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# Stable isotope analysis to assess fractionation in five tissues types of yearling Snake River spring Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract

Stable isotope analysis use allows for the quantification of habitat impact in highly migratory species such as Chinook salmon (*O. tshawytscha*). To provide baseline stable isotope (SI) values and discrimination factors for Snake River spring Chinook, we investigated the caudal fin, dorsal muscle, liver, plasma, and red blood cell tissue of hatchery reared spring/summer juveniles from the McCall Hatchery in Idaho. Understanding tissue specific SI values and fractionation factors, as well as variation in a diet-controlled study, will help future researchers choose tissue types appropriately. Baseline SI will allow for comparison to future field studies to provide insight into the role of different habitats and available prey in the migration of Snake River yearlings. The tissue types listed above, as well as hatchery feed, were analyzed for baseline $\delta^{13}C$ and $\delta^{15}N$. These values provided $\delta^{13}C$ and $\delta^{15}N$ values in different tissue types, so that fractionation and SI values may be compared with further field study. We found that tissue types often had high variation in both $\delta^{13}C$ and $\delta^{15}N$ SI values, were statistically different for both $\delta^{13}C$ and $\delta^{15}N$, and were overall poorly correlated between tissue types. Physical factors, such as fork length (FL), weight, fish condition, and C:N ratio also poorly described the variation within tissue types. Further laboratory study with more variables controlled (growth, temperature, size) could provide a more accurate depiction of SI interactions.

Introduction

Historically, the Columbia River Basin (CRB) is known for its abundance of salmonid species (*Oncorhynchus* spp.), as salmon have great economic, ecologic, and cultural significance (Quinn 2005). Prior to European settlement, the CRB had the largest Pacific salmon runs in the world (Weitkamp et al. 2012). Due to increased anthropogenic activity, runs have been reduced to 10% of their historical levels, and half the salmon populations in the CRB are extirpated from native reaches (PFMC 2011; Gustafson et al. 2017). The most at-risk populations are listed under U.S Endangered Species Act (which includes 5 subgroups of Steelhead, 1 subgroup of Coho, Chum, and Sockeye, and 5 subgroups of Chinook Salmon) (Weitkamp et al. 2012). Due to the rapid decrease of wild salmon, large-scale hatchery rearing programs were implemented as compensation. Hatchery rearing has become so prolific that, in CRB Chinook Salmon (*O. tshawytscha*), approximately 80% of population is comprised of hatchery-reared fish (Weitkamp et al. 2012).

Stock-specific success of yearling Chinook Salmon is often measured by adult returns, which has been positively correlated with juvenile abundance in the estuary, mean size at capture, and marine growth rates (Tomaro et al. 2012). The Snake River's spring/summer stock has seen a massive population decline since the installation of hydroelectric dams in the CRB (Williams 1989). Overall juvenile mortality is estimated to be 40-60% during emigration, as individuals must maneuver up to nine dams en route to the ocean (Raymond 1988). Due to vast migration distance and slow growth rates in colder waters, interior stocks enter the Columbia River estuary nearly a month later than other major stock groups, and the Snake River spring Chinook yearlings are, on average, 10-20 mm shorter than other stocks (Weitkamp et al. 2015). Later entry and more strenuous migrations could be negatively affecting adult returns; however, assessment of a highly migratory species such as Chinook proves challenging, resulting in a lack of understanding about the actual mechanisms behind juvenile survivorship (Weitkamp et al. 2015).

Possibly the least understood length of Snake River yearling migration is the role of the Columbia River estuary. Physical properties such as salinity, tidal stage, temperature, and depth are inherently variable over small spatial and temporal scales (Weitkamp et al. 2012). These physical attributes of the estuary can be influenced by basin scale phenomena such as the Pacific Decadal Oscillation, North Pacific Gyre Oscillation, and more local events such as upwelling that may affect survivorship in juveniles (Miller et al. 2014). Miller et al. (2014) found that the Columbia River plume area and volume was positively correlated with juvenile mass at capture during marine residence. Due to the immense variability within the system, the importance of the Columbia River estuary in the survival of Snake River yearling Chinook remains generally unknown. However, estuaries can provide juvenile salmon productive foraging opportunities, historically decreased pressure from predation, and an environmental intermediate between fresh and salt water (Simenstad et al. 1982; Thorpe 1994; Bottom et al. 2005). Within an estuary, healthy wetlands provide increased habitat and prey availability, which can increase early marine growth rates and survivorship (Gray et al. 2002; Magnusson & Hilborn 2003).

Due to the highly migratory nature of salmon, quantifying the importance of certain environments can prove to be a challenge. Yearling salmon reside in estuaries for a relatively short period of time, creating difficulty in tracking foraging habits and prey selection. Stable isotope analysis has recently been applied to fisheries research; it's application as an

ecological tracer of migratory animals allows for the quantification of how different environments impact individuals (Maier & Simenstad 2009, Heady & Moore 2013; Vander Zanden et al. 2015). Analysis of the ratios of stable isotopes in various tissues yields fractionation factors and $\delta^{15}N$ and $\delta^{13}C$ values. Fractionation/discrimination factors illustrate a shift in the isotopic ratio between the food source and the tissue type due to metabolic processes. Incorporation rates of diet isotopic signatures, or tissue turnover rates, are also based on the different metabolic pathways for individual tissue types. In Steelhead (*O. mykiss*), turnover rates in tissues can vary greatly, from 12.9 days in fin tissue to 40.0 days in scale tissue (Heady & Moore 2013). Stable isotope fractionation is useful in quantifying the incorporation of prey signals from resident time in the Columbia River estuary, specifically through analysis of fast-turnover tissues such as fin, plasma, and liver. $\delta^{13}C$ SI signatures will allow us to track carbon through the food web, and sources of organic compounds. $\delta^{15}N$ signatures allow us to track trophic position, with individuals in higher trophic levels expressing higher $\delta^{15}N$ levels as heavier nitrogen isotopes are assimilated into tissue more than lighter isotopes. Enrichment in the SI values is a result of the metabolism of heavier nitrogen isotopes. Typical trophic fractionation is 3.4‰ for $\delta^{15}N$ between prey and predator (Post 2002).

