A dietary study of *Moroteuthis ingens* and other Southern Ocean squid species: combined stomach contents and fatty acid analyses

by

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"EMBRIAGADO por la evidencia del prodigio, en aquel momento se olvidó de la frustración de sus empresas delirantes y del cuerpo de Melquíades abandonado al apetito de los calamares."

"INTOXICATED by the evidence of the miracle, he forgot at that moment about the frustration of his delirious undertakings and Melquíades' body, abandoned to the appetite of the squids."

Gabriel García Márquez
Cien años de soledad (One hundred years of solitude) (1967)

"NO shortage of explanations for life's mysteries. Explanations are two a penny these days. The truth, however, is altogether harder to find."

Salman Rushdie
The ground beneath her feet (1999)

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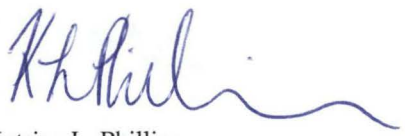

Katrina L. Phillips

ABSTRACT

The squid fauna are a key component of the Southern Ocean ecosystem, although unfortunately little is known of their distribution, biology and ecology. While the biomass of squid in the Southern Ocean must impose a large amount of predatory pressure on lower trophic levels, few dietary data exist for any of the Southern Ocean species. Conventional dietary analyses of squid are fraught with many sources of bias, and therefore this study has employed complementary stomach contents and fatty acid analyses to investigate aspects of the diet of Southern Ocean squid. The diet of the onychoteuthid *Moroteuthis ingens* was largely comprised of myctophid fish, and the digestive gland of *M. ingens* was rich in lipid and found to be an ideal source of fatty acid dietary tracers that are unmodified from the diet. Fatty acid dietary tracers were then applied in combination with stomach contents analyses to investigate the temporal, spatial and size-related dietary patterns of *M. ingens*. Using these complementary techniques, the diet of *M. ingens* was found to vary significantly on an interannual and seasonal basis within the vicinity of Macquarie Island, largely due to fluctuations in the proportions of myctophid fish species in the diet. *Moroteuthis ingens* also exhibited spatial variations in diet among Macquarie Island, the Falkland Islands, the Chatham Rise and Campbell Plateau (the latter two sites located within New Zealand waters). The diet at the New Zealand sites was characterised by a proportion of temperate myctophid species not identified at other sites, whereas squid collected from the Falkland Islands consumed a much greater proportion of cephalopod prey. Lipid class and fatty acid analyses also indicated that the diet varied considerably between the Chatham Rise and Campbell Plateau, most likely due to the influences of different oceanographic regimes. Stomach contents and fatty acid analyses of squid collected from the Falkland Islands indicated that *M. ingens* switches from a crustacean- and cephalopod-based diet to a fish- and cephalopod-diet at around 200 mm mantle length. Lipid class and fatty acid analyses of the other Southern Ocean squid indicated that oegopsid species accumulate a large amount of lipid in the digestive gland. This lipid has been determined to be of dietary origin, and will thus provide a rich source of fatty acid dietary tracers applicable to future food-web studies of squid. Further consideration of the mode of lipid and fatty acid storage in the digestive gland will be required before squid fatty acid profiles can be reliably applied to dietary studies of higher trophic predators.

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Acronyms

AA	arachidonic acid
ADWM	Antarctic Deep Water Mass
AFMA	Australian Fisheries Management Authority
APF	Antarctic Polar Front
BSTFA	N,O-bis-(trimethylsilyl)-trifluoroacetamide
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DA	discriminant analysis
DAGE	diacylglyceryl ether
DG	digestive gland
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EEZ	Exclusive Economic Zone
EPA	eicosapentaenoic acid
FAME	fatty acid methyl ester
FFA	free fatty acid
FIGFD	Falkland Islands Government Fisheries Department
FO	frequency occurrence
GC	gas chromatograph(y)
GMT	generic mapping tools
IASOS	Institute of Antarctic and Southern Ocean Studies
LRL	lower rostral length
MDS	multidimensional scaling
ML	mantle length

MS	mass spectrometry
MUFA	monounsaturated fatty acid
NI	numerical importance
NIWA	National Institute of Water and Atmospheric Research
OL	otolith length
PFZ	Polar Frontal Zone
PL	phospholipid
PSI	percentage similarity index
PUFA	polyunsaturated fatty acid
SD	standard deviation
SAF	sub-Antarctic Front
SAT	saturated fatty acid
SAW	sub-Antarctic Water
SL	standard length
SST	sea surface temperatures
ST	sterol
STF	Subtropical Front
TAG	triacylglycerol
TLC–FID	thin-layer chromatography–flame-ionization detector
TM	total mass
TSE	total solvent extract
WE	wax ester

Authorities for cephalopod species mentioned within the text of this thesis

Stoloteuthis leucoptera	Verrill, 1878
Stoloteuthis spp.	Verrill, 1881
Sepia officinalis	Linné, 1758
Loligo gahi	d'Orbigny, 1835
Sepioteuthis australis	Quoy & Gaimard 1832
Watasenia scintillans	Berry, 1911
Gonatopsis borealis	Sasaki, 1923
Gonatus antarcticus	Lönnberg, 1898
Moroteuthis ingens	Smith, 1881
Moroteuthis robsoni	Adam, 1962
Illex argentinus	Castellanos, 1960
Illex coindetii	Vérany, 1839
Martialia hyadesi	Rochebrune & Mabille, 1887
Sthenoteuthis oualaniensis	Lesson, 1830
Sthenoteuthis pteropus	Steenstrup, 1855
Todarodes angolensis	Adam, 1962
Todarodes filippovae	Adam, 1975
Todarodes spp.	Girard, 1890
Todaropsis eblanae	Girard, 1890

Chapter 1. General Introduction

1.1 SQUID AND THE SOUTHERN OCEAN

Squid (Mollusca: Cephalopoda) are among the most ancient of all existing groups of nekton (Lubimova 1985): they are highly mobile, strictly carnivorous and exclusively marine (Nixon 1987). Within the Southern Ocean, the squid fauna are an intriguing group of animals that demonstrate an incredible diversity of body form and, presumably, ecological function (Fig. 1.1). Approximately twenty species are permanent residents within the cool, circumpolar waters that extend northward from the Antarctic continent to the southern coastlines of Australia, New Zealand, South America and South Africa. These species represent nine families and thirteen genera, six of which are monotypic and endemic to the Southern Ocean (*Psychroteuthis*, *Alluroteuthis*, *Batoteuthis*, *Neoteuthis*, *Mesonychoteuthis* and *Kondakovia*) (Filippova 1972). This degree of endemism is unusual for squid, and thus distinguishes the Southern Ocean fauna from all other cephalopod populations (Filippova 1972 after Akimushkin 1983).

Squid are almost ubiquitous within the Southern Ocean, existing on the continental shelves and slopes of the major landmasses and scattered sub-Antarctic islands, and also within the open ocean from the euphotic zone to bathypelagic depths. Most species from the suborder Oegopsida - the oceanic squids - occupy the Antarctic Deep Water Mass (ADWM) (Lubimova 1985), and from within this environment provide an essential food resource for many marine predators including fish (Jackson et al. 2000a; Xavier et al. 2002), toothed whales (Clarke & Goodall 1994; Clarke & Roeleveld 1998), otariid and phocid seals (Bester & Laycock 1985; Daneri et al. 1999; Green & Burton 1993), penguins and other seabirds (Cherel et al. 1996; Thompson 1994). The diet of some albatross, in particular the wandering albatross (*Diomedea exulans*) and grey-headed albatross (*Diomedea chrysostoma*), is mostly composed of dead or dying squid scavenged from the ocean's surface (Cherel & Weimerskirch 1999; Waugh et al. 1999; Weimerskirch et al. 1997). As such, squid represent an important mechanism by which energy is transferred from the pelagic nekton to the top trophic predators of the Southern Ocean.

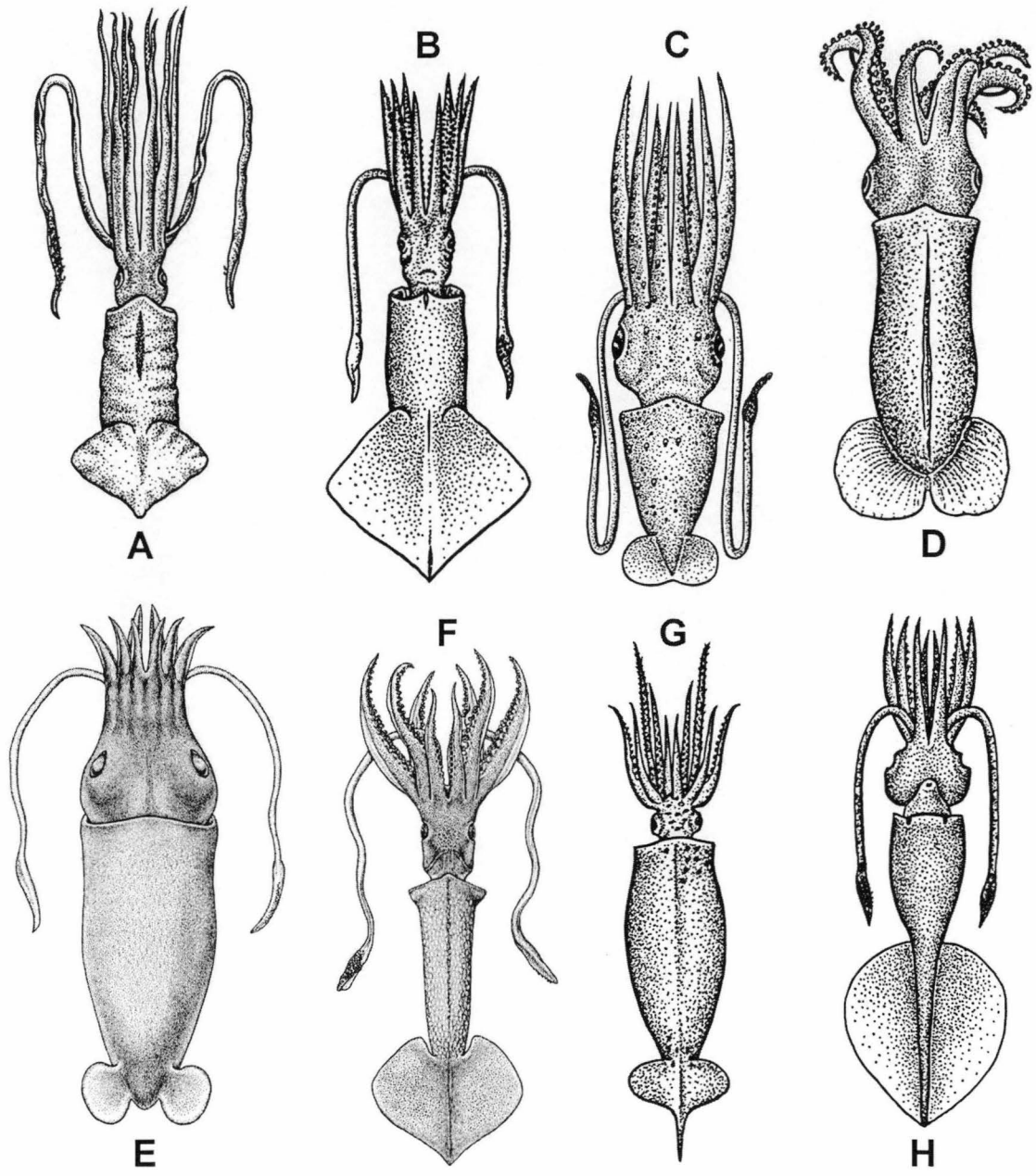

Fig. 1.1. Diversity in body form of some Southern Ocean squid. A: *Kondakovia longimana*; B: *Psychroteuthis glacialis*; C: *Histioteuthis eltaninae*; D: *Alluroteuthis antarctica*; E: *Bathyteuthis abyssicola*; F: *Brachioteuthis* cf *picta*; G: *Batoteuthis skolops*; H: *Mesonychoteuthis hamiltoni* (ventral view). Ilustrations A-D and G-H from Nesis (1987), E-F from Mangold et al. (1998).

Much of our existing knowledge of the diversity, distribution and size-range of Southern Ocean squid has been obtained indirectly from dietary studies of higher predators (for example, see (Cherel & Weimerskirch 1999; Clarke 1983; Rodhouse et al. 1996). Cephalopods themselves have rarely attracted the attention of major ecological studies in the Southern Ocean (Okutani 1994), and as a consequence, less is known of squid than of any other marine fauna in the ecosystem (Lubimova 1985). While the importance of squid as prey has been firmly established, the role of squid as predators in the Southern Ocean is largely undescribed.

1.2 SQUID AS PREDATORS IN THE SOUTHERN OCEAN

Squid in general are remarkably voracious predators, opportunistically consuming a broad range of prey that varies in both size and shape (Boyle & Boletzky 1996; Nixon 1987). This versatility in prey capture is implemented by unique feeding appendages: eight arms and two tentacles - all prehensile and many armed with toothed sucker rings and formidable hooks - that together comprise the brachial crown (Rodhouse & Nigmatullin 1996) (Fig. 1.2). The brachial crown facilitates the capture of prey that may equal or even exceed the mantle length of the squid itself (Mangold 1983), so that a wider spectrum of prey is available to squid than to contemporary fish predators. The standing stock of squid south of the Antarctic Polar Front (APF) has been estimated to exceed 35 million tonnes (Clarke 1983), and some Southern Ocean species may consume in excess of 10% of their body weight per day (Jackson et al. 1998a), thus the impact of predation by squid throughout the Southern Ocean must be considerable. To date, however, the impact of squid on lower trophic levels has received little attention and remains poorly understood.

Prior to 1986, stomach contents data were only available for around 200 squid from the Southern Ocean (Kock 1987) and since this time, larger numbers of stomachs have been examined for only a handful of species (Ivanovic & Brunetti 1994; Jackson et al. 1998a; Kear 1992; Lu & Williams 1994b; Mouat et al. 2001; Nemoto et al. 1988; Rodhouse et al. 1992). Mesopelagic fish of the myctophid family appear to be the main prey group of squid, at least within the Polar Frontal Zone (PFZ) that extends between the APF and sub-Antarctic Front (SAF) (Kock 1987; Lubimova, 1985; Rodhouse & White 1985).

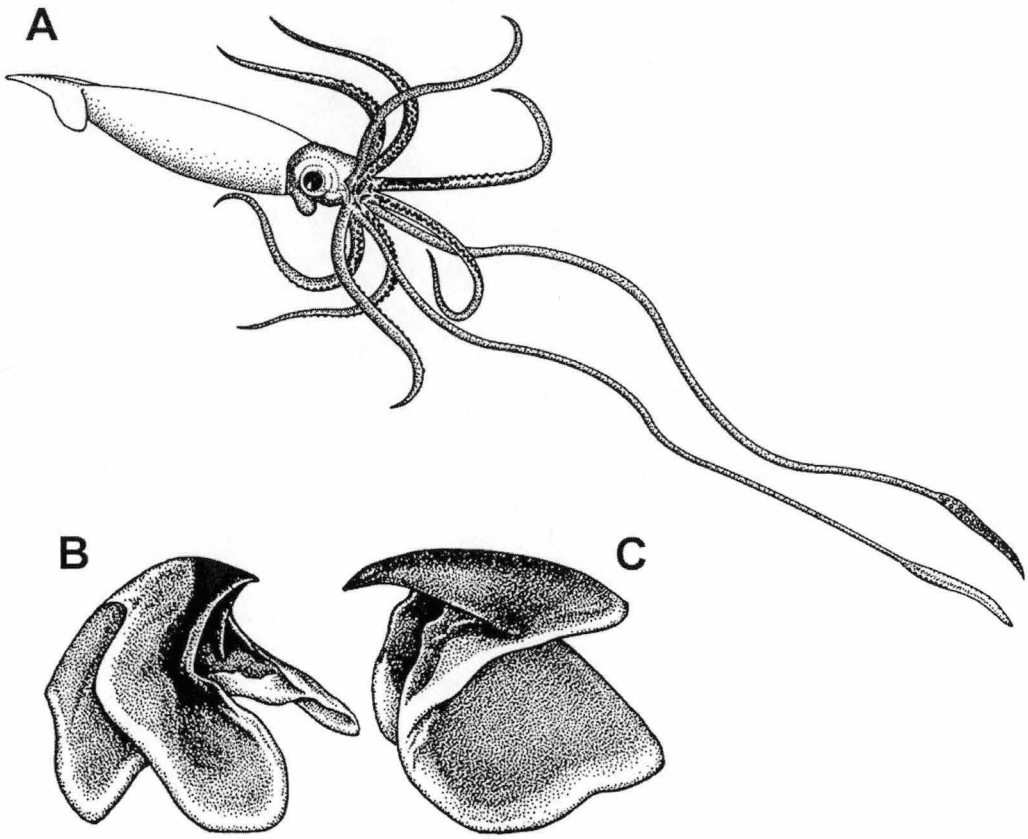

Fig. 1.2. Feeding apparatus of squid. A: *Architeuthis* sp., demonstrating the eight arms and two tentacles of the brachial crown; B: lower beak of *Moroteuthis ingens*; C: upper beak of *M. ingens*. Illustrations from Mangold et al. (1998).

Rodhouse & Nigmatullin (1996) suggested that within the open ocean, most predation pressure on myctophids derives from squid, and Lubimova (1985) and Rodhouse & White (1995) have independently proposed the existence of a copepod – myctophid – squid – higher predator food web within the Southern Ocean that is largely independent of keystone zooplankton species such as Antarctic krill (*Euphausia superba*). In contrast, some studies have identified *E. superba* and other krill species to be important prey for Southern Ocean squid (Kear 1992; Lu & Williams 1994b; Nemoto et al. 1988; Nemoto et al. 1985). It is of note, however, that such results were often obtained from squid that had been collected in nets designed and set to catch krill. As previously noted, squid are opportunistic predators and are known to feed even when captured by fishing gear (Breiby & Jobling 1985; Dawe et al. 1997; Kear 1992; Lipinski 1987; Piatkowski et al. 2001; Rodhouse & Nigmatullin 1996). Therefore, stomach contents can be highly correlated with the species composition of a trawl net or jigging gear and may not represent normal dietary composition.

1.3 DIETARY STUDIES OF SQUID

In addition to abnormal feeding in the presence of sampling gear, several other problems are associated with dietary studies of squid. Squid use their chitinous beaks and radula to finely bite prey into small pieces prior to ingestion, so that it can pass through the narrow diameter of their oesophagus (Piatkowski et al. 2001; Rodhouse & Nigmatullin 1996) (Fig. 1.2). As a result, stomach contents sometimes contain nothing more than a slurry of prey remains that cannot be identified visually (Kear 1992). Furthermore, squid often reject hard parts of prey, such as the heads of larger fish (Dawe et al. 1997; Lordan et al. 1998; Piatkowski et al. 2001; Rodhouse & Nigmatullin 1996), so that diagnostic structures such as fish otoliths are not ingested. Consequently, prey identification can be constrained, and sometimes remains cannot be identified beyond broad prey categories. A further problem is that while the heads of larger fish species may be rejected, smaller fish and other prey may be consumed whole (Lipinski 1987). Consequently, the proportion of these smaller prey species in the diet can be overestimated.

Conventional dietary studies of squid stomach contents are also affected by those general problems that affect dietary studies of all marine predators. Most importantly,

instantaneous sampling of squid stomach contents provides no information on long-term dietary trends. In addition, no dietary data can be obtained from empty stomachs; squid are thought to have a rapid digestion rate (Lipinski 1987), and therefore must be captured within several hours after feeding if identifiable remains are to be retrieved for diet analysis. To address the many problems and biases associated with conventional diet analysis, alternative or "complementary" methods should be sought in order to obtain a clearer understanding of the dietary composition of squid in the Southern Ocean.

1.4 COMPLEMENTARY METHODS OF DIET ANALYSIS

Several methods of complementary diet analysis are applicable to squid, and these may be split into two groups: a) methods that can identify prey to species level using visually unidentifiable remains from the stomach contents, and b) methods that rely on biomarkers or "dietary tracers" that have been accumulated within a tissue or an organ. Some of these methods are briefly discussed and compared below.

1.4.1 Serological and genetic techniques

Both serological and genetic analyses of stomach contents have the potential to identify prey remains to species level. Serological techniques have been previously applied to dietary studies of adult and juvenile squid (Kear 1992; Venter et al. 1999). Stomach contents are screened with an antisera from a potential prey species, and a positive reaction confirms the presence of that prey species within the stomach contents. However, similar prey species may cross-react with the antisera, and thus produce a false-positive result (Kear 1992). Furthermore, antisera are not reproducible as no two antisera have exactly the same properties (Symondson 2002), and antiseral production is expensive and time-consuming.

Genetic techniques for identifying prey species in the stomach contents of marine predators use the polymerase chain reaction (PCR) to amplify prey DNA (Jarman et al. 2002). The primers for the PCR may be specific to an individual prey species, or to a group of prey. Should the prey type be present in the stomach contents, the primers attach to the appropriate DNA which is replicated many times to generate enough copies of the fragment to allow it to be isolated and identified using gel electrophoresis. DNA-

based methods are still in their infancy and still have several disadvantages. These include the cost of developing species- or group-specific primers, and difficulties associated with the quantification of diet composition (S. Jarman personal communication).

Serological and genetic techniques may provide additional information on diet composition in those circumstances when prey has been finely macerated and can no longer be identified visually, or when a squid has rejected the identifiable hard body parts (such as the heads of fish). However, these techniques can only produce an "instantaneous snapshot" of diet, and provide no advantage over conventional analysis when squid have been feeding artificially in sampling gear, or when squid have not fed for several hours and thus have empty stomachs. The issue of feeding in sampling gear is of particular significance to studies of Southern Ocean squid. Until research programs begin to direct their attention to the specific collection of squid, samples can only be collected as by-catch from other research or commercial activities. Extensive research continues to be conducted on *E. superba*, and to date, krill surveys remain the most likely source of squid within the APF. Possibly the only way to assess the true importance of *E. superba* and other euphausiids in the diet of Southern Ocean squid species will be to use complementary methods of dietary analysis that are *not* dependent on stomach contents.

1.4.2 Stable isotopes and fatty acid dietary tracers

Stable isotopes and fatty acid dietary tracers have been applied to numerous studies of plankton and marine vertebrate species. Isotopic studies compare the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ between a predator and potential prey. While the isotopic composition of a predator generally reflects that of its diet, $\delta^{13}C$ is usually enriched by about 1% relative to the diet, whereas $\delta^{15}N$ can be enriched by around 3% (Deniro & Epstein 1978; Deniro & Epstein 1980; Wada 1987). Therefore, isotopic ratios can be used to estimate the trophic level of a marine predator, and could be determined for the various tissues of a squid. However, stable isotopes have not yet been extensively applied to the Southern Ocean ecosystem.

Fatty acids are the building blocks of lipids, a diverse group of carbon-based compounds that are essential components of every living cell (Sargent et al. 1987). Lipids may take the form of structural phospholipids and sterols, or they may be stored as several different forms of neutral lipid (eg triacylglycerol, wax ester, or diacylglyceryl ether) for energetic, reproductive or buoyancy purposes (Allen 1976). In the marine environment, fatty acids consist of a (usually) even-numbered carbon chain of 14 - 24 carbon atoms, with terminal carboxyl and methyl groups. Fatty acids vary not only in the number carbons, but also in the number of double bonds and the arrangement of double bonds and any side-chains (Fig. 1.3). Given their structural diversity and biological specificity, fatty acids are particularly useful biomarkers in food-web studies (Sargent et al. 1987), and fatty acid profiles may be obtained from any tissue type.

Many novel fatty acids exist in the marine environment that can only be synthesised by certain phytoplankton and algal species (Ackman 1980; Sargent 1976), and which become essential dietary components to higher organisms. Long-chain polyunsaturated fatty acids (PUFA) are primary examples of such essential dietary components. In addition to the long-chain PUFA, many other fatty acids are also derived largely from diet (Iverson 1993), so that the lipid reserves of a predator at least partly reflects that of its prey (Sargent 1976). Fatty acid dietary tracers have been explored in a number of diverse organisms such as copepods, euphausiids and other marine invertebrates, fish, penguins and other seabirds, seals and whales (Cripps et al. 1999; Graeve et al. 1994; Hansen & Cheah 1969; Horgan & Barrett 1985; Iverson 1993; Mourente & Tocher 1993; Raclot et al. 1998; Reinhardt & Van Vleet 1984), and their usefulness is now well recognised (Lea et al. 2002a). Fatty acid techniques have not previously been extensively applied to squid, although Navarro & Villanueva (2000) have established that long-chain PUFA are essential dietary requirements for juvenile cephalopods.

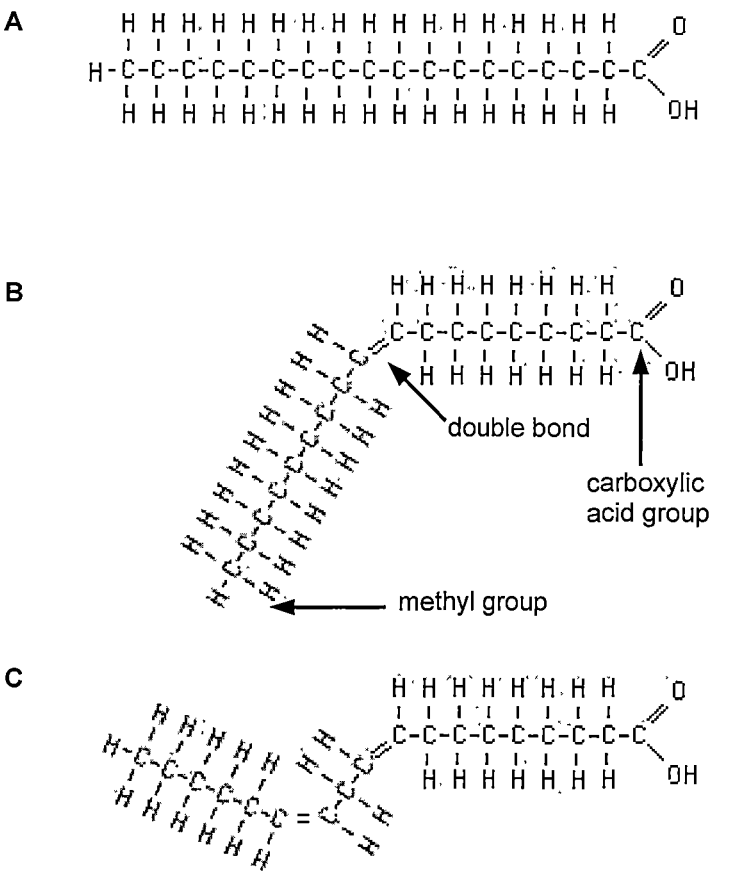

Fig. 1.3. Examples of fatty acids with 0, 1 or >1 double bond between consecutive carbon atoms: A: saturated fatty acid (SAT) (18:0: stearic acid); B: monounsaturated fatty acid (MUFA) (18:1n9: oleic acid); C: polyunsaturated fatty acid (PUFA) (18.2n6: linoleic acid). Fatty acid images obtained from the University of Utah Health Sciences Centre.

Extensive research on fatty acid biological markers has been conducted by CSIRO Marine Research and collaborating institutes for over a decade. Given that the appropriate facilities and expertise were available in Hobart, fatty acid techniques were selected to complement stomach contents analyses in this dietary study of Southern Ocean squid. It is important to note that current applications of stable isotope and fatty acid analyses to dietary studies are unable to identify individual prey species in the diet of a marine predator, unlike serological and genetic techniques. However, squid are generalist predators, as in fact are most marine predators (Link 2002), so assessing the proportion of an individual prey species in the diet may not be entirely relevant or necessary. It is likely to be more ecologically valid to identify important prey groups using fatty acid dietary tracers, and to monitor ontogenetic, temporal, spatial or inter-species shifts in fatty acid composition, in order to obtain an understanding of squid trophodynamics in the Southern Ocean.

1.5 THE ONYCHOTEUTHID SQUID *MOROTEUTHIS INGENS*

Most of the squid examined as part of this study were obtained as by-catch from commercial fishing or research activities conducted within regions of the sub-Antarctic Southern Ocean. The majority of specimens represented a single species, the onychoteuthid *Moroteuthis ingens* (Smith, 1881), otherwise known as the warty squid or greater hooked squid. A large proportion of this study is therefore concerned with *M. ingens*.

Large numbers of *M. ingens* have been previously collected from New Zealand waters and from the Patagonian Shelf, and consequently more biological and ecological information is available for this species than for most of the Southern Ocean squid. It has a circumpolar distribution in the Southern Ocean that generally extends between the APF and Subtropical Front (STF) (Kubodera et al. 1998), although it may enter the APF (Lubimova 1985). Within its distribution, it is associated with the ocean floor in the lower sublittoral and bathypelagic zone (Nesis 1987). *M. ingens* is a large, muscular species with rugose skin covered with fleshy warts, and it has 14 pairs of large chitinous hooks on each tentacular club (Kubodera et al. 1998) (Fig. 1.4). Hence, the common names for *M. ingens* derive from these taxonomic features. While initially considered to

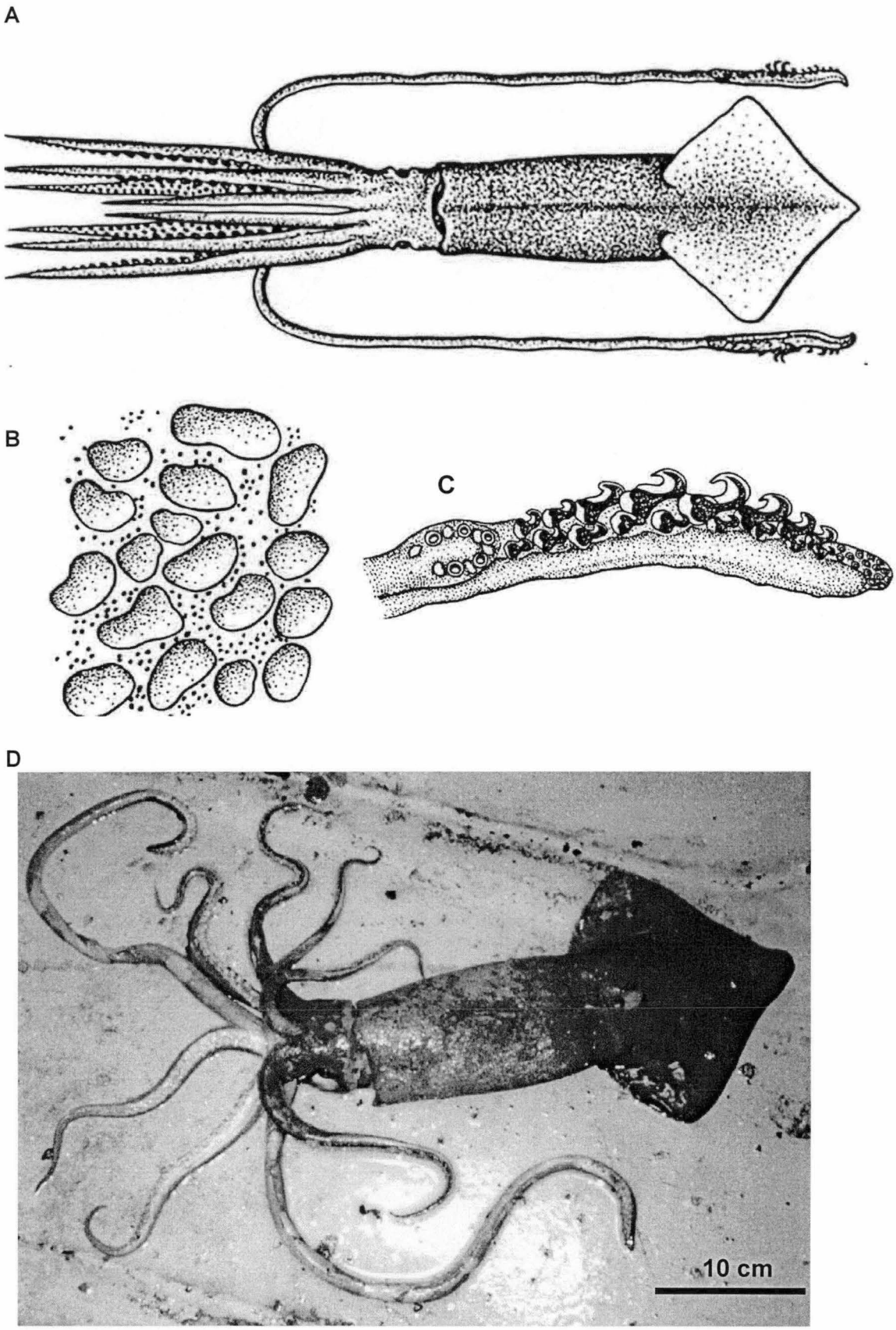

Fig. 1.4. A: *Moroteuthis ingens* from Nesis (1987) with detail of B: rugous, warty skin and C: tentacular club. D: A female specimen of *M. ingens*, maturity stage III, collected by trawl from the Falkland Islands during October 2001.

be rare, the prevalence of *M. ingens* in the diet of sperm whales indicates that this species is in fact abundant in the sub-Antarctic (Filippova 1972). *M. ingens* also constitutes an important prey item for at least four mammal, seventeen bird and thirteen fish species (Jackson et al. 1998b).

While juvenile and immature squid are found on the continental shelves of southern New Zealand and the sub-Antarctic islands (Cherel & Weimerskirch 1999; Jackson et al. 1998b), females are believed to undertake an ontogenetic migration to waters deeper than 700 m where they mature and spawn (Jackson 1997). Females reach a significantly larger size than males, and may attain a mantle length (ML) of 56 cm (Lipinski & Linkowski 1986) and a mass that is 5 times greater than that of male counterparts (Jackson 1997). As females approach their maximum size the ovary undergoes rapid development, prior to a spawning event that is associated with extensive tissue breakdown and inevitable death (Jackson 1997; Jackson & Mladenov 1994). Thus *M. ingens* appears to be a classic example of a "terminal spawner". The life cycle of *M. ingens* is completed within approximately one year (Jackson 1997).

Spawning is thought to occur mostly during the austral winter (Jackson et al. 2000b). Large amounts of tissue ammonia cause spent individuals to float to the ocean's surface after spawning (Lu & Williams 1994a), where they are scavenged by large numbers of albatross (Cherel & Weimerskirch 1999). Dietary information from the wandering albatross has confirmed winter spawning events around the Crozet archipelago, and has also indicated that the abundance and growth of *M. ingens* varies considerably on an interannual basis (Cherel & Weimerskirch 1999). This may be related to interannual fluctuations in the food resources of *M. ingens*.

A previous dietary study of *M. ingens* has indicated that temperate myctophid species are an important prey group (Jackson et al. 1998a), although one specimen collected by Nemoto et al. (1988) at an unidentified location had consumed *E. superba*. In total, published data have existed for only 38 specimens, 37 of which were collected in New Zealand waters and thus represented the northern-most distribution of *M. ingens*. Almost

no dietary information is available for *M. ingens* throughout its most common habitat: the sub-Antarctic Southern Ocean.

1.6 OBJECTIVES OF THIS STUDY

The objectives of this study were four-fold:

I. to expand upon current knowledge of the diet of Southern Ocean squid;

II. to establish which tissue or tissues would be best suited to complementary dietary analysis using fatty acid dietary tracers;

III. to use the combined techniques of stomach contents and fatty acid analyses to explore temporal, spatial and size-related dietary changes in a Southern Ocean squid species; and

IV. to consider how fatty acid dietary tracers may be applied to future predator-prey studies in the Southern Ocean.

Chapter 2. Materials and Methods

The materials and methods used throughout most of this study are described in this chapter, although some specific details are provided within each results chapter.

2.1 SQUID COLLECTION

2.1.1 Macquarie Island and Heard Island

All squid collected from around Macquarie Island and Heard Island were by-catch specimens from Australia's commercial fishery for Patagonian toothfish (*Dissostichus eleginoides*) fishery. Squid were collected on a strictly opportunistic basis between January 1995 and June 2000 by Australian Fisheries Management Authority (AFMA) fisheries observers posted on board the commercial trawlers *Austral Leader* and *Southern Champion.* Both vessels are owned and operated by Austral Fisheries Pty Ltd. *Austral Leader* is exclusively licensed to fish for *D. eleginoides* within Australia's Exclusive Economic Zone (EEZ) surrounding Macquarie Island, whereas both *Austral Leader* and *Southern Champion* are licensed to fish within Australia's EEZ surrounding Heard Island. The majority of squid collected from the Heard Island region were obtained from *Southern Champion.*

Squid were frozen whole at –20° C and transported to the Australian Antarctic Division, Hobart. Once transferred to the laboratories of the Institute of Antarctic and Southern Ocean Studies (IASOS) at the University of Tasmania, squid were thawed overnight prior to dissection and identified to species level where possible (Fischer & Hureau 1985; Nesis 1987). Further details are provided in Chapters 3 – 5 and Chapter 7.

2.1.2 Great Oyster Bay, Tasmania

A small number of the Southern calamary, *Sepioteuthis australis* (Quoy & Gaimard, 1832), were collected from Great Oyster Bay in south-eastern Tasmania (41° S, 148° E) during March 2000. Squid were collected by hand-line and jig on the shallow (<10m) inshore spawning grounds. Dissections were completed in the field on the day of collection, and

digestive gland and mantle tissue was frozen and transported to the laboratories of CSIRO Marine Research, Hobart. Tissue samples were stored at –20° C prior to lipid and fatty acid analysis. Further details are provided in Chapter 7.

2.1.3 The Chatham Rise and Campbell Plateau

Squid were trawled from within New Zealand's EEZ by the research vessel *Tangaroa*, operated by the National Institute of Water and Atmospheric Research (NIWA). Squid were collected from two research cruises, conducted firstly over the Chatham Rise between October 2000 – November 2000, and secondly over the Campbell Plateau between November 2000 – December 2000. Whole squid were frozen onboard and transferred to Hobart, where they were stored at –20° C. They were stored temporarily at the Hobart Cold Store facility, and transferred to IASOS as required. Squid were thawed overnight prior to dissection, and identified to species level where possible (Fischer & Hureau 1985; Nesis 1987). Further details are provided in Chapters 5 and 6.

2.1.4 The Falkland Islands

Specimens of *Moroteuthis ingens* were collected as by-catch from research surveys conducted by the Falkland Islands Government Fisheries Department (FIGFD) during October 2001. Squid were trawled by the fisheries patrol vessel *Dorada*, and dissected onboard within two hours of collection. Tissue and stomach samples were frozen and transported by air to Hobart, where they were stored at –20° C at either the laboratories of CSIRO Marine Research (prior to lipid and fatty acid analysis) or IASOS (prior to stomach contents analysis). See Chapters 5 and 6 for further details.

2.2 SQUID DISSECTIONS

Unless already stated otherwise, squid were dissected at the laboratories of IASOS. In all cases, the dorsal mantle length (ML) was recorded to the nearest mm, excluding those specimens with extensive damage to the mantle. Total mass was recorded to the nearest 0.1 g, again excluding those specimens that had been extensively damaged. An incision was then made along the ventral mantle to expose the internal organs. The gender was

determined, and each individual was allocated a maturity stage after Lipinski (1979) (Table 2.1). The stomach fullness was recorded using a 5-stage subjective scale (Jackson et al. 1998a after Zuev et al. 1985), and the stomach was removed intact and stored frozen in an individual plastic zip-lock bag until the contents could be analysed at a later date. The digestive gland was removed intact and weighed to the nearest 0.1 g, before being frozen and stored at –20° C in an individual plastic zip-lock bag prior to lipid and fatty acid analysis. Results were not recorded for badly damaged stomachs and digestive glands, nor were samples taken from these particular specimens. A piece of tissue approximately 1 cm^2 in size was removed from the ventral mantle, wrapped in aluminium foil and stored at –20° C for lipid and fatty acid analysis.

Table 2.1. Lipinski's universal scale, after Lipinski (1979)

	Male	Female
I **Juvenile**	SC visible as a whole unit spot only	NG as fine transparent strips. The remaining sexual organs invisible
II **Immature**	Parts of SC visible	Sexual organs translucent/white. Oviduct meander visible. Ovary visible as homogenous structure
III **Prepatory**	White streak on vas deferens (might be inconspicuous)	Sexual organs not translucent, oviduct meander extended. Immature ova visible, NG enlarged
IV **Maturing**	Vas deferens extended. White particles in Needam's sac. Testis structure present (fine grooves and ridges visible on surface	NG large, covering most internal organs. Mature ova (yellow) in ovary. No mature ova in oviduct
V **Mature**	SS contains tightly packed spermatophores. Testis structure present	Mature ova in oviduct. Secretion of NG
VI **Spent**	Degenerating spermatophores and SC. Testis structure disappears	Few if any eggs in oviduct and ovary

SC: spermatophoric complex; SS: spermatophoric sac; NG: nidamental glands

Further dissection was required for squid collected from Macquarie Island, Heard Island, the Chatham Rise and the Campbell Plateau as part of an auxiliary study. Reproductive organs were removed from these specimens and weighed to the nearest 0.1 g. Individual masses were recorded for the ovary, oviduct, oviducal glands and nidamental glands (females) and testis (males). A combined mass was recorded for all other male reproductive organs. Mantle and fin weights were also recorded to the nearest 0.1 g. These data are not directly

related to the objectives of this study and are provided in Appendix I. Statoliths were retrieved from each specimen, and stored dry in plastic wells pending increment analysis.

2.3 STOMACH CONTENTS ANALYSES

Stomachs were thawed, then cut open so that contents could be rinsed through a 500 μm mesh sieve. Small portions of the contents were examined at a time, until all contents had been sorted thoroughly. A heaped teaspoon was placed in a petri dish with a small amount of water, and sorted under dissection microscope. Remains were identified to one of three broad prey categories: fish, cephalopod or crustacean.

Identifiable hard parts were removed and stored appropriately. The sagittal otoliths of fish prey were dried and stored in small plastic bags, and identified to species level where possible (Smale et al. 1995; Williams & McEldowney 1990; D. Williams personal communication). Otoliths were sorted into left and right, and the number of individual fish per species was determined from the greatest number of either left or right otoliths. Cephalopod beaks were stored in 75% ethanol, prior to identification to genus or, where possible, species level (Clarke 1986; K. Evans personal communication). Other cephalopod remains such as gladii, funnel-locking cartilage, hooks and sucker-rings were stored in 75% ethanol. Statoliths were stored dry in small plastic bags. Exoskeleton remains from crustacean prey were stored in 75% ethanol and identified to the lowest possible taxon (G. Hosie and J. Kitchener personal communication).

2.4 LIPID EXTRACTION AND FATTY ACID ANALYSIS

Lipid extractions and fatty acid analysis was conducted at the organic laboratories of CSIRO Marine Research, Hobart. All samples were extracted overnight using a modified Bligh & Dyer (1959) extraction in a one-phase methanol:chloroform:water solvent mixture (2:1:0.8 v/v/v). Phases were separated the following day by addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol:chloroform:water). Lipids were recovered in the lower chloroform phase, and the solvent removed under vacuum to give the total solvent extract (TSE); these were weighed to obtain total lipid content (% wet mass). All samples were

made up to a known volume in chloroform and stored at -20° C. An aliquot of the TSE was analysed with an Iatroscan Mark V TH10 thin layer chromatograph (TLC) flame ionisation detector (Iatroscan Laboratories, Chiyoda-ku, Japan) to determine the proportion of major lipid classes. A polar solvent system (60:17:0.1 v/v/v ratio of hexane:diethyl ether:acetic acid) was used to resolve hydrocarbons, triacylglycerols, free fatty acids, sterols and phospholipids, while a non-polar solvent system (96:4 v/v hexane:ether) was used to determine wax esters and diacylglyceryl ethers. Peaks were quantified with DAPA Scientific Software (Kalamunda, Western Australia).

An aliquot of the TSE was transmethylated at 80° C for 2 hours in a 10:1:1 (v/v/v) mixture of methanol:hydrochloric acid:chloroform to produce fatty acid methyl esters (FAME). FAME were partitioned by the addition of water and extracted with 4:1 hexane:chloroform (v/v, 3 x 2ml), the solvent was then removed under a stream of nitrogen, and FAME were silylated at 60° C overnight in N,O-bis-(trimethylsilyl)-triflouracetamide (BSTFA) (Nichols et al. 1994). FAME were then reduced under nitrogen and stored in chloroform at –20° C. FAME were analysed by gas chromatography using a Hewlett Packard 5890A GC equipped with a HP-5 cross-linked methyl silicone fused capillary column (50 m x 0.32 mm i.d.), a flame ionisation detector (FID), a split/splitless injector and a HP 7673A auto sampler. Helium was the carrier gas, and pressure was maintained at 65kPa. Samples were injected in splitless mode with an oven temperature of 50° C, and temperature was ramped to 150° C at 30° C/minute, then to 250° C at 2° C/minute, and finally to 300° C at 5° C/minute. Peaks were quantified with Waters Millennium software (Milford, Massachusetts). A previously characterized laboratory FAME standard was routinely run with sample batches to both assist with peak identification and assess GC performance, particularly the response for PUFA. Confirmation of component identification was achieved by gas chromatography-mass spectrometry (GC-MS) analysis using a Finnigan Thermoquest GCQ GC-MS fitted with an on-column injector. The GC-MS was operated in scan mode, with an ionizing voltage of 70 eV. The GC was fitted with a column similar to that described above.

2.5 SOFTWARE

Statistical analyses were conducted in either SYSTAT 9 (SYSTAT Software Inc., Chicago, Illanois) or SPSS 10.0 for Macintosh (SPSS Inc, Chicago, Illanois) (see following chapters for specific details). All maps were produced using Generic Mapping Tools (GMT) 3.4.2 (Wessel & Smith 1991), and enhanced using Adobe Illustrator 9.0 (Adobe Systems Incorporated, San Jose, California). Most graphs were produced in Origin 7.0 (OriginLab, Northampton, Massachusetts), except Figures 5.3, 6.4, 7.2 & 7.3 (Microsoft Excel 98), Fig. 3.3 (SYSTAT 9) and Fig. 6.3 (SPSS 10.0). Graphs included in Appendix I were also produced in SPSS 10.0. All figures included in Chapter 1 were produced in Adobe Photoshop 6.0 (Adobe Systems Incorporated, San Jose, California). Tables were produced in Microsoft Excel 98.

