Journal of Helminthology

cambridge.org/jhl

The monogenean *Paradiplozoon ichthyoxanthon* behaves like a micropredator on two of its hosts, as indicated by stable isotopes

B. Sures[1,2,3], M. Nachev[1,2], B.M. Gilbert[3], Q.M. Dos Santos[3], M.A. Jochmann[2,4], D. Köster[4], T.C. Schmidt[2,4] and A. Avenant-Oldewage[3]

[1]Aquatic Ecology, University of Duisburg-Essen, Universitätsstr. 5, 45141 Essen, Germany; [2]Centre for Water and Environmental Research, University of Duisburg-Essen, Universitätsstr. 5, 45141 Essen, Germany; [3]Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park 2006, Johannesburg, South Africa and [4]Instrumental Analytical Chemistry, University of Duisburg-Essen, Universitätsstr. 5, 45141 Essen, Germany

Research Paper

Cite this article: Sures B, Nachev M, Gilbert BM, Dos Santos QM, Jochmann MA, Köster D, Schmidt TC, Avenant-Oldewage A (2019). The monogenean *Paradiplozoon ichthyoxanthon* behaves like a micropredator on two of its hosts, as indicated by stable isotopes. *Journal of Helminthology* **93**, 71–75. https://doi.org/10.1017/S0022149X17001195

Received: 27 October 2017
Accepted: 30 November 2017
First published online: 22 May 2018

Author for correspondence:
B. Sures, E-mail: bernd.sures@uni-due.de

Abstract

The analysis of stable isotopes of carbon and nitrogen has been used as a fingerprint for understanding the trophic interactions of organisms. Most of these studies have been applied to free-living organisms, while parasites have largely been neglected. Studies dealing with parasites so far have assessed the carbon and nitrogen signatures in endoparasites or ectoparasites of different hosts, without showing general trends concerning the nutritional relationships within host–parasite associations. Moreover, in most cases such systems involved a single host and parasite species. The present study is therefore the first to detail the trophic interactions of a freshwater monogenean–host model using $\delta^{13}C$ and $\delta^{15}N$, where a single monogenean species infects two distinctly different hosts. Host fishes, *Labeobarbus aeneus* and *Labeobarbus kimberleyensis* from the Vaal Dam, South Africa, were assessed for the monogenean parasite *Paradiplozoon ichthyoxanthon*, individuals of which were removed from the gills of the hosts. The parasites and host muscle samples were analysed for signatures of $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyser connected to an isotope ratio mass spectrometer. Host fish appear to use partly different food sources, with *L. aeneus* having slightly elevated $\delta^{13}C$ signatures compared to *L. kimberleyensis*, and showed only small differences with regard to their nitrogen signatures, suggesting that both species range on the same trophic level. Carbon and nitrogen signatures in *P. ichthyoxanthon* showed that the parasites mirrored the small differences in dietary carbon sources of the host but, according to $\delta^{15}N$ signatures, the parasite ranged on a higher trophic level than the hosts. This relationship resembles predator–prey relationships and therefore suggests that *P. ichthyoxanthon* might act as a micropredator, similar to blood-sucking arthropods such as mites and fleas.

Introduction

Stable-isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are increasingly used to study food-web architecture. In ecology, the isotopic discrimination values of $\delta^{13}C$ and $\delta^{15}N$ are applied as unique fingerprints, which allow for a determination of food sources and trophic interactions between organisms, respectively (Fry, 2006; Wada, 2009). For example, investigations of stable isotopes of nitrogen have shown that consumers are enriched in the heavy nitrogen isotope (^{15}N) with an average $\Delta\delta^{15}N$ of 3.4‰ per trophic level, and therefore it can be used to determine the position of an organism within food webs (Minagawa & Wada, 1984; Vander Zanden *et al.*, 1997). Carbon stable-isotope composition can deliver information about the food source, such as insect larvae, molluscs, algae, vegetation and detritus (Post *et al.*, 2000). Accordingly, application of stable-isotope analyses (SIA) might also be helpful to elucidate trophic relationships between a parasite and its associated host (Sabadel *et al.*, 2016; Nachev *et al.*, 2017; Yohannes *et al.*, 2017).

