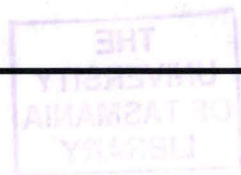


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THE LIPID COMPOSITION OF  
PATAGONIAN TOOTHFISH  
FROM THE  
MACQUARIE ISLAND REGION  
ECOLOGICAL AND DIETARY  
IMPLICATIONS WITHIN A REGIONAL  
FOOD WEB

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**GRADUATE DIPLOMA OF ANTARCTIC AND SOUTHERN OCEAN  
STUDIES WITH HONOURS**  
UNIVERSITY OF TASMANIA

**BACHELOR OF APPLIED SCIENCE**  
ROYAL MELBOURNE INSTITUTE OF TECHNOLOGY

SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF TASMANIA  
**APRIL 2004**

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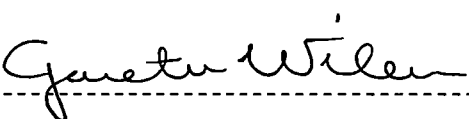
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# LIST OF COMMON ABBREVIATIONS

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ABBREVIATION	TRANSLATION
FA	Fatty Acid
SUFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
BrFA	Saturated Fatty Acid
AA	Arachidonic Acid
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
DAGE	Diacylglycerol Ether
WE	Wax Ester
TAG	Triacylglycerol
FFA	Free Fatty Acid
ST	Sterol
PL	Polar Lipid
TL	Total Length
TW	Total Weight
WW	Wet Weight
FRDC	Fisheries Research and Development Corporation
CSIRO	Commonwealth Scientific Industrial Research Organisation
IUU	Illegal, Unreported and Unregulated
GC	Gas Chromatography
FID	Flame Ionising Detection
TLC	Thin Layer Chromatography
LDA	Linear Discriminant Analysis
MDS	Multi Dimensional Scaling

# ABSTRACT

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Recent commercial interest in the Patagonian toothfish (*Dissostichus eleginoides*) in the Macquarie Island region has heightened the need for a better understanding of the life history of this species. Of particular interest are dietary considerations. However, despite the importance of toothfish in the deep-sea food web of this and other Southern Ocean regions, relatively little is known of their diet. Current knowledge is based upon the examination of stomach contents, a technique that fails to identify with any certainty long-term dietary composition. The determination of lipid composition may provide a simple and effective means of determining the diet of toothfish, one that complements traditional techniques. Specifically, many higher order species are thought to deposit fatty acids derived from prey into storage with little or no modification. Constituent fatty acids of a predator may therefore represent a temporal integration of diet. The lipid composition of toothfish was investigated in relation to both physical (sex, age/size) and fishing parameters (region, season, depth, time of day). Each parameter was compared using a variety of statistical methods including multidimensional scaling and linear discriminant analysis. Significant variations in total lipid content, lipid class composition and especially fatty acid composition between fish of different sizes (ages) points to a potential shift in diet as fish mature. Eicosapentaenoic and docosahexaenoic acids were those fatty acids most responsible for the observed variation with size. The influence of the remaining parameters on lipid composition is less pronounced. To determine whether these variations in lipid composition were due to possible variations in diet, a comparison with potential prey species was undertaken. This involved the examination of 21 species of midwater fish, including 8 species of Myctophidae, and 6 species of squid. Many of these species are known to contribute to the diet of toothfish in the Macquarie Island region. The results of statistical analysis suggest that the diet of Patagonian toothfish at Macquarie Island may vary to a far greater extent, relative to size increases, than is suggested by stomach content analysis. For instance smaller sized toothfish are more closely linked to squid, based on fatty acid composition, than are larger toothfish. Most notable with regards to potential fish prey is that the fatty acid compositions of Myctophidae are more closely related to larger rather than smaller toothfish, possibly indicating a shift in dietary preference as toothfish mature.

# ACKNOWLEDGEMENTS

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A special thanks to Austral Fisheries and the captain and crew of the *MV Austral Leader* and to FRDC who partially funded this project

This thesis is also dedicated to the memories of Theo and John, two friends sadly missed

**“Tell me what you eat and I  
will tell you what you are”**

**Anthelme Brillat-Savarin**

**The Physiology Of Taste, 1825**

**“Man is what he eats”**

**Ludwig Feuerbach**

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# CHAPTER 1

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## GENERAL INTRODUCTION

### 1.1 INTRODUCTION

The commencement of fishing for Patagonian toothfish (*Dissostichus eleginoides* Smitt 1898, hereafter referred to as toothfish) in the 1980s signaled a new era in the development of deepsea fisheries in the Southern Ocean. The factors counting against the capture of toothfish as an economically viable venture, namely the isolated nature and discontinuous distribution of the known population, were eroded by the dramatic collapse in worldwide fishing stocks. The best known example is the failure of the Atlantic cod (*Gadus morhua*) fishery in the Northern Atlantic in the 1990's (Fu et al. 2001; Haedrich et al. 2001). With what was an effectively self-managed fishery, the risk of overexploitation represented a tangible threat to the sustainability of toothfish stocks in the Southern Ocean (Haedrich et al. 2001). The scientific data necessary to construct a management framework, considered an essential component of proper fisheries management (Frank and Brickman 2001; Gordon 2001), did not initially exist for toothfish.

The fishing of toothfish within Australian waters, in the regions of Macquarie and Heard Islands, took significantly longer to take hold than other regions of the Southern Ocean. Exploratory fishing in the Macquarie Island region began in the 1994-1995 fishing season under the supervision of the Australian Fisheries Management Authority (AFMA) (Williams and Lamb 2001). Growing concern over the potentially deleterious impacts of these operations led to the commissioning of a report by the Fisheries Research and Development Corporation (FRDC), in association with partners Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine Research, Australian Antarctic Division (AAD) and Austral Fisheries Pty Ltd., into the ecological sustainability of such a fishery (He and Furlani

2001). The report covered all aspects of toothfish biology vital to the proper management of the fishery in the Macquarie Island region, including key population parameters (age validation, growth, age of maturity, mortality), distribution and relative abundance.

A cornerstone of the FRDC study was the identification of food chain linkages between the fishery, toothfish and the main bird and mammal species, primarily through the examination of stomach and faecal contents (Goldsworthy et al. 2001b; Goldsworthy et al. 2001c; Goldsworthy et al. 2001d; Goldsworthy et al. 2002). Although these traditional methods of dietary determination provide invaluable information, they are subject to a number of acknowledged yet unavoidable biases. Amongst these is a failure to identify with any certainty long-term dietary composition and bias due to differential digestion of prey items (Antonelis et al. 1987; Harwood and Croxall 1988; Chou et al. 1995).

Ways to ameliorate these problems were sought through the application of developing techniques that explore predator-prey interaction through the identification of biological markers (biomarkers) rather than prey remains. Fatty acids, biological components that perform a range of important functions, represent one such biomarker. Many fatty acids are readily transferred by dietary interactions within the food web from prey to predator. In many higher order species, dietary derived fatty acids are thought to be incorporated into storage with little or no modification (e.g. Iverson 1993). Observed differences in fatty acid composition between species and even between individuals of the same species are consequently due, in part, to dietary differences.

An initial attempt at assessing the diet of Macquarie Island toothfish using fatty acid tracers was included in the FRDC study (Wilson and Nichols 2001). Although providing a promising insight into the potential application of this technique to a dietary study of toothfish, this initial study was hampered by a lack of information regarding the fatty acid composition of potential prey species. Subsequent research has seen a rapid advancement in the number

of prey species for which fatty acid compositions exist as well as expansion in the scope of the toothfish data set, particularly with respect to size, geographical location and year of collection.

## **1.2 DEEPSEA FISHERIES OF THE SOUTHERN OCEAN**

The commercial capture of deepsea fish in the Southern Ocean, once restrained by the practical limitations of fishing the open oceans, has increased dramatically in recent times. The escalating interest in deep-sea stocks is partly a consequence of the worldwide collapse of the inshore fisheries that have historically formed the basis of the industry (Haedrich et al. 2001). Inshore fisheries, usually contained within or near to the margins of the continental shelf, are generally within easy reach of ports. This ease of access has left many inshore fisheries prone to overexploitation, a situation with disastrous implications for the entire marine ecosystem. In response, fishers have eventually sought out those fish stocks, situated on the continental slope, that were previously considered economically marginal.

Steady improvements in fishing technology have overcome prior restrictions to operating in the deepsea environment (e.g. Haedrich et al. 2001). These have included the development of suitable fishing gear, including longlines and specialised trawl nets, the construction of vessels capable of spending long periods at sea and handling often harsh weather conditions, increased understanding of fish behaviour, technological advances in fish detection, and improvements in refrigeration that have allowed the delivery of suitably 'fresh' fish to market. These factors, combined with ever increasing market pressures, mean that very few regions of the ocean are considered off-limits to fishers, both practically and economically. A number of deepsea species that, until recently, were not investigated to any great extent by fishers are now considered to be commercially viable.

## **Patagonian toothfish and Orange Roughy Fisheries of the Southern Ocean**

Fisheries based upon deepsea species are relatively new, with large-scale development beginning in the 1970s (Haedrich et al. 2001). Based beyond the continental shelf, they have redefined the way in which the industry traditionally operates. In contrast to the support offered to local communities by inshore fishing, deepsea operations usually operate outside national boundaries, sometimes relying on flags of convenience to evade international laws aimed at legislating fishing of the high seas (Willock 2002). The isolated nature of the deepsea and its large area has meant that patrolling and regulating fisheries for species such as toothfish has remained inherently difficult and generally unsuccessful (Pearce 1996).

The past three decades have seen the development of several important deep-sea fisheries in the Southern Ocean. Of greatest commercial interest in sub-Antarctic waters are the fisheries for orange roughy (*Hoplostethus atlanticus*) and Patagonian toothfish (*Dissostichus eleginoides*). The fillets of toothfish are commercially prized for their large size, absence of pin bones and soft flesh, all of which contribute to their high market value (Nichols et al. 1994a). A contributing factor to the difficulty in tracing the movement of illegally caught toothfish is the multitude of common names used in the international marketplace (Table 1.1).

The development of both fisheries continues to invoke political and environmental debate. The exploitation of both Patagonian toothfish and orange roughy illustrate the main issues surrounding fishing in the deep ocean. The discovery, development and subsequent decline of this fishery has closely followed the trajectory of other exploited fish species. A period of extremely high catch rates is quickly followed by a rapid decline in fish numbers, sometimes to the point of commercial extinction (Clark 1999). The rapid rise and fall of fish stocks, with high landings of fish in the first few seasons before a dramatic drop-off in returns, has been repeated throughout the worlds oceans. Perhaps the most dramatic examples have been from the North Atlantic, particularly the fisheries for Atlantic cod (*Gadus morhua*). In all

**Table 1.1.** A selection of country-specific common names for *Dissostichus eleginoides* used in the international marketplace

Country	Common Name
United Kingdom	Australian Sea Bass, Antarctic Sea Bass
USA, Canada	Chilean Sea Bass, Poor Man's Lobster
France	Légine Austral
Japan	Mero
Chile, Spain, Argentina	Merluza negra, Bacalao de profundidad

cases over-fishing and habitat degradation are cited as the main causes of the decline in fish stocks (Frank and Brickman 2001).

A defining characteristic of deepsea fisheries is that commercial exploration often precedes scientific investigation. Data essential to proper management, including population structure, location and life history, is often obtained after commercial exploitation has already commenced (Frank and Brickman 2001; Gordon 2001; Tuck et al. 2001a). Such information consequently rarely reflects the state of the fishery prior to anthropogenic influence. In many cases initial fish populations have already drastically declined before knowledge of the fishery has been suitably collated. Thus, catch-rates continue to decline, even after the introduction of a management strategy. The application of 'backwards logic' to fisheries management has lead to a number of negative effects not only to target species, but throughout the entire marine ecosystem. The need for a scientific basis to the management of fish stocks has been clearly evidenced by the rapid decline in orange roughy numbers in Australian and New Zealand waters (Clark 1999). The disastrous overexploitation of orange roughy from Tasmanian seamounts in the 1990s was a consequence of the initial overestimation of both virgin fish biomass and mortality rates (Pankhurst 1999).

Management of Patagonian toothfish in the Australian fisheries based around Macquarie, Heard and McDonald Islands has been directly influenced by the experienced gained from the overexploitation of orange roughy. Both

fisheries are strictly managed by regulatory bodies (e.g. AFMA, CCAMLR<sup>1</sup>) using the precautionary approach to fishing and aided by an ongoing program of scientific research (e.g. FRDC, CSIRO; AAD): Amongst the measures implemented in these fisheries are restrictions on the number of vessels permitted to operate, the use of trawl nets rather than longlines to reduce the incidental mortality of seabirds and the ability to close grounds where necessary based upon monitoring of catches using the total allowable catch (TAC) quota system (Sainsbury and de la Mare 2001).

Despite this attempt at regulation, the isolated nature of both fisheries and the high value of the catch has attracted illegal, unregulated and unreported (IUU) activities that may account for up to 50% of the total catch of toothfish in the Southern Ocean (Willock 2002). The impact of IUU fishing on these regions is substantial, directly accounting for declines in not only the stocks of target species but also the numbers of seabird and by-catch species as well as degradation of the ocean floor environment (Frank and Brickman 2001; Kock 2001). The decline in fish stocks affected by these impacts may prove irreversible.

### **1.3 DISTRIBUTION, BIOLOGY AND FORAGING ECOLOGY OF THE PATAGONIAN TOOTHFISH IN THE SOUTHERN OCEAN**

First described by Smitt in 1898, Patagonian toothfish are amongst the largest fish residing in the Southern Ocean. The unique ecology of toothfish, especially their aggregation in large numbers around deepsea mounts and valleys, has contributed to the attractiveness of this species to commercial concerns. Numerous other characteristics contribute to the unique nature of

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<sup>1</sup> Although Macquarie Island is not included in the regions covered by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR), certain aspects of the Convention apply to the fishery. Resolution 10/XII states that all signature countries, including Australia, must ensure that flag vessels harvesting stocks which are also found in the Convention area (such as Patagonian toothfish) do so responsibly and with due respect for Conservation Measures adopted by the Commission

toothfish including the absence of a swimbladder, the extreme depths at which they reside and the relative isolation of populations.

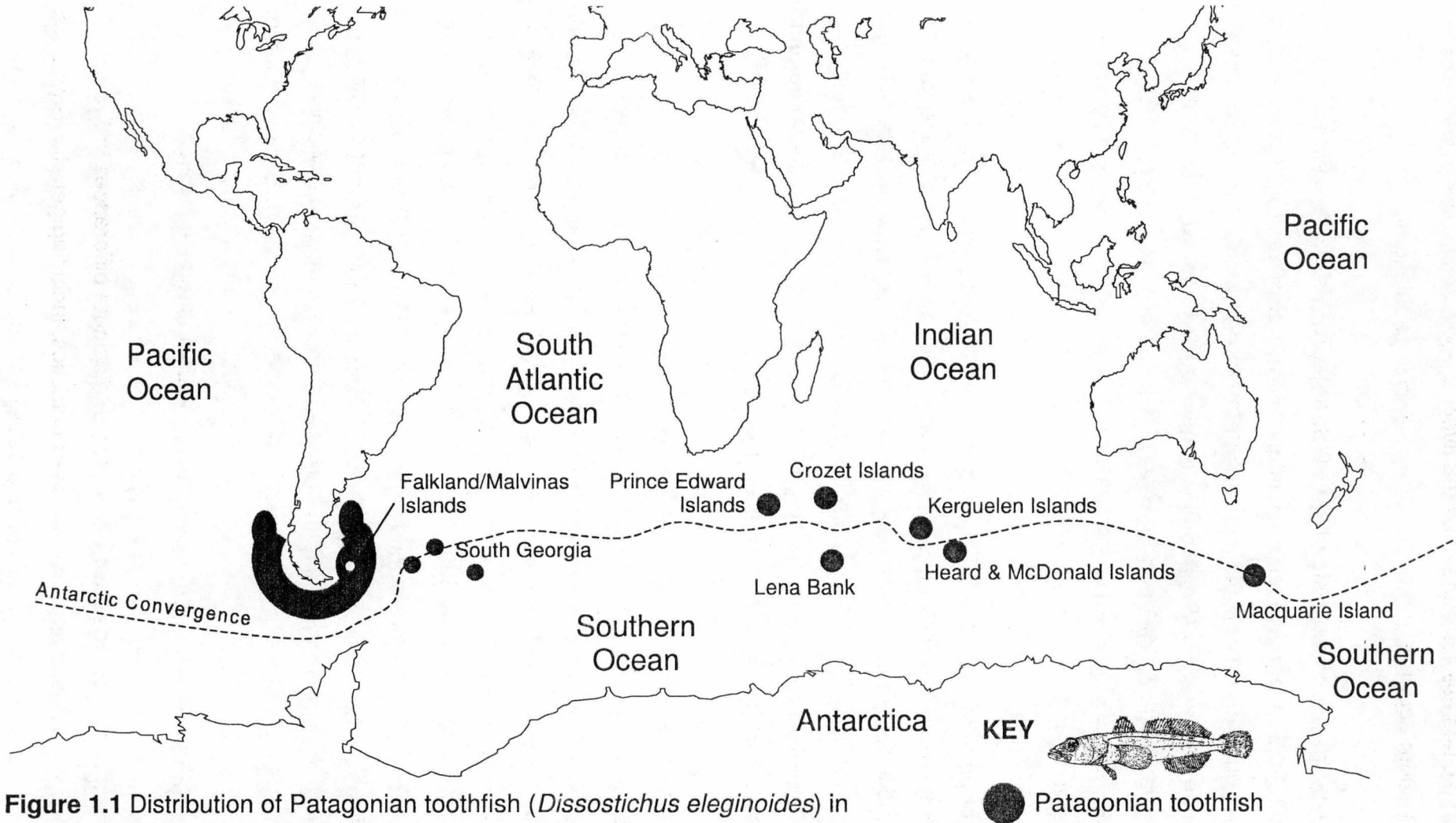
### **Distribution of Patagonian toothfish in the Southern Ocean**

Distribution of toothfish is a discontinuous band confined exclusively to sub-temperate and sub-Antarctic latitudes of the Southern Ocean (Figure 1.1). Northern and southern distribution is restricted to approximately 30°S and 60°S respectively. Distribution of adults within the water column is spread over a range of depths from 600-3500m and is highly dependent upon region.

Major populations have been identified from the coast of the South American continent, shelves and seamounts associated with a number of sub-Antarctic islands, and in the region of the Antarctic Peninsula. These populations are generally separated into two distinct groups, the southwest Atlantic and sub-Antarctic Indian Ocean (García de la Rosa et al. 1997). Specifically, toothfish in the southwest Atlantic region are reported from the coasts of southern Chile (Arrizaga et al. 1996; Parkes et al. 1996) and Argentina (García de la Rosa et al. 1997), the Falkland Islands (Coggan et al. 1996; Des Clers et al. 1996; Collins et al. 1999; Smith and McVeagh 2000), South Georgia (Zhivov and Krivoruchko 1990; McKenna Jr 1991; Evseenko et al. 1995; Parkes et al. 1996; García de la Rosa et al. 1997) and larvae have been reported from the Scotia Sea (Yefremenko 1979). Indian Ocean populations are confined to the sub-Antarctic islands of Prince Edward (Vukhov 1972), Kerguelen and Crozet (Vukhov 1972; Duhamel and Hureau 1985; Diano 1989; Chikov and Mel'nikov 1991), Heard and McDonald (Williams and Ensor 1988; Williams and de la Mare 1995; Smith and McVeagh 2000) and Macquarie (Smith and McVeagh 2000).

### **Patagonian toothfish fisheries in Australian waters**

The Australian toothfish fishery is based around two main fishing grounds in the Southern Ocean (Figure 1.1). The Heard (53°01'S: 73°23'E) and McDonald Islands (53°03'S: 72°36'E) form part of the Kerguelen-Gaussberg ridge and lie 300km south of the Antarctic Convergence (Williams 1983).



**Figure 1.1** Distribution of Patagonian toothfish (*Dissostichus eleginoides*) in the Southern Ocean (based upon Willock 2002)

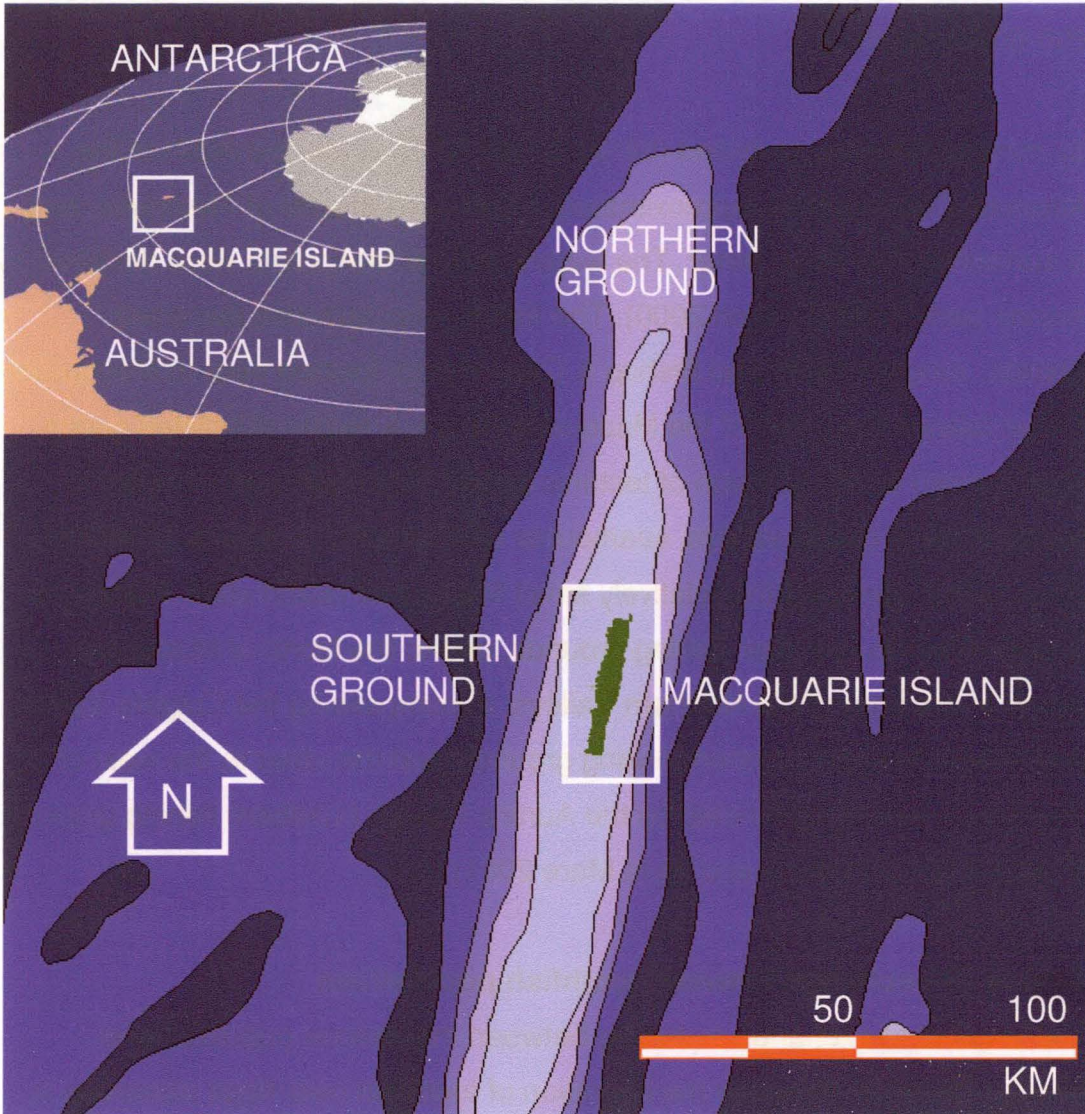
Both Islands are surrounded by relatively shallow shelf water of between 200-600m depth.

By contrast Macquarie Island (54°30'S: 158°55'E) is located north of the Antarctic Convergence, approximately 1500km southeast of Tasmania, and is surrounded by deep water. The waters around Macquarie were declared a marine park in 2000 (Anon 2000). The fishery also includes two small islands, Judge & Clerk and Bishop & Clerk Island, located near to Macquarie. These islands form part of the Macquarie Ridge, a region of uplifted ocean crust formed by the collision of tectonic plates. The ridge also includes a series of deep ocean valleys, seamounts and troughs, in excess of 5000m deep, which surround the relatively small Macquarie Island shelf. Fishing is concentrated in two main fishing grounds, one to the North of Macquarie Island in the area of the Northern Valleys, encompassing the Colgate Valley and Grand Canyon (northern fishing ground), and the other to the west of Macquarie Island, dominated by the Aurora Trough (southern fishing ground) (Figure 1.2) (Sainsbury and de la Mare 2001).

### **Degree of exchange between toothfish populations**

The amount of exchange occurring between the various populations is largely unknown. However the migration of toothfish over large distances is, at least with respect to adults, unlikely. Toothfish are essentially a localised, sedentary species with limited migratory abilities due to their weak swimming ability (Smith and McVeagh 2000). A notable exception is the apparent incidental migration of a single toothfish from the Southern Atlantic Ocean to Greenland, presumably by following the path of cold deepwater currents (Møller et al. 2003). The extent to which toothfish may regularly utilise currents of this type to migrate large distances is uncertain. The migration of larvae to other populations is more conceivable than for adult toothfish, although evidence to support this mode of mixing remains scarce.

A number of methods have been used to elucidate differences between populations based upon certain characteristics, including genetic (Smith and McVeagh 2000), enzymatic (Diano 1989) and morphological



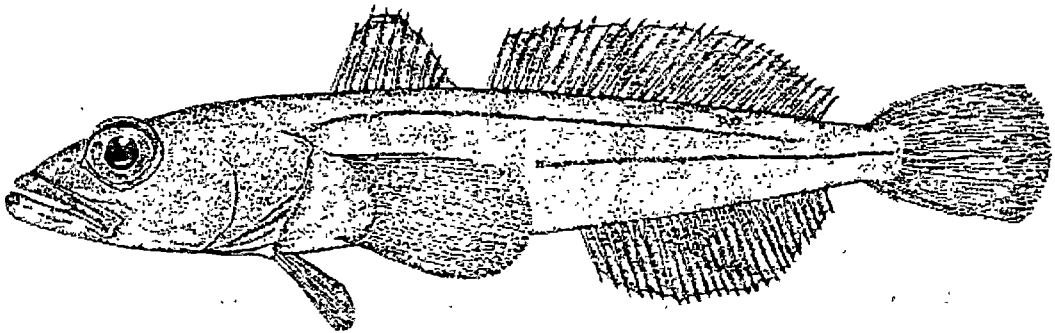
**Figure 1.2** Approximate positions of southern and northern fishing grounds relative to Macquarie Island (map courtesy of Simon Goldsworthy)

(Williams and de la Mare 1995). All support the view that toothfish are separated into distinctly segregated populations that experience little or no contact with toothfish from other regions.

### **Toothfish size and morphological characteristics**

Patagonian toothfish are the largest member of the family Nototheniidae. The maximum size documented is for a female toothfish from the Falkland Islands

measuring 225cm total length and 131kg estimated mass (Des Clers et al. 1996). Interpretation of otolith data currently places the maximum age of toothfish at 53 years (Horn 2002). A degree of sexual dimorphism has been suggested in certain stocks, such as those from South Georgia and Argentinean continental slope (García de la Rosa et al. 1997). These differences, however, may be due to differences in sampling regimes. Differences in both maximum size and stock size structure have also been observed for toothfish from different areas, although this is likely a consequence of exogenous constraints (primary production, oceanographic features) rather than purely endogenous biological factors. Toothfish morphology is characterised by a large body size, absence of a swimbladder, prominent mouth and eyes, dark skin, and a white, soft flesh devoid of pin bones (Figure 1.3).



**Figure 1.3** Patagonian toothfish, *Dissostichus eleginoides* (Source: Gon and Heemstra 1990)

### **Life history**

Many toothfish life history parameters remain unresolved. It appears that toothfish from different populations have slightly different life histories, possibly related to oceanographic features such as temperature. Generalised patterns of reproduction, spawning, and growth have been determined using evidence collected from several populations.

Along with a closely related Antarctic species, *Dissostichus mawsoni*, toothfish are amongst the most fecund of the nototheniids (Evseenko et al. 1995). Onset of sexual maturity is variable, occurring between 7-10 years (fish length of 64-94cm) in males and 9-12 years (85-115cm) in females (Zhivov and Krivoruchko 1990). Absolute toothfish fecundity increases with length and weight (Chikov and Mel'nikov 1991.)

Depending upon region, toothfish spawn between March and April on the continental slope at around 500m depth (Des Clers et al. 1996). Eggs are deposited demersally on rocky substrate (Zhivov and Krivoruchko 1990), hatching between August and November (Evseenko et al. 1995). Larvae and postlarvae develop pelagically in the upper 200m of the water column (García de la Rosa et al. 1997). Juveniles remain in the pelagic zone, living in depths of between 70-1500m over the shelf and drop-off (Zhivov and Krivoruchko 1990; García de la Rosa et al. 1997). Upon reaching 15-20cm in length or approximately 1 year, juveniles begin to migrate towards a demersal existence in shallower water (Duhamel and Hureau 1985; Des Clers et al. 1996). Adult fish continue to migrate into deeper water as they mature, evidenced by an increase in mean fish length with increasing depth (Zhivov and Krivoruchko 1990; Coggan et al. 1996).

### **Foraging ecology of toothfish**

Diet is region specific and changes as fish mature to deeper waters. Most dietary information is based upon the identification of prey in stomach contents, and is therefore affected by the acknowledged biases of this technique. Toothfish feed upon a wide diversity of prey items. Larvae consume small zooplankton, mainly euphausiids, whilst postlarvae and juveniles feed upon larger zooplankton species (Duhamel and Hureau 1985; García de la Rosa et al. 1997). Adults, toothfish aged greater than 3 years, feed primarily on fish species, including numerous myctophid species, with squid and zooplankton as secondary prey items (Duhamel and Hureau 1985; McKenna Jr 1991; Kozlov 1995; García de la Rosa et al. 1997).

## 1.4 LIPIDS AS DIETARY INDICATORS

An area of great importance in the study of ecosystems is the determination of diet or foraging ecology. Although the need to determine diet is unquestionable, this rarely translates into a practical response. The inherently difficult nature of study in the oceanic environment has limited exploration to only a fraction of the ecosystem. Compounding this is the fact that observation of marine organisms in their natural state is often difficult. With the exception of certain marine mammals and seabirds, the majority of species spend the entirety of their lives at sea, breeding, foraging and interacting. Although the relative biomass of the oceans is immense, the distribution of species within it is not well understood. Large aggregations of species are rare and unpredictable, the notable exception being commercially harvested fish (e.g. orange roughy).

### **Traditional methods of dietary determination**

The investigation of diet commonly relies heavily on two techniques; direct observation of feeding events, and the analysis of stomach contents. Direct observation involves firsthand witnessing of the capture of prey by a predator. Stomach content analysis requires the meticulous sorting and identification of indigestible body parts, including otoliths, statoliths and squid beaks, and relating these to potential prey species. Whilst each method has its relative merits, both are difficult to undertake, costly and time consuming. The results obtained from stomach content analysis in particular are virtually impossible to verify without avoiding any number of well-acknowledged biases associated with this technique. Amongst these are a failure to identify with certainty long-term dietary compositions and bias introduced by the differential digestion of prey items (see Antonelis et al. 1987; Harwood and Croxall 1988; Rodhouse and Nigmatullin 1996; Phillips et al. 2001).

Observation for the majority of marine populations involves the invasive sampling and removal of specimens. In some cases, these specimens can then be maintained in controlled environments (for instance aquaria), however most are sacrificed during the processes of inspection and analysis.

Modern techniques have been developed which attempt to observe marine organisms in conditions reflecting as closely as possible their natural state, including underwater filming (Collins et al. 1999; Yau et al. 2001; Yau et al. 2002) and satellite tracking. Associated with these sophisticated techniques are several notable disadvantages. They are expensive and difficult to perform whilst suffering from many of the same problems as the more traditional techniques, providing at best only a cursory insight into foraging ecology.

### **Fatty acids as dietary indicators**

The use of alternate and complementary methods may overcome some of the disadvantages of these traditional techniques. One such alternate method is the use of biological or biochemical markers including fatty acids. Analysis of the fatty acid composition of toothfish could provide a simple and effective means of determining diet. Combinations of fatty acids (also termed “signature lipids”) are readily transferred within the foodweb from predator to prey. Whilst numerous fatty acids can be synthesised *de novo* from non-lipoidal sources (e.g. protein and carbohydrate) a large number can only have arisen from dietary sources and are termed ‘essential’. Essential fatty acids are therefore particularly useful as signature lipids. Most of the long-chain polyunsaturated fatty acids (PUFA) are synthesised almost exclusively by primary producers at the base of the foodchain (Graeve et al. 1994; Nichols et al. 1994a; Dunstan et al. 1999). In higher order species, these dietary derived fatty acids are thought to be incorporated into storage tissues with little or no modification (e.g. Iverson 1993; Navarro et al. 1995; Iverson et al. 1997; Kirsch et al. 1998). Identification of signature lipid combinations in the lipid deposits of an organism provides potential evidence of foraging events and represents, to some extent, a temporal integration of diet. Observed differences in fatty acid composition between individuals of the same species are consequently due, in part, to differences in diet.

The use of fatty acids as dietary indicators is a relatively novel concept. The continuing development of more sophisticated analytical techniques (e.g. gas chromatography-mass spectrometry), and statistical methods for treating the

vast data sets created (e.g. principle component analysis, classification trees) has lead to the increasing relevance of fatty acids in dietary studies.

### **Fatty acid studies of the Southern Ocean**

Although signature fatty acids have been applied to the investigation of dietary interactions across a broad range of species and environments, it is the marine ecosystem that has most benefited from the application and development of this technique. Investigations into the lipid composition of marine organisms have included species from almost every trophic level and oceanic region. The degree of investigation varies greatly and has predominantly focussed upon higher level predators and those species of either commercial interest (e.g. fish) or a perceived threat to these commercial interests (e.g. seals). Rather than a detailed analysis of constituent fatty acids, the main objective of many of these studies has been the determination of proximal lipid composition (lipid class and total content).

The proximal lipid composition of marine organisms has been applied to the study of metabolic energy flow within foodwebs (Tierney et al. 2002), energy storage (Hochachka et al. 1975; Semmens 1998), the structural role of lipids in cells and buoyancy regulation (Clarke et al. 1984; Phleger 1991; Phleger 1998; Hagen et al. 2000), areas of research that generally do not require detailed information on fatty acid composition. Recently however, fatty acids have begun to play an important role in the detailed investigation of these topics as well as in research directed toward physiological processes, including reproduction (e.g. Cavalli et al. 2001), neurological development and the functioning of the central nervous system (Dumont et al. 1992; Dumont et al. 1994).

The use of lipids as indicators of foraging ecology first came to prominence with the work of Ackman et al. (1965) on fin whales (*Balaenoptera physalus*) from the North Atlantic where differences in the lipid composition of blubber in Arctic and Antarctic populations were related to possible dietary differences. At the present time, the fatty acid composition of relatively few marine species from sub-Antarctic regions have been determined. The

complexity of intermediary trophic interactions for sub-Antarctic species has limited dietary determination in the majority of fish and squid species to traditional techniques. Previous studies into the use of fatty acids as dietary tracers in the Southern Ocean have so far been restricted primarily to either top-level marine predators (namely whales, seals and seabirds e.g. Raclot et al. 1998; Brown et al. 1999), primary producers (phytoplankton and bacteria e.g. Nichols et al. 1993; Graeve et al. 1994) and lower-end consumers (zooplankton e.g. Kattner et al. 1994; Kattner and Hagen 1995). Detailed fatty acid studies incorporating mid-level consumers of the Southern Ocean, ostensibly fish and squid, have only recently emerged (e.g. Phillips et al. 2001; Lea et al. 2002; Phillips et al. 2002).

#### *Primary producers: phytoplankton, bacteria and particulate organic matter*

Knowledge of the lipid composition of primary producers is generally poor, particularly for the Southern Ocean. Given that phytoplankton are thought to produce many of the essential Omega-3 and Omega-6 PUFA (Graeve et al. 1994; Nichols et al. 1994a; Dunstan et al. 1999), the lack of attention given to those organisms at the base of the foodweb has resulted in a large gap in the understanding of fatty acid transfer from lower to higher trophic levels. Nichols et al. (1989) identified a number of unusual fatty acids in particulate matter of the Southern Ocean that may prove of use as signature lipids.

#### *Invertebrates*

The invertebrate fauna of the Southern Ocean is diverse, but is dominated by a relative few number of species of mainly copepods and euphausiids. Antarctic krill, *Euphausia superba*, are arguably the best studied of all Southern Ocean organisms. The position of krill as a corner-stone species, particularly in Antarctic waters, has meant that almost every aspect of their biology, including lipid composition, has been studied extensively. The fatty acid composition of *E. superba* appears to be highly sensitive to seasonal changes in diet. The fatty acid composition of wild populations of *E. superba* have been related to periods of omnivorous foraging (Falk-Petersen et al.

2000). Captive studies of *E. superba* have also been used to explore the use of fatty acids in the detection of carnivorous (Cripps and Atkinson 2000) and algal (Virtue et al. 1993a) feeding activities. Falk-Petersen et al. (2000) also examined a further krill, *E. crystallorophias*, and found a fatty acid composition consistent with a predominantly herbivorous diet.

The fatty acid composition of various Southern Ocean copepods have also been examined in relation to dietary studies. Graeve et al. (1994) classified various Antarctic copepods (*Calanoides acutus*, *Rhincalanus gigas*, *Metridia gerlachei*) as either herbivorous or omnivorous based upon lipid composition. A further study by Kattner and Hagen (1995) further explored the relationship between copepod diet and lipid composition and categorised wild populations of various phytoplankton-consuming Arctic and Antarctic copepods (*C. acutus*, *R. gigas*) into three broad dietary groups (diatom, dinoflagellate and omnivorous feeders) by the presence of fatty acids unique to these phytoplankton groups.

The use of lipids for dietary studies of Antarctic pteropods and amphipods has also been assessed. Interannual variations in the lipid composition of the pteropods *Clione limacina* and *C. pyramidata* were attributed to possible fluctuations in the composition of phytoplankton in the diet (Phleger et al. 2001). A similar interannual variation in the lipid composition of various Antarctic amphipods (*Themisto gaudichaudii*, *Hyperia macrocephalus*, *Primno macropa*, *Eusirus perdentatus*, *Orchomeme rossi*) was also related to diet (Nelson et al. 2001).

Finally, an inclusive study by Phleger et al. (1998) used fatty acid composition to distinguish between the herbivorous and carnivorous diets of a number of Antarctic zooplankton, including the amphipod *T. gaudichaudii*, the krill *E. tricantha* and *E. frigida* and the scyphomedusan *Periphylla periphylla*.

### *Mid-level consumers: fish and squid*

Although still grossly under-represented, factors such as the increasing commercialisation of many species has shifted the focus of current lipid research onto species of fish and squid in the Southern Ocean. Phleger et al. (1997) related differences in the lipid composition of two Antarctic populations of the myctophid *Electrona antarctica* to dietary sources. Lipid studies of midwater fish from sub-Antarctic regions are also beginning to emerge. Lea et al. (2002) determined the lipid composition of various myctophids and the nototheniid *Champscephalus gunnari* from the Kerguelen Plateau and Macquarie Island region and discussed the implications of these findings to marine predators.

Understanding of the biology of Southern Ocean squid has greatly benefited from lipid studies. Phillips et al. (2001) used fatty acids to explore the trophic interactions between the squid *Moroteuthis ingens* and myctophid fish in the Macquarie Island region.

### *Higher predators: marine mammals*

The work of Iverson (1993) on the lipid composition of milk from Californian sea lions and Harbour seals rejuvenated interest in the use of dietary lipids, especially in the study of marine mammals. This and subsequent research rested upon work stretching back to the study of Fin whales by Ackman et al. (1965). Importantly, the effectiveness of this technique to the study of marine mammals has been called into question by several researchers (Grahl-Nielsen and Mjaavatten 1991; Grahl-Nielsen 1999; Grahl-Nielsen et al. 2000), although this claim has also been repudiated (e.g. Smith et al. 1999).

The application of signature lipids to Southern Ocean marine mammals has been less pronounced. Later research by Iverson et al. (1997) applied the same technique used in relation to Californian sea lions and Harbour seals to the study of lactating Antarctic fur seal (*Arctocephalus gazella*) diet. A major shift in the fatty acid composition of milk in these seals was related to a

possible change in diet from krill to fish. Brown et al. (1999) also examined the milk of Antarctic fur seals as well as Southern elephant seals (*Mirounga leonina*). Similarities were observed in the fatty acid composition of krill and krill-eating fish and fur seals, whilst the fatty acid composition of Southern elephant seals were closer to larger fish such as nototheniids (including Patagonian toothfish) and myctophids.

## 1.5 PROJECT OBJECTIVES

The main objectives of this study are:

1. Determine the lipid composition of toothfish in the Macquarie Island region and to compare these profiles over a range of sizes (ages), fishing grounds, fishing seasons, fishing depths and between sexes
2. Determine the lipid composition of potential fish and squid prey including those species identified from stomach content analysis
3. Assess likely physiological functions of lipid in toothfish and potential prey
4. Assess the dietary interaction and the long-term diet of toothfish based on a comparison of the fatty acid profiles of toothfish and potential prey including the use of statistical analysis.

# CHAPTER 2

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## LIPID COMPOSITION OF THE MESOPELAGIC AND BATHYPELAGIC FISH ASSEMBLAGE OF THE MACQUARIE ISLAND REGION

### 2.1 INTRODUCTION

Mesopelagic and bathypelagic fish, collectively known as midwater fish, are important components of sub-Antarctic regions of the Southern Ocean marine ecosystem. Characterised by a relatively small shelf and a vast system of deep ocean valleys, seamounts and troughs, sub-Antarctic Macquarie Island supports a unique and diverse midwater fish assemblage (Williams et al. 2001). Lying within the Antarctic polar frontal zone, Macquarie Island is exposed to the seasonal productivity, typical of many sub-Antarctic Islands, that drives and sustains the growth of zooplankton (Goldsworthy et al. 2001a). It is upon this zooplankton community, consisting of various species of copepod, euphausiid and amphipod, that midwater fish primarily forage (Gaskett et al. 2001).

In terms of total biomass and species diversity, midwater fish perform a crucial role in the dietary interactions of numerous foodwebs. The total annual consumption of midwater fish in the Macquarie Island region by predators and fishing operations is estimated to be over 250 000 tonnes per year (Goldsworthy et al. 2001d). Fish of the mesopelagic family Myctophidae, in particular, are often cited as important prey for a number of higher predators especially seabirds (Kozlov 1995; Cherel et al. 1996; Goldsworthy et al. 2001d) and squid (Rodhouse and White 1995; Rodhouse and Nigmatullin 1996; Phillips et al. 2001). Although the importance of midwater fish to the diet of marine mammals is not as well defined, studies on Southern elephant seals (Green and Burton 1993), fur seals (Green et al.

1990) and Hooker's sea lions (McMahon et al. 1999) from the Macquarie Island region suggest that a number of fish species, including myctophids, are commonly consumed by these predators. The importance of midwater fish in the diet of other fish species (including cannibalism) is considered to be minor (Kozlov 1995), with the exception of larger predatory fish, such as Patagonian toothfish (*Dissostichus eleginoides*) (Goldsworthy et al. 2002).

The development of a commercial fishery for Patagonian toothfish has hastened the need for a better understanding of the dietary interactions operating within the Macquarie Island foodweb. Included within the scope of such an investigation is a need to determine the foraging ecology of midwater fish. Gaskett et al. (2001) investigated the diet of 23 commonly occurring species of mesopelagic and bathypelagic fish from the Macquarie Island region using stomach content analysis. Five trophic guilds were identified within this midwater population, based upon the varying importance of copepods, euphausiids, amphipods and, to a lesser degree, fish in the diet. Dietary studies relying upon the identification of prey remains in stomach contents are, however, prone to several well-acknowledged and unavoidable biases when applied in isolation. Amongst these are a failure to identify with certainty long-term dietary compositions and bias introduced by the differential digestion of prey items (Antonelis et al. 1987; Harwood and Croxall 1988; Rodhouse and Nigmatullin 1996; Phillips et al. 2001).

A technique with increasing application to dietary studies is the use of lipid, and in particular fatty acid, composition to assess predator-prey interaction. For instance Phillips et al. (2001) recently assessed the dietary interaction of squid and myctophids in the Macquarie Island region using a combination of traditional (stomach contents) and developing (dietary lipids) techniques. The use of dietary lipids in the study of midwater fish has so far been predominantly limited to oceanic regions other than the sub-Antarctic. Saito and Murata (1998) applied lipids in a dietary investigation of several Northern hemisphere myctophids. Phleger et al. (1997) related differences in the lipid composition of two Antarctic populations of the myctophid *Electrona antarctica* to dietary sources.

Lipid studies of midwater fish from sub-Antarctic regions are beginning to emerge. Lea et al. (2002) determined the lipid composition of various myctophids and the nototheniid *Champsocephalus gunnari* from the Kerguelen Plateau and discussed the implications of these findings to marine predators. Included within their study was a comparison with several myctophid species from the Macquarie Island region.

The lipid composition of 21 species of midwater fish from the Macquarie Island region were determined in the current investigation, many of which were included in the dietary study of Gaskett et al. (2001). The purpose of this study is to determine whether similarities in diet based upon stomach content analysis are reflected in similarities in lipid (primarily fatty acid) composition. Secondly, the lipid composition of species, included in this study, and collected in other regions of the Southern Ocean will be compared to investigate the potential effect of regional variations in diet on lipid composition.

Finally, a lack of lipid compositional data concerning invertebrates from the lower trophic levels (i.e. the prey of higher predators) has hampered the application of the signature lipid technique in regions such as Macquarie Island. It is therefore anticipated that the results of this and other studies (e.g. Phillips et al. 2001; Lea et al. 2002) will further the understanding of dietary interactions within the Macquarie Island marine ecosystem.

## **2.2 MATERIALS AND METHODS**

### **Sample collection**

Pelagic fish samples (n=84) were collected as part of joint fishing activities of the *FV Austral Leader*, a deep-sea trawler operated by Austral Fisheries Pty. Ltd. (Fremantle, Western Australia) and the CSIRO research vessel *RV Southern Surveyor*. Fishing took place over the Southern Hemisphere summers of 1998-1999 and 1999-2000 in fishing grounds of the Macquarie

Island region (54°30' S; 158°55' E), approximately 1500km SW of Tasmania, Australia.

Immediately after capture, samples were identified to species level, where possible, frozen at -20°C and analysed within 3 months where logistics allowed. Prior to analysis total length (TL, tip of snout to tip of caudal fin) and total weights (TW, wet mass) were determined before whole fish samples were thawed and then ground. A sub-sample (1-2g) was removed for lipid extraction and analysis.

### **Lipid extraction and class determination**

Total lipids were extracted using the one-phase method of Bligh and Dyer (1959). After phase separation, total lipids were recovered from the lower chloroform phase and concentrated *in vacuo* (rotary evaporator, 40°C). Samples were stored at -20°C prior to analysis. Lipid class profiles were determined by TLC-FID using an Iatroscan MkV TH10 analyser (Volkman and Nichols 1991). Wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA), sterol (ST) and polar lipid (PL) components were resolved in a polar solvent system consisting of hexane-diethyl ether-acetic acid (60:17:0.2 v/v). The presence or absence of WE and diacylglyceryl ethers (DAGE) was confirmed by the use of a non-polar solvent system (hexane-diethyl ether; 96:4 v/v). Peak areas were quantified using commercially available chromatography software (DAPA Scientific Software, Kalamunda, Western Australia).

### **Fatty acid and fatty alcohol determination**

Fatty acid methyl esters (FAME) were prepared by addition of methanol (MeOH), concentrated hydrochloric acid (HCl) and chloroform (CHCl<sub>3</sub>) (3mL 10:1:1, v/v/v, 80°C/2hr) to an aliquot of the total lipid. After the addition of 1mL of water, resulting FAME was extracted (3x) with 4:1 v/v hexane/CHCl<sub>3</sub>. Chloroform containing C<sub>19</sub> FAME internal standard was then added. FAME fractions were treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA 50μL, 60°C, 2hr) to convert any sterols present to their corresponding

trimethylsilyl (TMS) ethers and any wax esters to their corresponding fatty alcohols.

Gas chromatographic analysis was performed on a Hewlett Packard 5890A GC equipped with a flame ionisation detector, a cross-linked methyl silicone (HP5) fused silica capillary column (50m length  $\times$  0.32  $\mu$ m internal diameter), employing hydrogen as the carrier gas. Samples were injected (HP7673 auto-injector) at 50°C in the splitless mode, with a 2-min. venting time (Nichols et al. 1993). The injector and detector were maintained at 290°C and 310°C respectively. The temperature of the oven was increased after 1 min. to 150°C at 30°C min<sup>-1</sup>, then to 250°C at 2°C min<sup>-1</sup> and finally to 300°C at 5°C min<sup>-1</sup> (Nichols et al. 1993). Peak areas were quantified using Millennium 32 Chromatography Manager (Waters Corporation, Milford, Massachusetts, USA). Identification of individual fatty acids was based upon a comparison of relative retention times with those obtained for authentic and laboratory standards. GC-mass spectrometry (Fisons MD800 system and Finnigan Thermoquest GCQ fitted with an on-column injector, both configured as above) was used for confirmation of components from representative samples (Nichols et al. 1993).

### **Statistical analyses**

All statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, Illinois, USA). Variations in total lipid content, lipid class and individual fatty acid compositions in relation to varying size were compared using two-sample *t*-tests (95% confidence interval).

Multivariate statistics were also used to analyse total fatty acid and lipid class composition. Hierarchical clustering (Pearson's average linkage) was used to compare the average fatty acid composition of the various species. Non-metric multi-dimensional scaling (MDS; Kruskal loss function) was employed to compare the fatty acid and lipid class composition of the various fish species in two dimensions in order to assess whether groupings existed within the data set.

## 2.3 RESULTS

### Species composition and size

A total of 84 fish samples were collected for analysis in this study, representing 11 families and 21 species (Table 2.1). This included 8 species of Myctophidae, 3 species of Stomiidae, 2 species of Gonostomatidae and single species from the families Melamphidae, Astronesthidae, Sternoptychidae, Bathylagidae, Nemichthyidae, Carapidae, Phosichthyidae and Zoarcidae. Several authors classify the astronesthid *Borostomias antarcticus* and Stomiidae species together into a single family, Stomiidae (e.g. Gon and Heemstra 1990). However, for the purposes of this study, *Borostomias antarcticus* has been placed into a separate family (Astronesthidae) according to the grouping of Miller (1993).

Specimens ranged greatly in total length (TL) and total weight (TW), both between and within species (Table 2.1). TW was below 50g in all species with the exception of *Gymnoscopelus bolini*, which contained the heaviest specimen analysed as well as the greatest range of TW within a single species (range 50.2-89.6g). Another myctophid, *Gymnoscopelus braueri*, contained the lightest specimen analysed (0.1g).

TL in all species was all less than 300mm, with the exception of *Labichthys yanoi* (range 460-610mm) and *Echiodon cryomargarites* (320mm). A specimen of *G. braueri* represented the smallest TL (35mm), whilst the largest range of TL within a single species occurred in *Stomias gracilis* (range 80-290mm).

A sufficient range of sizes were available to separate specimens of *Borostomias antarcticus* (range 74-220mm TL), *Bathylagus antarcticus* (range 68-140mm TL) and *S. gracilis* (range 80-290mm TL) into adults and juveniles (Table 2.5). Of these species, however, only the sample size of *Bathylagus antarcticus* and *S. gracilis* allowed for the statistical comparison of adults and juveniles.

**Table 2.1** Summary of fish species included in the current study from Macquarie Island showing details of family and species name, total wet weight and length (data presented as range of values, n=number of samples) and habitat

Family	Family common name	Species	n	Total weight (g)	Total length (mm)	Habitat <sup>1</sup>
Stomiidae	Scaly Dragonfishes	<i>Stomias boa boa</i>	2	25.1-34.4	230-270	meso-bathypelagic
		<i>Stomias gracilis</i>	5	0.3-14.0	80-290	mesopelagic
		<i>Chauliodus sloani</i>	2	10.9-17.6	210-250	bathypelagic
Astronesthidae	Snaggletooths	<i>Borostomias antarcticus</i>	8	0.5-32.3	74-220	bathypelagic
Melamphidae	Bigscales, Ridgeheads, Crested Bigscales	<i>Poromitra crassiceps</i>	1	1.8	65	meso-bathypelagic
Sternoptychidae	Hatchetfishes	<i>Sternoptyx sp.</i>	1	4.3	60	meso-bathypelagic
Bathylagidae	Deepsea smelts, Blacksmelts	<i>Bathylagus antarcticus</i>	11	0.5-13.6	58-140	meso-bathypelagic
Myctophidae	Lanternfishes	<i>Electrona carlsbergi</i>	5	8.6-10.8	100-110	epi-mesopelagic
		<i>Electrona antarctica</i>	4	0.4-3.2	42-85	epi-mesopelagic
		<i>Lampanyctus achirus</i>	1	0.7	140	bathypelagic
		<i>Gymnoscopelus nicholsi</i>	2	18.8-21.2	140-150	epi-mesopelagic
		<i>Gymnoscopelus fraseri</i>	8	1.1-6.8	60-100	epi-mesopelagic
		<i>Gymnoscopelus braueri</i>	8	0.1-0.5	35-47	epi-mesopelagic
		<i>Gymnoscopelus bolini</i>	3	50.2-89.6	200-220	epi-mesopelagic
		<i>Protomyctophum bolini</i>	10	1.0-1.7	58-66	mesopelagic
Nemichthyidae	Snipe Eels	<i>Labichthys yanoi</i>	2	4.9-23.5	460-610	bathypelagic
Carapidae	Carapids, Pearlfishes	<i>Echiodon cryomargarites</i>	1	9.8	320	mesopelagic
Phosichthyidae	Lightfishes	<i>Phosichthys argenteus</i>	1	5.0	135	meso-bathypelagic
Gonostomatidae	Bristlemouths, Lightfishes	<i>Cyclothone sp.</i>	3	0.2-0.8	40-60	bathypelagic
		<i>Diplophos rebainsi</i>	1	22.7	240	bathypelagic
Zoarcidae	Eelpouts	<i>Melanostigma gelatinosum</i>	5	0.7-0.9	80-100	epi-mesopelagic

<sup>1</sup>Source: Gon and Heemstra (1990), Miller (1993), Kock (1992), Pakhomov et al. (1996), Kozlov and Tarverdiyeva (1989), Kozlov (1995)

**Total lipid content**

Total lipid contents were determined in 83 specimens (Tables 2.2-2.5). Lipid content, in individual specimens, ranged from a high of 20.4% WW (*Electrona antarctica*) to a low of <1%WW (*Phosichthys argenteus*). Variation within an individual species was greatest in *Gymnoscopelus braueri* (range 4.8-17.1% WW). Average lipid content within families varied little in stomiatids (Table 2.2) and gonostomiatids (Table 2.4), but showed greater variation across the eight myctophid species (Table 2.3).

Fish could be separated into three main groups, based on average lipid content. The first group consisted of those species with an average of <5% WW lipid and included members of various families; juvenile *Borostomias antarcticus*, juvenile *Bathylagus antarcticus*, juvenile *Stomias gracilis*, *Melanostigma gelatinosum*, *Phosichthys argenteus*, *Poromitra crassiceps*, *Sternoptyx* sp., and *Echiodon cryomargarites*.

The second group, with an average total lipid content between 5-10% WW, contained a number of myctophid species (*Electrona carlsbergi*, *Gymnoscopelus braueri*, *Gymnoscopelus fraseri*, *Gymnoscopelus nicholsi* and *Lampanyctus achirus*), a species of stomiatid (*Chauliodus sloani*), the nemichthyid *Labichthys yanoi* and adults of both *Borostomias antarcticus* and *Bathylagus antarcticus*.

