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

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**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 was 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 its prey species rather than 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.

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**Key words:** diet, *Gonatus antarcticus*, higher predators, *Moroteuthis robsoni*, *Sepioteuthis australis*, *Todarodes* spp.

## Introduction

Squid occupy a significant ecological niche in the Southern Ocean (Rodhouse & White 1995). However, we currently know little about the parameters required for adaptation to these polar waters. Lipid analysis of cephalopods can provide insights into biochemical, physiological and ecological requirements of these animals. For example, analyses of total lipid and lipid classes (that is, relative proportions of structural versus storage and other neutral lipid) of the digestive gland of some cephalopods have revealed that lipid stored in this organ is unlikely to be mobilised during sexual maturation (Blanchier & Boucaud-Camou 1984, Clarke *et al.* 1994) or long-term starvation (Castro *et al.* 1992). However, the digestive gland of *Illex argentinus* (Castellanos, 1960) is estimated to store enough lipid in the digestive gland to fuel a spawning migration of up to 21 days. More recent studies have investigated the relationship between cephalopod lipid content and diet.

Abolmosova *et al*. (1990) used the total lipid content of digestive glands from *Sthenoteuthis pteropus* (Steenstrup, 1855) as an index for comparing food availability in geographically distant regions. Digestive gland lipid content has also been used to identify important prey groups of the Southern Ocean squid *Moroteuthis ingens* (Smith, 1881), by using the fatty acid components of the lipid as dietary tracers (Phillips *et al*. 2001). The use of fatty acids as dietary tracers in the marine environment has been in practice since the 1960s (Ackman & Eaton 1966, Sargent 1976), and the technique has been used to explore dietary relationships in a number of diverse marine organisms (Mourente & Tocher 1993, Graeve *et al.* 1994, Kirsch *et al.* 1998, Cripps *et al.* 1999, Navarro & Villanueva 2000). It is based on the assumption that many fatty acids in the marine environment, particularly polyunsaturated fatty acids (PUFA), can only be biosynthesized by certain phytoplankton and macroalgae species and become essential dietary components to higher trophic levels. Phytoplankton and macroalgae species are often characterized by very distinct ratios of fatty acids, and these ratios influence the fatty acid profiles of higher organisms and are thus useful tools for providing information on food webs (Graeve *et al*. 2002). The digestive gland of cephalopods is probably an ideal source of fatty acid dietary tracers, as dietary lipid is likely to be deposited here with little or no modification of lipid content, such as chain elongation or desaturation of PUFA (Phillips *et al*. 2001). Thus this technique has potential future applications to dietary studies of cephalopods.

It is more difficult to interpret the influence of diet on fatty acid composition in a tissue where biosynthesis and modification of fatty acids is likely to occur, such as in the muscle or blubber tissue of marine vertebrates. Conflicting

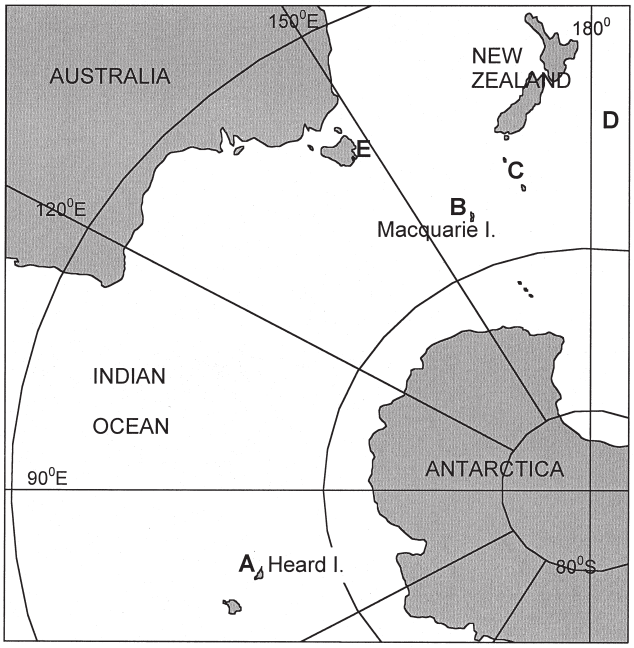


**Fig. 1.** Approximate locations of collection sites of Southern Ocean squid. Map courtesy of the Australian Antarctic Division. A = Heard Island, B = Macquarie Island, C = Southern Plateau, D = Chatham Rise, E = Great Oyster Bay, Tasmania.

