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Deciphering the complex trophic relationship of the black-spotted croaker (Teleostei: Sciaenidae) and its parasites using stable isotope analysis

Megan Porter[a], **Diane P. Barton**[a], **Shokoofeh Shamsi**[a], **David A. Crook**[b,c], **and Jo Randall**[b,d]

[a]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia; [b]Research Institute for the Environment and Livelihoods, Charles Darwin University, Casuarina, NT 0810, Australia; [c]Department of Primary Industries, Narrandera Fisheries Centre, Narrandera, NSW 2700, Australia; [d]Australian Institute of Marine Science, Arafura Timor Research Facility, Casuarina, NT 0810, Australia

Corresponding author: **Megan Porter** (email: mporter@csu.edu.au)

Abstract

The stable isotope values of nitrogen (δ^{15}N) and carbon (δ^{13}C) have been widely used in ecological studies to decipher the trophic relationships and interactions that occur between living organisms. The aim of this study was to determine the trophic relationship between a commercially important tropical Australian marine fish (*Protonibea diacanthus* (Lacepède, 1802)) (Sciaenidae) and its associated parasites, through stable isotope analysis of nitrogen and carbon (δ^{15}N and δ^{13}C). We examined the stable isotope ecology of four parasitic organisms: adult ectoparasitic copepods, and endoparasitic adult digeneans and nematodes and plerocercoids. Nitrogen in endoparasites was consistently depleted when compared with the host; however, digeneans expressed nitrogen signatures almost equivalent to those of the host. Ectoparasitic copepods were the only parasite that was substantially enriched in nitrogen compared with the host. All adult parasitic organisms were carbon depleted when compared with the host tissue associated with the site of infection; however, plerocercoids were enriched. Our findings emphasize the complexity of parasite–host interactions and the varying values of isotopic discrimination between parasite type, life-cycle stage, and location in host.

Key words: helminth, copepod, *Protonibea diacanthus*, black-spotted croaker, marine fish, isotope, parasite ecology

Introduction

Parasitism is a complex life history strategy that is ubiquitous across all ecosystems. Parasites are critical components of biodiversity, playing an integral role in ecosystem functioning and food web dynamics (Lõhmus and Björklund 2015). These highly diverse organisms can augment the flow of energy in food webs and manipulate host interactions, sometimes even causing trophic cascades (Lafferty et al. 2008).

Stable isotope analysis provides inference on the ecological interactions of organisms, to understand how organisms assimilate energy from resources, and to determine the trophic position of consumers within their food web (Fry 2006; Gilbert et al. 2020*a*; Huston et al. 2021). Trophic discrimination factors for nitrogen and carbon are used in ecological studies to characterize trophic relationships and determine pathways of nutrient assimilation. Specifically, the trophic discrimination factor of nitrogen is used to determine trophic levels, while the trophic discrimination factor of carbon is used to identify the dietary source of carbon that an organism consumes, based on stable isotope data (Riekenberg et al. 2021; Sabadel and MacLeod 2022).

Despite the growing use of stable isotopes in ecological studies, and the influence that parasites have on the number of trophic links within ecosystems, the inclusion of parasitic relationships within food web studies remains scarce (Thompson et al. 2005; Arias-Gonzalez and Morand 2006; Lafferty et al. 2006; Kuris et al. 2008; Lafferty et al. 2008; Sabadel et al. 2019). The cryptic habitats within hosts, their minute size, and the multidisciplinary requirements for their identification often make parasitic organisms easy to omit from these studies (Lafferty et al. 2008; Riekenberg et al. 2021). In addition, interpreting host–parasite relationships using isotopes has proven challenging, with host–parasite systems rarely showing consistent isotopic patterns (Lafferty et al. 2008; Thieltges et al. 2019; Kamiya et al. 2020; Huston et al. 2021). The varying trophic discrimination of host–parasite relationships can be attributed to the combined effects of the unique feeding ecologies of different types of parasites, the host metabolic effects due to parasitism, and the patterns of enrichment that may change due to the different stages present in often complex parasite life cycles (Huston et al. 2021; Riekenberg et al. 2021).

Host–parasite isotope signatures often express inconsistent patterns depending on the parasite's biology and the host–parasite system investigated (Taccardi et al. 2020; Huston et al. 2021; Riekenberg et al. 2021). Parasites assimilate nutrients via many strategies; for example, some parasites may feed on intestinal contents rather than on host tissue, whereas others selectively absorb biochemical compounds through attachment in or on different host tissues (Lafferty et al. 2008). Endoparasites, such as cestodes and nematodes, which acquire nutrients through assimilation of host metabolites, are often depleted in ^{15}N and ^{13}C isotopes when compared with fish hosts (Gilbert et al. 2020*a*; Riekenberg et al. 2021). Discrimination patterns can, however, alter depending on the life stage of the endoparasite, and the site of residence in the host. For example, larval nematodes may behave as active feeders and express predator-like discrimination patterns of enrichment when compared with fish hosts (Nachev et al. 2017). Larval cestodes, on the other hand, are thought to have a somewhat commensalistic feeding strategy and tend to express similar isotopic signatures to hosts (Nachev et al. 2017; Gilbert et al. 2020*a*, 2020*b*). Ectoparasites, unlike endoparasites, are more commonly represented with a predator–prey relationship with both marine and freshwater fish hosts. Copepods are ectoparasites and in many studies have offered similar isotopic enrichment patterns when compared with marine fish hosts, a finding that aligns with other blood-feeding ectoparasites (Iken et al. 2001; Deudero et al. 2002; Gilbert et al. 2020*a*). There are similar reports in freshwater host–parasite systems with monogenean ectoparasites also acting as micropredators, being enriched in nitrogen, and therefore residing at a higher trophic level than their fish hosts (Sures et al. 2019). Despite the similar patterns observed for some types of ectoparasites, many do not conform to expectations in a traditional predator–prey interaction (Taccardi et al. 2020).

