Stable isotope analysis spills the beans about spatial variance in trophic structure in a fish host – parasite system from the Vaal River System, South Africa

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Abstract

Background

Stable isotope analysis offers a unique tool for comparing trophic interactions and food web architecture in ecosystems. This approach is based on analysis of the stable isotope ratios of carbon (13 C/ 12 C) and nitrogen (15 N/ 14 N) in organisms. Studies comparing stable isotope enrichment in hosts and parasites have shown that parasites are variably enriched in stable isotopes relative to the host.

Methods

Sharptooth catfish (*Clarias gariepinus*) were collected from six sites along the Vaal River, South Africa and were assessed for ectoparasites and endoparasites. *Lamproglena clariae* (Copepoda), *Tetracampus ciliotheca* and *Proteocephalus glanduligerus* (Cestoda), and larval *Contracaecum* sp. (Nematoda) were collected from the gills, intestine and mesenteries, respectively. Signatures of δ 13 C and δ 15 N were analysed in host muscle tissue and parasites using bulk stable isotope analysis.

Results

Stable isotope enrichment was variable between parasites and the host fish, with *L. clariae* and the host sharing similar δ 15 N signatures and the endoparasites being depleted in both δ 13 C and δ 15 N relative to the host. Spatial differences in enrichment of stable isotopes were also identified. Fish and parasites collected from below the Vaal River Barrage were more enriched in the 15 N isotope than hosts and parasites collected from other sites. The opposite was identified for 13 C isotope fractionation.

Conclusion
Differences in stable isotope enrichment in parasites infecting *C. gariepinus* could be related to the feeding strategy of each parasite species collected. Enrichment of $\delta$ 15 N in *L. clariae* would relate to the micropredatory nature of this parasite, which feeds on whole blood of the host fish. Depleted isotope levels in endoparasites could relate to their absorptive feeding strategy on metabolic by-products of the host. Spatial differences in both host and parasite tissues identified likely resulted from differences in the diet of the host and related with availability of prey items for the host fish.

**Background**

Since the late 1970’s analysis of natural levels of stable isotopes of nitrogen ($^{15}$N/$^{14}$N) and carbon ($^{13}$C/$^{12}$C) have been used as a unique fingerprint for studying trophic relationships of organisms [1–3]. Differences in trophic levels between organisms have been assessed through comparison of the nitrogen stable isotope ratio ($\delta^{15}$N), with consumers being enriched in $^{15}$N by an average of 3.4‰ per trophic level [4, 5]. Information regarding the food sources incorporated into the diet by consumers is delineated by comparing enrichment of the stable carbon isotope [3]. Differences in carbon isotope signatures between sources of food and consumers are slight and only account for 1–2‰ [2, 6]. Stable isotope analysis (SIA) therefore provides a useful means for assessing complex ecological interactions by tracing energy flow through communities [3, 7]. Study of trophic interactions between organisms using stable isotopes has mostly been applied for free-living organisms, whereas, comparisons incorporating parasites have lagged behind [8, 9]. In ecosystems, parasites are functionally important in shaping and stabilising the structure of food webs [8, 10–12]. Parasites are in turn also affected by the
structure of food webs [13] as many parasites rely on trophic interactions between organisms for their transmission [14]. Notwithstanding the functional importance of parasites, they have received comparatively less attention in food web studies and as a result our understanding of the ecology of these organisms is lacking [8, 15]. Until recently, due to the fact that parasites derive nutrition from a host organism the host–parasite relationship has been likened to a predator–prey association [8, 11, 13, 14]. According to this premise, parasites should occupy at least a single trophic level above their host in a manner similar to how consumer organisms are trophically distinct from their food sources [13]. However, unlike a typical predator which feeds on multiple prey organisms, parasites only derive nourishment from a single host at a time in their life cycle [13]. Additionally, parasites have developed different feeding strategies which encompass active feeding on host tissues, assimilation of nutrients derived from the metabolism of the host and sharing of resources with the host [8, 16]. Variable isotope fractionation may further serve as an indication of selective feeding strategies developed by parasites [17]. Generally, parasites which are more enriched than the host actively consume host tissue, in a manner similar to a predator feeding on a prey organism. While those which are generally $^{15}$N depleted feed by assimilating metabolic by-products present in intestinal contents of the host [13, 18]. As a result of unique feeding strategies adopted by parasites, patterns of stable isotope enrichment have similarly been shown to be variable between parasite taxa. For instance, cestodes [18–26], acanthocephalans [8, 27] and trematodes [27–30] are generally depleted in the $^{15}$N isotope compared to their hosts, while some nematodes [18, 19, 22, 31], monogeneans [9] and ticks [32] are $^{15}$N enriched relative to their hosts. However,
the pattern of stable isotope fraction between hosts and parasites is not clear cut, as in some cases stable isotope fractionation has been found to vary between related parasite taxa infecting the same host [33] or between the same parasite taxa infecting different hosts [18]. Studies have also found that stable isotope enrichment is affected geographically [34]. Along with the differences in stable isotope fractionation being related to the feeding strategies of parasites, observed variances may further be linked to the high selectivity for specific microhabitats adopted by many parasite taxa.

