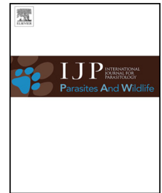
IJP: Parasites and Wildlife 12 (2020) 134–141

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw



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

Beric M. Gilbert[a,e], Milen Nachev[b,c], Maik A. Jochmann[c,d], Torsten C. Schmidt[c,d], Daniel Köster[d], Bernd Sures[a,b,c], Annemariè Avenant-Oldewage[a,*]

[a] Department of Zoology, University of Johannesburg, 524 Auckland Park, Johannesburg, 2006, South Africa
[b] Aquatic Ecology, University of Duisburg-Essen, Universitätsstr. 5, 45141, Essen, Germany
[c] Centre for Water and Environmental Research, University of Duisburg-Essen, Universitätsstr. 5, 45141, Essen, Germany
[d] Instrumental Analytical Chemistry, University of Duisburg-Essen, Universitätsstr. 5, 45141, Essen, Germany
[e] Spectrum Analytical Facility, University of Johannesburg, 524 Auckland Park, Johannesburg, 2006, South Africa

ARTICLE INFO

Keywords:
Lamproglena
Cestoda
Copepoda
Food webs
Nematoda
Trophic interactions

ABSTRACT

Stable isotope analysis offers a unique tool for comparing trophic interactions and food web architecture in ecosystems based on analysis of stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in organisms. *Clarias gariepinus* were collected from six sites along the Vaal River, South Africa and were assessed for ectoparasites and endoparasites. *Lamproglena clariae* (Copepoda), *Tetracampos ciliotheca* and *Proteocephalus glanduligerus* (Cestoda), and larval *Contracaecum* sp. (Nematoda) were collected from the gills, intestine and mesenteries, respectively. Signatures of $\delta^{13}C$ and $\delta^{15}N$ were analysed in host muscle tissue and parasites using bulk stable isotope analysis. Variable stable isotope enrichment between parasites and host were observed; *L. clariae* and the host shared similar $\delta^{15}N$ signatures and endoparasites being depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to the host. Differences in stable isotope enrichment between parasites could be related to the feeding strategy of each parasite species collected. Geographic and spatial differences in enrichment of stable isotopes observed in hosts were mirrored by parasites. As parasites rely on a single host for meeting their nutritional demands, stable isotope variability in parasites relates to the dietary differences of host organisms and therefore variations in baseline stable isotope signatures of food items consumed by hosts.

1. Introduction

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 (Kelly, 2000; Peterson and Fry, 1987; Post, 2002). 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 (Minagawa and Wada, 1984; Vander Zanden et al., 1997). Information regarding the food sources incorporated into the diet by consumers is delineated by comparing enrichment of the stable carbon isotope (Post, 2002). Differences in carbon isotope signatures between sources of food and consumers are slight and only account for 1–2‰ (DeNiro and Epstein, 1978; Kelly, 2000). Stable isotope analysis (SIA) provides a useful means for assessing complex ecological interactions by tracing energy flow through communities (Post, 2002; Wada, 2009). Study of trophic interactions between organisms using stable isotopes has mostly been applied for free–living organisms, whereas, comparisons incorporating macroparasites have lagged behind (Nachev et al., 2017; Sures et al., 2019).

In ecosystems, parasites are functionally important in shaping and stabilising the structure of food webs (Marcogliese, 2003; Nachev et al., 2017; Poulin, 2010; Sabadel et al., 2019). The functional importance of parasites is represented by their ubiquity in ecosystems and proportion of the global biomass (Dobson et al., 2008; Kuris et al., 2008; Selbach et al., 2020), and their ability to regulate host populations makes parasites important in determining host community structure (Marcogliese and Cone, 1997). Parasites are in turn also affected by the structure of food webs (Lafferty et al., 2008) as many parasites rely on

* Corresponding author.
E-mail address: aoldewage@uj.ac.za (A. Avenant-Oldewage).

https://doi.org/10.1016/j.ijppaw.2020.05.011
Received 8 April 2020; Received in revised form 22 May 2020; Accepted 23 May 2020
2213-2244/ © 2020 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

trophic interactions between organisms for their transmission (Marcogliese and Cone, 1997). Notwithstanding the functional importance of parasites in ecosystems, they have received less attention in food web studies and as a result our understanding of the ecology of these organisms is limited (Nachev et al., 2017; Thompson et al., 2005). 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 (Lafferty et al., 2008; Marcogliese and Cone, 1997; Nachev et al., 2017; Poulin, 2010). 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 (Lafferty et al., 2008). 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 (Lafferty et al., 2008). 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 (Goater et al., 2014; Nachev et al., 2017). Variable isotope fractionation may further serve as an indication of selective feeding strategies developed by parasites (Xu et al., 2007).