Stable isotope analysis of the Snake River yearling migration through the CRB will estimate a quantitative time frame of $\delta^{15}N$ and $\delta^{13}C$ values, and fractionation rates at each sampling site. Nitrogen ratios will illustrate the trophic level of ingested prey, and carbon ratios will indicate the marine or aquatic food source signatures in order to track foraging shifts in the migrating juveniles. Understanding SI values in individual tissue types and how tissue types relate to each other can help researchers choose which tissue type will help answer their research questions. Previous field studies have shown that fin and muscle are well correlated (Hayden et al. 2017), however little is know about the relationship and variation between tissue types when diet is controlled for.

Stable isotope field data illustrates a point in time that provides insight into the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in various tissue types, which can be related to marine influence and trophic level of prey items. However, without a baseline for comparison, conclusion from field data will be hard to support. By identifying and providing tissue specific fractionation rates, and initial $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in five different tissue types, and levels of inherent variation within a controlled system, this study will researchers greater ability to draw conclusions by comparison with baseline values and individual variations.

*The goals of this study were as follows:*

*(i)* *Identify tissue specific discrimination factors and individual variation between tissue types*

*(ii)* *Characterize and evaluate factors impacting variation within and among tissue types*

*(iii)* *Provide baseline tissue-specific discrimination rates and $\delta^{15}N$ and $\delta^{13}C$ values from hatchery yearlings as a metric to interpret field data to contribute to the future study to track Snake River spring yearlings throughout their migration and further understand the importance of estuarine residence in the success of Snake River spring yearlings.*

## Materials and Methods

*Idaho Stock Collection*

To determine baseline stable isotope values, Snake River spring Chinook yearlings were sampled at their migratory origin, McCall hatchery on the Snake River in Idaho. While there are 6-8 hatcheries operating on the Upper Snake River, samples were only collected from the McCall hatchery. Caudal fin, plasma, red blood cells, dorsal muscle, and liver tissue samples were collected on site and preserved with dry ice to be further processed in the laboratory. In the lab, caudal fin, liver, and dorsal muscle tissue samples were rinsed with deionized water, weighed, and dried at 60 °C for two days. These samples were then ground to a homogenized powder using mortar and pestle (Levin & Currin 2012). Whole blood, plasma and red blood cells were directly transferred into tin capsules for stable isotope analysis. All individuals included in this study were females, as all males were used in a related project on early maturation.

*Stable Isotope Analysis*

To quantify the incorporation of the estuarine signals in tissue samples, Carbon and Nitrogen discrimination factors were measured by analyzing the stable isotope ratios in muscle, fin, liver, red blood cell, and blood plasma tissue as well as hatchery food. Tissue samples were prepared based on Levin & Currin (2012) and $\delta^{15}N$ and $\delta^{13}C$ values were measured in the Stable Isotope Laboratory (http://stable-isotope.coas.oregonstate.edu/) at Oregon State University on a Carlo Erba NA1500 coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer. $\delta^{13}C$ was calibrated daily using the international standards USGS40 (glutamic acid 40, $\delta^{13}C$ = -26.389 permil vs VPDB) and the lab standard SIL Sucrose ($\delta^{13}C$ = -11.85 permil vs VPDB). The $\delta^{15}N$ was calibrated daily using the international standards USGS40 (glutamic acid 40, VPDB, $\delta^{15}N$ = -4.52 permil vs air) and the international standard IAEA-N2 (ammonium sulfate, $\delta^{15}N$ = +20.3 permil vs air). Typical error for $\delta^{15}N$ is $\pm 0.2$ mil$^{-1}$, and typical error for $\delta^{13}C$ is $\pm 0.1$ mil$^{-1}$.

Samples from the McCall hatchery stock tissue provide baseline $\delta^{15}N$ values to track trophic levels of fish diet, with higher nitrogen levels correlating with higher trophic levels. $\delta^{13}C$ values indicative of the dietary carbon were compared to hatchery feed for analysis of fractionation once incorporated into tissue. Samples of hatchery food (predominantly composed of marine lipids) were analyzed to illustrate baseline stable isotope fractionation rates in tissue types listed above. Comparing baseline carbon and nitrogen values/fractionation show how tissue types relate, while initial values will allow us to compare samples from below Bonneville Dam and samples from the mouth of the estuary. Fin tissue provides integrated diet signal across 7-10 days (Heady & Moore 2013) allowing for a more accurate future analysis of foraging habits of juvenile salmon during estuarine inhabitance. Muscle tissue has a turnover rate of around 39 days, RBC around 38 days, liver around 16 days, and plasma around 14 days (Heady & Moore 2013). While incorporation rates have been previously explored in other salmonid species, SI values and variation within and among tissue types in juvenile Chinook is for the most part unknown.

Samples of hatchery food used at McCall hatchery were analyzed for the isotopic signature. Bio-Olympic salmonid feed was weighed, dried, and pulverized as outlined previously. All fish within this study were fed the same feed, minimizing variation due to diet that may impact tissue SI values.

Lipids present in tissue have a different isotopic signature than tissue itself, impacting the accuracy of SI values (Post et al. 2007). Correcting for lipid content is often preformed when lipid content is directly measured in each tissue type. As we did not directly measure lipid content across tissue types, we relied on results and procedures from previous studies (Post et al. 2007, Litz et al. 2016). Based on results from Post et al. (2007), lipid corrections factors were applied to $\delta^{13}C$ values for all samples. Samples were corrected with the formula: $\Delta\delta^{13}C = -3.32 + 0.99 * C{:}N$. We chose to lipid correct all values instead of only ones with C:N ratios below 3.5, as values below 3.5 made up a small portion of the population within each tissue (RBC=9.5%, Muscle=26.2%, Fin=0%, Liver=0%, Plasma=0%).