evidence exists in the literature about the effectiveness of deriving dietary information of marine vertebrates, principally pinnipeds, from fatty acid analyses (Grahl-Nielsen & Mjaavatten 1991, Grahl-Nielsen 1999, Smith *et al.* 1997, Smith *et al.* 1999). However, over the past decade the technique has been increasingly applied to dietary studies of these animals in both the northern and southern hemispheres, as at-sea foraging data is very difficult to obtain from conventional methods. Analyses can be conducted on small milk or blubber samples, so intrusive techniques such as stomach lavage and lethal sampling are no longer required. While many marine vertebrates (including pinnipeds) prey on cephalopods, very few data are available on the fatty acid composition of squid for inclusion in such predator-prey comparisons.

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. Species include one myopsid, the loliginid *Sepioteuthis australis* (Quoy & Gaimard, 1832), an endemic species from temperate coastal waters around Australia and New Zealand that contributes to a small commercial fishery (Triantafillos & Adams 2001). The other three species are oegopsids thought to be distributed between the Subtropical Convergence and the Antarctic Polar Front, and include the onychoteuthid *Moroteuthis robsoni* (Adam, 1962), the ommastrephid *Todarodes* spp. (Girard, 1890) and the gonatid *Gonatus antarcticus* (Lönnberg, 1898). These data may not be directly applicable to dietary studies of higher predators. However, comparisons of mantle and digestive gland fatty acid content and composition will assist researchers to apply squid fatty acid data to studies of higher predators, and alert them to some of the implications involved with this.

## Materials and methods

### *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. 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-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 (D) the Chatham Rise between October–November 2000, and from (C) the Southern Plateau between November –December 2000. The Tasmanian Aquaculture and Fisheries Institute (TAFI) collected nine squid with hand lines from (E) Great Oyster Bay in south-eastern Tasmania in March 2000. All squid were frozen after collection at -20°C and returned to Hobart for dissection and analysis. Individuals were allocated a maturation stage according to a subjective 5-point scale (after Lipinski 1979).

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 sub-Antarctic (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 mantle length (ML) (Kubodera *et al.* 1998). However, Sands (2000) suggested that two,

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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 thought to represent the large variant. The taxonomic status of *M. robsoni* requires review.

### *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 (*c.* 1 g) taken from the ventral mantle was collected from most specimens, and the whole digestive gland of each animal was collected where possible. These were stored frozen at -20°C and retained for lipid and fatty acid analysis. Whole digestive glands were homogenised in a hand-held blender and a 0.25–0.5 g subsample was taken for lipid extraction. Mantle tissue samples were ground in a mortar and pestle prior to extraction.

All tissue samples were extracted overnight using a method modified from Bligh & Dyer (1959) 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 MK V TH10 thin layer chromatograph flame ionisation detector (TLC-FID) analyser to determine the proportion of lipid classes. A polar solvent system (60:17:0.1 v/v/v ratio of hexane:ether:acetic acid) was used to resolve triacylglycerols, free fatty acids, sterols and phospholipid, while a non-polar solvent system (96:4 v/v ratio of hexane:ether) was used to determine diacylglyceryl ethers and wax esters. Peaks were quantified with DAPA Scientific Software.

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 under nitrogen, then silylated at 60°C overnight in N,O-bis-(trimethylsilyl)-triflouracetamide (BSTFA). 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, an 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 $min^{-1}$, then to 250°C at 2°C $min^{-1}$, and finally to 300°C at 5°C $min^{-1}$. 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. Peaks were quantified with Waters Millennium software (Milford, MA, USA).

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 free fatty acid (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 *et al.* 1985, Hayashi & Yamamoto 1987, Kawasaki *et al*. 1994, Hayashi 1996, 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 we believe more likely to elevate FFA levels in this organ, especially when compared to low levels of FFA in the phospholipid (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.

## Results

### *General biology*

In total, 21 female and 8 male squid were analysed. All female squid were immature, while some male squid were mature (Table I). 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 II). 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.

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

| Species | Site | Year | 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 | 1998–99 | | 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 | 1998–99 | | | 1 | 1 | | | | | |
| | B | 1995 | | | 1 | 1 | | | | | |
| | | 1998–99 | | | 1 | 1 | | | | | |
| | | 2000 | | | | 1 1 | | | | | |
| | D | 2000 | | | 3 | 3 | | | | 1 | 1 |
| Total | | | | | | 21 | | | | | 8 |

**Table II.** Ranges of mantle length (ML), mantle mass (M), digestive gland mass (DG) 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 |

### *Total lipid and lipid class data - 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 III). The major lipid class in all species was phospholipid (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 IV). Sterol (ST) represented the only other lipid class with values greater than 1.5% of total lipid.

