Validation of lipid extracted and corrected methods for stable isotope food web analyses, South Africa

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Abstract

Stable isotope analysis is ubiquitous as a method to investigate food-web dynamics at various scales in aquatic ecology. Most studies make use of dorsal muscle tissue, which involves lethal sampling of the fish. The sampling of muscle tissue is often followed by chemical lipid extraction pre-treatment before stable isotope analysis. In this study we tested whether stable light isotope results obtained from fin tissue were comparable to those from muscle, and we investigated whether lipid correction could be used as a substitute for lipid extraction. Various lipid correction equations were evaluated. Based on our results, we propose ethical and efficient methods of sample collection and preparation for stable isotope analysis of freshwater fish. We found that dorsal muscle and fin tissue samples could yield similar interpretations of freshwater food-web dynamics in South Africa, demonstrating that fin clippings might be more widely applied as a nonlethal sampling method for stable isotope studies. Existing lipid correction equations either over- or underestimated true lipid extracted $\delta^{13}C$ values, therefore an amended lipid correction equation is proposed as it was successfully tested against a population of wild fish. The errors arising from existing lipid correction equations suggest that site-specific calibration should be employed.

Keywords: correction equations, fish tissue analysis, food web, freshwater fish, lipid normalisation, nonlethal sampling

Introduction

The isotopic composition of an organism is the integrated result of its food resources over time (Kilham et al. 2009, Boecklen et al. 2011, Ben-David and Flaherty 2012), and many ecological studies use stable isotopes of nitrogen and carbon to infer dietary information (De Niro and Epstein 1981, O'Reilly et al. 2002, Middelburg 2014, Therrien et al. 2011), identify energy sources (Thompson et al. 2005), infer trophic structures (Peterson and Fry 1987, Woodborne et al. 2012) and elucidate food web functionality (Fry 1991, Post 2002, Woodborne et al. 2012, Matich and Heithaus 2013, Middelburg 2014). In aquatic ecology, fish are often considered top predators that reflect broad patterns of ecological functionality (Hanisch et al. 2010, Maureaud et al. 2019) and the ubiquitous adoption of stable isotope analysis (SIA) has led to the development of diverse methods and laboratory procedures (McMonnaughey and McRoy 1979, Elsdon et al. 2010, Jardine et al. 2011, Taylor et al. 2017). The interpretation of stable isotope results depends on the techniques (muscle or fin tissue sampling and lipid extraction or lipid correction pre-treatment) that are employed (Sotiropoulos et al. 2004). While methods may be consistent within studies, there is a need for a standardised approach to compare results between studies. In addition to being feasible and repeatable, methods must also be ethical (Jardine et al. 2003, Kelly et al. 2006).

One source of variability between SIA studies arises from the use of different tissue types. Differences in tissue composition and their associated metabolic routing leads to different stable isotope values in different tissues of the same organism (Therrien et al. 2011). For freshwater fish, muscle tissue is most used, followed by fin, liver, blood and gut analysis, with tissue type selection determined by the research question and the logistics of sampling (Pinnegar and Polunin 1999, Kelly et al. 2006, Murry et al. 2006, Hanisch et al. 2010, Abrantes et al. 2012). Sampling of muscle tissue is invasive and lethal if not conducted with muscle biopsy (Kelly et al. 2006, Jardine et al. 2011, Henderson et al. 2016), while isotope data obtained from fin tissue can be comparable to that of dorsal muscle tissue and is less invasive and non-lethal (Murry et al. 2006, Willis et al. 2010, Hette-Tronquart et al. 2012, Kambikambi et al. 2019). The use of fin tissue also extends the use of SIA studies to the sampling of threatened or endangered species by decreasing mortality risk (Sanderson et al. 2009).

In addition to tissue type, variation in lipid content within a tissue type between individuals of the same species ("fat" vs "thin fish"), can result in strong enrichment/depletion of $\delta^{13}C$ values as lipids are composed of triglycerides that are synthesised through different metabolic pathways from the protein component of muscle (Babayan 1987). Lipids are stored

in multiple organs, each with different lipid concentrations (Sotiropoulos et al. 2004, Therrien et al. 2011, Taylor et al. 2017) and the effect of chemical lipid extraction can change measured $\delta^{13}C$ values (Spector and Yorek 1985, Henderson and Tocher 1987). The variable effect brought about by variations in lipid content can be addressed through chemical lipid extraction of samples (Hobson and Welch 1992, Sotiropoulos et al. 2004, Ingram et al. 2007). The change in $\delta^{13}C$ values between lipid extracted and non-lipid extracted samples affects the interpretation of an aquatic food web structure (Murry et al. 2006). In a trophic cascade an organism may represent a dietary item, and if the entire organism is consumed (lipids included), then non-lipid extracted data should be used to interpret the diet of higher trophic levels. Because lipid extracted and non-lipid extracted isotope data hold value, but cannot be measured on the same samples, a full food web analysis becomes costly and time-consuming (Greer et al. 2015). Arithmetic correction or normalisation methods have been proposed for predicting lipid extracted $\delta^{13}C$ values from the non-extracted $\delta^{13}C$ values, potentially halving the analytical load of a food web study (Appendix – Table A) (McMonnaughey and McRoy 1979, Post et al. 2007, Taylor et al. 2017, Skinner et al. 2016).

A range of chemical lipid extraction or lipid normalisation methods have been demonstrated for freshwater ecological studies (Appendix - Table B). In 12 studies (Appendix - Table B), six conclude that $\delta^{13}C$ and $\delta^{15}N$ values should be analysed separately and lipid extraction should only be used for $\delta^{13}C$ analyses (Pinnegar and Polunin 1999, Sotiropoulos et al. 2004, Logan 2008, Elsdon et al. 2010, Fagan et al. 2011, Abrantes et al. 2012), two suggest the use of lipid extracted data for both $\delta^{13}C$ and $\delta^{15}N$ values (Trueman et al. 2005, Ingram et al. 2007), two conclude that a lipid corrected equation should be applied rather than lipid extraction (Post et al. 2007, Taylor et al. 2017), one concludes that when the lipid content in samples is high, lipid extraction is necessary (Skinner et al.2016) and another (Murry et al. 2006) rejects the use of lipid extraction and correction. Apart from the effect of lipids, variability induced by size classes, species and diet composition should be evaluated. Most of these variables are subjective to specific conditions and caused inconsistent conclusions between studies (Kurle 2009, Therrien et al. 2011, Olin et al. 2013, Drago et al. 2015, Busst and Britton 2016).