The number of studies dealing with the stable-isotope composition of carbon and nitrogen in hosts and their parasites remains scarce, with investigations on some of the major parasite taxa, such as monogeneans, even being absent. So far, SIA of host–parasite associations has shown different patterns. Endoparasite species, such as cestodes and acanthocephalans, were found to be depleted in the heavier nitrogen isotope in comparison to the tissues of their hosts, instead of being enriched as expected for predators (Boag *et al.*, 1998; Ikken *et al.*, 2001; Pinnegar *et al.*, 2001; Deudero *et al.*, 2002; Power & Klein, 2004; Persson *et al.*, 2007; Behrmann-Godel & Yohannes, 2013; Navarro *et al.*, 2014; Nachev *et al.*, 2017). Also, studies on digeneans (sporocysts) provided similar results (Dubois *et al.*, 2009; Doi *et al.*, 2010). In contrast, ectoparasites, such as

CAMBRIDGE UNIVERSITY PRESS

different parasitic arthropods (Boag *et al.*, 1998; Deucett *et al.*, 1999; Voigt & Kelm, 2006; Schmidt *et al.*, 2011), were ^{15}N-enriched, similar to predators that feed on their prey.

As no information is available on stable-isotope signatures in monogeneans, the aim of the present study was to provide the first data on stable-isotope ratios of carbon and nitrogen in a monogenean with respect to its fish host. As a host–parasite system, the parasite *Paradiplozoon ichthyoxanthon* Avenant-Oldewage (in Avenant-Oldewage *et al.*, 2014) was selected as it occurs on the gills of two different fish species: the largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist & Thompson, 1913) and the smallmouth yellowfish, *Labeobarbus aeneus* (Burchell, 1822). The fish species differ in their habitat preferences and diet but both occur in the Orange-Vaal River system in South Africa. The largemouth yellowfish prefers flowing water but may also occur in dams. It is a predatory species, initially feeding on insects and small crustaceans but becoming piscivorous above a fork length of 300 mm (Skelton, 2001), and it can even prey on small *L. aeneus*. The smallmouth yellowfish frequents clear, flowing water with sandy and rocky river beds. Larvae feed on microscopic organisms but larger fish are omnivorous and may feed on benthic invertebrates, including bivalve molluscs, algae, vegetation and detritus (Skelton, 2001). Using these host–parasite systems, we provide the first information about the trophic interaction between a monogenean and its hosts. Moreover, as the parasite species occurs on hosts having different feeding regimes, the study attempted to understand whether the difference in biology of the fish host is reflected in the stable-isotope signatures of the hosts and eventually influences the stable-isotope composition in the monogenean.

Materials and methods

Fish and parasite sampling

During a single survey of the Vaal Dam, South Africa, in summer (January 2016), *L. aeneus* specimens ($n = 7$) were collected by means of gill nets (mesh size: 45–190 mm) around UJ Island (26°52′33.62″S; 28°10′25.76″E) (fig. 1). Incidentally, *L. kimberleyensis* ($n = 7$) that died in the nets were removed from the nets and afterwards used for sampling, whereas live specimens were released in accordance with permits from the Gauteng Department of Agriculture for collection of fish. Live and dead fish were transported back to a field laboratory on the island, and live fish were maintained in aerated plastic containers containing dam water. Thereafter, fish were euthanized by severing the spinal cord posterior to the head. The weight and the total length of each fish were determined and Fulton's fish condition factor (K) was calculated to estimate differences in nutritional status between individuals or species of fish, according to Heincke (1908) (see also Nash *et al.*, 2006):

$$K = 100 \times \mathrm{SW}/\mathrm{TL}^3$$

where SW = fish weight and TL = fish total length. Subsequently, the gills were excised and assessed for *P. ichthyoxanthon*. The parasites were removed and frozen (−20°C) before being returned to the laboratory.

