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Research

Parasites and stable isotopes: a comparative analysis of isotopic discrimination in parasitic trophic interactions

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Stable isotopes are widely used to identify trophic interactions and to determine trophic positions of organisms in food webs. Comparative studies have provided general insights into the variation in isotopic composition between consumers and their diet (discrimination factors) in predator–prey and herbivore–plant relationships while other major components of food webs such as host–parasite interactions have been largely overlooked. In this study, we conducted a literature-based comparative analysis using phylogenetically-controlled mixed effects models, accounting for both parasite and host phylogenies, to investigate patterns and potential drivers in $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors in metazoan parasitic trophic interactions. Our analysis of 101 parasite–host pairs revealed a large range in $\Delta^{13}C$ (–8.2 to 6.5) and $\Delta^{15}N$ (–6.7 to 9.0) among parasite species, with no significant overall depletion or enrichment of ^{13}C and ^{15}N in parasites. As previously found in other trophic interactions, we identified a scaling relationship between the host isotopic value and both discrimination factors with $\Delta^{13}C$ and $\Delta^{15}N$ decreasing with increasing host $\delta^{13}C$ and $\delta^{15}N$, respectively. Furthermore, parasite phylogenetic history explained a large fraction (>60%) of the observed variation in the $\Delta^{15}N$ discrimination factor. Our findings suggest that the traditional isotope ecology framework (using an average $\Delta^{15}N$ of 3.4‰) applies poorly to parasitic trophic interactions. They further indicate the need for a scaled rather than a fixed trophic discrimination factor framework along gradients of host $\delta^{15}N$. We also identified several conceptual and methodological issues which should to be considered in future research to help integrate parasitic interactions into a holistic isotope ecology framework across diverse trophic interactions.

Keywords: comparative analysis, discrimination factor, parasite–host interactions, stable isotope analysis, trophic fractionation, trophic interactions

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Introduction

Trophic interactions are pivotal in driving population dynamics, community structure and the functioning of food webs (McCann 2011, Hanley and La Pierre 2015). Underlying an understanding of the manifold effects of trophic interactions on ecological processes is the knowledge of who eats whom. In this respect, the analysis of naturally occurring stable isotopes has gained increasing popularity (Layman et al. 2012, Traugott et al. 2013). This method makes use of the differences (discrimination factor, Δ) between isotopic ratios of naturally occurring stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) between consumers and their resources, due to a process called isotopic fractionation (Fry 2006). The difference in $\delta^{13}C$ between consumers and their diet ($\Delta^{13}C$) is used to identify the diet source of carbon (e.g. terrestrial versus marine primary producers; Hobson 1986), and the difference in $\delta^{15}N$ (trophic enrichment, $\Delta^{15}N$) is used to determine a consumer's trophic position (Vander Zanden et al. 1997). The latter is calculated based on the empirically derived average difference between consumers and their resources known as the trophic discrimination factor, or $\Delta^{15}N$. In predator–prey and herbivore–plant trophic interactions, the difference between one trophic level and the next is assumed to be equivalent, on average, to a $\Delta^{15}N$ of about 3.4‰ (Minagawa and Wada 1984, Vander Zanden et al. 1997, Post 2002).

This average discrimination factor is often used to determine the trophic position of a consumer, but it is generally acknowledged that the actual values of $\Delta^{15}N$ (and also those of $\Delta^{13}C$) vary widely across individual consumer–resource relationships (Post 2002, McCutchan et al. 2003, Caut et al. 2009). Based on published data examining trophic discrimination factors of consumers, several comparative analyses indicate that isotope discrimination can differ depending on consumer and resource taxonomic affiliation, their environment (marine, freshwater, terrestrial), as well as the type of tissue analysed (Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003, Caut et al. 2009, Perkins et al. 2013). The general type of consumer diet (carnivorous, herbivorous or detrivorous; Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003), and their feeding mode (plant fluid feeders versus others; McCutchan et al. 2003) may, among others, also affect discrimination factors. From a methodological perspective, the extraction of lipids prior to stable isotope analysis has been shown to affect carbon isotope measurements as lipids are depleted in ^{13}C during biosynthesis, thus lowering $\Delta^{13}C$ in bulk samples (Sweeting et al. 2006, Post et al. 2007, Tarroux et al. 2010). Additionally, recent comparative studies have further identified a negative scaling of $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors with the stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$, respectively) of the resources consumed (Caut et al. 2009, Hussey et al. 2014). As $\delta^{15}N$ generally scales positively with trophic position, this means that $\Delta^{15}N$ discrimination factors are not constant within food webs, but instead, decrease with increasing trophic level (Hussey et al. 2014). This scaling pattern has important implications because it suggests that a scaled framework of $\Delta^{15}N$ discrimination along gradients of resource $\delta^{15}N$ will be more appropriate for correct identification of trophic levels of consumers within food webs compared to a traditionally fixed trophic discrimination factor (Hussey et al. 2014).

Although patterns of isotopic composition and discrimination factors in consumer–resource interactions in general, and their suitability as proxies for trophic position in particular, have received much interest, comparative studies have so far mainly focussed on predator–prey and herbivore–plant relationships. In contrast, much less is known about isotopic discrimination in other trophic relationships such as in parasite–host interactions, in which parasites are consumers feeding on their hosts as the resource. There are various classifications of what organisms and life styles fall under the term 'parasite', ranging from broad definitions including all organisms that have a durable relationship with their host, from which they gain energetic benefits at the expense of the host (Combes 2001), to more exclusive definitions based on the number of victims attacked (always only one per life-cycle stage) and the fitness impacts on victims (Lafferty and Kuris 2002). For example, while blood-sucking animals such as mosquitos, leeches, lampreys and vampire bats are considered by some authors to be parasites, others classify them as micropredators as they may feed on more than a single victim during a life cycle stage (Lafferty and Kuris 2002). Likewise, sub-categories of the term 'parasite' have been suggested based on the impact on victims. For example, typical parasites (e.g. helminths) feed on a single victim without necessarily killing their hosts, while parasitoids, although also only feeding on a single victim, require the death of their sole host for the development of their larval stages (Lafferty and Kuris 2002). Further definitional issues arise from the distinction of parasitism from commensalism. While parasites, by definition, have a negative effect on their hosts, commensals gain benefits from their hosts but do not affect them in either a negative or positive way (Begon et al. 2005). Some of these benefits may be nutritional but contrary to parasites, commensals do not feed directly on their hosts but rather on leftovers of their hosts' meals so that there is no direct energy transfer from hosts to commensals (Goater et al. 2013).

