

**TWO-TISSUE STABLE ISOTOPE ANALYSIS TO
ELUCIDATE ISOTOPIC INCORPORATION AND TROPHIC
NICHE PATTERNS FOR CHUBBYHEAD BARB
*ENTEROMIUS ANOPLUS***

A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

By

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December 2017

ABSTRACT

Knowledge of trophic ecology underpins conservation and management of threatened species. Stable isotope analysis has been widely used as a more objective approach for elucidating the trophic positions of freshwater fishes. Until recently, stable isotope analysis for trophic ecology studies in freshwater fishes largely utilised white muscle tissue. This sampling approach, however, involves either euthanasia or muscle biopsy procedures that may be inappropriate for small-sized and endangered fishes. These concerns raised the need to explore and validate the utility of non-lethal alternatives such as fin clips, mucus and scales. The present study investigated the use of caudal fin tissue as a potential non-lethal alternative to muscle tissue for trophic studies on the chubbyhead barb *Enteromius anoplus*. The chubbyhead barb was selected as a model taxon for the present study because it is closely related or comparable in body size to a number of highly threatened small-bodied minnows in southern Africa. The chubbyhead barb was also considered an ideal species for this study because it is widespread, abundant and classified as Least Concern on the IUCN list of threatened species.

The study used a two-pronged approach based on laboratory and field experiments. A laboratory experiment was conducted to quantify isotopic turnover rates and diet-tissue discrimination factors (DTDFs/ Δ) for both muscle and fin tissues. This involved feeding chubbyhead barb two diets with distinct carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values, and monitoring the temporal isotopic incorporation patterns into the two tissues. These patterns were assessed by applying least squares non-linear one- and two-compartment isotopic kinetics models. Model comparisons, based on Akaike information criterion (AIC), revealed that one-compartment models described isotopic incorporation patterns better than two-compartment models for both muscle and fin tissues. For $\delta^{13}\text{C}$, relatively short and comparable turnover rates were observed for muscle and fin tissues, which suggests that fin tissue could potentially provide similar inference as muscle tissue when assessing short term dietary patterns for

chubbyhead barb. In contrast to $\delta^{13}\text{C}$, turnover rates for $\delta^{15}\text{N}$ between muscle and fin tissue were different for both diets. Specifically, stable isotope incorporation turnover rate was faster in muscle tissue for animals that were fed on isotopically enriched diets compared to fin tissue. Conversely, stable isotope incorporation into fin tissue was faster in animals fed on isotopically depleted diets compared to muscle tissue. This suggests that knowledge of animal diet is critical when inferring fin tissue $\delta^{15}\text{N}$ turnover rates, particularly when extrapolating both short and long term dietary patterns.

Diet-tissue discrimination factors were influenced by diet type, with the fish fed on isotopically enriched diet having lower DTDFs than animals fed on isotopically depleted diets. This variation may be explained by the protein quality hypothesis, which suggests that the DTDFs of consumers will decrease as protein quality increases. When $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values were averaged across diets in muscle and fin tissue, the values were 0.74 ‰ and 0.64 ‰, respectively, for $\Delta^{13}\text{C}$, and 5.53 ‰ and 5.83 ‰, respectively, for $\Delta^{15}\text{N}$. This appeared to be consistent with studies on other taxa for $\Delta^{13}\text{C}$ (0-1 ‰), but for $\Delta^{15}\text{N}$ (3-5 ‰) the results of this study were higher than those reported for other taxa. These results suggest that investigating appropriate DTDFs for both muscle and fin tissues is important in trophic ecology studies of these minnows.

A field-based study was conducted to investigate temporal dynamics in food web patterns for chubbyhead barb in the wild within the headwaters of the Koonap River, a tributary of the Great Fish River, in the Eastern Cape, South Africa. This was achieved by collecting and comparing stable isotope data for chubbyhead barb and its potential food sources on a seasonal scale. There was a discernible difference in both the composition of carbon and nitrogen isotope values for basal food sources and macroinvertebrate communities, which suggests that this headwater stream was subject to temporal changes in food web dynamics. For chubbyhead barb, comparison of its isotopic niche sizes on a temporal scale based on both muscle and fin

tissue showed differences across seasons. Furthermore, isotopic niche sizes inferred from fin tissue were larger than those inferred from muscle tissue during winter and spring, whereas during summer and autumn the isotopic niche sizes inferred from muscle and fin tissue were generally comparable. This suggests the likely influence of different metabolic and physiological processes that these two tissues undergo on a temporal scale. Therefore, difference in tissue type, and their associated metabolic pathways should be considered when using fin tissue as a substitute for muscle tissue on broad temporal scales. The results from this study indicated that caudal fin tissue has the potential to be a substitute for muscle in trophic studies of chubbyhead barb *Enteromius anoplus*, as well as other related small bodied endangered minnow species from South Africa.

TABLE OF CONTENTS

ABSTRACT.....	i
TABLE OF CONTENTS	iv
LIST OF FIGURES	vi
LIST OF TABLES	viii
ACKNOWLEDGEMENTS	x
CHAPTER 1.....	1
General Introduction	1
2.1 Stable isotope analysis in trophic ecology studies	1
2.2 Endangered freshwater fish in South Africa.....	5
2.3 Aims and Objectives	9
2.4 Thesis outline.....	12
CHAPTER 2.....	13
General materials and methods	13
2.5 Why the Koonap River?	13
2.6 Study system	14
2.7 Site characteristics	15
2.8 General approach to stable isotope analysis.....	17
CHAPTER 3.....	19
Isotopic incorporation patterns of dorsal white muscle and caudal fin tissue for the chubbyhead barb <i>Enteromius anoplus</i>	19
3.1 Introduction.....	19
3.2 Materials and methods	21
3.3 Results	28
3.4 Discussion.....	43
CHAPTER 4.....	48
Tracking seasonal dietary shifts in wild chubbyhead barb <i>Enteromius anoplus</i> using muscle and fin tissues: a comparative approach.....	48
4.1 Introduction.....	48
4.2 Materials and methods	50
4.3 Results	52
4.4 Discussion.....	60
CHAPTER 5.....	64

General discussion	64
REFERENCES.....	68

LIST OF FIGURES

Figure 1.1: Chubbyhead barb <i>Enteromius anoplus</i> (Weber, 1897) collected in the headwaters of the Koonap River, Eastern Cape, South Africa.	10
Figure 1.2: Distribution range of chubbyhead barb <i>Enteromius anoplus</i> based on the Global Biodiversity Information Facility (GBIF) data portal.	11
Figure 2.1: Location of study site at the headwaters of the Koonap River.	13
Figure 2.2: The sampling reach in the upper Koonap River showing distinct seasonal changes in the riparian and bank-trailing vegetation in (a) winter (June 2016), (b) spring (September 2016), (c) summer (January 2017), and (d) autumn (April 2017).	15
Figure 2.3: The sampling reach in the upper Koonap River showing distinct seasonal changes in the riparian and bank-trailing vegetation in (a) winter (June 2016), (b) spring (September 2016), (c) summer (January 2017), and (d) autumn (April 2017).	16
Figure 3.1: Three experimental tanks showing two of the four treatments: control 1 and diet 2.	22
Figure 3.2: Tissue excision areas for caudal fin and dorsal white muscle.	25
Table 3.2: Sampling days of fish after switching to the two experimental diets.	25
Figure 3.3: Chubbyhead barb, <i>Enteromius anoplus</i> , white muscle and caudal fin tissue incorporation of (a) $\delta^{13}\text{C}$ from diet 1, (b) $\delta^{15}\text{N}$ from diet 1, (c) $\delta^{13}\text{C}$ from diet 2, and (d) $\delta^{15}\text{N}$ from diet 2. Solid circles represent muscle tissue whereas open circles represent caudal fin tissue. The experiment was carried out for a total of 180 days. Curves were fitted using the one-compartment model with the equation $\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-t/\tau}$	29
Figure 3.4: The average residence time of muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using the one-compartment model for diet 1 and 2.	32
Figure 3.5: Integration of isotopes of laboratory diet switch experiment by chubbyhead barb white muscle and caudal fin tissue for, (a) $\delta^{13}\text{C}$ in diet 1, (b) $\delta^{15}\text{N}$ in diet 1, (c) $\delta^{13}\text{C}$ in diet 2, and (d) $\delta^{15}\text{N}$ in diet 2. Solid circles represent muscle tissue whereas open circles represent caudal fin tissue. The experiment was carried out for a total of 180 days with diet switch at day 90. Curves were fit using the one-compartment model with the equation $\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-t/\tau}$	36
Figure 3.6: The average residence time for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before and after diet switch (trial 1 and 2) using the one-compartment model.	39

Figure 4.2: Stable isotope biplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the chubbyhead barb *Enteromius anoplus*, based on white muscle and caudal fin tissues, aquatic invertebrates and basal sources in winter, spring summer and autumn season. Samples were collected from the headwater of the Koonap River, Eastern Cape, South Africa. Alg = Algae; Mac = Macrophytes; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter Ara = Aranea; Dem = *Demoulinia*; Che = *Cheleocleon*; Ade = *Adenophlebia*; Tri = *Tricorythus*; Sig = *Sigara*; Lac = *Laccocoris*; Sim = *Simulium*; Aul = *Aulonogyrus*; Hyd = *Hydropsychinae*; Aes = *Aeshna*; Lyc = Lycosidae; Tet = *Tetragnatha*; Aes = *Aeshna*; Lib = Libellulidae; Les = *Lestes*; Hep = Heptageniidae; Hyd = Hydraticus; Ani = *Anisops*; Mic = *Micronecta*; Ple = *Plea*; Ger = Gerridae; Ath = Athericidae; Chi = Chironomidae; Hyd = Hydropsychidae; Bae = Baetidae; Tha = *Thalassius*; App = *Appasus*; Lym = *Lymnaea*; Anc = Ancyliidae; Tha = Thalassinae; Mic = *Microgomphus*; Tet = *Tetrathemis*; Ger = Gerrinae; Rha = *Rhagovelia*; Lac = Laccophilinae; Tel = Teloganodidae; Pse = *Pseudagrion*; Pot = *Potamonantes*; Anu = Anura.....57

Figure 4.3: Sample size-corrected standard ellipse areas (SEAc) for chubbyhead barb *Enteromius anoplus* based on muscle and fin tissues for the different season. The black dots represent the mode, whereas the red cross represents the mean. On the abscissa, the seasons were represented by values as winter (1), spring (2), summer (3) and autumn (4) for both tissues. 59

LIST OF TABLES

Table 1.1 List of species and genetic lineages of freshwater fishes from the Cape Fold Ecoregion of South Africa indicating their conservation statuses (adapted from Tweddle <i>et al.</i> , 2009; Ellender <i>et al.</i> , 2017).....	6
Table 3.1: Ingredients and energy values of the experimental diets.....	23
Table 3.3: The incorporation of ^{13}C and ^{15}N into muscle and fin tissue using one- and two-compartment models. All of the incorporation rates were best described using one compartment models as indicated by the lower (*) AIC values.	31
Table 3.4: Tissue comparison curves based on analysis of residual sum of squares (AoRSS) for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in diet 1 and 2. RSS and DF are the residual sum of squares and degrees of freedom, the ‘ <i>t</i> ’ and ‘ <i>i</i> ’ subscripts denote either total or each individual curve.	32
Table 3.5: Likelihood ratio test for muscle and fin tissue in diet 1 and 2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes. The parameters δX_{∞} denote the isotopic composition of the new diet, δX_0 is the isotopic composition of the tissues before the diet shift and τ is the average residence time. RSS is the residual sum of squares, χ^2 is the chi square distribution, <i>df</i> is the degrees of freedom and P is the P-value (a value of < 0.05 indicates that there is significant difference between two parameters).	33
Table 3.6: Mean carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of diet 1 and 2, and the muscle and fin tissues collected at the end of the experiment, and mean diet-tissue discrimination factors (Δ) in chubbyhead barb. Values are reported as mean $\pm$ SD and all values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are presented in ‰.	34
Table 3.7: The incorporation of ^{13}C and ^{15}N into muscle and fin tissue using one- and two-compartment models. All of the incorporation rates were best described using one-compartment models as indicated by the lower (*) AIC values.	38
Table 3.8: Tissue comparison curves based on analysis of residual sum of squares (AoRSS) for trial 1 and 2. Comparison of curves for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in diet 1 and 2. RSS and DF are the residual sum of squares and degrees of freedom, the ‘ <i>t</i> ’ and ‘ <i>i</i> ’ subscripts denote either total or each individual curve, and N/A is not applicable.	39
Table 3.9: Likelihood ratio test for muscle and fin tissue in trial 1 and 2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes. The parameters δX_{∞} denote the isotopic composition of the new diet, δX_0 is the isotopic composition of the tissues before the diet shift and τ is the average residence time.	

<i>RSS</i> is the residual sum of squares, χ^2 is the chi square distribution, <i>df</i> is the degrees of freedom and <i>P</i> is the P-value (a value of < 0.05 indicates that there is significant difference between two parameters).	40
Table 3.10: Mean carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values for trial 1 and 2, and the muscle and fin tissues collected at the end of the experiment, and mean trophic discrimination factors (Δ) in chubbyhead barb. Values are reported as mean $\pm$ SD and all values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are presented in ‰.	42
Table 4.1: Physico-chemical variables sampled during winter, spring, summer and autumn in the headwaters of the Koonap River, Eastern Cape, South Africa.	52
Table 4.2: Mean values of carbon and nitrogen stable isotopes ($\pm$ standard deviation) for the chubbyhead barb, macroinvertebrates, basal sources and other prey collected in the Koonap River in the winter, spring, summer and autumn seasons. The functional feeding trophic guild categories of the macroinvertebrates were based on Cummings <i>et al.</i> (2005).	55
Table 4.3: Comparison of seasonal shifts in isotope niche size and position of muscle and fin tissue.	58

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisors, Dr Wilbert Kadye and Dr Albert Chakona for all their help, guidance and patience throughout this study. Thank you for the many drafts you read and expressing confidence in my abilities when I could only do the opposite. I greatly appreciate everything you have taught me.

Thank you to everyone who assisted with the study. The Albany Museum, Department of Freshwater Invertebrates for the help they provided in invertebrate identification. Thanks are due to Delsy Sifundza, Moqebello Morallana, Nyiko Mabasa, and Siyabonga Ndlovu for the help they provided during my field work. Thank you also goes out to Rhodes University Research Committee, Rufford Small Grants Foundation and the University of Zambia for financial assistance that made this Master's degree possible.

Most importantly, I would like to thank my mum, dad and sister for their endless love, support and encouragement. Special thanks are due to my mother, who has been a constant source of support, advice, and understanding. Thank you, mom.

CHAPTER 1

General Introduction

2.1 Stable isotope analysis in trophic ecology studies

Stable isotope analysis (SIA) is being widely used for studying food web structure because this technique has demonstrated its usefulness as an alternative, complementary, and often as a better approach to food web analysis compared to other conventional dietary analyses (Mcfadden *et al.*, 2006; Rybczynski *et al.*, 2008; Davis *et al.*, 2012; Kadye & Booth, 2012a; Huang *et al.*, 2013; Tsai *et al.*, 2015; Remon *et al.*, 2016). In contrast to conventional methods such as stomach contents, faecal and biomarker compound analyses, which provide a short-term view of recently ingested dietary items, SIA is considered to provide a long-term view of a consumer's diet (Peterson & Fry, 1987; Hesslein *et al.*, 1993; Vander Zanden *et al.*, 1997). In addition to inferring the diet and trophic position of consumers, SIA is crucial in extrapolating other aspects of the food web structure, such as individual level resource specialisation, food chain length and trophic niche size and structure for populations and communities (Vander Zanden *et al.*, 1999a, 2010; Bearhop *et al.*, 2004; Phillips *et al.*, 2005; Newsome *et al.*, 2007; Jackson *et al.*, 2011; Comas *et al.*, 2014; Galetti *et al.*, 2016). The application of SIA has, thus, led to an improved comprehension of, among other factors, trophic interactions, life histories and human-induced impacts in aquatic and terrestrial ecosystems (Hobson *et al.*, 1994; Vander Zanden *et al.*, 1999b; Banas *et al.*, 2009; Bergfur *et al.*, 2009; Bergamino *et al.*, 2014; Remon *et al.*, 2016; Nachev *et al.*, 2017).

Isotopes are forms of the same element that differ in the number of neutrons in the nucleus (Fry, 2006). For example, carbon and nitrogen comprise of lighter isotopic forms, ^{12}C and ^{14}N , and heavier stable isotopic forms, ^{13}C and ^{15}N , respectively. The heavier stable isotopes are usually relatively less abundant in nature. For example, only about 1 % of carbon is present as ^{13}C , whereas ^{12}C comprise approximately 99% of the carbon present (Rosman & Taylor, 1999; Fry, 2006). Similarly, the natural abundance of nitrogen comprises of approximately 99.63 % of ^{14}N , whereas only less than 1% is present as ^{15}N (Rosman & Taylor, 1999). Stable isotopes are naturally occurring isotopes of the same element that have different molecular weights, and these isotopes are radioactively inert. Both ^{13}C and ^{15}N are the two most frequently utilised stable isotopes in trophic ecology studies (e.g. Bearhop *et al.*, 1999; Pinnegar & Polunin, 1999; Post, 2002; Kelly *et al.*, 2006; German & Miles, 2010; Lv *et al.*,

2012; Gillespie, 2013; Hobson *et al.*, 2016; Fu *et al.*, 2017). These two stable isotopes are usually used concurrently in order to provide a robust interpretation of food web structure and trophic interactions in both aquatic and terrestrial ecosystems. An isotopic ratio mass spectrometer is used to determine differences in relative abundance of the heavy and light isotopes in biological samples (e.g. animal, plant and sediment matter), which is expressed as a ratio (e.g. $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) that is measured against a global reference standard and presented using the delta (δ) symbol as parts per thousand or per mil (‰) (Inger & Bearhop, 2008; Mazumder, 2013).

In trophic ecology, interpreting stable isotope data through SIA requires information on how a consumer's tissue and its diets' isotopic values are related (Tieszen *et al.*, 1983; Martínez Del Rio *et al.*, 2009; German & Miles, 2010; Vander Zanden *et al.*, 2015; Lattanzio & Miles, 2016; Franssen *et al.*, 2017). The two main aspects that are essential for this purpose are the diet-tissue discrimination factors (DTDFs/ Δ) and isotopic turnover rates. The DTDF is the difference in isotopic ratios between a consumer's tissue and its diet (Tieszen *et al.*, 1983; Martínez Del Rio *et al.*, 2009). Consumers are assumed to assimilate specific isotopic ratios into their tissues from their ingested prey. There is, thus, an expected increase in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the consumer tissue compared to their prey because of elimination of the lighter isotopes and selective retention of the heavier isotopes due to metabolism. Discrimination factors of 0 - 1 ‰ for $\Delta^{13}\text{C}$ and 3 - 5 ‰ for $\Delta^{15}\text{N}$ have been reported in several studies, and are most frequently utilised for SIA using muscle tissue (DeNiro & Epstein, 1978; Minagawa & Wada, 1984; Peterson & Fry, 1987; Vander Zanden *et al.*, 1997; Post, 2002). Due to their perceived conservative discrimination, carbon stable isotopes are used to infer the source of energy in the ecosystem (DeNiro & Epstein, 1978; Post, 2002; Inger & Bearhop, 2008). Nitrogen stable isotope values, on the other hand, are used to identify a consumer's trophic position in the ecosystem (Minagawa & Wada, 1984; Peterson & Fry, 1987; Cabana & Rasmussen, 1996; Vander Zanden *et al.*, 1997; Post, 2002).

Diet-tissue discrimination factors are also used to estimate the proportional contribution of various prey sources to a consumer's diet using stable isotope mixing models (Phillips & Gregg, 2001, 2003; Moore & Semmens, 2008; Jackson *et al.*, 2011; Phillips *et al.*, 2014). Accurate estimates of DTDFs are required in mixing models as a discrepancy of just 1 ‰ can greatly alter the estimated contributions of dietary sources (Bond & Diamond, 2011; Phillips *et al.*, 2014). Studies increasingly suggest that DTDFs can vary between and within species due to factors such as growth rate, tissue type and diet (Vanderklift & Ponsard, 2003; Caut *et al.*, 2009; Martínez Del Rio *et al.*, 2009; Florin *et al.*, 2011; Busst *et al.*, 2015). If DTDFs are

species, tissue and diet specific, then the use of the commonly cited DTDFs for use in mixing models is inappropriate. It is therefore, important to determine the appropriate DTDFs in order to avoid misinterpreting the food web patterns of a particular ecosystem.

Isotopic turnover rate is the time lag associated with the assimilation of prey stable isotopes into consumer tissue (Hesslein *et al.*, 1993). Like the DTDF, this can be affected by tissue type, isotope analysed, taxon, growth and metabolic rates, and diet of the consumer (Bosley *et al.*, 2002; Suzuki *et al.*, 2005; McIntyre & Flecker, 2006; Guelinckx *et al.*, 2007; Church *et al.*, 2009). This is because animal tissues, for example, are synthesised and replaced at different rates. As a result, less metabolically active tissues, such as bone and collagen, have been reported to have slower turnover rates than more metabolically active tissues, such as liver and blood (Tieszen *et al.*, 1983; Hobson & Clark, 1992; Bosley *et al.*, 2002; Sponheimer *et al.*, 2006; Kurle, 2009; Heady & Moore, 2013; Vander Zanden *et al.*, 2015). Since food sources for consumers are likely to change on a temporal scale, different tissues can be used to extrapolate an animal's short-term or long-term diet (Tieszen *et al.*, 1983; Heady & Moore, 2013). Both DTDFs and isotopic turnover rates can be determined by experimentally feeding an animal two or more isotopically distinct diets and monitoring the temporal changes in the isotopic values of the consumer tissues (Hesslein *et al.*, 1993; Bosley *et al.*, 2002; Herzka, 2005; Suzuki *et al.*, 2005; German & Miles, 2010; Devries *et al.*, 2015; Busst & Britton, 2016, 2018; Oliveira *et al.*, 2017).

In fishes, dorsal white muscle is the most preferred model tissue for SIA (Boecklen *et al.*, 2011). This has been attributed to the view that white muscle tissue is considered to be homogenous, displaying less variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and has minimal inorganic carbon and lipid content (Pinnegar & Polunin, 1999; Dalerum & Angerbjörn, 2005; Jardine *et al.*, 2005; Sweeting *et al.*, 2005; Logan *et al.*, 2008; Sinnatambay *et al.*, 2008). Despite its widespread use, sampling of white muscle in small bodied fishes often requires the animals to be euthanised. Although tissue biopsy procedures are feasible in larger bodied fish species, these procedures can cause either infections or severe damage that can be fatal (Galván *et al.*, 2015). This lethal sampling is therefore not ideal when studying highly threatened fish species.