Stable-isotope analyses

For SIA, seven specimens of *L. aeneus* and four specimens of *L. kimberleyensis* were considered, as only these fish harboured sufficient parasite material for analyses. Furthermore, low parasite intensities on *L. kimberleyensis* necessitated pooling of monogeneans to a total of two samples. Muscle tissue from infected fishes was removed during dissection and frozen at −20°C. Thereafter, muscle samples and parasites were freeze dried to weight consistency at −77°C under negative pressure (−80 kPa). For SIA, triplicates of each sample in the range of 200–700 µg (dry weight; DW) were weighed in 4 × 6 mm tin-foil capsules for solids (IVA Analysentechnik, Meerbusch, Germany). Samples were analysed using a vario PYRO Cube elemental analyser (EA) system (Elementar Analysensysteme, Langenselbold, Germany) in C/N mode. The EA was coupled to an IsoPrime 100 isotope ratio mass spectrometer (IRMS; Elementar Analysensysteme). The EA-IRMS results were obtained following the principle of identical treatment and the experimental procedures described by Werner & Brand (2001) and Nachev *et al.* (2017). All isotope ratios were reported in the δ-notation as differences of the isotope ratio of the sample and isotope ratio of an international reference substance by equation (1).

$$\delta^h \mathrm{E}_{s,ref} = \frac{R\left({}^h\mathrm{E}/{}^l\mathrm{E}\right)_s}{R\left({}^h\mathrm{E}/{}^l\mathrm{E}\right)_{ref}} - 1 \quad (1)$$

where $R({}^h\mathrm{E}/{}^l\mathrm{E})_{ref}$ denotes the ratio of the heavy and light isotope (here ^{13}C/^{12}C as well as ^{15}N/^{14}N) in the reference material, and $R({}^h\mathrm{E}/{}^l\mathrm{E})_s$ the ratio in the sample. As reference materials for normalization of the laboratory working standard, acetanilide, to the international scale, the USGS40 and USGS41 reference materials were used.

Statistical analyses

In order to calculate the trophic level difference (ΔTL) between the monogeneans and their hosts, the following equation was applied:

$$(\Delta\mathrm{TL}) = \delta^{15}\mathrm{N}_{\mathrm{parasite}} - \delta^{15}\mathrm{N}_{\mathrm{host}})/\mathrm{TEF} \quad (2)$$

with TEF, the trophic enrichment factor, ranging between 1.3 and 5.3‰.

The Spearman rank correlation was applied to evaluate a possible relationship between the isotopic composition and fish morphometry (length, weight, condition factor). The Wilcoxon matched pair test was used for comparisons between the isotope signatures of host tissues and parasites.

Results

The monogenean was enriched by approximately 2‰ in the heavier nitrogen isotope with respect to both fish hosts, suggesting that the monogeneans range on a 0.4–1.8 higher trophic level than the host (based on equation 2; see table 1 and fig. 2). Statistical analyses performed for the *L. aeneus–P. ichthyoxanthon* system ($n = 7$) revealed significantly higher nitrogen signatures in parasites (Wilcoxon matched pair test, $Z = 2.366$; $P < 0.05$). Due to the low number of *P. ichthyoxanthon* samples ($n = 2$), no statistical analysis could be performed for the *L. kimberleyensis–P. ichthyoxanthon* system. The carbon signatures of both parasite and host tissues showed similar values, as can be expected for a parasite that feeds on its host tissues. The low differences observed in the carbon signatures suggest that the fish species use largely overlapping food sources, with only small nutritional variations. These

https://doi.org/10.1017/S0022149X17001195 Published online by Cambridge University Press