The black-spotted croaker, *Protonibea diacanthus* (Lacepède, 1802) (Teleostei: Sciaenidae), is a large predatory marine fish distributed throughout tropical waters from northern Australia to the Arabian Gulf (Taillebois et al. 2017; Barton 2018). This species is an opportunistic predator commonly found in epibenthic fish assemblages of both inshore and nearshore estuarine and coastal waters of Australia. Sciaenids are highly valued as food fish globally, and in addition to many species being targeted by demersal fisheries, many of the larger species are popular targets for leisure fishing (Ramcharitar et al. 2006). In Australia, the black-spotted croaker is the largest sciaenid and represents a key fisheries resource, through its significant contributions to the commercial, recreational, and Indigenous fishing sectors. Recent research has found this sciaenid to serve as a host for many parasitic organisms, and it is with growing importance that the impacts of these infections are understood (Taillebois et al. 2017; Barton 2018). This study used the stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) to assess isotopic discrimination patterns between *Protonibea diacanthus* and four different types of its associated parasites. We examined internal and external parasites to decipher the potential influence of factors such as parasite type, developmental stage, and unique feeding ecologies on different host–parasite trophic interactions.

Materials and methods

Host and parasite collection

A total of 17 *Protonibea diacanthus* were collected off the coast of the Northern Territory of Australia for the study. Fish were collected during the later part of the wet season in March 2021 using hook and line capture and euthanasia via percussive stunning. These methods were approved by The Charles Darwin University (CDU) Animal Ethics Committee (AEC), approval number #A19009. Following capture, morphological parameters such as weight and total length were recorded (Supplementary Table S1). Fish were processed within 24 h of capture, with the heads and internal organs placed into plastic bags, frozen, and shipped to the Parasitology Laboratory at Charles Sturt University, Wagga Wagga, Australia. At the time of dissection, samples were thawed and examined for parasites following standardized techniques as described in Taillebois et al. (2017). Fish samples for stable isotope analysis were collected from muscle tissue (collected from the head of the fish, ventral to the gills), gill filaments, and stomach wall. Parasites, representative of a range of different infection sites within the host and potential feeding strategies, were also collected: adult copepods (*Lernanthropus paracruciatus* Boxshall, Bernot, Barton, Diggles, Yong, Atkinson-Coyle & Hutson, 2020) were collected from the gill filaments—gill tissue is used as the representative tissue associated with this parasite; adult digeneans (*Orientodiploproctodaeum* sp. Bhutta & Khan, 1970) and nematodes (*Cucullanidae* sp. Cobbold, 1864) were collected from the gastrointestinal tract—the stomach wall tissue is used as the representative tissue associated with these parasites; and plerocercoids (*Poecilancistrium* sp. Dollfus, 1929) were collected from within the gill arches—muscle is used as the representative tissue associated with these parasites (similarly to this study, cited infections with plerocercoids are also found throughout the body musculature, and muscle tissue is reported as the more appropriate tissue for isotopic comparison given the cartilaginous structure of the gill arches (Barton, unpublished data; Power and Klein 2004)). All fish and parasite samples were stored frozen at –20 °C until isotopic analysis.

Stable isotope analysis

Samples of parasite and fish tissue were analysed individually, according to parasite and tissue type. To make up the required sample size of 1–2 mg of dry weight for isotope analysis, parasites belonging to the same type, and collected from the same fish host, were combined as one sample.

Fish and parasite isotope samples were analysed at the Stable Isotope Laboratory of Griffith University, Brisbane. To prepare fish and parasite isotope samples, samples were freeze-dried using a VirTis Genesis 35XL freeze drying system and ground to a fine powder using a Retsch Mixer Mill MM400. Samples were then combusted in a Sercon Europa EA-GSL elemental analyser and the resulting N_2 and CO_2 gases were chromatographically separated and run through a Sercon Hydra 20-22 isotopic ratio mass spectrometer. The isotopic standard for carbon was referenced to PeeDee Belem-

nite, and the isotopic standard for nitrogen to atmospheric air. All isotope ratios were expressed in parts per thousand (‰) as differences in isotopic proportions of the sample and the standard, where R_{sample} and $R_{standard}$ are the isotope ratios of the sample and the standard, respectively.

$$(1) \quad \delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

Statistical analysis

Mean intensity of infection (±SE) for each parasite type was calculated following Bush et al. (1997) and this was a calculation of the total number of parasites of a particular species divided by the number of hosts infected with that parasite (Supplementary Table S1). Mean values of $\delta^{15}N$ and $\delta^{13}C$ (±SE) for fish muscle, gill, and stomach tissue types, and associated parasites, were calculated (Table 1).