Because of these serious conservation concerns, there has been a considerable shift towards developing and adopting non-lethal sampling approaches as alternatives to the traditional use of muscle tissue in isotopic studies (Church *et al.*, 2009; Heady & Moore, 2013; Willis *et al.*, 2013; Busst *et al.*, 2015). Several recent studies have used non-lethal sampling approaches, including mucus (Church *et al.*, 2009; Heady & Moore, 2013; Burgess *et al.*, 2017; Shigeta *et al.*, 2017), scales (Perga & Gerdeaux, 2005; Sinnatambay *et al.*, 2008; Fincel *et al.*,

2012; Trembaczowski, 2012; Cano-Rocabayera *et al.*, 2014; Busst & Britton, 2018; Vašek *et al.*, 2017), blood (McIntyre & Flecker, 2006; German & Miles, 2010) and fins (Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Jardine *et al.*, 2011; Graham *et al.*, 2013; Willis *et al.*, 2013; Busst *et al.*, 2015; Busst & Britton, 2016, 2018; Franssen *et al.*, 2017) as alternatives to white muscle. The use of these non-lethal tissues enables researchers to study highly threatened species as this approach is perceived to have less impact on population sizes either when the study animals are not euthanized and when mortalities are unlikely.

Stable isotope turnover rates for blood and mucus tissue have been found to be faster than those for muscle tissue (MacNeil *et al.*, 2005; Church *et al.*, 2009), making them less ideal for establishing long-term dietary utilisation patterns. The utility of scales, on the other hand, is usually limited to fish species that possess large unembedded scales that can be removed with relative ease (Tyus *et al.*, 1999). Consequently, fins have been identified as the most ideal non-lethal alternative for stable isotope analysis in fishes (Van Doornik *et al.*, 1999; Jardine *et al.*, 2005; Suzuki *et al.*, 2005; Dietrich & Cunjak, 2006; Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Hanisch *et al.*, 2010). For most fish species, fin tissue has been found to be the most isotopically similar to muscle tissue, making fins viable surrogates for muscle tissue (Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Tronquart *et al.*, 2012; Willis *et al.*, 2013; Galván *et al.*, 2015). Furthermore, a number of studies on various species using fin clippings have found no significant adverse effects on important aspects of fish biology and behaviour, such as movement, growth, survival and sexual maturity (Tyus *et al.*, 1999; Pratt & Fox, 2002; Vander Haegen *et al.*, 2005; Dietrich & Cunjak, 2006).

While congruence in isotopic composition between muscle and fin tissues has been found (Sanderson *et al.*, 2009; Hanisch *et al.*, 2010; Jardine *et al.*, 2011), some studies have reported an array of variables that should be considered when using fin tissue as a surrogate for muscle tissue (Kelly *et al.*, 2006; Fincel *et al.*, 2012; Tronquart *et al.*, 2012; Graham *et al.*, 2013; Willis *et al.*, 2013; Cano-Rocabayera *et al.*, 2014; Busst *et al.*, 2015; Galván *et al.*, 2015). For example, in some studies, the fin muscle relationship has been shown to vary with the size of the fish (Willis *et al.*, 2013; Galván *et al.*, 2015). In other studies, correction models for converting fin values to muscle values are needed (Kelly *et al.*, 2006), which in some cases need to be species specific (Busst *et al.*, 2015), and in other cases, population specific (Fincel *et al.*, 2012; Galván *et al.*, 2015). This necessitates the need for comparing and validating muscle and fin tissue relationships on a case by case basis, particularly for understudied regions, to avoid inferring erroneous trophic patterns.

2.2 Endangered freshwater fish in South Africa

Freshwater biodiversity and habitats are among the most diverse in the world (Balian *et al.*, 2008). Similar to their terrestrial and marine counterparts, freshwater ecosystems are threatened by increasing anthropogenic activities. The main threats to freshwater biodiversity include invasion by non-native species, habitat degradation, pollution, hydrological modification and overexploitation (Cambray, 2003; Allan *et al.*, 2005; Thieme *et al.*, 2005; Xenopoulos *et al.*, 2005; Dudgeon *et al.*, 2006). These threats have caused range reductions, population declines and localised extinctions of many freshwater fishes from disparate regions (Hall & Mills, 2000; Collares-Pereira & Cowx, 2004; Dudgeon *et al.*, 2006; Light & Marchetti, 2007; Clark *et al.*, 2009; Tweddle *et al.*, 2009; Vitule *et al.*, 2009; Shaiek *et al.*, 2016). For example, in the Mediterranean region, 56 % of the endemic freshwater fish species are threatened, 20 % are endangered, and 18 % are critically endangered due to a combination of these freshwater threats (Smith & Darwall, 2006). The common carp (*Cyprinus carpio*), a species commonly cultivated in aquaculture worldwide, is established in 91 of the 121 countries to which it has been introduced, and is recognised as having adverse ecological effects in 15 of these countries (Clavero & Garcia-Berthou, 2005). The introduction of Nile perch (*Lates niloticus*) into Lake Victoria, caused about 60 % (conservative estimate) of the native fish species to go extinct (Witte *et al.*, 1992). In the Erhai Lake, the second largest highland freshwater lake in China, the abundance of indigenous species has declined, with a majority of these being extirpated from the lake, whereas the number of exotic species has increased (Tang *et al.*, 2013). Miller *et al.*, (1989), concluded that invasive species were the second cause of extinction for North American fish.

It is estimated that freshwater environments are experiencing a higher degree of biodiversity loss than either terrestrial or marine environments (Ricciardi & Rasmussen, 1999; Sala *et al.*, 2000; Loh, 2002; Jenkins, 2003; Strayer & Dudgeon, 2010). This is concerning because more than 10,000 (40 %) of the planet's fish species reside in freshwater habitats (Lundberg *et al.*, 2000) yet these ecosystems represent a very small proportion (0.01 %) of the earth's water supply. Globally, the deliberate or accidental introduction of non-native fish species has been identified as a leading threat to indigenous fish populations through predation, competition, introduction of diseases and structural habitat changes (Cambray, 2003; Clavero & Garcia-Berthou, 2005; Vitule *et al.*, 2009; Cucherousset & Olden, 2011; Marr *et al.*, 2013; Tang *et al.*, 2013).

In South Africa, almost all the major river systems have been invaded by non-native fish

species (de Moor & Bruton, 1988; van Rensburg *et al.*, 2011; Ellender & Weyl, 2014). A total of 55 fishes have been introduced, with the majority of these, except 11 species, having established viable populations (Ellender & Weyl, 2014). The spread of non-native species has caused severe decline in the historical distribution ranges and population sizes of several narrow range endemic species in South Africa (e.g. Thieme, 2005; Clark *et al.*, 2009; Tweddle *et al.*, 2009; Ellender & Weyl, 2014; Weyl *et al.*, 2014; Ellender *et al.*, 2017). The Cape Fold Ecoregion (CFE), in particular, is a hotspot of threatened endemic freshwater fishes due to the introduction and spread of non-native invasive piscivores, including smallmouth bass *Micropterus dolomieu*, largemouth bass *M. salmoides*, spotted bass *M. punctulatus*, sharptooth catfish *Clarias gariepinus*, bluegill sunfish *Lepomis macrochirus*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* (Skelton, 2001; Cambray, 2003; Tweddle *et al.*, 2009; Weyl *et al.*, 2014; Ellender *et al.*, 2017). Additional indirect impacts on native fishes include competition for food and habitat by other non-native fish species, such as banded tilapia *Tilapia sparrmanii*, common carp *Cyprinus carpio* and smallmouth yellowfish *Labeobarbus aeneus* (Tweddle *et al.*, 2009; Kadye & Booth, 2012a; Weyl *et al.*, 2014). The multiple impacts on rivers and streams in the CFE have resulted in localised extirpations of a number of fish species in the region (Clarke, 2009; Chakona *et al.*, 2013; Ellender *et al.*, 2017). At present 12 out of the 21 formally described freshwater fish species in the region, which constitute 60 % of these fishes, are listed under highly threatened categories of the IUCN Red List of Threatened Species (Ellender *et al.*, 2017; Table 1.1). Almost all mainstem populations of native fishes in the CFE have been extirpated, and the remnant populations of these now only persist in headwater streams above physical barriers such as waterfalls and impoundments that prevent upstream movement of non-native species (Woodford *et al.*, 2005; Tweddle *et al.*, 2009; Weyl *et al.*, 2014; Ellender *et al.*, 2017).

Table 1.1 List of species and genetic lineages of freshwater fishes from the Cape Fold Ecoregion of South Africa indicating their conservation statuses (adapted from Tweddle *et al.*, 2009; Ellender *et al.*, 2017).

Family	species	Conservation status
Austroglanididae	<i>Austroglanis barnardi</i> (Skelton, 1981)	Endangered
	<i>Austroglanis gilli</i> (Barnard, 1943)	Vulnerable
Galaxiidae	<i>Galaxias zebratus</i> Castelnau, 1861	Data deficient
	<i>Galaxias</i> sp. “zebratus Breede”	Endangered
	<i>Galaxias</i> sp. “zebratus Goukou”	Vulnerable

Table 1.1 continued:

Cyprinidae	<i>Galaxias</i> sp. “zebratus Heuningnes”	Endangered
	<i>Galaxias</i> sp. “zebratus Klein”	Endangered
	<i>Galaxias</i> sp. “zebratus Mollis”	Not formally assessed
	<i>Galaxias</i> sp. “zebratus nebula”	Vulnerable
	<i>Galaxias</i> sp. “zebratus Rectognathus”	Not formally assessed
	<i>Galaxias</i> sp. “zebratus Riviersonderend”	Not formally assessed
	<i>Galaxias</i> sp. “zebratus Slender”	Not formally assessed
	<i>Labeo seeberi</i> Gilchrist & Thompson, 1911	Endangered
	<i>Labeobarbus capensis</i> (A. Smith, 1841)	Least concern
	<i>Labeobarbus seeberi</i> (Gilchrist & Thompson, 1913)	Vulnerable
	<i>Pseudobarbus afer</i> (Peters, 1864)	Endangered
	<i>Pseudobarbus swartzi</i>	Endangered
	<i>Pseudobarbus senticeps</i> (Smith 1936)	Critically Endangered
	<i>Pseudobarbus asper</i> (Boulenger, 1911)	Endangered
	<i>Pseudobarbus burchelli</i> Smith, 1841	Critically Endangered
	<i>Pseudobarbus</i> sp. “burchelli Breede”	Near Threatened
	<i>Pseudobarbus</i> sp. “burchelli Heuningnes”	Critically Endangered
	<i>Pseudobarbus burgi</i> (Boulenger, 1911)	Endangered
	<i>Pseudobarbus phlegethon</i> (Barnard, 1938)	Endangered
	<i>Pseudobarbus skeltoni</i> Chakona & Swartz 2013	Endangered
	<i>Pseudobarbus tenuis</i> (Barnard, 1938)	Near Threatened
	<i>Pseudobarbus</i> sp. “tenuis Keurbooms”	Endangered
	<i>Pseudobarbus verlorene</i> Chakona, Swartz & Skelton, 2014	Endangered
	<i>‘Pseudobarbus’ capensis</i> (Smith, 1841)	Endangered
	<i>‘Pseudobarbus’ calidus</i> (Barnard, 1938)	Vulnerable
	<i>‘Pseudobarbus’ erubescens</i> (Skelton, 1974)	Critically Endangered
	<i>‘Pseudobarbus’ serra</i> (Peters, 1864)	Endangered
	<i>Enteromius anoplus</i> (Weber, 1897)	Least concern
	<i>Enteromius</i> sp. “pallidus south”	Least concern
Anabantidae	<i>Sandelia capensis</i> (Cuvier 1831)	Data deficient

<i>Sandelia sp.</i> “capensis Breede”	Not formally assessed
<i>Sandelia sp.</i> “capensis Agulhas”	Not formally assessed

For effective management of threatened freshwater fishes, comprehensive information on ecological aspects, such as food web dynamics and trophic interactions, is a critical requirement (e.g. Hussey *et al.*, 2012). Such information can be used to complement other ecological studies, such as habitat relationships (Davis *et al.*, 2012; Kadye & Booth, 2012b), in order to predict potential impacts associated with species introductions and habitat alterations (Cucherousset *et al.*, 2012; Remon *et al.*, 2016) and identify appropriate mitigatory measures to prevent biodiversity loss (Dudgeon, 2010; Strayer & Dudgeon, 2010). The current limited knowledge on the ecology of stream fishes in the CFE has been identified as a major impediment to implementing effective conservation measures to protect the remnant populations of endemic fish species in this region. There is therefore need to provide such ecological information, particularly for headwater streams which constitute the remaining refugia for the unique aquatic taxa of this region.

In headwater streams, food web and trophic dynamics are considered to be an interplay between allochthonous and autochthonous energy inputs, with fishes, which generally form the higher trophic consumer groups, indicating the functioning of these energy pathways. Several stream trophic function models have been proposed to predict how energy flows within these ecosystems. For example, the river continuum concept (Vannote *et al.*, 1980), which demonstrates the structural changes in food web patterns along a river system, postulates that within headwater streams, energy flow is driven by allochthonous matter (leaf litter, branches) than by autochthonous matter (periphyton, microalgae, cyanobacteria). This concept further postulates how dominant sources of energy influence the composition of consumer groups, such as the dominance of shredder invertebrate community and insectivorous fishes in allochthonous-driven food webs in headwater streams, and dominance of grazer invertebrate community and benthivorous fishes in autochthonous-driven mid reaches of rivers (Greathouse & Pringle, 2006; Tomanova *et al.*, 2007).

Despite the widespread use of many stream models, including the river continuum concept, there is uncertainty over the applicability of such models in tropical (Greathouse & Pringle, 2006) and southern temperate streams, such as within the CFE where headwater streams are characterised by high spatial and temporal heterogeneity (de Moor & Day, 2013). Given that within the CFE most threatened freshwater fishes are minnows that occur in

headwater streams, there is need for ecological studies that both elucidate food web dynamics and explore alternative research approaches that minimise detrimental effects on the integrity of these species. Understanding the food web dynamic is critical in order to provide ecological information on biotic interactions, which can be used in conjunction with studies on species habitat interrelationships (Kadye & Booth, 2012b; Kadye *et al.*, 2016) to inform conservation and guide management strategies for threatened species that occur in headwater streams. Similarly, exploring alternative non-lethal research approaches for trophic-ecology based studies is critical in order to enhance ecological studies of threatened species.

The present study explores the use and validity of fin tissue as a non-lethal alternative to muscle tissue for SIA in small bodied fish. Although studies elsewhere have explored the use of fin tissue (Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Jardine *et al.*, 2011; Graham *et al.*, 2013; Willis *et al.*, 2013; Busst *et al.*, 2015; Galván *et al.*, 2015; Vašek *et al.*, 2017), there is need to assess the applicability of such an approach on small bodied and endangered freshwater fish species, such as those of the genera *Pseudobarbus* and *Enteromius* that occur in many headwater streams of the CFE where ecological information on such species is lacking. As caudal fin tissue usually has the largest surface area compared to other fins in small bodied minnows found in Southern Africa, it was chosen as the non-lethal alternative to muscle tissue.

2.3 Aims and Objectives

The primary aim of the present study was to explore and validate the use of fin tissue as a non-lethal alternative to muscle tissue for stable isotope analysis in order to assess the food web patterns of *Enteromius anoplus* (Weber, 1897), within a southern temperate stream in South Africa. *Enteromius anoplus*, commonly referred to as chubbyhead barb (Figure 1.1), is a small bodied cyprinid minnow that is widely distributed in many freshwater habitats throughout South Africa (Figure 1.2). As using threatened or endangered species for this study would result in a further reduction of their numbers, this species was chosen as a model taxon for the study because it is comparable in size to most of the highly threatened small bodied stream fishes in the CFE (Table 1.1). Similarities have been found in some regional models used to quantify relationships between fin and muscle tissue (Tronquart *et al.*, 2012). Therefore, using a proxy species gives an indication of what the stable isotopic values of fin and muscle tissue would be in similar endangered species from the same region. For example, a study by Busst & Britton, (2016) used the European barbel (*Barbus barbus*) and chub (*Squalis cephalus*) to be representative of Eurasian omnivorous freshwater fishes. Furthermore, chubbyhead barb is

listed as a species of least concern in the IUCN Red List (Skelton, 2001; Cambray, 2007). Chubbyhead barb is also known to acclimatise and survive in a wide range of environments, such as small streams, large rivers and lakes (Cambray & Bruton, 1985; Skelton, 2001; Cambray, 2007).



Figure 1.1: Chubbyhead barb *Enteromius anoplus* (Weber, 1897) collected in the headwaters of the Koonap River, Eastern Cape, South Africa.

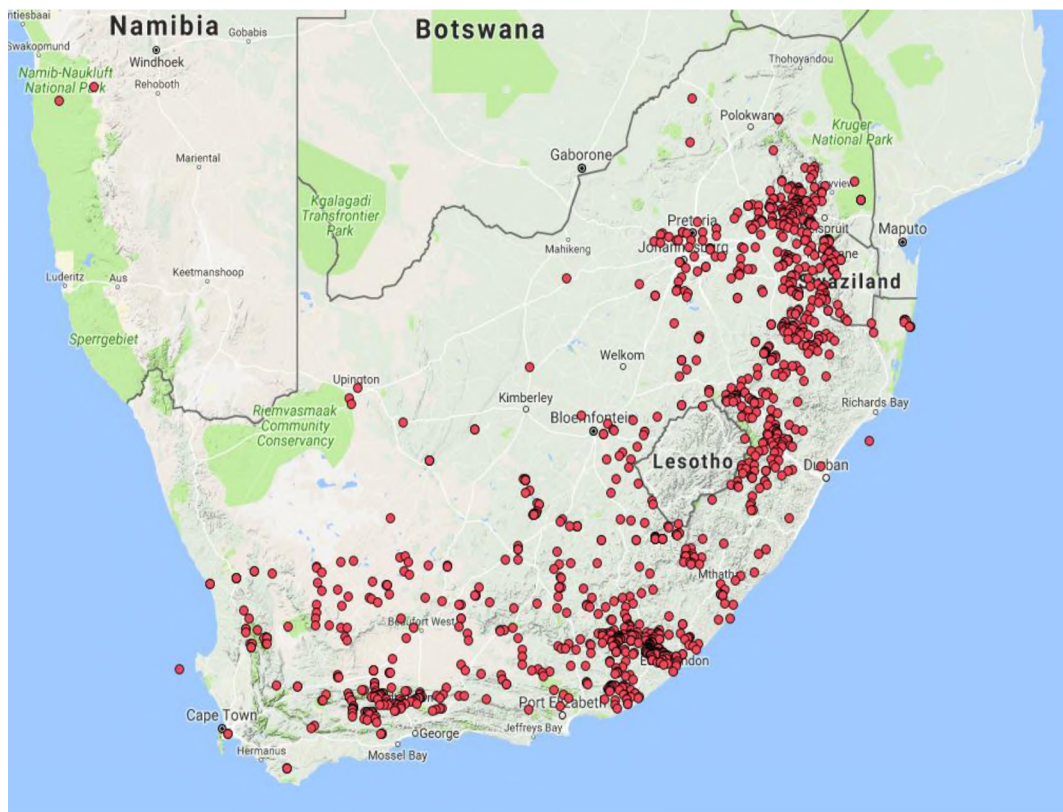


Figure 1.2: Distribution range of chubbyhead barb *Enteromius anoplus* based on the Global Biodiversity Information Facility (GBIF) data portal.

Since interpreting field-based SIA relies on laboratory experimentation in order to provide reasonable inferences on aspects such as tissue turnover rates and DTDFs, this study used a two-pronged approach based on laboratory and field experiments. Firstly, a laboratory experiment was carried out to quantify the isotopic turnover rates and DTDFs of fin and muscle tissue for chubbyhead barb. Secondly, a field experiment was conducted to investigate the food web dynamics of chubbyhead barb in the headwaters of the Koonap River, a tributary of the Great Fish River in the Eastern Cape, South Africa. Chubbyhead barb was the only fish species occurring in these headwaters, a pattern which mirrors the conditions for some of the threatened small-bodied species that occur in headwater streams across the CFE. The study species and field site therefore presented an opportunity to evaluate food web studies using fin and muscle tissues under conditions that would be comparable to some of the endangered cyprinid minnows in South Africa.

Due to the contrasting views on the comparisons between white muscle and fin tissues for different species that have been reported elsewhere, the objective of this study was to explore the suitability of using fin tissue as a non-lethal sampling strategy in trophic studies of chubbyhead barb using SIA. This study, therefore, aimed to contribute to the information

currently available on the use of SIA to elucidate the trophic ecology of freshwater taxa. To achieve this objective, this study addressed the following research questions: To what extent do isotopic turnover rates and DTDF's for caudal fin tissue compare with those of muscle tissue for chubbyhead barb? Can the different body tissues be used to reliably infer temporal changes in food web dynamics and trophic structure using SIA for chubbyhead barb within a headwater stream?

2.4 Thesis outline

To address the above aims and objectives, this thesis is structured into five chapters. Chapter 1 provides an overview introduction to the subject matter. Chapter 2 provides a detailed description of the *in-situ* field aspects and the general *ex situ* laboratory research approaches used to address the study objectives. Chapter 3 presents the findings of a controlled feeding laboratory-based experiment on chubbyhead barb using two isotopically distinct diets. This was done to achieve the following objectives: (1) To determine the turnover rates for caudal fin and white muscle tissue for chubbyhead barb; and (2) to determine tissue-specific $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values. Chapter 4 focuses on a field experiment where SIA-based temporal dynamics of primary producers and their consumers were assessed in order to infer chubbyhead barb's food web structure based on muscle and fin tissues in the headwaters of the Koonap River. The specific objectives of this field study were: (1) To identify the organic matter sources and supporting consumers in the headwaters of the Koonap River; and (2) To determine temporal changes in food sources and consumer communities in relation to the trophic ecology of chubbyhead barb. Lastly, Chapter 5 discusses the principal findings of the study and their implications towards trophic ecology studies of the species and other small bodied species in the CFE.