As the actual magnitude of impact on the host, and the presence or absence of a direct energy transfer, is not always known, the distinction between a commensal and parasitic life style of a given organism can be difficult to determine. In this case, stable isotope analysis has been proposed as a useful tool to identify the exact trophic relationship, also because gut content analyses are often difficult in the case of minute food items and small parasites (Parmentier and Das 2004, Becker et al. 2013). Stable isotope analysis has also been used to investigate the trophic ecology of typical parasites, such as helminths, for which the parasitic status is not in question (Deudero et al. 2002, Behrmann-Godel and Yohannes 2015). Finally, some studies utilised stable isotope analysis to infer the trophic position of parasites in complete

food webs (Iken et al. 2001). In most of these applications of stable isotope analysis, inference has been based on the assumption that the fixed trophic discrimination factor $\Delta^{15}N$ of 3.4‰, empirically derived from predator–prey and herbivore–plant studies, also holds true for parasitic organisms. However, a first qualitative review of the available data suggested that this assumption may not work well for parasites as they can be depleted, enriched, or without a difference, in ^{15}N (Lafferty et al. 2008). In addition, some studies to date have explicitly investigated discrimination variation for specific parasite–host systems, typically for hymenopteran endoparasites, and have reported lower, and variable, discrimination values compared with the widely accepted average trophic discrimination factor of 3.4‰ (Langellotto et al. 2005, Yarnes et al. 2005, Perkins et al. 2014). Likewise, $\Delta^{13}C$ seems to show a larger variation among parasite–host systems (Deudero et al. 2002) than the suggested average $\Delta^{13}C$ of 1‰ empirically derived from predator–prey and herbivore–plant systems (DeNiro and Epstein 1981, Peterson and Fry 1987, France and Peters 1997). These findings indicate the need to examine the trophic complexity of relationships between parasites and their hosts more closely. A quantitative, comparative analysis of the variation in $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors in parasites, with the aim to identify the factors driving them, could resolve these issues but, to date, is missing. In addition, the possibility that parasites show the same negative scaling of $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors with the stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$, respectively) of their resources, as observed in predator–prey interactions (Caut et al. 2009, Hussey et al. 2014), has never been investigated. This not only limits our understanding of the generality of this scaling pattern, but also compromises a critical evaluation of the suitability of stable isotope analysis for studying the trophic ecology of parasite–host interactions. Given the increasing interest in the role of parasitic trophic interactions in food web topology and energetics (Lafferty et al. 2008, Dunne et al. 2013), a quantitative analysis of isotope discrimination patterns in parasites is desirable.

In this study, we compiled a data set from the published literature on stable isotope measurements of $\delta^{13}C$ and $\delta^{15}N$ in metazoan parasites and their hosts. Due to the durable and specific relationships involved, parasite–host interactions constitute quasi-experimental settings where the resource (the host) of the consumer (the parasite) is well known. Using a comparative approach, we aimed to 1) characterise the variation of $\Delta^{13}C$ and $\Delta^{15}N$ isotope discrimination factors between parasites and their hosts, 2) identify potential factors affecting $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors (host habitat, parasite habitat on the host, parasite feeding mode, sample size and lipid extraction method prior to isotope analysis) and 3) investigate whether the negative scaling between resource isotopic composition and isotope discrimination factors observed in predator–prey and herbivore–plant trophic interactions also holds true for parasitic trophic interactions. Our analysis provides a quantitative framework for identifying patterns in stable isotope discrimination factors of parasites and broadens our general understanding of trophic interactions.

Methods

Dataset compilation

We compiled a dataset from published studies which reported stable isotope ($\delta^{13}C$ and $\delta^{15}N$) measurements of metazoan parasites and their respective hosts (see Supplementary material Appendix 1 for search details and inclusion criteria). A typical study collected hosts in the field, sampled parasites from these hosts and analysed the stable isotope composition of host and parasite tissue. We employed a broad definition of the term 'parasite' which included organisms that were presumably deriving energy from their host by feeding on host blood, tissue or on specific compounds pre-digested by the host. Besides typical parasites (e.g. helminths), this also included organisms sometimes grouped under different categories such as parasitoids and micropredators (Lafferty and Kuris 2002). In addition, we included organisms that were presumed by the authors of a study to be deriving energy from their hosts in a parasitic manner (e.g. fish living in sea cucumbers) and for which the respective study used stable isotope analysis to infer the actual trophic relationship.

From all 35 articles that met the inclusion criteria, we retrieved the name and taxonomic affiliation of the host and the parasite species. We also noted the environment from which hosts were sampled (terrestrial, freshwater, marine), the habitat of the parasites, on or in their hosts (endoparasitic, ectoparasitic, gills), as well as their feeding mode (blood versus other). Finally, for each parasite–host combination, we retrieved the mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios for the parasite and its host from the respective publication and noted sample sizes and whether lipid extraction was done prior to the analyses or not (see Supplementary material Appendix 2 for details).

Phylogenetic comparative analyses

Prior to the analysis, we calculated carbon and nitrogen discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$, respectively) from the mean $\delta^{13}C$ and $\delta^{15}N$ of parasites and hosts by subtracting the $\delta^{13}C$ or $\delta^{15}N$ of the host from the respective $\delta^{13}C$ or $\delta^{15}N$ of a parasite. The resulting discrimination factors from different studies were analysed unweighted with respect to the sample size. We considered this to be appropriate because the exact meaning of a 'replicate' differed among studies (Supplementary material Appendix 2). Nonetheless, the use of unweighted means is unlikely to be a source of bias (Morrissey 2016).