The discrimination factors ($\Delta^h E$) of parasite and hosts were estimated by subtracting isotope signatures of host tissues from the signatures of parasites: host muscle tissue from cestodes, host gill tissue from copepods, and host stomach wall from both digeneans and nematodes. Separate discrimination factors were also calculated for each parasite group compared with host muscle to allow for comparison of discrimination values against previous studies (Deudero et al. 2002; Power and Klein 2004; Huston et al. 2021) (Table 1).

$$(2) \quad \Delta^h E = \delta^h E_{parasite} - \delta^h E_{host}$$

All statistical analyses were undertaken using RStudio (RStudio Team 2022). Data (Supplementary Table S5) were assessed for normality using Shapiro–Wilk test (Supplementary Table S4) and a graphical visualization (QQ plots). Normal data were assessed using a Pearson's correlation and not-normal data were assessed using Spearman's correlation. A general linear model was generated using the lme4 package (Bates et al. 2015) to test whether there were significant differences ($p < 0.05$) between $\delta^{15}N$ signatures and $\delta^{13}C$ signatures between host tissue and parasites (Supplementary Table S2). Differences in trophic level (ΔTL) between *Protonibea diacanthus* and parasites were calculated according to the following equation (Gilbert et al. 2020*a*):

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

For estimation of the trophic position of each parasite relative to *Protonibea diacanthus*, the average trophic enrichment factor (TEF = 3.4‰) was used, based on a mean of variable measurements ranging from 1.3‰ to 5.3‰ (Gilbert et al. 2020*a*). This number is related to the nitrogen compounds that are transferred from the host to the parasite (Barranco et al. 2020). This was applied for isotope signatures between parasites and the host tissues from which they were collected, and between parasites and host muscle (Supplementary Table S3).

Results

All parasite types were found to infect all 17 fish sampled, with the exception of one fish that was not infected with copepods. Nematodes had the highest mean intensity of infection in the fish sampled at 41.5 (±11.7), with cestodes and digeneans recording the next highest infections in fish at 33.4 (±3.3) and 23.9 (±5.9), respectively (Supplementary Table S1). The ectoparasitic copepods had the lowest mean intensity at 8.9 (±1.2) (Supplementary Table S1). The isotopic value of ^{15}N from the muscle tissue was consistently higher than the values in both gill and stomach tissues; however, the opposite was reported for ^{13}C, with signatures from fish muscle much lower than those values from fish gill and stomach tissues (Table 1 and Fig. 1). Cestodes had the highest difference in nitrogen composition compared against host muscle, with a significant isotopic depletion of 6.0‰ ($F_{[1,32]} = 457.40$; $p < 0.0001$; Table 1 and Supplementary Table S2). Cestodes had a significant isotopic carbon enrichment compared with host muscle ($F_{[1,30]} = 22.08$; $p < 0.0001$; Supplementary Table S2). Nematodes had a significant isotopic depletion in nitrogen compared against both stomach wall ($F_{[1,14]} = 25.12$; $p = 0.0002$; Table 1 and Supplementary Table S2) and muscle ($F_{[1,14]} = 31.35$; $p < 0.0001$; Table 1 and Supplementary Table S2), although the level of depletion was less for the stomach wall. Similarly, nematodes were significantly depleted in carbon in comparison to both stomach wall ($F_{[1,14]} = 20.99$; $p = 0.0004$; Table 1 and Supplementary Table S2) and muscle ($F_{[1,14]} = 6.04$; $p = 0.0276$; Table 1 and Supplementary Table S2). Digeneans had a nitrogen signature equivalent to that of the host stomach tissue, but differed slightly from the carbon signatures of host stomach tissue, with a depletion of 1.7‰ (Table 1). Copepods were the only parasite to be isotopically enriched when compared with host nitrogen values, with a significant ^{15}N enrichment of 1.2‰ ($F_{[1,29]} = 6.88$; $p = 0.0138$; Table 1 and Supplementary Table S2). Copepods were significantly depleted in ^{13}C when compared with host gill tissue ($F_{[1,30]} = 57.88$; $p < 0.0001$; Table 1 and Supplementary Table S2).

Discussion

To our knowledge, this is one of the first studies to use stable isotope analysis to examine the trophic ecology of parasites associated with tropical predatory marine fish in Australia. The present study showed *Protonibea diacanthus* to reside at a higher trophic level than endoparasites, although this was not the case for the ectoparasitic copepods that were 0.35 trophic levels above the host (Supplementary Table S3), an enrichment which, although low, is similar to that of a predator (Nachev et al. 2017; Thieltges et al. 2019).

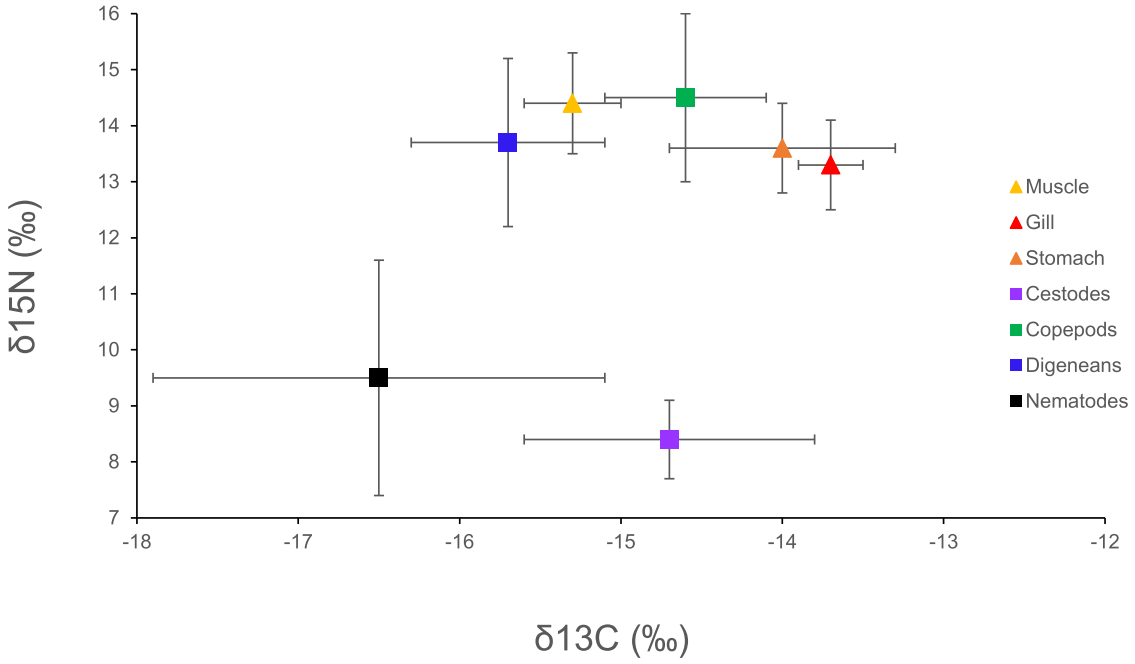
Adult nematodes extracted from the stomach and/or intestinal system of various marine fish were depleted in ^{15}N compared with host tissues (Table 2) (Iken et al. 2001; Deudero et al. 2002), similar to the nematodes in the present study. Adult parasites that inhabit the gastrointestinal tract of their host may acquire nutrients from digesta that has already been processed by the host. For parasites inhabiting the stomach, such as the cucullanid nematodes in this study,