Polyunsaturated fatty acids (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 V). PUFA were largely comprised of eicosapentaenoic acid (EPA) (20:5n3) and docosahexaenoic acid (DHA) (22:6n3); no other PUFA were at values exceeding 5% of total fatty acids. Saturated 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. Monounsaturated 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 III.** Total lipid content (% wet mass) of squid mantle and digestive gland tissue. Values are mean ± SD. *= total lipid data unavailable for all samples, figures in parenthese indicate no. of mantle samples available for fatty acid analysis.

| Species | *n* | mantle % total lipids (wet mass) | *n* | digestive gland % 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 |

### *Total lipid and lipid class data - 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 III). 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 IV). Triacylglycerol (TAG) was the major neutral lipid in *M. robsoni* and some individuals of *S. australis* and *Todarodes* spp. In contrast, diacylglyceryl ether (DAGE) was the major lipid class in *G. antarcticus*

**Table IV.** Percentage lipid class (of total lipids) in squid. Values are mean ± SD. Only data from squid collected in 2000 are included.

| a) Mantle tissue<br>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 tissue<br>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 |

WE = wax ester, DAGE = diacylglyceryl ether, TAG = triacylglycerol, FFA = free fatty acids, ST = sterol and PL = phospholipid.

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**Table V.** Percentage fatty acids (of total fatty acids) in squid mantle tissue.

| 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 |

Values are mean ± SD. AA = arachidonic acid, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SAT = saturated fatty acids. All bonds are cis-oriented unless stated otherwise.

**Table VI.** Percentage fatty acids (of total fatty acids) in squid digestive gland tissue.

| 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 |

Values are mean ± SD. AA = arachidonic acid, DHA = docosahexaenoic acid, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SAT = saturated fatty acids. All bonds are cis-oriented

contributing a mean value of 44.9 ± 6.8% to total lipid (unpublished data) and for remaining *Todarodes* spp. 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 wax ester (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 VI). 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*.

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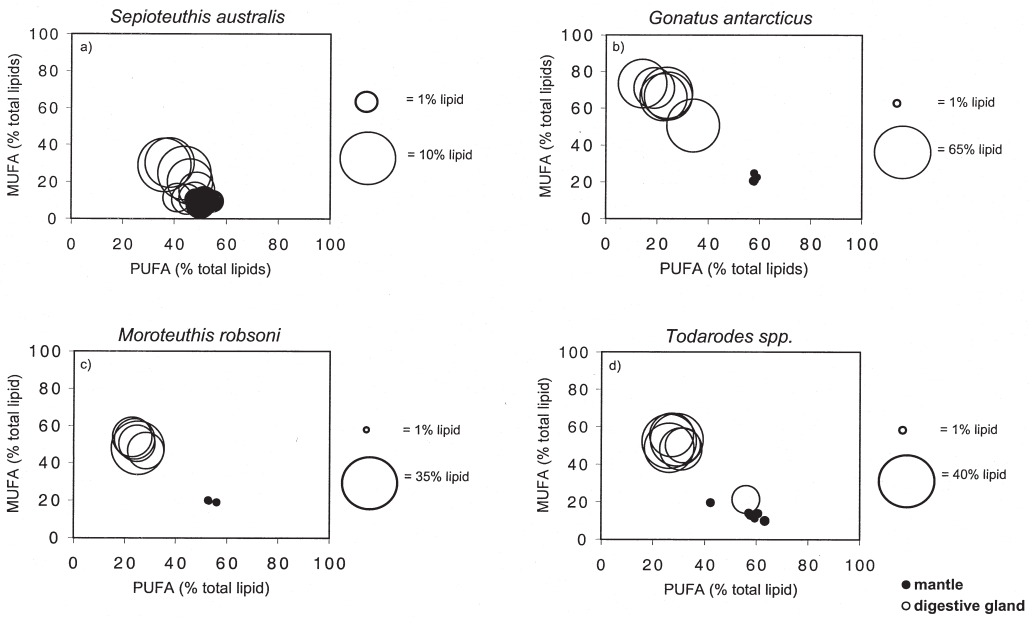


**Fig. 2.** Bubble plot of MUFA and PUFA content (% of total lipids) and total lipid content (% wet mass) of mantle and digestive gland tissue of **a.** *Sepioteuthis australis*, **b.** *Gonatus antarcticus*, **c.** *Moroteuthis robsoni* and **d.** *Todarodes* spp. Bubble size represents total lipid content. Note that the scale of bubble size changes for each species.

