Identifying a Non-Lethal Fatty Acid Sampling Method in Fish

by

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Abstract

Fatty acid profiling is crucial for understanding fish health and diet.This study evaluates the potential of fish fin tissues as non-lethal alternatives to muscle tissue for fatty acid profiling. Lipid extractions from muscle, adipose fin, inner-caudal, and outer-caudal tissues were analyzed using gas chromatography. Principal component analysis (PCA) and Euclidean distance analyses revealed a strong similarity between adipose and muscle tissue. Inner-caudal tissue showed a strong correlation with muscle, with moderate intra-fin variability. Outer-caudal tissue exhibited the most distinct fatty acid profile and high intra-fin composition variability, suggesting it provides a useful alternative. This study demonstrates that adipose fin tissue provides a reliable non-lethal alternative for fatty acid profiling in fish, given its strong correlation with muscle tissue, minimal variation, and consistency in fatty acid composition and concentration. Non-lethal fatty acid sampling methods support ecological research and conservation efforts, laying the foundation for more sustainable fish health and dietary studies.

Dedication

To my family, this project would not have been possible without your unwavering support and belief in me. I couldn't have done it without your encouragement.

Acknowledgements

This project was made possible by the help and support of many. Great thanks are extended to those at Research and Productivity Council (RPC), Fredericton, especially Attiq Rehman, Bruce Phillips and Jill Hay. A great thanks to the members of the Samways lab, especially Dr. Kurt Samways and Ben Andrews. Finally, I extend my gratitude to Maddison Brown for her invaluable guidance throughout this project.

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List of Symbols, Nomenclature, or Abbreviations

ALA	Alpha-linolenic acid
ANOVA	Analysis of Variance
C	Celcius
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
e.g.	(exempli gratia) for example
EPA	Eicosapentaenoic acid
FA	Fatty acid
g	Gram
i.e.	(id est) that is
L	Litre
mg	Milligram
mL	Millilitre
nd	Not detected
PCA	Principal component analysis
PC1	Principal component 1
PC2	Principal component 2
PERMANOVA	Permutational analysis of variance
PRIMER	Plymouth Routines in Multivariate Ecological Research
RPC	Research and Productivity Council
SD	Standard deviation
SIMPER	Similarity percentage
µ	Micro (10^{-6}, as a prefix)
°	Degree (temperature as a prefix)
%	Per cent (per hundred

Introduction

Over the past few decades, a significant number of fish species have become threatened, endangered, or extinct (Millennium Ecosystem Assessment, 2005). Major pressures such as habitat loss, overfishing, invasive species, and climate change, among others, have emerged, posing significant threats to various fish populations (Arthington et al., 2016). These pressures have not only led to population declines, but have also significantly altered food webs for many fish species, with decreased resource availability due to anthropogenic impacts and climate change (Donohue et al., 2017; Water et al., 2013). Consequently, the overall health and condition of fish, including energy reserve, have been adversely affected (Chiaramonte et al., 2016; Brodersen et al., 2011). In response to these alarming conditions, there has been a heightened emphasis on the conservation and preservation of many fish species (Reid et al., 2018).

Lipids serve as one of the primary sources of energy for a variety of organisms and provide significant insights into their overall well-being (Kainz and Fisk, 2009). As essential organic compounds, lipids improve the nutritional status of aquatic food webs and the health and fitness of aquatic organisms (Samways et al., 2017; Kainz and Fisk, 2009). Lipids are predominantly comprised of essential compounds known as fatty acids. Understanding fatty acid composition can yield a wealth of information about aquatic organism health and function (Heintz et al., 2004). Unlike other dietary compounds, fatty acids are generally not degraded and retain their original form in consumer tissues (Budge et al., 2006).

Fatty acid profiling is a valuable tool for assessessing the overall health of fish (Arts and Kohler, 2009), determining trophic position and dietary structure (Budge et al., 2006; Samways et al., 2017; Twinning et al., 2020; Xu et al., 2020), and understanding predator-prey

relationships (Iverson et al., 2004; Parrish et al., 2015; Peron et al., 2023). In aquaculture, analyzing fatty acid profiles provides insights into health factors such as immune response, cardiac function, stress levels, and overall energy availability. These factors are crucial for the well-being of individuals in the aquaculture industry (Olivia-Teles, 2012) and can also be applied to better understand declining wild populations.

Fatty acid profiling in fish typically requires lethal sampling to obtain tissues such as liver or muscle (Miller et al., 2006). These tissues are generally preferred for fatty acid profiling due to their high lipid concentrations as nutrient storage areas, as well as their physiological functions (Shewfelt, 1981). However, the critical status of many fish species, makes reliance on lethal sampling problematic, raising legal, ethical, and logistical concerns. Consequently, non-lethal sampling methods are emerging as viable alternatives and are expected to become integral to ichthyology sampling programs (Jeffries et al., 2021). For several years, non-lethal sampling has facilitated other bioindicator fields, such as genetic sequencing, to better understand population dynamics, as well as stable isotope analysis, to determine food web structure (Samways et al., 2018) and trophic positions (Jefferies et al., 2021). While a range of ecological disciplines have investigated this sampling approach (Thorstensen et al., 2022), there remains limited knowledge regarding its application as a sampling method for fatty acid profiling in fish (Tocher et al., 2010).

Atlantic salmon (*Salmo salar)* are a well-studied anadromous fish species historically distributed along the east coast of North America and along the west coast of Europe (COSEWIC, 2006). Fatty acid analysis has been used in various ways to understand Atlantic salmon populations, trophic ecology, conservation measures, and aquaculture escapes (Skilbrei et al., 2015; Samways et al., 2017; Parzanini et al., 2020). In Canada, Atlantic salmon are not

only ecologically important , but also culturally significant playinga vital role in many traditional practices for Indigenous communities across eastern Canada (Gross, 1998). Despite significant conservation efforts, wild populations are experiencing a rapid decline due to fishing pressure, habitat degradation, and climate change (Thorstad et al., 2021) resulting in many populations becoming endangered. This makes Atlantic salmon an ideal model for evaluating the effectiveness of non-lethal methods in assessing fatty acid profiles.

This study uses muscle tissue as a baseline to compare fatty acid concentrations in adipose and caudal fin tissues. The adipose fin was selected because, although it is unique to Salmonids, it is a thick fleshy tissue believed to provide a representative fatty acid profile. To establishing a methodology for non-lethal fatty acid sampling in fishes, it was essential to select another tissue that is present in all fish species. Therefore, the caudal fin was chosen as it is a common, non-lethal sample routinely collected (Nicola and Cordone, 2011). Since the entire caudal fin was available during sample collection, an inner portion of caudal tissue was opportunistically selected as an additional sampling location to determine if there were differences in fatty acids profiles along the length fin.

The purpose of this study is to identify a tissue in fish (using Atlantic salmon as the model species) that can be collected non-lethally and non-invasively to allow for fatty acid analysis. Given the vulnerable status of many fish stocks, the goal is to integrate this method into future fatty acid profiling studies, to prevent unnecessary euthanasia or harm to endangered fish species. The objective of this study is to assess whether the differences in fatty acid compositions and concentrations in different fish fin tissues are comparable to muscle tissue and provide a representative profile that is as informative as muscle tissue. This information can be applied to future studies to enhance the efficacy of in a non-lethal fatty acid profiling.

Methods

Sample Collection

In August 2024, Atlantic Salmon tissue samples were collected from a salmon processing plant. Since these fish originated from a commercial operation, all fish received the same diet and were of the same age range, relative size and genetic family. A total of 11 fish were haphazardly selected and had a muscle sample taken from the area immediately anterior to the operculum (Figure 1). The muscle sample location was based solely on the availability of tissue remaining post processing. In addition, the adipose fin and the entire caudal fin were collected (Figure 1). Although, in the wild, the whole adipose fin and/or caudal fin would not be sampled, since these fish were already sacrificed, whole fins were collected. As the entire caudal fin was made available during sample collection, the inner and outer sections of the caudal fin were opportunistically selected as sampling locations to better understand fatty acid differences along the length of the caudal fin (Figure 1).