The final group contained the remaining stomiatids (*Stomias boa boa* and adult *Stomias gracilis*) and myctophids (*Electrona antarctica*, *Gymnoscopelus bolini* and *Protomyctophum bolini*) as well as both gonostomatid species (*Cyclothone* sp. and *Diplophos rebainsi*), the total lipid content of which exceeded 10% WW.

Of those species with both a sufficient number of specimens ( $n>2$ ) and a diverse enough range of sizes, appreciable increases in lipid content with increasing length and weight were observed in *S. gracilis*, *Borostomias antarcticus*, *Bathylagus antarcticus*, *G. fraseri*, *G. braueri*, *G. bolini*, *Cyclothone* sp., *M. gelatinosum* and *P. bolini*. Lipid contents were negatively

**Table 2.2** Total lipid content (percent composition, wet weight) and lipid class composition (percentage of total lipid content) of various species of the family Stomiidae from the Macquarie Island region (all data presented as mean  $\pm$  standard deviation)

Species	n	Total lipid content	Lipid class					Total
			WE	TAG	FFA	ST	PL	
<i>Stomias gracilis</i> <sup>j</sup>	2	4.3 $\pm$ 0.8	14.9 $\pm$ 14.0	30.5 $\pm$ 21.8	39.9 $\pm$ 2.9	6.7 $\pm$ 1.2	8.1 $\pm$ 3.7	100.0
<i>Stomias gracilis</i> <sup>a</sup>	3	14.2 $\pm$ 2.0	0.0	90.4 $\pm$ 3.5	7.6 $\pm$ 3.2	0.9 $\pm$ 0.2	1.1 $\pm$ 0.3	100.0
<i>Stomias boa boa</i>	2	10.2 $\pm$ 2.6	0.0	91.9 $\pm$ 1.5	4.0 $\pm$ 1.2	0.9 $\pm$ 0.5	3.3 $\pm$ 2.2	100.0
<i>Chauliodus sloani</i>	2	9.7 $\pm$ 2.6	0.0	71.2 $\pm$ 2.3	22.6 $\pm$ 2.4	3.6 $\pm$ 0.4	2.5 $\pm$ 0.5	100.0

WE=wax ester; TAG=triacylglycerol; FFA=free fatty acid; ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol); PL=polar lipid; n=number of samples; j=juveniles (<200mm total length); a=adults (>200mm total length)

**Table 2.3** Total lipid content (percent composition, wet weight) and lipid class composition (percentage of total lipid content) of various species of the family Myctophidae from the Macquarie Island region (all data presented as mean  $\pm$  standard deviation)

Species	n	Total lipid content	Lipid class					Total
			WE	TAG	FFA	ST	PL	
<i>Electrona antarctica</i>	3	17.5 $\pm$ 3.6	86.2 $\pm$ 4.6	2.1 $\pm$ 1.1	4.4 $\pm$ 1.0	1.6 $\pm$ 0.3	5.7 $\pm$ 2.5	100.0
<i>Electrona carlsbergi</i>	5	9.5 $\pm$ 2.2	0.3 $\pm$ 0.2	64.2 $\pm$ 8.8	5.0 $\pm$ 2.8	1.6 $\pm$ 0.7	29.0 $\pm$ 7.3	100.0
<i>Gymnoscopelus bolini</i>	3	14.5 $\pm$ 1.8	85.1 $\pm$ 10.3	8.0 $\pm$ 7.8	3.9 $\pm$ 1.1	1.2 $\pm$ 0.9	1.7 $\pm$ 0.5	100.0
<i>Gymnoscopelus braueri</i>	8	9.7 $\pm$ 3.8	66.5 $\pm$ 20.7	0.9 $\pm$ 1.3	22.4 $\pm$ 16.0	4.7 $\pm$ 3.2	5.5 $\pm$ 2.4	100.0
<i>Gymnoscopelus fraseri</i>	8	7.6 $\pm$ 4.4	1.0 $\pm$ 2.0	53.6 $\pm$ 26.7	17.6 $\pm$ 11.3	4.0 $\pm$ 2.5	23.9 $\pm$ 15.4	100.0
<i>Gymnoscopelus nicholsi</i>	2	7.1 $\pm$ 2.0	0.0	80.7 $\pm$ 6.2	10.4 $\pm$ 3.0	1.2 $\pm$ 0.4	7.8 $\pm$ 2.8	100.0
<i>Protomyctophum bolini</i>	10	11.0 $\pm$ 3.4	1.0 $\pm$ 1.0	49.8 $\pm$ 21.0	17.7 $\pm$ 11.2	3.2 $\pm$ 2.1	28.3 $\pm$ 21.2	100.0
<i>Lampanyctus achirus</i>	1	6.1	2.7	20.4	27.9	8.8	40.2	100.0

WE=wax ester; TAG=triacylglycerol; FFA=free fatty acid; ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol); PL=polar lipid; n=number of samples

**Table 2.4** Total lipid content (percent composition, wet weight) and lipid class composition (percentage of total lipid content) of various species of the family Gonostomatidae from the Macquarie Island region (all data presented as mean  $\pm$  standard deviation)

Species	n	Total lipid content	Lipid class					Total
			WE	TAG	FFA	ST	PL	
<i>Cyclothone</i> sp.	3	12.3 $\pm$ 2.4	86.7 $\pm$ 1.9	6.4 $\pm$ 2.7	3.6 $\pm$ 2.3	1.5 $\pm$ 1.0	1.7 $\pm$ 0.3	100.0
<i>Diplosis rebaini</i>	1	17.0	96.6	0.8	1.3	0.4	1.0	100.0

WE=wax ester; TAG=triacylglycerol; FFA=free fatty acid; ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol); PL=polar lipid; n=number of samples

**Table 2.5** Total lipid content (percent composition, wet weight) and lipid class composition (percentage of total lipid content) of miscellaneous fish species from the Macquarie Island region (all data presented as mean  $\pm$  standard deviation)

Species	n	Total lipid content	Lipid class					Total
			WE	TAG	FFA	ST	PL	
<i>Borostomias antarcticus</i> <sup>j</sup>	5	1.5 $\pm$ 0.4	19.6 $\pm$ 10.7	10.3 $\pm$ 7.5	12.6 $\pm$ 7.8	10.8 $\pm$ 4.9	46.7 $\pm$ 7.9	100.0
<i>Borostomias antarcticus</i> <sup>a</sup>	1	9.6	59.4	34.1	3.2	1.1	2.2	100.0
<i>Bathylagus antarcticus</i> <sup>j</sup>	6	2.2 $\pm$ 0.5	1.6 $\pm$ 0.7	6.3 $\pm$ 4.1	35.9 $\pm$ 1.8	11.4 $\pm$ 0.8	44.9 $\pm$ 5.6	100.0
<i>Bathylagus antarcticus</i> <sup>a</sup>	5	6.3 $\pm$ 1.4	0.0 $\pm$ 0.0	82.9 $\pm$ 5.4	8.2 $\pm$ 3.7	1.8 $\pm$ 0.7	7.2 $\pm$ 3.0	100.0
<i>Labichthys yanoi</i>	2	8.7 $\pm$ 0.4	88.7 $\pm$ 6.0	4.5 $\pm$ 3.6	4.1 $\pm$ 1.9	1.5 $\pm$ 0.6	1.2 $\pm$ 0.1	100.0
<i>Melanostigma gelatinosum</i>	5	3.7 $\pm$ 0.8	3.5 $\pm$ 2.7	11.0 $\pm$ 5.8	29.9 $\pm$ 5.4	8.4 $\pm$ 2.0	47.2 $\pm$ 8.3	100.0
<i>Phosichthys argenteus</i>	1	1.0	2.4	20.2	0.1	11.9	65.3	100.0
<i>Poromitra crassiceps</i>	1	1.3	1.5	8.6	0.0	8.9	80.9	100.0
<i>Sternoptyx</i> sp.	1	1.9	0.7	8.6	0.0	12.6	78.1	100.0
<i>Echiodon cryomargarites</i>	1	2.0	19.7	7.1	18.2	10.2	44.7	100.0

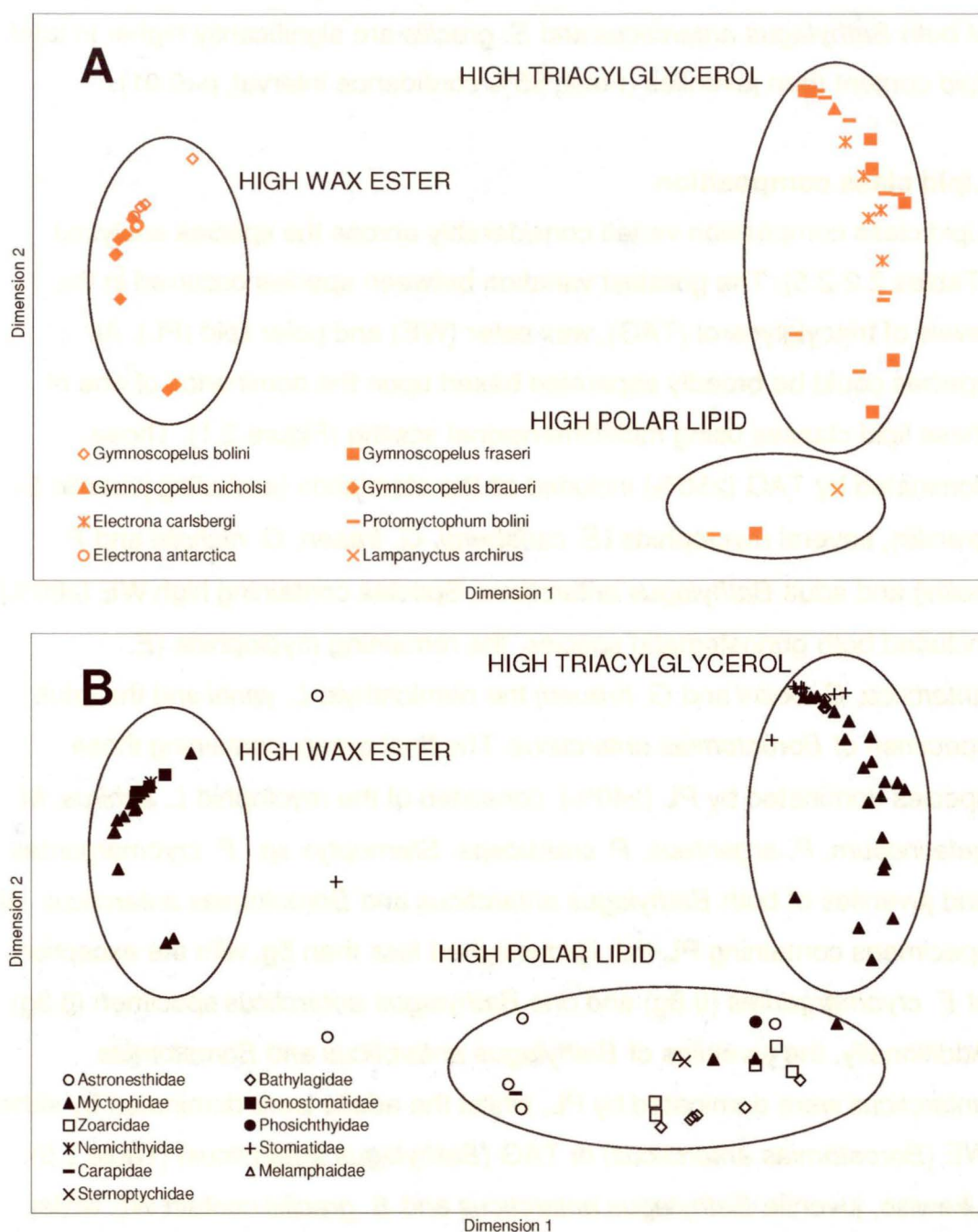
WE=wax ester; TAG=triacylglycerol; FFA=free fatty acid; ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol); PL=polar lipid; n=number of samples; j=juveniles (*Borostomias antarcticus* <200mm; *Bathylagus antarcticus* <100mm total length); a=adults (*Borostomias antarcticus* >200mm total length; *Bathylagus antarcticus* >100mm total length)

correlated in relation to increases in length and weight in *E. antarctica*. Adults of both *Bathylagus antarcticus* and *S. gracilis* are significantly higher in total lipid content than juveniles (*t*-test, 95% confidence interval,  $p < 0.01$ ).

### Lipid class composition

Lipid class composition varied considerably across the species analysed (Tables 2.2-2.5). The greatest variation between species occurred in the levels of triacylglycerol (TAG), wax ester (WE) and polar lipid (PL). All species could be broadly separated based upon the dominance of one of these lipid classes using multidimensional scaling (Figure 2.1). Those dominated by TAG ( $\geq 50\%$ ) included all the stomiatids (excluding juvenile *S. gracilis*), several myctophids (*E. carlsbergi*, *G. fraseri*, *G. nicholsi* and *P. bolini*) and adult *Bathylagus antarcticus*. Species containing high WE ( $\geq 60\%$ ) included both gonostomatid species, the remaining myctophids (*E. antarctica*, *G. bolini* and *G. braueri*) the nemichthyid *L. yanoi* and the adult specimen of *Borostomias antarcticus*. The final group, containing those species dominated by PL ( $\geq 40\%$ ), consisted of the myctophid *L. achirus*, *M. gelatinosum*, *P. argenteus*, *P. crassiceps*, *Sternoptyx* sp., *E. cryomargarites* and juveniles of both *Bathylagus antarcticus* and *Borostomias antarcticus*. All specimens containing PL-rich lipid weighed less than 5g, with the exception of *E. cryomargarites* (9.8g) and one *Bathylagus antarcticus* specimen (8.3g). Additionally, the juveniles of *Bathylagus antarcticus* and *Borostomias antarcticus* were dominated by PL, whilst the adults were dominated by either WE (*Borostomias antarcticus*) or TAG (*Bathylagus antarcticus*) (Table 2.5). Likewise, juvenile *Bathylagus antarcticus* and *S. gracilis* contain WE whilst adults do not. Statistical analysis of *Bathylagus antarcticus* and *S. gracilis* supports these observations, revealing a significant difference (*t*-test, *Bathylagus antarcticus*  $p < 0.001$ , *S. gracilis*  $p < 0.05$ ) in the content of all lipid classes between adults and juveniles.

Several individual specimens separated from the main groups when analysed using MDS (Figure 2.1). A juvenile specimen of *S. gracilis* separated from the other juvenile specimen due to a higher relative level of



**Figure 2.1** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of fish in this study from the Macquarie Island region **A**=grouped by myctophid species only; **B**=grouped by all families

WE (24.8% versus 5.0%). Two specimens of *Borostomias antarcticus* also separated from the other specimens; a juvenile specimen that contained higher WE levels (32.6%) than the other juveniles (average  $20.6 \pm 3.5\%$ ), and the single adult specimen that contained intermediate levels of both WE (59.4%) and TAG (34.1%).

A number of fish species also contained elevated levels (>10%) of free fatty acid (FFA). These include two species of stomiatid (juvenile *S. gracilis*, *C. sloani*), several myctophids (*G. braueri*, *G. fraseri*, *G. nicholsi*, *P. bolini*), juveniles of both *Borostomias antarcticus* and *Bathylagus antarcticus*, and *M. gelatinosum*. With the exception of *C. sloani*, *Borostomias antarcticus* and *Bathylagus antarcticus*, elevated fatty acid levels were confined to specimens with a TW of less than 1.5g. This is exemplified by the substantially elevated FFA content of juvenile *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis* compared to the levels observed in adults. *S. gracilis* also contained the highest FFA of any species (average  $39.9 \pm 2.9\%$ ).

The content of sterol (ST, predominantly cholesterol based upon GC analysis) also varied considerably. In a number of species ST was present at levels >10%, namely juvenile *Bathylagus antarcticus* (average  $11.4 \pm 0.8\%$ ), juvenile *Borostomias antarcticus* (average  $10.8 \pm 4.9\%$ ), *P. argenteus* (11.9%), *Sternoptyx sp.* (12.6%) and *E. cryomargarites* (10.2%). With the exception of *P. argenteus* and *Sternoptyx sp.*, the high ST levels of these species were associated with elevated FFA. For the remaining species, ST levels were generally less than 2%.

Little association between family and lipid class was observed. Both gonostomatids contained >85% WE and low contents of the other lipid classes. All stomiatids contained >70% TAG, with the exception of juvenile *S. gracilis* that contained appreciable amounts of WE ( $14.9 \pm 14.0\%$ , absent in the other stomiatid species), high levels of FFA ( $39.9 \pm 2.9\%$ ) and intermediate TAG content ( $30.5 \pm 21.8\%$ ). Myctophid lipid class composition varied significantly between the eight species analysed (Figure 2.1). WE (>65%) dominated the lipid class composition of three myctophid species, *E.*

*antarctica*, *G. bolini* and *G. braueri*, whilst contributing <3% to the remaining myctophid species. High levels of both TAG ( $\geq 50\%$ ) and PL ( $\geq 24\%$ ) characterised *E. carlsbergi*, *G. fraseri*, *P. bolini* and *L. achirus*. *G. nicholsi* contained no WE, low PL levels ( $7.8 \pm 2.8\%$ ) and high TAG ( $80.7 \pm 6.2\%$ ).

### Fatty acid composition

The fatty acid composition of the species analysed was dominated, with the exception of a small number of specimens, by monounsaturated fatty acids (MUFA) (Tables 2.6-2.11). MUFA content ranged from a high of 90% in *D. rebaini* (Table 2.7) to a low of 29.2% in *P. crassiceps* (Table 2.11). The greatest variation within a single species occurred in *Borostomias antarcticus* (37.9-71.9%), reflecting an increase in overall MUFA content from juveniles to adults (Table 2.10). Continuing this trend, MUFA content was also greater in the adults of *Bathylagus antarcticus* and *S. gracilis*. This was confirmed by statistical differences (*t*-test, 95% confidence interval,  $p < 0.05$ ) in the composition of several MUFA in *Bathylagus antarcticus* (16:1 $\omega$ 7c, 16:1 $\omega$ 7t, 17:1, 20:1 $\omega$ 9c, 20:1 $\omega$ 7c, 22:1 $\omega$ 9c, 24:1 $\omega$ 9c) and *S. gracilis* (14:1 $\omega$ 5c, 26:1 $\omega$ 7c, 18:1 $\omega$ 7c, 24:1 $\omega$ 9c).

The MUFA 18:1 $\omega$ 9c was the single most important fatty acid in the majority of species, dominating the composition of all stomiatids, all myctophids (excepting *G. nicholsi*), both gonostomatids, adults of both *Borostomias antarcticus* and *Bathylagus antarcticus* and a number of other species including *L. yanoi*, *M. gelatinosum*, *E. cryomargarites*. 18:1 $\omega$ 9c content was highest in *D. rebaini* (76.3%) and lowest in *P. crassiceps* (11.7%). Another MUFA, 20:1 $\omega$ 9c, dominated the fatty acid composition of the myctophid *G. nicholsi* (average  $22.7 \pm 1.0\%$ ). Other important MUFA included 16:1 $\omega$ 7c (range across all species 2.4-16%), 18:1 $\omega$ 7c (range 2.8-8.8%), 20:1 $\omega$ 9c (range 1.5-22.7%) and 22:1 $\omega$ 11c (range 0.2-6.0%).

Polyunsaturated fatty acids (PUFA) dominated the composition of *P. crassiceps* (43.4%), *Sternoptyx* sp. (41.8%) and juvenile *Bathylagus*

**Table 2.6** Fatty acid composition (% composition of total fatty acids) of various Stomiidae included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty Acid	Species				
	<i>Stomias</i>	<i>Stomias gracilis</i>			<i>Chauliodus</i>
	<i>boa</i> <i>boa</i>	juvenile	adult	average	<i>sloani</i>
	(n=2)	(n=2)	(n=3)	(n=5)	(n=3)
14:0	1.3 $\pm$ 0.6	1.1 $\pm$ 0.2	5.2 $\pm$ 1.0	3.6 $\pm$ 2.4 <sup>s</sup>	3.5 $\pm$ 1.7
15:0	0.3 $\pm$ 0.0	0.4 $\pm$ 0.2	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.3 $\pm$ 0.0
16:0	11.4 $\pm$ 5.7	18.8 $\pm$ 1.3	14.9 $\pm$ 0.1	16.4 $\pm$ 2.2	12.2 $\pm$ 1.9
17:0	0.7 $\pm$ 0.3	0.7 $\pm$ 0.0	0.5 $\pm$ 0.0	0.6 $\pm$ 0.1	0.5 $\pm$ 0.0
18:0	2.2 $\pm$ 0.1	4.0 $\pm$ 0.7	2.0 $\pm$ 0.6	2.8 $\pm$ 1.2	2.2 $\pm$ 0.3
20:0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
22:0	0.2 $\pm$ 0.0	0.5 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2	0.2 $\pm$ 0.1
14:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1 <sup>s</sup>	0.1 $\pm$ 0.0
16:1 $\omega$ 9c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
16:1 $\omega$ 7c	7.0 $\pm$ 0.1	4.8 $\pm$ 0.2	8.4 $\pm$ 0.1	7.0 $\pm$ 2.0 <sup>s</sup>	9.4 $\pm$ 1.5
16:1 $\omega$ 7t	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.0
17:1	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.0
18:1 $\omega$ 9c	39.9 $\pm$ 3.0	22.9 $\pm$ 3.1	34.4 $\pm$ 0.6	29.8 $\pm$ 6.5	31.9 $\pm$ 8.6
18:1 $\omega$ 7c	5.8 $\pm$ 0.1	3.2 $\pm$ 0.4	5.2 $\pm$ 0.8	4.4 $\pm$ 1.2 <sup>s</sup>	4.5 $\pm$ 1.0
18:1 $\omega$ 5c	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.0	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1
18:1	0.5 $\pm$ 0.2	0.1 $\pm$ 0.2	0.3 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
19:1	0.4 $\pm$ 0.1	0.1 $\pm$ 0.2	0.3 $\pm$ 0.0	0.3 $\pm$ 0.2	0.3 $\pm$ 0.0
20:1 $\omega$ 11c	1.0 $\pm$ 0.2	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.3
20:1 $\omega$ 9c	8.3 $\pm$ 0.4	2.9 $\pm$ 1.6	7.4 $\pm$ 1.1	5.6 $\pm$ 2.7	4.7 $\pm$ 0.3
20:1 $\omega$ 7c	0.6 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.3 $\pm$ 0.0
22:1 $\omega$ 11c	3.1 $\pm$ 0.4	4.2 $\pm$ 1.6	3.4 $\pm$ 0.9	3.7 $\pm$ 1.1	3.5 $\pm$ 3.0
22:1 $\omega$ 9c	1.4 $\pm$ 0.1	2.1 $\pm$ 0.3	1.4 $\pm$ 0.3	1.7 $\pm$ 0.4	1.5 $\pm$ 1.1
22:1 $\omega$ 7c	0.2 $\pm$ 0.0	0.3 $\pm$ 0.1	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
24:1 $\omega$ 11c	0.7 $\pm$ 0.2	1.6 $\pm$ 1.3	0.9 $\pm$ 0.5	1.2 $\pm$ 0.8	1.2 $\pm$ 1.3
24:1 $\omega$ 9c	0.9 $\pm$ 0.1	2.1 $\pm$ 0.3	1.2 $\pm$ 0.3	1.5 $\pm$ 0.6 <sup>s</sup>	1.1 $\pm$ 0.6
18:3 $\omega$ 6	0.1 $\pm$ 0.0	0.0 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0
18:4 $\omega$ 3	1.0 $\pm$ 0.1	1.1 $\pm$ 0.4	1.1 $\pm$ 0.0	1.1 $\pm$ 0.2	1.4 $\pm$ 0.3
18:2 $\omega$ 6	1.7 $\pm$ 0.2	1.1 $\pm$ 0.1	1.7 $\pm$ 0.2	1.5 $\pm$ 0.4 <sup>s</sup>	1.9 $\pm$ 0.4
AA	0.4 $\pm$ 0.2	0.9 $\pm$ 0.2	0.4 $\pm$ 0.1	0.6 $\pm$ 0.3	0.6 $\pm$ 0.0
EPA	2.2 $\pm$ 0.5	6.1 $\pm$ 0.6	2.4 $\pm$ 0.6	3.9 $\pm$ 2.1 <sup>s</sup>	4.6 $\pm$ 1.2
20:3 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
20:4 $\omega$ 3	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.7 $\pm$ 0.0
20:2 $\omega$ 6	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0
C <sub>21</sub> PUFA	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0
22:5 $\omega$ 6	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0
DHA	3.7 $\pm$ 1.2	15.5 $\pm$ 0.4	2.9 $\pm$ 1.5	8.0 $\pm$ 6.9 <sup>s</sup>	8.1 $\pm$ 2.6
22:4 $\omega$ 6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0
DPA	0.3 $\pm$ 0.1	0.8 $\pm$ 0.0	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.7 $\pm$ 0.2
SFA	16.1 $\pm$ 5.9	25.4 $\pm$ 1.2	23.3 $\pm$ 0.5	24.1 $\pm$ 1.4	19.1 $\pm$ 0.6
BrFA	1.4 $\pm$ 0.3	0.6 $\pm$ 0.2	0.4 $\pm$ 0.0	0.5 $\pm$ 0.2	0.3 $\pm$ 0.0
MUFA	72.0 $\pm$ 3.7	47.2 $\pm$ 1.2	66.4 $\pm$ 1.9	58.7 $\pm$ 10.6	61.7 $\pm$ 4.6
PUFA	10.5 $\pm$ 2.6	26.9 $\pm$ 0.2	10.0 $\pm$ 2.4	16.7 $\pm$ 9.4	18.9 $\pm$ 3.9
TOTAL	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples; juveniles= <200mm total length; adults= >200mm total length; <sup>s</sup>significant variation (*t*-test, 95% confidence interval, *p*<0.05)

**Table 2.7** Fatty acid composition (% composition of total fatty acids) of various Gonostomatidae included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty acid	Species	
	<i>Cyclothone</i> sp. (n=3)	<i>Diplophos rebainsi</i> (n=1)
14:0	1.2 $\pm$ 0.1	0.2
15:0	0.1 $\pm$ 0.0	0.3
16:0	5.0 $\pm$ 0.6	1.9
17:0	1.3 $\pm$ 0.1	1.0
18:0	1.9 $\pm$ 0.3	0.7
20:0	0.0 $\pm$ 0.0	0.0
22:0	0.4 $\pm$ 0.0	0.1
14:1 $\omega$ 5c	0.3 $\pm$ 0.0	0.1
16:1 $\omega$ 9c	0.1 $\pm$ 0.0	0.2
16:1 $\omega$ 7c	7.5 $\pm$ 0.7	4.7
16:1 $\omega$ 7t	0.2 $\pm$ 0.1	0.2
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.1
17:1	0.4 $\pm$ 0.0	0.2
18:1 $\omega$ 9c	29.7 $\pm$ 2.2	76.3
18:1 $\omega$ 7c	6.3 $\pm$ 1.3	2.9
18:1 $\omega$ 5c	0.7 $\pm$ 0.0	0.2
18:1	0.7 $\pm$ 0.0	0.7
19:1	0.3 $\pm$ 0.0	0.3
20:1 $\omega$ 11c	1.1 $\pm$ 0.1	0.2
20:1 $\omega$ 9c	9.3 $\pm$ 1.0	2.5
20:1 $\omega$ 7c	0.7 $\pm$ 0.1	0.2
22:1 $\omega$ 11c	3.0 $\pm$ 0.5	0.6
22:1 $\omega$ 9c	1.7 $\pm$ 0.2	0.3
22:1 $\omega$ 7c	0.3 $\pm$ 0.1	0.0
24:1 $\omega$ 11c	0.7 $\pm$ 0.1	0.1
24:1 $\omega$ 9c	1.4 $\pm$ 0.3	0.4
18:3 $\omega$ 6	0.2 $\pm$ 0.1	0.0
18:4 $\omega$ 3	1.1 $\pm$ 0.1	0.2
18:2 $\omega$ 6	1.4 $\pm$ 0.2	1.2
AA	0.5 $\pm$ 0.0	0.5
EPA	8.2 $\pm$ 0.6	1.1
20:3 $\omega$ 6	0.2 $\pm$ 0.0	0.1
20:4 $\omega$ 3	0.8 $\pm$ 0.1	0.3
20:2 $\omega$ 6	0.3 $\pm$ 0.0	0.1
C <sub>21</sub> PUFA	0.2 $\pm$ 0.0	0.1
22:5 $\omega$ 6	0.3 $\pm$ 0.1	0.0
DHA	11.5 $\pm$ 0.6	1.7
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.1
DPA	0.7 $\pm$ 0.0	0.3
SFA	9.9 $\pm$ 0.8	4.2
BrFA	0.2 $\pm$ 0.0	0.1
MUFA	64.4 $\pm$ 0.6	90.0
PUFA	25.4 $\pm$ 1.3	5.8
TOTAL	100.0 $\pm$ 0.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples

**Table 2.8** Fatty acid composition (% composition of total fatty acids) of various Myctophidae (genus *Gymnoscopelus*) included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty Acid	Species			
	<i>Gymnoscopelus bolini</i> (n=3)	<i>Gymnoscopelus braueri</i> (n=8)	<i>Gymnoscopelus fraseri</i> (n=3)	<i>Gymnoscopelus nicholsi</i> (n=2)
14:0	0.9 $\pm$ 0.6	0.8 $\pm$ 0.5	1.5 $\pm$ 1.0	1.7 $\pm$ 0.4
15:0	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2	0.4 $\pm$ 0.1	0.1 $\pm$ 0.0
16:0	4.3 $\pm$ 1.4	8.5 $\pm$ 4.2	17.4 $\pm$ 3.1	10.4 $\pm$ 0.4
17:0	0.8 $\pm$ 0.3	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1	0.2 $\pm$ 0.0
18:0	1.1 $\pm$ 0.2	2.5 $\pm$ 0.9	4.3 $\pm$ 0.2	4.8 $\pm$ 0.4
20:0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.1	0.4 $\pm$ 0.1
22:0	0.2 $\pm$ 0.2	0.3 $\pm$ 0.2	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1
14:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
16:1 $\omega$ 9c	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0
16:1 $\omega$ 7c	5.2 $\pm$ 0.6	5.1 $\pm$ 0.8	3.3 $\pm$ 0.9	2.5 $\pm$ 0.2
16:1 $\omega$ 7t	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.0
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0
17:1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.2 $\pm$ 0.0
18:1 $\omega$ 9c	30.5 $\pm$ 0.4	38.7 $\pm$ 11.0	17.6 $\pm$ 1.4	20.7 $\pm$ 0.5
18:1 $\omega$ 7c	8.8 $\pm$ 0.7	4.7 $\pm$ 0.8	5.8 $\pm$ 0.5	8.2 $\pm$ 0.4
18:1 $\omega$ 5c	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.0	0.5 $\pm$ 0.0
18:1	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.4 $\pm$ 0.0	0.3 $\pm$ 0.0
19:1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.2	0.5 $\pm$ 0.1	0.2 $\pm$ 0.0
20:1 $\omega$ 11c	1.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.2	0.8 $\pm$ 0.2
20:1 $\omega$ 9c	17.2 $\pm$ 2.4	4.3 $\pm$ 1.0	11.0 $\pm$ 3.1	22.7 $\pm$ 1.0
20:1 $\omega$ 7c	0.9 $\pm$ 0.0	0.4 $\pm$ 0.1	0.5 $\pm$ 0.0	1.0 $\pm$ 0.1
22:1 $\omega$ 11c	6.0 $\pm$ 2.8	1.4 $\pm$ 0.3	2.3 $\pm$ 1.1	5.4 $\pm$ 0.0
22:1 $\omega$ 9c	2.4 $\pm$ 0.7	1.2 $\pm$ 0.4	1.3 $\pm$ 0.5	2.5 $\pm$ 0.4
22:1 $\omega$ 7c	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.0	0.5 $\pm$ 0.1
24:1 $\omega$ 11c	1.7 $\pm$ 0.7	0.6 $\pm$ 0.1	1.0 $\pm$ 0.5	1.2 $\pm$ 0.0
24:1 $\omega$ 9c	1.7 $\pm$ 0.4	2.0 $\pm$ 0.5	2.2 $\pm$ 0.3	2.1 $\pm$ 0.6
18:3 $\omega$ 6	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.8 $\pm$ 0.2	0.1 $\pm$ 0.0
18:4 $\omega$ 3	0.8 $\pm$ 0.4	2.1 $\pm$ 0.6	0.5 $\pm$ 0.0	0.3 $\pm$ 0.0
18:2 $\omega$ 6	1.6 $\pm$ 0.3	1.8 $\pm$ 0.3	2.0 $\pm$ 0.2	3.3 $\pm$ 0.2
AA	0.6 $\pm$ 0.2	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4 $\pm$ 0.0
EPA	2.8 $\pm$ 1.5	5.3 $\pm$ 2.0	7.1 $\pm$ 0.5	2.1 $\pm$ 0.1
20:3 $\omega$ 6	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0
20:4 $\omega$ 3	1.5 $\pm$ 0.2	0.7 $\pm$ 0.1	1.1 $\pm$ 0.2	0.2 $\pm$ 0.1
20:2 $\omega$ 6	0.8 $\pm$ 0.2	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
C <sub>21</sub> PUFA	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1
22:5 $\omega$ 6	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0	0.0 $\pm$ 0.1
DHA	3.6 $\pm$ 0.8	12.3 $\pm$ 5.5	11.4 $\pm$ 1.0	4.4 $\pm$ 0.1
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0
DPA	0.7 $\pm$ 0.4	0.6 $\pm$ 0.2	0.7 $\pm$ 0.1	0.4 $\pm$ 0.0
SFA	7.5 $\pm$ 2.0	13.2 $\pm$ 5.8	24.4 $\pm$ 3.9	17.9 $\pm$ 0.3
BrFA	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	1.5 $\pm$ 0.1	0.8 $\pm$ 0.3
MUFA	79.2 $\pm$ 5.5	62.1 $\pm$ 12.9	48.9 $\pm$ 3.4	69.5 $\pm$ 0.3
PUFA	13.1 $\pm$ 3.5	24.5 $\pm$ 7.2	25.2 $\pm$ 1.4	11.7 $\pm$ 0.2
TOTAL	100.0	100.0	100.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples

**Table 2.9** Fatty acid composition (% composition of total fatty acids) of various Myctophidae included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty Acid	Species			
	<i>Electrona antarctica</i> (n=3)	<i>Electrona carlsbergi</i> (n=3)	<i>Protomyctophum bolini</i> (n=10)	<i>Lampanyctus achirus</i> (n=1)
14:0	1.7 $\pm$ 0.1	1.5 $\pm$ 0.6	4.5 $\pm$ 0.6	1.6
15:0	0.1 $\pm$ 0.0	0.4 $\pm$ 0.0	0.5 $\pm$ 0.0	0.3
16:0	5.8 $\pm$ 1.1	18.1 $\pm$ 0.7	20.4 $\pm$ 2.0	17.4
17:0	1.2 $\pm$ 0.1	0.4 $\pm$ 0.1	0.7 $\pm$ 0.1	0.3
18:0	2.0 $\pm$ 0.3	3.9 $\pm$ 0.1	3.2 $\pm$ 0.4	4.1
20:0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1
22:0	0.2 $\pm$ 0.0	0.6 $\pm$ 0.6	0.3 $\pm$ 0.1	0.1
14:1 $\omega$ 5c	0.4 $\pm$ 0.1	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1
16:1 $\omega$ 9c	0.3 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2
16:1 $\omega$ 7c	16.0 $\pm$ 1.8	5.7 $\pm$ 0.7	8.0 $\pm$ 1.1	5.8
16:1 $\omega$ 7t	0.2 $\pm$ 0.2	0.8 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2
17:1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.0	0.4 $\pm$ 0.1	0.7
18:1 $\omega$ 9c	36.6 $\pm$ 0.9	23.7 $\pm$ 1.9	22.1 $\pm$ 1.4	22.8
18:1 $\omega$ 7c	4.0 $\pm$ 0.5	3.9 $\pm$ 0.4	5.2 $\pm$ 0.7	4.1
18:1 $\omega$ 5c	0.5 $\pm$ 0.0	0.7 $\pm$ 0.0	0.8 $\pm$ 0.1	0.5
18:1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.0	0.4 $\pm$ 0.1	0.3
19:1	0.3 $\pm$ 0.0	0.4 $\pm$ 0.3	0.4 $\pm$ 0.0	0.3
20:1 $\omega$ 11c	0.6 $\pm$ 0.1	0.7 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5
20:1 $\omega$ 9c	3.4 $\pm$ 0.6	4.8 $\pm$ 1.5	5.7 $\pm$ 1.1	5.6
20:1 $\omega$ 7c	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4
22:1 $\omega$ 11c	3.4 $\pm$ 0.9	3.6 $\pm$ 1.3	2.2 $\pm$ 0.5	2.1
22:1 $\omega$ 9c	1.9 $\pm$ 0.4	1.9 $\pm$ 0.5	1.2 $\pm$ 0.3	0.9
22:1 $\omega$ 7c	0.5 $\pm$ 0.1	0.4 $\pm$ 0.0	0.3 $\pm$ 0.1	0.2
24:1 $\omega$ 11c	1.4 $\pm$ 0.5	1.1 $\pm$ 0.4	0.7 $\pm$ 0.2	0.5
24:1 $\omega$ 9c	1.5 $\pm$ 0.2	1.7 $\pm$ 0.3	2.0 $\pm$ 0.3	2.2
18:3 $\omega$ 6	0.1 $\pm$ 0.0	1.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.2
18:4 $\omega$ 3	1.5 $\pm$ 0.1	0.4 $\pm$ 0.0	1.0 $\pm$ 0.1	0.4
18:2 $\omega$ 6	1.5 $\pm$ 0.1	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	1.0
AA	0.5 $\pm$ 0.0	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1	1.1
EPA	4.1 $\pm$ 0.4	6.5 $\pm$ 1.2	5.6 $\pm$ 1.4	5.2
20:3 $\omega$ 6	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1
20:4 $\omega$ 3	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5
20:2 $\omega$ 6	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2
C <sub>21</sub> PUFA	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	0.1 $\pm$ 0.0	0.3
22:5 $\omega$ 6	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0	0.1
DHA	6.7 $\pm$ 0.9	10.6 $\pm$ 3.4	8.8 $\pm$ 2.8	17.3
22:4 $\omega$ 6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1
DPA	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.8
SFA	11.0 $\pm$ 1.3	25.2 $\pm$ 0.8	29.7 $\pm$ 2.6	23.9
BrFA	0.3 $\pm$ 0.0	1.4 $\pm$ 0.3	0.3 $\pm$ 0.2	1.3
MUFA	72.7 $\pm$ 0.9	51.0 $\pm$ 4.5	51.2 $\pm$ 2.2	47.6
PUFA	16.0 $\pm$ 1.2	22.4 $\pm$ 4.7	18.8 $\pm$ 4.4	27.3
TOTAL	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples

**Table 2.10** Fatty acid composition (% composition of total fatty acids) of *Borostomias antarcticus* and *Bathylagus antarcticus* included in this study from Macquarie Island (data presented as mean standard  $\pm$  deviation)

Fatty Acid	Species					
	<i>Borostomias antarcticus</i>			<i>Bathylagus antarcticus</i>		
	juvenile (n=7)	adult (n=1)	average (n=8)	juvenile (n=6)	adult (n=5)	average (n=11)
14:0	1.6 $\pm$ 0.8	1.7	1.7 $\pm$ 0.7	2.1 $\pm$ 0.7	1.9 $\pm$ 0.6	2.0 $\pm$ 0.6
15:0	0.3 $\pm$ 0.1	0.2	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1
16:0	13.8 $\pm$ 2.0	8.8	13.1 $\pm$ 2.6	16.4 $\pm$ 0.6	15.4 $\pm$ 0.8	15.9 $\pm$ 0.8
17:0	1.0 $\pm$ 0.5	1.0	1.0 $\pm$ 0.5	0.4 $\pm$ 0.0	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
18:0	3.8 $\pm$ 1.1	2.0	3.6 $\pm$ 1.2	5.1 $\pm$ 0.5	4.0 $\pm$ 0.4	4.6 $\pm$ 0.7 <sup>s</sup>
20:0	0.0 $\pm$ 0.0	0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
22:0	0.1 $\pm$ 0.2	0.3	0.2 $\pm$ 0.2	0.1 $\pm$ 0.0	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
14:1 $\omega$ 5c	0.0 $\pm$ 0.0	0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
16:1 $\omega$ 9c	0.2 $\pm$ 0.0	0.2	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
16:1 $\omega$ 7c	2.9 $\pm$ 0.8	5.6	3.3 $\pm$ 1.3	3.0 $\pm$ 1.3	4.7 $\pm$ 0.6	3.8 $\pm$ 1.3 <sup>s</sup>
16:1 $\omega$ 7t	0.3 $\pm$ 0.1	0.2	0.3 $\pm$ 0.1	0.6 $\pm$ 0.1	1.1 $\pm$ 0.1	0.8 $\pm$ 0.3 <sup>s</sup>
16:1 $\omega$ 5c	0.2 $\pm$ 0.1	0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1
17:1	0.6 $\pm$ 0.2	0.2	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1 <sup>s</sup>
18:1 $\omega$ 9c	17.6 $\pm$ 1.5	36.9	20.4 $\pm$ 7.4	16.5 $\pm$ 1.8	18.8 $\pm$ 2.9	17.5 $\pm$ 2.5
18:1 $\omega$ 7c	2.8 $\pm$ 0.8	4.7	3.1 $\pm$ 1.0	4.0 $\pm$ 0.7	3.7 $\pm$ 0.2	3.8 $\pm$ 0.5
18:1 $\omega$ 5c	0.6 $\pm$ 0.1	0.6	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
18:1	0.3 $\pm$ 0.1	0.5	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
19:1	0.4 $\pm$ 0.0	0.4	0.4 $\pm$ 0.0	0.5 $\pm$ 0.1	0.4 $\pm$ 0.3	0.4 $\pm$ 0.2
20:1 $\omega$ 11c	1.0 $\pm$ 0.7	1.7	1.1 $\pm$ 0.7	0.2 $\pm$ 0.0	0.4 $\pm$ 0.2	0.3 $\pm$ 0.2
20:1 $\omega$ 9c	10.7 $\pm$ 5.0	11.0	10.8 $\pm$ 4.5	2.8 $\pm$ 0.2	7.8 $\pm$ 1.7	5.1 $\pm$ 2.8 <sup>s</sup>
20:1 $\omega$ 7c	0.4 $\pm$ 0.1	0.5	0.4 $\pm$ 0.1	0.8 $\pm$ 0.2	1.6 $\pm$ 0.2	1.1 $\pm$ 0.4 <sup>s</sup>
22:1 $\omega$ 11c	3.8 $\pm$ 2.0	5.0	3.9 $\pm$ 1.8	1.1 $\pm$ 0.3	4.1 $\pm$ 1.4	2.4 $\pm$ 1.8
22:1 $\omega$ 9c	0.9 $\pm$ 0.2	1.7	1.1 $\pm$ 0.3	1.4 $\pm$ 0.4	3.4 $\pm$ 0.5	2.3 $\pm$ 1.1 <sup>s</sup>
22:1 $\omega$ 7c	0.4 $\pm$ 0.1	0.2	0.4 $\pm$ 0.1	0.2 $\pm$ 0.0	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
24:1 $\omega$ 11c	0.4 $\pm$ 0.1	1.0	0.5 $\pm$ 0.2	0.6 $\pm$ 0.1	1.4 $\pm$ 0.6	1.0 $\pm$ 0.6
24:1 $\omega$ 9c	2.8 $\pm$ 0.5	1.2	2.6 $\pm$ 0.8	2.3 $\pm$ 0.2	1.2 $\pm$ 0.3	1.8 $\pm$ 0.7 <sup>s</sup>
18:3 $\omega$ 6	0.2 $\pm$ 0.2	0.1	0.2 $\pm$ 0.2	0.3 $\pm$ 0.1	0.7 $\pm$ 0.2	0.5 $\pm$ 0.3 <sup>s</sup>
18:4 $\omega$ 3	0.4 $\pm$ 0.1	0.5	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1
18:2 $\omega$ 6	1.2 $\pm$ 0.2	1.4	1.2 $\pm$ 0.2	0.8 $\pm$ 0.1	1.1 $\pm$ 0.2	0.9 $\pm$ 0.2
AA	1.3 $\pm$ 0.3	0.5	1.2 $\pm$ 0.4	2.0 $\pm$ 0.3	0.9 $\pm$ 0.3	1.5 $\pm$ 0.6 <sup>s</sup>
EPA	5.1 $\pm$ 1.2	2.7	4.7 $\pm$ 1.4	7.0 $\pm$ 0.7	7.6 $\pm$ 1.2	7.3 $\pm$ 1.0
20:3 $\omega$ 6	0.2 $\pm$ 0.1	0.1	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
20:4 $\omega$ 3	0.8 $\pm$ 0.2	0.6	0.8 $\pm$ 0.2	0.5 $\pm$ 0.1	0.8 $\pm$ 0.1	0.6 $\pm$ 0.2 <sup>s</sup>
20:2 $\omega$ 6	0.2 $\pm$ 0.1	0.2	0.2 $\pm$ 0.1	0.3 $\pm$ 0.0	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
C <sub>21</sub> PUFA	0.4 $\pm$ 0.1	0.1	0.3 $\pm$ 0.1	0.8 $\pm$ 0.2	0.5 $\pm$ 0.2	0.7 $\pm$ 0.2 <sup>s</sup>
22:5 $\omega$ 6	0.1 $\pm$ 0.1	0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1 <sup>s</sup>
DHA	20.6 $\pm$ 3.9	6.5	18.6 $\pm$ 6.4	24.7 $\pm$ 4.6	11.4 $\pm$ 1.1	18.6 $\pm$ 7.7 <sup>s</sup>
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.1	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0
DPA	1.0 $\pm$ 0.2	0.9	1.0 $\pm$ 0.2	1.4 $\pm$ 0.3	1.1 $\pm$ 0.3	1.3 $\pm$ 0.3
SFA	20.8 $\pm$ 2.4	14.0	19.8 $\pm$ 3.4	24.5 $\pm$ 0.7	22.6 $\pm$ 1.2	23.6 $\pm$ 1.3
BrFA	1.0 $\pm$ 0.5	0.2	0.9 $\pm$ 0.6	1.4 $\pm$ 0.2	1.7 $\pm$ 0.3	1.5 $\pm$ 0.3
MUFA	46.5 $\pm$ 6.2	71.9	50.1 $\pm$ 11.1	35.6 $\pm$ 4.3	50.3 $\pm$ 1.5	42.3 $\pm$ 8.3
PUFA	31.7 $\pm$ 4.3	13.9	29.2 $\pm$ 7.8	38.5 $\pm$ 4.8	25.4 $\pm$ 1.9	32.6 $\pm$ 7.7
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples; <sup>s</sup>significant variation (*t*-test, 95% confidence interval, *p*<0.05); refer to main body of text for details on sizing

**Table 2.11** Fatty acid composition (% composition of total fatty acids) of miscellaneous fish species included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty Acid	Species					
	<i>Labichthys yanoi</i> (n=2)	<i>Melanostigma gelatinosum</i> (n=5)	<i>Phosichthys argenteus</i> (n=1)	<i>Poromitra crassiceps</i> (n=1)	<i>Sternoptyx sp.</i> (n=1)	<i>Echiodon cryomargarites</i> (n=1)
14:0	1.3 $\pm$ 0.5	2.3 $\pm$ 0.8	1.3	1.5	1.6	2.6
15:0	0.1 $\pm$ 0.0	0.6 $\pm$ 0.1	0.5	0.3	0.5	0.4
16:0	4.0 $\pm$ 0.2	16.1 $\pm$ 0.8	20.7	17.4	19.8	13.5
17:0	1.0 $\pm$ 0.2	0.7 $\pm$ 0.0	0.9	0.5	0.6	1.3
18:0	1.3 $\pm$ 0.2	4.4 $\pm$ 0.5	6.1	5.3	4.7	2.2
20:0	0.0 $\pm$ 0.0	0.5 $\pm$ 1.0	0.1	0.3	0.1	0.0
22:0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.1	0.7	0.2	0.1	0.4
14:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0	0.0	0.0	0.0
16:1 $\omega$ 9c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2	0.1	0.2	0.3
16:1 $\omega$ 7c	8.3 $\pm$ 1.6	6.4 $\pm$ 1.7	2.8	2.6	2.4	5.5
16:1 $\omega$ 7t	0.1 $\pm$ 0.0	0.5 $\pm$ 0.1	0.2	0.1	0.3	0.2
16:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.4 $\pm$ 0.1	0.1	0.2	0.3	0.2
17:1	0.2 $\pm$ 0.0	0.8 $\pm$ 0.1	0.6	0.3	0.7	0.5
18:1 $\omega$ 9c	43.2 $\pm$ 2.6	19.6 $\pm$ 2.3	17.9	11.7	14.5	25.4
18:1 $\omega$ 7c	4.8 $\pm$ 0.6	5.1 $\pm$ 0.4	3.7	3.7	4.2	4.3
18:1 $\omega$ 5c	0.6 $\pm$ 0.0	0.7 $\pm$ 0.1	0.3	0.6	0.6	0.6
18:1	0.6 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2	0.3	0.3	0.3
19:1	0.6 $\pm$ 0.0	0.7 $\pm$ 0.3	0.4	0.7	0.6	0.4
20:1 $\omega$ 11c	1.8 $\pm$ 0.4	0.5 $\pm$ 0.2	0.3	0.2	0.1	0.5
20:1 $\omega$ 9c	8.7 $\pm$ 1.1	6.0 $\pm$ 1.1	2.4	1.8	1.5	4.5
20:1 $\omega$ 7c	1.2 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2	0.4	0.3	0.3
22:1 $\omega$ 11c	2.7 $\pm$ 0.4	0.7 $\pm$ 0.3	1.1	1.1	0.2	1.6
22:1 $\omega$ 9c	1.5 $\pm$ 0.2	0.9 $\pm$ 0.1	0.6	0.7	0.4	0.8
22:1 $\omega$ 7c	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0	0.1	0.4	0.2	0.3
24:1 $\omega$ 11c	1.4 $\pm$ 0.4	0.3 $\pm$ 0.1	0.6	0.9	0.2	0.6
24:1 $\omega$ 9c	1.2 $\pm$ 0.0	2.0 $\pm$ 0.3	4.0	3.3	2.3	2.2
18:3 $\omega$ 6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0	0.2	0.5	0.1
18:4 $\omega$ 3	0.2 $\pm$ 0.0	1.8 $\pm$ 0.3	0.5	0.4	0.4	0.6
18:2 $\omega$ 6	1.3 $\pm$ 0.1	1.4 $\pm$ 0.1	0.9	0.8	2.0	1.6
AA	0.8 $\pm$ 0.1	1.4 $\pm$ 0.4	1.6	2.8	2.2	1.8
EPA	3.2 $\pm$ 1.0	7.4 $\pm$ 1.2	4.0	9.1	7.6	9.2
20:3 $\omega$ 6	0.2 $\pm$ 0.0	0.0 $\pm$ 0.1	0.1	0.1	0.2	0.2
20:4 $\omega$ 3	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4	0.4	0.6	0.5
20:2 $\omega$ 6	0.3 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2	0.2	0.3	0.2
C <sub>21</sub> PUFA	0.2 $\pm$ 0.0	0.3 $\pm$ 0.1	0.4	0.7	0.2	0.4
22:5 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1	0.3	0.2	0.3
DHA	6.3 $\pm$ 1.7	15.1 $\pm$ 3.3	24.5	27.3	26.5	14.5
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0	0.0	0.1	0.1
DPA	0.9 $\pm$ 0.5	0.7 $\pm$ 0.1	1.1	1.1	1.0	0.8
SFA	8.0 $\pm$ 0.4	24.8 $\pm$ 2.0	30.4	25.4	27.5	20.4
BrFA	0.2 $\pm$ 0.0	0.3 $\pm$ 0.1	0.2	2.0	1.4	0.9
MUFA	77.7 $\pm$ 4.2	45.7 $\pm$ 4.8	35.6	29.2	29.3	48.6
PUFA	14.2 $\pm$ 3.9	29.2 $\pm$ 4.9	33.8	43.4	41.8	30.1
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3); DPA=docosapentaenoic acid (22:5 $\omega$ 3); SFA=saturated fatty acid; BrFA=branched fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; n=number of samples

*antarcticus* (average  $38.5 \pm 4.8\%$ ) and, after MUFA, was the second most important fatty acid class in the majority of the remaining species. PUFA levels were highest in *P. crassiceps* and lowest in *D. rebaini* (5.8%), whilst variation within a single species was greatest in *Borostomias antarcticus* (range 13.9-37.5%). The PUFA docosahexaenoic acid (DHA, 22:6 $\omega$ 3) was present at high levels in many species and was the single most abundant fatty acid (>20%) in *P. argenteus* (24.5%), *P. crassiceps* (27.3%), *Sternoptyx* sp. (26.5%), and juveniles of both *Bathylagus antarcticus* (average  $24.7 \pm 4.6\%$ ) and *Borostomias antarcticus* (average  $20.6 \pm 3.9\%$ ). Levels of DHA ranged from a high of 27.3% in *P. crassiceps* to a low of 1.7% in *D. rebaini*. Other important PUFA included eicosapentaenoic acid (EPA, 20:5 $\omega$ 3; range 1.1-9.2%), 18:2 $\omega$ 6 (range 0.8-3.3%) and arachidonic acid (AA, 20:4 $\omega$ 6; range 0.4-2.8%). The increase in MUFA with increasing age was matched by a proportional decrease in PUFA content. For example, the decrease in PUFA from juvenile to adult fish observed for *S. gracilis*, *Bathylagus antarcticus* and *Borostomias antarcticus* was primarily due to a substantial variation (approximately 15%) in DHA levels. T-testing confirmed significant differences ( $p < 0.05$ ) in the amounts of several PUFA in juveniles and adults of both *Bathylagus antarcticus* (18:3 $\omega$ 6, AA, 20:4 $\omega$ 3, C<sub>21</sub> PUFA, 22:5 $\omega$ 6, DHA) and *S. gracilis* (18:2 $\omega$ 6, EPA, DHA).

Saturated fatty acids (SFA) were the second most common fatty acid class in several species including the myctophids *E. carlsbergi* (average  $25.2 \pm 0.8\%$ ), *P. bolini* (average  $29.7 \pm 2.6\%$ ) and *G. nicholsi* (average  $17.9 \pm 0.3$ ) and the stomiatids *S. boa* (average  $16.1 \pm 5.9\%$ ), *C. sloani* (average  $19.1 \pm 0.6\%$ ) and adult *S. gracilis* (average  $23.3 \pm 0.5\%$ ). SFA levels were highest in *P. argenteus* (30.4%) and lowest in *D. rebaini* (4.2%). The SFA that contributed the most to overall fatty acid composition was 16:0, which ranged from 1.9% (*D. rebaini*) to 20.7% (*P. argenteus*). Of the remaining SFA, 14:0 (range 0.2-5.2%) and 18:0 (range 0.7-6.1%) were also major fatty acid components. The composition and content of SFA in adults and juveniles of *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis* did not vary greatly. However, significant differences ( $t$ -test,  $p < 0.05$ ) were detected in levels of 14:0 in *S. gracilis* and 18:0 in *Bathylagus antarcticus*.

Branched fatty acids (BrFA) were minor components of total fatty acid composition, representing <2% for all species (range 0.1-2.0%).