Chapter 3. The digestive gland of *Moroteuthis ingens* as a source of fatty acid dietary tracers

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3.1 ABSTRACT

The diet of the sub-Antarctic onychoteuthid squid *Moroteuthis ingens* was assessed using stomach contents analyses and fatty acids as complementary dietary tracers. The contents of 54 stomachs (50 collected from near Macquarie Island and 4 from around Heard Island) were examined visually, and prey remains were identified to species level where possible. Myctophid fish were the most common prey in the stomach contents of *M. ingens* and were identified in 59% of stomachs. In total, teleost fish remains were found in 96% of stomachs. The lipid class and fatty acid profiles of digestive gland and mantle tissue were analysed for 5-6 squid from each area, in addition to 4 stomach fluid samples taken from Heard Island animals. Mantle tissue was low in lipid, and contained high levels of phospholipids and polyunsaturated fatty acids (PUFA). Digestive gland tissue had a high lipid content, mean value of 26.8% ± 12.9% wet mass in Macquarie Island squid and 41.7% ± 8.5% wet mass in Heard Island squid, and was rich in triacylglycerol (TAG) and monounsaturated fatty acids (MUFA). Stomach fluid generally contained high levels of TAG, although one sample was high in wax ester (WE). Stomach fluid was also characterised by high MUFA levels. The fatty acid profiles of the digestive gland of *M. ingens* grouped with those of the stomach fluid and some myctophid species in multivariate analyses, indicating that the digestive gland is a source of fatty acid dietary tracers. Thus the fatty acid profile of the digestive gland strongly supports findings from stomach contents analyses that myctophids, particularly TAG-rich species, are an important prey group of *M. ingens* at Macquarie and Heard Islands. This powerful combination of techniques has the potential to increase our knowledge of the feeding ecology of squids in the Southern Ocean.

3.2 INTRODUCTION

Dietary studies of oceanic squids have been mostly restricted to conventional stomach content analyses. Many sources of bias are associated with conventional techniques which have been discussed elsewhere (Dawe et al. 1997; Jackson et al. 1998a; Kear 1992; Lipinski 1987; Lordan et al. 1998; Rodhouse & Nigmatullin 1996), and as a result, Kear (1992) warns against using conventional stomach contents analyses in isolation to determine important prey items. The objective of this study was to establish how fatty acid dietary tracers can be used to complement stomach content analyses of the sub-Antarctic squid *Moroteuthis ingens*.

The application of fatty acid dietary tracers to studies of squid will require that squid possess a lipid reserve that is influenced by diet. However, a unique feature of the cephalopods is that they have a protein-based metabolism. While protein is used for locomotion, structural support, energy, oxygen transport and osmoregulation, lipid use is largely constrained to cell membrane structure and hormone production (Lee 1994). Lipid is not stored for reproductive purposes or as an energy reserve during starvation (Blanchier & Boucaud-Camou 1984; Castro et al. 1992; Clarke et al. 1994), in contrast to most marine animals. The mantle and other flesh of squid contains little lipid, usually less than 2% wet mass (Nash et al. 1978; Vleig 1984); furthermore, this lipid is composed primarily of phospholipids (PL) (De Koning 1993; Ha 1982; Hayashi 1989; Hayashi 1996; Hayashi & Kawasaki 1990; Hayashi et al. 1990; Jangaard & Ackman 1965; Sugiyama et al. 1989). Lee et al. (1971) suggested that PL fatty acids are not affected by diet, but instead reflect biosynthetic pathways due to the specific structural functions of PL.

Despite the fact that squid have few requirements for lipid, many species do possess a significant reserve of lipid that is stored within the digestive gland (Hayashi et al. 1990; Hayashi & Yamamoto 1987a). The function of lipid in the digestive gland is unclear. The digestive gland plays a key role in the digestive processes of cephalopods (Boucaud-Camou & Roper 1995), and it is a site for active absorption from the diet (Boucher-Rodoni & Boucaud-Camou 1987). The digestive gland also has an important excretory

absorb it from the diet (Ballantyne et al. 1981; Mommsen & Hochachka 1981; Vonk 1962), and Semmens (1998) suggests that the digestive gland simply stores lipid on a temporary basis prior to excretion. In contrast, Clarke et al. (1979) suggest that high lipid levels found within the digestive gland may assist with the maintenance of neutral buoyancy, and thus lipid may be stored for a specific purpose over a longer time-frame.

In either scenario outlined above, it appears certain that digestive gland lipid is of dietary origin and, due to catabolic limitations, is largely unmodified from the diet. For instance, Castro et al. (1992) noted that the fatty acid composition of the digestive gland of *Sepia officinalis* (Linné, 1758) was highly correlated with the fatty acid composition of a prawn diet. Hence this organ lends itself to the study of fatty acid dietary tracers. To my knowledge, digestive gland fatty acids have not been specifically used as dietary tracers in any previous studies of cephalopod diets. This chapter compares the fatty acid profiles of digestive gland and mantle tissue with published profiles of prey species that have been identified in the stomach contents of squid collected from Macquarie and Heard Islands. Comparisons are also drawn between the fatty acid profiles of material taken directly from the stomach contents.

3.3 MATERIALS AND METHODS

3.3.1 Sample description and preparation

Eighty-two individuals of *M. ingens* (51 females and 31 males) were collected from commercial fishing vessels by Australian Fisheries Management Authority (AFMA) observers from 2 geographically distant localities in the Southern Ocean. During the periods December 1997 – January 1998 and October 1998 – January 1999, seventy-four squid were collected near Macquarie Island (Pacific Ocean Sector) between 158°50'E, 52°22'S and 158°23'E, 55°23'S in 500 - 1198 m depth. Eight individuals were collected during March and April 1999 in the vicinity of Heard/McDonald Islands (Indian Ocean Sector), between 74°17'E, 50°45'S and 74°47'E, 52°55'S in 500 - 600 m depth (Fig. 3.1). All squid were frozen on board and returned to Hobart for analysis.

3.3.2 Stomach contents analyses

Intact stomachs were collected from 54 individuals; stomachs that had been badly damaged were discarded. A small fluid portion (containing no hard parts) was drained from stomachs collected from Heard Island and retained for lipid and fatty acid analysis. The stomach contents were then rinsed through a 500 μm sieve, and examined using methods described in Section 2.3, Chapter 2. The state of digestion of the stomach contents was determined on a 6-point subjective scale (Jackson et al. 1998a after Zuev et al. 1985).

3.3.3 Lipid extraction and fatty acid analysis

Whole digestive glands and a small mantle tissue sample (taken from the ventral mantle) were retained for lipid and fatty acid analysis. These were stored frozen at –20° C along with the stomach fluid samples collected from Heard Island individuals. 6 digestive glands, 6 mantle samples and 4 stomach fluid samples from Heard Island were randomly selected for analysis, and 5 digestive glands and 6 mantle samples were analysed from Macquarie Island. Entire digestive glands were homogenised in a mortar and pestle, and a 1 g subsample was taken for lipid extraction. 1 g mantle tissue samples were homogenised in a mortar and pestle prior to extraction, and 1 g was taken from each stomach fluid sample for lipid extraction. All other procedures related to lipid extraction and fatty acid analysis are described in Section 2.4, Chapter 2.

3.3.4 Statistical analyses

Percent frequency occurrence (% FO) in diet was determined as the number of stomachs containing prey species *h* as a percentage of the total number of stomachs. Two-tailed t-tests assuming equal variance were used to determine significant differences within a 95% confidence interval. Fatty acid profiles were compared by cluster analysis, using Pearson's correlation coefficient and average linkage. Pearson's correlation coefficient and non-metric multidimensional scaling (MDS) were also used to compare fatty acid profiles in two dimensions, using the Kruskal Loss Function. All multivariate analyses were conducted using SYSTAT 9 (SYSTAT Software Inc., Chicago, Illanois).

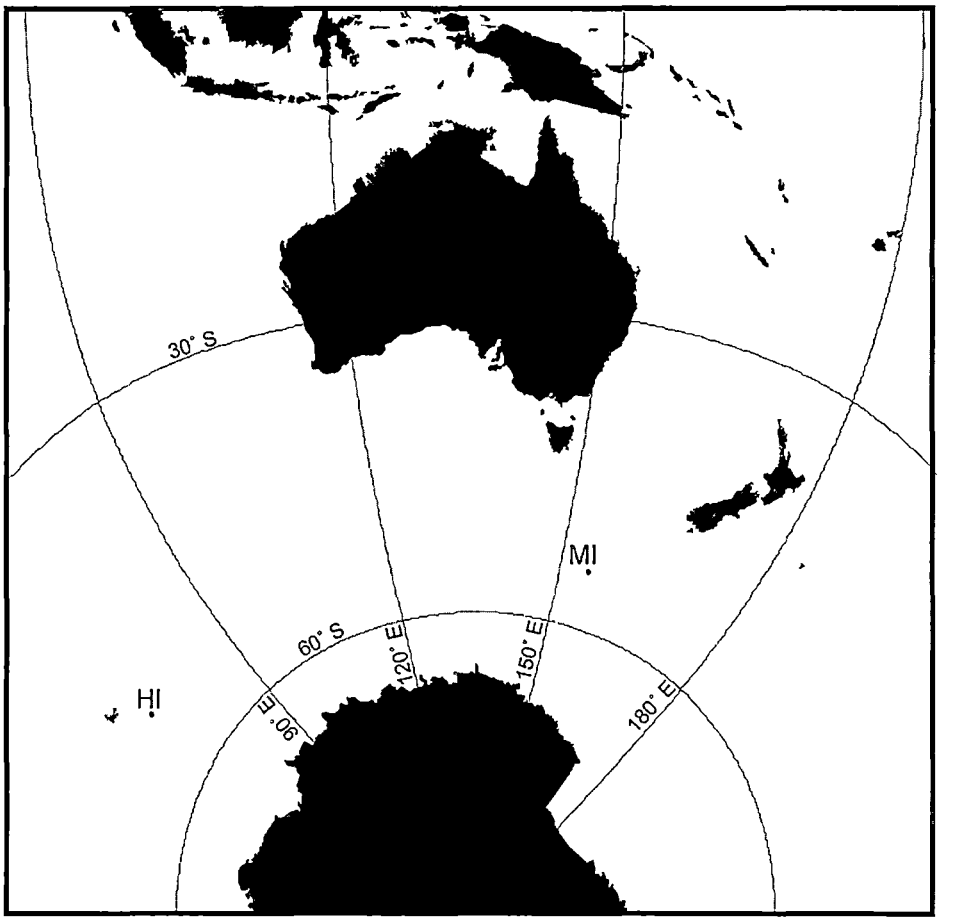

Fig. 1. The location of sampling sites, Macquarie Island (MI) and Heard Island (HI), in the sub-Antarctic Southern Ocean.

3.4 RESULTS

3.4.1 General Biology

Females were significantly larger in mantle length (ML) ($p < 0.02$, mean ML=286 mm, max ML=432 mm) and total mass (TM) ($p < 0.005$, mean TM=783 g, max TM = 2613 g) than males (mean ML=257 mm, max ML=360 mm; mean TM=538 g, max TM=1436 g). Most males were fully mature, as only 7 specimens were in various stages of immaturity. Conversely no females were mature. Nidamental glands were small and undeveloped, oviducts and oviducal glands were very small and transparent. An exponential relationship was observed between both TM and digestive gland mass and ML (Fig. 3.2).

3.4.2 Stomach contents

Ingested food was determined to accumulate in the stomach of *M. ingens*, previously referred to as the caecum by Jackson et al. (1998a). The spiral caecum is small and lacks a caecal sac in this species. Most stomachs were only moderately full with a fullness score of 1 to 3 (Table 3.1). Some stomachs contained large amounts of tissue and bone but no or few otoliths or eye lenses, which suggests that the heads of the fish prey had not been consumed. However, some stomachs contained large numbers of otoliths. Two females contained 75 and 76 otoliths representing at least 38 fish, while one male contained 55 otoliths representing at least 27 fish. These data indicate large consumption rates by individual squid. All stomach contents were in a moderate to high state of digestion, with no pieces of intact tissue available for prey identification (Table 3.1). Most tissue was in an amorphous condition, while most hard parts (bones, scales, otoliths, gladii and beaks) were clean of tissue, thin and eroded. Myctophid fish were the most common prey identified in stomach contents (59% frequency of occurrence). At least 12 myctophid species were identified in the prey spectrum; some myctophid otoliths (eg *Protomyctophum* spp.) could not be identified to species level. In total, bony fish remains were found in 96% of stomachs (Table 3.2), and appear to be the major prey of *M. ingens* at Macquarie and Heard Island based on stomach contents data.

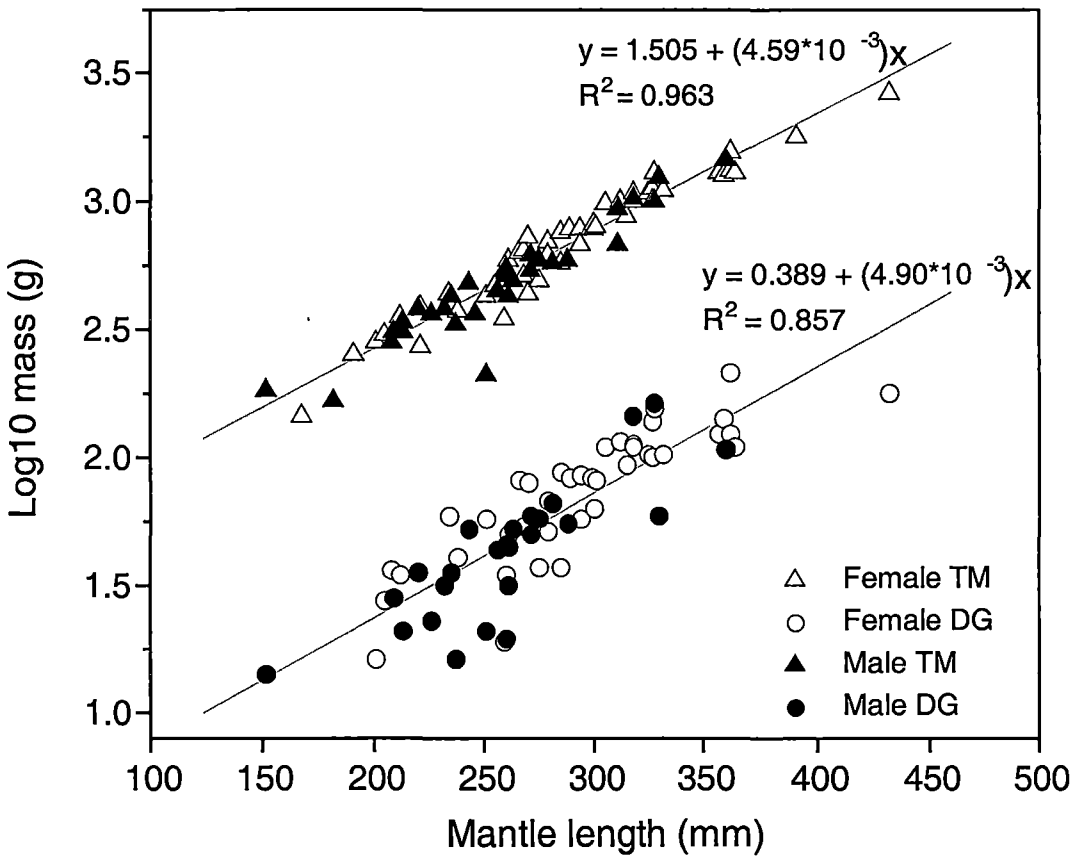

Fig. 3.2. *Moroteuthis ingens*: Total mass (TM) and digestive gland mass (DG) versus mantle length (ML). Linear regression lines are provided for combined female and male TM:ML and DG:ML relationships.

Table 3.1. The fullness and digestion stages of stomachs collected from *Moroteuthis ingens*

Digestion/Fullness stage	1	2	3	4	5	6
Number of stomachs at each fullness stage	15	11	19	7	3	n/a
Number of stomachs at each digestion stage	0	0	14	28	10	4

n/a. not applicable

Other fish species identified from otoliths belonged to the Bathylagidae family (*Bathylagus antarcticus*) and Paralepididae family (*Magnisudis prionosa*). *Protomyctophum* spp., *B. antarcticus* and *Gymnoscopelus braueri* were found in the greatest number of stomachs (percent frequency occurrence of 21%, 19% and 19% respectively), whereas the largest number of otoliths belonged to *Gymnoscopelus* spp., *Krefftichthys anderssoni* and *Electrona subaspera* (n = 85, 69 and 55 respectively) (Table 3.2).

Squid remains were found in 47% of stomachs, however, these rarely contributed to the bulk of material in the stomach and only 2 stomachs contained squid remains alone. Most squid beaks were deformed and not intact. Crustacean prey was found in 9% of stomachs, represented by small amphipods and copepods. Crustaceans were in a high state of digestion with only the outer skeleton remaining. The small size and low frequency occurrence of these remains suggests that crustaceans were not targeted prey items and may have been secondarily ingested by *M. ingens* from the stomachs of fish prey.

3.4.3 Lipid classes and fatty acids

3.4.3.1 Mantle tissue

Mantle tissue had a mean lipid content of 1.5% ± 0.1% wet mass in Macquarie Island squid and similarly 1.5% ± 0.1% in Heard Island animals (Table 3.3). The major lipid class in animals from both areas was PL, which comprised 77-83% of total lipid (Table 3.4).

Table 3.2. Prey species identified in the stomach contents of *Moroteuthis ingens*

Prey item	%FO	N	%TO
Family Bathylagidae			
Bathylagus antarcticus	19	19	5
Family Myctophidae			
Electrona antarctica	11	17	4
Electrona carlsbergii	6	5	1
Electrona subaspera	17	55	13
Electrona spp.	2	2	1
Gymnoscopelus braueri	19	43	10
Gymnoscopelus fraseri	9	23	5
Gymnoscopelus hintonoides	2	4	1
Gymnoscopelus nicholsi	15	21	5
Gymnoscopelus piabilis	9	10	2
Gymnoscopelus spp.	11	85	20
Hintonia sp.	2	2	1
Krefftichthys anderssoni	15	69	16
Protomyctophum bolini	11	15	4
Protomyctophum normani	6	10	2
Protomyctophum spp.	21	40	9
Family Paralepididae			
Magnisudis prinosa	4	4	1
Total Fish	**96**		
beaks	34		
statoliths	17		
Total Squid	**47**		
Hyperiid amphipods			
Hyperiella dilata	2		
Solopes sp.	2		
Gameriid amphipods	2		
Unidentified copepods	8		
Total Crustaceans	**9**		

%FO: % frequency occurrence; N. number of otoliths; %TO: % of total otoliths

Sterols (ST), almost exclusively cholesterol (data not shown), and free fatty acids (FFA) contributed 11.5% ± 0.9% and 9.9% ± 4.0% to total lipid content in Heard Island animals, respectively. ST were the only other major lipid class in Macquarie Island squid, and comprised 12.3% ± 1.6% of total lipids. The fatty acid profiles of mantle tissue were dominated by PUFA, which comprised over 50% of fatty acid content (Table 3.5). The main PUFA were eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3); no other PUFA were above 2.5%. The saturated fatty acid (SAT) 16:0 was also abundant (26%).

Table 3.3. Total lipid content (% wet mass) of the mantle and digestive gland tissue of *Moroteuthis ingens* from Macquarie and Heard Islands, and of the stomach fluid of *M. ingens* from Heard Island. Values are means ± SD

Sample	Total lipid content (% wet mass)
Mantle	
Macquarie Island	1 5 ± 0 1
Heard Island	1 5 ± 0 1
Digestive Gland	
Macquarie Island	26.8 ± 12.9
Heard Island	41.7 ± 8.5
Stomach Fluid	
Heard Island	16.8 ± 10 6

3.4.3.2 Digestive Gland

Lipid class and fatty acid profiles of the digestive glands were very different from those of mantle tissue. Total lipid content (% wet mass) was an order of magnitude greater than the lipid content of the mantle, and was also highly variable. Total digestive gland lipid content in Macquarie Island animals ranged between 15.9% and 47.4% wet mass, with a mean value of 26.8% ± 12.9% wet mass (Table 3.3). Total digestive gland lipid content in Heard Island squid ranged between 33.5% and 56.7% wet mass, with a mean value of 41.7% ± 8.5% wet mass (Table 3.3). TAG was the major lipid class, contributing 75.0% ± 17.5% and 91.4% ± 1.6% to total lipid in squid from Macquarie and Heard Islands respectively (Table 3.4). FFA were moderately high, comprising 11.7% ± 6.9% of total lipids in Macquarie Island squid and 5.4 ± 1.2% in Heard Island squid. High FFA levels in the digestive gland have been reported for several other squid species (Hayashi et al. 1985; Hayashi & Yamamoto 1987b; Hayashi 1996; Kawasaki et al. 1994; Wako et al. 1993) and may be largely due to the enzymatic activity of the digestive gland rather than an artefact of storage. Major fatty acids in the digestive gland were 16:0, 18:1n9 and 20:1n9 (Table 3.5). MUFA were the major class of fatty acid.

Table 3.4. Percent lipid class (of total lipids) of the mantle and digestive gland of *Moroteuthis ingens* from Macquarie and Heard Islands, and stomach fluid of *M. ingens* from Heard Island. Mean values with standard deviations are given for mantle and digestive tissue, whereas minimum and maximum content of each lipid class is given for stomach fluid

	Mantle		Digestive gland		Stomach fluid
	Macquarie Island	Heard Island	Macquarie Island	Heard Island	Heard Island
	n =6	*n* =6	*n* =5	*n* =6	*n* =4
Lipid Class					min – max
WE	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.9	0.7 ± 0.4	0.0 – 53.8
DAGE	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 1.1	0.4 ± 0.5	0.0 – 0.0
TAG	1.9 ± 1.7	1.9 ± 0.6	75.0 ± 17.5	91.4 ± 1.6	17.9 – 87.5
FFA	2.7 ± 0.7	9.9 ± 4.0	11.7 ± 6.9	5.4 ± 1.2	3.5 – 5.6
ST	12.3 ± 1.6	11.5 ± 0.9	3.9 ± 3.1	0.7 ± 0.2	0.2 – 0.8
PL	83.1 ± 2.1	76.7 ± 4.3	5.8 ± 7.7	1.6 ± 0.5	6.9 – 22.5

n : sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid.

Table 3.5. Percent fatty acids (of total fatty acids) of the mantle and digestive gland tissue of *Moroteuthis ingens* from Macquarie and Heard Islands, and of stomach fluid of *M ingens* from Heard Island. Values are means ± SD

	Mantle		Digestive gland		Stomach fluid
	Macquarie Island	Heard Island	Macquarie Island	Heard Island	Heard Island
Fatty acid	*n* =6	*n* =6	*n* =5	*n* =6	*n* =4
14.0	1.7 ± 0.3	1.8 ± 0.3	3.4 ± 0.5	3.5 ± 0.3	5.1 ± 1.4
16.0	25.8 ± 0.6	25.7 ± 0.6	17.8 ± 1.6	15.8 ± 0.7	16.5 ± 3.0
17:0	0 5 ± 0.1	0 4 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
18:0	2.7 ± 0.2	2.3 ± 0.1	3.2 ± 1.8	3.3 ± 0.2	2.7 ± 0.2
16:1n7	0.8 ± 0.1	1.4 ± 0.1	5.0 ± 1.2	5.2 ± 0.8	7.9 ± 2.0
18:1n9	3.7 ± 0.2	4.6 ± 0.4	27.3 ± 4.2	20.5 ± 1.2	23.4 ± 3.0
18:1n7	1.9 ± 0.2	2.0 ± 0.1	5.1 ± 1.2	3.6 ± 0.3	4.6 ± 0.1
18:1n5	0.4 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.2
20:1n9	5 5 ± 0.9	5.5 ± 0.1	8.8 ± 2.5	9.2 ± 1.5	6.4 ± 2.1
20:1n7	0.1 ± 0.0	0.3 ± 0.5	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
22:1n11	0.3 ± 0.1	0.5 ± 0.1	3.6 ± 0.4	5.0 ± 1.1	2.4 ± 1.0
22:1n9	1.4 ± 0.2	1.3 ± 0.1	1.9 ± 0.2	2.2 ± 0.3	1.7 ± 0.4
22:1n7	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.3	0.2 ± 0.1
24:1n11	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.2	1.3 ± 0.2	0.8 ± 0.3
24:1n9	0.3 ± 0.1	0.3 ± 0.0	1.0 ± 0.3	1.4 ± 0.2	1.0 ± 0.2
C16 PUFA	0.0 ± 0 0	0.0 ± 0.0	0.5 ± 0.1	3.3 ± 2.3	2.0 ± 3 0
18:4n3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.5 ± 0.1	0.9 ± 0.4
18:2n6	0.2 ± 0.0	0.2 ± 0.0	1.3 ± 0.2	0.9 ± 0.3	1.2 ± 0.2
20.4n6 (AA)	2.1 ± 0.1	1.3 ± 0.2	0.7 ± 0.3	0 6 ± 0.1	0.5 ± 0.1
20:5n3 (EPA)	13.6 ± 0.4	14.7 ± 0.2	4.4 ± 1.9	7.9 ± 1.2	9.0 ± 3.7
20:4n3	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.1
20:2n6	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.6	0.3 ± 0.0	0.2 ± 0.1
22:5n6	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.2	0.2 ± 0.1	0.0 ± 0 0
22:6n3 (DHA)	35.2 ± 1.3	33 9 ± 0.5	6.9 ± 4.2	9.1 ± 0.9	7.7 ± 0.9
22:5n3 (DPA)	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.2	0.9 ± 0.1	0.9 ± 0.2
Sum SAT	**31.2 ± 0.7**	**30.9 ± 0.5**	**25.4 ± 1.4**	**23.4 ± 1.1**	**25.7 ± 2.3**
Sum MUFA	**15.5 ± 1.4**	**17.6 ± 0.9**	**56.6 ± 6.9**	**51.1 ± 1.9**	**50.5 ± 4.6**
Sum PUFA	**53.1 + 1.6**	**51.5 + 0.5**	**17.3 + 7.0**	**25.0 + 1.8**	**23.8 + 4.6**

n : sample size; AA: arachadonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

3.4.3.3 Stomach fluid

Total lipid content was highly variable between individuals, with a minimum content of 4.8% wet mass and a maximum of 28.5% wet mass. The mean lipid content was 16.8% ± 10.6% wet mass (Table 3.3). The proportions of lipid classes were also variable between individuals. Major lipid classes were TAG and WE; three stomach fluid samples contained large amounts of TAG (81.7%-87.5% of total lipid) whereas the fourth was rich in WE (53.8% of total lipid) (Table 3.4). Interestingly, however, there was relatively little variation in the fatty acid profiles between individuals despite these differences in lipid class content. Fatty acid profiles are dominated by MUFA with 16:0 and 18:1n9 again being major components. EPA and DHA levels were also relatively high (Table 3.5).

3.4.4 Comparisons with potential prey

The fatty acid profiles of mantle and digestive gland tissue of *M. ingens* from Macquarie and Heard Islands, and stomach fluid from Heard Island squid, have been compared to published fatty acid profiles of eleven myctophid species using cluster analysis and MDS (Fig. 3.3). In addition, comparisons have been made with adult specimens of the Antarctic krill *Euphausia superba,* often cited to be an important prey item of squid (including *M. ingens*) in the Southern Ocean (Nemoto et al. 1985). Collection and publication details of potential prey species are presented in Table 3.6. Multivariate comparisons were made using a restricted selection of fatty acids, as governed by those suites available in the literature. This selection comprised 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n11, 24:1n11, 18:2n6, EPA and DHA.

Table 3.6. Collection details of potential prey species of *Moroteuthis ingens* included in fatty acid comparisons

Species	Collection site	Reference
Myctophid fish		
Electrona antarctica	Kerguelen Island & Macquarie Island	Lea et al. (2002)
Electrona carlsbergi	Kerguelen Island	Lea et al. (2002)
Electrona subaspera	Kerguelen Island	Lea et al. (2002)
Gymnoscopelus braueri	Elephant Island	Phleger et al. (1999)
Gymnoscopelus fraseri	Kerguelen Island & Macquarie Island	Lea et al. (2002)
Gymnoscopelus nicholsi	Kerguelen Island	Lea et al. (2002)
Gymnoscopelus opisthopterus	Elephant Island	Phleger et al. (1999)
Gymnoscopelus piabilis	Kerguelen Island	Lea et al. (2002)
Krefftichthys anderssoni	Elephant Island	Phleger et al. (1999)
Protomyctophum bolini	Possession Island	Raclot et al. (1998)
Protomyctophum tenisoni	Kerguelen Island & Macquarie Island	Lea et al. (2002)
Zooplankton		
Euphausia superba	Elephant Island, 1997 & 1998	Phleger et al. (2002)

Four main groups were defined at a distance of 0.25 in the cluster analysis. Group A comprised *E. superba*, while Group B contained mantle tissue and the myctophids *Gymnoscopelus fraseri* and *Protomyctophum tenisoni* collected from the vicinity of Macquarie Island. Remaining TAG-rich myctophid species were clustered in Group C together with the digestive gland tissue and stomach fluid, including specimens of *G. fraseri* and *P. tenisoni* collected from the vicinity of Kerguelen Island. Group D was comprised of three WE-rich myctophid species. These groupings were also represented in a scatterplot of MDS (Fig. 3.3b).

3.5 DISCUSSION

3.5.1 The diet of Moroteuthis ingens *- stomach content analyses*

Stomach contents of squid from Macquarie and Heard Islands confirmed that *M. ingens* is a myctophid feeder, as shown by Jackson et al. (1998a), rather than a euphausiid feeder as suggested by Nemoto et al. (1985). Other species of fish such as *B. antarcticus* were also commonly consumed. Only 4 stomachs were available from Heard Island squid, and consequently it was not feasible to analyse the sample sets separately and investigate spatial differences in the occurrence of prey frequency. Whilst many species

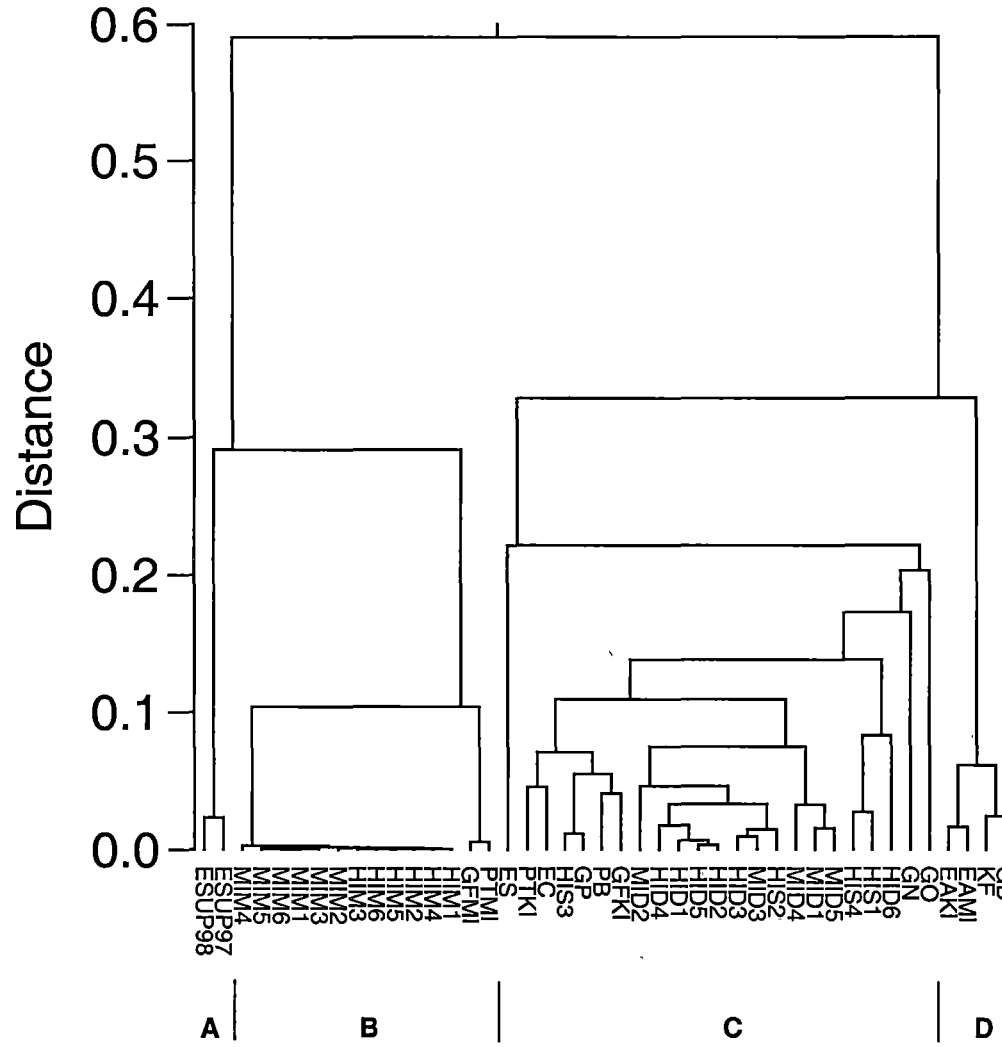

Fig. 3.3a. Dendrogram of cluster analysis comparing mantle, digestive gland tissue and stomach fluid from *Moroteuthis ingens* with myctophid species and *Euphausia superba* using a suite of fatty acids. MIM: Macquarie Island mantle; HIM: Heard Island mantle; MID: Macquarie Island digestive gland; HID: Heard Island digestive gland; HIS: Heard Island stomach fluid; EAKI: *Electrona antarctica* (Kerguelen Island); EAMI: *E. antarctica* (Macquarie Island); EC: *E. carlsbergi*; ES: *E. subaspera*; GB: *Gymnoscopelus braueri*; GFKI: *G. fraseri* (Kerguelen Island); GFMI: *G. fraseri* (Macquarie Island); GN: *G. nicholsi*; GO: *G. opisthopterus*; GP: *G. piabilis*; KF: *Krefftichthys anderssoni*; PB: *Protomyctophum bolini*: PTKI: *P. tenisoni* (Kerguelen Island); PTMI: *P. tenisoni* (Macquarie Island); Esup97: *Euphausia superba* 1997; Esup98: *Euphausia superba* 1998.

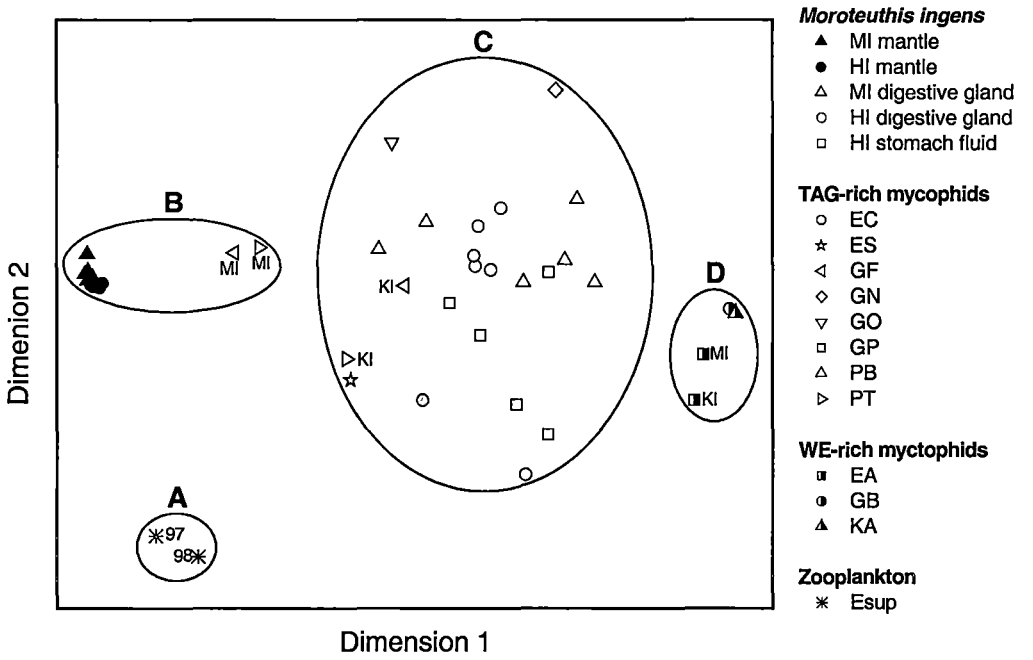

Fig. 3.3b. Scatterplot of multidimensional scaling (MDS) for data compared in cluster analysis (Fig. 3.3a); stress = 0.051, proportion of variance = 0.991. Cluster groups identified in Fig. 3.3a are superimposed. MI: Macquarie Island; HI: Heard Island; KI: Kerguelen Island; EA: *Electrona antarctica*; EC: *E. carlsbergi*; ES: *E. subaspera*; GB: *Gymnoscopelus braueri*; GF: *G. fraseri*; GN: *G. nicholsi*; GO: *G. opisthopterus*; GP: *G. piabilis*; KA: *Krefftichthys anderssoni*; PB: *Protomyctophum bolini*; PT: *P. tenisoni*; Esup: *Euphausia superba*; 97: 1997; 98: 1998. Axis scales are arbitrary in non-metric MDS and are therefore omitted.

of fish and other pelagic organisms have a circumpolar distribution in the Southern Ocean, Macquarie and Heard Island are found on opposite sides of the APF that acts as a biological barrier to many organisms. Therefore the composition of the diet of *M. ingens* is likely to differ between these two areas.

Fewer prey species were identified from the stomach contents of *M. ingens* from New Zealand (Jackson et al. 1998a), although this may be related to the smaller number of samples examined during the previous study. Several species of teleost were common prey items of New Zealand specimens that were not identified in the Macquarie and Heard Island squid. In fact, *Gymnoscopelus* spp. and *Electrona* spp. were the only prey items identified in both the study by Jackson et al. (1998a) and the current study. Therefore, it appears that the diet of *M. ingens* is markedly different at the northern limits of its distribution compared to its sub-Antarctic habitat.

3.5.2 The lipid composition of the digestive gland, stomach and prey items

No stomachs from Macquarie Island squid had been suitably processed to facilitate fatty acid analysis of fluid portions. However, the fluid from all four stomachs collected from Heard Island were analysed, and were found to be variable in lipid composition. Three stomachs contained large amounts of TAG while one stomach was rich in WE (Table 3.4). The majority of myctophids accumulate TAG as their major storage lipid, including *Electrona carlsbergi*, *E. subaspera*, *G. fraseri*, *Gymnoscopelus nicholsi*, *Gymnoscopelus piabilis*, *Gymnoscopelus opisthopterus* and *P. tenisoni* (note that *G. opisthopterus* and *P. tenisoni* were not identified in the stomach contents during this study, although may have been represented by some *Gymnoscopelus* spp. and *Protomyctophum* spp. otoliths). A smaller number of species store large amounts of WE with only trace amounts of TAG (Phleger et al 1999; Saito & Murata 1998). *Electrona antarctica*, *G. braueri* and *K. anderssoni* are species rich in WE. Therefore high TAG and WE levels in the stomach fluid may be accounted for by a myctophid diet.

The digestive gland tissue grouped with stomach fluid from Heard Island squid in cluster analysis and MDS (Fig. 3.3). Both the digestive gland and the stomach fluid are characterised by high MUFA content, and the major lipid component of the digestive

gland is TAG. Overall these findings suggest that there is little modification of fatty acids (for example desaturation) in the transport of lipids from the stomach to the digestive gland. The digestive gland of *M. ingens* also groups with the TAG-rich myctophids *E. carlsbergi*, *E. subaspera*, *G. nicholsi* and *G. piabilis*, and *P. tenisoni* and *G. fraseri* from the vicinity of Kerguelen Island. *P. bolini* was also included in this group, although no lipid class data were available for this species. Thus multivariate analyses of fatty acid profiles indicate that dietary lipid uptake and subsequent digestive gland lipid composition is influenced by the consumption of these myctophid species.

Specimens of *G. fraseri* and *P. tenisoni* collected from the vicinity of Macquarie Island did not group with digestive gland or stomach fatty acid profiles. These myctophids were characterised by higher levels of EPA and DHA, and lower levels of certain MUFA, when compared to their Kerguelen Island counterparts (Lea et al. 2002b), and were thus more similar to mantle tissue. *G. fraseri* and *P. tenisoni* may not be important dietary components of *M. ingens* at Macquarie Island, relative to the diet at Heard Island. Spatial variations in fatty acid composition of *G. fraseri* and *P. tenisoni* may be related to food availability, or to the size and age of fish included in the analysis (Lea et al. 2002b). Clearly, such parameters will affect interpretations drawn from predator-prey comparisons of fatty acid composition, and therefore need to be further examined in food-web studies.

Three WE-rich myctophid species - *G. braueri*, *K. anderssoni* and *E. antarctica* - were grouped together, independent of any other tissue or prey type (Fig. 3.3). The fatty acid profile of these species is less similar to that of the digestive gland when compared to other myctophid species, and suggest that these prey items make lesser contributions to the diet of *M. ingens*. These findings initially appear to contradict those of stomach contents analyses, as *G. braueri* and *K. anderssoni* were two of the most common prey items identified (the former by percent frequency occurrence, the latter by percent of total otoliths). However, *K. anderssoni* is possibly the smallest myctophid distributed in the sub-Antarctic, reaching a maximum of 71 mm standard length (Hulley 1990). The importance of small items in the diet can be over-estimated using indices such as % FO (Hyslop 1980), as a larger number must be consumed to meet dietary requirements.

Furthermore, while small fish are often consumed whole, squid often reject the heads of larger species (Lipinski 1987; Rodhouse & Nigmatullin 1996), so that the prevalence of small fish such as *K. anderssoni* in the stomach contents is further distorted. The use of fatty acids as dietary tracers will expose such sources of error when quantifying important prey items from stomach contents, particularly when hard body parts are eroded and cannot be reliably used to estimate standard length and mass of prey, or when identification can be difficult.

Alternatively, the representation of species with distinctive lipid profiles (such as WE-rich myctophids) may be obscured in the fatty acid profile of the digestive gland, thus limiting its usefulness as an indicator of important prey groups. However, fatty alcohols were found only in small amounts in the digestive gland (data not shown), indicating that the diet of these squid was low in WE and that TAG-rich species are more commonly consumed.

The Antarctic krill *E. superba* did not group with any other potential prey species or any sample of *M. ingens* tissue (Fig. 3.3). This supported the results from stomach contents analyses that euphausiid species are not a dietary component of *M. ingens* around Macquarie and Heard Islands.

3.5.3 Lipid and fatty acid composition of mantle tissue

The mantle tissue of *M. ingens* contained low lipid content, which was characterised by high levels of PL and PUFA. These data agree well with those in the literature, where PUFA dominate the flesh tissue of other squid species such as *Illex illecebrosus* (LeSueur, 1821), *Loligo beka* (Sasaki, 1929), *Loligo vulgaris* (Lamarck, 1798), *Gonatopsis borealis* (Sasaki, 1923), *Berryteuthis magister* (Berry, 1913), *Gonatopsis makko* (Okutani & Nemoto, 1964), and *Moroteuthis robusta* (Dall in Verrill, 1876) (De Koning 1993; Ha 1982; Hayashi 1989; Hayashi & Kawasaki 1990; Hayashi et al. 1990; Hayashi & Yamamoto 1987a; Jangaard & Ackman 1965). PUFA are often associated with structural lipids, in particular phospholipids (Sargent 1976); therefore the role of lipid in the mantle of *M. ingens* is likely to be largely structural.

The origin of PUFA in the mantle tissue of squids is presently unclear. PUFA will be selectively absorbed from the diet and transferred to the mantle without further modification, or alternatively, squid may have some metabolic capacity for chain elongation and desaturation of dietary fatty acids transported to the mantle. Lipid in squid mantle tissue may fulfil specific structural requirements, and due to selective uptake and/or modification of fatty acids, the profile of the mantle is less likely to reflect that of important prey items. Similarly, PL fatty acids in the copepod *Calanus helgolandicus* are not influenced by diet due to their specific structural role (Lee et al. 1971). This is illustrated in Fig. 3.3, where mantle fatty acid composition is clearly independent of the fatty acid composition of prey items, the digestive gland and stomach fluid.

3.5.4 Future applications of fatty acid dietary tracers to studies of squid

Mantle tissue of *M. ingens* is not generally suited to studies of fatty acid dietary tracers, although it may be of use as a "control" tissue when the affects of dietary lipid uptake are being compared between groups. In contrast, the digestive gland and material from the stomach may be applied to future dietary studies of *M. ingens* and other Southern Ocean squid using fatty acid dietary tracers, as both closely represent the fatty acid composition of the diet. However, use of the digestive gland may have significant advantages over further fatty acid analyses of stomach contents. As stated in Chapter 1, it is preferable to develop a complementary method of dietary analysis that is not dependent on stomach contents, as stomach contents are vulnerable to instantaneous events such as feeding in sampling gear. Furthermore, future complementary analyses of stomach contents are likely to employ DNA-based techniques, rather than fatty acid dietary tracers (Symondson 2002). The lipid content of the digestive gland of *M. ingens* has been observed to exceed that of the stomach, and thus indicates that lipid is accumulated here over a considerable period of time. The fatty acid composition of the digestive gland appears likely to characterize a history of dietary uptake, and will thus provide a long-term representation of diet that cannot be obtained by either fatty acid or DNA-based analyses of stomach contents.

3.5.5 Implications for squid ecology in the Southern Ocean

Myctophids are known to be the major prey item of other squid species in the Southern Ocean, such as the ommastrephid *Martialia hyadesi* (Rochebrune & Mabille, 1887). After studying the stomach contents of this species collected from the Atlantic Sector of the Southern Ocean, Rodhouse et al. (1992) proposed that a copepod-myctophid-*M. hyadesi*-higher predator food-chain may be operating in the Antarctic oceanic ecosystem, largely independent of the keystone Antarctic krill species *E. superba.* Soviet research had also indicated the presence of a copepod-myctophid-squid-higher predator food-web (Lubimova 1985). Stomach contents and fatty acid analyses of *M. ingens* indicate that a similar food chain is important on the continental slopes near Macquarie and Heard Islands, that is copepod-myctophid-*M. ingens*-higher predator. Squid are thought to have replaced fish as the major nektonic predators in the epipelagic zone of the Southern Ocean (Rodhouse & White 1995). Therefore, if many Southern Ocean species depend heavily on myctophids and other mesopelagic fish, the copepod-myctophid-squid-higher predator food chain may be one of the most widespread and overlooked food chains in the Southern Ocean.

Many dietary studies of squid are hampered by the possibility that squid have been feeding in sampling gear, and consequently that stomach contents do not represent natural feeding behaviour (Breiby & Jobling 1985). This study was not likely to be affected by this source of bias, as all stomach contents were in a moderate to high state of digestion. This indicates that *M. ingens* had not fed recently prior to capture. The similarities between the digestive gland lipid composition and that of the stomach fluid also suggests that, with this species, the range of prey items identified from stomach contents provided a good representation of natural feeding behaviour.