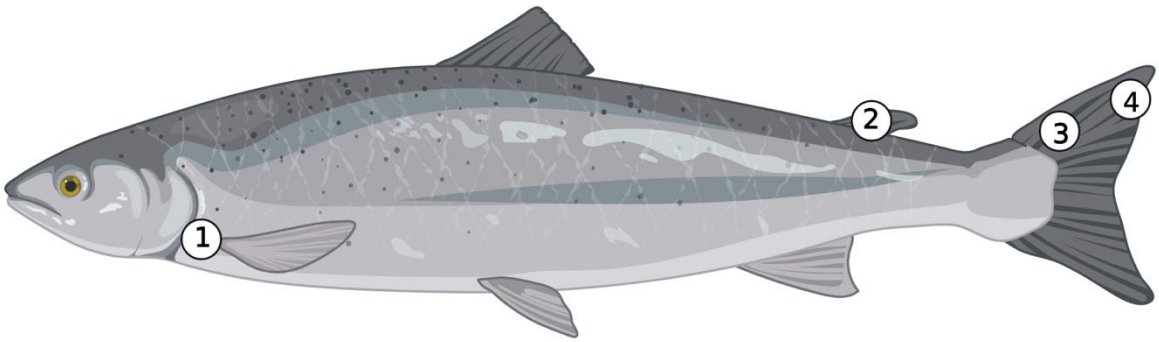

Figure 1. Tissue sample locations on Atlantic salmon (*Salmo salar)* included (1: Muscle 2: Adipose fin 3: Inner caudal fin 4: Outer caudal fin).

Lipid Extraction

Lipid extractions were conducted at the Research and Productivity Councils (RPC) facility in Fredericton, New Brunswick. Lipids were extracted from the collected tissues using a modified version of the Mojonnier method (RPC, 2024; Hooi et al., 2004; Nielsen, 2024). One gram of each tissue sample was homogenized and transferred to Mojonnier flasks, where 100mg of pyrogallic acid and 200µl of glycerol triundecanoate internal standard were added to all samples. Next, 2mL of ethanol and 10mL of 100% HCl were added to all flasks, which were then placed in a 75°C hot water bath of for complete digestion. Flasks were then removed and cooled with the addition of 10mL of ethanol. Once at room temperature, the lipids were separated from the mixture using three separate additions of ethyl ether and petroleum ether. The top, clear layer, which contained the lipids, was decanted into labeled beakers for each sample. Beakers with the lipid fractions were then placed in a 53°C hot water bath at to facilitate the evaporation of ethers from the lipids. Beakers were then removed from the hot water bath and the remaining fat was transferred to culture tubes using petroleum ether rinses. Under a stream of nitrogen, the petroleum ether was evaporated, and 2mL of 1% sulfuric acid in methanol and 1mL of toluene were added. Samples were stored in a 50°C oven overnight to allow for methylation. After approximately 18 hours, the samples were cooled to room temperature, and 5mL of water, along with 2mL of hexane, were added to each sample. The upper layer, consisting of the lipids, was transferred to new culture tubes containing sodium sulfate, using hexane rinses. Each sample was then transferred to new culture tubes and diluted to 10mL with hexane. Finally, 1.5mL of the solution was prepared in vials for gas chromatography. The fatty acid methyl esters were analyzed using an Agilent Technologies 7890B Gas Chromatography system with Auto-Sampler 7693 (Agilent Technologies, Santa Clara, California, USA) at RPC. Glycerol trindecanoate

internal standard, added during lipid extraction, was used to determine the relative concentration of fatty acid peaks.

Statistical Analysis

A Principal Component Analysis (PCA) was conducted to identify the similarities in fatty acid composition (% of total) and concentration (g/100g) between tissues and to determine which fatty acids accounted for major sources of variation within the data, therefore, minimizing redundancy. The Euclidean distance for each sample to the group centroid was calculated to provide a metric of fatty acid profile variability within each tissue (Layman et al. 2007). The centroid was defined as the mean PC1 and PC2 values for all samples in each tissue. The Euclidean distances (amount of variation) were compared between tissues using an analysis of variance (ANOVA).

Using the statistical software PRIMER, fatty acid composition and concentration were compared between and within each tissue. A permutational multivariate analysis of variance (PERMANOVA) was conducted to analyze variations in fatty acid profiles across tissues. In the PERMANOVA, Tissue (four levels: muscle, adipose, inner caudal, and outer caudal) was the fixed factor, while Sample (the error term) was the random factor. Similarity percentage (SIMPER) analysis, which measures the top 90% of contributing variables, was used to calculate the average sample dissimilarity of each fatty acid between and within tissues. Addtionally, a linear regression was performed to assess the strength and nature of the relationship between each tissue and muscle.

Results

Principal component analyses (PCA) based on the fatty acid profiles characterized variation between tissues in two-dimensional ordination. The PCA based on fatty acid composition (% of total) showed that the first principal component axis (PC1) accounted for 76.6% of the variance, effectively distinguishing the outer-caudal tissue from other tissues (Figure 2). The fatty acids with high positive loading on PC1 included 18:2n6, 18:3n3 and 18:1n9, while those with high negative loadings were 18:0, 20:4n6 and 22:6n3. The second principal component axis (PC2) accounted for an additional 8.2% of variance, with high positive loadings 18:1n7 and 20:5n3 and high negative loadings 22:5n3 and 22:6n3. This analysis indicates a significant difference between the outer-caudal tissue and other tissues, whereas adipose and inner-caudal tissues exhibit considerable overlap. The outer-caudal tissue is characterized by fatty acids such as C16:0, C18:0, C20:4n6, C20:5n3, C22:5n3 and C22:6n3. In contrast, the adipose, inner-caudal and muscle tissues are characterized by C14:0, C16:1n7, C18:1n9, C18:2n6, C18:3n3 and C20:2n6.

A PCA based on fatty acid concentration (g/100g) revealed differential variation between tissue. PC1 accounted for majority of the variance (96.8%), demonstrating the greatest differentiation between tissues, with all fatty acids showing high positive loadings (Figure 3). PC2 accounted for additional variance of 1.4%, with 22:6n3 exhibiting a high positive loading and 14:0 demonstrating a strong negative loading. Outer-caudal had strong negative PC1 scores, indicating low fatty acid concentrations (Figure 3). In contrast, muscle tissue displayed the highest PC1 scores, with the greatest variance, indicating the greatest concentration of fatty acids of all tissue (Figure 3).