## Discussion

### *Digestive gland lipid composition - implications for food-web studies*

In this study we observed marked differences in the total lipid, lipid class and fatty acid content of the digestive gland between one myopsid and three oegopsid squid species (Fig. 2). Lipid stored in the digestive gland is likely to be of dietary origin (Blanchier & Boucaud-Camou 1984, Abolmasova *et al.* 1990, Clarke *et al.* 1994, Semmens 1998), as opposed to other sources such as biosynthesis and fatty acid modification, and differences between myopsid and oegopsid lipid content are potentially related to dietary differences between these groups of squid. In addition, different modes of lipid storage (such as the storage of low-density oils as a buoyancy adaptation in *G. antarcticus* (Clarke *et al*. 1979)) may account for differences in total lipid and lipid class data. Large amounts of DAGE in the digestive gland have been previously reported for other gonatid squid (Hayashi & Yamamoto 1987, Hayashi 1989, Hayashi & Kawasaki 1990), in addition to the onychoteuthid *M. robusta* (Hayashi *et al.* 1990).

Lipid deriving from the digestive gland of oegopsid species was composed of large amounts of MUFA, with the exception of one individual of *Todarodes* spp. MUFA are the dominant fatty acids in myctophid fish, which are likely to be common prey items of the three oegopsid species (Rodhouse *et al.* 1992, Rodhouse & Nigmatullin 1996), 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 characterized by relatively abundant PUFA, and this may reflect the different spectrum of fish prey available to this coastal, inshore species. Unfortunately, little dietary data are currently available for this species to confirm this.

Comparisons of stomach content and digestive gland fatty acid content of other squid species confirm 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 is suspected that squid have been feeding in sampling gear so that stomach contents data provides unreliable information on 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

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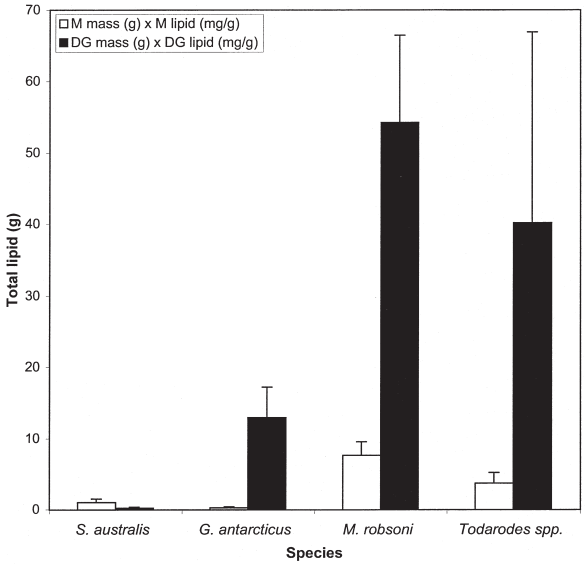


**Fig. 3.** Mean calculated total lipid content of the entire mantle and digestive glands of *Sepioteuthis australis*, *Gonatus antarcticus*, *Moroteuthis robsoni* and *Todarodes* spp. M = mantle, DG = digestive gland. Error bars are standard deviations.

serological analysis of stomach contents (Kear 1992) or genetic identification of prey remains, 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.

The same properties that define the usefulness of these lipids as a tool to squid biologists may also hamper the effectiveness of lipids to explore the diets of animals that prey on cephalopods. 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 II), 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. 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 II). Therefore the absolute lipid content of the digestive gland of *G. antarcticus*, *M. robsoni* and *Todarodes* spp. greatly exceeds that of the mantle (Fig. 3) (large error bars for *M. robsoni* and *Todarodes* spp. in Fig. 3 result not only from variability in digestive gland mass, but also from fluctuating lipid content of the digestive gland). Therefore, an important point 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. An oegopsid squid predator would ingest more lipid from secondary prey items, which has been 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 Vlieg (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 (G.A. Wilson, personal communication 2001). The total lipid content and fatty acid composition of whole homogenised 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 1993, Iverson *et al.* 1997, Smith *et al.* 1997, Raclot *et al.* 1998). 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 2001). 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 our findings for three species of Southern Ocean oegopsids, squid flesh data 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. 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

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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.

In summary, we observed marked differences 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:

a) 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 thus eliminating biases associated with instantaneous sampling of diet, and;

b) 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.

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