In this study we use stable isotope analyses of carbon and nitrogen to investigate the effects of different tissue types and lipid extraction for freshwater fish species from southern Africa. We hypothesise that stable light isotope data from fin tissue, will be comparable to muscle tissue, and predict that lipid correction can be used as a substitute for lipid extraction.

Lipid extracted values are compared with those obtained from various lipid correction equations to assess the applicability of their parameters. We make use of farmed fish where diets are known and validate a revised model on a population of wild fish.

Materials and methods

Fish collection and tissue sampling

This study was approved by the Animal Ethics Screening Committee (AESC) of the University of Pretoria, South Africa (AESC NAS037/2019). A total of 7 different fish species were sampled in April 2019 from four commercial fish farms (Farms 1 – 4). These included *Clarias gariepinus* (n = 8), *Coptodon rendalli* (n=15), *Cyprinus carpio* (n=7), *Enteromius trimaculatus* (n=6), *Oreochromis mossambicus* (n=42), *Oreochromis niloticus* (n=35) and *Pseudocrenilabrus philander* (n=2). These species were chosen because they are widely distributed in fish farms throughout southern Africa, have broad dietary niches, and have previously been part of food web studies in southern Africa (Kotze *et al*. 1999, Turker and Brune 2002, Woodborne *et al*. 2012).

Fish were euthanized on-site following the methodology described by Fernandes *et al*. (2017) and stored in a freezer at -10°C for transport to the stable isotope laboratory in the Mammal Research Institute of the University of Pretoria, Pretoria, South Africa. In the laboratory, fish were thawed and rinsed with distilled water and total length (TL, mm), standard length (SL, mm) and mass (W, g) were recorded (Appendix - Table C). Fin samples were collected from the distal part of the dorsal fin (Sanderson et al. 2009) and muscle samples were removed subcutaneously from epaxial muscles below the dorsal fin (Jardine et al. 2003). Individual fish with a mass below 5g were analysed as 'whole fish' (Vander Zanden et al. 1997) and from these samples fin and muscle samples were not collected separately. These 'whole fish' were only used to compare lipid extraction versus no lipid extraction and were not used to compare between tissue type.

Dorsal fin and muscle tissue samples were dried for 48 hours and whole fish samples were dried for 72 hours at 70°C as per Woodborne *et al*. (2012). Dried samples were homogenized with a mortar and pestle and Beadbug® (Benchmark Scientific, Sayreville NJ, U.S.A.) microtube homogenizer. Homogenized samples were subsampled so that approximately half could be used for chemical lipid extraction and the remainder analysed with no additional pre-treatment.

Where available, the fish feed on farms were also collected and analysed but the relative ratios of different feeds and feeding regimes confounded any diet to tissue discrimination investigations.

Wild fish collection

Fish samples were collected from the Olifants River, South Africa (23°59'21.8" S, 31°49'35.6" E) (Permit number: SANPARK 012/16, Ethics number: EC031-16). A total of 45 fish were collected from eight different species, including *Hydrocynus vittatus (N=16*), *Clarias gariepinus (N=15*), *Oreochromis mossambicus N=1*), *Labeo molybdinus (N=1*), *Labeo cylindricus (N=7*), *Labeobarbus marequensis (N=1*), *Glossogobius giuris (N=3*) and *Chiloglanis paratus (N=1*). The fish were collected using rod and reel and electrofishing (SAMUS 452). Fish were euthanized following the methods of Fernandes *et al*. (2017) and dorsal muscle samples were collected and frozen on site in a freezer at -10°C. Sample analyses and lipid extraction was identical to those used for farmed fish. Wild fish samples were obtained independently and here, only muscle tissue was analysed, samples were subdivided similar to farmed fish and analysed as both lipid extracted and non-lipid extracted.

Lipid extraction pre-treatment

Lipids were extracted in ±10 ml of a 2:1 chloroform: methanol solution as per Connan *et al*. (2019) per the standard operating procedures of the stable isotope laboratory of the University of Pretoria. After mixing, the sample settled for 60 seconds before excess 2:1 chloroform: methanol solution (containing the dissolved lipids) was removed. This was repeated three times before the sample was rinsed with distilled water and dried at 70°C for 24 hours. All tissues, including fin tissues, were lipid extracted to ensure the removal of any residues that could containing lipids.

Stable isotope analyses

All samples for isotopic analyses were weighed into D1006 Tin Capsules on a Mettler Toledo MX5 microbalance (Thermo Fisher, Bermen, Germany). One aliquot of 0.55-0.60mg from individual samples were used for isotopic analyses. All samples were analysed using a Flash EA 1112 Series elemental analyser coupled to a Delta V plus isotope ratio Mass spectrometer by a Conflo IV interface. All samples were measured with a laboratory standard (Merck Gel: $\delta^{13}C$ = -20.57‰, $\delta^{15}N$ = 6.8‰, C% = 43.83, N% = 14.64 and Valine: $\delta^{13}C$ = -10.57‰, $\delta^{15}N$ = -6.15‰, C% = 50.35, N% = 11.86%) and blanks were measured after every

12 unknown samples. Duplicates samples were measured after every 10-15 samples. All results are reported against Vienna Pee-Dee Belemnite (VPDB) for carbon isotope values and atmospheric nitrogen (Air) for nitrogen isotope values. Measurements of stable carbon and stable nitrogen isotope ratios are represented as: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000)$ where R represents $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively (Peterson and Fry 1987, O'Leary 1984).

