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Note

The diet of deepwater sharks and the benefits of using DNA identification of prey

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ABSTRACT

Stomachs from the sharks *Dalatias licha*, *Centrophorus squamosus*, *Centroscymnus owstoni*, *Centroselachus crepidater*, *Proscymnodon plunketi*, and *Galeorhinus galeus* were sampled from three research trawl surveys on Chatham Rise, east of New Zealand. Between 14 and 50 stomachs were examined for each species, of which 8–62% were empty. Prey were visually identified in 80 stomachs, and by DNA barcoding in a further 28 stomachs. The use of DNA methods allowed the identification of chunks of flesh found in the stomachs of *D. licha* and *P. plunketi*, and nearly doubled the rate of data accumulation for *D. licha*, *C. squamosus*, and *C. owstoni*. Between 84 and 223 stomachs were estimated to be needed to measure 90% of the extrapolated total prey richness. The prey of *D. licha*, *C. squamosus*, and *P. plunketi* were predominantly benthic or demersal fishes and cephalopods. The prey of *C. owstoni* and *C. crepidater* were predominantly mesopelagic fishes and squids. *G. galeus* foraged throughout the water column. Scavenging of discards from commercial fishing vessels was likely in *C. squamosus*, *P. plunketi*, and *G. galeus*. The diet of all species except *C. crepidater* was dominated by the commercially important benthopelagic species hoki *Macruronus novaezelandiae*.

1. Introduction

Many deepwater sharks are taken as bycatch in commercial fisheries (Blackwell and Stevenson, 2003; Last and Stevens, 2009). Around New Zealand the recorded catches of deepwater sharks increased substantially during the 1980s and 1990s (Francis, 1998; Blackwell and Stevenson, 2003). Because sharks have a relatively low initial stock size, low growth rate, moderate to high age at maturity, and low fecundity, they have a high intrinsic vulnerability to overfishing (Francis, 1998; Stevens et al., 2000; Barker and Schluessel, 2005). Nevertheless, the biology, ecology, and stock status of many deepwater sharks remains poorly known (Blackwell and Stevenson, 2003; Last and Stevens, 2009).

The Chatham Rise is a submarine ridge which runs eastwards for about 1000 km from the east coast of the South Island of New Zealand, rising from depths of about 3000 m to 50 m at the western end, and sea level at the eastern end. The subtropical front, a permanent oceanographic feature where warm and more saline subtropical water from the north meets subantarctic water from the south, extends eastwards along Chatham Rise (Heath, 1985; Uddstrom and Oien, 1999). Chatham Rise is a relatively productive area (Murphy et al., 2001; Nodder et al., 2003), and the demersal fish assemblage has the highest species richness found in New Zealand waters, with species richness higher on the northern slope and increasing with depth to a peak at about 1000 m (Leathwick et al., 2006).

Sharks account for a small proportion of deepwater (200–800 m) demersal trawl catches on Chatham Rise, although occasional large catches (>0.5 t) occur, presumably as a consequence of shark aggregations (Wetherbee, 2000). This study investigated the diet of the less common deepwater sharks as part of a wider study of trophic structure in deepwater demersal assemblages on Chatham Rise, in a research programme aimed at an ecosystem approach to fisheries management (Francis et al., 2007). The species were *Dalatias licha* Bonnaterre 1788, *Centrophorus squamosus* Bonnaterre 1788, *Centroscymnus owstonii* Garman 1906, *Centroselachus crepidater* Barbosa du Bocage & de Brito Capello 1864, *Proscymnodon plunketi* Waite 1909, and *Galeorhinus galeus* Linnaeus 1758. With the exception of *P. plunketi*, restricted to the southwest Pacific and southern Indian Oceans, all species occur worldwide (Last and Stevens, 2009). On Chatham Rise, *C. crepidater* is the most common of the six species but, along with *C. squamosus*, *C. owstoni*, and *P. plunketi*, is not commercially important (Wetherbee, 2000; Blackwell and Stevenson, 2003). *D. licha* has been valued for its flesh (Francis, 1998), but may be the least common species (Wetherbee, 2000). *G. galeus* has been caught in middle-depth trawl surveys, but it is primarily a shallower water species that is targeted by

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commercial and recreational fisheries, and is the only shark of these six species that is subject to fisheries management measures in New Zealand (Francis, 1998; Ministry of Fisheries, 2009).