*Statistical Analysis*

To investigate whether $\delta^{13}C$ and $\delta^{15}N$ stable isotope values differ statistically between tissue types, an analysis of variance (ANOVA) was run with tissue type as a categorical factor with carbon, nitrogen and C:N as response variables. Analyzing variation between tissue types illustrated how tissue specific SI values and discrimination factors differ when diet is controlled. A post hoc Tukey multiple mean comparison was run to show which tissues were significantly different or similar. All data was checked for trends, normal distribution and homoscedasticity. Directionally skewed data were transformed accordingly to meet the assumption of normal distribution within an analysis of variance.

FL, weight, and especially fish condition are often used as metrics for growth rate and fish health (Miller et al. 2011, Tomaro et al. 2012, Miller et al. 2013). Fish condition is a metric determined by FL and weight, calculated by $[(100{,}000*\text{Weight})/(FL^3)]$. Fish condition is useful when FL and weight do not provide an accurate fish health metric on their own. In order to explain variability between individuals within the data, correlation were run between stable isotope values and ratios, and an individual's physical attributes. Correlation coefficients were calculated between all physical factors and stable isotope values in all tissue types to help visualize degrees of variation within the dataset.

Due to variation in carbon isotope discrimination rates in lipids, $\delta^{13}C$ values in all tissues were corrected for lipid content based on Post et al. (2007). To determine a cutoff for lipid correction, and investigate the impact of lipid correction on the interpretation of data, correlations between corrected and uncorrected, and physical variables were run.

Statistics were run in RStudios (version 1.0.143).

Results

This study included 42 juvenile Snake River spring stock yearling Chinook salmon from the McCall Hatchery on the Upper Snake River in Idaho. Average fork length was 119.6 mm, with a range from 105-141 mm. Average weight of individuals was 19.90 grams, with a minimum of 12 grams and maximum of 31.3 grams. Average fish condition of the population was 1.15 with a range from 0.98 to 1.34. Means across stable isotope values and tissue types, along with standard deviations and coefficients of variance can be found in Table 1.

There was a statistically significant difference in $\delta^{13}C$ values between tissue types as determined by a one-way ANOVA and post hoc Tukey-Kramer analyses ($F(4,232)=201.5$, $p<0.001$). Liver and fin $\delta^{13}C$ are statistically different, with a percentage difference of 12.4 % enrichment $mil^{-1}$ (95% confidence limits, p-value<0.001). Average percent difference between muscle and fin was 3.3% enrichment $mil^{-1}$, with statistically significant difference between means (95% confidence limits, p-value<0.001). Plasma-Fin, RBC-Fin, Muscle-Liver, Plasma-Liver, RBC-Liver, Plasma-Muscle, and RBC-Plasma were all significantly different in $\delta^{13}C$ values, with average percent differences of 18.7%, 6.2%, -8.1%, 5.4%, -5.8%, 14.7%, and -10.6% respectively (values in percent $mil^{-1}$, 95% confidence limits, p-values<0.001). RBC and muscle had an average percent difference of 2.3% $mil^{-1}$, and were statistically different (95% confidence limits, p-value=0.003). As a lipid correction was applied to all the $\delta^{13}C$ values, an ANOVA was run to compare means of lipid corrected carbon SI values between tissue types. Average percent differences are displayed to visualize the variance between tissue types (Table 3).

$\delta^{15}N$ values between tissue types showed a significant difference, although post hoc analysis determined that two means were not significantly different. Liver-Fin, RBC-Fin, Muscle-Liver, Plasma-Liver, Plasma-Muscle, RBC-Plasma, and RBC-Muscle were all significantly different (p-value<0.001, average percent differences in Table 2). Plasma-Fin and RBC-Liver were not statistically different within 95% confidence limits.

Ratio of carbon and nitrogen percentages were statistically different between groups based on results from a one way ANOVA ($F(4,205)= 226.8$, p-value<0.001). RBC-Muscle, RBC-Fin, and Muscle-Fin were not statistically different (Table 4), but RBC-Muscle and RBC-Fin means were closer than Muscle-Fin values (p-values of 0.80, 0.40 and 0.03 respectively). Plasma and liver had higher C:N ratios than the other tissue types, but were poorly correlated (p-value<0.001).

One sample of the hatchery grade salmonid feed used at the McCall Hatchery was analyzed to determine tissue-specific fractionation/discrimination factors. Bio-Olympic hatchery food has a $\delta^{13}C$ of -22.15 $mil^{-1}$ and a lipid corrected $\delta^{13}C$ of -19.43 $mil^{-1}$. A more negative $\delta^{13}C$ value is indicative of the high amount of fatty fish oils sourced from marine fish in the feed. More positive $\delta^{15}N$ values indicate a higher trophic level. Trophic enrichment of $\delta^{15}N$ is around 3.4‰ per trophic level. Tissue-specific fractionation factors for both $\delta^{13}C$ and $\delta^{15}N$ can be found in Table 6. Fin tissue was the closest tissue to full trophic enrichment (3.25‰) but all the tissues were around the theoretical 3.4‰ (Post 2002).

Variation within and between tissue types yielded interesting results as we explored possible reasons for such a high degree of variation (Figure 1). The assumption that tissue types can be used as proxies for each other was not supported within the data. Individuals with the highest carbon values within muscle tissue could have the lowest carbon values in another tissue type. Antithetical to results from Hayden et al. (2017), muscle and fin were poorly correlated, and described little variance in each other (Figure 2).

Variation within tissue types (Table 1) resulted in poor correlations between tissue types for both $\delta^{13}C$ and $\delta^{15}N$ values (Figure 2 & 3), and C:N ratios (Figure 4). For the most part, tissue types are poor predictors of each other for all three response variables. Statistically, $\delta^{13}C$

values in red blood cells and liver tissue are correlated ($R^2$=0.28, p-value<0.001). A linear model of $\delta^{15}N$ values in plasma and liver describes around 35% of the variation between the two, with a statistically significant correlation (p-value<0.001). Muscle and fin are also statistically correlated ($R^2$= 0.23, p-value=0.0015) in $\delta^{15}N$ values. The remaining tissue type comparisons were poorly correlated in the three response variables (Table 5). Three individuals within our sample population had markedly high (less negative) $\delta^{13}C$ values. Values were within three standard deviations of the mean, and correlations were not significantly impacted by the inclusion or exclusion of these points.