Generally, parasites which are more enriched than the host actively consume host tissue resulting in isotope enrichment reminiscent of a predator feeding on a prey organism (Sures et al., 2019). While those which are generally ^{15}N depleted feed by assimilating metabolic by-products present in intestinal contents of the host (Deudero et al., 2002; Lafferty et al., 2008). 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 (Behrmann-Godel and Yohannes, 2015; Boag et al., 1998; Deudero et al., 2002; McGrew et al., 2015; Navarro et al., 2014; Neilson et al., 2005; Persson et al., 2007; Pinnegar et al., 2001; Power and Klein, 2004), acanthocephalans (Nachev et al., 2017; Zhang et al., 2018) and trematodes (Doi et al., 2010; Dubois et al., 2009; Iken et al., 2001; Zhang et al., 2018) are generally depleted in the ^{15}N isotope compared to their hosts, while some nematodes (Boag et al., 1998; Deudero et al., 2002; Neilson et al., 2005; O'Grady and Dearing, 2006), monogeneans (Sures et al., 2019) and ticks (Schmidt et al., 2011) 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 (Demopoulos and Sikkel, 2015) or between the same parasite taxa infecting different hosts (Deudero et al., 2002). Studies have also found that stable isotope enrichment is affected geographically (Gómez-Díaz and González-Solís, 2010). Along with the differences in stable isotope fractionation being related to the feeding strategies of parasites, observed variances may result from 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 (Gilbert et al., 2020; Sures et al., 2019). Sures et al. (2019) found that the monogenean, *Paradiplozoon ichthyoxanthon*, was enriched in ^{15}N isotope relative to the host fishes, *Labeobarbus kimberleyensis* and *Labeobarbus aeneus*. Following on, Gilbert et al. (2020) showed that the cestode, *Schyzocotyle acheilognathi*, also found infecting the *L. kimberleyensis*, was depleted in the heavier nitrogen isotope (^{15}N) 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.

We hypothesize that within the *Clarias gariepinus* – parasite system there will be variability in the enrichment factors of ectoparasites and endoparasites in relation to the host fish; where ectoparasites will be enriched in ^{15}N relative to the host and the opposite will be observed for endoparasites. The variability in isotope enrichment in parasites will be related to differences in feeding patterns and mechanisms parasites use to assimilate nutrients from the host fish. Further to this, spatial geographic variability in the host fish stable isotope levels will be mirrored in parasites and given the expansiveness of the Vaal River we expect to find that ^{15}N and ^{13}C levels in hosts and parasites will show similar geographic patterns. To test these hypotheses, *C. gariepinus* were collected from six different sites along the Vaal River in South Africa and muscle tissue and parasites were analysed for stable isotopes of carbon and nitrogen. *Clarias gariepinus* is a known omnivore and has been found to feed on a wide variety of prey items ranging from plant material to invertebrates and small vertebrates in aquatic environments (Skelton, 2001). This varied diet has further been suggested as a factor leading to the diversity of parasites found infecting this fish species (Crafford and Avenant-Oldewage, 2009). In light of this, we therefore further hypothesize that variability in stable isotope levels in *C. gariepinus* will be related to the size of the fish, with larger fish being enriched in ^{15}N isotope compared to smaller sized fish.

2. Methods

2.1. Host and parasite collection

A total of 49 *C. gariepinus* were collected from six sites along the Vaal River, South Africa (Fig. 1). The Vaal River is the third largest river in South Africa and is an economically and ecologically important aquatic ecosystem in South Africa due to its importance in providing water for agricultural, urban and industrial activities occurring within the catchment area (Braune and Rogers, 1987). The river originates in the Mpumalanga Province and flows in a south-west direction across the interior of the country and eventually joins the Orange River in the Northern Cape Province near the town of Douglas (Braune and Rogers, 1987). The Vaal River, being 1120 km long, is the largest tributary of the Orange River with six major impoundments. Our sampling sites represent three of the available impoundments (Vaal Dam (26°53′40.3″S 28°08′44.1″E), Bloemhof Dam (27°40′42.3″S 25°40′25.2″E) and Douglas Weir (29°01′23.9″S 23°53′09.7″E)) and three riverine sites (Vaal River below Grootdraai Dam (26°55′17.9″S 29°16′52.5″E), Vaal River below the Vaal Barrage (26°44′23.8″S 27°38′08.9″E) and the Vaal River below Vaalharts Weir (28°05′56.9″S 24°51′12.8″E)). 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 gill nets (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 (Heincke, 1908):