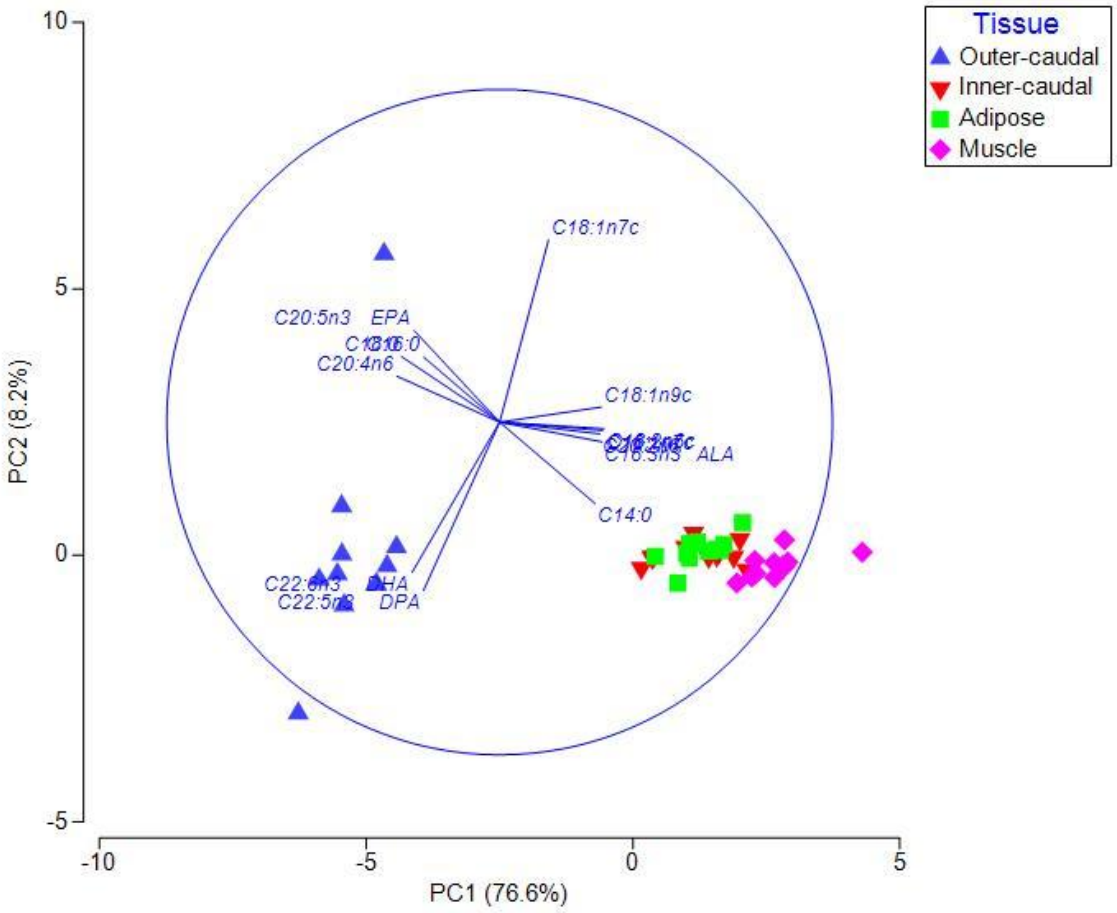

Figure 2. Principle component analysis (PCA) of muscle (pink diamonds), adipose (green squares), inner-caudal (red inverted triangles) and outer-caudal (blue triangles) fatty acid composition (% of total). The eigenvectors (represented by individual fatty acids and corresponding vector length) illustrate the importance of that fatty acid's contribution to the tissue on the first two principal component axes.

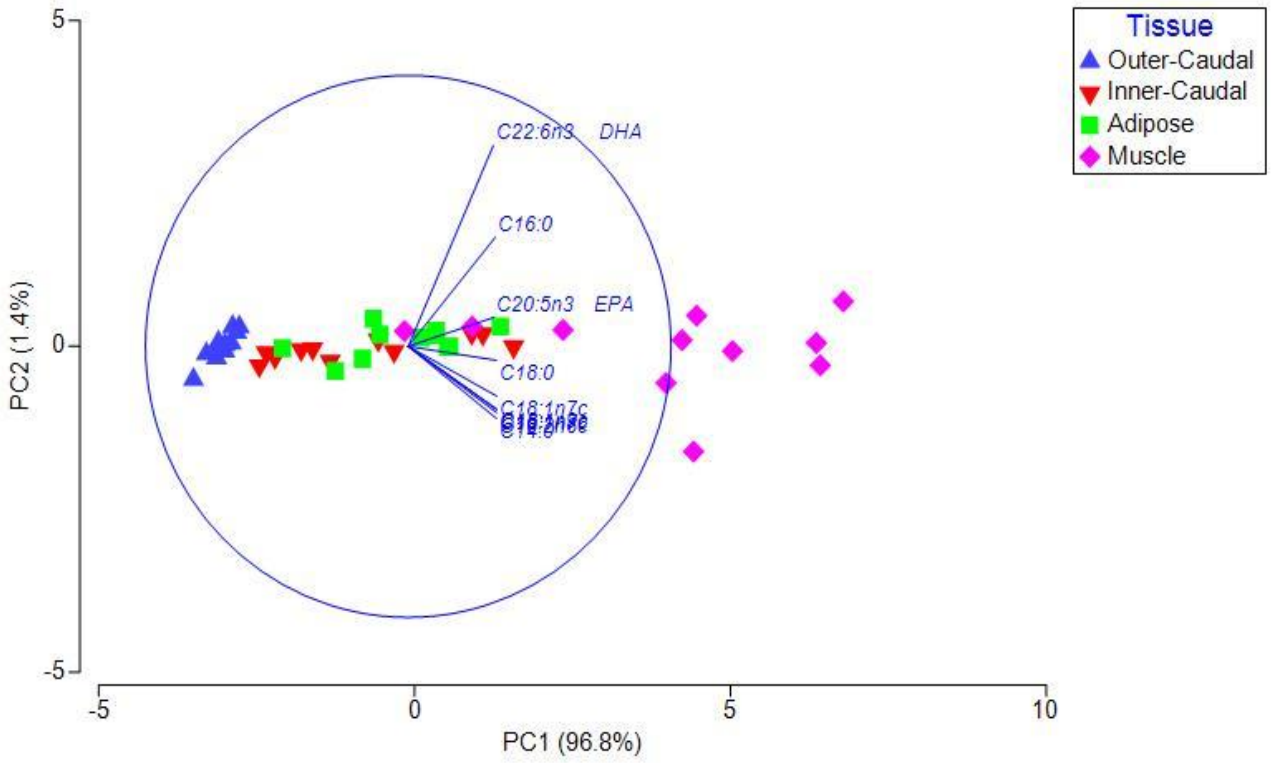

Figure 3. Principle component analysis (PCA) of muscle (pink diamonds), adipose (green squares), inner-caudal (red inverted triangles) and outer-caudal (blue triangles) fatty acid abundance (g/100g). The eigenvectors (represented by individual fatty acids and corresponding vector length) illustrate the importance of that fatty acid's contribution to the tissue on the first two principal component axes.

The variation in fatty acid composition, measured as the median Euclidean distance to group centroid, was lowest in the outer-caudal tissue (mean Euclidian distance of 0.24) and greatest in the inner-caudal tissue (mean Euclidian distance of 4.02) (Figure 4). Comparing between tissues, variation in fatty acid composition was significantly different between tissues ($F_{3,40}$=6.294, p=0.001). All tissues had significantly different amounts of variation between replicate samples, expect for inner-caudal and muscle (t_{10}=0.021, p=0.49) (Figure 4).

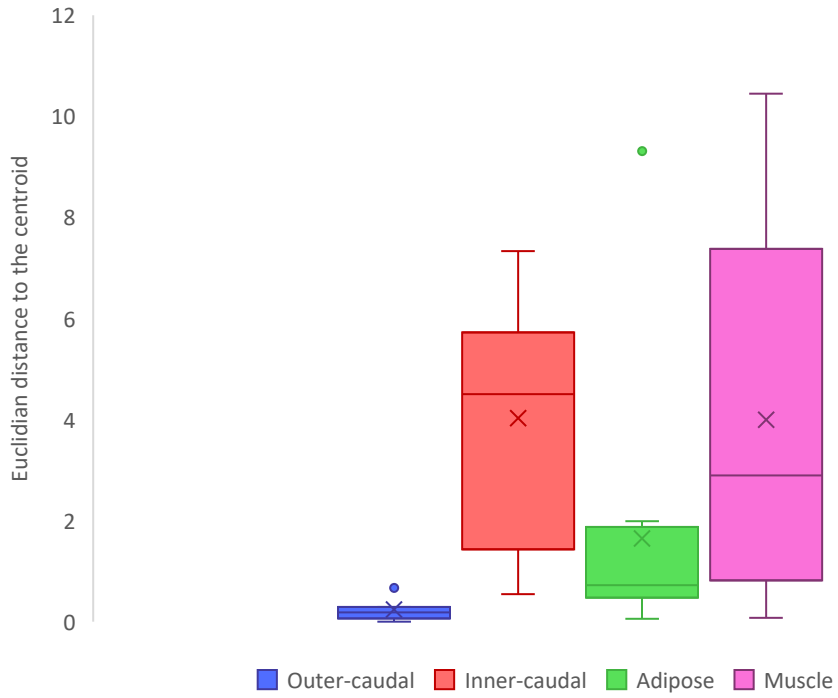

Figure 4. Box and whisker plot of the Euclidean distance to the centroid for fatty acid composition (% of total) for each tissue; outer-caudal, inner-caudal, adipose, and muscle.