Lipid correction estimations

Three different lipid correction equations (McMonnaughey and McRoy 1979, Post et al. 2007, Taylor et al. 2017) (Appendix – Table A) were applied to the non-lipid extracted data and compared to the observed lipid extracted data to determine the effectiveness and applicability of the different lipid correction equations.

Parameters of the best-fit correction equation were adjusted to minimise residuals between lipid extracted and lipid corrected data. The adjusted parameters of the best fit equation were applied to dorsal muscle isotopic data of wild fish from the Olifants River, Kruger National Park.

Statistical analyses

Data were analysed in R through the Rstudio© interface (R core team 2019). A Kruskal-Wallis test was performed to test the significance between lipid extracted and non-lipid extracted as well as between lipid extracted and lipid corrected data. A Hotelling's t-test was used to test the significance between lipid extracted and non-lipid extracted data between the different tissue types. A two-sample t-test was conducted between the lipid corrected and lipid extracted $\delta^{13}C$ values per species, and between lipid extracted and lipid corrected $\delta^{13}C$ for wild fish populations.

Results

Of the 115 fish from the farms, 59 were analysed as whole fish and 56 were subsampled for both muscle and fin tissue. There were significant variations in $\delta^{13}C$ ($p<0.001$) and $\delta^{15}N$ ($p<0.001$) values between fish from different farms (Kruskal Wallis Test) (Figure 1) and variation in $\delta^{13}C$ and $\delta^{15}N$ was greater between farms than between species (Table 1). Any effect of size is confounded by the differences in diet between farms and relationships could not be established between $\delta^{13}C$ and $\delta^{15}N$ values and a fishes standard length in the current study (Figure 1). Subsequently, methodologies are compared across a broad range of $\delta^{13}C$ and $\delta^{15}N$ values for muscle (Min: -25.38‰, 6.21‰, Max: -14.77‰, 25.51‰), fin tissue (Min: -24.5‰, 6.78‰, Max: -12.8‰, 25.01‰) and whole fish samples (Min: -27.17‰, 7.43‰, Max: -15.99‰, 27.77 ‰).

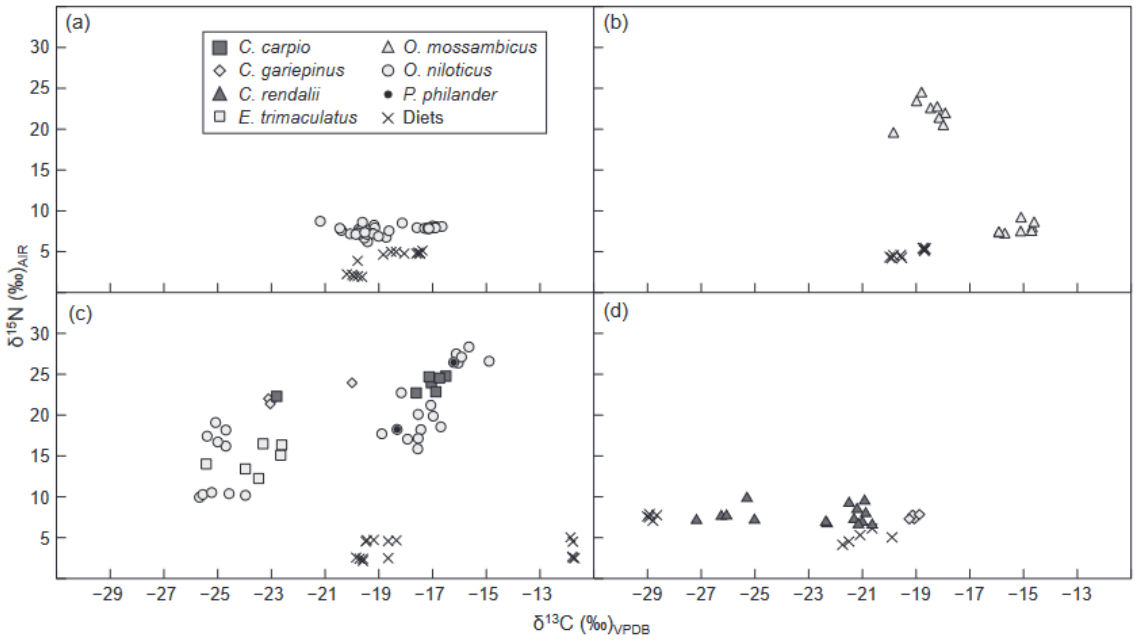

Figure 1: A comparison between the non-lipid-extracted δ13C and δ15N values for different fish species and their respective diets on each farm (panels 'a–d' represent four different farms). Note the deviation from diet for Oreochromis mossambicus from Farm B and that of all fish from Farm C

Table 1: The mean ± SD $\delta^{13}C$ and $\delta^{15}N$ of fish collected at different fish farms and the different types of feed that was fed.

Farm	Feed	Mean $\delta^{13}C$ ± SD	Mean $\delta^{15}N$ ± SD
1	A	-20.03 ± 0.66	8.03 ± 0.46
	B	-19.69 ± 0.21	7.19 ± 0.15
	C	-18.36 ± 0.08	20.83 ± 0.15
2	D	-15.68 ± 0.20	7.70 ± 0.15
	E	-18.29 ± 0.04	20.83 ± 0.11
3	F	-22.58 ± 0.50	21.96 ± 0.10
	G	-20.40 ± 0.06	18.87 ± 1.23
	H	-25.09 ± 0.48	17.42 ± 0.48
4	I	-20.40 ± 0.14	7.98 ± 0.32
	J	-21.42 ± 0.73	7.88 ± 0.77

Comparing results from fin and muscle tissue resulted in no significant difference between the isotopic composition of a sample when measured from dorsal muscle or dorsal fin tissue ($\delta^{13}C$ and $\delta^{15}N$ combined) (Lipid extracted: Hotellings t-test, T^2= 1.7211, df1=2, df2= 109, p= 0.1837, non-lipid extracted: T^2= 0.8311 df1=2, df2= 109, p= 0.4383). Whether or not interpretations are made from fin or muscle tissue samples will not influence the interpretation of food web dynamics from the analysed samples.