Table 1. Stable isotope composition of host tissues and parasites, including fractionation patterns.

	Isotope signatures $\delta^{15}N$				Isotope signatures $\delta^{13}C$				$\Delta^{h}E$ (parasite-tissue)		$\Delta^{h}E$ (parasite-muscle)	
	Mean (±SE)	SD	Min.	Max.	Mean (±SE)	SD	Min.	Max.	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$
Muscle	14.4 (±0.2)	0.9	12.6	15.9	-15.3 (±0.1)	0.3	-15.7	-14.9				
Gill	13.3 (±0.2)	0.8	12.1	14.7	-13.7 (±0.1)	0.2	-14.0	-13.2				
Stomach	13.6 (±0.2)	0.8	12.4	15.0	-14.0 (±0.2)	0.7	-15.1	-12.5				
Cestodes	8.4 (±0.2)	0.7	7.3	9.5	-14.7 (±0.2)	0.9	-17.0	-12.4	-6.0*	0.6*	NA	NA
Copepods	14.5 (±0.4)	1.5	12.9	17.2	-14.6 (±0.1)	0.5	-15.3	-13.7	1.2*	-0.9*	0.1	0.7*
Digeneans	13.7 (±0.4)	1.5	11.1	16.5	-15.7 (±0.1)	0.6	-16.8	-15.0	0.1	-1.7*	-0.7	-0.4
Nematodes	9.5 (±0.8)	2.1	7.0	12.6	-16.5 (±0.5)	1.4	-18.4	-14.4	-4.1*	-2.5*	-4.9*	-1.2*

*Values are statistically significant ($p < 0.05$) (see Supplementary Table S2).

Fig. 1. Mean isotope ratios and standard deviations (SD) for $\delta^{15}N$ and $\delta^{13}C$ of the black-spotted croaker (*Protonibea diacanthus*) tissues and parasites.



in addition to ingestion of elements that have been previously processed by the host, they are using the food before it is purged into the intestine (Iken et al. 2001). Hence, the isotopic signatures of the nematodes and the host fish will likely differ either as a result of specific enzyme properties or selective feeding of the parasite on the available food, meaning that signatures seen in adult nematodes may in fact coincide more closely with signatures of prey items present in the fish's stomach.

The digeneans in our study showed very low ^{15}N discrimination values, which were similar to the host tissue values: these findings are in agreement with other studies that recorded neutral values for digenean species (Table 2) (Iken et al. 2001; Huston et al. 2021). The digeneans examined in this study inhabit the pyloric caecae, which are found immediately posterior to the stomach. The food passing through, therefore, has already been partially enzymatically processed by the fish, so parasites will assimilate host-metabolized nitrogen likely derived from the host diet (Iken et al. 2001; Kmentová et al. 2020; Riekenberg et al. 2021), as evidenced by the similar trophic level of digeneans and *Protonibea diacanthus*. The plerocercoids in this study were isotopically depleted in nitrogen compared with *Protonibea diacanthus*. This pattern of depletion was also shown between the encysted plerocercoids *Triaenophorus nodulosus* (Pallas, 1781) Rudolphi, 1793 and *Schistocephalus solidus* (Müller, 1776) Steenstrup, 1857, and their respective fish hosts (Table 2) (Pinnegar et al. 2001; Behrmann-Godel and Yohannes 2015). Despite host tissue responses to plerocercoids, studies suggest that the cyst wall in which these organisms become encapsulated is permeable to certain nutrients and that absorption of simple molecules (such as amino acids, fatty acids, etc.) is possible (Kanaya et al. 2019). This process, known as pinocytosis, has been described in the tegument of plerocercoids (Barrett 1981), and hinders the absorption of certain compounds and nutrients, including stable isotopes, ultimately leading to isotopic depletion against host tissues (Maule and Marks 2006; Behrmann-Godel and Yohannes 2015)

Table 2. Summary of stable isotope ratios (δ^{15}N and δ^{13}C) for parasite–host relationships.