$$K = 100 \times \left(\frac{Fish\ weight}{Total\ Length^3}\right) \tag{1}$$

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

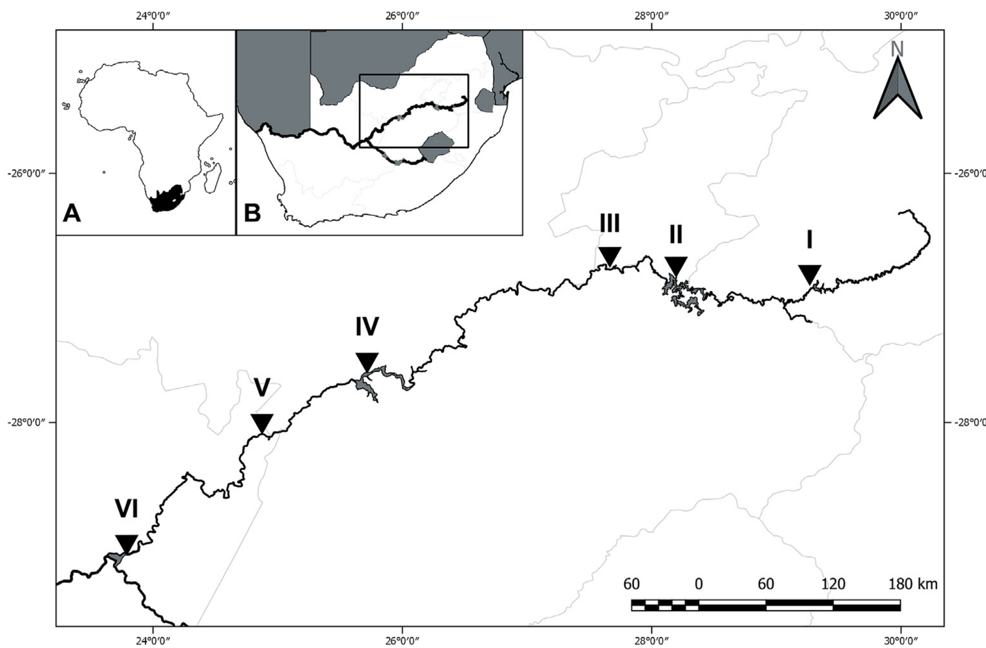


Fig. 1. Map of the Vaal River showing the position of sampling sites (I: below Grootdraai Dam; II: Vaal Dam; III: below Vaal River Barrage; IV: Bloemhof Dam; V: below Vaalharts Weir; VI: Douglas Weir) along the Vaal River. The block (B) indicates the position of the Vaal River within South Africa and insert A indicates the position of South Africa shaded on the African continent.

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, *Tetracampos ciliotheca* (Cestoda) and *Proteocephalus glanduligerus* (Cestoda) from the intestine, and larval *Contracaecum* sp. (Nematoda) which were found encysted in the mesenteries of the intestine.

2.2. 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. (2017) 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 differences in isotopic proportion of the sample and internal reference standard (acetanilide AcAn) by equation (2).

$$\delta^{h}E_{s,ref} = \frac{E)_s}{E)_{ref}} - 1 \tag{2}$$

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.

2.3. Statistical analysis

Differences in trophic level (ΔTL) of hosts and parasites were assessed using isotope levels for host muscle tissue and parasites according to following equation (3):

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

Trophic level differences were determined using the average trophic enrichment factor (TEF = 3.4‰) by Minagawa and Wada (1984). 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. (1997). 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.

3. Results

Enrichment of stable isotopes in host fish and parasites, parasite mean intensities and host length and weight varied among sampling sites in the Vaal River (Table 1). 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) among the *C. gariepinus* samples along the Vaal River. Host fish collected from the Vaal River Barrage were most enriched with ^{15}N 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 ^{13}C isotope. To better

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		Number of hosts	TL [cm]	W [kg]	K	Parasite mean intensity (± SE)	$\delta^{15}N$	$\delta^{13}C$	Δ^hE (parasite – host)	
									$\Delta^{15}N$	$\Delta^{13}C$
Vaal River below Grootdraai Dam	*Clarias gariepinus*	5	71.7(± 26.5)	2.92(± 2.76)	0.01(± 0.01)		14.1(± 2.66)	−18.74(± 0.76)		
	Lamproglena clariae (adult)					6.67(± 3.15)	16.28(± 1.05)	−18.76(± 1.17)	2.18	0.02
	Lamproglena clariae (egg)						16.38(± 0.64)	−19.73(± 1.29)	0.1[a]	0.97[a]
	Tetracampos ciliotheca					2.00(± 2.00)	13.19(± 0.00)	−19.08(± 0.00)	−0.91	0.34
	Contracaecum sp. (larvae)					6.00(± 1.00)	15.79(± 0.06)	−19.27(± 0.92)	1.69	0.53
Vaal Dam	*Clarias gariepinus*	10	53.8(± 21.7)	1.36(± 1.43)	0.01(± 0.01)		15.77(± 2.06)	−19.97(± 0.82)		
	Lamproglena clariae (adult)					8.57(± 4.47)	16.35(± 2.58)	−19.82(± 0.51)	0.58	−0.15
	Lamproglena clariae (egg)						16.49(± 2.58)	−20.21(± 0.58)	0.14[a]	0.39[a]
	Tetracampos ciliotheca					3.00(± 0.00)	13.49(± 0.27)	−19.94(± 2.94)	−2.28	−0.03
Vaal River below Vaal River Barrage	*Clarias gariepinus*	10	61.9(± 13.4)	1.99(± 1.29)	0.01(± 0.01)		24.16(± 1.01)	−23.19(± 1.15)		
	Lamproglena clariae (adult)					3.80(± 1.13)	23.59(± 1.33)	−23.46(± 1.86)	−0.57	0.27
	Lamproglena clariae (egg)						23.58(± 1.55)	−23.63(± 2.32)	−0.01[a]	0.17[a]
	Tetracampos ciliotheca					2.50(± 0.681)	14.36(± 1.70)	−26.86(± 2.78)	−9.8	3.67
Bloemhof Dam	*Clarias gariepinus*	11	50.3(± 17.1)	1.20(± 1.60)	0.01(± 0.01)		16.24(± 1.16)	−18.49(± 0.64)		
	Lamproglena clariae (adult)					3.91(± 1.96)	15.28(± 0.00)	−17.42(± 0.00)	−0.96	−1.1
	Tetracampos ciliotheca					10.3(± 6.60)	13.15(± 1.26)	−20.69(± 0.64)	−3.09	2.2
Vaal River below Vaalharts Weir	*Clarias gariepinus*	8	53.03(± 9.53)	0.89(± 0.36)	0.01(± 0.01)		15.65(± 0.37)	−20.85(± 1.51)		
	Lamproglena clariae (adult)					4.29(± 1.44)	16.91(± 0.00)	−19.45(± 0.00)	1.26	−1.4
	Lamproglena clariae (egg)						21.8(± 0.00)	−22.2(± 0.00)	4.89[a]	2.75[a]
	Tetracampos ciliotheca					1.50(± 0.50)	11.77(± 0.01)	−20.04(± 1.77)	−3.88	−0.81
	Proteocephalus glanduligerus					1.00(± 0.00)	12.14(± 0.00)	−18.38(± 0.00)	−3.51	−2.47
	Contracaecum sp. (larvae)					13.0(± 3.49)	12.66(± 0.48)	−20.64(± 1.35)	−2.99	−0.21
Douglas Weir	*Clarias gariepinus*	5	73.5(± 4.34)	2.67(± 0.68)	0.01(± 0.01)		15.71(± 0.38)	−24.57(± 0.28)		
	Tetracampos ciliotheca					4.67(± 2.73)	12.2(± 0.06)	−28.09(± 1.29)	−3.51	3.52
	Proteocephalus glanduligerus					3.00(± 0.00)	13.35(± 0.00)	−26.33(± 0.00)	−2.36	1.76
	Contracaecum sp. (larvae)					29.0(± 3.62)	11.41(± 1.07)	−22.47(± 1.45)	−4.3	−2.1