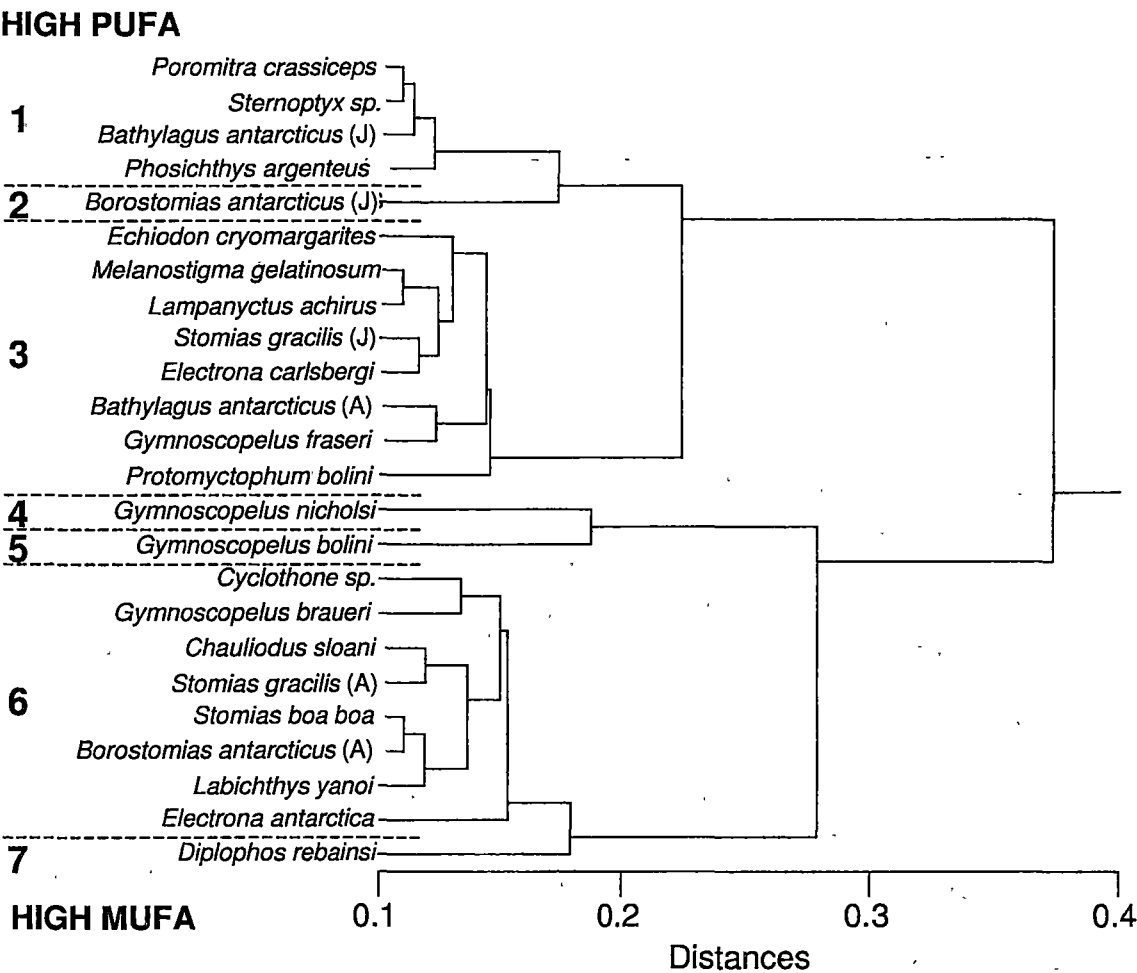
## **Multivariate statistical analysis of fatty acid composition**

### *Hierarchical clustering analysis*

Hierarchical clustering analysis (HCA) produced several distinct groupings of midwater fish based upon the average fatty acid composition of each species (Figure 2.2), especially in the relative proportions of both PUFA and MUFA. When groups are placed in the order 1,2,3,6,4,5,7 a trend toward increasing MUFA and decreasing PUFA is observed. For instance total PUFA levels for those species belonging to group 1 (range 33.8-43.4%) are considerably higher than those in group 7 (*D. rebainsi*, 5.8%), due mainly to a decrease in DHA (group 1, range 24.5-27.3%; group 7, 1.7%). Similarly, total MUFA increases from group 1 (range 29.2-35.6%) to group 7 (90.0%), primarily a consequence of an increase in 18:1 $\omega$ 9c (group 1, 11.7-17.9%; group 7, 76.3%). Stomiatis and myctophids were, to some extent, grouped together by HCA. With the exception of juvenile *S. gracilis* (group 3,) all the stomiatis clustered into group 6. Myctophids were clustered into several groups; *L. achirus*, *E. carlsbergi*, *G. fraseri* and *P. bolini* clustered into group 3, *G. nicholsi* group 4, *G. bolini* group 5, *G. braueri* and *E. antarctica* into group 6. Both gonostomatis clustered into separate groups. Additionally, adults and juveniles of *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis* clustered into different groups.

### *Multidimensional scaling*

Multidimensional scaling (MDS) analysis separated species into a number of groups based upon the proportion of MUFA and PUFA, similar to that observed for HCA (Figure 2.3). The largest grouping is of those species with



**Figure 2.2** Hierarchical cluster dendrogram based on Pearson's correlation coefficient (average linkage) for the average fatty acid composition of 21 species of midwater fish from the Macquarie Island region. Species are separated into groups 1-7 by horizontal dashed lines (J=juveniles; A=adults; PUFA=polyunsaturated fatty acid; MUFA=monounsaturated fatty acid)

intermediate levels of both total MUFA and PUFA, and contains many of the same species contained within groups 2 and 3 of HCA. Similarly, a second large clustering of species with high total MUFA, coincides with groups 6 and 7 of HCA. *G. bolini* and *G. nicholsi* form distinct groups, mainly due to elevated levels of 20:1 $\omega$ 9c relative to all other species (average, *G. bolini* 17.2 $\pm$ 2.4%; *G. nicholsi* 22.7 $\pm$ 1.0%), supporting the observations of HCA. The separation of *D. rebaini* is not as defined as that obtained from HCA, although it does group close to the same species as it did in HCA. Three of the species from group 1 of HCA, *P. argenteus*, *Sternoptyx sp.* and *P. crassiceps* also cluster closely in the MDS plot. Juvenile *Bathylagus*

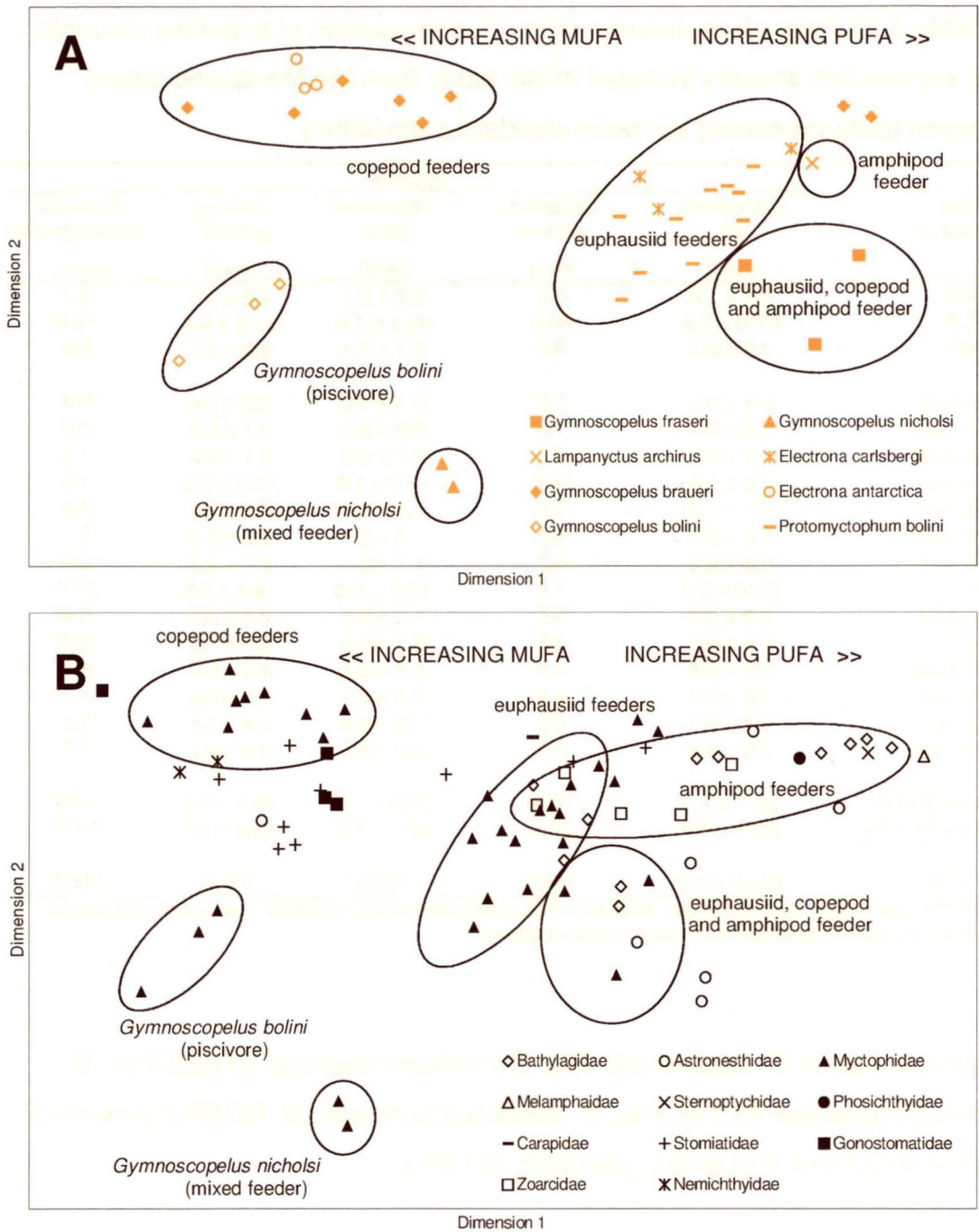
*antarcticus* is spread more widely, though several specimens also group close to these three species. Once again, juveniles and adults of *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis* are contained within different groups.

MDS of only myctophids produced a much clearer separation of species, similar to that observed using MDS, and consistent with dietary information (Figure 2.3). *E. antarctica* and *G. braueri* (6 of 8 specimens), those species for which copepods are important dietary components, separated from other myctophid species mainly on the basis of higher levels of MUFA (e.g. 18:1 $\omega$ 9c). Two specimens of *G. braueri* also grouped close to both euphausiid and amphipod feeders. These prey, along with amphipods, are important dietary components of *G. braueri*. A large collection of myctophids clustered, that consisted of species with potentially overlapping diets. These included euphausiid feeders (*E. carlsbergi*, *P. bolini*), amphipod feeders (*L. archirus*) and mixed euphausiid, copepod and amphipod feeders (*G. fraseri*). The two remaining species, *G. bolini* (fish feeder) and *G. nicholsi* (mixed feeder) are separated from all other myctophids, consistent with the distinct diet of these species.

### **Fatty alcohol composition**

Fatty alcohol composition, in those species containing WE, consisted entirely of saturated and monounsaturated fatty alcohols (SATAlc and MUFAlc respectively) (Tables 2.12-2.14). MDS produced a scattered array of data points, highlighting the great variation in fatty alcohol composition between all species (Figure 2.4). Within-species variation in fatty alcohol composition was minimal, with the exception of *Borostomias antarcticus* (especially amongst juveniles).

SATAlc (range across all species 18.1-75.4%) dominated the fatty alcohol composition of the myctophids *E. antarctica* (average 50.2 $\pm$ 0.2%) and *G. braueri* (average 58.6 $\pm$ 7.5%) and the gonostomatid *D. rebainsi* (75.4%). The SATAlc 16:0 (range across all species 10.9-60.9%) was the most common



**Figure 2.3** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of various fish families included in this study from the Macquarie Island region (PUFA=polyunsaturated fatty acid; MUFA=monounsaturated fatty acid) **A**=grouped by myctophid species only; **B**=grouped by all families

**Table 2.12** Fatty alcohol composition (% composition of total fatty alcohols) of various fish species included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty Alcohol	Species				
	<i>Cyclothone sp.</i>	<i>Diplophos rebaini</i>	<i>Labichthys yanoi</i>	<i>Stomias gracilis</i> <sup>1</sup>	<i>Echiodon cryomargarites</i>
	(n=3)	(n=1)	(n=2)	(n=2)	(n=1)
14:0	9.1 $\pm$ 1.6	5.6	3.7 $\pm$ 0.7	4.5 $\pm$ 0.9	3.1
16:0	27.6 $\pm$ 5.4	60.9	25.1 $\pm$ 1.8	27.9 $\pm$ 4.4	10.9
18:0	3.0 $\pm$ 0.4	9.0	5.1 $\pm$ 0.1	4.0 $\pm$ 2.1	2.9
16:1 $\omega$ 9	0.3 $\pm$ 0.0	0.2	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.2
16:1 $\omega$ 7	7.6 $\pm$ 0.5	1.6	0.8 $\pm$ 0.1	3.1 $\pm$ 0.9	2.6
16:1 $\omega$ 5	0.7 $\pm$ 0.0	0.3	0.2 $\pm$ 0.0	4.1 $\pm$ 3.3	1.4
18:1 $\omega$ 9	7.8 $\pm$ 1.0	16.6	18.2 $\pm$ 1.8	13.2 $\pm$ 2.0	3.0
18:1 $\omega$ 7	7.3 $\pm$ 1.7	2.8	7.8 $\pm$ 0.3	7.9 $\pm$ 3.0	4.6
18:1 $\omega$ 5	1.2 $\pm$ 0.1	0.2	1.4 $\pm$ 0.1	4.9 $\pm$ 2.3	2.1
20:1 $\omega$ 11	0.0 $\pm$ 0.0	0.3	1.1 $\pm$ 0.1	2.1 $\pm$ 2.9	0.0
20:1 $\omega$ 9	21.4 $\pm$ 3.8	1.8	10.5 $\pm$ 2.6	14.1 $\pm$ 3.6	27.1
20:1 $\omega$ 7	0.9 $\pm$ 0.2	0.1	1.6 $\pm$ 0.3	0.4 $\pm$ 0.5	0.9
22:1 $\omega$ 11	5.9 $\pm$ 0.3	0.3	8.5 $\pm$ 0.9	5.8 $\pm$ 1.2	20.9
22:1 $\omega$ 9	3.3 $\pm$ 0.6	0.2	5.7 $\pm$ 0.1	4.3 $\pm$ 0.7	8.0
22:1 $\omega$ 7	0.5 $\pm$ 0.1	0.2	2.3 $\pm$ 2.1	0.4 $\pm$ 0.6	1.3
24:1 $\omega$ 11	0.8 $\pm$ 0.1	0.2	3.6 $\pm$ 0.0	3.4 $\pm$ 0.5	3.2
24:1 $\omega$ 9	2.8 $\pm$ 0.3	0.0	4.2 $\pm$ 0.5	0.0 $\pm$ 0.0	7.8
Sum SATAlc	39.7 $\pm$ 7.0	75.4	33.9 $\pm$ 1.2	36.4 $\pm$ 5.6	16.9
Sum MUFAIc	60.3 $\pm$ 7.0	24.6	66.1 $\pm$ 1.2	63.6 $\pm$ 5.6	83.1
TOTAL	100.0 $\pm$ 0.0	100.0	100.0	100.0	100.0

SATAlc=saturated fatty alcohols; MUFAIc=monounsaturated fatty alcohols; n=number of samples;

<sup>1</sup> *Stomias gracilis* juveniles only (total length <200mm)

fatty alcohol in *D. rebaini* (60.9%), *Cyclothone* (average 27.6 $\pm$ 5.4%), *G. braueri* (average 45.7 $\pm$ 8.4%), *E. antarctica* (average 39.3 $\pm$ 0.6%), juvenile *S. gracilis* (27.9 $\pm$ 4.4%) and *L. yanoi* (25.1 $\pm$ 1.8%).

MUFAIc dominated the fatty alcohol of the remaining species; juvenile and adult *Borostomias antarcticus* (average 82.6 $\pm$ 14.2% and 72.7% respectively), *L. yanoi* (average 66.1 $\pm$ 1.2%), juvenile *S. gracilis* (average 63.6 $\pm$ 5.6%), *G. bolini* (average 72.0 $\pm$ 2.3%) and *Cyclothone sp.* (average 60.3 $\pm$ 7.0%). Major MUFAIc included 20:1 $\omega$ 9 (range across all species 1.8-29.2%), which was also the most common fatty alcohol in adult *Borostomias antarcticus* (21.2%) and *G. bolini* (average 26.2 $\pm$ 3.4%). The MUFAIc 22:1 $\omega$ 11 (range across all species 0.3-29.2%) dominated the fatty alcohol composition of juvenile *Borostomias antarcticus* (average 29.2 $\pm$ 13.7%), whilst 18:1 $\omega$ 9 (range across

all species 3.0-28.1%) dominated *E. antarctica* ( $28.1 \pm 1.2\%$ ). Fatty alcohol composition varied greatly between adult and juvenile *Borostomias antarcticus*, particularly in the relative proportions of 16:0, 18:1 $\omega$ 9 and 22:1 $\omega$ 11 (Table 2.14).

**Table 2.13** Fatty alcohol composition (% composition of total fatty alcohols) of various Myctophidae included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

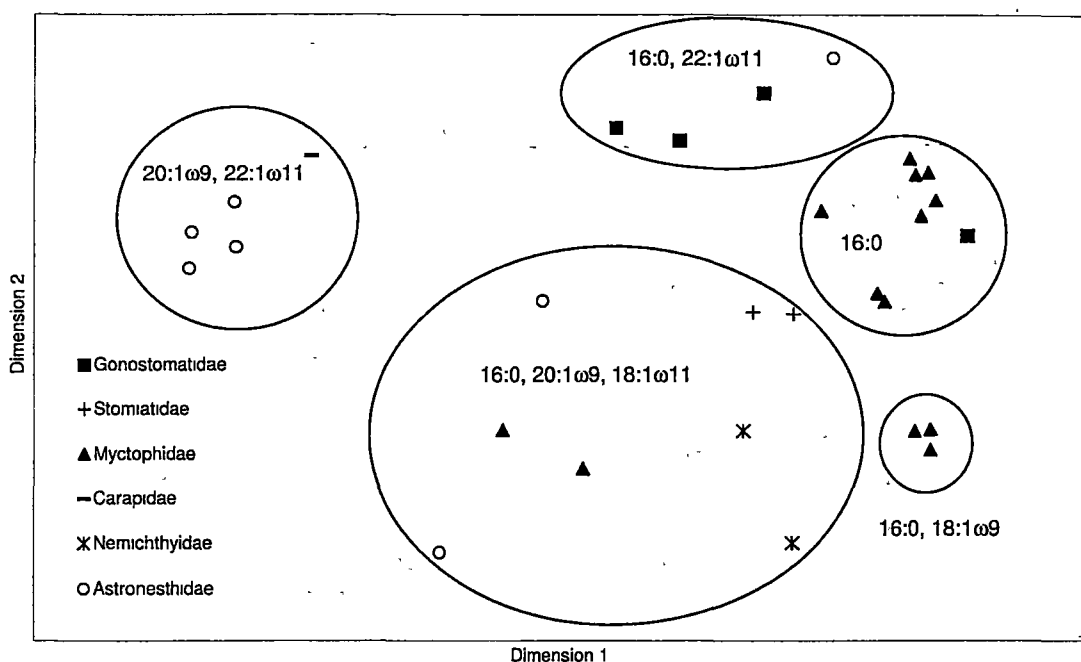
Fatty Alcohol	Species		
	<i>Electrona antarctica</i> (n=3)	<i>Gymnoscopelus bolini</i> (n=2)	<i>Gymnoscopelus braueri</i> (n=8)
14:0	8.3 $\pm$ 0.8	1.3 $\pm$ 0.4	10.7 $\pm$ 1.6
16:0	39.3 $\pm$ 0.6	17.5 $\pm$ 1.5	45.7 $\pm$ 8.4
18:0	2.6 $\pm$ 0.3	8.4 $\pm$ 0.4	2.2 $\pm$ 0.5
16:1 $\omega$ 9	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.3 $\pm$ 0.1
16:1 $\omega$ 7	5.5 $\pm$ 0.2	0.7 $\pm$ 0.3	6.7 $\pm$ 1.0
16:1 $\omega$ 5	0.6 $\pm$ 0.0	0.2 $\pm$ 0.1	1.0 $\pm$ 0.4
18:1 $\omega$ 9	28.1 $\pm$ 1.2	14.6 $\pm$ 1.2	14.9 $\pm$ 2.3
18:1 $\omega$ 7	5.6 $\pm$ 0.2	9.5 $\pm$ 1.8	4.3 $\pm$ 1.2
18:1 $\omega$ 5	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1	1.0 $\pm$ 0.3
20:1 $\omega$ 11	0.3 $\pm$ 0.3	2.7 $\pm$ 0.1	0.1 $\pm$ 0.1
20:1 $\omega$ 9	5.5 $\pm$ 1.0	26.2 $\pm$ 3.4	9.2 $\pm$ 3.9
20:1 $\omega$ 7	0.3 $\pm$ 0.0	1.6 $\pm$ 0.1	0.2 $\pm$ 0.1
22:1 $\omega$ 11	0.8 $\pm$ 0.3	7.7 $\pm$ 1.5	1.1 $\pm$ 0.5
22:1 $\omega$ 9	0.9 $\pm$ 0.1	3.9 $\pm$ 0.4	1.2 $\pm$ 0.4
22:1 $\omega$ 7	0.1 $\pm$ 0.0	1.1 $\pm$ 0.2	0.1 $\pm$ 0.1
24:1 $\omega$ 11	0.5 $\pm$ 0.1	1.1 $\pm$ 0.1	1.2 $\pm$ 0.6
24:1 $\omega$ 9	0.3 $\pm$ 0.5	2.3 $\pm$ 0.0	0.0 $\pm$ 0.0
Sum SATAlc	50.2 $\pm$ 0.2	27.1 $\pm$ 2.3	58.6 $\pm$ 7.5
Sum MUFAIc	49.8 $\pm$ 0.2	72.9 $\pm$ 2.3	41.4 $\pm$ 7.5
TOTAL	100.0	100.0	100.0

SATAlc=saturated fatty alcohols; MUFAIc=monounsaturated fatty alcohols; n=number of samples

**Table 2.14** Fatty alcohol composition (% composition of total fatty alcohols) of *Borostomias antarcticus* included in this study from the Macquarie Island region (data presented as mean standard  $\pm$  deviation)

Fatty alcohol	<i>Borostomias antarcticus</i>		
	juvenile (n=6)	adult (n=1)	average (n=7)
14:0	4.0 $\pm$ 3.9	3.0	3.9 $\pm$ 3.6
16:0	12.0 $\pm$ 9.8	20.4	13.2 $\pm$ 9.5
18:0	1.4 $\pm$ 0.7	4.0	1.8 $\pm$ 1.2
16:1 $\omega$ 9	0.6 $\pm$ 0.5	0.2	0.5 $\pm$ 0.5
16:1 $\omega$ 7	3.4 $\pm$ 3.8	2.1	3.2 $\pm$ 3.5
16:1 $\omega$ 5	1.4 $\pm$ 0.8	0.4	1.3 $\pm$ 0.8
18:1 $\omega$ 9	5.9 $\pm$ 6.5	11.8	6.8 $\pm$ 6.3
18:1 $\omega$ 7	4.8 $\pm$ 2.4	4.6	4.8 $\pm$ 2.2
18:1 $\omega$ 5	2.3 $\pm$ 2.2	0.9	2.1 $\pm$ 2.1
20:1 $\omega$ 11	0.3 $\pm$ 0.5	0.0	0.3 $\pm$ 0.5
20:1 $\omega$ 9	26.8 $\pm$ 9.8	21.2	26.0 $\pm$ 9.2
20:1 $\omega$ 7	0.7 $\pm$ 0.1	0.9	0.7 $\pm$ 0.1
22:1 $\omega$ 11	29.2 $\pm$ 13.7	14.6	27.1 $\pm$ 13.7
22:1 $\omega$ 9	3.2 $\pm$ 0.7	7.0	3.7 $\pm$ 1.6
22:1 $\omega$ 7	0.4 $\pm$ 0.2	1.2	0.5 $\pm$ 0.4
24:1 $\omega$ 11	1.0 $\pm$ 1.7	3.2	1.4 $\pm$ 1.8
24:1 $\omega$ 9	2.6 $\pm$ 1.5	4.7	2.9 $\pm$ 1.6
Sum SATAlc	17.4 $\pm$ 14.2	27.3	18.8 $\pm$ 13.5
Sum MUFAlc	82.6 $\pm$ 14.2	72.7	81.2 $\pm$ 13.5
TOTAL	100.0	100.0	100.0

SATAlc=saturated fatty alcohols; MUFAlc=monounsaturated fatty alcohols; Alc=alcohol; n=number of samples; juvenile=total length <200mm; adult=total length >200mm; n=number of samples



**Figure 2.4** Scatterplot of multidimensional scaling (MDS) based upon the fatty alcohol composition of various fish families included in this study from the Macquarie Island region, highlighting the major fatty alcohols of each group

## 2.4 DISCUSSION

The range of midwater fish species in this study, including several non-myctophid species, is significant given the general preference for dietary studies to investigate predators occupying only the higher trophic levels. Midwater fish provide an important link between the primary production of phytoplankton (and dependent zooplankton) and higher predators (Pakhomov et al. 1996; Gaskett et al. 2001). In the Macquarie Island region these higher predators include the Patagonian toothfish (*Dissostichus eleginoides*), an ecologically and more recently commercially important fish species, and numerous marine mammals.

Investigating the lipid composition of midwater fish and other prey groups (e.g. squid and invertebrates) provides an invaluable insight into a commonly overlooked aspect of the marine ecosystem. The transfer of lipids, via the diet, from prey to predators is an important component of the maintenance of

energy flow from lower to higher trophic levels (Kozlov 1995; Pakhomov et al. 1996; Gaskett et al. 2001). The knowledge gained from examining lipid composition can be applied two-fold. Firstly, it can be used to examine the importance, energetically, of a potential prey species to a predator. Secondly, aspects of lipid composition, primarily fatty acids, can be applied to the study of dietary interactions and may provide clues to hitherto undetected predator-prey relationships.

The total lipid content of a species is to a certain degree a reflection of its calorific or energetic importance to a predator. Those species with high total lipid contents will generally pass on more metabolic energy to predators than species containing little lipid (excluding the importance of protein or carbohydrate). This observation is supported by the results of a study of the calorific content (a measure of the combined energy derived from carbohydrates, proteins and lipids) of mesopelagic fish from Macquarie Island by Tierney et al. (2002). The lipid-rich species from the present study, including the myctophids *Electrona antarctica* (17.5% WW) and *Gymnoscopelus bolini* (14.5% WW), are the same species identified as being the most calorie-rich. In terms of ecosystem functioning and trophodynamics, removal of those species with high lipid content (e.g. by commercial fishing) will potentially impact predators to a greater extent than removal of lipid poor species.

In reality however, the functioning of ecosystems is rarely this simple. The maintenance of ecosystem diversity is crucial to the ability of species contained within the foodweb to deal with the seasonal flux of primary production that impacts upon both the composition and biomass of their prey. Consequentially, few predators rely upon just one or two prey species, but rather a suite of prey. Predators therefore adapt their dietary composition in tune to shifts in the availability of prey species (McKenna Jr 1991).

The usual method of dietary analysis, the identification of prey within the contents of the stomach, has difficulty in detecting these shifts. Stomach contents are effectively a snapshot of diet, only reflecting recent dietary

events. Unless a population is continually and regularly sampled (an expensive, time-consuming and difficult undertaking) the detection of dietary variation using only stomach contents is impractical. The application of lipids, particularly fatty acids, to the determination of long-term diet may overcome some of the disadvantages associated with traditional techniques. Many fatty acids are readily transferred from prey to predators with little or no modification (e.g. Navarro et al. 1995; Kirsch et al. 1998). The lipid composition of a predator is therefore assumed to reflect, to some extent, a temporal integration of diet over a much longer time frame than stomach contents. The use of lipids as dietary indicators has been applied to the study of Southern Ocean fish (Phleger et al. 1997; Lea et al. 2002), seals (Brown et al. 1999), seabirds (Horgan and Barrett 1985), squid (Phillips et al. 2001; Phillips et al. 2002), amphipods (Nelson et al. 2001), and zooplankton (Phleger et al. 1998; Cripps and Atkinson 2000; Falk-Petersen et al. 2000).

In theory, the use of fatty acids as dietary indicators requires knowledge of the lipid composition of potential prey with which to draw comparisons. Currently, the lipid composition of zooplankton (primary prey for midwater fish) is poorly described, at least for sub-Antarctic regions such as Macquarie Island. Nonetheless, an improved understanding of diet can still be obtained. For example, fish that group together based on fatty acid composition, especially PUFA, may share a common diet. By cross-referencing the results of stomach content analysis and lipid composition, the likelihood of such a dietary interaction can be compared. The following discussion will compare the various elements of lipid composition to results obtained from stomach content analysis, where available.

### **Total lipid content and lipid class composition**

#### *Implications to energy storage and buoyancy*

The lipid content of fish included in this study ranges from levels that can be considered to be lipid-rich (e.g. *E. antarctica*, *D. rebaini*, *G. bolini*, adult *S. gracilis*) to those that can be described as lipid-poor (e.g. *P. argenteus*, *P.*

*crassiceps*, juvenile *Bathylagus antarcticus*). The varying levels of lipid stored by marine organisms has long been considered to reflect differing requirements for energy storage during times of reduced food availability (Friedrich and Hagen 1994; Bakes et al. 1995; Hagen et al. 2000; Kamler et al. 2001). Furthermore, the presence of large stores of WE (a long-term store of metabolic energy) rather than TAG in many of the species included in this study also points the use of lipid as an energy store.

More recent research has centred on the potentially significant role played by large lipid stores in the buoyancy regulation of Southern Ocean fish, including the swim-bladderless notothenioids (Clarke et al. 1984; Eastman 1985; Eastman 1988; Friedrich and Hagen 1994; Phleger et al. 1999b; Hagen et al. 2000; Kamler et al. 2001), myctophids (Phleger et al. 1999a; Kamler et al. 2001) and deep-sea oreos (Bakes et al. 1995). The presence of large stores of low density lipid, such as WE, allows fish to maintain position in the water column whilst expending little energy (Phleger 1991; Phleger 1998). Lipids (including WE), as an alternative to swim bladders, also have the advantage of being essentially non-compressible and are therefore beneficial to vertically migrating species (e.g. during diurnal migration).

Knowing the amount of lipid stored by fish can also provide information regarding life history. In contrast to the buoyancy requirements of many pelagic fish species, benthic species have little or no capacity to migrate within the water column and, consequently, less need for lipid stores enabling neutral buoyancy. For instance pelagic notothenioids tend to have higher lipid contents than those that are benthic (Friedrich and Hagen 1994; Kamler et al. 2001). The applicability of these findings to the present study is unclear. The low lipid content of many species included in this study, and their reliance on TAG rather than WE, suggests that lipid does not play a large role in either the long-term storage of energy or buoyancy regulation in these fish. As all the fish examined are predominately midwater rather than benthic species, one might expect that they would store greater quantities of lipid than is observed. However, there are many other strategies to maintain neutral

buoyancy such as reduction of heavy protein and bone tissues and reduction of heavy ions to create low density body fluids.

Many species examined do possess the pre-requisites that would enable them to control buoyancy through the use of lipids, namely a high WE and total lipid content. These include several myctophid (*E. antarctica*, *G. bolini* and *G. braueri*), both gonostomatids (*Cyclothone sp.* and *D. rebaini*) and the nemichthyid *L. yanoi*. Although all these species live within pelagic waters, the degree to which they migrate within the water column at Macquarie Island, and would therefore benefit from the storage of WE, is unclear. Williams et al. (2001) found that *G. braueri* from Macquarie Island shows strong upward movement at night from below 500m to the 500-0m depth range. The migration of *E. antarctica* is thought to be seasonal rather than diurnal (Sabourenkov 1990). Understanding of the life history of the remaining species is incomplete.

Elevated FFA levels were a prominent feature of many species (up to 39.9% in *S. gracilis*). The fact that this was confined to specimens of small size, generally less than 1.5g TW, suggests that the smaller mass of these samples left them at greater risk of degradation due to thawing, heating etc. prior to storage. Whilst the effect of this degradation on lipid class composition is striking and significant, total lipid content is likely to have remained unaffected. Similarly, fatty acid composition appears to have remained largely unchanged. This is evidenced by the fact that PUFA levels (highly sensitive to prolonged exposure to oxygen) are actually highest in many of the specimens with elevated FFA content. For instance, both FFA and PUFA content is substantially higher in juveniles of *S. gracilis*, *Bathylagus antarcticus* and *Borostomias antarcticus* than in adults.

#### *Variation in total lipid content and lipid class composition with fish size*

The increase in lipid content from juvenile to adult stages observed in *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis* is probable evidence of two mutually occurring factors. Firstly, increasing lipid stores

have been associated with the development of gonads in several Southern Ocean fish species (Friedrich and Hagen 1994; Montgomery et al. 1999; Kamler et al. 2001). Secondly, if lipid content is related to the maintenance of neutral buoyancy in these species, then the increased accumulation of lipid with age compensates for the higher density of adult fish. This pattern of lipid accumulation has been observed in a number of Antarctic species including *Pleuragramma antarcticum* (Eastman 1988; Friedrich and Hagen 1994) and *Trematomus lepidorhinus* (Friedrich and Hagen 1994).

Lipid class composition also varied relative to size, particularly a decrease in PL with increasing size in *Bathylagus antarcticus*, *Borostomias antarcticus* and *Stomias gracilis*. The dietary significance of this shift is relatively minor and is more a reflection of the accumulation of larger quantities of TAG-rich lipid in larger fish. As PL are mainly found as structural components of membranes, the accumulation of TAG contributes to a proportionate drop in their levels.

### **Dietary implications of fatty alcohol composition**

Determining the dietary origin of fatty alcohols (derived from WE) is difficult. Whilst dietary accumulation is considered the main source of fatty alcohol in a number of marine fish, including deep-sea oreos (Bakes et al. 1995) and myctophids (Phleger et al. 1997; Phleger et al. 1999a), the degree to which fish simply accumulate or actively modify fatty alcohols and WE from the diet is poorly understood. Nothing is known of the lipid composition of copepods in the Macquarie Island region, typical sources of WE in Antarctic waters (Graeve et al. 1994; Kattner et al. 1994). One possible explanation for the high levels of WE found in many fish species, other than a WE-rich diet, is synthesis (Phleger et al. 1997). Rather than transferring WE directly from the diet into tissue, species such as myctophids may reincorporate dietary-derived fatty alcohols into WE within the body. The potential for these species to *selectively* accumulate certain fatty alcohols in preference to others is therefore plausible.

Although the species included in the present study are characterised by a varied array of fatty alcohol compositions (Figure 2.4), this is not necessarily indicative of a dietary influence. In the absence of data pertaining to the lipid class composition of potential prey such copepods, the amount and composition of WE available to these fish species from dietary sources remains unknown.

### **Dietary implications of fatty acid composition**

The substantial variation in fatty acid composition, particularly PUFA, across the range of midwater fish species included in this study points to a possible underlying dietary influence (Figures 2.2 and 2.3). The ability of marine fish to convert short-chain omega-3 PUFA (e.g. 18:3 $\omega$ 3) to longer-chain omega-3 PUFA (e.g. DHA, DPA, EPA) is limited (Sargent et al. 1993; Ruyter et al. 2000; Koven et al. 2001). The majority of these PUFA must therefore have originated from dietary sources. Evidence supporting the deposition of many dietary derived PUFA into the flesh of fish with minimal modification is supplied by various captive studies (e.g. Navarro et al. 1995; Kirsch et al. 1998). Given that variation in the composition of long-chain PUFA (particularly EPA and DHA) is likely to be indicative of dietary variation, the wide-ranging PUFA compositions of those species included in the present study (varying by ~40% across all the species examined) add support to the observations of available stomach content analyses. Although detailed dietary information concerning midwater fish from Macquarie Island is restricted almost entirely to myctophids, it is likely that the general range of prey consumed by fish would be similar across all species. Myctophids consume a complex array of euphausiids, copepods, amphipods, decapods, ostracods, mysids, gastropods, chaetognaths, cnidarians, cephalopods and fishes, the combination of which appears to be species specific (Gaskett et al. 2001).

Determining the dietary source of the constituent fatty acids of midwater fish is severely curtailed by a lack of lipid compositional data on these potential prey species. Reliance is instead placed upon identifying similarities in fatty acid composition between species for which dietary information exists (e.g.

myctophids) and those for which the diet is unknown, and debating the likelihood that they share similar diets.

Of the 21 midwater species included in this study, 14 were also included in the study of Gaskett et al. (2001). Five trophic guilds were identified from stomach contents, although the results of cluster analysis are restricted to myctophid species due to the limited sample sizes of other species (Table 2.15). Assuming that the dietary differences attributed to each of these trophic guilds was great enough to be reflected in variations in dietary fatty acids, then the grouping patterns of each of these dietary aspects would be expected to be similar. That is, species with similar diets and therefore fatty acid compositions should theoretically group together. Figures 2.2 and 2.3 provide evidence of the existence of such a pattern, particularly with regards to myctophids.

### *Myctophidae*

Of the eight myctophid species analysed, seven were assigned to trophic guilds by Gaskett et al. (2001) using hierarchical clustering analysis (HCA). For comparative purposes, HCA was also used to classify these same myctophids based on their fatty acid compositions (Figure 2.2). Comparison of the grouping patterns obtained reveals substantial correspondence between these two methods of dietary assessment (Table 2.15). Firstly, myctophids are present in four out of the seven groups identified from fatty acid composition, similar to the five trophic guilds identified from stomach content analysis.

Secondly, the distribution of myctophid species throughout these four groups closely resembles that of the trophic guilds (Table 2.15). That is, many of the same species are grouped together based on both stomach content and fatty acid composition. *E. antarctica* and *G. braueri*, species for which copepods are the most important dietary component (50.2% and 31.5% of the diet respectively), share the same HCA group (group 6, Figure 2.2) and also form a distinct group based on MDS (Figure 2.3). *G. bolini* is placed in it's own

**Table 2.15** Comparison of the grouping of various midwater fish species by cluster analysis using stomach content analysis (Gaskett et al. 2001) and average fatty acid composition (this study)

Species	Trophic guild <sup>1</sup>	Dominant fatty acids <sup>2</sup>	Cluster grouping <sup>3</sup>
<i>Electrona carlsbergi</i>	E*	18:1 $\omega$ 9c, 16:0, DHA, EPA, 16:1 $\omega$ 7c	3
<i>Protomyctophum bolini</i>	E*	18:1 $\omega$ 9c, 16:0, DHA, 20:1 $\omega$ 9c, EPA	3
<i>Gymnoscopelus braueri</i>	C*	18:1 $\omega$ 9c, DHA, 16:0, EPA, 16:1 $\omega$ 7c	6
<i>Electrona antarctica</i>	C*	18:1 $\omega$ 9c, 16:1 $\omega$ 7c, DHA, 16:0, EPA	6
<i>Lampanyctus achirus</i>	A*	18:1 $\omega$ 9c, 16:0, DHA, 16:1 $\omega$ 7c, 20:1 $\omega$ 9c	3
<i>Melanostigma gelatinosum</i>	A	18:1 $\omega$ 9c, 16:0, DHA, EPA, 16:1 $\omega$ 7c	3
<i>Bathylagus antarcticus</i> <sup>a</sup>	A	18:1 $\omega$ 9c, 16:0, DHA, 20:1 $\omega$ 9c, EPA	3
<i>Bathylagus antarcticus</i> <sup>j</sup>	A	DHA, 18:1 $\omega$ 9c, 16:0, EPA, 18:0	1
<i>Gymnoscopelus bolini</i> <sup>*</sup>	P*	18:1 $\omega$ 9c, 20:1 $\omega$ 9c, 18:1 $\omega$ 7c, 22:1 $\omega$ 11c, 16:1 $\omega$ 7c	5
<i>Echiodon cryomargarites</i>	P	18:1 $\omega$ 9c, DHA, 16:0, EPA, 16:1 $\omega$ 7c	3
<i>Phosichthys argenteus</i>	P	DHA, 16:0, 18:1 $\omega$ 9c, 18:0, EPA + 24:1 $\omega$ 9c	1
<i>Borostomias antarcticus</i> <sup>a</sup>	P	18:1 $\omega$ 9c, 20:1 $\omega$ 9c, 16:0, DHA, 16:1 $\omega$ 7c	6
<i>Borostomias antarcticus</i> <sup>j</sup>	P	DHA, 18:1 $\omega$ 9c, 16:0, 20:1 $\omega$ 9c, EPA	2
<i>Stomias gracilis</i> <sup>a</sup>	P	18:1 $\omega$ 9c, 16:0, 16:1 $\omega$ 7c, 20:1 $\omega$ 9c, 14:0	6
<i>Stomias gracilis</i> <sup>j</sup>	P	18:1 $\omega$ 9c, 16:0, DHA, EPA, 16:1 $\omega$ 7c	3
<i>Gymnoscopelus fraseri</i>	E / C / A*	18:1 $\omega$ 9c, 16:0, DHA, 20:1 $\omega$ 9c, EPA	3
<i>Gymnoscopelus nicholsi</i>	E / C / A	20:1 $\omega$ 9c, 18:1 $\omega$ 9c, 16:0, 18:1 $\omega$ 7c, 22:1 $\omega$ 11c,	4

<sup>1</sup>inferred from stomach contents (\*confirmed by cluster analysis) by Gaskett et al.(2001); E=euphausiid feeder; C=copepod feeder; A=amphipod feeder; P=piscivore feeder; <sup>2</sup>representing the five most prevalent fatty acids in descending order of dominance; <sup>3</sup>determined from hierarchical cluster analysis of average fatty acid composition (refer to Figure 2.2); j=juvenile; a=adult (refer to text for details on sizing); EPA=eicosapentaenoic acid (20:5 $\omega$ 3); DHA=docosahexaenoic acid (22:6 $\omega$ 3)

group on the basis of HCA (Group 5) and MDS, reflecting the fact that it also singularly occupies a trophic guild (piscivore). The grouping of the other myctophids by HCA and MDS are more ambiguous. *E. carlsbergi* and *P. bolini* (euphausiid consumers) are placed together in Group 3 and also group closely using MDS. However, they are also grouped with *L. achirus* (amphipod feeder) and *G. fraseri* (euphausiid, copepod and amphipod feeder) using HCA and MDS, even though these two myctophids are placed into separate trophic guilds on the basis of their stomach contents (Table 2.15). This could indicate a greater degree of dietary overlap in these four species than is revealed by stomach content analysis.

Although *G. nicholsi* was not placed into a trophic guild by Gaskett et al. (2001), its diet appears to be quite distinct from the other myctophids, characterized by a reliance on copepods, euphausiids and other unidentified crustaceans. This fact is reflected in the separation of *G. nicholsi* into a distinct group based on both HCA (Group 4) and MDS.

#### *Remaining midwater species*

Of the 13 non-myctophid species analysed in this study, the stomach contents of only 6 were determined by Gaskett et al. (2001). Due to limited sample sizes, none of these 6 species were placed into trophic guilds. Grouping of these species based on diet is, however, still possible. For instance *M. gelatinosum* and *Bathylagus antarcticus* consume mainly amphipods (>90% of diet), whilst *E. cryomargarites*, *P. argenteus*, *Borostomias antarcticus* and *S. gracilis* rely primarily on (unspecified) fish (>70% of diet). Comparison is complicated by the fact that *S. gracilis*, *Borostomias antarcticus* and *Bathylagus antarcticus* have been separated into juveniles and adults in the current study. Gaskett et al. (2001) failed to specify whether they used adults, juveniles or a mixture of both ages classes in their analysis. The assumption has therefore been made that stomach contents were of adult fish only.

Dealing firstly with species for which dietary information exists, comparison of the two methods of dietary determination (Figure 2.2) again reveals a great deal of concurrence. The amphipod feeders *M. gelatinosum* and *Bathylagus antarcticus* (adults only) are not only grouped together by HCA (Group 3), they also group with the myctophid *L. achirus*, also identified by stomach content analysis as primarily amphipod consumers (Table 2.15). MDS also groups these species, though the separation from other fish species is not as distinct.

These species in turn grouped amongst a number of other species with wide-ranging diets, including the euphausiid feeders *E. carlsbergi* and *P. bolini*, the piscivore *E. cryomargarites* and the mixed feeder *G. fraseri*. The species contained within Group 3 (Figure 2.2) may therefore represent a group relying upon varying proportions of copepods, euphausiids, amphipods and fish rather than relying upon a single prey group. Although juvenile *S. gracilis* also grouped with these species, its diet is unclear. That fact that it grouped closest to *E. carlsbergi* in cluster analysis (Figure 2.2) and within the same group as *E. carlsbergi* using MDS analysis (Figure 2.3) raises the possibility that the diet of juvenile *S. gracilis* is dominated by euphausiids.

The fish identified as piscivorous feeders by stomach content analysis are spread across a number of groups. *E. cryomargarites* (Group 3) and *P. argenteus* (Group 1) and *G. bolini* (Group 5) are all present in separate groups, whilst adults of both *S. gracilis* and *Borostomias antarcticus* are included in the same grouping (Group 6). The exact composition of the fish included in the diet of these species is not specified by Gaskett et al. (2001). However it is clear, given the range of fatty acid compositions encountered in the present study, that a diet based upon different combinations of fish prey would consequentially produce a considerable range of fatty acid compositions in the predator species.

Although dietary information is unavailable for the remaining fish species examined in this study, several features are apparent. The species contained within HCA Group 1, *P. crassiceps*, *Sternoptyx* sp., juvenile *Bathylagus*

*antarcticus* and *P. argenteus* must all share a diet rich in PUFA, particularly DHA (>24%). This could be a single DHA-rich prey species, or perhaps a combination of numerous DHA-rich prey. Whilst juvenile *Borostomias antarcticus* occupy their own group (Group 2), they are present on the same branch as Group 1 and share a similar fatty acid composition. A lower DHA content ( $\approx 20\%$ ) and elevated level of 20:1 $\omega$ 9c ( $\approx 10\%$ ) suggests that, although juvenile *Borostomias antarcticus* possibly overlap with the diet of the fish from Group 1, they perhaps additionally forage upon MUFA-rich prey.

The remaining species are all characterised by high levels of MUFA (>60%) and are split between two groups using HCA (Figure 2.2), but tend to associate in the same grouping using MDS (Figure 2.3). HCA Group 6 *Cyclothone* sp., *C. sloani*, *S. boa* *boa* and *L. yanoi* are placed in HCA Group 6, a group that also contains a number of fish that are classified as either piscivores (adults of both *Borostomias antarcticus* and *S. gracilis*) or copepod feeders (*G. braueri* and *E. antarctica*) on the basis of stomach contents. The clearer grouping of these species with *E. antarctica* and *G. braueri* by MDS suggests that copepods may dominate the diet.

*D. rebainsi* is singularly placed in Group 7. The fatty acid composition of *D. rebainsi* is exceptional in that its fatty acid composition is dominated by 18:1 $\omega$ 9c (76.3%). The influence of biosynthesis and or bioaccumulation in this species may be substantial compared to other species, as levels of 18:1 $\omega$ 9c in excess of 30% are rare for marine species.

### **Dietary implications of the variation in fatty acid composition with fish size**

A significant decrease in PUFA (up to 80%) and increase in MUFA (up to 60%) was associated with increasing size in those species for which juvenile and adult stages were assessed, *Stomias gracilis*, *Bathylagus antarcticus* and *Borostomias antarcticus*. Similar shifts in fatty acid composition with increasing fish size, also related to dietary changes, have been reported in other species (e.g. Budge et al. 2002). Considerable differences in the diet of adult and juvenile fish are also apparent for other Southern Ocean species,

including myctophids (Kozlov and Tarverdiyeva 1989; Sabourenkov 1990; Pakhomov et al. 1996) nototheniids (Duhamel and Hureau 1985; Williams 1985; Arrizaga et al. 1996; García de la Rosa et al. 1997; Pakhomov 1997) and channichthyids (Duhamel and Hureau 1985).

Although diet is likely to change as fish increase in size, the corresponding shift in fatty acid composition can be explained by other factors. Fish tend to selectively deposit PUFA such as DHA and EPA into the PL fraction in order to maintain membrane fluidity (Dunstan et al. 1999; Cahu et al. 2000). Decreasing PL levels are also associated with the increasing size of *S. gracilis*, *Bathylagus antarcticus* and *Borostomias antarcticus* due to the deposition of larger quantities of TAG-rich lipid in adults, partly explaining the observed decrease in PUFA in large fish.

### **Comparison with fatty acid composition of potential prey**

Confirming whether the variations in fatty acid composition between these groups of fish are due to dietary factors requires a knowledge of the lipid composition of the various species of copepod, euphausiid and amphipod that are thought to contribute to the diet. Unfortunately, detailed lipid compositional data on zooplankton from the Macquarie Island region does not exist. Current knowledge of the lipid composition of Southern Ocean euphausiids (Virtue et al. 1993a; Virtue et al. 1995; Phleger et al. 1998; Cripps and Atkinson 2000; Falk-Petersen et al. 2000), copepods (Graeve et al. 1994; Kattner et al. 1994; Kattner and Hagen 1995) and amphipods (Phleger et al. 1998; Nelson et al. 2001) is restricted to primarily Antarctic species.

The lipid composition of Antarctic zooplankton is likely to differ substantially to their sub-Antarctic equivalents due to many factors. The so-called 'latitudinal effect' is well described by many authors (e.g. Dunstan et al. 1999). Many marine species residing in high latitudes contain proportionally greater levels of omega-3 PUFA than more temperate species, a consequence of cold-water adaptation (PUFA enable cellular membranes to remain fluid and thus active at low temperature).

Thus any comparison based upon the use of Antarctic rather than sub-Antarctic species of prey would be speculative. A case in-point, and one that actually contradicts the 'latitudinal effect', is the significantly higher DHA levels (average 21.3%) of the temperate euphausiid *Nyctiphanes australis* (Virtue et al. 1995) compared to the Antarctic species *Euphausia superba* ( $\approx 10\%$ ) (e.g. Phleger et al. 1998; Falk-Petersen et al. 2000) due to dietary differences.

### **Regional variations in fatty acid composition**

Lipid compositional data of midwater fish from other regions is essentially limited to myctophid species inhabiting Antarctic rather than sub-Antarctic waters. An exception is the study of Lea et al. (2002) that examined a number of myctophid species, also analysed in this study, from Macquarie Island and the Kerguelen Plateau. These included *E. antarctica* and *G. fraseri* (from both regions) and *E. carlsbergi* and *G. nicholsi* (Kerguelen Plateau only). Two further myctophid species from the sub-Antarctic have also been investigated. *P. bolini* was analysed in a dietary study of king penguins (*Aptenodytes patagonicus*) from Ile de la Possession, Crozet Peninsula (Raclot et al. 1998) and *G. nicholsi* in an investigation into Antarctic fur seal (*Arctocephalus gazella*) and Southern elephant seal (*Mirounga leonina*) diet at Bird Island, South Georgia (Brown et al. 1999).

With respect to Antarctic populations, Phleger et al. (1997; 1999a) determined the lipid composition of several myctophids from Elephant Island, Antarctic Peninsula (*E. antarctica*, *G. braueri*, *G. nicholsi* and *E. carlsbergi*) and Eastern Antarctica (*E. antarctica* again). Comparison with the results of these studies is complicated by the fact that analysis was performed on a number of different body sections rather than whole specimens, as is the case with the current study. For the purposes of this investigation, the results obtained from flesh sections (flesh includes all body sections except viscera, neurocranium and vertebral centra) were compared. Reinhardt and Van Vleet (1986) also analysed *E. antarctica*, *G. nicholsi* and *E. carlsbergi* from Croker Passage, Antarctic Peninsula as well as *P. bolini* and the bathylagid

*Bathylagus antarcticus*. Although the lipid class composition of flesh from these species was determined, fatty acid analysis was limited to either viscera or larvae making them unsuitable for comparisons. Donnelly et al. (1990) determined the proximate composition, including lipid content, of *Bathylagus antarcticus*, *E. antarctica*, *G. braueri*, *G. nicholsi* and *P. bolini* in Antarctic waters (Weddell and Scotia Sea).

### *Electrona antarctica*

The lipid composition of *E. antarctica* in this study closely resembles that previously determined from Macquarie Is. by Lea et al. (2002). Although total lipid content is comparable ( $17.5 \pm 3.6\%$  WW this study versus  $14.5 \pm 1.1\%$  WW), lipid class composition differed slightly with respect to PL ( $5.7 \pm 2.5\%$  this study versus  $14.2 \pm 4.7\%$ ). PUFA composition was slightly lower in this study, due mainly to a variation in the level of EPA ( $4.1 \pm 0.4\%$  this study versus  $6.8 \pm 0.1\%$ ) whilst MUFA content (mainly 18:1 $\omega$ 9c) was slightly higher ( $36.6 \pm 0.9\%$  this study versus  $32.1 \pm 1.5\%$ ). Little difference in fatty alcohol composition was observed. The difference in fatty acid composition may reflect the size differences apparent between these two studies. The *E. antarctica* included in the current study are slightly larger (average TL 62.3mm) than those analysed by Lea et al. (2002) (average TL 57.7mm), perhaps leading to a difference in diet between these two size groups.

With regards to Antarctic populations of *E. antarctica*, total lipid content from this study is close to the findings from Croker Passage (range 17.1-20.7% WW), and Kerguelen Plateau ( $13.7 \pm 1.6\%$  WW), though is higher than the range reported by Donnelly et al. (1990) in the Scotia and Weddell Seas (8.9-13.7% WW). *E. antarctica* from the current study are closer in lipid class composition to fish from Elephant Is. and Eastern Antarctica (WE >85%) (Phleger et al. 1997) than those from either Croker Passage or Kerguelen Island. *E. antarctica* from Croker Passage and Kerguelen Plateau contain less WE (range, Croker Passage 34.7-61.8%; Kerguelen Plateau  $71.6 \pm 8.2\%$ )

and considerably more TAG (range, Croker Passage 8.2-27.4%; Kerguelen Plateau  $17.3 \pm 10.3\%$  ) and PL (Croker Passage only, 19.8-42.5%) than fish from any of the other locations, although Phleger et al. (1997) attributed the elevated TAG in the Croker Passage population to a methodological error on the part of Reinhardt and Van Vleet (1986). Several differences in fatty acid composition are also apparent. Levels of total MUFA are lower ( $72.7 \pm 0.9\%$ , principally 18:1 $\omega$ 9c, 16:1 $\omega$ 7c and 18:1 $\omega$ 7c) and PUFA higher ( $16.0 \pm 1.2\%$ , especially DHA and EPA) in Macquarie Is. *E. antarctica* than either the Eastern Antarctic (MUFA  $82.2 \pm 1.2\%$ ; PUFA  $7.8 \pm 0.5\%$ ) or Elephant Is. (MUFA  $79.8 \pm 2.4\%$ ; PUFA  $9.8 \pm 2.7\%$ ) populations.

The variation in PUFA levels, in particular, point to a significant difference in diet between *E. antarctica* from the Antarctic and sub-Antarctic. Copepods dominate the diet of *E. antarctica* populations stretching from the sub-Antarctic (including Macquarie) Island to the Antarctic continent (Williams 1985; Kozlov and Tarverdiyeva 1989; Sabourenkov 1990; Pakhomov et al. 1996). Slight variations in diet are however apparent. Sabourenkov (1990) observed the growing importance of euphausiids in the adult *E. antarctica* (at an average TL of approximately 90mm) in Antarctic waters. As the TL of the *E. antarctica* from Elephant Island ranged from 96-100mm, the diet of these specimens may be dominated by euphausiids rather than copepods. This is at odds with the high proportion of MUFA in the Elephant Island population, more readily associated with copepods (e.g. Kattner et al. 1994) rather than PUFA-rich euphausiids (e.g. Virtue et al. 1993b; Phleger et al. 1998; Cripps and Atkinson 2000). *E. antarctica* from East Antarctica on the other hand were of similar size to the specimens from the current study (TL range 57-72mm) The differences in fatty acid composition between these two populations can more easily be attributed to dietary differences.

### *Electrona carlsbergi*

The total lipid content of *E. carlsbergi* from Croker Passage (11.2%, Reinhardt and Van Vleet 1986), and the two Macquarie Island studies

( $9.5 \pm 2.2\%$  in this study versus  $12.2 \pm 3.9\%$ ) reveals little variation relative to geographical location.

Lipid class composition is also relatively consistent between the various locations. In particular *E. carlsbergi* from this study closely resembles fish from Elephant Island (Phleger et al. 1999a). Fish from all regions are dominated by TAG ( $>64\%$ ), substantial quantities of PL ( $>17\%$ ) and trace amounts of WE (excepting Croker Passage,  $6.7\%$ ). Slight differences are, however, apparent. Most notably, PL are considerably less in both the Kerguelen Plateau and Croker Passage ( $\approx 17\%$ ) relative to Macquarie and Elephant Islands ( $29.0 \pm 7.3\%$  and  $24.5 \pm 14.7\%$  respectively).