Phylogenetic comparative analyses were carried out in the statistical software environment R ver. 3.3.1 (<www.r-project.org>). We used the MCMCglmm package (Hadfield 2010) to account for correlated structures arising from study identity, species identity and host and parasite phylogenetic

relationships, by including these factors as random terms in mixed effects models (for details of phylogenetic tree construction see Supplementary material Appendix 3).

We implemented a null intercept-only model, which only contained those random-effects listed above, to estimate the overall trend of the discrimination factors. Then, an information theoretic approach was applied to compare the contribution of the fixed effects predictors which included the host isotopic value, host habitat (marine, freshwater, terrestrial), host sample size, parasite habitat on the host (ectoparasitic, endoparasitic, gills), parasite feeding mode (blood versus other), parasite sample size and lipid extraction prior to analysis (yes versus no). Through an exhaustive search of fixed effects combinations, the 'consensus model' was chosen based on the predictors that appeared in the majority of the 10 models with the lowest deviance information criterion (DIC) values. The effects of predictors that appeared in the consensus model were then evaluated based on the consensus model, while other predictors were omitted from further analyses. The amount of variance accounted for by the random effects was calculated for the null model, while the amount of variance accounted for by both fixed and random effects was calculated for the consensus model. The proportion of total variance explained by all fixed effects in a mixed effects model is known as marginal R^2, whereas the proportion of total variance explained by all fixed and random effects is termed conditional R^2 (Nakagawa and Schielzeth 2013). Finally, separate mixed effects models, with taxonomic groups as fixed effects, were analysed for nitrogen discrimination factors where a strong phylogenetic signal was detected. All models were run in triplicates and we report the mean of the posterior distributions to control for MCMC errors except during the model selection procedure.

Data deposition

Data available from doi: <http://dx.doi.org/10.4121/uuid:73327bf9-ead9-4b9c-a6b5-dd3ba1170163> (Thieltges et al. 2019).

Results

The final dataset consisted of 35 studies providing 101 effect sizes based on $\delta^{13}C$ and $\delta^{15}N$ measurements from a diverse array of parasite–host pairs from marine (61 pairs), freshwater (20) and terrestrial (20) ecosystems (for data set see Thieltges et al. 2019). Most parasite species in the data set were arthropods (50 parasite–host pairs), followed by helminths (41), lophotrochozoans (polychaetes and bivalves) and vertebrates (both 5). In the following sections, we present the results of the analyses separately for $\Delta^{13}C$ and $\Delta^{15}N$.

Patterns in $\Delta^{13}C$ discrimination factors

The $\Delta^{13}C$ discrimination factors between parasites and their hosts ranged widely from −8.2 to 6.5, thus showing evidence for strong depletion, as well as strong enrichment, of ^{13}C in parasites compared to their hosts (Fig. 1a). The average estimated $\Delta^{13}C$ discrimination factor reported in the literature was −0.259, however the CI for $\Delta^{13}C$ spanned zero, indicating no significant evidence for enrichment, nor depletion, of ^{13}C between hosts and parasites over the entire data set (n = 101, posterior mean = −0.259, CI = −1.338 to 0.801; Fig. 1a).

When investigating the drivers of this large among-parasite variation of discrimination factors, we found a significant negative relationship between the host isotopic $\delta^{13}C$ and isotope discrimination $\Delta^{13}C$ (Fig. 2a, Table 1a), indicating the higher the host $\delta^{13}C$, the smaller the isotopic difference

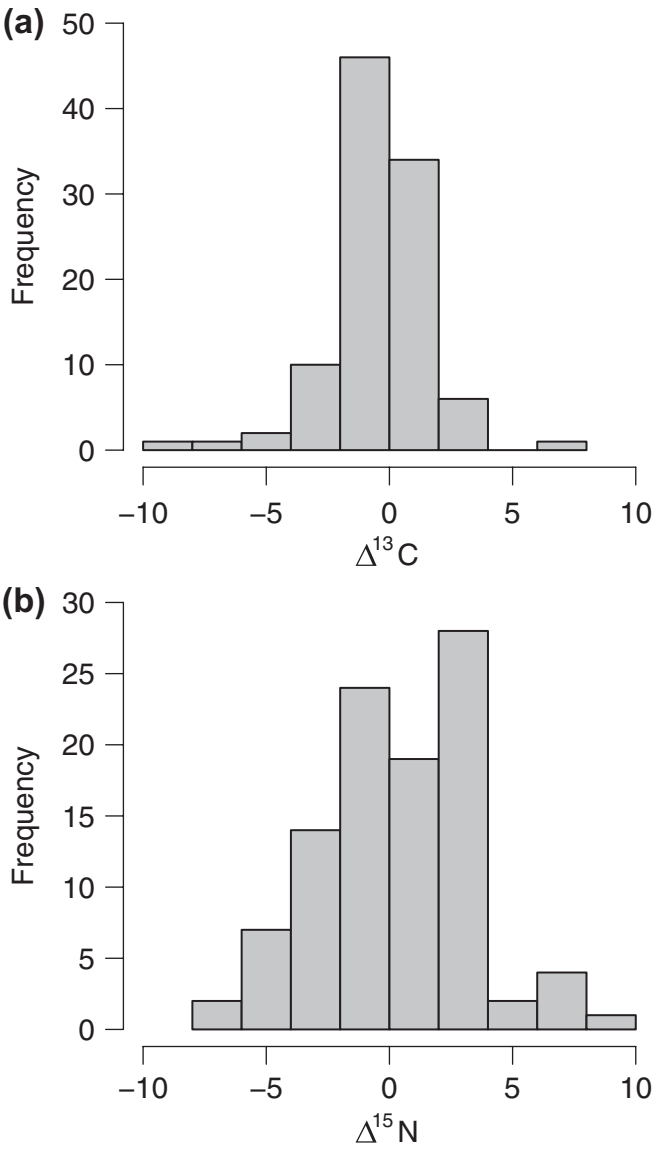

Figure 1. Frequency distribution of (a) $\Delta^{13}C$ and (b) $\Delta^{15}N$ discrimination factors in parasitic trophic interactions (calculated as $\delta^{13}C$ or $\delta^{15}N$ of a parasite minus the respective $\delta^{13}C$ or $\delta^{15}N$ of its host). n = 101 parasite–host pairs.