[a] Delta values for ^{15}N and ^{13}C calculated from difference in isotope signatures between eggs and adults of *L. clariae*.

understand the observed geographic differences in stable isotope composition in *C. gariepinus* hosts; host size, weight and condition factor were compared as a means of determining if dietary differences were a contributing factor. 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 (Kruskal-Wallis $H = 8.44$, df = 5, P = 0.134) or host sex (Mann-Whitney $U = 101$, P = 0.220). 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 δ^{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 length and weight, 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).

Parasite mean intensity was variable between the different sampling sites. 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. Intensity of parasites infecting *C. gariepinus* were compared with host length and weight, 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: $\tau = 0.297$, P = 0.019; weight: $\tau = 0.324$, P = 0.012).

Similarly to host fish, ^{15}N and ^{13}C were variably enriched and depleted in the parasite taxa collected in the present study and the stable isotope composition in parasites reflected those of the host fish along the Vaal River. Generally, endoparasites were depleted in ^{15}N and enriched in ^{13}C compared to *C. gariepinus* hosts. For cestodes, *T. ciliotheca* Δ^{15}N varied from 0.91‰ below the Grootdraai Dam to 9.8‰ at the site below the Vaal Barrage and in *P. glanduligerus* Δ^{15}N ranged from 2.36‰ to 3.51‰ at Douglas Weir and below Vaalharts Weir, respectively. *Contracaecum* sp. larvae collected below the Grootdraai Dam were enriched in ^{15}N 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‰. Differences in nitrogen and carbon stable isotopes between adult female copepods and hosts were not significant (δ^{15}N: Wilcoxon matched pair test, $Z = -0.314$, P = 0.754; δ^{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‰.

Geographic differences in ^{15}N enrichment in parasites were not significant 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). For *Contracaecum* sp. differences in ^{15}N were significant (Kruskal-Wallis $Z = 7.12$, df = 2, P = 0.028) among the sampling sites. In the case of *P. glanduligerus*, the low number of specimens collected (n = 2) hampered any statistical comparison. 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, geographic differences were only significant for *T. ciliotheca* (Kruskal-Wallis Z = 14.4, df = 5, P = 0.013). Differences among *L. clariae* adults, eggs and *Contracaecum* sp. larvae were not significant.

4. Discussion

In the present study distinct spatial-geographic differences in stable isotope fractionation patterns were observed for *C. gariepinus* and some parasites from the Vaal River. In host fish, highest ^{15}N 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 ^{13}C 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, behavioural differences of *C. gariepinus* and ecological differences at each sampling site along the Vaal River. *Clarias gariepinus* 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 (Skelton, 2001; Willoughby and Tweddle, 1978). Willoughby and Tweddle (1978) demonstrated through analysis of the stomach contents of *C. gariepinus* from Shire Valley, Malawi, were highly variable regardless of whether or not *C. gariepinus* were inhabiting the same and different environments. As stable carbon isotope ratios serve as a representation of the nutrient sources in food webs (Fry and Sherr, 1984), comparison of differences in the enrichment of ^{13}C isotope can be considered a good indicator of spatial variation in baseline isotope levels (Gómez-Díaz and González-Solís, 2010; Hobson, 2005). The variability of δ^{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. In addition to a highly diverse diet, *C. gariepinus* have been found to undergo a shift in diet as they grow (Kadye and Booth, 2012; Willoughby and Tweddle, 1978). The correlations observed in *C. gariepinus* stable isotope levels and host size (length and weight) support a shift in the foraging habits and food items consumed by this fish species with growth. In addition to dietary variation with growth, Kadye and Booth (2012) showed that 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. *Clarias gariepinus* serves as a host for a wide variety of endoparasite and ectoparasite taxa (Crafford and Avenant-Oldewage, 2009) and this could likely result from the variable diet and wide distribution of this fish species. Comparison between host size (length and weight) and parasite intensity indicate that larger fish harbour more parasites than smaller ones. This concept has been well documented in previous studies (see Patterson and Ruckstuhl, 2013).