Fatty acid composition is similarly consistent. All populations are characterised by high PUFA ( $>20\%$ ) and MUFA ( $>40\%$ ). The content of various individual PUFA do, however, show some variation. *E. carlsbergi* from Elephant Island are particularly rich in DHA ( $14.7 \pm 8.2\%$ ) compared to fish from Macquarie Island and the Kerguelen Plateau ( $\approx 10\%$ ). Similarly, EPA is present at higher levels at Elephant Island and the Kerguelen Plateau ( $\approx 10\%$ ) than the Macquarie Island population ( $6.5 \pm 1.2\%$ ). The level of 18:1 $\omega$ 9c is approximately 5-10% higher in the Macquarie Island population ( $23.7 \pm 1.9\%$ ) than in fish from either the Kerguelen Plateau ( $17.8 \pm 0.4\%$ ) or Elephant Island ( $15.4 \pm 2.2\%$ ).

The diet of *E. carlsbergi* is heavily influenced by geographical location. Copepods dominate the diet of populations from the Bellinghausen, d'Urville and Kosmonavtov (Kozlov and Tarverdiyeva 1989) and Lazarev Seas (Pakhomov et al. 1996) as well as South Georgia and the Scotia Sea between 20-40°W (Sabourenkov 1990). Euphausiids are the dominant prey consumed by *E. carlsbergi* from Macquarie Island (Gaskett et al. 2001) and the Argentine Depression, Scotia Sea (Oven et al. 1990). Regional variations in the diet of *E. carlsbergi* could thus explain the observed variation in PUFA.

*Gymnoscopelus braueri*

Total lipid content of *G. braueri* from this study ( $9.7 \pm 3.8\%$  WW) is comparable to populations from the Weddell and Scotia Seas ( $11.1$ – $13.7\%$  WW Donnelly et al. 1990). The dominance of WE in *G. braueri* from this study is similar to that of fish from Elephant Island. However FFA content (this study  $22.4 \pm 16.0\%$ , Elephant Is.  $0.5 \pm 0.5\%$ ) is considerably greater and PL lower in the Macquarie Island population ( $5.5 \pm 2.4\%$  versus  $23.7 \pm 7.5\%$ ). Large differences in fatty acid composition between the two populations are also evident. The higher PUFA content of Macquarie Island *G. braueri* ( $24.5 \pm 7.2\%$ ) compared to those from Elephant Is. ( $6.3 \pm 0.5\%$ ) is due mainly to variations in DHA (Macquarie Is.  $12.3 \pm 5.5\%$ ; Elephant Is.  $2.1 \pm 0.0\%$ ) and EPA (Macquarie Is.  $5.3 \pm 2.0\%$ ; Elephant Is.  $0.9 \pm 0.1\%$ ). Consequently MUFA content is greatly reduced at Macquarie Is. ( $62.1 \pm 12.9\%$ ) compared to Elephant Island ( $80.3 \pm 2.7\%$ ), especially in the levels of  $18:1\omega9c$  (Macquarie Is.  $38.7 \pm 11.0\%$ ; Elephant Is.  $55.0 \pm 0.6\%$ ). Differences in fatty alcohol composition are also evident, especially the higher levels of  $20:1\omega9Alc$  in Macquarie ( $9.2 \pm 3.9\%$ ) compared to Elephant Island ( $3.8 \pm 1.1\%$ ).

Although the diet of *G. braueri* is poorly described, variations related to both region and fish size have been described. The diets of *G. braueri* from Macquarie Island (Gaskett et al. 2001) and small fish (TL 50–80mm) from the Kosmonavtov Sea, Antarctica (Kozlov and Tarverdiyeva 1989) are dominated by copepods. Larger fish from the Kosmonavtov Sea consume a mixture of euphausiids (mainly *Euphausia superba*) and copepods, whilst in the region of the Lazarev Sea (Kozlov and Tarverdiyeva 1989) and Prydz Bay, Eastern Antarctica (Williams 1985, TL 41–150mm) the diet consists almost entirely of *E. superba*. Based on these observations, the *G. braueri* specimens (standard length 77–93mm) included in the study of Phleger et al. (1999a) are more likely to forage upon euphausiids. By contrast, the smaller sized specimens included in this study (TL 35–47mm) are far more likely to consume copepods, supporting the observations of stomach content analysis. Again, the variation in DHA and EPA content between the

Macquarie and Elephant Island populations of *G. braueri* could lie in these dietary differences.

### *Gymnoscopelus nicholsi*

Total lipid contents of *G. nicholsi* from the Kerguelen Plateau ( $18.0 \pm 2.3\%$ WW) and Weddell-Scotia Seas ( $23.3\%$ WW) are considerably higher than that of the Macquarie Island population (this study,  $7.1 \pm 2.0\%$ WW) despite similar TW and TL. Lipid class composition was reasonably consistent, characterised by high TAG ( $>90\%$ ) at both Elephant Island and the Kerguelen Plateau. The lower TAG levels from this study ( $80.7 \pm 6.2\%$ ) is most likely a consequence of the elevated FFA ( $17.6 \pm 11.3\%$ ) in the Macquarie Island populations relative to the two other locations. Reinhardt and Van Vleet (1986) reported that flesh of *G. nicholsi* from the Croker passage contained considerable amounts of WE ( $19.6\%$ ), a lipid class not detected in the other populations. This is likely to be a consequence of the same methodological error suggested by Phleger et al. (1997) in relation to *E. antarctica*.

Fatty acid composition varies greatly between each of the sampling locations. *G. nicholsi* from Bird Island (Brown et al. 1999) are distinguished by a high PUFA content ( $40.8\%$ ), mainly levels of DHA ( $12.3\%$ ), EPA ( $12.6\%$ ) and  $20:4\omega3$  ( $3.8\%$ ), that far exceed those from the other populations (DHA  $<9\%$ ; EPA  $<7\%$ ;  $20:4\omega3$   $<1\%$ ). The fatty acid composition of *G. nicholsi* in this study most closely matches that of Elephant Island, with some variation in MUFA (Elephant Is.  $58.9\%$ ; Macquarie Is.  $69.5 \pm 0.3\%$ ) and SFA ( $29.2\%$ ;  $17.9 \pm 0.3\%$ ). The PUFA content of the Kerguelen Plateau ( $17.5 \pm 0.5\%$ ) population is considerably higher than Macquarie Island (this study,  $11.7 \pm 0.2\%$ ), especially EPA (Kerguelen Plateau  $6.7 \pm 1.1\%$ ; Macquarie Is.  $2.1 \pm 0.1\%$ ).

The variation in fatty acid composition, especially PUFA, suggests the possibility of significant geographical variation in the diet of *G. nicholsi*.

However, euphausiids almost totally dominate the diet of most populations (Williams 1985; Kozlov and Tarverdiyeva 1989; Oven et al. 1990; Sabourenkov 1990). An exception is the study of Pakhomov (1996) who suggested that euphausiid consumption by *G. nicholsi*, rather than a continuous event, only occurs during certain periods and within specific regions. The diet of *G. nicholsi* in the study of Pakhomov (1996) is instead dominated by copepods. The diet of *G. nicholsi* at Macquarie Island is unclear due to the high proportion (>50%) of unidentified crustacea in the stomach contents (Gaskett et al. 2001), making it difficult to conclude whether regional variations in diet may have influenced fatty acid composition.

### *Gymnoscopelus fraseri*

The two studies incorporating *G. fraseri* from Macquarie Island reveal differing total lipid contents ( $7.6 \pm 4.4\%$  in this study versus  $3.6 \pm 0.4\%$  Lea et al. 2002). This is most likely a consequence of the larger specimens analysed in the current study (TL  $83.0 \pm 15.7\text{mm}$  in this study versus  $50.7 \pm 1.2\text{mm}$ ). The high total lipid content of *G. fraseri* from the Kerguelen Plateau ( $11.6 \pm 2.9\%$ ) similarly reflects the larger size of these specimens (TL  $71.7 \pm 5.8\text{mm}$ ) relative to those analysed by Lea et al. (2002).

Lipid class composition is consistent between the two Macquarie Island studies, characterised by high levels of TAG (>40%), PL (>24%) and FFA ( $\approx 20\%$ ) and small quantities of WE (<1%). Consequentially, the lipid class composition of *G. fraseri* from this study also contrasts with that of the Kerguelen Plateau (TAG  $78.1 \pm 5.4\%$ , FFA  $7.1 \pm 1.4\%$  and PL  $12.4 \pm 4.0\%$ ). As suggested by Lea et al. (2002), the higher FFA levels in specimens from Macquarie Island are probably the result of differences in storage conditions rather a direct dietary influence.

Fatty acid composition of *G. fraseri* also varies with location and between the two Macquarie Island studies and is most likely due to the range of *G. fraseri* sizes. PUFA is highest in the Macquarie Island study of Lea et al. (2002)

( $36.6 \pm 1.1\%$ ), followed by the current study ( $25.2 \pm 1.4\%$ ) and then finally the Kerguelen Plateau ( $23.4 \pm 2.3\%$ ). DHA is responsible for the greatest variation, ranging from  $20.5 \pm 0.1$  at Macquarie Island (Lea et al. 2002) to  $8.8 \pm 1.2\%$  at the Kerguelen Plateau. MUFA content is greatest in this study (total MUFA  $48.9 \pm 3.4\%$ ; primarily  $18:1\omega9C$ ,  $17.6 \pm 1.4\%$ ), followed by the Kerguelen Plateau ( $46.4 \pm 6.5\%$ ;  $18:1\omega9C$ ,  $13.7 \pm 0.8\%$ ) and then Macquarie Island (Lea et al. 2002) ( $33.8 \pm 3.4\%$ ;  $18:1\omega9c$ ,  $13.3 \pm 0.4\%$ ). Overall PUFA content, therefore, increases with increasing size of *G. fraseri*, associated with a concomitant decrease in MUFA. The diet of *G. fraseri* in regions other than Macquarie Island is currently unknown, making it impossible to speculate whether or not dietary differences may have contributed to the observed variation in fatty acid composition.

#### *Protomyctophum bolini*

*P. bolini* contains far more lipid at Macquarie Island ( $11.0 \pm 3.4\%WW$ ) than the Weddell-Scotia Sea population ( $4.0\%WW$ ), despite similar sized specimens. The lipid class composition of *P. bolini* from Croker Passage contrasts strongly with the results of this study. For instance the levels of WE in Croker Passage specimens ( $7.7\%$ ) exceed those detected in Macquarie Island fish ( $1.0 \pm 1.0\%$ ). PL content is conversely higher in this study ( $28.3 \pm 21.2\%$ ) compared to Croker Passage ( $10.0\%$ ). The variation in TAG (Macquarie Is.  $49.8 \pm 21.0\%$ ; Croker Passage  $82.3\%$ ) can be explained in part by the elevated FFA levels of Macquarie Island *P. bolini* ( $17.7 \pm 11.2\%$ ).

A comparison with the fatty acid composition of *P. bolini* from Ile de la Possession (Raclot et al. 1998) reveals minor variations in both MUFA and PUFA. The specimen from Ile de la Possession contains slightly higher levels of DHA ( $11.7\%$ ) and EPA ( $6.3\%$ ), and a lower content of  $18:1\omega9c$  ( $15.7\%$ ) than fish from the Macquarie Island population (DHA  $8.8 \pm 2.8\%$ ; EPA  $5.6 \pm 1.4\%$ ;  $18:1\omega9c$   $22.1 \pm 1.4\%$ ).

The diet of *P. bolini* varies greatly with region. The diet of fish from Macquarie Island population is dominated by euphausiids (Gaskett et al. 2001), whilst specimens collected below South Africa near the sub-tropical convergence consume mainly copepods (Pakhomov et al. 1996). The varied fatty acid composition of *P. bolini* from Macquarie Island and Ile de la Possession could conceivably be due to a similar difference in diet between these two regions.

### *Bathylagus antarcticus*

A regional comparison of lipid class data is limited to just one other region, Croker Passage (Reinhardt and Van Vleet 1986). The level of PL in the specimen from Croker Passage (34.2%) is similar to the levels observed in juvenile *Bathylagus antarcticus* in the present study ( $44.9 \pm 5.6\%$ ). Levels of TAG (Croker Passage, 60.7%) are, however, closer to values for adult specimens included in the current study ( $82.9 \pm 5.4\%$ ). The content of WE (Croker Passage, 5.1%) is higher than in either the juveniles ( $1.6 \pm 0.7\%$ ) or adults (no WE) from Macquarie Island. Little potential dietary information can be gained from these results, compounded by the fact that the stomach contents of populations other than Macquarie Island are yet to be examined.

## **2.5 CONCLUSIONS**

The wide-ranging lipid composition of midwater fish from the Macquarie Island region may reflect diversity in both the biological function of lipid in this fish assemblage, as well as diet. The potential dietary overlap of many species, suggested by stomach content analysis and in many cases confirmed by fatty acid data, has significant implications for the region and underlines the inclusive nature of marine ecosystems.

# CHAPTER 3

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## LIPID COMPOSITION OF SQUID FROM THE MACQUARIE ISLAND REGION: BIOLOGICAL FUNCTION AND IMPLICATIONS FOR DIETARY INTERACTIONS

### 3.1 INTRODUCTION

Despite their importance, many biological, physiological and ecological aspects of Southern Ocean squid, including species composition, distribution and biomass, are poorly understood (Rodhouse 1989; Lu and Williams 1994a). Squid are widely distributed in this vast region of ocean and in certain areas, namely the Antarctic Polar Frontal Zone, are thought to occupy the ecological niche usually dominated by epipelagic fish (Rodhouse and White 1995). This lack of knowledge is due in part to the well documented ability of squid to escape nets (Piatkowski and Hagen 1994), the logistically difficult study environment of the Southern Ocean and the limited range of commercially viable squid species, traditionally a valuable source of research data and material.

Equally apparent is the lack of research directed towards the determination of biochemical compounds, including lipids. The almost singular reliance of squid on amino acid metabolism (Lee 1994) has meant that the role played by lipids in cephalopods is often overlooked (Navarro and Villanueva 2000). This is especially surprising given the high levels of lipid found in the digestive gland tissue of several species. For example, the digestive glands of several Northern Hemisphere gonatid squid contain high levels of diacylglycerol ethers (DAGE) that are thought to play a major role in buoyancy regulation and/or energy storage (Hayashi et al. 1985; Hayashi and Yamamoto 1987; Hayashi 1989; Hayashi and Kawasaki 1990; Hayashi

et al. 1990). The large lipid stores present in many cephalopod species are thought to be essential to the proper functioning of mechanisms as diverse as neurological development during early life (Navarro and Villanueva 2000), functioning of the central nervous system (Dumont et al. 1992; Dumont et al. 1994), reproduction (Blanchier and Boucaud-Camou 1984), buoyancy (Clarke et al. 1979) and energy storage (Castro et al. 1992; Semmens 1998). Lipid composition may also be useful as a descriptive tool in the evaluation of certain nutritional, metabolic and energetic parameters (Lawson et al. 1998). For instance it has been suggested that the lipid content of squid digestive glands is a proximal indicator of the trophicity, or lipid potential, of the collection region (Abolmasova et al. 1990; Semmens 1998). An increase in the relative lipid content of this organ would therefore be correlated to an increase in the availability of dietary lipid in a given region.

Squid are recognized as important prey for a number of Macquarie Island predators. Amongst these are Southern Elephant seals (Green and Burton 1993), Fur seals (Green et al. 1990), Hooker's sea lions (McMahon et al. 1999), and Patagonian toothfish (Goldsworthy et al. 2002). The diet of a variety of seabird species from Macquarie Island also include squid (summarised in Goldsworthy et al. 2001d). Squid are also important dietary components of these predators in other regions of the Southern Ocean, including Patagonian toothfish (Arrizaga et al. 1996; García de la Rosa et al. 1997; Pilling et al. 2001; Xavier et al. 2002), whales (Nemoto et al. 1985; Clarke and Goodall 1994; Slip et al. 1995; Clarke and Roeleveld 1998; Clarke and Roper 1998), seabirds (Cherel and Ridoux 1992; Thompson 1994; Cherel et al. 1996; Rodhouse et al. 1998; Cherel and Weimerskirch 1999), and marine mammals (Rodhouse et al. 1992).

Lipid profiling techniques are rapidly finding a use as a means of determining aspects of diet. Assessment of squid diet is essential in furthering the understanding of trophic interactions within food webs, as well as the broader implications of energy flow within oceanic systems. In spite of this need, diet has so far received only rudimentary attention for most squid species. Moreover, diet is usually assessed by stomach content analysis, a technique

with a number of inherent and well acknowledged shortcomings if used in isolation. These include a failure to identify with any certainty long-term dietary composition and bias due to differential digestion of prey items (Antonelis et al. 1987; Harwood and Croxall 1988; Rodhouse et al. 1992; Jackson 1995; Rodhouse and Nigmatullin 1996; Phillips et al. 2001). The use of lipid compounds, including fatty acids, fatty alcohols and glyceryl ether diols as an adjunct to traditional techniques of dietary determination has gained increasing interest in recent times, including for Southern Ocean squid species. For example Phillips et al. (2001) used fatty acids to explore the trophic interactions between the squid *Moroteuthis ingens* and myctophid fish in the Macquarie Island region.

This chapter examines the lipid composition of six squid species collected from the Macquarie Island region; *Moroteuthis ingens*, *Mastigoteuthis* sp., *Histioteuthis eltaninae*, *Kondakovia longimana*, *Galiteuthis glacialis* and *Gonatus antarcticus*, representing squid from five different families. For a number of these species (*K. longimana*, *Mastigoteuthis* sp., *H. eltaninae*), this study represents the first time that lipid composition has been reported. For others (*M. ingens*, *G. antarcticus*, *G. glacialis*) the data presented is an expansion of the current understanding regarding the lipid composition of these squid, especially in relation to the influence of temporal, growth and geographical factors. The main aims of this study are twofold. Firstly, the lipid composition of these squid will be related to its possible role in biological function, particularly in relation to buoyancy and energy storage. Secondly, potential dietary interactions will be explored by comparison of the fatty acid composition of squid and their prey, mainly myctophids, and identifying similarities.

## **3.2 MATERIALS AND METHODS**

### **Sample description and preparation**

All squid were collected from the Macquarie Island region (54°30'S; 158°55'E) between 1995 and 1998 by operations of the commercial trawler

*Austral Leader* (Austral Fisheries Pty. Ltd., Fremantle, Western Australia). The duration of net tows ranged from 4-5 hours at varying trawl depths. A total of 19 specimens were collected from 6 species of varying total lengths, mantle lengths and total weights (Table 3.1). Stomachs were removed for content analysis and the remainder of the sample stored in -20°C freezers until analysis. Samples were analysed within 3 months where possible.

Whole specimens of *Moroteuthis ingens*, *Histioteuthis eltaninae*, *Mastigoteuthis sp.* and *Galiteuthis glacialis* were thawed and ground before removal of a 1-2g sub-sample for lipid extraction. Two of the three specimens of *K. longimana* were dissected into mantle, tentacle and digestive gland tissue (DG) which were ground separately before removal of 1-2g for lipid extraction. The remaining specimen of *K. longimana* was ground whole.

The digestive glands were already ruptured in four of the five *Gonatus antarcticus* specimens, therefore these samples were analysed as whole animals. Sub-samples of tissue were obtained from blended whole specimens (referred to as whole animal tissue). The intact DG and mantle of the remaining specimen were separated and blended independently.

### **Lipid extraction**

Total lipids were extracted overnight from a sub-sample (approximately 1g) of squid tissue using the modified one-phase Bligh-Dyer (1959) method. After phase separation total lipids were recovered from the lower chloroform phase and concentrated *in vacuo* (rotary evaporator, 40°C). Samples were stored at -20°C prior to analysis.

### **Lipid class determination**

Lipid class profiles were determined by TLC-FID using an Iatroscan MkV TH10 analyser (Volkman and Nichols 1991), using the method described in Nelson et al. (2001). Wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA), sterol (ST), diacylglycerol (DG) and polar lipid (PL) were resolved in a polar solvent system of hexane-diethyl ether-acetic acid (60:17:0.2 v/v). The presence of WE and diacylglycerol ether (DAGE) was confirmed by the use

of a non-polar solvent system (hexane-diethyl ether; 96:4 v/v). Peak areas were quantified using commercially available chromatography software (DAPA Scientific Software, Kalamunda, Western Australia, Australia).

### **Fatty acid and glyceryl ether diol determination**

Fatty acid methyl esters (FAME) were prepared by addition of MeOH/HCl/CHCl<sub>3</sub> (3mL 10:1:1, v/v, 80°C/2hr) to an aliquot of the total lipid. After the addition of 1mL of water, FAME were extracted (3x 2ml) with 4:1 v/v hexane/CHCl<sub>3</sub>. Chloroform containing C<sub>19</sub> FAME internal standard was then added. FAME fractions were treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA 50μL, 60°C, overnight) to convert glyceryl ether diols (derived from DAGE), alcohols (derived from WE) and ST to their corresponding trimethylsilyl (TMS) ethers.

Gas chromatographic analysis was performed on a Hewlett Packard 5890A GC equipped with a flame ionisation detector, a cross-linked methyl silicone (HP5) fused silica capillary column (50 m length × 0.32 μm internal diameter), employing hydrogen as the carrier gas. Samples were injected (HP7673 auto-injector) at 50°C in the splitless mode, with a 2-min. venting time (Nichols et al. 1993). The injector and detector were maintained at 290°C and 310°C respectively. The temperature of the oven was increased after 1 min. to 150°C at 30°C min<sup>-1</sup>, then to 250°C at 2°C min<sup>-1</sup> and finally to 300°C at 5°C min<sup>-1</sup> (Nichols et al. 1993). Peak areas were quantified using Millennium 32 Chromatography Manager (Waters Corporation, Milford, Massachusetts, USA). Identification of individual fatty acids was based upon a comparison of relative retention times with those obtained for authentic and laboratory standards. GC-mass spectrometry (Fisons MD800 system and Finnigan Thermoquest GCQ fitted with an on-column injector, both configured as above) was used for confirmation of components from representative samples (Nichols et al. 1993).

**Potential prey species**

The fatty acid compositions of several myctophids (*Gymnoscopelus fraseri*, *G. nicholsi*, *G. bolini*, *G. braueri*, *Electrona antarctica*, *E. carlsbergi*, *Protomyctophum bolini* and *Lampanyctus archirus*) and the bathylagid *Bathylagus antarcticus* were compared to those of squid. A number of these myctophids and *Bathylagus antarcticus* have previously been identified by Phillips et al. (2001) as potentially important prey species for squid (*Moroteuthis ingens*) in the Macquarie Island region. All potential prey specimens were collected from the Macquarie Island region and were analysed as per above (refer to Chapter 2 for further details).

**Statistical analysis**

All statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, IL, USA). Non-metric multi-dimensional scaling (MDS; Kruskal loss function) was employed to compare fatty acid, glyceryl ether diol and lipid class composition in two dimensions between the various body sections of *Gonatus antarcticus* and *Kondakovia longimana*.

MDS was also employed to compare the total fatty acid profiles of squid and potential prey species. Potential prey that clustered close to squid using MDS were then compared using linear discriminant analysis (LDA; tolerance=0.01, classification and jackknifed classification matrix, Wilk's lambda, automatic forward stepping [F to enter=4, F to remove=3.9]) of total fatty acid profiles. LDA allowed for the identification of those fatty acids (predictors) most responsible for differences between Patagonian toothfish and potential prey. The jack-knifed classification matrix is included as a means of cross-validating the normal classification matrix. Considerable difference in the percentage of correct classifications between these two matrices would suggest potential difficulties in correctly classifying the data.

### 3.3 RESULTS

#### Squid size

Squid sizes varied greatly across the species examined (Table 3.1). Both total length (TL, mantle and tentacles) and total weight (TW, minus stomach mass) was greatest in a specimen of *Kondakovia longimana* (1050mm, 2131g) and lowest in a specimen of *Galiteuthis glacialis* (280mm, 35g), although TL was not measured in *Mastigoteuthis sp.* or *Histioteuthis eltaninae*. Mantle length (ML) ranged from a low of 110mm in *Galiteuthis glacialis* to 190mm in *Gonatus antarcticus* although ML was not determined, once again, in *Mastigoteuthis sp.* or *H. eltaninae* as well as *K. longimana*. The largest range of size within a species was observed in *Moroteuthis ingens* that varied greatly in TL (325-1000mm), ML (180-360mm) and TW (145-686g).

*G. antarcticus* and *K. longimana* both contained prominent digestive glands (DG). The DG of the single specimen of *G. antarcticus* in which the gland was still intact accounted for 64g (34% by mass) of the total wet weight (187g). The weights of the DG from the two *K. longimana* specimens were not measured.

#### Total lipid content and lipid class composition

##### *Whole specimens*

The total lipid content (wet weight, WW) of whole specimens ranged from a low of  $2.6 \pm 0.6\%$  WW (range 1.9-2.7%) in *Galiteuthis glacialis* to a high of  $17.5 \pm 7.2\%$  WW in *G. antarcticus* (Table 3.2). The lipid contents of *K. longimana* (13.5% WW) and *M. ingens* (mean  $8.4 \pm 2.9\%$  WW, range 4.6-12.1%) were also high, whilst those of *H. eltaninae* (mean  $5.6 \pm 1.6\%$  WW, range 3.4-6.9%) and *Mastigoteuthis sp.* (mean 3.4% WW) were lower. Variations in lipid content within species were minimal, with the exception of *G. antarcticus* (range 8.1-27.7%). A positive correlation between increasing total lipid content and TW of *G. antarcticus* was also observed.

**Table 3.1** Comparison of cephalopod (squid) species analysed from the Macquarie Island region including total length (mantle and tentacles) and total wet weight of specimens (data presented as range of values)

Family	Species	Common name	n	Total length (mm)	Mantle length (mm)	Total weight (g)
Onychoteuthidae	<i>Moroteuthis ingens</i>	Warty Squid	5	325-1000	180-360	145-686
	<i>Kondakovia longimana</i>		3	700-1050	-	226-2321
Mastigoteuthidae	<i>Mastigoteuthis sp.</i>	Jewel Squid	1	-	-	37
Histioteuthidae	<i>Histioteuthis eltaninae</i>		3	-	-	62-91
Cranchiidae	<i>Galiteuthis glacialis</i>		2	280-450	110-290	35-152
Gonatiidae	<i>Gonatus antarcticus</i>		5	340-470	190-300	132-217

**Table 3.2** Distribution of total lipid content (% composition of tissue, wet weight) and lipid class composition (% composition of total lipid content) throughout whole and body sections of various squid species from the Macquarie Island region (data presented as mean  $\pm$  standard deviation)

Species	Body section	n	Total lipid content	Lipid class						Total
				WE	DAGE	TAG	FFA	ST	PL	
<i>M. ingens</i>	Whole	5	8.4 $\pm$ 2.9	2.2 $\pm$ 0.8	0.0	41.1 $\pm$ 18.5	26.1 $\pm$ 10.6	4.7 $\pm$ 2.6	25.9 $\pm$ 11.3	100.0
<i>Mastigoteuthis sp.</i>	Whole	1	3.4	0.0	0.0	21.4	5.2	6.4	67.0	100.0
<i>H. eltaninae</i>	Whole	3	5.6 $\pm$ 1.6	1.2 $\pm$ 0.8	0.0	32.4 $\pm$ 19.9	20.4 $\pm$ 6.4	8.1 $\pm$ 2.1	37.9 $\pm$ 22.7	100.0
<i>G. glacialis</i>	Whole	2	2.3 $\pm$ 0.6	0.1 $\pm$ 0.2	0.0	1.4 $\pm$ 0.5	1.7 $\pm$ 1.0	14.9 $\pm$ 1.8	81.8 $\pm$ 3.5	100.0
<i>K. longimana</i>	Whole	1	13.5	0.8	0.0	44.4	38.3	2.8	13.7	100.0
	Tentacle	2	1.3 $\pm$ 0.0	0.0	0.0	4.1 $\pm$ 3.9	4.0 $\pm$ 3.7	15.3 $\pm$ 0.7	76.7 $\pm$ 8.4	100.0
	Mantle	2	1.4 $\pm$ 0.3	0.0	0.0	2.5 $\pm$ 1.1	6.2 $\pm$ 0.4	13.5 $\pm$ 1.6	77.8 $\pm$ 1.0	100.0
	Digestive Gland	2	38.0 $\pm$ 1.3	3.1 $\pm$ 0.0	0.0	71.2 $\pm$ 4.6	21.4 $\pm$ 7.0	1.5 $\pm$ 0.2	3.0 $\pm$ 2.1	100.0
<i>G. antarcticus</i>	Whole	4	17.5 $\pm$ 7.2	0.8 $\pm$ 0.2	60.5 $\pm$ 10.3	14.3 $\pm$ 6.7	12.3 $\pm$ 8.1	6.0 $\pm$ 3.4	6.1 $\pm$ 6.5	100.0
	Mantle	1	3.2	0.0	0.0	21.6	10.6	13.9	60.7	100.0
	Digestive Gland	1	47.7	0.6	71.0	4.0	12.1	9.7	2.6	100.0

WE=wax ester, DAGE=diacylglyceryl ether, TAG=triacylglycerol, FFA=free fatty acid, ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol), PL=polar lipid, n=number of samples

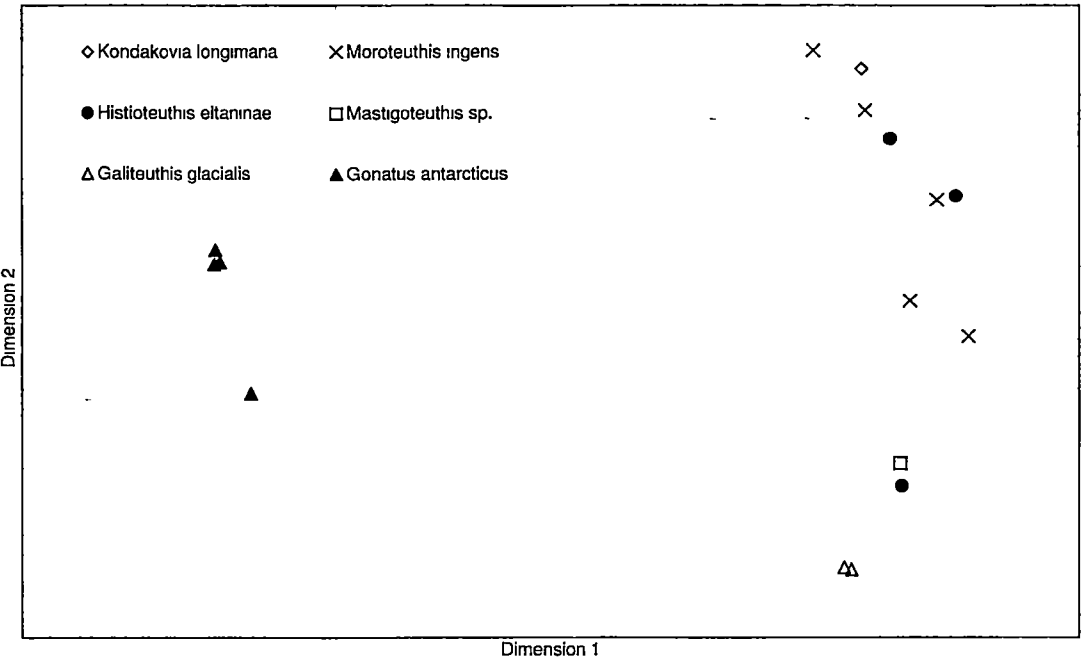
Lipid class composition varied considerably between species, as well as between individuals of the same species (Table 3.2). This is supported by the wide scattering of data produced by MDS (Figure 3.1). The majority of the differences in lipid class composition between species can be attributed to variations in the levels of TAG and PL with the exception of *G. antarcticus*, the only species that contained DAGE (mean  $60.5 \pm 10.3\%$ , range 49.4-74.3%). The remaining squid species can be broadly separated into two groups based upon the levels of TAG and PL in whole specimens. The first group consists of those species containing high levels of TAG and includes *M. ingens* (mean  $41.1 \pm 18.5\%$ , range 18.5-63.7%) and *K. longimana*. (44.4%). The second group contains species in which PL represented the dominant lipid class and comprises *Mastigoteuthis* sp. (67.0%), *H. eltaninae* (mean  $37.9 \pm 22.7\%$ , range 24.5-64.1%) and *G. glacialis* (mean  $81.8 \pm 3.5\%$ , range 79.4-84.3%).

The relative levels of FFA also contributed greatly to differences between the species. *M. ingens*, *H. eltaninae* and *K. longimana* contained FFA levels in excess of 20%, whilst in the remaining species FFA content was <13%. WE was present in all species with the exception of *Mastigoteuthis* sp., although levels were <3% in all whole specimens. ST was a minor component of all squid (<10% of total composition), with the exception of *G. glacialis* (mean  $14.9 \pm 1.8\%$ ; range 13.7-16.2%). In addition, lipid class composition was particularly variable between individuals of *M. ingens* and *H. eltaninae*, especially with respect to levels of TAG, FFA and PL.

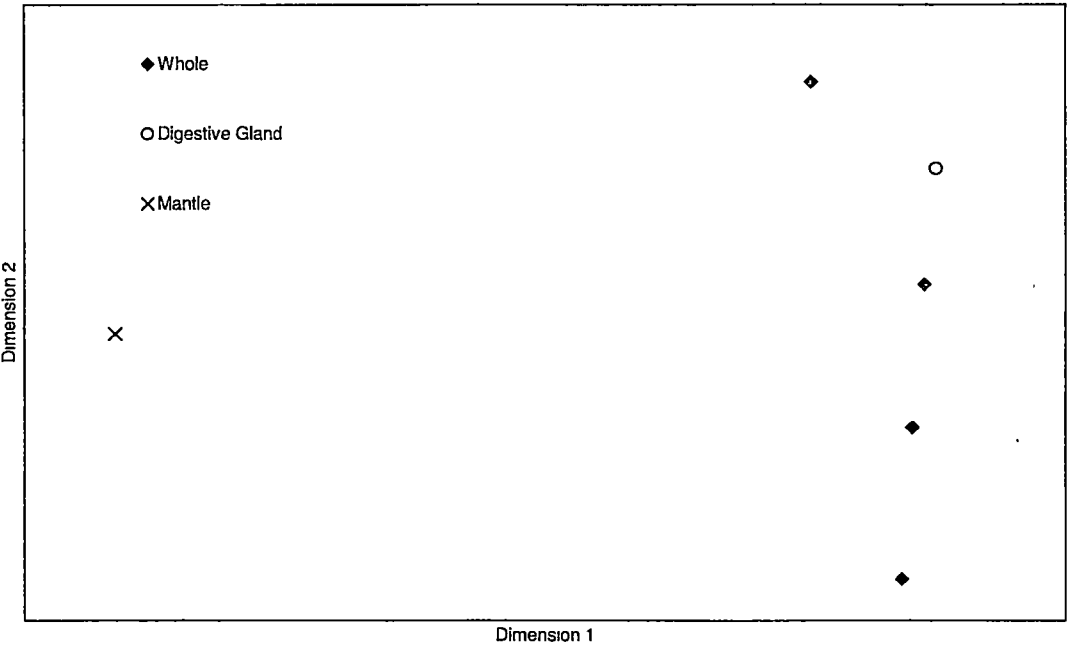
### *Gonatus antarcticus*

The total lipid content of *G. antarcticus* varied considerably between DG and mantle tissues, ranging between 47.7% WW in the DG and 3.2% WW in the mantle, compared to  $17.5 \pm 7.2\%$  WW in whole specimens (Table 3.2).

The lipid class composition of *G. antarcticus* DG reflected that of whole specimens whereas the composition of mantle tissue was considerably different (Table 3.2), an observation supported by MDS (Figure 3.2). Both the



**Figure 3.1** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of whole specimens of various squid from the Macquarie Island region



**Figure 3.2** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of various body sections of *Gonatus antarcticus* from the Macquarie Island region

DG (71.0%) and whole specimens (60.5%) were characterized by high levels of diacylglyceryl ether (DAGE), a lipid class absent from the mantle. Similarly, WE was present in small quantities (~1%) in both whole specimens and the DG, but was undetected in mantle tissue. In comparison, high PL levels (60.7%) distinguished the mantle from either the DG (2.6%) or whole specimens. ST levels were also higher in the mantle (13.9%) compared to whole specimens (6.0%) and the DG (9.7%). Triacylglycerol (TAG) levels were higher in both mantle tissue (21.6%) and whole specimens (14.3%) than the DG (4.0%). Both body regions and whole specimens contained FFA at levels between ~10-12%.

### *Kondakovia longimana*

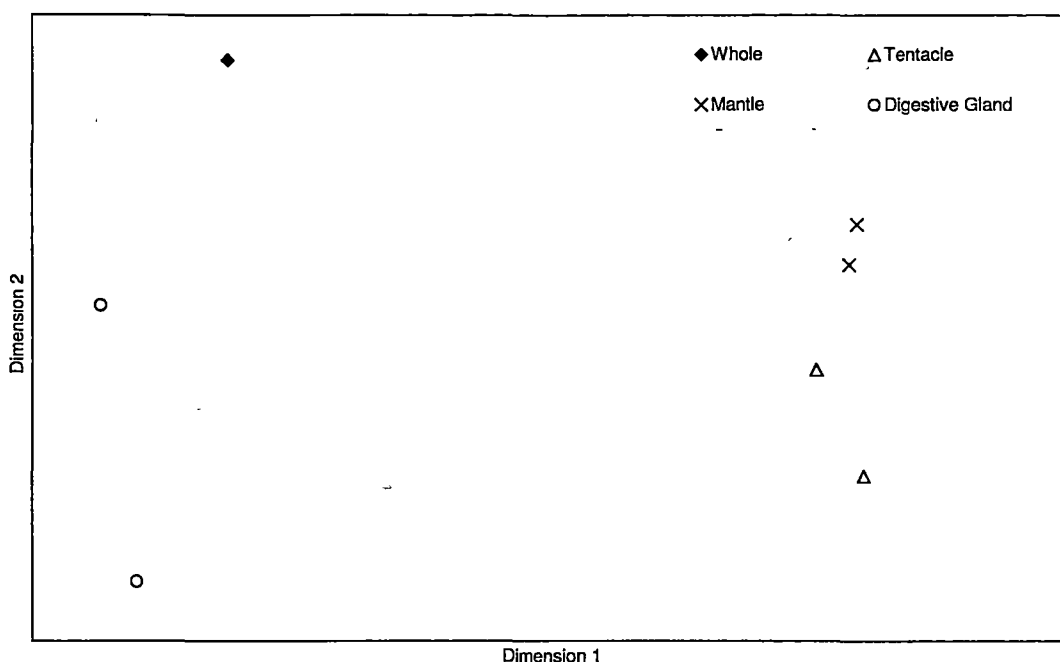
Lipid content varied substantially between the body sections of *K. longimana*, and was greatest in the digestive gland (mean  $38.0 \pm 1.3\%$  WW; range 37.1-38.9%), well above the whole specimen (13.5% WW), and lowest in the tentacle (mean  $1.3 \pm 0.0\%$  WW) and mantle (mean  $1.4 \pm 0.3\%$  WW, range 1.2-1.6%) (Table 3.2).

A marked variation in lipid class composition between the body sections of *K. longimana* was also observed. MDS produced a close association between the mantle and tentacle, whilst the DG was further separated (Figure 3.3). None of the body sections shared lipid class compositions matching that of the whole specimen. The DG was dominated by TAG ( $71.2 \pm 4.6\%$ ; range 67.9-74.4%) and FFA ( $21.4 \pm 7.0\%$ ; range 16.4-26.3%). This contrasted with the tentacle and mantle, where PL accounted for >76% of the lipid class composition and TAG and FFA were each <6%.

## **Fatty acid composition**

### *Whole specimens*

The fatty acid composition of whole specimens varied considerably across the species analysed (Tables 3.3-3.5). In particular *G. antarcticus* (Table 3.4)



**Figure 3.3** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of various body sections of *Kondakovia longimana* from the Macquarie Island region

did not contain several fatty acids (16:1 $\omega$ 7t, 18:1, 20:1 $\omega$ 11c, 22:1 $\omega$ 7c, 24:1 $\omega$ 11c, 24:1 $\omega$ 9c, 18:3 $\omega$ 6) that were generally present in most other species. Within-species variation was minimal.

MUFA dominated the composition of all species, with the exception of *G. glacialis*, and was highest in *G. antarcticus* (mean 68.7 $\pm$ 5.9%) and lowest in *G. glacialis* (20.9 $\pm$ 0.7%). Included amongst the major constituent MUFA (>1% of total fatty acid composition) common to all species (only whole specimens) were 16:1 $\omega$ 7c (range across all species 1.6-4.7%), 18:1 $\omega$ 9c (range 13.1-30.4%), 18:1 $\omega$ 7c (range 3.4-5.1%), 20:1 $\omega$ 9c (range 4.5-15.8%), 22:1 $\omega$ 11 (excepting *G. glacialis* and *K. longimana*, range 0.2-7.9%) and 22:1 $\omega$ 9c (excepting *K. longimana*, range 0.8-4.3). Other major MUFA included 24:1 $\omega$ 9c (mean 1%) and 24:1 $\omega$ 11c (mean 1%) in *Mastigoteuthis* sp., 20:1 $\omega$ 11c in *H. eltaninae* (mean 1.4 $\pm$ 0.2%), *G. glacialis* (mean 1.2 $\pm$ 1.7%) and *Mastigoteuthis* sp. (mean 1.0%), and 16:1 $\omega$ 9c in *H. eltaninae* (mean 6.3 $\pm$ 2.2%) and *Mastigoteuthis* sp. (mean 2.7%).

**Table 3.3** Fatty acid composition (% composition of total fatty acids) of various squid species (whole specimens) included in this study from the Macquarie Island region (data presented as mean  $\pm$  standard deviation)

Fatty Acid	Squid species			
	<i>Moroteuthis ingens</i> (n=5)	<i>Mastigoteuthis</i> sp. (n=1)	<i>Histioteuthis eltaninae</i> (n=3)	<i>Galiteuthis glacialis</i> (n=2)
14:0	3.1 $\pm$ 0.6	0.7	0.8 $\pm$ 0.2	2.7 $\pm$ 0.4
15:0	0.4 $\pm$ 0.1	0.2	0.2 $\pm$ 0.0	0.4 $\pm$ 0.0
16:0	19.2 $\pm$ 2.2	16.5	12.7 $\pm$ 1.8	24.9 $\pm$ 1.7
17:0	0.4 $\pm$ 0.1	0.6	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1
18:0	3.6 $\pm$ 0.5	4.3	3.5 $\pm$ 1.1	2.4 $\pm$ 0.1
20:0	0.1 $\pm$ 0.0	0.2	0.3 $\pm$ 0.1	0.0 $\pm$ 0.0
22:0	0.0 $\pm$ 0.0	0.1	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0
14:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
16:1 $\omega$ 9c	0.5 $\pm$ 0.4	2.7	6.3 $\pm$ 2.2	0.5 $\pm$ 0.1
16:1 $\omega$ 7c	4.1 $\pm$ 0.9	2.2	3.2 $\pm$ 0.3	1.6 $\pm$ 0.1
16:1 $\omega$ 7t	0.6 $\pm$ 0.4	0.1	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0
17:1	0.3 $\pm$ 0.1	0.2	0.3 $\pm$ 0.1	0.0 $\pm$ 0.0
18:1 $\omega$ 9c	19.3 $\pm$ 2.1	13.1	13.3 $\pm$ 0.5	5.3 $\pm$ 1.0
18:1 $\omega$ 7c	4.0 $\pm$ 0.7	3.9	3.7 $\pm$ 0.4	3.5 $\pm$ 0.5
18:1 $\omega$ 5c	0.7 $\pm$ 0.1	0.6	0.7 $\pm$ 0.2	0.3 $\pm$ 0.0
18:1	0.3 $\pm$ 0.1	0.3	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0
19:1	0.4 $\pm$ 0.1	0.6	0.4 $\pm$ 0.1	0.0 $\pm$ 0.0
20:1 $\omega$ 11c	0.9 $\pm$ 0.2	1.0	1.4 $\pm$ 0.2	1.2 $\pm$ 1.7
20:1 $\omega$ 9c	7.9 $\pm$ 1.2	12.0	7.8 $\pm$ 0.4	6.3 $\pm$ 1.8
20:1 $\omega$ 7c	0.5 $\pm$ 0.1	0.6	0.4 $\pm$ 0.0	0.2 $\pm$ 0.0
22:1 $\omega$ 11c	2.4 $\pm$ 0.5	3.0	3.4 $\pm$ 0.5	0.2 $\pm$ 0.0
22:1 $\omega$ 9c	1.6 $\pm$ 0.3	4.3	3.1 $\pm$ 0.3	1.0 $\pm$ 0.0
22:1 $\omega$ 7c	0.4 $\pm$ 0.3	1.2	6.0 $\pm$ 4.8	0.1 $\pm$ 0.1
24:1 $\omega$ 11c	0.5 $\pm$ 0.1	1.0	0.8 $\pm$ 0.1	0.0 $\pm$ 0.0
24:1 $\omega$ 9c	0.8 $\pm$ 0.1	1.0	0.7 $\pm$ 0.2	0.2 $\pm$ 0.0
18:3 $\omega$ 6	0.0 $\pm$ 0.0	0.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
18:4 $\omega$ 3	0.7 $\pm$ 0.1	0.1	0.5 $\pm$ 0.1	0.0 $\pm$ 0.0
18:2 $\omega$ 6	1.0 $\pm$ 0.2	0.7	0.7 $\pm$ 0.1	0.3 $\pm$ 0.0
AA	1.0 $\pm$ 0.2	1.1	0.9 $\pm$ 0.2	1.9 $\pm$ 0.4
EPA	8.6 $\pm$ 1.4	10.2	9.9 $\pm$ 1.5	19.3 $\pm$ 1.1
20:3 $\omega$ 6	0.2 $\pm$ 0.0	0.1	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0
20:4 $\omega$ 3	0.6 $\pm$ 0.2	0.4	0.5 $\pm$ 0.0	0.2 $\pm$ 0.0
20:2 $\omega$ 6	0.4 $\pm$ 0.1	0.8	0.9 $\pm$ 0.1	0.5 $\pm$ 0.1
C <sub>21</sub> PUFA	0.2 $\pm$ 0.1	0.2	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0
22:5 $\omega$ 6	0.1 $\pm$ 0.0	0.2	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0
DHA	13.3 $\pm$ 2.2	13.6	14.1 $\pm$ 1.5	25.6 $\pm$ 4.4
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.1	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0
DPA	0.7 $\pm$ 0.3	0.7	0.8 $\pm$ 0.0	0.3 $\pm$ 0.0
SFA	26.9 $\pm$ 2.5	22.7	18.0 $\pm$ 2.9	30.8 $\pm$ 2.2
BR	0.6 $\pm$ 0.1	0.8	0.7 $\pm$ 0.3	0.4 $\pm$ 0.0
MUFA	45.4 $\pm$ 5.3	48.1	52.3 $\pm$ 5.5	20.9 $\pm$ 0.7
PUFA	27.0 $\pm$ 3.0	28.4	29.0 $\pm$ 2.8	48.0 $\pm$ 2.9
TOTAL	100.0	100.0	100.0	100.0

AA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid, SFA=saturated fatty, acid BR=branched fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, c=*cis* double bond orientation, t=*trans* double bond orientation, n=number of specimens

**Table 3.4** Fatty acid composition (% composition of total fatty acids) of various body sections of *Gonatus antarcticus* included in this study from the Macquarie Island region (data presented as mean  $\pm$  standard deviation)

Fatty Acid	Body section		
	Whole Specimen (n=4)	Digestive Gland (n=1)	Mantle Tissue (n=1)
14:0	2.3 $\pm$ 1.2	1.9	0.3
15:0	0.1 $\pm$ 0.0	0.1	0.2
16:0	4.7 $\pm$ 1.0	4.2	7.9
17:0	0.3 $\pm$ 0.1	0.2	0.5
18:0	1.7 $\pm$ 0.2	1.9	2.6
20:0	0.2 $\pm$ 0.1	0.1	1.1
22:0	0.2 $\pm$ 0.0	0.1	0.1
14:1 $\omega$ 5c	0.1 $\pm$ 0.0	0.1	0.0
16:1 $\omega$ 9c	0.3 $\pm$ 0.1	0.9	0.2
16:1 $\omega$ 7c	4.7 $\pm$ 1.2	5.0	2.1
16:1 $\omega$ 5c	0.1 $\pm$ 0.1	0.1	0.1
17:1	0.3 $\pm$ 0.1	0.3	0.2
18:1 $\omega$ 9c	30.4 $\pm$ 6.5	40.2	20.7
18:1 $\omega$ 7c	5.1 $\pm$ 0.5	6.7	4.5
18:1 $\omega$ 5c	0.2 $\pm$ 0.1	0.2	0.3
19:1	0.2 $\pm$ 0.1	0.3	0.0
20:1 $\omega$ 9c	15.8 $\pm$ 2.4	12.4	13.2
20:1 $\omega$ 7c	0.7 $\pm$ 0.1	0.7	0.1
22:1 $\omega$ 11c	7.9 $\pm$ 3.9	3.2	3.1
22:1 $\omega$ 9c	3.0 $\pm$ 0.7	1.8	3.0
18:4 $\omega$ 3	0.6 $\pm$ 0.1	0.7	1.2
18:2 $\omega$ 6	1.5 $\pm$ 0.3	1.5	0.5
AA	0.8 $\pm$ 0.2	0.9	1.9
EPA	5.7 $\pm$ 1.3	5.2	11.4
20:3 $\omega$ 6	0.3 $\pm$ 0.1	0.4	0.3
20:4 $\omega$ 3	1.0 $\pm$ 0.3	1.1	0.6
20:2 $\omega$ 6	0.6 $\pm$ 0.1	0.9	1.2
C <sub>21</sub> PUFA	1.1 $\pm$ 1.5	0.4	0.3
22:5 $\omega$ 6	0.1 $\pm$ 0.1	0.1	0.2
DHA	8.9 $\pm$ 1.6	7.3	21.4
22:4 $\omega$ 6	0.1 $\pm$ 0.0	0.0	0.1
DPA	1.1 $\pm$ 0.4	1.1	0.9
SFA	9.5 $\pm$ 2.2	8.5	12.7
BrFA	0.1 $\pm$ 0.1	0.1	0.0
MUFA	68.7 $\pm$ 5.9	71.9	47.5
PUFA	21.7 $\pm$ 3.9	19.6	39.9
TOTAL	100.0	100.0	100.0

AA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid, SFA=saturated fatty acid, BR=branched fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, c=*cis* double bond orientation, t=*trans* double bond orientation, n=number of specimens

**Table 3.5** Fatty acid composition (% composition of total fatty acids) of various body sections of *Kondakovia longimana* included in this study from the Macquarie Island region (data presented as mean  $\pm$  standard deviation)

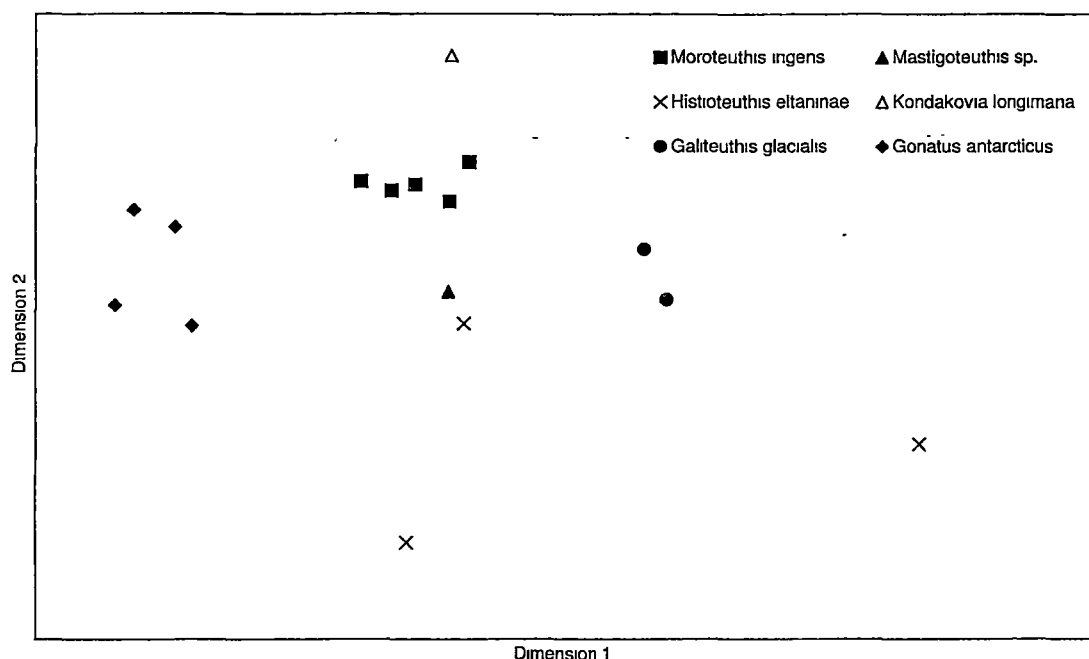
Fatty Acid	Body section			
	Whole (n=1)	Mantle. (n=2)	Tentacle (n=2)	Digestive Gland (n=2)
14:0	3.3	2.9 $\pm$ 1.3	2.8 $\pm$ 0.6	5.6 $\pm$ 2.1
15:0	0.5	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.3 $\pm$ 0.0
16:0	27.5	29.8 $\pm$ 3.0	29.3 $\pm$ 1.2	18.3 $\pm$ 0.2
17:0	0.7	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.2
18:0	2.4	3.6 $\pm$ 0.0	3.6 $\pm$ 0.2	2.7 $\pm$ 0.1
20:0	0.2	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
14:1 $\omega$ 5c	0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
16:1 $\omega$ 9c	0.6	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	1.0 $\pm$ 0.6
16:1 $\omega$ 7c	4.3	2.0 $\pm$ 0.3	1.9 $\pm$ 0.2	7.0 $\pm$ 0.3
16:1 $\omega$ 7t	0.4	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.5 $\pm$ 0.5
16:1 $\omega$ 5c	0.4	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.4 $\pm$ 0.0
17:1	0.6	0.4 $\pm$ 0.1	0.3 $\pm$ 0.0	0.5 $\pm$ 0.0
18:1 $\omega$ 9c	20.5	6.9 $\pm$ 0.2	6.8 $\pm$ 1.6	25.0 $\pm$ 2.9
18:1 $\omega$ 7c	3.4	5.0 $\pm$ 1.7	4.9 $\pm$ 1.4	7.1 $\pm$ 0.9
18:1 $\omega$ 5c	1.1	0.6 $\pm$ 0.2	0.6 $\pm$ 0.3	0.7 $\pm$ 0.3
18:1	0.4	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.3 $\pm$ 0.0
19:1	0.5	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.3 $\pm$ 0.0
20:1 $\omega$ 11c	0.6	2.2 $\pm$ 3.1	0.0 $\pm$ 0.0	0.7 $\pm$ 0.4
20:1 $\omega$ 9c	4.5	6.1 $\pm$ 4.8	8.8 $\pm$ 1.3	7.1 $\pm$ 1.9
20:1 $\omega$ 7c	0.5	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.5 $\pm$ 0.0
22:1 $\omega$ 11c	0.8	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	1.9 $\pm$ 1.1
22:1 $\omega$ 9c	0.8	1.7 $\pm$ 0.3	1.7 $\pm$ 0.3	1.2 $\pm$ 0.3
22:1 $\omega$ 7c	0.5	0.4 $\pm$ 0.3	0.3 $\pm$ 0.2	1.3 $\pm$ 0.6
24:1 $\omega$ 11c	0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.4 $\pm$ 0.2
24:1 $\omega$ 9c	0.4	0.2 $\pm$ 0.2	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2
18:3 $\omega$ 6	0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0
18:4 $\omega$ 3	2.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.8 $\pm$ 0.0
18:2 $\omega$ 6	1.3	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	1.7 $\pm$ 0.7
AA	0.5	2.2 $\pm$ 0.2	2.2 $\pm$ 0.0	0.6 $\pm$ 0.1
EPA	9.5	13.3 $\pm$ 0.3	13.7 $\pm$ 1.4	5.8 $\pm$ 1.8
20:3 $\omega$ 6	0.2	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
20:4 $\omega$ 3	0.6	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.6 $\pm$ 0.1
20:2 $\omega$ 6	0.5	1.3 $\pm$ 0.2	1.1 $\pm$ 0.2	0.4 $\pm$ 0.1
C <sub>21</sub> PUFA	0.5	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0
22:5 $\omega$ 6	0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.3 $\pm$ 0.0
DHA	8.2	15.7 $\pm$ 3.0	16.1 $\pm$ 0.1	4.0 $\pm$ 0.2
22:4 $\omega$ 6	0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.0 $\pm$ 0.0
DPA	0.3	0.6 $\pm$ 0.0	0.6 $\pm$ 0.1	0.5 $\pm$ 0.0
SFA	34.5	37.2 $\pm$ 4.1	36.7 $\pm$ 1.5	27.5 $\pm$ 2.2
BR	1.2	0.4 $\pm$ 0.2	0.2 $\pm$ 0.3	0.9 $\pm$ 0.1
MUFA	40.6	27.7 $\pm$ 0.1	27.6 $\pm$ 2.4	56.4 $\pm$ 4.9
PUFA	23.8	34.6 $\pm$ 3.7	35.5 $\pm$ 1.2	15.2 $\pm$ 2.8
TOTAL	100.0	100.0	100.0	100.0

AA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid, SFA=saturated fatty acid, BR=branched fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, c=*cis* double bond orientation, t=*trans* double bond orientation, n=number of specimens

PUFA dominated the fatty acid composition of *G. glacialis* (mean  $48.0 \pm 2.9\%$ ) and was the second most prevalent group in the remaining squid species (all species 21.7-48.0%, whole specimens only) with the exception of *K. longimana* and *G. glacialis* (both SFA). PUFA composition was lowest in *G. antarcticus* (mean  $21.7 \pm 3.9\%$ ). Docosahexaenoic acid (DHA, 22:6 $\omega$ 3, range across all species 8.2-25.6%) and eicosapentaenoic acid (EPA, 20:5 $\omega$ 3, range 5.7-19.3%) were major PUFA of all species. Other major PUFA included arachidonic acid (AA, 20:4 $\omega$ 6) in *M. ingens* (mean  $1.0 \pm 0.2\%$ ), *Mastigoteuthis* sp. (mean 1.1%) and *G. glacialis* (mean  $1.9 \pm 0.4\%$ ), 18:2 $\omega$ 6 in *M. ingens* (mean  $1.0 \pm 0.2\%$ ), *K. longimana* (mean 1.3%) and *G. antarcticus* (mean  $1.5 \pm 0.3\%$ ), 20:4 $\omega$ 3 (mean  $1.0 \pm 0.3\%$ ), C<sub>21</sub> PUFA (mean  $1.1 \pm 1.5\%$ ) and docosapentaenoic acid (DPA, 22:5 $\omega$ 3, mean  $1.1 \pm 0.4\%$ ) in *G. antarcticus* and 18:4 $\omega$ 3 in *K. longimana* (mean 2.1%). A positive correlation was also observed, across all species examined, between PL and the relative proportions of EPA and DHA.