Diet studies of deepwater sharks have been limited by low catch rates, compounded by the majority of sharks having empty stomachs (Garrick, 1959; Mauchline and Gordon, 1983; Ebert et al., 1992). Furthermore, prey items may be substantially fragmented, or digested, making identification difficult. Consequently the diets of deepwater sharks are often described from only a few ($n<50$) specimens (Mauchline and Gordon, 1983; Ebert et al., 1992; Cortés, 1999). In this study we evaluate the use of DNA barcoding for the identification of fish prey items in order to maximise the information collected from these relatively rare sharks.

DNA techniques are widely used in identification studies including fish products (Bartlett and Davidson, 1992; Smith et al., 2008), and allow the identification of poorly preserved and small tissue samples, but are a relatively new approach for fish prey identification (Rosel and Kocher, 2002, Smith et al., 2005). Early molecular methods used in fish gut content identification relied on the development of genus-specific DNA primers to amplify a few target species (Jarman et al., 2002; Rosel and Kocher, 2002, Jarman and Wilson, 2004, Parsons et al., 2005), although the mitochondrial cytochrome *b* gene was applied to the identification of prey items in large pelagic fishes (Smith et al., 2005). The rapid developments in molecular biology have provided more sophisticated DNA tools for identification of prey items (Deagle et al., 2009; King et al., 2008; Soininen et al., 2009), but all are dependent on matching sequences from unknown prey items against a database of reference sequences.

A global DNA-based barcode identification system is being developed for all animal species, and the Barcode Of Life Database (BOLD) provides a universal tool for the identification of fish specimens and their gut contents. The barcode system is based on DNA diversity in a single gene region (a section of the mitochondrial DNA cytochrome *c* oxidase I gene, COI); unknown samples are identified by comparing their DNA barcode sequences against a DNA database of COI sequences derived from reference specimens. Hebert and co-workers (Hebert et al., 2003a, 2003b) have demonstrated that the COI region is appropriate for discriminating between closely related species across diverse animal phyla, and this has been verified in marine fishes (Ward et al., 2009). DNA barcodes have been obtained for over 6000 species of fish, including ca. 450 species from the New Zealand Exclusive Economic Zone (EEZ), and for ca. 200 species of macroinvertebrates, and the COI sequences deposited in BOLD, providing a database for identification of shark prey items in New Zealand waters.

2. Materials and methods

2.1. Specimen collection

Samples of *D. licha*, *C. squamosus*, *C. owstoni*, *C. crepidater*, *P. plunketi*, and *G. galeus* were obtained from stratified-random research bottom trawl surveys on Chatham Rise during December 2004–January 2005, December 2005–January 2006, and December 2006–January 2007 (Stevens et al., 2009). The sampling consisted of ca.100 bottom trawl tows per year in 26 strata defined by location and depth covering 146 855 km^2 at depths between 200 and 800 m. The trawl net was towed at each station for ca. 3 nautical miles, at a speed of 3.5 knots, during daylight hours. After capture, each shark was measured for sex, total length (TL) to the nearest full cm below, and total weight to the nearest 5 g. A random sub-sample of sharks were then sampled for stomachs, the size of which was determined by the available time. Sharks with obviously regurgitated or everted stomachs were not sampled. At sea, the stomachs from larger sharks (ca. >50 cm TL) were sealed by fixing a cable-tie around the oesophagus, then the oesophagus was cut in front of the tie, the intestines cut below the pyloric sphincter, and the stomach removed, labelled, frozen at $-20\,^{\circ}C$ and returned to the laboratory; smaller sharks were frozen whole, and the stomach dissected in the laboratory.

2.2. Gut analyses

In the laboratory, the stomachs and contents were thawed, a qualitative estimate made of stomach fullness and overall prey digestion state, and the stomach contents rinsed with water using a 500 μm steel sieve to remove fluid and very fine material. Recognizable prey items were identified to the lowest taxon possible, using reference guides and a reference collection of preserved specimens and hard parts (otoliths and cephalopod beaks) held by NIWA, Wellington. For each prey taxon, the number of prey individuals was estimated, and wet weight recorded to the nearest 0.01 g after removal of surface water by blotting paper.