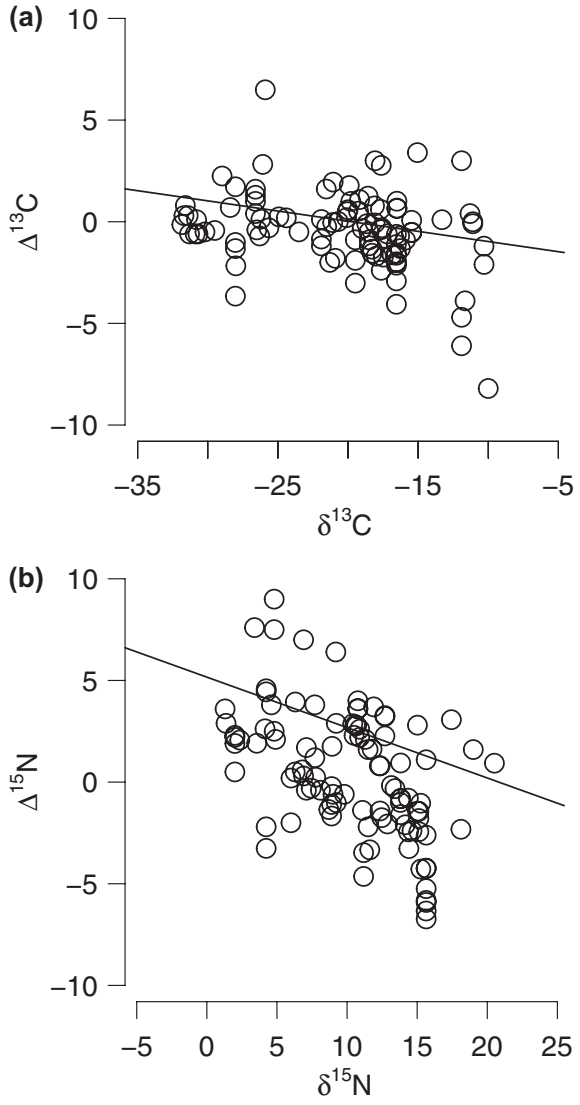

Figure 2. Relationship between (a) host $\delta^{13}C$ and $\Delta^{13}C$ and (b) host $\delta^{15}N$ and $\Delta^{15}N$ in parasitic trophic interactions. The lines show significant regressions from mixed models (carbon: $y = -0.100x + 0.101$; nitrogen: $y = -0.248x + 2.601$).

between host and parasite. Because $\Delta^{13}C$ and $\delta^{13}C$ are mathematically related, such an association may be due to a so-called 'spurious correlation' (Kenney 1982, Jackson and Somers 1991, Auerswald et al. 2010). However, a randomisation test indicated that the observed association was not the result of a spurious correlation (Supplementary material Appendix 4 Fig. A1a). Other biological and methodological variables investigated (host habitat, parasite habitat on the host, parasite feeding mode, sample size and lipid extraction method prior to isotope analysis) did not display significant associations with $\Delta^{13}C$ (Table 1a, Supplementary material Appendix 5 Table A1). We note that the large amounts of variance were either due to parasite species identity (null model: 30.13%; consensus model: 23.44%, Table 2), or unaccounted for in the mixed-effects models (residuals were 41.95 and 42.38% for null and consensus model, respectively, Table 2, Supplementary material Appendix 5 Table A3), indicating that, while the carbon discrimination factors seem to vary substantially among parasite-species, values are difficult to predict a priori.

Patterns in $\Delta^{15}N$ discrimination factors

As for $\Delta^{13}C$ discrimination factors, the $\Delta^{15}N$ discrimination factors of parasites reported in the literature ranged widely from −6.7 to 9.0, hence including strong depletion, as well as strong enrichment, of ^{15}N in parasites compared to their hosts (Fig. 1b). Parasites showed a tendency for enrichment in ^{15}N compared to their respective hosts (Fig. 1b), with the estimated mean $\Delta^{15}N$ being 1.726, but this did not differ significantly from zero (n = 101, posterior mean = 1.726, CI = −1.906 to 5.446).

When investigating the drivers of the observed among-parasite variation of discrimination factors, $\Delta^{15}N$ was affected by the environment from which parasite–host pairs were sampled, with parasites from freshwater habitats showing significantly higher $\Delta^{15}N$ discrimination compared to those from marine habitats (Table 1b). Furthermore, as for $\Delta^{13}C$, the $\Delta^{15}N$ discrimination factors of parasites scaled negatively with the $\delta^{15}N$ isotope composition of the host (Fig. 2b, Table 1b). Here again, a randomisation test demonstrated that the observed negative relationship was not the result of a 'spurious correlation' (Supplementary material Appendix 4 Fig. A1b). Other biological and methodological variables investigated (parasite habitat on the host, parasite feeding mode, sample size and lipid extraction method prior to isotope analysis) did not significantly explain the variation in $\Delta^{15}N$ (Table 1b, Supplementary material Appendix 5 Table A2). Since the majority of variance in nitrogen discrimination factors was accounted for by the parasites' phylogenetic history (null model: 62.21%; consensus model: 69.63%; Table 2, Supplementary material Appendix 5 Table A3), we explored nitrogen discrimination factors in different parasite taxa in subsequent analyses. The average $\Delta^{15}N$ values were significantly positive in parasitic arthropods (n = 50, posterior mean = 1.762, CI = 0.148–3.299) and parasitic vertebrates (n = 5, posterior mean = 5.302, CI = 2.656–8.228), however they were not significantly different from zero in the other parasitic groups (Fig. 3a). Within parasitic arthropods, significant enrichment was found in arachnids (mites and ticks; n = 2, posterior mean = 5.806, CI = 2.388–9.300), fleas (n = 6, posterior mean = 2.858, CI = 0.046–5.821) and lice (n = 6, posterior mean = 3.408, CI = 0.509–6.594; Fig. 3b). Within helminths, cestodes showed a significant depletion in ^{15}N compared to their hosts (n = 14, posterior mean = −2.126, CI = −4.158 to −0.222; Fig. 3c).