Due to the high amount of variation exhibited above, physical attributes were poorly correlated with stable-isotope values (Figure 5). FL, fish condition, and fish weight described little variation within each stable isotope population. As FL was not well correlated with most tissue types and SI values, it was not included as a covariate in the ANOVA model.

Discussion

Our findings have many implications on our understanding of tissue specific stable isotope values in multiple tissue types of juvenile Chinook. The most significant of these being that our results suggest that there is inherently a very high level of variation within some tissue types compared to others suggesting that factors outside of diet influence stable isotope ratios. Secondly, contrary to previous field study, variation within an individual tissue does a poor job of explaining variation within another, suggesting that factors influencing stable isotope value affect tissue types differently. Thirdly, fish metrics (FL, weight, fish condition, C:N) did not explain variation within tissue types, indicating that sources for variation could be factors that were not controlled for in our experimentation, or inherent variation in metabolic pathways between individuals and tissue types.

Variation of $\delta^{13}C$ and $\delta^{15}N$ Within Tissue Types

High levels of variation within tissue types suggests that factors other than diet could influence tissue specific stable isotope values. As this experiment was not a laboratory study, many factors that were not controlled for could influence stable isotope values. One possible explanation for the variation within both $\delta^{13}C$ and $\delta^{15}N$ values could be that hatchery practices were not consistent across all individuals. Individuals were collected from two different rearing ponds, however if this factor was significantly impacting fractionation factors, then we would expect to see some bifurcation between those two ponds. Means were compared between ponds, as pond origin was recorded, and no significant difference was found. Another factor that could be causing high variation within tissue types is that batches of the Bio-Oregon feed used in the McCall Hatchery could have different isotopic signatures. While we assumed that batches of feed would have little variation due to identical ingredients, individual batches could vary in the amount of marine sourced components. Although all individuals were within the same genetic stock group, and reared within the same environment, some of the variation seen within tissue types could relate to variation in tissue metabolism between individuals. The oxidative capacity to metabolize proteins is often represented by growth rates in fishes (Houlihan et al. 1993). Individuals with higher growth rates are expected to have more protein-metabolism infrastructure, allowing for the correlation between growth and protein synthesis (Houlihan et al. 1993). While there is often negligible variation in the protein composition of tissues, the inability to control for growth

rate could be contributing to the variation within tissue types. The variability within tissues could also be due to faulty methodology in analyzing samples. Some of the samples were ground using a marble mortar and pestle, while the rest were ground using agate. The use of marble did change the stable isotope values of the tissue, but all tissues ground with the marble were corrected for using an acidification process. It is possible that the acidification process did not remove the residuals from the marble mortar, thus skewing the data away from the actual tissue specific value, causing high variation within tissues.

One possible explanation for variation in fin tissue could be the difference in isotopic values between the membranous epithelial and the cartilaginous rays. Hayden et al. (2015) found that in two salmonid species, fin-ray components are depleted around 1‰ in terms of carbon relative to the fin membrane.

Variation of $\delta^{13}C$ and $\delta^{15}N$ Between Tissues

Results from this study indicated high variation in fractionation rates between tissue types, indicating that physiological processes are impacting fractionation factors between tissues. Biochemical pathways to metabolize different tissues vary considerably, and tissues are comprised of different proteins, amino acids, and carbohydrates, all which could be affecting the tissue specific fractionation factors. The structural differences between fish muscle and tissue could be one variable creating such variation. Fish muscle is a homogeneous protein-based matrix while fin is a matrix of cartilaginous rays and membranous epithelium. Hayden et al. (2017) concluded that this structural difference between tissues could be resulting in the enrichment differences between muscle and fin. The same biochemical factors causing this disparity could explain variation between the other tissue types. While it was predicted that tissues would inherently have varying isotopic signatures, our study found little correlation between tissue types, a contrary results to previous studies investigating isotopic relationships between tissues for both $\delta^{13}C$ and $\delta^{15}N$ (Jardine et al. 2005, Sanderson et al. 2009 ,Fincle et al. 2012).

Selectivity of Tissue Type

The results of this study suggest that understanding variation within tissue types for both $\delta^{13}C$ and $\delta^{15}N$ values could help researchers make educated decisions when selecting tissue types in future studies. Depending on the focus of the study, some tissues are a more appropriate choice than others. If diet assessment and $\delta^{15}N$ fractionation are of interest, it is advantageous to select a tissue such as fin that has low variability of $\delta^{15}N$ in a controlled setting. If $\delta^{13}C$ and the sources of organic carbons within the food web are the focus, the selecting fin would not be appropriate as variation is incredibly high, but tissues such as red blood cells or muscle would provide lower degrees of variation. If both $\delta^{13}C$ and $\delta^{15}N$ were of interest, selecting muscle would give researchers greater power in comparison to field data.

Conclusion

This experiment attempted to shed insight into tissue-specific fractionation factors and stable isotope values within five different tissue types of juvenile Chinook Salmon, and provide baseline data for comparison with later field study. While our results were variable,

controlled studies such as these are vital to validate results from field studies that utilize stable isotope analysis. Collecting and analyzing stable isotope data is relatively easy to accomplish, and without understanding of baseline values, conclusions could miss the food web interactions actually occurring. Beyond this study, there is a general need for further laboratory studies into tissue-specific fractionation factors in multiple tissue types across species. Until underlying interactions causing variation in $\delta^{13}C$ and $\delta^{15}N$ are further explored, evaluating the impact of estuarine habitats in migrating juvenile yearling Chinook will prove difficult. Sampling from multiple hatcheries that feed into the headwaters of the Snake River could shed further insight into the actual variation in fractionation factors of different tissue types by expanding the sample size and possibly identifying factors within the McCall hatchery that could cause such high levels of variation. Increasing accuracy in baseline fractionation factors will prove useful in understanding food web linkages within the Columbia River estuary.