To assess the rate at which new prey were being identified, the cumulative number of individual prey types (prey richness) was plotted against the cumulative number of non-empty stomachs. The mean and 95% confidence interval (2.5th and 97.5th quantile) were calculated from 1000 curves based upon different random orders of the stomachs. An asymptotic curve was fitted to the mean values, having the form $H=an/(1+bn)$, where a and b are constants, n is the number of stomachs sampled, and the asymptote is given by a/b (Dunn, 2009). The contribution of different prey items to the diet was determined by the numerical importance (%N), frequency of occurrence (%O), and weight (%W) (Hyslop, 1980). The index of relative importance (IRI), which incorporates the previous three indices, was calculated as IRI=%O (%N+%W), and expressed as a percentage (%IRI, Cortés, 1997).

2.3. DNA analyses

Small samples of muscle tissue (200–500 mg) were taken from all prey items that could not be identified with reference guides, and where sufficient muscle tissue remained. Total genomic DNA was extracted from each muscle tissue sub-sample by homogenisation and digestion with proteinase-K at 55 °C for 4 h. After digestion, DNA was extracted with a standard phenol–chloroform–ethanol procedure (Taggart et al., 1992). Approximately 600 base pairs (bp) of the 5′ region of the CO1 gene were amplified using the primer pair FishF2 and FishR2 (Ward et al., 2005) for each muscle tissue sub-sample. Amplifications were carried out using an initial denaturation of 94 °C for 1 min; 35 cycles of 94 °C for 60 s, 57 °C for 90 s, and 72 °C for 60 s, followed by an extension at 72 °C for 5 min, using a Cetus 9600 DNA thermocycler (Perkin-Elmer Corporation, Connecticut, USA). PCR products were purified using the QIAquick gel extraction kit (Qiagen Pty Ltd., Doncaster, Victoria, Australia). Sequences were determined using the ABI Taq DyeDeoxy™ Terminator Cycle Sequencing Kit according to the Manufacturer's directions (Applied Biosystems Inc., Foster City, California, USA) and run on an ABI prism autosequencer. DNA sequences were edited in CHROMAS (Technelysium, Queensland), and aligned in CLUSTAL in MEGA version 3 (Kumar et al., 2004). Sequences from the shark gut contents were aligned against BOLD entries. Sequence

Table 1
Statistics for sharks sampled from the Chatham Rise, combined for the three surveys. No. tows is the number of tows from which samples were taken. Sampling rate is expressed as the proportion of the total sample which yielded usable diet information. Stomachs with everted or obvious regurgitated stomach contents were not sampled and are not included in these statistics.

	D. licha	*C. squamosus*	*C. owstoni*	*C. crepidater*[a]	*P. plunketi*	*G. galeus*
Samples						
n sampled	36	42	50	27	14	25
Median TL (min.–max.)	51 (41–137)	113 (46–142)	84 (65–120)	70 (38–92)	94 (72–117)	144 (109–160)
No. tows	23	10	5	3	9	14
Median depth (min.–max.)	514 (404–799)	672 (607–799)	751 (626–794)	792 (649–799)	611 (460–730)	264 (206–430)
Non-empty stomach samples						
n	19	26	19	19	12	23
Median TL (min.–max.)	55 (41–137)	104 (46–142)	83 (78–116)	66 (38–90)	94 (72–115)	144 (109–160)
n, all prey unidentifiable	3	2	3	0	2	0
No. prey identified by DNA	6	13	9	0	4	5
n, all identified prey used DNA	6	11	7	0	3	1
Sampling method and rates						
Visual only	0.28	0.31	0.18	0.70	0.50	0.88
Visual and DNA	0.44	0.57	0.32	0.70	0.71	0.92
Prey richness at asymptote	9	21	28	10	16	36
n for 90% of asymptote	90	209	223	84	117	171

[a] No *C. crepidater* gut contents were tested for DNA.

Table 2
Number of stomachs by qualitative stomach fullness for deepwater sharks from Chatham Rise. Stomachs with everted or obvious regurgitated stomach contents were not sampled and are not included in these statistics.

	D. licha	*C. squamosus*	*C. owstoni*	*C. crepidater*	*P. plunketi*	*G. galeus*
Empty	17	16	31	8	2	2
Trace	4	7	3	13	2	1
1/4–3/4 full	14	17	12	5	9	11
Full	1	2	4	1	1	11
Proportion empty	0.47	0.38	0.62	0.30	0.14	0.08

divergences were calculated using the Kimura two parameter (K2P) distance model (Kimura, 1981).

