienoic	0.4	0.016 (0.012)	0.007 (0.002)
20:3(n6)	Eicosatrienoic_3n6	0.28	0.0032 (0.0014)	0.0022 (0.0005)
22:1(n9)	Erucic	0.46	0.006 (0.003)	0.008 (0.004)
20:3(n3)	Eicosatrienoic_3n3	0.69	0.0032 (0.0018)	0.0025 (0.0007)
20:4(n6)	Arachidonic	0.15	0.089 (0.022)	0.070 (0.016)
22:2(n6)	Docosadienoic	0.87	0.013 (0.004)	0.013 (0.004)
20:5(n3)	Eicosapentaenoic_EPA	0.19	0.36 (0.05)	0.32 (0.05)
24:01:00	Nervonic	0.34	0.012 (0.005)	0.013 (0.004)
22:4(n3)	Docosatetraenoic	0.47	0	0.0026
22:5(n3)	Docosapentaenoic	0.34	0.0039 (0.0016)	0.0031 (0.0015)
22:6(n3)	Docosahexaenoic_DHA	0.78	0.91 (0.06)	0.94 (0.13)

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