Parasite taxon	Host	Site of infection	Parasite feeding strategy	^{15}N	^{13}C	Reference
Nematoda	*Protonibea diacanthus*	Gastrointestinal system	Ingestion of host digesta	Depleted[a] Depleted[b]	Depleted[a] Depleted[b]	Present study
Nematoda	*Chalinura profundicola*	Gastrointestinal system	Ingestion of host digesta	Depleted	Depleted	Iken et al. (2001)
Nematoda	*Coryphaenoides armatus*	Gastrointestinal system	Ingestion of host digesta	Depleted	Enriched	Iken et al. (2001)
Nematoda	*Gadus morhua* and *Merlangius merlangus*	Gastrointestinal system	Ingestion of host digesta	Depleted	Depleted	Deudero et al. (2002)
Digenea	*Protonibea diacanthus*	Pyloric caeca	Ingestion of host digesta	Depleted[a] Similar[b]	Depleted[a] Similar[b]	Present study
Digenea	*Chalinura leptolepsis*	Gastrointestinal system	Ingestion of host digesta	Similar	Similar	Iken et al. (2001)
Digenea	*Kyphosus bigibbus*	Gastrointestinal system	Ingestion of host digesta	Similar	Depleted	Huston et al. (2021)
Cestoda plerocercoid	*Protonibea diacanthus*	Peritoneal cavity and gill arches	Selective absorption	Depleted[b]	Similar[b]	Present study
Cestoda plerocercoid	*Perca fluviatilis*	Liver	Selective absorption	Depleted	Depleted	Behrmann-Godel and Yohannes (2015)
Cestoda plerocercoid	*Gasterosteus aculeatus*	Peritoneal cavity	Selective absorption	Depleted	Similar	Pinnegar et al. (2001); Power and Klein (2004)
Cestoda plerocercoid	*Gadus ogae*	Musculature	Selective absorption	Depleted	Similar	Power and Klein (2004)
Cestoda adult	*Salvelinus fontinalis*	Intestine	Selective absorption	Depleted	Similar	Power and Klein (2004)
Cestoda adult	*Salmo salar*	Intestine	Selective absorption	Depleted	Enriched	Persson et al. (2007)
Copepoda	*Protonibea diacanthus*	Gill filament	Blood feeding	Enriched[a] Similar[b]	Depleted[a] Enriched[b]	Present study
Copepoda	*Coryphaenoides armatus*	Gill filament	Blood feeding	Enriched	Similar	Iken et al. (2001)
Copepoda	*Acanthurus bahianus*	Gill filament	Blood feeding	Enriched*	Depleted*	Jenkins et al. (2020)
Copepoda	*Acanthurus coeruleus*	Gill filament	Blood feeding	Enriched*	Enriched*	Jenkins et al. (2020)
Copepoda	*Gadus morhua* and *Merlangius merlangus*	Gill filament and musculature	Blood feeding	Depleted	Depleted	Deudero et al. (2002)

[a]Values compared with respective host tissue (see the "Materials and methods" section). [b]Values compared with host muscle. *Value compared against host blood not host tissue.

The copepods, which are blood-feeding ectoparasites attached to the gill filaments of the host, were ^{15}N-enriched relative to host gill tissue. This finding correlates with the feeding pattern of the parasite and corroborates stable ^{15}N fractionation patterns in other blood-feeding ectoparasites (Table 2) (Deudero et al. 2002; Sures et al. 2019; Jenkins et al. 2020). In contrast to our *a priori* expectations that any parasite feeding directly from host tissues should be enriched in ^{15}N compared with the host (as would be the case in a classic predator–prey relationship), copepods and many other blood-feeding ectoparasites infecting marine fish have been reported to exhibit variable isotopic patterns (Deudero et al. 2002; Demopoulos and Sikkel 2015; Gilbert et al. 2020*a*). Ectoparasites, such as copepods, primarily feed on whole blood and although an essential source of nutrients, ectoparasites cannot directly uptake amino acids and instead must break down complex blood proteins via transamination. This biochemical process, and the absence of fish whole blood available for analysis in this study, would explain why some ectoparasites are enriched in ^{15}N with respect to their host (Table 2) (Jenkins et al. 2020). However, enrichment patterns can be dissimilar and can, in fact, be adjusted according to nutrient availability and time of extraction, as the isotopic values of progressing parasite life stages and long-term residents approach those of their hosts (Deudero et al. 2002; Dean et al. 2011; Hernández-Arciga et al. 2016). A study focusing on a blood-feeding copepod reported similar patterns of ^{15}N enrichment (Table 2) (Iken et al. 2001) as those recorded with the *Lernanthropus paracruciatus* copepods. This pattern was also reported from the copepod *Caligus atromaculatus* Wilson C.B., 1913 (Jenkins et al. 2020). *Clavella adunca* (Strøm, 1762), a copepod that embeds into host musculature, highlighted the variable isotopic relationships possible between ectoparasites and hosts reporting an isotopic depletion compared with hosts (Table 2) (Deudero et al. 2002). Despite previous studies describing these variable relationships, blood-feeding copepods infecting marine fish are most consistently expressing enrichment patterns similar to that of a predator.

The analysis of parasite carbon isotope signatures can also be effective in food web studies, allowing researchers to identify the specific diet source of carbon that the parasite has

consumed (Fry 2006; Sabadel et al. 2019; Gilbert et al. 2020*b*; Sabadel and MacLeod 2022). The isotopic turnover rates, and subsequent signatures of carbon (δ^{13}C), are used as tracers of diet sources within an organism, or its host (Vander Zanden et al. 1997; Guillemin et al. 2022). The nematodes and digeneans, both collected from the gastrointestinal tract, showed ^{13}C depletion when compared with both stomach and muscle tissues from *Protonibea diacanthus*. Carbon depletion was also reported from the intestinal digenean *Enenterum* sp. Linton, 1910 compared with host muscle (Table 2) (Huston et al. 2021). One study from Iken et al. (2001) reported nematodes to have either carbon depletion or carbon enrichment against host muscle, depending on the host from which they were collected (Table 2). Comparison of the diets of the two host fish *Chalinura profundicola* Nybelin, 1957 and *Coryphaenoides armatus* (Hector, 1875) from this study, showed the former (hosts with enriched carbon levels) to have a diet dominated by benthic crustaceans and algae (both with high δ^{13}C values), whereas the latter (carbon-depleted hosts) preferred cephalopods (Iken et al. 2001; Froese and Pauly 2022). Thus, the basis of the host's source of food, and the host's resulting level of dietary carbon, may also influence the relationship between the parasite and the host tissues. In cestodes, there is often a close correspondence between the fatty acid composition of the parasite and its surroundings (Pinnegar et al. 2001; Deudero et al. 2002; Behrmann-Godel and Yohannes 2015) likely explaining why encysted plerocercoids are often reported to have no substantial difference to the carbon signatures expressed by hosts (Table 2).