Lipid extraction had a significant effect on both $\delta^{13}C$ and $\delta^{15}N$ for muscle ($p<0.001$, $p<0.001$ for $\delta^{13}C$ and $\delta^{15}N$ respectively) and whole fish ($p<0.001$, $p=0.04$ for $\delta^{13}C$ and $\delta^{15}N$ respectively) samples (Two-sampled t-test). For fins, lipid extraction had a significant effect on $\delta^{13}C$ ($p=0.042$), but not on $\delta^{15}N$ values ($p= 0.69$) (Two-sampled t-test) (Figure 2).

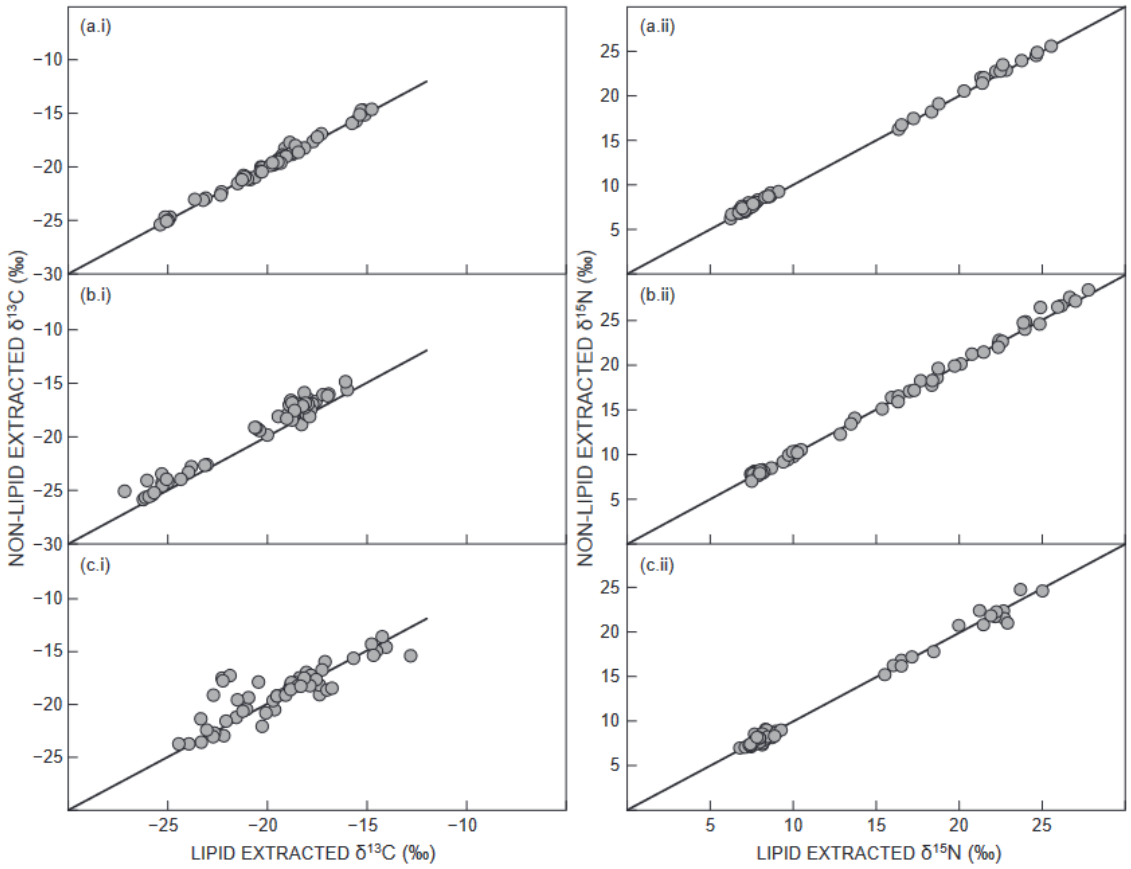

Figure 2: Lipid extracted $\delta^{13}C$ and $\delta^{15}N$ plotted against non-lipid extracted values for the different tissue types. A1 and 2) Muscle, B1 and 2) Whole fish and C1 and 2) Dorsal fin. The solid line represents a 1:1 relationship of no difference.

Considering all tissue types together, there was a significant difference between lipid extracted $\delta^{13}C$ values and lipid corrected $\delta^{13}C$ values for each correction equation (Two-sampled t-test) (Figure 3A: t = -102.27, p<0.001, Figure 3B: t = -7.99, p<0.001, Figure 3C: t = -10.37, p<0.001). The parameters *D* and *I* from McConnaughey and McRoy (1979) (equation 1) were adjusted to achieve a best fit model between lipid extracted and corrected values with an R-squared of 0.94 that conforms to a 1:1 relationship through the origin (Figure 3D). The amended formula is:

$$\delta^{13}C' = \delta^{13}C + D\left(I + \frac{3.90}{1+\frac{287}{L}}\right) \qquad \text{Equation 1}$$

where $\delta^{13}C'$ represents the lipid corrected value, $\delta^{13}C$ represent the original ^{13}C value, D = 0.8105 and I = -0.704. The adjusted D parameter is derived from the slope of the relationship (between protein and lipids) and the I parameter is the x-intercept. Equation 1 was tested for fish fin and muscle tissue samples with C:N ratios between 3.8 and 6.9.