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 ^{15}N stable isotope in cestodes and larval nematodes is in line with previous findings for other cestodes

and larval nematodes (Iken et al., 2001; Pinnegar et al., 2001) infecting fish hosts, and for some cestodes infecting rabbit hosts (Boag et al., 1998). 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 (Boag et al., 1998; McGrew et al., 2015; Navarro et al., 2014; Persson et al., 2007; Pinnegar et al., 2001; Power and Klein, 2004) 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 (Smyth and McManus, 1989). As a result of transamination of complex proteins, the ^{15}N stable isotope is retained in the tissues of the host (Macko et al., 1986) and as a result endoparasites are depleted in heavier isotopes.

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 (Deudero et al., 2002; Nachev et al., 2017). 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. (2016) 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. (2016) 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 or assimilating nutrients from the host. Additionally, Nachev et al. (2017) 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. Stable isotope fractionation observed in the present study indicated that endoparasite taxa infecting *C. gariepinus* occupy relatively similar trophic levels. These findings are comparable to other reports for similar host – parasite systems (Behrmann-Godel and Yohannes, 2015; Deudero et al., 2002; Power and Klein, 2004) and indicate that nutrients assimilated by the host and parasites are from similar sources (Power and Klein, 2004).

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 $\Delta^{15}N$ values 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 (Boag et al., 1998; Doucett et al., 1999; Schmidt et al., 2011; Sures et al., 2019; Voigt and Kelm, 2006). 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* (Deudero et al., 2002; Iken et al., 2001; Pinnegar et al., 2001). Iken et al. (2001) found that a copepod infecting gills of *Coryphaenoides armatus* was enriched by 2.7‰ in ^{15}N relative to the host. Pinnegar et al. (2001) 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 (Brooker et al., 2007). In an assessment of isotope fractionation between several copepods infecting the gills of their hosts, Deudero et al. (2002) 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. (2001) and Deudero et al. (2002) infected the gills and feed on the blood of the host fishes. However, Shotter (1971) 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 (Baud et al., 2004; Goedknegt et al., 2018; Gretsy and Quarmby, 1991). Gretsy and Qyarmby (1991) found that adult *Mytilicola intestinalis* were enriched by 3‰ relative to the intestine of European blue mussel host (*Mytilus edulis*). Goedknegt et al. (2018) 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. (2004) 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. An additional aspect to consider for parasitic copepods is the effect of changes in the life style of these parasites from juvenile stages to adults. 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.

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 and likely relates to differences in nutrient acquisition strategies by each parasite taxon analysed. Variability in the signatures reported between the different parasite taxa analysed are similar to other studies which found similar co-infections (Deudero et al., 2002; Iken et al., 2001; Pinnegar et al., 2001). Mature, adult female *L. clariae* consume whole blood and epithelial cells which they acquire from the secondary gill filaments of the host fish (Moll and Avenant-Oldewage, 2017; Tsotetsi et al., 2005). 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 (Moll and Avenant-Oldewage, 2017; Molnár et al., 2018; Tsotetsi et al., 2005). 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 blood meals (Deudero et al., 2002). 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.

In the present study, variable stable isotope fractionation patterns of

parasites infecting *C. gariepinus* hosts corresponded or mirrored geographic isotope enrichment of the host among the collection sites along the Vaal River. 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 (Caut et al., 2009; DeNiro and Epstein, 1981, 1978). As such variability in the diet of the host should be reflected more prominently in the stable isotope composition of endoparasites than in ectoparasites (Deudero et al., 2002). 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 in their endoparasites. Iken et al. (2001) 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. (2002) similarly noted that stomach nematodes did not reflect isotopic differences observed in host fishes. Sures et al. (2019) showed that in a monogenean – host fish system from the Vaal Dam, the micropredatory nature of *P. ichthyoxanthon* resulted in mirroring the 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 (Deudero et al., 2002; Gómez-Díaz and González-Solís, 2010). Geographic differences observed in stable isotope enrichment patterns of parasite taxa in the present study mirrored those of the host and are likely related to differences in food items utilised by the host fish as feeding strategies of the parasites do not change between the different sites. 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 $\delta^{13}C$ and $\delta^{15}N$ levels of organisms has previously been documented (Hobson, 2005). In parasites, Gómez-Díaz and González-Solís (2010) 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. (2015) for *Hyperia curticephala* infecting the scyphomedusa, *Chrysaora plocamia*. It should also be noted that unevenness of the distribution 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.

5. Conclusions

Results of the analysis of stable isotopes of carbon and nitrogen in *C. gariepinus* and ectoparasite and endoparasite taxa collected from the Vaal River are mostly consistent with patterns already described for similar host-parasite systems. 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 ^{15}N isotope compared to their host. These trends were in line with previous studies on similar parasite taxa. Geographic and spatial differences in ^{15}N and ^{13}C isotope levels in host fish and some parasite species reflect differences in baseline isotope signatures across the host distribution. The differences observed in stable isotopes of *C. gariepinus* tissue further reflected the omnivorous feeding biology of the host fish. In the case of the parasite taxa analysed, variation in ^{15}N and ^{13}C isotope levels mirrored those of the host fish at each site and related to the fact that parasites only derive nutrition from a single source, being the host. For specific parasite taxa, comparison between sites showed a degree of variation in stable isotope levels, which in turn may be related to variable resource partitioning in the hosts as well as difference relating to the feeding biology of the host fish. Unlike their hosts, parasites may reflect more accurately baseline isotope levels for specific sampling sites given that they acquire all nutrition from a single host organism. Along with a shorter lifespan than the host, large changes in isotope levels would likely not be reflected in parasites unlike host fish which could move large distances between sites.