The current study found a significant difference between parasite and host carbon signatures; however, the differences were consistently low, a finding that would suggest an overall similarity between parasite and host isotope signatures. Many studies have also reported this pattern, with the larval plerocercoids *Ligula intestinalis* (Linnaeus, 1758) Gmelin, 1790 and *Schistocephalus solidus,* both from the peritoneal cavity of their freshwater hosts, representing cases of insignificant differences in host–parasite carbon signatures (Table 2) (Pinnegar et al. 2001; Deudero et al. 2002; Power and Klein 2004). Larval stages, such as in this study, are encysted within host tissues and may be closer in physiology to larval digenean metacercaria, than to adult cestodes, with studies of adult cestodes reporting inconsistent isotopic carbon signatures against host tissues (Table 2) (Power and Klein 2004; Persson et al. 2007). Larval digenean metacercaria, such as those from freshwater fish hosts *Lepomis macrochirus* Rafinesque, 1819 (Centrarchidae) and *Lepomis gibbosus* (Linnaeus, 1758) (Centrarchidae), reported an isotopic depletion relative to host tissues (Zhang et al. 2018; Kanaya et al. 2019; Huston et al. 2021), a finding that is consistent with parasites that obtain nutrients via absorption. The cyst wall that both plerocercoids and digenean metacercaria become encapsulated acts as a diffusion barrier, ultimately hindering these organisms from the direct uptake of certain nutrients, resulting in depletion of stable isotopes (Behrmann-Godel and Yohannes 2015)

The copepods reported in this study showed significant levels of ^{13}C depletion with respect to the hosts gills. This significant relationship was not observed against host muscle, which rather highlighted variable carbon signatures when compared with the copepods, showing that location of infection is important to consider when determining the biological significance of these results. The ^{13}C depletion observed in copepods in this study is in line with expectations surrounding blood-feeding parasites and the high lipid content of their diets. Lipid synthesis discriminates against ^{13}C in favour of the lighter isotope ^{12}C and because of this, fish tissues that contain a large amount of lipid tend to be depleted in the heavier carbon isotope with respect to other tissues (Deudero et al. 2002; Sabadel et al. 2022). Fish blood and liver possess a relatively high lipid content and thus these tissues are typically ^{13}C depleted. Parasites feeding on these lipid-rich tissues are therefore expected to be depleted in ^{13}C. Patterns in this study are consistent with other blood-feeding parasites in marine systems, including the copepod *Lernaeocera branchialis* (Linnaeus, 1767) that has been shown to cause significant reductions in fat content of infected marine hosts (Table 2) (Khan 1988; Deudero et al. 2002). Patterns of both carbon depletion and enrichment have been observed between the copepod *Caligus atromaculatus* and its two marine hosts; however, these findings are not comparable as signatures were compared with host blood rather than tissues (Table 2) (Jenkins et al. 2020). The copepods in our study, with the isotopic enrichment observed, and blood-feeding strategy, reside at a higher trophic level than the host.

The growing body of literature showing a lack of a consistent pattern in isotopic signatures between parasites and their hosts indicates that parasites are trophically diverse and adaptive, and these organisms should be viewed in greater detail as having unique characteristics within food webs (Iken et al. 2001; Taccardi et al. 2020). Fundamental information regarding the trophic ecology of parasitic relationships remains relatively scarce across taxa and the findings of our study reiterate that parasites are fundamental components of ecosystems and should not be ignored (Marcogliese and Cone 1997; Sukhdeo 2012; Dunne et al. 2013; Taccardi et al. 2020).

This study supports previous literature, suggesting that neither δ^{15}N nor δ^{13}C patterns in fish host–parasite relationships conform to the long-assumed standards that all parasites behave as predators and survive at the cost of their host (Taccardi et al. 2020). Such a pattern exemplifies the need for careful consideration of the life-cycle stage and species-specific biology when examining trophic relationships in parasites, and additional consideration into host factors such as biology and potential environmental influence should be addressed. Given the variable patterns of isotope discrimination presented here, there is a need for future studies that examine specific factors that influence discrimination of stable isotopes between parasites and their hosts.

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Data availability

All data produced for this study are provided in the manuscript. Electronic supplementary material and raw data are available on the online version (Supplementary Materials 1, 2, 3, 4, and 5).

Author information

Author ORCIDs

Megan Porter https://orcid.org/0000-0003-1404-1391

Author contributions

Conceptualization: MP, DPB, JR
Data curation: MP
Formal analysis: JR
Funding acquisition: MP, JR
Investigation: MP
Methodology: MP
Resources: MP
Supervision: DPB, SS
Writing – original draft: MP
Writing – review & editing: MP, DPB, SS, DAC, JR

Competing interests

The authors declare that there are no competing interests.

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Ethics approval

Ethics approval for this study was provided by The Charles Darwin University (CDU) Animal Ethics Committee (AEC), approval number #A19009.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/CJZ-2022-0126.

References

Arias-Gonzalez, J.E., and Morand, S. 2006. Trophic functioning with parasites: a new insight for ecosystem analysis. Mar. Ecol. Prog. Ser. (Halstenbek), **320**: 43–53. doi:10.3354/meps320043.

