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Dietary fatty acid analyses of the squid *Idioteuthis cordiformis*: further evidence for predation on deepwater sharks

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ABSTRACT: *Idioteuthis cordiformis* is the largest deepwater mastigoteuthid squid in the southern Pacific Ocean. Signature fatty acid (FA) and lipid class analysis was carried out on the digestive gland, fins and caecum oil of 18 individuals of *I. cordiformis* caught in the waters off southern Australia during late 2004. Lipid classes varied between the tissues and oil samples, with sex not being an important factor. The presence of hydrocarbons within the digestive gland and caecum was noteworthy, as high proportions of this lipid class are generally only common in the livers of many deepwater sharks. Monounsaturated FAs dominated the digestive gland and caecum oil, while the fin had high values of both saturated and polyunsaturated FAs. FA profiles of *I. cordiformis* were compared to profiles of potential prey species (sharks, small and large fish, crustaceans and squid) using Bray-Curtis similarity coefficients. Analysis of the digestive gland and caecum oil FA profiles revealed a close match with the following prey: myctophids (*Lampanyctodes australis*, *Electrona paucirastra*, *Symbolophorus barnardi*), the dragon fish *Stomias boa*, the smooth oreo *Pseudocyttus maculatus* and the deepwater sharks *Etmopterus baxter*, *Dalatias licha*, *Centroselachus crepidater*, *Centroscymnus coelopsis* and *Centrophorus zeehaani*. The fin FA profile did not match closely to any potential prey and was most similar to other squid mantle tissue. Based on the results of this study, *I. cordiformis* has a broad diet spectrum of teleost fish and deepwater sharks and occupies a high trophic position.

KEY WORDS: Mastigoteuthidae · Cephalopod · Fatty acid · Squid · Diet

1. INTRODUCTION

Cephalopod growth and population levels are generally recognized as being robust, with populations responding rapidly to environmental change due to features of their biology and physiology (Jackson & O'Dor 2001, Jackson & Domeier 2003). Moreover, squids occupy the middle of many food chains, thus exerting a significant impact on other elements of the food web: they exert top-down control of prey and are themselves important prey of apical predators and potentially act as keystone species in marine ecosystems (Coll et al. 2013). These features, along with the potential decline of their teleost predators and competitors, have resulted in the continued increase in cephalopod populations (Doubleday et al. 2016), although it appears this increase has levelled off or even declined in recent years (FAO 2020). It is expected that sustained fishing pressure in concert with climate change will continue to favor the expansion of squid populations (Coll et al. 2013). Therefore, cephalopods are deemed important in most marine

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ecosystems and likely play a significant part in shaping ecosystems. However, our understanding of the role of cephalopods, especially in ecosystem models, is lacking in many instances (de la Chesnais et al. 2019). The function of cephalopods in many marine ecosystems is likely more significant than currently understood.

Due to the difficulty in observing deepwater species in their natural environment along with the numerous challenges associated with collecting individuals for study, it is essential to have indirect methods for undertaking trophic analysis. Such methodologies have included isotopic, fatty acid (FA), heavy metal and genetic analyses for collecting such information (Jackson et al. 2007, Hoving et al. 2014, Xavier et al. 2015). These methodologies are especially useful for species for which it is difficult to obtain stomach contents or when stomach contents fail to provide the full picture. FA signature analysis and the quantifying of lipid class data offers a means to infer recent feeding history, trophic position and information needed for ecosystem models. This is based on the fact that dietary FAs can be transferred up the food chain relatively unmodified so that the FA composition of the consumer will reflect the FA composition of the prey (Couturier et al. 2020). Biochemical tracers are particularly helpful in that they integrate the feeding history of individuals over varying time periods. Lipids and FAs have particularly rapid turnover rates and can therefore be used to observe trophic changes over weeks to months rather than months to seasons (Pethybridge et al. 2018). Iverson (2009) highlighted 3 features of FAs that make them particularly suitable as trophic tracers: (1) there are biochemical limitations to how much organisms can modify FAs, as these are generally not degraded and are taken up by tissues in their basic form (especially for more complex organisms like vertebrates higher up the food chain); (2) individual isomers and 'families' of FAs bioaccumulate through the food chain and can be traced back to their origin in the food web; and (3) FAs are typically stored and accumulate over time, thus providing dietary integration as mentioned above.

FA signature analysis has now been routinely applied to a large number of marine species (e.g. Iverson 2009, Parrish et al. 2015, Parzanini et al. 2018, Pethybridge et al. 2018, Couturier et al. 2020). It also provides a means to explore predation on species that would be difficult to study otherwise, such as great white sharks (Pethybridge et al. 2014) and whale sharks (Marcus et al. 2016, Cárdenas-Palomo et al. 2018). FA analysis has also been successfully applied in the dietary analysis of the Southern Ocean squids *Nototoarus goulidi*, *Todarodes filippovae*, *Moroteuthis* (*Moroteuthopsis*) *ingens*, *Moroteuthis* (*Onykia*) *robsoni*, *Gonatus antarcticus* and *Sepioteuthis australis* (Phillips et al. 2001, 2002, Pethybridge et al. 2012, 2013). More recently, FA profiling has been used to explore energy reproductive investment of *Illex argentinus* (Lin et al. 2019) and *Dosidicus gigas* (Chen et al. 2020).

Squids may be especially well suited for dietary studies using FA signature analysis. This suitability is due to their protein-based metabolism in conjunction with their inability to metabolize lipids well and subsequent excretion of excess lipids by the digestive gland (DG) (Semmens 1998, Jackson & O'Dor 2001). Therefore, it is unlikely that squids modify ingested lipids or FAs and consequently will more closely reflect the FA composition of their prey. This would be especially relevant for lipids removed directly from the digestive system such as those found in the caecum.

Mastigoteuthidae are a family of deepwater squid for which the ecology and trophic dynamics are poorly known. Based on the distribution of the vacuoles in the body and arms which provide buoyancy, it has been suggested that mastigoteuthids hover vertically in the water column with their long tentacles hanging passively downward, whereby passing prey may become entangled (Boyle & Rodhouse 2005). Few studies have determined the feeding or trophic interactions of deepwater mastigoteuthids. However, Braid & Bolstad (2014) explored the feeding ecology of *Idioteuthis cordiformis* in New Zealand waters based on 4 specimens. For 2 of these specimens, DNA analysis on gut content analysis suggested predation on the shark *Deania calcea* and the teleost *Lutjanus* sp. This indicates that *I. cordiformis* is potentially a much more active predator than previously suggested. Documentation of a squid preying on a shark is unusual and modifies our understanding of the trophic dynamics of large deepwater squid.