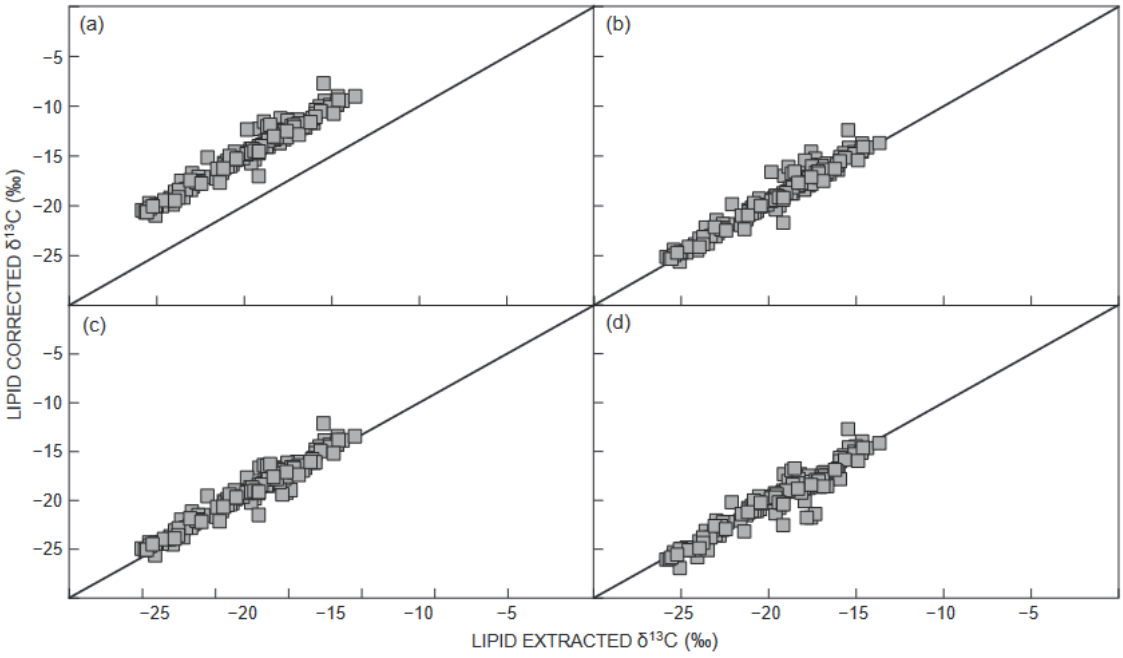

Figure 3: The relationship between the lipid corrected and lipid extracted $\delta^{13}C$ values for the different lipid correction equations. A) McConnaughey and McRoy (1979), B) Post et al. (2007), C) Taylor et al. (2017) and D) Adjusted McConnaughey and McRoy (1979) parameters. The solid line represents a 1:1 relationship or the line of no difference.

Lipid extracted $\delta^{13}C$ and the lipid corrected $\delta^{13}C$ values using Equation 1 did not differ significantly between species and the equation can be applied irrespective of the species or tissue type (*C. carpio*: t = 2.12, p=0.76, *C. gariepinus*: t=2.07, p=0.86, *C. rendalii*: t=2.01, p=0.91, *E. trimaculatus*: t= 2.23, p = 0.95, *O. mossambicus*: t= 1.97, $p > 0.05$ (p = 0.87), *O. niloticus*: t=1.98, p=0.97) (Two-sampled t-test) (Figure 4). When Equation 1 is applied to muscle samples from a wild fish population (donated samples) there is no significant difference (t = 1.18, p= 0.24) between the true lipid extracted $\delta^{13}C$ and lipid corrected $\delta^{13}C$ values from non-lipid extracted samples (Figure 5) (Two-sampled t-test) (i.e. whether or not the lipid correction is performed mathematically or through chemical lipid extraction, the results are statistically indistinguishable).

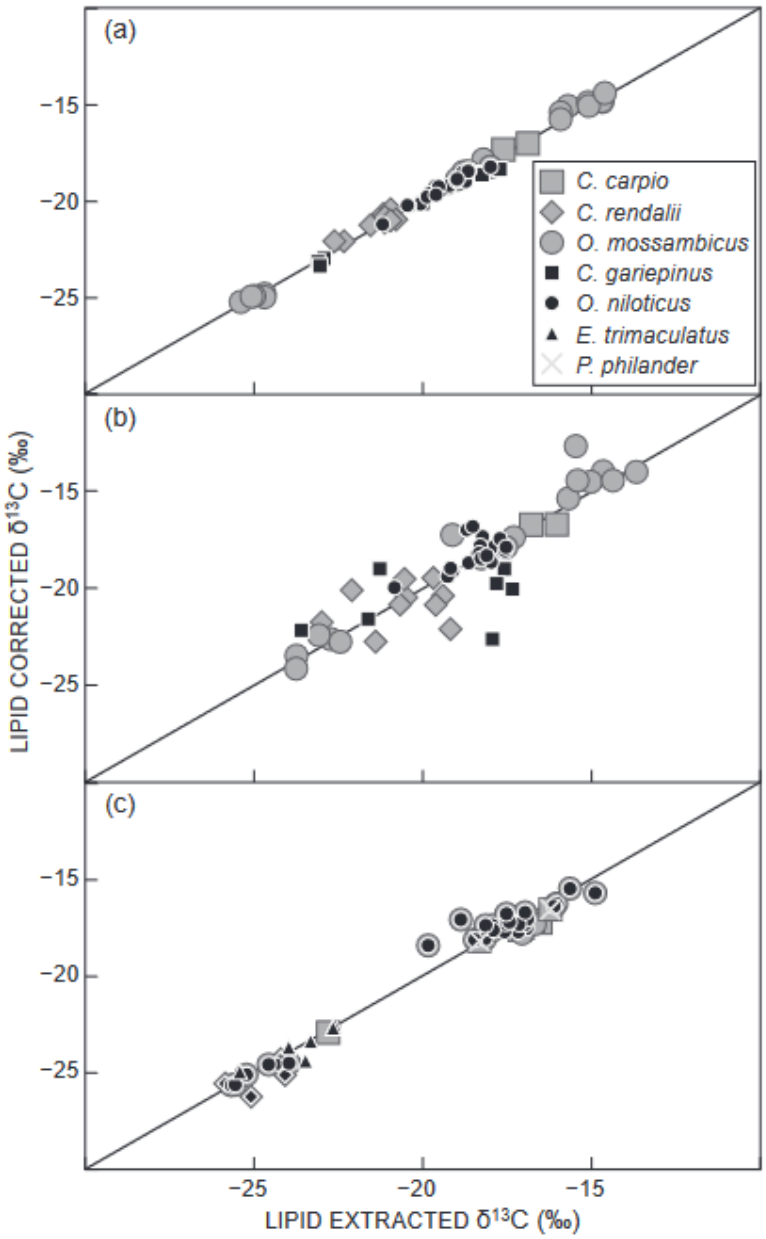

Figure 4: The relationship between the lipid extracted and lipid corrected $\delta^{13}C$ values using Equation 1 per species and per tissue type (A: Muscle, B: Dorsal fin, C: Whole fish).