Discussion

Our analyses revealed large variation in $\Delta^{13}C$ and $\Delta^{15}N$ isotope discrimination factors among the parasite species

Table 1. Results of the 'consensus' mixed effects models evaluating the effect of predictors on discrimination factors of (a) carbon and (b) nitrogen isotopes. All continuous variables are centred in the model. In any case where the 95% credible interval (CI) does not cross zero the difference is considered significant (in bold).

Model	Posterior mean	Lower 95% CI	Higher 95% CI
(a) Carbon			
Parasite habitat: Ectoparasite	0.100	−1.196	1.313
Parasite habitat: Endoparasite	−0.460	−1.672	0.556
Parasite habitat: Gills	−0.847	−2.445	0.676
Host isotopic value	**−0.100**	**−0.172**	**−0.021**
Host sample size	0.012	−0.019	0.047
Parasite sample size	0.001	−0.014	0.016
Pairwise comparisons (Parasite habitat)			
Ectoparasite – Endoparasite	0.560	−0.594	1.690
Ectoparasite – Gills	0.948	−0.577	2.456
Endoparasite – Gills	0.388	−1.053	2.307
(b) Nitrogen			
Host habitat: Freshwater	2.601	−0.934	6.280
Host habitat: Marine	1.170	−2.265	4.678
Host habitat: Terrestrial	1.285	−2.390	5.104
Host isotopic value	**−0.248**	**−0.366**	**−0.123**
Host sample size	−0.037	−0.078	0.004
Parasite sample size	0.003	−0.011	0.016
Pairwise comparisons (Host habitat)			
Freshwater – Marine	**1.432**	**0.015**	**2.900**
Freshwater – Terrestrial	1.317	−0.818	3.327
Marine – Terrestrial	−0.115	−2.851	2.036

included, with no overall indication of significant depletion or enrichment of parasites compared to their hosts. A closer look at potential predictors of this variation revealed that both $\Delta^{13}C$ and $\Delta^{15}N$ scaled negatively with host $\delta^{13}C$ and $\delta^{15}N$, respectively. Additionally, parasites sampled from freshwater habitats showed significantly higher $\Delta^{15}N$ discrimination compared to those from marine habitats. Importantly, we detected a strong influence of parasite phylogenetic history on $\Delta^{15}N$ discrimination, with a taxonomic sub-group analytical approach revealing significant enrichment in some arthropods and vertebrates while cestodes showed significant depletion.

The absence of a significant average enrichment of ^{13}C and ^{15}N in parasites compared to their hosts contrasts with the typical average enrichment observed in comparative analyses of predator–prey, and herbivore–plant, trophic interactions which have revealed average trophic discrimination of 3.4‰ for $\Delta^{15}N$ (Minagawa and Wada 1984, Vander Zanden et al. 1997, Post 2002) and 1.0‰ for $\Delta^{13}C$ (DeNiro and Epstein 1981, Peterson and Fry 1987, France and Peters 1997). In

Table 2. Percentage of the overall variance accounted for by the different fixed and random factors in the null and consensus models for carbon and nitrogen isotope discrimination factors of parasites (95% credibility intervals in parentheses). From the consensus models, for carbon and nitrogen respectively, we report that marginal R^2 was 0.076 and 0.083, and conditional R^2 was 0.576 and 0.909.

	Carbon		Nitrogen	
Effects	Null model	Consensus model	Null model	Consensus model
Fixed				
Habitat (only for nitrogen)				
Host isotopic value				
Parasite habitat (only for carbon)	NA	7.60 (4.92–9.42)	NA	8.25 (5.16–10.84)
Host sample size				
Parasite sample size				
Random				
Parasite species name	30.13 (0.008–61.63)	23.44 (0.007–69.07)	2.23 (0.001–10.63)	1.57 (0.001–8.57)
Parasite phylogeny	3.12 (0.004–15.73)	4.65 (0.004–20.85)	62.21 (27.57–91.82)	69.63 (6.01–192.74)
Host species name	6.50 (0.005–23.43)	7.59 (0.004–29.52)	8.06 (0.006–17.49)	5.58 (0.005–15.04)
Host phylogeny	9.90 (0.005–41.05)	8.78 (0.005–43.95)	8.07 (0.001–31.11)	2.14 (0.001–11.52)
Study ID	8.40 (0.005–27.45)	5.56 (0.003–24.48)	6.81 (0.001–20.92)	3.72 (0.001–14.37)
Residual variance	41.95 (11.68–79.86)	42.38 (14.00–81.74)	12.61 (2.41–25.71)	9.11 (2.31–20.68)