Squid, including *M. ingens*, are often described as krill feeders in the Southern Ocean (Nemoto et al. 1985) based on stomach contents analyses. However, many squid have been collected in surface waters (0-200 m) in nets designed to catch *E. superba* (Nemoto et al. 1988) which is likely to have biased dietary studies and hence our perception of the feeding ecology of squid in the Southern Ocean. Based on a study of feeding of the sperm whale *Physeter macrocephalus* on squid, Nemoto et al (1988) suggests that there

are several unidentified links in the food web between *E. superba*, squid and sperm whales. The thickness of sperm whale blubber was not found to increase seasonally in Antarctic waters relative to increased zooplankton production, in contrast to the blubber thickness of baleen whales. The fatty acid profile of *E. superba* showed no similarities to those of *M. ingens* in this study, although this was expected as the distribution of *E. superba* does not extend as far north as Macquarie and Heard Islands and it was not identified in stomach contents. However, it should be possible to collect squid, such as other species of onychoteuthids, from Antarctic waters where *E. superba* is distributed and determine from combined stomach content and digestive gland lipid analyses whether *E. superba* comprises a major component of the diet, or if it is only taken opportunistically.

3.6 CONCLUSIONS

Fatty acid composition of the digestive gland of *M. ingens* is markedly similar to the fatty acid composition of material from the stomach contents and to published fatty acid profiles of prey species. Thus dietary lipid is deposited in the digestive gland of this squid with little or no modification from the diet. Both stomach contents and fatty acid analyses have identified *M. ingens* to be a myctophid feeder around Macquarie and Heard Islands. The use of digestive gland fatty acids as dietary tracers has potential applications to future studies of squid, particularly where squid have been suspected to feed in sampling gear so that error has been introduced to stomach contents analyses.

ADDENDUM

The research within the original manuscript and this resultant Chapter was conducted by myself. Co-authors G. D. Jackson and P. D. Nichols fulfilled supervisory roles as my Ph. D. supervisors at IASOS, University of Tasmania, and provided constructive criticism on the text. J. Finn and B. McGrath are acknowledged for their assistance with squid dissections. J. Kitchener completed the identification of crustacean prey, and D. Williams verified the identification of some uncommon otoliths. Fig. 3.1 was adapted from a map created by T. van Ommen.

Chapter 4. Temporal variations in the diet of *Moroteuthis ingens* at Macquarie Island

ADAPTED FROM: Phillips KL, Jackson GD & Nichols PD (2002) Temporal variations in the diet of the squid *Moroteuthis ingens* at Macquarie Island: stomach contents and fatty acid analyses. Marine Ecology Progress Series 256:135-149.

4.1 ABSTRACT

The onychoteuthid squid *Moroteuthis ingens* was collected as by-catch from a commercial trawl fishery in the vicinity of Macquarie Island, within the Pacific sector of the Southern Ocean. Squid were collected during three austral summers and one austral winter between January 1995 and June 2000. Stomach contents and fatty acid profiles of both mantle and digestive gland tissues were used to determine if the diet of *M. ingens* was subject to temporal variations in this region. Discriminant analysis (DA) of stomach contents data and digestive gland fatty acid data indicated that the diet varied significantly on an interannual basis, most likely due to an increase in the consumption of the myctophid *Gymnoscopelus nicholsi* during the summer of 1999 relative to the summers of 1995 and 2000. Comparisons with oceanographic data reveal that the summer of 1999 was a period of warmer sea surface temperature and reduced primary production compared to the summers of 1995 and 2000. Fluctuations in oceanographic conditions may have underpinned variations in the availability of prey during the study period. DA of digestive gland fatty acid data also indicated that the diet varied significantly between the summer and winter of 2000. Stomach contents data indicate that the myctophid *Electrona carlsbergi* replaced *Krefftichthys anderssoni* as a key prey species for *M. ingens* during the winter period, as has been observed for other marine predators in the sub-Antarctic Southern Ocean. A comparison of methods reveals that, while fatty acid analyses greatly aid the interpretation of stomach contents data, both techniques are subject to limitations and are best used in combination.

4.2 INTRODUCTION

Cephalopods are a key intermediate component of the sub-Antarctic Southern Ocean ecosystem, occupying a niche that is characterised by epipelagic fish in oceans at lower latitude (Rodhouse & White 1995). The consumption of squid and other cephalopods by toothed whale, seal and seabird species has been estimated to exceed 30 million tonnes per year (Clarke 1983), so that cephalopods represent an important mechanism by which energy is transferred to top trophic levels. Cephalopods have rarely attracted the attention of major research programs conducted in the Southern Ocean (Okutani 1994), and it is likely that they comprise a much greater proportion of the biomass of the midwater community than most ecosystem models allow (Clarke 1996). It has become important to increase the number of ecological studies of cephalopods, particularly studies of trophic interactions between cephalopods and their prey (Piatkowski et al. 2001). The ecological role of squid as consumers in the Southern Ocean is poorly documented; prior to 1986, published results were available for fewer than 200 specimens (Kock 1987). While the number of studies has increased markedly since this time, it has been possible to collect only basic descriptive dietary data for most species examined so far. Very few published studies have been able to investigate ecological variations in the diet of a Southern Ocean squid species, for example over space or time.

This study investigates the diet of the onychoteuthid squid *Moroteuthis ingens*, an ecologically important species that has been collected as by-catch from the commercial Patagonian toothfish (*Dissostichus eleginoides*) fishery around Macquarie Island within the PFZ. Squid populations have been observed to fluctuate widely from year to year (Piatkowski et al. 2001), possibly in response to parameters such as prey availability. Jackson et al. (1998b) and Cherel & Weimerskirch (1999) have suggested that the population size of *M. ingens* fluctuates on an interannual basis over the Patagonian Shelf and within waters surrounding the Crozet Archipelago, and Cherel & Weimerskirch (1999) have related interannual variations in the size of this species to poor food availability. Previous dietary studies have shown that *M. ingens* consumes large amounts of myctophid fish (Jackson et al. 1998a; Phillips et al. 2001), as do other squid distributed in the PFZ (Kock 1987; Lubimova 1985; Rodhouse et al. 1992). Myctophids are sparsely distributed over large areas of the Southern Ocean, but can be found in dense

concentrations in restricted areas, particularly within the PFZ (Filin et al. 1991; Kozlov 1995). The movement of these shoals of myctophids over time is highly variable and dependent on oceanographic conditions (Filin et al. 1991), and their presence or absence may significantly impact the diet of a predator such as *M. ingens* at a given location.

The dietary composition of other myctophid predators, such as the king penguin, Antarctic fur seal and Patagonian toothfish, have been observed to fluctuate on an interannual and seasonal basis (Cherel et al. 1996; Goldsworthy et al. 2002; Hindell 1988; Lea et al. 2002a; Rodhouse et al. 1998). To my knowledge, however, this has not been previously demonstrated for a Southern Ocean squid species. Specimens of *M. ingens* were collected during three austral summer fishing seasons, in addition to one exploratory season conducted during the austral winter, thus presenting an opportunity to investigate interannual and seasonal variations in the diet of this species.

4.3 MATERIALS AND METHODS

4.3.1 Squid collection

One hundred and sixty-eight specimens of *M. ingens* were collected from numerous locations on the continental slope surrounding Macquarie Island (54°30'S – 158°55'E; Fig. 4.1) over three summer periods and one winter period between January 1995 and June 2000. The squid were collected by Australian Fisheries Management Authority (AFMA) observers on board the commercial trawler *Austral Leader*. Fifty-two squid were collected during the 1994/1995 austral summer (5 January 1995 - 10 February 1995); these are referred to as "1995 summer" squid. 1995 summer squid were collected from twelve locations around the island at depths between 910 m and 990 m. Fifty-seven squid were collected from twenty-two locations during the 1998/1999 austral summer ("1999 summer" squid) (16 October 1998 - 18 January 1999) at depths between 251 m and 1078 m. Twenty-eight squid were collected during the 1999/2000 austral summer ("2000 summer squid") (5 January 2000 - 4 February 2000) from six locations

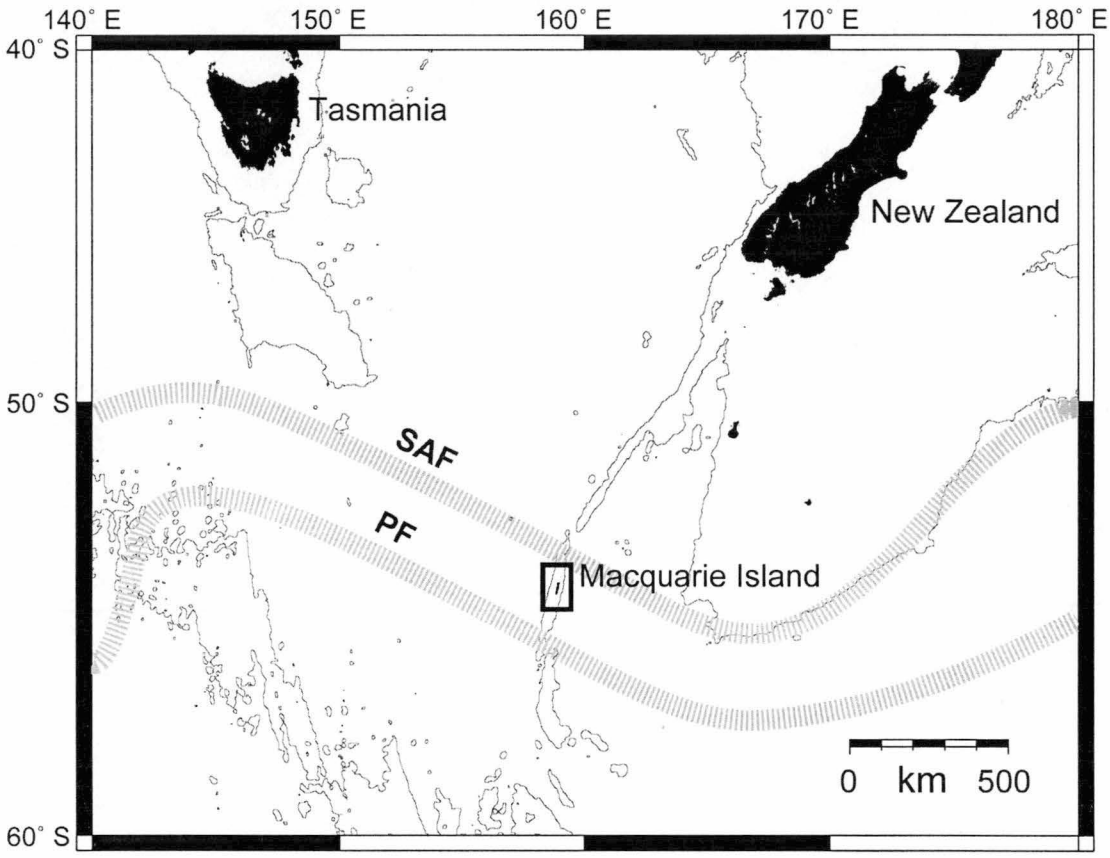

Fig 4.1. The location of Macquarie Island relative to Tasmania (Australia) and New Zealand in the Pacific Ocean sector of the Southern Ocean. The 3000 m bathymetry contour and average locations of the sub-Antarctic Front (SAF) and Polar Front (PF) are depicted.

at depths ranging from 508 m – 1145 m, and thirty-one squid were collected during the 2000 austral winter ("2000 winter squid") (21 June - 28 June) from eleven locations around the island at depths between 611 m – 965 m.

4.3.2 Stomach contents analyses

The stomach contents data of 1999 summer squid were presented in Chapter 3, in combination with stomach contents data from a small number of squid collected during the 1997 – 1998 summer and also four specimens from Heard Island. Data from the 1999 summer only are included within this Chapter to facilitate temporal comparisons. Stomachs from all intact squid collected during the 1995 summer, 2000 summer and 2000 winter were examined for contents, according to the protocol described in Section 2.3, Chapter 2. After identifying prey remains to fish, cephalopod or crustacean level, the prey category that comprised the majority of the contents for an individual stomach was recorded as the "primary prey item" for that stomach.

Otolith identification was completed according to the protocol described in Section 2.3, Chapter 2. However, many otoliths recovered during this study were eroded, particularly otoliths from the genus *Gymnoscopelus*. Otoliths of *Gymnoscopelus nicholsi* can be difficult to distinguish from otoliths of *Gymnoscopelus piabilis*, and otoliths of *Gymnoscopelus fraseri* may be difficult to distinguish from *Gymnoscopelus bolini* (Williams & McEldowney 1990; D. Williams personal communication). This is particularly so when otoliths have been eroded after partial digestion. Therefore, these otoliths were not identified to species level, but were grouped as either *G. nicholsi/piabilis* or *G. fraseri/bolini*. When in good condition, otolith lengths (mm) were measured by image analysis with a Hitachi HV-C20 camera and Optimus version 6.2 (Media Cybernetics, Bothell, Washington), and regression equations from Williams & McEldowney (1990) were used to estimate standard lengths of fish prey. Regression equations for *G. nicholsi* and *G. fraseri* were used to represent *G. nicholsi/piabilis* and *G. fraseri/bolini*, respectively. When large amounts of fish remains were found (such as scales, bones and flesh), but without (or with very few) eye lenses or otoliths, it was assumed that fish heads may have been discarded and the incidence was recorded.

4.3.3 Lipid extraction and fatty acid analysis

Lipid was extracted from fifteen 1995 summer squid (eight female, seven male), twenty 1999 summer squid (thirteen female, seven male), ten 2000 summer squid (six female, four male) and five 2000 winter squid (all female). Only females of maturity stage III and males of maturity stage V were selected for fatty acid analysis; additionally, animals with damaged digestive glands were excluded from analyses. Lipid extraction and fatty acid analysis was conducted using the protocols described in Section 2.4, Chapter 2.

4.3.4 Statistical analyses

The abundance of prey in the stomach contents of *M. ingens* was described by percent frequency occurrence (% FO), percent numerical importance (% NI) and total number (N). The % FO was determined from the number of stomachs in season *k* containing prey species *h* as a percentage of the total number of stomachs examined for season *k*, and % NI was determined as the number of prey species *h* consumed during season *k* as a percentage of the total number of prey items consumed in season. A prey diversity index was determined as the total number of identifiable fish, cephalopod and crustacean species per individual stomach. Analyses of stomach contents data were conducted by excluding those prey species that occurred rarely in the diet of *M. ingens*; furthermore, unidentified fish, cephalopod and crustacean categories were excluded from analysis as these did not consistently represent a particular prey type. Dietary overlap between seasons was determined from stomachs containing identifiable hard remains using a percentage similarity index (%PSI) adapted from Goldsworthy et al. (2001a):

$$\%\mathrm{PSI}ij = 100 \times (1.0 - 0.5\sum_{h=1}^{s} | Shi - Shj |)$$

where Shi is the proportion by numerical importance of prey species or category h in season i, Shj is the proportion by numerical importance of prey species or category h in season j, and s is the number of prey species or categories. Dietary overlap was compared using two different groupings of prey. Firstly, h represented each of 10 individual fish and cephalopod species that passed the arbitrary selection criteria of ≥5% NI in two or more groups (*Electrona carlsbergi*, *Electrona subaspera*, *Gymnoscopelus*

braueri, *G. nicholsi/piabilis*, *Gymnoscopelus* spp., *Krefftichthys anderssoni*, *Lampanyctus achiris*, *Protomyctophum* spp. and *Stoloteuthis* sp.); secondly, *h* represented one of four prey categories defined as either myctophid fish, other fish, sepiolid cephalopods or other cephalopods. Crustacean prey could not be included in analyses of dietary overlap as the number of crustacean prey consumed by an individual squid could not be accurately determined from exoskeleton remains.

Complete linear canonical discriminant analysis (DA) and linear canonical DA with forward-stepwise entry of variables were used to classify individuals to season using both prey species presence-absence data and fatty acid mg g^{-1} data. Each data set was used to investigate 1) dietary variations between the 1995, 1999 and 2000 summer and 2) dietary variations between the 2000 summer and 2000 winter. Arbitrary selection criteria were set where a prey species must be abundant at or above 5% FO in at least 2 groups. Therefore, comparisons between the summer groups were made using 12 prey variables; *Electrona antarctica, E. carlsbergi, G. braueri, G. fraseri/bolini, G. nicholsi/piabilis, Gymnoscopelus* spp., *K. anderssoni, Protomyctophum* spp., *Bathylagus antarcticus, Stoloteuthis* sp. and calanoid copepods; comparisons between the 2000 summer and 2000 winter were made using only 4 prey variables; *E. carlsbergi, G. nicholsi/piabilis, Protomyctophum* spp. and *Stoloteuthis* sp.

Fatty acids that contributed a mean of less than 0.5% (of total fatty acids) were excluded from DA. For digestive gland tissue, analyses were performed using 14:0, 16:0, 18:0, br17:1, 16:1n7, 18:1n9, 18:1n7, 18:1n5, 20:1n9, 20:1n7, 22:1n11, 22:1n9, 24:1n11/9, 18:2n6, 20:4n6, 20:5n3, 20:4n3, 22:6n3 and 22:5n3; for mantle tissue 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n11, 22:1n9, 24:1n11/9, 20:4n6, 20:5n3, 22:6n3, 22:5n3 and C23 PUFA. No fatty acid data from Chapter 3 were included in this study, as the previous data-set could not be converted to mg g^{-1} values.

One-way ANOVA with Tukey's post-hoc test, ANCOVA and two-tailed t-tests assuming unequal variance were also conducted on general biological data, stomach contents data, total lipid and fatty acid data. All statistical analyses were conducted using SPSS 10.0 for Macintosh (SPSS Inc, Chicago, Illanois).

4.4 RESULTS

4.4.1 General biology

The ML (mm) and TM (g) was recorded for each individual, excluding seven squid collected during the 2000 winter (Table 4.1). Females had a significantly greater mean ML and TM than males ($p < 0.01$ in each case, except during the 2000 winter where $p < 0.05$ for ML), as has been previously documented for this sexually-dimorphic species (Jackson & Mladenov 1994; Lipinski & Linkowski 1986). No significant differences in ML were observed for either sex between seasons. Regression of the log of TM against the covariate ML showed that there were also no significant differences in TM between seasons for either sex. During summer periods, all females were immature and the modal maturity was stage III, whereas most males were mature (maturity stage V). During the winter, almost half the females were of maturity stage II, although the modal maturity was again stage III. No mature males were collected during winter.

4.4.2 Stomach contents

Most stomachs collected during the 1995 summer, 2000 summer and 2000 winter contained some contents although few were completely full; modal fullness was consistently recorded as stages 1 and 2. Most stomach contents were in a medium to high state of digestion. A large number of prey species were identified (Table 4.2); however, nearly 50% of stomachs with identifiable hard remains contained only one prey species (Fig. 4.2). The prey diversity index for individual stomachs did not vary significantly between summers and the 2000 winter, and the mean prey diversity index over the collection period was low at 2.5.

Table 4.1. Average mantle length and total mass of *Moroteuthis ingens* collected over three summers and one winter period.

	Females			Males		
		ML mm	TM g		ML mm	TM g
	n	ave ± SD	ave ± SD	*n*	ave ± SD	ave ± SD
1995 summer	26	320.6 ± 44.6	1018.0 ± 369.6	26	278.5 ± 38.8	602.0 ± 210.9
1999 summer	31	303.9 ± 40.9	906.7 ± 334.8	26	256.7 ± 36.0	511.1 ± 209.6
2000 summer	23	325.3 ± 45.7	1093.7 ± 450.9	5	261.2 ± 22.4	489.0 ± 123.5
2000 winter	18	279.1 ± 82.6	866.1 ± 612.6	6	216.8 ± 21.5	312.2 ± 94.7

n: sample size; ML: mantle length; TM: total mass.

Fish were most commonly identified as the primary prey (Table 4.2), with at least 21 species identified. Only three fish species were identified in every collection period: *E. carlsbergi*, *G. nicholsi/piabilis* and *Protomyctophum* spp. A total of fourteen species of myctophid fish were identified, in addition to another 7 fish species from 7 different families that generally contributed only minor amounts to the diet (with the exception of *B. antarcticus* and *Paradiplospinus gracilis*). The number of fish prey consumed by *M. ingens* during the 2000 winter (7 species) was considerably reduced from the number consumed during summer periods (11 – 16 species). Standard lengths (SL) of fish prey were estimated for 9 species (Fig. 4.3). *M. ingens* consumes a large range of fish of different sizes, from small individuals of *K. anderssoni* (20-50 mm SL) to individuals of the elongate *P. gracilis* that exceeded 400 mm SL.

Cephalopod prey was consumed during every season, although they were not often the primary prey item (Table 4.2). Lower rostral length (LRL) measurements were not recorded due to the small size of the cephalopod beak remains, and thus no estimates of cephalopod prey size are available. Most beaks were no larger than 3 mm crest length, thus precluding accurate measurement of the LRL using callipers. The most common species consumed was the sepiolid *Stoloteuthis* sp., particularly during the 1995 summer when it was identified in 25% of stomachs and comprised 74% of the identifiable cephalopod remains. This species may represent a Macquarie Island population of *Stoloteuthis leucoptera* (Verrill, 1878), which has been collected from the Kerguelen plateau in the Indian Ocean sector of the Southern Ocean (Nesis 1987). The Australian Antarctic Division has collected specimens of *Stoloteuthis* sp. in the vicinity of Macquarie Island (unpublished data). However, to my knowledge, the presence of this or similar sepiolid species at Macquarie Island has not been previously published, either from pelagic surveys or from prey remains retrieved from vertebrate predators.

Crustacean remains were identified in relatively few stomachs, and only comprised the primary prey group in one individual stomach collected during the 2000 winter (Table 4.2). Most crustacean remains were highly digested and could not be identified beyond broad group categories. Most remains appeared to be from natant decapods and calanoid

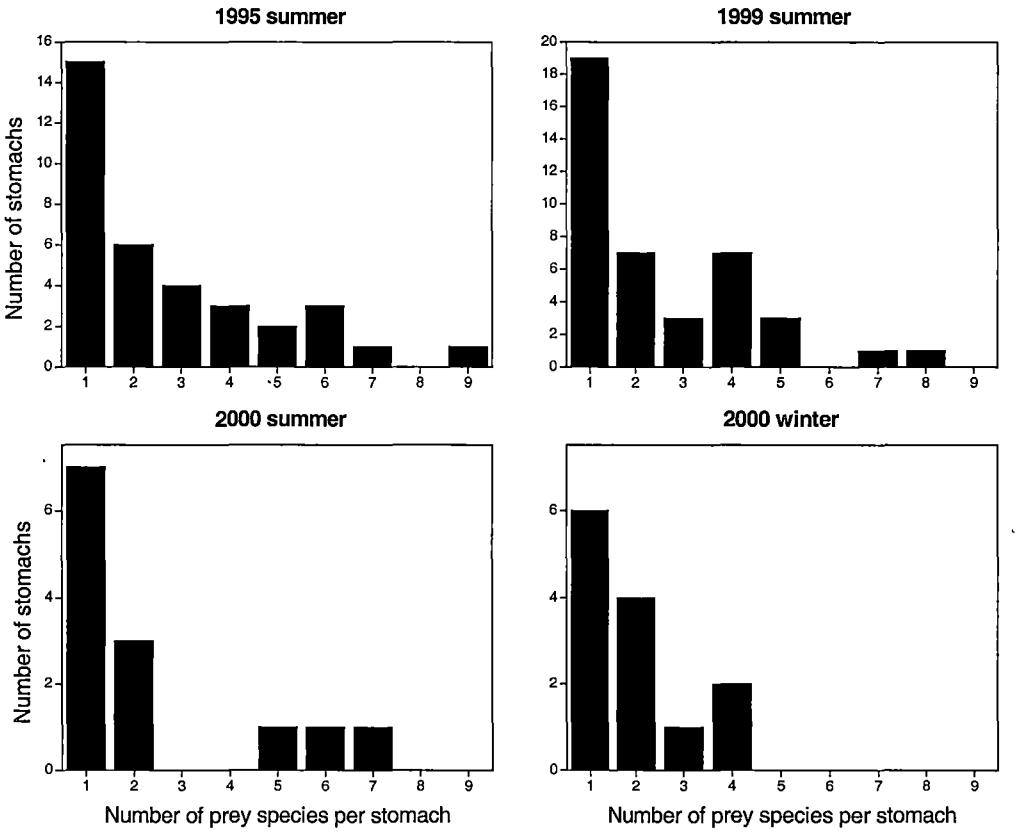

Fig. 4.2. Frequency distribution of the number of prey species identified in the stomachs of *Moroteuthis ingens* from Macquarie Island.

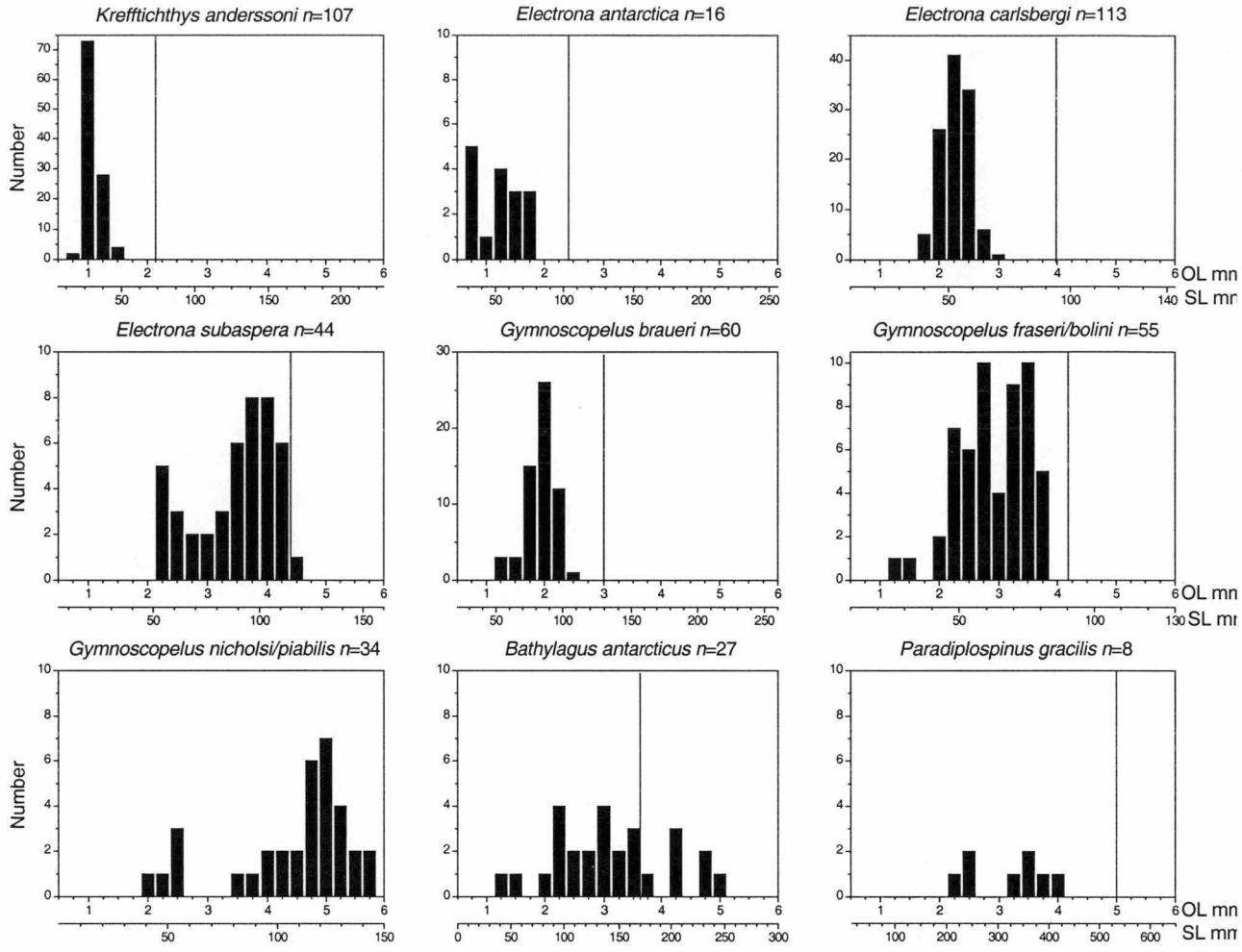

Fig. 4.3. Otolith lengths (OL) and estimated standard lengths (SL) of nine species of fish consumed by *Moroteuthis ingens* at Macquarie Island. Vertical lines indicate the maximum SL recorded for most fish species (*Gymnoscopelus nicholsi* attains 160 mm) (Hulley 1990; Smith & Heemstra 1986), *n* = number of otoliths measured per species.

Table 4.2. Total number (N), %NI and %FO of prey categories and prey species consumed by *Moroteuthis ingens* over three summers and one winter period

a) Fish prey	1995 summer *n* = 52			1999 summer *n* = 50			2000 summer *n* = 20			2000 winter *n* = 31		
Prey item	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO
FAMILY MYCTOPHIDAE												
Electrona antarctica	7	2	6	16	5	12						
Electrona carlsbergi	72	23	19	6	2	6	2	3	10	39	48	13
Electrona subaspera	21	7	21	30	9	16	1	1	5			
Electrona spp.	1	0 3	2	2	1	2	1	1	5			
Gymnoscopelus braueri	21	7	2	30	9	18	14	19	15			
Gymnoscopelus fraseri/bolini	10	3	8	28	9	12				2	2	6
Gymnoscopelus hintonoides				3	1	2						
Gymnoscopelus nicholsi/piabilis	2	1	4	28	9	24	19	26	25	4	5	6
Gymnoscopelus opisthopterus	2	1	2									
Gymnoscopelus spp.				42	13	10	9	12	25	1	1	3
Hintonia spp.				2	1	2						
Krefftichthys anderssoni	83	27	19	46	14	16	5	7	15			
Lampanyctus achiris	14	5	6							11	14	13
Protomyctophum bolini	1	0.3	2	15	5	12						
Protomyctophum normani							1	1	5			
Protomyctophum spp.	4	1	8	34	10	22	1	1	5	10	12	6
OTHER FISH												
Bathylagus antarcticus	24	8	21	17	5	18				8	10	10
Persparsia kopua	1	0.3	2									
Macrourus carinatus ?										1	1	3
Poromitra crassiceps							1	1	5			
unidentified Channichthyid	1	0.3	2				6	8	5			
Paradiplospinus gracilis	2	1	4				9	12	15			
Unidentified	13	4	12				1	1	5			
TOTAL FISH	**279**	**90**	**85**	**299**	**92**	**96**	**70**	**95**	**75**	**76**	**94**	**58**
%FO as primary prey			77			78			65			58
%FO fish heads discarded			19			6			15			0

N total number; NI: numerical importance; FO. frequency occurrence, *n* = total number of stomachs

Table 4.2. continued.

b) Cephalopod and crustacean prey	1995 summer *n* = 52			1999 summer *n* = 50			2000 summer *n* = 20			2000 winter *n* = 31		
Prey item	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO
CEPHALOPODS												
Brachioteuthis spp.				3	1	6						
Cranchiid spp.				1	0.3	2				1	1	3
Mastigoteuthis spp.	1	0.3	2									
Megalocranchia spp.	1	0.3	2									
Moroteuthis ingens	1	0.3	2				1	1	5			
Octopoteuthis spp.										1	1	3
Sepiolidae	3	1	4									
Stoloteuthis spp.	23	7	25	6	2	12	1	1	5	2	2	6
Unidentified	2	1	4	16	5	8	2	3	10	1	1	3
TOTAL CEPHALOPODS	**31**	**10**	**42**	**26**	**8**	**47**	**4**	**5**	**35**	**5**	**6**	**19**
%FO as primary prey			12			13			10			3
CRUSTACEANS												
amphipods						4						
calanoid copepods			12			8						
mysids			2									
natant decapods			4						10			3
TOTAL CRUSTACEANS			**13**			**9**			**5**			**10**
%FO as primary prey			0			0			0			3

N: total number; NI: numerical importance; FO: frequency occurrence; *n* = total number of stomachs.

copepods, of which at least three species were consumed over the study period. Myctophid fish consume large amounts of copepods (Gaskett et al. 2001; Pakhomov et al. 1996), therefore such crustacean remains may derive from secondary ingestion via myctophids rather than from direct predation.

Indices of prey abundance (N, % NI and % FO) were found to vary considerably between sampling periods. Abundant prey species in the diet of *M. ingens* during the 1995 summer were *E. carlsbergi*, *K. anderssoni*, *B. antarcticus* and *Stoloteuthis* sp (Table 4.2). *K. anderssoni* was also an important prey during the summer of 1999, in addition to *E. subaspera*, *G. braueri* and *G. nicholsi/piabilis*. During the 2000 summer, abundant prey species were *G. braueri*, *G. nicholsi/piabilis*, *Gymnoscopelus* spp. and *P. gracilis*, whereas *E. carlsbergi*, *L. achiris* and *Protomyctophum* spp. were important species during the 2000 winter. Indices of dietary overlap using 10 prey species categories indicated that the 1999 summer and 2000 summer were most similar, with a %PSI of 64.50% (Table 4.3a). Dietary overlap between the 1995 summer and the 1999 summer was less at 59.57%, and the overlap between 1995 summer and 2000 summer was reduced to 43.22%. The overlap between the 2000 summer and 2000 winter was 30.15%.

When the overlap between sampling periods was determined using 4 prey categories instead of individual species, indices of overlap were much higher and different patterns were also observed (Table 4.3b). The 1999 summer and 2000 winter were most similar with an overlap of 92.7%, whereas the 1999 summer and 2000 summer were least similar with an overlap of 80.5%. Inconsistent trends in dietary overlap between 10 prey species and 4 prey categories indicate that this technique is highly sensitive to the method of grouping (Goldsworthy et al. 2001a). Nevertheless, it appears from both methods of grouping that the diet of *M. ingens* changed markedly from the 2000 summer to the 2000 winter.

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Table 4.3. Dietary overlap of *Moroteuthis ingens* between three summers and one winter period.

a) dietary overlap using 10 prey species categories				
%PSI	1995	1999	2000s	2000w
1995	100.0			
1999	49.6	100.0		
2000s	24.5	41.8	100.0	
2000w	43.0	24.9	12.8	100.0

b) dietary overlap using 4 prey group categories				
%PSI	1995	1999	2000s	2000w
1995	100.0			
1999	90.5	100.0		
2000s	87.8	80.5	100.0	
2000w	92.0	92.7	87.8	100.0

%PSI: Percent Similarity Index; s: summer; w: winter.

Complete linear DA of stomach contents data indicated that the summer diet of *M. ingens* varied on an interannual basis (Wilks' lambda = 0.470, Approx. F = 2.866, $p < 0.01$). Two prey variables were incorporated into forward-stepwise DA of the same data to distinguish between years: *G. nicholsi/piabilis* and *Gymnoscopelus* spp. (Wilks' lambda = 0.760, Approx. F = 6.240, $p < 0.01$). Forward-stepwise DA assigned 53.9% of individuals to the correct summer (53.9% cross-validated) (Table 4.4a). While 94% of individuals from the 1995 were assigned to the correct year, only 24% and 39% of individuals were correctly assigned to the 1999 and 2000 summer, respectively. This largely reflects the fact that very few 1995 summer squid had consumed either *G. nicholsi* or *Gymnoscopelus* spp.; those that had were incorrectly assigned to the 2000 summer. In contrast, 1999 and 2000 summer squid that had not consumed *G. nicholsi* and *Gymnoscopelus* spp. were incorrectly assigned to the 1995 summer. Squid that had consumed only *G. nicholsi/piabilis* were assigned to the 1999 summer, whereas squid that had consumed *Gymnoscopelus* spp. and perhaps also *G. nicholsi/piabilis* were assigned to the 2000 summer.

Complete linear DA indicated that the diet of *M. ingens* did not vary between the 2000 summer and winter (Wilks' lambda = 0.830, Approx F = 1.073, $p < 0.5$), in contrast to results obtained for the PSI. Complete linear DA assigned 61.5% of squid to the correct season (26.9% cross-validated) (Table 4.4b). Forward-stepwise DA could not be used to compare 2000 summer and winter stomach contents data.

4.4.3 Lipid and fatty acid analysis

Mantle tissue was low in lipid, with mean contents between 1.3 ± 0.1% wet mass (2000 winter) and 1.6 ± 0.3% wet mass (1995 summer) (Table 4.5). Digestive gland tissue was lipid-rich, with a content between 35.9 ± 12.4% wet mass (1999 summer) and 48.3 ± 11.9% wet mass (2000 winter) (Table 4.5). No significant differences in mantle or digestive gland total lipid content were observed between groups. Mantle tissue is characterised by large amounts of PUFA, principally DHA (22:6n3: comprising between 35.6 ± 2.9% and 37.7 ± 2.3% of total fatty acids) and EPA (20:5n3: comprising between 13.2 ± 0.8% and 13.5 ± 0.2% of total fatty acids) (Table 4.6).

Table 4.5. Total lipid content (% wet mass) of mantle and digestive gland tissue of *Moroteuthis ingens* . Values are means ± SD

Total lipid		Mantle		Digestive gland
% wet mass	*n*	ave ± SD	*n*	ave ± SD
1995 summer	15	1.6 ± 0.3	15	44.7 ± 15.1
1999 summer	20	1.5 ± 0.2	20	35.9 ± 12.4
2000 summer	10	1.4 ± 0.2	10	44.8 ± 11.2
2000 winter	5	1.3 ± 0.1	5	48.3 ± 11.9

n : sample size.

No other PUFA comprised more than 2.5% of the total fatty acids. Sum PUFA values varied between 52.8 ± 2.6% and 55.5 ± 2.9% of total fatty acids, between the 1999 summer and 2000 summer respectively. Sum SAT values ranged from 29.6 ± 1.6% of total fatty acids in 2000 winter females, to 30.8 ± 2.7% of total fatty acids in 1999 summer. SAT were largely comprised of 16:0. MUFA were the least abundant class of fatty acids, and comprised between 14.6 ± 1.2% and 16.1 ± 1.9% of total fatty acids. 18:1n9 and 20:1n9 were major MUFA.

Digestive gland fatty acid profiles were dominated by large amounts of MUFA, which contributed from 52.2 ± 4.6% up to 60.2 ± 5.6% of total fatty acids, between the 2000 summer and 1999 summer respectively (Table 4.7). MUFA were largely comprised of

Table 4.4. Discriminant analysis of identifiable hard remains from the stomach contents of *Moroteuthis ingens* , comparing a) the summers of 1995, 1999 and 2000 and b) the summer and winter of 2000.

	a) 1995, 1999 & 2000 summer					Standardised discriminant	
Allocated	Predicted group membership				Prey species used	function coefficients	
group	1995	1999	2000	*n*	in separation	Function 1	Function 2
1995	33	2		35	GN/P	0.614	0.790
1999	26	10	5	41	GS	0.813	-0.583
2000	4	4	5	13			
	53.9% of allocated cases correctly classified						

	b) 2000 summer & winter				Standardised discriminant
Allocated	Predicted group membership			Prey species used	function coefficients
group	2000s	2000w	*n*	in separation	Function 1
2000s	11	2	13	EC	0.676
2000w	8	5	13	GN/P	-0.894
				PS	0.352
				ST	0.352
	61.5% of allocated cases correctly classified				

n : sample size; GN/P: *Gymnoscopelus nicholsi* ; GS: *Gymnoscopelus* spp.; EC: *Electrona carlsbergi* ; PS: *Protomyctophum* spp.; ST: *Stoloteuthis* spp.

Table 4.6. Percentage fatty acids (of total fatty acids) of the mantle tissue of *Moroteuthis ingens* from three summers and one winter period. Values are means ± SD.

Fatty acid	1995 summer *n* =15	1999 summer *n* =20	2000 summer *n* =10	2000 winter *n* =5
14:0	1.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.3	1.7 ± 0.2
16:0	24.8 ± 1.4	25.8 ± 2.3	24.6 ± 2.2	24.8 ± 0.8
18:0	2.4 ± 0.3	2.4 ± 0.3	2.5 ± 0.1	2.4 ± 0.1
16:1n7	0.8 ± 0.3	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
18:1n9	3.5 ± 0.6	3.9 ± 0.7	3.1 ± 0.3	3.0 ± 0.2
18:1n7	1.9 ± 0.5	1.9 ± 0.2	1.5 ± 0.2	1.8 ± 0.2
20:1n9	5.3 ± 0.3	5.6 ± 0.4	5.8 ± 0.7	5.6 ± 0.2
22:1n11	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.0	0.3 ± 0.0
22:1n9	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.2	1.7 ± 0.1
24:1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
20:4n6 (AA)	1.8 ± 0.2	1.9 ± 0.2	2.0 ± 0.1	2.0 ± 0.3
20:5n3 (EPA)	13.3 ± 0.4	13.2 ± 0.8	13.3 ± 0.7	13.5 ± 0.2
22:6n3 (DHA)	37.7 ± 2.0	35.6 ± 2.9	37.7 ± 2.3	36.7 ± 0.8
22:5n3 (DPA)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
C23 PUFA	0.6 ± 0.3	0.3 ± 0.4	0.6 ± 0.4	0.9 ± 0.2
SUM SAT	**29.6 ± 1.6**	**30.8 ± 2.7**	**29.7 ± 2.4**	**29.7 ± 0.8**
SUM MUFA	**15.0 ± 1.3**	**16.1 ± 1.9**	**14.6 ± 1.2**	**15.1 ± 0.6**
SUM PUFA	**55.1 ± 2.2**	**52.8 ± 2.6**	**55.5 ± 2.8**	**55.0 ± 0.8**

n : sample size; AA: arachidonic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table 4.7. Percentage fatty acids (of total fatty acids) of the digestive gland of *Moroteuthis ingens* from three summers and one winter period. Values are means ± SD

Fatty acid	1995 summer *n* =15	1999 summer *n* =20	2000 summer *n* =10	2000 winter *n* =5
14:0	3.1 ± 0.6	3.1 ± 0.6	3.1 ± 0.3	3.5 ± 0.2
16:0	15.6 ± 1.8	14.8 ± 1.4	16.2 ± 1.9	14.7 ± 1.0
18:0	3.1 ± 0.5	3.6 ± 0.7	4.0 ± 0.5	3.2 ± 0.3
br17:1	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
16:1n7	4.7 ± 1.1	3.9 ± 1.2	4.0 ± 0.7	4.3 ± 0.4
18:1n9	23.2 ± 2.6	25.6 ± 4.4	21.5 ± 2.3	20.5 ± 1.6
18:1n7	4.2 ± 0.7	5.0 ± 0.7	3.3 ± 1.1	4.7 ± 0.5
18:1n5	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.0
20:1n9	8.9 ± 1.7	11.5 ± 2.3	10.8 ± 3.2	11.1 ± 1.2
20:1n7	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
22:1n11	5.3 ± 1.5	5.7 ± 2.8	5.3 ± 1.5	5.4 ± 0.6
22:1n9	2.8 ± 0.8	2.3 ± 0.6	2.4 ± 0.6	2.8 ± 0.2
24:1	3.2 ± 1.4	2.9 ± 1.1	2.7 ± 1.4	3.1 ± 1.1
18:2n6	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.1
20:4n6 (AA)	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
20:5n3 (EPA)	5.5 ± 0.9	4.0 ± 1.3	5.8 ± 1.5	5.7 ± 0.6
20:4n3	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.0
22:6n3 (DHA)	9.9 ± 2.2	7.1 ± 2.7	10.6 ± 2.7	10.0 ± 1.7
22:5n3 (DPA)	0.8 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	1.0 ± 0.1
SUM SAT	**22.7 ± 2.2**	**22.4 ± 2.1**	**24.3 ± 2.2**	**22.3 ± 1.1**
SUM MUFA	**55.0 ± 3.6**	**60.2 ± 5.5**	**52.2 ± 4.6**	**54.9 ± 3.5**
SUM PUFA	**21.1 ± 3.2**	**16.2 ± 4.4**	**22.4 ± 4.8**	**21.7 ± 2.4**

n : sample size; AA: arachidonic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

18:1n9 (with values between 20.5 ± 1.6% and 25.6 ± 4.4% of total fatty acids). The major SAT was 16:0, as observed in mantle tissue. Sum SAT values ranged between 22.3 ± 1.1% and 24.3 ± 2.2% of total fatty acids (between 2000 winter and 2000 summer, respectively), and similar values of sum PUFA were observed. PUFA levels were lowest in the 1999 summer (16.2 ± 4.4% of total fatty acids) and highest in 2000 summer (22.4 ± 4.8% of total fatty acids), and were comprised largely of DHA and EPA.

4.4.4 Interannual and seasonal comparisons of fatty acid profiles

Forward-stepwise DA of digestive gland fatty acid data provided firm evidence that dietary intake varied between summers (Wilks' lambda = 0.161, Approx. F = 11.324, $p < 0.01$), with 93.3% cases correctly assigned (88.9% cross-validated) using only five fatty acid variables (Table 4.8a). The first discriminant function accounted for 79.9% of variability in the data, and clearly separated 1999 summer squid from the 1995 and 2000 summer squid (Fig. 4.4). This function can be interpreted as a contrast between proportions of 18:1n7 and proportions of DHA (Table 4.8a), with the 1999 summer squid having significantly higher levels of 18:1n7 than 1995 or 2000 summer squid (F = 16.46, $p < 0.01$) and significantly lower levels of DHA than 1995 or 2000 summer squid (F = 8.44, $p < 0.01$) (Table 4.7). Sum values of MUFA and PUFA were significantly different between summers (MUFA: F = 6.19, $p < 0.01$; PUFA: F = 5.68, $p < 0.01$). The interannual trends in 18:1n7 and DHA are reflected in sum MUFA and PUFA values; that is, mean MUFA levels were highest during the 1999 summer, whereas mean PUFA abundance was lowest during this season (Table 4.7).

The second discriminant function accounted for the remaining 21.1% of variability in the fatty acid data, and separated the 1995 summer squid from the 2000 summer squid (Fig. 4.4). This function can be interpreted as a contrast between proportions of 18:0 and 18:1n7. Proportions of 18:0 were significantly different between 1995 and 2000 summer squid (F = 5.33, $p < 0.01$) (Table 4.7) and squid with high levels of 18:0 were assigned to the 2000 summer (Table 4.8a). Proportions of 18:1n7 were significantly larger in 1995 summer squid compared to 2000 summer squid (F = 16.46, $p < 0.01$) (Table 4.7) so that squid with higher 18:1n7 levels were assigned to the 1995 summer (Table 4.8a).