In South Africa, to date, two studies have been performed to compare the trophic relationship between parasites and their host fish from the Vaal Dam (Sures et al. 2019; Gilbert et al. UNDER REVIEW)[9]. Sures et al. [9] found that the monogenean, Paradiplozoon ichthyoxanthion, was enriched in $^{15}$N isotope relative to the host fishes, Labeobarbus kimberleyensis and Labeobarbus aeneus. Following on, Gilbert et al. (UNDER REVIEW) showed that the cestode, Schyzocotyle acheilognathi, also found infecting the Largemouth yellowfish (L. kimberleyensis), was depleted in the heavier nitrogen isotope relative to the host fish. From both studies, the differences in isotopic enrichment of $^{15}$N accounted for a difference of two trophic levels above and below the host fish respectively. In the present study, stable isotope fractionation and enrichment was compared in a host-parasite model where a single fish host is infected by different parasite taxa. The aims of this study were therefore to analyse and compare the enrichment and fractionation of stable isotopes of carbon and nitrogen in the Sharptooth catfish (Clarias gariepinus) and ectoparasites and endoparasites from the Vaal River. In addition, as samples were collected from six different locations along the Vaal River, stable isotope levels were compared in
order to better understand geographic and spatial changes in isotopic enrichment patterns in the host-parasite system.

Methods

Host and parasite collection

A total of 49 Sharptooth catfish (Clarias gariepinus) were collected from six sites along the Vaal River (Fig. 1). The sites represent three impounded sites (Vaal Dam, Bloemhof Dam and Douglas Weir) and three riverine sites (Vaal River below Grootdraai Dam, Vaal River below the Vaal Barrage and the Vaal River below Vaalharts Weir). The upper most site was located below the Grootdraai Dam in the Mpumalanga Province and the last site was located in the Douglas Weir before the confluence of the Vaal River and Orange River in the Northern Cape Province.

During the surveys at each site, C. gariepinus were caught using gillnets (mesh size: 45–190 mm) and transported in 160 L plastic containers filled with aerated water from each site to a field laboratory. Collections of fish were performed in accordance with permits from relevant national government organisations (Mpumalanga Tourism and Parks Agency: MPB. 5555; Gauteng Department of Agriculture and Rural Development: CPE000125; the Department of Economic Development, Tourism and Environmental Affairs: 01/34287; Northern Cape Nature Conservation: FAUNA 1120/2016) and following approval from the Ethics Committee of the University of Johannesburg (reference number: 2016-5-03). Fish were then euthanized by severing the spine posterior to the head. Weight and total length of C. gariepinus were recorded for determination of condition factor (K) for comparison of the nutritional status between host individuals from the same site and between sampling locations [35]:
where the weight of fish in kilograms (kg) and total length of the fish in centimetres (cm). Following collection of morphometric data the fish were euthanized and dissected, the intestines, mesenteries and gills were removed and assessed for parasites with a stereo microscope. Muscle tissue of the host was also collected using a ceramic knife and plastic forceps, and along with parasites, were frozen at -20 °C before returning to the laboratory. Parasites collected included Lamproglena clariae (Copepoda) from the gills, Tetracampus ciliotheca (Cestoda) and Proteocephalus glanduligerus (Cestoda) from the intestine, and larval Contracaecum sp. (Nematoda) which were found encysted in the mesenteries of the intestine.

Stable isotope analysis

In the laboratory, parasites were defrosted and cleaned in fresh saline (0.092% w/v) to remove any host tissue and debris from the microhabitat. In the case of L. clariae, egg strings were removed from adult females. The parasites were then refrozen (-20 °C) and along with host tissue, dried to weight consistency in a freeze dryer (Martin-Christ Gefriertrocknungsanlagen, GmbH; Germany). Host muscle and parasites tissue were homogenised and triplicates of each sample were weighed (0.4–0.8 mg) into 4 × 6 mm tin capsules for analysis of stable isotopes of carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N). Analysis of samples was performed following procedures described by Nachev et al. [8] in C/N mode using a Vario PYRO Cube elemental analyser (EA) system (Elementar Analysensysteme, Langenselbold, Germany) coupled with to an IsoPrime 100 isotope ratio mass spectrometer (IRMS; Elementar Analysensysteme). All isotope ratios were reported in δ-notation as

$$K = 100 \times \left( \frac{Fish\ weight}{Total\ Length^3} \right)$$ (1)
differences in isotopic proportion of the sample and internal reference standard (acetanilide AcAn) by Eq. (2).

$$\delta^{13}E_{2,ref} = \frac{R\left(h_{E}/l_{E}\right)}{R\left(h_{E}/l_{E}\right)_{ref}} - 1$$ (2)

Ratios of $^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N isotopes are expressed as $R\left(h_{E}/l_{E}\right)_{ref}$ in the reference material and $R\left(h_{E}/l_{E}\right)_{s}$ in the sample. Normalisation of samples and working standard, acetanilide, was done against the international scale, USGS40 and USGS41 reference materials (International Atomic Energy Agency). Normalized delta values for both stable isotopes were reported against the VPDB scale for carbon and Air for nitrogen.