The importance of SFA (range across all species 9.5-34.5%) was slightly less than that of PUFA for all species, with the exception of *K. longimana* (mean 34.5%, whole specimen only) in which SFA was greater than PUFA. SFA content was lowest in *G. antarcticus* (mean  $9.5 \pm 2.2\%$ ). The major SFA of all species examined were 16:0 (range across all species 4.7-27.5%, whole specimens only) and 18:0 (range 1.7-4.3%). 14:0 was also a major component of all species (range 0.7-3.3%) with the exception of *Mastigoteuthis* sp. and *H. eltaninae*. BrFA accounted for <1% of the total fatty acid composition of all species with the exception of *K. longimana* (mean 1.2%).

MDS confirmed the range of fatty acid compositions across the range of species (Figure 3.4). With the exception of *H. eltaninae*, MDS clustered those species with more than one specimen in close proximity to one another.

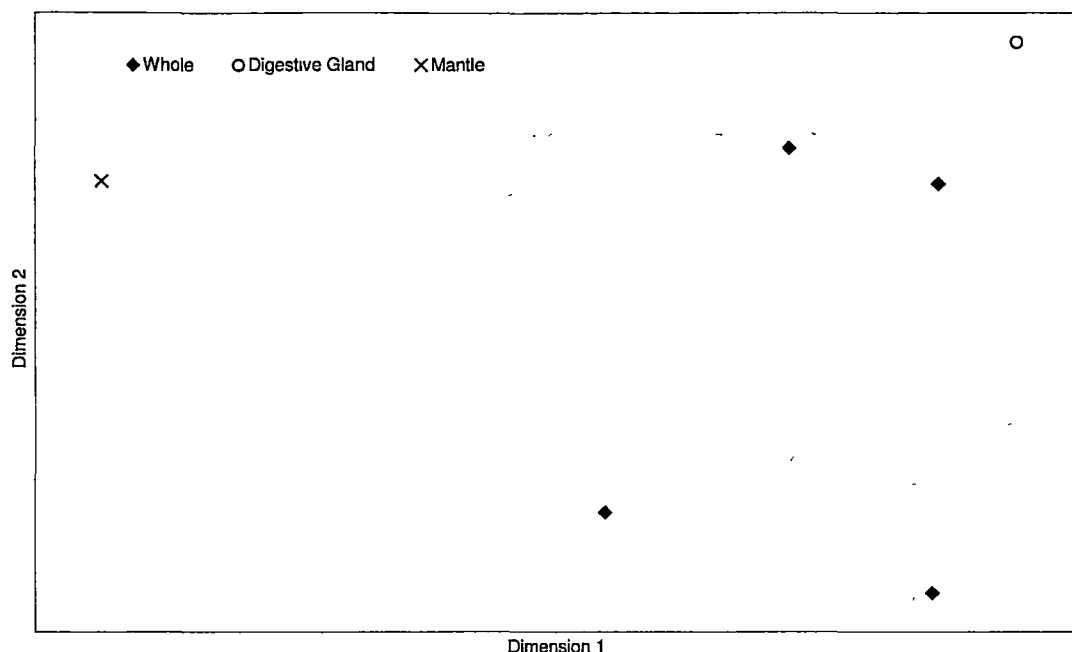


**Figure 3.4** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of whole specimens of various squid from the Macquarie Island region

#### *Gonatus antarcticus*

Comparison of the fatty acid composition of the DG and mantle of *G. antarcticus* revealed considerable differences (Table 3.4). The fatty acid composition of the DG was comparable to that of whole specimens, with MUFA (71.9%) and PUFA (19.6%) dominating, while SFA (8.5%) were present at lower levels. Major constituent fatty acids of the DG included 18:1 $\omega$ 9c (40.2%), 20:1 $\omega$ 9c (12.4%), DHA (7.3%), EPA (5.2%), 16:1 $\omega$ 7c (5.0%), 16:1 $\omega$ 7c (5.0%), 16:0 (4.2%) and 22:1 $\omega$ 11c (3.2%), the levels of which are similar to those observed in whole specimens.

This contrasted sharply with the fatty acid composition of the mantle, which differed appreciably from that of both the DG and whole specimens. This is confirmed by MDS analysis whereby the mantle separated from both whole specimens and the DG (Figure 3.5). The fatty acid composition of the mantle is dominated by comparable levels of MUFA (47.5%) and PUFA (39.9%), with lower levels of SFA (12.7%) and an absence of branched fatty acids. The relative proportion of PUFA in the mantle is roughly double that of the



**Figure 3.5** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of various body sections of *Gonatus antarcticus* from the Macquarie Island region

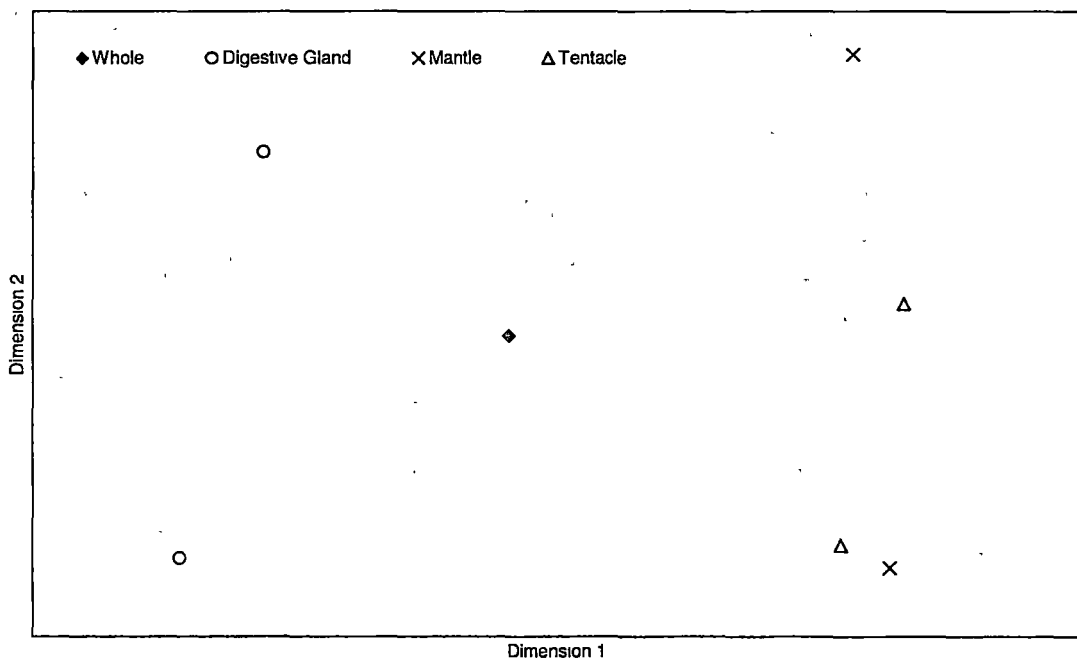
DG and whole specimens. The major components of the mantle included DHA (21.4%), 18:1 $\omega$ 9c (20.7%), 20:1 $\omega$ 9c (13.2%), EPA (11.4%) 16:0 (7.9%), 22:1 $\omega$ 11c (3.1%), 22:1 $\omega$ 9c (3.0%) and 18:0 (2.6%). In particular, levels of DHA and EPA far exceed those of the DG or whole specimens, whilst the MUFA is considerably lower.

#### *Kondakovia longimana*

Following the trend observed in *G. antarcticus*, the fatty acid composition of *K. longimana* varied substantially between body sections (Table 3.5). The composition of the tentacles and mantle were almost indistinguishable, and were dominated by approximately equal levels of SFA (tentacle  $36.7 \pm 1.5\%$ ; mantle  $37.2 \pm 4.1\%$ ) and PUFA (tentacle  $35.5 \pm 1.2\%$ ; mantle  $34.6 \pm 3.7\%$ ). MUFA levels were of slightly less importance (tentacle  $27.6 \pm 2.4\%$ ; mantle  $27.7 \pm 0.1\%$ ) and BrFA contributed  $<0.5\%$ . The composition of individual fatty acids was similarly comparable. Major components ( $>1\%$  of total fatty acid composition) included 16:0 (tentacle  $29.3 \pm 1.2\%$ ; mantle  $29.8 \pm 3.0\%$ ), DHA

( $16.1 \pm 0.1\%$ ;  $15.7 \pm 3.0\%$ ), EPA ( $13.7 \pm 1.4\%$ ;  $13.3 \pm 0.3\%$ ),  $18:1\omega 9c$  ( $6.8 \pm 1.6\%$ ;  $6.9 \pm 0.2\%$ ),  $20:1\omega 9c$  ( $8.8 \pm 1.3\%$ ;  $6.1 \pm 4.8\%$ ),  $18:1\omega 7c$  ( $4.9 \pm 1.4\%$ ;  $5.0 \pm 1.7\%$ ),  $18:0$  ( $3.6 \pm 0.2\%$ ;  $3.6 \pm 0.0\%$ ),  $14:0$  ( $2.8 \pm 0.6\%$ ;  $2.9 \pm 1.3\%$ ), AA ( $2.2 \pm 0.0\%$ ;  $2.2 \pm 0.2\%$ ),  $16:1\omega 7c$  ( $1.9 \pm 0.2\%$ ;  $2.0 \pm 0.3\%$ ),  $22:1\omega 9c$  ( $1.7 \pm 0.3\%$ ;  $1.7 \pm 0.3\%$ ),  $20:2\omega 6$  ( $1.1 \pm 0.2\%$ ;  $1.3 \pm 0.2\%$ ) and, in mantle only,  $20:1\omega 11c$  ( $2.2 \pm 3.1\%$ ).

The fatty acid composition of the DG was considerably different to that of either the mantle or tentacles (Table 3.5 and Figure 3.6). Composition was dominated by MUFA ( $56.4 \pm 4.9\%$ ), followed by SFA ( $27.5 \pm 2.2\%$ ), PUFA ( $15.2 \pm 2.8\%$ ), and BrFA contributed  $<1.0\%$ . Major fatty acids included  $18:1\omega 9c$  ( $25.0 \pm 2.9\%$ ),  $16:0$  ( $18.3 \pm 0.2\%$ ),  $20:1\omega 9c$  ( $7.1 \pm 1.9\%$ ),  $18:1\omega 7c$  ( $7.1 \pm 0.9\%$ ),  $16:1\omega 7c$  ( $7.0 \pm 0.3\%$ ), EPA ( $5.8 \pm 1.8\%$ ),  $14:0$  ( $5.6 \pm 2.1\%$ ), DHA ( $4.0 \pm 0.2\%$ ),  $18:0$  ( $2.7 \pm 0.1\%$ ),  $22:1\omega 11c$  ( $1.9 \pm 1.1\%$ ),  $18:2\omega 6$  ( $1.7 \pm 0.7\%$ ),  $22:1\omega 7c$  ( $1.3 \pm 0.6\%$ ),  $22:1\omega 9c$  ( $1.2 \pm 0.3\%$ ) and  $16:1\omega 9c$  ( $1.0 \pm 0.6\%$ ). The levels of DHA and EPA in particular were substantially lower in the DG compared to the mantle and tentacles, whilst  $18:1\omega 9c$  was higher.



**Figure 3.6** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of various body sections of *Kondakovia longimana* from the Macquarie Island region

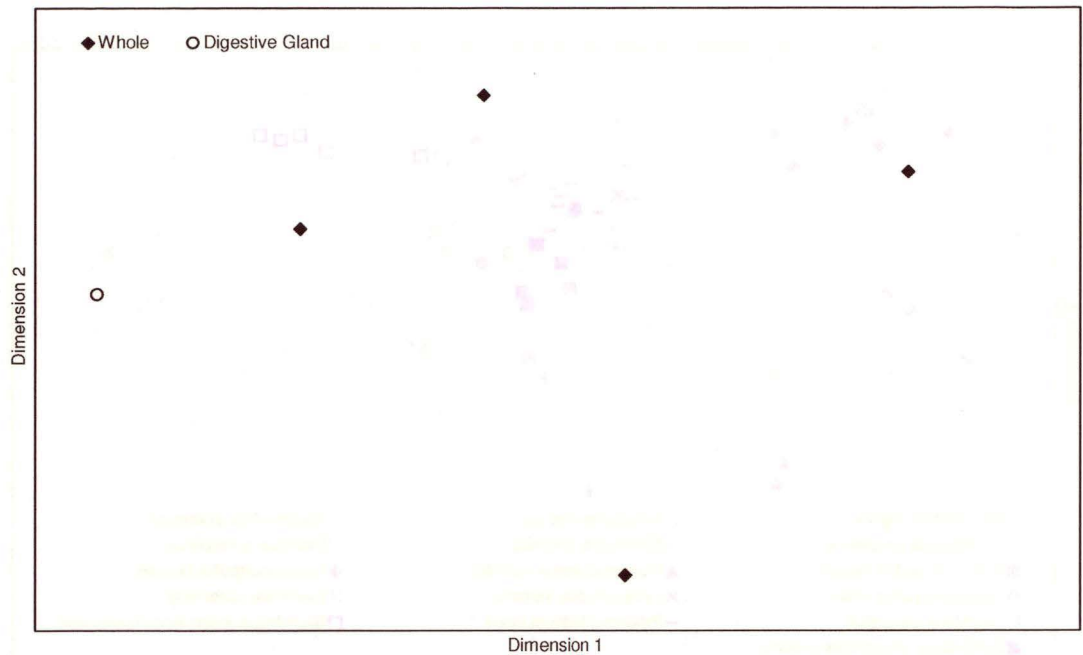
**Glyceryl ether diol composition derived from DAGE**

The glyceryl ether diol (GED) composition of whole specimens of *G. antarcticus* and the DG were similar (Table 3.6 and Figure 3.7). Both contained high levels of saturated GED (whole specimens, mean  $54.4\% \pm 3.5$ ; DG 48.0%) and monounsaturated GED (mean  $43.2\% \pm 3.3$ ; 49.2%), whilst the proportions of branched and polyunsaturated GED were low. The overall GED profile was similar and major GED included 16:0 (whole specimen, mean  $38.0\% \pm 3.4$ ; DG 33.0%), 18:1 $\omega$ 9 (mean  $23.2\% \pm 4.3$ ; 29.4%) and 18:0 (mean  $11.4\% \pm 1.1$ ; 8.9%).

**Table 3.6** Glyceryl ether diol composition (% composition of total glyceryl ether diols) of *Gonatus antarcticus* from the Macquarie Island region with respect to various tissues (data presented as mean  $\pm$  standard deviation)

Glyceryl ether diol	Whole Specimen (n=4)	Digestive Gland (n=1)
14:0	$3.1 \pm 1.3$	4.2
15:0	$0.8 \pm 0.0$	1.1
16:0	$38.0 \pm 3.4$	33.0
17:0	$1.0 \pm 0.1$	0.7
18:0	$11.4 \pm 1.1$	8.9
19:0	$0.1 \pm 0.1$	0.2
i17:0	$0.8 \pm 0.1$	1.1
a17:0	$0.7 \pm 0.2$	1.1
i18:0	$0.5 \pm 0.1$	0.6
16:1	$2.2 \pm 1.0$	2.1
18:1 $\omega$ 9	$23.2 \pm 4.3$	29.4
18:1 $\omega$ 7	$7.8 \pm 0.6$	9.4
18:1	$5.4 \pm 2.0$	2.5
19:1	$0.1 \pm 0.1$	0.2
20:1	$4.6 \pm 1.1$	5.6
18:2	$0.4 \pm 0.2$	0.0
Saturates	$54.4 \pm 3.5$	48.0
Branched	$2.0 \pm 0.3$	2.7
Monounsaturates	$43.2 \pm 3.3$	49.2
Polyunsaturates	$0.4 \pm 0.2$	0.0
TOTAL	100.0	100.0

i=iso branching, a=anteiso branching



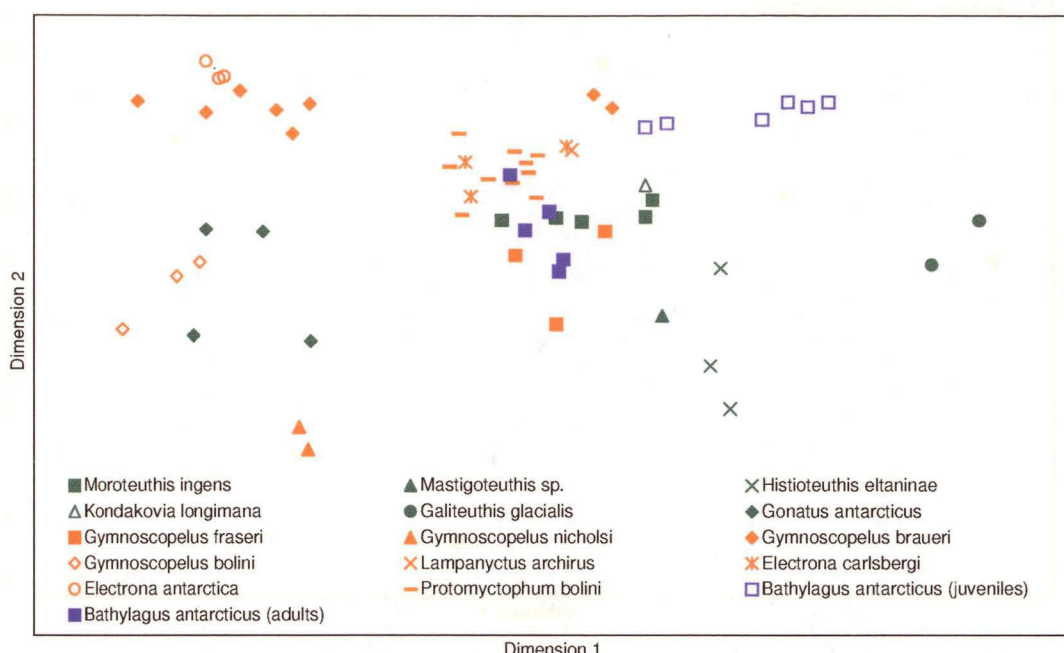
**Figure 3.7** Scatterplot of multidimensional scaling (MDS) based upon the glyceryl ether diol composition of various body sections of *Gonatus antarcticus* from the Macquarie Island region

### Comparison with potential prey species

#### Multidimensional scaling

MDS of fatty acid profiles for all squid and a number of potential prey species produced two main groupings (Figure 3.8). The first group consisted of the squid *G. antarcticus* clustering close to the myctophid *G. bolini*, and to a lesser extent with the myctophids *G. nicholsi*, *G. braueri* and *E. antarctica*. These species were characterised by levels of MUFA >60%. Those that cluster closest to *G. antarcticus* (*G. bolini* and *G. nicholsi*) shared high levels of certain fatty acids, including 18:1 $\omega$ 9c (~20-30%) and 20:1 $\omega$ 9c (~15-20%).

The second group identified by MDS contained two squid, *M. ingens* and *K. longimana*, clustered with the myctophids *P. bolini*, *G. fraseri*, *E. carlsbergi*, *L. archirus* and adults of the bathylagid *Bathylagus antarcticus*. These species were characterised by MUFA levels accounting for ~40-50% of total



**Figure 3.8** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of various squid species and potential fish prey included in this study from the Macquarie Island region

composition and approximately equal proportions of PUFA (~20-30%) and SFA (~25-35%). Major fatty acids common to all these species included 18:1 $\omega$ 9c (~20%), 16:0 (~15-25%), DHA (~10-20%) and EPA (~5-10%). The squid species *H. eltaninae*, *Mastigoteuthis sp.* and *G. glacialis* were also separated from all other squid as well as other potential prey using MDS.

#### Linear discriminant analysis

LDA was used to compare those squid (i.e. *G. antarcticus*, *M. ingens*, *K. longimana*) and fish species that grouped together using MDS (Table 3.7). The dissimilarity in fatty acid composition was of such a magnitude that LDA, in all cases, classified a low proportion (0%) of squid and potential prey into the same group. The exact composition and number of fatty acids predictors leading to this classification varied between each of the squid species, however, relatively few were major components (>1% of total composition).

**Table 3.7** Comparison of the percentage of correctly classified squid and potential fish prey by linear discriminant analysis (normal and jackknifed classification, automatic, forward stepping, F to enter=4, F to remove=3.9) relative to total fatty acid composition

Squid	Potential Prey <sup>1</sup>	LDA <sup>2</sup>	JK <sup>3</sup>	Predictors <sup>4</sup>
<i>Moroteuthis ingens</i>	<i>Gymnoscopelus fraseri</i>	0	0	18:1ω7c*, 22:1ω11c*, 24:1ω9c
	<i>Electrona carlsbergi</i>	0	0	14:0*, 20:0, 20:1ω7c, 18:3ω6
	<i>Protomyctophum bolini</i>	0	0	15:0, 20:0, 14:1ω5c, 16:1ω7t, 22:1ω9c*, 22:1ω7c, 20:4ω3, 20:2ω6, DPA
	<i>Lampanyctus archirus</i>	0	0	16:1ω7t, 24:1ω9c, 20:2ω6
	<i>Bathylagus antarcticus</i>	0	0	16:1ω9c, 20:1ω7c, 18:2ω6*, 22:5ω6
<i>Kondakovia longimana</i>	<i>Gymnoscopelus fraseri</i>	0	75	18:1, DHA*
	<i>Electrona carlsbergi</i>	0	0	14:1ω5c, 18:4ω3*
	<i>Lampanyctus archirus</i>	ID	ID	ID
	<i>Protomyctophum bolini</i>	0	0	16:0, 17:1, 18:1, 18:3ω6, AA, EPA*, 22:4ω6
	<i>Bathylagus antarcticus</i>	0	0	22:0, 22:1ω9c, 18:4ω3*
<i>Gonatus antarcticus</i>	<i>Gymnoscopelus bolini</i>	0	29	22:0, 16:1ω7t, 20:1ω11c
	<i>Gymnoscopelus braueri</i>	0	0	17:0, 20:0
	<i>Gymnoscopelus nicholsi</i>	0	0	22:0, 18:3ω6
	<i>Electrona antarctica</i>	0	43	22:1ω7c, 18:3ω6, 22:4ω6

<sup>1</sup>closest grouping prey determined from multidimensional scaling of entire fatty acid profile;

<sup>2</sup>linear discriminant analysis, normal classification (0%=no similarity between squid and prey);

<sup>3</sup>jack-knifed classification; <sup>4</sup>representing fatty acids most responsible for differences between squid and potential prey; \*major fatty acids of respective squid species;

ID=insufficient data

*M. ingens* and *L. archirus* were separated by three fatty acids, although none represented major components. Similarly, *M. ingens* could be differentiated

from *G. fraseri* by just three fatty acids. However two of these, 18:1ω7c (4.0%) and 22:1ω11c (2.4%), represented major components. Both *E. carlsbergi* and *Bathylagus antarcticus* were separated from *M. ingens* by four fatty acids. In both cases, only one of these fatty acids represented a major component, namely 14:0 (3.1%) in *E. carlsbergi* and 18:2ω6 (1%) in *Bathylagus antarcticus*. The fatty acid composition of *P. bolini* differed from that of *M. ingens* by a large number (n=9) of predominately minor components, with the exception of 22:1ω9c (1.6%).

*K. longimana* differed from potential fish prey by small numbers of fatty acids, many of these were major components. For instance, although *K. longimana* and *G. fraseri* only differed by two fatty acids, one of these was DHA (8.2%) an important component. Similarly, *P. bolini* was separated from *K. longimana* by a number of mainly minor fatty acids with the exception of EPA (9.5%). *Bathylagus antarcticus* and *E. carlsbergi* were differentiated from *K. longimana* by just three and two fatty acids respectively, however included amongst these components was 18:4 $\omega$ 3 (2.1%).

In contrast to the other two squid species, only minor fatty acids were responsible for the separation of *G. antarcticus* and the potential prey species *G. bolini*, *G. braueri*, *G. nicholsi* and *E. antarctica* (Table 3.7). *G. antarcticus* and *G. bolini*, the closest clustering prey, were separated based on two minor SFA (17:0 and 20:0).

### 3.4 DISCUSSION

Although lipid compositional data is available for a number of squid, the majority of species studied so far have been from the Northern Hemisphere. The general biology of Southern Ocean squid species is poorly understood with only broad geographic distribution and population size structures given sufficient attention to date (e.g. Nemoto et al. 1985; Lu and Williams 1994a; Jackson 1995). The paucity of information has left a large gap in the understanding of metabolic energy flow in the oceans of the Southern hemisphere, especially given the important role played by squid in these regions. The few studies which have determined the lipid content of Southern Ocean squid species (e.g. Vlieg 1984; Piatkowski and Hagen 1994) have focussed primarily on proximate and lipid class composition, with little or no attention given to either the specific fatty acid composition or its distribution throughout various tissues. The recent work of Phillips et al. (2001; 2002) has for the first time provided detailed lipid and fatty acid data on several squid species in the Macquarie Island region for incorporation into comprehensive trophic studies.

The difficulty faced in obtaining squid samples for analysis is the principal reason for the lack of lipid data currently available. More recently, heightened interest in the health benefits of seafood, including fish and squid; has lead to the targeting of previously marginal fish species by commercial ventures in the Southern Ocean (Rodhouse 1989). Lipid information is now available for Southern Ocean species, both targets and by-catch, previously overlooked (e.g. Sinclair et al. 1992; Nichols et al. 1994b; Dunstan et al. 1999). With specific reference to the Macquarie Island region, deepsea trawling operations for the Patagonian toothfish (*Dissostichus eleginoides*) have resulted in the capture of squid as by-catch and is the source of the samples obtained for this study. However, the vast majority of commercial species are from temperate, inshore environments, limiting the available study material to species from these regions. The availability of squid samples for this study, although limited, is therefore symptomatic of the recent shift towards exploration of distant, sub-Antarctic and Antarctic fisheries.

### **Distribution of lipid between squid body sections**

Although only the body sections of *G. antarcticus* and *K. longimana* were examined in this study, several important aspects of lipid composition are apparent. Namely, *G. antarcticus* and *K. longimana* are characterised by prominent, lipid rich-digestive glands (>38%) and comparatively lipid-poor (<4%) mantles and, for *K. longimana* only, tentacles. The lipid class and fatty acid composition of the mantle-tentacle and the DG of these two species also differs markedly, most obvious in the high level of PUFA (especially EPA and DHA), mainly incorporated into PL, in the mantle compared to the DG.

The absence of appreciable quantities of lipid in the mantle and tentacles is an apparently ubiquitous characteristic of squid, one that has historically lead researchers to the general conclusion that lipids perform little or no energetic function in squid (e.g. Hochachka et al. 1975; Lee 1994). Nonetheless, the extraordinary lipid content of *G. antarcticus* and *K. longimana* observed in this and other studies is adding to the growing reevaluation of the importance of lipids in the biology of squid. Recent investigation has revealed that

prominent, lipid-rich DGs may be a more common feature of squid than previously thought (e.g. Phillips et al. 2001; Phillips et al. 2002).

### **Comparison with previous studies**

#### *DAGE-rich squid*

The lipid-rich, DAGE dominated DG of *G. antarcticus* is unusual amongst the majority of squid species analysed to date. However, a number of noteworthy exceptions to the general notion that squid are low in both oil content and DAGE have been previously observed, including for several other species of gonatid squid. Phillips et al (2002) reported substantial quantities of DAGE in the digestive glands of two species collected in the Macquarie Island region, *Moroteuthis robsoni* (5.0%) and *Todarodes spp.* (16.4%). *G. antarcticus* was also examined in the study of Phillips et al. (2002), however a description of the lipid class composition of either the mantle or the DG were not included. All three species contained substantial quantities of lipid in the DG. The DGs of *M. robsoni* and *Todarodes spp.* contained 22.3% and 29.7% lipid (wet weight) respectively, whilst the lipid content of *G. antarcticus* (54.3%) was comparable to that observed in this study (47.7%). Fatty acid composition of the DG in these three species was dominated by MUFA (range across all species 47.0-66.1%), PUFA (22.8-32.4%) and SFA (10.7-23.1%). In contrast, mantle tissue was dominated by PUFA (range across all species 53.0-58.1%), with smaller proportions of MUFA (13.6-21.8%) and SFA (19.9-29.7%). Total lipid content of *G. antarcticus* mantle was 1.6%, lower than that observed in the current study (3.2%). The overall fatty acid compositions of the DG of *G. antarcticus* in the current study and that of Phillips et al. (2002) are comparable, although some conspicuous differences are apparent with regards to the mantle. PUFA levels in the mantle of *G. antarcticus* were higher in the study of Phillips et al (2002) compared to this study, due mainly to elevated levels of DHA (36.3% versus 21.4% in this study). This may partly be a consequence of the lower lipid content of the mantle in the study of Phillips et al. (2002), due to the fact that the proportion of PUFA-rich PL

generally increases with decreasing lipid content. This cannot be confirmed as the lipid class composition of the mantle was not reported.

The lipid composition of *G. antarcticus* is also comparable to that previously reported for numerous Northern hemisphere squid, including the gonatids *Beryteuthis magister* (Hayashi et al. 1985), *Gonatopsis makko* (Hayashi and Kawasaki 1990) and *Gonatopsis borealis* (Hayashi 1989), as well as the Onychoteuthidae *Morotheuthis robusta* (Hayashi et al. 1990). All species contain digestive glands (livers) relatively high in lipid (range across all species 16.7-54.5%) and DAGE (10.3-84.7%) and mantle tissue containing low lipid (0.5-1.5%). The proximate composition of two further lipid rich gonatid species, *Gonatus fabricii* (Clarke et al. 1979; Lawson et al. 1998) and *Gonatus steenstrupi* (Clarke et al. 1985) have been investigated in relation to energy density and buoyancy.

*Potential dietary source of DAGE in the digestive gland of Gonatus antarcticus*

The fatty acid composition of *G. antarcticus* differed substantially from that of the other squid species, but was similar to that of the myctophids *E. antarctica*, *G. bolini*, *G. braueri* and *G. nicholsi*. From the limited information available, the diet of *G. antarcticus* is dominated by *Euphausia superba*, at least in the Antarctic (Nemoto et al. 1988). Although the diet of *G. antarcticus* at Macquarie Island is unknown, the predatory capabilities of this species are likely to match those of other large squid such as *M. ingens*. It is therefore plausible that *G. antarcticus* forage upon such prey as myctophids.

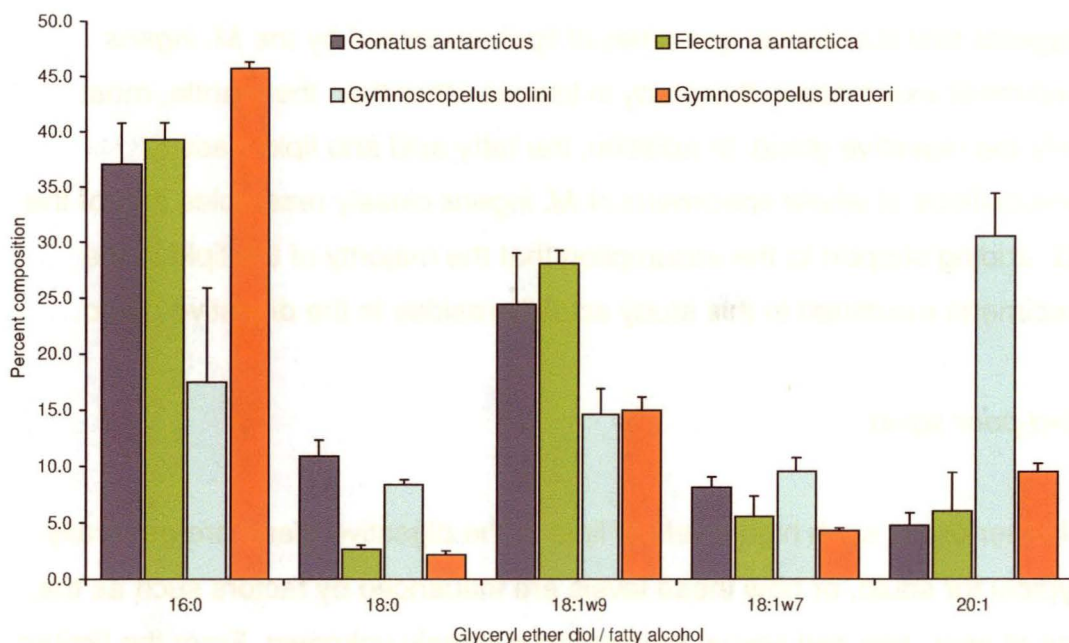
The possibility of DAGE originating in the diet of *G. antarcticus* appears unlikely. Significant quantities of DAGE do not occur in any of the potential prey species from Macquarie Island, including myctophids, and the DAGE-containing Antarctic pteropod species reported by Phleger et al. (2001) are similarly not known to contribute directly to the diet of *G. antarcticus*. The formation of large DAGE deposits is therefore surprising given the limited

dietary source of DAGE in the Southern Ocean as well as the limited capacity for lipid synthesis by squid.

A possible alternative source of DAGE is the conversion of dietary derived wax ester to glyceryl ethers via an unspecified metabolic pathway in the digestive gland of squid, analogous to that found in the liver of dogfish (*Squalus acanthias*) by Malins and Sargent (1971). For instance a study of *Berryteuthis magister*, a species containing up to 60% DAGE of the total lipids in the digestive gland, found 41% wax ester in the stomach contents compared to only 3% DAGE (Hayashi and Yamamoto 1987). Unlike DAGE, large quantities of WE are found in many myctophid and copepods (e.g. Kattner et al. 1994) species in the Southern Ocean. Three of the four myctophid species that have fatty acid compositions comparable to that of *G. antarcticus* (*E. antarctica*, *G. bolini* and *G. braueri*) also contain >60% of their total lipid as WE, similar to the levels of DAGE observed in *G. antarcticus*. Additionally, the composition of major fatty alcohols (derived from WE) in two of the WE-containing myctophid species (*E. antarctica* and *G. braueri*) and the glyceryl ether diols (derived from DAGE) in *G. antarcticus* are also comparable (Figure 3.9). A third myctophid, *G. bolini*, contained lower 16:0 GED and higher 20:1 GED than *G. antarcticus* and the other myctophids. This difference suggests that *G. bolini* is may be of less dietary importance to *G. antarcticus* than the other two species. Conversion of the WE obtained from these potential dietary components is conceivably the source of DAGE present in the digestive gland of *G. antarcticus*.

#### *Other lipid-rich squid*

Lipid-rich DG have also been reported for numerous other squid species. For example, the offal (incorporating digestive organs) of *Nototodarus sloanii*, *Sepioteuthis bilineata* and *M. ingens* collected from New Zealand contains lipid in excess of 30% WW (Vlieg 1984) and lipid-rich digestive glands are also common in Northern hemisphere species, such as *Watasenia scintillans* (Kawasaki et al. 1994). The lipid-rich DG of *K. longimana* (38.0%) examined



**Figure 3.9** Comparison of the composition of major fatty alcohols (derived from wax esters) in selected myctophids and glyceryl ether diols (derived from diacylglycerol ethers) in the squid *Gonatus antarcticus*

in this study is the first time this characteristic has been reported for this species.

Contrasting with the gonatids, TAG dominates the lipid composition of many of these squid and they contain little or no DAGE. For instance, the level of lipid stored in the digestive gland of *M. ingens* ranges between ~40% (Heard Island) and ~30% (Macquarie Island) mainly in the form of TAG (75-90%) (Phillips et al. 2001). Although TAG also dominated the lipid composition of the *M. ingens* specimens examined in the current study (41.5%), they did not contain prominent digestive glands, perhaps pointing to the influence of seasonal, developmental or dietary factors. The different approaches taken by each of these studies (i.e. whole specimens in this study versus separation into DG and mantle) does not allow for direct comparison of either lipid class or fatty acid composition. However, the lipid content of whole specimens of *M. ingens* in the current study (8.4%) was substantially higher

than that of the mantle tissue (1.5%) observed by Phillips et al. (2001). This suggests that substantial quantities of lipid are stored by the *M. ingens* specimens examined in this study in tissues other than the mantle, most likely the digestive gland. In addition, the fatty acid and lipid class compositions of whole specimens of *M. ingens* closely resembles that of the DG, adding support to the assumption that the majority of the lipid in the specimens examined in this study actually resides in the digestive gland.

### *Lipid-poor squid*

Whether or not such high levels of lipid in the digestive gland are generally atypical for squid, or how these levels are influenced by factors such as the time of year, age and sexual maturation is largely unknown. From the limited available data, temperate species residing in the southern hemisphere appear to be less likely to store such immense quantities of lipid. The DG of adults and juveniles of the loliginid *Sepioteuthis lessoniana*, collected from the waters of far-north Queensland, contained approximately 10% lipid (Semmens and Moltschaniwskyj 2000). Sex and sexual maturity also appear to have no effect on the amount of lipid stored in the DG of *S. lessoniana* (Semmens 1998). The DG of another loliginid, *Sepioteuthis australis* collected from Tasmanian waters, contained <10% lipid, mainly in the form of PL (Phillips et al. 2002).

Lipid-poor squid are also found in Antarctic and sub-Antarctic waters. Whole specimens of the cranchiid *Galiteuthis glacialis* collected from the Weddell Sea contain less than 1% lipid (Piatkowski and Hagen 1994), lower than the levels observed in this study (2.3%). Importantly though, the specimens of *G. glacialis* examined by Piatkowski and Hagen (1994) represented early developmental stages (ML <20mm), whereas those included in this study represent adult stages (ML 110-290mm). The tendency for lipid content to accumulate with increasing size (age) was also observed in this study for *G. antarcticus*. The low lipid contents of *Mastigoteuthis* sp. (3.4%) and *H. eltaninae* (5.6%) may accordingly reflect the juvenile size of these specimens (TW <100g).

### *Role of lipid-rich digestive gland*

The presence of large lipid stores in some species is likely to be partly a consequence of the unique foraging ecology of squid. Their rapid growth rate has particularly led to the development of an equally voracious diet in order to sustain the high metabolism typical of many squid (Ballantyne et al. 1981; Lee 1994; Rodhouse and Nigmatullin 1996). Assuming that only protein is utilised extensively for energy, the excess supply of lipid needs to be either directly excreted or stored in an organ or tissue. The prominent digestive gland of species such as *G. antarcticus* (almost 50% lipid and representing 17% of total body weight) and *K. longimana* suggests that some squid favour the deposition and storage of lipid rather than its excretion.

Two reasons most likely account for the preference of storage over excretion. Firstly, squid may take advantage of the relatively low density of lipid in a passive manner, for example to provide buoyancy (Clarke et al. 1979; 1985). The storage of large quantities of low density lipid is one of the strategies employed by squid to achieve neutral buoyancy identified by Clarke et al. (1985). The remaining methods include gas filled chambers, the substitution (within the body) of sulphate ions from seawater with chloride ions, the presence of large amounts of muscular tissue to enable continuous swimming and an elevated concentration of ammonium in mantle tissue. Secondly, lipid may be involved more dynamically in squid function, acting as an energy store for reproduction or long-term survival.

### *Buoyancy*

A link between the presence of large amounts of lipid, whether as a part of complex organs or as extracellular reservoirs, and the potential role of these lipids in buoyancy, are well documented for a number of marine fish (e.g. Phleger 1991; Phleger 1998) but are scarce for squid. Although large lipid reserves are not uncommon in squid, the majority of research on squid buoyancy has focussed on the abundant, ammonium rich species (e.g.

Clarke et al. 1979). In particular, the presence of vast quantities of DAGE in *G. antarcticus* presents substantive evidence for its application in buoyancy regulation. This is based upon the fact that DAGE provides more static lift than the equivalent amount of TAG (Phleger 1991; Phleger 1998) and is metabolised at a lower rate. In particular, Malins and Barone (1970) concluded that DAGE is present in dogfish (*Squalus acanthias*) primarily as a buoyancy regulator. High levels of DAGE are also found in the livers of several other species, including deepsea sharks (Hayashi and Takagi 1981; Deprez et al. 1990; Bakes and Nichols 1995; Kang et al. 1998), the ratfish (*Hydrolagus novaezealandiae*) (Hayashi and Takagi 1980) and in Antarctic pteropods (Phleger et al. 2001). For example the DAGE-rich livers of certain deepsea shark species contain in excess of 50% total lipid, of which up to 90% is present as DAGE, despite the limited capacity for DAGE metabolism of sharks (Wetherbee and Nichols 2000).

The degree to which the vast lipid store of *K. longimana* DG is applied to the regulation of buoyancy is not clear. Most lipid is present as TAG, more readily associated with the storage of energy. The high levels of ammonium found in the tissue of *K. longimana* (Lu and Williams 1994b) also suggests that this squid is unlikely to exclusively rely upon lipid to maintain buoyancy.

### *Energy Storage*

The possibility of the lipid-rich DGs of species such as *G. antarcticus* and *K. longimana* acting as an energy store appears superficially attractive when the large amount of lipid present is considered. The actual degree to which cephalopods utilise lipid for metabolic purposes, however, varies greatly. The cuttlefish *Sepia officinalis* uses the lipid store of the digestive gland to provide energy during short periods of starvation (Castro et al. 1992). Nonetheless, the bulk of the metabolic energy is still provided by the muscle proteins, and the overall influence of stored lipid is considered to be minimal. This contrasts with the loliginid squids *Sepioteuthis lessoniana* and *Photololigo* sp., where lipid is immediately excreted from the digestive gland and appears to play no role in providing energy (Semmens 1998). Low levels of lipid do

appear to be an essential nutritional requirement of squid, especially at the paralarval stage (Navarro and Villanueva 2000), though the levels observed in the digestive gland of *G. antarcticus* and *K. longimana* far exceed these.

Several other factors count against squid using the lipid of the digestive gland as a source of energy. The acknowledged metabolic preference of squid for amino acids (Hochachka et al. 1975; Lee 1994) and their limited capacity for fatty acid metabolism (Ballantyne et al. 1981) would appear to limit the use of lipid by squid. Additionally, with specific reference to *G. antarcticus*, it is more common for lipid energy stores to consist mainly of TAG, a form that is both more energetically favourable and less difficult to metabolise than DAGE. Finally, assuming that *G. antarcticus* converts WE into DAGE in preference to storing it unmodified, the transformation of dietary derived lipid in this manner is an inefficient and seemingly unnecessary metabolic step.

#### *Potential role of mantle and tentacle lipids*

The variation in lipid composition observed between the body sections of these squid is likely to be significant in the context of physiological function. Given that the vast majority of dietary lipid is deposited into the digestive gland (Semmens 1998), and that the fatty acid composition of this lipid is not reflected in the mantle, certain fatty acids appear to be selectively deposited from the digestive gland into the mantle, specifically into the polar lipid portion (Phillips et al. 2001).

The absence of prominent digestive glands in *Mastigoteuthis* sp. and *G. glacialis* examined in this study also suggests that the bulk of lipid in these species must originate from the mantle and tentacles. Although prominent DGs were also not observed in *M. ingens* or *H. eltaninae*, the higher than usual lipid content of these species suggests the presence of a developing DG. The relatively low lipid content of *Mastigoteuthis* sp. and *G. glacialis*, and the low content of tentacle and mantle in *G. antarcticus* and *K. longimana*, discounts the use of these tissues as a major lipid repository, adding support to the apparent metabolic preference of squid for amino acids (Lee 1994). In

addition, polar lipids, a class more directly involved in the structure of cell membranes than energy storage, dominate the lipid class composition of the mantle and are a common feature of numerous squid species. The function of lipid in the mantle is unclear, although it is involved in both a structural role and most likely a range of biological activities distinct from those performed in the digestive gland. The polar lipids and PUFA present in the mantle tissue are integral to the development and functioning of various neurological and physiological systems. Navarro and Villanueva (2000) found that polar lipids and PUFA, particularly DHA, were important to the early development of reared European squid *Loligo vulgaris* due to incorporation of these components into cell membranes. The rapid growth rates of most squid would also necessitate a constant supply of polar lipid throughout the life cycle to allow for the formation of new tissue. High levels of polar lipid, DHA and EPA are also found in the central nervous system of the cephalopod *Sepia officinalis*, particularly the optic lobes and the supraoesophageal mass (Dumont et al. 1992; 1994).

### **Dietary implications of lipid composition**

With the exception of *M. ingens* (Phillips et al. 2001), the diet of squid in the Macquarie Island region is poorly described. Based upon observations from other regions, the diet of squid is generally restricted to medium-sized midwater fish (e.g. myctophids) and crustaceans such as euphausiids, amphipods and copepods. *M. ingens*, for example, predominately forages upon myctophid fish (mainly of the genus *Gymnoscopelus* and *Electrona*) in the Macquarie Island region (Phillips et al. 2001).

The fatty acid compositions of available myctophid species and the bathylagid *Bathylagus antarcticus* (see Chapter 2) are similar to that of three squid, *G. antarcticus*, *K. longimana* and *M. ingens*. The fatty acid compositions of the remaining squid (*H. eltaninae*, *Mastigoteuthis* sp. and *G. glacialis*) share less similarity with the potential prey species examined. This may indicate a reliance on prey species in these squid for which lipid data does not currently exist, such as invertebrates.

Both onychoteuthid species, *K. longimana* and *M. ingens*, associate with a similar range of potential fish prey (using MDS), though the degree of dietary overlap between these species is unknown. *M. ingens* consumes many of these species at Macquarie Island, including the myctophids *G. fraseri*, *P. bolini* and *E. antarctica*, and *Bathylagus antarcticus* (Phillips et al. 2001). The diet of *K. longimana* is dominated by euphausiids in Antarctic waters (Nemoto et al. 1985; Lu and Williams 1994a; Lu and Williams 1994b), but dietary descriptions from sub-Antarctic waters (including Macquarie Island) do not exist. Although LDA identified a number of differences in the fatty acid compositions of *K. longimana* and *M. ingens* and potential fish prey, the majority of these differences are due to either minor fatty acid components (<1% of the total fatty acid composition) or components that are easily synthesised.

### 3.5 CONCLUSIONS

Several unique characteristics of squid are apparent from the lipid data collected. The mantle and tentacles of most of the squid examined in this study are characterised by a low lipid content. However, the high lipid content of the DGs of *G. antarcticus* and *K. longimana* is in contrast to the generally held view that squid are low in lipid. The presence of large stores of DAGE in *G. antarcticus* also parallels the findings of studies of Northern Hemisphere gonatid squid. The role of this DAGE is unclear, especially given the preferential metabolism of protein over lipids in squid. Studies on other species containing DAGE-rich organs, particularly the livers of certain deep-sea sharks, suggest that DAGE plays a role in maintaining neutral buoyancy rather than energy storage. The source of DAGE in the digestive gland of *G. antarcticus* also raises important dietary questions. Assuming that DAGE cannot be directly synthesized by *G. antarcticus*, a possible source is the conversion of wax esters in such prey as the myctophids *E. antarctica* and *G. braueri*.

The two onychoteuthidae, *M. ingens* and *K. longimana*, share similar fatty acid compositions with each other as well as a number myctophid species and the bathylagid fish *Bathylagus antarcticus*. The contrasting fatty acid composition of *H. eltaninae*, *Mastigoteuthis sp.* and *G. glacialis* suggests that myctophids may not play as important a role in the diet of these squid. Instead, other fish or invertebrates for which fatty acid data does not currently exist may be more commonly consumed.

# CHAPTER 4

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## VARIATION IN LIPID COMPOSITION BETWEEN BODY SECTIONS OF PATAGONIAN TOOTHFISH

### 4.1 INTRODUCTION

The large size of Patagonian toothfish (*Dissostichus eleginoides*), reaching in excess of 200cm and 100kg (Des Clers et al. 1996), excludes the practical use of whole fish samples when determining lipid composition (total lipid, lipid class and fatty acid). This necessitates the use of a flesh sub-sample from a single body section that, as closely as possible, reflects the lipid composition of the entire specimen. The distribution of lipid in many fish, including Patagonian toothfish, is poorly described at present. This presents a problem when it comes to the selection of the tissue or body section that best approximates the lipid composition of the entire fish.

Determining whole animal profiles is essential when attempting to use lipids as dietary tracers as large variation between different tissues is not uncommon. Studies on Antarctic toothfish (*Dissostichus mawsoni*), a closely related congener species, suggest that significant variation in lipid composition is likely. For example Eastman (1988) and Clarke et al. (1984) describe the presence of several large lipid deposits, concentrated close to the centre of buoyancy, in the subcutaneous, white axial muscle and red pectoral muscle tissues of Antarctic toothfish. Whether or not this partitioning exists in Patagonian toothfish is unknown.

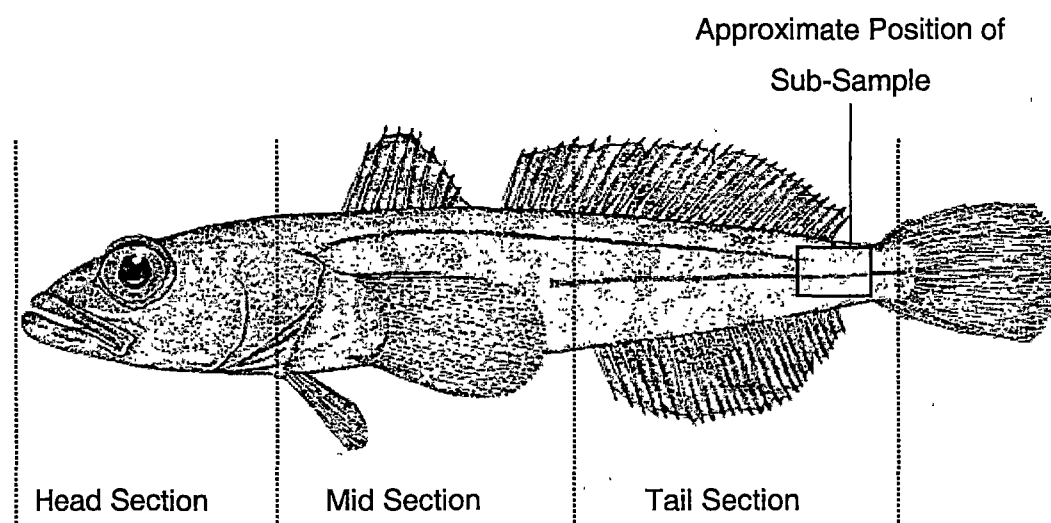
The purpose of this chapter is, firstly, to determine the degree to which lipid composition differs with body section and, secondly, to ascertain which body section most closely approximates the lipid composition of Patagonian

toothfish as a whole. Comparison with the results of similar studies of Antarctic toothfish and other Southern Ocean fish species will also be made.

## 4.2 MATERIALS AND METHODS

### Sample collection and preparation

A toothfish specimen collected during the 1998-1999 fishing season from the Macquarie Island region was dissected into six body sections, specifically head, mid and tail sections, skin (including fins) stomach (including contents) and ovaries (Figure 4.1). The lipid composition of each region was then determined.



**Figure 4.1.** Approximate positions of the main body regions analysed for lipid composition from *Dissostichus eleginoides*, including the sub-sample used as a proxy (modified from Gon and Heemstra 1990)

### Lipid extraction and class analysis

Total lipids were extracted in duplicate using the one-phase method of Bligh and Dyer (1959). After phase separation, total lipid was recovered from the lower chloroform phase and concentrated in vacuo (rotary evaporator, 40°C). Samples were stored at -20°C prior to analysis and analysed within 3 months of capture. Lipid class profiles were determined by TLC-FID using an latroscan MkV TH10 analyser (Volkman and Nichols 1991). Wax ester (WE),

triacylglycerol (TAG), free fatty acid (FFA), sterol (ST), diacylglycerol (DAG) and polar lipid (PL) components were resolved in a polar solvent system consisting of hexane-diethyl ether-acetic acid (60:17:0.2 v/v). The presence or absence of WE and diacylglycerol ethers (DAGE) was confirmed by the use of a non-polar solvent system (hexane-diethyl ether; 96:4 v/v). Peak areas were quantified using commercially available chromatography software (DAPA Scientific Software, Kalamunda).

### **Fatty acid analysis**

Fatty acid methyl esters (FAME) were prepared by addition of methanol (MeOH), concentrated hydrochloric acid (HCl) and chloroform ( $\text{CHCl}_3$ ) (3mL 10:1:1, v/v/v, 80°C/2hr) to an aliquot of the total lipid. After the addition of 1mL of water, resulting FAME was extracted (3x) with 4:1 v/v hexane/ $\text{CHCl}_3$ . Chloroform containing C19 FAME internal standard was then added. FAME fractions were treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA 50 $\mu\text{L}$ , 60°C, 2hr) to convert any sterols present to their corresponding trimethylsilyl (TMS) ethers.

Gas chromatographic (GC) analysis was performed on a Hewlett Packard 5890A GC equipped with a flame ionisation detector, fitted with a cross-linked methyl silicone (HP1) fused silica capillary column (50m length  $\times$  0.32  $\mu\text{m}$  internal diameter), employing hydrogen as the carrier gas. Samples were injected (HP7673A auto-injector) at 50°C in the splitless mode, with a 2-min. venting time (Nichols et al. 1993). The injector and detector were maintained at 290°C and 310°C respectively. After 1 min. the oven was raised to 150°C at 30°C min<sup>-1</sup>, then to 250°C at 2°C min<sup>-1</sup> and finally to 300°C at 5°C min<sup>-1</sup> (Nichols et al. 1993). Peak areas were quantified using Millennium 32 Chromatography Manager (Waters Corporation, Milford, Massachusetts, USA). Identification of individual fatty acids and sterols was based upon a comparison of relative retention times with those obtained from previous studies in addition to laboratory standards.