3. Results

Between 14 and 50 shark specimens were sampled for each species (Table 1). *G. galeus* was the largest species sampled, and was captured only in relatively shallow tows. *P. plunketi* was the least common species sampled, with an average of only four specimens collected per survey. The effective sample sizes for examining diet were reduced because many stomachs were empty; between 8% and 62% of the stomachs were empty of prey (Table 2). Stomachs were rarely full, except for *G. galeus* (Table 2).

COI sequences 387–667 base pairs in length were obtained from 37/41 gut content samples. The preliminary digestion does not appear to denature the DNA to an extent that eliminates recovery of moderate length (ca. 500 bp) sequences from gut contents. All of the gut content samples aligned with New Zealand fish species and not with marine invertebrates or mammals (Table 3).

The prey identified using DNA were the only prey found in 28 stomachs (Table 1). Including DNA identification allowed the identification of chunks of flesh that formed a component of the diet of *D. licha* and *P. plunketi*. DNA identification also increased the sample size, and it almost doubled the rate at which data were collected for three species (Table 1). The sample sizes achieved were too small to provide a full description of diet (Fig. 1), but suggested the richest diet would be found for *G. galeus*, and the least rich found for *D. licha* (Table 1). At the present sampling rate, without using DNA prey identification, 90% of the estimated mean asymptotic prey richness would be achieved in between 14 and 56 years depending on species; and this would be reduced to 14–36 years when including DNA identifications.

A large proportion of the prey remained unidentified; these prey were predominantly well-digested remains of fishes (bones and scales) (Table 4). The prey of *D. licha* was the least rich (Table 1) and consisted almost entirely of fishes, of which hoki (*Macruronus novaezelandiae*) were most frequently identified (Table 4). Other fish prey included elasmobranchs, of which *Deania calcea* was identified. The occurrence of salps in one stomach may have been through incidental ingestion. The teleost fish prey included pieces of flesh, and in one case also part of a liver, suggesting either the prey were scavenged, or live prey were attacked but not entirely ingested.

The prey of *C. squamosus* consisted entirely of fishes, of which hoki were dominant (Table 4). The other fish prey were a mixture of demersal species (the eels *Bassanago* spp., sea perch *Helicolenus* spp., and oreo dory *Neocyttus rhomboidalis*), pelagic species (sea bream *Brama brama*, and jack mackerel *Trachurus* spp.), and ubiquitous species (*Squalus* sp.). One stomach contained only jack mackerel heads and tails, where were presumably scavenged discards from a commercial fishing vessel.

The prey of *C. owstoni* consisted of mainly fishes, with small proportions of crustaceans, squids and salps. The fish prey were predominantly hoki, with some mesopelagic sea bream (*B. australis* and *B. brama*) and slender tuna (*Allothunnus fallai*), and a demersal rattail (*Coelorinchus bollonsi*). The crustacean prey was the pelagic shrimp *Oplophorus novaezeelandiae*, and the cephalopod prey included the mesopelagic squids *Onykia ingens* and Octopoteuthiidae.

The prey of *C. crepidater* consisted largely of fishes, but these were mostly well digested, consisting of only fish eyes, bones, and scales.

Table 3
Predator, prey identification, number of prey items, number of nucleotides, and Barcode Of Life Database (BOLD) % base match, for prey items identified from Chatham Rise sharks.

Predator	Prey	*n*	Sequence bp	% identity BOLD
C. squamosus	*Bassanago hirsutus*	2	644, 654	99.8, 100
	Bassanago spp.	1	659	99.8
	Brama brama	1	660	99.4
	Helicolenus spp.	1	645	98.6
	Macruronus novaezelandiae	6	387–667	97.1–100
	Neocyttus rhomboidalis	1	665	100
	Trachurus murphyi	1	632	100
C. owstoni	*Allothunnus fallai*	1	658	99.8
	Brama australis	1	631	99.7
	Brama brama	2	625, 635	99.8
	Macruronus novaezelandiae	5	507–652	99.3–100
P. plunketi	*Bassanago hirsutus*	1	642	100
	Macruronus novaezelandiae	1	636	100
	Trachurus murphyi	2	631, 634	96.1, −99.1
D. licha	*Deania calcea*	1	634	100
	Macruronus novaezelandiae	5	515–658	94–100
G. galeus	*Argentina elongate*	1	641	99.8
	Brama brama	1	659	100
	Helicolenus spp.	2	634, 649	99.3, 100
	Macruronus novaezelandiae	1	659	100