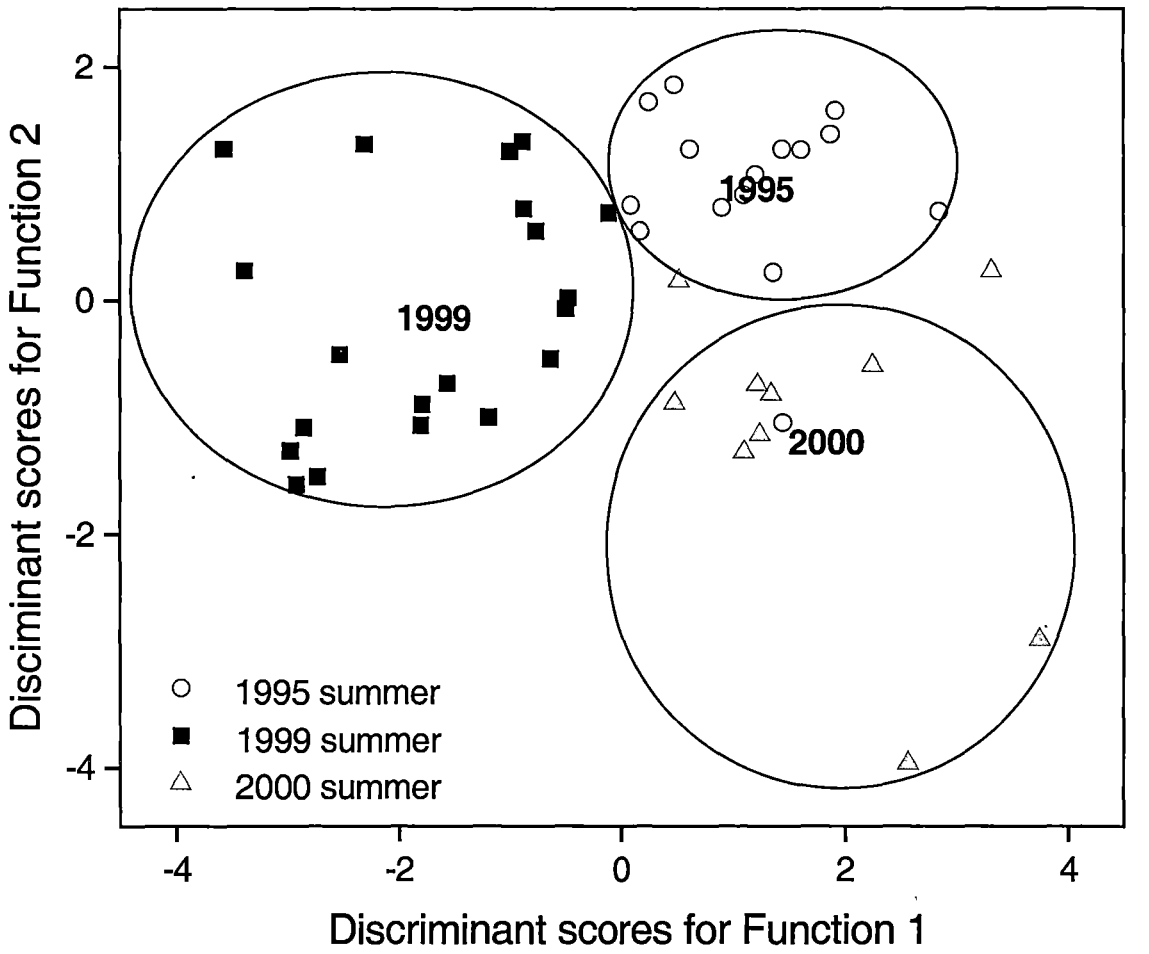

Fig. 4.4. Discriminant scores of fatty acid data (mg g^{-1}) from the digestive gland of *Moroteuthis ingens* from Macquarie Island. Year-group labels in the figure are positioned over respective group centroids.

Table 4.8. Discriminant analysis of digestive gland fatty acid data of *Moroteuthis ingens* , comparing a) the summers of 1995, 1999 and 2000 and b) the summer and winter of 2000

a) 1995, 1999 & 2000 summer						Standardised discriminant	
Allocated	Predicted group membership				Fatty acids used	function coefficients	
group	1995	1999	2000	*n*	in separation	Function 1	Function 2
1995	14		1	15	14:0	1.787	-0.344
1999	1	19		20	18:0	0.59	-1.321
2000	1		9	10	18:1n7	-2.242	2.242
					20:4n6 (AA)	-1.637	-1.816
					22:6n3 (DHA)	1.749	1.245
	93.3% of allocated cases correctly classified						

b) 2000 summer & winter				Standardised discriminant	
Allocated	Predicted group membership			Fatty acids used	function coefficients
group	2000s	2000w	*n*	in separation	Function 1
2000s	10		10	20:1n9	-1.036
2000w		5	5	20:1n7	1.689
	100% of allocated cases correctly classified				

AA: arachidonic acid; DHA: docosahexaenoic acid.

Fatty acid composition of the digestive gland were found to vary between the 2000 summer and winter using forward-stepwise DA (Wilks' lambda = 0.287, Approx. F = 14.902, $p < 0.01$). Two MUFA, 20:1n9 and 20:1n7, were used to distinguish the 2000 summer from the 2000 winter with 100% of cases correctly assigned (86.7% cross-validated) (Table 4.8b). Hence fatty acid data supports findings from the %PSI that a considerable shift in diet occurred between the 2000 summer and 2000 winter.

Interannual variations were also observed in the fatty acid composition of mantle tissue using complete linear DA (Wilks' lambda = 0.164, Approx. F = 2.741, $p < 0.01$). Forward-stepwise DA indicated that 18:1n7, 20:1n9 and DHA were largely responsible for interannual variations in mantle composition. These three fatty acid discriminators correctly assigned 75.6% of individuals (73.3% cross-validated). The first discriminant function was largely a contrast between proportions of 18:1n7 and 20:1n9, where individuals with lower proportions of 18:1n7 and higher proportions of 20:1n9 were allocated to the 2000 summer (Table 4.9). The second discriminant function was a contrast between proportions of 18:1n7 and DHA; individuals with higher proportions of 18:1n7 were assigned to the 1999 summer whereas individuals with higher proportions of DHA were assigned to the 1995 summer (Table 4.9).

Complete linear DA indicated that there was little seasonal variation in the fatty acid composition of mantle tissue between the 2000 summer and winter (Wilks' lambda = 0.200, Approx. F = 2.220, $p < 0.5$). Forward-stepwise DA functions could not be constructed to discriminate between the 2000 summer and 2000 winter using mantle fatty acid profiles.

4.5 DISCUSSION

Comparisons of stomach content and digestive gland fatty acid data for *M. ingens* have allowed three main conclusions to be drawn regarding temporal variations in diet composition at Macquarie Island. Firstly, interannual and seasonal comparisons of digestive gland fatty acids reveal significant differences in fatty acid composition between collection periods, indicating that the dietary intake of lipid by *M. ingens* varied

Table 4.9. Discriminant analysis of mantle fatty acid data of *Moroteuthis ingens* , comparing the summers of 1995, 1999 and 2000

Allocated group	Predicted group membership 1995	1999	2000	*n*	Fatty acids used in separation	Standardised discriminant function coefficients Function 1	Function 2
1995	9	5	1	15	18:1n7	7.039	-1.675
1999	1	17	2	20	20:1n9	-9.242	0.657
2000		2	8	10	22:6n3 (DHA)	2.541	1.873
75.6% of allocated cases correctly classified							

DHA: docosahexaenoic acid.

between three summers and also between a consecutive summer and winter. Secondly, stomach contents provide good information on the broad prey spectrum of *M. ingens*, the diversity of prey consumed by an individual and provide some information on what prey species may be responsible for temporal variations in dietary lipid uptake. Thirdly, neither technique should be used in isolation to determine interannual and seasonal variations in the diet of *M. ingens*.

4.5.1 Interannual and seasonal variations in diet

Based on the findings of previous studies and the results from Chapter 3, it is assumed that lipid stored in the digestive gland of *M. ingens* is of strictly dietary origin (Abolmasova et al. 1990; Blanchier & Boucaud-Camou 1984; Clarke et al. 1994; Semmens 1998; Shchepkin et al. 1982). There was sufficient between-year variation in the fatty acid profiles of digestive glands collected over three summers to assign >93% of individuals to the correct summer. That is, dietary intake of fatty acids by *M. ingens* differed considerably between the summers of 1995, 1999 and 2000, most likely as a result of changes in the proportions of available prey over the study period (Fig. 4.4).

The considerable shift in diet that occurred during the 1999 summer, as indicated by significantly higher levels of 18:1n7 and MUFA, may have resulted from an increased intake of certain myctophid species during this period. These fish are unique in that their lipids are characterised by large amounts of MUFA as opposed to PUFA (Saito & Murata 1998). Even within the myctophid group, some species such as *G. nicholsi* are particularly rich in both lipid and MUFA (Lea et al. 2002b; Phleger et al. 1999). Lea et al. (2002b) noted that levels of 18:1n7 were particularly elevated in *G. nicholsi* compared with other myctophid species, and that levels of DHA were relatively low. This pattern was also generally reflected in myctophid species studied by Phleger et al. (1999). The discriminators 18:1n7 and DHA were both largely responsible for separating 1999 summer squid from 1995 and 2000 summer squid (Table 4.8a). *G. nicholsi/piabilis* and *Gymnoscopelus* spp. were the only prey variables incorporated into forward-stepwise DA of stomach contents to distinguish between year groups. Fatty acid profiles therefore indicate that many otoliths identified in 1999 stomachs are representative of *G. nicholsi* rather than *G. piabilis*. With this assumption, comparisons of fatty acid and stomach

contents analyses indicate that interannual variations in the diet of *M. ingens* were largely due to an increased consumption of *G. nicholsi* during the 1999 summer relative to other years.

Interannual variations in the availability of myctophids have affected other marine predators in the Southern Ocean. Evidence from fatty acid analysis indicates that, within the vicinity of Kerguelen Island, the consumption of *G. nicholsi* by the Antarctic fur seal *Arctocephalus gazella* increased during the 2000 summer relative to the 1999 summer. In contrast, faecal analysis indicated that *Protomyctophum tenisoni* was consumed in larger numbers during the 1999 summer (Lea et al. 2002a). At South Georgia, the diet of the King penguin *Aptenodytes patagonicus* has been subject to interannual variations in composition during summer, largely dependent on the scarcity or abundance of key myctophid species such as *K. anderssoni* (Olsson & North 1997; Rodhouse et al. 1998). The proportions of myctophids in the diet of the Patagonian toothfish has also varied on an interannual basis at Macquarie Island (Goldsworthy et al. 2002), although no information is available for individual myctophid species. Larger amounts of PUFA in the digestive gland of *M. ingens* during the 1995 and 2000 summers may be related to higher proportions of non-myctophid fish such as *B. antarcticus* and *P. gracilis* in the diet.

The comparison of several oceanographic parameters suggests that environmental conditions around Macquarie Island were different during the summer of 1999, relative to the summers of 1995 and 2000 (Table 4.10). The 1999 summer was characterised by warmer sea surface temperatures (SST) around the island and a substantial decrease in primary production. Thus environmental fluctuations have feasibly underpinned temporal variations in prey availability over the study period. (Goldsworthy et al. 2001b) suggest that cooler sea surface temperatures (SST) may be related to increased primary production at Macquarie Island. While the affects of decreased primary production and warmer SST on nektonic fish and squid communities are presently unknown, DA of digestive gland fatty acid profiles and stomach contents data suggest that such environmental fluctuations have had an affect on the dietary structure of *M. ingens*.

Table 14.10. Oceanographic parameters around Macquarie Island recorded over the study period. Data are from Goldsworthy et al. (2001b).

Season	mean summer SST relative to the 1981-2000 mean	ocean colour (chlorophyll a) data
summer 1995	cooler	no data available
summer 1999	warmer	no significant local primary production
summer 2000	cooler	homogenous local primary production

SST: sea surface temperature.

Analysis of digestive gland fatty acid profiles of *M. ingens* provides evidence for a highly significant switch in prey between the summer and winter of 2000. Two MUFA (20:1n9 and 20:1n7) were required to assign individuals to the summer or winter period with 100% accuracy. The 20:1 MUFA are particularly important components of myctophid MUFA content (Saito & Murata 1998). While TAG is the major lipid class of many myctophid species, some species contain WE as their major lipid class (Phleger et al. 1999; Saito & Murata 1996). The relative proportions of MUFA vary between these groups of myctophids, with several TAG-rich myctophid species *Gymnoscopelus opisthopterus*, *G. nicholsi* and *E. carlsbergi* containing much larger amounts of 20:1 MUFA than the WE-rich myctophids *G. braueri* and *K. anderssoni* (Phleger et al. 1999). The amounts of 20:1n9 and 20:1n7 were both significantly larger in the digestive gland of *M. ingens* during winter compared to summer ($p < 0.05$ and $p < 0.01$, respectively). Comparisons with stomach contents data suggest that increased 20:1n9 and 20:1n7 during winter may reflect a switch of dominant prey species from WE-rich species such as *K. anderssoni* during summer to TAG-rich species such as *E. carlsbergi* during winter.

K. anderssoni was absent from the diet of *M. ingens* during the 2000 winter, whereas *E. carlsbergi* was more abundant in terms of N, % NI and % FO compared to the 2000 summer. These findings support evidence from the stomach contents of king penguins that *E. carlsbergi* replaces *K. anderssoni* as the dominant prey item of this predator during winter at Macquarie Island (Hindell 1988). Other analyses of seasonal variations in stomach contents between the 2000 summer and winter are ambiguous. On one hand, the %PSI suggests that there is little overlap in diet. In contrast, DA of stomach contents data suggests that, to a large extent, diet did not vary over this period.

4.5.2 A comparison of methods

This study has confirmed that while fatty acid analysis complement stomach contents analysis, neither technique should be used in isolation to assess temporal variations in diet. It was not possible to incorporate fatty acid data of prey species into the analyses. Samples of myctophids are typically difficult to access (Lea et al. 2002a), particularly as by-catch from commercial trawlers, and have not been collected as part of this study. Although published fatty acid profiles are available for some Southern Ocean myctophid species (Lea et al. 2002b; Phleger et al. 1999; Phleger et al. 1997; Raclot et al. 1998; Reinhardt & Van Vleet 1986), data are only available for two species from Macquarie Island; *E. antarctica* and *G. fraseri* (Lea et al. 2002a). The fatty acid profiles of myctophids are prone to considerable spatial variations (Phleger et al. 1997; Lea et al. 2002a) and it was not valid to include data from geographically distant sites into an analysis of temporal variation of the lipids of *M. ingens* at Macquarie Island. Furthermore, almost no fatty acid data exists for the cephalopod prey of *M. ingens*, and without further identification of crustacean prey, no crustacean fatty acid data could be included in temporal analyses. Fatty acid data have clearly shown that the diet of *M. ingens* at Macquarie Island has varied on both an interannual and seasonal basis during the study period. However, due to the current paucity of data for potential prey, fatty acid data presently provides very little information on the relative proportions of individual prey species in the diet at a given time.

In contrast, while stomach contents provide good information on the species composition of the diet, temporal variations are much more difficult to interpret in these data compared to fatty acid data. Although *M. ingens* was shown to consume a broad range of prey (Table 4.2), the prey diversity index for individual stomachs was very low; those stomachs with identifiable prey remains contained a mean of 2.5 prey species, and almost half of these stomachs contained only 1 prey species (Fig. 4.2). Thus the data contained a large amount of within-group variation, and it was necessary to conduct between-group comparisons with few prey variables. For example, only four prey species were consistently present in the diet over the study period; the myctophids *E. carlsbergi*, *G. nicholsi/piabilis* and *Protomyctophum* spp. and the cephalopod *Stoloteuthis* sp. Contrasting findings from the %PSI and DA of stomach contents may be

largely due to methods used to reduce the stomach contents dataset (by either grouping prey or excluding prey species from analyses according to arbitrary selection criteria) so that groups could be more easily compared.

Indices of prey abundance used in stomach contents analyses must be treated with caution; indices such % NI overestimate the importance of small prey items that are consumed in large numbers, such as *K. anderssoni* and *E. carlsbergi* (Hyslop 1980). This source of error is compounded by the fact that squid may often reject the heads of larger fish (Collins & Pierce 1996). This may strongly bias against the larger prey species, but rarely affect the small species such as *K. anderssoni* and *E. carlsbergi.* Based on the absence of otoliths and eye lenses combined with large amounts of fish remains, it appeared that heads of fish had been rejected by up to 19% of individual squid. One final problem that obstructed the temporal analyses in the diet of *M. ingens* from stomach contents data is that was not possible to include estimates of crustacean remains as a proportion of the diet. On most occasions, the number of crustaceans in a sample could not be determined from exoskeleton remains.

4.5.3 Prey size of Moroteuthis ingens

Individuals of *M. ingens* that were collected at depths between 250 m and 1200 m had consumed a large size range of fish prey, including the larger cohort of a number of species such as *E. subaspera*, *G. fraseri/bolini*, *G. nicholsi/piabilis* and *B. antarcticus* (Fig. 4.3). Interestingly, however, *M. ingens* had only consumed specimens of *K. anderssoni*, *E. antarctica* and *E. carlsbergi* and *G. braueri* that were within the size-range of juveniles (Hulley 1990). Comparisons with the standard length of other fish prey suggest that the absence of adults of these latter species is not due to size-selectivity on behalf of *M. ingens*, but instead due to the absence of shoals of adult fish around Macquarie Island during the study period.

In contrast to the size range of fish prey, the cephalopod prey of *M. ingens* at Macquarie Island are very small. Although LRL of cephalopod beaks were not measured during this study, all beaks were estimated to have a crest length of less than 3 mm and thus derive from very small individuals. While *M. ingens* can prey on relatively large fish,

the size of cephalopod prey is a limiting constraint and only small individuals are taken as prey. Similarly, Collins & Pierce (1996) found that individual squid of the species *Loligo forbesi* could only consume squid much smaller than themselves.

4.5.4 Mantle fatty acid profiles

Some interannual variation in the fatty acid composition of mantle tissue is apparent; for example, trends in the proportions of 18:1n7 and DHA as observed in the digestive gland during the 1999 summer were also reflected in mantle tissue. However, fatty acids incorporated into mantle tissue largely fulfil specific requirements, and selective uptake of fatty acids by mantle tissue will lead to a degree of homogeneity in mantle tissue fatty acid composition over time. That is, while temporal variations in dietary uptake of fatty acids may have some influence the fatty acid composition of mantle tissue, other processes such as modification or biosynthesis of fatty acids may also account for mantle fatty acid composition, independent of temporal variations in diet. Therefore, mantle tissue is not affected by temporal variations in dietary lipid uptake to the extent of digestive gland tissue, and fatty acid profiles of the mantle tissue cannot be so easily related to temporal variations in the environment of *M. ingens*.

4.6 CONCLUSIONS

Interannual and seasonal variation the diet of *M. ingens* at Macquarie Island have been detected using a combined approach of stomach contents and fatty acid analyses. A considerable shift in dietary lipid uptake was observed during the 1999 summer relative to the 1995 and 2000 summers, and both fatty acid and stomach contents data indicate that this was due to an increased consumption of *G. nicholsi* at this time. Comparisons with oceanographic data confirm that conditions around the island differed during the 1999 summer relative to other summers, and changing oceanographic conditions may have affected prey distribution and food availability to *M. ingens*. Fatty acid and stomach content analyses suggest that, during the 2000 winter, *E. carlsbergi* replaced *K. anderssoni* as an important prey species, as observed for other marine predators in the Southern Ocean.

ADDENDUM

The research within the original manuscript and this resultant Chapter was conducted by myself. Co-authors G. D. Jackson and P. D. Nichols fulfilled supervisory roles as my Ph. D. supervisors at IASOS, University of Tasmania, and provided constructive criticism on the text. C. Sands and T. McArthur are acknowledged for their assistance with squid dissections. J. Kitchener completed the identification of crustacean prey, and D. Williams verified the identification of some uncommon otoliths.

Chapter 5. Spatial variations in the diet of *Moroteuthis ingens* between four sites in the Southern Ocean

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5.1 ABSTRACT

Specimens of the onychoteuthid squid *Moroteuthis ingens* were collected from four sites in the Southern Ocean: Macquarie Island, the Falkland Islands, the Chatham Rise (New Zealand) and the Campbell Plateau (New Zealand). Spatial variations in squid diet between these areas were investigated using stomach contents and lipid and fatty acid profiles. Myctophid fish were prominent prey items at all sites, and the diet at New Zealand sites comprised temperate myctophid species that were not identified at other sites. The diet at the Falkland Islands differed considerably from other sites due to the large proportion of cephalopod prey that had been consumed by *M. ingens*. This is likely to be due to the absence of key myctophids, such as *Electrona carlsbergi*, and the abundance of smaller squid such as *Loligo gahi* and juvenile *M. ingens* over the Patagonian Shelf. Stomach contents data could not be used effectively to determine dietary differences between the Chatham Rise and Campbell Plateau, largely due to differences in sample sizes between these sites. Lipid class and fatty acid profiles of the digestive gland indicated that the diet of *M. ingens* differed significantly between the Chatham Rise and Campbell Plateau, despite the relative proximity of these sites. Differences in total lipid content indicate that this was due to a reduction in food availability to *M. ingens* at the Campbell Plateau. The highly productive waters of the STF pass over the Chatham Rise, whereas the Campbell Plateau is situated in less productive sub-Antarctic water. Differences in oceanographic conditions are likely to have driven dietary variations between these two sites.

5.2 INTRODUCTION

Squid are voracious, opportunistic predators that exploit a wide variety of prey. These highly mobile animals are able to adapt to spatial variations in food resources, to the extent that Rodhouse & Nigmatullin (1996) have suggested that patchy prey distributions have driven the evolution of migratory behaviour of some species, so that feeding effort in productive areas can be maximised. Geographic variations in dietary composition have been recorded for a number of ommastrephid species: *Illex illecebrosus* in the region of Newfoundland (Ennis & Collins 1979), *Todaropsis eblanae* (Girard, 1890) and *Illex coindetii* (Vérany, 1839) in Galician waters (Rasero et al. 1996), *Illex argentinus* (Castellanos, 1960) on the Patagonian Shelf and along the coasts of Argentina and Brazil (Ivanovic & Brunetti 1994; Santos & Haimovici 2000), and *Martialia hyadesi* off the coast of Argentina and in the Scotia Sea in the vicinity of the PFZ (Ivanovic et al. 1998; Rodhouse et al. 1992).

A number of studies conducted over the past decade have made valuable contributions to existing knowledge of the diets of squid distributed within the Southern Ocean (Ivanovic & Brunetti 1994; Jackson et al. 1998a; Kear 1992; Lu & Williams 1994b; Mouat et al. 2001; Nemoto et al. 1988; Rodhouse et al. 1992). However, most of these studies have been located in the south-west region of the Atlantic sector. Few reports describe spatial variations in the diet of a squid species throughout different regions of the Southern Ocean. Many of the species found in the Southern Ocean have a circumpolar distribution, are found throughout a vast expanse of habitat, and are likely to feed on patchily distributed prey and thus exhibit spatial variations in diet.

We have investigated the diet of the onychoteuthid squid *Moroteuthis ingens* from four sites in the Southern Ocean: the Falkland Islands in the south-west Atlantic, and Macquarie Island, the Chatham Rise and the Campbell Plateau in the south-west Pacific. The latter two sites are both located within the Exclusive Economic Zone (EEZ) of New Zealand. Squid distributed between the APF and the STF feed largely on myctophid fish (Kock 1987; Lubimova 1985; Rodhouse & White 1995) and *M. ingens* is no exception, consuming a number of species in southern New Zealand waters and at Macquarie Island (Jackson et al. 1998a; Phillips et al. 2001). Comparisons of published data reveal that,

while myctophids provide the bulk of the diet in both regions, there is little overlap in prey species between Macquarie Island and New Zealand. To date, no published data are available on the composition of the diet of *M. ingens* at the Falkland Islands.

In this study, fatty acid dietary tracers are extended to complement stomach content analysis of spatial variation in the diet of *M. ingens*. Squid are thought to undergo an ontogenetic shift in diet with size, from a crustacean-based diet as juveniles to a fish-based diet as adults (Rodhouse & Nigmatullin 1996). To ensure that spatial variations in diet were not correlated with ontogenetic differences in squid samples between sites, only large and muscular individuals of *M. ingens* (female stage II and III, and male stage IV and V) were examined as part of this study.

5.3 MATERIALS AND METHODS

5.3.1 Squid collection

Individuals of *M. ingens* were collected from three geographically distant locations in the sub-Antarctic Southern Ocean. One hundred and three squid were collected by Australian Fisheries Management Authority (AFMA) observers on board the commercial trawler *Austral Leader* in the vicinity of Macquarie Island over three seasons spanning the periods 5 January – 10 February 1995, 5 January – 4 February 2000 and 21 June – 28 June 2000 (Fig. 5.1a). Macquarie Island squid were collected between 53.73°S-55.12°S and 158.52°E-159.06°E at depths between 508-988 m. Ninety-one squid were trawled from two sites in the vicinity of New Zealand by the Research Vessel *Tangaroa*. Nineteen squid were collected from the Chatham Rise (44.05°S-44.35°S, 177.45°W-178.27°E) between 27 October 2000 – 4 November 2000 at depths between 941-1026 m, and seventy-two squid were collected between 27 November 2000 – 8 December 2000 from a site on the north-west Campbell Plateau (48.46°S-51.36°S, 167.30°E-174.50°E) between depths of 530-723 m (Fig. 5.1b). During the period 7 October 2001 – 31 October 2001, thirty-one squid were collected from the continental shelf and slope

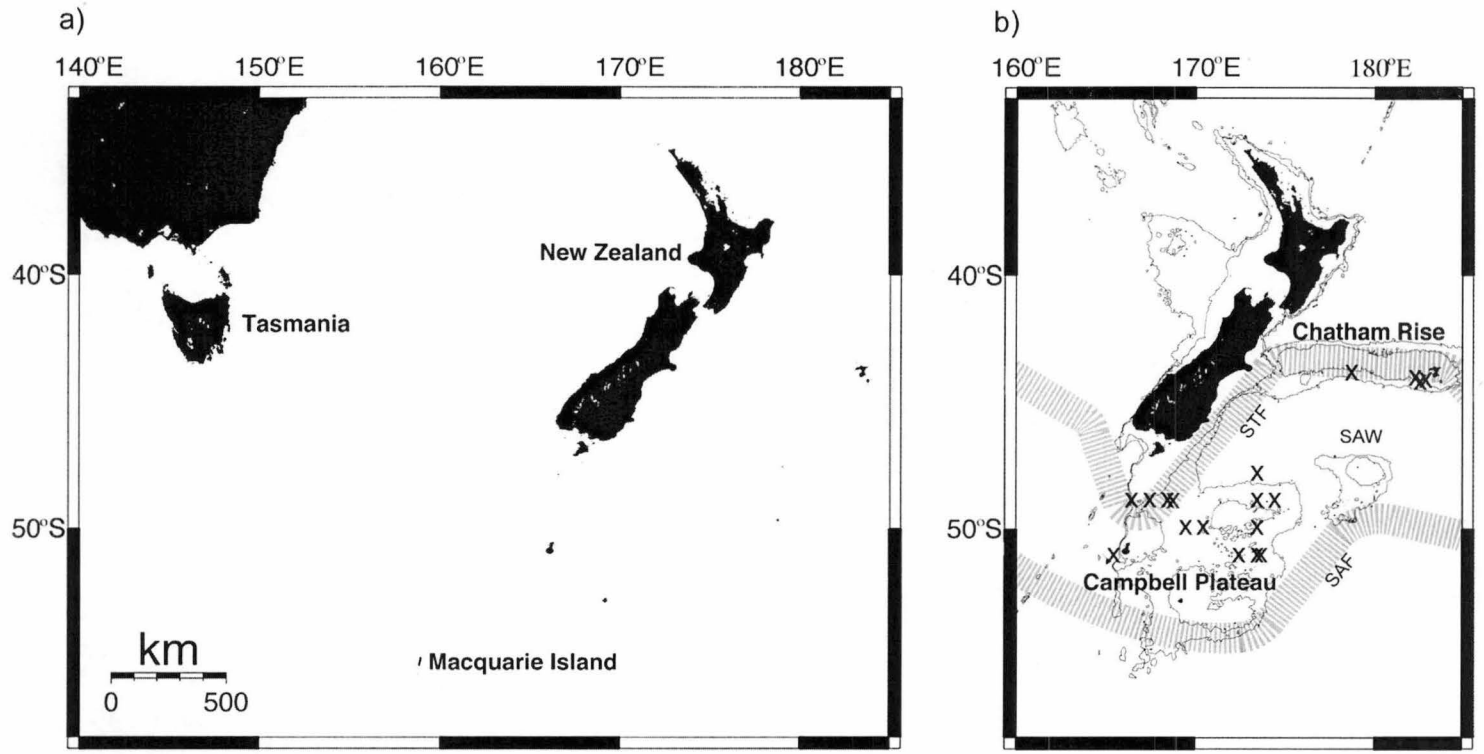

Fig. 5.1. a) The location of Macquarie Island in relation to Tasmania (Australia) and New Zealand. b) The Chatham Rise and Campbell Plateau, depicted by 500m and 1000m bathymetry contours. Collection sites of *Moroteuthis ingens* are represented by **x**. Oceanic fronts have been adopted from Heath (1981). STF, Subtropical Front; SAW, sub-Antarctic Water; SAF, sub-Antarctic Front.

of the Falkland Islands (48.30°S –53.24°S, 57.13°W-61.79°W) by the Fisheries Patrol Vessel *Dorada*, between depths of 137-472 m and additionally at 945 m. Squid collected from the Falkland Islands were dissected *in situ* and samples were frozen at –20° C and returned to Hobart for analysis. All other squid were frozen at –20° C after collection and returned to Hobart for dissection and analysis. Upon dissection, squid were assigned a maturity stage after Lipinski (1979).

5.3.2 Stomach contents analyses

Stomach contents analyses and identification of prey remains were largely conducted using the protocols described in Section 2.3, Chapter 2. Some *Gymnoscopelus* otoliths from the stomachs of Macquarie Island and New Zealand squid were eroded, and were thus allocated to the species pairs *G. nicholsi/piabilis* or *G. fraseri/bolini* as described in Section 4.3.2, Chapter 4.

After identifying prey remains to fish, cephalopod or crustacean level, the prey category that comprised the majority of the contents for an individual stomach was recorded as the "primary prey item" for that stomach. When large amounts of fish remains were found (such as scales, bones and flesh), but without (or with very few) eye lenses or otoliths, it was assumed that fish heads may have been discarded and the incidence was recorded. Larval nematode parasites of the genus *Anisakis* were removed from the stomach wall and contents, and the number of infestations per stomach recorded.

5.3.3 Lipid extraction and fatty acid analysis

Lipid was extracted from thirty Macquarie Island squid (nineteen female, eleven male), seven Chatham Rise squid (three female, four male), eight Campbell Plateau squid (four female, four male) and ten Falkland Islands squid (seven female, three male). All female squid selected for fatty acid analysis were immature (stage III), whereas all male squid were fully mature (stage V). Lipid extraction and fatty acid analyses were conducted using the protocols described in Section 2.4, Chapter 2.

5.3.4 Statistical analyses

The abundance of a prey species in the stomach contents of *M. ingens* was described by percent frequency occurrence (% FO) and percent numerical importance (% NI). The % FO was determined from the number of stomachs from site *k* containing prey species *h* as a percentage of the total number of stomachs examined from site *k*. The % NI was determined as the number of prey species *h* consumed at site *k* as a percentage of the total number of prey items consumed at site *k*.

General biology data were compared by ANCOVA, and total lipid and lipid class data were compared by ANOVA and Tukey's post-hoc test. Fatty acid data were compared using the multivariate techniques of complete linear canonical discriminant analysis (DA) and linear canonical DA with forward-stepwise entry of variables. Fatty acids that contributed a mean of less than 0.5% (of total fatty acids) at one or more sites were excluded from statistical analyses; thus for digestive gland tissue, analyses were performed using 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n11, 22:1n9, 24:1n11/9, 18:2n6, 20:4n6, 20:5n3, 20:4n3, 22:6n3 and 22:5n3; for mantle tissue 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n9, 20:4n6, 20:5n3, 22:6n3 and c23 PUFA. All fatty acid data were converted to mg g^{-1} values prior to discriminant analyses. All statistical analyses were conducted using SPSS 10.0 for Macintosh (SPSS Inc, Chicago, Illanois).

Note: Stomach content and fatty acid data from specimens collected from Macquarie Island during the 1998 – 1999 summer have not been included in this spatial comparison: a) to reduce the size of the Macquarie Island sample set, which is currently much larger than sample sets from other sites, and b) because temporal comparisons drawn from Chapter 4 indicated that the diet of *M. ingens* during the 1999 summer was anomalous from the diet at other times.

5.4 RESULTS

5.4.1 General Biology

All specimens of *M. ingens* included in this study were large individuals that, with the exception of three squid from Macquarie Island, exceeded 200 mm dorsal ML (Fig. 5.2). Immature females were on average larger than mature males, as previously documented for this sexually dimorphic species (Jackson & Mladenov 1994; Lipinski & Linkowski 1986). Regression of the log of TM (g) against the covariate ML showed that there were no significant differences in TM between sites (ANCOVA: df = 3, F = 0.12, $p > 0.95$). However, the mass of the digestive gland varied significantly between sites. The natural log of digestive gland mass was regressed against the covariate ML, and ANCOVA indicated that digestive gland mass was dependent on collection site (df = 3, F = 25.35, $p < 0.001$). The digestive glands of squid collected from the Campbell Plateau were generally smaller than those from other sites, particularly Macquarie Island (Fig. 5.2).

5.4.2 Stomach contents

At least 10% of all stomachs collected from each site were empty. At the Chatham Rise, approximately one third (32%) of stomachs examined contained no contents (1). Only three categories of prey were identified in the stomach contents collected at each site: fish prey, cephalopod prey and crustacean prey. Fish comprised the greatest proportion of the diet of *M. ingens* at all sites. Fish remains occurred in over 60% of all stomachs, and represented the primary prey group in more than 50% of stomachs collected from all sites (Table 5.1). While cephalopod remains occurred in at least 15% and up to 55% of stomachs collected, the importance of cephalopods as a primary prey group varied markedly between the Falkland Islands and other sites. Cephalopods did not generally form the bulk of the prey consumed by *M. ingens* at most sites, (% FO as primary prey ≤6%) (Table 5.1); instead, they were usually consumed in conjunction with larger amounts of fish. Importantly, however, cephalopods occurred as the primary prey item in over 20% of stomachs at the Falkland Islands and thus represent a much larger proportion of the diet at this site.

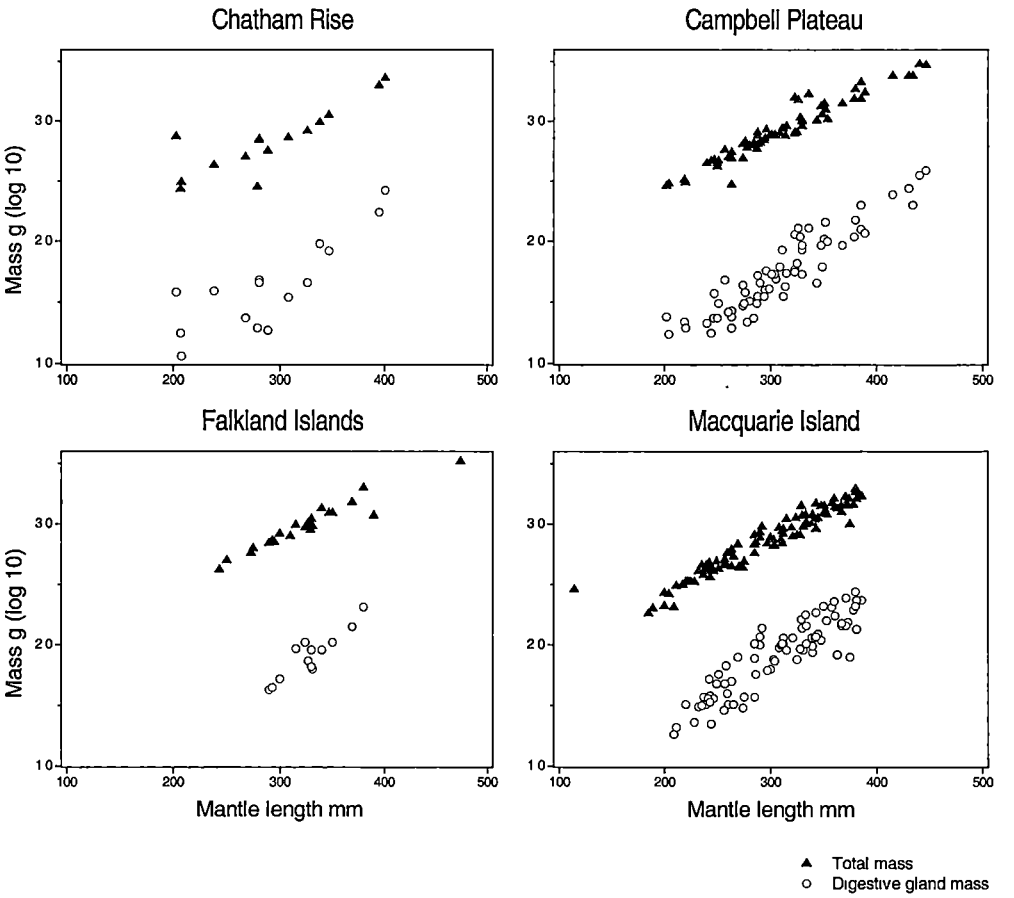

Fig. 5.2. Total mass and digestive gland mass versus mantle length for *Moroteuthis ingens*.

Table 5.1. % FO of major prey categories in the diet of *Moroteuthis ingens* from four sites in the Southern Ocean. %FO of empty stomachs, infestation of *Anisakis* spp. and other data are also shown.

	Chatham Rise (NZ)	Campbell Plateau (NZ)	New Zealand pooled sites	Falkland Islands	Macquarie Island
n	19	72	91	31	103
%FO empty stomachs	32	13	16	10	21
%FO of prey categories					
Fish prey	63	86	81	71	75
Cephalopod prey	16	21	20	55	34
Crustacean prey	11	15	14	3	11
%FO as primary prey					
Fish prey	58	81	76	65	69
Cephalopod prey	5	3	3	23	6
Crustacean prey	5	4	4	3	0
%FO fish heads discarded	0	10	8	0	13
***Anisakis* spp. infestation**					
mean number/stomach	7	9	9	1	6
% FO	95	96	96	39	90

n: sample size; %FO: % frequency occurrence; NZ: New Zealand

Few whole crustaceans were recovered, and it was not possible to identify remains other than attributing them to either zooplankton or prawn-like natant decapods. Crustaceans comprised the smallest proportion of the diet of *M. ingens* at all sites except the Campbell Plateau; here they occurred in 3-15% of all stomachs and represented the primary prey group in 5% of stomachs or less (Table 5.1).

The loading of the parasite *Anisakis* spp. in the stomach wall and contents of *M. ingens* in terms of mean number per stomach and % FO was also investigated (Table 5.1). Using both indices, it appears that far fewer stomachs are infested with *Anisakis* spp. at the Falkland Islands compared to other sites. The parasite loadings together with the finding that cephalopods comprise a far greater proportion of the diet of *M. ingens* at the Falkland Islands indicates that *M. ingens* is a predator of markedly different trophic pathways between the Pacific and Atlantic Sectors of the Southern Ocean.

In total, at least 45 prey species were consumed by *M. ingens* across the study areas, confirming the generalist nature of this predator (Table 5.2). The number of prey items identified at a site was strongly dependent on the number of stomachs examined (Fig.

5.3). For example, only 6 prey items were identified in stomachs collected from the Chatham Rise, compared to 19 identified from the Campbell Plateau samples, implying that the diet of *M. ingens* at the Campbell Plateau is more diverse than at the Chatham Rise despite the relative proximity of these sites. However, this is likely to be a direct effect of unequal sample size (Campbell Plateau: $n = 72$; Chatham Rise: $n = 19$). Therefore, stomach contents could not be used to assess dietary differences between the Chatham Rise and Campbell Plateau, and data from these sites have also been combined and presented as pooled New Zealand data.

Despite the large number of prey items identified overall, only 2-3 species at any one site occurred in more than 10% of stomachs and comprised more than 10% NI (Table 5.2), suggesting that *M. ingens* may prey on certain core species at a given location and supplements the diet on an opportunistic basis. Such species are referred to as dominant prey species (ie., those that exceed the arbitrary criteria of 10% FO and 10% NI at a site). The diet was composed of three dominant prey species at the Chatham Rise (*Electrona carlsbergi*, *Lampanyctodes hectoris* and *Protomyctophum normani*), compared to two dominant prey species at the Campbell Plateau (*E. carlsbergi* and *L. hectoris*) and Macquarie Island (*E. carlsbergi* and *Krefftichthys anderssoni*), and three species at the Falkland Islands including two cephalopod species (*Gymnoscopelus nicholsi/piabilis*, *Loligo gahi* and *M. ingens*).

The most prolific of the prey groups present in the diet of *M. ingens* across the study area were myctophid fish; in total, more than 16 types of myctophid otoliths were identified. At least one myctophid was a dominant prey species of *M. ingens* at each site (see above). With the exception of the macrourids, no other fish families were represented by more than one species, and many non-myctophid species were represented by only one individual. Similarly, most of the cephalopod prey species were represented by very few individuals. Exceptions to this were *L. gahi* and *M. ingens* included in the diet at the Falkland Islands, where they were dominant prey species, and *Stoloteuthis* spp. at Macquarie Island and the Campbell Plateau. *Stoloteuthis* spp. or other sepiolid species were consumed at every site except the Chatham Rise; similarly, cannibalism on *M. ingens* occurred at each site except the Chatham Rise.

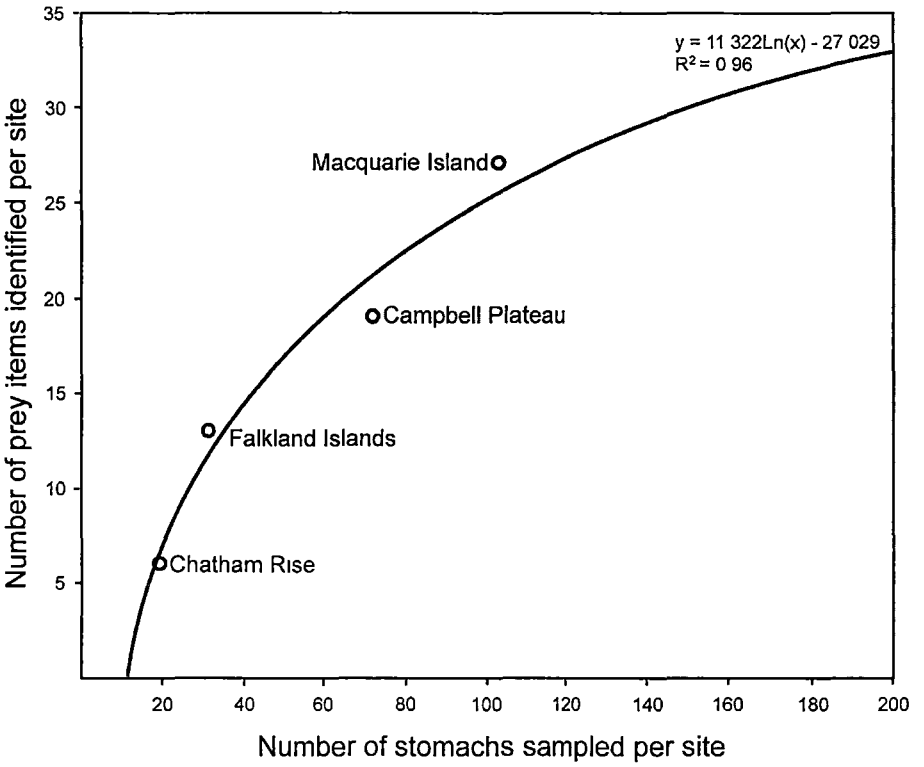

Fig. 5.3. The number of prey items identified per site versus the number of stomachs of *Moroteuthis ingens* sampled per site. A logarithmic line has been fitted to the data.

Table 5.2. Prey species identified in the diet of *Moroteuthis ingens* from four sites in the Southern Ocean

a) Myctophid prey	New Zealand Chatham Rise			New Zealand Campbell Plateau			New Zealand pooled sites			Falkland Islands			Macquarie Island		
	n =19			*n* =72			*n* =91			*n* =31			*n* =103		
PREY SPECIES	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO
Family Myctophidae															
Electrona antarctica				2	0	3	2	0	2				7	2	3
Electrona carlsbergi	32	52	21	182	39	40	214	41	36				113	24	16
Electrona subaspera													22	5	12
Electrona spp.													2	0	2
Gymnoscopelus braueri										1	1	3	35	8	4
Gymnoscopelus fraseri/bolini													12	3	6
Gymnoscopelus microlampus				1	0	1	1	0	1						
Gymnoscopelus nicholsi/piabilis				24	5	6	24	5	4	29	42	35	25	5	9
Gymnoscopelus opisthopterus													2	0	1
Gymnoscopelus spp.				6	1	7	6	1	5				10	2	6
Hintonia candens				1	0	1	1	0	1						
Krefftichthys anderssoni				3	1	3	3	1	2				88	19	13
Lampanyctodes hectoris	11	18	16	165	36	17	176	34	16						
Lampanyctus achiris										1	1	3	25	5	7
Metelectrona ventralis	2	3	5				2	0	1						
Protomyctophum bolini				6	1	4	6	1	3				1	0	1
Protomyctophum normani	9	15	11	21	5	13	30	6	12				1	0	1
Protomyctophum spp.				12	3	13	12	2	10				15	3	7
Symbolophorus (cf *barnardi*)				8	2	4	8	2	3						
unidentified myctophid				1	0	1	1	0	1						

n: sample size; N: total number; %NI: % numerical importance; %FO: % frequency occurrence

Table 5.2. continued

b) Other fish prey	New Zealand Chatham Rise *n* =19			New Zealand Campbell Plateau *n* =72			New Zealand pooled sites *n* =91			Falkland Islands *n* =31			Macquarie Island *n* =103		
PREY SPECIES	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO
Family Bathylagidae															
Bathylagus antarcticus													32	7	14
Family Searsiidae															
Persparsia kopua													1	0	1
Family Gonostomatidae															
Photichthys argenteus	4	6	11	6	1	6	10	2	7	1	1	3			
Family Paralepididae															
Magnisudis prionosa										2	3	3			
Family Moridae															
Physiculus spp.				1	0	1	1	0	1						
Family Macrouridae															
Coelorhynchus acanthiger	1	2	5				1	0	1						
Coelorhynchus spp.				1	0	1	1	0	1	1	1	3			
Macrouris carinatus ?				1	0	1	1	0	1				1	0	1
Famlily Melamphaidae															
Poromitra crassiceps													1	0	1
Family Gobiidae															
cf *Gobionotothen angustifrons*										1	1	3			
Family Channichthyidae															
unidentified Channichthyid													7	2	2
Family Gempylidae															
Paradiplospinus gracilis													11	2	5
Unidentified Fish	2	3	5	3	1	4	5	1	4	1	1	3	14	3	7

n: sample size; N: total number; %NI: % numerical importance; %FO: % frequency occurrence

Table 5.2. continued

c) Cephalopod and crustacean prey	New Zealand Chatham Rise *n* =19			New Zealand Campbell Plateau *n* =72			New Zealand pooled sites *n* =91			Falkland Islands *n* =31			Macquarie Island *n* =103		
PREY SPECIES	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO	N	%NI	%FO
Cephalopods															
Sepiolidae										2	3	6	3	1	2
Stoloteuthis spp.				8	2	7	8	2	5				26	6	16
Loligo gahi										15	22	23			
Illex argentinus										1	1	3			
Ommastrephid spp.				1	0	1	1	0	1						
Cranchiid spp.													1	0	1
Gonatus antarcticus										3	4	6			
Mastigoteuthis spp.													1	0	1
Megalocranchia spp.													1	0	1
Moroteuthis ingens				2	0	3	2	0	2	10	14	16	2	0	2
Octopoteuthis spp.													1	0	1
Unidentified octopus										1	1	3			
Unidentified cephalopods	1	2	5	2	0	3	3	1	3				5	1	5
Crustaceans															
zooplankton			0			7			5			3			6
natant decapods			11			1			3			0			5

n: sample size; N: total number; %NI: % numerical importance; %FO: % frequency occurrence

When Chatham Rise and Campbell Plateau data were combined as pooled New Zealand data, two prey were present in the diet across the three geographic regions - the species pair *G. nicholsi/piabilis* and conspecifics of *M. ingens* resulting from cannibalism. Four prey species were consumed by *M. ingens* at both the Falkland Islands and Macquarie Island. Similarly, four species were consumed by *M. ingens* at both the Falkland Islands and the pooled New Zealand sites. In contrast, ten species were included in the diet of *M. ingens* at both Macquarie Island and the combined New Zealand sites. That is, comparisons of prey species provides further evidence that the diet of *M. ingens* is markedly different at the Atlantic site compared to the sites located in the Pacific sector of the Southern Ocean.