Statistical analysis

Differences in trophic level ($\Delta TL$) of hosts and parasites were assessed using isotope levels for host muscle tissue and parasites according to following Eq. (3):

$$\Delta TL = \frac{\delta^{15}N_{parasite} - \delta^{15}N_{host}}{TEF}$$ (3)

Trophic level differences were determined using the average trophic enrichment factor (TEF = 3.4‰) by Minagawa and Wada [4]. To compare trophic enrichment of parasites, the same equation was applied.

Statistical analyses of the data was performed using SPSS version 25 for Windows (Statistical Package for the Social Sciences, SPSS Inc., USA). The normality of the data was assessed using the Shapiro-Wilk test and as data were not normally distributed all comparisons were performed using non-parametric tests. To compare the stable isotope levels between hosts and parasites the Kruskal-Wallis test was applied. In cases where differences in $\delta^{15}$N and $\delta^{13}$C values were significantly different the Wilcoxon matched pair test was used between hosts and parasite taxa.

Differences in stable isotope enrichment in hosts and parasites between different
sites were analysed using the Kruskal-Wallis test and where differences were significant the Mann-Whitney test was applied as a post hoc test to the Kruskal-Wallis test. Mean intensity of parasites infecting C. gariepinus was calculated according to Bush et al. [36]. Comparisons of spatial variation in host morphometrics and parasite mean intensity were performed using the Kruskal-Wallis test. To assess the magnitude of the effect of host size on the isotope enrichment and parasite intensity, and host isotope enrichment on parasite intensity the Kendall’s’ Tau test was used.

Results

Enrichment of stable isotopes of carbon and nitrogen, parasite infection intensities and host morphometrics in the C. gariepinus – parasite system varied spatially among the different sites in the Vaal River (Table 1). Differences in $^{15}$N enrichment were significant between species (Kruskal-Wallis $H = 50.4$, df = 5, $P < 0.001$), whereas, differences in $\delta^{13}$C were not significant (Kruskal-Wallis $H = 6.75$, df = 5, $P = 0.24$). There were significant differences in the composition of $\delta^{15}$N (Kruskal – Wallis, df = 5, $Z = 21.54$, $P = 0.001$) and $\delta^{13}$C (Kruskal – Wallis, $Z = 28.96$, df = 5, $P < 0.001$) between the fish along the Vaal River. Host fish collected from the Vaal River Barrage were most enriched with heavier nitrogen compared with fish from the other sites, whereas, hosts collected below the Grootdraai Dam had lowest $\delta^{15}$N values. Fish collected from Douglas Weir showed highest depletion in $^{13}$C and those collected from Bloemhof Dam ($\delta^{13}$C = 18.49‰) and below Grootdraai Dam ($\delta^{13}$C = 18.74‰) were the most enriched in the carbon stable isotope. Based on isotopic discrimination values, stable isotope enrichment of parasites infecting C. gariepinus
was also variable between different sites. Comparison between the different sampling locations showed that stable isotope composition patterns in parasites reflected those of hosts.

![Table 1](image-url)

**Table 1**
Stable isotope composition, host morphometry and mean intensity (± SE) of parasites for selected host – parasite system for six sampling sites along the Vaal River.

| Site                        | Clarias gariepinus | Lamprolena clariae (adult) | Lamprolena clariae (egg) | Tetracampus ciliotheca | Contracecum sp. (larvae) | Clarias gariepinus | Lamprolena clariae (adult) | Lamprolena clariae (egg) | Tetracampus ciliotheca | Contracecum sp. (larvae) |
|-----------------------------|--------------------|---------------------------|-------------------------|------------------------|-------------------------|--------------------|---------------------------|-------------------------|------------------------|-------------------------|
| Vaal River below Grootdraai Dam | 5                 | 2.92 (± 2.76)            | 0.01 (± 0.01)           | 14.1 (± 2.66)         | 14.38 (± 0.64)         | 8.67 (± 3.15)     | 16.28 (± 1.05)            | -18.76 (± 1.17)         | 2.18                  | 0.1*                     |
| Vaal Dam                    | 10                | 5.38 (± 1.43)            | 0.01 (± 0.01)           | 15.77 (± 2.06)        | 16.38 (± 0.64)         | 8.57 (± 4.47)     | 16.35 (± 2.58)            | -19.82 (± 0.51)         | 0.58                  | -0.15                   |
| Vaal River below Vaal River Barrage | 10               | 6.91 (± 1.29)            | 0.01 (± 0.01)           | 24.16 (± 1.01)        | 16.49 (± 2.58)         | 3.00 (± 0.00)     | 13.49 (± 0.27)            | -2.94 (± 2.94)          | -2.28                 | -0.03                   |
| Vaal River below Vaal River Barrage | 10               | 6.91 (± 1.29)            | 0.01 (± 0.01)           | 24.16 (± 1.01)        | 23.59 (± 1.33)         | 3.80 (± 1.13)     | 23.59 (± 1.33)            | -23.46 (± 1.86)         | -0.57                 | 0.27                    |
| Vaal River below Vaal River Barrage | 10               | 6.91 (± 1.29)            | 0.01 (± 0.01)           | 24.16 (± 1.01)        | 23.58 (± 1.55)         | 2.50 (± 0.681)    | 14.36 (± 1.70)            | -26.86 (± 2.78)         | 9.8                   | 3.67                    |
| Location          | Species                  | N  | C  | Isotope (‰)       | C  | Isotope (‰)       |
|-------------------|--------------------------|----|----|-------------------|----|-------------------|
| **Bloemhof Dam**  | Clarias gariepinus       | 11 |    | 50.3(±17.1)       | 1.20(±1.60) | 0.01(±0.01)       |
|                   | Lamproglenia clariae (adult) |   |    | 3.91(±1.96)       | 15.28(±0.00) | -17.42(±0.00)     |
|                   | Tetracampus ciliotheca   |   |    | 10.3(±6.60)       | 13.15(±1.26) | -20.69(±0.64)     |
| **Vaal River**    | Clarias gariepinus       | 8  |    | 53.03(±9.53)      | 0.89(±0.36) | 0.01(±0.01)       |
| below Vaalharts Weir | Lamproglenia clariae (adult) |   |    | 4.29(±1.44)       | 16.91(±0.00) | -19.45(±0.00)     |
|                   | Lamproglenia clariae (egg) |   |    | 21.8(±0.00)       | -22.2(±0.00) | 4.89*               |
|                   | Tetracampus ciliotheca   |   |    | 1.50(±0.50)       | 11.77(±0.01) | -20.04(±1.77)     |
|                   | Proteoccephalus glanduligerus |   |    | 1.00(±0.00)       | 12.14(±0.00) | -18.38(±0.00)     |
|                   | Contracecum sp. (larvae) |   |    | 13.0(±3.49)       | 12.66(±0.48) | -20.64(±1.35)     |
| **Douglas Weir**  | Clarias gariepinus       | 5  |    | 73.5(±4.34)       | 2.67(±0.68) | 0.01(±0.01)       |
|                   | Tetracampus ciliotheca   |   |    | 4.67(±2.73)       | 12.2(±0.06)  | -28.09(±1.29)     |
|                   | Proteoccephalus glanduligerus |   |    | 3.00(±0.00)       | 13.35(±0.00) | -26.33(±0.00)     |
|                   | Contracecum sp. (larvae) |   |    | 29.0(±3.62)       | 11.41(±1.07) | -22.47(±1.45)     |