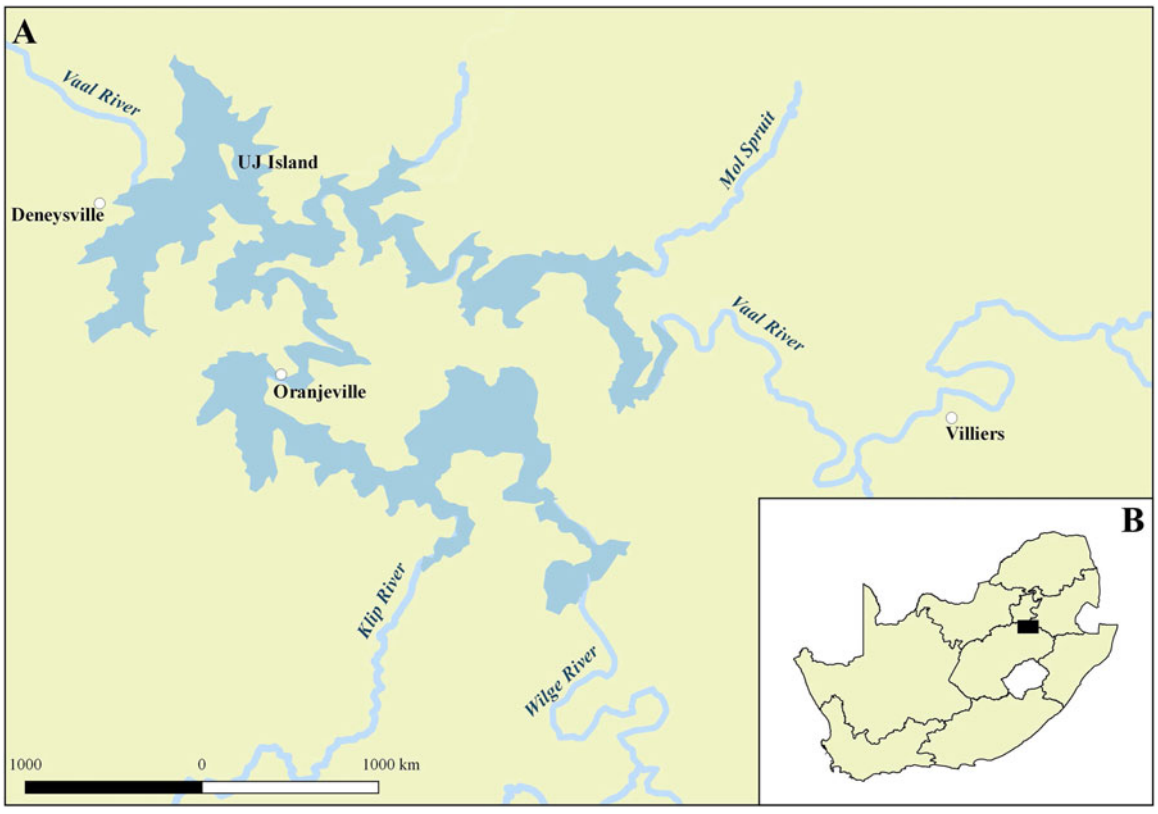

Fig. 1. Map of the Vaal Dam and feeder rivers, showing the position of UJ Island (A) within the map of South Africa (B) The black block in (B) indicates the location of (A).

trends are mirrored in the stable-isotope signatures of the monogeneans collected from the respective fish species. No significant relationships between the stable-isotope composition of the fish host and monogeneans, nor between the fish morphometric parameters and isotope composition of parasites, were found.

Discussion

In the present study, signatures of stable isotopes for carbon and nitrogen for a monogenean–host system are presented for the first time. Regarding nitrogen, the monogenean *P. ichthyoxanthon* was enriched by approximately 2‰ with respect to both of its host species. This difference corresponds to an average isotope fractionation of one trophic level, as consumers are $\delta^{15}N$ enriched in the range from 1.3 to 5.3‰ with respect to their diet (Minagawa & Wada, 1984). Other flatworms, such as adult cestodes, have shown contrasting patterns, in being depleted in the heavier nitrogen isotope compared to the host (Boag *et al.*, 1998; Ikken *et al.*, 2001; Pinnegar *et al.*, 2001; Deudero *et al.*, 2002; Power & Klein, 2004; Persson *et al.*, 2007; Behrmann-Godel & Yohannes, 2013; Navarro *et al.*, 2014; McGrew *et al.*, 2015). However, studies on adult digeneans and other monogeneans are currently lacking and therefore it is not possible to provide any pattern for the Platyhelminthes. A reasonable explanation for the different stable-isotope signatures between *P. ichthyoxanthon* and its host species could be its nutritional mode. Diplozoids feed on blood, similar to a predator feeding on its prey. Interestingly, other parasite taxa, such as nematodes, that feed on host tissues are also enriched in the heavier nitrogen isotope (Boag *et al.*, 1998; Neilson *et al.*, 2005; O'Grady & Dearing, 2006; Nachev *et al.*, 2017). More specifically, our results corroborate isotope fractionation in other blood-

Table 1. Data on fish morphometry and stable-isotope composition of carbon and nitrogen in selected host–parasite systems.