5.4.3 Lipid and fatty acid profiles

5.4.3.1 Digestive gland

A large amount of within-site and between-site variability in total lipid content of the digestive gland was observed, with the lowest lipid content recorded at the Campbell Plateau (13.9 ± 5.8% wet mass) and the highest recorded at the Chatham Rise (48.0 ± 22.7% wet mass) (Table 5.3a). The lipid content of the digestive gland varied significantly over the study area ($df = 52$, $F = 14.25$, $p < 0.001$). Post-hoc tests show that no significant differences were observed between the Chatham Rise, Falkland Islands or Macquarie Island, whereas highly significant differences were observed between the Campbell Plateau and all other sites.

The lipid class composition of the digestive gland also varied largely within-sites and between-sites. In general, digestive glands from the Chatham Rise, Falkland Islands and Macquarie Island all contained large amounts of TAG (>74% of total lipid). In contrast, FFA was the most abundant lipid class in digestive glands from the Campbell Plateau (39.3 ± 16.9% of total lipids), followed by ST (26.4 ± 15.1% of total lipids) then TAG (19.6 ± 26.0% of total lipids) (Table 5.3a). Levels of wax-ester (WE), TAG, FFA and ST were significantly different between sites ($df = 28$, $F = 12.35$, $p < 0.001$; $df = 27$, $F = 19.88$, $p < 0.001$; $df = 32$, $F = 4.13$, $p < 0.05$; $df = 32$, $F = 16.20$, $p < 0.001$, respectively).

Post-hoc tests show that while no significant differences in WE, TAG or ST levels were found between the Chatham Rise, Falkland Islands or Macquarie Island, levels of these respective lipid classes were highly significant between the Campbell Plateau and all other sites. Levels of FFA were significantly different between the Campbell Plateau and the Falkland Islands, and between the Campbell Plateau and Macquarie Island, but not between any other site-pairs.

The most abundant fatty acids in digestive gland tissue (>10% of total fatty acids at all sites) were 16:0, 18:1n9 and DHA (22:6n3). Considerable variation in values for individual fatty acids was observed between sites. Generally, the fatty acid composition of the digestive gland tissue was characterised by large amounts of MUFA (>45% of total fatty acids), whereas SAT and PUFA were present in relatively equal amounts (20.7 ± 1.2% - 25.6 ± 3.7% and 21.6 ± 3.7% - 26.8 ± 7.0% of total fatty acids, respectively) (Table 5.4).

Digestive glands collected from the Campbell Plateau contained unusually large amounts of PUFA (36.4 ± 6.8% of total fatty acids) and correspondingly reduced levels of MUFA (39.0 ± 6.9% of total fatty acids). Elevated PUFA values in Campbell Plateau digestive glands were largely due to increased levels of DHA (21.2 ± 4.9% of total fatty acids). The DHA levels from Campbell Plateau samples were more than double the amount recorded in Macquarie Island digestive glands (10.2 ± 2.3% of total fatty acids). Levels of EPA (20:5n3) were also highest in digestive glands from the Campbell Plateau (8.8 ± 2.0% of total fatty acids compared with 5.6 – 7.1% of total fatty acids at other sites). Digestive glands sampled from the Falkland Islands recorded the highest levels of all MUFA of chain-length 20-24 carbons, whereas Chatham Rise digestive glands contained high levels of 18:1n9.

Table 5.3. Total lipid content and lipid class composition of a) digestive gland and b) mantle tissue of *Moroteuthis ingens* from four sites in the Southern Ocean. Total lipid data are given as % wet mass and lipid class data are given as % of total lipids. Values are means ± SD

a) Digestive gland	Chatham Rise	Campbell Plateau	Falkland Islands	Macquarie Island
n	7	8	10	32
total lipid	48.0 ± 22.7	13.9 ± 5.8	39.8 ± 5.9	45.4 ± 12.3
n	7	8	10	15
WE	4.6 ± 7.3	12.0 ± 5.1	1.6 ± 1.2	0.8 ± 0.5
DAGE	1.6 ± 1.4	0.9 ± 0.0	0.9 ± 0.7	1.0 ± 0.7
TAG	73.9 ± 29.7	19.6 ± 26.0	90.0 ± 5.0	82.5 ± 9.1
FFA	28.6 ± 29.0	39.3 ± 16.9	12.0 ± 22.7	8.8 ± 3.0
ST	6.0 ± 7.6	26.4 ± 15.1	2.2 ± 3.6	1.6 ± 0.8
PL	8.9 ± 9.7	9.9 ± 5.2	2.5 ± 3.3	6.0 ± 7.5

b) Mantle	Chatham Rise	Campbell Plateau	Falkland Islands	Macquarie Island
n	7	8	10	32
total lipid	1.0 ± 0.4	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.3
n	7	8	10	15
FFA	1.7 ± 0.5	1.8 ± 0.4	1.3 ± 0.3	5.0 ± 3.5
ST	4.8 ± 0.9	3.9 ± 0.5	3.6 ± 0.4	12.1 ± 3.9
PL	93.5 ± 1.1	94.3 ± 0.7	95.1 ± 0.5	82.9 ± 7.1

n: sample size; WE: wax ester; DAGE: diacyglyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid.

Table 5.4. Fatty acid composition (as % of total fatty acids) of the digestive gland of *Moroteuthis ingens* from four sites in the Southern Ocean. Only those fatty acids with a mean value exceeding 0.5% at all sites are shown. Values are means ± SD

Fatty acid	Chatham Rise	Campbell Plateau	Falkland Islands	Macquarie Island
n	7	8	10	30
14:0	2.7 ± 0.6	1.6 ± 0.6	2.7 ± 0.5	3.2 ± 0.5
16:0	17.6 ± 2.6	15.6 ± 1.0	13.9 ± 1.0	15.6 ± 1.8
18:0	4.3 ± 0.7	5.3 ± 1.0	3.4 ± 0.2	3.4 ± 0.6
16:1n7	3.3 ± 1.7	1.9 ± 1.0	2.9 ± 0.4	4.4 ± 0.9
18:1n9	21.0 ± 5.0	15.0 ± 4.5	18.6 ± 2.1	22.2 ± 2.5
18:1n7	3.6 ± 0.4	2.3 ± 0.7	3.5 ± 0.4	3.9 ± 1.0
20:1n9	9.5 ± 3.6	7.9 ± 1.8	12.7 ± 1.9	9.9 ± 2.4
22:1n11	3.3 ± 0.4	3.8 ± 1.3	7.3 ± 1.3	5.3 ± 1.4
22:1n9	1.4 ± 0.4	2.2 ± 0.5	2.7 ± 0.4	2.7 ± 0.7
24:1	1.9 ± 0.5	4.0 ± 1.3	4.4 ± 0.9	3.0 ± 1.3
18:2n6	0.7 ± 0.4	0.6 ± 0.2	0.9 ± 0.2	1.0 ± 0.2
20:4n6 (AA)	1.1 ± 0.5	1.4 ± 0.5	0.8 ± 0.1	0.7 ± 0.1
20:5n3 (EPA)	7.1 ± 3.0	8.8 ± 2.0	6.0 ± 1.1	5.6 ± 1.1
20:4n3	1.0 ± 0.3	0.7 ± 0.2	1.0 ± 0.2	0.7 ± 0.2
22:6n3 (DHA)	12.4 ± 3.9	21.2 ± 4.9	11.5 ± 1.6	10.2 ± 2.3
22:5n3 (DPA)	1.8 ± 0.8	0.9 ± 0.2	1.1 ± 0.1	0.9 ± 0.2
Sum SAT	**25.6 ± 3.7**	**23.4 ± 1.8**	**20.7 ± 1.2**	**23.2 ± 2.2**
Sum MUFA	**46.2 ± 7.4**	**39.0 ± 6.9**	**54.9 ± 4.1**	**54.1 ± 4.0**
Sum PUFA	**26.8 ± 7.0**	**36.4 ± 6.8**	**23.6 ± 3.2**	**21.6 ± 3.7**

n : sample size; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

5.4.3.2 Mantle

The lipid content of mantle tissue was ≤ 1.5% wet mass (Table 5.3b), and varied significantly between sites (df = 54, F = 6.90, $p < 0.005$). The lipid content of mantle tissue from the Chatham Rise was significantly different to mantle tissue from the Falkland Islands and Macquarie Island; no other significant differences were observed. Mantle lipid was composed of very large amounts of PL, comprising 82.9 ± 7.1% of total lipids at Macquarie Island, and over 93% of total lipids at all other sites (Table 5.3b). ST contributed 12.1 ± 3.9% to total lipids at Macquarie Islands, and elsewhere comprised less than 5% of total lipids. Levels of FFA, ST and PL in mantle tissue varied significantly between sites (df = 39, F = 7.54, $p < 0.001$; df = 39, F = 33.42, $p < 0.001$; df = 39, F = 21.13, $p < 0.001$). Significant differences existed for each lipid class between Macquarie Island other sites, but no other significant differences were observed.

Lipid from the mantle tissue was characterised by large amounts of PUFA, exceeding 55% of total fatty acids at all sites (Table 5.5). SAT comprised approximately 29% of total fatty acids, whereas MUFA contributed less than 15% to total fatty acids at each site. The most abundant fatty acids were 16:0, EPA and DHA. Unlike digestive gland fatty acid profiles, fatty acid content of mantle tissue was similar between sites with no large differences for individual fatty acids.

5.4.4 Between-sites comparison of fatty acid profiles

5.4.4.1 Fatty acid profiles of the digestive gland

Complete linear DA indicated that the digestive gland fatty acid profiles of *M. ingens* varied between the sample sites (Wilks' lambda = 0.016, Approx. F = 6.875, $p < 0.001$). Seven fatty acid variables were incorporated into forward-stepwise DA of the same data in order to distinguish between sites: 16:0, 22:1n11, 22:1n9, 24:1, 20:4n6 (arachidonic acid: AA), EPA and docosapentaenoic acid (DPA). Forward-stepwise DA assigned 94.5% of individuals to the correct site (89.1% cross-validated) (Wilks' lambda = 0.035, Approx. F = 13.630, $p = 0.001$) (Table 5.6a). Box's M test for homogeneity of variance showed strong evidence ($p = 0.001$) that the site groups did not have a common

covariance structure, suggesting that a quadratic discriminant rule would have greater discriminating power. However, with 7 to 30 observations per group, there were an insufficient number of cases to justify quadratic discriminant analysis. The misclassification rate from the linear rule was only 5.5% (10.9% cross-validated), and the linear discriminant rule was considered acceptable.

Table 5.5. Fatty acid composition (as % of total fatty acids) of the mantle tissue of *Moroteuthis ingens* from four sites in the Southern Ocean. Only those fatty acids with a mean value exceeding 0.5% at all sites are shown. Values are means ± SD

Fatty acid	Chatham Rise	Campbell Plateau	Falkland Islands	Macquarie Island
n	7	8	10	30
14:0	1.4 ± 0.2	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
16:0	23.5 ± 1 3	24.8 ± 0.5	24.5 ± 1.3	24.7 ± 1.6
18:0	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
16:1n7	0.6 ± 0.3	0.6 ± 0 1	0.6 ± 0.1	0.8 ± 0.2
18:1n9	3.1 ± 0.2	3.1 ± 0.2	2.6 ± 0.2	3.3 ± 0.5
18:1n7	1.5 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.7 ± 0.4
20:1n9	5.6 ± 0.4	5.2 ± 0.3	5.8 ± 0.4	5.5 ± 0.5
22:1n9	1.7 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.2
20:4n6 (AA)	2.4 ± 0.4	2.0 ± 0 2	1.9 ± 0.2	1.9 ± 0.2
20:5n3 (EPA)	13.6 ± 0.4	13.5 ± 0.3	13.9 ± 0.5	13.3 ± 0.5
20:4n3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
22:6n3 (DHA)	37.7 ± 1.4	38.9 ± 0.9	38.8 ± 1.7	37.5 ± 1.9
22:5n3 (DPA)	0.7 ± 0.1	0.5 ± 0.0	0 5 ± 0.1	0.5 ± 0 1
C23 PUFA	1.3 ± 0.4	1.1 ± 0.1	0.7 ± 0.3	0.6 ± 0.3
Sum SAT	**28.4 ± 1.4**	**29.6 ± 0.5**	**29.1 ± 1.4**	**29.7 ± 1.8**
Sum MUFA	**14.3 ± 0.8**	**13.3 ± 0.4**	**13.9 ± 0.5**	**14.9 ± 1.1**
Sum PUFA	**57.0 ± 1.2**	**56.9 ± 0.8**	**56.8 ± 1.5**	**55.2 ± 2.3**

n: sample size; AA: arachidonic acid; EPA· eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

The first discriminant function accounted for 53.8% of variability in the digestive gland fatty acid data, and can be interpreted as a contrast between proportions of 22:1n9, EPA and DPA (Table 5.6a). Squid from both the Chatham Rise and Campbell Plateau were characterised by low levels of 22:1n9, which may have separated the New Zealand sites from the Falkland Islands and Macquarie Island along Function 1 (Table 5.4; Fig. 5.4). Relatively lower levels of 22:1n9 and EPA, and relatively greater levels of DPA, were important to characterise Chatham Rise squid independent from Campbell Plateau squid along Function 1 (Table 5.4; Fig. 5.4).

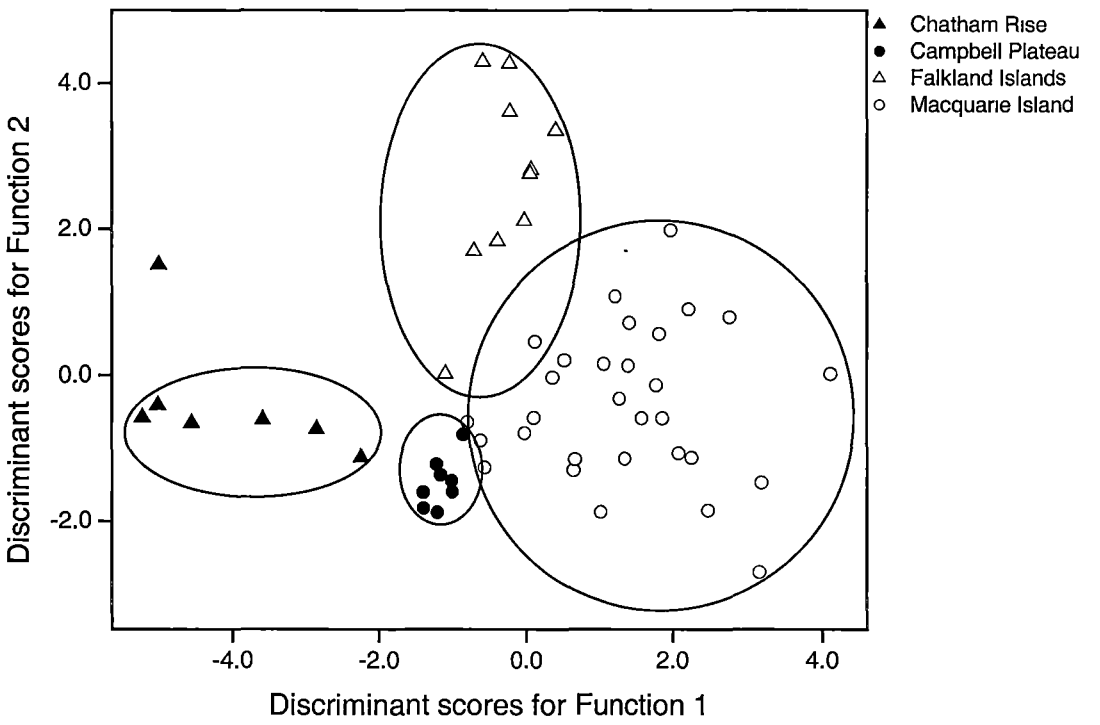

Fig. 5.4. Discriminant scores of fatty acid data from the digestive gland of *Moroteuthis ingens*.

Table 5.6. Results of forward-stepwise discriminant analysis: site allocation for a) digestive gland fatty acid data and b) mantle tissue fatty acid data

	a) Digestive gland						Standardized discriminant		
	Predicted Site Membership					Fatty acid used	function coefficients		
Assigned Site	CR	CP	FI	MI	*n*	in separation	Function 1	Function 2	Function 3
CR	6	1			7	16:0	-0.013	-0.395	0.924
CP		8			8	22:1n11	-0.053	1.923	-0.516
FI		1	9		10	22:1n9	1.583	-1.483	0.609
MI		1		29	30	24:1	-0.57	0.232	-0.396
						20:4n6 (AA)	-0.623	0.198	0.595
						20:5n3 (EPA)	1.536	0.03	-0.932
94.5% of cases correctly predicted to belong to assigned si						22:5n3 (DPA)	-1.703	0.222	0.448

	b) Mantle tissue						Standardized discriminant	
	Predicted Site Membership					Fatty acid used	function coefficients	
Assigned Site	CR	CP	FI	MI	*n*	in separation	Function 1	Function 2
CR	5		2		7	14:0	2.567	-1.826
CP		5	3		8	20:4n6 (AA)	-1.855	2.546
FI	2	3	5		10			
MI	2	5	10	13	30			
50.9% of cases correctly predicted to belong to assigned site								

n : total number; CR: Chatham Rise; CP: Campbell Plateau; FI: Falkland Islands; MI: Macquarie Island.
AA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid.

The second discriminant function accounted for 28.3% of variance in the DA, and may be interpreted as a contrast between 22:1n11 (Table 5.6a). Squid with relatively large amounts of 22:1n11 in the digestive gland were predicted to belong to the Falkland Islands group, whereas squid with moderate levels of 22:1n11 were predicted to belong to the Macquarie Island group (Table 5.4; Fig. 5.4).

The third discriminant function accounted for the remaining 17.9% of variance. Proportions of 22:1n9, 24:1 and EPA were used to distinguish between Macquarie Island squid and those collected from the Campbell Plateau. Levels of 24:1 and EPA were relatively larger in squid collected from the Campbell Plateau (Table 5.4), and these variables were given a negative coefficient in the third discriminant function (Table 5.6a). In contrast, levels of 22:1n9 were relatively greater in squid from Macquarie Island, and this variable was assigned a positive coefficient in the third discriminant function (Table 5.6a).

5.4.4.2 Fatty acid profiles of the mantle

Complete linear DA indicated that the fatty acid composition of mantle tissue was also dependent on site (Wilks' lambda = 0.159, Approx F. = 2.858, $p < 0.001$). Only 50.9% of individuals were assigned to the correct site by forward-stepwise DA (40% cross-validated) (Wilks' lambda = 0.694, Approx. F = 3.337, $p < 0.005$) (Table 5.6b). Two fatty acid variables were incorporated into forward-stepwise DA of mantle tissue: 14:0 and AA (Table 5.6b). However, it is not clear how proportions of these fatty acid variables were used to distinguish between the sites.

5.5 DISCUSSION

Stomach contents and digestive gland fatty acid profiles varied markedly between the areas studied, demonstrating that *M. ingens* is able to successfully exploit a different spectrum of prey across its distribution. Stomach contents provide clear evidence for dietary differences over large spatial scales: ie. between the Falkland Islands and Pacific Ocean sites, and to a lesser extent between Macquarie Island and New Zealand sites.

However, it was not possible to assess dietary differences between the Chatham Rise and Campbell Plateau using stomach contents, at least in part due to the small number of samples collected from the Chatham Rise. Lipid and fatty acid profiles indicate that the diet of *M. ingens* varies over smaller spatial scales, particularly between the Chatham Rise and the Campbell Plateau. Stomach contents and fatty acid data are discussed separately.

5.5.1 Stomach contents – spatial variation in diet

At the four sites included in this study, *M. ingens* fed largely on 2-3 dominant prey species and supplemented the diet with a range of other prey. At Macquarie Island and New Zealand sites, dominant prey species and supplementary prey species are often myctophid fish. Dominant prey species are likely to be the most abundant species in a given area that *M. ingens* is able to consume. Cephalopods in general are able to "opportunistically exploit the most abundant and available prey" (Boyle & Boletzky 1996); for example, *Illex argentinus* has also been shown to consume those prey types with which it has the greatest encounter rate (Mouat et al. 2001).

At both New Zealand sites, dominant prey items are the myctophids *Lampanyctodes hectoris* and *Electrona carlsbergi*, and additionally *Protomyctophum normani* at the Chatham Rise. *L. hectoris* is an abundant myctophid of southern Africa, south-east Australia and New Zealand (Young et al. 1988). The diet of *M. ingens* in New Zealand waters is characterised by a component of temperate myctophids, such as *L. hectoris* and also *Symbolophorus* spp., whose distributions extend past the STF into sub-Antarctic water but not south of the SAF (Fig. 5.1b). *E. carlsbergi* is distributed in sub-Antarctic waters between the STF and the APF (Hulley 1990), and is likely to be the most abundant myctophid in the Deep Scattering Layer of the Pacific Ocean at depths between 50-150 m (Linkowski 1983). This species is commonly consumed by *M. ingens* near New Zealand and Macquarie Island. *E. carlsbergi* is also reported to be one of the most common dietary items of *Martialia hyadesi* near South Georgia in the Atlantic Sector of the Southern Ocean (Rodhouse et al. 1992).

In contrast, the absence of *E. carlsbergi* from the diet of *M. ingens* at the Falkland Islands indicates that *E. carlsbergi* is not abundant at this location compared to other areas of the Southern Ocean, at least during the sampling period of this study. Interannual and seasonal variation in the abundance of myctophids, including *E. carlsbergi*, has been observed in the diet of *M. ingens* and other marine predators at Macquarie Island (Goldsworthy et al. 2002; Hindell 1988). Spatial comparisons of the diet of *M. ingens* may require multi-year and seasonal data from all sites, as suggested by Goldsworthy et al. (2002) when commenting on spatial variation in the diet of the Patagonian toothfish *Dissostichus eleginoides*. However, *E. carlsbergi* was not a common dietary component of the Southern Opah *Lampris immaculatus* sampled from the Falkland Islands between September 1993 and November 1994 (Jackson et al. 2000a). Only one individual of *E. carlsbergi* was consumed during this period, suggesting that this species may not be abundant in the area regardless of interannual and seasonal fluctuations.

Interestingly, the only three species that were above 10% FO and 10% NI in the diet of *L. immaculatus* were the same species that were above 10% FO and 10% NI in the diet of *M. ingens* at the Falkland Islands: *Gymnoscopelus nicholsi* (cf *G. nicholsi/piabilis*), *Loligo gahi* and *M. ingens* (Jackson et al. 2000a). *M. ingens* was the most frequent prey species consumed by *L. immaculatus*, occurring in 93% of stomachs. *G. nicholsi/piabilis*, *L. gahi* and *M. ingens* may provide the basis of the diet for several pelagic predators around the Falkland Islands. Alternatively, the stomach contents of *L. immaculatus* may have contained a considerable number of items that had been secondarily ingested from its prey *M. ingens*. It appears that that secondary ingestion of prey remains from squid stomachs may bias stomach contents data of higher predators.

Two of the dominant prey species of *M. ingens* at the Falkland Islands were cephalopods, a phenomenon that was not observed at any other site. *M. ingens* may adapt to a largely cephalopod-based diet when suitable fish prey, such as *E. carlsbergi*, are not abundant. There is evidence that *M. ingens* and other squid species may only consume cephalopods that are from younger cohorts and/or much smaller than themselves (Collins & Pierce 1996) (Section 4.5.3, Chapter 4). Therefore, the

consumption of large numbers of cephalopods - other than sepiolids and occasional conspecifics – may be largely precluded at Macquarie Island and New Zealand. However, a large and commercially exploited population of the small loliginid *L. gahi* exists around the Falkland Islands. Hatfield & Rodhouse (1994) found that many specimens of *L. gahi* collected during October and within the same depth range as *M. ingens* were no larger than 16 cm ML. Therefore, large numbers of *L. gahi* ≤ 16cm ML may have been available to *M. ingens* at the Falkland Islands during the sampling period. Populations of analogous loliginids do not exist at the Chatham Rise, Campbell Plateau or Macquarie Island. In addition, large numbers of juvenile *M. ingens* are present on the Patagonian Shelf during October-December (Jackson et al. 1998b) (Chapter 6), whereas juvenile *M. ingens* were not collected from Macquarie Island, the Chatham Rise or the Campbell Plateau. It appears that the absence of key myctophid species such as *E. carlsbergi* on the Patagonian Shelf, and the presence of large populations of small squid (*L. gahi* and juvenile *M. ingens*), has influenced the dietary structure of *M. ingens* at the Falkland Islands so that the diet at this location includes a much larger cephalopod component compared to the three sites in the Pacific Sector.

5.5.2 Lipid and fatty acid data – digestive gland

The digestive gland fatty acid composition of *M. ingens* is clearly dependent on site (Fig. 5.4). By assuming that cephalopod digestive gland lipid content is derived solely from dietary intake (Abolmasova et al. 1990; Blanchier & Boucaud-Camou 1984; Clarke et al. 1994; Semmens 1998), stomach contents data has been complemented with fatty acid profiling to demonstrate that the diet of *M. ingens* varies considerably between regions of the Southern Ocean. Fatty acid data of potential prey has *not* been used to determine which species may be responsible for between-groups variations in dietary lipid uptake as samples of sub-Antarctic mesopelagic fish such as myctophids are scarce (Lea et al. 2002b). While some fatty acid data are available for species from Macquarie Island and the Falkland Islands (Lea et al. 2002b; Wilson 2003; Chapter 6), it was not possible to obtain specimens from the Chatham Rise or Campbell Plateau. Spatial variation in fatty acid content, in addition to temporal and size variation, has been recorded for some fish prey of *M. ingens* (Lea et al. 2002b; Phleger et al. 1997) in addition to other species of fish (Iverson et al. 2002), and may affect the interpretation of fatty acid data (Section

3.5.2, Chapter 3). Until such data become available that are consistent with the location, time of capture and size of prey identified as part of this study, it is not presently feasible to use published data from other regions of the Southern Ocean to explore spatial variation in dietary lipid uptake of *M. ingens.*

5.5.3 Lipid and fatty acid data – the Campbell Plateau

Clear trends in the fatty acid, lipid class composition and total lipid content of the digestive gland of *M. ingens* indicate dietary differences between sites. Digestive glands collected from the Falkland Islands are enriched in MUFA of C 20 or longer, a characteristic of some copepod-based food-webs (Saito & Murata 1998), whereas digestive glands from the Campbell Plateau contain very large amounts of DHA and relatively high levels of EPA. Long-chain PUFA such as EPA and DHA can only be biosynthesised by certain macroalgae and phytoplankton species and are essential dietary components to higher predators (Ackman 1980; Sargent 1976). Deposition of EPA and DHA in a predator can be dependent on trophic pathways. Large amounts of EPA and DHA in digestive glands collected from the Campbell Plateau indicate that the diet of *M. ingens* at this site differs from other sites, including the Chatham Rise.

Digestive glands from Campbell Plateau specimens also differ from those collected from other sites with a lipid class composition that is characterised by large amounts of FFA instead of TAG. Large amounts of TAG have been previously recorded in the digestive gland of *M. ingens* from Macquarie Island (Phillips et al. 2001) and in the digestive glands of other onychoteuthids (Hayashi et al. 1990; Phillips et al. 2001). Elevated FFA have been linked to oxidation of lipids under inappropriate storage conditions (Pizzocaro et al. 1980), but appear to be a characteristic of squid digestive glands regardless of storage temperature (Hayashi 1996; Hayashi et al. 1985; Hayashi & Yamamoto 1987b; Kawasaki et al. 1994; Wako et al. 1993). Excluding possible variations in storage conditions onboard different vessels, the methodology has remained constant throughout this study and elevated FFA levels in digestive glands from Campbell Plateau squid are not correlated with the period of storage. Additionally, PUFA levels were very high in Campbell Plateau digestive glands suggesting that production of FFA has not degraded

these fatty acids. Therefore, large amounts of FFA in the digestive glands of squid from the Campbell Plateau are not considered to be an artefact of methodology.

Digestive glands collected from the Campbell Plateau contained significantly less lipid than those from other sites. The mass of the digestive gland relative to ML also differed significantly between sites and was lowest at the Campbell Plateau, probably as a direct consequence of reduced lipid content. Shchepkin et al. (1982) stated that large amounts of lipid were found in digestive glands of squid with access to abundant food resources, and that this lipid was dominated by TAG as opposed to other lipid classes. Abolmasova et al. (1990) suggested that lipid content of the digestive gland of squid may be used as an indicator of food availability in different regions, and found that digestive gland lipid content was reduced in regions where the abundance of *Sthenoteuthis pteropus* was low. Similarly, Blanchier & Boucaud-Camou (1984) found that lipid levels in the digestive gland of the cuttlefish *Sepia officinalis* were reduced during a period when this species was rare, probably due to severe winter conditions.

Comparisons with published data indicate that depleted lipid stores and low TAG levels in digestive glands from the Campbell Plateau reflect a reduction in food availability to *M. ingens*. In contrast, large amounts of lipid and TAG in the digestive glands of squid from the Chatham Rise potentially result from abundant food resources at this site. The STF is a major oceanic boundary that passes over the Chatham Rise, separating subtropical water to the north from sub-Antarctic water (SAW) to the south (Heath 1981). All Chatham Rise squid were collected from a region strongly influenced by the STF (Fig. 5.1b). In contrast, a large proportion of the Campbell Plateau lies to the south of the STF, within the cooler SAW. While some squid were collected from a region of the Campbell Plateau that is also influenced by the STF, the majority were collected from SAW, including those selected for lipid and fatty acid analysis (51.36°S, 173.42°E). The STF is a region of higher biological productivity than SAW and supports a greater biomass of phytoplankton and zooplankton, particularly during spring when samples of *M. ingens* were collected (Bradford-Grieve et al. 1999; Nodder & Alexander 1998). A reduction in food availability to *M. ingens* at the Campbell Plateau, as determined from

lipid content and class composition of the digestive gland, is consistent with reduced productivity of SAW.

ADDENDUM

The research within the original manuscript and this resultant Chapter was conducted by myself. Co-authors G. D. Jackson and P. D. Nichols fulfilled supervisory roles as my Ph. D. supervisors at IASOS, University of Tasmania, and provided constructive criticism on the text. C. Sands is acknowledged for his assistance with squid dissections. D. Williams verified the identification of some uncommon otoliths, and T. van Ommen assisted with the preparation of Fig. 5.1.

Chapter 6. Size-related dietary changes observed in *Moroteuthis ingens* at the Falkland Islands

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6.1 ABSTRACT

Dietary composition of the onychoteuthid squid *Moroteuthis ingens* at the Falkland Islands was related to predator size, as shown by stomach contents and fatty acid profiling. Comparisons were made between two size classes of squid: those of ML < 200 mm and those of ML > 200 mm. Smaller squid had frequently consumed crustaceans and cephalopods; fish were of secondary importance. Larger squid consumed mostly fish and moderate proportions of cephalopods, but had rarely consumed crustaceans. These findings were supported by comparisons drawn between fatty acid profiles of the digestive gland and those of potential prey species. Fatty acid analyses indicated that the crustaceans *Euphausia lucens*, *Munida gregaria* and *Themisto gaudichaudii* were important prey items of smaller squid, whereas stomach content and fatty acid analyses indicated that *Gymnoscopelus nicholsi* of around 100 mm standard length represented much of the fish prey of larger squid.

6.2 INTRODUCTION

Many studies have established that cephalopods change their diet with increasing size, to incorporate a different and usually broader spectrum of prey (for reviews see Mangold 1983; Nixon 1987; Rodhouse & Nigmatullin 1996). Previous studies of prey selectivity have been conducted almost exclusively on the Loliginidae and Ommastrephidae (eg Breiby & Jobling 1985; Coelho et al. 1997; Collins & Pierce 1996; Ivanovic & Brunetti 1994; Lipinski 1987; Pierce et al. 1994; Quetglas et al. 1999), and with few exceptions (Quetglas et al. 1999), small and/or juvenile squid have been found to consume mostly crustaceans and then switch to a fish- and cephalopod-based diet as they grow. These changes in diet have been related to the energetic advantages of a fish diet compared to a crustacean diet (Pierce et al. 1994), in addition to the simple principle that a wider size-range of prey is available to larger squid, in part due to allometric development of the brachial crown (Rodhouse & Nigmatullin 1996).

While squid are recognised to be a key ecological component of the Southern Ocean (Rodhouse & White 1995), less is known of this group than of any other marine fauna in the region (Lubimova 1985), particularly in the context of squid as predators (Kock 1987). *Moroteuthis ingens*, a member of the cosmopolitan Onychoteuthidae, has in recent times been collected as by-catch from several commercial fisheries operating within the Southern Ocean. These industries have subsequently made relatively large samples of *M. ingens* available to research. *M. ingens* is an endemic species of the Southern Ocean with a circumpolar distribution in the sub-Antarctic. It is an abundant and ecologically important squid that inhabits the bathypelagic zone, although it is found in relatively shallow water over the Patagonian shelf (Filippova 1972; Jackson et al. 1998b; Lubimova 1985).

Previous dietary studies of large, adult specimens have identified myctophids as the major prey of *M. ingens* (Lubimova 1985; Jackson et al. 1998b; Chapter 3), although spatial comparisons have revealed that cephalopods comprise a relatively greater component of the diet around the Falkland Islands (Chapter 5). This study explores changes in diet with increasing size of *M. ingens* at the Falkland Islands, using stomach contents and complementary fatty acid profiling. For the first time, fatty acid dietary

tracers have been applied to the study of small, juvenile specimens of *M. ingens* so that size-related changes in dietary lipid uptake may be examined in an onychoteuthid species.

6.3 MATERIALS AND METHODS

6.3.1 Sample collection

Sixty specimens of *M. ingens* were obtained during a four-week research cruise conducted by the Falkland Islands Government Fisheries Department (FIGFD) on board the Fisheries Patrol Vessel *Dorada*, from 4 October 2001 to 31 October 2001. *M. ingens* was collected from various regions on the Patagonian Shelf at depths of 43 – 945 m (Fig. 6.1). Potential prey species of *M. ingens* were also collected during this cruise. Squid and fish samples were collected by either a Stealth 153 mid-water rope trawl equipped with Super-V doors, or a two-panel bottom trawl fitted with Super-V doors. Zooplankton samples were collected by Isaacs Kidd midwater trawl (0.5 mm mesh). The mantle and digestive glands of twenty-seven specimens of *M. ingens*, in additional to thirteen whole potential prey species, were frozen and transported by air to Hobart where they were stored at –20° C, prior to lipid and fatty acid analysis. The sample sizes of both *M. ingens* and prey species were constrained by the difficulty of transporting frozen samples from the Falkland Islands to Hobart.

An additional fifty stomachs from *M. ingens* were collected on an opportunistic basis by fisheries observers posted on commercial boats operating over the Patagonian Shelf during September 2001.

6.3.2 Stomach contents analyses

Most stomachs were sorted either at FIGFD or on board *Dorada*. Remaining stomachs were returned frozen to Hobart and processed at the laboratories of the Institute of Antarctic and Southern Ocean Studies (IASOS), University of Tasmania. Stomach contents analyses were conducted using protocols described in Section 2.3, Chapter 2.

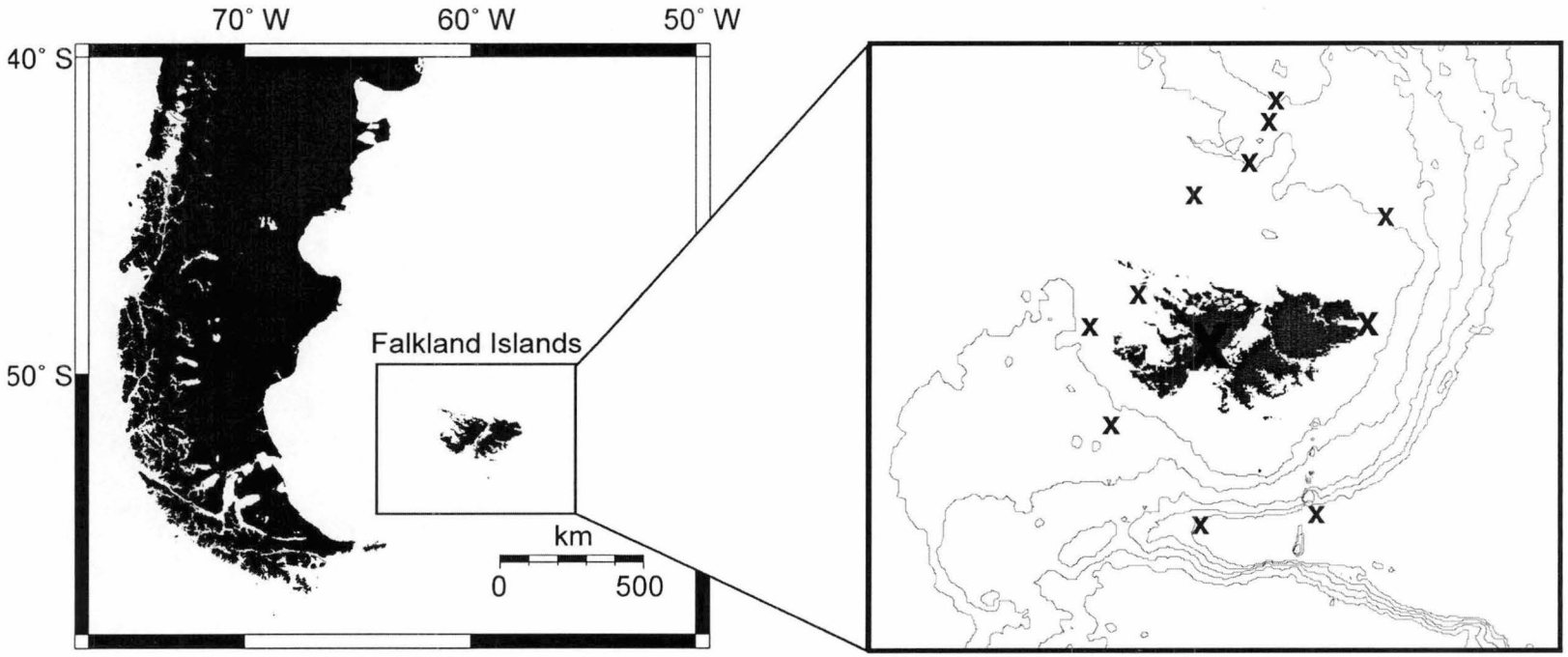

Fig. 6.1. Collection sites of *Moroteuthis ingens* around the Falkland Islands, south-west Atlantic. Sites are shown only for the vessel *Dorada*. 200 m bathymetry contours are given to a depth of 1000 m.

In addition, the prey category that comprised the majority of the contents in a stomach was described as the primary prey group for that stomach. Otoliths were sorted into left and right, and the number of individual fish per species was determined from the greatest number of either left or right otoliths. Otoliths of *Gymnoscopelus nicholsi* were measured using a Wild M29.5 stereo dissecting microscope and SigmaScan Pro image analysis software (SPSS Science, Chicago, Illanois). Regression equations for the standard length of *G. nicholsi* were taken from Williams & McEldowney (1990). When large proportions of fish remains were found (such as scales, bones and flesh), but without (or with very few) eye lenses or otoliths, it was assumed that fish heads may have been discarded and the incidence was recorded. Other prey identifications were completed using the protocols described in Section 2.3, Chapter 2.

6.3.2 Lipid extraction and fatty acid analysis

Lipid was extracted from the mantle and digestive gland of twenty-seven specimens of *M. ingens* of known ML, in addition to thirteen potential prey species. A small tissue sample (approximately 1 g) was taken from the ventral mantle of *M. ingens* and ground in a mortar and pestle prior to extraction. Whole digestive glands from *M. ingens* were homogenised in a hand-held blender, and a 0.25-0.5g subsample was taken for lipid extraction. Prey species were either homogenised whole, or a sample was taken (eg, from the tail flesh of fish) and then homogenised (Table 6.1). A subsample of approximately 1 g was taken from homogenised prey samples for lipid analysis. All lipid extraction and fatty acid analysis was conducted using the protocols described in Section 2.4, Chapter 2.

6.3.4 Statistical analyses

The abundance of identifiable prey species in the stomach contents of *M. ingens* were described by percent frequency occurrence (% FO), percent numerical importance (% NI) and total number (N). The % FO was determined from the number of stomachs from size class k containing prey species h as a percentage of the total number of stomachs examined from size class k. The % NI was determined as the number of prey species h consumed by size class k as a percentage of the total number of prey items consumed by

Table 6.1. Details of potential prey species included in fatty acid analyses. Prey length represents carapace length for crustaceans, standard length for fish and mantle length for cephalopods.

Species	Code	*n*	N	Prey length mm	Tissue type
Crustaceans					
Euphausia lucens	EL	>10	2		whole specimens
Munida gregaria	MG	5	2		whole specimens
Thymops birsteini	TB	1	1	45	thoracic + tail flesh
Themisto gaudichaudii	TG	>10	2		whole specimens
Fish					
Electrona subaspera	ES	1	2	185 - 240	whole specimens
Gymnoscopelus bolini	GB	1	2		tail flesh
Gymnoscopelus nicholsi s	GN	1	2	100 - 110	whole specimens
Gymnoscopelus nicholsi l	GN	1	2	140 - 150	whole specimens
Paradiplospinus gracilis	PG	1	1	310	whole specimen
Photichthys argenteus	PA	1	2		tail flesh
Salilota australis	SA	1	2	115 - 140	whole specimens
Icichthys australis	IA	1	1		tail flesh
Arctozenus risso	AR	1	1	300	whole specimen
Cephalopods					
Loligo gahi	LG	1	2	126 - 142	whole specimens

n : number of animals per sample; N: number of samples; s: small; l: large.

size class *k*. Note that the number of crustaceans within a stomach could not usually be determined, and as a consequence N and % NI do not include crustacean remains.

Fatty acid profiles were compared using the multivariate technique of non-metric multidimensional scaling (MDS) and linear discriminant analysis (DA). Fatty acids that contributed a mean of less than 0.5% (of total fatty acids) were excluded from statistical analyses: thus for digestive gland tissue, analyses were performed using 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n11, 22:1n9, 24:1, 18:2n6, 20:4n6, 20:5n3, 20:4n3, 22:6n3, 22:5n3 and C 23 PUFA; for mantle tissue 14:0, 16:0, 18:0, 16:1n7, 18:1n9, 18:1n7, 20:1n9, 22:1n9, 20:4n6, 20:5n3, 22:6n3 and C 23 PUFA. MDS was performed in two dimensions on mg g^{-1} fatty acid data that had been standardised to the range 0-1, and correlation matrices were constructed using Euclidean distances. DA was performed on mg g^{-1} fatty acid data, and results were confirmed by analysis of % composition data (not shown). Throughout this study, two-tailed *t*-tests assuming equal variance were used to determine significant differences within a 95% CI. All statistical analyses were conducted using SPSS 10.0 for Macintosh (SPSS Science, Chicago, Illanois).