The population of *I. cordiformis* in New Zealand waters appears to have shown a substantial decline resulting from deepwater fishing and it is subsequently uncommonly encountered in New Zealand waters (Freeman et al. 2010, 2013). However, it was regularly captured in deepwater trawls off Tasmania, which formed the basis for the present study. FA signature analysis was especially well suited to this species, given that ingested prey contents were rare and individuals regularly had distended caeca with fluid consisting of a distinct water and lipid component.

The taxonomy of the family Mastigoteuthidae (Braid et al. 2014, 2017), including the genus *Idioteuthis*, requires more investigation. There have been at least 2 species identified from the Southern Ocean and Japan (Braid et al. 2017). In the present study, we follow the taxonomy of Braid et al. (2017) and Braid & Bolstad (2014) and will be referring to the individuals caught in Tasmanian waters off mainland Australia as *I. cordiformis*.

The focus of this study was to undertake lipid class and signature FA analyses to explore the potential diet and trophic history of *I. cordiformis* in waters off Tasmania, Australia. The abundance of oil within the caecum was especially conducive for taking a forensic approach to determine the prey of this large deepwater squid. Furthermore, the previous documentation of this squid potentially preying on sharks in New Zealand waters provided a basis for exploring potential shark prey for this species in Australian waters.

2. MATERIALS AND METHODS

2.1. Specimen collection

Individuals of *Idioteuthis cordiformis* used in this study (5 males, 13 females) were collected off the coast of Southern Tasmania, including in the region near Maatsuyker Island, in October (n = 5), November (n = 5) and December (n = 8) 2004. Individuals ranged in mantle length from 250 to 560 mm and in total weight from 663 to 6890 g (Table 1). Individuals were obtained as bycatch of the deepwater trawler 'Adriatic Pearl', a commercial vessel which targeted orange roughy *Hoplostethus atlanticus*. The gear used was a cut-away wing demersal trawl with a headline height of 6 m. Trawling depth was typically between 700 and 1200 m. All individuals were kept on ice onboard the vessel and frozen after arrival at port and then defrosted in the laboratory at a later date for processing.

2.2. Lipid extraction

Lipid class and FA analyses of *I. cordiformis* DG, caecum fluid and fin were undertaken at the CSIRO Marine Laboratories in Hobart, Tasmania, Australia. DG samples (~1 g) were taken from the mid-region of the DG, after being homogenized with a spoon. Similarly, approximately 11–15 mg of caecum oil and approximately 1 cm^2 of fin were also collected. Once dissected, all samples were subsequently stored in a −80°C freezer prior to lipid extraction.

Quantitative lipid extraction on all samples was undertaken overnight via a modified Bligh & Dyer (1959) one-phase methanol-chloroform-water extraction (2:1:0.8 v/v/v). The following day, chloroform and water were added to separate the phases (final solvent ratio, 1:1:0.9 v/v/v methanol:chloroform:water). Once separation of the upper aqueous and lower chloroform phases was formed, solvents were removed *in vacuo* from the lower chloroform phase by roto-evaporation at ~40°C to recover a total solvent extract (TSE). The TSE was weighed in small glass vials (1.5 ml) and concentrated to dryness via a stream of inert nitrogen gas to obtain total lipid content (% and mg g^{-1} wet mass). The total lipid extract (TLE) was then prepared to a known volume with chloroform and stored at −20°C prior to lipid class and FA analyses.

2.3. Lipid class determination

Lipid classes were determined by spotting in duplicate 1 µl of TLE along with standards of known quantities of wax esters (WEs), hydrocarbons (HCs), triacylglycerols (TAGs), free FAs (FFAs), sterols (STs) and phospholipids (PLs) on silica gel SIII chromarods. A polar solvent system (60:17:0.1 v/v/v hexane: diethyl ether:acetic acid) was used to resolve the

Table 1. Collection data of *Idioteuthis cordiformis* used in lipid class and signature fatty acid analyses. DG: digestive gland; C: caecum oil; F: fin

ID	Sex	Mantle length (mm)	Total weight (g)	Date (2004)	Tissue sampled
201	F	490	5260	22 Oct	DG, C
210	F	325	2320	29 Nov	DG, C
243	F	560	5356	8–12 Dec	DG, C
246	F	560	6840	8–12 Dec	DG, C
249	F	530	4143	8–12 Dec	DG, C
251	F	495	6585	8–12 Dec	DG, C
259	F	355	2077	8–12 Dec	DG, C
260	F	400	3400	8–12 Dec	DG, C
223	F	270	1025	22 Oct	DG, C, F
224	F	430	4420	22 Oct	DG, C, F
225	F	530	4855	22 Oct	DG, C, F
234	F	500	6890	22 Oct	DG, C
244	F	295	1412	29 Nov	DG, C
252	M	250	663	8–12 Dec	DG, C, F
258	M	270	797	8–12 Dec	DG, C
200	M	300	1617	29 Nov	DG
233	M	320	1432	29 Nov	DG, C, F
245	M	280	1058	29 Nov	DG, C, F

lipid classes in the DG, caecum fluid and fin samples. Immediately after drying in an oven for 25 min, samples were analysed by way of an Iatroscan MK V TH10 thin layer chromatography-flame ionization detector (TLC-FID) analyser. For the DG and caecum fluid, a non-polar solvent (96:4 v/v hexane:ether) was also used to separate HCs from WEs and diacylglyceryl ethers from TAGs. DAPA Scientific Software was used to quantify lipid class peaks.

2.4. FA analyses

An aliquot of the TLE was transmethylated with 3 ml of methanol:hydrochloric acid:chloroform (10:1:1, v/v/v) at 100°C for 2 h to generate FA methyl esters (FAMEs). Milli-Q water (1 ml) was then added to separate the FAMEs, which were subsequently extracted using hexane and chloroform (4:1 v/v, 3 × 1.5 ml). A stream of nitrogen gas was used to concentrate the FAMEs which were then silylated by the addition of 50 µl of N-O-bis (trimethylsilyl)-triflouracetamide (BSTFA). After samples were left overnight at 50–60°C, they were reduced under a nitrogen stream the following morning. An internal injection standard (C_{19} FAME) was then added to all the FAME samples.

Gas chromatographic (GC) analyses on FAMEs were performed using an Agilent Technologies 6890N GC with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), a flame ionization detector (FID), a split/splitless injector as well as an Agilent Technologies 7683 auto sampler and injector (Phleger et al. 2005). Quantification of FAME peaks was undertaken using Agilent Technologies Chemstation software. A GC-mass spectrometer (GC-MS) was used to confirm identification of FA components of selected samples for all tissues (DG, caecum fluid and fin). The GC-MS was a Finnigan ThermoQuest GCQ GC-mass spectrometer with a capillary system similar to the GC machine, fitted with an on-column injector which used Thermoquest Scalibur software (Phleger et al. 2005).