CHAPTER 2

General materials and methods

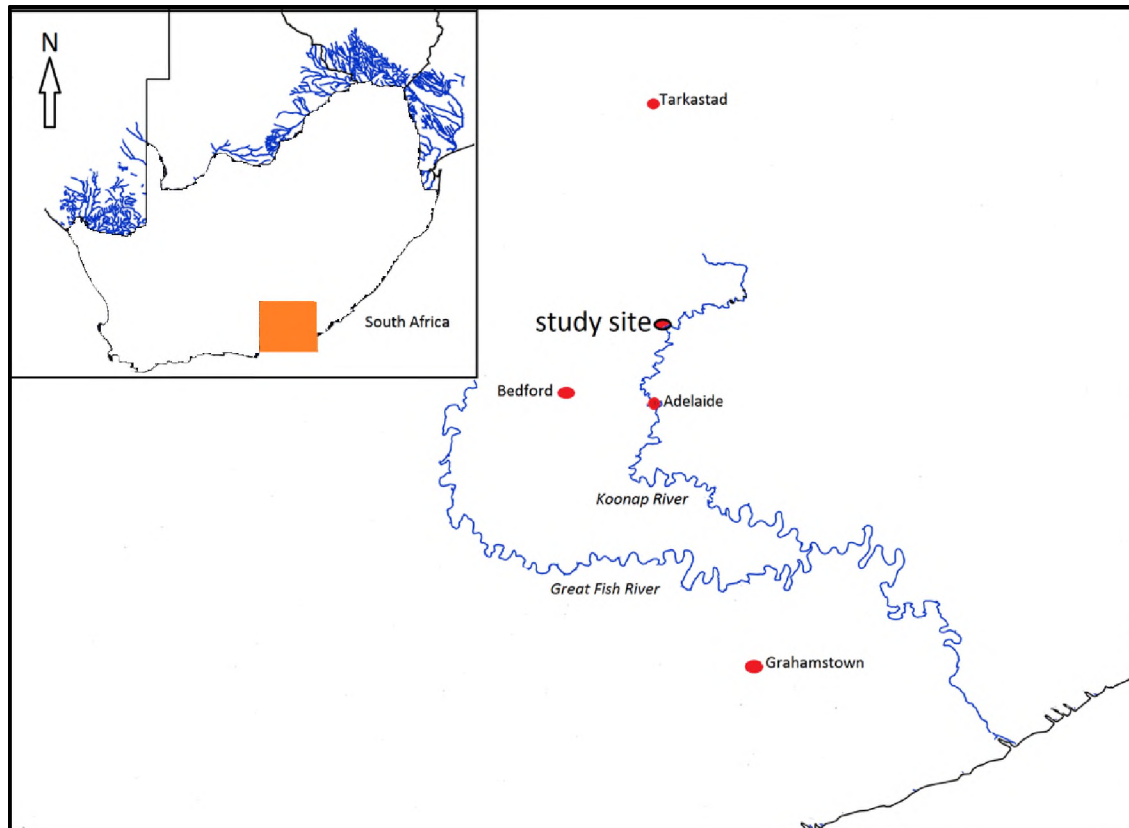


Figure 2.1: Location of study site at the headwaters of the Koonap River.

2.5 Why the Koonap River?

The headwater streams of the Amatola-Winterberg Highlands ecoregion, like the Cape-Fold Ecoregion (CFE), host a rich aquatic fauna with a number of endemic species (Thieme *et al.*, 2005). Also, similar to the CFE, this ecoregion faces serious threats from alien invasive species, flow modifications and over exploitation (Thieme *et al.*, 2005). The headwaters of the Koonap River provide an excellent opportunity to study a small indigenous fish species in environmental conditions that are similar to those of small endangered fishes in the CFE. These streams are subject to both spatial and temporal variation in environmental conditions. In most of the Koonap River's headwater streams, *Enteromius anoplus* occurs as the only species (Kadye & Booth, 2013). Other fish species, such as the longfin eel (*Anguilla mossambica*), Eastern Cape Rocky (*Sandelia bainsii*) and the non-native invasive predator sharptooth catfish (*Clarias gariepinus*) are found in the lower sections of the river (Kadye, 2011).

2.6 Study system

The Koonap River is a tributary of the Great Fish River, in the Eastern Cape, South Africa. It originates from the southern slopes of the Winterberg Mountains and it flows through mountainous terrain to join the lower reaches of the Great Fish River (Department of Water Affairs, 2005). The Koonap River has a total catchment area of 3360 km² and is approximately 220 km long.

Geology and climate

The dominant geological group is the sedimentary Beaufort group of the Karoo super group, which consists of shales, sandstones and mudstones (O'Keeffe & De Moor, 1988; Hoare & Bredenkamp, 1999; Hoare *et al.*, 2006; Kadye, 2011). The climate of the region is warm temperate with an annual precipitation of 650 mm of rainfall that falls mainly during summer (December to February) and autumn (March to May) (Hoare & Bredenkamp, 1999; Thieme *et al.*, 2005). The upper reaches of the Koonap River may have rainfall of up to 800 mm per annum (O'Keeffe & De Moor, 1988). The region has an average daily maximum air temperatures of 19 °C in winter and 29 °C in summer (van Zyl, 1994). Snow fall may occur at high altitudes within the Winterberg with frost occurring regularly between April and September (Kadye, 2011).

Vegetation

At high altitude, a mixture of fynbos heath and montane grasslands is the dominant vegetation type, with the highland peaks covered in both wet and dry Afromontane forest (Thieme *et al.*, 2005). At lower altitudes, the montane forests are replaced by scrub forests and rolling grasslands. A variety of woody trees (e.g. *Pappea capensis*, *Zanthoxylum capense*, *Ozoroa mucronata*), shrubs (e.g. *Putterlickia pyracantha*, *Felicia muricata*), spinescent shrubs (e.g. *Euphorbia bothae*, *Euphorbia tetragona*) and succulent plants (e.g. *Portulacaria afra*, *Delosperma ecklonis*) are found in this area (Hoare *et al.*, 2006). Shorter, dry forest species are found on steeper slopes and gorges while riparian valleys tend to be thickly forested (Thieme *et al.*, 2005).

2.7 Site characteristics

The field study area (Figure 2.1) consisted of a 200 m stretch of stream in the headwaters of the Koonap River north the town of Adelaide. The stream was narrow with rocky substrata. The stream bank was lined with trees and grasses. The major land use pattern around the sampling locality is cattle and sheep livestock ranching.



Figure 2.2: The sampling reach in the upper Koonap River showing distinct seasonal changes in the riparian and bank-trailing vegetation in (a) winter (June 2016), (b) spring (September 2016), (c) summer (January 2017), and (d) autumn (April 2017).

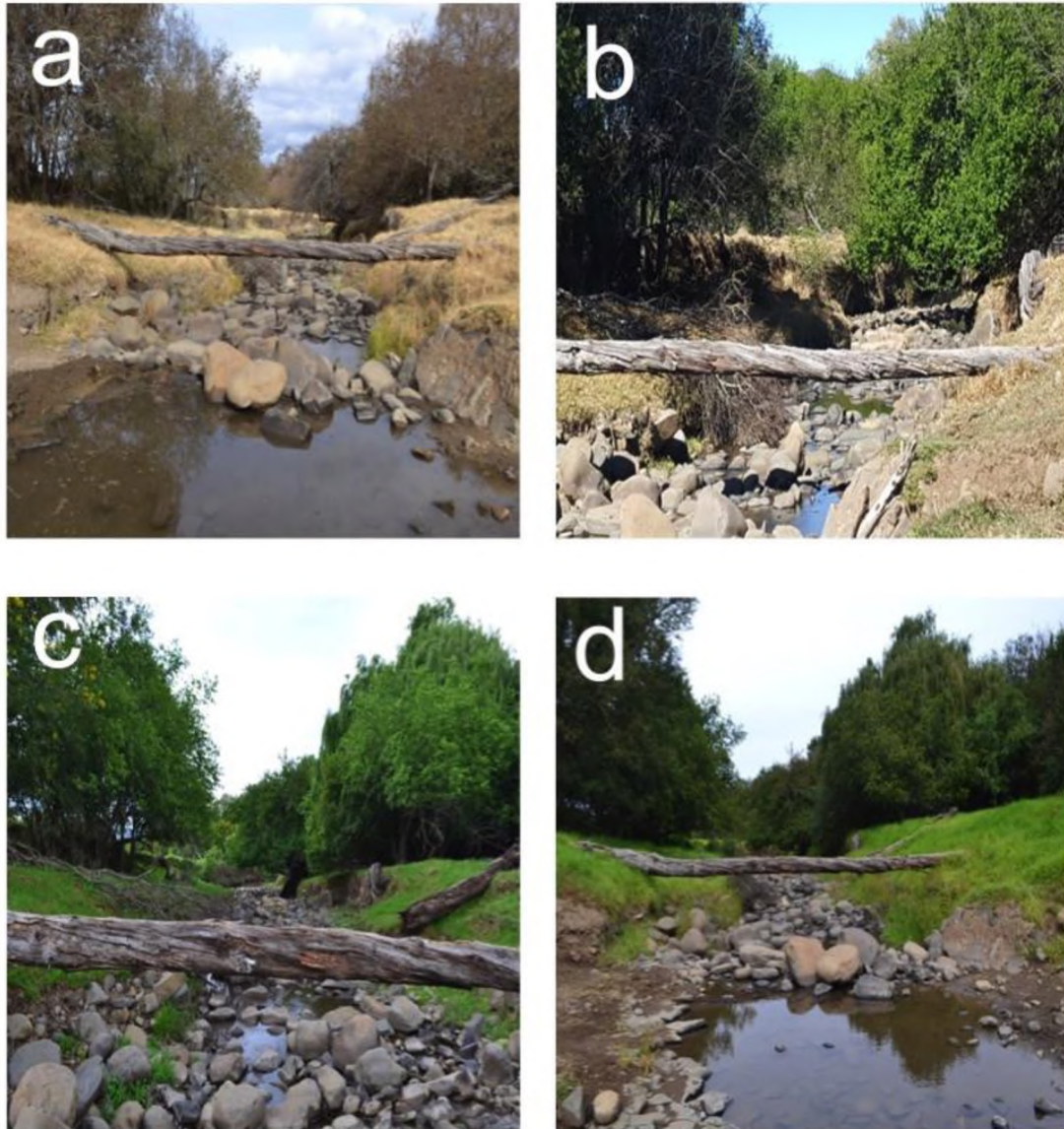


Figure 2.3: The sampling reach in the upper Koonap River showing distinct seasonal changes in the riparian and bank-trailing vegetation in (a) winter (June 2016), (b) spring (September 2016), (c) summer (January 2017), and (d) autumn (April 2017).

In the winter season, the stream water depth was low with little or no flow occurring. The riparian cover was predominantly dry with most trees having lost their leaves (Figures 2.2a and 2.3a). In the stream, allochthonous material, in the form of leaves, twigs and branches, was visually abundant. Little autochthonous material (algae) was observed in the stream during this season. In the spring, stream water depth and flow was low but higher than in the winter season. Cobble and boulder substratum in the deeper parts of the stream were colonised by epilithic algae (Figures 2.2b and 2.3b). Some trees in the riparian zone had new leaf growth whereas,

the grasses were still predominantly dry. Allochthonous material was visually less abundant in the stream.

In the summer, stream flow was higher than in the previous seasons. Autochthonous matter (epilithic algae and macrophytes) was visually abundant in the stream. The riparian zone was lush (Figures 2.2c and 2.3c), and there was less visible in-stream allochthonous material during this season. In the autumn season, stream flow was high. Less algae and macrophytes were also present in the stream. Although the riparian vegetation (both trees and grass) was lush green, there was evidence of senescence (Figures 2.2d and 2.3d).

2.8 General approach to stable isotope analysis

Stable isotope analysis was conducted on the samples that were collected for both the laboratory experiment (Chapter 3) and field study (Chapter 4). For the laboratory experiment, these samples included muscle and fin tissues for chubbyhead barb, and formulated experimental diets. Field study samples included muscle and fin tissues for chubbyhead barb, macroinvertebrates (both aquatic and terrestrial) and basal food sources, such as autochthonous (algae and macrophytes) and allochthonous (terrestrial-derived organic matter).

Stable isotope analysis was done to determine the sample $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ ratios. Isotopic analysis was done on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany) housed at the Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria. Aliquots of approximately 0.6 to 0.65 mg of muscle and fin tissue samples were weighed into tin capsules that had been pre-cleaned in toluene. Two laboratory running standards (Merck Gel: $\delta^{13}\text{C} = -20.26\text{‰}$, $\delta^{15}\text{N} = 7.89\text{‰}$ and DL-Valine: $\delta^{13}\text{C} = -10.57\text{‰}$, $\delta^{15}\text{N} = 6.15\text{‰}$) and a blank sample were run after every 11 unknown samples. Data corrections were done using the values obtained for the Merck Gel during each run while the values for the DL-Valine standard provided the $\pm$ error for each run. These running standards were calibrated against international standards: National Institute of Standards and Technology (NIST): NIST 1557b (Bovine liver), NIST 2976 (mussel tissue) and NIST 1547 (peach leaves). The ratios were reported as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in per mille (‰), relative to Vienna Pee Dee Belemnite and atmospheric nitrogen standards, respectively, according to the formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [R_{\text{sample}} - R_{\text{standard}}] - 1] \times 1000$$

where $R = {}^{13}\text{C}:{}^{12}\text{C}$ or ${}^{15}\text{N}:{}^{14}\text{N}$ respectively (Peterson & Fry, 1987).

Recent studies have shown that tissue differences in lipid content can potentially introduce a source of bias because lipids are generally depleted in $\delta^{13}\text{C}$ (Post *et al.*, 2007; Michaud *et al.*, 2013). This bias can be reduced either by chemical extraction to remove lipids from tissues or by mathematical corrections that are based on tissue C:N ratios (Post *et al.*, 2007). Previous studies on minnows in this region have shown that tissue C:N ratios are generally low (Kadye & Booth, 2013), and that mathematical normalisation was often sufficient (Kadye *et al.*, 2016). In this study, both muscle and fin tissue C:N ratios were generally found to be low ($< 6\text{‰}$) and comparable, hence these tissues were analysed without modifications.

CHAPTER 3

Isotopic incorporation patterns of dorsal white muscle and caudal fin tissue for the chubbyhead barb *Enteromius anoplus*

3.1 Introduction

Stable carbon and nitrogen isotope ratios in animal tissues can be used to make inferences about dietary patterns and trophic relationships in freshwater food webs. Their utility as a tool for inferring these dietary patterns and trophic relationships depends upon an understanding of the rates at which stable isotopes incorporate from the diet into consumer tissue. This requires information of the diet-tissue discrimination factors (DTDFs/ Δ) and isotope turnover rates (DeNiro & Epstein, 1978; Gannes *et al.*, 1997; Martínez del Río & Wolf, 2005). It is generally recognised that the isotopic composition of a particular diet is reflected in the consumer tissue after accounting for the diet to tissue difference of heavier isotopes due to the processes of metabolism and assimilation (DeNiro & Epstein, 1978). This difference in stable isotope values between a consumer's tissue and its diet is referred to as diet to tissue discrimination (Tieszen *et al.*, 1983; Martínez Del Río *et al.*, 2009) and is used in mixing models to quantify the proportional contributions of various food sources to a consumers diet (Jackson *et al.*, 2011; Phillips *et al.*, 2014). Previous experimental studies have reported low diet to tissue discrimination for the carbon stable isotope ($\Delta^{13}\text{C} = 0 - 1 \text{ ‰}$), whereas the nitrogen stable isotope has been found to have relatively high discrimination values ($\Delta^{15}\text{N} = 3 - 5 \text{ ‰}$) (DeNiro & Epstein, 1978; Minagawa & Wada, 1984; Peterson & Fry, 1987; Vander Zanden *et al.*, 1997; Post, 2002). Recent studies in aquatic ecosystems have, nevertheless, shown that the DTDFs for fishes vary among different species, body tissues and regions (Vander Zanden & Rasmussen, 2001; Caut *et al.*, 2009). This prompts the need for determining species and tissue specific DTDF values in order to provide reliable and region-specific inferences of food web interactions. Diet-tissue discrimination factors can be determined from laboratory experiments by switching consumers between alternative diets that differ in stable isotope ratios. These experiments require that the consumer tissue reach isotopic equilibrium with the new diet in order to obtain accurate DTDFs.

Isotope turnover rate is the time lag associated with the assimilation of prey stable isotopes into consumer tissue (Hesslein *et al.*, 1993). Similar to DTDFs, isotope turnover rate can differ among both organisms and tissues within individual organisms due to factors such as growth and catabolic tissue turnover (Tieszen *et al.*, 1983; Dalerum & Angerbjörn, 2005;

Martínez del Río & Wolf, 2005; Logan *et al.*, 2008; German & Miles, 2010; Heady & Moore, 2013). For example, in fishes, metabolically active tissues such as liver and plasma proteins have been found to have faster turnover rates than metabolically less active tissues such as bone collagen and red blood cells (Hesslein *et al.*, 1993; Suzuki *et al.*, 2005; Church *et al.*, 2009; Heady & Moore, 2013). These intraspecific tissue differences can therefore be used to infer temporal variation of a consumer's diet by sampling different types of tissues in a single individual. Furthermore, assessing isotopic turnover rates provides an opportunity to examine the changes in the isotopic composition of an animal's diet, such as due to seasonal change, when animals migrate, or due to anthropogenic effects such as species invasion (Vander Zanden *et al.*, 1999a; Perga & Gerdeaux, 2005; Guelinckx *et al.*, 2007; Hamaoka *et al.*, 2016; Remon *et al.*, 2016). These turnover rates can be determined under laboratory conditions by experimentally feeding the study species a diet of known isotopic composition and then examining the temporal assimilation patterns of isotopes into the tissues as they equilibrate to the new diet (Suzuki *et al.*, 2005; Guelinckx *et al.*, 2007; Martínez Del Río & Anderson-Sprecher, 2008; German & Miles, 2010; Hamaoka *et al.*, 2016).

Several mathematical models have been proposed to quantify the rates of isotopic incorporation in animal tissues. These include one-compartment and multi-compartment models. One-compartment models have been traditionally used for studying species trophic relationships and dietary use patterns (Tieszen *et al.*, 1983; Hobson & Clark, 1992; Carleton *et al.*, 2006). These models assume that dietary isotopes are incorporated and replaced at the same rate within a consumer's tissue. More recently, however, some studies have argued that one-compartment models could be an oversimplification of a complex process and they may sometimes bias estimates of the time taken for different isotopes to be assimilated into body tissues (Cerling *et al.*, 2007).

The reaction progress variable approach, which was proposed by Cerling *et al.* (2007), determined the number of compartments needed for isotopic incorporation in consumer tissue and the size and rate constant of each compartment, also referred to as a "pool". While agreeing that the reaction progress variable is a good approach for assessing the number of compartments needed for a particular isotopic incorporation data set, Martínez Del Río & Anderson-Sprecher (2008), nevertheless, explored the utility of one- and multiple-compartment models to explain isotope incorporation data. These authors complemented the use of the reaction progress variable by comparing model goodness-of-fit with Akaike information criterion (AIC) values to evaluate the most appropriate models of isotopic incorporation. Consequently, there has been a shift towards the integration of one- and multi-

compartment models in order to determine which models best explain isotopic incorporation patterns (Carleton *et al.*, 2008; Tsahar *et al.*, 2008; Heady & Moore, 2013).

Although DTDFs and turnover rates have been studied for different fish species, there are, however, uncertainties on the use of appropriate DTDFs and turnover rates to quantify food webs because differences have been found among fishes (Suzuki *et al.*, 2005; McIntyre & Flecker, 2006; German & Miles, 2010; Heady & Moore, 2013; Willis *et al.*, 2013; Busst & Britton, 2016, 2018; Franssen *et al.*, 2017; Shigeta *et al.*, 2017). Furthermore, there is a paucity of empirical information on both DTDFs and isotope incorporation rates that can be used to infer trophic dynamics for freshwater fishes in understudied regions, such as in southern Africa. Due to the occurrence of several threatened freshwater fishes in southern Africa, particularly for biodiversity hotspots such as the Cape Fold Ecoregion (CFE) (Abell *et al.*, 2008; Tweddle *et al.*, 2009; Ellender *et al.*, 2017), there is need to empirically determine DTDFs and isotope turnover rates, particularly exploring the utility of non-lethal tissues as surrogates for muscle tissue for studies involving imperilled species.

The present study used the chubbyhead barb *Enteromius anoplus* as a surrogate headwater stream fish species to compare patterns of temporal variation in this species' diet based on different body tissues. Furthermore, this study aimed to validate the use of fin tissue as a non-lethal sampling strategy for small bodied freshwater fishes in trophic ecology studies. This was achieved by conducting a laboratory feeding experiment on chubbyhead barb using two diets, each with distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and then quantifying the incorporation rates by determining whether one- or two-compartment models best described isotope turnover for different body tissues. Overall, the laboratory experiment had the following objectives: (1) To explore and compare the isotopic incorporation patterns for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ into white dorsal muscle and caudal fin tissues of chubbyhead barb using two diets and; (2) To determine the $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values for the different body tissues.

3.2 Materials and methods

Experimental design and dietary treatments

On the 27th of June 2016, 250 individuals of *Enteromius anoplus* were captured with a seine net from the headwaters of the Koonap River (Figure 2.1). Upon capture, fish were placed in a 200 L drum with aerated river water and transported to the freshwater laboratory at the Department of Ichthyology and Fisheries Science at Rhodes University. At the laboratory, the

fish were randomly assigned to five aquaria, each with a length, width and height of 90 cm × 32 cm × 35 cm respectively. The fish were fed daily on standard commercial aquarium fish flakes from the 27th of June 2016 to the 2nd of September 2016 (67 days) to allow for acclimation to the experimental conditions.

After the acclimation period, on the 2nd of September 2016, the fish were transferred to experimental tanks (Figure 3.1). The experimental tanks consisted of 20 identical units, each with a length, width and height of 30 cm × 23 cm × 24 cm, respectively. Each experimental tank contained an under-gravel bed and airlift oxygenated system. Each tank was further divided into two areas of open water and pipe refuge (half cylinder pipe 110 mm in diameter and 150 mm long). The aquaria were covered with a fine meshed gauze to prevent the fish from accidentally jumping out of the tanks.



Figure 3.1: Three experimental tanks showing two of the four treatments: control 1 and diet 2.

Two diets with different stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values were developed in the laboratory (Table 3.1). Diet 1, a fishmeal based diet, had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of -14.49 ± 0.01 ‰ and 10.27 ± 0.26 ‰, respectively, whereas diet 2, a soya based diet, had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of -26.77 ± 0.09 ‰ and 0.76 ± 0.03 ‰, respectively. The experimental diets were oven-dried and stored in a blast freezer at -25 °C for the duration of the experiment. At

the beginning of the experiment, the 20 experimental tanks were randomly assigned to two treatments and two control groups, which were designated as diet 1, diet 2, control 1 and control 2, each consisting of five replicate tanks. Each experimental tank was stocked with nine individuals of *Enteromius anophus*. The remainder of the fish (reserves) were left in four acclimation tanks with 18 fish in each tank, with each acclimation tank divided into diet 1, diet 2, control 1 and control 2. Fish were fed once daily to satiation with the food dispersed evenly throughout each tank. The experiment was therefore divided into two parts: (1) control group whereby fish were fed on either diet 1 or diet 2 for the whole duration of the experiment (180 days); and (2) treatment group whereby fish were first fed on either diet 1 or diet 2 for a duration of 90 days (trial 1), then switched diet 2 and diet 1, respectively, from day 90 to day 180 (trial 2).