Euclidian distances to the centroid for fatty acid concentration was lowest in the outer-caudal tissue (mean Euclidian distance of 0.11) and greatest in muscle tissue (mean Euclidian distance of 12.14) (Figure 5). Comparing between tissues, variation in fatty acid concentration was also significantly different between tissues ($F_{3,40}$=4.145, p=0.01). There was no difference in the amount of variation between inner-caudal tissues and either adipose tissue (t_{10}=1.702, p=0.06), nor muscle tissue (t_{10}=-1.419, p=0.09). All other tissues had significantly different amount of variation from one another (Figure 5)

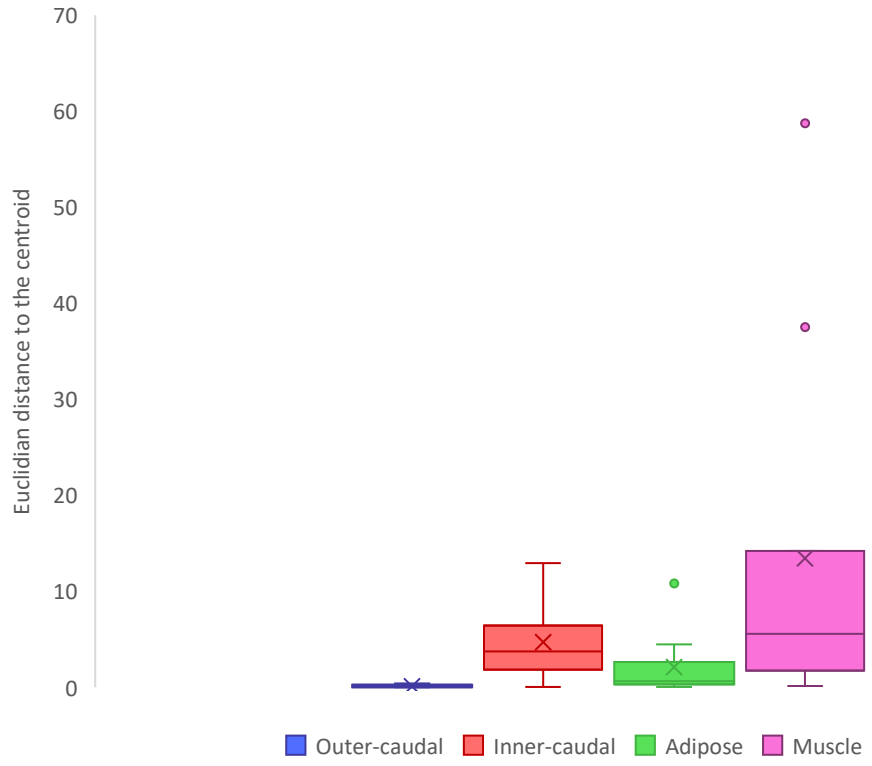

Figure 5. Box and whisker plot of the Euclidean distance to the centroid fatty acid concentration (g/100g) for each tissue; outer-caudal, inner-caudal, adipose, and muscle.

A total of 29 interrelated fatty acids were detected and identified across all tissues for both fatty acid composition (Table 1) and concentration (Table 2). Permutational Multivariate Analysis of Variance (PERMANOVA) showed that fatty acid composition varied significantly between tissues ($F_{3,40}$=19.692, p=0.0001). Pairwise comparisons showed significant differences between tissues, except between inner-caudal and adipose (t_{20}=6.434, p=0.1296), suggesting some level of similarity between tissues. Outer-caudal versus muscle (t_{20}=6.115, p=0.0001) showed the largest between group difference, while inner-caudal versus muscle (t_{20}=2.807, p=0.0003) and adipose versus muscle (t_{20}=2.362, p=0.0013) show a much smaller, yet significant difference (Table 1). Other significant between group comparisons include outer-caudal versus

inner-caudal (t_{20}=4.7035, p=0.0001) and outer-caudal versus adipose (t_{20}=5.5026, p=0.0001), which both showed moderate differences (Table 1).

A PERMANOVA conducted on fatty acid concentration also identified significant differences between tissues ($F_{3,40}$ = 25.656, p=0.0001) (Table 2). All between tissue comparisons were statistically significant, except for inner-caudal versus adipose (t_{20}=1.106, p=0.2722) (Table 2). Outer-caudal versus muscle (t_{20}=7.4393, p=0.0001) showed the greatest between tissue difference, while inner-caudal versus muscle (t_{20}=4.7688, p=0.0002) and adipose versus muscle (t_{20}=4.4801, p=0.0001) showed weaker between tissue differences. (Table 2) Other significant between group comparisons included outer-caudal versus inner-caudal (t_{20}=3.2775, p=0.0001) and outer-caudal versus adipose (t_{20}=5.2529, p=0.0001), which both showed moderate differences (Table 2).

Table 1. Fatty acid percent composition of muscle, adipose fin, inner-caudal and outer-caudal tissue samples.

FAs	Muscle (±SD)	Adipose (±SD)	Inner-Caudal (±SD)	Outer-Caudal (±SD)
∑Saturates	23.47±2.03	26.23±1.35	25.13±1.44	34.36±5.51
C12:0	nd	nd	nd	nd
C14:0	3.04±0.23	2.69±0.28	2.42±0.31	0.97±0.65
C15:0	0.23±0.02	0.19±0.10	0.10±0.12	nd
C16:0	15.63±2.40	18.02±0.58	17.03±0.72	23.12±5.55
C17:0	0.21±0.01	0.17±0.11	0.10±0.12	nd
C18:0	4.25±0.27	5.12±0.36	5.44±0.78	10.27±1.40
C20:0	0.11±0.06	0.04±0.07	0.04±0.07	nd
C22:0	nd	nd	nd	nd
∑Monounsaturates	44.12±0.41	40.26±0.20	40,48±0.18	26.88±0.85
C14:1n5c	0.04±0.05	nd	nd	nd
C16:1n7c	7.39±0.41	6.66±0.50	6.38±0.68	2.67±0.39
C18:1	0.31±0.04	0.22±0.12	0.10±0.12	nd
C18:1n9c	32.81±1.87	29.85±1.23	30.62±1.02	20.45±2.44
C18:1n7c	2.89±0.15	2.86±0.10	2.87±0.11	2.43±0.90
C20:1	0.34±0.05	0.27±0.10	0.17±0.16	nd
C22:1n11	0.02±0.04	nd	nd	nd
C22:1n9	0.14±0.05	0.06±0.08	0.04±0.08	nd
C24:1n9	0.18±0.04	0.34±0.16	0.30±0.22	1.33±0.88
∑Polyunsaturates†	39.17±0.05	32.56±0.25	33.40±0.06	38.64±8.66
∑Omega-6	26.39±0.17	17.41±0.16	18.04±0.15	15.54±0.59
C18:2n6c	15.86±0.96	13.80±0.75	14.17±0.67	6.41±0.88
C18:3n6	0.28±0.04	0.25±0.09	0.17±0.13	nd
C20:2n6	0.90±0.06	0.92±0.05	0.96±0.05	nd
C20:3n6	0.49±0.10	0.57±0.15	0.68±0.28	1.22±1.13
C20:4n6	7.91±1.15	1.85±0.57	2.05±0.57	7.91±1.15
C22:2n6	0.95±0.12	0.02±0.03	0.01±0.04	nd
∑Omega-3	12.78±0.27	15.15±0.69	15.36±0.30	23.1±4.12
C18:3n3 ALA	2.02±0.16	1.68±0.12	1.78±0.20	0.14±0.24
C18:4n3	0.58±0.05	0.55±0.05	0.40±0.26	nd
C20:3n3	0.11±0.07	0.06±0.08	0.06±0.09	nd
C20:5n3 EPA	3.47±0.50	5.26±0.72	5.15±0.88	7.69±1.72
C22:5n3 DPA	1.79±0.15	2.13±0.27	2.28±0.35	3.31±1.14
C22:6n3 DHA	4.806±1.10	5.47±01.80	5.69±0.82	11.96±4.37
(n-3/n-6)	0.48	0.87	0.85	1.49
20:5n3/22:6n3	0.72	0.96	0.91	0.64
20:4n6/22:6n3	1.65	0.34	0.36	0.66