2.5. Prey FA comparisons

The FA profiles of potential prey of *I. cordiformis* used in this study were obtained from the literature (Table 2). Possible crustacean, fish and squid prey species that are more commonly consumed by other squid (e.g. Jackson et al. 1998, Nichols et al. 1998, Phillips et al. 2001, Pethybridge et al. 2013) in southeast Australian and Southern Ocean waters as well as other common deepwater species were included. Additionally, *Deania calcea* and *Lutjanus* sp., which were recently identified via DNA barcoding from *I. cordiformis* specimens in New Zealand waters (Braid & Bolstad, 2014), were also included in the analysis. We also chose FA profiles from the livers of other dogfish/sharks containing high relative levels of HCs due to the presence of HCs in some *I. cordiformis* samples.

2.6. Statistical analyses

FAs are expressed as a percentage of total FAs, and only FAs present in greater than trace amounts (0.5%) were incorporated in the analyses. Due to the multivariate nature of the FA and lipid class data, 3-way full factorial permutational multivariate analysis of variance (PERMANOVA) models using 9999 permutations based on a Bray-Curtis resemblance matrix were used to determine differences among the samples using sex and tissue type as fixed factors and individual nested within sex as a random factor. When sex was deemed not an important factor, both the sex × tissue interaction and sex as a main effect were removed one at a time, based on their high p-values and sex having a negative estimate for its component of variation. Simpler models were then run with tissue type as a fixed factor and individual as a random factor. Pairwise comparisons were used to determine differences between the levels of a significant factor when applicable. Significant patterns were then visualized using ordination plots such as non-metric multidimensional scaling (nMDS) or principal coordinates analysis (PCO). SIMPER determined which FA or lipid classes contributed to the similarities or dissimilarities within and between the groups.

The nMDS was also used to visually explore relationships between *I. cordiformis* and potential prey. Only FAs present at ≥1% of the total FAs in *I. cordiformis* were used for prey comparisons. Similarity between individual *I. cordiformis* tissue samples to individual prey species was determined using the highest coefficients in the Bray-Curtis similarity matrix. The percentage of nearest neighbor similarities were then determined for the DG, caecum oil and fin. Subsequently, the average coefficient for each prey species and corresponding *I. cordiformis* tissues were ranked to determine which potential prey species were likely to feature as prey based on tissue type.

Table 2. Potential prey species used in this study for analysis of their lipid classes and fatty acid profiles

Species	Number	Common name/group	Author/source
Shark (liver)			
Centroselachus crepidater	10	Longnose velvet dogfish	Pethybridge et al. (2010a)
Centroscymnus coelopsis	2	Portuguese dogfish	Pethybridge et al. (2010a)
Deania calcea	6	Birdbeak dogfish	Pethybridge et al. (2010a)
Centrophorus zeehaani	10	Southern dogfish	Pethybridge et al. (2010a)
Etmopterus baxteri	10	New Zealand lanternshark	Pethybridge et al. (2010a)
Dalatias licha	3	Kitefin shark	Pethybridge et al. (2010a)
Large fish			
Hoplostethus atlanticus	2	Orange roughy	Bakes et al. (1995)
Macruronus novaezelandiae	3	Blue grenadier	Nichols et al. (1998)
Genypterus blacodes	3	Pink ling	Nichols et al. (1998)
Rexea solandri	3	Gemfish	Nichols et al. (1998)
Pseudocyttus maculatus	5	Smooth oreo	Bakes et al. (1995)
Lutjanus malabaricus	3	Malabar blood snapper	Nichols et al. (1998)
Lutjanus vitta	3	Brownstripe red snapper	Nichols et al. (1998)
Small fish			
Electrona antarctica	3	Myctophid	Phleger et al. (1997)
Lampanyctodes australis	4	Myctophid	Pethybridge et al. (2010b)
Electrona paucirastra	3	Myctophid	Pethybridge et al. (2010b)
Symbolophorus barnardi	4	Myctophid	Pethybridge et al. (2010b)
Stomias boa	3	Scaly dragonfish	Pethybridge et al. (2010b)
Crustaceans			
Euphausia sp.	1	Temperate krill	Pethybridge et al. (2010b)
Systellaspis debilis	2	Caridean shrimp	Pethybridge et al. (2010b)
Acanthephyra sp.	5	Caridean shrimp	Pethybridge et al. (2010b)
Sergia potens	5	Sergestid shrimp	Pethybridge et al. (2010b)
Squid			
Histioteuthis atlantica (whole)	2	Squid	Pethybridge et al. (2010b)
Todarodes filippovae (mantle only)	42	Squid	Pethybridge et al. (2010b)
Octopoteuthis megaptera	3	Squid	Pethybridge et al. (2010b)
Moroteuthis ingens (mantle only Macquarie Island)	6	Squid	Phillips et al. (2001)

All statistical analyses were conducted using Primer v7 software (Primer-E) on untransformed FA data and fourth-root transformed lipid class data. All FA data were standardized by summing all profiles to 100% using sample totals. This allowed comparisons to be independent of varying sample sizes used in different studies from where mean values were obtained. FAs, lipid classes and total lipids are expressed as means ± SD.

3. RESULTS

3.1. Lipid composition

There were large differences in percent total lipid based on tissue type. The caecum oil had the highest lipid content (100%) followed by the DG (mean ± SD: 10.2 ± 2.3%) and the fin (0.8 ± 0.1%) (Table 3).