* delta values for 15N and 13C calculated from difference in isotope signatures between eggs and adults of L. clariae.

Parasite enrichment patterns showed that in most comparisons, endoparasites were depleted in 15N compared to the host fish and adult L. clariae. For cestodes, T. ciliotheca Δ15N varied from 0.91‰ below the Grootdraai Dam to 9.8‰ at the site below the Vaal Barrage and in P. glanduligerus Δ15N ranged from 2.36‰ to 3.51‰.
at Douglas Weir and below Vaalharts Weir, respectively compared to host muscle tissue. For Contracaecum sp. larvae collected below the Grootdraai Dam were enriched by 1.69‰ relative to the host fish, whereas, specimens collected below Vaalharts Weir and in Douglas Weir were depleted in $^{15}$N compared to the host. For $^{13}$C, enrichment patterns mostly showed that cestodes and Contracaecum sp. were enriched relative to C. gariepinus hosts. Except for T. ciliotheca collected in the Vaal Dam and below Vaalharts Weir; P. glanduligerus from below Vaalharts Weir and Contracaecum sp. collected from below Vaalharts Weir and Douglas Weir sites which were depleted in $^{13}$C compared with the hosts.

Adult female L. clariae were variably enriched and depleted in both $^{15}$N and $^{13}$C isotopes. In instances where adult female L. clariae were enriched in $^{15}$N isotope, differences accounted for 0.58–2.18‰ higher than in host muscle tissue. Samples from below the Vaal Barrage and Bloemhof Dam were depleted in $^{15}$N by 0.57‰ and 0.96‰ respectively. Differences in $^{15}$N stable isotope indicate that despite the mixed fractionation patterns, adult L. clariae and C. gariepinus hosts share similar trophic levels. For differences in $^{13}$C isotope, L. clariae were depleted at Vaal Dam, Bloemhof Dam and below Vaalharts Weir by 0.15–1.4‰, whereas, adult copepods collected from below the Vaal Barrage were enriched by 0.27‰. Despite the mixed fractionation patterns for $^{13}$C stable isotope, L. clariae and the host fish share similar stable carbon isotope levels. Differences in nitrogen and carbon stable isotopes between adult female copepods and hosts were not significant ($\delta^{15}$N: Wilcoxon matched pair test, $Z = -0.314, P = 0.754$; $\delta^{13}$C: Wilcoxon matched pair test, $Z = -1.098, P = 0.272$). Eggs of adult L. clariae were analysed and compared
with isotope signatures in adult parasites as larval stages derive nutrients from the adult organism and not from the host fish. Stable isotope fractionation patterns between adult *L. clariae* and egg strings were variable; with Δ^{15}N for egg strings being enriched from 0.1‰ below Grootdraai Dam to 4.89‰ below Vaalharts Weir or shared similar signatures for ^{15}N signatures relative to adult parasites as in the case of samples collected below the Vaal River Barrage. Differences in δ^{15}N between egg strings and adult *L. clariae* were not significant (Wilcoxon matched pair test, Z = -0.459, P = 0.646). For the Δ^{13}C, egg strings were consistently enriched compared to adult *L. clariae* by 0.17–2.75‰.