Tables and Figures

**Table 1.** Population averages, standard deviations, and coefficients of variance of stable isotope values in Fin, Liver, Muscle, Red Blood Cell (RBC), including Lipid Corrected Carbon (LC Carbon).

| Tissue Type | $\delta^{13}C$ (per mil, VDPB) | | Lipid Corrected $\delta^{13}C$ | | $\delta^{15}N$ (per mil, Air) | | C:N | |
|---|---|---|---|---|---|---|---|---|
| | *Mean(±SD)* | *CV* | *Mean(±SD)* | *CV* | *Mean(±SD)* | *CV* | *Mean(±SD)* | *CV* |
| Fin | -18.53(1.12) | 6.04 | -17.83(1.38) | 7.74 | 12.72(0.14) | 1.10 | 4.06(0.30) | 7.39 |
| Liver | -20.83(0.63) | 3.02 | -17.90(0.89) | 4.97 | 11.84(0.23) | 1.94 | 6.31(1.40) | 22.2 |
| Muscle | -19.16(0.31) | 1.61 | -18.91(0.23) | 1.22 | 12.87(0.22) | 1.71 | 3.61(0.19) | 5.26 |
| RBC | -19.60(0.32) | 1.63 | -19.18(0.17) | 0.89 | 11.95(0.36) | 3.01 | 3.79(0.22) | 5.80 |
| Plasma | -21.93(0.38) | 1.73 | -18.11(0.64) | 3.53 | 12.60(0.25) | 1.98 | 7.22(0.63) | 8.73 |
| Bio-Olympic | -22.15(-) | - | -19.43(-) | - | 9.59(-) | - | 6.10(-) | - |

**Table 2.** Post hoc Tukey multiple comparisons of means results of $\delta^{15}N$ SI values between all 5 tissue types.

| Tissue Comparison | % Difference | $\delta^{15}N$ Enrichment |
|---|---|---|
| Liver-Fin | -6.9* | - |
| Muscle-Fin | 1.2+ | + |
| Plasma-Fin | -0.9 | - |
| RBC-Fin | -6.1* | - |
| Muscle-Liver | 8.7* | + |
| Plasma-Liver | 6.4* | + |
| RBC-Liver | 0.9 | + |
| Plasma-Muscle | -2.1* | - |
| RBC-Muscle | -7.1* | - |
| RBC-Plasma | -5.2* | - |

*p-value<0.001
+p-value<0.05

**Table 3.** Post hoc Tukey multiple comparisons of means results of lipid corrected $\delta^{13}C$ SI values between all tissue types. Average difference has been calculated into % difference.

| Tissue Comparison | % Difference | $\delta^{13}C$ Enrichment |
|---|---|---|
| Liver-Fin | 0.4 | + |
| Muscle-Fin | 6.1* | + |
| Plasma-Fin | 1.6 | + |
| RBC-Fin | 7.6* | + |
| Muscle-Liver | 5.6* | + |
| Plasma-Liver | 1.1 | + |
| RBC-Liver | 7.1* | + |
| Plasma-Muscle | -4.2* | - |
| RBC-Muscle | 1.4 | + |
| RBC-Plasma | 5.9* | + |

*p-value<0.001

**Table 4.** Post hoc Tukey multiple mean comparisons of C:N ratios between tissue types with 95% confidence intervals.

| Tissue Comparisons | % Difference | Direction of Change |
|---|---|---|
| Liver-Fin | 55.4* | + |
| Muscle-Fin | -11.1+ | - |
| Plasma-Fin | 77.8* | + |
| RBC-Fin | -6.65 | - |
| Muscle-Liver | -42.8* | - |
| Plasma-Liver | 14.4* | + |
| RBC-Liver | -40.1* | + |
| Plasma-Muscle | 100* | + |
| RBC-Muscle | 4.71 | + |
| RBC-Plasma | -47.5* | - |

*p-value<0.001
+p-value<0.05

**Table 5.** $R^2$ values of correlations between tissue types across all response variables. Statistically significant results are marked.

| Tissue Type Comparison | $\delta^{13}C$ | $\delta^{15}N$ | C:N |
|---|---|---|---|
| Liver-Fin | <0.03 | <0.03 | <0.03 |
| Muscle-Fin | <0.03 | 0.225** | <0.03 |
| Plasma-Fin | <0.03 | <0.03 | <0.03 |
| RBC-Fin | 0.03 | <0.03 | 0.04 |
| Muscle-Liver | <0.03 | <0.03 | 0.031 |
| Plasma-Liver | <0.03 | 0.35* | 0.14+ |
| RBC-Liver | 0.277* | 0.05 | <0.03 |
| Plasma-Muscle | <0.03 | 0.045 | <0.03 |
| RBC-Muscle | 0.122+ | 0.14+ | <0.03 |
| RBC-Plasma | 0.046 | 0.078 | <0.03 |

*p-value<0.001
**p-value<0.01
+p-value<0.05

**Table 6.** Tissue specific fractionation rates for Nitrogen and Carbon stable isotopes compared with Bio-Olympic hatchery fish food.

| Tissue Type | $\delta^{13}C$ (‰ enrichment) | Lipid Corrected $\delta^{13}C$ | $\delta^{15}N$ (‰ enrichment) |
|---|---|---|---|
| Fin | 3.6‰ | 1.6‰ | 3.13‰ |
| Liver | 1.3‰ | 1.5‰ | 2.25‰ |
| Muscle | 3.0‰ | 0.5‰ | 3.28‰ |
| RBC | 2.6‰ | 0.25‰ | 2.36‰ |
| Plasma | 0.2‰ | 1.3‰ | 3.01‰ |