The percentage composition of the different lipid classes varied widely (Table 3). The main lipid classes in the DG of *Idioteuthis cordiformis* in decreasing order of proportions were FFAs, PLs, TAGs and WEs. The fin was dominated by PLs, whereas the caecum oil was dominated by TAGs and WEs. Levels of FFAs were low in the fin and caecum oil (<1%). However, FFAs contributed a relatively high proportion in the

Table 3. Mean (±SD) percentage lipid class composition (as % of total lipids) and total lipid content (as % of wet weight) of the digestive gland, fin and caecum oil from *Idioteuthis cordiformis* collected from Tasmanian waters. DAGE: diacylglycerol ether; FFA: free fatty acid; HC: hydrocarbon; PL: phospholipid; ST: sterol; TAG: triacylglycerol; WE: wax ester

Lipid class	Digestive gland (n = 18)	Fin (n = 6)	Caecum oil (n = 17)
HC	1.5 ± 3.5	0.0 ± 0.0	4.0 ± 7.3
WE	13.7 ± 6.9	0.0 ± 0.0	18.1 ± 23.6
DAGE	1.0 ± 1.2	0.0 ± 0.0	3.0 ± 6.8
TAG	22.6 ± 12.6	0.0 ± 0.0	72.9 ± 24.3
FFA	30.4 ± 14.6	0.8 ± 0.9	0.4 ± 0.4
ST	3.5 ± 3.1	5.2 ± 0.7	1.6 ± 0.7
PL	27.4 ± 8.0	94 ± 1.3	0.0 ± 0.0
Total lipid	10.2 ± 2.3	0.8 ± 0.1	100

DG (30.4 ± 14.6%), which suggests potential tissue breakdown since collection or by natural catabolism processes occurring in the DG. Although a relatively minor contributor, the presence of HCs in 10 DG (1.5 ± 3.5%) and 9 caecum oil (4.0 ± 7.3%) samples is noteworthy.

Lipid classes showed significant random differences between individuals (PERMANOVA pseudo-F = 5.293, df = 17, 21, p = 0.0003) along with distinct differences between the *I. cordiformis* tissues (DG, caecum and fin) (PERMANOVA pseudo-F = 274.1, df = 2, 21, p = 0.0001). Pairwise comparisons revealed that all tissues were significantly different from one another (t = 10.6–19.2, all p < 0.001).

Although there were significant individual differences, SIMPER revealed high similarities within tissue groups: the fins had the highest group similarity (97%), followed by the DG (88%) and caecum oil (82%). The lipid class patterns which discriminated between the tissue groups can be observed in the nMDS plot. The main discriminating lipid classes (SIMPER) between the fin and caecum oil, which had a dissimilarity of 74%, was due to the fin's abundance of PLs, with lower levels of TAGs and WEs. These lower levels of TAGs and WEs were also the main discriminating lipid classes between the fin and DG (dissimilarity of 48%). Although the DG and caecum oil had the lowest dissimilarity, the higher levels of PLs in the DG and to a lesser extent FFAs helped discriminate between the DG and caecum oil (Fig. 1). The higher levels of HCs in the caecum oil relative to the DG also contributed about 10% to their dissimilarity.

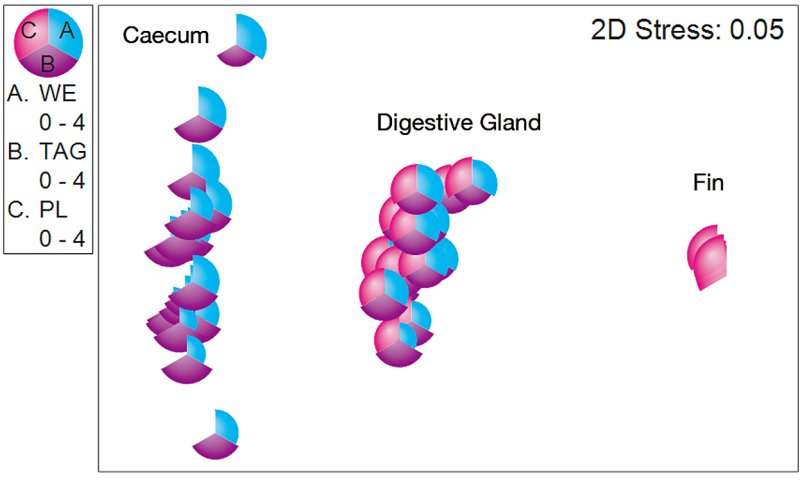

Fig. 1. Nonmetric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarities on fourth-root transformed percentages with a superimposed segmented bubble plot representing the relative amount of wax esters (WE), triacylglycerols (TAG) and polar lipids (PL) in the caecum oil, digestive gland and fin of *Idioteuthis cordiformis* (0–4 represents a fourth-root scale)

3.2. FA profiles

Overall, 25 FAs (accounting for 98–99% of total FAs) were detected in the DG, fin and caecum oil in greater than trace amounts (>0.5%). The following FAs dominated samples in decreasing order of relative abundance in the DG: 20:1ω9/ω11, 18:1ω9c, 22:6ω3, 16:0, 20:5ω3, 22:1ω11/ω13c, 18:1ω7c, 18:0; the fin: 16:0, 22:6ω3, 20:5ω3, 20:1ω9)/ ω11,18:0; and the caecum oil: 18:1ω9c, 20:1ω9/ω11, 16:0, 22:6ω3, 16:1ω7c (Table 4).

Monounsaturated FAs (MUFAs) dominated both the DG (58.9 ± 5%) and caecum oil (63.0 ± 5%), while the fin had high values of both saturated (SAT FAs; 38.0 ± 3.9%) and polyunsaturated FAs (PUFAs; 41.7 ± 5.4%) (Table 4). These FA classes revealed significant individual differences (PERMANOVA pseudo-F = 3.04, df = 17, 21, p = 0.0009) along with distinct differences between the tissues (PERMANOVA pseudo-F = 225.99, df = 2, 21, p = 0.0001). Pairwise comparisons revealed all tissues were different from each other (t = 4.9–13.7, all p < 0.001). The first PCO axis, which accounted for 97% of the variation between the samples, revealed that differences between the tissues were predominantly due to the high correlations between proportions of PUFAs and SAT FAs in the DG and caecum oil, while the fin was correlated with higher levels of MUFAs and lower levels of PUFAs and SAT FAs (Fig. 2).

There were also significant differences between the FA profiles of individuals (PERMANOVA pseudo-F = 3.18, df = 17, 21, p = 0.0001), although SIMPER revealed within-group similarities ranging from 87% for caecum oil to 91% for fins. There were more definite differences between *I. cordiformis* tissues (DG, caecum and fin) (PERMANOVA pseudo-F = 139.12, df = 2, 21, p = 0.0001). Pairwise comparisons indicated that all tissues were significantly different from one another (t = 10.4–10.7, all p < 0.001). SIMPER revealed that the main discriminating FA resulting in dissimilarity between the fin and the DG (43%) and the caecum oil (50%) was the 6- to 9-fold lower abundance of 18:1ω9c in the fin. Moreover, the fin, with an approximate 2- to 3-fold higher abundance of 16:0 and 20:5ω3 (eicosapentaenoic acid, EPA) and a 2-fold

Table 4. Mean (±SD) fatty acid (FA) composition (% of total FA) of digestive gland, fin and caecum oil from *Idioteuthis cordiformis*. SAT: saturated fatty acid; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; c: cis-configured; br: branched chain; i: iso branched; a: anteiso branched