### **Statistical analysis**

Non-metric multi-dimensional scaling (MDS; Kruskal loss function) was employed to compare fatty acid and lipid class composition in two dimensions between the various body sections. All statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, Illinois, USA).

## **4.3 RESULTS**

### **Toothfish size and apportioning of body sections**

The toothfish specimen analysed had a mass of 292g (total weight) and measured 300mm (tip of caudal fin to tip snout) in total length. The mid section accounted for the majority of toothfish body mass (34.9%) (Table 4.1). The tail (22.9%), head (19.9%) and skin (16.4%) also represented large proportions of the total mass whilst the stomach (2.8%) and ovaries (3.0%) contributed only a small proportion of total mass.

### **Total lipid content**

Total lipid content varied substantially with toothfish body section on both a wet weight (WW) and dry weight (DW) basis (Table 4.1). Lipid content was greatest in the mid section (14.2% WW, 54.1% DW) and skin (13.4% WW, 35.0% DW). The head (4.1% WW, 13.0% DW), ovaries (4.3% WW) and tail (4.9% WW, 18.1% DW) had considerably lower levels of total lipid. The lipid content of the stomach (7.7% WW) was comparable to the calculated value of whole fish (9.4% WW). With respect to the main body sections (head, mid and tail) an increase in total lipid content of approximately 10% was observed from both the tail and head toward the mid section.

### **Lipid class composition**

The body sections analysed can be separated into two main groupings based upon their lipid class composition (Table 4.1). The first group was dominated by high levels of triacylglycerol (TAG, range 89.9-94.3%) and includes the skin, head, mid and tail sections. Levels of TAG were highest in the skin

**Table 4.1.** Distribution of total lipid content (% composition of tissue, wet weight) and lipid class composition (% composition of total lipid content) throughout various body sections of a *Dissostichus eleginoides* specimen (data presented as mean of duplicates)

Body Region	Percent of Body Mass	Lipid Content (WW)	Lipid Content (DW)	Lipid Class				
				TAG	FFA	ST	PL	Total
Head Section	19.9	4.1	13.0	90.1	5.0	1.6	3.3	100.0
Mid Section	34.9	14.2	54.1	89.9	3.1	2.1	4.9	100.0
Tail Section	22.9	4.9	18.1	90.2	5.3	1.3	3.2	100.0
Skin	16.4	13.4	35.0	94.3	3.6	1.0	1.1	100.0
Stomach	2.8	7.7	nd	63.9	9.7	9.6	16.9	100.0
Ovaries	3.0	4.3	nd	66.3	7.2	8.1	18.4	100.0
Whole Fish <sup>1</sup>	100.0	9.4	-	90.0	3.8	2.0	4.1	100.0

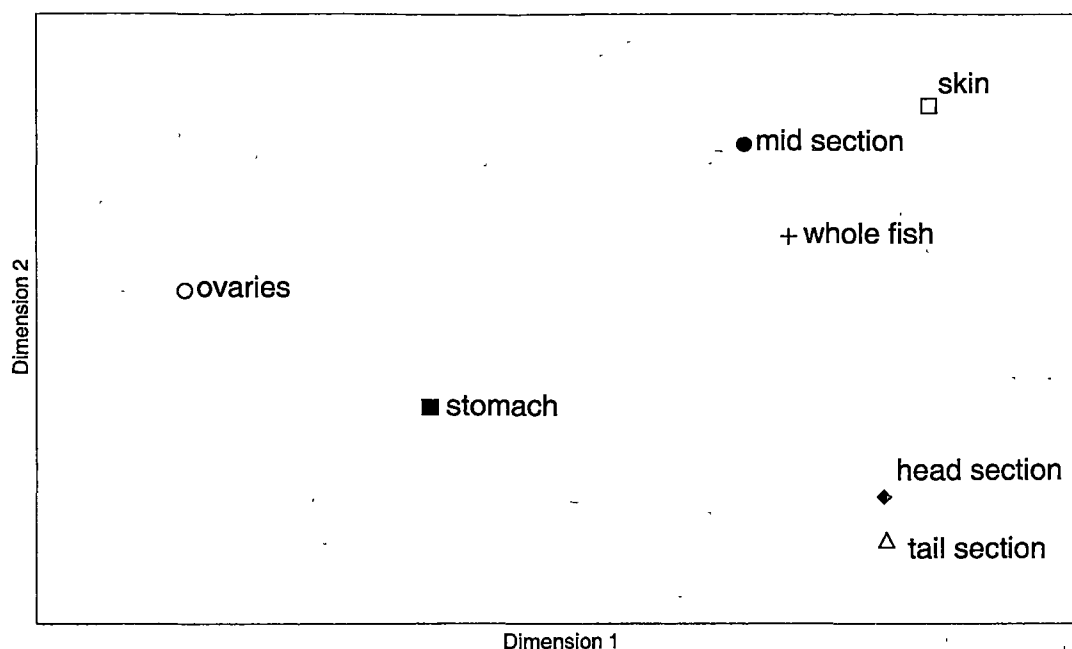
TAG=triacylglycerol, FFA=free fatty acid, ST=sterol (mainly cholesterol, may contain traces of diacylglycerol), PL=polar lipid, WW=wet weight, DW=dry weight, nd=not determined, <sup>1</sup>values for whole fish were determined using the total lipid content and weight distribution (percent of body mass) of each body section

(94.3%) and lowest in the stomach (63.9%). The stomach and ovaries are categorized into a second group and were characterised by high polar lipid levels (PL, 16.9% and 18.4% respectively). PL levels ranged from a high of 18.4% in the ovaries to a low of 1.1% in the skin. The stomach also contained the greatest levels of both free fatty acid (FFA, 9.7%) and sterols (ST, 9.6%). FFA was lowest in the mid section (3.1%) and ST in the skin (1.0%).

Multidimensional scaling separated the various body sections into three main groupings based on lipid class composition (Figure 4.2). The mid section and skin were closely correlated with the calculated value of whole fish, whilst the head and tail sections were distanced further from whole fish. Once again the grouping of the stomach and ovaries is distinct from the other body sections.

### Fatty acid composition

A total of 43 individual fatty acids were detected in the toothfish samples. Monounsaturated fatty acid (MUFA) dominated the fatty acid composition of all the body sections (>57%) with the exception of the ovaries where



**Figure 4.2.** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of various body sections of *Dissostichus eleginoides* (values for whole fish were determined using the total lipid content and weight distribution of each body section)

saturated fatty acid (SFA) dominated (38.3%) (Table 4.2). Levels of MUFA were greatest in the head section (66.2%) and lowest in the ovaries (31.3%). SFA also contributed a major proportion of the fatty acid composition (>21% in all body sections) and levels were greatest in the ovaries and least in the stomach (21.5%). Polyunsaturated fatty acid (PUFA) content was extremely variable, ranging from a high of 27.6% in the ovaries to a low of 8.6% in the head section. The total composition of branched fatty acid (BrFA) was comparable for all body sections (range 2.0-2.8%). As was observed in the grouping of body sections relative to lipid class, the head, mid and tail sections and skin are very similar in fatty acid composition (high MUFA, range 57.0-66.2%) and is distinct from that of the stomach and ovaries (elevated PUFA, 19.5% and 27% respectively).

The composition of individual fatty acids also varied substantially. Nearly all of the variation in fatty acid composition between body sections can be attributed to the substantially elevated levels of the PUFA docosahexaenoic

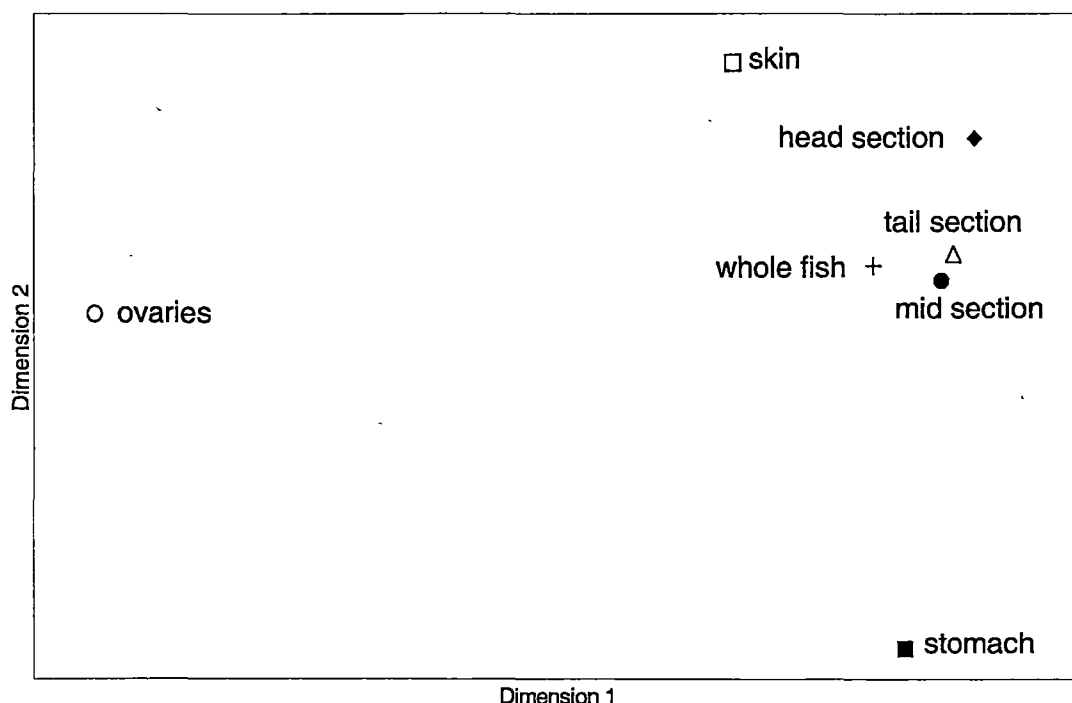
**Table 4.2.** Fatty acid composition (% composition of total fatty acids, wet weight) of various body sections of a *Dissostichus eleginoides* specimen (data presented as the mean of duplicates)

Fatty acid	Body section						
	Head	Mid	Tail	Skin	Stomach	Ovaries	Whole Fish <sup>1</sup>
14:0	5.1	4.6	4.7	6.4	3.0	4.9	5.0
15:0	0.5	0.3	0.4	0.6	0.3	0.7	0.4
16:0	15.5	15.3	15.4	20.0	13.2	27.9	16.5
17:0	0.2	0.1	0.2	0.2	0.2	0.3	0.2
18:0	1.8	1.8	1.8	2.3	4.7	4.3	2.0
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:0	0.1	0.1	0.1	0.1	0.1	0.0	0.1
14:1 $\omega$ 5c	0.2	0.2	0.2	0.2	0.1	0.1	0.2
16:1 $\omega$ 9c	0.4	0.4	0.3	0.4	0.5	0.4	0.4
16:1 $\omega$ 7c	10.5	9.5	9.8	13.3	6.6	10.6	10.4
16:1 $\omega$ 5c	0.3	0.3	0.3	0.4	0.2	0.4	0.3
17:1	0.2	0.2	0.2	0.2	0.1	0.2	0.2
18:1 $\omega$ 9c	40.9	38.1	39.0	31.6	34.4	9.1	36.5
18:1 $\omega$ 7c	3.3	5.7	5.8	0.2	6.0	0.2	4.2
18:1 $\omega$ 5c	0.7	0.7	0.7	0.9	0.6	1.0	0.7
18:1	0.1	0.1	0.1	0.2	0.1	0.3	0.1
19:1	0.3	0.3	0.3	0.5	0.1	0.1	0.4
20:1 $\omega$ 11c	0.5	0.5	0.4	3.0	0.4	0.4	1.1
20:1 $\omega$ 9c	5.0	4.8	4.9	3.7	4.2	5.4	4.6
20:1 $\omega$ 7c	0.3	0.3	0.3	0.4	0.3	0.4	0.3
22:1 $\omega$ 11c	1.6	1.9	1.5	1.6	1.1	1.0	1.7
22:1 $\omega$ 9c	0.8	0.8	0.8	0.9	0.6	0.6	0.8
22:1 $\omega$ 7c	0.1	0.1	0.1	0.1	0.2	0.1	0.1
24:1 $\omega$ 11c	0.4	0.4	0.3	0.2	0.3	0.1	0.3
24:1 $\omega$ 9c	0.5	0.5	0.4	0.2	1.0	0.7	0.4
C16 PUFA	0.1	0.1	0.1	0.2	0.1	0.1	0.1
18:3 $\omega$ 6	0.1	0.1	0.1	0.1	0.1	0.1	0.1
18:4 $\omega$ 3	0.9	0.9	0.9	1.1	0.6	1.2	0.9
18:2 $\omega$ 6	2.6	2.7	2.4	2.8	2.2	3.3	2.7
AA	0.2	0.3	0.3	0.3	1.6	2.0	0.4
EPA	1.6	2.2	2.0	2.0	5.1	9.1	2.2
20:3 $\omega$ 6	0.1	0.1	0.1	0.0	0.2	0.4	0.1
20:4 $\omega$ 3	0.2	0.3	0.2	0.3	0.3	0.7	0.3
20:2 $\omega$ 6	0.1	0.2	0.1	0.2	0.2	0.3	0.2
C21 PUFA	0.1	0.1	0.1	0.1	0.2	0.2	0.1
DHA	2.2	3.9	3.4	2.3	8.8	10.0	3.5
DPA	0.1	0.2	0.1	0.1	0.3	0.3	0.1
SFA	23.1	22.3	22.6	29.6	21.5	38.3	24.3
BrFA	2.1	2.0	2.1	2.8	2.0	2.8	2.2
MUFA	66.2	64.7	65.4	58.0	57.0	31.3	62.7
PUFA	8.6	11.0	9.9	9.5	19.5	27.6	10.7
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0

AA=arachidonic acid (20:4 $\omega$ 6), EPA=eicosapentaenoic acid (20:5 $\omega$ 3), DHA=docosahexaenoic acid (22:6 $\omega$ 3), DPA=docosapentaenoic acid (22:5 $\omega$ 3), SFA=saturated fatty acid, BrFA=branched fatty acid, MUFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid, c=*cis* double bond orientation; t=*trans* double bond orientation, <sup>1</sup>values for whole fish were determined using the total lipid content and weight distribution (percent of body mass) of each body region

acid (DHA) and eicosapentaenoic acid (EPA) in the stomach and ovaries. DHA comprised 8.8% and 10.0% of the total fatty acids in the stomach and ovaries respectively, whilst EPA represented 5.1% and 9.1%. The MUFA 18:1 $\omega$ 9c was the single most important fatty acid component in all the body sections (>31%) with the exception of the ovaries where 16:0 (27.9%) dominated. Levels of 18:1 $\omega$ 9c were greatest in the head section (40.9%) and least in the ovaries (9.1%). Other major components included 16:0 (range 13.2-27.9%), 16:1 $\omega$ 7c (range 6.6-13.3%), DHA (range 2.2-10.0%), EPA (range 1.6-9.1%), 14:0 (range 3.0-6.4%), 18:1 $\omega$ 7c (range 0.2-6.0%), 20:1 $\omega$ 9c (range 3.7-5.4%), 18:0 (range 1.8-4.7%) and 18:2 $\omega$ 6c (range 2.2-3.3%).

MDS analysis produced a spread of data similar to that for lipid class composition (Figure 4.3). However in this case the fatty acid compositions of the mid and tail sections are those most closely correlated with that of whole fish. The ovaries and stomach are once again substantially separated from each other and whole fish when compared using MDS.



**Figure 4.3.** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of various body sections of *Dissostichus eleginoides* (values for whole fish were determined using the total lipid content and weight distribution of each body region)

## 4.4 DISCUSSION

### Total lipid content

With the exception of the stomach (7.7% WW), none of the body sections contained lipid at levels comparable to that calculated for whole fish (9.4% WW, Table 4.1). However, the stomach is particularly unsuited as a proxy as its lipid content is inextricably linked to the prey items it contains. Ideally, the lipid content of the proxy should remain relatively constant, independent of exogenous factors such as changes in diet. The lipid content of the stomach is therefore affected by both dietary intake and the state of digestion of the contents of the stomach. The stomach is, consequently, unlikely to accurately reflect the long-term lipid content of the entire fish. For similar reasons, the lipid content of the ovary is significantly affected by both reproductive status and the stage of maturity, also making it unsuited for use as a proxy.

On this basis skin (13.4% WW), the body section next closest to the predicted content of whole fish following the stomach, would appear to make the best proxy indicator of overall lipid content. The mid section (14.2%) is a poor indicator as its lipid content is considerably greater (4.8% above) than that of whole fish. Similarly the content of the head (4.1%) is less than half that of whole fish and would appear to be a poor predictor. The tail section however may be of some use as a proxy. Although the lipid content of the tail section (4.9%) is also substantially lower than whole fish (4.5% below), it is similar in distance to skin (4.0% above) from the predicted value of the entire fish. The tail section may therefore be equally as applicable as a predictor of lipid content than skin and, additionally, its collection much simpler.

The high lipid content observed in the skin is comparable, though lower in absolute value, to the extensive subcutaneous deposits of lipid (74.1% WW) reported for the Antarctic toothfish, *Dissostichus mawsoni* (Clarke et al. 1984). Similarly, the increase in lipid content of approximately 10% from both the head and tail towards the mid section (centre of buoyancy) also reflects the results of Clarke et al. (1984), suggesting a possible role in buoyancy regulation for the lipid deposits of Patagonian toothfish.

A comparison of total lipid content with other fish species from the Southern Ocean is difficult because of the limited availability of published data. Of these studies, even fewer have explored the variation in lipid content between different body sections. The limited results available suggest that Patagonian toothfish are a relatively lipid rich species. For instance the lipid content in white muscle (the tissue most commonly analysed and comparable to the head, mid and tail sections of this study) in the majority of studied species is almost without exception well below 2.0% WW. Clarke et al. (1984), in the same study that included the Antarctic toothfish, examined two other Antarctic nototheniid species, *Trematomus bernacchii* and *Pagothenia borchgrevinki*. The white muscle of these species contained 1.1% WW and 1.9% WW total lipid respectively, considerably lower than in either *D. eleginoides* or *D. mawsoni*. The lipid content of the skin is also lower in *P. borchgrevinki* (3.5% WW) compared to both toothfish species. The lipid contents of muscle tissue from a further two nototheniid species, *Notothenia coriiceps* (1.2% WW) and *Lepidonotothen nudifrons*. (0.9% WW) (Kamler et al. 2001), are also well below the levels determined for Patagonian toothfish in this study.

Data is more readily available for whole fish comparison. Based upon the result of several studies, Patagonian toothfish fall within the middle range of lipid contents determined for Southern Ocean fish, comparable to several species including adult *Pleuragramma antarcticum* (10.2%WW, Friedrich and Hagen 1994), *Protomyctophum bolini* (8.5%WW, Reinhardt and Van Vleet 1986), *Gymnoscopelus piabilis* (9.2%WW, Raclot et al. 1998), and *Lampichthys procerus* (8.8%WW, Raclot et al. 1998).

### **Lipid class composition**

Minimal variation in lipid class composition is apparent between body sections, with the exception of the stomach and ovaries. The head, mid, tail sections and skin are almost identical to the calculated lipid class composition of whole fish and would appear to be well suited as proxies. The stomach and ovaries, besides differing considerably in lipid class composition to whole fish, are rejected as potential proxies for the same reasons

mentioned previously with respect to lipid content. MDS identified the mid section as the best proxy for determining lipid classes (Figure 4.2). The skin, followed by the head and tail, are the body sections next most similar to whole fish. The closer clustering of skin is surprising given that both TAG and PL differ substantially in skin relative to whole fish whilst in the head and tail their levels are comparable. The apparent reason for the closer grouping of skin by MDS is that levels of FFA in whole fish and skin are comparable, whilst in the head and tail sections they are higher relative to whole fish.

The distribution of lipid composition with body region is similar to that reported for Antarctic toothfish by Clarke et al. (1984). The skin and subcutaneous tissue of Antarctic toothfish contains >95% TAG, similar to the levels observed in this study (94.3%). High TAG levels are also present in the head, mid and tail sections of both toothfish species, though the relative amounts differ considerably (89.9-90.2% in Patagonian toothfish versus 46.6-68.9% in Antarctic toothfish). The lower levels of TAG in Antarctic toothfish are offset by elevated PL levels (20.8-48.2%) relative to Patagonian toothfish (3.2-4.9%). Additionally the decrease in TAG and increase in PL from the snout to the tail reported for Antarctic toothfish was not observed for Patagonian toothfish in this study.

As is the case with lipid content, the majority of studies of lipid class composition in Southern Ocean fish are restricted to the examination of either the whole fish or white muscle. Clarke et al. (1984), Reinhardt and Van Vleet (1986) and Phleger et al. (1997; 1999a) observed substantial variation in the lipid class composition of various body sections (including the skeleton, neurocranium, viscera, white muscle/flesh, red muscle, liver, spleen, heart, skin, testis and serum) in a number of species. Comparisons with the present study are unfortunately restricted to the white muscle/flesh and skin. Of those species examined by the various authors only *Gymnoscopelus nicholsi* (Phleger et al. 1999a) contained levels of TAG (93.7%) and PL (2.8%) in the white muscle comparable to those present in Patagonian toothfish. In several other species, including *Bathylagus antarcticus* (60.7%), *Electrona carlsbergi* (75.3% and 74.8%), *Gymnoscopelus opisthopterus* (75.2%) and

*Protomyctophum bolini* (82.3%) TAG also dominated lipid class composition. However, these species also contained significant amounts of WE and/or PL, well above the levels determined for Patagonian toothfish. The absence of WE in Patagonian toothfish therefore sets it apart from most other Southern Ocean deepsea fish. Elevated levels of WE in flesh are believed to be associated with buoyancy regulation in many of these species, particularly in myctophids (e.g. Phleger et al. 1997; Phleger et al. 1999a). With respect to skin, the one species for which analysis of this body section was performed, *Pagothenia borchgrevinki* (Clarke et al. 1984), shows very few similarities with the skin of Patagonian toothfish. Levels of PL (42.8%) and free sterol (25.8%) are elevated relative to Patagonian toothfish, while TAG is substantially reduced (20.0%).

### **Fatty acid composition**

As is the case with lipid class composition, the head, mid and tail sections are all acceptable proxies of Patagonian toothfish fatty acid composition. This contrasts with skin, which varies substantially in SFA content from the calculated composition of whole fish (29.6% in skin, 24.3% in whole fish) and would make a poor proximal indicator. Once again the stomach and ovaries vary dramatically in their fatty acid compositions from the calculated profile of whole fish, particularly the elevated levels of DHA and EPA, and are also unacceptable proxies. MDS analysis supports these observations. All three of the main body sections (head, mid and tail) are closely associated with the calculated fatty acid composition of whole fish (Figure 4.3).

The study of Clarke et al. (1984) did not include fatty acid data for Antarctic toothfish, therefore comparison with the results of this study is impossible. With regards to other Southern Ocean fish, both the total and constituent fatty acid levels of MUFA and PUFA in white muscle show great variation across a range of species. However, in general, the white muscle of Patagonian toothfish contains relatively lower levels of PUFA and higher levels of MUFA in comparison to other species. For example in a study of several *Trematomus* species by Phleger et al. (Phleger et al. 1999b), white muscle PUFA exceeded 33% in all cases (compared to 8.6-11.0% in

Patagonian toothfish), predominantly DHA, whilst MUFA (less than 50% in all *Trematomus* species) are well below the levels of Patagonian toothfish (64.7-66.2%).

### **Choice of proxy indicator**

Although the results suggest that no one body section can be relied upon to provide an accurate approximation of the overall lipid composition of Patagonian toothfish, a reasonable proxy can still be chosen. The large size of the majority of the toothfish samples collected for this study requires that tissue from only one part of the body can be analysed for lipid composition. Initial analysis suggests that the stomach is the best estimate of total lipid content, whilst tissue from the mid section most closely matches both lipid class and fatty acid composition (Table 4.3). The proxy indicator to be chosen, however, will ideally consist of a single body section in order to simplify the processes of collection and analysis. The body section chosen from this study will therefore represent a compromise between the degree of accuracy that it provides with regard to lipid composition and the ease with which it can be sampled.

A number of factors, directly influencing lipid composition, must additionally be considered when choosing the proxy. As previously discussed, reliance upon the stomach as an indicator of lipid content is problematic due to the influence of the stomach contents. The choice of skin also presents the problem of difficult sample collection and preparation. Any of the main body sections (head, mid and tail) are acceptable proxies of lipid class and fatty acid composition, but are generally unsuited as predictors of lipid content (Table 4.3). The tail section though has the advantage of much simpler collection and is additionally a better proximal indicator of the total lipid content than either the mid or head body sections. On this basis, flesh from the tail section is the body section most suited as an overall indicator of lipid composition of Patagonian toothfish for this study.

**Table 4.3.** Comparison of body sections best and least applicable as proxy indicators of total lipid, lipid class and fatty acid composition in *Dissostichus eleginoides* (based upon closeness to calculated values for whole fish)

Lipid component	Best proxy indicators	Worst proxy indicators
Total lipid content	stomach, skin, tail	mid, ovaries, head
Lipid class <sup>1</sup>	mid, skin, head, tail	stomach, ovaries
Fatty acid <sup>1</sup>	mid, tail, head, skin	stomach, ovaries

<sup>1</sup>determined from multidimensional scaling

4.5 CONCLUSIONS

In common with other Southern Ocean species such as Antarctic toothfish, the lipid composition of Patagonian toothfish is heavily dependent upon the location of the tissue within the body. Lipid content in particular varies substantially with body section. The marked increase in lipid content in the middle body section suggests that lipid deposits may perform an important role in the maintenance of buoyancy. The skin also contains large quantities of lipid, consistent with the subcutaneous deposits that are a notable characteristic of Antarctic toothfish. Lipid class and fatty acid composition are less varied, excepting those body sections that are organs (ovaries and stomach). The main body sections, represented by the tail, mid and head, provide the bulk of the tissue (78% total body mass) and share similar lipid class and fatty acid compositions. Although quantitative (i.e. lipid content) differences between the main body sections are apparent, this qualitative similarity suggests that the selective deposition of fatty acids into different body sections is minimal.

# CHAPTER 5

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## VARIATION IN THE LIPID COMPOSITION OF PATAGONIAN TOOTHFISH FROM THE MACQUARIE ISLAND REGION IN RELATION TO SEVERAL BIOLOGICAL AND SAMPLING PARAMETERS

### 5.1 INTRODUCTION

The Patagonian toothfish (*Dissostichus eleginoides*) is an important component of the sub-Antarctic fish community. Its large size, well characterised distribution and high market value have seen a steady increase in the commercial catch of this species in the last decade, particularly in the waters of the sub-Antarctic islands that support the vast majority of this species (Williams and Lamb 2001). Several biological features leave toothfish prone to over-exploitation including low fecundity, late onset of sexual maturity (Chikov and Mel'nikov 1991), longevity and a moderate growth rate (Horn 2002). A direct result has been a marked decrease in toothfish biomass in many areas of the Southern Ocean (e.g. Haedrich et al. 2001; Tuck et al. 2001b) through the combined action of sanctioned fishing operations (regulated by government bodies) and illegal, unregulated and unreported fishing that may account for up to 50% of the total catch (Willock 2002).

The complex system of seamounts and canyons surrounding Macquarie Island is home to a commercially exploited toothfish population. In 1997 the Fisheries Research Development Corporation (FRDC) commissioned a study into the potential of developing an ecologically sustainable fishery for toothfish around Macquarie Island (He and Furlani 2001). An important component of the FRDC study was establishing the ecological interactions of

toothfish with both their prey and potential predators, essential to attaining an understanding of trophic structures within regional food webs and consequently ensuring the proper management of the fishery. The Macquarie Island region is well suited to a comparative study of toothfish diet. The population is split between two main regions separated by a distance of approximately 60km, one to the west of the island, the other to the north on the Macquarie Ridge (Goldsworthy et al. 2002). On the basis of both genetic (Reilly et al. 2001) and tag-recapture studies (Williams and Lamb 2001), each of these geographically isolated regions are thought to support distinct, non-migratory sub-populations of toothfish. The influence on toothfish diet of this separation is therefore potentially significant.

Toothfish have previously been identified as important dietary components of Hooker's sea lions in the Macquarie Island region (McMahon et al. 1999). The FRDC study sought to expand the assessment of trophic interactions between toothfish and potential predators to include various seal and seabird species and (Goldsworthy et al. 2001d). The results suggest that Macquarie Island seals and seabirds do not prey upon toothfish to any great extent.

The exact dietary composition of toothfish is, however, less certain. Current dietary information available for toothfish from the Macquarie Island region is based upon the examination of stomach contents (Goldsworthy et al. 2001c; Goldsworthy et al. 2002), a technique that has a number of inherent problems. Amongst these is a failure to identify with any certainty long-term dietary composition and bias due to differential digestion of prey items problems (Antonelis et al. 1987; Harwood and Croxall 1988; Chou et al. 1995). More recently, underwater video observation has been used to observe the foraging of toothfish *in situ*, though these observations are restricted to mainly scavenging events rather than true predation (e.g. Collins et al. 1999; Yau et al. 2002). Despite these disadvantages, the complexity of intermediary trophic interactions for sub-Antarctic species has limited dietary determination in the majority of fish species to traditional techniques.

The use of alternate and complementary methods may help overcome the disadvantages of traditional methods of determining diet. The analysis of fatty acid composition is one technique that has received growing recognition as a potentially simple and effective means of determining diet. At the present time, the fatty acid compositions of relatively few marine species from sub-Antarctic regions have been determined (Piatkowski and Hagen 1994). Previous studies into the use of fatty acids as dietary tracers in Southern Ocean ecosystems have so far been restricted primarily to either top-level marine predators (namely seals and whales) (e.g. Raclot et al. 1998; Brown et al. 1999), primary producers (phytoplankton, bacteria) (e.g. Nichols et al. 1993; Graeve et al. 1994) and lower-end consumers (zooplankton) (e.g. Kattner et al. 1994; Kattner and Hagen 1995).

Lipid studies of large, mid to top-level predatory fish in the Southern Ocean have predominantly been of Antarctic species such as the Antarctic toothfish, *Dissostichus mawsoni* (Ohno et al. 1976; Clarke et al. 1984; Eastman 1985; Nachman 1985; Eastman 1988). These investigations concentrated on the role of lipids in the maintenance of neutral buoyancy rather than their use as dietary indicators. Few truly inclusive dietary studies, incorporating traditional methods in addition to fatty acids, have been attempted for Southern Ocean fish. A comparative study of myctophids and mackerel icefish (*Champscephalus gunnari*) by Lea et al. (2002) used fatty acids to examine dietary interactions within the Macquarie and Kerguelen Islands food-webs. Phillips et al. (2001) assessed the predation upon myctophids by the squid *Moroteuthis ingens* in the Macquarie Island region by similar means. Brown et al. (1999) linked the diets of Southern elephant seals and toothfish in the South Georgia region based on common fatty acid compositions.

More recently, heightened interest in the health benefits of fish and the increasing commercialisation of previously marginal marine species (including toothfish) has combined to provide some lipid compositional information (e.g. Sinclair et al. 1992; Nichols et al. 1994a; Dunstan et al. 1999). However, the vast majority of commercial fish species are from temperate, inshore environments and their use in dietary studies is limited to

these regions. Reinhardt & Van Vleet (1986) and Friedrich & Hagen (1994) have studied a number of non-commercial Antarctic fish species, emphasising the possible influence of diet on lipid composition, but these studies were hampered by the lack of lipid data available for prey species.

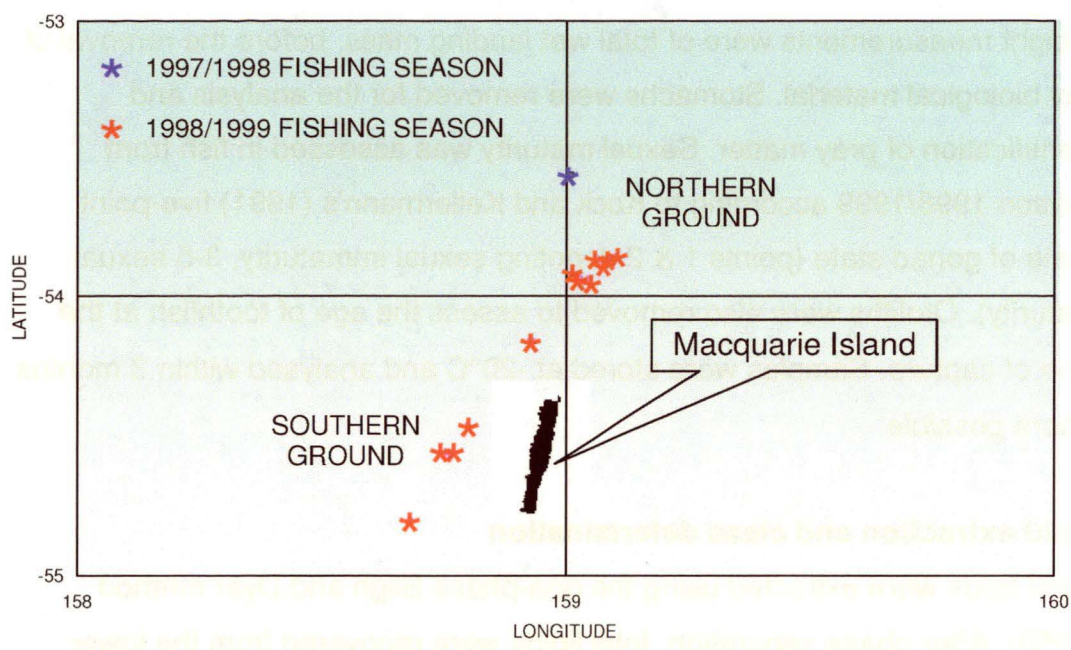
The use of fatty acids as signature lipids may therefore contribute to an expansion in current understanding of toothfish diet in the Macquarie Island region. The main objectives of this study are firstly to determine the total lipid content, lipid class and fatty acid composition of toothfish in the Macquarie Island. Secondly, variations in lipid composition relative to a number of sampling parameters, namely toothfish size, fishing ground, fishing season, fishing depth, time of day and sex, will be assessed using statistical analysis.

## **5.2 MATERIALS AND METHODS**

### **Sample collection**

Toothfish samples were collected as part of commercial fishing activities of the *Austral Leader*, a deep-sea trawler operated by Austral Fisheries Pty. Ltd. (Fremantle, Western Australia). Fishing took place over the Southern Hemisphere summers of 1997-1998 and 1998-99 in fishing grounds of the Macquarie Island region (54°30'S; 158°55'E), approximately 1500km SW of Tasmania. Samples were collected from two main fishing grounds, one to the North of Macquarie Island in the area of the Northern Valleys, encompassing the Colgate Valley and Grand Canyon (northern fishing ground), and the other to the west of Macquarie Island, dominated by the Aurora Trough (southern fishing ground) (Figure 5.1).

Fish of varying sizes were collected from trawls over a range of trawl depths, locations and times of day (Table 5.1). A total of 259 samples were collected for lipid analysis, 83 from season 1997-1998 and 178 from season 1998-1999. Samples from the lower tail section (1-2g) were removed for lipid analysis, frozen at -20°C and analysed within 3 months when possible. Previous investigation (Chapter 4)



**Figure 5.1** Position of northern and southern fishing grounds relative to Macquarie Island

**Table 5.1** Summary of *Dissostichus eleginoides* samples collected from the Macquarie Island Region during the fishing seasons 1997/1998 and 1998/1999 from two fishing grounds at various times of the day

Fishing Season	Samples (n)			Trawls <sup>2</sup> (n)				Time of Day <sup>1</sup>	
	N	S	Total	N	S	Total	Depth Range (m)	Night	Day
1997/1998	83	0	83	18	0	18	770-1253	51	32
1998/1999	119	57	176	15	5	20	622-1855	81	95
Total	102	57	259	33	5	38	622-1855	132	127

N=northern fishing ground; S=southern fishing ground; <sup>1</sup>night (1800-0600hr) day (0600-1800hr); <sup>2</sup>refer to main body of text for location of fishing grounds

found that lower tail flesh was an acceptable proxy for overall toothfish lipid composition (total lipid content, lipid class and fatty acid composition).

**Determination of size, stomach contents, age, sex and sexual maturity**

Upon capture biological data including toothfish length, weight, sex and stomach fullness were recorded. Both total (tip of snout to tip of caudal fin) and standard (tip of snout to base of caudal fin) lengths were determined.

Weight measurements were of total wet landing mass, before the removal of any biological material. Stomachs were removed for the analysis and identification of prey matter. Sexual maturity was assessed in fish from season 1998/1999 according to Kock and Kellermann's (1991) five-point scale of gonad state (points 1 & 2 denoting sexual immaturity, 3-5 sexual maturity). Otoliths were also removed to assess the age of toothfish at the time of capture. Samples were stored at -20°C and analysed within 3 months where possible.

### **Lipid extraction and class determination**

Total lipids were extracted using the one-phase Bligh and Dyer method (1959). After phase separation, total lipids were recovered from the lower chloroform phase and concentrated *in vacuo* (rotary evaporator, 40°C). Lipid class profiles were determined by TLC-FID using an Iatroscan MkV TH10 analyser (Volkman and Nichols 1991). Wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA), sterol (ST) and polar lipid (PL) components were resolved in a polar solvent system consisting of hexane-diethyl ether-acetic acid (60:17:0.2 v/v). The presence or absence of WE and diacylglycerol ethers (DAGE) was confirmed by the use of a non-polar solvent system (hexane-diethyl ether; 96:4 v/v). Peak areas were quantified using commercially available chromatography software (DAPA Scientific Software, Kalamunda, Western Australia).

### **Fatty acid determination**

Fatty acid methyl esters (FAME) were prepared by addition of methanol (MeOH), concentrated hydrochloric acid (HCl) and chloroform (CHCl<sub>3</sub>) (3mL 10:1:1, v/v/v, 80°C/2hr) to an aliquot of the total lipid. After cooling and the addition of 1mL of water, resulting FAME were extracted (3x) with 4:1 v/v hexane/CHCl<sub>3</sub>. Chloroform containing C<sub>19</sub> FAME internal standard was then added. FAME fractions were treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, 50µL, 60°C, 2hr) to convert any sterols present to their corresponding trimethylsilyl (TMS) ethers.

Gas chromatographic (GC) analysis was performed on a Hewlett Packard 5890A GC equipped with a flame ionisation detector, a cross-linked methyl silicone (HP5) fused silica capillary column (50 m length  $\times$  0.32  $\mu$ m internal diameter), employing hydrogen as the carrier gas. Samples were injected (HP7673A auto-injector) at 50°C in the splitless mode, with a 2-min. venting time (Nichols et al. 1993). The injector and detector were maintained at 290°C and 310°C respectively. After 1 min. the oven was raised to 150°C at 30°C min<sup>-1</sup>, then to 250°C at 2°C min<sup>-1</sup> and finally to 300°C at 5°C min<sup>-1</sup> (Nichols et al. 1993). Peak areas were quantified using Millennium 32 Chromatography Manager (Waters Corporation, Milford, Massachusetts, USA). Identification of individual fatty acids and sterols was based upon a comparison of relative retention times with those obtained from previous studies in addition to authentic and laboratory standards. GC-mass spectrometry (FISONS MD800 system and Finnigan Thermoquest GCQ fitted with an on-column injector, both configured as above) was used for confirmation of components from representative samples (Nichols et al. 1993).

### **Statistical analyses**

Statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, Illinois, USA). Variations in total lipid content, lipid class and individual fatty acid compositions in relation to the various sampling parameters were compared using two-sample *t*-tests (95% confidence interval).

Multivariate statistics were also used to analyse total fatty acid and lipid class composition. Non-metric multi-dimensional scaling (MDS; Kruskal loss function) was employed to compare the fatty acid and lipid class composition of toothfish in two dimensions to assess whether groupings existed within the data set.

Linear discriminant analysis (LDA; tolerance=0.01, complete estimation, classification and jackknifed classification matrix, Wilk's lambda) was used to

determine which, if any, of the sampling parameters were likely to be responsible for the observed grouping of toothfish fatty acid and lipid class data. LDA also enabled the identification of those variables (fatty acids and lipid classes) most responsible for the differences amongst groups.

Automatic forward stepping (F to enter=4, F to remove=3.9) was used when analysing fatty acid composition. The jack-knifed classification matrix is included as a means of cross-validating the normal classification matrix.

Considerable difference in the percentage of correct classifications between these matrices would suggest potential difficulties in correctly classifying the data.

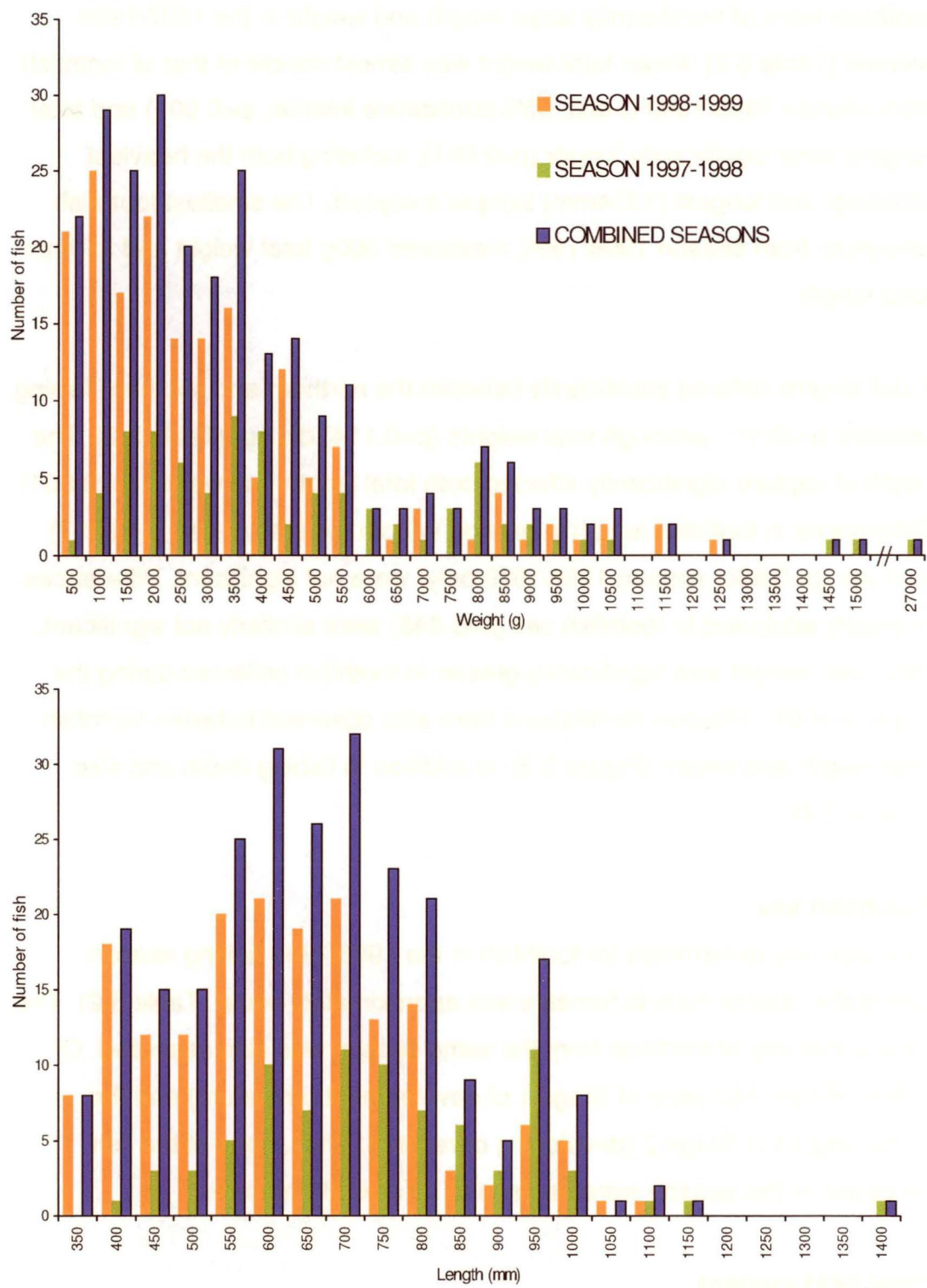
The scale of LDA classification is between 0 and 100%. A classification of 0% means that there is no similarity in the lipid class or fatty acid composition of toothfish taken from the two grouping variables (e.g. fish sampled from northern or southern grounds). A classification of 100% means that toothfish from both grouping variables have the same lipid class or fatty acid composition.

## **5.3 RESULTS**

### **Toothfish size**

Examination of length-frequency and weight frequency (combined seasons) reveals a relatively normal (unimodal) size distribution and emphasises the prevalence of smaller sized fish (total length <1000mm, total weight <5000g) in this study (Figure 5.2). Most of the toothfish analysed fall within the length range 350-1000mm with the greatest length frequency (n=33) occurring in the 650-700mm class. Weight distribution is also skewed toward the smaller size classes, particularly between 2000-5000g, and few toothfish weighing over 12000g have been included in this study. The weight class 2000-2500g contains the greatest number of toothfish (n=30).

A comparison of size distribution between the fishing seasons reveals that



**Figure 5.2** Weight-frequency and length-frequency distribution of *Dissostichus eleginoides* samples included in this study from the Macquarie Island region

toothfish were of significantly larger length and weight in the 1997/1998 season (Table 5.2). Mean total weight was almost double of that of toothfish from season 1998/1999 ( $t$ -test, 95% confidence interval,  $p<0.001$ ) and total lengths were significantly longer ( $p<0.001$ ), including both the heaviest (26900g) and longest (1354mm) sample analysed. The smallest toothfish analysed, from season 1998/1999, measured 300g total weight and 310mm total length.

Total lengths differed significantly between the northern and southern fishing grounds ( $p<0.01$ ), although total weights ( $p=0.114$ ) did not (Table 5.2). The depth of capture significantly affected both total length and weight ( $p<0.001$ ). Differences in toothfish length in relation to both the time of day ( $p=0.157$ ) and sex ( $p=0.630$ , season 1998/1999 only) were not significant. Differences in weight attributed to toothfish sex ( $p=0.848$ ) were similarly not significant. However, weight was significantly greater in toothfish collected during the night ( $p<0.01$ ). Positive correlations were also observed between toothfish total length and weight (Figure 5.3), in addition to fishing depth and size (Figure 5.4).

### **Toothfish sex**

Sex was only determined for toothfish in the 1998/1999 fishing season, where the ratio of male to females was approximately equal (Table 5.2). The sexual maturity of toothfish from the same season was also assessed. Of 176 toothfish, 162 were at Stage 1 of development (immature) and the remaining 14 at Stage 2 (developing or resting). Thus, none of the fish analysed in this season were deemed to be sexually mature.

### **Total lipid content**

Total lipid content averaged 12.0% WW (wet weight) over all toothfish analysed ( $n=246$ ) and ranged from 0.8-24.8% WW (Table 5.3). Lipid content was highly influenced by several of the sampling parameters. Significant differences in lipid content based upon sex ( $p<0.001$ ), total weight ( $p<0.001$ ), total length ( $p<0.001$ ), time of day ( $p<0.01$ ), fishing depth ( $p<0.001$ ) and fishing ground ( $p<0.001$ ) were detected by  $t$ -tests. Lipid content did not vary

**Table 5.2** Total length and weight of *Dissostichus eleginoides* from the Macquarie Island region included in this study with respect to toothfish sex and various fishing parameters (data presented as mean  $\pm$  standard deviation)

(i) Toothfish sex

Toothfish	All	Sex <sup>2</sup>		
Size Parameter <sup>1</sup>	Toothfish (n=259)	Male (n=91)	Female (n=85)	t
Length (mm)	642 $\pm$ 180	609 $\pm$ 147	597 $\pm$ 191	ns
Weight (g)	3421 $\pm$ 3116	2815 $\pm$ 2158	2888 $\pm$ 2841	ns

(ii) Fishing parameters

Toothfish	All	Fishing Season			Fishing Ground <sup>3</sup>			Time of Day <sup>4</sup>			Fishing Depth		
Size Parameter <sup>1</sup>	Toothfish (n=259)	1997/1998 (n=83)	1998/1999 (n=176)	t	North (n=202)	South (n=57)	t	Day (n=127)	Night (n=132)	t	<1000m (n=79)	>1000m (n=180)	t
Length (mm)	642 $\pm$ 180	723 $\pm$ 175	603 $\pm$ 169	s	664 $\pm$ 161	564 $\pm$ 220	s	626 $\pm$ 132	657 $\pm$ 216	ns	534 $\pm$ 201	689 $\pm$ 147	s
Weight (g)	3421 $\pm$ 3116	4630 $\pm$ 3871	2850 $\pm$ 2504	s	3591 $\pm$ 3053	2816 $\pm$ 3286	ns	2862 $\pm$ 1856	3958 $\pm$ 3900	s	2332 $\pm$ 2955	3898 $\pm$ 3072	s

t=two-sample t-test, 95% confidence interval; s=significant ( $p<0.05$ ); ns=non-significant ( $p>0.05$ ); n=number of samples; <sup>1</sup>total length and total wet weight; <sup>2</sup>sex only determined in fishing season 1998/1999; <sup>3</sup>refer to main body of text for location of fishing grounds; <sup>4</sup>0600-1800hrs (day) 1800-0600hrs (night)

**Table 5.3** Total lipid content (percent composition, wet weight) of *Dissostichus eleginoides* from Macquarie Island in relation to various biological and fishing parameters (data presented as mean  $\pm$  standard deviation):

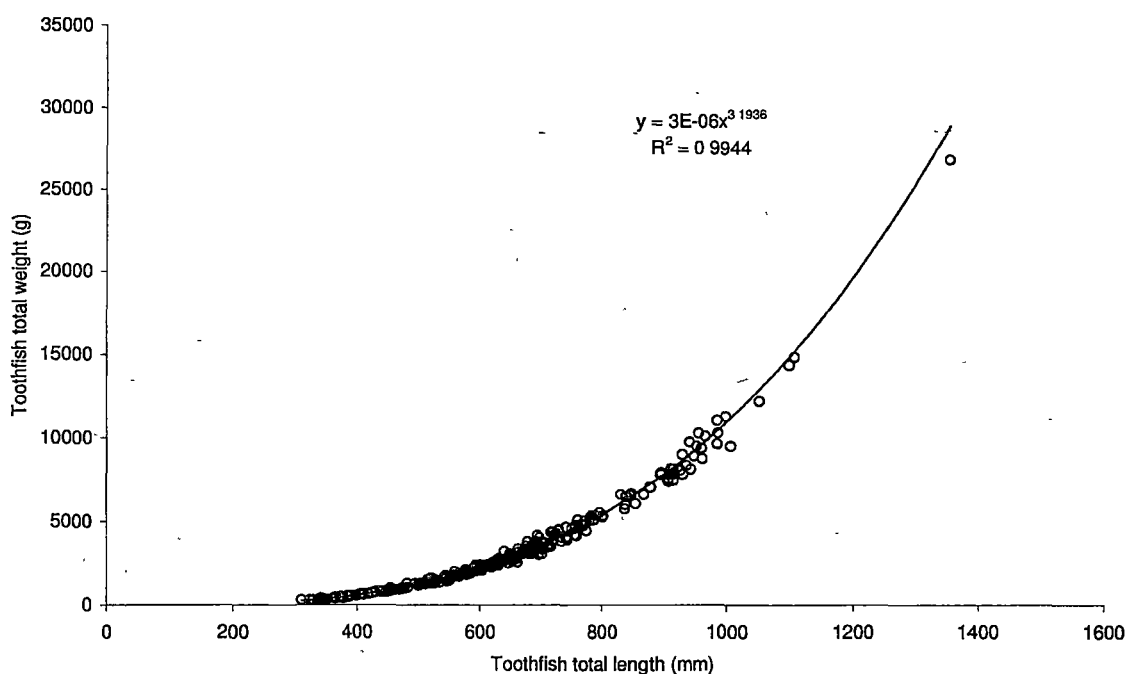
(i) Biological Parameters

	All Toothfish (n=246)	Total Length			Total Weight			Sex <sup>1</sup>		
		<500mm (n=56)	>500mm (n=188)	<i>t</i>	<1000g (n=51)	>1000g (n=193)	<i>t</i>	Male (n=90)	Female (n=83)	<i>t</i>
Total Lipid (ww)	12.0 $\pm$ 5.7	5.3 $\pm$ 4.8	13.9 $\pm$ 4.2	s	4.8 $\pm$ 4.4	13.9 $\pm$ 4.3	s	13.7 $\pm$ 5.6	10.1 $\pm$ 6.4	s

(ii) Fishing Parameters

	Fishing Season			Fishing Ground <sup>2</sup>			Time of Day <sup>3</sup>			Fishing Depth		
	1997/1998 (n=71)	1998/1999 (n=175)	<i>t</i>	North (n=187)	South (n=57)	<i>t</i>	Day (n=118)	Night (n=126)	<i>t</i>	<1000m (n=79)	>1000m (n=165)	<i>t</i>
Total Lipid (ww)	12.0 $\pm$ 4.0	12.0 $\pm$ 6.2	ns	13.3 $\pm$ 4.8	7.7 $\pm$ 6.3	s	13.1 $\pm$ 4.6	10.9 $\pm$ 6.3	s	7.7 $\pm$ 5.9	14.0 $\pm$ 4.2	s

*t*=two-sample *t*-test, 95% confidence interval; s=significant ( $p<0.05$ ); ns=non-significant ( $p>0.05$ ); n=number of samples; ww=wet weight; <sup>1</sup>sex only determined in fishing season 1998/1999; <sup>2</sup>refer to main body of text for location of fishing grounds; <sup>3</sup>0600-1800hrs (day) 1800-0600hrs (night)



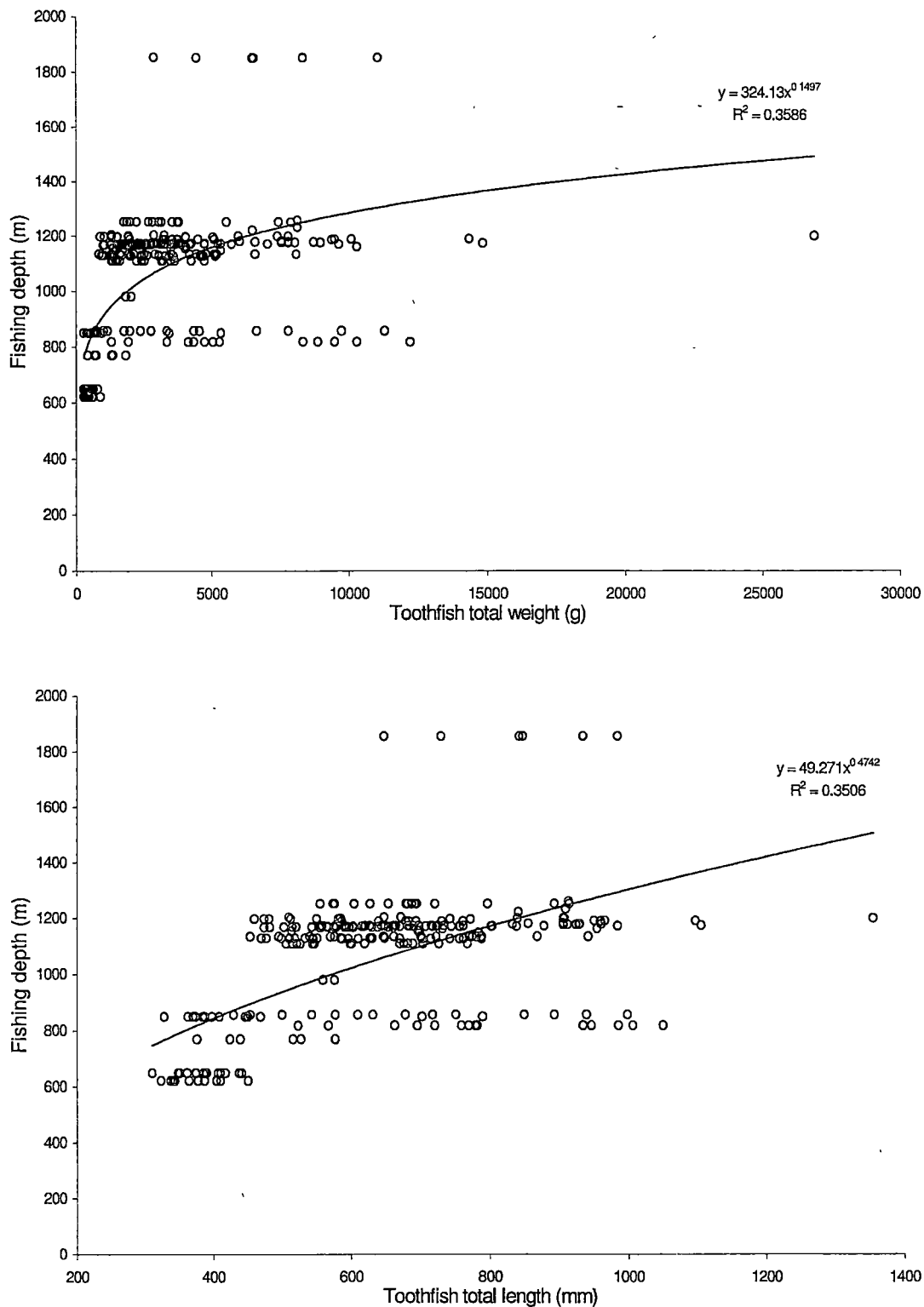
**Figure 5.3** Relationship between the total length and weight of *Dissostichus eleginoides* analysed in this study from the Macquarie Island region (n=259)

significantly between fishing seasons ( $p > 0.05$ ). Variations in lipid content were greatest in relation to increasing total length and weight (Figure 5.5). A positive correlation was also observed between fishing depth and lipid content (Figure 5.6).