Acknowledgements

The authors would like to acknowledge the University of Johannesburg for funding (SASOLD-0107 awarded to AAO) for the collection of samples from the Vaal Dam and providing infrastructure for the preparation of samples for analysis. The Burroughs–Wellcome Trust is thanked for providing 50% of funding for travel to the University of Duisburg-Essen by BMG. AAO research trust fund for the remaining 50%. Gregg van Rensburg is thanked for his assistance in collecting samples in the field.

References

Baud, A., Cuoc, C., Grey, J., Chappaz, R., Alekseev, V., 2004. Seasonal variability in the gut ultrastructure of the parasitic copepod *Neoergasilus japonicus* (Copepoda, Poecilostomatoida). Can. J. Zool. 82, 1655–1666. https://doi.org/10.1139/z04-149.
Behrmann-Godel, J., Yohannes, E., 2015. Multiple isotope analyses of the pike tapeworm *Triaenophorus nodulosus* reveal peculiarities in consumer-diet discrimination patterns. J. Helminthol. 89, 238–243. https://doi.org/10.1017/S0022149X13000849.
Boag, B., Neilson, R., Robinson, D., Scrimgeour, C.M., Handley, L.L., 1998. Wild rabbit host and some parasites show trophic-level relationships for $\delta^{13}C$ and $\delta^{15}N$: a first report. Isot. Environ. Health Stud. 34, 81–85. https://doi.org/10.1080/10256019708036335.
Braune, E., Rogers, K.H., 1987. The Vaal River Catchment: Problems and Research Needs, A Report of the Committee for Inland Water Ecosystems. National Programme for Ecosystems Research, Pretoria.
Brooker, A.J., Shinn, A.P., Bron, J.E., 2007. A Review of the biology of the parasitic copepod *Lernaeocera branchialis* (L., 1767) (Copepoda: pennellidae). Adv. Parasitol. 65, 297–341. https://doi.org/10.1016/S0065-308X(07)65005-2.
Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: margolis et al. revisited. J. Parasitol. 83, 575–583. https://doi.org/10.2307/3284227.
Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453. https://doi.org/10.1111/j.1365-2664.2009.01620.x.
Crafford, D., Avenant-Oldewage, A., 2009. Application of a fish health assessment index and associated parasite index to *Clarias gariepinus* (Teleostei: clariidae) in the Vaal River system, South Africa. Afr. J. Aquat. Sci. 34, 261–272. https://doi.org/10.2989/AJAS.2009.34.3.8.984.
Demopoulos, A.W.J., Sikkel, P.C., 2015. Enhanced understanding of ectoparasite-host trophic linkages on coral reefs through stable isotope analysis. Int. J. Parasitol. Parasites Wildl. 4, 125–134. https://doi.org/10.1016/j.ijppaw.2015.01.002.
DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochem. Cosmochim. Acta 45, 341–351. https://doi.org/10.1016/0016-7037(81)90244-1.
DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochem. Cosmochim. Acta 42, 495–506.
Deudero, S., Pinnegar, J.K., Polunin, N.V.C., 2002. Insights into fish host-parasite trophic relationships revealed by stable isotope analysis. Dis. Aquat. Org. 52, 77–86. https://doi.org/10.3354/dao052077.
Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: how many parasites? How many hosts? Proc. Natl. Acad. Sci. U.S.A. 105, 11482–11489. https://doi.org/10.1073/pnas.0803232105.
Doi, H., Yurlova, N.I., Vodyanitskaya, S.N., Kanaya, G., Shikano, S., Kikuchi, E., 2010. Estimating isotope fractionation between cercariae and host snail with the use of isotope measurement designed for very small organisms. J. Parasitol. 96, 314–317. https://doi.org/10.1645/ge-2245.1.
Doucett, R.R., Giberson, D.J., Power, G., 1999. Parasitic association of *nanocladius* (Diptera: chironomidae) and *Pteronarcys biloba* (plecoptera: pteronarcyidae): insights from stable-isotope analysis. North Am. Benthol. Soc. 18, 514–523.
Dubois, S.Y., Savoye, N., Sauriau, P.G., Billy, I., Martinez, P., de Montaudouin, X., 2009. Digenean trematodes-marine mollusc relationships: a stable isotope study. Dis. Aquat. Org. 84, 65–77. https://doi.org/10.3354/dao2022.
Fry, B., Sherr, E.B., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. 27, 13–47.
Gilbert, B.M., Nachev, M., Jochmann, A., Schmidt, T.C., Köster, D., Sures, B., Avenant-Oldewage, A., 2020. You are how you eat: differences in trophic position of two parasite species infecting a single host according to stable isotopes. Parasitol. Res. https://doi.org/10.1007/s00436-020-06619-1.

Goater, T.M., Goater, C.P., Esch, G.W., 2014. Parasitism: the Diversity and Ecology of Animal Parasites, Second. Cambridge University Press, New York.