	TL (cm)	*W* (g)	*K*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
L. aeneus	38.3 (±3.5)	587.2 (±164.0)	1.1 (±0.1)	−21.10 (±0.32)	16.06 (±0.45)
P. ichthyoxanthon	–	–	–	−21.19 (±0.46)	18.11 (±0.44)
$\Delta^{h}E$ (host–parasite)	–	–	–	−0.09	−2.05
L. kimberleyensis	35.8 (±6.7)	432.5 (±204.6)	1.0 (±0.2)	−20.61 (±0.28)	16.42 (±0.32)
P. ichthyoxanthon	–	–	–	−20.83 (±0.35)	18.73 (±0.18)
$\Delta^{h}E$ (host–parasite)	–	–	–	−0.22	−2.31

TL, total length; *W*, weight; *K*, Fulton's condition factor.

https://doi.org/10.1017/S0022149X17001195 Published online by Cambridge University Press

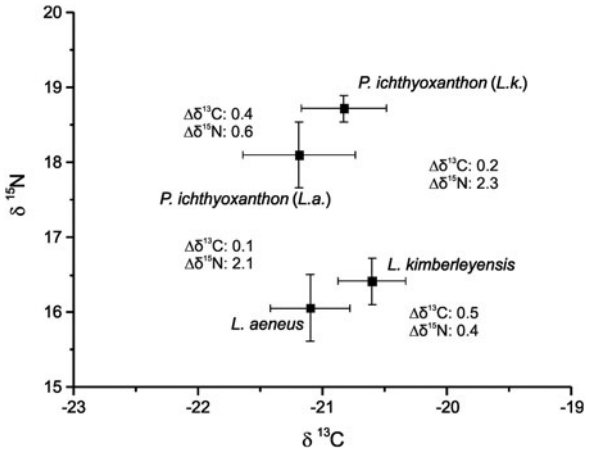

Fig. 2. Means and standard deviations of $\delta^{13}C$ vs. $\delta^{15}N$ for muscle tissue of selected fish hosts (*L. aeneus* and *L. kimberleyensis*) and their monogenean parasite *P. ichthyoxanthon*.

sucking ectoparasites, such as parasitic insects and ticks (Boag *et al.*, 1998; Voigt & Kelm, 2006; Schmidt *et al.*, 2011), where the parasites act as micropredators. The active feeding mode combined with the ectoparasitic lifestyle determines the isotopic fractionation of monogeneans and other parasites with a similar nutrition strategy. In contrast, parasites such as cestodes and acanthocephalans have no digestive system and synthesize no digestive enzymes. They are unable to catabolize macromolecules, such as lipids and proteins, or to synthesize several macromolecules, such as fatty acids, nucleic acids, amino acids and many others. They therefore rely entirely on an appropriate supply from their hosts (Barrett, 1981). Accordingly, they assimilate metabolically reprocessed molecules derived from the host, which are depleted in ^{15}N and therefore show opposite patterns to those found for monogeneans, which feed directly on their hosts. Regarding nutritional relationships, monogeneans therefore have more similarities to ectoparasitic arthropods than to other endoparasitic flatworms.

Comparing the fish species, the difference in carbon isotope signatures was rather low, with a $\Delta\delta^{13}C$ of 0.5‰. This corroborates the suggestion that the diet of the species largely overlaps, with probably a small preference for an omnivorous feeding strategy in *L. aeneus* versus a predatory lifestyle in *L. kimberleyensis* (Skelton, 2001). However, these principal nutritional differences do not lead to differences in the trophic level of the fishes, as their $\Delta\delta^{15}N$ was found to be 0.4‰. This probably indicates that in both fish species a large proportion of the diet consists of macroinvertebrates. Given their different habitat preferences the specific macroinvertebrate species they prey on are likely to be different. Moreover, *L. aeneus* also feeds on detritus and algae, whereas the diet of *L. kimberleyensis* includes fish, which may occasionally be *L. aeneus*. The partly overlapping diet might be due to the size of the fish. The piscivorous mode of nutrition develops fully in *L. kimberleyensis* when it grows bigger than 300 mm (Skelton, 2001). Given the fact that the average size of *L. kimberleyensis* was even smaller than the average size of *L. aeneus* in our study indicates that piscivority in *L. kimberleyensis* was not fully developed.