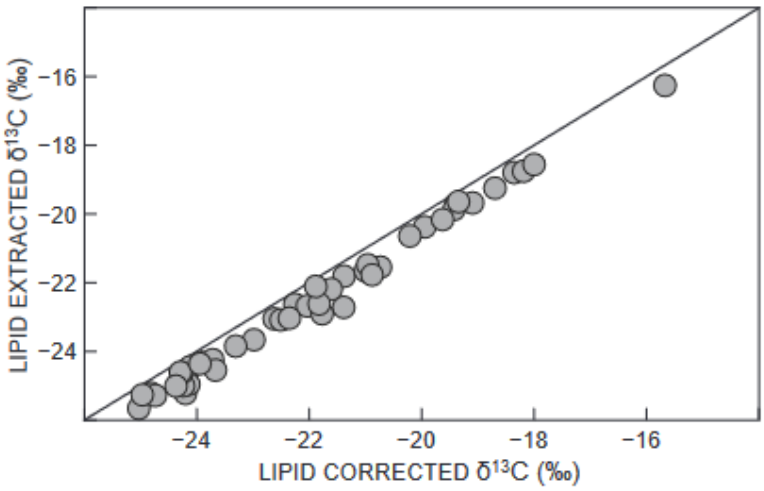

Figure 5: The relationship between lipid extracted and lipid corrected $\delta^{13}C$ values from a population of wild fish from the Olifants River, South Africa. Lipid corrections are based on the adjusted McConnaughey and McRoy (1979) equation derived in this study.

Discussion

These results support the non-lethal sampling of fins as a viable alternative to dorsal muscle tissues and lipid corrections could be an alternative to chemical lipid extraction if specific corrections are considered. In the analyses of farmed fish inter farm $\delta^{15}N$ variation was greater than interspecific variation and Farms B and C had $\delta^{15}N$ values that are characteristic of fish from a eutrophic impoundment (Rau et al. 1981, Wayland and Hobson 2001). Consultation with the farm owners revealed that fish from Farm B with elevated nitrogen values were bought from Farm C a week prior to sampling. Farm C is located close to a large, highly polluted water body (precise details omitted to protect the anonymity of the farm) and the current study suggests that these fish may have been locally caught and not reared on the farm. Nonetheless, this allowed for a comparison of lipid extraction on an overly broad $\delta^{13}C$ and $\delta^{15}N$ range.

The first objective of this research was to assess whether non-lethal fin clips are an adequate alternative to muscle tissue in the isotopic analysis of fish. The Hotelling's t-test combines the relative isotope ratios of carbon and nitrogen to compare the isotopic composition as inferred from muscle tissue when compared to that derived from fin tissue. No significant difference in trophic interpretation is reached whether fin or muscle tissue is used. Although the sample size is limited, this study supports the use of dorsal fin tissue as an alternative to

lethal muscle tissue in fish food web studies. This is similar to others that have supported the use of non-lethal fin tissue and muscle samples due to strong correlation between their respective isotopic composition (Kelly et al. 2006, Hanisch et al. 2010, Hette-Tronquart et al. 2012, Kambikambi et al. 2019, Hicks et al. 2021). Muscle biopsy is a technique that can be used with no lasting effects on the survival of fish (Bøe et al. 2020) but here and elsewhere (Hicks *et al.* 2021, Hette-Tronquart 2012) it is demonstrated that the similarity between the isotopic composition of muscle and fin tissue supports the sue of the less invasive method (Sanderson et al. 2009). Some degree of error might exist between muscle and fin tissue due to variation in tissue turnover rates and diet switching experiments will be required to quantify the variation in tissue turnover times between these two tissue types. Future studies should consider which tissue type to use to answer the research question in mind as short and long term dietary integration between tissue types will yield different isotopic compositions, especially in the case of ecological perturbations, such as algal blooms.

The second objective was to assess if mathematical lipid corrections provide a valid alternative to costly and time-consuming lipid extraction protocols. Chemical lipid extraction is known to cause changes in $\delta^{13}C$ values (Appendix - Table A), while theoretically, no changes should have occurred in the $\delta^{15}N$ values (Sotiropoulos et al. 2004, Trueman et al. 2005, Murry et al. 2006, Post et al. 2007). In line with previous studies (Pinnegar and Polunin 1999, Sotiropoulos et al. 2004, Trueman et al. 2005, Logan et al. 2008, Skinner et al. 2016), the lipid extraction process affected the $\delta^{13}C$ values of all the tissue types, but in our study $\delta^{15}N$ values were also affected for all tissues except dorsal fin tissue. Solvents that are commonly used for lipid extraction such as chloroform-methanol (1:1) (used in this study) or dichloromethane-methanol (2:1) are not lipid specific and may cause some removal of nitrogenous components (Radin 1981, Murry et al. 2006, Connan et al. 2019). The main concern is that a change in the $\delta^{15}N$ values can affect the interpretation of the food web through $\delta^{15}N$ enrichment, increasing the apparent trophic level of some individuals (Sotiropoulos et al. 2004, Murry et al. 2006). Additional studies are needed to determine the specific mechanism causing ^{15}N enrichment through chemical lipid extraction. Lipid correction does not influence the $\delta^{15}N$ values as only $\delta^{13}C$ values are included in correction equations, and the results from this study indicate that accurate lipid correction equations are less likely to influence trophic interpretation than chemical lipid extraction. Studies using lipid extraction, should preserve the $\delta^{15}N$ values from non-lipid extracted samples. By using lipid correction equations, the whole-tissue isotope

values are retained and can be used in the interpretation of trophic cascades where lipids must be included as dietary contributors to higher trophic levels (Woodborne et al. 2012).