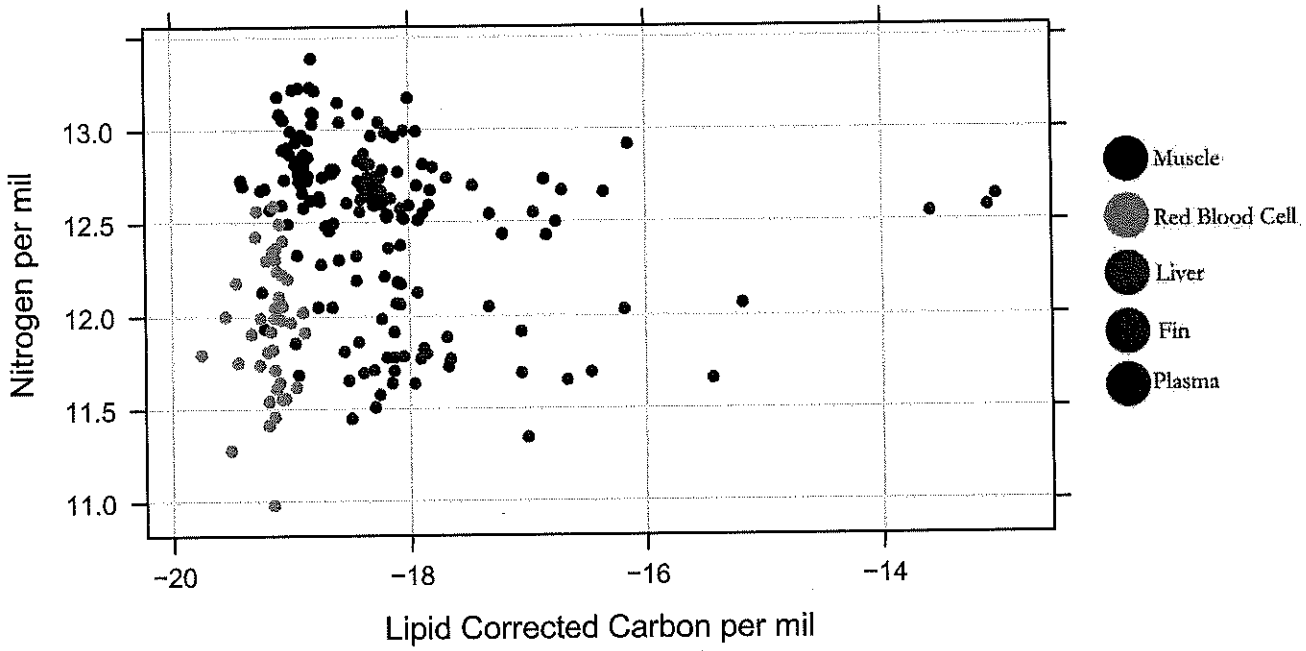



**Figure 1.** Grouped scatter plot of entire population (n=42) of juvenile spring run Chinook salmon based on lipid corrected carbon (LCC) and nitrogen (N) SI values in five tissue types. No values fell outside of three standard deviations from means; therefore no values were considered outliers.