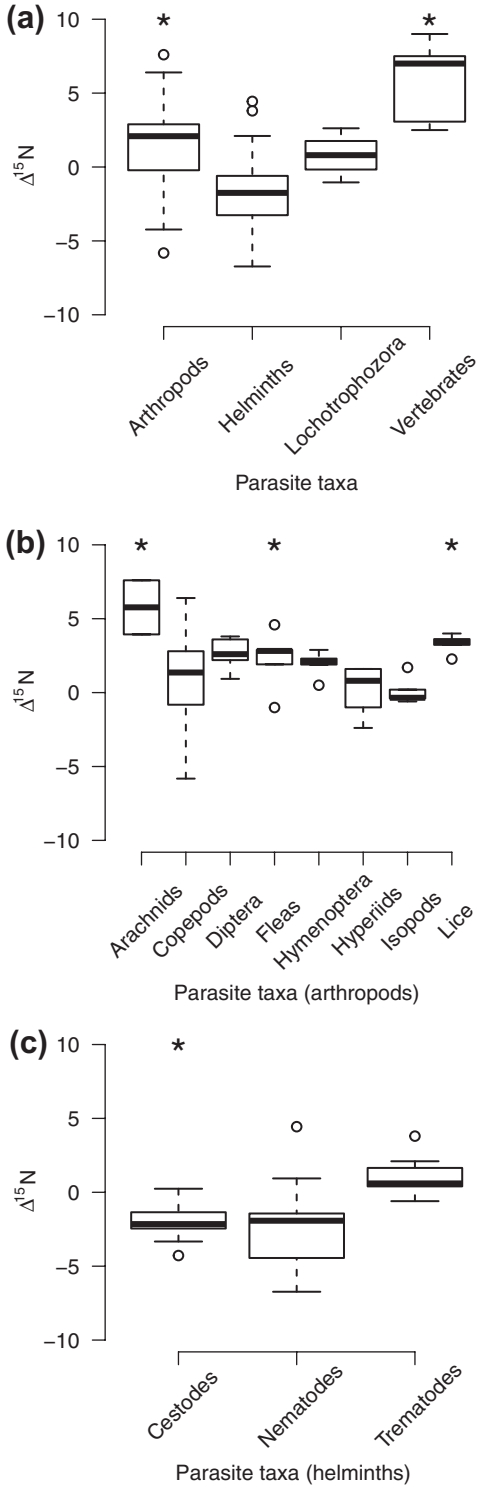

Figure 3. Effects of parasite taxonomic affiliation on trophic enrichment $\Delta^{15}N$ at the (a) phylum level, (b) among arthropods and (c) among helminths. Statistically significant signs of discrimination are indicated by asterisks.

contrast, we found averages of 1.726‰ and −0.259‰ for $\Delta^{15}N$ and $\Delta^{13}C$, respectively, which did not significantly differ from zero. While it is generally acknowledged that discrimination factors in non-parasitic trophic interactions also often diverge from the above averages and therefore species- and system-specific values may often be more appropriate for any inference (Post 2002, Caut et al. 2009, Martínez del Rio et al. 2009), this difference in averages is still distinct. The contrasting pattern may have several explanations and has generally important implications for the use of stable isotopes in the study of parasite trophic ecology. First of all, the lack of support for an enrichment in ^{13}C and ^{15}N of parasites compared to their hosts could suggest that many parasite species included in the analysis are actually not parasites but rather commensals which are not feeding directly on their hosts. For some species, this may be true. For example, the stable isotope analyses conducted with several pearlfish species living inside sea cucumbers or starfish, suggest a commensal lifestyle rather than parasitic, although the expectation, given the natural history of the species, suggested otherwise (Parmentier and Das 2004). However, for most of the parasite species in the dataset their parasitic status is not in question as they belong to typical parasitic taxa such as helminths, fleas, lice and parasitoids that all are well known to feed on their hosts. Nevertheless, most of these typical parasitic taxa showed large variation in discrimination factors, ranging from negative to positive $\Delta^{13}C$ and $\Delta^{15}N$. Only a few parasite taxonomic groups showed significant average enrichment in ^{15}N compared to their hosts and one group, the cestodes, even showed significant depletion. In addition, only three of the parasite groups showed significant enrichment (arachnids, fleas and lice), with mean isotope discrimination values of around 3.4‰, similar to those observed in predator–prey, and herbivore–plant, trophic relationships. In all other groups any detected significant mean isotope discrimination values were either much lower or higher than those previously described for other consumer–resource relationships. This suggests that the average discrimination factors observed in predator–prey and herbivore–plant trophic interactions ($\Delta^{15}N$ of 3.4‰ and $\Delta^{13}C$ of 1.0‰) do not hold true as a general indicator for trophic relationships between parasites and their hosts.

The reasons for the deviating discrimination patterns in parasites are most likely twofold, and either relate to the feeding ecology of parasites, or to potential methodological issues. Although feeding on their hosts, parasites may differ from predators in metabolic processes and they may often feed only on specific host tissues, or on specific compounds pre-digested by the host rather than ingesting the entire host (like predators often do with their prey). For example, most helminths have only limited ability to biosynthesise amino acids (Köhler and Voigt 1988), and thus, many endoparasites absorb ^{15}N-depleted amino acids from the intestines of their hosts (Barrett 1981, Hare et al. 1991). In addition, helminths can often re-utilise ^{15}N-depleted ammonia that they have excreted or taken up from host blood to synthesise amino acids (Barrett 1981). These metabolic pathways can lead to lower isotope discrimination than observed in predators and herbivores, and can even lead to the significant depletion in ^{15}N of cestodes compared to their hosts, as observed in our

analysis. Likewise, we speculate that if parasites selectively feed on specific host tissue, or host blood, which is relatively depleted in ^{15}N, this may lead to lower isotope discrimination factors than is observed in predators, which usually consume their prey as a whole. Selective feeding may also affect $\Delta^{13}C$ discrimination factors. Host tissues with high lipid content (e.g. blood, liver) are usually ^{13}C-depleted relative to other tissues (Focken and Becker 1998, Pinnegar and Polunin 1999, Pinnegar et al. 2001), so that selective feeding on these lipid-rich tissues may lead to lower $\Delta^{13}C$ discrimination than in predators. Further effects on isotope discrimination factors, both in regard to $\Delta^{13}C$ and $\Delta^{15}N$, may arise in cases where parasites do not only feed on host tissue but also on non-host resources such as the general gut content of their hosts or on epiphytic and intestinal bacteria (Goedknegt et al. 2018). Apart from a few parasites of medical or veterinarian importance, our knowledge of the actual feeding processes (which tissues and components are consumed), and the biochemical pathways, within various parasite taxa is very limited, and clearly deserves further investigation to help elucidate the observed variation in discrimination factors among parasite taxa. Our models indicate that whether or not the parasite feeds on blood versus other host tissues does not significantly affect isotopic discrimination overall, but we acknowledge that a better resolution of feeding tissues would be desirable in future comparative studies.