Fatty acid	Digestive gland (n = 18)	Fin (n = 6)	Caecum oil (n = 17)
14:0	0.9 ± 0.3	1.8 ± 0.2	1.6 ± 0.3
16:0	10.7 ± 1.4	28.4 ± 3.1	9.9 ± 1.7
17:0	0.5 ± 0.1	0.9 ± 0.2	0.3 ± 0.1
18:0	4.4 ± 0.7	6.3 ± 0.6	1.7 ± 0.3
Total SAT	**17.2 ± 2.2**	**38.0 ± 3.9**	**14.4 ± 2.1**
16:1ω7c	3.4 ± 0.8	0.9 ± 0.2	7.9 ± 1.7
16:1ω9	3.2 ± 0.6	0.2 ± 0.2	0.6 ± 0.2
17:1ω8+a17:0	0.6 ± 0.1	0.0 ± 0.0	1.1 ± 0.2
18:1ω5c	0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.2
18:1ω7c	4.6 ± 0.7	2.7 ± 0.5	4.8 ± 0.8
18:1ω9c	18.1 ± 2.7	2.7 ± 0.4	27.7 ± 2.8
19:1	0.7 ± 0.2	0.2 ± 0.2	0.5 ± 0.2
20:1ω7	0.8 ± 0.1	0.2 ± 0.2	0.8 ± 0.2
20:1ω9/ω11	18.2 ± 2.8	9.0 ± 2.3	11.7 ± 4.2
22:1ω9c	3.1 ± 0.8	2.8 ± 0.3	1.8 ± 1.0
22:1ω11ω13c	4.9 ± 2.2	0.6 ± 0.5	4.8 ± 2.6
24:1ω9c	0.9 ± 0.4	0.5 ± 0.1	0.9 ± 0.4
Total MUFA	**58.9 ± 5.0**	**20.1 ± 3.0**	**63.0 ± 5.0**
18:2ω6	0.4 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
20:2ω6	0.7 ± 0.1	0.7 ± 0.1	0.3 ± 0.1
18:4ω3	0.0 ± 0.1	0.0 ± 0.0	0.6 ± 0.3
20:4ω3	0.9 ± 0.2	0.0 ± 0.0	1.1 ± 0.3
20:4ω6 AA	2.1 ± 0.6	1.1 ± 0.1	1.1 ± 0.5
20:5ω3 EPA	6.0 ± 1.6	17.3 ± 1.9	5.5 ± 2.0
22:5ω3	1.2 ± 0.3	0.0 ± 0.0	1.0 ± 0.2
22:6ω3 DHA	10.8 ± 2.7	20.7 ± 3.6	8.9 ± 2.4
Total PUFA	**22.9 ± 4.9**	**41.7 ± 5.4**	**20.5 ± 5.0**
br17:1	0.5 ± 0.1	0.0 ± 0.0	1.4 ± 1.8
18:1ω7/18:1ω9	0.3 ± 0.0	10. ± 0.1	0.2 ± 0.0
EPA/DHA	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.2
16:1ω7/16:00	0.3 ± 0.1	0.0 ± 0.0	0.8 ± 0.3
ω3/ω6	5.3 ± 0.8	15.3 ± 2.2	6.9 ± 1.4
Others[a]	2.0 ± 0.1	1.2 ± 0.1	1.6 ± 0.1

[a]Other fatty acids include those <0.5%: i15:0, 15:0, i17:0, i18:0, 20:0, 22:0, 24:0, 16:1ω5c, 20:1ω 5, 22:1ω 7c, 24:1ω 7c, 24:1ω 11/ ω 13c, 16:2ω 4, 18:2, 18:3ω 3, 20:3ω 6, 22:4ω 6, 21:5ω3, 22:5ω 6

higher abundance of 22:6ω3 (docosahexaenoic acid, DHA) compared to the DG and caecum oil, also contributed to the dissimilarity. Additionally, in comparison to the DG, the fin also had comparatively lower levels of 20:1ω9/ω11 while the caecum oil had an 8-fold higher level of 16:1ω7 than the fin (Fig. 3).

Dissimilarity between the DG and caecum oil was low (23%). The main discriminating FAs were higher levels of 18:1ω9 and 16:1ω7, but lower levels of 20:1ω9/ω11 in the caecum relative to the DG.

3.3. Prey comparisons

Potential prey of *I. cordiformis* were grouped according to crustaceans, small fish, large fish, sharks and squid (Table 2). Upon examining the Bray-Curtis similarity coefficients between each FA profile of the DG of *I. cordiformis* and potential FA prey profiles, it was revealed that more than half of the highest coefficients (61%) were closest to 2 myctophid profiles (*Lampanyctodes australis*, 44%; *Electrona paucirastra*, 17%), while the remainder were closest to the smooth oreo (*Pseudocyttus maculatus*, 22%), 2 species of sharks (*Etmopterus baxteri*, 6%; *Dalatias licha*, 6%) and the dragonfish (*Stomias boa*, 6%). In contrast, *S. boa* was closest to 53% of caecum oil samples, while *L. australis* only accounted for 12% of caecum oil samples. The remaining 35% were closest to 2 deepwater shark species (*D. licha*, 29%; *Centroselachus crepidater*, 6%). Interestingly, of the 9 caecum oil samples that contained HCs mentioned previously, 6 caecum oil FA profiles were closest to a shark profile, and for the 3 that were not, sharks were a close second to *S. boa*. The FA profile of the *Todarodes filippovae* mantle had the highest coefficients associated with 100% of *I. cordiformis* fin samples (Fig. 4).

Similar results were obtained when the Bray-Curtis similarity coefficients for each prey species corresponding to either DG, fin or caecum oil were averaged then ranked. The ranked similarities based on average coefficients revealed that the DG had the highest similarity (≥80%) to 2 small fish, *L. australis* and *E. paucirastra*, as well as the large fish *P. maculatus*. SIMPER revealed that the FAs 18:1ω9, 20:1ω9/ω11, DHA and 16:0 always had the highest contribution (62–72%) to the similarity between the fish and DG. Average ranked similarity (≥75%) was followed closely by the small fish *S. boa*, the shark *D. licha* and the small fish *S. barnardi*. The main difference between the FA contribution to the similarity between the DG and the fish prey groups as opposed to the shark-dominated prey group was the lower contribution of DHA in the shark group.

