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# Feeding ecology of the largest mastigoteuthid squid species, *Idioteuthis cordiformis* (Cephalopoda, Mastigoteuthidae)

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**ABSTRACT:** The squid *Idioteuthis cordiformis* is consumed by several apex predators, but nothing is known of its own feeding ecology. Its anomalous size and morphology within the family Mastigoteuthidae suggest that its diet may also be unusual, but material suitable for dietary studies has not been available previously. Herein, using several opportunistically collected specimens from New Zealand waters, gut contents were examined using DNA barcoding, revealing that this species feeds upon birdbeak dogfish *Deania calcea* and, apparently, snapper *Lutjanus* sp. Stable-isotope analysis of  $^{15}\text{N}$  and  $^{13}\text{C}$  confirmed that *D. calcea* occupied a trophic level immediately below that of the squid. These data suggest that this squid may prey actively upon relatively large pelagic species; alternatively, it could be net feeding and/or scavenging. *I. cordiformis* has  $^{15}\text{N}$  signatures comparable to those of the colossal squid and higher than those of the giant squid, which could indicate a high trophic position; however, further research is needed to conclusively distinguish predation from scavenging.

**KEY WORDS:** *Idioteuthis cordiformis* · Mastigoteuthidae · New Zealand · DNA barcoding · Stable isotopes

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## INTRODUCTION

The largest species in the family Mastigoteuthidae is *Idioteuthis cordiformis*; many times larger than any other species in this family, it attains more than 1 m in mantle length and 75 kg in mass (S. O'Shea pers. comm.). It is known from Sumatra (Chun 1908), Japan (Sasaki 1929), the Philippines (Voss 1963) and New Zealand, where it was recently listed as nationally endangered (Hitchmough et al. 2005). However, after the re-evaluation of the threat classification system, the status for *I. cordiformis* was elevated to nationally critical (Freeman et al. 2010), meaning that it is considered to be within the top 10 species in

New Zealand in the greatest danger of local extinction. This benthic, bathypelagic species, found around northern New Zealand, is associated with seamounts, including those in the Bay of Plenty and on the Chatham Rise (Freeman et al. 2010), where this species has been caught between 750 and 1500 m (Braid 2013). Its occurrence on seamounts makes it vulnerable to deep-sea fishing activities that target these structures. Although fishing is believed to have strong effects on the New Zealand *I. cordiformis* population (Freeman et al. 2010), the actual impacts have not been verified due to limited data.

A variety of different marine species are known to consume *I. cordiformis*. It has been found in the diets

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of blue marlin *Makaira nigricans* (Shimose et al. 2012), sperm whales *Physeter macrocephalus* (Evans & Hindell 2004), swordfish *Xiphias gladius* (Watanabe et al. 2009) and neon flying squid *Ommastrephes bartramii* (Watanabe et al. 2008). Conversely, very little is known of the diet of any mastigoteuthid, although one species (*Mastigoteuthis psychrophila*) has been reported to consume euphausiids (Kear 1992). The tentacular morphology of most mastigoteuthids (unexpanded clubs with microscopic suckers) suggests that they are passive predators, dangling their tentacles in search of prey (Dilly et al. 1977). However, the diet of *I. cordiformis* (a mastigoteuthid with unusually large tentacular suckers for this family, making the tentacle club more comparable to those of large ommastrephids) remains completely unknown.

Previously, morphological analysis of the gut contents has been successful for some large squids (Bolstad & O'Shea 2004, Ibáñez et al. 2008, Stewart et al. 2013), but this approach relies on identifiable hard parts, which are often lacking in squid gut contents, since any items that successfully traverse the esophagus have been finely masticated by the beak (Jackson et al. 2007). However, DNA analysis can identify squid prey from even fragmentary remains and soft tissue (e.g. Braley et al. 2010, Braid et al. 2012). Gut contents identified by either method can give insight into specific recently consumed prey, while stable isotopes can give a more general, long-term view of the trophic role of a species and have been used to study the trophic interactions of other squids (e.g. Cherel & Hobson 2005, Cherel et al. 2008). Stable isotopes for nitrogen can be used to infer trophic position because the tissue of predators is enriched with  $^{15}\text{N}$  compared to their prey, while carbon, which remains relatively constant, can be used to geographically pinpoint sources of primary productivity.