Table 3.1: Ingredients and energy values of the experimental diets.

	Diet 1	Diet 2
Ingredient		
Fishmeal	461.2	0.0
Soya	0.0	643.7
Maize	536.6	0.0
Rice	0.0	289.2
Sunflower oil	0.0	64.9
Vitamix	2.2	2.2
Total (g)	1000.0	1000.0
Protein (%)	35.00	35.00
Protein digestible (%)	30.23	33.67
Energy level (MJ/kg)	18.73	18.73
Energy level (kcal/kg)?	4477.65	4476.64
Isotope values		
$\delta^{13}\text{C}$ (‰)	-14.49 ± 0.01	-26.77 ± 0.09
$\delta^{15}\text{N}$ (‰)	10.27 ± 0.26	0.76 ± 0.03

During the course of the experiment, the fish were kept in clean filtered water with water temperature maintained at 20 °C and dissolved oxygen was kept at saturation level. The tanks were cleaned (a minimum of once a week) whenever food and faeces accumulated to limit algal and microbial growth in the tanks as these potential food sources would confound the results. This involved either siphoning out debris, faeces and a percentage of the water or removing the fish from the tanks, scrubbing the tanks and changing the water. The laboratory was

designed to simulate a 12-hour day and night photoperiod using timer controlled fluorescent lights. The day period was illuminated from 06:00 to 18:00 hours, whereas the night period commenced from 18:00 to 06:00 hours. Any dead fish were removed from the aquaria, and disposed of following the department of Ichthyology and Fisheries Science guidelines.

Sample collection and preparation

Before the switch to experimental diets (day zero), 12 fish were euthanised in order to determine the initial $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for the muscle and caudal fin tissues. After the switch to experimental diets, fish from each control and treatment group of diet 1 and 2 were then randomly selected, sacrificed and sampled at specific time intervals to track the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope ratios of fish over time. Three fish were randomly sampled (each fish from a different tank on the specific sampling day) from each control and treatment group for both diet 1 and 2. The fish were sampled on days 5, 10, 20, 30, 60 and 90 days (Table 3.2). After 90 days, on the 30th of November 2016, the treatment group fish that were fed diet 1 were switched to diet 2, whereas those that were fed diet 2 were switched to diet 1. The fish in the control groups 1 and 2 continued to be fed the same diets for the duration of the experiment. Three fish were sampled (each fish from a different tank on the specific sampling day) in each control and treatment group of diet 1 and 2 per sample. After the second diet switch, the fish were sampled after 5, 10, 20, 30, 60 and 90 days after diet switch (Table 3.2). The experiment was concluded after 180 days on 28th February 2017.

During each sampling occasion, fish were euthanised with a lethal dose of 2-phenoxyethanol. Each fish was weighed (wet weight (WW), g) and measured for total length (TL, mm), standard length (SL, mm). White muscle tissue samples were removed from the dorsal region of each fish above the lateral line and anterior to the dorsal fin. Whole caudal fins were also excised from each fish (Figure 3.2). These samples were dried at 60 °C for 48 hours, after which they were ground and homogenised into a fine powder using a mortar and pestle. The ground samples were packed in Eppendorf tubes. They were then sent for stable isotope analysis at the Stable Isotope Laboratory, Mammal Research Institute at University of Pretoria, South Africa.



Figure 3.2: Tissue excision areas for caudal fin and dorsal white muscle.

Table 3.2: Sampling days of fish after switching to the two experimental diets.

sampling Phase	
Before diet switch	2-09-2016 to 30-11-2016
1 (DAY 5)	06-09-2016
2 (DAY 10)	11-09-2016
3 (DAY 20)	21-09-2016
4 (DAY 30)	1-10-2016
5 (DAY 60)	31-10-2016
6 (DAY 90)	30-11-2016
After diet switch	30-11-2016 to 28-02-2017
7 (DAY 5)	05-12-2016
8 (DAY 10)	10-12-2016
9 (DAY 20)	20-12-2016
10 (DAY 30)	30-12-2016
11 (DAY 60)	29-01-2017
12 (DAY 90)	28-02-2017

Stable isotope analysis

The procedure for stable isotope analysis is described in Chapter 2.

Statistical analysis

In order to examine isotopic incorporation rates, Martínez Del Rio & Anderson-Sprecher (2008) approach was used to compare how different tissue turnover models fitted the data from the laboratory diet switch experiment. Specifically, one- and two- compartment non-linear models were fitted to estimate isotopic incorporation rates for caudal fin and muscle tissues. The two models were fitted using the following equations:

One-compartment model

$$\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-t/\tau}$$

Two-compartment model

$$\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0) \times (pe^{-t/\tau_1} + (1-p)e^{-t/\tau_2}).$$

For both equations, δX_t was the measured isotopic value of a given element (either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) for a tissue at time t , δX_0 was the isotopic composition of the tissues before the switch to the new diet and δX_∞ was the isotopic composition of the tissues after the switch to new diet. The turnover rate was estimated as the average residence time (τ) or the reciprocal of the fractional incorporation rate $\tau = 1/\lambda$ with half-lives calculated as $t_{1/2} = \tau \ln(2)$ for one-compartment models (Carleton *et al.*, 2008; Martínez Del Rio & Anderson-Sprecher, 2008). For the two-compartment model, each portion's average residence time, τ_1 and τ_2 , and p , the proportional contribution of τ_1 to the overall turnover within a tissue at time t was estimated. To assess the weight of evidence in favour of a one- or two-compartment model, the Akaike information criterion, corrected for small samples (AICc) for each of the models was calculated as:

$$AIC_c = n \left[\ln(2\pi) + 1 + \ln \left(\frac{SSE}{n} \right) \right] + 2K + \frac{2K(K+1)}{(n-K-1)},$$

where n equals the number of observations, K was the number of parameters that were estimated in each model (4 and 6 parameters for the one- and two- compartment models, respectively), and SSE is the error sum of squares. The model with the lowest AIC value was considered to be better in explaining the data. All analyses were done using non-linear least squares routine in Microsoft Excel version 15.35. Additionally, complementary model fits were done in R (R Development Core Team 2017) using the `minpack.lm` package for solving nonlinear least-squares problems by a modification of the Levenberg-Marquardt algorithm, with support for lower and upper parameter bounds.

Analysis of residual sum of squares (AoRSS) was used to compare the isotopic incorporation patterns of muscle and fin tissues. This analysis was used to test the H_0 that isotope incorporation rates of muscle and fin tissues was statistically similar, thereby producing coincident isotope incorporation curves. This was done based on the following procedure. Firstly, for each data set (muscle and fin tissue data), non-linear least squares curve fitting was done and the associated individual residual sum of squares (RSS_i) and degrees of freedom (DF_i) were calculated for either $\delta^{13}C$ or $\delta^{15}N$. Secondly, for all data, a separate non-linear least squares curve fitting was done and the associated total residual sum of squares (RSS_t) and the degrees of freedom (DF_t) were calculated. Finally, the F -statistic was calculated as:

$$F = \frac{\frac{RSS_t - \sum RSS_i}{DF_t - \sum DF_i}}{\frac{\sum RSS_i}{\sum DF_i}},$$

where the RSS and DF were the residual sum of squares and degrees of freedom, respectively. In all cases, DF were calculated as $N-P$, where N was the sample size and P was the number of parameters that were estimated by the model. The ‘ t ’ and ‘ i ’ subscripts denote either the total or each individual estimates, respectively (Chen *et al.*, 1992; Haddon, 2001). Significance testing (P -values) was based on comparison of the calculated and critical F values, and their associated DF based on an F distribution. Where the null hypothesis of similar isotope incorporation rates was rejected, likelihood ratio tests (Kimura, 1980; Haddon, 2001) were then used to test the H_0 on equality of isotopic parameters (δX_0 , δX_∞ and τ) between muscle and fin tissue for both isotopes and diets. The likelihood ratio test was calculated based on a χ^2 statistical test given as:

$$\chi^2_{DF} = -N \times \ln \left(\frac{\sum RSS_i}{RSS_t} \right),$$

where DF was the degrees of freedom, N was the total number of observations from both curves combined, RSS_i was the residual sum of squares obtained from non-linear curve fitting of separate data sets, and RSS_t was the residual sum of squares obtained from non-linear least squares curve fitting when one of the hypothesized parameter was similar (for example when either δX_0 , δX_∞ or τ were constrained as equal for both muscle and fin data) (Haddon, 2001). Both AoRSS and likelihood ratio tests were conducted using Microsoft Excel version 15.35. Discrimination factors were calculated as follows:

$$\delta^y X \text{ or } \Delta^y X = \text{mean} \delta^y X_{\text{tissue}} - \delta^y X_{\text{diet}}$$

where $\delta^y X$ is the diet-tissue discrimination factor of either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, $\delta^y X_{\text{tissue}}$ is the isotopic composition of the consumers tissue and $\delta^y X_{\text{diet}}$ is the isotopic composition of the diet (Greer *et al.*, 2015).

3.3 Results

Isotope incorporation based on control diets

For the control diets, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ incorporation into muscle and fin tissues generally changed asymptotically over time. For diet 1, which was enriched in both ^{13}C and ^{15}N , isotope incorporation revealed progressive enrichment of both muscle and fin tissues (Figure 3.2a and b). For muscle tissue, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from $-20.51 \pm 0.49 \text{ ‰}$ and $11.17 \pm 0.21 \text{ ‰}$ at the start of the experiment, to $-18.56 \pm 0.30 \text{ ‰}$ and $12.46 \pm 0.30 \text{ ‰}$ at the end of the experiment, respectively. By comparison, fin tissue showed a relatively higher change than muscle tissue, whereby $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increased from $-20.63 \pm 0.19 \text{ ‰}$ and $11.34 \pm 0.14 \text{ ‰}$ at the start of the experiment, to $-17.25 \pm 0.60 \text{ ‰}$ and 13.15 ± 0.15 at the end of the experiment. In contrast, for diet 2, which was depleted in both ^{13}C and ^{15}N , isotope incorporation indicated progressive depletion in both muscle and fin tissues over time (Figure 3.3c and d). Muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from $-20.51 \pm 0.49 \text{ ‰}$ and $11.17 \pm$

0.21 ‰ at the start of the experiment, to -21.32 ± 0.06 ‰ and 10.40 ± 0.18 ‰ at the end of the experiment, respectively. Fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from -20.63 ± 0.19 ‰ and 11.34 ± 0.14 ‰ at the start of the experiment, to -22.67 ± 0.34 ‰ and 9.83 ± 0.26 ‰ at the end of the experiment, respectively.

For both diets, isotope incorporation in muscle and fin tissues was best supported by one-compartment models, which had lower AIC values compared to two-compartment models (Table 3.3). Fin tissue had a higher average residence time than muscle tissue in diet 1 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, whereas, in diet 2, muscle tissue had the higher average residence times for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Figure 3.4). For fin tissue, in both diet 1 and 2, $\delta^{15}\text{N}$ had a higher average residence time (130 and 72 days, respectively) than $\delta^{13}\text{C}$ (70 and 59 days, respectively). In muscle tissue, $\delta^{13}\text{C}$ had a higher average residence time for diet 1 (57 days), whereas in diet 2, $\delta^{15}\text{N}$ had a higher average residence time (300 days) when compared to $\delta^{13}\text{C}$ (61 days).

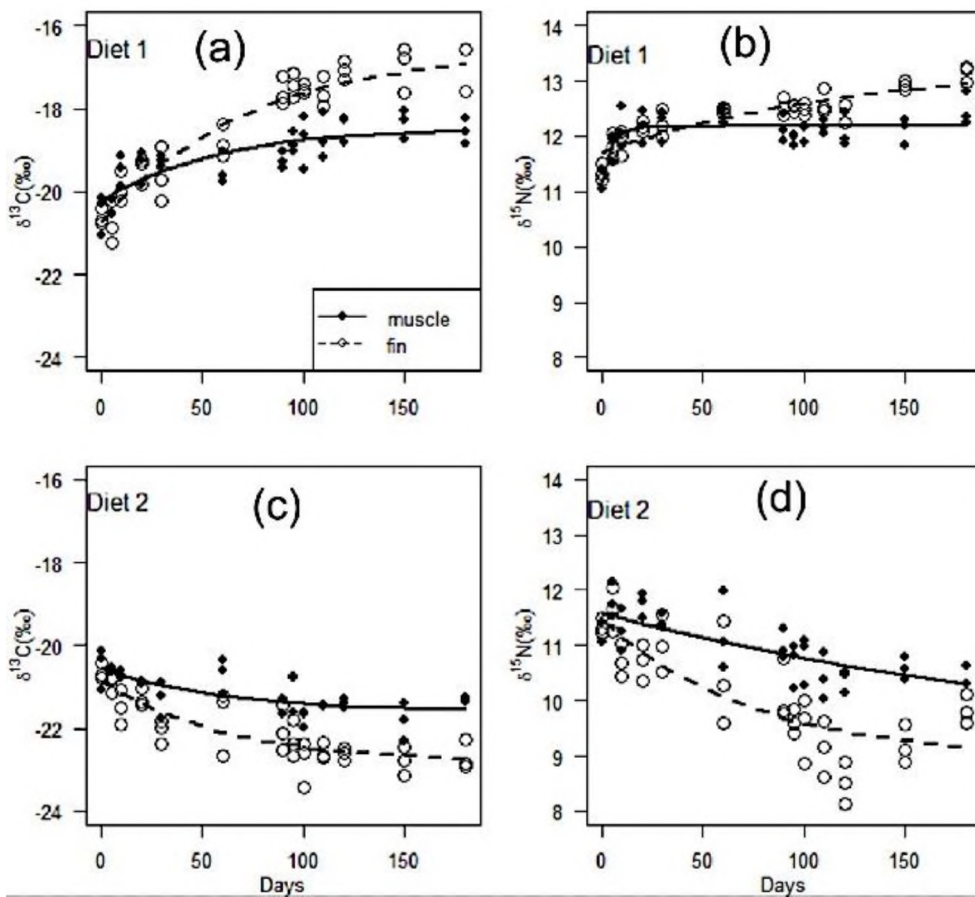


Figure 3.3: Chubbyhead barb, *Enteromius anoplus*, white muscle and caudal fin tissue incorporation of (a) $\delta^{13}\text{C}$ from diet 1, (b) $\delta^{15}\text{N}$ from diet 1, (c) $\delta^{13}\text{C}$ from diet 2, and (d) $\delta^{15}\text{N}$ from diet 2. Solid circles represent muscle tissue whereas open circles represent caudal fin

tissue. The experiment was carried out for a total of 180 days. Curves were fitted using the one-compartment model with the equation $\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-t/\tau}$.

Comparison of isotope incorporation patterns revealed a relatively higher change in the stable isotope values for fin than muscle tissue. Analysis of residual sum of squares (AoRSS) on diet 1 and 2 indicated that isotopic incorporation curves differed significantly between muscle and fin tissue for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes ($P < 0.05$) (Table 3.4). This was further corroborated by likelihood ratio tests on pair-wise comparisons of parameters between muscle and fin tissue isotope incorporation models (Table 3.5). For $\delta^{13}\text{C}$, comparison of isotope incorporation pattern tissues revealed that $\delta^{13}\text{C}_\infty$ values were significantly lower ($\chi^2_1 = 52.83$, $P < 0.001$) in muscle tissue ($\delta^{13}\text{C}_\infty = -18.45 \text{ ‰}$) than fin tissue ($\delta^{13}\text{C}_\infty = -16.62 \text{ ‰}$) for diet 1. In comparison, for $\delta^{15}\text{N}$, isotope incorporation pattern into muscle and fin tissues indicated significant differences in $\delta^{15}\text{N}_\infty$ ($\chi^2_1 = 42.29$, $P < 0.001$), $\delta^{15}\text{N}_0$ ($\chi^2_1 = 19.07$, $P < 0.001$) and τ ($\chi^2_1 = 6.91$, $P = 0.01$) (Table 3.5). For fin tissue, the model estimated values for the parameters $\delta^{15}\text{N}_\infty$, $\delta^{15}\text{N}_0$ and τ were 13.38 ‰, 11.71 ‰ and 130.78 days (half-life = 90.11 day), respectively, whereas those for muscle were 12.18 ‰, 11.17 ‰ and 4.28 days (half-life = 2.97 days), respectively (Table 3.3; Table 3.5). A similar trend was observed in the isotopic incorporation for diet 2 whereby $\delta^{13}\text{C}$, isotope incorporation into muscle and fin tissue showed significant differences in $\delta^{13}\text{C}_\infty$ values ($\chi^2_1 = 40.92$, $P < 0.001$) with fin tissue having lower $\delta^{13}\text{C}_\infty$ values ($\delta X_\infty = -22.82 \text{ ‰}$) than muscle tissue ($\delta X_\infty = -21.61 \text{ ‰}$). In comparison, for $\delta^{15}\text{N}$, isotope incorporation pattern into muscle and fin tissues indicated no significant differences in either $\delta^{15}\text{N}_\infty$ ($\chi^2_1 = 0.00$, $P > 0.05$), $\delta^{15}\text{N}_0$ ($\chi^2_1 = 1.54$, $P > 0.05$) or τ ($\chi^2_1 = 0.25$, $P > 0.05$).

Table 3.3: The incorporation of ^{13}C and ^{15}N into muscle and fin tissue using one- and two- compartment models. All of the incorporation rates were best described using one compartment models as indicated by the lower (*) AIC values.

Diet	Isotope	Tissue	One-compartment	AICc1	Half-life (days)	Two-compartment	AICc2
1	C	Fin	$-16.62 - 4.13e^{-t/69.99}$	50.16 *	48.51	$-16.69 - 15.88(0.27e^{-t/64.09} + -0.07e^{-t/15.32})$	55.51
1	C	Muscle	$-18.45 - 1.78e^{-t/56.67}$	49.92 *	39.28	$-18.32 - 3.49(0.37e^{-t/97.19} + 0.54e^{-t/15.22})$	53.35
1	N	Fin	$13.38 - 1.66e^{-t/130.14}$	29.06 *	90.11	$13.10 - 2.76(0.38e^{-t/129.25} + 0.45e^{-t/17.64})$	30.92
1	N	Muscle	$12.18 - 1.01e^{-t/4.28}$	30.22 *	2.97	$12.18 - 1.29(0.50e^{-t/5.49} + 0.44e^{-t/4.23})$	35.73
2	C	Fin	$-22.82 + 1.97e^{-t/58.65}$	48.93 *	40.65	$-23.01 + 3.97(0.41e^{-t/95.58} + -0.345e^{-t/17.94})$	53.61
2	C	Muscle	$-21.61 + 1.06e^{-t/61.32}$	43.53 *	42.50	$-15 - 6.61(0.5e^{-t/2.49} + 0.04e^{-t/61.37})$	48.98
2	N	Fin	$8.96 + 2.51e^{-t/72.10}$	60.02 *	49.98	$9.03 + 8.87(0.32e^{-t/60.45} + -0.21e^{-t/15.73})$	65.26
2	N	Muscle	$8.72 + 2.86e^{-t/299.52}$	45.01 *	207.61	$7 + 1.73(0.5e^{-t/7.98} + -3.83e^{-t/297.05})$	50.46

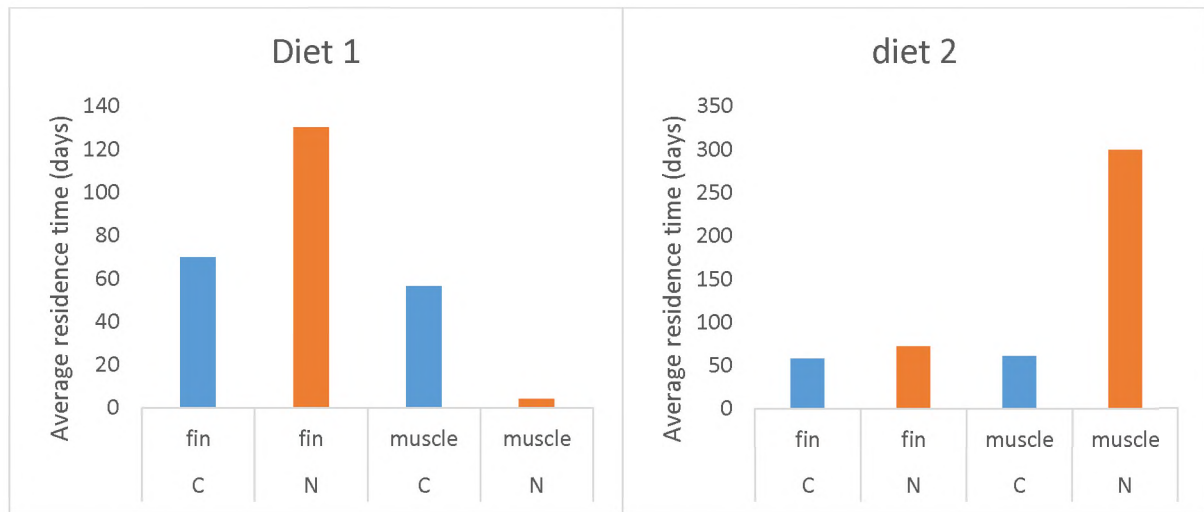


Figure 3.4: The average residence time of muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using the one-compartment model for diet 1 and 2.

Table 3.4: Tissue comparison curves based on analysis of residual sum of squares (AoRSS) for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in diet 1 and 2. RSS and DF are the residual sum of squares and degrees of freedom, the '*t*' and '*i*' subscripts denote either total or each individual curve.