The caecum oil had the highest ranked similarity (≥80%) with *S. boa* and the myctophid *L. australis* and also with the demersal shark *D. licha*. This was followed by a grouping of 3 demersal sharks (76–79%) in descending order of similarity (*Centroscymnus coelopsis*, *Centrophorus zeehaani*, *Centroselachus crepidater*). SIMPER showed that 16:0, 18:1ω9 and 20:1ω9/ω11 were the FAs that dominated the caecum oil and all the prey it clustered most closely to.

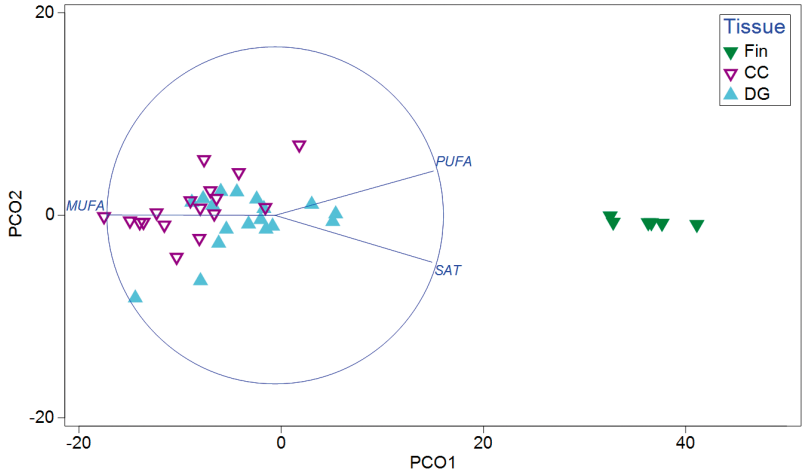

Fig. 2. Principal coordinates analysis (PCO) using a Bray-Curtis similarity on standardized percentages of saturated fatty acids (SAT), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of the caecum oil (CC), digestive gland (DG) and fin of *Idioteuthis cordiformis*. Vector overlays represent correlations >0.8 between the PCO variables

The fin differed from the DG and caecum oil in that the prey similarity did not overlap with any potential prey species identified with the DG or caecum oil. The fin was most similar to the mantles of the squids *T. filippovae* (84%) and *Moroteuthis ingens* (82%). SIMPER revealed that DHA, 16:0 and EPA were the FAs dominating the similarity between the *I. cordiformis* fin and the other squid mantles.

4. DISCUSSION

In this study, trophic level and prey composition were determined for *Idioteuthis cordiformis* based on indirect lipid class and signature FA analyses.

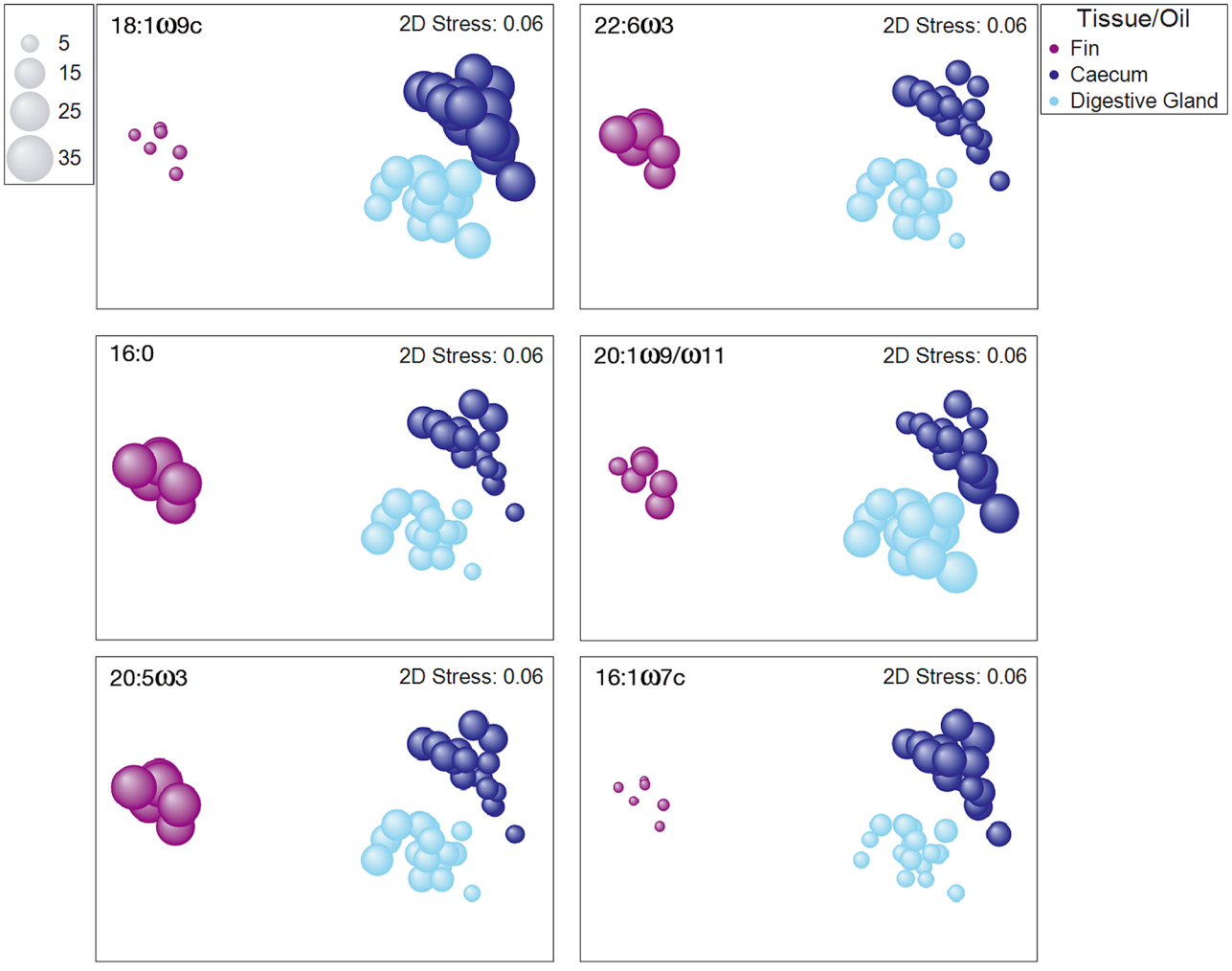

Fig. 3. Nonmetric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarities on standardized percentages of the 6 most important discriminating fatty acids between the fin, caecum oil and digestive gland of *Idioteuthis cordiformis* as identified by SIMPER. Bubble plot size represents relative percentages of the fatty acids