This research aimed to gain the first known insight into the feeding ecology of *I. cordiformis* by analy-

zing (1) gut contents from fresh specimens using DNA barcoding, and (2) food sources and trophic position using stable isotopes  $^{13}\text{C}$  and  $^{15}\text{N}$ .

## MATERIALS AND METHODS

This study was based on opportunistically collected samples because fresh or frozen material was required. Four frozen individuals of *Idioteuthis cordiformis* were available for gut content and stable isotope analysis; one lacked collection data, and the others were collected from West Norfolk Ridge by the R/V 'Tangaroa', during the 2003 New Zealand and Australia Norfolk Ridge and Lord Howe Rise Biodiversity Voyage (NORFANZ). Specimens were kept frozen at  $-20^{\circ}\text{C}$  until analysis (Table 1). Individual prey items (pieces of scale, bone or tissue) were removed and stored individually in 100% EtOH. Eight items were chosen from each gut for analysis based on morphological differences (different tissue types or colours). The DNA barcode region (Hebert et al. 2003) was amplified for 8 items from each of 2 gut contents of 2 individuals (2 were empty) using primers and methods following Braid et al. (2012), except that DNA was extracted using the Xytogen Animal Extraction Kit and sequenced with Folmer et al. (1994) primers. Edited sequences were uploaded to the Barcode of Life Data Systems (BOLD) under the project name '*Idioteuthis cordiformis* Gut Contents' (project code DBICG) and compared against the BOLD COI Species Database (Ratnasingham & Hebert 2007) in January 2014. Species-level identifications were made using a tree-based identification method by examining neighbor-joining trees.

Muscle samples from the fins of the 4 *I. cordiformis* specimens were used for stable-isotope analysis for carbon and nitrogen (Table 1). Approximately 700 mg of fin tissue was dried at  $60^{\circ}\text{C}$  for 48 h. The

Table 1. Stable isotope analysis of New Zealand *Idioteuthis cordiformis* gut contents. No collection data were available for NIWA 84390; gear type was orange roughy trawl (ORT) or ratcatcher (RTC); trophic level was estimated based on the equation from Cherel et al. (2008). ML: mantle length; indet.: sex indeterminate

| Specimen ID   | Sex    | ML (mm) | Latitude ( $^{\circ}\text{S}$ ) | Longitude ( $^{\circ}\text{E}$ ) | Depth (m) and gear type | Date (d/mo/yr) | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | Estimated trophic level |
|---------------|--------|---------|---------------------------------|----------------------------------|-------------------------|----------------|---------------------------|---------------------------|-------------------------|
| NMNZ M.306356 | Indet. | 405     | 34.24                           | 168.35                           | 1195–1200 (ORT)         | 3/06/2003      | 16.121                    | -18.160                   | 5.9                     |
| NMNZ M.306355 | ♂      | 513     | 34.57                           | 168.94                           | 1013–1340 (RTC)         | 3/06/2003      | 16.258                    | -18.043                   | 6                       |
| NMNZ M.306358 | ♂      | 549     | 33.78                           | 167.49                           | 1431–1460 (ORT)         | 29/05/2003     | 15.538                    | -18.284                   | 5.7                     |
| NIWA 84390    | ♀      | 820     | –                               | –                                | –                       | –              | 16.602                    | -17.831                   | 6.1                     |

tissue was ground using a mortar and pestle under liquid nitrogen. Samples were sent to the Waikato Stable Isotope Unit, University of Waikato, Hamilton, New Zealand. Analysis was performed using a fully automated Europa Scientific 20/20 isotope analyzer.  $\delta^{13}\text{C}$  was calculated using precalibrated C4 sucrose that is cross-referenced to PeeDee belemnite.  $\delta^{15}\text{N}$  was calculated using a urea standard traceable to atmospheric nitrogen. Trophic levels were calculated according to the equation  $\text{TL} = [(\delta^{15}\text{N}_x - 3.4)/3.2] + 2.0$  from Chérel et al. (2008), where TL is trophic level and x is the individual squid.