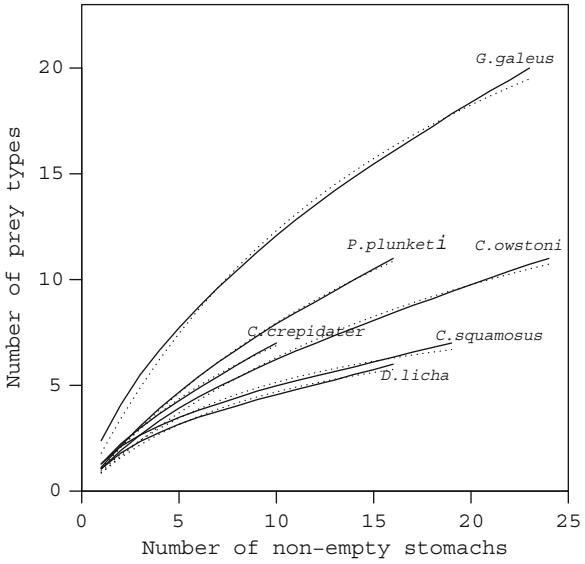

Fig. 1. The cumulative number of prey types identified with increasing sample size. The dotted lines indicate the fitted curves from which asymptotic prey richness was estimated.

The only fish prey species identified was a single mesopelagic lightfish (*Diplophos* sp). Squids were also eaten, and included the mesopelagic *Chiroteuthis* sp. The copepods eaten were presumably incidental. *C. crepidater* was the only shark where hoki was not identified as prey.

The prey of *P. plunketi* consisted entirely of fishes, of which hoki, sea perch, and eels were most frequently identified. Other fish prey included jack mackerels, and Lucifer's dogfish (*Etmopterus lucifer*). The prey were often incomplete, and four of the ten stomachs consisted of between one and six pieces of fish flesh. One stomach, however, included an entire hoki. The jack mackerel consisted of heads and tails, and were presumably scavenged discards from a commercial fishing vessel.

The diet of *G. galeus* was the most rich (Table 1), and consisted largely of fishes, with some crustaceans, cephalopods, and salps (Table 4). Hoki were the dominant fish prey, and were identified in just over half the stomachs. One stomach contained 13 similar sized, complete, and very fresh hoki; it is possible that these were eaten in the trawl net. The other fish prey were a mixture of demersal sea perch, red cod (*Pseudophycis bachus*) and rattail (*C. bollonsi*), benthic flatfish (Pleuronectiformes) and opalfish (*Hemerocoetes* sp.), and pelagic jack mackerel, sea bream, and argentine (*Argentina elongata*). The crustacean prey were benthic scampi (*Metanephrops challengeri*) and mesopelagic shrimp (*Notopandalus magnoculus*). The cephalopod prey included both benthic octopus and mesopelagic squids (*O. ingens* and *Nototodarus* spp.). The occurrence of salps in two stomachs may have been incidental ingestion.

4. Discussion

Whether a sample is considered large enough to adequately describe diet depends on the level of taxonomic detail to which the prey species are identified, and the statistic used to measure diet breadth, which may be cumulative prey richness (as used here) or prey diversity. We expect fewer samples to be required to adequately describe prey diversity compared to prey richness (Dunn, 2009), and fewer samples to be required when taxonomic identification of prey is less detailed. Although as few as 15–30 non-empty stomach samples may be considered adequate to describe prey diversity for some shark species (Alonso et al., 2002; Lucifora et al., 2006), we do not consider the sample sizes achieved in this study to be large enough, although we do consider them indicative of prey and feeding behaviour.

Five of the six sharks were found to have eaten primarily hoki, which is the most abundant and commercially important demersal fish species on Chatham Rise (Ministry of Fisheries, 2009). The only species which was not found to eat hoki, *C. crepidater*, was found to have eaten mesopelagic squid and fish, and so may compete for food resources with hoki (Bulman and Blaber, 1986). It seems likely that hoki were being predated directly, given their abundance as a potential prey, but some hoki were identified in a *G. galeus* stomach which may have been eaten in the net. Prey potentially eaten in the net were noted when the stomach contents were analysed, but the source of prey was not certain, and any bias in dietary composition was therefore

Table 4
Total non-empty stomach contents composition for sharks from the Chatham Rise (visual and DNA results combined). %O, percentage frequency of occurrence; %W, percentage of weight; %N, percentage of total number of prey; %IRI, percentage index of relative importance.