6.4 RESULTS

6.4.1 Size classes

A bimodal distribution was observed in the ML of both females and males (Fig. 6.2). Modes in female ML were recorded at 125-150 mm and 350-375 mm, and modes in male ML were recorded at 75-100 mm and 300-325 mm. According to frequency distributions of ML (Fig. 6.2), squid were sorted into two size classes for dietary, lipid class and fatty acid comparisons; a small size class containing squid of ML < 200 mm, and a large size class where ML > 200 mm. On some occasions, stomachs were collected from certain individuals but ML was not recorded. In these instances, squid were assigned to either the small or large size class according to their maturity stage. Based on the mean and range of ML recorded for each maturity stage (Fig. 6.3), stage I females and stage I and II males were assigned to the small size class, whereas stage II and III females and stage IV and V males were assigned to the large size class

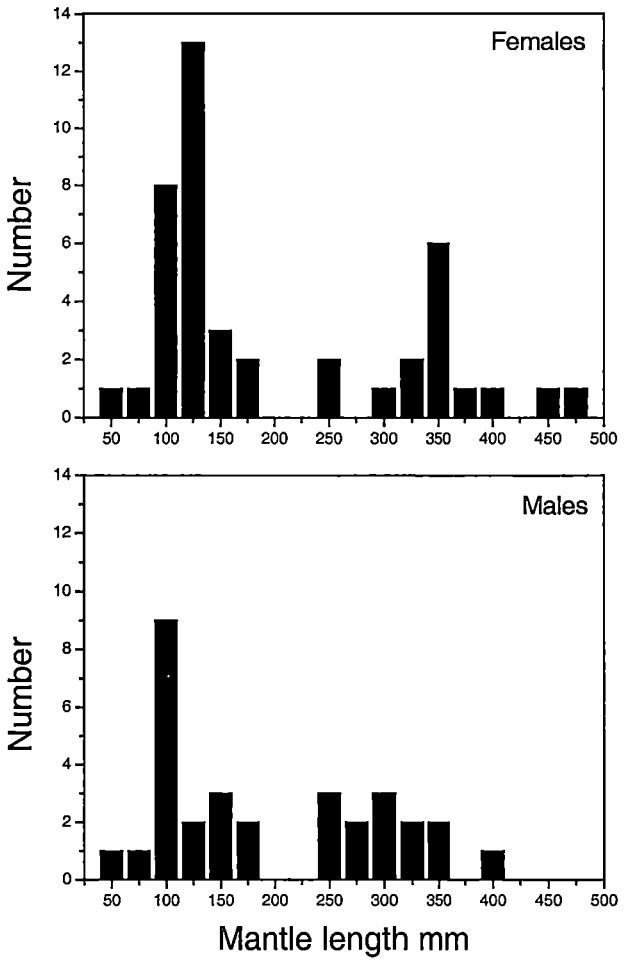

Fig. 6.2. Mantle length frequency distributions for female and male specimens of *Moroteuthis ingens* from the Falkland Islands.

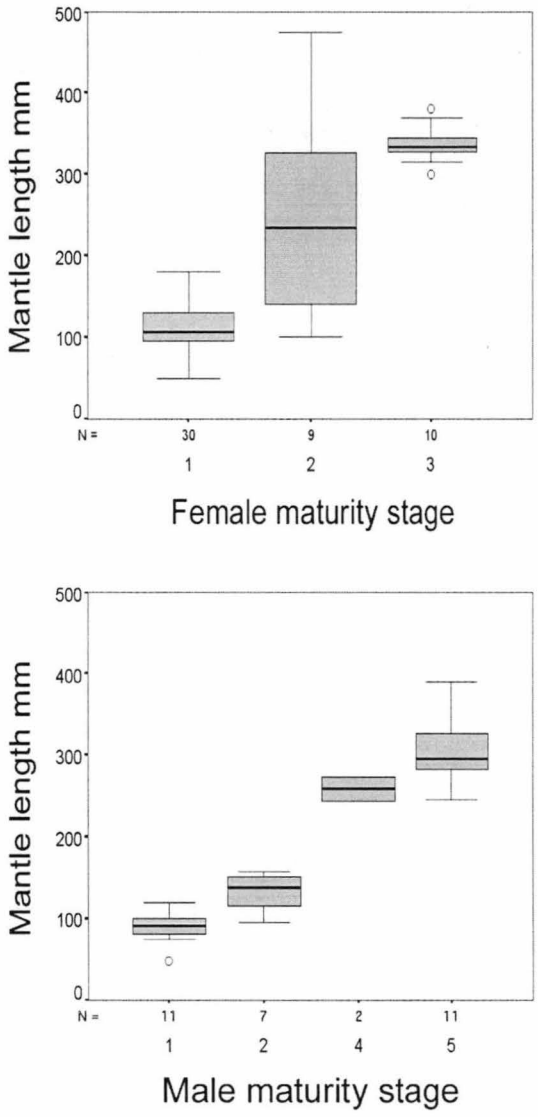

Fig. 6.3. Box-and-whisker plots of the mantle length range for each maturity stage of *Moroteuthis ingens*. The box represents the interquartile range (containing 50% of values) and black bars within each box represent mean values. Whiskers indicate minimum and maximum values, and circles represent outliers. N = number of squid per maturity stage.

(no other maturity stages were recorded as part of this study). A large proportion of variation in ML was recorded for females of maturity stage II, providing a potential source of error for the allocation of stage II females to the correct size class. However, it was necessary to allocate only three stage II females according to their maturity stage rather than known length, and thus this source of error was minimal. No significant differences were observed between the depth distributions of the small and large size class of *M. ingens* ($p < 0.7$).

6.4.2 Stomach contents

A marked contrast in diet was observed between the two size classes of *M. ingens*. The stomach contents of squids of ML < 200 mm were dominated by highly macerated crustacean remains (40.9% FO), which comprised the primary prey category in 36.4% of stomachs (Fig. 6.4). Due to the degree of maceration, crustacean remains could not be identified. However, they were generally red in colour and often contained small black eyes, indicating that they had derived from euphausiid-like prey. Cephalopod prey were also frequently represented in the stomach contents (33.3% FO), and comprised the primary prey category in 30.3% of stomachs (Fig. 6.4). While the absence of intact lower beaks precluded the identification of most cephalopod remains, very small numbers of *Loligo gahi*, *M. ingens* and Octopus spp. were identified (Table 6.2). Fish were of less importance in the diet of the small size class (25.8% FO), and represented the primary prey category in only 16.7% of stomachs (Fig. 6.4). The myctophid *G. nicholsi* was the most frequently consumed fish species (Table 6.2). Fish heads were assessed to have been discarded by 6.1% of individuals. Some fragments of unidentified calcareous material, probably deriving from a sedentary benthic invertebrate, were also found in a small number of stomachs. These were classified as "other" remains (Fig. 6.4). Empty stomachs were collected from 15.2% of individuals from the small size class.

The consumption of crustaceans by squids of ML > 200 mm was much less than for the small size class of *M. ingens*. Crustacean remains were present in only 2.3% of stomachs, and on these occasions represented the primary prey category (Fig. 6.4).

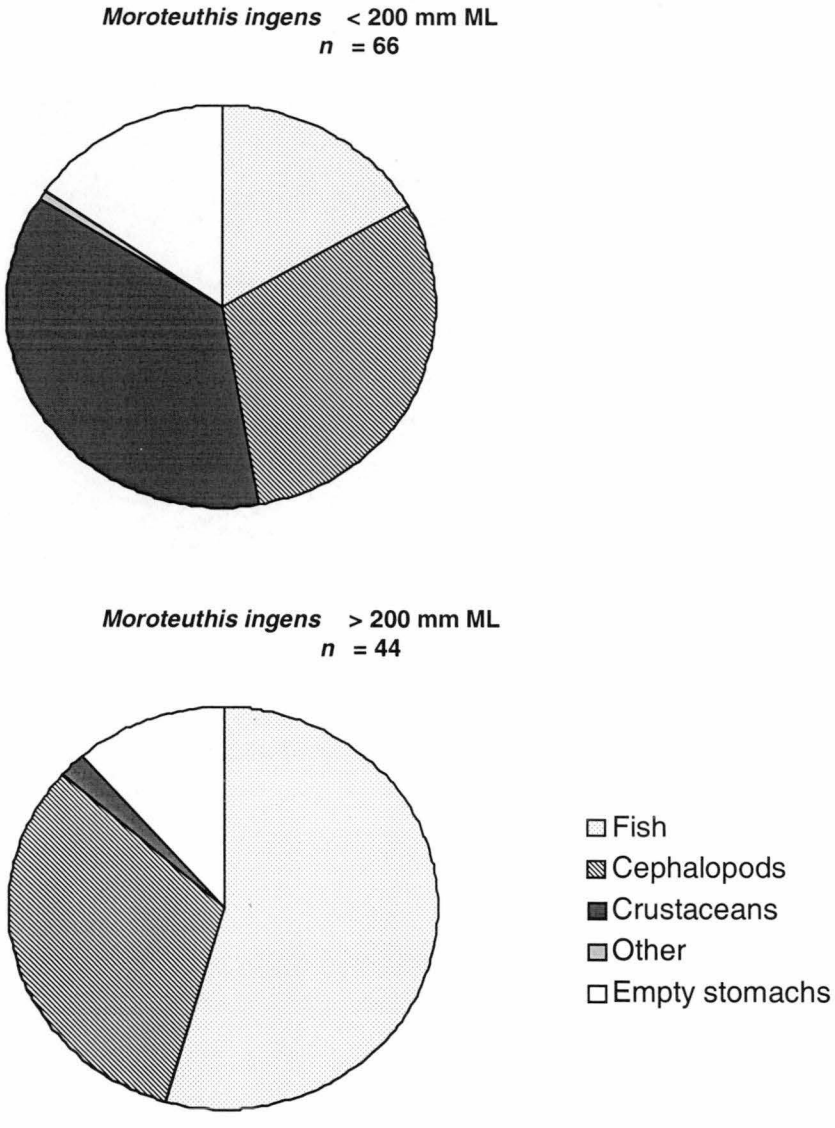

Fig. 6.4. The % frequency occurrence (%FO) of each major prey category of *Moroteuthis ingens* as primary prey. The %FO of empty stomachs is also given. *n:* total number of stomachs per size class; ML: mantle length.

Table 6.2. The N, NI and FO of fish and cephalopod species consumed by *Moroteuthis ingens*

	< 200mm ML			> 200mm ML		
PREY SPECIES	N	%NI	%FO	N	%NI	%FO
Fish						
Family Bathylagidae						
Bathylagus antarcticus				1	0 6	2.3
Family Gonastomatidae						
Photichthys argenteus				1	0.6	2.3
Family Myctophidae						
Electrona subaspera				1	0.6	2 3
Gymnoscopelus braueri				1	0.6	2.3
Gymnoscopelus nicholsi	7	29.2	7.6	76	48.1	31.8
Gymnoscopelus spp.				10	6.3	2.3
Lamponyctus achiris				1	0.6	2.3
Protomyctophum bolini				5	3.2	2.3
Protomyctophum spp.	1	4.2	1.5	3	1.9	2.3
Family Paralepididae						
Magnisudis prionosa				3	1.9	4.5
Family Macrouridae						
Coelorhynchus spp.				1	0.6	2.3
Family Nototheniidae						
Juvenile Notothenioidei/Blennioidei?	9	37.5	3.0			
Family Gobiidae						
cf Gobionotothen angustifrons				1	0 6	2.3
UNIDENTIFIED	1	4.2	1.5	1	0.6	2.3
Cephalopods						
Sepiolid				2	1.3	2.3
Loligo gahi	1	4.2	1.5	16	10 1	18.2
Illex argentinus				1	0.6	2.3
Gonatus antarcticus				3	1.9	4.5
Moroteuthis ingens	2	8.3	1.5	30	19.0	20 5
Octopus spp.	3	12.5	1.5	1	0.6	2.3
UNIDENTIFIED			28 8			20.5

N. total number; NI: numerical importance; FO: frequency occurrence.

Cephalopods were consumed by a large proportion of individuals in the > 200 mm ML size class (59.1% FO), and comprised the primary prey category in 31.8% of stomachs (Fig. 6.4). *L. gahi* and *M. ingens* were the most frequently consumed species of cephalopod prey (Table 6.2). The remains of at least 14 individual conspecifics were found in the stomach of one squid, demonstrating the voracious predatory nature of *M. ingens*. Small numbers of a variety of other cephalopod species were also consumed (Table 6.2). All lower beak remains were very small and could not be measured with hand-held callipers, thus no estimates of cephalopod prey size were obtained. Fish were the most important prey category of the large size class of *M. ingens*. The FO of fish remains was 59.1%, and fish represented the primary prey category in 54.5% of stomachs (Fig. 6.4). As observed in the stomach contents of the small size class, *G. nicholsi* was the most frequently consumed fish prey species. At least 10 other species of fish were identified in the diet of the large size class; however, these prey species were usually represented by only one individual (Table 6.2). Fish heads were estimated to have been discarded by 4.5% of individuals.

Otoliths of *G. nicholsi* retrieved from the stomach contents varied significantly in length between the small and large size classes ($p < 0.020$), although some overlap was observed (Fig. 6.5). The mean length of otoliths consumed by the small size class was 3.49 mm (± 0.63 SD), and otolith lengths varied between 2.23 mm and 4.47 mm. These otolith lengths were equivalent to a mean standard length (SL) of 79 mm, and a minimum and maximum SL of 43 mm and 107 mm, respectively. Otoliths consumed by the large size class were of mean length 3.90 mm (± 0.50 SD) and varied between 2.44 mm and 4.96 mm. Thus the estimated mean SL of *G. nicholsi* consumed by the large size class was 91 mm, and the minimum and maximum SL recorded were 49 mm and 121 mm, respectively.

6.4.3 Lipid class and fatty acid data of Moroteuthis ingens

The mantle tissue of squid from the small size class contained significantly more lipid than squid from the large size class (mean values 1.7 ± 0.2% and 1.5 ± 0.1%, respectively; $p < 0.001$) (Table 6.3), although lipid content of the mantle was

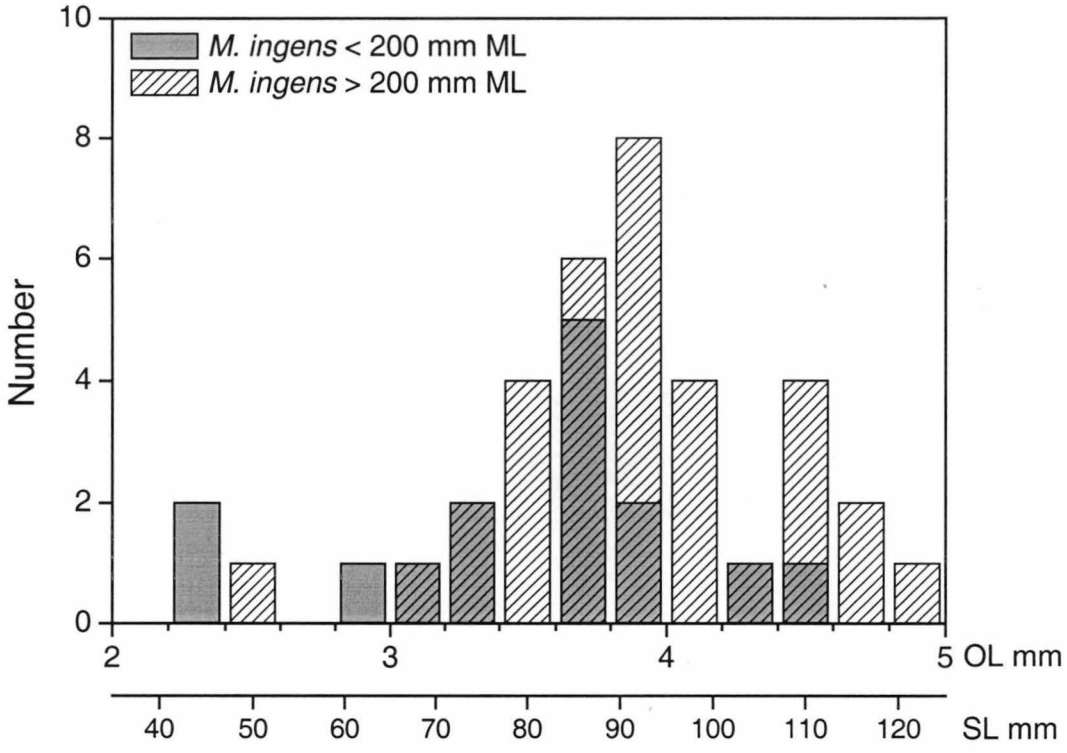

Fig. 6.5. Length frequency distribution of the otoliths of *Gymnoscopelus nicholsi*. OL: otolith length, SL: standard length; ML: mantle length.

consistently low and never exceeded 2% wet mass. Mantle lipid was comprised primarily of PL, which exceeded 90% of total lipid in both the small and large size classes (Table 6.3). Mantle fatty acids were consistently rich in PUFA regardless of size class, largely due to very high proportions of DHA (22:6n3) that represented around 40% of total fatty acids (Table 6.4a). Large proportions of 16:0 and EPA (20:5n3) were also present. The fatty acid composition of mantle tissue was very similar between the small and large size classes (Table 6.4a).

Table 6.3. Lipid content (% wet mass) and lipid class composition (% of total lipids) of mantle and digestive gland tissue of *Moroteuthis ingens* . Values are means ± SD.

% Composition	< 200mm ML	> 200mm ML
Mantle *n*	14	13
FFA	4.6 ± 1.9	1.5 ± 0.6
ST	3.9 ± 0.3	3.6 ± 0.4
PL	91.5 ± 2.0	94.9 ± 0.9
Total Lipid	**1.7 ± 0.2**	**1.5 ± 0.1**
Digestive gland *n*	13	13
WE	9.4 ± 3.6	1.8 ± 1.4
DAGE	0.0 ± 0.0	1.2 ± 1.0
TAG	1.8 ± 0.3	87.3 ± 12.0
FFA	54.0 ± 10.7	6.2 ± 7.0
ST	13.7 ± 9.7	1.6 ± 2.3
PL	22.6 ± 4.9	1.8 ± 1.8
Total Lipid	**7.5 ± 1.9**	**36.4 ± 9.0**

n: sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid.

The lipid content of the digestive glands of squid from the large size class greatly exceeded that of squid from the small size class (mean values 36.4 ± 9.0% and 7.5 ± 1.9%, respectively; $p < 0.001$) (Table 6.3). Lipid class composition also differed between the small and large size class. Large proportions of FFA were recorded in digestive glands from the small size class. WE, ST and PL were also recorded at levels between 10-20% of total lipid (Table 6.3). In contrast, digestive glands from the large size class contained a high proportion of TAG (Table 6.3), as has been previously recorded for *M. ingens* (Phillips et al. 2001; Chapter 3). Small proportions of FFA were also recorded. No other lipid class was recorded above a mean of 2% of total lipids in digestive glands from the large size class (Table 6.3). The fatty acid composition of

Table 6.4. The fatty acid profiles (% of total fatty acids) of a) mantle and b) digestive gland tissue of *Moroteuthis ingens* . Values are means ± SD

a)	Mantle tissue	
	< 200mm ML	> 200mm ML
Fatty acid	*n* =14	*n* =13
14:0	1.7 ± 0.2	1.5 ± 0.2
16:0	24.3 ± 1.6	24.5 ± 1.2
18:0	1.3 ± 0.1	2.3 ± 0.2
16:1n7	1.2 ± 0.2	0.6 ± 0.1
18:1n9	2.7 ± 0.3	2.6 ± 0.2
18:1n7	2.1 ± 0.4	1.6 ± 0.1
20:1n9	3.7 ± 0.6	5.7 ± 0.4
22:1n9	1.1 ± 0.2	1.4 ± 0.1
20:4n6 (AA)	1.1 ± 0.2	1.9 ± 0.2
20:5n3 (EPA)	13.9 ± 1.2	13.7 ± 0.6
22:6n3 (DHA)	40.9 ± 1.8	39.3 ± 1.9
C23 PUFA	1.1 ± 0.4	0.6 ± 0.3
SUM SAT	**28.2 ± 1.7**	**29.0 ± 1.3**
SUM MUFA	**12.9 ± 0.6**	**13.7 ± 0.5**
SUM PUFA	**58.6 ± 1.7**	**57.1 ± 1.5**

b)	Digestive gland	
	< 200mm ML	> 200mm ML
Fatty acid	*n* =13	*n* =13
14:0	2.1 ± 0.7	2.6 ± 0.5
16:0	20.2 ± 3.3	13.9 ± 0 9
18:0	4.2 ± 0.5	3.5 ± 0.4
16:1n7	2.9 ± 1.3	2.8 ± 0.6
18:1n9	9.0 ± 1.2	18.7 ± 3.2
18:1n7	4.1 ± 1.2	3.3 ± 0.5
20:1n9	3.5 ± 2.4	12.6 ± 1.7
22:1n11	1.5 ± 2.4	7.7 ± 2.0
22:1n9	1.2 ± 0.8	3.0 ± 0.9
24:1	2.3 ± 2.1	4.8 ± 1.5
18:2n6	0.9 ± 0.4	0.9 ± 0.3
20:4n6 (AA)	1.9 ± 0.6	0.8 ± 0.1
20:5n3 (EPA)	16.5 ± 3.1	5.9 ± 1.2
20:4n3	0.4 ± 0.1	0.9 ± 0.2
22:6n3 (DHA)	22.5 ± 5.4	11.4 ± 1.6
22:5n3	0.9 ± 0.3	1.1 ± 0.1
C23 PUFA	0.7 ± 0.2	0.4 ± 0.1
SUM SAT	**27.5 ± 4.1**	**20.6 ± 1.1**
SUM MUFA	**26.2 ± 6.5**	**55.5 ± 4.3**
SUM PUFA	**45.4 ± 6.4**	**23.1 ± 3.5**

n : sample size; AA: arachadonic acid; EPA: eicosapentaenoic acid; DHA: docosapentaenoic acid; SAT: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ML: mantle length.

the digestive gland varied markedly between the small and large size classes. While fatty acid profiles of digestive glands from the small size class were characterised by high proportions of EPA, DHA and 16:0, digestive glands from the large size class contained relatively larger proportions of 18:1n9, 20:1n9, 22:1n11 and 24:1 (Table 6.4b). Comparisons of sum values revealed that digestive glands from the small size class were characterised by large proportions of PUFA, whereas digestive glands from the large size class were characterised by high levels of MUFA (Table 6.4b).

6.4.4 Lipid class and fatty acid profiles of potential prey

A large amount of variability was recorded in the total lipid content and lipid class composition of prey species. The lipid content of crustacean prey was generally low, and was comprised mostly of either PL or TAG (Table 6.5a). The lipid of *Euphausia lucens* contained high levels of FFA. Elevated FFA levels in *E. lucens* are most likely to be a product of storage at –20° C. Crustacean species generally contained high proportions of PUFA such as EPA and DHA, and relatively high levels of 16:0 and 18:1n9 (Table 6.6a).

The lipid content and class composition of myctophid prey varied markedly between and within species (Table 6.5b). The myctophids *Electrona subaspera* and *G. nicholsi* contained large proportions of TAG. Two size classes of *G. nicholsi* were analysed: a smaller size class containing individuals of SL 100–110 mm, and a larger class with individuals of SL 140-150 mm (Table 6.1). Notably, the larger specimens of *G. nicholsi* contained significantly more TAG than smaller specimens ($p < 0.02$), in addition to a significantly greater proportion of lipid ($p < 0.01$). In contrast to the other myctophids, the dominant lipid class of *Gymnoscopelus bolini* was WE (Table 6.5b). *G. bolini* also contained a relatively large amount of lipid. All myctophid species contained high levels of MUFA such as 18:1n9 and 20:1n9, particularly the WE-rich *G. bolini* (Table 6.6b).

Table 6.5. The total lipid content (% wet mass) and lipid class composition (% of total lipids) of potential prey species of *Moroteuthis ingens* : a) crustaceans, b) myctophid teleosts and c) non-myctophid teleosts and squid. Values are means ± SD

a) % Composition	EL *n* =2	TB *n* =1	TG *n* =2	MG *n* =2
WE		0.0	1.0 ± 0.1	0.0 ± 0.0
DAGE		0.0	0.1 ± 0.0	0.0 ± 0.0
TAG	0.2 ± 0.1	0.2	83.3 ± 0.4	0.7 ± 0.5
FFA	63.1 ± 2.3	0.8	8.2 ± 0.1	11.5 ± 0.1
ST	2.2 ± 0.1	3.5	0.3 ± 0.0	2.2 ± 0.1
PL	34.5 ± 2.3	95.5	7.1 ± 0.4	85.6 ± 0.6
Total lipid	**1.0 ± 0.7**	**0.9**	**2.5 ± 0.0**	**0.8 ± 0.3**

b) % Composition	ES *n* =2	GB *n* =2	GN small *n* =2	GN large *n* =2
WE	0.0 ± 0.0	92.8 ± 8.6	0.0 ± 0.0	0.0 ± 0.0
DAGE	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
TAG	91.1 ± 4.3	6.2 ± 8.3	55.8 ± 8.0	97.9 ± 0.1
FFA	2.3 ± 1.0	0.2 ± 0.1	7.1 ± 0.9	1.0 ± 0.2
ST	0.3 ± 0.2	0.0 ± 0.0	2.4 ± 0.3	0.1 ± 0.0
PL	6.3 ± 3.1	0.8 ± 0.2	34.6 ± 7.4	1.0 ± 0.2
Total lipid	**3.3 ± 0.1**	**10.1 ± 0.1**	**3.2 ± 0.1**	**16.4 ± 0.5**

c) % Composition	PA *n* =2	SA *n* =2	PG *n* =1	AR *n* =1	IA *n* =1	LG *n* =2
WE	0.0 ± 0.0	0.2 ± 0.1	0.2	82.1	0.0	0.1 ± 0.0
DAGE	0.0 ± 0.0		0.0	0.1	50.8	0.0 ± 0.0
TAG	91.1 ± 4.4	0.3 ± 0.1	95.7	4.7	30.5	0.1 ± 0.0
FFA	1.1 ± 1.1	53.1 ± 0.6	1.0	3.0	8.2	6.6 ± 0.9
ST	0.2 ± 0.1	6.6 ± 0.1	0.2	0.3	0.5	5.2 ± 0.1
PL	7.7 ± 3.2	39.8 ± 0.7	2.9	9.8	10.0	88.1 ± 1.0
Total lipid	**3.8 ± 0.3**	**1.8 ± 0.7**	**9.1**	**4.0**	**3.1**	**2.3 ± 0.2**

n : sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid. See Table 1 for prey species codes.

The fatty acids 16:0 and DHA were present at moderately large but variable proportions (Table 6.6b).

With the exception of *Paradiplospinus gracilis*, the remaining fish species contained variable but relatively low levels of lipid (Table 6.5c). The major lipid class of *Photichthys argenteus* and *Paradiplospinus gracilis* was TAG, whereas WE were present in large quantities in *Arctozenus risso* and the lipid of *Icychthys australis* contained large proportions of diacylglyceryl ether (DAGE) and moderate levels of TAG (Table 6.5c). The lipid of *Salilota australis* contained large proportions of FFA. Most of these fish species contained high levels of MUFA, particularly 18:1n9 (Table 6.6c). The exception, *S. australis*, was characterised by a large proportion of PUFA, largely due to high DHA levels. The squid *L. gahi* contained low levels of lipid, mostly in the form of PL and including large proportions of the long-chain PUFA EPA and DHA (Table 6.5c and Table 6.6c).

6.4.5 Comparisons with prey

MDS was used to sort the digestive glands and potential prey species into groups with similar fatty acid profiles. Two groups were identified by this analysis (stress = 0.12, R^2 = 0.94) (Fig. 6.6), in addition to three outliers: one digestive gland each from the small and large size class of *M. ingens*, and one individual *G. bolini*. Group A contained the digestive glands from squid of ML < 200 mm, in addition to *L. gahi*, *S. australis* and all crustacean species. Group B contained the digestive glands from squid of ML > 200 mm, and all other fish species. Thus MDS clearly separated the digestive glands of *M. ingens* into small and large size classes, and it also grouped digestive glands with the important prey types of each size class as identified from stomach contents. Of interest is the apparent dissimilarity in fatty acid composition between the two size classes of *G. nicholsi* (Fig. 6.6). Digestive glands of the larger size class of *M. ingens* contained less PUFA, largely due to a reduction in DHA, and a correspondingly greater proportion of MUFA (Table 6.6b).

DA was used to test the group membership of the prey species. Forward-stepwise DA assigned 100% of prey species (100% cross-validated) to the correct group identified

Table 6.6. The fatty acid profiles (% of total fatty acids) of potential prey species of *Moroteuthis ingens* : a) crustaceans, b) myctophid teleosts and c) non-myctophid teleosts. Values are means ± SD

a) Fatty acid	EL *n* =2	TB *n* =1	TG *n* =2	MG *n* =2
14:0	2.7 ± 0.1	1.4	2.9 ± 0.1	2.1 ± 0.2
16:0	17.3 ± 1.1	14.7	17.3 ± 0.2	14.7 ± 0.3
18:0	1.0 ± 0.0	4.2	2.4 ± 0.0	3.4 ± 0.2
16:1n7	2.0 ± 0.0	2.2	5.8 ± 0.1	4.9 ± 0.5
18:1n9	9.3 ± 0.2	12.1	14.3 ± 0.0	11.3 ± 0.5
18:1n7	4.7 ± 0.1	4.1	2.7 ± 0.0	5.1 ± 0.1
20:1n9	0.6 ± 0.0	3.8	1.6 ± 0.0	1.0 ± 0.1
22:1n11	0.0 ± 0.0	0.8	0.4 ± 0.3	0.1 ± 0.0
22:1n9	0.4 ± 0.0	0.7	0.4 ± 0.2	0.2 ± 0.1
24:1	0.2 ± 0.2	0.4	0.6 ± 0.0	0.3 ± 0.0
18:2n6	3.3 ± 0.1	1.0	1.0 ± 0.0	3.0 ± 0.2
20:4n6 (AA)	1.2 ± 0.0	3.6	1.4 ± 0.2	1.7 ± 0.1
20:5n3 (EPA)	24.0 ± 0.4	24.9	24.4 ± 0.1	25.2 ± 1.1
20:4n3	0.5 ± 0.0	0.3	0.4 ± 0.0	0.9 ± 0.2
22:6n3 (DHA)	24.1 ± 0.6	18.7	14.6 ± 0.1	16.9 ± 1.9
22:5n3	0.6 ± 0.1	0.6	1.0 ± 0.0	0.7 ± 0.0
C23 PUFA	0.0 ± 0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
SUM SAT	**22.2 ± 1.4**	**22.0**	**23.2 ± 0.2**	**21.3 ± 0.5**
SUM MUFA	**19.0 ± 0.2**	**25.9**	**28.1 ± 0.1**	**24.6 ± 1.0**
SUM PUFA	**58.1 ± 1.2**	**51.3**	**48.4 ± 0.2**	**53.5 ± 0.6**

b) Fatty acid	ES *n* =2	GB *n* =2	GN small *n* =2	GN large *n* =2
14:0	2.3 ± 0.2	0.7 ± 0.5	2.6 ± 0.3	7.6 ± 0.2
16:0	16.1 ± 0.1	5.3 ± 1.0	17.3 ± 0.3	16.4 ± 0.2
18:0	3.3 ± 0.2	4.2 ± 4.1	4.1 ± 0.5	4.1 ± 0.2
16:1n7	5.5 ± 0.2	4.7 ± 0.4	2.5 ± 0.3	6.4 ± 0.3
18:1n9	23.2 ± 3.4	28.8 ± 7.2	17.6 ± 2.4	25.1 ± 0.3
18:1n7	3.8 ± 0.3	6.2 ± 0.8	3.7 ± 0.5	8.2 ± 0.8
20:1n9	6.1 ± 0.9	19.8 ± 6.0	7.9 ± 2.1	10.6 ± 0.6
22:1n11	2.8 ± 0.7	4.3 ± 5.1	5.3 ± 2.6	0.9 ± 0.3
22:1n9	1.0 ± 0.1	1.4 ± 1.2	1.8 ± 0.2	0.5 ± 0.1
24:1	1.7 ± 0.5	3.6 ± 1.1	4.9 ± 0.1	0.5 ± 0.2
18:2n6	0.9 ± 0.2	0.9 ± 0.1	0.5 ± 0.6	0.7 ± 0.1
20:4n6 (AA)	1.1 ± 0.3	0.8 ± 0.1	0.9 ± 0.1	0.4 ± 0.0
20:5n3 (EPA)	7.8 ± 0.1	2.8 ± 1.4	5.4 ± 0.5	5.4 ± 0.0
20:4n3	0.6 ± 0.0	1.3 ± 0.3	0.6 ± 0.1	0.9 ± 0.2
22:6n3 (DHA)	17.0 ± 4.9	8.3 ± 1.8	18.3 ± 1.4	6.3 ± 1.0
22:5n3	1.3 ± 0.0	0.6 ± 0.3	1.1 ± 0.1	0.4 ± 0.6
C23 PUFA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SUM SAT	**22.8 ± 0.2**	**10.6 ± 5.7**	**25.0 ± 0.7**	**28.9 ± 0.1**
SUM MUFA	**46.1 ± 5.5**	**72.0 ± 9.7**	**45.8 ± 1.2**	**54.8 ± 0.2**
SUM PUFA	**30.5 ± 5.4**	**17.3 ± 4.0**	**28.6 ± 0.5**	**15.8 ± 0.0**

n : sample size; AA: arachadonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SAT: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. See Table 1 for prey species codes.

Table 6.6. continued

c) Fatty acid	PA *n* =2	SA *n* =2	PG *n* =1	AR *n* =1	IA *n* =1	LG *n* =2
14:0	5.9 ± 3.5	0.7 ± 0.1	3.9	2.1	2.9	2.4 ± 0.2
16:0	17.7 ± 3.5	16.4 ± 0.9	18.7	9.5	17.2	23.7 ± 0.2
18:0	2.0 ± 0.1	5.2 ± 0.3	3.2	2.1	3.8	3.7 ± 0.1
16:1n7	9.1 ± 2.1	2.1 ± 0.0	7.4	10.2	4.4	0.7 ± 0.1
18:1n9	22.0 ± 4.5	15.2 ± 0.0	25.2	24.8	25.4	3.2 ± 0.1
18:1n7	4.6 ± 1.7	3.0 ± 0.3	4.1	3.5	2.5	2.4 ± 0.0
20:1n9	7.2 ± 5.1	1.8 ± 0.3	8.2	4.8	6.0	5.3 ± 0.3
22:1n11	2.2 ± 1.0	0.4 ± 0.3	4.3	2.6	2.8	0.0 ± 0.0
22:1n9	1.1 ± 0.1	0.5 ± 0.1	1.8	1.2	7.1	0.2 ± 0.0
24:1	1.6 ± 0.6	1.6 ± 0.1	2.7	2.3	0.2	0.2 ± 0.0
18:2n6	1.2 ± 0.2	0.7 ± 0.0	1.0	1.8	0.1	0.4 ± 0.1
20.4n6 (AA)	0.7 ± 0.1	4.2 ± 0.3	0.7	1.3	1.8	1.7 ± 0.2
20:5n3 (EPA)	4.7 ± 1.2	8.8 ± 0.2	4.3	9.2	6.7	18.2 ± 0.1
20:4n3	0.5 ± 0.0	0.3 ± 0.0	0.8	1.1	0.2	0.2 ± 0.0
22:6n3 (DHA)	12.0 ± 0.5	31.7 ± 0.4	6.7	14.6	12.8	33.1 ± 0.3
22:5n3	0.6 ± 0.1	2.3 ± 0.3	0.9	1.1	0.8	0.5 ± 0.0
C23 PUFA	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0	0.0	0.0 ± 0.0
SUM SAT	**27.2 ± 7.4**	**22.9 ± 1.3**	**27.3**	**15.0**	**24.8**	**30.9 ± 0.3**
SUM MUFA	**50.5 ± 9.4**	**26.4 ± 0.5**	**56.2**	**51.8**	**51.0**	**13.6 ± 0.0**
SUM PUFA	**21.5 ± 1.8**	**50.2 ± 0.8**	**16.0**	**32.8**	**23.8**	**55.2 ± 0.4**

n : sample size; AA: arachadonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SAT: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. See Table 1 for prey species codes.

from MDS (Fig. 6.6) (Wilks' lambda = 0.222, Approx. F = 16.650, $p < 0.001$). The fatty acid variables used to distinguish between the two groups were 18:1n9 and EPA. A comparison of the proportions of 18:1n9 and EPA reveals that prey species with high proportions of EPA were assigned to Group A, whereas species containing higher proportions of 18:1n9 were assigned to Group B (Tables 6.6 & 6.7). The fatty acids 18:1n9 and EPA were then incorporated into complete linear DA of digestive gland data from *M. ingens*. Digestive glands were originally allocated to either the small or large *M. ingens* size class, and complete linear DA assigned 96.2% of digestive glands (96.2% cross-validated) to the correct group (Wilks' lambda = 0.151, Approx. F = 64.658, $p <$

Table 6.7. Standardised canonical discriminant function coefficients from forward-stepwise DA of prey fatty acid profiles

FA variable	Function 1
18:1n9	1.016
EPA (20:5n3)	-0.708

FA: fatty acid; EPA: eicosapentaenoic acid.

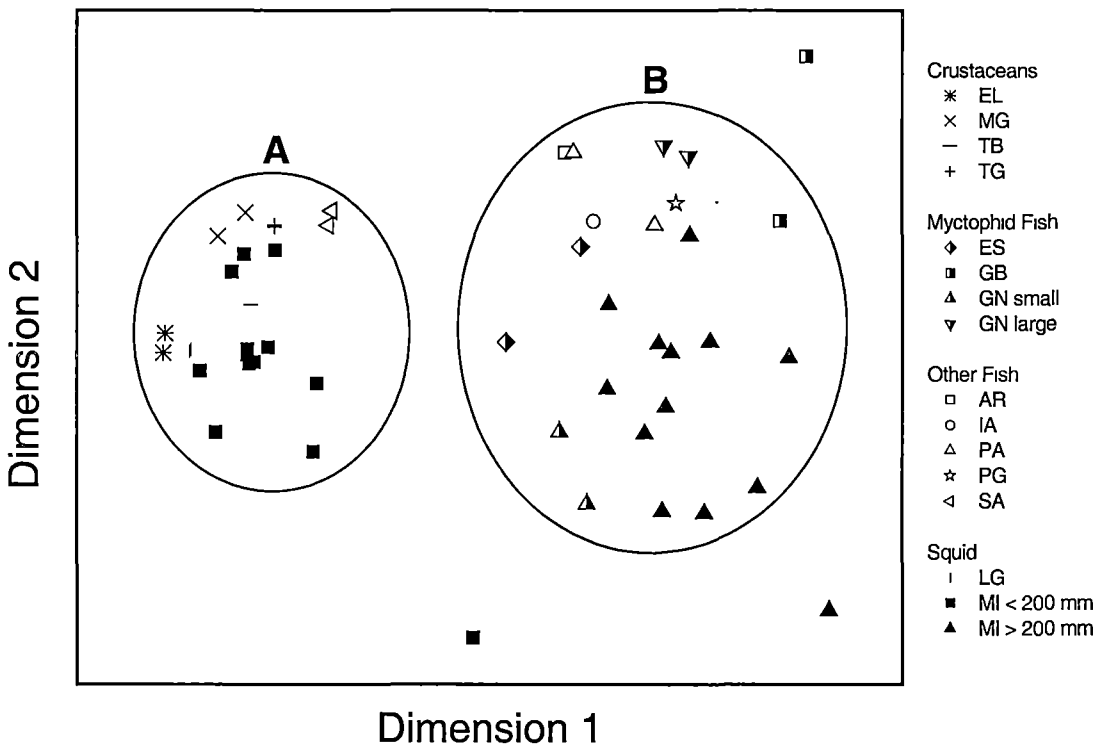

Fig. 6.6. Scatterplot of multidimensional scaling (MDS) of digestive gland fatty acid profiles from the small and large size classes of *Moroteuthis ingens*, together with profiles from potential prey species. Stress = 0.12, $R^2 = 0.94$. ML: *Moroteuthis ingens*; see Table 6.1 for all other species codes. Axis scales are arbitrary in non-metric MDS and are therefore omitted.

0.001). One digestive gland from the large size class was incorrectly assigned to the small size class using the fatty acid variables 18:1n9 and EPA.

6.5 DISCUSSION

The stomach contents and fatty acid profiles of the digestive gland clearly indicate that the diet of *M. ingens* changes from a crustacean and cephalopod diet to a fish and cephalopod diet at around 200 mm ML. The sample sizes were too small to finely resolve changes in diet with size, eg to identify at what size range fish begin to be included in the diet, and at what size crustaceans stop being consumed on a regular basis. However, the largest squid to consume crustacean prey was a male of maturity stage V (likely to exceed 200 mm ML), whereas the smallest squid to have consumed fish prey was of 90 mm ML. Therefore, changes in diet composition were likely to be gradual. Ivanovic & Brunetti (1994) observed that *Illex argentinus* also switched from a crustacean diet to a fish diet at around 200 mm ML on the Bonaerensis Shelf off Argentina, although this trend was not clear in squid collected from the Patagonian Shelf.

The diet of *M. ingens* at the Falkland Islands appears to differ greatly from other squid species for which data is available. Samples of *L. gahi* and *I. argentinus* from around the Falklands were found to feed mostly on crustacean prey regardless of size (Guerra et al. 1991; Ivanovic & Brunetti 1994; Mouat et al. 2001), in direct contrast with *M. ingens*. Thus considerable niche separation in diet may exist between *M. ingens* and commercial squid species over the Patagonian Shelf, and caution may be required when extrapolating known ecology of loliginid and ommastrephid squid to the ecology of non-commercial but important species in the Southern Ocean.

A characteristic of size selectivity by squid is that, while the maximum size of prey increases with squid size, larger squid continue to consume small prey throughout their life history (Rodhouse & Nigmatullin 1996). This trend is apparent in the consumption of *G. nicholsi* by *M. ingens*: the modal length of *G. nicholsi* increased with predator size, but larger squid were also found to have consumed small specimens of *G. nicholsi*. Fish

heads may have been rejected by the small size class of *M. ingens*, thus skewing the range of otolith sizes consumed by these squid. However, it appears that fish heads were discarded in similar proportions by small and large squid, and this source of bias may have been relatively constant throughout the study. While no other fish species were deemed to have any significance in the diet of *M. ingens* at the Falkland Islands, it was not possible to examine interannual or interseasonal variations in diet. Other species of fish may become important prey at different times of year.

Although differences in total lipid content were observed in mantle tissue between the small and large size class, the lipid and fatty acid composition of the mantle remained largely unchanged during the growth of *M. ingens*. This observation supports findings from previous chapters that lipid fulfils specific requirements in the mantle of *M. ingens*, with a large proportion of fatty acids being either biosynthesised, modified or selectively absorbed from the diet.

This study has demonstrated that the digestive gland of both small and large specimens of *M. ingens* contains lipid that is remarkably similar in fatty acid composition to that of potential prey. The lipid content of the digestive gland of *M. ingens* is significantly lower in the small size class. This may be due to the lower lipid content of the major prey group (crustaceans) of small squid compared to the major prey group (fish) of large squid. Alternatively, digestive gland lipid may be accumulated over a prolonged period of time in squid of larger size. Laboratory trials would provide important information on the affect of size and other factors on lipid storage in the digestive gland. However, it is unlikely that such trials could be conducted on a bathypelagic oegopsid such as *M. ingens*, and functions of lipid storage in common laboratory species such as loliginids may differ markedly from that of oceanic species.

Differences in digestive gland lipid class composition between small and large size classes were indicative of dietary differences. *G. nicholsi* was the most important prey of the large size class and was rich in TAG, thus resulting in a high proportion of TAG in the digestive glands of the large size class. The small specimens of *M. ingens* contained large proportions of FFA in the digestive gland. Storage conditions, storage periods and

extraction techniques remained constant during this study; therefore, differences in FFA levels were considered to be real and not an artefact of methodology. Large proportions of FFA in the digestive gland of *M. ingens* from the small size class may result from the ingestion of dietary lipid from some species of potential prey, such as *E. lucens* and *S. australis*, that may demonstrate high lipase activity. Euphausiids and certain other species demonstrate very high lipase activity, which results in the rapid degradation of biomolecules and production of FFA, unless specimens are stored frozen at –80° C within minutes of collection (Saether et al. 1986; C. Phleger, personal communication). Unfortunately, it was not possible to access facilities that would allow storage at –80° C at either the time of collection or during transport to Australia. Regardless, all samples that had high levels of FFA also contained a large proportion of PUFA (mean values 45 – 58% of total fatty acids), therefore production of FFA was unlikely to have affected PUFA levels.

The fatty acid profiles of digestive glands from the small size class of *M. ingens* support observations from stomach contents that crustaceans were the main prey of squid under 200 mm ML. Results from MDS suggested that *E. lucens*, *Munida gregaria* and *Themisto guadichaudii* were important prey species, information that could not be obtained from the highly macerated stomach contents (aptly described as an "unidentifiable slurry" of prey remains by Kear [1992]). *L. gahi* was also grouped with the small size class of *M. ingens*, even though many *L. gahi* were consumed by the large size class. *L. gahi* may have been grouped with the small size class for several reasons; firstly, very few cephalopod remains could be identified from the stomach contents of the small size class, and many of these remains were perhaps derived from *L. gahi*. A higher degree of maceration must be required from smaller squid so that food particles can pass through the oesophagus, and very few lower beaks survived this process intact thus precluding identification. Secondly, *L. gahi* consumes mostly *Euphausia* spp. (Guerra et al. 1991), and its fatty acid profile was strongly influenced by that of its prey (Fig. 6.6). The digestive gland of squid contains a large proportion of "secondary" fatty acids derived from prey, and these secondary fatty acid profiles may lead to a misinterpretation of the importance of cephalopods in the diet of a higher predator (Phillips et al. 2002, see Chapter 7).

Salilota australis was not identified in the stomach contents of *M. ingens*, even though MDS grouped this fish prey with the small size class (Fig. 6.6). The fatty acid profile of *S. australis* were very similar to that of one of its important prey species, the amphipod *T. gaudichaudii* (Arkhipkin et al. 2001). *S. australis* may have been placed in Group A for this reason alone, and not because it contributes to the diet of *M. ingens*. While further stomach contents analyses may identify *S. australis* in the diet of *M. ingens*, it remains important to combine fatty acid analyses of digestive gland tissue with conventional diet analysis so that misinterpretation of dietary composition can be avoided.

Digestive glands from the large size class grouped with fish that had a high MUFA content. Saito & Murata (1998) have suggested that high MUFA levels are characteristic of myctophid species, and that non-myctophid fish contain higher levels of PUFA. This study has shown otherwise, and further analyses of Southern Ocean fish species are required to elucidate this. While some fish included in fatty acid comparisons were not identified in the stomach contents from the Falkland Islands, *P. gracilis* and *G. bolini* have been identified in stomachs of squid collected from Macquarie Island (Chapter 4). Fatty acid evidence indicates that these species were also consumed by *M. ingens* at the Falkland Islands, although larger numbers of stomach analyses will be required to confirm this. In general, digestive gland fatty acid profiles were most closely associated with the fatty acid profiles of *G. nicholsi* of SL 100 – 110 mm. This agrees very well with the size of *G. nicholsi* identified from the stomach contents of the large size class of *M. ingens* (modal SL of 91 mm, maximum SL of 121 mm) (Fig. 6.5). These results demonstrate that it is important to include the appropriate size range of prey into fatty acid analyses of diet, as fatty acid profiles of both prey and predator are influenced by size.