## RESULTS

Of the 4 *Idioteuthis cordiformis* individuals, only 2 contained gut contents suitable for DNA analysis, while the others contained only red-orange oil. Of the 16 samples extracted, 10 PCR amplicons were obtained, and from these, 2 barcodes were successfully sequenced and identified as prey. One sequence was a 100% match to the shark *Deania calcea*. The other sequence was a 100% match to *Lutjanus* sp. (this sequence was a 100% match to BOLD records for 2 species: *L. lemniscatus* and *L. fulvus*). *I. cordiformis* showed high trophic levels, ranging from 5.7 to 6.1 (Table 1).

## DISCUSSION

This study is of particular importance because *Idioteuthis cordiformis* specimens rarely have gut contents (S. O'Shea pers. comm.), and such contents have not been previously reported; the only other known gut contents were otoliths from orange roughy *Hoplostethus atlanticus* anecdotally observed in a single specimen (D. Stevens pers. comm.). This specimen was a female of 510 mm mantle length collected from the Challenger Plateau, and the otoliths were from 2 fish that were both approximately 30 cm in standard length; this may represent net feeding because the *I. cordiformis* was collected during an orange roughy survey. Lu & Williams (1994) experienced similar difficulties in examining *Mastigoteuthis psychrophila* for gut contents: of 19 specimens, 12 had empty guts and the rest were missing their viscera. In our specimens and in preserved material, a large amount of red-orange oil was found in the stomachs (see Braid 2013, Fig. 59C); Phillips et al. (2001) found a similarly high oil content in the gut fluid of *Moroteuthis ingens*. Their fatty acid analysis

of this fluid suggested that it was from myctophid fishes, which are known to contain a high level of oil (Phleger et al. 1999).

The shark sequence was unexpected because mastigoteuthids are generally believed to be passive predators (Dilly et al. 1977) that likely—based on the diet of *M. psychrophila*—consume euphausiids (Kear 1992). The sequence of the birdbeak dogfish *Deania calcea* matched most closely with those of specimens from the Tasman Sea, which is near the capture location for these *I. cordiformis* specimens (Norfolk Ridge). *Deania calcea*, which is relatively abundant in the same depths where *I. cordiformis* is often collected (Zintzen et al. 2011), was also caught during the NORFANZ voyage (Clark et al. 2003). While initially surprising, it is not unreasonable to find sharks as prey of a cephalopod. Dogfish skin has been found in the gut contents of another large squid in New Zealand waters, *Onychia ingens* (J. McKinnon pers. comm.). The considerable maceration of the gut contents prevented any estimation of prey size; however, a large squid could consume a small shark, and reports also exist of squid actively preying on animals of sizes comparable to, or even larger than, themselves (e.g. MBARI 2013). The shark may also have been scavenged. Scavenging would be a rare primary feeding strategy, since to date only one cephalopod has been revealed as a detritivore (the vampire squid *Vampyroteuthis infernalis*; see Hoving & Robison, 2012). Another possibility is that the squids analysed here had fed on prey caught in the same trawl net, as has been reported for other large squids, such as *Dosidicus gigas* (Ibáñez et al. 2008).

The snapper sequence (*Lutjanus* sp.) is from a shallow-water reef species (Newman & Williams 1996). However, the 2 potential species matches for the sequence from *I. cordiformis* gut contents—*L. lemniscatus* and *L. fulvus*—are both found in the Great Barrier Reef (Newman & Williams 1996), which is west of the Norfolk Ridge. Some deep-sea fishes, such as rattails (*Coryphaenoides armatus* and *C. yaquinae*), have been shown to scavenge shallow-water carrion (Drazen et al. 2008). However, the presence of snapper could also be due to a different kind of sampling bias: secondary predation can be a significant source of error with molecular prey detection (Harwood et al. 2001, Sheppard et al. 2005) and could also potentially explain the *Lutjanus* sequence. More research is needed to conclusively determine the feeding behavior of *I. cordiformis*.