Notes: Values (mean ± SD) are percentages of total FAs (nd = not detected). SD of ∑ calculated using pooled standard deviation. † Totals include Omega-6 and Omega-3 fatty acids.

Table 2. Fatty acid concentrations of muscle, adipose fin, inner-caudal and outer-caudal tissue samples.

FAs	Muscle (±SD)	Adipose (±SD)	Inner-Caudal (±SD)	Outer-Caudal (±SD)
∑Saturates	2.29±0.03	1.12±0.01	0.903±0.01	0.28±0.001
C12:0	nd	nd	nd	nd
C14:0	0.30±0.08	0.11±0.03	0.09±0.05	0.008±0.006
C15:0	0.02±0.006	0.009±0.005	0.005±0.006	nd
C16:0	1.52±0.46	0.77±0.21	0.61±0.31	0.19±0.08
C17:0	0.02±0.006	0.008±0.005	0.005±0.006	nd
C18:0	0.42±0.12	0.22±0.06	0.19±0.08	0.08±0.02
C20:0	0.01±0.006	0.002±0.003	0.003±0.004	nd
C22:0	nd	nd	nd	nd
∑Monounsaturates	4.37±0.12	1.70±0.02	1.48±0.04	0.21±0.0002
C14:1n5c	0.005±0.005	nd	nd	nd
C16:1n7c	0.72±0.20	0.28±0.08	0.24±0.13	0.02±0.004
C18:1	0.03±0.01	0.01±0.006	0.005±0.007	nd
C18:1n9c	3.26±1.01	1.27±0.36	1.11±0.59	0.16±0.04
C18:1n7c	0.29±0.09	0.12±0.03	0.10±0.05	0.02±0.007
C20:1	0.03±0.01	0.01±0.004	0.008±0.008	nd
C22:1n11	0.002	nd	nd	nd
C22:1n9	0.01±0.008	0.003±0.004	0.002±0.004	nd
C24:1n9	0.02±0.004	0.01±0.007	0.01±0.009	0.01±0.008
∑Polyunsaturates†	3.08±2.46	1.37±0.47	1.20±0.65	0.31±0.42
∑Omega-6	1.84±0.04	0.74±0.005	0.65±0.01	0.12±0.00005
C18:2n6c	1.57±0.48	0.59±0.17	0.52±0.28	0.05±0.01
C18:3n6	0.03±0.008	0.01±0.004	0.008±0.007	nd
C20:2n6	0.09±0.03	0.04±0.01	0.03±0.02	0.0005±0.002
C20:3n6	0.05±0.02	0.02±0.007	0.02±0.006	0.009±0.009
C20:4n6	0.09±0.02	0.08±0.01	0.07±0.02	0.06±0.01
C22:2n6	0.009±0.01	0.001±0.003	0.0007±0.002	nd
∑Omega-3	1.24±0.005	0.63±0.001	0.54±0.002	0.91±0.0003
C18:3n3 ALA	0.20±0.06	0.07±0.02	0.07±0.04	0.001±0.002
C18:4n3	0.06±0.02	0.02±0.007	0.02±0.01	nd
C20:3n3	0.01±0.008	0.003±0.004	0.004±0.005	nd
C20:5n3 EPA	0.34±0.10	0.22±0.06	0.17±0.07	0.06±0.01
C22:5n3 DPA	0.18±0.05	0.09±0.02	0.08±0.03	0.03±0.01
C22:6n3 DHA	0.45±0.11	0.23±0.06	0.20±0.08	0.10±0.04
(n-3/n-6)	0.67	0.85	0.84	1.60
20:5n3/22:6n3	0.76	0.96	0.85	0.60
20:4n6/22:6n3	0.20	0.35	0.35	0.60

Notes: Values (mean ± SD) are concentrations (g/100g) of FAs (nd = not detected). SD of ∑ calculated using pooled standard deviation. † Totals include Omega-6 and Omega-3 fatty acids.

Fatty acid composition varied among each of the tissues. Other than outer-caudal, all tissues had the greatest portion of their fatty acid composition from monosaturated fatty acids, which was largely driven by 18:1n9. This fatty acid accounted for 32.81%, 29.85% and 30.62% in muscle, adipose and inner-caudal respectively (Table 1). The greatest portion of outer-caudal composition was composed of saturated and polyunsaturated fatty acids. The primary contributing fatty acid in outer-caudal was saturated fatty acid 16:0, contributing 23.12% to this tissue's overall makeup (Table 1). While polyunsaturated fatty acids, made up of omega-3 and omega-6 fatty acids, also contributed a large portion to tissue composition, this was driven by the omega-6 group, rather than omega-3's for muscle, adipose and inner-caudal, with 26.39%, 17.41% and 18.04% contribution respectively (Table 1). While in the outer-caudal tissue, the polyunsaturated group composition was driven by omega-3 fatty acids (23.1%) (Table 1).

Concentrations of each fatty acid and/or fatty acid groups within each tissue followed a similar pattern to composition. All tissues, except for outer-caudal, had the highest concentrations of monounsaturated fatty acids, of which 18:1n9 was the largest contributor with 3.26 g/100, 1.27 g/100g, and 1.11 g/100g for muscle, adipose, and inner-caudal respectively (Table 2). As with composition, inner-caudal showed the highest concentrations of saturated and polyunsaturated fatty acid groups, with 16:0 making up the largest fatty acid concentration (0.19 g/100g) (Table 2). A similar trend to fatty acid composition was found where, the polyunsaturated fatty acid concentration was driven by the omega-6 fatty acids, in muscle, adipose and inner-caudal, having greater concentrations of omega-6 fatty acids than omega-3 fatty acids (1.84 g/100g, 0.74 g.100g, and 0.6 5g/100g: respectively) (Table 2). The outer-caudal showed greater concentrations of omega-3 fatty acids (with 0.91 g/100g) than omega-6's (Table 2).

A similarity percentage analysis (SIMPER) was conducted on fatty acid composition to evaluate the average dissimilarity within and between tissue groups. The SIMPER analysis revealed minimal dissimilarity within tissue groups (Figure 6). Outer-caudal showed the highest level of dissimilarity of all tissue groups, with an average squared distance of 15.84, indicating a more diverse fatty acid composition between tissue samples. Muscle and inner-caudal displayed moderate levels of dissimilarity among the four tissues, with average squared distances of 12.02 and 10.80; respectively. Adipose fin tissue displayed the least amount of dissimilarity, with an average squared distance of 7.83, indicating a more uniform fatty acid composition among tissue samples (Figure 6). Fatty acid 18:1n7 was driving the dissimilarity between outer-caudal tissue samples highest, making up 21.5% of the dissimilarity, whereas dissimilarity in the inner-caudal tissue was driven by fatty acids 20:3n3, 20:0 and 17:0 (11.49% 10.48%, and 10.18% of dissimilarity; respectively). In adipose tissue. the primary fatty acid contributing to dissimilarity was 20:3n3 (14.78%) and in muscle tissue dissimilarity was driven by fatty acid 22:1n11 (30.66%).