	Dalatias licha				*Centrophorus squamosus*				*Centroscymnus owstoni*			
	%O	%W	%N	%IRI	%O	%W	%N	%IRI	%O	%W	%N	%IRI
Osteichthyes												
Allothunnus fallai	–	–	–	–	–	–	–	–	6.3	13.1	5.3	3.6
Argentina elongata	–	–	–	–	–	–	–	–	–	–	–	–
Bassanago sp.	–	–	–	–	4.2	3.4	3.3	0.7	–	–	–	–
Bassanago hirsutus	–	–	–	–	8.3	11.6	6.7	3.7	–	–	–	–
Brama australis	–	–	–	–	–	–	–	–	6.3	1.1	5.3	1.2
Brama brama	–	–	–	–	4.2	3.6	6.7	1.0	12.5	18.7	10.5	11.3
Coelorinchus bollonsi	–	–	–	–	–	–	–	–	6.3	10.7	5.3	3.1
Diplophos sp.	–	–	–	–	–	–	–	–	–	–	–	–
Helicolenus spp.	–	–	–	–	4.2	3.2	3.3	0.7	–	–	–	–
Hemerocetes sp.	–	–	–	–	–	–	–	–	–	–	–	–
Macruronus novaezelandiae	31.3	18.3	23.1	30.8	33.3	51.7	36.7	71.6	25.0	34.9	26.3	47.5
Neocyttus rhomboidalis	–	–	–	–	4.2	0.4	3.3	0.4	–	–	–	–
Pleuronectiformes	–	–	–	–	–	–	–	–	–	–	–	–
Pseudophycis bachus	–	–	–	–	–	–	–	–	–	–	–	–
Trachurus declivis	–	–	–	–	8.3	13.9	6.7	4.2	–	–	–	–
Trachurus murphyi	–	–	–	–	4.2	4.5	3.3	0.8	–	–	–	–
Fish scales	–	–	–	–	4.2	< 0.1	3.3	0.3	–	–	–	–
Otoliths unidentified	–	–	–	–	–	–	–	–	–	–	–	–
Fish unidentified	50.0	9.0	34.6	52.0	25.0	2.6	23.3	15.8	25.0	13.2	21.1	26.6
Chondrichthyes												
Deania calcea	6.3	63.4	3.8	10.0	–	–	–	–	–	–	–	–
Etmopterus lucifer	–	–	–	–	–	–	–	–	–	–	–	–
Squalus sp.	–	–	–	–	4.2	5.2	3.3	0.9	–	–	–	–
Egg case	6.3	0.1	3.8	0.6	–	–	–	–	–	–	–	–
Elasmobranch unidentified	6.3	8.4	3.8	1.8	–	–	–	–	–	–	–	–
Crustacea												
Copepoda	–	–	–	–	–	–	–	–	–	–	–	–
Metanephrops challengeri	–	–	–	–	–	–	–	–	–	–	–	–
Notopandalus magnoculus	–	–	–	–	–	–	–	–	–	–	–	–
Oplophorus novaezeelandiae	–	–	–	–	–	–	–	–	6.3	< 0.1	5.3	1.0
Cephalopoda												
Chiroteuthis sp.	–	–	–	–	–	–	–	–	–	–	–	–
Onykia ingens	–	–	–	–	–	–	–	–	6.3	6.8	5.3	2.3
Nototodarus spp.	–	–	–	–	–	–	–	–	–	–	–	–
Octopoda	–	–	–	–	–	–	–	–	–	–	–	–
Octopoteuthiidae	–	–	–	–	–	–	–	–	6.3	0.8	5.3	1.2
Unidentified squid	–	–	–	–	–	–	–	–	6.3	0.6	5.3	1.1
Salpida												
Iasis zonaria	–	–	–	–	–	–	–	–	–	–	–	–
Unidentified Salpida	6.3	0.7	30.8	4.7	–	–	–	–	6.3	< 0.1	5.3	1.0
Total (*n* stomachs, weight (g), *n* prey)	16	893	26	–	24	3665	30	–	16	1540	19	–

Table 4. (*continued*)