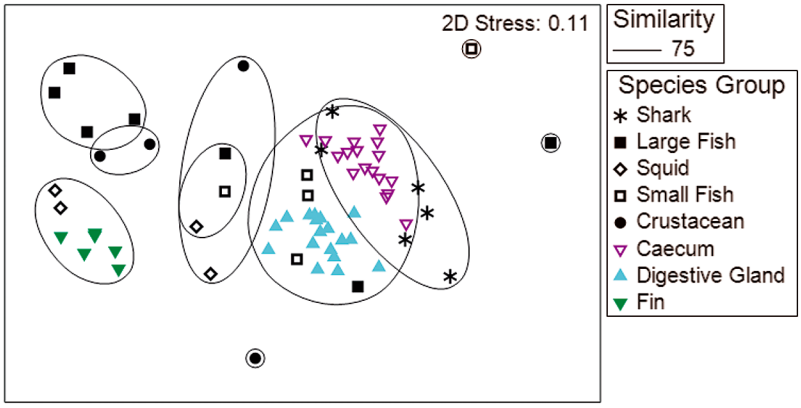

Fig. 4. Nonmetric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarities on standardized percentages of the fatty acid signatures of potential prey which are grouped according to shark, large fish, squid, small fish or crustacean, along with the fatty acid profiles of the caecum oil, digestive gland and fin of *Idioteuthis cordiformis* individuals. Ellipses represent 75% similarity

The individuals captured in this study were particularly well suited for this indirect analysis, as there was an obvious lack of ingested prey remains in these specimens, which was also observed in a larger number of other specimens (G. D. Jackson unpubl. data). The lipid content examined in the 2 tissues and caecum oil revealed the different and unique properties of each. Our study has shown that there is very little, if any, diagnostic prey value in the lipids found in the fin tissue. The fin tissue reflects the PL composition, which serves a structural role in the cell membranes (Parzanini et al. 2018). The fin tissue grouping with the mantle tissue of other cephalopods reflects the similar role these tissues play in different squid species. Lipids from both the DG and the caecum oil provided the highest level of information for the recent dietary history of *I. cordiformis*. These lipids extracted directly from the digestive system provided useful diagnostic information which would have likely represented recent ingestion of relatively unmodified prey lipids. Both the DG and caecum had relatively high levels of TAGs and WEs, with the highest levels in the caecum, particularly for TAGs. TAGs are predominantly used for energy storage, while WEs are used for buoyancy (Parzanini et al. 2018). The WEs in the caecum might be retained for buoyancy purposes. However, the high levels of TAGs (72.9%) in the caecum may simply represent unmetabolized lipids diverted from the DG resulting from the protein-based metabolism of squid (Jackson & O'Dor 2001).

Experimental work with cephalopods has found that FA profiles can reflect the diet of the squid *Loliguncula brevis* (Stowasser et al. 2006) and paralarvae of *Octopus vulgaris* (Navarro & Villanueva 2003) in as little as 10 d and the cuttlefish *Sepia officinalis* within 14 d (Fluckiger et al. 2008). These findings likely reflect the rapid digestion of cephalopods, especially evident for FA profiles that are obtained from DG samples. We would expect that the analysis of oil from the caecum would also reflect relatively recent feeding in *I. cordiformis*, and the DG probably within the last several weeks.

The presence of HCs in both the DG and caecum (Table 3) provides compelling evidence of dietary selection. The detection of high proportions of HCs is generally uncommon in deep-sea organisms. In a survey of 139 species of 8 phyla from the North Atlantic deep sea, Parzanini et al. (2018) found that HC was one lipid class which was not abundant in any of the phyla, accounting for a mean proportion of <1.7% and subsequently not included in the analysis of that study. The variation in the percentage of HCs within *I. cordiformis* is apparent from the large SD values (Table 3), with some individuals with zero HCs (Table 3). However, individuals of *I. cordiformis* had HC values as high as 12.6% for the DG and 28.3% for the caecum oil in our study.

The source of the HCs ingested by *I. cordiformis* is therefore an important question to consider. In a study of deepwater sharks, Pethybridge et al. (2011) commented that HCs are mainly associated with chondrichthyans inhabiting depths greater than 200 m and that it is mainly in the form of squalene, which is metabolically inert and believed to function largely for buoyancy. Pethybridge et al. (2011) also found the highest proportions of HCs in 2 deepwater sharks, *Deania calcea* and *Centrophorus zeehaani*. This observation is intriguing in the light of the DNA barcoding results of Braid & Bolstad (2014), who found a match for *D. calcea* in gut contents of *I. cordiformis* in New Zealand waters. A useful analysis in the future would be to test specifically for the presence of squalene in the caecum oil of *I. cordiformis*.

The lipid class data for *I. cordiformis* was compared to published lipid class data for 5 other Southern Ocean squid species (Table 5). In these studies, both polar and non-polar solvent systems were used that should have detected the presence of HCs. However, out of the 6 species examined, only *I. cordiformis* and *Todarodes filippovae* had HCs. The presence of HCs in *I. cordiformis* could be due to shark feeding as suggested by Braid & Bolstad (2014). However, the

Table 5. Mean (±SD) percentage lipid class composition of the digestive gland of female *Idioteuthis cordiformis* compared to the digestive gland of 5 other Southern Ocean squid species. Lipid class abbreviations are as defined in Table 3

Species Lipid class	*I. cordiformis* female (n = 13)	*Todarodes filippovae* (n = 43)	*Nototodarus gouldi* (n = 150)	*Moroteuthis ingens* (n = 5)	*Moroteuthis robsoni* (n = 5)	*Sepioteuthis australis* (n = 8)
HC	1.8 ± 4.0	1.5 ±2.2	Not reported[a]	Not reported	Not reported	Not reported
WE	12.8 ± 7.1	3.7 ± 2.3	13.1 ± 5.1	2.0 ± 0.9	1.3 ± 0.7	0.6 ± 0.6
DAGE	0.8 ± 0.6	2.6 ±3.4	Not reported[a]	1.6 ± 1.1	5.0 ± 7.1	0.0 ± 0.0
TAG	19.8 ± 11.6	79.2 ± 9.5	46.1 ± 17.6	75 ± 17.5	79.9± 6.3	26.3 ± 25.6
FFA	32.9 ± 15.5	5.1 ± 3.5	13.2 ± 6.3	11.7 ± 6.9	3.8 ± 2.0	8.2 ± 6.7
ST	3.2 ± 3.5	2.0 ± 1.9	9.0 ± 5.1	3.9 ± 3.1	1.4 ± 0.4	12.9 ± 7.9
PL	28.5 ± 8.9	5.9 ± 4.5	18.6 ± 5.4	5.8 ± 7.7	8.6 ± 0.3	52.0 ± 20.8
Source	This study	Pethybridge et al. (2013)	Pethybridge et al. (2012)	Phillips et al. (2001)	Phillips et al. (2002)	Phillips et al. (2002)
Ocean region	Tasmania	Tasmania	South Australia / Western Victoria	Macquarie Island	New Zealand Southern Plateau / Chatham Rise	Tasmania shallow inshore