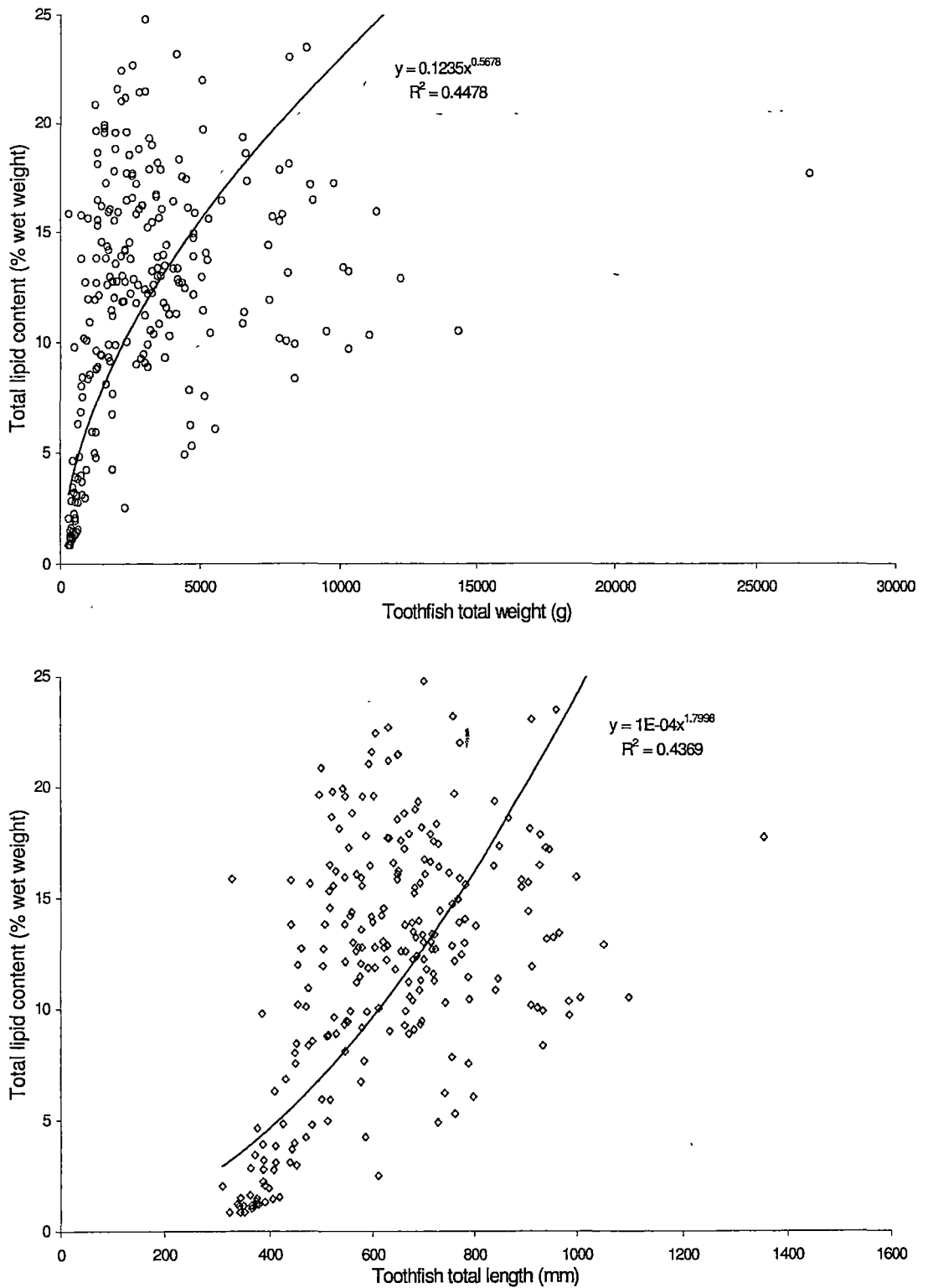
### Lipid class composition

A total of 230 toothfish samples were analysed for lipid class composition (Table 5.4). Triacylglycerol (TAG) dominated class composition (mean  $91.6 \pm 11.6\%$ , range 33.0-98.9%, all toothfish), followed by free fatty acid (FFA; mean  $6.1 \pm 8.8\%$ , range 0.6-54.1%), polar lipids (PL; mean  $1.6 \pm 3.0\%$ , range 0.0-25.1%) and sterols (ST, primarily cholesterol; mean  $0.7 \pm 1.5\%$ , range 0.0-13.7%).

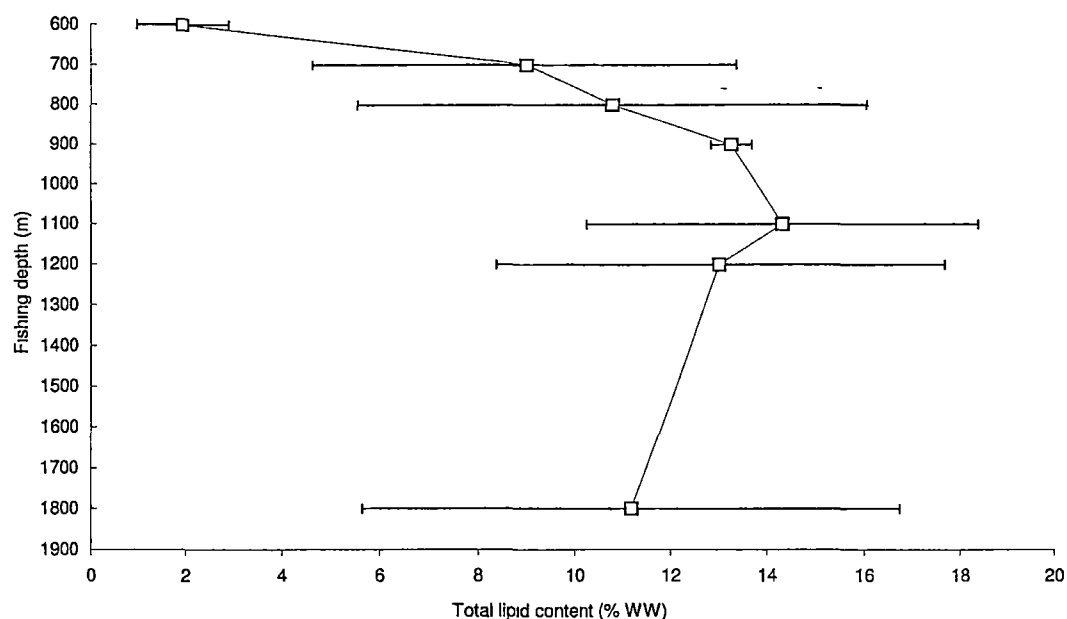
Significant differences ( $t$ -test,  $p < 0.05$ ) existed in the content of all lipid classes on the basis of time of day, fishing ground, fishing season, fishing depth, total length and total weight, with the exception of PL in relation to fishing season ( $p = 0.082$ ). The sex of toothfish did not significantly influence



**Figure 5.4.** Variation in *Dissostichus eleginoides* size (total length and weight) included in this study with increasing fishing depth in the Macquarie Island region



**Figure 5.5** Relationship between the total lipid content, total length and weight of *Dissostichus eleginoides* included in this study from the Macquarie Island region



**Figure 5.6** Total lipid content (% wet weight, averaged over 100m depth intervals, bars represent standard deviation) versus fishing depth (m) for *Dissostichus eleginoides* samples included in this study from the Macquarie Island region

the composition of any lipid classes ( $p > 0.1$ ). A positive correlation was observed between TAG content and increasing length, weight and fishing depth, whilst FFA content was negatively correlated. Levels of PL and ST remained constant, regardless of variations in size and fishing depth.

MDS analysis of lipid class composition revealed one major grouping of toothfish, with a wide scattering of the remaining samples (Figure 5.7). LDA identified fishing ground (normal classification 15%; jackknifed classification 15%), fishing depth (classification 15%; jackknifed classification 16%), total length (classification 9%; jackknifed classification 11%) and total weight (classification 7%; jackknifed classification 7%) as having a significant influence on lipid class composition. The sex of toothfish, however, did not significantly influence lipid class composition (classification 43%; jackknifed 45%).

**Table 5.4** Lipid class composition (percentage of total lipid content) of *Dissostichus eleginoides* from the Macquarie Island region in relation to various biological and fishing parameters (data presented as mean  $\pm$  standard deviation)

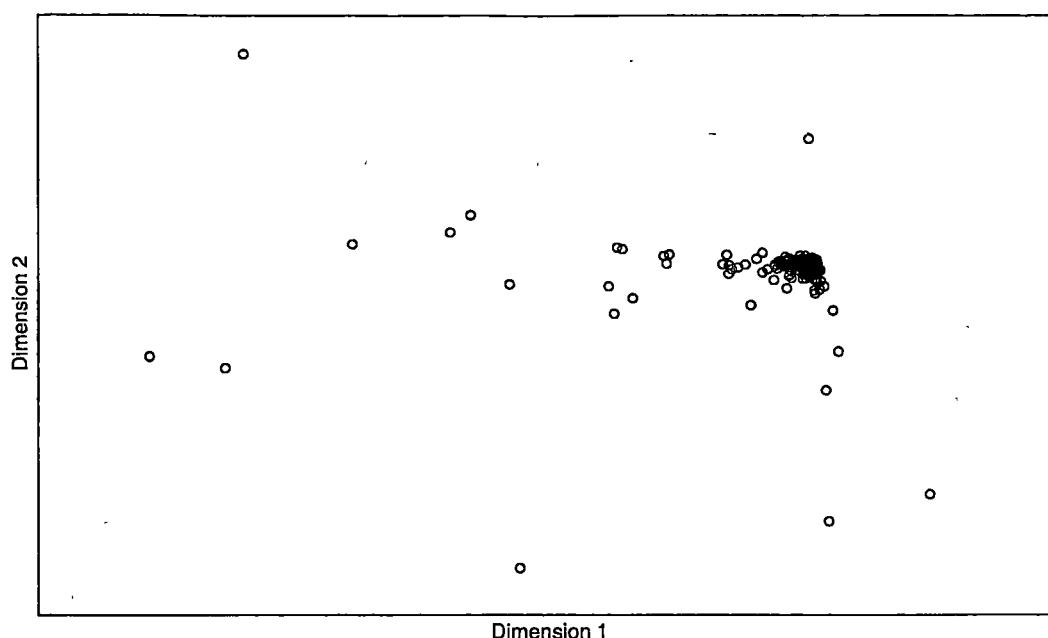
(i) Biological Parameters

Lipid Class	All Toothfish (n=230)	Total Length			Total Weight			Sex <sup>1</sup>		
		<500mm (n=39)	>500mm (n=189)	t	<1000g (n=33)	>1000g (n=195)	t	Male (n=78)	Female (n=67)	t
TAG	91.6 $\pm$ 11.6	75.2 $\pm$ 19.6	94.9 $\pm$ 4.4	s	72.1 $\pm$ 19.8	94.8 $\pm$ 4.4	s	91.2 $\pm$ 13.1	89.0 $\pm$ 15.0	ns
FFA	6.1 $\pm$ 8.8	18.3 $\pm$ 14.9	3.5 $\pm$ 3.6	s	20.5 $\pm$ 15.1	3.6 $\pm$ 3.6	s	6.1 $\pm$ 9.5	8.0 $\pm$ 11.7	ns
ST/DG	0.7 $\pm$ 1.5	2.1 $\pm$ 2.5	0.5 $\pm$ 1.0	s	2.4 $\pm$ 2.7	0.5 $\pm$ 0.9	s	0.9 $\pm$ 1.8	1.0 $\pm$ 1.9	ns
PL	1.6 $\pm$ 3.0	4.4 $\pm$ 6.0	1.1 $\pm$ 1.4	s	5.0 $\pm$ 6.3	1.1 $\pm$ 1.4	s	1.8 $\pm$ 3.4	1.9 $\pm$ 3.7	ns
Total	100.0	100.0	100.0		100.0	100.0		100.0	100.0	

(ii) Fishing Parameters

Lipid Class	Fishing Season			Fishing Ground <sup>2</sup>			Time of Day <sup>3</sup>			Fishing Depth		
	1997/1998 (n=83)	1998/1999 (n=147)	t	North (n=185)	South (n=43)	t	Day (n=110)	Night (n=118)	t	<1000m (n=55)	>1000m (n=173)	t
TAG	93.9 $\pm$ 4.9	90.2 $\pm$ 14.0	s	94.3 $\pm$ 5.1	79.7 $\pm$ 21.1	s	89.0 $\pm$ 14.0	94.3 $\pm$ 5.4	s	81.3 $\pm$ 19.4	94.8 $\pm$ 3.7	s
FFA	4.5 $\pm$ 4.3	7.0 $\pm$ 10.6	s	4.1 $\pm$ 4.2	14.6 $\pm$ 16.1	s	7.8 $\pm$ 11.3	4.2 $\pm$ 4.6	s	13.7 $\pm$ 14.8	3.7 $\pm$ 3.4	s
ST/DG	0.4 $\pm$ 0.3	1.0 $\pm$ 1.8	s	0.5 $\pm$ 1.0	1.8 $\pm$ 2.5	s	1.0 $\pm$ 2.0	0.5 $\pm$ 0.4	s	1.6 $\pm$ 2.3	0.5 $\pm$ 1.0	s
PL	1.3 $\pm$ 1.8	1.9 $\pm$ 3.6	ns	1.1 $\pm$ 1.5	3.8 $\pm$ 5.8	s	2.2 $\pm$ 4.0	1.0 $\pm$ 1.2	s	3.5 $\pm$ 5.2	1.1 $\pm$ 1.5	s
Total	100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0	

TAG=triacylglycerol; FFA=free fatty acid; ST=sterol; DG=diacylglycerol; PL=polar lipid; n=number of samples; t= two-sample t-test, 95% confidence interval; s=significant (p<0.05); ns=non-significant (p>0.05); n=number of samples; <sup>1</sup>sex only determined in fishing season 1998/1999; <sup>2</sup>refer to main body of text for location of fishing grounds; <sup>3</sup>0600-1800hrs (day) 1800-0600hrs (night)



**Figure 5.7** Scatterplot of multidimensional scaling (MDS) based upon the lipid class composition of *Dissostichus eleginoides* included in this study from Macquarie Island region (n=230)

LDA also revealed that TAG was the lipid class most responsible, and PL the least responsible, for the classification of toothfish into separate groupings for all the sampling parameters examined, with the exception of total length. FFA and ST were the most and least responsible lipid classes respectively for classification by total length.

### Fatty acid composition

#### *All toothfish*

Examination of the fatty acid profile of toothfish from both seasons revealed a total of 45 identifiable components. The profile is dominated by monounsaturated fatty acids (MUFA), accounting for on average  $65.6 \pm 7.6\%$  (range 27.4-75.8%) of the total fatty acids (Table 5.5, all toothfish). Saturated (SFA; mean  $22.1 \pm 1.8\%$ , range 16.4-27.8%) and polyunsaturated fatty acids (PUFA; mean  $11.6 \pm 7.3\%$ , range 0.7-47.9%) were present at lower levels,

**Table 5.5** Fatty acid composition (% composition of total fatty acids) of *Dissostichus eleginoides* included in this study from the Macquarie Island region with respect to various biological parameters (data presented as mean  $\pm$  standard deviation)

Fatty Acid	All	Total Weight		Total Length		Sex <sup>1</sup>	
	Toothfish (n=259)	<1000g (n=47)	>1000g (n=212)	<500mm (n=56)	>500mm (n=203)	Male (n=91)	Female (n=85)
14:0	3.5 $\pm$ 1.1	2.8 $\pm$ 1.2	3.7 $\pm$ 1.0	3.0 $\pm$ 1.3	3.7 $\pm$ 1.0	3.2 $\pm$ 1.2	3.2 $\pm$ 1.2
15:0	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3	0.4 $\pm$ 0.4	0.4 $\pm$ 0.1
16:0	14.4 $\pm$ 1.2	15.3 $\pm$ 1.3	14.1 $\pm$ 1.1	15.2 $\pm$ 1.3	14.1 $\pm$ 1.0	14.3 $\pm$ 1.2	14.6 $\pm$ 1.4
17:0	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
18:0	3.0 $\pm$ 0.6	3.3 $\pm$ 0.8	2.9 $\pm$ 0.5	3.2 $\pm$ 0.8	2.9 $\pm$ 0.5	2.9 $\pm$ 0.5	3.2 $\pm$ 0.6
20:0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1
22:0	0.2 $\pm$ 0.4	0.3 $\pm$ 0.4	0.2 $\pm$ 0.4	0.3 $\pm$ 0.4	0.2 $\pm$ 0.4	0.2 $\pm$ 0.4	0.3 $\pm$ 0.6
14:1 $\omega$ 5c	0.2 $\pm$ 0.2	0.2 $\pm$ 0.5	0.2 $\pm$ 0.1	0.2 $\pm$ 0.4	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.4
16:1 $\omega$ 9c	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2
16:1 $\omega$ 7c	8.1 $\pm$ 1.6	6.4 $\pm$ 2.6	8.5 $\pm$ 0.9	6.7 $\pm$ 2.6	8.5 $\pm$ 0.9	7.8 $\pm$ 1.4	7.6 $\pm$ 2.1
16:1 $\omega$ 7t	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0
17:1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
18:1 $\omega$ 9c	32.9 $\pm$ 5.3	25.9 $\pm$ 7.4	34.3 $\pm$ 3.3	27.6 $\pm$ 8.0	34.2 $\pm$ 3.2	33.3 $\pm$ 5.5	31.3 $\pm$ 6.7
18:1 $\omega$ 7c	5.2 $\pm$ 0.6	5.3 $\pm$ 0.6	5.2 $\pm$ 0.6	5.3 $\pm$ 0.6	5.2 $\pm$ 0.6	5.5 $\pm$ 0.5	5.4 $\pm$ 0.8
18:1 $\omega$ 5c	0.7 $\pm$ 0.4	0.6 $\pm$ 0.1	0.7 $\pm$ 0.4	0.6 $\pm$ 0.1	0.7 $\pm$ 0.4	0.7 $\pm$ 0.6	0.7 $\pm$ 0.1
18:1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
19:1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0
20:1 $\omega$ 11c	0.7 $\pm$ 0.3	0.6 $\pm$ 0.2	0.7 $\pm$ 0.3	0.6 $\pm$ 0.2	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3	0.6 $\pm$ 0.3
20:1 $\omega$ 9c	7.4 $\pm$ 1.5	6.8 $\pm$ 1.7	7.5 $\pm$ 1.4	6.7 $\pm$ 1.8	7.6 $\pm$ 1.3	7.7 $\pm$ 1.6	7.4 $\pm$ 1.8
20:1 $\omega$ 7c	0.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.7 $\pm$ 0.2	0.4 $\pm$ 0.1	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2
22:1 $\omega$ 11c	3.2 $\pm$ 0.9	2.5 $\pm$ 0.9	3.6 $\pm$ 2.6	2.5 $\pm$ 0.9	3.7 $\pm$ 2.7	3.5 $\pm$ 2.6	2.9 $\pm$ 0.9
22:1 $\omega$ 9c	1.9 $\pm$ 0.7	1.4 $\pm$ 0.4	2.1 $\pm$ 0.7	1.3 $\pm$ 0.4	2.1 $\pm$ 0.7	1.9 $\pm$ 0.8	1.7 $\pm$ 0.6
22:1 $\omega$ 7c	0.2 $\pm$ 0.1	0.1 $\pm$ 0.0	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
24:1 $\omega$ 11c	0.8 $\pm$ 0.3	0.6 $\pm$ 0.2	0.8 $\pm$ 0.3	0.6 $\pm$ 0.2	0.8 $\pm$ 0.3	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3
24:1 $\omega$ 9c	1.0 $\pm$ 0.4	1.0 $\pm$ 0.3	1.0 $\pm$ 0.4	1.0 $\pm$ 0.4	1.0 $\pm$ 0.3	1.0 $\pm$ 0.4	0.9 $\pm$ 0.4
18:3 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
18:4 $\omega$ 3	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.8 $\pm$ 0.2
18:2 $\omega$ 6	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2	1.2 $\pm$ 0.2	1.4 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2
AA	0.7 $\pm$ 0.6	1.3 $\pm$ 0.9	0.5 $\pm$ 0.4	1.2 $\pm$ 0.9	0.5 $\pm$ 0.4	0.6 $\pm$ 0.6	0.8 $\pm$ 0.6
EPA	2.8 $\pm$ 1.6	5.1 $\pm$ 2.4	2.3 $\pm$ 0.7	4.6 $\pm$ 2.5	2.3 $\pm$ 0.8	2.7 $\pm$ 1.8	3.4 $\pm$ 1.9
20:3 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
20:4 $\omega$ 3	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
20:2 $\omega$ 6	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
C <sub>21</sub> PUFA	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
22:5 $\omega$ 6	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
DHA	4.8 $\pm$ 5.0	12.0 $\pm$ 8.0	3.2 $\pm$ 1.4	10.7 $\pm$ 8.0	3.2 $\pm$ 1.4	4.5 $\pm$ 4.9	6.4 $\pm$ 6.7
22:4 $\omega$ 6	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.1	0.0 $\pm$ 0.0
DPA	0.3 $\pm$ 0.2	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2
SFA	22.1 $\pm$ 1.8	22.6 $\pm$ 1.6	22.0 $\pm$ 1.8	22.5 $\pm$ 1.6	22.0 $\pm$ 1.8	21.6 $\pm$ 2.0	22.3 $\pm$ 2.0
BrFA	0.8 $\pm$ 0.5	1.1 $\pm$ 0.4	0.7 $\pm$ 0.5	1.0 $\pm$ 0.4	0.7 $\pm$ 0.5	0.9 $\pm$ 0.5	1.0 $\pm$ 0.5
MUFA	65.5 $\pm$ 7.6	54.1 $\pm$ 11.6	68.1 $\pm$ 2.7	56.0 $\pm$ 11.8	68.1 $\pm$ 2.7	66.3 $\pm$ 8.1	62.6 $\pm$ 9.7
PUFA	11.6 $\pm$ 7.3	22.2 $\pm$ 11.4	9.2 $\pm$ 2.5	20.4 $\pm$ 11.5	9.2 $\pm$ 2.5	11.3 $\pm$ 7.5	14.1 $\pm$ 9.4
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0

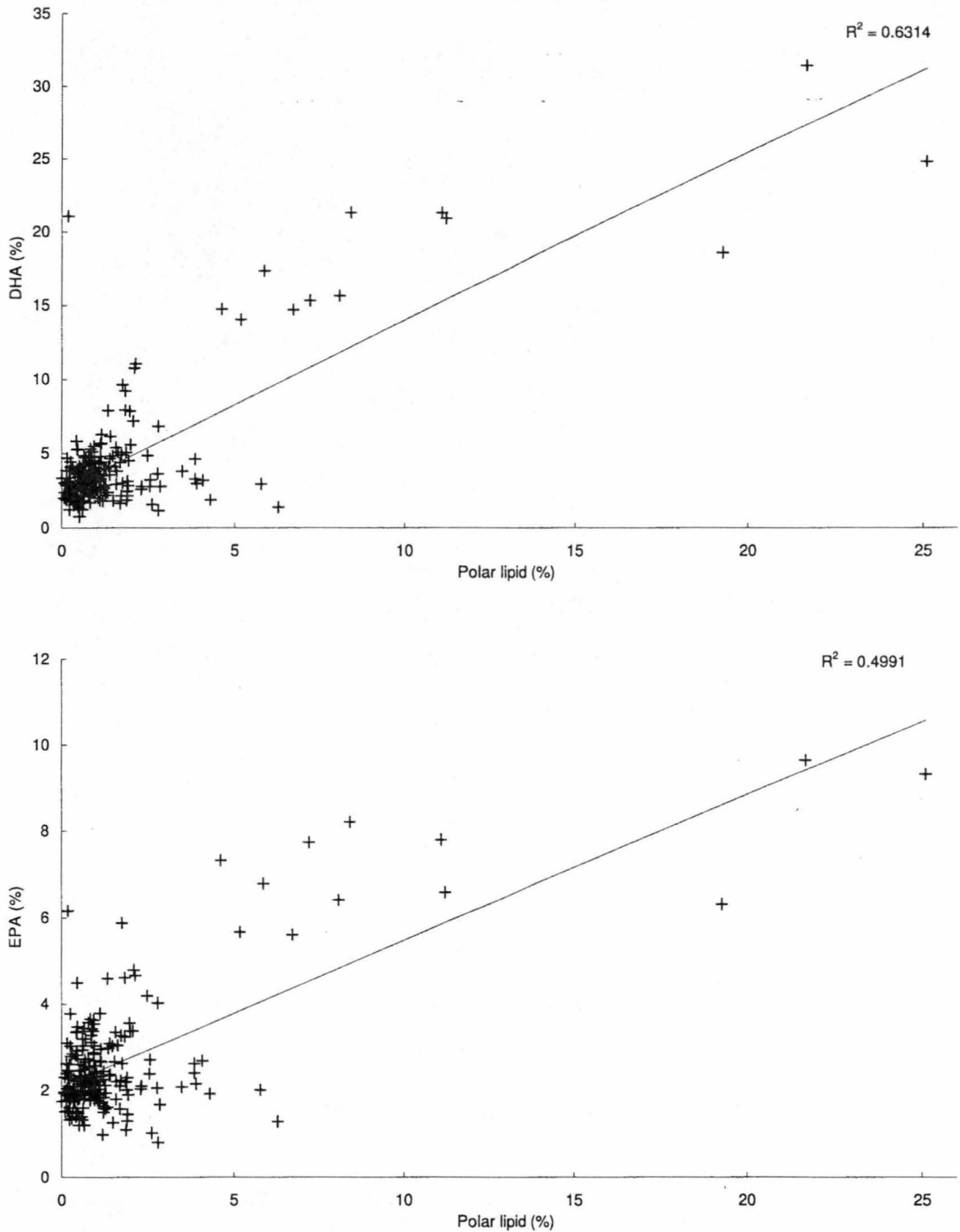
AA=arachidonic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid;  
DPA=docosapentaenoic acid; SFA=saturated fatty acid; BrFA=branched fatty acid  
MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation;  
t=*trans* double bond orientation; <sup>1</sup>sex only determined in fishing season 1998/1999

whilst branched fatty acids (BrFA) comprised a minor component (mean  $0.8 \pm 0.5\%$ , range 0.2-2.2%). Major fatty acids, those representing  $>1\%$  of total composition, and in descending order of importance (composition), included 18:1 $\omega$ 9c (mean  $32.9 \pm 5.3\%$ , range 11.5-42.4%), 16:0 (mean  $14.4 \pm 1.2\%$ , range 11.6-18.7%), 16:1 $\omega$ 7c (mean  $8.1 \pm 1.6\%$ , range 2.2-16.9%), 20:1 $\omega$ 9c (mean  $7.4 \pm 1.5\%$ , average 2.9-13.3%), 18:1 $\omega$ 7c (mean  $5.2 \pm 0.6\%$ , average 0.5-6.7%), docosahexaenoic acid (DHA [22:6 $\omega$ 3],  $4.8 \pm 5.0\%$ , range 0.0-31.5%), 14:0 (mean  $3.5 \pm 1.1\%$ , range 0.2-5.5%), 22:1 $\omega$ 11c (mean  $3.2 \pm 0.9\%$ , range 0.4-7.2%), 18:0 (mean  $3.0 \pm 0.6\%$ , range 1.8-5.1%), EPA (eicosapentaenoic acid [20:5 $\omega$ 3], mean  $2.8 \pm 1.6\%$ , range 0.0-10.7%), 22:1 $\omega$ 9c (mean  $1.9 \pm 0.7\%$ , range 0.6-7.4%), 18:2 $\omega$ 6 (mean  $1.3 \pm 0.2\%$ , range 0.0-2.0%) and 24:1 $\omega$ 9c (mean  $1.0 \pm 0.4\%$ , range 0.0-2.0%). Combined, these 12 fatty acids represented ~90% of the total composition. The variation in fatty acid composition was principally due to DHA and 18:1 $\omega$ 9c, the levels of which fluctuated by over 30%. Of the major PUFA, levels of DHA and EPA were positively correlated with PL (Figure 5.8).

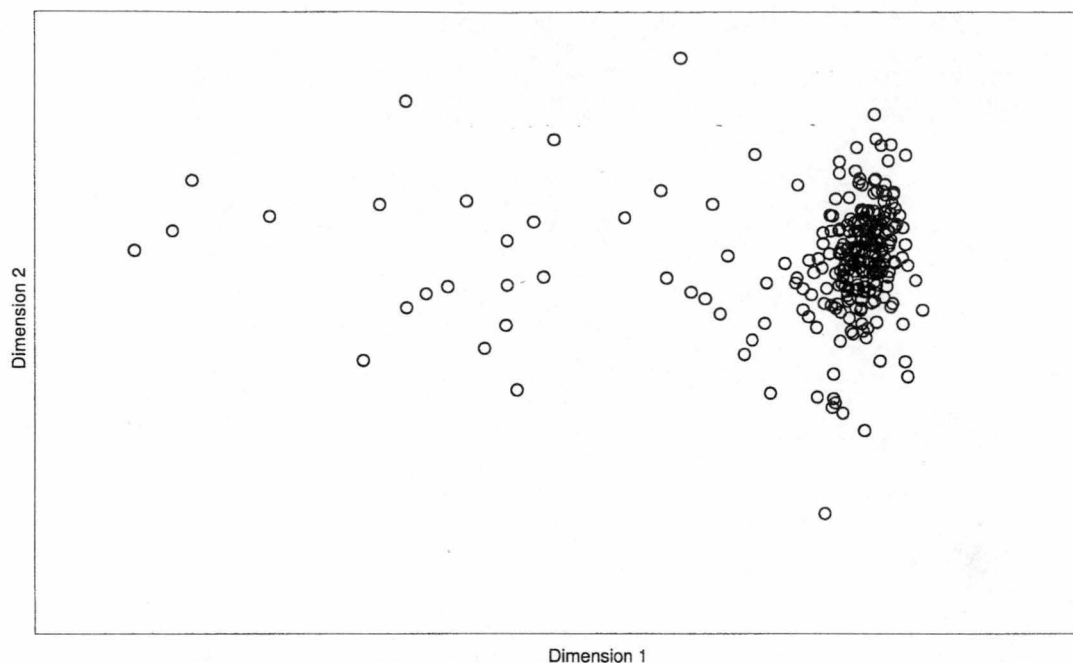
MDS analysis of toothfish fatty acid composition demonstrates a single, large grouping of toothfish ( $n=212$ ), with significant variation in the distribution of the remaining samples (Figure 5.9).

#### *Fatty acid variation relative to toothfish size*

The size of toothfish, measured by total weight (TW) and length (TL), had a marked influence on fatty acid composition (Table 5.5). Increases in TW and TL were the parameters most closely associated with variations in fatty acid composition. In particular levels of total PUFA were considerably greater, and total MUFA lower, in toothfish less than 1000g/500mm compared to those weighing greater than 1000g/500mm. Levels of DHA, for example, were approximately 10% greater in toothfish  $<1000\text{g}/500\text{mm}$ , whilst levels of 18:1 $\omega$ 9c approximately 10% lower.



**Figure 5.8** Comparison of the percent composition of polar lipid versus DHA (docosahexaenoic acid, 22:6 $\omega$ 3) and EPA (eicosapentaenoic acid, 22:5 $\omega$ 3) in *Dissostichus eleginoides* samples from Macquarie Island included in this study



**Figure 5.9.** Scatterplot of multidimensional scaling (MDS) based upon the fatty acid composition of *Dissostichus eleginoides* included in this study from the Macquarie Island region (n=259)

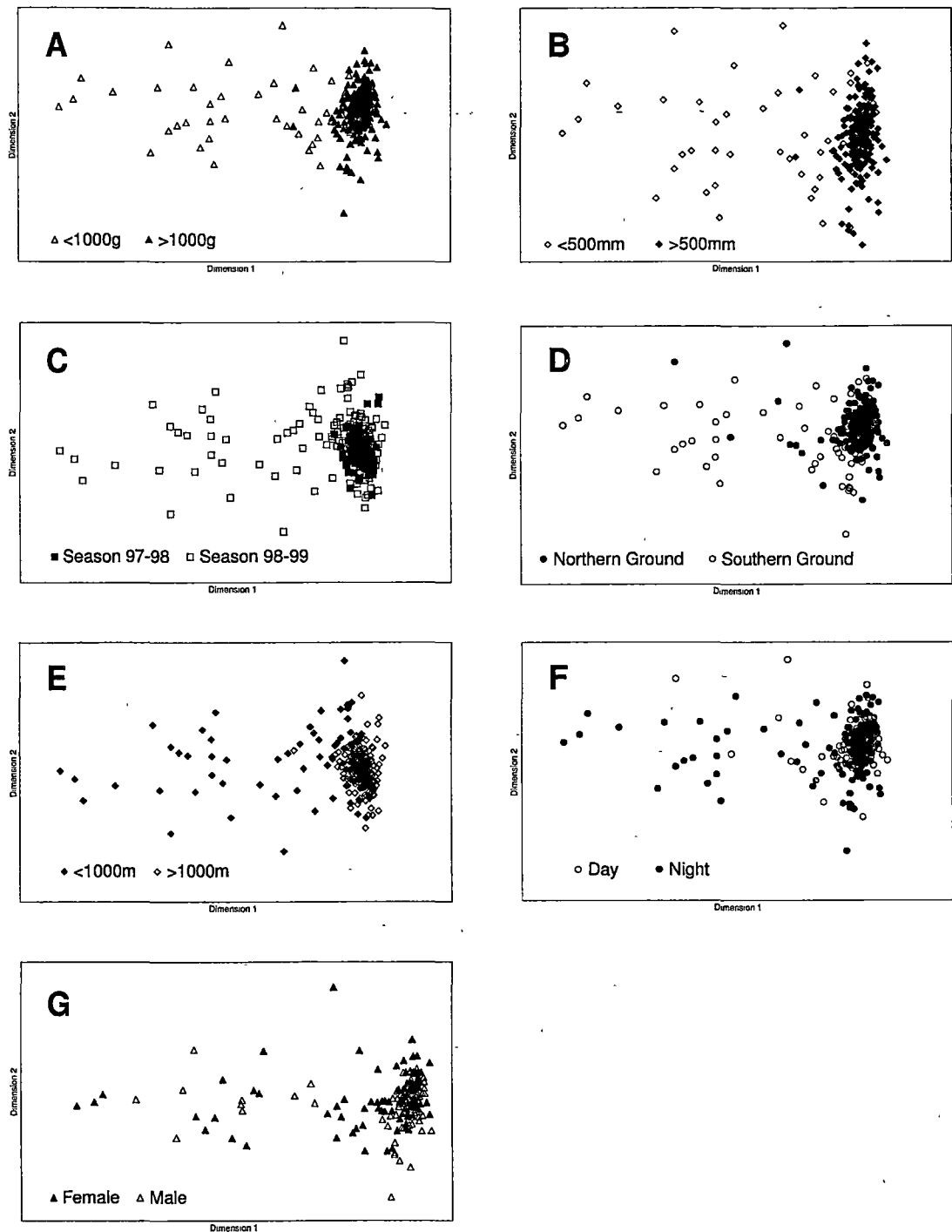
The results of *t*-test analysis indicate significant variation in the composition of 33 of the 45 fatty acids relative to changes in TW, whilst 31 of the 45 fatty acids also varied significantly with increasing TL (Tables 5.6). All PUFA (excepting 18:3 $\omega$ 6, 18:4 $\omega$ 3, 20:2 $\omega$ 6 and C<sub>21</sub> PUFA), MUFA (excepting 14:1 $\omega$ 5c, 16:1 $\omega$ 9c, 16:1 $\omega$ 5c, 18:1, 19:1 and 24:1 $\omega$ 9c), SFA and BrFA (excepting i15:0 and a15:0) varied significantly with increasing TW, amounting to 96.1% of the total fatty acid composition. The fatty acids exhibiting differences relative to TL are also similar to those varying with respect to TW. These include the same BrFA, as well as most of the same PUFA (in addition to 20:2 $\omega$ 6), MUFA (excluding 17:1, 18:1 $\omega$ 7c, 18:1 $\omega$ 5c and including 19:1) and SFA (excluding 22:0), totaling 90% of the total fatty acid composition.

MDS supports the clear separation of toothfish based upon TW and TL (Figure 5.10). LDA revealed that similarity in the fatty acid composition of toothfish from the two weight groups (3%) and length groups (4%) is low,

**Table 5.6** Results of *t*-tests (two-sample, 95% confidence interval) for individual fatty acids of *Dissostichus eleginoides* included in this study from the Macquarie Island region in relation to various sampling parameters

Fatty Acid	Fishing Season	Fishing Ground <sup>1</sup>	Time of Day <sup>2</sup>	Fishing Depth	Total Length	Total Weight	Toothfish Sex <sup>3</sup>
14:0	s	s	ns	s	s	s	ns
15:0	ns	s	ns	s	s	s	ns
16:0	ns	s	ns	s	s	s	ns
17:0	ns	s	ns	s	s	s	ns
18:0	ns	s	s	s	s	s	s
20:0	ns	ns	ns	ns	s	s	ns
22:0	ns	s	ns	s	ns	s	ns
i15:0	s	ns	s	ns	ns	ns	ns
a15:0	s	ns	s	ns	ns	ns	ns
i16:0	s	s	ns	s	s	s	s
i17:1	s	s	ns	s	s	s	s
i17:0	s	s	ns	s	s	s	s
a17:0	s	s	ns	s	s	s	ns
14:1ω5c	ns	s	ns	ns	ns	ns	ns
16:1ω9c	ns	ns	ns	s	ns	ns	ns
16:1ω7c	s	s	s	s	s	s	ns
16:1ω7t	s	ns	s	s	s	s	ns
16:1ω5c	ns	ns	ns	ns	ns	ns	ns
17:1	ns	s	s	s	ns	s	s
18:1ω9c	s	s	s	s	s	s	ns
18:1ω7c	s	ns	ns	s	ns	s	ns
18:1ω5c	ns	s	ns	ns	ns	s	ns
18:1	s	ns	ns	ns	ns	ns	ns
19:1	s	ns	ns	ns	s	ns	ns
20:1ω11c	s	ns	ns	ns	s	s	ns
20:1ω9c	s	ns	ns	ns	s	s	ns
20:1ω7c	s	s	ns	s	s	s	ns
22:1ω11c	ns	s	ns	s	s	s	s
22:1ω9c	ns	s	ns	s	s	s	ns
22:1ω7c	s	s	ns	s	s	s	s
24:1ω11c	s	s	s	s	s	s	ns
24:1ω9c	s	s	s	ns	ns	ns	ns
18:3ω6	ns	s	s	s	ns	ns	ns
18:4ω3	s	s	ns	ns	ns	ns	s
18:2ω6	s	s	ns	s	s	s	ns
AA	ns	s	s	s	s	s	ns
EPA	s	s	s	s	s	s	s
20:3ω6	s	s	s	s	s	s	s
20:4ω3	s	s	s	s	s	s	ns
20:2ω6	ns	s	ns	s	s	ns	ns
C <sub>21</sub> PUFA	ns	s	ns	s	ns	ns	ns
22:5ω6	ns	s	ns	s	s	s	ns
DHA	s	s	s	s	s	s	s
22:4ω6	s	s	s	s	s	s	ns
DPA	s	s	s	s	s	s	ns

AA=arachidonic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; PUFA=polyunsaturated fatty acid; n=significant difference ( $p < 0.05$ ); ns=non-significant difference ( $p > 0.05$ ); c=*cis* double bond orientation; t=*trans* double bond orientation; i=*iso* branching; a=*anti-iso* branching; <sup>1</sup>northern and southern ground (see main body of text for location of grounds); <sup>2</sup>day (0600-1800hrs) night (1800-0600hrs); <sup>3</sup>sex only determined in season 1999/1999



**Figure 5.10** Scatterplots of multidimensional scaling (MDS) based on the total fatty acid composition of *Dissostichus eleginoides* included in this study from Macquarie Island in relation to various biological and fishing parameters (**A** = toothfish weight; **B** = toothfish length; **C** = fishing season; **D** = fishing ground; **E** = fishing depth; **F** = time of day; **G** = toothfish sex; refer to main body of text for details of each parameter)

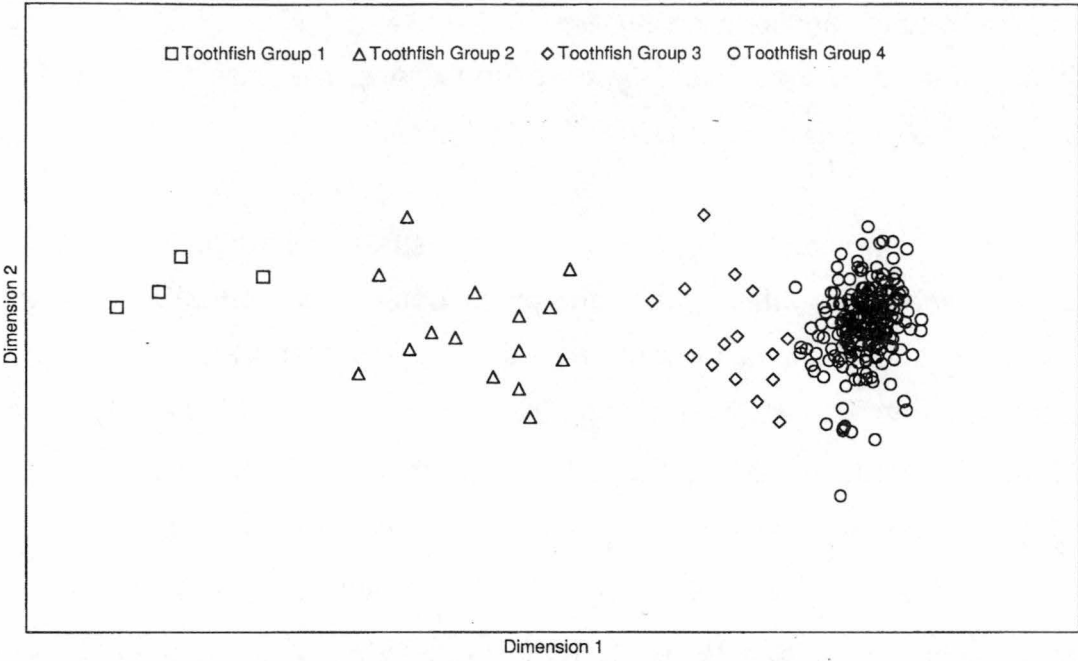
primarily due to variations in several MUFA and PUFA (Table 5.7). A number of major fatty acids (predictors) were responsible for the dissimilarity (EPA, DHA, 24:1 $\omega$ 9c, 20:1 $\omega$ 9c, 18:1 $\omega$ 7c, 18:0).

Further refinement of this separation is demonstrated by the ability to place toothfish within four arbitrary size groupings, determined from MDS (Figure 5.11). The size of toothfish contained within each grouping was as follows; group 1 (mean TL 346 $\pm$ 18mm, range 323-365mm; mean TW 350 $\pm$ 41g, range 300-400g); group 2 (mean TL 363 $\pm$ 29mm; range 310-418mm; mean TW 433 $\pm$ 105g, range 300-650g), group 3 (mean TL 423 $\pm$ 64mm; range 363-610mm; mean TW 760 $\pm$ 489g, range 400-2350g) and group 4 (mean TL 681 $\pm$ 159mm; range 376-1354mm; mean TW 3852 $\pm$ 3121g, range 450-26900g). LDA identified a low degree of similarity (2%) in the fatty acid composition of toothfish from each of these four size groups (Table 5.8). Only five predictors contributed to this segregation, including two major PUFA, EPA and DHA. Examination of only EPA and DHA using LDA produced a comparable classification (Table 5.8). This was further supported by the direct comparison of EPA and DHA levels (Figure 5.12) that revealed the grouping of toothfish by size similar to that obtained by MDS.

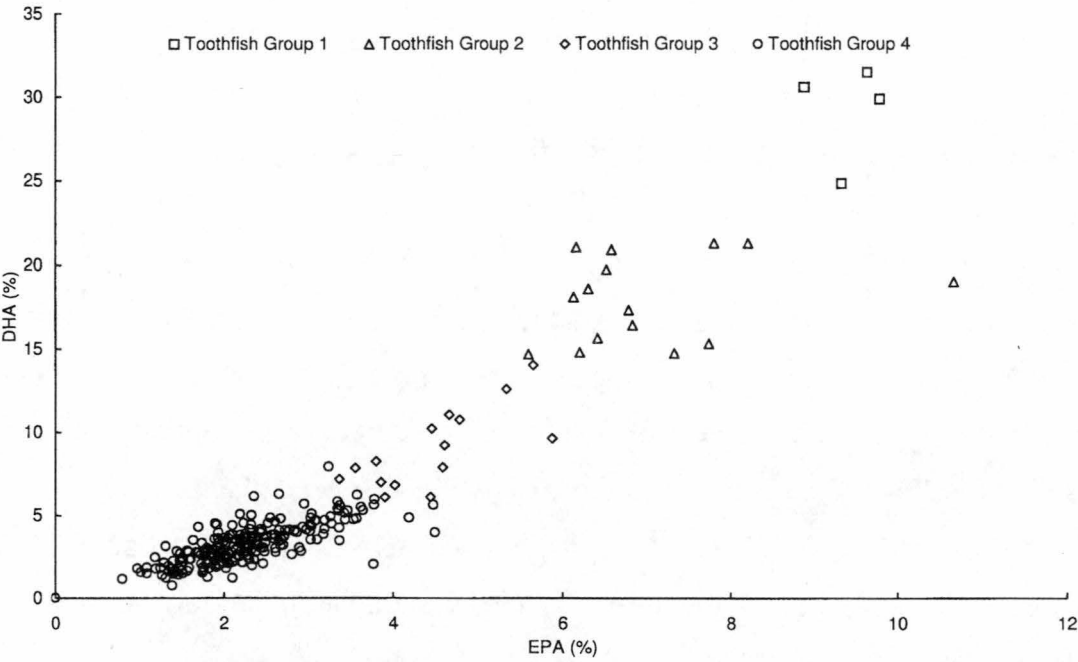
#### *Fatty acid variation relative to toothfish sex*

Fatty acid composition does not appear to differ considerably between male and female toothfish (Table 5.5). This is supported by the fact that *t*-testing identified only 11 of the 45 fatty acids as differing significantly between male and female toothfish (Table 5.6). The specific sources of variation were several PUFA (18:4 $\omega$ 3, EPA, 20:3 $\omega$ 6, DHA), MUFA (17:1, 22:1 $\omega$ 11c, 22:1 $\omega$ 7c), BrFA (i16:0, i17:1, i17:0) and the SFA 18:0. These fatty acids represented only 16% of the total fatty acid composition, although several were major fatty acids (EPA, DHA, 18:0).

Little separation of male and female toothfish into could be distinguished from the plot of MDS (Figure 5.10). Discriminant analysis of toothfish from the



**Figure 5.11** Scatterplot of multidimensional scaling (MDS) based on the total fatty acid composition of *Dissostichus eleginoides* emphasising the separation of toothfish into various size groups (group 1= mean 346mm/350g, group 2= mean 363mm/433g, group 3= mean 423mm/760g, group 4= mean 681mm/3852g)



**Figure 5.12** Comparison of the relative percent composition of EPA and DHA of *Dissostichus eleginoides* emphasising the separation of toothfish into various size groups (group 1= mean 346mm/350g, group 2= mean 363mm/433g, group 3= mean 423mm/760g, group 4= mean 681mm/3852g)

**Table 5.7** Comparison of the results of linear discriminant analysis (automatic forward stepping, F to enter=4, F to remove=3.9) of the fatty acid composition of *Dissostichus eleginoides* in relation to various fishing and biological parameters (0%=no similarity in fatty acid composition)

Parameter	Normal	Jack-knifed	Fatty Acid Predictors
Toothfish Weight	3%	4%	EPA <sup>A</sup> , DPA, 22:1ω7c, 20:3ω6, 20:1ω9c <sup>A</sup> , 18:3ω6, 24:1ω9c <sup>A</sup> , 18:0 <sup>A</sup> , 22:1ω11c, 20:2ω6, 22:4ω6
Toothfish Length	4%	4%	DPA, DHA <sup>A</sup> , 22:1ω7c, 22:4ω6, 20:3ω6, 20:4ω3, 18:1ω7c <sup>A</sup> , 24:1ω9c <sup>A</sup> , 20:2ω6, 19:1
Toothfish Sex	36%	39%	18:0 <sup>A</sup> , 22:4ω6, 22:1ω7c, AA
Fishing Season	1%	0%	a15:0, i15:0, i16:0, i17:1, a17:0, 20:3ω6, 18:4ω3, 14:0 <sup>A</sup> , 18:0 <sup>A</sup> , 22:0, 24:1ω11c, 22:1ω11c, 20:1ω7c, 20:2ω6, 19:1
Fishing Ground	10%	11%	20:1ω7c, 24:1ω9c <sup>A</sup> , 24:1ω11c, 20:4ω3, 18:4ω3, 16:1ω9c, 20:3ω6, 18:3ω6, 18:1, 22:0
Time of Day	35%	35%	16:1ω7t, 18:1ω9c <sup>A</sup>
Fishing Depth	9%	10%	20:3ω6, i17:0, 24:1ω9c <sup>A</sup> , 16:1ω9c, 20:1ω7c, 18:3ω6, 22:1ω9c <sup>A</sup> , 20:1ω9c <sup>A</sup> , 18:1, EPA <sup>A</sup> , 20:2ω6, DPA

<sup>A</sup>representing major fatty acids (>1% total fatty acid composition)

**Table 5.8.** Comparison of the percentage of correctly classified *Dissostichus eleginoides* (separated into various size groups) by linear discriminant analysis (normal and jackknifed classification, automatic, forward stepping, F to enter=4, F to remove=3.9) relative to total fatty acid composition and DHA and EPA only

Toothfish Size Group <sup>1</sup>	All Fatty Acids		EPA and DHA Only	
	Normal	Jackknifed	Normal	Jackknifed
1	0	25	0	25
2	0	0	0	0
3	7	2	7	7
4	1	1	1	2
Total	2	3	2	2
Predictors	18:3ω6, EPA, DHA, 22:4ω6, DPA		EPA, DHA	

<sup>1</sup>group 1=mean 346mm/350g; group 2=mean 363mm/433g; group 3=mean 423mm/760g; group 4=mean 681mm/3852g

1998/1999 season further supports the results of *t*-testing (Table 5.7). Significant differences in the fatty acid composition of male and female toothfish could not be detected using LDA. A high proportion of male and female toothfish shared similar fatty acid compositions (normal classification 36%; jack-knifed classification 39%). The predictors responsible for this classification were predominantly minor components, with only 18:0 representing a major fatty acid.

#### *Fatty acid variation relative to fishing season*

The fatty acid composition of toothfish varies considerably with fishing season (Table 5.9). Basis of the results of *t*-testing, significant variations were observed in 27 of the 45 component fatty acids (Table 5.6). All the BrFA and the majority of both PUFA (with the exception of 18:3 $\omega$ 6, AA, 20:2 $\omega$ 6, C<sub>21</sub> PUFA and 22:5 $\omega$ 6) and MUFA (excepting 14:1 $\omega$ 5c, 16:1 $\omega$ 9c, 16:1 $\omega$ 5c, 17:1, 18:1 $\omega$ 5c, 22:1 $\omega$ 11c and 22:1 $\omega$ 9c) accounted for the significant differences detected. Of the SFA, however, only 14:0 varied significantly. Combined, these 27 fatty acids amounted to 72.9% of the total composition.

Comparison of the fatty acid composition of toothfish from northern and southern fishing grounds using LDA reveals little similarity (Table 5.7), due to a large number of fatty acids, of which only 2 (14:0, 18:0) were major components. A clear separation based on season was also apparent from MDS plotting (Figure 5.10).