Isotope	Tissue	Comparison	RSS _{<i>t</i>}	DF	RSS _{<i>i</i>}	DF	F	P - value
between								
Diet 1								
^{13}C	Muscle	Fin	32.79	75	13.86	72	8.20	<0.001
^{15}N	Muscle	Fin	7.25	75	4.19	72	4.37	<0.001
Diet 2								
^{13}C	Muscle	Fin	26.29	75	11.21	72	8.29	<0.001
^{15}N	Muscle	Fin	35.16	75	17.64	72	5.96	<0.001

Table 3.5: Likelihood ratio test for muscle and fin tissue in diet 1 and 2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes. The parameters δX_∞ denote the isotopic composition of the new diet, δX_0 is the isotopic composition of the tissues before the diet shift and τ is the average residence time. RSS is the residual sum of squares, χ^2 is the chi square distribution, df is the degrees of freedom and P is the P-value (a value of < 0.05 indicates that there is significant difference between two parameters).

isotope		Full model	Coincident	δX_∞	δX_0	τ
Diet 1						
^{13}C	RSS	13.86	32.79	27.28	13.87	14.36
	χ^2 value		67.18	52.83	0.10	2.78
	df		3	1	1	1
	P-value		<0.001	<0.001	0.75	0.10
^{15}N	RSS	4.16	7.25	7.14	5.30	4.54
	χ^2 value		43.35	42.29	19.07	6.91
	df		3	1	1	1
	P		<0.001	<0.001	<0.001	0.01
Diet 2						
^{13}C	RSS	11.21	26.69	18.79	13.88	11.48
	χ^2 value		67.68	40.29	0.10	1.88
	df		3	1	1	1
	P-value		<0.001	<0.001	0.75	0.16
^{15}N	RSS	17.63	35.15	17.64	17.99	17.69
	χ^2 value		53.81	0.00	1.54	0.23
	df		3	1	1	1
	P-value		0.00	0.92	0.21	0.61

The fishmeal based diet 1 had lower $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values than the soya based diet 2. In both diet 1 and diet 2, $\Delta^{15}\text{N}$ values were higher than $\Delta^{13}\text{C}$ values. For the fishmeal based diet 1, fin tissue had higher $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values than muscle tissue. The estimates for muscle tissue $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values were $3.96 \pm 0.30 \text{ ‰}$ and $1.98 \pm 0.40 \text{ ‰}$, respectively. Those for fin tissue were $-2.62 \pm 0.44 \text{ ‰}$ and $2.61 \pm 0.43 \text{ ‰}$, respectively. For diet 2, muscle tissue had higher $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values than fin tissue. The estimates for muscle tissue $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values were $5.24 \pm 0.37 \text{ ‰}$ and $9.69 \pm 0.21 \text{ ‰}$, respectively. Those for fin tissue were $4.05 \pm 0.28 \text{ ‰}$ and $8.45 \pm 0.68 \text{ ‰}$, respectively.

Table 3.6: Mean carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of diet 1 and 2, and the muscle and fin tissues collected at the end of the experiment, and mean diet-tissue discrimination factors (Δ) in chubbyhead barb. Values are reported as mean $\pm$ SD and all values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are presented in ‰.

Isotope	Day range	Equation $\Delta^y X_{\text{tissue-diet1}}$	DTDF $\Delta^y X_{\text{tissue-diet1}}$	Equation $\Delta^y X_{\text{tissue-diet2}}$	DTDF $\Delta^y X_{\text{tissue-diet2}}$
Muscle					
$\delta^{13}\text{C}$	180	$-18.44 \pm 0.30 + 14.49 \pm 0.01$	-3.96 ± 0.30	$-14.49 \pm 0.01 + 26.77 \pm 0.09$	5.24 ± 0.37
$\delta^{15}\text{N}$	180	$-14.49 \pm 0.01 - 10.27 \pm 0.26$	1.98 ± 0.40	$-14.49 \pm 0.01 - 0.76 \pm 0.03$	9.69 ± 0.21
Fin					
$\delta^{13}\text{C}$	180	$-17.11 \pm 0.44 + 14.49 \pm 0.01$	-2.62 ± 0.44	$-14.49 \pm 0.01 + 26.77 \pm 0.09$	4.05 ± 0.28
$\delta^{15}\text{N}$	180	$-14.49 \pm 0.01 - 10.27 \pm 0.26$	2.61 ± 0.43	$-14.49 \pm 0.01 - 0.76 \pm 0.03$	8.45 ± 0.68

Isotope incorporation based on two-trial treatments

In trial 1 (0-90 days), isotopic incorporation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in fin and muscle tissue showed asymptotic change over time (Figure 3.5a-d). For diet 1, which was enriched in both ^{13}C and ^{15}N , isotope incorporation revealed progressive enrichment of both muscle and fin tissues (Figure 3.5a and b). In muscle tissue, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from $-20.47 \pm 0.21 \text{ ‰}$ and $10.97 \pm 0.22 \text{ ‰}$ at the start of the trial, to $-19.31 \pm 0.27 \text{ ‰}$ and $12.17 \pm 0.12 \text{ ‰}$ at the end of the trial, respectively. By comparison, fin tissue showed a relatively higher change in isotopic incorporation than muscle tissue for $\delta^{13}\text{C}$, whereby $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increased from $-20.75 \pm 0.08 \text{ ‰}$ and $11.21 \pm 0.20 \text{ ‰}$ at the start of the trial, to $-18.12 \pm 0.34 \text{ ‰}$ and $12.54 \pm 0.46 \text{ ‰}$ at the end of the trial. For diet 2 (Figure 3.5c and d), which was depleted in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, muscle isotopic incorporation indicated progressive depletion for $\delta^{13}\text{C}$ and relatively constant incorporation for $\delta^{15}\text{N}$. Furthermore, muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from $-20.47 \pm 0.21 \text{ ‰}$ and $10.97 \pm 0.22 \text{ ‰}$ at the start of the trial, to $-21.58 \pm 0.87 \text{ ‰}$ and $10.78 \pm 0.30 \text{ ‰}$ at the end of the trial, respectively. Fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values changed from $-20.75 \pm 0.08 \text{ ‰}$ and $11.21 \pm 0.20 \text{ ‰}$ at the start of the trial, to $-22.48 \pm 0.71 \text{ ‰}$ and $9.38 \pm 0.20 \text{ ‰}$ at the end of the trial.

In trial 2 (90-180 days) isotopic incorporation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, in diet 1 and 2, generally showed a shift towards a new equilibrium for both muscle and fin tissue (Figure 3.5a-d). For $\delta^{13}\text{C}$, one-compartment models revealed that a switch from isotopically enriched diet 1 to isotopically depleted diet 2 was associated with a shift in $\delta^{13}\text{C}_\infty$ from -19.24 ‰ to -20.04 ‰ and -17.12 ‰ to -19.25 ‰ in muscle and fin tissues, respectively (Table 3.7). Similarly, for $\delta^{15}\text{N}_\infty$, a switch from diet 1 to diet 2 indicated a shift from 12.19 ‰ to 11.17 ‰ in muscle tissue, whereas the shift in fin tissue was indeterminate. In contrast, a switch from isotopically depleted diet 2 to isotopically enriched diet 1 revealed a shift from -21.51 ‰ to -19.92 ‰ for muscle $\delta^{13}\text{C}_\infty$ and from -22.44 ‰ to -20.61 ‰ for fin $\delta^{13}\text{C}_\infty$. A similar pattern was observed for $\delta^{15}\text{N}_\infty$ whereby a switch from isotopically depleted to isotopically enriched diets revealed a shift from 6.39 ‰ to 10.45 ‰ for fin tissue, whereas the shift in muscle tissue was indeterminate.

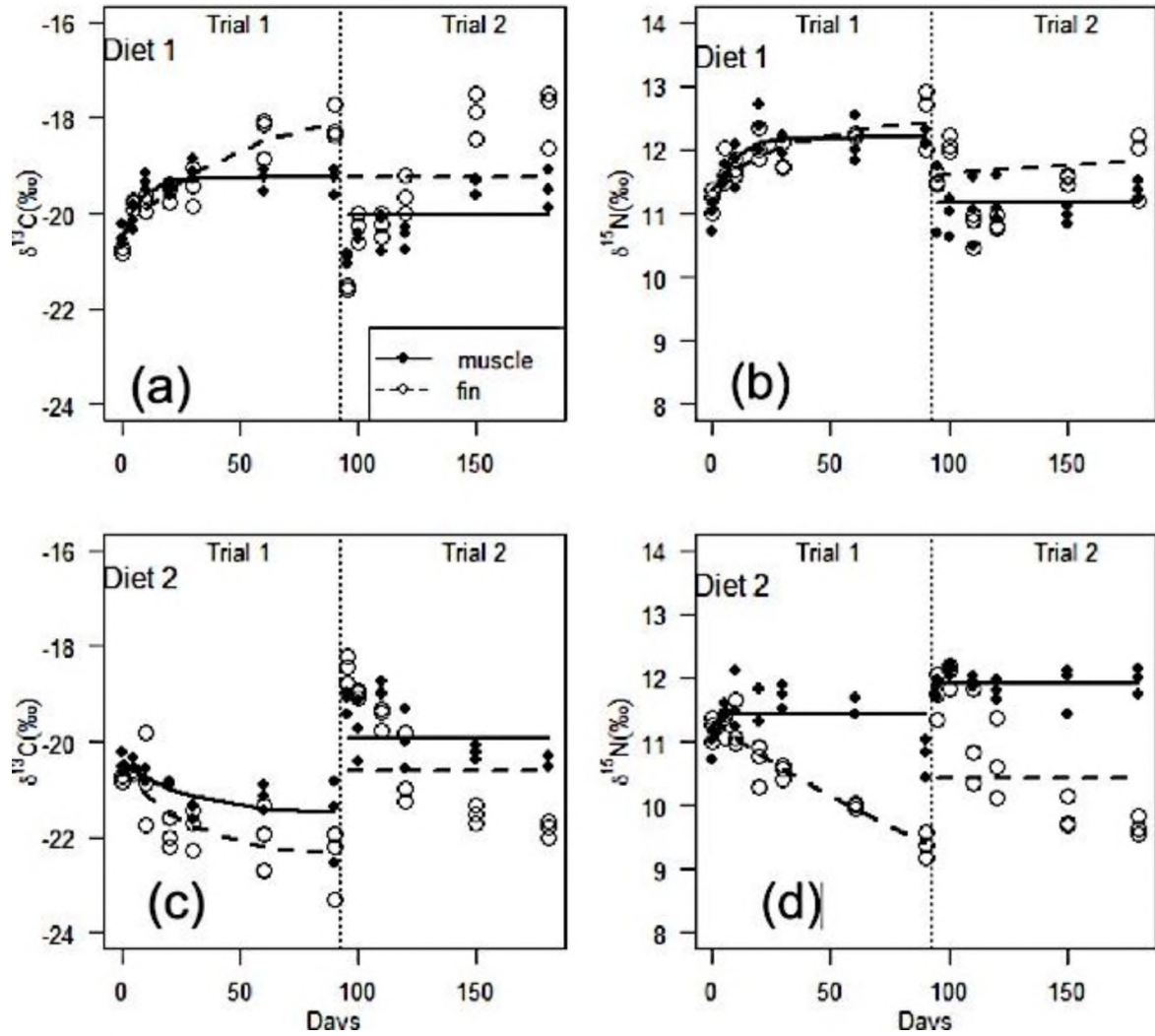


Figure 3.5: Integration of isotopes of laboratory diet switch experiment by chubbyhead barb white muscle and caudal fin tissue for, (a) $\delta^{13}\text{C}$ in diet 1, (b) $\delta^{15}\text{N}$ in diet 1, (c) $\delta^{13}\text{C}$ in diet 2, and (d) $\delta^{15}\text{N}$ in diet 2. Solid circles represent muscle tissue whereas open circles represent caudal fin tissue. The experiment was carried out for a total of 180 days with diet switch at day 90. Curves were fit using the one-compartment model with the equation $\delta X_t = \delta X_\infty - (\delta X_\infty - \delta X_0)e^{-t/\tau}$.

For both trial 1 and 2, isotope incorporation in muscle and fin tissues was best supported by one-compartment models, which had lower AIC values compared to two-compartment models (Table 3.7). For trial 1 in diet 2, $\delta^{15}\text{N}$ values for muscle tissue was indeterminate. This was also the case for trial 2, $\delta^{15}\text{N}$ values for fin tissue in diet 1 and muscle tissue in diet 2. For trial 1, for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, fin tissue had a higher average residence time than muscle tissue for diet 1 and 2 (Figure 3.6). For diet 1, fin tissue had average residence times of 50.45 and 32.02 days for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively, whereas muscle tissue had average residence times of 7.51 and 6.91 days respectively. For diet 2, fin tissue had average residence times of 28.02

and 186.3 days for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively, whereas muscle tissue had an average residence time of 26.08 days for $\delta^{13}\text{C}$ and an indeterminate residence time for $\delta^{15}\text{N}$. In trial 2, for both diet 1 and 2, muscle tissue, muscle tissue had a higher average residence time (8.09 and 12.18 respectively) than fin tissue (7.67 and 9.05 respectively) for $\delta^{13}\text{C}$. For $\delta^{15}\text{N}$, muscle tissue had an average residence time of 22.61 days whereas the average residence time of fin tissue was indeterminate. For diet 2, fin tissue had an average residence time of 11.13 whereas the average residence time of muscle tissue was indeterminate.

Analysis of residual sum of squares (AoRSS) before and after diet switch (trial 1 and 2) indicated that isotopic incorporation curves differed significantly between muscle and fin tissue for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($P < 0.05$) (Table 3.8). The likelihood ratio tests on pair-wise comparisons of parameters between muscle and fin tissue isotope incorporation models were used for parameters that were determined by the models (Table 3.9). For trial 1, $\delta^{13}\text{C}$, comparison of isotope incorporation pattern tissues indicated significant differences in $\delta^{13}C_{\infty}$ ($\chi^2_1 = 22.35$, $P < 0.001$) and τ ($\chi^2_1 = 15.18$ $P = 0.001$) for diet 1. In comparison, for $\delta^{15}\text{N}$, isotope incorporation pattern into muscle and fin tissues indicated significant differences in $\delta^{15}N_0$ ($\chi^2_1 = 4.21$, $P < 0.05$) and τ ($\chi^2_1 = 4.97$ $P < 0.05$). For diet 2, $\delta^{13}\text{C}$, comparison of isotope incorporation pattern tissues indicated significant differences in $\delta^{13}C_{\infty}$ ($\chi^2_1 = 9.26$, $P < 0.001$). For $\delta^{15}\text{N}$ for trial 1(diet 1) and trial 2 (diet 1 and 2) comparison of isotope incorporation pattern could not be carried out for the reasons stated in the paragraph above. For trial 2, $\delta^{13}\text{C}$, comparison of isotope incorporation pattern tissues indicated significant differences in $\delta^{13}C_{\infty}$ ($\chi^2_1 = 17.64$, $P < 0.001$) for diet 1. For $\delta^{13}\text{C}$, diet 2, isotope incorporation into muscle and fin tissue indicated significant differences in $\delta^{13}C_{\infty}$ ($\chi^2_1 = 7.78$, $P < 0.001$)

Table 3.7: The incorporation of ^{13}C and ^{15}N into muscle and fin tissue using one- and two- compartment models. All of the incorporation rates were best described using one-compartment models as indicated by the lower (*) AIC values.

Diet	Isotope	Tissue	One-compartment	AICc1	Half-life (days)	Two-compartment	AICc2
Trial 1							
1	C	Fin	$-17.12 - 3.32e^{-t/50.45}$	25.70 *	34.97	$-17.41 - 6.07(0.44e^{-t/68.80} + 0.18e^{-t/6.14})$	30.01
1	C	Muscle	$-19.24 - 1.28e^{-t/7.51}$	20.79 *	5.21	$-19.22 - 5.10(0.51e^{-t/5.71} + -0.70e^{-t/9.91})$	29.13
1	N	Fin	$12.50 - 1.13e^{-t/32.02}$	18.49 *	22.19	$12.16 - 1.19(0.50e^{-t/17.98} + 0.42e^{-t/4.22})$	26.50
1	N	Muscle	$12.19 - 1.23e^{-t/6.95}$	22.33 *	4.81	$12.20 - 2.02(0.52e^{-t/7.69} + 0.16e^{-t/5.19})$	30.97
2	C	Fin	$-22.44 + 1.89e^{-t/28.02}$	32.81 *	18.08	$-22.27 + 3.51(0.46e^{-t/26.25} + 0.06e^{-t/12.29})$	41.44
2	C	Muscle	$-21.51 + 1.12e^{-t/26.08}$	18.98 *	19.42	$-21.31 + 3.15(0.57e^{-t/15.44} - 0.65e^{-t/58.84})$	27.47
2	N	Fin	$6.39 + 4.93e^{-t/183.60}$	20.11 *	127.26	$9.82 + 3.81(0.56e^{-t/25.27} - 0.42e^{-t/4.92})$	27.21
2	N	Muscle	indeterminate	N/A	indeterminate	indeterminate	N/A
Trial 2							
1	C	Fin	$-19.25 - 2.31e^{-t/7.67}$	48.15 *	5.30	$-19.59 + 23.00(0.50e^{-t/4} + -0.50e^{-t/4})$	56.71
1	C	Muscle	$-20.04 - 0.88e^{-t/8.09}$	24.74 *	5.61	$-19.31 - 10.67(1.97e^{-t/36.40} + 1.00e^{-t/8.00})$	33.53
1	N	Fin	indeterminate	N/A	indeterminate	indeterminate	N/A
1	N	Muscle	$11.17 - 1.18e^{-t/22.61}$	27.03 *	15.67	$11.09 - 1.35(0.59e^{-t/9.82} + 0.35e^{-t/4.83})$	35.59
2	C	Fin	$-20.61 + 2.33e^{-t/9.05}$	47.48 *	6.29	$-20.17 - 1.81(0.50e^{-t/7.48} + 0.5e^{-t/8.00})$	56.03
2	C	Muscle	$-19.92 + 0.76e^{-t/12.18}$	36.28 *	8.44	$-19.74 - 3.26(0.49e^{-t/8.00} + 0.50e^{-t/8.00})$	44.84
2	N	Fin	$10.45 + 1.56e^{-t/11.13}$	42.69 *	7.71	$10.83 + 5.76(0.49e^{-t/6.27} + 0.50e^{-t/8.00})$	51.25
2	N	Muscle	indeterminate	N/A	indeterminate	indeterminate	N/A

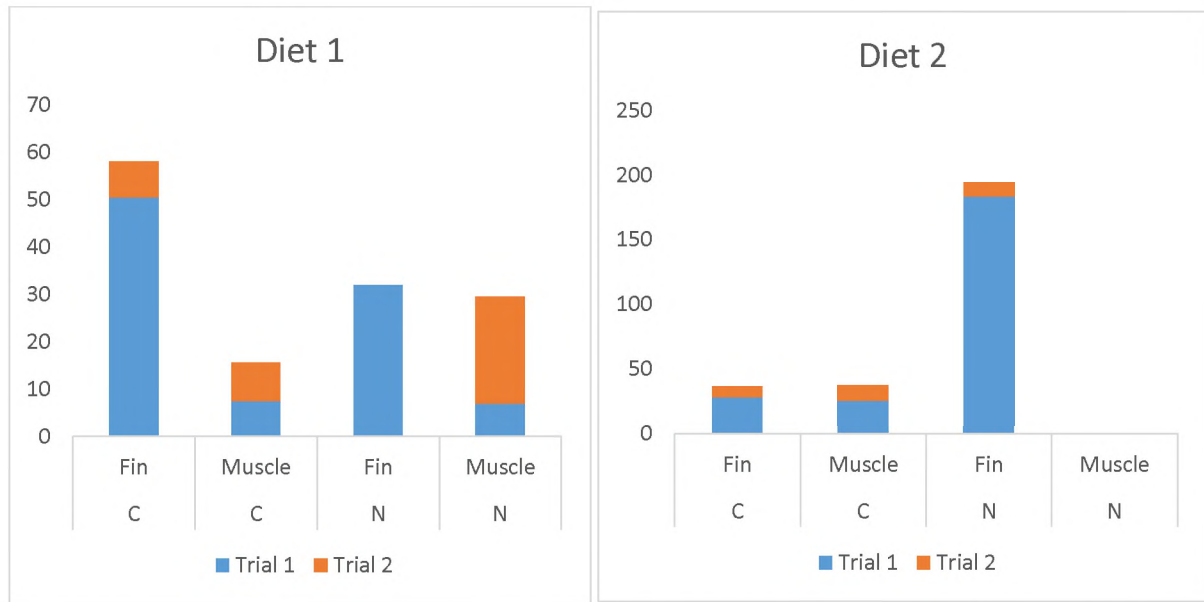


Figure 3.6: The average residence time for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ before and after diet switch (trial 1 and 2) using the one-compartment model.

Table 3.8: Tissue comparison curves based on analysis of residual sum of squares (AoRSS) for trial 1 and 2. Comparison of curves for muscle and fin tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in diet 1 and 2. RSS and DF are the residual sum of squares and degrees of freedom, the ' t ' and ' i ' subscripts denote either total or each individual curve, and N/A is not applicable.

Isotope	Diet	Tissue	Comparison	RSS _{<i>t</i>}	DF	RSS _{<i>i</i>}	DF	F	P-value
between									
Trial 1									
^{13}C	1	Muscle	Fin	6.70	39	3.27	36	6.28	<0.001
^{15}N	1	Muscle	Fin	3.51	39	2.61	36	2.01	0.01
^{13}C	2	Muscle	Fin	11.68	39	8.15	36	2.59	<0.001
^{15}N	2	Muscle	Fin	N/A	N/A	N/A	N/A	N/A	N/A
Trial 2									
^{13}C	1	Muscle	Fin	13.54	39	39.34	36	3.93	<0.001
^{15}N	1	Muscle	Fin	N/A	N/A	N/A	N/A	N/A	N/A
^{13}C	2	Muscle	Fin	13.69	39	9.32	36	2.81	<0.001
^{15}N	2	Muscle	Fin	N/A	N/A	N/A	N/A	N/A	N/A

Table 3.9: Likelihood ratio test for muscle and fin tissue in trial 1 and 2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes. The parameters δX_∞ denote the isotopic composition of the new diet, δX_0 is the isotopic composition of the tissues before the diet shift and τ is the average residence time. RSS is the residual sum of squares, χ^2 is the chi square distribution, df is the degrees of freedom and P is the P-value (a value of < 0.05 indicates that there is significant difference between two parameters).

Isotope	Diet		Full model	Coincident	δX_∞	δX_0	τ
Trial 1							
^{13}C	1	RSS	3.27	6.70	5.56	4.70	3.28
		χ^2 value		30.09	22.35	15.18	0.17
		df		3	1	1	1
		P-value		<0.001	<0.001	<0.001	0.67
^{15}N	1	RSS	2.61	2.99	2.75	2.93	2.88
		χ^2 value		5.83	2.24	4.97	4.21
		df		3	1	1	1
		P		0.12	0.13	0.03	0.04
^{13}C	2	RSS	8.15	11.68	10.17	8.16	8.21
		χ^2 value		15.09	9.25	0.00	0.27
		df		3	1	1	1
		P-value		<0.001	<0.001	0.95	0.60
^{15}N	2	N/A					
Trial 2							
^{13}C	1	RSS	6.42	14.80	10.47	6.43	7.12
		χ^2 value		30.09	17.64	0.10	3.79
		df		3	1	1	1
		P-value		<0.001	<0.001	0.75	0.05
^{15}N	1	N/A					
^{13}C	2	RSS	5.92	13.69	7.99	6.06	6.29
		χ^2 value		27.28	7.87	-2.02	-0.72
		df		3	1	1	1
		P-value		<0.001	<0.001		
^{15}N	2	N/A					

For trial 1, diet 2 had higher $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values than diet 1 (Table 3.10).