However, spatial differences in ^{15}N enrichment were not significant between the different sites for parasites. Highest enrichment for adults (Kruskal-Wallis Z = 8.5, df = 4, P = 0.075) and eggs (Kruskal-Wallis Z = 7.36, df = 3, P = 0.061) of *L. clariae*, and *T. ciliotheca* (Kruskal-Wallis Z = 10.1, df = 5, P = 0.073) were observed for samples collected from the site below the Vaal River Barrage. For *Contracaecum* sp. (Kruskal-Wallis Z = 7.12, df = 2, P = 0.028), specimens collected from the site below the Grootdraai Dam showed significantly higher ^{15}N enrichment than nematodes infecting catfish hosts from below the Vaalharts Weir and at Douglas Weir. In the case of *P. glanduligerus*, the low number of specimens collected (n = 2) hampered any statistical comparison, but the specimen collected at Douglas Weir showed higher enrichment for both stable isotopes analysed compared with the specimen collected below Vaalharts Weir. In terms of enrichment of ^{13}C, highest enrichment factors were found for adults and eggs of *L. clariae* from below the Vaal River Barrage. For endoparasites, highest enrichment was observed for those collected from Douglas Weir, but these differences were not significant for *Contracaecum* sp.
Differences in $^{13}$C enrichment were only significant for T. ciliotheca collected from Douglas Weir compared with other sites (Kruskal-Wallis $Z = 14.4$, df = 5, $P = 0.013$). Host fish size (total length) and weight were significantly different between different sites (Kruskal-Wallis $H = 11.5$, df = 5, $P = 0.042$), while the condition factor showed no differences between sites. Comparison of host size (length and weight) showed that smaller hosts were less enriched in $^{15}$N than larger hosts and the opposite was observed for $^{13}$C which was higher in smaller hosts than larger ones. Nitrogen stable isotope enrichment in C. gariepinus was positively but not significantly correlated with total length (Kendall’s tau, $\tau = 0.135$, $P = 0.247$) and weight (Kendall’s tau, $\tau = 0.214$, $P = 0.071$) of host fish. Correlations between host morphometry and $^{13}$C enrichment were significant and negative (total length: Kendall’s tau, $\tau = -0.361$, $P = 0.002$; weight: Kendall’s tau, $\tau = -0.382$, $P = 0.001$). Condition factor was higher in female fish than in males, however, differences between host sex and sites were not significant (host sex: Mann-Whitney $U = 101$, $P = 0.220$; sampling sites: Kruskal-Wallis $H = 8.44$, df = 5, $P = 0.134$).

The mean intensities of parasite taxa collected were variable between the sampling sites (Table 1). Highest mean intensity of L. clariae was recorded at the Vaal Dam. Mean intensities of T. ciliotheca and Contracaecum sp. were highest at Bloemhof Dam and Douglas Weir respectively. Proteocephalus glanduligerus were only collected from fish below Vaalharts Weir and Douglas Weir where, in both instances, only a single cestode was recovered from either site. Differences in parasite intensities were significantly different between the sampling sites for L. clariae (Kruskal-Wallis $H = 13.9$, df = 5, $P = 0.016$) and Contracaecum sp. (Kruskal-Wallis $H$
= 27.9, df = 5, P ≤ 0.001), but not for T. ciliotheca (Kruskal-Wallis H = 6.03, df = 5, P = 0.303) and P. glanduligerus (Kruskal-Wallis H = 4.04, df = 5, P = 0.543). Intensity of parasites infecting C. gariepinus were compared with host morphometry and showed intensity for all taxa collected was positively related with total length and weight of the host fish. However, only the intensity of T. ciliotheca correlated significantly with host size (Kendall’s tau, length: τ = 0.297, P = 0.019; weight: τ = 0.324, P = 0.012). Intensities of Contracaecum sp. (Kendall’s tau, length: τ = 0.185, P = 0.156; weight: τ = 0.161, P = 0.225) and L. clariae (Kendall’s tau, length: τ = 0.057, P = 0.640; weight: τ = 0.055, P = 0.656) were not significantly correlated with host size. The low number of P. glanduligerus collected hampered comparisons with host morphometrics. As the condition factor did not differ between different sites, comparisons between K and host stable isotope enrichment factors was not performed.