While the use of lipid correction equations holds some advantage, there is a caveat whether the corrections are universally applicable. The three lipid correction equations that are widely used were compared against lipid extracted $\delta^{13}C$ data and were found to either over- or underestimate the true values. The current study demonstrates that widely applied lipid correction equations must be adjusted, but whether this is applicable at a regional level (southern Africa) (Taylor et al. 2017), or whether the corrections are more site, species or size specific needs further assessment.

In the current study the *D* and *I* parameters of the McConnaughey and McRoy (1979) equation were recalibrated (D = 0.8105 and I = -0.704) from farmed fish populations (this study) and tested against a wild freshwater fish population (donated samples) in southern Africa. The data obtained from chemical lipid extracted samples from wild fish were indistinguishable from those obtained through mathematical lipid correction using the parameters from the current study. The result is an improvement on the McConnaughey and McRoy (1979) equation and whilst size and species-specific effects are negated in the current study there is still a slight offset (non-significant) that may imply the need for site- specific calibration. Future studies could use a subset of samples for lipid extraction to calibrate the correction equation to use on their entire dataset, limiting the need for lipid extraction of all samples.

Conclusion

Water resources around the world are increasingly under pressure through processes of eutrophication (Jeppesen et al. 2010). This is more acute in areas like southern Africa where evaporative potential often exceeds precipitation, and so perennial rivers and impoundments are critical in agricultural production and economic development. Stable light isotope analysis provides a means of assessing the ecological impacts of eutrophication processes, but it has traditionally required a broad range of samples from an entire food web. This normally entails sacrificing large numbers of fish if muscle tissue is the selected sample type. Fin regeneration is demonstrated if whole fins are not removed (Thompson and Blankenship 1997). This research demonstrates that the sampling of dorsal fin tissue is a non-lethal alternative to dorsal muscle tissue and allows for the same interpretation of food webs during SIA approaches.

Ecological studies require both the whole tissue (lipid present) and derived (no lipids) isotopic values to trace energy flow between individuals within a food web (Logan et al. 2008). Lipid extraction processes are costly and time consuming, but they can be substituted by lipid correction equations. Whether the lipid correction equations are universally applicable is not clear, and there may be some error that arises from the use of different solvents in the lipid extraction process. The widely accepted lipid correction equations were shown to have systematic errors when applied to a population of farmed fish with widely varying $\delta^{15}N$, $\delta^{13}C$ and C/N ratios. Recalibration of the equations yielded a statistically indistinguishable inferred lipid corrected isotope dataset when compared with the corresponding lipid extracted dataset in a wild population of freshwater fishes. The dataset used to recalibrate the lipid correction equations, and the dataset used to test the approach are completely independent of one another, and the improvement achieved in the recalibration suggests that the correction equations should be at least regionally tested before being applied.

The research presented here substantiates a more ethical approach to fish isotope analysis because the sampling of fin clips is non-lethal but provides results that are comparable with muscle analysis. The verification of lipid correction equations substantially reduces the cost and time spent in the laboratory, and this enhances the utility of stable isotope analysis as a cornerstone technique in the face of growing pressure on freshwater ecological systems.

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Appendix

Table A: Summary of literature relating to isotopic lipid correction equations of aquatic species.

Reference	Lipid percentage equation	Lipid corrected equation
McMonnaughey and McRoy 1979	$L = \frac{93}{1+(0.246*(C:N)-0.775)^{-1}}$	$\delta^{13}C' = \delta^{13}C + D * (I + \frac{3.9}{1+\frac{287}{L}})$, with D=6‰ and I= -0.207
Post *et al*. 2007	δ	$\delta^{13}C'$ = $\delta^{13}C$ value without lipids present $\delta^{13}C$ = $\delta^{13}C$ value with lipids present $^{13}C' = \delta^{13}C - 3.32 + (0.99 * C: N)$
Abrantes *et al*. 2012	L = -20.54 + 7.24 * C: N	$\Delta\delta^{13}C = -0.47 + 0.13 * L$ $\Delta\delta^{13}C = -3.32 + 0.99 * C: N$
Taylor *et al*. 2017	$L = \frac{93}{1+(0.246*(C:N)-0.775)^{-1}}$	$\delta^{13}C' = \delta^{13}C + D*\left(I + \frac{3.9}{1+\frac{287}{L}}\right)$ with D = 4.46‰ and I = 0
Logan *et al*. 2008		$\delta^{13}C' - \delta^{13}C = \frac{a*C:N+b}{C:N+c}$
Kiljunen *et al*. 2006	$L = \frac{93}{1+(0.246*(C:N)-0.775)^{-1}}$	$\delta^{13}C' = \delta^{13}C + D * (I + \frac{3.9}{1+\frac{287}{L}})$, with D=7.018‰ and I= 0.048
Skinner *et al*. 2016	L = -20.54 + (7.24*C: N)	$\delta^{13}C' = \delta^{13}C + D * (I + \frac{3.9}{1+\frac{287}{L}})$, with D=7‰ and I= -0.207

Table B: Summary of literature relating to isotopic chemical lipid extraction vs. non-lipid extraction and the effect on $\delta^{13}C$ and $\delta^{15}N$ on freshwater fish species.