Goedknegt, M.A., Shoesmith, D., Jung, A.S., Luttikhuizen, P.C., van der Meer, J., Philippart, C.J.M., van der Veer, H.W., Thieltges, D.W., 2018. Trophic relationship between the invasive parasitic copepod *Mytilicola orientalis* and its native blue mussel (*Mytilus edulis*) host. Parasitology 145, 814–821. https://doi.org/10.1017/S0031182017001779.

Gómez-Díaz, E., González-Solís, J., 2010. Trophic structure in a seabird host-parasite food web: insights from stable isotope analyses. PloS One 5, e10454. https://doi.org/10.1371/journal.pone.0010454.

Gretsy, K.A., Quarmby, C., 1991. The trophic level of *Mytilicola intestinalis* Steuer (Copepoda: poecilostomatoida) in *Mytilus edulis* L., as determined from stable isotope analysis. Bull. Plankton Soc. Jpn. 363–371.

Heincke, F., 1908. Bericht über die Untersuchungen der Biologischen Anstalt auf Helgoland zur Naturgeschichte der Nutzfische. Die Beteiligung Deutschlands an der Int. Meeresforsch. 4/5, 67–155.

Hobson, K.A., 2005. Using stable isotopes to trace long-distance dispersal in birds and other taxa. Divers. Distrib. 11, 157–164. https://doi.org/10.1111/j.1366-9516.2005.00149.x.

Iken, K., Brey, T., Wand, U., Voigt, J., Junghans, P., 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog. Oceanogr. 50, 383–405. https://doi.org/10.1016/S0079-6611(01)00062-3.

Kadye, W.T., Booth, A.J., 2012. Integrating stomach content and stable isotope analyses to elucidate the feeding habits of non-native sharptooth catfish *Clarias gariepinus*. Biol. Invasions 14, 779–795. https://doi.org/10.1007/s10530-011-0116-6.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1–27. https://doi.org/10.1139/cjz-78-1-1.

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E., Lafferty, K.D., 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature 454, 515–518. https://doi.org/10.1038/nature06970.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R., Thieltges, D.W., 2008. Parasites in food webs: the ultimate missing links. Ecol. Lett. 11, 533–546. https://doi.org/10.1111/j.1461-0248.2008.01174.x.

Macko, S.A., Fogel Estep, M.L., Engel, M.H., Hare, P.E., 1986. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochem. Cosmochim. Acta 50, 2143–2146.

Marcogliese, D.J., 2003. Food webs and biodiversity: are parasites the missing link? J. Parasitol. 89, S106–S113.

Marcogliese, D.J., Cone, D.K., 1997. Food webs: a plea for parasites. Trends Ecol. Evol. 12, 320–325. https://doi.org/10.1016/S0169-5347(97)01080-X.

McGrew, A.K., O'Hara, T.M., Stricker, C.A., Castellini, J.M., Beckmen, K.B., Salman, M.D., Ballweber, L.R., 2015. Ecotoxicoparasitology: understanding mercury concentrations in gut contents, intestinal helminths and host tissues of Alaskan gray wolves (*Canis lupus*). Sci. Total Environ. 536, 866–871. https://doi.org/10.1016/j.scitotenv.2015.07.106.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of δ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochem. Cosmochim. Acta 48, 1135–1140.

Moll, J., Avenant-Oldewage, A., 2017. Morphology of the digestive system of *Lamproglena clariae* Fryer, 1956 (Crustacea: Copepoda), a gill parasite of African catfish *Clarias gariepinus* (Burchell, 1822). Invertebr. Zool. 14, 45–52. https://doi.org/10.15298/invertzool.14.1.07.

Molnár, K., Avenant-Oldewage, A., Sellyei, B., Varga, A., Székely, C., 2018. Histopathological changes on the gills of asp (*Aspius aspius*) and European catfish (*Silurus glanis*) caused by *Lamproglena pulchella* and a *Lamproglena* sp. (Copepoda: lernaeidae), respectively. J. Fish. Dis. 41, 33–39. https://doi.org/10.1111/jfd.12667.

Moravec, F., Jansen van Rensburg, C., Van As, L.L., 2016. Larvae of *Contracaecum* sp. (nematoda: anisakidae) in the threatened freshwater fish *Sandelia capensis* (anabantidae) in South Africa. Dis. Aquat. Org. 120, 251–254. https://doi.org/10.3354/dao03033.

Nachev, M., Jochmann, M.A., Walter, F., Wolbert, J.B., Schulte, S.M., Schmidt, T.C., Sures, B., 2017. Understanding trophic interactions in host-parasite associations using stable isotopes of carbon and nitrogen. Parasites Vectors 10, 90. https://doi.org/10.1186/s13071-017-2030-y.

Navarro, J., Albo-Puigserver, M., Coll, M., Saez, R., Forero, M.G., Kutcha, R., 2014. Isotopic discrimination of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) in a host-specific holocephalan tapeworm. J. Helminthol. 88, 371–375. https://doi.org/10.1017/S0022149X13000126.

Neilson, R., Boag, B., Hartley, G., 2005. Temporal host-parasite relationships of the wild rabbit, *Oryctolagus cuniculus* (L.) as revealed by stable isotope analyses. Parasitology 131, 279–285. https://doi.org/10.1017/S0031182005007717.