The greatest degree of dissimilarity in fatty acid composition was between tissue types (Figure 6). The outer-caudal and muscle tissue had the greatest dissimilarity between all tissues (130.16 average squared distance), with contribution to dissimilarity equally distributed between most fatty acids. Adipose and muscle displayed the lowest levels of dissimilarity, with 14:1n5 and 22:1n11 contributing 15.66%, 14.56%, and 12.38%; respectively. Inner-caudal and muscle showed moderate levels of dissimilarity, with 14:1n5 and 22:1n11 also contributing the greatest to the differences between the tissues (11.87% and 11.04%; respectively). Dissimilarity of outer-caudal and adipose, as well as outer-caudal and inner-caudal, was driven by relatively equal

contributions from each fatty acid. Inner-caudal and adipose dissimilarity was driven by 17:0, 15.0, and 20:3n3 (11.24%, 11.07%, and 10.87%; respectively).

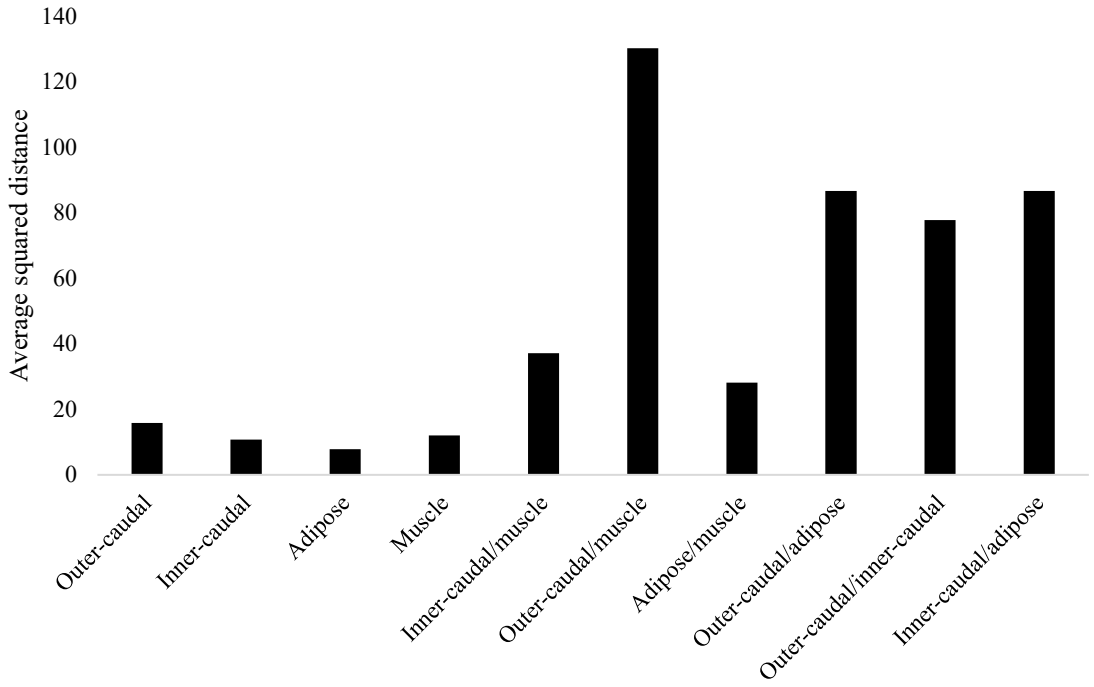

Figure 6. Within group average squared distance for each tissue, based fatty acid composition (% of total), using average squared distance, as calculated using similarity percentage analysis.

A similarity percentage analysis (SIMPER) conducted on fatty acid abundance also revealed dissimilarity within and between tissues (Figure 7). Minimal dissimilarity was exhibited within the outer-caudal group, with an average squared distance of only 2.06, while inner-caudal and adipose tissues showed moderate levels of dissimilarity, with average squared distances of 9.01 and 5.28; respectively. Muscle tissue showed the most dissimilarity with an average squared distance of 23.35 (Figure 7). Dissimilarity within outer-caudal fatty acid concentrations was

largely driven by the fatty acid C24:1n9, contributing 58.37% to the overall dissimilarity. Dissimilarity with inner-caudal tissue was driven by 24:1n9 (15.19%), whereas dissimilarity within adipose tissue was driven by 20:4n6 and 24:1n9 (11.94% and 15.88%: respectively). The two main fatty acids contributing to dissimilarity in muscle tissue were 14:1n5 and 22:2n6, contributing 11.36% and 11.94% respectively.

Substantial differences in fatty acid concentrations were observed between between the various tissue types. Outer-caudal and muscle tissues showed the largest degree of dissimilarity of all between group comparison, with an average squared distance of 150.94, with the dissimilarity being driven by all fatty acids in a relative equal contribution. Inner-caudal and muscle tissues had a high level of dissimilarity, with an average squared distance of 96.31, as did adipose and muscle tissues with an average squared distance of 78.27 Inner-caudal and adipose showed the least amount of dissimilarity of all between group comparisons, with an average squared distance of 14.67, to which 24:1n9 and 20:4n6 contributed the most towards dissimilarity (14.76% and 10.99%; respectively). Outer-caudal and adipose tissues showed lower levels of dissimilarity which was driven by 24:1n9 and 20:5n3 with 8.26% and 7.96% contribution; respectively. Outer-caudal and inner-caudal also showed low levels of dissimilarity driven by 24:1n9, contributing 11.23% (Figure 7).

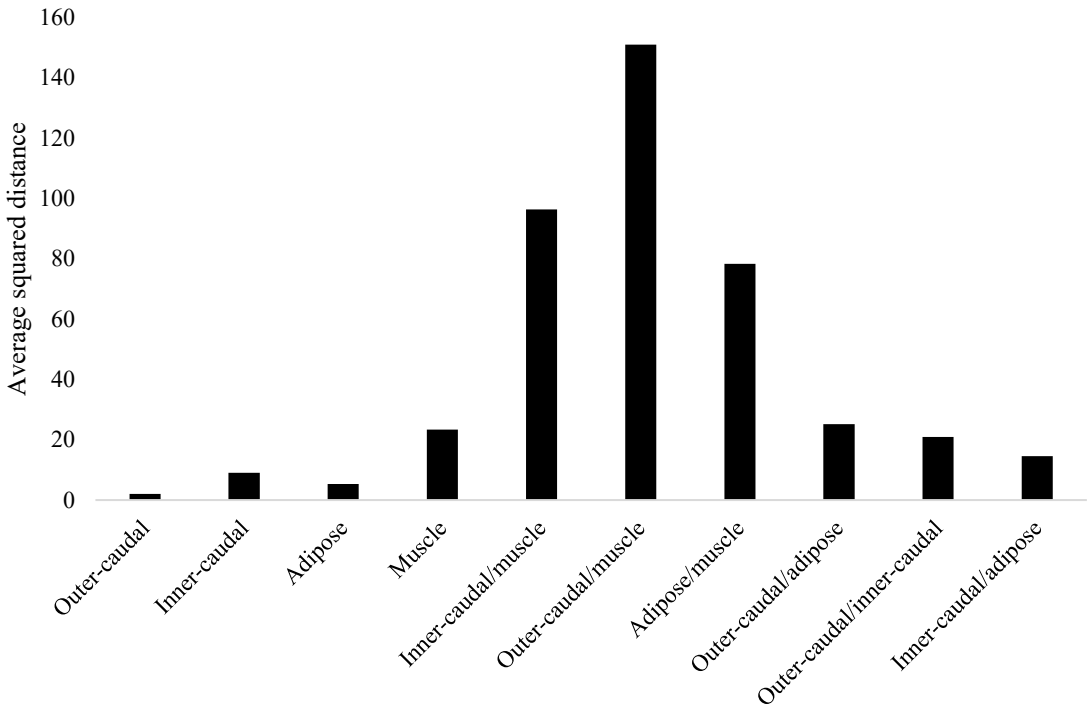

Figure 7. Within group average squared distance for each tissue, based fatty acid abundance (g/100g), using Euclidean distance, as calculated using similarity percentage analysis.