Discussion

In the present study distinct spatial differences in stable isotope fractionation patterns were observed for C. gariepinus and parasites from the Vaal River. In host fish, highest δ¹⁵N levels were recorded from samples collected below the Vaal River Barrage, whereas, samples collected from the site below Grootdraai Dam showed lowest stable nitrogen isotope levels. The opposite was observed for ¹³C enrichment in hosts among the different sites, with lowest levels observed in C. gariepinus collected below the Vaal River Barrage. These differences may be related to the variability in the diet of C. gariepinus as a result of availability of prey items at each sampling site along the Vaal River. The Sharptooth catfish is a typical omnivore, feeding on a wide variety of organic matter with prey items varying from birds,
reptiles, other fish species, including smaller C. gariepinus, to macroinvertebrates and plant material [37]. As stable carbon isotope ratios serve as a representation of the nutrient sources in food webs [38], comparison of differences in the enrichment of $^{13}$C isotope can be considered a good indicator of spatial variation in baseline isotope levels [34, 39]. The variability of $\delta^{13}$C in muscle tissue of C. gariepinus between sampling sites suggests that a wide range of dietary items are consumed by this fish species. The present results further support a dietary shift in C. gariepinus inhabiting the Vaal River. Negative correlation between host morphometry and $\delta^{13}$C indicate that the foraging habits and food sources utilised change as fish grow. This was further supported by a positive correlation between host length and weight with $\delta^{15}$N values. In a previous study, Kadye and Booth [40] showed that although there is an apparent dietary shift in C. gariepinus with fish growth, there is a high dietary overlap between fish of different size classes which can be related to the omnivorous feeding strategy of this fish species. In the present study collections were performed in spring which coincided with the period when C. gariepinus prepare to begin spawning in the summer following rains [37]. Differences in condition factor (K) between male and female host fish ($K_{\text{female fish}} > K_{\text{male fish}}$) can therefore be related to the larger weight of gravid female fish. Spatially, there was no difference in the condition factor of the hosts between the different sampling points along the Vaal River and therefore with the levels of stable nitrogen and carbon isotopes. Clarias gariepinus serves as a host for a wide variety of endoparasite and ectoparasite taxa [41] and this could likely result from the variable diet and wide distribution of this fish species. In the present study the association between parasite intensity and host morphometry was variable.
Comparisons with P. glanduligerus were not performed due to the low number of samples obtained. Positive correlation between the size and weight of C. gariepinus hosts and L. clariae, T. ciliotheca and larval Contracaecum sp. indicate that larger fish harbour more parasites than smaller ones. This was especially so for T. ciliotheca infecting C. gariepinus which showed a significant and positive correlation with host morphometry. The concept of larger fish harbouring higher parasite intensity has been well documented in previous studies (see [42]).

Isotopic discrimination of $^{15}$N indicated that all nematode larvae and cestodes collected from C. gariepinus were depleted relative to the host fish from all sites. Depletion of the heavier stable nitrogen isotope in cestodes and larval nematodes is in line with previous findings for other cestodes and larval nematodes [20, 29] infecting fish hosts, and for some cestodes infecting rabbit hosts [19]. Differences in $\delta^{15}$N between larval nematodes and cestodes with host muscle tissue accounted for shifts of approximately one to two trophic levels, respectively. These observed differences are in the range for other host – cestode and larval nematode systems analysed [19–21, 23, 24, 26] and can be related to the mode of nutrient acquisition. For cestodes, lack of a digestive system has meant that the tegument of these organisms has become specially modified for the accumulation of molecules derived from the hosts’ metabolism [43]. As a result of transamination of complex proteins, the heavier stable nitrogen isotope is retained in the tissues of the host [44] and as a result endoparasites are depleted in heavier isotopes. Comparison of stable nitrogen isotope levels between endoparasites showed that larval Contracaecum sp. has the least depletion. This can be related to the fact that the larval stages of these nematodes are not actively feeding on host tissue or metabolic compounds as
adult cestodes do.

Results for isotopic discrimination between host muscle tissue and Contracaecum sp. larvae in the present study are in line with previous studies for other larval nematodes encysted in the peritoneal cavity of host fish [8, 18]. In the case of larvae of Contracaecum sp. infecting C. gariepinus, nematodes were encysted in the mesenteries of the viscera in the region of the intestinal tract. According to Moravec et al. [45] these larval stages exhibit low pathogenicity and along with the current $^{13}$C and $^{15}$N isotope data indicate that the nematodes are not actively feeding on fish hosts. Rather Moravec et al. [45] suggests that these larval nematodes develop to the third stage in the egg in the water column which is then ingested by a fish which functions as a paratenic host. Thus, it is plausible that the lack of a relationship between host stable isotopes and larval nematodes is the result of C. gariepinus being a paratenic host to Contracaecum sp. larvae which are not actively feeding on the host. Additionally, Nachev et al. [8] indicated that the slow growth rate of some nematode larvae is a contributing factor resulting in the lack of a relationship between host stable isotopes and larval nematodes. In order to determine if these larval stages derive some nutrition from paratenic hosts, comparisons of stable isotopes for different larval stages will have to be performed.

In South Africa, studies have indicated that larval Contracaecum sp. are widespread and occur frequently in C. gariepinus [see 39, 40]. Despite this, $^{15}$N fractionation observed in the present study indicated that endoparasite taxa infecting C. gariepinus occupy relatively similar trophic levels. Similarly $\delta^{13}$C for all endoparasite taxa fall within the 1–2‰ range of enrichment factors reported in previous studies [21, 38, 47]. For cestodes and encysted larval nematodes,
differences in carbon stable isotope signatures are comparable to other reports for similar host – parasite systems [18, 21, 25] and indicate that nutrients assimilated by the host and parasites are from similar sources [21].