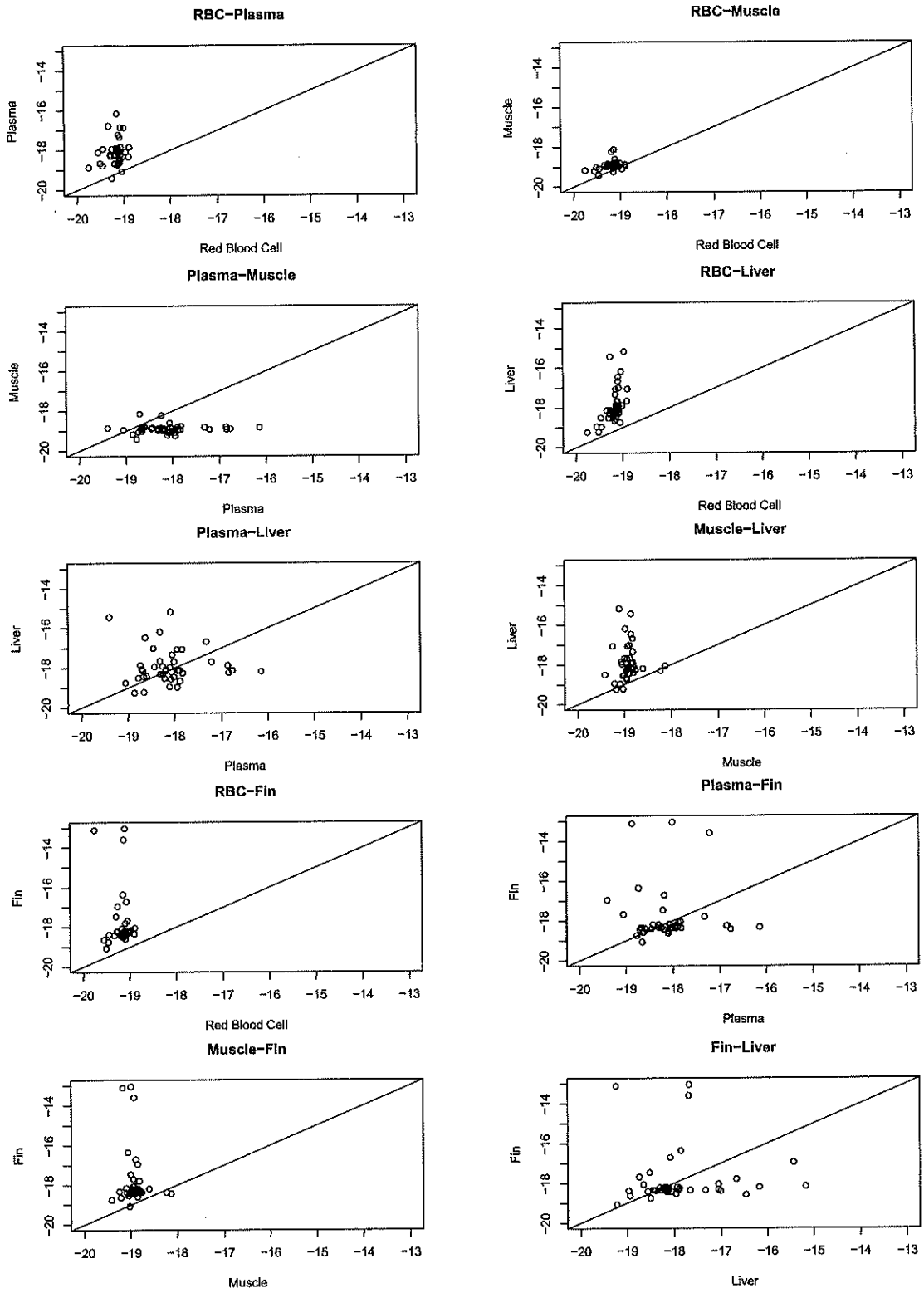



**Figure 2.** Correlations in $\delta^{13}C$ between 5 different tissue types. 1 to 1 correlation line added for reference.