Linear regressions of each tissue against muscle were conducted on both the fatty acid composition and concentration data sets. There was a strong relationship between adipose fin and muscle tissues for both fatty acid composition and concentration (R^2=0.98, p<0.0001 and R^2=0.98, p<0.0001; respectively) (Figure 8). There was also a strong relationship between inner-caudal and muscle tissues for both fatty acid composition and concentration (R^2=0.99, p<0.0001 and R^2=0.99, p<0.0001; respectively) (Figure 9). While the relationships between outer-caudal and muscle tissue for either fatty acid composition and concentration were not as strong as either adipose to muscle or inner-caudal to muscle, they were significant (R^2=0.64, p<0.001 and R^2=0.62, p<0.0001 for fatty acid composition and concentration respectively) (Figure 10).

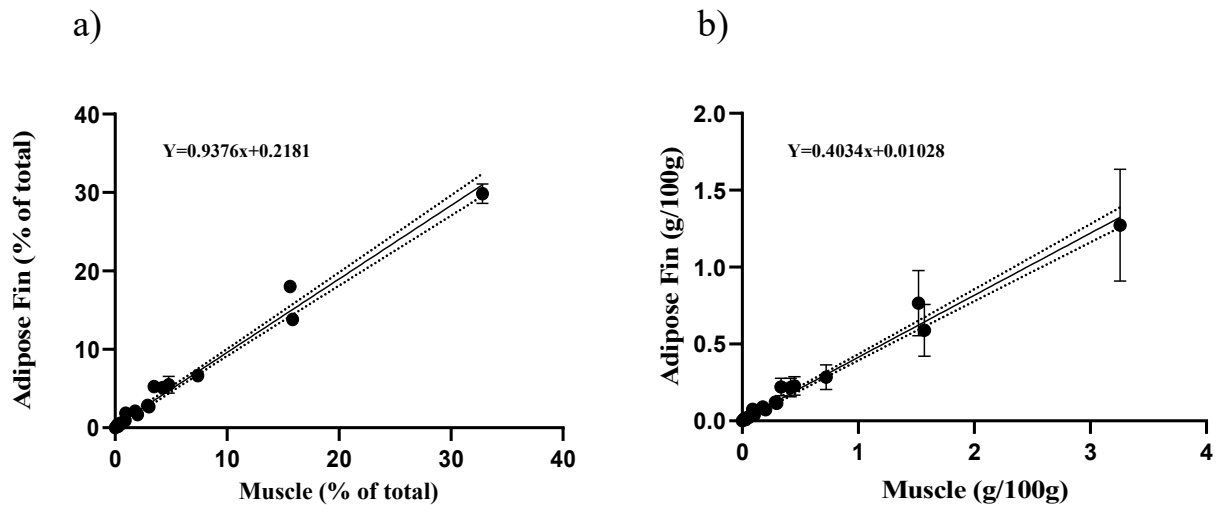

Figure 8. Relationship between adipose fin and muscle tissue fatty acids for: a) concentrations (% of total) and b) abundance (g/100g). The solid line represents the line of best fit and the dotted lines represent the 95% confidence interval, with each dot representing a single fatty acid and error bars representing standard deviation.

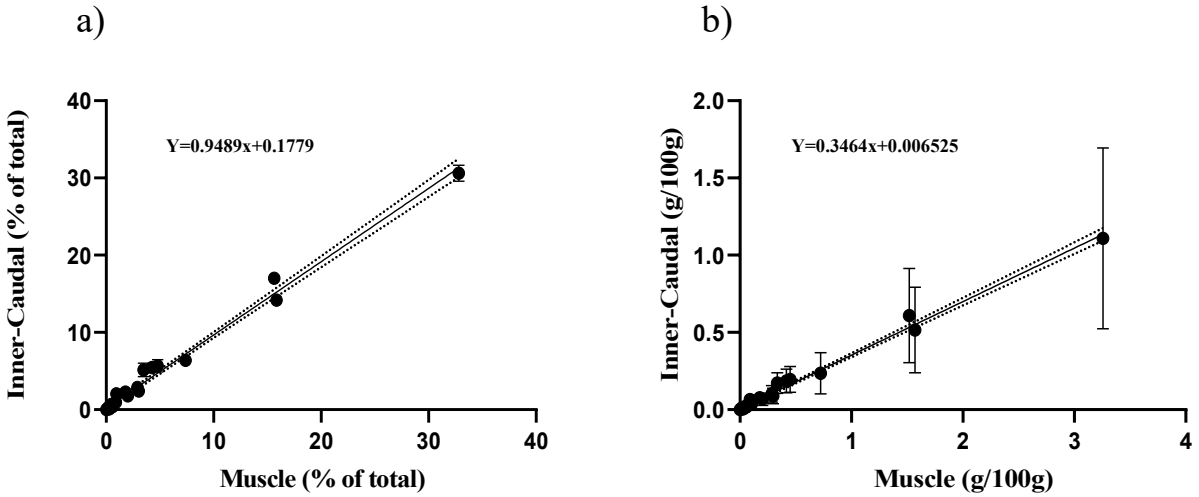

Figure 9. Relationship between inner-caudal and muscle tissue fatty acids for: a) concentrations (% of total) and b) abundance (g/100g). The solid line represents the line of best fit and the dotted lines represent the 95% confidence interval, with each dot representing a single fatty acid and error bars representing standard deviation.

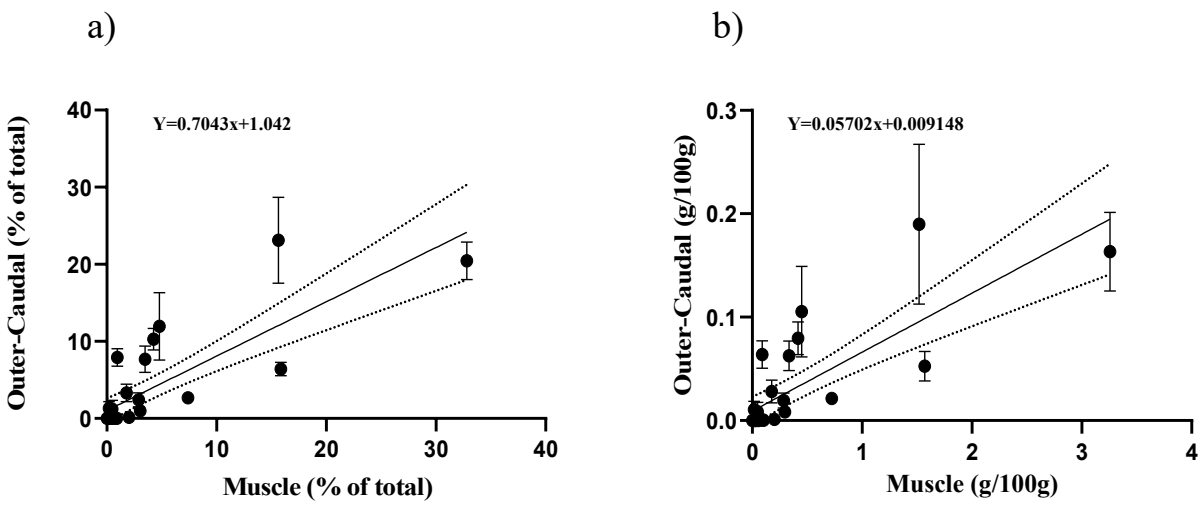

Figure 10. Relationship between outer-caudal and muscle tissue fatty acids for: a) concentrations (% of total) and b) abundance (g/100g). The solid line represents the line of best fit and the dotted lines represent the 95% confidence interval, with each dot representing a single fatty acid and error bars representing standard deviation.