Reference	Lipid extraction	Tissue type	$\delta^{13}C$ change	$\delta^{15}N$ change
Abrantes *et al.* 2012	Chloroform:methanol:water (1:2:0.8)	Muscle	Yes	Yes
Elsdon *et al.* 2010	Dichloromethane:methanol (1:1)	Muscle	Yes	Yes
Fagan *et al.* 2011	Chloroform:methanol (2:1)	Muscle	Yes	Yes
Ingram *et al.* 2007	Chloroform: methanol: water (4:2:1)	Muscle Liver	-	Yes (small)
Logan *et al.* 2008	Chloroform:methanol (2:1)	Liver Muscle Gonad Whole fish	Yes	Yes
Murry *et al.* 2006	Dichloromethane: methanol (1:1).	Muscle	Yes	Yes
Pinnegar and Polunin 1999	Methanol: Chloroform: Water (10:5:4)	White muscle Red muscle Heart Liver Whole fish	Yes	No
Post *et al.* 2007	Methanol: Chloroform (1:1)	Muscle Whole fish	Yes	Yes
Skinner *et al.* 2016	Chloroform:methanol:water (8:4:3) (Deionized water)	Muscle Liver	Yes	No (liver) Yes (Muscle)
Sotiropoulos *et al.* 2004	Chloroform: methanol (1:1)	Muscle Whole fish	Yes	Yes (small)
Taylor *et al.* 2017	Chloroform:methanol (2:1)	White muscle	Yes	-
Trueman *et al.* 2005	Methanol: chloroform: water (10:5:4)	Muscle Liver Gut content	Yes	Yes

Table C: Table indicating the different species collected from the various fish farms and the total length, standard length and weigh of each individual collected.

Species	Fish farm	Total Length (mm)	Standard Length (mm)	Weight (g)
C. carpio	3	74	57	4.43
C. carpio	3	90	73	4.91
C. carpio	3	73	60	4.71
C. carpio	3	55	45	2.13
C. carpio	3	50	44	2.00
C. carpio	3	108	85	13.7
C. carpio	3	110	84	12.79
C. gariepinus	3	601	525	>2000
C. gariepinus	3	640	563	>2000
C. gariepinus	3	543	485	>2000
C. gariepinus	3	551	497	>2000
C. gariepinus	4	76	65	4.22
C. gariepinus	4	92	80	7.98
C. gariepinus	4	88	75	5.23
C. gariepinus	4	90	85	8.47
C. rendalli	4	39	30	1.00
C. rendalli	4	41	32	1.18
C. rendalli	4	37	30	0.89
C. rendalli	4	35	28	0.72
C. rendalli	4	35	28	0.73
C. rendalli	4	210	175	168
C. rendalli	4	250	240	>2000
C. rendalli	4	251	205	>2000
C. rendalli	4	210	170	122
C. rendalli	4	146	112	46
C. rendalli	4	240	200	>2000
C. rendalli	4	268	218	>2000
C. rendalli	4	142	110	40
C. rendalli	4	240	204	>2000
C. rendalli	4	164	134	80
E. trimaculatus	3	60	47	1.51
E. trimaculatus	3	48	38	0.74
E. trimaculatus	3	56	44	1.01

E. trimaculatus	3	58	45	1.17
E. trimaculatus	3	55	48	1.07
E. trimaculatus	3	49	39	0.78
O. mossambicus	3	205	175	130
O. mossambicus	2	160	130	36
O. mossambicus	2	165	135	52
O. mossambicus	2	151	120	52
O. mossambicus	2	162	130	56
O. mossambicus	2	120	95	23
O. mossambicus	2	148	120	44
O. mossambicus	2	109	85	22
O. mossambicus	3	235	200	>2000
O. mossambicus	3	211	180	194
O. mossambicus	3	229	199	>2000
O. mossambicus	3	273	225	>2000
O. mossambicus	2	136	109	44
O. mossambicus	3	73	55	5.82
O. mossambicus	3	77	60	5.9
O. mossambicus	3	50	40	1.12
O. mossambicus	3	60	46	2.01
O. mossambicus	3	70	55	4.9
O. mossambicus	3	35	30	0.64
O. mossambicus	3	55	44	2.24
O. mossambicus	3	77	53	4.05
O. mossambicus	3	73	56	4.22
O. mossambicus	3	60	57	3.68
O. mossambicus	3	67	53	3.97
O. mossambicus	3	65	50	2.6
O. mossambicus	3	72	55	4.73
O. mossambicus	3	70	55	4.34
O. mossambicus	3	54	44	1.96
O. mossambicus	3	53	38	1.48
O. mossambicus	3	40	30	0.82
O. mossambicus	3	60	46	2.58
O. mossambicus	3	45	34	1.02
O. mossambicus	3	45	34	1.02
O. mossambicus	2	80	62	7.29
O. mossambicus	2	81	65	7.19
O. mossambicus	2	57	52	3.33
O. mossambicus	2	79	60	6.14

O. mossambicus	2	80	73	7.01
O. mossambicus	2	90	70	8.74
O. mossambicus	2	89	69	9.13
O. mossambicus	2	80	62	6.32
O. mossambicus	2	90	70	10.3
O. mossambicus	2	109	86	13.68
O. niloticus	1	205	171	110
O. niloticus	1	255	205	>2000
O. niloticus	1	210	198	168
O. niloticus	1	240	206	194
O. niloticus	1	210	164	140
O. niloticus	1	201	169	120
O. niloticus	1	222	190	182
O. niloticus	1	200	163	108
O. niloticus	1	185	154	70
O. niloticus	1	282	234	>2000
O. niloticus	1	20	15	0.098
O. niloticus	1	23	19	0.183
O. niloticus	1	16	14	0.07
O. niloticus	1	20	17	0.142
O. niloticus	1	15	10	0.08
O. niloticus	1	20	18	0.19
O. niloticus	1	21	17	0.14
O. niloticus	1	21	18	0.15
O. niloticus	1	23	19	0.15
O. niloticus	1	22	18	0.16
O. niloticus	1	151	126	30
O. niloticus	1	174	147	64
O. niloticus	1	155	125	60
O. niloticus	1	150	123	54
O. niloticus	1	136	110	40
O. niloticus	1	175	150	66
O. niloticus	1	135	114	20
O. niloticus	1	178	140	80
O. niloticus	1	180	153	63
O. niloticus	1	42	36	1.09
O. niloticus	1	52	41	2.31
O. niloticus	1	34	28	0.48
O. niloticus	1	40	33	0.82
O. niloticus	1	40	34	0.93

O. niloticus	1	38	32	0.74
O. niloticus	1	39	32	0.65
O. niloticus	1	39	37	0.75
P. philander	3	61	49	3.42
P. philander	3	50	39	1.32