6.6 CONCLUSIONS

Size-related dietary changes have now been confirmed for *M. ingens*, and ecologically important onychoteuthid species distributed in the sub-Antarctic Southern Ocean. Further development of the combined application of stomach contents and fatty acid profiling may identify more clearly the important prey species of squid of ML < 200 mm; such prey are currently very difficult to determine by visual techniques given the degree of maceration of both crustacean and cephalopod remains. Digestive gland fatty acid dietary tracers combined with conventional techniques have provided a powerful technique for dietary studies of both large and small, juvenile specimens of *M. ingens.*

ADDENDUM

The research within the original manuscript and this resultant Chapter was conducted by myself. Co-authors G. D. Jackson and P. D. Nichols fulfilled supervisory roles as my Ph. D. supervisors at IASOS, University of Tasmania, and provided constructive criticism on the text. Scientific observers from the Falkland Islands Government Fisheries Department are acknowledged for the identification of prey species collected during this study. D. Williams verified the identification of some uncommon otoliths.

Chapter 7. Lipid and fatty acid composition of the mantle and digestive gland from four Southern Ocean squid species: implications for food-web studies

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7.1 ABSTRACT

Lipid content, lipid class and fatty acid composition of four Southern Ocean cephalopod species - the myopsid *Sepioteuthis australis* and three oegopsids, *Gonatus antarcticus*, *Moroteuthis robsoni* and *Todarodes* spp – were analysed. The lipid content of the digestive gland was consistently greater than that of the mantle, and an order of magnitude greater in oegopsid species. The lipid class and fatty acid composition of the mantle and digestive gland also differed markedly in each species. Digestive gland lipid is likely to be of dietary origin, and large amounts of lipid in the digestive gland of oegopsids may accumulate over time. Thus the digestive gland is a rich source of fatty acid dietary tracers and may provide a history of dietary intake. However, the absolute amount of dietary lipid in the digestive gland of oegopsid species exceeds the absolute lipid content of mantle tissue. Therefore the overall lipid "signature" of an oegopsid may more closely resemble that of its prey species rather than of its mantle tissue. When lipid techniques are used in dietary analysis of teuthophagous predators, squid may not be represented by a unique signature in analyses and their importance in the diets of predators may be underestimated.

7.2 INTRODUCTION

Over the past thirty years, fatty acid profiles have been used to examine a number of interactions within the marine environment. Fatty acid biomarkers have been extensively applied to determine trophic linkages in microbial, phytoplankton and zooplankton communities (eg Bottino 1974; Falk-Petersen et al. 2002; Graeve et al. 2002; Nelson et al. 2001; Reuss & Poulsen 2002; Zhukova & Kharlamenko 1999). They have also been used to qualify and quantify the essential dietary requirements of aquaculture and fisheries stocks of commercial interest (Dunstan et al. 1996; Johnsen et al. 2000; Kirsch et al. 1998; Klungsøyr et al. 1989; Navarro et al. 1995; St John & Lund 1996). More recently, fatty acids have been used to examine the diet of some of the top marine predators (Brown et al. 1999; Horgan & Barrett 1985; Iverson et al. 1997; Lea et al. 2002a; Raclot et al. 1998; Smith et al. 1997). One of the earliest examples of this was when Hansen & Cheah (1969) reported that the fatty acid composition of sperm whales was largely derived from the fatty acid composition of cephalopod prey. However, since this time and regardless of the importance of cephalopods to many marine predators, very few fatty acid data have been collected from cephalopod species for inclusion into predator-prey comparisons.

Interpreting the influence of diet on the fatty acid composition of a tissue becomes more complex where biosynthesis and modification of fatty acids is likely to occur, such as in the muscle or blubber tissue of marine vertebrates. Some authors do not consider that the fatty acid composition of higher marine predators, principally pinniped seals, can be easily related to dietary lipid uptake (Grahl-Nielsen 1999; Grahl-Nielsen & Mjaavatten 1991). Despite these reservations, fatty acid analysis has been increasingly applied to dietary studies of seals and other marine mammal predators in both the northern and southern hemispheres. The technique is attractive because it may provide an assessment of at-sea foraging behaviour, which can not usually be determined from conventional methods. Fatty acid analyses can also be conducted on small milk or blubber samples, so that intrusive techniques such as stomach lavage and lethal sampling are no longer required.

This study reports the total lipid, lipid class and fatty acid composition of mantle and digestive gland tissue from four squid species from the Southern Ocean. These data may not be directly applicable to dietary studies of higher predators. However, comparisons of mantle and digestive gland fatty acid composition will assist researchers to incorporate squid fatty acid data into studies of higher predators, and will alert them to some of the implications involved with this. The squid species examined during this study include one myopsid, the loliginid *Sepioteuthis australis*, an endemic species from temperate coastal waters around Australia and New Zealand that contributes to a small commercial fishery (Trantafillos & Adams 2001). The other three species are oegopsids thought to be distributed between the STF and the APF, and include the onychoteuthid *Moroteuthis robsoni*, the ommastrephid *Todarodes* spp. and the gonatid *Gonatus antarcticus*.

7.3 MATERIALS AND METHODS

7.3.1 Squid collection

Twenty-nine squid representing four species from four families were collected from various regions of the Southern Ocean between January 1995 and December 2000 (Fig. 7.1). Eleven squid were collected from the commercial fishing trawlers *Austral Leader* and *Southern Champion* by Australian Fisheries Management Authority (AFMA) observers from two areas: (A) in the vicinity of Heard/McDonald Islands between October 1998 and March 1999, and (B) near Macquarie Island during the periods January 1995-February 1995, February 1998, October 1998-January 1999 and February 2000. Nine squid were collected from trawl gear by the National Institute of Water and Atmospheric Research (NIWA) vessel *Tangaroa* from (C) the Campbell Plateau between November 2000 – December 2000, and from (D) the Chatham Rise between October 2000 –November 2000. Nine squid were collected in <10 m water with hand lines from (E) Great Oyster Bay in southeastern Tasmania in March 2000. All squid were frozen after collection at –20° C and returned to Hobart for dissection and analysis.

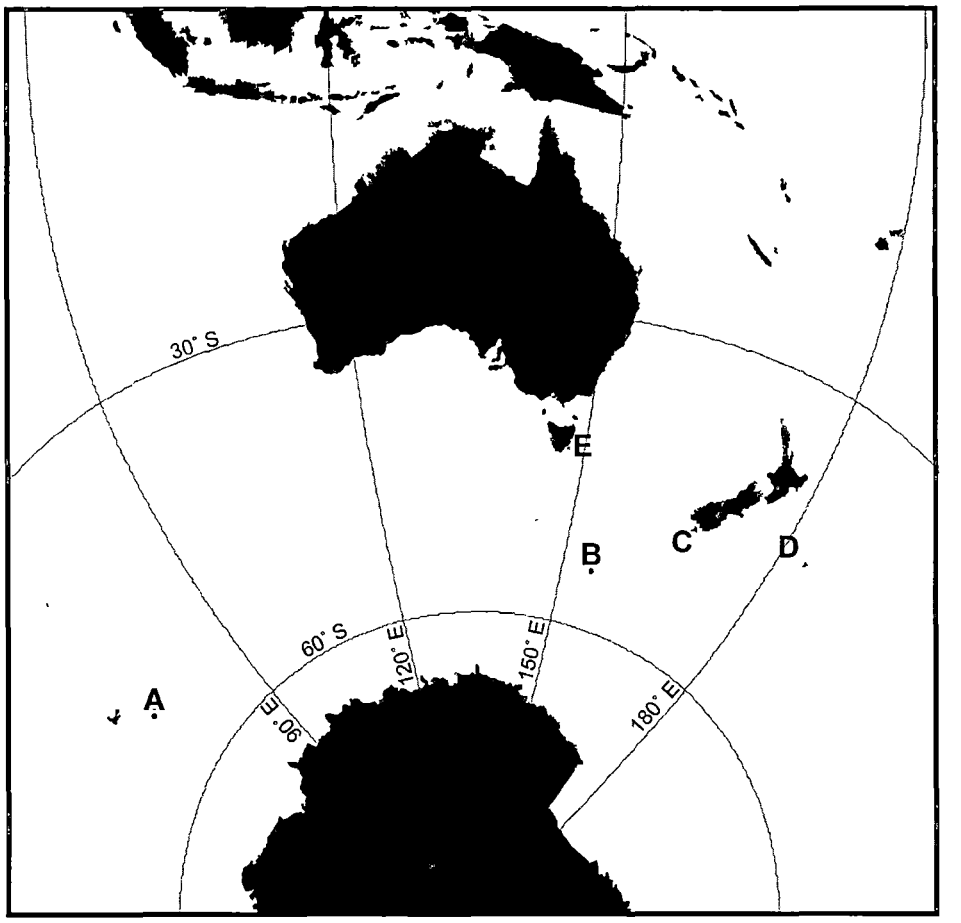

Fig. 7.1. Approximate locations of the collection sites of Southern Ocean squid. A: Heard Island; B: Macquarie Island; C: Campbell Plateau; D: Chatham Rise; E: Great Oyster Bay, Tasmania.

The species descriptions and distributions of *Todarodes* in the Southern Ocean are unclear. Recent studies suggest that *Todarodes angolensis* (Adam, 1962) and *Todarodes filippovae* (Adam, 1975) represent two separate species and that their distribution may overlap. However, the validity of *T. filippovae* has often failed to be recognised (Nesis 1987), and subsequently many biologists have referred to all *Todarodes* collected in the southern hemisphere as *T. angolensis* (Dunning 1998). While the distribution of *T. filippovae* is associated with the Subtropical Convergence and extends north to 32°S, *T. angolensis* is thought to be distributed south of 43°S and extend into the subantarctic (Dunning 1998). Therefore, specimens collected in this study are likely to be *T. angolensis*. However, due to uncertainties in the taxonomic status and zoogeography of the *Todarodes* family in the Southern Ocean, all specimens collected as part of this study have been referred to as *Todarodes* spp. Meanwhile, *Moroteuthis robsoni* has hitherto been considered to obtain a maximum size of around 470 mm ML (Kubodera et al. 1998). However, Sands (2000) suggested that two, phylogenetically distinct morphotypes of this species exist, including a large variant that is significantly larger than specimens previously described for this species. Specimens of *M. robsoni* used in this study were of ML 630-775 mm, thus significantly exceeding the maximum size suggested by Kubodera et al. (1998), and are thought to represent the large variant. The taxonomic status of *M. robsoni* requires review.

7.3.2 Lipid extraction and fatty acid analysis

Due to damage from collection gear to individuals, mantle lengths, mantle and digestive gland masses and samples could not be taken from every animal collected; therefore sample sizes (*n*) vary within species categories. A small tissue sample (approximately 1 g) taken from the ventral mantle was collected from most specimens, and the whole digestive gland of each animal was collected where possible.

Some specimens of *Todarodes* spp. and all specimens of *G. antarcticus* had been stored at -20° C for a number of years prior to analysis. Pizzocaro et al. (1980) found that auto-oxidation of sardine oil, particularly of PUFA, was significantly increased unless the oil was stored at or below –30° C. Therefore, due to elevated FFA levels in both mantle and digestive gland tissues of *G. antarcticus* and some *Todarodes* spp.

specimens, lipid class data for these individuals have been omitted. FFA were present in the digestive glands of *S. australis* and *Todarodes* spp., at levels that fall within the range of values reported for digestive glands of *Illex argentinus* which had been stored at –70° C (Wako et al. 1993). Production of FFA in the digestive gland appears to be a feature of many species of squid (Hayashi 1996; Hayashı et al. 1985; Hayashi & Yamamoto 1987b; Kawasaki et al. 1994; Phillips et al. 2001), even when the storage temperatures are below that recommended by Pizzocaro et al. (1980) to prevent auto-oxidation of lipid (Wako et al. 1993). The enzymatic nature of this organ, more than storage conditions at –20° C, is more likely to elevate FFA levels in this organ, especially when compared to low levels of FFA in the PL-rich mantle tissue which had been stored for the same amount of time. High PUFA levels were maintained in both mantle and/or digestive gland tissue of all species.

All lipid and fatty acid analyses were conducted using the protocols described in Section 2.4, Chapter 2.

7.4 RESULTS

7.4.1 General biology

In total, 21 female and 8 male squid were analyzed. All female squid were immature, while some male squid were mature (Table 7.1). The smallest squid were specimens of *S. australis* and *G. antarcticus*, with minimum ML of 150 mm and 153 mm respectively, while the largest squid were *M. robsoni*. The mean mantle mass and digestive gland mass for each species are presented, in addition to the ratio of the mass of the mantle to the digestive gland (Table 7.2). The ratio of mantle mass to digestive gland mass was greatest in *S. australis* (range of 10.3-19.0). In contrast, the relative mass of the digestive gland was largest in *G. antarcticus*, with a minimum ratio of mantle to digestive gland of 0.1 and a maximum ratio of 1.2.

Table 7.2. Ranges of mantle length, mantle mass, digestive gland mass and the ratio of mantle mass to digestive gland mass of squids analysed in this study

Species	ML mm	M g	DG g	M/DG
Sepioteuthis australis	150–270	57–256	3–15	10.3–19.0
Gonatus antarcticus	153–224	4–73	20–60	0.1–1.2
Moroteuthis robsoni	630–775	1591–2311	325–520	4.4–4.9
Todarodes spp.	280–510	167–811	28–329	2.5–6.2

ML: mantle length; M: mantle mass; DG: digestive gland mass; M/DG: ratio of mantle mass to digestive gland mass.

7.4.2 Total lipid and lipid class data

7.4.2.1 Mantle tissue

Mantle tissue was low in lipid, with lipid content ranging between 0.8 ± 0.1% wet mass in *M. robsoni* and 1.9 ± 0.6% wet mass in *S. australis* (Table 7.3). The major lipid class in all species was PL, present at a minimum of 84.4 ± 3.7% of total lipids in *S. australis* and a maximum of 89.4 ± 1.5% of total lipids in *Todarodes* spp. (Table 7.4). ST represented the only other lipid class with values greater than 1.5% of total lipid.

PUFA were the most abundant class of fatty acids in mantle tissue of all species, with sum values between 51.5 ± 2.5% and 58.1 ± 0.6% of total fatty acids in *S. australis* and *G. antarcticus* respectively (Table 7.5). PUFA were largely comprised of EPA (20:5n3) and DHA (22:6n3); no other PUFA were at values exceeding 5% of total fatty acids. SAT were dominated by 16:0 in all species, with the sum of SAT ranging from 19.9 ± 2.2 % (*G. antarcticus*) to 38.6 ± 2.4% (*S. australis*) of total fatty acids. MUFA comprised between 9.6 ± 0.9% and 21.8 ± 1.9% of total fatty acids in *S. australis* and *G. antarcticus* respectively, and were represented largely by the MUFA 20:1n9.

Table 7.1. Collection site, year of capture, sex and maturity stage of squids analysed in this study

Species	Site	Year	Maturity Stage										
			Females					Males					
			1	**2**	**3**	**?**	**total**	**2**	**3**	**4**	**5**	**?**	**total**
Sepioteuthis australis	E	2000	1	4			**5**	1	1	1	1		**4**
Gonatus antarcticus	A	1998-1999		2			**2**						
	B	1995			4	1	**5**						
Moroteuthis robsoni	C	2000			1		**1**				1	1	**2**
	D	2000			1		**1**					1	**1**
Todarodes spp.	A	1998-1999			1		**1**						
	B	1995			1		**1**						
		1998-1999			1		**1**						
		2000				1	**1**						
	D	2000			3		**3**				1		**1**
Total							**21**						**8**

A: Heard Island; B: Macquarie Island; C: Campbell Plateau; D: Chatham Rise; E: Great Oyster Bay, Tasmania. Maturity stages determined after Lipinski (1979).

Table 7.3. Total lipid content (% wet mass) of squid mantle and digestive gland tissue. Values are means ± SD. *= total lipid data unavailable for all samples, figures in parenthese indicate the number of mantle samples available for fatty acid analysis

		mantle		digestive gland
Species	*n*	% total lipids (wet mass)	*n*	% total lipids (wet mass)
Sepioteuthis australis	9	1.9 ± 0.6	8	6.6 ± 3.1
Gonatus antarcticus	5	1.6 ± 0.3	6	54.3 ± 9.1
Moroteuthis robsoni	3	0.8 ± 0.1*(5)	5	22.3 ± 8.0
Todarodes spp.	8	1.2 ± 0.2	7	29.7 ± 11.7

n : sample size.

Table 7.4. Percentage lipid class (of total lipids) of (a) mantle tissue and (b) digestive gland tissue in squid. Values are means ± SD. Only data from squid collected in 2000 are included

(a)		mantle					
Species	*n*	WE	DAGE	TAG	FFA	ST	PL
Sepioteuthis australis	9	0.1 ± 0 2	0 0 ± 0.0	0.6 ± 0.3	0.1 ± 0.2	14.9 ± 3.6	84.4 ± 3 7
Moroteuthis robsoni	5	0.0 ± 0.0	0 0 ± 0.0	0.4 ± 0.7	1.1 ± 0.7	11.0 ± 2.2	87 5 ± 2.8
Todarodes spp.	4	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.7	1.5 ± 1.2	8 8 ± 1.6	89 4 ± 1.5

(b)		digestive gland					
Species	*n*	WE	DAGE	TAG	FFA	ST	PL
Sepioteuthis australis	8	0.6 ± 0.6	0.0 ± 0.0	26.3 ± 25.6	8.2 ± 6.7	12.9 ± 7.9	52.0 ± 20 8
Moroteuthis robsoni	5	1.3 ± 0.7	5.0 ± 7.1	79.9 ± 6.3	3.8 ± 2.0	1.4 ± 0.4	8.6 ± 3.0
Todarodes spp.	4	3.2 ± 1.3	16.4 ± 16.1	42.3 ± 30.1	20.9 ± 23.4	5.0 ± 4.9	12.2 ± 9.1

n : sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol; FFA: free fatty acids; ST: sterol; PL: phospholipid.

7.4.2.2 Digestive gland

Lipid content of digestive gland tissue was highly variable between species, with a minimum content of 6.6 ± 3.1% in *S. australis* and a maximum content of 54.3 ± 9.1% wet mass in *G. antarcticus* (Table 7.3). Lipid class composition of the digestive gland was variable between- and also within-species, notably in *S. australis* and *Todarodes* spp. Neutral lipids represented the major lipid classes in all squid except some individuals of *S. australis*, where PL was the major lipid class (Table 7.4). TAG was the major neutral lipid in *M. robsoni* and some individuals of *S. australis* and *Todarodes* spp. In contrast, DAGE was the major lipid class in *G. antarcticus* contributing a mean value of 44.9 ± 6.8% to total lipid (unpublished data) and for remaining *Todarodes* spp. Large amounts of DAGE in the digestive gland have been previously reported for other gonatid squid (Hayashi 1989; Hayashi & Kawasaki 1990; Hayashi & Yamamoto 1987b), in addition to the onychoteuthid *M. robusta* (Hayashi et al. 1990). FFA were present in all species, with a minimum of 8.2 ± 6.7% of total lipids in *S. australis* and a maximum of 20.9 ± 23.4% of total lipids in *Todarodes* spp. Small amounts of WE were found in the digestive glands of all species.

In contrast to mantle tissue, MUFA were the major fatty acid class in the digestive glands of three species, the exception being *S. australis* where MUFA only contributed 19.0 ± 8.2% to total fatty acids (Table 7.6). Total MUFA values of the oegopsids were between 47.0 ± 11.7% and 66.1 ± 8.0% in *Todarodes* spp. and *G. antarcticus*, respectively and were represented largely by 18:1n9 and 20:1n9, in addition to 22:1n11 and 16:1n7 in *G. antarcticus*. PUFA were the major fatty acid class in *S. australis* with a sum value of 43.3 ± 4.5% of total fatty acids. PUFA comprised between 10.7 ± 2.6% and 23.1 ± 1.4% of total fatty acids in all other species, and were largely represented by EPA and DHA. Values of SAT fell between 10.7 ± 2.6% of total fatty acids in *G. antarcticus* and 37.0 ± 6.4% of total fatty acids in *S. australis*. Major SAT were 16:0, in addition to 18:0 in *S. australis*.

Table 7.5. Percentage fatty acids (of total fatty acids) in squid mantle tissue. All bonds are *cis* -oriented unless stated otherwise. Values are means ± SD

Fatty acid	*Sepioteuthis australis* *n* =9	*Gonatus antarcticus* *n* =5	*Moroteuthis robsoni* *n* =5	*Todarodes* spp *n* =8
14:0	2.7 ± 1.1	1.8 ± 0.4	1.3 ± 0.3	0.9 ± 0.3
15:0	0.8 ± 0.1	0.2 ± 0 0	0.3 ± 0.0	0.4 ± 0.1
16:0	28.0 ± 1.7	15 8 ± 1.7	20.5 ± 0.9	23.3 ± 2.7
17·0	1.2 ± 0.1	0.3 ± 0.0	0 6 ± 0.1	0.7 ± 0 2
18:0	5.8 ± 0.6	1 8 ± 0.2	3.7 ± 0.1	4.3 ± 0.7
20:0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
14:1n5	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0 0 ± 0.0
16·1n9	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:1n7	0.7 ± 0.1	1.4 ± 0.2	0.5 ± 0.1	0.3 ± 0 1
16:1n7t	0.1 ± 0 0	0.2 ± 0 0	0.1 ± 0.0	0.1 ± 0.0
16:1n5	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0 1 ± 0.0
18:1n9	2.9 ± 0 3	5.4 ± 0.7	3.7 ± 0.5	1.7 ± 0.5
18:1n7	1.4 ± 0.2	1 9 ± 0.9	1.5 ± 0.2	1.3 ± 0.2
18:1n5	0.1 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
19:1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
20 1n9	2.3 ± 0.2	6.6 ± 1.2	9.7 ± 0.2	7.0 ± 0.9
20:1n7	0.1 ± 0 0	0 1 ± 0 1	0.2 ± 0.1	0.1 ± 0.0
22:1n11	0.1 ± 0.0	0.6 ± 0.1	0.3 ± 0.1	0 5 ± 0.8
22:1n9	0.7 ± 0.1	2.4 ± 0.5	2 7 ± 0 5	1.4 ± 0.5
22:1n7	0.1 ± 0.0	0.3 ± 0.4	0.1 ± 0.0	0.1 ± 0.0
24:1n11/9	0.3 ± 0.1	1.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.4
18:2n6	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
20:4n6 (AA)	2.9 ± 0.5	2.0 ± 0.4	4.1 ± 0.8	1.1 ± 0.2
20:5n3 (EPA)	14.9 ± 0.9	15.6 ± 0.7	13.6 ± 0.9	14.2 ± 1.4
20:4n3	0.2 ± 0.0	0.2 ± 0.1	0 3 ± 0 1	0 1 ± 0 0
20:2n6	0.2 ± 0.0	1 2 ± 0 1	0.4 ± 0.0	0.3 ± 0.2
22:4n6	0.4 ± 0.3	0.1 ± 0.0	0.3 ± 0.0	0.0 ± 0.0
22:5n6	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0
C21 PUFA	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
22:6n3 (DHA)	30.8 ± 2.6	36.3 ± 0 6	32.2 ± 1.5	39.6 ± 5.3
22:5n3 (DPA)	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.1	0.4 ± 0.1
C23 PUFA	0.2 ± 0.3	0.8 ± 0.4	0.4 ± 0.3	0.3 ± 0.3
C24 PUFA	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Sum SAT	**38.6 ± 2.4**	**19.9 ± 2.2**	**26.5 ± 1.0**	**29.7 ± 3.4**
Sum MUFA	**9.6 ± 0.9**	**21.8 ± 1.9**	**20.3 ± 1.1**	**13.6 ± 2.9**
Sum PUFA	**51.5 ± 2.5**	**58.1 ± 0.6**	**53.0 ± 1.7**	**56.5 ± 6.1**

n : sample size; AA: arachidonic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA. eicosapentaenoic acid, MUFA. monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SAT: saturated fatty acids.

Table 7.6. Percentage fatty acids (of total fatty acids) in squid digestive gland tissue. Values are means ± SD

Fatty acid	*Sepioteuthis australis* *n* =8	*Gonatus antarcticus* *n* =6	*Moroteuthis robsoni* *n* =5	*Todarodes* spp. *n* =7
14:0	2.5 ± 2.0	3.1 ± 1.1	2.5 ± 0.3	1.8 ± 0.6
15.0	0.8 ± 0.1	0.1 ± 0.0	0.5 ± 0.1	0.4 ± 0.1
16:0	21.5 ± 4.6	5.0 ± 1.5	15.6 ± 1.0	12.7 ± 2.2
17:0	1.3 ± 0.5	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0 3
18:0	10.3 ± 3.2	1.7 ± 0.4	3.9 ± 0.5	4.1 ± 0.9
20:0	0.2 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
22:0	0.4 ± 0.3	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
14:1n5	0.3 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
16:1n9	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	1.2 ± 1.4
16:1n7	3.0 ± 2.4	6.4 ± 2.1	3.9 ± 0.4	2.8 ± 1.6
16:1n5	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
18:1n9	8.5 ± 5.1	27.2 ± 12 7	25.4 ± 3.1	18.9 ± 5.9
18:1n7	2.3 ± 0.8	5.9 ± 1.5	3.1 ± 0.4	3.2 ± 0.7
18:1n5	0.1 ± 0 1	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.1
19:1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
20:1n9	2.1 ± 0.9	13.3 ± 2.1	10.6 ± 1.6	10 7 ± 2 5
20:1n7	0.1 ± 0.1	0 7 ± 0.1	0.6 ± 0.1	0.7 ± 0.3
22:1n11	0.3 ± 0.3	5.9 ± 1.2	2 5 ± 0.8	3.3 ± 1 5
22:1n9	0.4 ± 0.3	2.6 ± 0.4	1.4 ± 0.2	1.8 ± 0.6
22:1n7	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.5	1.1 ± 2.8
24:1n11	0.7 ± 0.4	2.6 ± 0.7	0.4 ± 0.2	2 0 ± 1.1
C16 PUFA	0.3 ± 0.2	1.9 ± 2.9	0.1 ± 0 0	0.5 ± 0.8
18:3n6	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.3
18:4n3	0.9 ± 0.7	0.8 ± 0.4	0.5 ± 0.1	0.3 ± 0 1
18:2n6	1.1 ± 0.6	1.9 ± 0.5	0.7 ± 0.1	0.8 ± 0.1
20:4n6 (AA)	4.3 ± 1.2	0.8 ± 0.2	1.2 ± 0.3	1.2 ± 0.3
20:5n3 (EPA)	13.4 ± 3.3	5.8 ± 2.4	5.7 ± 0.9	7.9 ± 2.3
20:4n3	0.6 ± 0.4	0.8 ± 0.2	1.0 ± 0.1	0.9 ± 0.2
20:2n6	0.6 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.6 ± 0.3
22:4n6	0.6 ± 0.9	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
22.5n6	0.7 ± 0.6	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.2
C21 PUFA	0.2 ± 0 2	0.4 ± 0.1	0 2 ± 0.0	0.3 ± 0.1
22:6n3 (DHA)	18.6 ± 3.2	7.8 ± 3.0	13.0 ± 1.2	17.4 ± 8.1
22:5n3 (DPA)	1.3 ± 0.6	1.0 ± 0.3	1.4 ± 0.2	1.1 ± 0.3
C23 PUFA	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0 2 ± 0.1
C24 PUFA	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.1
Sum SAT	**37.0 ± 6.4**	**10.7 ± 2.6**	**23.1 ± 1.4**	**19.6 ± 2.8**
Sum MUFA	**19.0 ± 8.2**	**66.1 ± 8.0**	**50.3 ± 3.1**	**47.0 ± 11.7**
Sum PUFA	**43.3 ± 4.5**	**22.8 ± 6.6**	**24.9 ± 2.0**	**32.4 ± 10.6**

n : sample size; AA: arachidonic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SAT: saturated fatty acids.

7.5 DISCUSSION

7.5.1 Lipid composition of the digestive gland

The total lipid, lipid class and fatty acid composition of the digestive gland of the myopsid *S. australis* differed markedly from that of three oegopsid species (Fig. 7.2; Table 7.6). Lipid stored in the digestive gland is likely to be of dietary origin (Abolmasova et al. 1990; Blanchier & Boucaud-Camou 1984; Clarke et al. 1994; Semmens 1998), as opposed to other sources such as biosynthesis and fatty acid modification. Therefore, differences between the lipid content and composition of the digestive gland are potentially related to dietary differences between myopsid and oegopsid squid. In addition, modes of lipid storage may account for differences in total lipid and lipid class data. The digestive gland of certain loliginid species appears to excrete excess dietary lipid (Semmens 1998), which may explain the low but variable lipid composition of the digestive gland of *S. australis*. The lipid content and composition of digestive gland of the Loliginidae and other myopsids may be highly correlated with the time elapsed since the last meal, and also with the lipid class and fatty acid composition of the last meal.

In contrast, the oegopsid species included in this study were found to contain large amounts of lipid in the digestive gland. In general, the digestive gland of other oepopsids such as the enoploteuthid *Watasenia scintillans* (Berry, 1911), the gonatid *Gonatopsis borealis*, the ommastrephids *Berryteuthis magister*, *Illex argentinus*, *Illex illecebrosus* and *Sthenoteuthis oualaniensis* (Lesson, 1830) and the onychoteuthids *Moroteuthis ingens* and *Moroteuthis robusta* contains at least 20% lipid (wet weight) and sometimes up to 60% lipid (Clarke et al. 1994; Hayashi 1989; Hayashi et al. 1990; Hayashi & Yamamoto 1987a; Jangaard & Ackman 1965; Kawasaki et al. 1994; Phillips et al. 2001; Shchepkin et al. 1982; Wako et al. 1993). Thus the digestive gland of oegopsid squid is likely to accumulate lipid from many meals. The function of lipid stored in the digestive gland of oegopsids is not clear, although it may enhance buoyancy (Clarke et al. 1979). Regardless, the physiological processes of digestive gland lipid storage differ considerably between myopsid and oegopsid squid.

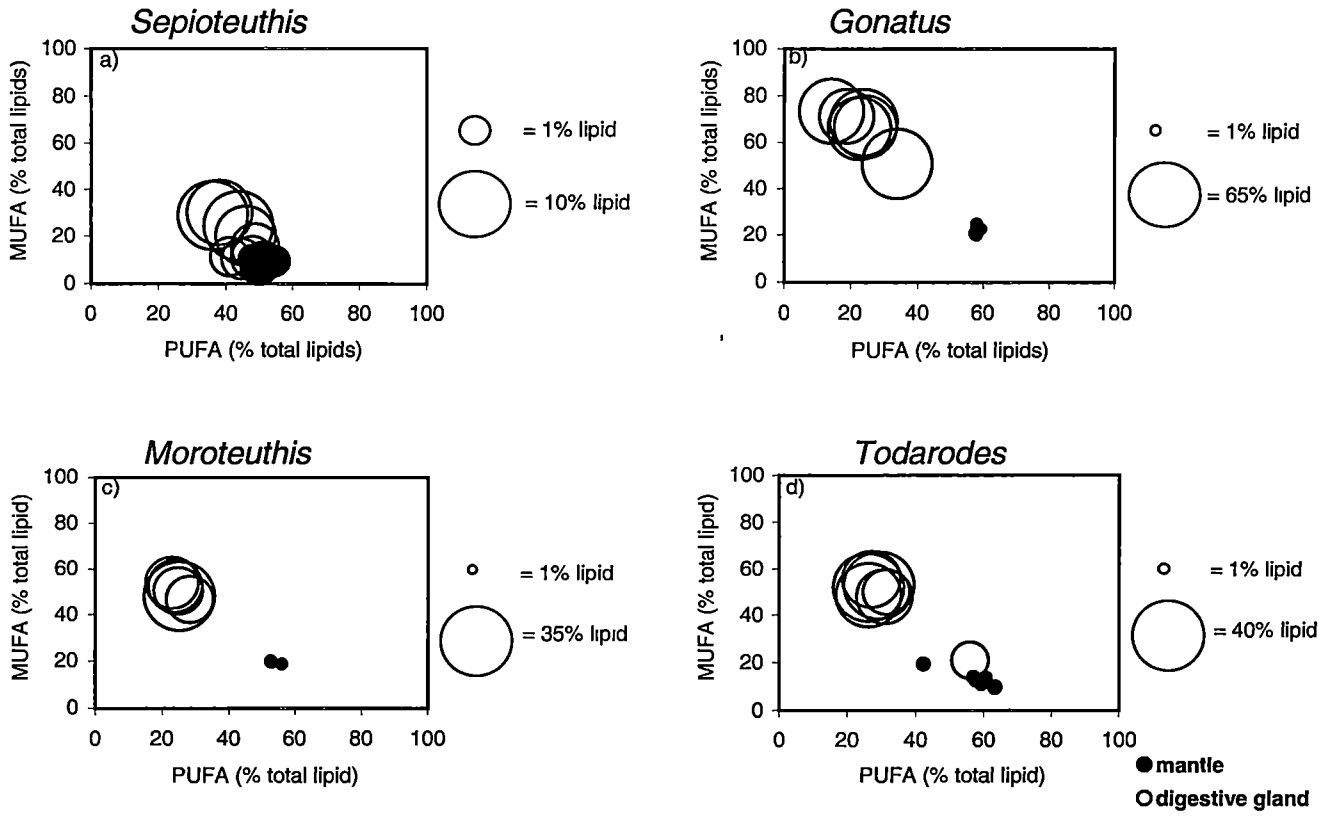

Fig. 7.2. Bubble-plot of MUFA and PUFA content (% of total lipids) and total lipid content (% wet mass) of mantle and digestive gland tissue of a) *Sepioteuthis australis*, b) *Gonatus antarcticus*, c) *Moroteuthis robsoni* and d) *Todarodes* spp. Bubble size represents total lipid content. Note that the scale of bubble size is specific for each species.

7.5.2 Dietary influence on the fatty acid composition of the digestive gland

With the exception of one individual of *Todarodes* spp., lipid deriving from the digestive gland of oegopsid species was composed of large amounts of MUFA. MUFA are the dominant fatty acids in myctophid fish, which are likely to be common prey items of the three oegopsid species (Kock 1987; Lubimova 1985; Rodhouse & White 1995; Rodhouse et al. 1992), and thus contribute large amounts of MUFA to digestive gland lipid content. The fatty acid content of myctophids is quite unusual, as the fatty acid profiles of other marine fish generally contain higher levels of PUFA (Saito & Murata 1998). In contrast, the digestive gland lipid content of *S. australis* is characterised by relatively abundant PUFA, and this may reflect that a different spectrum of fish prey is available to this coastal, inshore species. Unfortunately, little dietary data are currently available for *S. australis*.

Comparisons drawn between the fatty acid composition of stomach contents and the digestive gland of other squid species have established that lipid in the digestive gland is very likely to derive from the diet with little or no modification prior to deposition (Hayashi et al. 1990; Phillips et al. 2001). While fatty acid data from squid stomach contents can provide an "instantaneous snapshot" of prey lipid composition, digestive gland lipid content has the power to provide a history of prey lipids consumed over time. This is particularly so in oegopsid species such as *G. antarcticus*, *M. robsoni* and *Todarodes* spp., where total lipid content of the digestive gland is very high and represents accumulation of dietary lipids over a substantial period of time. This technique has many future applications to dietary studies of squid, and may be particularly useful when it appears that squid have fed in sampling gear so that stomach contents do not represent normal predatory behaviour (Rodhouse & Nigmatullin 1996). Fatty acid analysis of digestive gland fatty acids has a major advantage over other complementary techniques of squid diet analysis, such as serological analysis of stomach contents (Kear 1992) or genetic identification of prey remains (Jarman et al. 2002), as these techniques can only provide information on stomach contents over the short-term and are biased by the effects of net-feeding. In addition, fatty acid analysis of the digestive gland can also be used even when a squid stomach is empty.

7.5.3 The application of squid fatty acid profiles to dietary studies of higher predators

The same properties that define the usefulness of digestive gland lipids to squid biologists may also impede the application of squid fatty acid profiles to dietary studies of higher predators. This is particularly so when the total lipid content of the digestive gland is considered. In this study, the mantle of *S. australis* was found to be 10.3 to 19.0 times the mass of the digestive gland (Table 7.2), and the lipid content of the digestive gland was relatively low. For individual specimens of *S. australis*, the absolute lipid content of the mantle is greater than that of the digestive gland (Fig. 7.3). Conversely, the lipid content of the digestive gland of the other three species was an order of magnitude greater than that of mantle tissue. The ratio of the mass of mantle to digestive gland was also much smaller in these species, with the mantle only several times the mass, or in the case of *G. antarcticus*, less than the mass of the digestive gland (Table 7.2). Therefore the absolute lipid content of the digestive gland of *G. antarcticus*, *M. robsoni* and *Todarodes* spp. greatly exceeds that of the mantle (Fig. 7.3) (large error bars for *M. robsoni* and *Todarodes* spp. in Fig. 7.3 result not only from variability in digestive gland mass, but also from fluctuating lipid content of the digestive gland). Therefore, an important feature is that fatty acids in the digestive gland - derived from oegopsid squid prey - are in greater absolute abundance than fatty acids in the mantle tissue. A predator of oegopsid squid would ingest more lipid from secondary prey items (as stored in the digestive gland of the squid) than from the mantle tissue of the squid itself.

Although other flesh tissue such as the arms, tentacles and fins would add to the absolute amount of lipid ingested from mantle tissue, data from Nash et al. (1978) and Vleig (1984) suggest that the total lipid and fatty acid content of these tissues would not vary greatly from that of mantle tissue. Therefore the lipid content of the digestive gland is still likely to exceed the lipid content of the mantle, head, tentacles and fins combined. Data from whole, homogenised squid were not available from this study, however other unpublished analyses of homogenised specimens of *G. antarcticus* have been completed (Wilson 2003). The total lipid content and fatty acid composition of whole homogenised

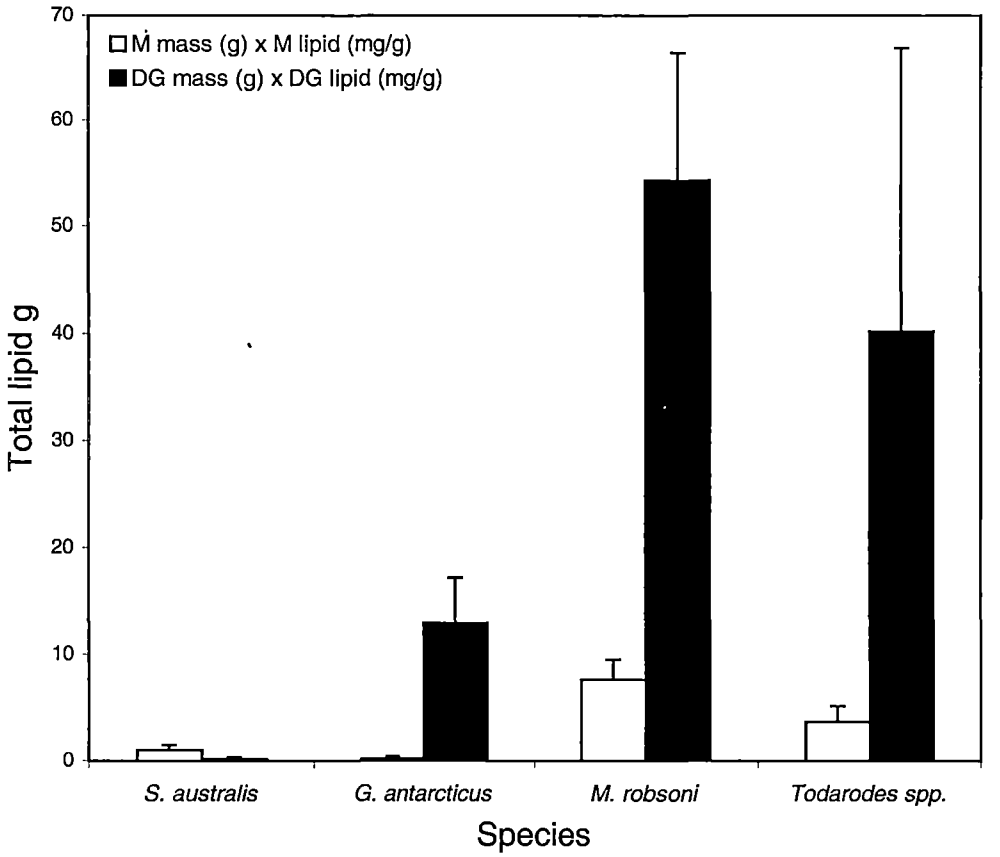

Fig. 7.3. Mean calculated total lipid content of the entire mantle and digestive glands of *Sepioteuthis australis*, *Gonatus antarcticus*, *Moroteuthis robsoni* and *Todarodes* spp. Error bars are standard deviations. M: mantle, DG: digestive gland.

squid is similar to that of the digestive gland, while the total lipid content and fatty acid composition of mantle tissue is distinctly different.

In the context of dietary lipid studies of teuthophagous predators, blubber, milk and muscle samples from a number of higher predators have been analysed with the aim of identifying major prey groups (Horgan & Barrett 1985; Iverson et al. 1997; Iverson 1993; Raclot et al. 1998; Smith et al. 1997). However, when squid data have been included in these analyses, it is often unclear whether fatty acid data were obtained from whole homogenised squid, flesh tissue only, or from squid remains retrieved from the stomach contents of a predator (Y. Cherel, personal communication). If squid data is low in total lipid content (around 1% wet mass) and dominated by PUFA (Iverson 1993, Iverson et al. 1997), it is likely to have been extracted from flesh tissue only. Based on the findings for three species of Southern Ocean oegopsids presented in this study, squid flesh data alone is not suitable for inclusion in these analyses. Such data does not represent the lipid composition of a squid as ingested by a predator, and consequently it is highly likely that squid will be interpreted as having little importance in the diet.

When whole, homogenised squid are used to represent potential prey items in fatty acid studies of higher predators, it will be important to consider the large amount of "secondary" fatty acids stored in the digestive gland. Squid may not be effectively represented as a distinct prey group in analyses as their lipid signature may be very similar to (or in the case of lipid-rich species, masked by) other potential prey items such as myctophid fish. Therefore, the dietary importance of squid as a prey group may be difficult to interpret and isolate from other prey groups. Furthermore, results from Chapter 6 suggest that the total lipid content and fatty acid profile of the digestive gland of squid changes considerably with increasing size. Ideally, then, squid fatty acid profiles should be obtained from animals that are of the same size class as those consumed by the higher predator in question. Given that squid obtained from research nets and other sampling gear are usually significantly smaller than those consumed by higher predators (Clarke 1983), it may be difficult from the outset to obtain squid that are appropriate for inclusion into such predator-prey studies.

These implications could constrain the use of fatty acids to assess the importance, or inclusion over space and time, of squid prey items in the diet of higher predators. Given the fact that our general knowledge of squid trophodynamics in the Southern Ocean is poor, it is important to identify and attempt to understand such biases associated with food-web studies. A combination of techniques, such as fatty acid analysis of blubber or muscle, and DNA analysis of stomach contents or faecal remains, may provide a more robust representation of the inclusion of squid in the diets of higher predators.

7.6 CONCLUSIONS

Marked differences were observed in the total lipid, lipid class and fatty acid content between the myopsid squid *S. australis*, and three oegopsid species *G. antarcticus*, *M. robsoni* and *Todarodes* spp. Large amounts of dietary lipid in the digestive gland of oegopsid species have two important implications for the use of fatty acids as dietary tracers in food-webs associated with squid:

V. This technique has a promising future for applications to dietary studies of oegopsid squid, as digestive gland lipid content may provide a history of prey fatty acids consumed over a period of time and thus eliminate biases associated with instantaneous sampling of diet, and;

VI. Due to the abundance of prey lipids in the digestive gland of oegopsids, (that exceed mantle lipid content by more than an order of magnitude), it is extremely important to consider the type of squid fatty acid data included in dietary studies of higher predators. Squid may not be identifiable as a separate group in such analyses, but may be grouped with major prey species such as myctophid fish. Thus food-web studies based on fatty acid analyses become considerably more difficult to interpret in higher trophic levels.

ADDENDUM

The research within the original manuscript and this resultant Chapter was conducted by myself. Co-authors G. D. Jackson and P. D. Nichols fulfilled supervisory roles as my Ph. D. supervisors at IASOS, University of Tasmania, and provided constructive criticism on the text. C. Sands is acknowledged for his assistance with squid dissections.

Chapter 8. Summary and recommendations

8.1 DIETARY COMPOSITION OF *MOROTEUTHIS INGENS*

Moroteuthis ingens conforms to almost all the patterns in feeding behaviour that are typical of squid. It consumes large amount of crustaceans as a juvenile, then begins to incorporate greater proportions of fish into the diet with increasing size. It is, however, an inherent opportunist that is capable of capturing a wide variety of prey throughout its lifetime, so that adult specimens can continue to exploit crustacean prey whenever possible. *M. ingens* is also able to adapt to temporal and spatial variations in food resources. Undoubtedly, myctophids contribute a large proportion to the diet of *M. ingens* throughout much of its life cycle and distribution. It also exerts a degree of cannibalism on conspecifics, and preys upon many other species of cephalopod including sepiolids and octopus. *M. ingens* is a voracious predator and can consume a large number of prey items at a time: on one occasion, the remains of at least 89 fish were retrieved from a single stomach, whilst another individual had consumed at least 14 squid. In summary then, this squid is perhaps best described by the words of Boyle and Boletsky (1996) as a predator that "opportunistically [exploits] the most abundant and available prey".

In the context of the ecology of the sub-Antarctic Southern Ocean, *M. ingens* must place a large amount of predatory pressure on mesopelagic fish populations, particularly myctophids. This in itself is not a novel finding. Whilst providing no specific details, Lubimova (1985) and Kock (1987) have clarified that, in general, squid distributed within the Southern Ocean are consumers of mesopelagic fish, not zooplankton such as *Euphausia* spp. The most widely acknowledged dietary study to demonstrate this was conducted by Rodhouse et al. (1992), who confirmed that the ommastrephid *Martialia hyadesi* consumed large amounts of myctophids in the Scotia Sea. This lead to a subsequent and important publication that redescribed the ecological niche of squid within the Southern Ocean, at least within the epipelagic zone (Rodhouse & White 1995). However, these hypotheses have not yet become established in more general syntheses of the Southern Ocean ecosystem, and squid are still often depicted to be

zooplankton feeders in ecosystem models (for example, see He & Furlani [2001] and Knox [1994]). This study has provided further evidence that squid within the Southern Ocean are largely piscivorous, and it is important that this information becomes more widely incorporated into ecological models of the Southern Ocean.

8.2 STOMACH CONTENTS

In total, at least 55 prey species were identified in the stomach contents of *M. ingens* during the course of this study. I was therefore able to establish the diverse nature of the diet of *M. ingens* from stomach contents analyses, and also obtain some information on the size-range of prey. However, one of the greatest obstacles that I encountered was the incorporation of stomach contents data into statistical comparisons, for example to examine variations in diet over time or between sites. Due to the diversity of the diet, it was only possible to compare the relative proportions of a handful of species between groups. The contributions of many prey species had to be ignored as they were simply absent from all but one group. That is, it was necessary to impose arbitrary selection criteria in an attempt to reduce group data sets into a comparable format – selection criteria that may have been insensitive to real trends in dietary variation.