Regarding the copepod, L. clariae, fraction of both $^{15}$N and $^{13}$C showed that the parasites were variably enriched and depleted in both stable isotopes compared with host muscle tissue. Based on differences in the heavier nitrogen isotope this did not correspond to differences in trophic position and therefore host and female copepods likely occupy similar trophic positions. Instances where adult female L. clariae were $^{15}$N enriched relative to host muscle tissue correlates with the feeding pattern of the parasite and corroborates stable $^{15}$N isotope fractionation patterns in other haemophagous ectoparasites [9, 19, 32, 48, 49]. The variability in $^{15}$N fractionation between C. gariepinus and L. clariae observed in the present study further corroborates findings for other parasitic copepods, which parasitise the gills of the host fishes and share similar feeding biology as L. clariae [18, 20, 29]. Iken et al. [29] found that a copepod infecting gills of Coryphaenoides armatus was enriched by 2.7‰ in $^{15}$N relative to the host. Pinnegar et al. [20] found that Lernaeocera branchialis, infecting the gills of the host fish, Platichthys flesus, were depleted in $^{15}$N stable isotope by 0.81‰ relative to the host. Unlike L. clariae which feeds on blood from the gill filaments, L. branchialis feeds on blood from the bulbous arteriosus of the heart of infected fish hosts, where it macerates host tissue with its mandibles and feeds on host blood [see 45]. In an assessment of isotope fractionation between several copepods infecting the gills of their hosts, Deudero et al. [18] showed high interspecific variation in $^{15}$N enrichment between copepod species feeding on the same host fish. For instance, they found that Clavella adunca
feeding on both cod and whiting were depleted in $^{15}$N stable isotope but L. branchialis feeding on whiting or haddock were enriched in $^{15}$N but were depleted in $^{15}$N when parasitising cod. Similarly with L. clariae, the copepods analysed by Pinnegar et al. [20] and Deudero et al. [18] infected the gills and feed on the blood of the host fishes. However, Shotter [51] indicated that the mandibles of Clavella uncinata, a similar species to C. adunca, were too weak to tear tissue but instead function in gathering material by scraping superficial tissue toward the mouth, with little blood comprising the diet and intestinal contents. In some instances, studies have indicated adult female copepods are significantly enriched in $^{15}$N relative to the host organism [52–54]. Gretsy and Qyarmby [52] found that adult Mytilicola intestinalis were enriched by 3‰ relative to the intestine of European blue mussel host (Mytilus edulis). Goedknegt et al. [54] similarly found that adult Mytilicola orientalis were enriched in $^{15}$N stable isotope relative to the adductor muscle of the host mussel, M. edulis, by 1.2‰. In both instances the higher $^{15}$N enrichment of both species could be related to the parasites feeding directly on the intestinal tissue of the host. In a seasonal study on the gut ultrastructure and contents in conjunction with stable carbon and nitrogen isotope analysis of Neoergasilus japonicus, Baud et al. [53] showed that adult female parasites infecting the fins of the host fish, Perca fluviatilis, were enriched in the $^{15}$N stable isotope by 3.7‰ relative to the host muscle tissue, indicating that the parasite feeds on host tissue. The high variability in $^{15}$N enrichment of parasitic copepods may be related to the variability in stable isotope enrichment of host tissues consumed by parasites as well as the isotopic enrichment of the host resulting from dietary variation in cases where parasitic copepods feed on similar host tissues. In the present study, spatially
variable $^{15}$N fractionation patterns of L. clariae infecting the same host corresponded or mirrored spatial $^{15}$N isotope enrichment of the host among the collection sites along the Vaal River. In most instances, adult L. clariae were slightly enriched in $^{15}$N stable isotope relative to the host fish tissues (VRGD, VD and VRHD), but were also slightly depleted relative to the host fish at sites below the Vaal River Barrage and at Bloemhof Dam. Adult L. clariae and egg strings shared similar stable isotope signatures. High standard deviations in the stable isotope levels of L. clariae could be related to spatial differences in the food sources consumed by the host fish. Additionally, during their life cycles many parasitic Copepoda undergo drastic morphological changes from free swimming larval stages which are able to move between hosts to sedentary parasitic adults. It is possible that during the free swimming larval stages, copepodite stages may feed on hosts of variable isotope enrichment as well as incorporate other sources of nutrition before becoming parasitic on a host fish. The variation in the diet during the free swimming larval stages may then further result in variations in stable isotope enrichment observed in adult organisms. According to predictions of stable isotope fractionation between consumers and prey items, geographic variation in the ratios of stable isotopes of carbon and nitrogen should be reflected in a predictable manner in relation to the isotope signatures of the host [6, 47, 55]. As such variability in the diet of the host should be reflected more prominently in the stable isotope composition of endoparasites more so than ectoparasites [18]. However, from the results of the present study, variations in the host diet were more closely mirrored in the isotope enrichment of L. clariae than endoparasites. Iken et al. [29] observed no similarity in isotope
enrichment in trematodes feeding on intestinal tissue of the host fishes, Chalinura profundicola and Chalinura leptolepis, while a copepod feeding on a gastropod host, Oneirphanta mutabilis, did exhibit similarity in isotope fractionation. Deudero et al. [18] similarly noted that stomach nematodes did not reflect isotopic differences observed in host fishes. Sures et al. [9] showed that in a monogenean – host fish system from the Vaal Dam, the micropredatory nature of P. ichthyoxanthon resulted in mirroring in isotope fractionation between two yellowfish hosts.