Discussion

The goal of this study was to identify a tissue in fish, that could be sampled non-lethally, with a fatty acid profile representative of muscle tissue, with the purpose of using fatty acids as a biomarker for ecological studies. This study identified strong relationships in both fatty acid composition and concentration between muscle and fin tissues (*i.e.,* adipose, inner-caudal, and outer-caudal) in Atlantic salmon. The results showed that adipose fin tissue is an excellent candidate as a fatty acid biomarker in salmonids, with a high degree of correlation to muscle tissue composition and concentration of fatty acids. Additionally, outer-caudal fin tissue could be an ideal non-lethal tissue sample for fatty acid analysis in other finned fish, therefore meeting this study's objective.

The adipose fin tissue proved to be a representative tissue from which to assess fatty acids non-lethally. PCA results demonstrated strong overlap with muscle tissue, and Euclidean distance analysis indicated minimal variability between adipose tissue samples. The PERMANOVA analyses conducted on adipose and muscle tissue composition and concentration further supported the overlap between tissues, indicating great similarity. The minimal intra-adipose fin variability was further demonstrated by the SIMPER analysis, which showed the lowest within-group dissimilarity (average squared distance = 7.83), highlighting its consistency and reliability, compared to all other tissues. This highlights the precision of fatty acid sampling in adipose fin tissue, making it the most reliable tissues to sample. The Linear regression analysis revealed a strong, predictive relationship between adipose and muscle tissue, suggesting that adipose tissue provides the most representative non-lethal alternative for fatty acid profiling in fish populations.

Inner-caudal tissue also showed a strong relationship with muscle tissue. The PCA results positioned inner-caudal tissue closer to muscle than outer-caudal tissue, however it demonstrated more variability than adipose tissue. While PERMANOVA results confirmed small differences between inner-caudal and muscle tissue composition and concentration, the overall correlation between the two remained high. The variance in fatty acid composition and concentration along the caudal fin suggests that careful attention should be paid to sampling location to avoid potential inconsistencies.

Outer-caudal tissue proved to be more dissimilar to the other tissues, but may still serve as a valuable tissue for non-lethal fatty acid sampling in finned fish. This tissue exhibited the most distinct fatty acid profile, showed the highest intra-fin variability in fatty acid composition, while its concentration remained relatively consistent. The correlation between outer-caudal and muscle tissue was significant but lower than other tissues ($R^2 = 0.64$ for composition, $R^2 = 0.62$ for concentration). In ecological studies, R^2 values can vary greatly, however values between 0.20 to 0.50 are considered acceptable or good (Lin and Wiegand, 2023). Therefore, the outer-caudal tissue remains a promising candidate to serve as a viable non-lethal alternative for fatty acid sampling in ecological studies.

A consistent and strong relationship between muscle and adipose fin tissue has identified adipose fin as a non-lethal tissue alternative in Atlantic salmon. Due to minimal intra-adipose fin variability, it could be argued that adipose fin tissue may be more desirable than muscle tissue for sampling purposes. While the accuracy and consistency of this method in other salmonids is yet to be explored, the results from this study suggest, significant potential for use of these non-lethal fatty acid sampling methods. The relatively strong relationship between outer-caudal and

muscle tissues supports the development of a minimally invasive non-lethal sampling method that could be applicable not only to Atlantic salmon but also to other finned fish species. The models identified through linear regressions can be used to correct for both composition and concentration. The strength of the relationship found between outer-caudal and muscle tissue demonstrates its value as an ecological monitoring tool.

Although inner-caudal tissue cannot be non-lethally sampled, this study demonstrated the intra-fin variability in fatty acid composition and concentration along the caudal fin, providing additional insight into non-lethal fatty acid sampling, beyond the objective. Muscle tissue, being one of the most common tissues for fatty acid analysis in fish, is a homogenous sample in comparison to fish fin (i.e., bone, connective tissue, and skin). The area from which outer-caudal was sampled contained fin rays, which are composed mainly of bone (calcium carbonate) and collagen (Flammang, 2014). As a result, this section of fin lacks great concentrations of fatty acids. These observations allow for speculations on the relationship between fatty acid composition and concentration and the anatomical make-up of the caudal fin. The area from which inner-caudal was sampled was within closer proximity to muscle tissue, which is where the majority of lipids are stored in Atlantic salmon (Schneider & Sulner, 2006; Aursand et al., 1994). As a result, inner-caudal fatty acid trends were more closely related to those of muscle tissue. The heterogenous caudal fin tissue increases the potential for intra-individual variability along the length of the fin, which highlights the importance of sampling location consistency, but has rarely been studied. One study looking at isotope ratios along the length of Atlantic salmon similarly found differences between the tip and the base of the fin, indicating different isotope biomarker composition along the caudal fin (Hayden et al., 2015).

Current stock assessment objectives include gaining estimates of abundance, total stock biomass, biological processes, environmental conditions impacting the stock, harvest rates and natural mortality. This information is collected through both fishery-dependent and fisheries-independent methodologies (Government of Canada, 2024). However, these stock assessments often overlook the physical condition of the individuals contributing the stock. Implementing non-lethal fatty acid sampling (e.g., the collection of adipose outer-caudal fin tissue) to enhance stock assessments, would allow for better understanding of the physiological condition of the individuals and would provide further insights into survival and reproductive capacity, enhancing pre-existing estimates. Collecting this additional information during stock assessment could also impact fisheries management decisions, potentially leading to adjustments of harvest rates, seasonal closures and size limits.

Despite the growing interest in identifying non-lethal sampling methods in ecology and more specifically ichthyology, research aimed at non-lethal sampling for the purpose of fatty acid profiling has yet to be conducted, prior to this study (Jeffries et al., 2021). Having identified tissues that can be used to accurately provide a representative fatty acid profile through non-lethal methods, will allow fatty acid studies to occur on populations that could not previously be sampled. This may also increase fatty acid biomarker studies overall. Because of this methodology, more frequent and larger-scale studies can be conducted across different habitats, populations, or regions. This will be especially insightful in areas of high biodiversity to gain a greater understanding of predator-prey relationships, temporal changes in food availability, or indicating environmental or chemical stressors (Filimonona et al., 2016; Fonseca et al., 2022). This will in turn, allow for a deeper understanding of dietary composition, enhanced food web monitoring, migration patterns, and total metabolism (Lovern, 1935; Saddler et al., 1972).

Understanding these patterns will provide a foundation for ecological decision makers in order to further promote conservation and protection of these species (Cooke et al., 2017).

This study demonstrates that adipose fin tissue provides a reliable non-lethal alternative for fatty acid profiling in fish, given its strong correlation with muscle tissue, minimal variation, and consistency in fatty acid composition and concentration. While outer-caudal tissue exhibited greater variability, its consistency in fatty acid concentration suggests it may still serve as a viable non-lethal sampling option. The consistent, strong relationship between muscle and fin tissues facilitates the use of to be used to correct for both composition and concentration, allowing direct comparisons to published and future studies where muscle was used. The findings of this study have significant implications for ecological research and conservation, providing a foundation for non-lethal monitoring of fatty acid profiles in fish populations. This methodology will allow greater insight into fish diet, climate change and ecosystem changes, as well as overall energy availability. The methods identified in this research are hoped to become the new gold standard for fatty acid sampling in the conservation and protection of fish species. Future research should explore the applicability of these methods to other species, further supporting the use of non-lethal techniques in ecological studies and fisheries management.

Contributions to Thesis

During this project, I was responsible for collecting tissue samples from a salmon processing plant, as well as conducting lipid extractions for all samples. Following methodology, I conducted all statistical analyses included in this study and explained my project through writing my thesis.

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