The difference between the fish species can be explained by differences in their diet and habitat preferences. Although the difference is rather small, it seems to be sufficient to influence the carbon stable-isotope composition of the parasite, as the carbon signatures of the parasites mirror those of their fish hosts. This can be explained by the micropredatory way of feeding of *P. ichthyoxanthon*. When fish feed on different food sources the associated differences in carbon isotopes will be present in the blood that carries nutrients. As the monogeneans feed on their host's blood, they are therefore provided with these differences in carbon isotopes. Given the overlap in the hosts' diet, the resulting difference concerning the nitrogen isotopes is $\Delta\delta^{15}N$ of 0.6‰, which is higher than that of the host species. This specifically stresses the fact that the same parasite species may display different stable-isotope signatures depending on its particular host species. Therefore, the trophic position of a parasite with a broad species specificity may change, depending on the trophic position of its hosts. Although most monogeneans are known to be strictly species specific (Rohde, 1993), diplozoons display varying stenospecificity (Le Brun *et al.*, 1988), with *P. ichthyoxanthon* occurring in two species of the genus *Labeobarbus* (Avenant-Oldewage & Milne, 2014).

In conclusion, our data clearly show that the stable-isotope signatures of micropredatory parasites mirror the feeding strategy of their hosts. Care should be taken in future studies to consider the feeding regime of the hosts when interpreting stable-isotope data. This may even refer to a host species changing diet due to migration, seasonal changes or availability of food.

Financial support. Our thanks are due the National Research Foundation South Africa and UJ Faculty Research Committee for funding the fieldwork (NRF grant no. 80789).

Conflict of interest. None.

Ethical standards. The capture of fish was in accordance with permits from the Gauteng Department of Agriculture for collection of fish (permit number: CPE3000123). Slaughter of fish was in accordance with guidelines set out by the South African National Animal Ethics Council and approval by the University of Johannesburg Ethics committee (Protocol number: 9 April 2013).

References

Avenant-Oldewage A and Milne SJ (2014) Aspects of the morphology of the juvenile life stages of *Paradiplozoon ichthyoxanthon* Avenant-Oldewage, 2013 (Monogenea: Diplozoidae). *Acta Parasitologica* **59**, 247–254.

Avenant-Oldewage A, Le Roux LE, Mashego SN and Jansen Van Vuuren B (2014) *Paradiplozoon ichthyoxanthon* n. sp. (Monogenea: Diplozoidae) from *Labeobarbus aeneus* (Cyprinidae) in the Vaal River, South Africa. *Journal of Helminthology* **88**, 166–172.

Barrett J (1981) *Biochemistry of parasitic helminths*. London, Macmillan.

Behrmann-Godel J and Yohannes E (2013) Multiple isotope analyses of the pike tapeworm *Triaenophorus nadulosus* reveal peculiarities in consumer-diet discrimination patterns. *Journal of Helminthology* **89**, 238–243.

Boag B, Neilson R, Robinson D, Scrimgeour CM and Handley LL (1998) Wild rabbit host and some parasites show trophic-level relationships for $\delta^{13}C$ and $\delta^{15}N$: A first report. *Isotopes in Environmental and Health Studies* **34**, 81–85.

Deudero S, Pinnegar JK and Polunin NVC (2002) Insights into fish host–parasite trophic relationships revealed by stable isotope analysis. *Diseases of Aquatic Organisms* **52**, 77–86.

Doi H, Yurlova NI, Vodyanitskaya SN, Kanaya G, Shikano S and Kikuchi E (2010) Estimating isotope fractionation between cercariae and host snail with the use of isotope measurement designed for very small organisms. *Journal of Parasitology* **96**, 314–317.

Doucett RR, Giberson DJ and Power G (1999) Parasitic association of *Nanocladius* (Diptera: Chironomidae) and *Pteronarcys biloba* (Plecoptera: Pteronarcyidae): insights from stable-isotope analysis. *Journal of the North American Benthological Society* **18**, 514–523.

https://doi.org/10.1017/S0022149X17001195 Published online by Cambridge University Press

Dubois SY, Savoye N, Sauriau PG, Billy I, Martinez P and de Montaudouin X (2009) Digenean trematodes–marine mollusc relationships: a stable isotope study. *Diseases of Aquatic Organisms* **84**, 65–77.

Fry B (2006) *Stable isotope ecology*, New York, Springer-Verlag.

Heincke F (1908) Bericht über die Untersuchungen der Biologischen Anstalt auf Helgoland zur Naturgeschichte der Nutzfische. *Die Beteiligung Deutschlands an der Internationalen Meeresforschung 1908* **4/5**, 67–155.