Barranco, V.S., Van der Meer, M.T.J., Kagami, M., Van den Wyngaert, S., Van de Waal, D.B., Van Donk, E., and Gsell, A. 2020. Trophic position, elemental ratios and nitrogen transfer in a planktonic host–parasite–consumer food chain including a fungal parasite. Oecologia, **194**(4): 541–554. doi:10.1007/s00442-020-04721-w. PMID: 32803339.

Barrett, J. 1981. Biochemistry of Parasitic Helminths, Red Globe Press, London.

Barton, D.P. 2018. Notes on the diet of the black-spotted croaker (*Protonibea diacanthus*) across northern Australia. North. Territ. Nat. **28**: 61–69.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**(1): 1–48. doi:10.18637/jss.v067.i01.

Behrmann-Godel, J. and Yohannes, E. 2015. Multiple isotope analyses of the pike tapeworm *Triaenophorus nodulosus* reveal peculiarities in consumer–diet discrimination patterns. J. Helminthology **89**(2): 238–243. doi:10.1017/S0022149X13000849.

Bush, A.O., Lafferty, K.D., Lotz, J.M., and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. **83**(4): 575–583. doi:10.2307/3284227. PMID: 9267395.

Dean, S., DiBacco, C., and McKinley, R.S. 2011. Assessment of stable isotopic signatures as a means to track the exchange of sea lice (*Lepeophtheirus salmonis*) between host fish populations. Can. J. Fish. Aquat. Sci. **68**(7): 1243–1251. doi:10.1139/f2011-039.

Demopoulos, A.W.J., and Sikkel, P.C. 2015. Enhanced understanding of ectoparasite-host trophic linkages on coral reefs through stable isotope analysis. Int. J. Parasitol. Parasites Wildl. **4**(1): 125–134. PMID: 25830112.

Deudero, S., Pinnegar, J.K., and Polunin, N.V.C. 2002. Insights into fish host–parasite trophic relationships revealed by stable isotope analysis. Dis. Aquat. Organ. **52**(1): 77–86. doi:10.3354/dao052077. PMID: 12517008.

Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., et al. 2013. Parasites affect food web structure primarily through increased diversity and complexity. PLoS Biol. **11**(6): e1001579. doi:10.1371/journal.pbio.1001579. PMID: 23776404.

Froese,, R., and Pauly, D. (*Editors*). 2022. Fishbase. World wide web electronic publication [online]. Available from www.fishbase.org.

Fry, B. 2006. Stable isotope ecology. Springer, New York, NY.

Gilbert, B.M., Nachev, M., Jochmann, M.A., Schmidt, T.C., Köster, D., Sures, B., and Avenant-Oldewage, A. 2020a. 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. Int. J. Parasitol. Parasites Wildl. **12**: 134–141. PMID: 32547919.

Gilbert, B.M., Nachev, M., Jochmann, M.A., Schmidt, T.C., Köster, D., Sures, B., and Avenant-Oldewage, A. 2020b. You are how you eat: differences in trophic position of two parasite species infecting a single host according to stable isotopes. Parasitol. Res. **119**(4): 1393–1400. doi:10.1007/s00436-020-06619-1. PMID: 32030511.

Guillemin, T.A., Pepperell, J.G., Gaston, T., and Williamson, J.E. 2022. Deciphering the trophic ecology of three marlin species using stable iso-