	Centroselachus crepidater				*Proscymnodon plunketi*				*Galeorhinus galeus*			
	%O	%W	%N	%IRI	%O	%W	%N	%IRI	%O	%W	%N	%IRI
Osteichthyes												
Allothunnus fallai	–	–	–	–	–	–	–	–	–	–	–	–
Argentina elongata	–	–	–	–	–	–	–	–	4.3	0.6	0.7	0.1
Bassanago sp.	–	–	–	–	–	–	–	–	–	–	–	–
Bassanago hirsutus	–	–	–	–	10.0	10.0	7.7	2.9	–	–	–	–
Brama australis	–	–	–	–	–	–	–	–	–	–	–	–
Brama brama	–	–	–	–	–	–	–	–	4.3	0.3	0.7	0.1
Coelorinchus bollonsi	–	–	–	–	–	–	–	–	4.3	7.3	1.5	0.5
Diplophos sp.	5.3	82.9	2.3	8.6	–	–	–	–	–	–	–	–
Helicolenus spp.	–	–	–	–	10.0	21.8	7.7	4.8	26.1	18.9	8.9	10.3
Hemerocetes sp.	–	–	–	–	–	–	–	–	8.7	0.1	2.2	0.3
Macruronus novaezelandiae	–	–	–	–	20.0	11.7	23.1	11.2	52.2	56.3	27.4	62.0
Neocyttus rhomboidalis	–	–	–	–	–	–	–	–	–	–	–	–
Pleuronectiformes	–	–	–	–	–	–	–	–	4.3	0.5	1.5	0.1
Pseudophycis bachus	–	–	–	–	–	–	–	–	4.3	4.8	1.5	0.4
Trachurus declivis	–	–	–	–	10.0	1.4	7.7	1.5	4.3	2.4	1.5	0.2
Trachurus murphyi	–	–	–	–	10.0	1.4	7.7	1.5	–	–	–	–
Fish scales	26.3	0.1	18.2	9.3	–	–	–	–	4.3	< 0.1	0.7	< 0.1
Otoliths unidentified	5.3	< 0.1	2.3	0.2	–	–	–	–	13.0	< 0.1	11.1	2.1
Fish unidentified	68.4	7.4	47.7	72.7	60.0	38.5	38.5	74.5	47.8	5.2	26.7	21.6
Chondrichthyes												
Deania calcea	–	–	–	–	–	–	–	–	–	–	–	–
Etmopterus lucifer	–	–	–	–	10.0	15.3	7.7	3.7	–	–	–	–
Squalus sp.	–	–	–	–	–	–	–	–	–	–	–	–
Egg case	–	–	–	–	–	–	–	–	–	–	–	–
Elasmobranch unidentified	–	–	–	–	–	–	–	–	–	–	–	–
Crustacea												
Copepoda	5.3	< 0.1	11.4	1.2	–	–	–	–	–	–	–	–
Metanephrops challengeri	–	–	–	–	–	–	–	–	4.3	0.1	0.7	0.1
Notopandalus magnoculus	–	–	–	–	–	–	–	–	4.3	< 0.1	2.2	0.1
Oplophorus novaezeelandiae	–	–	–	–	–	–	–	–	–	–	–	–
Cephalopoda												
Chiroteuthis sp.	5.3	< 0.1	2.3	0.2	–	–	–	–	–	–	–	–
Onykia ingens	–	–	–	–	–	–	–	–	8.7	< 0.1	1.5	0.2
Nototodarus spp.	–	–	–	–	–	–	–	–	13.0	0.6	3.0	0.7
Octopoda	–	–	–	–	–	–	–	–	4.3	< 0.1	0.7	< 0.1
Octopoteuthiidae	–	–	–	–	–	–	–	–	–	–	–	–
Unidentified squid	15.8	9.6	15.9	7.8	–	–	–	–	13.0	2.8	2.2	0.9
Salpida												
Iasis zonaria	–	–	–	–	–	–	–	–	4.3	< 0.1	0.7	< 0.1
Unidentified Salpida	–	–	–	–	–	–	–	–	4.3	0.1	4.4	0.3
Total (*n* stomachs, weight (g), *n* prey)	19	129	44	–	10	807	13	–	23	14541	135	–

difficult to determine. In addition, some hoki heads only were found in *G. galeus*, and some pieces of hoki flesh were found in *D. licha*; these occurrences may indicate some incomplete ingestion, or perhaps scavenging. Jack mackerel heads and/or tails were found in the stomachs of *C. squamosus*, *C. plunketi*, and *G. galeus*, and were almost certainly scavenged discards from commercial fishing vessels. Therefore scavenging of other species certainly seems possible. However, incomplete ingestion of prey, where the shark takes bites out of larger prey, has previously been suspected for *D. licha* (Last and Stevens, 2009). Deep sea oreo dories (Oreosomatidae) and orange roughy (*Hoplostethus atlanticus*) have been observed with crescents of flesh removed, typically in the dorso-posterior region, that have subsequently healed over. *D. licha* could be a potential culprit for these attacks, although the relatively abundant and sympatric Baxter's dogfish *Etmopterus baxteri* might also be responsible. Orange roughy have also previously been found in the stomachs of *C. owstoni* and *C. squamosus* (Wetherbee, 2000).