Nitrogen, which becomes enriched as it passes through the food chain, can be analysed to provide

insight into the trophic level at which animals are feeding (Fry 2006). *I. cordiformis*, rather unexpectedly, had an estimated trophic level comparable to that of the colossal squid *Mesonychoteuthis hamiltoni* and higher than that of the giant squid *Architeuthis dux* (Cherel et al. 2008), indicating that it occupies a high trophic position in the system. Another mastigoteuthid, *M. psychrophila*, which is relatively small, was also previously found to have higher  $\delta^{15}\text{N}$  values than those of the giant squid (Cherel & Hobson 2005). Relatively elevated  $\delta^{15}\text{N}$  values can be caused by scavenging, which was found in the gastropod *Cyclope neritea* (Camusso et al. 1998).

Unlike nitrogen, the isotope value for carbon remains relatively constant as it moves through the food chain, making carbon a helpful tool for tracing the origin of primary production for a system (Fry 2006). Carbon and nitrogen stable-isotope values have been previously identified for *D. calcea* specimens from southeastern Australia (Pethybridge 2010). These isotope values place *D. calcea* one trophic position lower than *I. cordiformis*, with a similar carbon value (Pethybridge 2010), which supports it as potential prey of *I. cordiformis* (Fig. 1).

This is the first investigation into the ecology of any mastigoteuthid using stable isotopes and DNA barcoding for gut-content analysis. DNA barcoding did successfully identify prey items, and stable-isotope values indicated that this species could be a top predator; however, the gut contents revealed herein could reflect scavenging or net feeding (Ibáñez et al. 2008, Hoving & Robison 2012). Cephalopod trophic interactions in the Southern Ocean are poorly known (Cherel & Hobson 2005); however, it is important to study the ecological role that this species plays to assess the impacts that its population reduction could have on other deep-sea species around New Zealand. Future research should investigate the stable-isotope values and gut contents of other organisms within the local ecosystem to better understand the trophic interactions between species and help predict the impacts that could occur from disturbances.

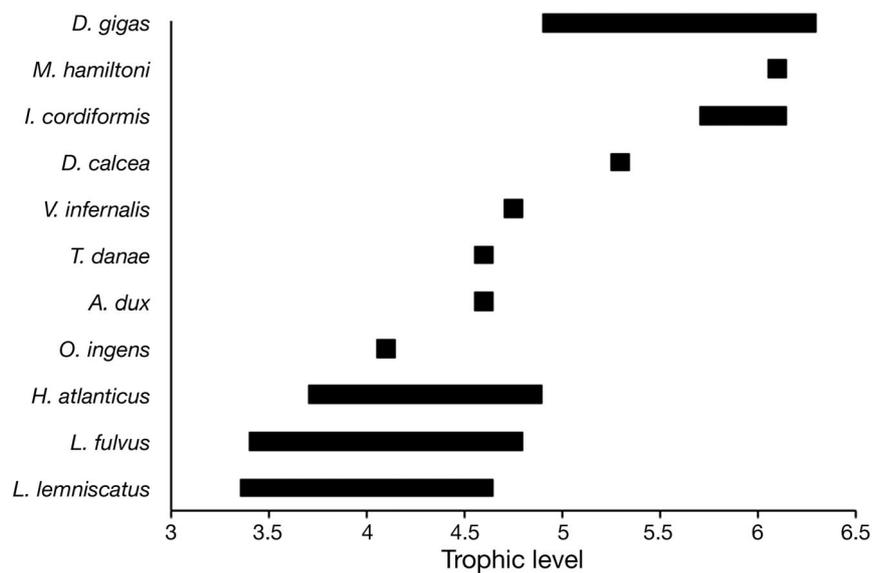


Fig. 1. Trophic level for *Idioteuthis cordiformis*, other squids, and fish prey items of *I. cordiformis*. Trophic levels from Cherel et al. (2008) are included for *Architeuthis dux*, *Mesonychoteuthis hamiltoni*, *Onykia ingens* and *Taningia danae*. Trophic levels were calculated from nitrogen stable isotope levels ( $^{15}\text{N}$ ) using the equation from Cherel et al. (2008) for *Deania calcea* (Pethybridge 2010), *Dosidicus gigas* (Ruiz-Cooley et al. 2006), *I. cordiformis* and *Vampyroteuthis infernalis* (Cherel et al. 2009). Trophic levels for *Hoplostethus atlanticus* and the *Lutjanus* species are from Froese & Pauly (2011)

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