Any selective feeding on host tissue by parasites could lead to methodological issues confounding the determination of discrimination factors. While parasite species were usually analysed as a whole in the studies included in our analysis, only specific tissues from hosts were usually sampled. As the stable isotope composition often differs among tissues of the same individual (DeNiro and Epstein 1981, Hobson and Clark 1992, Caut et al. 2009), the specific tissue used as a reference will inevitably affect the calculation of discrimination factors in consumers (Perkins et al. 2013). Hence, in cases where a parasite species actually feeds on other host tissues than the ones used for the analyses (or on non-host resources), this may lead to discrepancies in the measurements of isotope discrimination. Likewise, using different tissues of parasites (instead of whole organism sampling as in most of the studies included in our analysis) may lead to different discrimination factors. As outlined in the methods, we matched the host tissues used for our analyses with the putative feeding source of each parasite species, but in many cases the available tissue type was simply limited to what had been analysed by the authors (whole organisms for parasites and often muscle tissue for hosts). The resulting potential mismatch of host and parasite tissues in our analysis is thus unavoidable, but it may have confounded some of the discrimination factors. It is possible that such potential inaccuracies in determining the 'real' discrimination factors in parasites may, at least partly, underlie the absence of a universal pattern in our study. In other words, there may well be a universal pattern but the available data to date may not be good enough to test for it. More research into the role of appropriate tissue comparisons of both parasites and hosts will be needed to evaluate the magnitude of this potential confounding methodological issue, and to develop standardised sampling protocols.

While no overall enrichment or depletion patterns in discrimination factors were observed, the statistical analyses identified two factors to be significant predictors of $\Delta^{13}C$ and $\Delta^{15}N$. In the case of $\Delta^{15}N$, the discrimination factor was affected by the general environment from which parasite–host pairs were sampled, with parasites from freshwater habitats showing significantly higher $\Delta^{15}N$ discrimination, compared to those from marine habitats. These results reflect earlier findings from a comparative study of discrimination factors in consumer–resource interactions determined under experimental conditions (Vanderklift and Ponsard 2003). Here, discrimination factors of consumers were, on average, also higher in freshwater compared to marine systems. However, the authors were questioning the validity of their results as they may have been confounded by the strong presence of detritivorous crustaceans and molluscs in the marine category, which all had relatively low discrimination factors, thus decreasing the average discrimination factors for marine environments (Vanderklift and Ponsard 2003). Similar confounding effects due to differences in the specific parasite–host pair composition between the two groups, may have also affected our analyses as the freshwater category mainly consisted of fish and a few invertebrate hosts, while in the marine category bird and invertebrate hosts were more abundant. Hence, whether there are true differences in isotopic discrimination in parasites among the different realms remains to be investigated.

Besides a potential effect of the general environment on $\Delta^{15}N$, our analyses also revealed that discrimination factors of both $\Delta^{13}C$ and $\Delta^{15}N$ scaled negatively with host $\delta^{13}C$ and $\delta^{15}N$, respectively, i.e. parasite species feeding on hosts with a relatively low isotope ratio demonstrated larger isotope discrimination factors than parasites feeding on hosts with relatively high values of $\delta^{13}C$ and $\delta^{15}N$. The same pattern of a decrease in isotope discrimination factors with resource $\delta^{13}C$ and $\delta^{15}N$ has been observed in comparative analyses on predator–prey and herbivore–plant trophic interactions (Caut et al. 2009, Hussey et al. 2014), as well as in studies of specific consumer–resource prey interactions (Caut et al. 2008, Dennis et al. 2010). A comparison of the slopes observed in these studies (Caut et al. 2008: C: −0.113 to −0.417, N: −0.141 to −0.311; Hussey et al. 2014: N: −0.27), with the ones observed in our analyses (C: −0.100, N: −0.248) indicates that the relationship seems consistent in parasitic trophic interactions. The underlying mechanism of the pattern observed in predator–prey and plant–herbivore trophic interactions is not well understood (Caut et al. 2009, Hussey et al. 2014) and the validity of a general discrimination scaling pattern has been questioned (for a discussion of the matter see Auerswald et al. 2010, Caut et al. 2010, Perga and Grey 2010). However, the fact that our analysis of parasitic trophic interactions identified the same negative scaling suggests that it may be a universal pattern in trophic interactions that could be driven by similar mechanisms. The proposition that the mechanism may simply be

an artefact resulting from the fact that the isotopic signal of the resource is included in both the independent ($\delta_{resource}$) and the dependent ($\Delta_{consumer} = \delta_{consumer} - \delta_{resource}$) factor in the analysis (Auerswald et al. 2010) does not seem to confound our analyses as randomisation tests did not find evidence for such 'spurious correlation' issues.

A more likely mechanism may relate to the effect of diet quality on isotope discrimination factors. The so-called diet quality hypothesis suggests that as diet quality (in the form of protein quality) increases, the trophic enrichment ($\Delta^{15}N$) of consumers will decrease (Roth and Hobson 2000, Robbins et al. 2005, 2010, Florin et al. 2011). This hypothesis has received empirical support (Robbins et al. 2005, 2010, Florin et al. 2011) and is supposed to result from increased nitrogen metabolism and excretion of consumers feeding on a low quality diet which is linked to preferential renal retention of ^{15}N causing higher $\delta^{15}N$ signatures in the consumer, which in turn elevates the consumer's isotope discrimination factor. In contrast, consumers feeding on a high protein quality diet do not have to elevate their nitrogen metabolism and excretion and consequently have lower $\Delta^{15}N$. As carnivores ingest higher quality protein than herbivores, this mechanism leads to a decrease in trophic discrimination factors with an increase in trophic levels within food webs (Robbins et al. 2005). These patterns are further supported by results from compound-specific isotope analysis of amino acids (CSIA-AA), a method using only specific compounds instead of the bulk isotopes traditionally studied (McMahon and McCarthy 2016, Ohkouchi et al. 2017, Ishikawa 2018). In experimental CSIA-AA studies under controlled feeding regimes, $\Delta^{15}N$ depends on diet quality, suggesting that the trophic discrimination factor in nature is more variable than previously thought (McMahon et al. 2015, Chikaraishi et al. 2015).