The $\Delta^{13}\text{C}$ values for muscle and fin tissue were -4.83 ± 0.27 and -3.63 ± 0.44 respectively for diet 1. The $\Delta^{15}\text{N}$ values for muscle and fin tissue were 1.90 ± 0.29 and 2.27 ± 0.26 respectively. By comparison, the $\Delta^{13}\text{C}$ values for muscle and fin tissue for diet 2 were 5.19 ± 0.88 and 4.29 ± 0.69 respectively, whereas those for $\Delta^{15}\text{N}$ were 10.00 ± 0.30 and 8.62 ± 0.05 respectively. The same pattern was observed in trial 2 with the diet 2 treatment having higher $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values than the diet 1 treatment. The $\Delta^{13}\text{C}$ values for muscle and fin tissue were -5.00 ± 0.38 and -3.43 ± 0.61 respectively for diet 1. The $\Delta^{15}\text{N}$ values for muscle and fin tissue were 1.11 ± 0.30 and 1.54 ± 0.59 respectively. By comparison, the $\Delta^{13}\text{C}$ for muscle and fin tissue for diet 2 were $6.33 \pm 0.16 \pm 0.88$ and 4.93 ± 0.20 respectively, whereas those for $\Delta^{15}\text{N}$ were 11.19 ± 0.21 and 8.91 ± 0.15 respectively.

Table 3.10: Mean carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values for trial 1 and 2, and the muscle and fin tissues collected at the end of the experiment, and mean trophic discrimination factors (Δ) in chubbyhead barb. Values are reported as mean $\pm$ SD and all values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are presented in ‰.

Isotope	Tissue	Day range	Equation $\Delta^y X_{\text{tissue-diet1}}$	DTDF $\Delta^y X_{\text{tissue-diet1}}$	Equation $\Delta^y X_{\text{tissue-diet2}}$	DTDF $\Delta^y X_{\text{tissue-diet2}}$
Trial 1						
$\delta^{13}\text{C}$	Muscle	90	$-19.31 \pm 0.30 + 14.49 \pm 0.01$	-4.83 ± 0.27	$-21.58 \pm 0.87 + 26.77 \pm 0.09$	5.19 ± 0.88
$\delta^{15}\text{N}$	Muscle	90	$12.17 \pm 0.12 - 10.27 \pm 0.26$	1.90 ± 0.29	$10.76 \pm 0.30 - 0.76 \pm 0.03$	10.00 ± 0.30
$\delta^{13}\text{C}$	Fin	90	$-18.12 \pm 0.44 + 14.49 \pm 0.01$	-3.63 ± 0.44	$-22.48 \pm 0.69 + 26.77 \pm 0.09$	4.29 ± 0.69
$\delta^{15}\text{N}$	Fin	90	$12.53 \pm 0.04 - 10.27 \pm 0.26$	2.27 ± 0.26	$9.38 \pm 0.05 - 0.76 \pm 0.03$	8.62 ± 0.05
Trial 2						
$\delta^{13}\text{C}$	Muscle	180	$-19.49 \pm 0.38 + 14.49 \pm 0.01$	-5.00 ± 0.38	$-20.44 \pm 0.13 + 26.77 \pm 0.09$	6.33 ± 0.16
$\delta^{15}\text{N}$	Muscle	180	$11.38 \pm 0.14 - 10.27 \pm 0.26$	1.11 ± 0.30	$11.96 \pm 0.20 - 0.76 \pm 0.03$	11.19 ± 0.21
$\delta^{13}\text{C}$	Fin	180	$-17.92 \pm 0.61 + 14.49 \pm 0.01$	-3.43 ± 0.61	$-21.83 \pm 0.18 + 26.77 \pm 0.09$	4.93 ± 0.20
$\delta^{15}\text{N}$	Fin	180	$11.81 \pm 0.54 - 10.27 \pm 0.26$	1.54 ± 0.59	$9.67 \pm 0.15 - 0.76 \pm 0.03$	8.91 ± 0.15

3.4 Discussion

Isotopic turnover patterns for chubbyhead barb

This study illustrated that isotopic incorporation patterns into both muscle and fin tissues of chubbyhead barb were characterised by asymptotic enrichment in fish that were fed isotopically enriched diets and asymptotic depletion in fish that were fed isotopically depleted diets. Isotopic incorporation patterns based on the control diets depicted a broad view of turnover rates in muscle and fin tissues based on different diets. On the other hand, isotopic incorporation patterns observed for two-trial 90-day diet switch experiment illuminated short-term temporal patterns that are likely to be observed in tissue stable isotope values for this species due to factors such as pronounced seasonal changes in prey availability. These patterns were generally consistent with those observed in several studies that have explored the use of kinetic isotopic incorporation models on different body tissues based on different diets in other animal taxa (Cerling *et al.*, 2007; Caut *et al.*, 2009; Kurle, 2009; Martínez Del Rio *et al.*, 2009; Cloyd *et al.*, 2015). Previous studies on fishes have, nevertheless, reported that isotopic incorporation patterns for different body tissues were supported differently by both one- and two-compartment models (German & Miles, 2010; Heady & Moore, 2013). By comparison, for chubbyhead barb, isotopic incorporation patterns were supported by one-compartment models in both muscle and fin tissues. This suggests the importance of first order isotope kinetics in isotope incorporation in both muscle and fin tissue for this species.

Previous studies have attributed the variation in isotopic turnover rates among tissues to differences in metabolic activity, such as tissue growth due to anabolism and catabolic turnover (Tieszen *et al.*, 1983; Hobson & Clark, 1992; Martínez del Río & Wolf, 2005; McIntyre & Flecker, 2006; Carleton *et al.*, 2008; Martínez Del Rio *et al.*, 2009; Vander Zanden *et al.*, 2015; Franssen *et al.*, 2017). In fishes, tissue turnover generally follows the pattern that more metabolically active tissues would exhibit faster stable isotopic turnover rates (Suzuki *et al.*, 2005; McIntyre & Flecker, 2006; Guelinckx *et al.*, 2007; Heady & Moore, 2013; Shigeta *et al.*, 2017). Fin tissue, with a layer of mucus that is probably regenerated continuously is expected to have a faster turnover rate than muscle tissue (Guelinckx *et al.*, 2007). Muscle tissue on the other hand has a relatively low protein turnover in fish (Heady & Moore, 2013). In this study, shorter half-lives were, nonetheless, observed in muscle than fin tissue for fish that were fed on isotopically enriched diets, whereas for fish that were fed on isotopically depleted diets, fin

tissue had shorter half-lives than muscle tissue. Thus, pattern of the more metabolic active tissue having faster turnover rates appears to be diet dependent in chubbyhead barb.

Tissues differ in their $\delta^{15}\text{N}$ values due to differences in their amino acid content and the isotopic composition of individual amino acids (Reich *et al.*, 2008). For $\delta^{13}\text{C}$, values between tissues may differ due to differences in lipid content and amino acid composition (Martínez Del Rio *et al.*, 2009). Fish use liver and skeletal (white and red) muscle as the main sites for lipid storage (Pinnegar & Polunin, 1999). Lipid synthesis is accompanied by depletion in $\delta^{13}\text{C}$ (DeNiro & Epstein, 1978), therefore muscle tissue which has more lipid content than fin tissue will have a faster turnover rate than fin tissue when fed on an isotopically enriched diet as a result of lipid synthesis. In this study, since the C:N ratios for fin and muscle tissue were relatively low (Chapter 2), it is likely that lipid content may not have been the sole source of variation between the two tissues (Post, 2002). Because of the small fin size of chubbyhead barb, both soft rays and membrane tissues were included. It is thus likely that the inclusion of this different parts may have had an influence on the overall amino acid profile of the fin tissue, and this aspect need further investigation. Macko *et al.*, (1986) suggested that as dietary protein increases, the percentage of nitrogen in the diet increases and more amino acids are catabolised for energy. Therefore, for chubbyhead barb, in protein rich diets, muscle tissue may have a higher rate of catabolism than fin tissue leading to a higher turnover rate.

Although there were significant differences in isotope incorporation rates, the observed patterns for $\delta^{13}\text{C}$ were comparable between fin and muscle tissues, whereas those for $\delta^{15}\text{N}$ differed between tissues for both control and treatment groups. Half-life differences for $\delta^{13}\text{C}$ between muscle and fin tissue were in the range of 39-48 days in the control groups and 5-8 days in the two-trial treatment groups after diet switch. An exception to this comparable trend was observed in the diet 1 treatment group during the first trial (half-lives of 35 days and 5 days for fin and muscle tissue respectively). These results suggest that caudal fin tissue can be a useful substitute for muscle tissue in field studies for chubbyhead barb using $\delta^{13}\text{C}$. In contrast to $\delta^{13}\text{C}$, considerable differences in turnover rates were observed between fin and muscle tissue for $\delta^{15}\text{N}$ in the two diets. In particular, fin tissue had longer turnover rates than muscle tissue in diet 1 (90 and 3 days for fin and muscle tissue respectively), whereas muscle tissue had longer turnover rates than fin tissue in diet 2 (50 and 208 days for fin and muscle tissue respectively). Therefore, diet considerations need to be taken when inferring trophic ecology based on $\delta^{15}\text{N}$ using fin tissue.

Other studies on different fish species have found differences in $\delta^{15}\text{N}$ half-life between muscle and fin tissue, although the differences of these trends have been found to be

inconsistent. For example, Heady and Moore (2013), calculated half-lives for muscle, fin and scales in rainbow trout *Oncorhynchus mykiss* and found that fin had the fastest $\delta^{15}\text{N}$ half-life (13 days), followed by muscle (39 days). In contrast, a study on juvenile barbel *Barbus barbus* found $\delta^{15}\text{N}$ half-life to be slower in fin tissue (95 days) and faster in muscle tissue (84 days) (Busst & Britton, 2018). Other studies have reported similar turnover rates between fin and muscle tissue (Suzuki *et al.*, 2005; McIntyre & Flecker, 2006). These observations highlight the importance of evaluating species/taxa specific isotope incorporation patterns in fishes (Busst & Britton, 2018).

Diet-tissue discrimination factors

In comparison to the most commonly referred diet-tissue discrimination factors of 0-1 ‰ for $\delta^{13}\text{C}$ and 3-5 ‰ for $\delta^{15}\text{N}$ (DeNiro & Epstein, 1978; Peterson & Fry, 1987; Post, 2002), this study showed that there was considerable variation in the DTDFs for muscle and fin tissue based on the different diets. Specifically, the DTDFs for fish fed on the soya based diet were higher ($\delta^{13}\text{C}$ range from 4.05 to 5.76, $\delta^{15}\text{N}$ range from 8.45 to 10.59) than the values mostly used in literature, whereas those for fishmeal based diet were lower ($\delta^{13}\text{C}$ range from -4.91 to -2.62, $\delta^{15}\text{N}$ range from 1.51 to 2.61) than literature based values. Certain biochemical reactions in an organism discriminate against the heavier isotopes causing a fractionation in the amounts of isotopes in tissues (Peterson & Fry, 1987). Incorporation rates are also likely to differ between feeding guilds. For example, herbivores that feed on plant based diets are likely to have different metabolic pathways to those of carnivores that feed on animal based diets, which potentially produces different isotopic fractionation values (Mill *et al.*, 2007; Sweeting *et al.*, 2007)

The marked differences in DTDFs between the fishmeal and soya based diets can be explained by the protein quality hypothesis which suggests that discrimination in stable isotopes of consumers and their prey will increase as protein quality decreases (Roth *et al.*, 2000; Florin *et al.*, 2011). Roth *et al.*, (2000) suggested that the diets of herbivorous animals contain amino acid mixtures different from that required by the consumer. Therefore, herbivores synthesise a large proportion of amino acids by transamination of keto acids derived from the carbohydrate part of their diet. In comparison, diets of carnivores are predominantly made up of proteins and lipids, therefore, the amino acid requirement is satisfied primarily by the diet. As a result, because carnivores assimilate higher quality protein they tend to have lower DTDFs than herbivores which assimilate lower quality protein (Mill *et al.*, 2007;

Sweeting *et al.*, 2007). Omnivorous fish, on the other hand, will have diet-tissue discrimination factors which vary considerably between animal and plant prey (Busst & Britton, 2016). In future experiments, it would be beneficial to check if the sex of the fish would have any effect on the isotopic incorporation rates. The sex of the fish and sex ratio per tank were not considered in this study. As size influences metabolism with smaller sized animals having faster metabolism and hence faster isotopic incorporation and turnover (Hobson & Clark, 1992; Martínez del Río & Wolf, 2005; McIntyre & Flecker, 2006; Martínez Del Río *et al.*, 2009; Vander Zanden *et al.*, 2015), it would also be beneficial in future studies to tag and weigh the study species before and after diet switch. As the determination of DTDFs depends on the consumer being in isotopic equilibrium with its diet, increasing the experimental time to allow for all the tissues to reach isotopic equilibrium with the diets would be advantageous.

Averaging the DTDFs in the control groups produced discrimination factors that were in line with the literature values for $\delta^{13}\text{C}$ (0.74 ‰ and 0.64 ‰ for fin and muscle tissue respectively), but higher than the literature values for $\delta^{15}\text{N}$ (5.53 ‰ and 5.83 ‰ for fin and muscle tissue respectively). Averaging of diet-tissue discrimination factors also showed that muscle tissue was enriched in $\delta^{15}\text{N}$ and depleted in $\delta^{13}\text{C}$ when compared with fin tissue. This general pattern of enrichment and depletion between muscle and fin tissue has been observed in other studies (Sanderson *et al.*, 2009; Graham *et al.*, 2013; Busst *et al.*, 2015; Busst & Britton, 2016) and is probably related to the variation in amino acid profiles between different tissues which can cause differences in discrimination factors (Reich *et al.*, 2008). The results from this experiment suggest that in predictive models for estimating the diet composition of omnivores like chubbyhead barb, the average values of diet-to tissue discrimination factors between animal based and plant based diets should be used. Alternatively, the diet-tissue discrimination factors of a diet containing 50:50 mix of plant and animal feed would have to be used.

To conclude, turnover rates are important for determining the rate at which an animal's dietary stable isotopes are incorporated into its tissue. Knowledge of turnover rates of tissues of a consumer species important as tissues with distinct turnover rates may represent diets integrated over various temporal scales (Hesslein *et al.*, 1993; Dalerum & Angerbjörn, 2005; Boecklen *et al.*, 2011). The results from this study indicated that for $\delta^{13}\text{C}$, fin and muscle tissue had comparable turnover rates, and therefore fin tissue could be used as a replacement for muscle tissue for this isotope for threatened small minnow species in this region. By comparison, $\delta^{15}\text{N}$ incorporated into muscle and fin tissue appeared to be influenced by diet type. In a low-quality protein diet, fin tissue had a shorter half-life, which in theory should

provide more recent dietary information, whereas muscle tissue with the longer half-life should provide longer term dietary information. The opposite is true for a high-quality protein diet. Key to the interpretation of stable isotope values in diet and trophic studies is the stepwise enrichment in isotopic values between a consumer and its diet known as the diet-tissue discrimination factor. These DTDFs are critical as they are used in mixing models to predict the proportional contribution of various food sources to a consumer's diet. The results from this study also indicated that although discrimination factors for chubbyhead barb were comparable between muscle and fin tissue, they were diet-dependent. Therefore, the DTDF values found in this study can be used on chubbyhead barb and potentially other small minnow species in this region if information on their diets in their specific systems is taken into account. More studies are needed to illustrate the mechanism through which dietary isotopic ratios influence the extent of isotopic incorporation in tissues.

CHAPTER 4

Tracking seasonal dietary shifts in wild chubbyhead barb *Enteromius anoplus* using muscle and fin tissues: a comparative approach

4.1 Introduction

Conservation of freshwater fishes in lotic habitats requires an understanding of both their trophic ecology and the ecosystem dynamics of their environment (Geist, 2011; Hussey *et al.*, 2012). Understanding the ecosystem dynamics and energy flow in lotic ecosystems is, however, challenging because many of these habitats are open systems with high spatial and temporal variability, which influence both the availability and utilisation of trophic resources (Grossman *et al.*, 1998; Thorp *et al.*, 2006). From a trophic ecology perspective, riverine fishes are assumed to have evolved biological traits that enable them to survive variable environmental conditions (Eros & Grossman, 2005). For example, stream fishes have evolved towards euryphagy and stenophagy feeding habits as adaptations to wide and narrow prey range, respectively. However, stream fishes that occur in headwaters are assumed to be facultative feeders, exhibiting a high dietary plasticity in response to available prey resources (Welcomme, 1985; Lowe-McConnell, 1987).

Several stream trophic function models have been proposed to predict how energy flows within lotic ecosystems (e.g. Vannote *et al.*, 1980; Ward & Stanford, 1983; Junk *et al.*, 1989; Boulton *et al.*, 1998). One of the most widely used model is the river continuum concept proposed by Vannote *et al.*, (1980). Specifically, this model postulates that many headwater streams are influenced by riparian vegetation, which both contributes large biomass of allochthonous detritus to the aquatic ecosystem and reduces autochthonous production by shading. This model, thus, hypothesizes that these headwater streams would be dominated by shredder and collector macroinvertebrate communities that utilize coarse particulate organic matter and fine particulate organic matter derived from the riparian zone. Because many headwater streams are generally characterised by invertivorous fishes (Vannote *et al.*, 1980), the composition and abundance of the macroinvertebrate fauna, and any corresponding spatial and temporal changes, are likely to influence the energy dynamics and trophic ecology of these fishes. Although the river continuum concept is primarily based on North American temperate systems, this model is often inferred to be universal, and therefore, assumed to be applicable to other regions, including southern tropical and temperate river systems (Greathouse &

Pringle, 2006; Tomanova *et al.*, 2006). However, due to the ecological differences in lotic ecosystems in different parts of the world, there is need to explore the validity of trophic dynamics models, such as the river continuum concept, and, where applicable, provide either complementary or contrasting alternatives, particularly for river systems in southern Africa where such studies are still lacking.

Stable isotope analysis has been widely used to study the trophic links between primary producers and higher trophic levels (Whitledge & Rabeni, 1997; Rybczynski *et al.*, 2008; Allen *et al.*, 2009; Bouillon *et al.*, 2011). In lotic ecosystems, stable isotope analysis has been used to show spatial and temporal shifts in the relative importance of autotrophic production versus detrital input and processing (Vizzini & Mazzola, 2003; Fenoglio *et al.*, 2015). For fishes, since some studies have shown that different body tissues may have either different or comparable isotope turnover rates (Kelly *et al.*, 2006; German & Miles, 2010; Heady & Moore, 2013; Willis *et al.*, 2013; Cano-Rocabayera *et al.*, 2014), the use of different body tissues in stable isotope-based trophic ecology has the potential to reveal contrasting temporal patterns. This requires using tissues that display different turnover rates on a seasonal timescale. Alternatively, where different body tissues are comparable, this provides an opportunity to explore alternative approaches that are context-dependent, such as the use of non-lethal body tissues for imperilled taxa.

The headwater sections of the Koonap River offer a unique opportunity to study trophic dynamics of fish in relation to their proximate habitat. The Koonap River is subject to both spatial and temporal variation in environmental conditions, with most habitats occurring in alternative states of connected habitats when there is flow during the wet season and as isolated pools during the dry season (Kadye & Booth, 2012c). These pronounced seasonal changes are likely to influence the energy input (allochthonous versus autochthonous production), which may influence the composition and energy pathways of higher trophic groups that include macroinvertebrates and fish. Chubbyhead barb *Enteromius anoplus* is a fish species known to adapt and persist in environmentally variable freshwater habitats (e.g. headwater streams such as that of the Koonap River) where it usually occurs as the only fish species (Cambray & Bruton, 1985; Kadye & Booth, 2013). The chubbyhead barb is also known to have a highly adaptable feeding behaviour, and has been reported to prey on a wide range of aquatic invertebrates in both lentic and lotic habitats (Cambray, 1983). The distribution pattern and feeding behaviour of chubbyhead barb is relatively comparable to that of some threatened indigenous small bodied fishes that are found within the Cape Fold Ecoregion (Tweddle *et al.*, 2009; Ellender *et al.*, 2017).

This chapter assessed the temporal food web dynamics for the chubbyhead barb based on two-tissue comparison by determining the relative importance of allochthonous and autochthonous food resources and other consumers in the headwaters of the Koonap River. In addition, Bayesian stable isotope analyses were used to determine both the trophic structure and the seasonal patterns of the chubbyhead barb's isotopic niche. The specific objectives of the field study were (1) To determine whether there were any temporal changes in both basal and prey sources for chubbyhead barb, and (2) To examine temporal changes in the isotopic trophic niche structure for chubbyhead barb based on different body tissues in the headwaters of the Koonap River.

4.2 Materials and methods

Study area and data collection

Seasonal sampling of fin and muscle tissues and potential prey items of *Enteromius anoplus* was done within a 200 m reach in the upper Koonap River (Figure 2.1). Course particulate organic matter (CPOM), fine particulate organic matter (FPOM), aquatic invertebrates, arachnids, algae, macrophytes and fish were collected from the field for stable isotopic measurements during four seasons between 27th June 2016 and 7th April 2017. The chubbyhead barb is the only fish species that occurs in the headwater section of the Koonap where the study was conducted. The site was sampled in the winter (27th June 2016), spring (27th September 2016), summer (11th January 2017) and autumn (7th April 2017) seasons (Figure 2.2 and 2.3). The environmental variables, temperature (°C), pH, total dissolved solids (ppm) and conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) were measured with a HANNA HI 98129 Combo meter. Turbidity (NTU) was measured using a HANNA HI 98703 turbidity meter.