An associated problem was that the number of prey species identified in a sample of stomachs (eg stomachs from one particular site) was directly related to the number of stomachs within that sample. Therefore, I suggest that it will be important to obtain approximately equal sample sizes before undertaking future studies of temporal and spatial variations in the diet of Southern Ocean squid. It will also be important to obtain sample sizes that are large enough to provide a robust representation of the diet - perhaps at least one hundred stomachs per subset would be required (see Fig. 5.3). Such numbers are, of course, very difficult (if not impossible) to obtain on an opportunistic basis from the by-catch of commercial fisheries or other activities. Therefore, targeted collections of these animals will be required to improve upon studies of temporal and spatial variations in the diet of *M. ingens* and other Southern Ocean squid.

8.3 LIPID AND FATTY ACID ANALYSES

Lipid and fatty acid analysis of the digestive gland has provided an effective complementary method for the dietary analysis of *M. ingens*. This is due to the large amount of lipid stored within this organ that is unmodified from the diet. Fatty acid profiles have proved to be particularly useful when investigating temporal and spatial variations in diet: that is, in those instances when variations in diet were difficult to determine statistically using stomach contents data. Dietary patterns could be robustly determined by multivariate analyses of fatty acid dietary tracers. In contrast, such dietary patterns are unlikely to be resolved using serological or genetic techniques that aim to identify the presence of singular prey species in the diet. Thus, fatty acid profiles from the digestive gland provide a unique suite of tools that have many future applications to dietary studies of squid.

Dietary assessment from digestive gland fatty acid profiles does, of course, have its limitations. Currently, it is not possible to quantify the proportion of individual prey species in the diet using this technique. Interpretation of dietary patterns must also be completed with some caution. It is important to consider several parameters when including data from potential prey species into comparisons of fatty acid profiles: a) that prey species were collected from the same location as the squid species in question; b) that the prey were within the size-range that is likely to be consumed by the squid, and c) potentially, that the prey were collected during the same period as the squid. Temporal, spatial and size-related variations in lipid and fatty acid composition have not only been recorded for *M. ingens* but also for other pelagic organisms. Therefore, it will be important that such factors are accounted for within a food-web study incorporating fatty acid dietary tracers.

Two approaches may be adopted in the immediate future to further improve the application of fatty acid dietary tracers to trophic studies of squid. Firstly, laboratory trials would significantly improve our understanding of the uptake, storage and turnover of dietary lipid in the digestive gland of squid. It is important to stress, however, that such physiological processes may differ considerably between different groups of squid, particularly between myopsids and oegopsids. While such experiments are likely to be

more successful with myopsid species eg from the loliginid family, it will be also important to attempt such experiments with oegopsid species.

Secondly, temporal, spatial and size-related variations in the prey of squid such as *M. ingens* are still poorly understood, despite several important studies such as those by Saito & Murata (1998) and Lea et al. (2002). A more thorough understanding of the lipid biochemistry of lower trophic levels will improve the incorporation of prey fatty acid profiles into dietary studies of squid, and provide for more robust interpretations of predator-prey relationships.

Finally, it is also important to carefully consider the modes of lipid storage in squid before cephalopod fatty acid data can be effectively included into dietary studies of higher predators – the rich source of "secondary" fatty acids stored in the digestive gland may obscure a unique squid signature in multivariate comparisons of fatty acid profiles. Effective application of squid fatty acid data to such studies will also require that squid are of the same size class as those consumed by the predator in question, as I have demonstrated that the fatty acid profiles of squid may vary considerably with size.

In conclusion, the combined use of stomach contents and digestive gland fatty acid analyses presents a powerful method that can improve our understanding of the diet and trophic ecology of Southern Ocean squid, whilst minimising the effects of instantaneous biases such as feeding in sampling gear. This combined approach has the potential to provide important trophic information on a key component of the Southern Ocean ecosystem, which has been previously considered to be somewhat of a "black box" in ecological models. In the future, stomach contents and fatty acid analyses may be used to further elucidate the trophic niches of squid in other regions around the world.

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Appendix I. Additional biological data for *Moroteuthis ingens* from various locations in the Southern Ocean

The data included in this appendix were collected by myself during the course of my Ph. D. study. At various stages, I was assisted by J. Finn, C. Sands, B. McGrath and T. McArthur. Data are from specimens of *Moroteuthis ingens* collected from Heard Island, Macquarie Island, the Chatham Rise (New Zealand) and the Campbell Plateau (NZ).

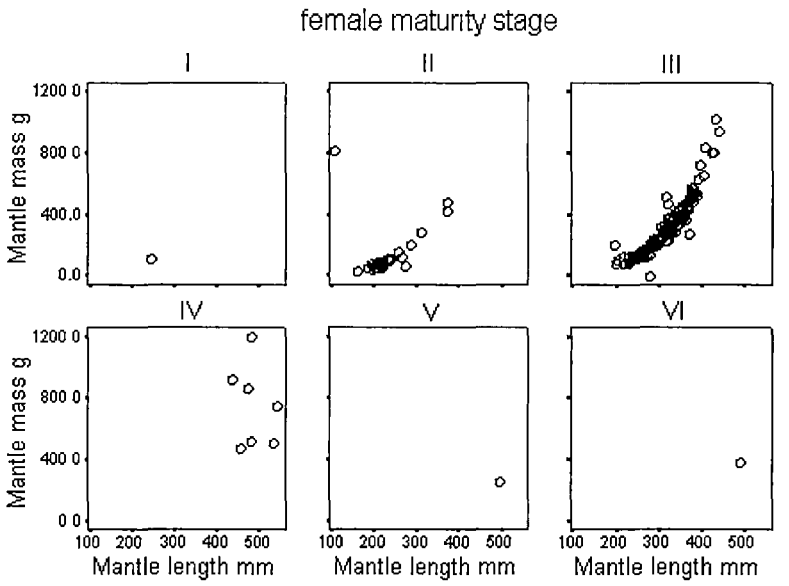

Fig. Ia. Mantle mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

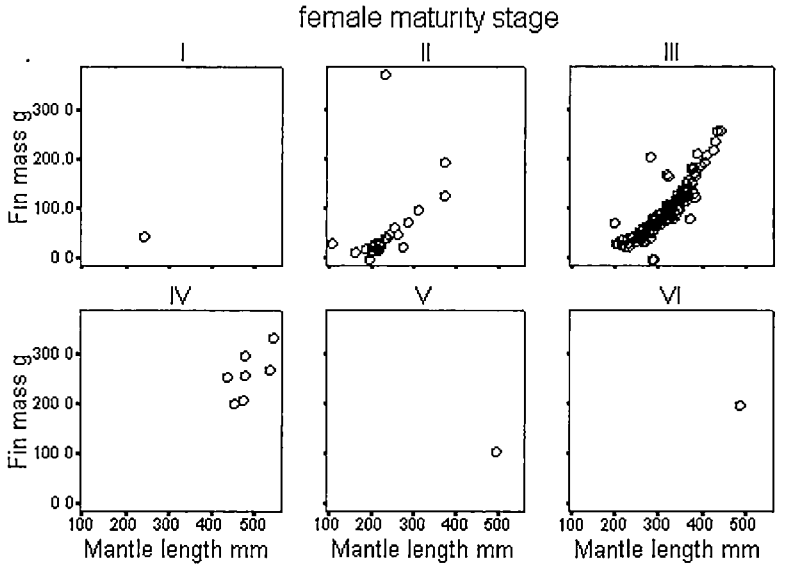

Fig. Ib. Fin mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

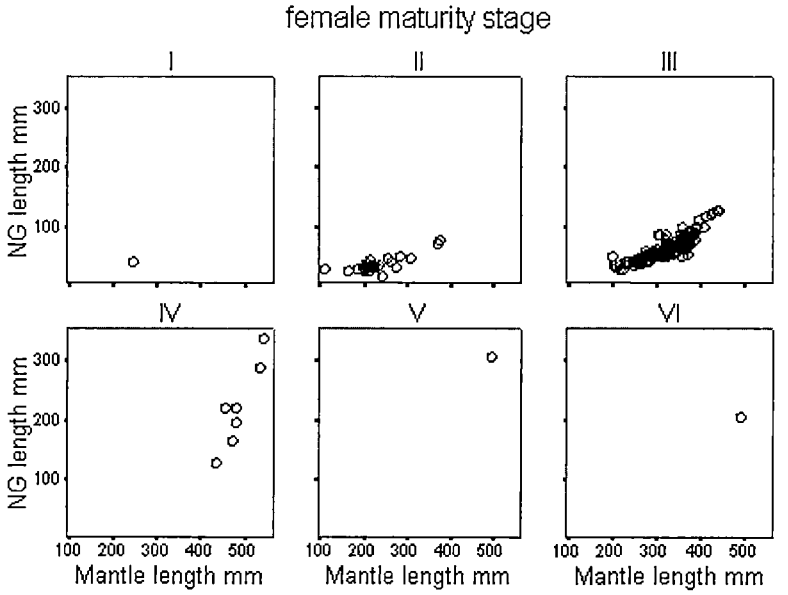

Fig. Ic. Nidamental gland (NG) length versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

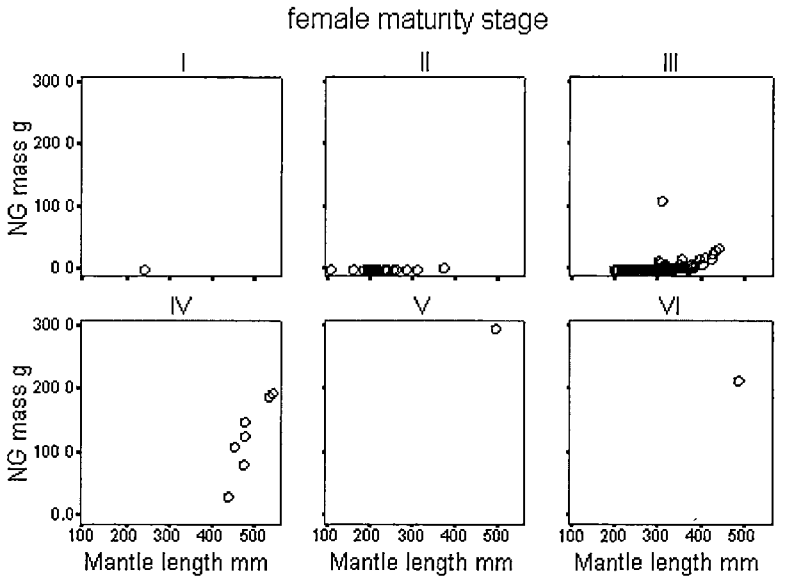

Fig. Id. Nidamental gland (NG) mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

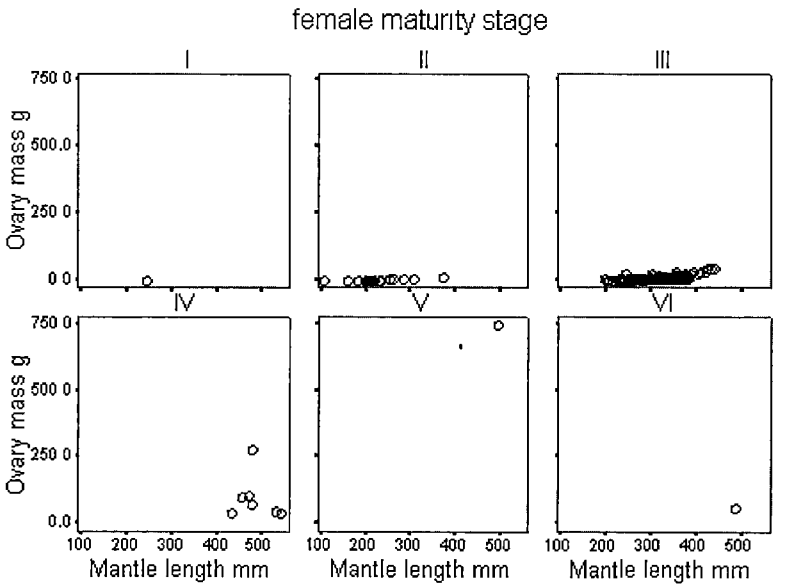

Fig. Ie. Ovary mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

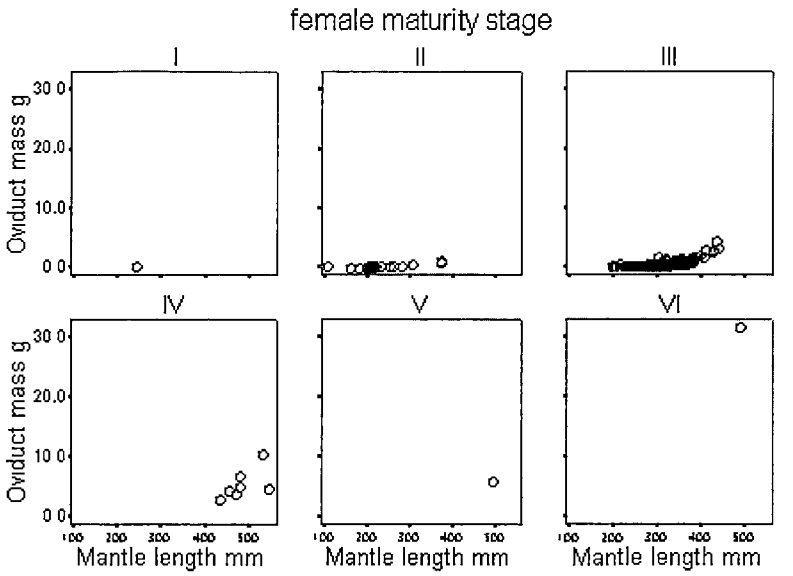

Fig. If. Oviduct mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean

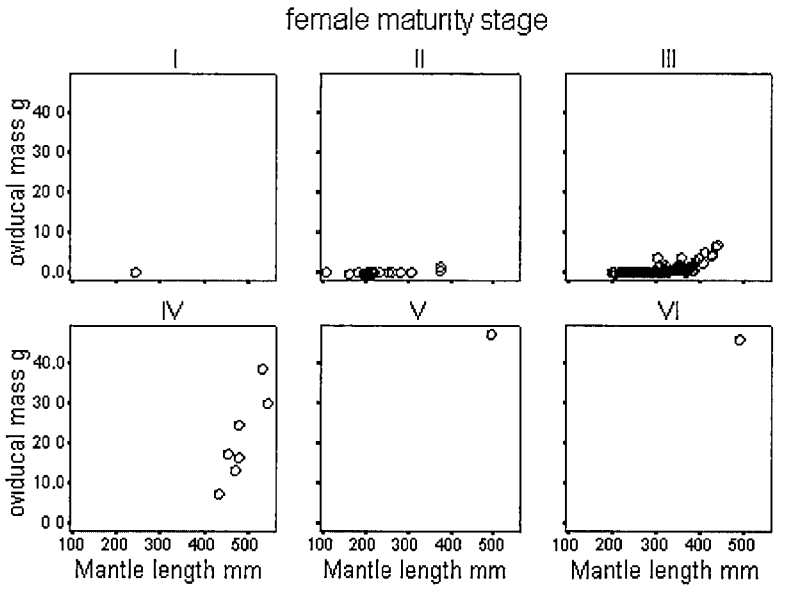

Fig. Ig. Oviducal mass versus mantle length for female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

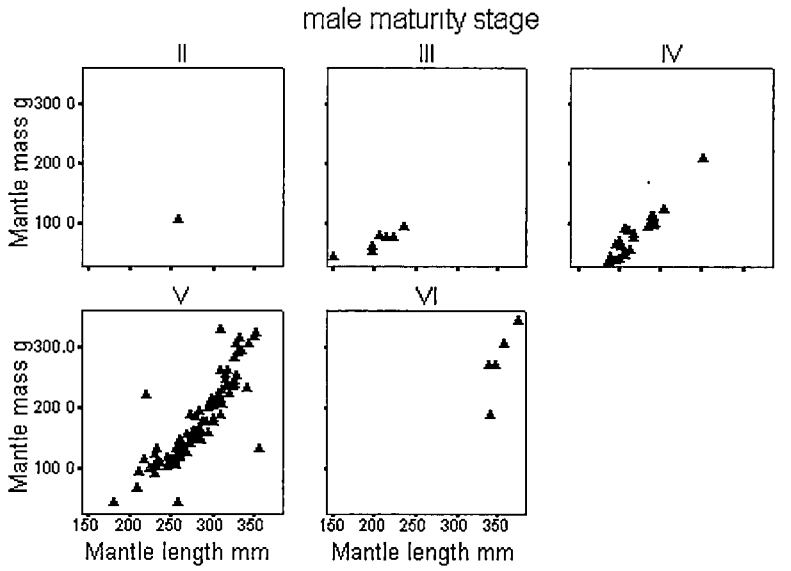

Fig. Ih. Mantle mass versus mantle length for male specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

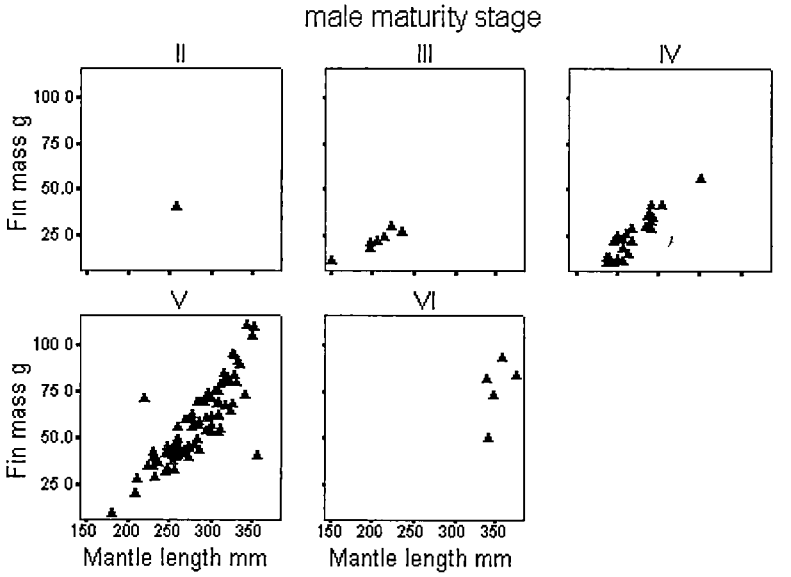

Fig. Ii. Fin mass versus mantle length for male specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

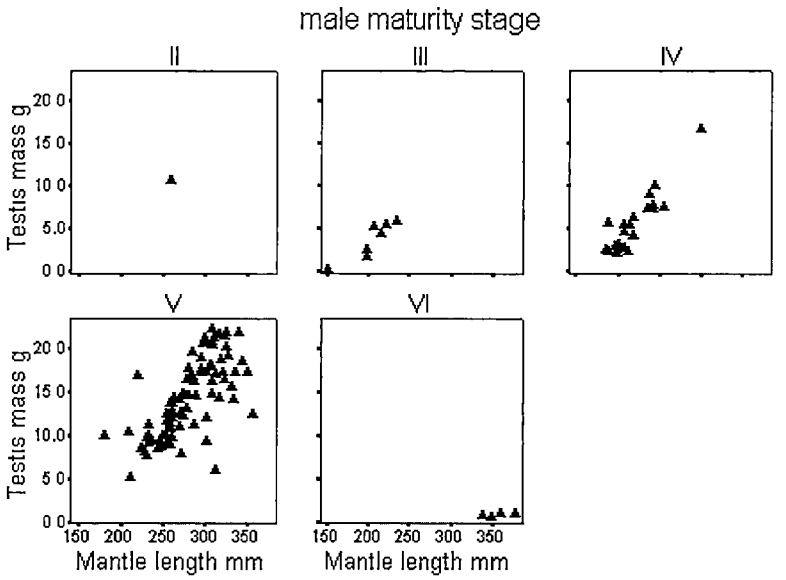

Fig. Ij. Testis mass versus mantle length for male specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

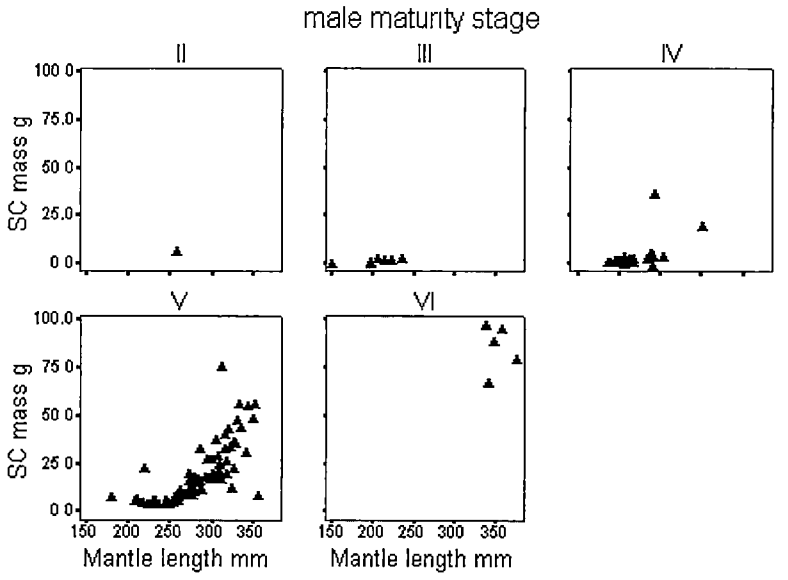

Fig. Ik. Spermatophoric complex (SC) mass versus mantle length for male specimens of *Moroteuthis ingens* from various locations in the Southern Ocean.

Table Ia. Biological data collected from female specimens of *Moroteuthis ingens* from various locations in the Southern Ocean

Maturity stage	*n*	min	max	mean ± SD
Mantle length mm				
I	1	249		
II	36	114	379	231.0 ± 49.9
III	139	203	446	319.7 ± 52.3
IV	7	440	549	491.0 ± 39.9
V	1	500		
VI	1	495		
Mantle mass g				
I	1	115.0		
II	36	4.1	826.0	136.7 ± 153.8
III	138	82.0	1030.4	323.0 ± 179.9
IV	7	477.5	1211.8	757 6 ± 271.3
V	1	262 0		
VI	1	394.1		
Fin mass g				
I	1	45.8		
II	35	15.3	195.0	40.2 ± 36.3
III	137	26.2	262.3	101.3 ± 51.3
IV	7	205.5	336.6	264.4 ± 46.0
V	1	106.8		
VI	1	202.5		
Nidamental gland length mm				
I	1	42		
II	36	18	81	39 ± 12
III	136	32	131	65 ± 21
IV	7	132	342	225 ± 72
V	1	311		
VI	1	208		

Maturity stage	*n*	min	max	mean ± SD
Nidamental gland mass g				
I	1	0.3		
II	34	0.1	4.2	0 6 ± 0.9
III	135	0.2	109.1	4.3 ± 10.5
IV	7	32.4	195.0	126.7 ± 58.1
V	1	295.6		
VI	1	215.4		
Ovary mass g				
I	1	0.1		
II	30	0.3	14 2	2.3 ± 3.3
III	134	1.5	44.7	9 8 ± 8.7
IV	7	39.1	276.3	97.3 ± 83 2
V	1	745 3		
VI	1	60.5		
Oviduct mass g				
I	1	0.1		
II	31	0.0	1.0	0.1 ± 0 1
III	125	0 1	4.3	0.6 ± 0.4
IV	7	2 9	10.6	5.5 ± 2.5
V	1	5.9		
VI	1	31.8		
Oviducal mass g				
I	1	0.1		
II	32	0.0	1.5	0.1 ± 0.1
III	132	0.0	7.2	0 8 ± 1.0
IV	7	7.5	39 3	21.5 ± 10.9
V	1	47.8		
VI	1	46.5		

Table Ib. Biological data collected from male specimens of *Moroteuthis ingens* from various locations in the Southern Ocear

Maturity stage	*n*	min	max	mean ± SD
Mantle length mm				
II	1	261		
III	7	152	237	205.4 ± 27.1
IV	25	189	303	221.6 ± 26.2
V	92	182	550	284.2 ± 37.5
VI	5	340	378	354.6 ± 15.3
Mantle mass g				
II	1	112.1		
III	7	49.4	100.9	75.6 ± 17.8
IV	25	41.4	214.5	85.6 ± 38.0
V	92	50.5	335.7	183.0 ± 66.0
VI	5	195.1	348.2	281.9 ± 56.7
Fin mass g				
II	1	43.0		
III	7	13.6	32.2	24.2 ± 6.0
IV	25	12.9	58.1	27.4 ± 11.7
V	89	10.7	112.2	58.4 ± 19.8
VI	5	51.6	95.5	78.1 ± 16.6
Testis mass g				
II	1	11.2		
III	7	0.8	6.3	4.1 ± 2.1
IV	24	2.5	17.1	6.2 ± 3.4
V	87	5.6	22.7	14.6 ± 4.4
VI	4	0.9	1.5	1.3 ± 0.3
Spermatophoric complex mass g				
II	1	7.4		
III	7	1.0	3.7	2.4 ± 1.1
IV	24	1.4	37.2	5.4 ± 7.8
V	90	4.9	76.9	19.6 ± 14.8
VI	5	68.8	98.2	86.8 ± 12.3

Appendix II. Lipid class and fatty acid data from mature and maturing female specimens of *Moroteuthis ingens* from the Chatham Rise, New Zealand

The following data were compiled by myself during the course of my Ph. D. study. C. Sands is acknowledged for his assistance with the dissection of squid.

Table IIa. Total lipid content (% wet mass) and lipid class composition (% of total lipid) of mantle and digestive gland of mature and maturing (stages IV - VI) female specimens of *Moroteuthis ingens* from the Chatham Rise, New Zealand

	Mantle *n* = 8				**Digestive gland *n* = 7**			
% composition	AVE ± SD		min	max	AVE ± SD		min	max
total lipid	1.7 ± 0.4		1.2	2.2	14.4 ± 7.8		3.2	27.0
WE					16.2 ± 20.4		3.0	59.0
DAGE					0.9 ± 1.8		0.0	4.8
TAG					3.2 ± 7.2		0.0	19.3
FFA	3.3 ± 1.5		1.6	5.8	25.7 ± 32.6		1.3	69.4
ST	12.9 ± 5.1		5.2	18.7	30.7 ± 28.2		0.8	63.3
PL	83.7 ± 5.5		76 6	92.5	23.4 ± 12.8		4.3	39.8

n : sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol, FFA: free fatty acid; ST: sterol; PL: phospholipid.

Table IIb. Fatty acid composition (% of total fatty acids) of the mantle and digestive gland of mature and maturing (stages IV - VI) female specimens of *Moroteuthis ingens* from the Chatham Rise, New Zealand

FATTY ACID	Mantle *n* = 8	Digestive gland *n* = 7
14:0	0.7 ± 0.2	1.3 ± 0.7
15:0	0.2 ± 0.1	0.2 ± 0.1
16:0	20.7 ± 1.5	10.5 ± 3.8
17:0	0.6 ± 0.1	0.7 ± 0.5
18:0	3.7 ± 1.5	4.4 ± 1.1
a15:0	0.0 ± 0.0	0.3 ± 0.5
br17:1	0.0 ± 0.0	0.4 ± 0.4
16:1n9c	0.1 ± 0.1	0.7 ± 0.3
16:1n7c	0.3 ± 0.2	0.8 ± 0.5
17:1	0.1 ± 0.0	0.2 ± 0.1
18:1n9c	3.6 ± 1.4	27.0 ± 9.0
18:1n7c	1.4 ± 0.2	3.4 ± 0.7
18:1n5c	0.3 ± 0.0	0.3 ± 0.2
20:1n9c	8.1 ± 1.0	15.4 ± 3.2
20:1n7c	0.1 ± 0.0	0.4 ± 0.3
22:1n11c	0.5 ± 0.2	2.5 ± 1.4
22:1n9c	4.2 ± 1.4	2.2 ± 0.6
22:1n7c	0.7 ± 0.9	0.3 ± 0.2
24:1	1.8 ± 0.7	2.6 ± 1.5
18:2n6c	0.1 ± 0.0	0.7 ± 0.5
20:4n6 (AA)	3.5 ± 0.8	3.6 ± 2.7
20:5n3 (EPA)	14.3 ± 0.7	8.7 ± 4.2
20:3n6c	0.0 ± 0.0	0.3 ± 0.2
20:4n3c	0.3 ± 0.6	0.6 ± 0.4
20:2n6c	0.7 ± 0.1	0.9 ± 0.5
22:4n6c	0.2 ± 0.1	0.1 ± 0.1
22:5n6c	0.2 ± 0.0	0.2 ± 0.1
22:6n3 (DHA)	31.2 ± 3.4	9.9 ± 4.5
22:5n3 (DPA)	0.7 ± 0.0	0.7 ± 0.4
C23 PUFA	0.8 ± 0.3	0.1 ± 0.1
SUM SAT	**26.0 ± 1.7**	**17.1 ± 4.2**
SUM MUFA	**21.6 ± 4.0**	**55.9 ± 11.6**
SUM PUFA	**52.2 ± 3.8**	**25.9 ± 9.8**

n : sample size; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

APPENDIX III. Lipid class and fatty acid data from other Southern Ocean squid species

These data were compiled by myself during the course of my Ph. D. study. C. Sands is acknowledged for his assistance with the dissection of squid. Data from this appendix have contributed to the following publication:

Bradshaw CJA, Hindell MA, Best NJ, Nichols PD, Phillips KL, Wilson G (2003) You are what you eat: estimating diet structure of southern elephant seals (*Mirounga leonina*) using blubber fatty acids. *Philosophical Transactions of the Royal Society of London* 270:1283-1292.

Table IIIa. Total lipid content (% wet mass) and lipid class composition (% of total lipids) of the mantle tissue of some Southern Ocean squid species

	Onychoteuthidae			
	Kondakovia longimana	*Moroteuthis knipovitchi*	*Moroteuthis*	Onychoteuthid spp.
% composition	AVE ± SD	AVE ± SD	*robsoni*	AVE ± SD
n	1	2	1	2
total lipid	1.76	1.6 ± 0.4	2.00	1.3 ± 0.8
n	2	2	1	2
WE	0.3 ± 0.4	0.0 ± 0.0	0.0	0.7 ± 1.0
DAGE	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
TAG	1.6 ± 2.3	0.3 ± 0.4	0.0	3.1 ± 2.2
FFA	0.6 ± 0.9	23.2 ± 6.2	28.3	0.0 ± 0.0
ST	9.1 ± 1.6	11.6 ± 0.6	13.8	7.2 ± 0.3
PL	88.3 ± 0.3	65.0 ± 6 0	57.9	89.0 ± 3.5

	Psychroteuthidae	**Brachioteuthidae**	**Ommastrephidae**	**Cranchiidae**
	Psychroteuthis	*Brachioteuthis*	*Martialia*	*Mesonychoteuthis*
% composition	*glacialis*	spp.	*hyadesi*	*hamiltoni*
n	1	1	1	1
total lipid	4.4	1.5	1.1	0.5
n	1	1	1	1
WE	0.1	0.0	0.0	0.0
DAGE	0.0	0.0	0.0	0.0
TAG	0.0	0.0	0.0	5.3
FFA	25.9	24.9	1.5	0.0
ST	10.8	16.2	8.8	8.1
PL	63.1	58.9	89 6	86.6

n : sample size; WE: wax ester; DAGE: diacylglyceryl ether; TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid.

Table IIIb. Total lipid content (% wet mass) and lipid class composition (% of total lipids) digestive gland of some Southern Ocean squid species

	Onychoteuthidae			
	Kondakovia longimana	*Moroteuthis*	*Moroteuthis*	Onychoteuthid spp.
% composition	AVE ± SD	*knipovitchi*	*robsoni*	AVE ± SD
n	2	1	1	2
total lipid	23.8 + 1.2	20.4	35.1	24.5 + 2.7
DG/SQ				
WE	6.5 ± 6.4	6.0	1.0	2.7 ± 0.1
DAGE	2.9 ± 3.4	1.5	2.5	2.4 ± 0.3
TAG	48.6 ± 1.8	40.6	71.2	71.0 ± 2.8
FFA	29.7 ± 4.5	41.4	22.0	18.4 ± 3.3
ST	2.6 ± 0.2	3.7	0.5	1.2 ± 0.2
PL	9.6 ± 3.5	6.8	2.7	4.4 ± 0.5

	Psychroteuthidae	**Brachioteuthidae**	**Ommastrephidae**	**Cranchiidae**
	Psychroteuthis	*Brachioteuthis*	*Martialia*	*Mesonychoteuthis*
% composition	*glacialis*	spp.	*hyadesi*	*hamiltoni*
n	1	1	1	1
total lipid	31.7	8.0	4.7	31.9
DG/SQ				2.0
WE	35.0	5.7	6.3	0.8
DAGE	2.6	0.0	0.0	0.0
TAG	32.3	30.8	0.0	51.0
FFA	21.2	27.0	25.4	43.5
ST	0.0	3.6	18.8	2.0
PL	8.8	32.9	49.5	0.7

n : sample size; DG: diacylglyceryl; SQ: squalene; WE: wax ester; DAGE: diacylglyceryl ether TAG: triacylglycerol; FFA: free fatty acid; ST: sterol; PL: phospholipid.

Table IIIc. Fatty acid composition (% of total fatty acids) of the mantle tissue of some Southern Ocean squid species. All bonds are cis-oriented unless stated otherwise

	Onychoteuthidae								
	Kondakovia longimana *n* =2			*Moroteuthis knipovitchi* *n* =2			*Moroteuthis robsoni*	Onychoteuthid spp. *n* =2		
Fatty acid	AVE	±	SD	AVE	±	SD	*n* =1	AVE	±	SD
14:0	2.0	±	0.0	1.6	±	0.1	1.7	1.3	±	0.1
15:0	0.3	±	0.1	0.3	±	0.1	0.3	0.4	±	0.0
16:0	24.0	±	3.5	19.4	±	1.0	22.3	24.8	±	1.3
17:0	0.4	±	0.2	0.4	±	0.0	0.5	0.6	±	0.0
18:0	2.4	±	0.2	2.0	±	0.1	2.4	3.3	±	0.2
14:1n5c	0.2	±	0.0	0.1	±	0.0	0.1	0.0	±	0.0
16:1n9c	0.1	±	0.0	0.1	±	0.0	0.1	0.1	±	0.0
16:1n7c	1.0	±	0.1	0.7	±	0.1	0.7	0.9	±	0.2
16:1n7t	0.1	±	0.0	0.1	±	0.1	0.1	0.1	±	0.0
16:1n5c	0.2	±	0.0	0.1	±	0.0	0.2	0.1	±	0.0
18:1n9c	3.0	±	1.4	2.3	±	0.4	2.9	4.3	±	0.9
18:1n7c	3.7	±	2.6	1.9	±	0.2	1.7	2.3	±	0.0
18:1n5c	0.5	±	0.1	0.3	±	0.0	0.4	0.5	±	0.0
19:1	0.1	±	0.0	0.1	±	0.0	0.1	0.1	±	0.1
20:1n11c	0.0	±	0.0	0.0	±	0.0	0.0	0.0	±	0.0
20:1n9c	6.1	±	0.9	7.0	±	0.5	5.8	8.0	±	0.0
20:1n7c	0 2	±	0.1	0.2	±	0.1	0.1	0.2	±	0.0
20:1n5c	0.0	±	0.0	0.0	±	0.0	0.0	0.0	±	0.0
22:1n11c	0.4	±	0.1	0.5	±	0.1	0.3	0.5	±	0.1
22:1n9c	1.4	±	0.0	1.4	±	0.3	1.7	1.7	±	0.2
24:1n11/9c	0.2	±	0.0	0.7	±	0.1	0.7	0.3	±	0.0
18:2n6c	0.4	±	0.1	0.7	±	0.1	0.2	0.3	±	0.0
20:4n6 (AA)	2.5	±	0.8	0.7	±	0.1	1.8	3.5	±	0.0
20:5n3 (EPA)	16.0	±	2.3	15.8	±	1.6	13.7	14.4	±	0.3
20:4n3c	0.1	±	0.0	0.1	±	0.0	0.1	0.2	±	0.0
20:2n6c	1.7	±	0.3	1.3	±	0.0	0.5	1.6	±	0.0
C21 PUFA	0.2	±	0.2	0.2	±	0.1	0.2	0.1	±	0.1
22:6n3 (DHA)	31.1	±	2.4	41.0	±	0.6	39.6	28.8	±	0.5
22:5n3 (DPA)	0.6	±	0.3	0.3	±	0.0	0.5	0.5	±	0.0
C23PUFA	0.2	±	0.1	0.2	±	0.0	0.6	0.2	±	0.2
C24 PUFA	0.1	±	0.1	0.1	±	0.0	0.1	0.1	±	0.0
SUM SAT	**29.2**	±	**3.9**	**23.8**	±	**1.0**	**27.4**	**26.5**	±	**1.0**
SUM MUFA	**17.4**	±	**0.0**	**15.6**	±	**0.1**	**14.9**	**20.3**	±	**1.1**
SUM PUFA	**53.3**	±	**4.1**	**60.5**	±	**1.0**	**57.6**	**53.0**	±	**1.7**

n : sample size; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table IIIc. continued

Fatty acid	**Psychroteuthidae** *Psychroteuthis glacialis* *n* =1	**Brachioteuthidae** *Brachioteuthis* sp. *n* =1	**Ommastrephidae** *Martialia hyadesi* *n* =1	**Cranchiidae** *Mesonychoteuthis hamiltoni* *n* =1
14·0	2.8	0.0	0 7	2 0
15·0	0.3	0.0	0 4	0.5
16:0	25 3	19.9	20.1	22.4
17.0	0 3	0 5	0 8	0.6
18·0	3 2	2.2	5.1	5 0
14:1n5c	0.2	0.0	0 0	0.1
16.1n9c	1.1	0.3	0 0	· 0 2
16:1n7c	2 6	0 8	0 2	1.4
16.1n7t	0.3	0.1	0.1	0 1
16·1n5c	0.4	0.2	0.1	0.1
18:1n9c	5.4	5.5	1 2	4.6
18:1n7c	6.2	6 4	1.3	2.1
18·1n5c	0.5	0 4	0.3	0.2
19:1	0.1	0 2	0.1	0 2
20.1n11c	0.0	0.0	0.0	0.0
20·1n9c	10 9	7.3	6.2	11.9
20:1n7c	0 3	0.2	0.1	0.2
20:1n5c	0.0	0 0	0.0	0 0
22 1n11c	1 3	0 2	0.2	0 6
22·1n9c	1.8	1 0	1.1	2 6
24:1n11/9c	1.0	0.6	0.5	0 6
18.2n6c	1.1	0.4	0 2	0 1
20.4n6 (AA)	0.3	2 3	0.8	2.1
20.5n3 (EPA)	21.5	17 0	16.3	17.6
20.4n3c	0 2	0 2	0.1	0 2
20.2n6c	0 1	0 6	0 6	0 2
C21 PUFA	0 4	0.3	0.2	0.1
22·6n3 (DHA)	9 8	31 5	42.1	22.7
22:5n3 (DPA)	0.9	0.5	0 3	0.6
C23PUFA	0.6	0 4	0.2	0 2
C24 PUFA	0 1	0 0	0.0	0 0
SUM SAT	**32.0**	**22.8**	**22.3**	**30.5**
SUM MUFA	**32.7**	**23.4**	**11.6**	**25.3**
SUM PUFA	**35.1**	**53.4**	**60.9**	**44.0**

n · sample size; AA: arachidonic acid; EPA· eicosapentaenoic acid, DHA: docosahexaenoic acid; DPA. docosapentaenoic acid; SAT: saturated fatty acid; MUFA· monounsaturated fatty acid; PUFA:

Table IIId. Fatty acid composition (% of total fatty acids) of the digestive gland of some Southern Ocean squid species

	Onychoteuthidae								
	Kondakovia longimana			*Moroteuthis knipovitchi*	*Moroteuthis robsoni*	Onychoteuthid spp.			
	n =2			*n* =1	*n* =1	*n* =2			
Fatty acid	AVE	±	SD			AVE	±	SD	
14:0	3.7	±	0.7	1.5	3.5	13.0	±	14.0	
15:0	0.3	±	0.1	0.2	0.4	0.3	±	0.1	
16:0	16.4	±	2.9	14.3	16.2	15.8	±	2.7	
17:0	0.3	±	0.2	0.4	0.3	0.3	±	0.0	
18:0	2.7	±	1.3	3.1	3.5	2.5	±	0.3	
20:0	0.1	±	0.0	0.3	0.3	0.1	±	0.0	
br17:1	0.3	±	0.5	0.0	0.0	0.6	±	0.1	
i17:0	0.1	±	0.0	0.3	0.3	0.2	±	0.0	
a17:0	0.1	±	0.1	0.2	0.2	0.1	±	0.0	
14:1n5c	0.1	±	0.0	0.0	0.1	0.0	±	0.0	
16:1n9c	1.0	±	0.6	2.1	0.4	0.3	±	0.1	
16:1n7c	4.6	±	0.7	2.7	4.1	5.4	±	1.1	
16:1n5c	0.3	±	0.1	0.1	0.3	0.3	±	0.1	
17:1	0.2	±	0.0	0.2	0.3	0.3	±	0.1	
18:1n9c	21.2	±	3.6	20.7	19.8	22.6	±	3.3	
18:1n7c	6.2	±	2.8	5.0	4.5	3.7	±	0.6	
18:1n5c	0.5	±	0.3	0.7	0.7	0.7	±	0.1	
19:1	0.1	±	0.1	0.0	0.0	0.1	±	0.0	
20:1n9c	8.3	±	4.1	13.7	10.8	7.2	±	0.1	
20:1n7c	0.5	±	0.0	0.4	0.4	0.4	±	0.0	
22:1n11c	2.0	±	1.1	7.2	5.3	2.5	±	0.2	
22:1n9c	1.4	±	0.5	3.1	2.5	1.1	±	0.1	
22:1n7c	0.4	±	0.5	0.0	0.0	0.7	±	0.1	
24:1n11c	0.8	±	0.7	0.0	0.0	0.7	±	0.1	
C16 PUFA	2.9	±	3.6	0.2	0.5	0.4	±	0.1	
18:3n6c	0.6	±	0.6	0.1	0.7	0.3	±	0.3	
18:4n3c	0.3	±	0.1	0.4	0.3	0.5	±	0.4	
18:2n6c	1.5	±	0.9	1.0	1.2	1.1	±	0.2	
20:4n6 (AA)	1.1	±	0.1	0.8	0.7	0.8	±	0.2	
20:5n3 (EPA)	9.5	±	5.7	4.9	5.7	7.1	±	1.7	
20:3n6c	0.3	±	0.0	0.2	0.2	0.2	±	0.0	
20:4n3c	0.6	±	0.2	0.5	0.8	0.6	±	0.1	
20:2n6c	0.6	±	0.1	0.6	0.5	0.1	±	0.0	
22:4n6c	0.0	±	0.0	0.1	0.1	0.1	±	0.0	
C21 PUFA	0.3	±	0.2	0.1	0.1	0.2	±	0.1	
22:6n3 (DHA)	9.8	±	0.3	9.9	9.0	8.6	±	2.0	
22:5n3 (DPA)	0.6	±	0.0	0.5	0.7	0.5	±	0.2	
C23PUFA	0.1	±	0.1	0.1	0.2	0.2	±	0.1	
SUM SAT	**23.5**	**±**	**3.9**	**20.2**	**24.9**	**32.2**	**±**	**10.9**	
SUM MUFA	**47.6**	**±**	**6.7**	**56.0**	**49.2**	**45.9**	**±**	**5.8**	
SUM PUFA	**28.1**	**±**	**11.2**	**19.4**	**20.6**	**20.8**	**±**	**4.9**	

n : sample size; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Table IIId. continued

Fatty acid	**Psychroteuthidae** *Psychroteuthis glacialis* *n* =1	**Brachioteuthidae** *Brachioteuthis* sp. *n* =1	**Ommastrephidae** *Martialia hyadesi* *n* =1	**Cranchiidae** *Mesonychoteuthis hamiltoni* *n* =1
14:0	3.5	0.7	0.0	1.7
15:0	0.1	0.1	0.0	0.3
16:0	6.4	12.7	13.4	11.9
17:0	0.8	0.3	1.6	0.3
18:0	0.8	3.4	7.9	3.1
20.0	0.1	0.2	0.0	0.2
br17:1	0.2	0.0	0.0	0.7
i17.0	0.0	0.1	0.0	0.3
a17:0	0.1	0.1	0.0	0.2
14:1n5c	0.3	0.0	0.0	0.0
16:1n9c	5.3	1.5	0.0	0.1
16:1n7c	6.8	0.8	0.6	4.5
16:1n5c	0.2	0.1	0.0	0.2
17:1	0.2	0.1	0.0	0.5
18:1n9c	19.4	24.1	9.8	27.3
18:1n7c	5.8	4.0	2.0	4.3
18:1n5c	0.4	0.4	0.0	0.5
19:1	0.0	0.2	0.0	0.3
20:1n9c	14 8	17 2	6.7	16.7
20:1n7c	0.7	0.4	0.0	0.9
22:1n11c	7.8	3 4	0.6	4.3
22:1n9c	3.0	1.4	0.8	1.7
22:1n7c	0.3	0.1	0.0	0.6
24:1n11c	1.4	2.0	0.6	1.3
C16 PUFA	6.8	0.0	0.0	0.3
18:3n6c	0.1	0.0	0.8	0.2
18:4n3c	0.6	0.3	0.0	0.2
18:2n6c	1.4	0.7	0.8	1.0
20:4n6 (AA)	0.4	1.6	3.4	1.1
20:5n3 (EPA)	6.7	11.8	17.7	4.3
20:3n6c	0.1	0.9	0.0	0.2
20:4n3c	0.2	1.0	0.6	0.8
20:2n6c	0.2	0.7	1.2	0.1
22:4n6c	0.0	0.0	0.0	0.1
C21 PUFA	0 2	0.3	0.3	0.2
22:6n3 (DHA)	3.9	8.5	30.0	8.0
22:5n3 (DPA)	0.4	0.7	0.7	1.0
C23PUFA	0.1	0.1	0.0	0.1
SUM SAT	**11.8**	**17.4**	**22.8**	**17.5**
SUM MUFA	**66.4**	**55.7**	**21.2**	**63.2**
SUM PUFA	**21.2**	**26.7**	**55.5**	**17.5**

n : sample size, AA: arachidonic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, DPA: docosapentaenoic acid; SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.