Spatial geographic differences observed at various trophic levels can be related to host – specific differences in ecology and behaviour [18, 34]. Regarding the spatial differences observed in isotopic enrichment patterns of parasite taxa in the present study, as the feeding strategies of the parasites do not change between the different sites, the differences in enrichment patterns observed are likely related to differences in food items utilised by the host fish. The spatial variability in stable isotopes in parasites infecting C. gariepinus therefore reflects spatial differences in the baseline isotope signatures across the distribution of the host. Geographical differences in δ¹³C and δ¹⁵N levels of organisms has previously been documented [39]. With regard to parasites, Gómez-Díaz and González-Solís [34] similarly observed spatial differences in the isotopic signatures of ectoparasites infecting two closely related shearwater hosts, Calonectris diomedea and Calonectris borealis, across the Mediterranean Sea and Northeast Atlantic. Low variation in stable isotope composition of parasites collected from the Vaal River can be related to the fact that parasites do not change the resources they utilise from the host and rather spatial differences in parasite stable isotope enrichment is related to the variance in host diet. This was similarly observed by Riascos et al. [56] for Hyperia curticephala infecting the scyphomedusa, Chrysaora plocamia. It should also be
noted that unevenness of the prevalence and abundance of parasites along the Vaal River likely lead to a lop-sided sampling design and as such spatial differences in parasite stable isotope enrichment observed in the present study should be confirmed following more even sample collection.

Comparison between the parasites infecting C. gariepinus showed that L. clariae were significantly and constantly enriched in $^{15}N$ stable isotope compared to the endoparasites. The shift in $\delta^{15}N$ observed for endoparasites and L. clariae overall accounted for a difference of approximately two trophic levels. Variability in the signatures reported between the different parasite taxa analysed are similar to other studies which have found similar co-infections [18, 20, 29]. The differences in $\delta^{15}N$ can be linked with differences in nutrient acquisition strategies by each parasite taxon. Mature, adult female L. clariae consume whole blood and epithelial cells which they acquire from the secondary gill filaments of the host fish [57, 58]. Mature female copepods attach to the gill filaments using their maxillipeds and feed on gill epithelium and blood using the maxillae which scrape cellular material toward the mouth and along with a negative pressure created by the muscular oesophagus, blood and cellular debris are sucked into the buccal cavity [57–59]. Unlike helminth endoparasites, some copepod ectoparasites are not able to accumulate simple amino acids across the keratinised body surface and instead must break down complex proteins which are accumulated in meals [18]. As a result these parasites are generally enriched in heavier $^{15}N$ stable isotopes in a manner similar to consumer organisms which resembles a predator–prey relationship.

Whereas, in the case of both cestodes and larval nematodes, feeding can be likened to that of a commensalistic scavenger, whereby, the parasites feed on left over by-
products of the hosts metabolism and in doing so pose little harm during feeding toward the host.

Conclusions

Findings of the present study indicate that parasites infecting C. gariepinus are variably enriched in stable isotopes of carbon and nitrogen. General trends in stable isotope fractionation show that the ectoparasite, L. clariae and host shared similar $\delta^{15}$N values, whereas, the endoparasite, T. ciliotheca, P. glanduligerus and Contracaecum sp. were depleted in the heavier nitrogen isotope. This deficit accounted for a difference of one to two trophic levels between endoparasites and C. gariepinus. As L. clariae and C. gariepinus shared similar isotope levels, both organisms occupy a similar trophic level. The patterns identified in the present study therefore corroborate previous studies in other similar host-parasite systems. In addition to the general trends identified, spatial differences in stable isotope enrichment patterns were observed for both the host and parasites. Spatial differences between the different collection sites could likely be the result of differences in the size of the host and with that differences in types of food items incorporated into the diet. Clarias gariepinus is an omnivorous and scavenging fish species and as such incorporates a wide variety of different prey items in the diet. This can additionally explain the wide variety of parasite taxa found infecting this fish species. Variation in intensity of parasites infecting C. gariepinus showed mixed correlations with $\delta^{15}$N and $\delta^{13}$C isotope levels. The observed pattern likely results from the differences in prey items available at the different locations along the Vaal River (in the case of endoparasites) as well as the foraging behaviour of the hosts at each site (in the case of L. clariae). As such, the present results further support
the usefulness of stable isotope comparisons in tracing the infection pathways of certain parasite taxa through the food web. This is especially so for parasites which require multiple hosts for successful completion of the life cycle. As parasites derive nutrition by either feeding on the host tissues or by assimilating simple biomolecules derived from the host, spatial differences in the enrichment patterns of the parasite taxa likely resulted from differences in host diet.

Declarations

Ethics approval and consent to participate

Research was undertaken following approval by the Research Ethics Committee of the University of Johannesburg (Ethics number: Gilbert 2016). Permits for the collection of fish sampled in the present study were obtained from relevant national government institutions (Mpumalanga Tourism and Parks Agency: MPB. 5555; Gauteng Department of Agriculture and Rural Development: CPE000125; the Department of Economic Development, Tourism and Environmental Affairs: 01/34287; Northern Cape Nature Conservation: FAUNA 1120/2016).

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Conceived and designed the experiments: BMG, MN, BS, AAO. Performed the experiments: BMG, MN, DK. Analysed the data: BMG, MN, DK. Contributed reagents/materials/analysis tools: MJ, TS, AAO. Wrote the paper: BMG, MN, BS, AAO

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Figures

Figure 1

Map of the Vaal River showing the position of sampling sites (I: below Grootdraai

Supplementary Files

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