During each sampling period, 15 individuals of chubbyhead barb were captured using a seine net and were kept in a bucket containing stream water. The fish were then euthanised using clove oil, after which total length and standard length were then measured. The caudal fin and white muscle tissues were excised and placed in Eppendorf tubes for stable isotope analysis. Course particulate organic matter was collected by disturbing substratum within an area of 1 m² and washing the dislodged organic matter into a 250 μm hand net. Fine particulate organic matter was collected by filtering 25 litres of stream water, first through a 250 μm net to remove course debris, then through a 100 μm hand net. Three replicate samples were collected for both CPOM and FPOM during each sampling period. Invertebrates were collected

from all available habitats using a hand net (250 μm mesh size). In shallow riffles, invertebrate samples were collected from a 1 m^2 area by either kicking to disturb the substratum or by selecting pebbles and washing attached invertebrates into a hand net (250 μm mesh size). Vegetated sections were sampled by repeatedly sweeping a hand over the vegetation for approximately one minute. The samples were sorted in the field using a combination of nested sieves and trays to isolate the macroinvertebrates from the detritus. The macroinvertebrates were sorted and identified to family level and stored on ice in the field. A reference sample of the macroinvertebrates was preserved in 70 % ethanol to verify the identification using a stereomicroscope upon a return to the laboratory. Epilithic algae were scrapped from the substrates using a scalpel blade and washed. Filamentous algae, macrophytes and other visible organic matter were hand-picked. Arachnids, which included web and ground spiders, were collected within a 1 metre wide section along the perimeter of the sampled reach. Web spiders were captured by sweeping a hand-held insect net, whereas ground spiders were hand-picked. All samples were kept on ice in the field.

The samples were then transported to the freshwater laboratory at the Department of Ichthyology and Fisheries Science at Rhodes University. In the laboratory, the ethanol preserved invertebrates were identified to the lowest taxonomic unit possible using a compound microscope. Invertebrates of the same taxonomic group were pooled to achieve sufficient mass for analysis. All fish, CPOM, FPOM, algae, macrophytes and invertebrate samples were dried in an oven at 60 $^{\circ}\text{C}$ for 48 hours, before being ground into a fine powder with a mortar and pestle. The ground samples were put in Eppendorf tubes and sent for stable isotope analysis at the Stable Isotope Laboratory, Mammal Research Institute at University of Pretoria in South Africa.

Stable Isotope analysis

The procedure for stable isotope analysis has been outlined in Chapter 2.

Statistical analysis

In order to compare isotope niche sizes for the different seasons, sample size-corrected standard ellipse area (SEAc) (Jackson *et al.*, 2011) were calculated for both muscle and fin tissue. To test the hypothesis of temporal shift in the isotope niche structure for the chubbyhead barb, Bayesian standard ellipse areas (SEA.B, expressed as ‰^2) (Jackson *et al.*, 2011) were

calculated for each season based on both muscle and fin tissues. Inter-seasonal SEA overlap was then compared based on the maximum likelihood approach (Jackson *et al.*, 2011). This approach examines the area of overlap between two specified groups based on maximum likelihood (ML) estimates of means and covariance matrices. To test whether the isotope niche sizes of muscle and fin tissues were similar for the respective seasons, the SEA.B based on the two tissues were compared by calculating the probability that the posterior distribution of fin tissue ellipses were larger than those of muscle tissue. This probability was calculated as a proportion expressed as:

$$\Pr\left\{\sum (SEA.B_{fin} > SEA.B_{muscle}) / SEA.B_{total}\right\},$$

where SEA.B were Bayesian posterior distributions for the respective tissues. All analyses were conducted in R (R Development Core Team 2017) using the packages SIAR and SIBER.

4.3 Results

The physico-chemical variables showed seasonal variation (Table 4.1). Water temperature was highest in summer at 22.3 °C and lowest in winter at 12.2 °C. The water pH was neutral in summer (7.3) and alkaline in the winter (8.05), spring (8.13) and autumn (9.3) seasons. Total dissolved solids and turbidity were the highest in the autumn and lowest in the winter. Conductivity ranged from 456 uS.cm⁻¹ in summer to 495 uS.cm⁻¹ in winter and autumn.

Table 4.1: Physico-chemical variables sampled during winter, spring, summer and autumn in the headwaters of the Koonap River, Eastern Cape, South Africa.

Physico-chemical variables	Winter (27/06/2016)	Spring (27/10/2016)	Summer (11/01 2017)	Autumn (7/04/2017)
Temperature (°C)	12.2	14.7	22.3	18.6
pH	8.1	8.1	7.3	9.3
Total dissolved solids (ppm)	270	283	305	401
Conductivity (uS.cm ⁻¹)	495	485	456	495
Turbidity (NTU)	2.9	4.9	10.5	22

Temporal changes in basal food sources and invertebrate composition

The potential basal food sources included both autochthonous (macrophytes and algae) and allochthonous (CPOM and FPOM) organic matter. In all seasons, macrophytes were generally most depleted in $\delta^{13}\text{C}$, with values ranging from -30.32 ± 3.21 ‰ in winter to -21.86 ± 7.79 ‰ in spring, whereas algae were most enriched in $\delta^{13}\text{C}$, with values ranging from -17.10 ± 0.09 ‰ in summer to -19.71 ‰ in both spring and autumn (Table 4.1). In comparison, for $\delta^{15}\text{N}$, CPOM were most depleted in $\delta^{15}\text{N}$ during all seasons with values ranging from 3.36 ± 2.00 ‰ in spring to 5.09 ± 1.64 ‰ in autumn, except in summer when FPOM was most depleted ($\delta^{15}\text{N} = 5.40 \pm 0.74$ ‰), whereas macrophytes and algae were generally most enriched in $\delta^{15}\text{N}$, with values ranging from 4.71 ± 0.75 ‰ to 8.60 ± 0.00 ‰. Thirty-eight different taxa that comprised aquatic invertebrates, arachnids and amphibian groups were collected in the four seasons (Table 4.2). Thirty-two of these belonged to the benthic macroinvertebrate group whereas five belonged to the arachnid group and one from the anuran group.

In the winter season, seven aquatic invertebrate orders were collected (Table 4.2). These included Ephemeroptera with four genera, Hemiptera with three genera, Diptera, Coleoptera, Odonata, Decapoda with one genus each, and Trichoptera with one family collected. The $\delta^{13}\text{C}$ values for these invertebrate taxa ranged from -26.65 ± 0.00 ‰ for the genus *Sigara* which was the most depleted, to -18.09 ± 0.00 ‰ for the genus *Potamonautes* which was the most enriched. In comparison, the $\delta^{15}\text{N}$ ranged from 5.46 ± 0.00 ‰ for the genus *Demoulinea* which was the most depleted to 8.96 ± 0.48 ‰ for the genus *Potamonautes* which was the most enriched (Figure 4.2).

In the spring season, seven aquatic invertebrate orders were collected. These included Ephemeroptera and Odonata with two genera and one family each, Hemiptera with two genera, Decapoda, Diptera and Coleoptera with one genus each and Trichoptera with one family collected. The $\delta^{13}\text{C}$ values for these invertebrate taxa ranged from -25.45 ± 0.00 ‰ for the family Heptageniidae which was the most depleted, to -19.25 ± 1.64 ‰ for the genus *Potamonautes* which was the most enriched (Figure 4.2). In comparison, the $\delta^{15}\text{N}$ ranged from 5.87 ± 0.01 ‰ for the genus *Cheleocleon* which was the most depleted to 8.97 ± 0.00 ‰ for the genus *Laccocoris* which was the most enriched.

In the summer season, nine aquatic invertebrate orders were collected (Table 4.2). These included Ephemeroptera with one genus and family, Hemiptera with five genera and one family, Diptera with one genus and two families, Basommatophora and Trichoptera with one

family, and Hygrophila, Coleoptera, Decapoda and Odonata with genus each. The $\delta^{13}\text{C}$ values for these invertebrate taxa ranged from $-23.29 \pm 0.54 \text{ ‰}$ for the family Baetidae which was the most depleted, to $-16.27 \pm 0.00 \text{ ‰}$ for the genus *Lymnae* which was the most enriched (Figure 4.2). In comparison, the $\delta^{15}\text{N}$ ranged from $5.10 \pm 0.00 \text{ ‰}$ for the genus *Appasus* which was the most depleted to $9.06 \pm 0.00 \text{ ‰}$ for the family Athericidae which was the most enriched.

In the autumn season, seven aquatic invertebrate orders were collected (Table 4.2). These included Ephemeroptera with three genera and one family, Coleoptera with two genera and one subfamily, Hemiptera with three genera and one subfamily, Odonata with four genera collected, Decapoda and Diptera with one genus each and Trichoptera with one family were collected. The $\delta^{13}\text{C}$ values for these invertebrate taxa ranged from $-24.46 \pm 0.00 \text{ ‰}$ for the subfamily Laccophilinae which was the most depleted, to $-18.47 \pm 0.50 \text{ ‰}$ for the genus *Potamonautes* which was the most enriched. In comparison, the $\delta^{15}\text{N}$ ranged from $6.09 \pm 0.00 \text{ ‰}$ for the subfamily Gerrinae which was the most depleted to $9.98 \pm 0.64 \text{ ‰}$ for the genus *Appasus* which was the most enriched (Figure 4.2)

Table 4.2: Mean values of carbon and nitrogen stable isotopes ($\pm$ standard deviation) for the chubbyhead barb, macroinvertebrates, basal sources and other prey collected in the Koonap River in the winter, spring, summer and autumn seasons. The functional feeding trophic guild categories of the macroinvertebrates were based on Cummings *et al.* (2005).

Order		Taxa	Winter		Spring		Summer		Autumn		Trophic guild	
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)		
Algae			-19.58±1.07	4.75±0.10	-19.7±3.08	4.7±0.75	-17.10±0.09	8.60±0.00	-19.71±4.46	6.45±0.39	primary producer	
Macrophytes			-30.32±3.21	6.15±0.76	-21.86±7.79	4.73±1.10	-29.35±0.21	6.39±0.38	-29.16±0.53	5.96±1.53	primary producer	
Course particulate organic matter			-27.70±1.23	3.77±2.62	-27.06±0.97	3.36±2.00	-26.19±0.32	5.62±0.25	-25.98± 0.28	5.09±1.64	secondary producer	
Fine particulate organic matter			-23.67±0.49	5.16±0.60	-19.87±0.28	4.76±0.13	-20.88±0.49	5.40±0.74	-22.13±0.35	5.69±0.10	secondary producer	
Fish	Cypriniformes	<i>E.anoplus</i> (Fin tissue)	-20.68±0.85	11.74±1.07	-21.56±0.88	11.92±0.38	-19.22±0.91	11.33±0.31	-20.45±1.09	11.48±0.43	omnivore	
		<i>E.anoplus</i> (muscle tissue)	-20.90± 0.69	11.70±0.63	-21.38±1.02	11.20±0.36	-19.26±0.85	11.58±0.31	20.55±1.17	12.4±0.39	omnivore	
Frogs	Anura				-21.16±0.00	6.46±0.00	-19.94±0.04	9.03± 0.27	-17.58± 0.81	8.68±0.15	omnivore	
Spiders	Araneae		-20.37±1.09	9.96±0.86							predator	
		Lycosidae			-20.94±0.00	11.15±0.00	-18.44± 0.17	10.13± 0.24	-19.44±0.00	10.54±0.00	predator	
Aquatic invertebrates		Thalassinae					-20.56±0.87	10.02±0.28			predator	
		<i>Thalassius</i>							-22.73± 0.00	8.52±0.00	predator	
		<i>Tetragnatha</i>			-22.35±0.00	10.16±0.00	-20.97±0.00	9.08±0.00	-21.32± 0.00	8.86±0.00	predator	
		Ancylidae					-17.26± 0.00	8.26±0.00			grazer	
	Basommatophora											
		Coleoptera	<i>Aulonogyrus</i>	-22.44±0.31	8.27±0.25			-22.35±0.00	7.46±0.00	-21.03±0.04	7.93±0.19	predator
			<i>Hydaticus</i>			-21.78±0.75	8.03±1.06	-22.69±0.00	7.24± 0.00	-22.54±0.00	8.89±0.00	predator
	Laccophilinae								-24.46±0.00	7.07±0.00	predator	
Decapoda	<i>Potamonautes</i>	-18.09±0.81	8.96±0.48	-19.25±1.64	8.13±0.11	-17.36±0.50	8.92±0.36	-18.47±0.50	8.28±0.11	omnivore		
Diptera	Athericidae					-19.46± 0.00	9.06±0.00			predator		
	Chironomidae					-22.20±0.00	6.75±0.00			grazer		

Table 4.2 continued:

Ephemeroptera	<i>Simulium</i>	-19.10±0.00	5.50±0.00	-19.61±0.00
	<i>Adenophlebia</i>	-21.49±0.00	7.91±0.00	-21.72±0.00
	Baetidae			
	<i>Cheleocleon</i>	-21.36±0.00	6.10±0.00	-25.04±3.16
	<i>Demoulinea</i>	-23.59±0.00	5.46±0.00	
Hemiptera	Heptageniidae			-25.45±0.00
	Teloganodidae			
	<i>Tricorythus</i>	-20.57±0.00	5.34±0.00	
	<i>Anisops</i>			-22.31±0.00
	<i>Appasus</i>			
	Gerridae			
	Gerrinae			
	<i>Laccocoris</i>	-18.52±0.00	7.32±0.00	-22.79±0.00
	<i>Micronecta</i>	-20.74±0.00	6.88±0.00	
	<i>Plea</i>			
Hygrophila	<i>Rhagovelia</i>			
	<i>Sigara</i>	-26.65±0.00	7.08±0.00	
	<i>Lymnaea</i>			
Odonata	<i>Aeshna</i>	-22.87±0.00	8.67±0.00	-23.70±0.97
	<i>Lestes</i>			-23.16±0.00
	Libellulidae			-21.93±0.97
	<i>Microgomphus</i>			
	<i>Pseudagrion</i>			
	<i>Tetrathemis</i>			
Trichoptera	Hydropsychidae	-20.14±0.07	7.80±0.05	-21.40±0.00

6.54±0.00	-22.28±0.00	6.67±0.00	-21.52±0.39	7.50±0.19	filterer
6.51±0.00	-22.27±0.09	7.93±0.01	-22.55±0.45	8.07±0.22	collector
	-23.29±0.54	8.09±0.36			collector
5.87±0.01			-23.01±2.10	8.14±1.02	collector
			-23.82±0.00	8.63±0.00	collector
6.86±0.00					collector
			-22.14±0.15	7.09±0.06	filterer
					filterer
7.97±0.00	-19.43±0.06	8.67±0.03	-21.47±0.43	7.87± 0.61	predator
	-21.60±0.00	5.10±0.00	-22.93±1.90	9.98± 0.64	predator
	-22.31± 0.00	8.18±0.00			predator
			-22.51±0.00	6.09±0.00	predator
8.97±0.00	-18.72±0.00	7.50±0.00			predator
	-20.84±0.00	6.06±0.00			predator
	-18.27±0.00	3.72±0.00			predator
			-20.94±0.00	8.79±0.00	predator
					predator
	-16.27±0.00	6.45±0.00			grazer
7.59±0.39			-22.18±1.41	8.86±0.06	predator
7.90±0.00	-22.62±0.04	8.55± 0.28			predator
6.84±0.03					predator
			-20.63±0.00	8.46±0.00	predator
			-21.72±0.00	8.75±0.00	filterer
			-19.53± 0.00	7.70±0.00	predator
7.83±0.00	-23.18±0.00	7.09±0.00	-20.74±0.25	9.44±0.19	filterer

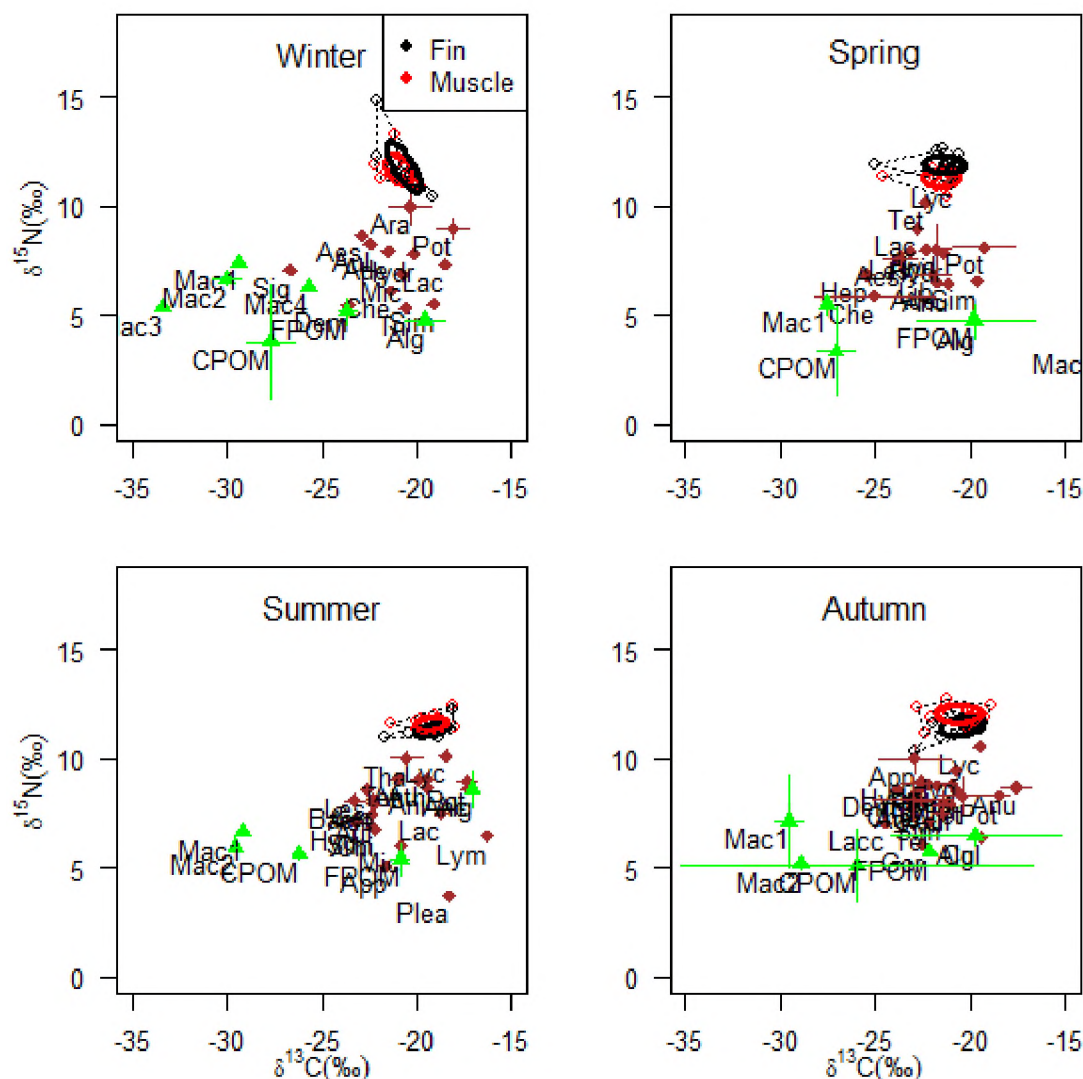


Figure 4.2: Stable isotope biplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the chubbyhead barb *Enteromius anoplus*, based on white muscle and caudal fin tissues, aquatic invertebrates and basal sources in winter, spring summer and autumn season. Samples were collected from the headwater of the Koonap River, Eastern Cape, South Africa. Alg = Algae; Mac = Macrophytes; CPOM = Course particulate organic matter; FPOM = Fine particulate organic matter Ara = Aranea; Dem = *Demoulinia*; Che = *Cheleocleon*; Ade = *Adenophlebia*; Tri = *Tricorythus*; Sig = *Sigara*; Lac = *Laccocoris*; Sim = *Simulium*; Aul = *Aulonogyrus*; Hyd = *Hydropsychinae*; Aes = *Aeshna*; Lyc = *Lycosidae*; Tet = *Tetragnatha*; Aes = *Aeshna*; Lib = *Libellulidae*; Les = *Lestes*; Hep = *Heptageniidae*; Hyd = *Hydaticus*; Ani = *Anisops*; Mic = *Micronecta*; Ple = *Plea*; Ger = *Gerridae*; Ath = *Athericidae*; Chi = *Chironomidae*; Hyd = *Hydropsychidae*; Bae = *Baetidae*; Tha = *Thalassius*; App = *Appasus*; Lym = *Lymnaea*; Anc = *Ancylidae*; Tha = *Thalassinae*; Mic = *Microgomphus*; Tet = *Tetrathemis*; Ger = *Gerrinae*; Rha = *Rhagovelia*; Lac = *Laccophilinae*; Tel = *Teloganodidae*; Pse = *Pseudagrion*; Pot = *Potamonantes*; Anu = *Anura*.

Temporal changes in chubbyhead barb's isotopic niche structure

The SEAc based on both muscle and fin tissue for the chubbyhead barb were variable across the different seasons. The SEAc based on fin tissue varied from 2.12 ‰² in winter, 1.36 ‰² in spring, 0.75 ‰² in summer to 1.52 ‰² in autumn (Figure 4.3). By comparison, SEAc based on muscle tissue were relatively smaller in winter (SEAc = 1.49 ‰²) and spring (SEAc = 1.05 ‰²) and relatively larger in summer (SEAc = 0.87 ‰²) and autumn (1.55 ‰²) than those derived from fin tissue. For the fin tissue the SEA.B showed a relatively high overlap between winter and spring seasons (SEA.B_{winter} = 12.68 ‰², SEA.B_{spring} = 8.17 ‰², overlap = 4.97 ‰²), autumn and winter seasons (SEA.B_{autumn} = 9.09 ‰², SEA.B_{winter} = 12.68 ‰², overlap = 5.10 ‰²), and relatively low overlap between spring and summer seasons (SEA.B_{spring} = 8.17 ‰², SEA.B_{summer} = 4.51 ‰², overlap = 1.65 ‰²) (Table 4.3). A similar pattern was observed for muscle tissue whereby the SEA.B showed a relatively high overlap between winter and spring seasons (SEA.B_{winter} = 8.91 ‰², SEA.B_{spring} = 6.30 ‰², overlap = 4.50 ‰²), autumn and winter seasons (SEA.B_{autumn} = 9.29 ‰², SEA.B_{winter} = 8.90 ‰², overlap = 5.87 ‰²), and relatively low overlap between spring and summer seasons (SEA.B_{spring} = 6.30 ‰², SEA.B_{summer} = 5.19 ‰², overlap = 1.89 ‰²).

Table 4.3: Comparison of seasonal shifts in isotope niche size and position of muscle and fin tissue.

	Fin tissue			Muscle tissue		
	Area 1	Area 2	Overlap	Area 1	Area 2	Overlap
Winter to spring	12.68	8.17	4.98	8.91	6.30	4.50
Spring to summer	8.17	4.51	1.65	6.30	5.19	1.89
Summer to autumn	4.51	9.09	3.81	5.19	9.29	3.69
Autumn to winter	9.09	12.68	5.10	9.29	8.90	5.87

Comparison of isotope niche sizes between fin and muscle tissues for the respective seasons revealed that the probability that SEA.B_{fin} > SEA.B_{muscle} was high in winter (0.87) and spring (0.75). This indicated that the isotope niche sizes inferred from fin tissue were larger than those inferred from muscle tissue during winter and spring. In contrast, the probability that SEA.B_{fin} > SEA.B_{muscle} was low in summer (0.39) and autumn (0.48). This indicated that the isotope niche sizes that were inferred from the two tissues were generally comparable in summer and autumn.