In 26 *C. squamosus*, we found predominantly benthic or demersal teleost prey, dominated by hoki, but including some elasmobranchs and likely scavenging; Ebert et al. (1992) found predominantly demersal teleosts, cephalopods, and a single crustacean in the diet of 18 specimens from South Africa, and Mauchline and Gordon (1983) found predominantly fishes, including Chondrichthyes, in 21 specimens from the North Atlantic. In 19 *D. licha*, we found predominantly teleosts, with some elasmobranchs, including *D. calcea*; Garrick (1959) found cephalopods and teleosts in the stomachs of 12 specimens from New Zealand, and Macpherson (1979) found teleosts, cephalopods, and natant decapods in 31 specimens from the Mediterranean Sea (*n* empty not given). In 19 *C. owstoni* we found a variety of mesopelagic and benthopelagic fishes, squids, crustacean and salp prey; in Japanese waters Yano and Tanaka (1984) examined 336 stomachs and found the majority were empty ($>50\%$; *n* empty not given) and the diet was largely mesopelagic fishes, macrourids, and squids, including, as in this study, fast-swimming mesopelagic *Brama* spp. In 19 *C. crepidater* we observed mesopelagic prey; in 98 stomachs from the North Atlantic, Mauchline and Gordon (1983) found squids and micronektonic fishes, and Ebert et al. (1992) found a similar diet in four specimens from southern Africa. In 12 *P. plunketi* we found predominantly demersal fish prey, including other elasmobranchs, and likely scavenging; a qualitative diet of mainly fishes and cephalopods is reported by Last and Stevens (2009). In 23 *G. galeus* we found benthic, demersal, and pelagic fish and invertebrate prey; the diet of *G. galeus* is comparatively well described, and comprises mostly fishes and cephalopods, but varies with shark size, season, and between regions (Lucifora et al., 2006 and references therein).

The sample sizes in this study were too small to allow examination of spatial, temporal, or biological (e.g., ontogenetic) variability in diet, and as a result the diet descriptions may be biased compared to other published reports. Ontogenetic and seasonal variability in diet has been demonstrated for *G. galeus* (Lucifora et al., 2006), and should be suspected for other shark species. Bias in the diet may also result from feeding in the net, and from partial (and unobserved) regurgitation of stomach contents.

The estimates of asymptotic prey richness are tentative given the low number of observations from which the asymptotes were predicted, and the unknown suitability of the asymptotic curves as models of cumulative prey richness for these species. The time taken to collect enough samples to measure 90% of the estimated asymptotic prey richness depends not only on the method used to identify prey, but on the sampling rate of sharks within each survey, and the number of surveys each year. Improvements to the sampling rate might be made by prioritising sharks for sample collection in future surveys of the study region. However, for rarer species such as *P. plunketi*, where only ca. 10 specimens have been caught per survey, the sample collection rate on Chatham Rise could only be substantially increased with additional survey time. The current cost of survey time vastly outweighs that of DNA barcoding of prey, making DNA barcoding a cost-effective way of increasing sampling rate.

We have demonstrated that DNA barcoding can be used to identify prey, and can greatly increase the rate of data accumulation. The use of DNA barcoding is also invaluable for species such as *C. plunketi* and *D. licha* where chunks of flesh are found in the stomach, as a result of taking bites out of larger prey (Last and Stevens, 2009). With this feeding method, visual identification of prey will be limited to species where characteristic remains are found (i.e., species with unusual morphology), but much of the diet will remain unidentifiable unless alternative prey identification methods are used. The BOLD will also become increasing useful as more invertebrate reference sequences are added, and we therefore recommend DNA barcoding be considered as a method for identifying prey of all rare fish-predator species. Rapid developments in molecular biology are providing high-throughput sequencing techniques that enable DNA identification of numerous taxa in large numbers of gut samples (Soininen et al., 2009); the major constraint being the high unit cost, which may decline as the new technologies become mainstream (King et al., 2008).

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