Similar mechanisms may be in place in the case of parasitic trophic interactions. Parasites (consumers) feeding on hosts at higher trophic levels (indicated by larger $\delta^{15}N$) may be feeding on a high protein quality diet, thus leading to lower isotopic discrimination compared to parasites feeding on hosts at lower trophic levels (indicated by smaller $\delta^{15}N$). The effect of diet quality on isotopic discrimination should in principle lead to a negative scaling of parasite discrimination factors with host $\delta^{15}N$. This rationale works best when all organisms are part of the same food web as in this case the isotopic composition ($\delta^{15}N$) of resources are roughly equivalent with food quality and trophic level within the food web. However, isotopic baselines can be expected to differ among food webs (Post 2002) and comparative analyses such as ours may compile data from systems with very different isotopic baselines, thus potentially blurring the strong link between resource $\delta^{15}N$ and food quality, and trophic level. It may be that this links is still retained to a certain degree in our data set nevertheless (i.e. hosts occupying low trophic levels having a relatively low $\delta^{15}N$) so that a discrimination scaling patterns appears despite the mixed isotopic baselines. However, in the absence of reliable data on the trophic level of all the host species in our data set, we can only hypothesise that diet quality effects underlie the observed discriminations scaling. Hence, whether this mechanism is really the driving force behind the negative scaling pattern observed in parasitic trophic interactions remains to be investigated. Compound-specific isotope analysis of amino acids (CSIA-AA) may be a promising tool in this respect, as it may help to not only reveal the exact trophic position of hosts but also of parasites (Steffan et al. 2013, Sabadel et al. 2019). In any case, the existence of a negative scaling of $\Delta^{15}N$ of parasite species with the $\delta^{15}N$ of their hosts suggests that a scaled rather than a fixed, discrimination factor framework may be needed to be able to include parasites in traditional isotopic food web studies.

Whatever the exact mechanisms, the observed absence of a general enrichment pattern, the lack of significant predictors, and the negative scaling of discrimination factors with host isotope ratios, suggest that the traditional framework of isotope discrimination patterns, developed based on predator–prey and herbivore–plant trophic interactions, is not easily transferable to parasitic trophic interactions. A solution to the lack of a universal reference point for parasites, such as the rule of thumb of a $\Delta^{15}N$ of 3.4‰, and a $\Delta^{13}C$ of 1‰, for other trophic interactions, may be to use species- or group-specific discrimination factors for inferring trophic relationships and trophic levels. This has increasingly been done for predator–prey and herbivore–plant interactions, as the average values mentioned above also do not seem to be universally applicable to all consumers (Gannes et al. 1997, Martínez del Rio et al. 2009). However, for parasites, species-specific determinations of isotope discrimination factors are often methodologically challenging as discussed above, and appropriate sampling schemes need careful consideration. In addition, most parasite discrimination factors available to date have been determined from field-based samples. It is well known that isotope discrimination factors in predator–prey and herbivore–plant trophic interactions can be affected by environmental conditions, stress levels of organisms, and many other factors (Boecklen et al. 2011). This is likely to also apply to parasitic trophic interactions. Although parasites sampled from their hosts can be considered to be a quasi-experimental situation, where the parasites have been feeding on the host as the known food source, it is currently unknown whether other factors related to the host and its environment may affect parasite isotope discrimination factors. This calls for more experimental work on parasitic trophic interactions and the potentially mediating environmental factors, similar to what has been increasingly applied in studies on other consumer–resource interactions (Gannes et al. 1997, Martínez del Rio et al. 2009, Wolf et al. 2009). Using compound-specific isotope analysis of amino acids (CSIA-AA) rather than the traditional bulk isotope analysis may provide further insights into the trophic position of parasites (Steffan et al. 2013, Sabadel et al. 2019). A better understanding of potential methodological issues and of the ecological drivers mediating discrimination factors in parasitic trophic interactions will allow for stronger inference in respect to feeding relationships of parasites and their trophic positions in food webs.

In conclusion, our comparative analysis suggests that the traditional framework of isotope discrimination patterns is currently not well suited for studying parasitic trophic interactions. Whether taxon-specific trophic discrimination factors can reliably be used as substitutes for the conventional $\Delta^{15}N$ of 3.4‰ will need further investigation, however, the strong effect of parasite phylogeny in our analysis suggests that for certain groups such as arthropods (lice, mites, ticks) and helminths (cestodes), further studies are likely to be fruitful in helping resolve a robust range for discrimination values, that would allow for greater inclusion of parasites in traditional isotopic food web studies. In addition, the negative scaling between parasite $\Delta^{15}N$ and host $\delta^{15}N$, observed in our analysis, suggests the need for a scaled (rather than a fixed trophic discrimination factor) framework of $\Delta^{15}N$ discrimination along gradients of host $\delta^{15}N$ for the appropriate identification of trophic positions of parasitic consumers within food webs. Finally, further research on potential methodological issues such as tissue mismatches in both parasite and hosts is needed, in addition to experimental work that identifies potentially mediating factors. We hope that our study will spark future research into the patterns and mechanisms of trophic discrimination in parasites to help develop a holistic framework of isotopic discrimination that integrates parasitic trophic interactions among other trophic interactions.

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Supplementary material (available online as Appendix oik-06086 at <www.oikosjournal.org/appendix/oik-06086>). Appendix 1–5.