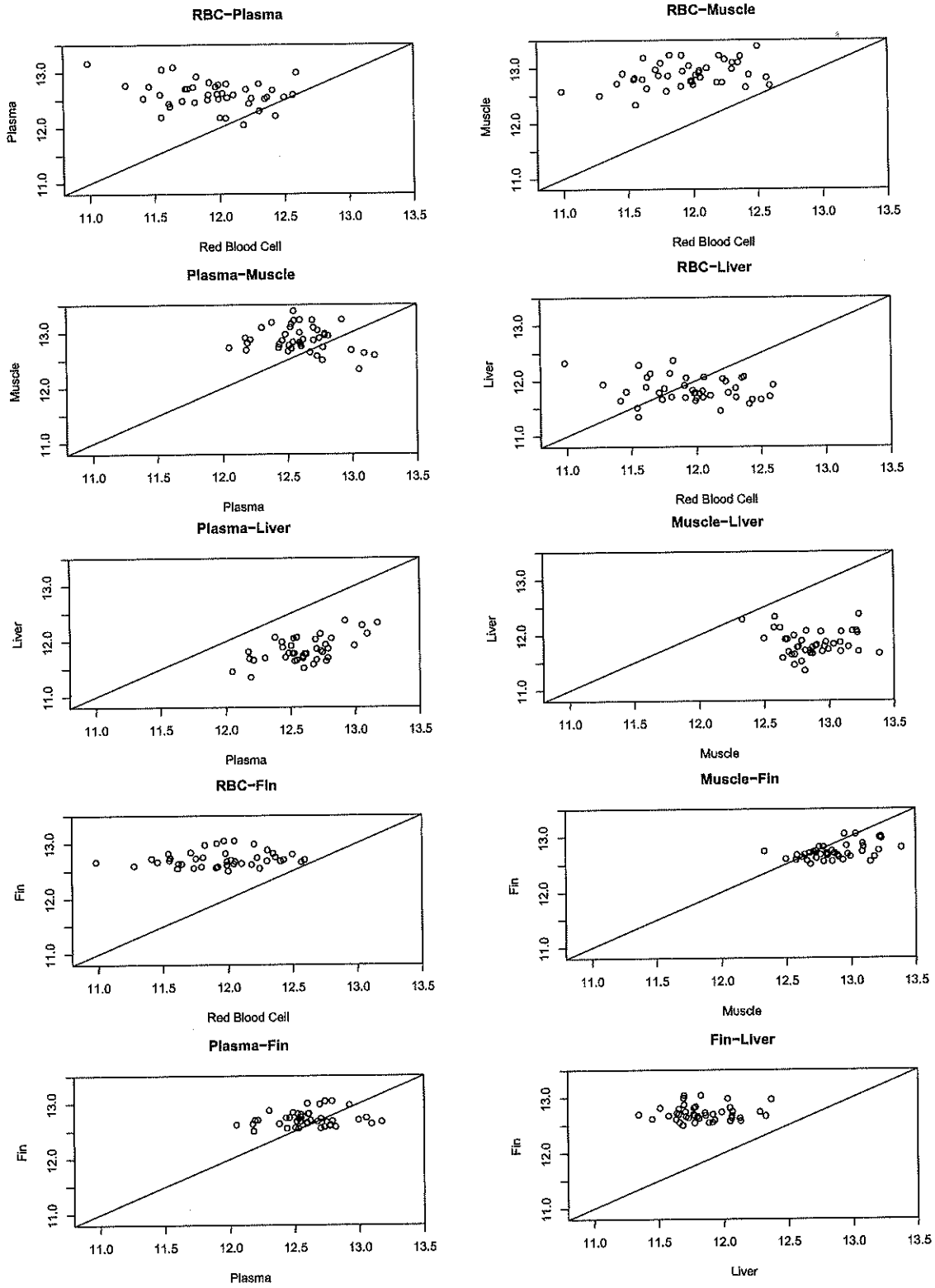



**Figure 3.** $\delta^{15}N$ correlations between tissue types with a one to one correlation line added for comparison.

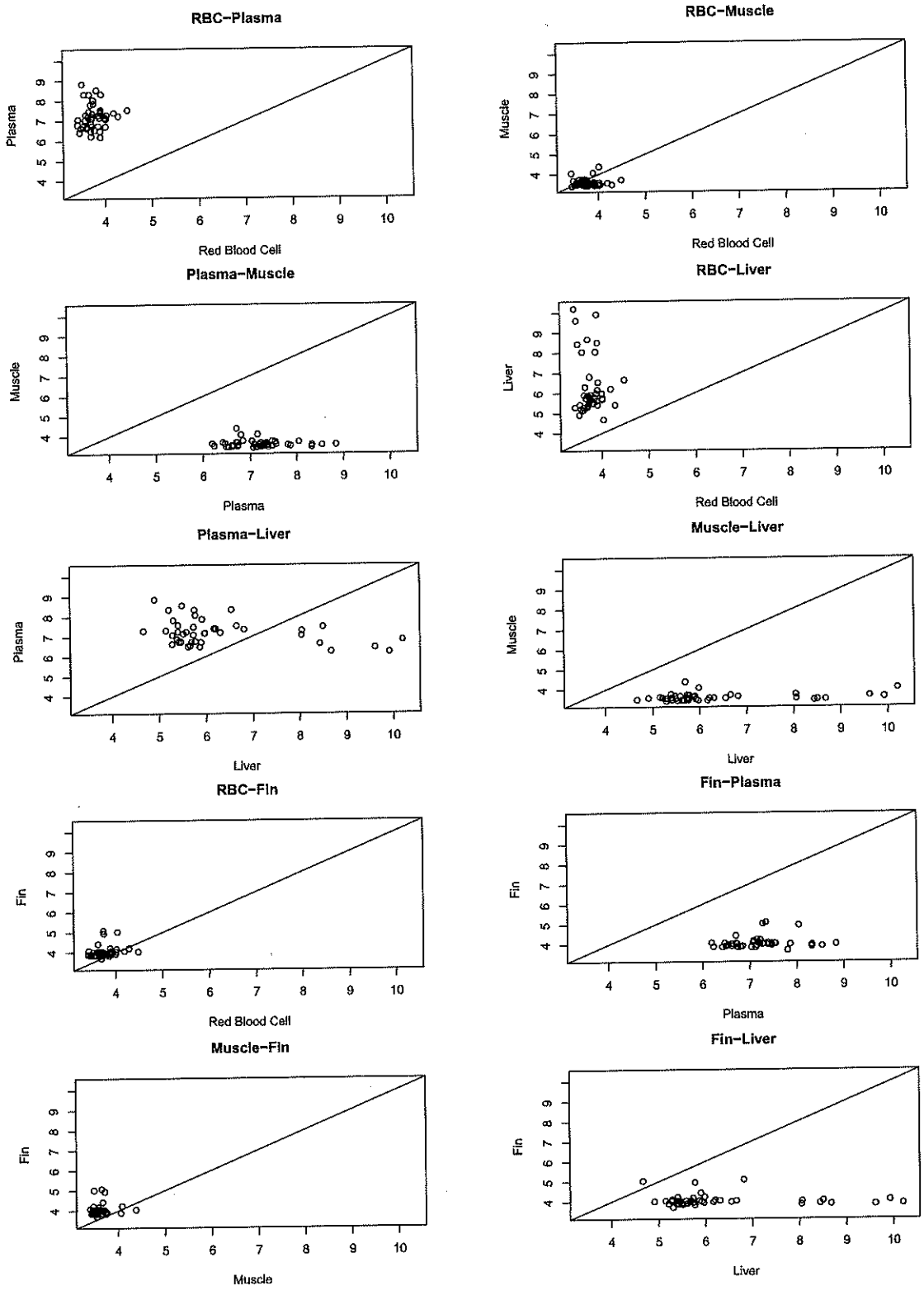
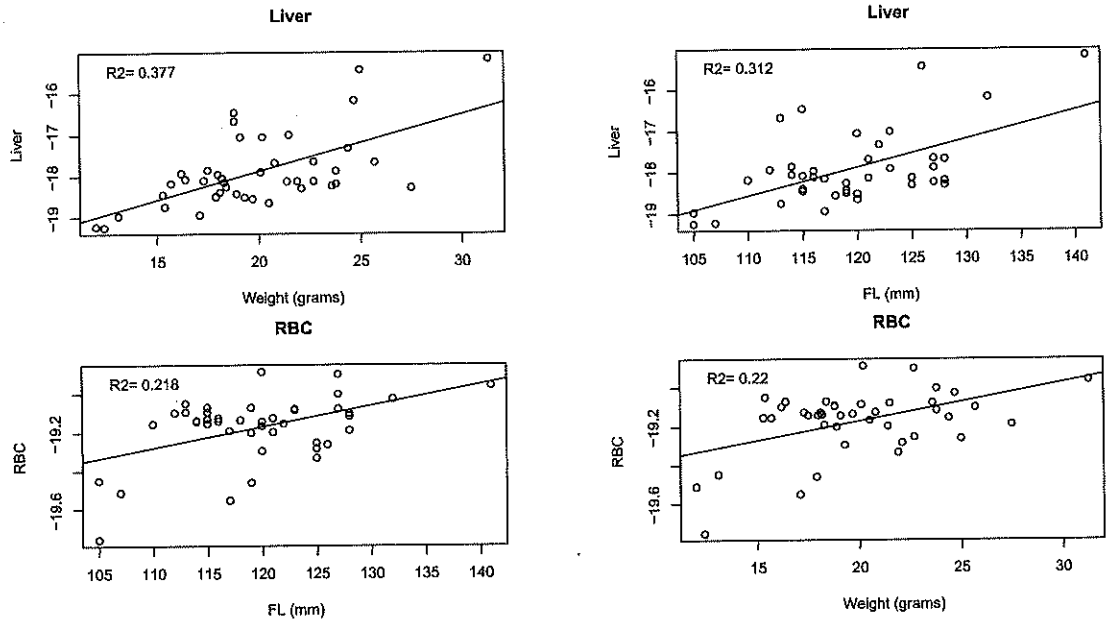



**Figure 4.** %Carbon to %Nitrogen ratios between each tissue type. Trend line is one to one for comparison.

(a) $\delta^{13}C$




(b) $\delta^{15}N$

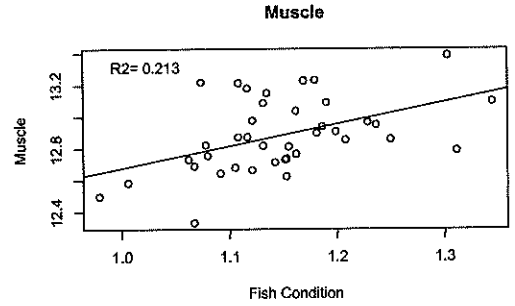



**Figure 5.** Correlations between SI values and physical attributes of individuals. Only plots of statistically significant correlations ($p<0.05$) included.

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