[a]Both a polar and a non-polar solvent developing system were used to detect the presence of HCs

presence of HCs in *T. filippovae* is unusual. Phleger (1998) commented that the North Pacific euchalon *Thaleichthyes pacificus* is the only known teleost containing appreciable amounts of squalene. In contrast, HCs have been shown to be associated with many deepwater shark species (Bakes & Nichols 1995, Pethybridge et al. 2010a, 2011, Phleger 1998, Wetherbee & Nichols 2000). The source of HCs in the DG of *T. filippovae* is currently uncertain and deserves more research.

Comparisons of FA profiles from potential prey with both the caecum oil and DG tissue revealed a more complete picture of the diet of *I. cordiformis*. As predicted by the lipid class data, the FA analysis also revealed a strong similarity between *I. cordiformis* and a variety of deepwater sharks (including *D. calcea* identified by Braid & Bolstad 2014), which have high levels of shark liver-derived HCs. However, there was also a close grouping with a variety of myctophids, the medium sized dragonfish *Stomias boa* and the large smooth oreo *Pseudocyttus maculatus*.

The nMDS scaling analysis revealed a relationships between *I. cordiformis* and potential prey. Based on the hypotheses we tested and what was known from other studies on deepwater Southern Ocean squid diet, potential prey FA profiles were chosen from the literature. We used a number of taxa from chondrichthyans, large and small teleosts, crustaceans and squid to provide an overview and preliminary assessment of potential prey species. Along with DNA barcode identification of predation on *D. calcea*, Braid & Bolstad (2014) also detected a match with 2 species of *Lutjanus* sp. with *I. cordiformis* in New Zealand. However, we did not find any clustering with the *Lutjanus* species selected in this study (although the species available in our study were different from the 2 detected in the New Zealand study). This is not surprising given that lutjanids apparently do not inhabit deepwater environments, but rather are found in shallow water tropical reef environments (Heupel et al. 2009).

The FA analysis suggests a broad prey spectrum of sharks and a range of sizes in fish prey. Interestingly, other than smooth oreo, none of the other common large deepwater teleosts were detected in the diet of *I. cordiformis.* There was also no grouping with crustaceans. This finding was somewhat surprising given that the caecum oil in our Tasmanian specimens and the New Zealand specimens (Braid & Bolstad 2014) was commonly bright orange. We suspected this might be related to carotenoid pigments from ingesting orange roughy *Hoplostethus atlanticus* or crustaceans. Although on 1 occasion crustacean remains were detected in the caecum of 1 individual, neither the crustacean nor orange roughy prey FA profile grouped with *I. cordiformis*. Currently, the reason for the bright orange (and sometimes rose colored) caecum oil is still unknown.

I. cordiformis appears to be a generalist feeder and shares components of its diet with other Southern Ocean squid species such as *Moroteuthis ingens* (Jackson et al. 1998, Phillips et al. 2001) *T. filippovae* (Pethybridge et al. 2013) and *Nototodarus gouldi* (Pethybridge et al. 2012), which all feed on a variety of small fish including myctophids. Myctophids are known to be an important food source for many oceanic squids (Boyle & Rodhouse 2005). The high degree of individual variation revealed by the nMDS plot indicated a broad range of predation by individuals. However, even though the diet of *I. cordiformis*

ranges from small fish to larger fish and sharks, stable isotope analysis has revealed that this species is a top predator. *I. cordiformis* had the highest nitrogen stable isotope ($\delta^{15}N$) values in the Tasmanian cephalopod community with a mean value of 15.8‰, which was even higher than sperm and pilot whales (Jackson 2017). This also agrees with $\delta^{15}N$ values reported by Braid & Bolstad (2014), which ranged from 15.1 to 16.6‰. Thus, it is likely that the biomass of sharks (and perhaps large fish species) may be appreciably greater than the smaller fish prey ingested by *I. cordiformis*.

FA signature analysis can also reveal other biological and habitat information for finer scale ecological studies. Certain FAs have been identified with specific habitats and physiology. *I. cordiformis* had high levels of 20:1ω11, which has been connected with mesopelagic feeding (Meyer et al. 2019). Additionally, Meyer et al. (2019) also found that EPA (20:5ω3) was associated with both muscles of fast-swimming fish and with cold water. This FA occurred at high levels in the fins of *I. cordiformis*. We also detected relatively high levels of DHA (22:6ω3), which is also linked with deep, coldwater habitats (Meyer et al. 2019). Relatively high levels of 18:1ω9 and 20:1ω9 have been found to be correlated with deep-sea and pelagic environments respectively. Furthermore, these 2 FAs were also identified as common in sharks of the families Squaliformes, Hexanchiformes and Lamniformes (Meyer et al. 2019). All the sharks used in our analysis were from the family Squaliformes, some of which were closely associated with *I. cordiformis*.

There are certain FAs present in the profile of *I. cordiformis* that associate this species with other deepwater and oceanic species. Pethybridge et al. (2013) compared FA profiles of a number of species inhabiting Southern Ocean, Antarctic and nearshore environments. They found that squid separated into 2 groups (a and b). *I. cordiformis* associated more closely with group-a, which included a number of other Southern Ocean pelagic and deepwater squid that shared high levels of 18:1ω9 and 20:1ω9 and moderate levels of 22:6ω3. This contrasted with group-b squid from Pethybridge et al. (2013), which included nearshore and Antarctic species. This difference was attributed to diet, with group-a squid being predominantly piscivorous compared to group-b squid, which consumed crustaceans.

New biochemical, molecular and genetic techniques continue to reveal insights into the diet of species which would otherwise be difficult to study. Our work on the diet of *I. cordiformis* in Tasmanian waters provides strong corroborating data to the New Zealand study (Braid & Bolstad 2014) that this species feeds on deepwater sharks such as *D. calcea*. Recent research has documented interactions with sharks and squid based on scarring on shark skin in the North Pacific off Hawaii (Papastamatiou et al. 2020) and Mexico (Becerril-García et al. 2020). Ongoing studies will help to document trophic interactions between large predators in the deep sea. It is also likely that some other species of large deepwater and oceanic squid may occupy higher trophic positions than previously realized.

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