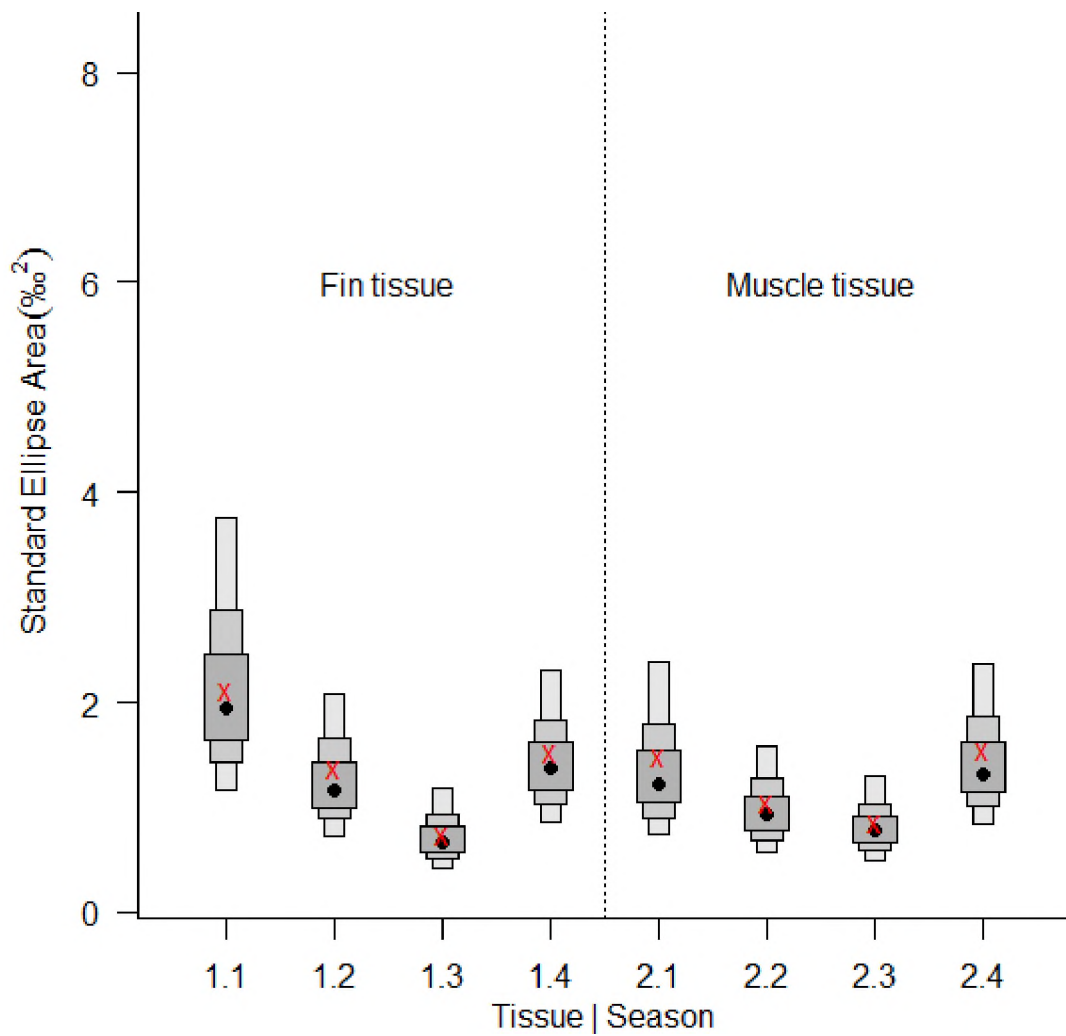


Figure 4.3: Sample size-corrected standard ellipse areas (SEAc) for chubbyhead barb *Enteromius anoplus* based on muscle and fin tissues for the different season. The black dots represent the mode, whereas the red cross represents the mean. On the abscissa, the seasons were represented by values as winter (1), spring (2), summer (3) and autumn (4) for both tissues.

4.4 Discussion

Temporal changes in basal food sources and invertebrate composition

The headwater stream food web of the Koonap River was characterised by seasonal variability in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of both basal food sources and consumer community. Specifically, pronounced seasonal differences were observed for allochthonous matter (CPOM and FPOM) and algae between relatively cold (winter and spring) and warm (summer and autumn) seasons. Similarly, pronounced seasonal variability was observed for the macroinvertebrate communities whereby the warm seasons were more speciose compared to cold seasons. The seasonal differences that were observed for these communities were in concordance with temporal patterns in macroinvertebrate community dynamics that have been observed in different freshwater habits in this region (O'Keeffe & De Moor, 1988; Mabidi *et al.*, 2016, 2017). Macroinvertebrate communities of this region reflect seasonal differences in both abundance and richness because many of these taxa's life histories are synchronised to respond to changes in temperature and hydro periods (de Moor & Day, 2013). This study therefore reflected temporal food web dynamics, which suggested that allochthonous matter was important as a basal food source across all seasons, whereas the importance of autochthonous matter (algae) appeared to be seasonal. Furthermore, temporal changes in macroinvertebrate communities suggest that likely response to changes in organic input across the seasons.

In the headwaters of the Koonap River, the most common macroinvertebrate functional feeding group (FFG) in all the seasons was the predator group (those that fed on other consumers), with the highest taxonomic richness being observed in the summer and autumn seasons, whereas the lowest richness was observed in the winter and spring seasons. Collectors (those that fed on FPOM from the stream bottom) and filterers (those that fed on FPOM from the water column using a variety of filters) were also common to all seasons but less taxonomically rich than predators. The highest taxonomic richness of collectors and filterers occurred in the autumn and winter seasons, which probably coincided with peak leaf litter fall, whereas the least taxonomic richness occurred in the spring and summer seasons. With less input of allochthonous material in the summer, autochthonous production took precedence and grazers (fed on algae and associated material) were present in the system. These temporal macroinvertebrate communities in the different seasons suggest that in the headwaters of the Koonap River, allochthonous production represents a major energetic input in the environment, which is in line with the RCC predictions (e.g. Vannote *et al.*, 1980).

Although there was general congruence with the RCC in relation to allochthonous organic matter input in this headwater stream, there was, however, a less diverse community of shredder macroinvertebrates (those that feed on CPOM). This is, nevertheless, not unique as other studies, particularly in tropical areas such as Hong Kong (Li & Dudgeon, 2009), New Zealand (Thompson & Townsend, 2000), Kenya (Dobson *et al.*, 2002), Brazil (Wantzen *et al.*, 2008; Moulton *et al.*, 2010) have observed this. It has been suggested that litter processing in these streams may be carried out by larger omnivores, such as decapod shrimps and crabs (Crowl *et al.*, 2001; March *et al.*, 2001; Dobson *et al.*, 2002; Moulton *et al.*, 2010). In the headwaters of the Koonap River, the freshwater crab *Potamonautes* sp. may fulfil the shredder role. Another factor that might explain the lack of shredder macroinvertebrate community is that at higher temperatures, increased microbial activity may be the leading processor of CPOM (Irons *et al.*, 1994). Therefore, macroinvertebrate shredder communities might be predominantly a feature of northern temperate streams on which the RCC was based on.

Temporal changes in chubbyhead barb's isotopic niche structure

Seasonal shifts in chubbyhead barb muscle and fin tissue isotopic niche (a proxy for trophic niche) were studied to determine if the same information would be gained from the two tissues. Differences in ellipse (isotopic niche space) size and orientation for muscle and fin tissue were observable on a $\delta^{13}\text{C}/\delta^{15}\text{N}$ biplot and calculated as the Bayesian standard ellipse areas (SEA). Statistical examination of muscle and fin tissue SEAc revealed that there were seasonal differences in isotopic niche sizes of muscle and fin tissue. The largest isotopic niche sizes for both muscle and fin tissue were observed in the winter and autumn, and the lowest in the spring and summer seasons. A smaller or bigger isotopic niche may reflect changes in resource availability due to the environmental changes occurring in the seasons (Newsome *et al.*, 2007). Because *Enteromius anoplus* is considered to displays omnivorous feeding behaviour (Skelton, 2001; Cambray, 2007), coupled with the fact that the summer season had high taxonomic diversity whereas the winter season had the lowest taxonomic diversity, the smaller isotopic niche sizes in the spring and summer seasons compared to winter season suggest differences in trophic resource use in relation to either prey availability or physiological processes.

The optimal foraging theory suggests that animals may select prey items that guarantee the highest intake of energy per unit time (Stephens & Krebs, 1986). Therefore, in the spring and summer, it is likely that chubbyhead barb's was influenced by a narrow prey range that yielded the highest energy input hence the smaller niche sizes in these seasons. When a more

profitable prey categories become scarce, an expansion of the trophic niche is expected as other prey categories are exploited to maximise the energy intake per time unit. This is likely to explain the large trophic niche sizes in the winter and autumn seasons. Elsewhere, seasonal shifts in the diet of *Enteromius anoplus* have been observed with the species mainly feeding on corixids in the summer and chironomid larvae in the winter and autumn seasons (Cambray, 1983). Another possible reason for the differential niche sizes in the seasons could be due to the physiological changes associated with reproduction. Studies on mammals have reported decreases in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values in pregnant or lactating females (Kurlle, 2002; Fuller *et al.*, 2005). A study by Michaud *et al.*, (2013) on the fish species Arctic charr (*Salvelinus alpinus*) indicated that reproductive status and gender had significant effects on the $\delta^{13}\text{C}$ values between muscle and liver tissue. *Enteromius anoplus* has a multiple spawning habit in November (end of spring) – January and February (end of summer)-March (Cambray & Bruton, 1985), which could cause resources to be allocated to reproduction leading to smaller niche sizes in fin and muscle tissue. This hypothesis however needs further testing to account for the different genders and whether in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ will be equally affected by reproduction.

The Bayesian standard ellipse areas (SEA.B) of fin and muscle tissues revealed varying degrees of isotopic niche overlap between seasons. For example, a seasonal shift from autumn to winter showed the highest trophic niche overlap, whereas spring-summer shift showed the lowest overlap. This suggests that there was pronounced seasonal change, particularly during summer compared to other seasons, in the trophic resource use by chubbyhead barb. This pattern was mirrored consistently by both muscle and fin tissues and coincides with the changes in macroinvertebrate taxa, particularly grazers that were observed in the summer. Although muscle and fin tissue reflect similar temporal patterns in isotopic trophic niche structure, Bayesian ellipses (SEA.B) revealed differences in trophic niche sizes that were inferred for specific seasons. Comparable niche sizes between fin and muscle tissue were observed in the summer and autumn, whereas in the winter and spring seasons, the niche sizes of the two tissues were different. Perga & Gerdeaux, (2005) suggest that due to the fact that fish may have a discontinuous pattern of growth (somatic growth, gonadic growth, basal metabolism) throughout the year, muscle tissue isotopic signatures may only reflect food consumed during periods of growth. Muscle isotope turnover has been found to depend mainly on growth (Hesslein *et al.*, 1993) and in their study on whitefish *Coregonus lavaretus*, they found that seasonal amplitude of isotope variation was two to three times higher in liver compared to muscle tissue, with only liver tissue responding to changes in isotopic composition of food sources in the winter and autumn seasons when growth was limiting. This could be the case for

chubbyhead barb with muscle tissue having a smaller isotopic niche size than fin tissue in the winter and spring seasons due to the lack of growth. Further information on the relative contribution of growth and catabolic metabolism to turnover in fin and muscle tissue is needed to accurately state that this is the case.

In conclusion, the headwater stream of the Koonap River was influenced by temporal changes in the food web dynamics. While certain aspects of the RCC appeared consistent, particularly the role of allochthonous organic inputs, there were notable disparities in the composition of certain macroinvertebrate groups, such as shredders. Although not conducted in this study the DTDFs deduced from the previous chapter could be used with the stable isotopic values for potential food sources found in this chapter in mixing models to elucidate the specific prey contributions to chubbyhead barb's diet. Both muscle and fin tissue reflected temporal changes in food web dynamics, albeit with differences that suggest tissue-specific differences in metabolism for different seasons. Both muscle and fin tissue reflected similar temporal patterns in isotopic trophic niche structure, which when coupled with the fact that comparable turnover rates were found for fin and muscle tissue (for $\delta^{13}\text{C}$), makes caudal fin tissue a good substitute for muscle tissue for trophic studies using stable isotopes, particularly in the summer and autumn seasons when muscle and fin tissue have comparable niche sizes.

CHAPTER 5

General discussion

The use of fin tissue as a replacement for muscle tissue in stable isotope ecological studies of fish has increasingly been investigated (Kelly *et al.*, 2006; Sanderson *et al.*, 2009; Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Willis *et al.*, 2013; Galván *et al.*, 2015; Vašek *et al.*, 2017; Busst & Britton, 2018). In the southern temperate region of Africa, the use of non-lethal fin tissue is particularly useful for trophic dynamic studies on small bodied and endangered freshwater fish species such as those of the genera *Enteromius* and *Pseudobarbus* that occur in many headwater streams of this region (Tweddle *et al.*, 2009; Weyl *et al.*, 2014; Ellender *et al.*, 2017). This study explored the utility of using caudal fin clips as a non-lethal alternative to dorsal white muscle in the assessment of representative fish species *Enteromius anoplus* trophodynamics.

Isotopic turnover rates provide information on the time required for isotopic incorporation of a new diet into consumer tissue, and this information is often inferred from diet-tissue isotopic kinetic models (Martínez Del Río *et al.*, 2009). In this study, the one-compartment non-linear least squares model (Carleton *et al.*, 2008) appeared to be the most plausible in estimating the turnover rates for both muscle and fin tissues compared to two-compartment models for chubbyhead barb. Comparable isotope incorporation rates were found for $\delta^{13}\text{C}$ between dorsal white muscle and caudal fin tissues (half-lives of less than 50 days were determined). Therefore, fin tissue $\delta^{13}\text{C}$ turnover rates found in this study can be used as an alternative to muscle tissue to assess short term dietary patterns of chubbyhead barb. Currently, there are a few comparative studies on $\delta^{13}\text{C}$ for different tissues. These few studies have, nevertheless, reported variable intra- and inter-specific tissue turnover rates. For example, Suzuki *et al.*, (2005)'s study on Japanese temperate bass *Lateolabrax japonicus* found that caudal fin and muscle tissue had comparable turnover rates (half-lives of 26 and 22 days respectively). In comparison, muscle tissue half-lives have been variable for other species, e.g. juvenile steelhead *Oncorhynchus mykiss*, sand goby *Pomatoschistus minutus* and juvenile marbled flounder *Pleuronectes yokohamae* had half-lives of 136, 25 and 17 days respectively (Guelinckx *et al.*, 2007; Church *et al.*, 2009; Hamaoka *et al.*, 2016).

In contrast to $\delta^{13}\text{C}$, marked differences in incorporation rates for $\delta^{15}\text{N}$ were found between fin and muscle tissue in the different diets, with muscle tissue having faster turnover rates than fin tissue for diet 1 (3 and 90 days respectively) and muscle tissue having slower

turnover rates than fin tissue for diet 2 (208 and 50 days respectively). Fin tissue $\delta^{15}\text{N}$ turnover rates should therefore be used with caution as they may vary depending on the particular diet of the consumer in the system and may either extrapolate long term (protein rich diet) or short term (protein poor diet) dietary patterns. Further experimentation is required to investigate the mechanisms that lead to muscle and fin tissue having varying incorporation rates in the different diets. Other studies on $\delta^{15}\text{N}$ isotopic incorporation rates have found varying results. For example, fin tissue has been found to have a faster turnover rate than muscle tissue in anadromous rainbow trout *Oncorhynchus mykiss* (13 and 39 days respectively) and leopard coral grouper *Plectropomus leopardus* (37 and 126 days respectively) (Heady & Moore, 2013; Matley *et al.*, 2016). On the other hand, comparable turnover rates were found between fin and muscle tissue in Japanese temperate bass *Lateolabrax japonicus* (21 and 19 days respectively) and barbel *Barbus barbus* (95 and 84 days respectively) (Suzuki *et al.*, 2005; Busst & Britton, 2018). Fin tissue has been found to have slower turnover rates than muscle tissue in armored catfish *Ancistrus triradiatus* (12 and 18 days respectively) (McIntyre & Flecker, 2006).

Diet-tissue discrimination factors (DTDFs) represent differences in isotopic values between a consumer and its diet. Differences in DTDFs were observed in this study with diet 1 (fishmeal based) having lower DTDFs than diet 2 (soya based). Other animals, including birds (Florin *et al.*, 2011), echinoderms (Blanchet-Aurigny *et al.*, 2012), Crustacea (Devries *et al.*, 2015) and fish (Busst & Britton, 2016) have exhibited significant decreases in DTDFs in response to being fed high quality protein diets. The high protein quality of diet 1 likely contributed to its lower DTDFs for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in muscle and fin tissue. The averaged DTDFs estimated from this study were, nonetheless, comparable to the values of 0-1 ‰ for $\delta^{13}\text{C}$, but differed from the values of 3-5 ‰ for $\delta^{15}\text{N}$ reported in literature (DeNiro & Epstein, 1978; Peterson & Fry, 1987; Kelly, 2000; Post, 2002). Other studies on fish species have found a wide range of DTDF values both in line and differing from the most frequently used literature values (Mill *et al.*, 2007; Sweeting *et al.*, 2007; German & Miles, 2010; Busst *et al.*, 2015).

Given the differences in turnover rates and DTDFs for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in different fish species, turnover rates predominantly appear to be species specific. This suggests that the use of published turnover rates and DTDFs should be used in cases where the study species is the same or similar (e.g. in terms of its diet, physiology and locality). Some authors have advocated for the use of regional models for the replacement of muscle with fin tissue (Tronquart *et al.*, 2012), whereas others suggest this should be done on a case by case basis (Willis *et al.*, 2013; Galván *et al.*, 2015). Given these considerations, the utility of the turnover

rates and DTDFs produced in this study should be limited to modelling the diet selection of chubbyhead barb and potentially other small-bodied cyprinid minnows in the southern temperate region. This is particularly so for endangered species that could benefit from non-lethal sampling techniques, such as *Enteromius treurensis*, *Pseudobarbus erubescens*, *P. afer*, *P. asper*, *P. burchelli*.

In addition to fin tissue having comparable turnover rates ($\delta^{13}\text{C}$) and DTDFs with muscle tissue, the applicability of fin tissue as a replacement for muscle tissue also requires an inference about trophic niche sizes within a particular habitat. For the *in-situ* study, the Koonap River was characterised by seasonal variability in discharge, temperature, turbidity pH in the different seasons. Predators appeared to be the most diverse functional feeding group. Similarly, collectors and filterers were also diverse, suggesting that primary allochthonous production represented a major energetic input in this environment. In contrast, grazers were poorly represented, which appeared consistent with the river continuum concept (Vannote *et al.*, 1980) which suggests that autochthonous matter is less abundant in headwater streams. Assessment of temporal trophic niche dynamics for chubbyhead barb revealed that fin and muscle tissues were both able to depict the temporal shift in isotopic niche structure. This seasonal shift was reflected by comparatively similar trophic niche structure in the summer and autumn seasons, but differently in the winter and spring seasons. This suggests that caution should be taken when inferring isotopic results from fin tissue in the winter and spring seasons. This is because different tissues are likely to undergo different metabolic and physiological processes on a temporal scale (Michaud *et al.*, 2013).

Isotopic incorporation rates have been found to be influenced by both tissue growth and catabolic processes that result in tissue replacement (Hobson & Clark, 1992; Hesslein *et al.*, 1993; Carleton & Del Rio, 2005; McIntyre & Flecker, 2006; Boecklen *et al.*, 2011; Franssen *et al.*, 2017). Turnover in small, rapidly growing animals is expected to be dominated by growth, whereas catabolic turnover is expected to dominate in larger slow growing animals (Martínez Del Rio *et al.*, 2009). In this study, predominantly adult chubbyhead barb were used to elucidate turnover rates without giving much attention to growth and catabolic turnover. It would be informative in future studies to determine the proportional contributions of growth and catabolic turnover to the turnover rates in muscle and fin tissue of chubbyhead barb and related species. For example, would the contributions of growth and catabolism be the same in juvenile and adult fish, and would these differences help explain the differences in niche sizes of muscle and fin tissue in the winter and spring seasons?

Studies on non-lethal alternatives to muscle tissue have used a variety of fin tissues. For example, caudal (Suzuki *et al.*, 2005; Sanderson *et al.*, 2009; German & Miles, 2010; Hanisch *et al.*, 2010; Jardine *et al.*, 2011; Heady & Moore, 2013; Galván *et al.*, 2015), dorsal (Valladares & Planas, 2012; Willis *et al.*, 2013; Vašek *et al.*, 2017), pectoral (McIntyre & Flecker, 2006; Andvik *et al.*, 2010; Fincel *et al.*, 2012), pelvic (Busst *et al.*, 2015; Busst & Britton, 2018) and adipose (Allen *et al.*, 2009) tissues have previously been assessed. It is assumed that all fins are isotopically equivalent, hence the lack of consensus on which fin tissue is best suited to act as a proxy for muscle tissue. Furthermore there is also no consensus on whether the addition (Kelly *et al.*, 2006; Hanisch *et al.*, 2010; Jardine *et al.*, 2011) or exclusion (Sanderson *et al.*, 2009) of hard spines in fin tissue has a discernible effect on the isotopic values. Fin tissue may not be an ideal substitute for small fishes if not enough tissue can be sampled for analysis without removing the whole fin. In small bodied minnows, caudal fin tissue usually has the largest surface area compared to other fins and is therefore considered the most appropriate for stable isotope analysis. Sanderson *et al.*, (2009) suggested that fin tissue is a useful proxy for muscle tissue in fish bigger than 65 mm. Because survival of the fish was not the goal for this study, the whole caudal fin was excised for use in stable isotope analysis. If fin tissue is to be used for stable isotopic trophic studies in endangered small bodied minnows in this region, it would be vital to ascertain how much fin tissue could be for stable isotope analysis without endangering the survival of the fish.

In conclusion, this study reports on one of the first examinations of isotopic incorporation rates and DTDFs of fish in the southern temperate region. These findings add to a growing body of literature on the use of non-lethal stable isotopic alternatives to muscle tissue by providing data on the small cyprinid species chubbyhead barb. The turnover rates and DTDFs inferred in this study could be useful for drawing inferences on chubbyhead barb diet in the wild, as well as for use in dietary studies relying on isotopic data collected from other small bodied cyprinid minnows in the southern temperate region of South Africa.

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