O'Grady, S.P., Dearing, M.D., 2006. Isotopic insight into host-endosymbiont relationships in Liolaemid lizards. Oecologia 150, 355–361. https://doi.org/10.1007/s00442-006-0487-z.

Patterson, J.E.H., Ruckstuhl, K.E., 2013. Parasite infection and host group size: a meta-analytical review. Parasitology 140, 803–813. https://doi.org/10.1017/S0031182012002259.

Persson, M.E., Larsson, P., Stenroth, P., 2007. Fractionation of δ^{15}N and δ^{13}C for Atlantic salmon and its intestinal cestode *Eubothrium crassum*. J. Fish. Biol. 71, 441–452. https://doi.org/10.1111/j.1095-8649.2007.01500.x.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Systemat. 18, 293–320.

Pinnegar, J.K., Campbell, N., Polunin, N.V.C., 2001. Unusual stable isotope fractionation patterns observed for fish host–parasite trophic relationships. J. Fish. Biol. 59, 494–503. https://doi.org/10.1006/jfbi.2001.1660.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Poulin, R., 2010. Network analysis shining light on parasite ecology and diversity. Trends Parasitol. 26, 492–498. https://doi.org/10.1016/j.pt.2010.05.008.

Power, M., Klein, G.M., 2004. Fish host-cestode parasite stable isotope enrichment patterns in marine, estuarine and freshwater fishes from northern Canada. Isot. Environ. Health Stud. 40, 257–266. https://doi.org/10.1080/10256010410001678062.

Riascos, J.M., Docmac, F., Reddin, C., Harrod, C., 2015. Trophic relationships between the large scyphomedusa *Chrysaora plocamia* and the parasitic amphipod *Hyperia curticephala*. Mar. Biol. 162, 1841–1848. https://doi.org/10.1007/s00227-015-2716-7.

Sabadel, A.J.M., Stumbo, A.D., MacLeod, C.D., 2019. Stable-isotope analysis: a neglected tool for placing parasites in food webs. J. Helminthol. 93, 1–7. https://doi.org/10.1017/S0022149X17001201.

Schmidt, O., Dautel, H., Newton, J., Gray, J.S., 2011. Natural isotope signatures of host blood are replicated in moulted ticks. Ticks Tick. Borne. Dis. 2, 225–227. https://doi.org/10.1016/j.ttbdis.2011.09.006.

Selbach, C., Soldánová, M., Feld, C.K., Kostadinova, A., Sures, B., 2020. Hidden parasite diversity in a European freshwater system. Sci. Rep. 10, 2694. https://doi.org/10.1038/s41598-020-59548-5.

Shotter, R.A., 1971. The biology of *Clavella uncinata* (muller) (Crustacea: Copepoda). Parasitology 63, 419–430.

Skelton, P., 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Struiker, Cape Town.

Smyth, J.D., McManus, D.P., 1989. The Physiology and Biochemistry of Cestodes. Cambridge University Press, Cambridge, UK.

Sures, B., Nachev, M., Gilbert, B.M., Dos Santos, Q.M., Jochmann, M.A., Köster, D., Schmidt, T.C., Avenant-Oldewage, A., 2019. The monogenean *Paradiplozoon ichthyoxanthon* behaves like a micropredator on two of its hosts, as indicated by stable isotopes. J. Helminthol. 93, 71–75.

Thompson, R.M., Mouritsen, K.N., Poulin, R., 2005. Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. J. Anim. Ecol. 74, 77–85. https://doi.org/10.1111/j.1365-2656.2004.00899.x.

Tsotetsi, A.M., Avenant-Oldewage, A., Mashego, S.N., 2005. Aspects of the pathology of *Lamproglena clariae* (Copepoda: lernaeidae) on gills of *Clarias gariepinus* from the Vaal River system, South Africa. Afr. Zool. 40, 169–178. https://doi.org/10.1080/15627020.2005.11407316.

Vander Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. Can. J. Fish. Aquat. Sci. 54, 1142–1158.

Voigt, C.C., Kelm, D.H., 2006. Host preference of the common vampire bat (*Desmodus rotundus*; Chiroptera) assessed by stable isotopes. J. Mammal. 87, 1–6. https://doi.org/10.1644/05-MAMM-F-276R1.1.

Wada, E., 2009. Stable δ^{15}N and δ^{13}C isotope ratios in aquatic ecosystems. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 85, 98–107. https://doi.org/10.2183/pjab/85.98.

Willoughby, N.G., Tweddle, D., 1978. The ecology of the catfish *Clarias gariepinus* and *Clarias ngamensis* in the Shire Valley, Malawi. J. Zool. 186, 507–534. https://doi.org/10.1111/j.1469-7998.1978.tb03936.x.

Xu, J., Zhang, M., Xie, P., 2007. Trophic relationship between the parasitic isopod *Ichthyoxenus japonensis* and the fish *Carassius auratus* as revealed by stable isotopes. J. Freshw. Ecol. 22, 333–338. https://doi.org/10.1080/02705060.2007.9665055.

Zhang, L., Vanderhorst, K., Kyser, K., Campbell, L., 2018. Diet assimilation trends and host-parasite relationships in two species of sunfish (*Lepomis*) revealed by stable isotope analyses of multiple tissues. Parasitol. Res. 117, 1043–1049. https://doi.org/10.1007/s00436-018-5781-2.