tope analysis in temperate waters off southeastern Australia. Front. Mar. Sci. **9**. doi:10.3389/fmars.2022.795436. PMID: 35450130.
Hernández-Arciga, U., Herrera M., L.G., and Morales-Malacara, J.B. 2016. Tracking host use by bat ectoparasites with stable isotope analysis. Can. J. Zool. **94**(5): 353–360. doi:10.1139/cjz-2015-0246.
Huston, D.C., Cribb, T.H., and Welicky, R.L. 2021. Stable isotope signatures of an acanthocephalan and trematode from the herbivorous marine fish *Kyphosus bigibbus* (Perciformes: Kyphosidae). J. Parasitol. **107**(5): 726–730. doi:10.1645/21-29. PMID: 34534332.
Iken, K., Brey, T., Wand, U., Voigt, J., and Junghans, P. 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog. Oceanogr. **50**(1–4): 383–405. doi:10.1016/S0079-6611(01)00062-3.
Jenkins, W.G., Demopoulos, A.W.J., Nicholson, M.D., and Sikkel, P.C. 2020. Stable isotope dynamics of herbivorous reef fishes and their ectoparasites. Diversity (Basel), **12**(11): 429. doi:10.3390/d12110429.
Kamiya, E., Urabe, M., and Okuda, N. 2020. Does atypical ^{15}N and ^{13}C enrichment in parasites result from isotope ratio variation of host tissues they are infected? Limnology, **21**(1): 139–149. doi:10.1007/s10201-019-00596-w.
Kanaya, G., Solovyev, M.M., Shikano, S., Okano, J., Ponomareva, N.M., and Yurlova, N.I. 2019. Application of stable isotopic analyses for fish host–parasite systems: an evaluation tool for parasite-mediated material flow in aquatic ecosystems. Aquat. Ecol. **53**(2): 217–232. doi:10.1007/s10452-019-09684-6.
Khan, R.A. 1988. Experimental transmission, development, and effects of a parasitic copepod, *Lernaeocera branchialis*, on Atlantic cod, *Gadus morhua*. J. Parasitol. **74**(4): 586–599. doi:10.2307/3282174. PMID: 3397819.
Kmentová, N., Bray, R.A., Koblmüller, S., Artois, T., De Keyzer, E.L.R., Gelnar, M., et al. 2020. Uncharted digenean diversity in Lake Tanganyika: Cryptogonimids (Digenea: Cryptogonimidae) infecting endemic lates perches (Actinopterygii: Latidae). Parasit. Vectors, **13**(1): 221–224.
Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., et al. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. Nature (London), **454**(7203): 515–518. doi:10.1038/nature06970.
Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., et al. 2008. Parasites in food webs: the ultimate missing links. Ecol. Lett. **11**(6): 533–546. doi:10.1111/j.1461-0248.2008.01174.x. PMID: 18462196.
Lafferty, K.D., Dobson, A.P., and Kuris, A.M. 2006. Parasites dominate food web links. Proc. Natl. Acad. Sci. U.S.A. **103**(30): 11211–11216. doi:10.1073/pnas.0604755103.
Lõhmus, M., and Björklund, M. 2015. Climate change: what will it do to fish–parasite interactions? Biol. J. Linn. Soc. **116**(2): 397–411. doi:10.1111/bij.12584.
Marcogliese, D.J., and Cone, D.K. 1997. Food webs: a plea for parasites. Trends Ecol. Evol. (Amsterdam), **12**(8): 320–325.
Maule, A.G., and Marks, N.J. 2006. Parasitic flatworms: molecular biology, biochemistry, immunology and physiology. CABI, Wallingford, UK.
Nachev, M., Jochmann, M.A., Walter, F., Wolbert, J.B., Schulte, S.M., Schmidt, T.C., and Sures, B. 2017. Understanding trophic interactions in host–parasite associations using stable isotopes of carbon and nitrogen. Parasit. Vectors, **10**(1).
Persson, M.E., Larsson, P., and 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**(2): 441–452. doi:10.1111/j.1095-8649.2007.01500.x.
Pinnegar, J.K., Campbell, N., and Polunin, N.V.C. 2001. Unusual stable isotope fractionation patterns observed for fish host–parasite trophic relationships. J. Fish Biol. **59**(3): 494–503.
Power, M., and 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**(4): 257–266. doi:10.1080/10256010410001678062. PMID: 15621744.
Ramcharitar, J., Gannon, D.P., and Popper, A.N. 2006. Bioacoustics of fishes of the family Sciaenidae (croakers and drums). Trans. Am. Fish. Soc. **135**(5): 1409. doi:10.1577/T05-207.1.
Riekenberg, P.M., Briand, M.J., Moléana, T., Sasal, P., van der Meer, M.T.J., Thieltges, D.W., and Letourneur, Y. 2021. Isotopic discrimination in helminths infecting coral reef fishes depends on parasite group, habitat within host, and host stable isotope value. Sci. Rep. **11**(1): 4638. doi:10.1038/s41598-021-84255-0. PMID: 33633261.
RStudio Team. 2022. RStudio: integrated development environment for R. PBC, Boston, MA.
Sabadel, A.J.M. Stumbo, A.D. and Macleod, C.D. 2019. Stable-isotope analysis: a neglected tool for placing parasites in food webs. J. Helminthology **93**: 1–7. doi:10.1017/s0022149x17001201
Sabadel, A.J.M., and MacLeod, C.D. 2022. Stable isotopes unravel the feeding mode–trophic position relationship in trematode parasites. J. Anim. Ecol. **91**(2): 484–495. doi:10.1111/1365-2656.13644. PMID: 34860441.
Sabadel, A.J.M., Cresson, P., Finucci, B., and Bennett, J. 2022. Unravelling the trophic interaction between a parasitic barnacle (*Anelasma squalicola*) and its host Southern lanternshark (*Etmopterus granulosus*) using stable isotopes. Parasitology, **149**(14): 1976–1984. doi:10.1017/S0031182022001299. PMID: 36076261.
Sukhdeo, M.V.K. 2012. Where are the parasites in food webs? Parasit. Vectors, **5**(1): 239.
Sures, B., Nachev, M., Gilbert, B.M., Santos, D., Q., M., Jochmann, M.A., et al. 2019. The monogenean *Paradiplozoon ichthyoxanthon* behaves like a micropredator on two of its hosts, as indicated by stable isotopes. J. Helminthol. **93**(1): 71–75. doi:10.1017/S0022149X17001195. PMID: 29785892.
Taccardi, E.Y., Bricknell, I.R., and Byron, C.J. 2020. Stable isotopes reveal contrasting trophic dynamics between host–parasite relationships: a case study of Atlantic salmon (*Salmo salar*) and parasitic lice (*Lepeophtheirus salmonis* and *Argulus foliaceus*). J. Fish Biol. **97**(6): 1821–1832. doi:10.1111/jfb.14546. PMID: 32944965.
Taillebois, L., Barton, D.P., Crook, D.A., Saunders, T., Taylor, J., Hearnden, M., et al. 2017. Strong population structure deduced from genetics, otolith chemistry and parasite abundances explains vulnerability to localized fishery collapse in a large sciaenid fish, *Protonibea diacanthus*. Evol. Appl. **10**(10): 978–993. doi:10.1111/eva.12499. PMID: 29151854.
Thieltges, D.W., Goedknegt, M.A., O'Dwyer, K., Senior, A.M., and Kamiya, T. 2019. Parasites and stable isotopes: a comparative analysis of isotopic discrimination in parasitic trophic interactions. Oikos, **128**(9): 1329–1339. doi:10.1111/oik.06086.
Thompson, R.M., Mouritsen, K.N., and 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**(1): 77–85. doi:10.1111/j.1365-2656.2004.00899.x.
Vander Zanden, M.J., Cabana, G., and 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**(5): 1142–1158. doi:10.1139/f97-016.
Zhang, L., Vanderhorst, K., Kyser, K., and 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**(4): 1043–1049. doi:10.1007/s00436-018-5781-2.