Ikken K, Brey T, Wand U, Voigt J and Junghans P (2001) Food web structure of the benthic community at the Procupine Abyssal Plain (NE Atlantic): a stable isotope analysis. *Progress in Oceanography* **50**, 383–405.

Le Brun N, Renaud F and Lambert A (1988) The genus *Diplozoon* (Monogenea, Polyopisthocotylea) in Southern France; speciation and specificity. *International Journal for Parasitology* **18**, 395–400.

McGrew AK, O'Hara TM, Stricker CA, Castellini JM, Beckmen KB, Salman MD and Ballweber LR (2015) Ecotoxicoparasitology: understanding mercury concentrations in gut contents, intestinal helminths and host tissues of Alaskan gray wolves. *Science of the Total Environment* **536**, 866–871.

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

Nachev M, Jochmann MA, Walter F, Wolbert JB, Schulte SM, Schmidt TC and Sures B (2017) Understanding trophic interactions in host–parasite associations using stable isotopes of carbon and nitrogen. *Parasites & Vectors* **10**, 90.

Nash RDM, Valencia AH and Geffen AJ (2006) The origin of Fulton's condition factor – setting the record straight. *Fisheries* **31**, 236–238.

Navarro J, Albo-Puigserver M, Coll M, Saet R, Forero MG and Kutcha R (2014) Isotopic discrimination of stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) in a host-specific holocephalan tapeworm. *Journal of Helminthology* **88**, 371–375.

Neilson R, Boag B and Hartley G (2005) Temporal host–parasite relationships of the wild rabbit, *Oryctolagus cuniculus* (L.) as revealed by stable isotope analyses. *Parasitology* **131**, 279–285.

O'Grady SP and Dearing MD (2006) Isotopic insight into host–endosymbiont relationships in Liolaemid lizards. *Oecologia* **150**, 355–361.

Persson ME, Larsson P and Stenroth P (2007) Fractioning of δ^{15}N and δ^{13}C for Atlantic salmon and its intestinal cestode *Eubothrium crissum*. *Journal of Fish Biology* **71**, 441–452.

Pinnegar JK, Campbell N and Polunin NVC (2001) Unusual stable isotope fractionation patterns observed for fish–parasite trophic relationship. *Journal of Fish Biology* **59**, 494–503.

Post DM, Pace ML and Hairston NG Jr (2000) Ecosystem size determines food-chain length in lakes. *Nature* **405**, 1047–1049.

Power M and Klein GM (2004) Fish host–cestode parasite stable isotope enrichment patterns in marine, estuarine and freshwater fishes from northern Canada. *Isotopes in Environmental and Health Studies* **40**, 257–266.

Rhode K (1993) *Ecology of marine parasites*. Wallingford, UK, CAB International.

Sabadel AJM, Woodward EMS, Van Hale R and Frew RD (2016) Compound-specific isotope analysis of amino acids: a tool to unravel complex symbiotic trophic relationships. *Food Webs* **6**, 9–18.

Schmidt O, Dautel H, Newton J and Gray JS (2011) Natural isotope signatures of host blood are replicated in moulted ticks. *Ticks and Tick-borne Diseases* **2**, 225–227.

Skelton P (2001) *Freshwater fishes of Southern Africa*. Cape Town, Struik Publishers.

Vander Zanden M, Cabana G and Rasmussen J (1997) Comparing the trophic position of littoral fish estimated using stable nitrogen isotopes (δ^{15}N) and dietary data. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1142–1158.

Voigt CC and Kelm DH (2006) Host preferences of bat flies: following the bloody path of stable isotopes in a host–parasite food chain. *Canadian Journal of Zoology* **84**, 397–403.

Wada E (2009) Stable δ^{15}N and δ^{13}C isotope ratios in aquatic ecosystems. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences* **85**, 98–107.

Werner RA and Brand WA (2001) Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry* **15**, 501–519.

Yohannes E, Grimm C, Rothhaupt KO and Behrmann-Godel J (2017) The effect of parasite infection on stable isotope turnover rates of δ^{15}N, δ^{13}C and δ^{34}S in multiple tissues of Eurasian perch *Perca fluviatilis*. *PLOS One* **12**, e0169058.