#### *Fatty acid variation relative to fishing ground*

Large variations are apparent in the fatty acid composition of toothfish from northern and southern fishing grounds (Table 5.9). *T*-tests indicate that 34 of the 45 fatty acids differed significantly between grounds (Table 5.6). Specifically, all PUFA, all SFA with the exception of 20:0 and several MUFA (14:1 $\omega$ 5c, 16:1 $\omega$ 7c, 17:1, 18:1 $\omega$ 9c, 18:1 $\omega$ 5c, 20:1 $\omega$ 7c, 22:1 $\omega$ 11c, 22:1 $\omega$ 9c, 22:1 $\omega$ 7c, 24:1 $\omega$ 11c & 24:1 $\omega$ 9c) are significantly different in toothfish from the

**Table 5.9** Fatty acid composition (% composition of total fatty acids) of *Dissostichus eleginoides* included in this study from the Macquarie Island region in relation to various fishing parameters (data presented as mean  $\pm$  standard deviation)

Fatty Acid	Fishing Season		Fishing Ground <sup>1</sup>		Time Of Day <sup>2</sup>		Fishing Depth	
	1997/1998 (n=83)	1998/1999 (n=176)	Northern (n=201)	Southern (n=58)	Night (n=132)	Day (n=127)	<1000m (n=79)	>1000m (n=180)
14:0	4.2 $\pm$ 0.4	3.2 $\pm$ 1.2	3.7 $\pm$ 1.0	2.8 $\pm$ 1.1	3.4 $\pm$ 1.1	3.6 $\pm$ 1.1	2.9 $\pm$ 1.2	3.8 $\pm$ 1.0
15:0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.3	0.4 $\pm$ 0.3	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3
16:0	14.2 $\pm$ 0.9	14.4 $\pm$ 1.3	14.3 $\pm$ 1.0	14.5 $\pm$ 1.7	14.3 $\pm$ 1.3	14.4 $\pm$ 1.1	14.6 $\pm$ 1.5	14.2 $\pm$ 1.0
17:0	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1
18:0	2.8 $\pm$ 0.5	3.1 $\pm$ 0.6	2.9 $\pm$ 0.5	3.3 $\pm$ 0.6	3.1 $\pm$ 0.6	2.9 $\pm$ 0.5	3.2 $\pm$ 0.7	2.9 $\pm$ 0.5
20:0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
22:0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.5	0.2 $\pm$ 0.3	0.4 $\pm$ 0.7	0.3 $\pm$ 0.5	0.2 $\pm$ 0.3	0.3 $\pm$ 0.6	0.2 $\pm$ 0.3
14:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.1 $\pm$ 0.2	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.3	0.2 $\pm$ 0.4	0.2 $\pm$ 0.1
16:1 $\omega$ 9c	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1
16:1 $\omega$ 7c	8.8 $\pm$ 0.7	7.8 $\pm$ 1.8	8.5 $\pm$ 1.1	6.5 $\pm$ 2.0	7.7 $\pm$ 1.8	8.5 $\pm$ 1.3	6.9 $\pm$ 2.2	8.6 $\pm$ 0.8
16:1 $\omega$ 7t	0.4 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1
16:1 $\omega$ 5c	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0
17:1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
18:1 $\omega$ 9c	33.8 $\pm$ 3.0	32.4 $\pm$ 6.2	34.3 $\pm$ 3.8	27.3 $\pm$ 6.8	31.4 $\pm$ 6.3	34.2 $\pm$ 3.8	28.3 $\pm$ 6.6	34.7 $\pm$ 3.2
18:1 $\omega$ 7c	4.9 $\pm$ 0.4	5.4 $\pm$ 0.6	5.2 $\pm$ 0.6	5.3 $\pm$ 0.6	5.2 $\pm$ 0.6	5.3 $\pm$ 0.7	5.4 $\pm$ 0.6	5.2 $\pm$ 0.7
18:1 $\omega$ 5c	0.6 $\pm$ 0.0	0.7 $\pm$ 0.4	0.7 $\pm$ 0.4	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.5	0.6 $\pm$ 0.1	0.7 $\pm$ 0.4
18:1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
19:1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.0	0.4 $\pm$ 0.1	0.4 $\pm$ 0.0
20:1 $\omega$ 11c	0.7 $\pm$ 0.2	0.7 $\pm$ 0.3	0.7 $\pm$ 0.2	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3	0.7 $\pm$ 0.2
20:1 $\omega$ 9c	7.1 $\pm$ 1.1	7.5 $\pm$ 1.7	7.3 $\pm$ 1.2	7.7 $\pm$ 2.3	7.5 $\pm$ 1.8	7.3 $\pm$ 1.2	7.6 $\pm$ 2.2	7.3 $\pm$ 1.1
20:1 $\omega$ 7c	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	0.6 $\pm$ 0.2
22:1 $\omega$ 11c	3.9 $\pm$ 3.2	3.2 $\pm$ 2.0	3.5 $\pm$ 2.7	3.0 $\pm$ 1.2	3.5 $\pm$ 2.7	3.4 $\pm$ 2.1	3.0 $\pm$ 1.1	3.6 $\pm$ 2.8
22:1 $\omega$ 9c	2.2 $\pm$ 0.5	1.8 $\pm$ 0.7	2.0 $\pm$ 0.7	1.6 $\pm$ 0.6	1.9 $\pm$ 0.6	2.0 $\pm$ 0.8	1.6 $\pm$ 0.5	2.1 $\pm$ 0.7
22:1 $\omega$ 7c	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
24:1 $\omega$ 11c	1.0 $\pm$ 0.2	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3	0.8 $\pm$ 0.3	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3
24:1 $\omega$ 9c	1.2 $\pm$ 0.2	0.9 $\pm$ 0.4	1.0 $\pm$ 0.3	1.1 $\pm$ 0.4	1.1 $\pm$ 0.4	1.0 $\pm$ 0.3	1.1 $\pm$ 0.4	1.0 $\pm$ 0.3
18:3 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
18:4 $\omega$ 3	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2
18:2 $\omega$ 6	1.2 $\pm$ 0.3	1.3 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2	1.2 $\pm$ 0.2
AA	0.6 $\pm$ 0.5	0.7 $\pm$ 0.6	0.5 $\pm$ 0.4	1.1 $\pm$ 0.8	0.8 $\pm$ 0.7	0.5 $\pm$ 0.4	1.0 $\pm$ 0.8	0.5 $\pm$ 0.4
EPA	2.2 $\pm$ 0.7	3.0 $\pm$ 1.9	2.3 $\pm$ 1.0	4.5 $\pm$ 2.2	3.2 $\pm$ 2.0	2.4 $\pm$ 1.1	4.2 $\pm$ 2.2	2.1 $\pm$ 0.7
20:3 $\omega$ 6	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
20:4 $\omega$ 3	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
20:2 $\omega$ 6	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
C <sub>21</sub> PUFA	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0
22:5 $\omega$ 6	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
DHA	3.5 $\pm$ 1.2	5.4 $\pm$ 5.9	3.4 $\pm$ 2.1	9.9 $\pm$ 8.1	6.0 $\pm$ 6.3	3.5 $\pm$ 2.4	8.9 $\pm$ 7.4	3.0 $\pm$ 1.2
22:4 $\omega$ 6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0
DPA	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1
SFA	22.4 $\pm$ 1.2	21.9 $\pm$ 2.0	22.1 $\pm$ 1.7	21.9 $\pm$ 2.2	22.1 $\pm$ 1.8	22.1 $\pm$ 1.8	22.0 $\pm$ 2.0	22.1 $\pm$ 1.8
BrFA	0.5 $\pm$ 0.1	0.9 $\pm$ 0.5	0.8 $\pm$ 0.5	0.9 $\pm$ 0.5	0.7 $\pm$ 0.4	0.9 $\pm$ 0.5	1.0 $\pm$ 0.5	0.7 $\pm$ 0.5
MUFA	67.6 $\pm$ 2.5	64.5 $\pm$ 9.0	67.7 $\pm$ 4.0	57.8 $\pm$ 11.9	63.8 $\pm$ 9.5	67.3 $\pm$ 4.6	59.1 $\pm$ 11.1	68.3 $\pm$ 2.7
PUFA	9.5 $\pm$ 2.5	12.6 $\pm$ 8.5	9.5 $\pm$ 3.6	19.3 $\pm$ 11.2	13.4 $\pm$ 9.1	9.8 $\pm$ 4.0	17.8 $\pm$ 10.5	8.9 $\pm$ 2.3
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

AA=arachidonic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; SFA=saturated fatty acid; BrFA=branched fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond orientation; <sup>1</sup>refer to main body of text for location of fishing grounds; <sup>2</sup>0600-1800hrs (day) 1800-0600hrs (night)

northern and southern grounds. These 34 fatty acids amounted to 84.5% of the total composition.

Discriminant analysis similarly points to a significant variation in fatty acid composition with fishing ground (Table 5.7), an observation also supported by MDS analysis (Figure 5.10). Only one major fatty acid (24:1 $\omega$ 9c) was included amongst the predictors of this classification.

#### *Fatty acid variation relative to time of day*

Toothfish caught at different times of the day exhibited an intermediate variation in fatty acid composition, relative to the other sampling parameters (Table 5.9). *T*-tests indicated that 17 of the 45 fatty acids detected differ significantly in toothfish caught during the day compared to the night (Table 5.6). Fatty acids responsible were a number of PUFA (18:3 $\omega$ 6, AA, EPA, 20:3 $\omega$ 6, 20:4 $\omega$ 3, DHA, 22:4 $\omega$ 6, DPA) and MUFA (16:1 $\omega$ 7c, 16:1 $\omega$ 7t, 17:1, 18:1 $\omega$ 9c, 24:1 $\omega$ 11c, 24:1 $\omega$ 9c) and 18:0, equal to 56.3% of the total composition.

LDA also failed to reveal as strong an association, as was observed for other parameters, between the time of day at which toothfish were collected and fatty acid composition (Table 5.7). A relatively high degree of similarity in fatty acid composition was observed in toothfish collected during the day and night (normal classification 35%; jack-knifed classification 35%). Classification was due to two fatty acids, of which only 18:1 $\omega$ 9c represented major components. MDS also failed to clearly separate toothfish (Figure 5.10).

#### *Fatty acid variation relative to fishing depth*

A strong relationship existed between fatty acid composition and the depth at which toothfish were caught (Tables 5.9). Significant differences were detected in 33 of the 45 fatty acids by *t*-testing (Table 5.6). Specifically the majority of PUFA (excepting 18:4 $\omega$ 3), SFA (excluding 20:0), MUFA (aside

from 14:1 $\omega$ 5c, 16:1 $\omega$ 5c, 18:1 $\omega$ 5c, 18:1, 19:1, 20:1 $\omega$ 11c, 20:1 $\omega$ 9c & 24:1 $\omega$ 9c) and BrFA (excepting i15:0 & a15:0) differed significantly with increasing fishing depth. Combined, these 33 fatty acids amounted to 87.7% of total composition, including a large number of major components.

LDA supports this correlation (normal classification 9%; jack-knifed classification 10%) due to differences in many MUFA and PUFA and the BrFA i17:0 (Table 5.7). Amongst these fatty acids were three major MUFA (20:1 $\omega$ 9c, 22:1 $\omega$ 9c and 24:1 $\omega$ 9c). MDS analysis, however, failed to distinguish clear differences in fatty acid composition between the two fishing depths (Figure 5.10).

## 5.4 DISCUSSION

The toothfish examined in this study show significant variation in total lipid content, fatty acid composition and, to a lesser extent, lipid class composition. In an attempt to determine which factors may have contributed to these variations, the effect of several different sampling parameters on lipid composition was assessed. The majority of biological (size, to a lesser extent sex) and fishing related parameters (ground, season, time of day, depth) examined in this study were associated with considerable variations in lipid composition. However, the fishing parameters investigated, on their own, are unlikely to have had such a dramatic and direct effect on lipid composition.

Rather, it is the size of toothfish (total length and weight) that exerts the greatest influence on lipid composition. Toothfish included in this study were chosen with the aim of, as closely as possible, serving as an accurate reflection of the Macquarie Island toothfish population. Of particular importance is size structure, especially given the possibility that toothfish diet, and therefore the intake of dietary lipids, is likely to vary significantly with size. However, several of the fishing parameters examined in this study are associated with significant differences in toothfish size, particularly season

and depth. The disproportionate size distribution of toothfish between the different seasons, grounds and fishing depths is the more likely cause of the variation observed. When examining differences in lipid composition in relation to the various fishing parameters, it is therefore essential to consider the size distribution of toothfish included in this study.

### **Variation in toothfish size in relation to various fishing parameters**

The overwhelming majority of toothfish specimens analysed in this study are substantially smaller than the known maximal size of toothfish. Few toothfish measuring over 1000mm were analysed and the length of the longest fish (1354mm) is considerably lower than the known maximum length attained by toothfish (~2000mm). The mass of the heaviest fish analysed (~26kg) is similarly substantially less than the upper size limit of toothfish (~100kg). Notwithstanding, the length and weight of toothfish analysed are consistent with the size distribution previously determined for the entire Macquarie Island toothfish population during 1998 (Constable et al. 2001). Catches in this season were dominated by fish of less than 1100mm, mostly between 500-900mm, similar to the overall distribution of toothfish included in this study (Figure 5.2).

Several other results are consistent with the known biology of toothfish. In agreement with studies of other toothfish populations, where the occurrence of sexual dimorphism is yet to be encountered, sex has no influence on size. Similarly, the significant size increase of toothfish with increasing depth in this study (Table 5.2 and Figure 5.4) is similar to the findings from several other toothfish populations. However a number of the other significant, and to some extent unexpected, variations in toothfish size require further explanation. Due to sampling and logistical constraints, a relatively small number of toothfish were included in this study (n=259), calling for a cautious assessment of size distribution. Although the results of this study suggest that the size of toothfish differ significantly in relation to season and location, such an assessment should be treated with care. For example although toothfish are significantly larger in the 1997/1998 season compared to 1998/1999, this is most likely a consequence of sampling bias. In particular,

studies of other toothfish populations (as well as the toothfish sampled in this study) have determined that toothfish size increases with fishing depth (e.g. Williams and de la Mare 1995; Agnew et al. 1999), meaning that variations in trawl depth will significantly effect overall size distribution. Examination of trawl depths in each fishing season reveals that in the 1997/1998 season over 90% of trawls were greater than 1000m, whilst in 1998/1999 this figure dropped to ~60%. The substantial bias toward deeper trawl depths in the 1997/1998 season is therefore reflected in the significantly larger size of toothfish in this season relative to those from 1998/1999. Other potential sources of bias include differences in the sample sizes for each season (1997/1998  $n=83$ ; 1998/1999  $n=176$ ). The same is true with regards to the significant difference in toothfish length between the northern and southern fishing grounds. In the northern ground, ~90% of all fish were sampled from trawl depths in excess of 1000m, whilst in the southern ground all toothfish were taken from trawl depths of less than 1000m. Once again, this is reflected in the presence of significantly longer and considerably heavier fish in the northern ground compared to the southern. Alternatively, if each of the fishing grounds is highly localised, as genetic (Reilly et al. 2001) and tag-release (Williams and Lamb 2001) studies suggest, then regional differences could conceivably lead to differences in size distribution. However, the maximum depths of each fishing ground are similar (~4000m), suggesting that size distribution in relation to depth between grounds would be comparable. The uneven size distribution of toothfish relative to several of the sampling parameters included in this study must therefore be taken into account when considering the reasons behind the observed variations in lipid composition.

### **Age and sexual maturity**

The age-length relationship formulated by Constable et al. (2001) for toothfish at Macquarie Island places the age of toothfish analysed in the current study in the range 3 to 22 years. Based upon an age estimate of 12-15 years for sexual maturity (equivalent to approximately 900mm), ~90% ( $n=229$ ) of the toothfish samples analysed in this study are likely to be

sexually immature. This virtually precludes the influence that reproductive potential may or may not have on lipid composition. The absence of sexually mature gonads in toothfish from the 1998/1999 season supports this assumption. Although the gonad state of fish from season 1997-1998 was not assessed, it seems likely that all but the larger fish analysed from this season (i.e. those longer than 900mm) are sexually immature.

### **Total lipid content and lipid class composition**

With the exception of fishing season, all the parameters assessed in this study produced a significant variation in total lipid content. The greatest influence on this variation appears to be the length and weight of toothfish. Total lipid content increased by an average of approximately 10% (range 1-25% wet weight) from the smallest to the largest sized specimens, indicating that proportionally more lipid is being deposited into storage tissue as toothfish age. Assuming that size is the primary influence on lipid content, the variations in lipid content observed with respect to the other parameters are largely a reflection of differences in toothfish size. For instance, the significant difference in lipid composition between the two fishing grounds can be partly explained by the predominance of larger, lipid-rich toothfish in the northern ground (Tables 5.2 and 5.3) rather than simply a dietary influence specifically related to geographical separation.

Lipid deposits are dominated by TAG (mean=91.6%) although lipid class composition varies markedly in relation to many of the sampling parameters examined. Once again, many of the significant variations in lipid class observed are likely to be mainly a consequence of toothfish size. TAG content varies by approximately 20% (range 33-99%) relative to toothfish size and is associated with a concomitant variation in FFA levels (Table 5.4). Rather than a direct dietary influence however, the high FFA content of smaller toothfish may be due to degradation of these samples post-storage.

*Role of lipid in buoyancy regulation*

The trend of increasing levels of TAG-rich lipid with increasing size is observed in many southern ocean species, including a number of nototheniids. For example the lipid content of toothfish is comparable to the levels observed in whole specimens of the nototheniid *Pleuragramma antarcticum* (Friedrich and Hagen 1994) and the Myctophidae *Electrona carlsbergi* and *Protomyctophum bolini* (Reinhardt and Van Vleet 1986). Accumulation of lipid in these and other Southern Ocean species is thought to be related to the regulation of neutral buoyancy, due mainly to the low density of lipid relative to seawater (e.g. Eastman 1988; Friedrich and Hagen 1994; Phleger 1998). Neutral buoyancy allows fish to maintain a constant position in the water column without the need to actively swim, a distinct energetic advantage (Phleger 1998). Many fish achieve neutral buoyancy by employing a swimbladder, a structure absent in toothfish (Eastman 1993).

Toothfish consequently require an alternate means of attaining and sustaining neutral buoyancy, specifically an adaptation in one or more body systems (Eastman 1988). The deposition of lipid could conceivably fill this requirement, particularly because the total lipid content of larger toothfish is at the higher end of the spectrum for Southern Ocean fish species. The increase in lipid content with toothfish size (also observed in several fish species analysed in Chapter 2) may reflect the need to compensate for the greater density of larger fish, in order to ensure the maintenance of neutral buoyancy (Friedrich and Hagen 1994)

*TAG as a store of energy*

Although the large lipid stores of toothfish could easily provide the static lift needed for neutral buoyancy, other factors must also be considered. For example, many of the fish that are assumed to use lipids as buoyancy regulators, including myctophids, favour the deposition of WE over TAG (e.g. Phleger et al. 1997; Phleger et al. 1999a). This is thought to be because of the slower metabolic turnover of WE relative to TAG, along with the fact that

WE provides more static lift than the equivalent amount of TAG. However, nototheniids, as a rule, do not contain appreciable amounts of WE. In contrast, toothfish may store significant quantities of TAG-rich lipid within tissue for use as an energy reserve when the availability of food is limited. The annual variation in primary production in the Macquarie Island region is, however, less dramatic than the episodic seasonal production of polar regions (Goldsworthy et al. 2001a). This seemingly excludes the availability of food as a limiting factor in toothfish survival. Significantly though, the proportion of toothfish captured in the Macquarie Island region with stomachs containing prey items is low. Goldsworthy et al. (2002) found that, based on the results of 3 fishing seasons at Macquarie Island (1995/96, 1997/1998, 1998/1999) on average only 58.6% of the toothfish sampled contained stomach contents and, of these, only 1% of contents were classified as fresh. Although this can be explained in part by the tendency of toothfish to regurgitate their stomach contents during capture (Pilling et al. 2001), foraging events may in fact be episodic, necessitating a reserve of energy in the form of lipid.

#### *Influence of sex on lipid content*

The onset of sexual maturity is also accompanied by a proportionally greater increase in the lipid content of one sex compared to the other in several fish species, including the nototheniids *Bathyrdraco marri* (Friedrich and Hagen 1994) and *Notothenia coriiceps* (Kamler et al. 2001). This enrichment is primarily due to the increased energy demand involved in gonad development. The lipid content of male toothfish is significantly greater than that of females, suggesting that sex influenced the total lipid content of toothfish included in this study. However, of the 176 toothfish from the 1998/1999 season for which sex was determined, none were found to be sexually mature. The variation observed can also not be explained by size differentiation between the sexes (Table 4.2). The variation observed may conceivably be due to a difference in the lipid demand of sexually immature males compared to females, related to factors such as relative gonad size or energetic requirements.

### Fatty acid composition

Fatty acid composition is affected by the same sampling bias observed with respect to lipid content and class composition. Apparent differences in fatty acid composition due to the fishing parameters examined are predominantly a reflection of size-disparity. This is supported by statistical evidence that shows that the composition of not only the vast majority of fatty acids differs between small and large toothfish, but that these fatty acids in the main are major components. As expected sex had no significant effect on fatty acid composition and, unlike the fishing parameters, this parameter was not influenced by size differences between male and females.

The major trend observed is a decrease in total PUFA and proportional increase in total MUFA with increasing toothfish size. The greatest variation apparent is the significant decrease in the relative proportions of EPA and DHA in larger toothfish, both of which varied by approximately 10%. As previously discussed in relation to midwater fish from Macquarie Island (Chapter 2), variations in essential PUFA could be related to a change in diet as toothfish mature. The likelihood of this is discussed further in Chapter 7. However, factors other than those related to diet cannot be discounted. Decreases in PUFA can partially be explained by the increase in total lipid content, and accompanying proportional drop in PL levels (Figure 5.8), in these larger specimens. Marine fish tend to selectively deposit PUFA such as DHA and EPA into the PL fraction in order to maintain membrane fluidity (Dunstan et al. 1999; Cahu et al. 2000). The increased deposition of TAG-rich lipid in aging toothfish leads to a concomitant decrease in PL and with it the relative levels of PUFA. A similar size-related shift in fatty acid composition has been reported for other wild fish populations (e.g. Budge et al. 2002; Iverson et al. 2002), including those of *Stomias gracilis*, *Bathylagus antarcticus* and *Borostomias antarcticus* examined in this study from Macquarie Island (Chapter 2).

Comparison with the results of previous studies is difficult given that the fatty acid composition of toothfish has only rarely been reported. Nichols et al. (1994a) determined the fatty acid composition of toothfish from Heard Island.

Although the TL and TW of this specimen was not reported, EPA (8.4%) and DHA (20.3%) levels are at the higher end of the results range determined in the current study (corresponding to a specimen with approximately 450g TW and 400mm TL), suggesting that similarly high levels of PUFA exist in toothfish populations other than Macquarie Island. Brown et al. (1999) determined the fatty acid composition of a single toothfish specimen (1189.0g TW) as part of a signature lipid study of Antarctic fur seals (*Arctocephalus gazella*) and Southern elephant seals (*Mirounga leonina*). The level of DHA in this specimen (5.0%) is roughly equivalent to that of similar sized toothfish in the current study (approximately  $3.2 \pm 1.4\%$ ). However, the relative proportion of EPA (9.8%) in the specimen examined by Brown et al. (1999) exceeds the levels of equivalent sized toothfish in the current study ( $2.3 \pm 0.7\%$ ) and is more in line with EPA levels observed in specimens of less than 1000g TW ( $5.1 \pm 2.4\%$ ). Whether or not this difference in fatty acid composition could be a consequence of regional variations in toothfish diet is difficult to assess given the limited sample size of Brown et al. (1999).

## **5.5 CONCLUSIONS**

The lipid composition of Patagonian toothfish from the Macquarie Island region was examined in relation to a number of fishing and biological parameters. Although total lipid content, lipid class composition and fatty acid composition appear to vary significantly in relation to many of these parameters, the majority of the variation observed is a consequence a disproportionate distribution of toothfish sizes within these parameters. Determining the potential impact of the various fishing parameters, particularly fishing season, ground and depth, is therefore difficult with the current data set. The overriding influence of size is evidenced by the fact that variation in all aspects of lipid composition is greatest in relation to variations in toothfish TL and TW.

The accumulation of TAG-rich lipid with increasing size is a common feature of marine fish and is most likely related to maintaining neutral buoyancy in swimbladder-less species such as toothfish. The dramatic increase in MUFA and decrease in PUFA in larger fish may point to a possible dietary influence. This is particularly true with regards to the large variations in the levels of essential PUFA such as DHA (up to 30%) and EPA (up to 10%) that must have originated from mainly dietary sources.

# CHAPTER 6

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## LIPID COMPOSITION OF ANTARCTIC TOOTHFISH: COMPARISON WITH PATAGONIAN TOOTHFISH AND POTENTIAL PREY

### 6.1 INTRODUCTION

Fish of the family Nototheniidae dominate the fish fauna of the Antarctic, representing approximately 100 predominately endemic species. The largest of this group is the Antarctic toothfish (*Dissostichus mawsoni*). The immense size of *D. mawsoni*, capable of reaching lengths of up to 175cm in length and 100kg in weight (Eastman and DeVries 2000; La Mesa and Vacchi 2001) is comparable to that of it's sub-Antarctic congener, *Dissostichus eleginoides* (Patagonian toothfish), but is unusual amongst the majority of Antarctic and sub-Antarctic fish.

Populations of Antarctic and Patagonian toothfish are sharply divided along the boundary of the Antarctic Convergence, though a small degree of overlap has resulted in misidentification in some regions (Vukhov 1972). Detailed catch data on *D. mawsoni* is lacking, however distribution appears to be circumpolar. Large populations are thought to aggregate in polar waters close to the Antarctic continent south of 60°. Populations in the Weddell Sea and particularly McMurdo Sound have provided much of the information currently available for this species. Biomass appears to vary greatly between summer and winter. Ekau (1990) reported an almost complete absence of *D. mawsoni* in the southern Weddell Sea during winter compared to the relatively high numbers observed during summer. Within the water column, *D. mawsoni* does not appear to reach the great depths inhabited by

Patagonian toothfish. In summer, *D. mawsoni* is found at depths of between 420-670m in the Weddell Sea (Ekau 1990).

Due to their relatively large size, predation on *D. mawsoni* is thought to be restricted mainly to marine mammals. Weddell seals consume *D. mawsoni* in McMurdo Sound (Dearborn 1965; Eastman 1988; Burns et al. 1998) and, more occasionally, in the Weddell Sea (Plotz 1986). The extent of predation by Weddell seals varies greatly, although most studies agree that the nototheniid *Pleuragramma antarcticum* far outweighs *D. mawsoni* in dietary importance (e.g. Testa et al. 1985). Other mammalian predators include Killer whales in McMurdo Sound (Eastman 1985; Eastman 1988) and Sperm whales closer to the Antarctic Convergence (Vukhov 1972).

Classified as a mid-water predator, *D. mawsoni* is believed to rely on a low diversity of prey species including a range of fish, cephalopods and epibenthic species such as mysid and caridean shrimps (Vukhov 1972; Eastman 1988). *P. antarcticum* is the main species consumed by weight in McMurdo Sound (Eastman 1985). In contrast to most other Antarctic nototheniids, reliance upon krill (*Euphausia superba*) by adult fish is thought to be low (Eastman 1985).

Like *D. eleginoides*, determining the lipid composition of *D. mawsoni* may potentially expand current understanding of the dietary interactions of this species in the Southern Ocean. The growing threat posed by commercial fishing (e.g. Horn 2002; Parker et al. 2002; Willock 2002) adds to the need for investigation into all aspects of *D. mawsoni* biology in order to minimise negative impacts on the Antarctic ecosystem. The lipid composition of *D. mawsoni* has been investigated almost entirely with respect to the role these compounds play in functions such as the maintenance of buoyancy (Clarke et al. 1984; Eastman 1985; Eastman 1988). Research to date has focussed primarily on proximate composition (lipid class and content) and its distribution throughout the body rather than the identification of fatty acids. As a consequence, the fatty acid composition of *D. mawsoni* has been determined only rarely (e.g. Ohno et al. 1976; Nachman 1985).

The purpose of this study is therefore to determine the lipid composition of *Dissostichus mawsoni* from waters adjacent to Casey Station, Antarctica and assess the applicability of these findings to dietary studies. This will firstly involve comparison with the lipid composition of potential prey species from the high-Antarctic. Secondly, the effect of regional differences in diet will be assessed using a comparison of the lipid composition of the cogenetic species *Dissostichus eleginoides* from the Macquarie Island region.

## 6.2 MATERIALS AND METHODS

### Sample Collection

*Dissostichus mawsoni* samples (n=12) were collected as part of scientific activities of the Australian Antarctic Division (AAD, Kingston, Tasmania, Australia). Fishing took place over the Southern Hemisphere summer of 1998-99 in waters adjacent to Casey Station (66°17'S; 110°32'E), a research base located on the Antarctic continent and maintained by the AAD.

After collection, whole specimens were frozen at -20°C and analysed within 3 months where possible. Approximately 1-2g of white muscle from at the base of the caudal fin was later removed for lipid analysis. Care was taken to avoid the thick subcutaneous oil layer.

### Lipid extraction and class determination

Total lipids were extracted using the one-phase Bligh and Dyer method (1959). After phase separation, total lipids were recovered from the lower chloroform phase and concentrated *in vacuo* (rotary evaporator, 40°C). Samples were stored at -20°C prior to analysis. Lipid class profiles were determined by TLC-FID using an Iatroscan MkV TH10 analyser (Volkman and Nichols 1991). Wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA), sterol (ST) and polar lipid (PL) components were resolved in a polar solvent system consisting of hexane-diethyl ether-acetic acid (60:17:0.2 v/v). The presence or absence of WE and diacylglycerol ethers (DAGE) was

confirmed by the use of a non-polar solvent system (hexane-diethyl ether; 96:4 v/v). Peak areas were quantified using commercially available chromatography software (DAPA Scientific Software, Kalamunda, Western Australia).

### **Fatty acid determination**

Fatty acid methyl esters (FAME) were prepared by addition of methanol (MeOH), concentrated hydrochloric acid (HCl) and chloroform ( $\text{CHCl}_3$ ) (3mL 10:1:1, v/v/v, 80°C/2hr) to an aliquot of the total lipid. After cooling and the addition of 1mL of water, resulting FAME were extracted (3x) with 4:1 v/v hexane/ $\text{CHCl}_3$ . Chloroform containing  $\text{C}_{19}$  FAME internal standard was then added. FAME fractions were treated with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, 50 $\mu\text{L}$ , 60°C, 2hr) to convert any sterols present to their corresponding trimethylsilyl (TMS) ethers.

Gas chromatographic (GC) analysis was performed on a Hewlett Packard 5890A GC equipped with a flame ionisation detector, fitted with a cross-linked methyl silicone (HP5) fused silica capillary column (50 m length  $\times$  0.32  $\mu\text{m}$  internal diameter), employing hydrogen as the carrier gas. Samples were injected (HP7673A auto-injector) at 50°C in the splitless mode, with a 2-min. venting time (Nichols et al. 1993). The injector and detector were maintained at 290°C and 310°C respectively. After 1 min. the oven was raised to 150°C at 30°C  $\text{min}^{-1}$ , then to 250°C at 2°C  $\text{min}^{-1}$  and finally to 300°C at 5°C  $\text{min}^{-1}$  (Nichols et al. 1993). Peak areas were quantified using Millennium 32 Chromatography Manager (Waters Corporation, Milford, Massachusetts, USA). Identification of individual fatty acids and sterols was based upon a comparison of relative retention times with those obtained from previous studies in addition to authentic and laboratory standards. GC-mass spectrometry (Finnigan Thermoquest GCQ fitted with an on-column injector and configured as above) was used for confirmation of components from representative samples.

### **Statistical analyses**

Statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, Illinois, USA). Variations in total lipid content, lipid class and individual fatty acid compositions between Antarctic and Patagonian toothfish were compared using two-sample *t*-tests (95% confidence interval).

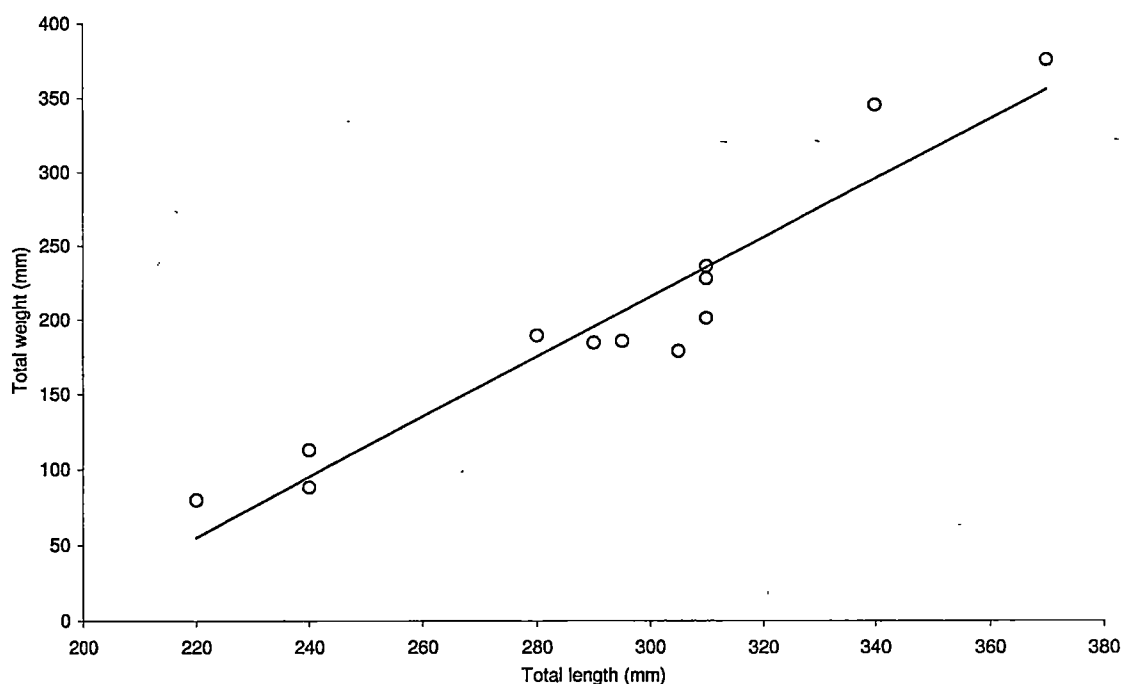
Multivariate statistics were also used to analyse fatty acid composition. Non-metric multi-dimensional scaling (MDS; Kruskal loss function) was employed to compare the fatty acid composition of Antarctic toothfish in two dimensions to assess whether groupings existed within the data set. MDS was also used as a comparative tool for examining differences in fatty acid composition between Antarctic and Patagonian toothfish.

Linear discriminant analysis (LDA; tolerance=0.01, complete estimation, classification and jackknifed classification matrix, Wilk's lambda) was used to determine if Antarctic and Patagonian toothfish could be differentiated on the basis of fatty acid profiles alone. LDA also allowed for the identification of those variables (fatty acids and lipid classes) most responsible for the differences amongst groups. Automatic forward stepping (F to enter=4, F to remove=3.9) was used when analysing fatty acid composition. The jackknifed classification matrix is included as a means of cross-validating the normal classification matrix. Considerable difference in the percentage of correct classifications between these matrices would suggest potential difficulties in correctly classifying the data.

## **6.3 RESULTS**

### **Size of Antarctic toothfish**

The total weight (TW, wet weight) of Antarctic toothfish analysed, ranged from 88.2 to 376.1g. Total length (TL, measured from the tip of snout to the tip of the caudal fin) ranged from 240 to 370mm. A linear relationship between TL and TW was observed (Figure 6.1).



**Figure 6.1** Relationship between total length (TL) and total weight (TW) of *Dissostichus mawsoni* samples from waters adjacent to Casey Station, Antarctica

### Lipid content and class composition

Total lipid content was low across all *D. mawsoni* samples analysed, (mean  $1.0 \pm 0.2\%$ ; range 0.7-1.3%) and appears to be independent of both TL and TW (Table 6.1).

The lipid class composition of all specimens (Table 6.1) was dominated by triacylglycerol (TAG, mean  $77.8 \pm 5.1\%$ ). The relative level of TAG ranged from 66.2 to 84.6%. Polar lipids (PL) contributed from 6.5-27.0% of the overall composition (mean  $13.1 \pm 5.9\%$ ), whilst sterols (ST, predominantly free cholesterol based upon GC analysis) were present in smaller amounts (mean  $6.7 \pm 1.1\%$ ). Wax ester (WE) was also present as a minor component (mean  $2.4 \pm 1.2\%$ ; range 1.1-4.7%). FFA was not detected in any of the specimens analysed. No relationship was observed between either TL or TW and the content of any lipid class.

**Table 6.1** Size (wet weight), total lipid content (percent composition, wet weight) and lipid class composition (percentage of total lipid content) of *Dissostichus mawsoni* from waters adjacent to Casey Station, Antarctica

Specimen number	Size		Lipid content and class				
	TL	TW	% Lipid	WE	TAG	ST	PL
1	310.0	228.2	1.3	n/a	n/a	n/a	n/a
2	310.0	236.5	0.7	1.8	75.6	8.9	13.7
3	290.0	184.8	1.0	2.0	79.5	7.8	10.7
4	220.0	79.9	0.9	1.6	83.5	6.8	8.1
5	305.0	179.3	0.9	2.9	84.6	6.1	6.5
6	295.0	185.9	0.8	1.8	74.9	8.0	15.3
7	340.0	345.7	1.0	1.4	78.2	6.3	14.1
8	240.0	88.2	1.0	4.2	79.4	6.1	10.3
9	240.0	112.8	1.0	1.8	66.2	5.0	27.0
10	280.0	189.8	1.3	4.7	79.0	7.2	9.1
11	310.0	201.4	0.9	2.8	81.4	6.2	9.6
12	370.0	376.1	1.0	1.1	73.6	5.8	19.6
Mean±SD	292.5±42.9	200.7±90.4	1.0±0.2	2.4±1.2	77.8±5.1	6.7±1.1	13.1±5.9

WE=wax ester; TAG=triacylglycerol; ST=sterol (predominantly cholesterol, may also contain trace amounts of diacylglycerol); PL=polar lipid; SD=standard deviation; n/a=not analysed; TL=total length (mm); TW=total weight (g)

### Fatty acid composition

Fatty acid composition was dominated by polyunsaturated fatty acids (PUFA, mean  $56.5 \pm 5.5\%$ ; range 45.9-63.0%), the proportion of which varied considerably between specimens (Table 6.2). The separation of specimens by multidimensional scaling (MDS) supports this observation and is principally due to differences in the composition and relative levels of PUFA (Figure 6.2). The PUFA 22:6 $\omega$ 3 (DHA, docosahexaenoic acid) was both the most prevalent fatty acid (mean  $32.7 \pm 5.0\%$ ) and accounted for the majority of variation between specimens (range 26.0-39.1%). EPA (eicosapentaenoic acid, 20:5 $\omega$ 3) also represented an important PUFA (mean  $17.9 \pm 1.9\%$ ), although levels varied considerably less than for DHA (range 14.7-18.9%).

Monounsaturated fatty acids (MUFA, mean  $21.7 \pm 5.8\%$ ; range 16.4-34.0%) and saturated fatty acids (SFA, mean  $21.6 \pm 3.0\%$ ; range 18.6-23.9%) contributed approximately equal portions to the total fatty acid composition. The MUFA 18:1 $\omega$ 9c (mean  $8.8 \pm 2.6\%$ ; range 5.5-12.7%), 18:1 $\omega$ 7c (mean  $5.3 \pm 0.5\%$ ; range 4.8-6.4%) and 16:1 $\omega$ 7c (mean  $3.1 \pm 1.3\%$ ; range 1.8-5.2%)

**Table 6.2** Fatty acid composition (% composition of total fatty acids) of *Dissostichus mawsoni* specimens included in this study from waters adjacent to Casey Station, Antarctica

Fatty acid	<i>Dissostichus mawsoni</i> specimen												Mean
	1	2	3	4	5	6	7	8	9	10	11	12	±sd
14:0	1.1	0.7	0.6	0.2	1.1	0.5	1.4	0.6	1.6	1.0	0.8	1.2	0.9 ± 0.4
15:0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2 ± 0.0
16:0	13.1	16.3	16.7	16.5	14.7	15.6	13.9	18.6	23.6	15.3	18.7	16.1	16.6 ± 2.8
17:0	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2 ± 0.1
18:0	3.2	3.8	3.8	3.4	3.3	3.9	2.9	4.0	3.8	3.3	4.0	3.9	3.6 ± 0.4
20:0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.1
22:0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.1
16:1ω9c	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.4	0.2 ± 0.1
16:1ω7c	3.4	3.5	1.9	1.8	3.1	1.9	4.2	1.9	2.4	5.2	2.5	5.2	3.1 ± 1.3
16:1ω7t	0.4	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.4	0.3	0.2	0.3 ± 0.1
16:1ω5c	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3 ± 0.0
17:1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.1
18:1ω9c	12.7	7.3	8.1	5.5	7.9	6.1	12.4	6.5	7.8	11.2	7.4	12.4	8.8 ± 2.6
18:1ω7c	5.0	4.8	4.5	5.8	5.0	5.2	5.0	5.3	5.9	6.4	5.1	5.2	5.3 ± 0.5
18:1ω5c	0.4	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2 ± 0.1
18:1	0.3	0.0	0.0	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.2	0.3	0.2 ± 0.1
19:1	0.3	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.1	0.3	0.2	0.3	0.2 ± 0.1
20:1ω11c	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1 ± 0.1
20:1ω9c	6.6	1.0	1.4	0.6	0.9	0.7	1.9	0.7	0.9	1.2	0.7	1.5	1.5 ± 1.7
20:1ω7c	0.4	0.1	0.1	0.3	0.2	0.2	0.2	0.2	0.1	0.7	0.2	0.2	0.3 ± 0.2
22:1ω11c	1.7	0.3	0.1	0.0	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.2	0.2 ± 0.5
22:1ω9c	0.8	0.1	0.2	0.1	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.4	0.3 ± 0.2
22:1ω7c	0.2	0.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1 ± 0.0
24:1ω11c	0.0	0.9	0.9	0.7	0.9	0.9	0.9	0.4	0.4	0.4	0.4	0.6	0.6 ± 0.3
24:1ω9c	0.8	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1 ± 0.2
18:3ω6	0.1	1.0	1.1	0.5	1.2	0.7	1.7	0.1	0.1	0.1	0.1	0.2	0.6 ± 0.6
18:4ω3	0.9	0.1	0.1	0.1	0.2	0.1	0.2	0.7	1.2	1.4	0.9	1.9	0.7 ± 0.6
18:2ω6	1.1	1.2	0.9	0.6	0.8	0.7	1.0	0.8	1.5	0.9	0.8	1.5	1.0 ± 0.3
AA	1.5	2.2	2.2	2.3	2.1	3.2	1.7	2.0	1.2	1.9	2.2	2.4	2.1 ± 0.5
EPA	14.7	22.5	17.9	18.9	17.3	19.7	16.2	17.6	17.7	18.7	16.8	17.2	17.9 ± 1.9
20:3ω6	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1 ± 0.0
20:4ω3	0.2	0.3	0.3	0.2	0.2	0.2	0.4	0.2	0.2	0.4	0.2	0.4	0.3 ± 0.1
20:2ω6	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0 ± 0.1
C <sub>21</sub> PUFA	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1 ± 0.2
22:5ω6	0.2	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1 ± 0.1
DHA	26.0	30.5	37.5	39.1	37.4	36.7	31.7	37.2	28.2	27.1	34.9	26.1	32.7 ± 5.0
22:4ω6	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1 ± 0.0
DPA	1.0	0.9	0.1	1.0	0.9	1.3	0.8	0.9	0.7	1.1	1.0	0.9	0.9 ± 0.3
SFA	18.6	21.2	21.4	20.5	19.5	20.3	18.6	23.6	29.5	20.1	23.9	21.5	21.6 ± 3.0
BrFA	1.5	0.1	0.0	0.1	0.2	0.1	0.4	0.1	0.2	0.4	0.6	0.1	0.3 ± 0.4
MUFA	34.0	18.9	18.3	16.4	20.0	16.7	26.9	16.7	19.3	27.6	18.2	27.6	21.7 ± 5.8
PUFA	45.9	59.7	60.2	63.0	60.3	63.0	54.0	59.6	51.0	51.9	57.3	50.7	56.4 ± 5.5
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0 ± 0.0

AA=arachidonic acid (20:4ω6); EPA=eicosapentaenoic acid (20:5ω3);

DHA=docosahexaenoic acid (22:6ω3); DPA=docosapentaenoic acid (22:5ω3);

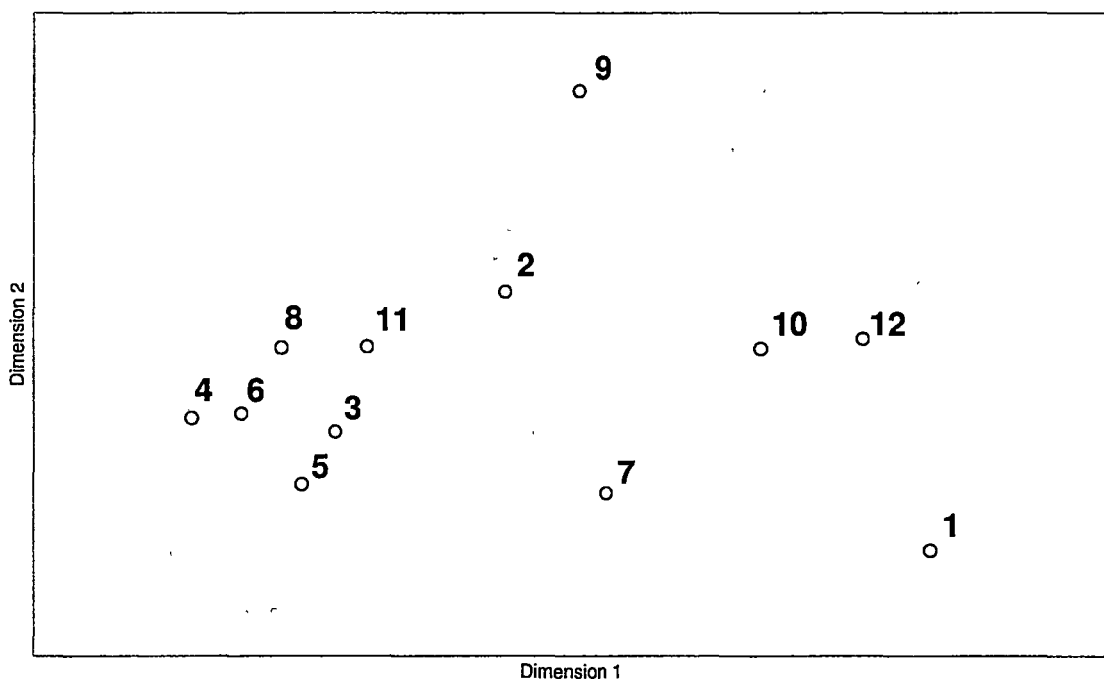
SFA=saturated fatty acid; BrFA=branched fatty acid MUFA=monounsaturated fatty acid;

PUFA=polyunsaturated fatty acid; c=*cis* double bond orientation; t=*trans* double bond

orientation; sd=standard deviation

represented major fatty acids. SFA content was dominated by 16:0 (mean  $16.6 \pm 2.8\%$ ; range 13.1-23.6%) and 18:0 (mean  $3.6 \pm 0.4\%$ ; range 2.9-4.0%). Branched fatty acid (BrFA) was a minor contributor to the total fatty acid composition of all specimens ( $\leq 1.5\%$ ).

The limited sample size did not allow for the exploration of fatty acid variation with size, however general trends in the content of predominant fatty acids could be ascertained. Levels of the MUFA 16:1 $\omega$ 7c and 18:1 $\omega$ 9c showed a general increase with increasing size (TL and TW), DHA, 18:1 $\omega$ 7c and 16:0 tended to decrease, whilst EPA and 18:0 remained relatively constant.



**Figure 6.2** Scatterplot of multidimensional scaling (MDS) based upon the complete fatty acid composition of *Dissostichus mawsoni* specimens from waters adjacent to Casey Station, Antarctica (numbers refer to individual specimens listed in Table 6.1)

## 6.4 DISCUSSION

The collection of cogenetic species such as *Dissostichus eleginoides* and *Dissostichus mawsoni* provides a unique opportunity to compare the effect of dietary differences on lipid composition. Although distribution of both toothfish species overlaps in certain regions (e.g. northern Ross Sea, Horn 2002), for the most part they are geographically isolated. This is likely to be the case for the toothfish populations compared in this study, with specimens collected from Macquarie Island (*D. eleginoides*) and Casey Station (*D. mawsoni*). A direct result of this separation of sample collection areas is that the diet of each toothfish species differs greatly, reflecting local differences in available prey. Added to this is the well-acknowledged influence of the high-Antarctic on fatty acid composition, including the elevated production of omega-3 polyunsaturated fatty acids (PUFA) by primary producers that are subsequently transferred up the foodweb to higher predators (e.g. Dunstan et al. 1999). The influence of regional differences in diet would therefore be anticipated to produce large variations in the fatty acid composition (derived from the diet) of Antarctic and Patagonian toothfish.

Morphologically, Patagonian and Antarctic toothfish are almost identical. Principal features include a prominent mouth, large eyes and enormous frame. Both species lack a swimbladders, in common with all nototheniids (e.g. Clarke et al. 1984). Physiological differences are, by comparison, significant. The extreme cold of high-Antarctic waters in particular has necessitated the development of many physiological adaptations in *D. mawsoni*. Most notable is the presence of antifreeze glycopeptides in the blood (AFGP) (DeVries et al. 1974; Eastman 1993; Metcalf et al. 1999). The effect of these adaptations on lipid composition, through either selective metabolism or modification of dietary fatty acids is unknown.

### **Total lipid content and lipid class composition**

Large lipid stores are a common feature of many high-Antarctic fish, including Antarctic toothfish. Accumulation of lipid is linked to biological purposes as diverse as the development of gonads (e.g. Donnelly et al. 1990; Friedrich

and Hagen 1994; Montgomery et al. 1999; Kamler et al. 2001), the storage of energy (e.g. Crockett and Sidell 1992; Lund and Sidell 1992; Bakes et al. 1995; Hagen et al. 2000), the transfer of oxygen to cells (e.g. Sidell 2001) and the maintenance of buoyancy (e.g. Phleger 1991; Phleger 1998).

A number of previous studies have explored the abundant and extensive distribution of lipid within various tissues of *D. mawsoni*. The complex system of subcutaneous and intra-muscular lipid stores found in *D. mawsoni* is thought to principally assist in the maintenance of neutral buoyancy (Clarke et al. 1984; Eastman 1985; Eastman 1988). Clarke et al. (1984) reported high total lipid contents (wet weight) in tissues including the skin (74%), liver (21%), red muscle (16-33%) and white muscle (7-23%). The large quantity of predominantly triacylglycerol (TAG) rich lipid contained within these stores provides the static lift that helps maintain buoyancy.

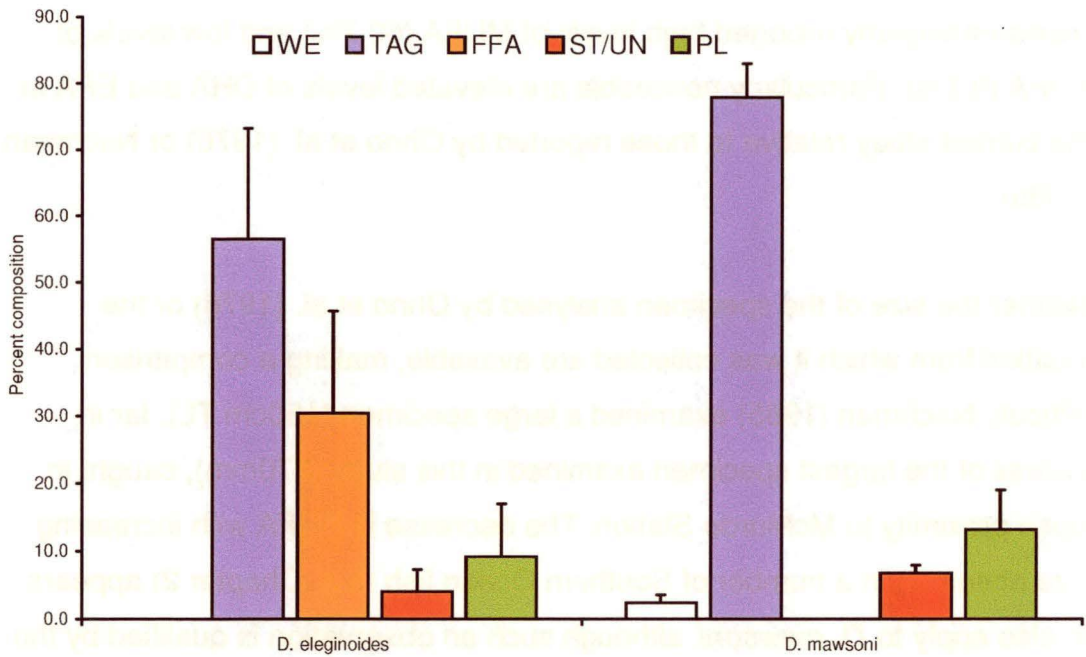
The lipid content of white muscle in the present study (Table 6.1, average  $1.0 \pm 0.2\%$ ) is far exceeded by the levels observed by Clarke et al. (1984). The large discrepancy may be a consequence of the increasing lipid content of white muscle from the tail to the centre of buoyancy observed by Clarke et al (1984). The sub-samples of white muscle used in the current study were taken from the base of the caudal fin, a body section in which Clarke et al. (1984) reported a rapid decline in total lipid content toward the tail (also observed for Patagonian toothfish, refer to Chapter 4). Therefore, the total lipid content determined in this study does not necessarily reflect the true content of whole specimens. Secondly, the specimens analysed in the present study are of *D. mawsoni* of less than 40cm TL, considerably smaller than the maximum size attained by this species (170cm TL, La Mesa and Vacchi 2001). Increasing TL is associated with increasing total lipid content in a number of Antarctic fish (e.g. Eastman 1988; Donnelly et al. 1990; Friedrich and Hagen 1994). The TL of the *D. mawsoni* specimens analysed by Clarke et al. (1984) were unfortunately not specified, making it impossible to determine whether size differences contributed to the lower total lipid levels seen in the present study.

The low amounts of total lipid present in the *D. mawsoni* specimens analysed in the present study would appear to largely discount the primary use of this store for either energy or buoyancy. The influence of gonad development can also be discounted as the specimens analysed in the current study are well below the age of sexual maturity (8 yrs, TL 90-100cm) reported by La Mesa and Vacchi (2001) and Eastman and DeVries (2000).

The domination of TAG (average  $77.8 \pm 5.1\%$ ) is in keeping with most other Antarctic nototheniids, as well as previous studies of *D. mawsoni* (Ohno et al. 1976; Clarke et al. 1984; Nachman 1985). The presence of wax ester (WE), although a minor component (average  $2.4 \pm 1.2\%$ ), is more unusual in nototheniids. Nachman (1985) reported low levels of WE (0.6%) in the specimen of *D. mawsoni* collected from McMurdo Sound, whilst the specimen of Ohno et al. (1976) was free of WE. The role of WE in the maintenance of buoyancy for several Southern Ocean fish is well described (see Phleger 1991; Phleger 1998). The small amounts of WE present in the specimens examined in this study could reflect a mechanism by which young fish utilise small amounts of WE to assist in the provision of buoyancy until total lipid content reaches a level that allows for the use of TAG-rich stores (e.g. the onset of sexual maturity). However, the presence of WE may once again reflect the large variation in lipid content (and possibly lipid class composition) within white muscle and the possible differences in lipid composition associated with this.

#### **Comparison of total lipid content and lipid class composition with Patagonian toothfish of equivalent size**

The total lipid content of equivalent sized *D. eleginoides* (TL <400mm, n=27, average  $2.7 \pm 3.2\%$  wet weight) is higher than for *D. mawsoni*, although much more variable (range 0.8-15.9%). Comparison of lipid class compositions of the two toothfish species is complicated by the fact that *D. eleginoides* of the same size are characterised by high free fatty acid levels (FFA, average  $30.4 \pm 15.3\%$ ), a lipid class absent in *D. mawsoni* (Figure 6.3). The high FFA levels present in those smaller *D. eleginoides* specimens used in this



**Figure 6.3** Comparison of the lipid class composition (percentage of total lipid content) of *Dissostichus eleginoides* (n=27) from the Macquarie Island region and *Dissostichus mawsoni* (n=12) from waters adjacent to Casey Station, Antarctica

comparison suggest a degree of deterioration either pre- or post-storage. Thus although TAG levels are significantly lower in *D. eleginoides* ( $56.4 \pm 16.7\%$  versus  $77.8 \pm 5.1\%$ ; *t*-test, 95% confidence interval,  $p < 0.001$ ), this is most likely due to sample degradation. Levels of sterol (ST) also differ significantly between both toothfish species (*t*-test,  $p < 0.05$ ), whilst the content of polar lipid (PL) is comparable (*t*-test,  $p > 0.05$ ). As previously mentioned an almost total reliance on TAG lipid storage is a characteristic common to all nototheniid species analysed so far, a feature that is also observed in both Antarctic and Patagonian toothfish.

#### Fatty acid composition: comparison with previous studies

PUFA dominated the fatty acid composition of *D. mawsoni* (total PUFA  $56.4 \pm 5.5\%$ ) examined in this study. This finding contrasts with the results of Ohno et al. (1976) in which monounsaturated fatty acids (MUFA, 66.9%; PUFA 9.3%) were the major components of skeletal muscle. A study by Nachman (1985) on the fatty acid composition of a whole specimen of *D.*

*mawsoni* similarly reported high levels of MUFA (60.7%) and low levels of PUFA (9.1%). Particularly noticeable are elevated levels of DHA and EPA in the current study relative to those reported by Ohno et al. (1976) or Nachman (1985).

Neither the size of the specimen analysed by Ohno et al. (1976) or the location from which it was collected are available, making a comparison difficult. Nachman (1985) examined a large specimen (150cm TL), far in excess of the largest specimen examined in this study (370mm), caught in close proximity to McMurdo Station. The decrease in PUFA with increasing size observed in a number of Southern Ocean fish (e.g. Chapter 2) appears to also apply to *D. mawsoni*, although such an observation is qualified by the low sample size available for comparison. The influence that different body sections and fish age may have on fatty acid composition is unknown with regards to *D. mawsoni*, but has been explored for other Southern Ocean fish. For example, fatty acid composition varies between different body sections, including the head, tail and skin, of Patagonian toothfish (Chapter 4) as well as with age (Chapter 5). Similarly, Lund and Sidell (1992) observed variations in the constituent fatty acids of serum, oxidative muscle and adipose tissue in nototheniids *Trematomus newnesi* and *Notothenia gibberifrons*. The differences in the fatty acid composition between the current study and Ohno et al. (1976) may therefore be reflecting differences in the age of fish, the type of tissue examined and the locations from which specimens were collected.

The high levels ( $\geq 10\%$ ) of both DHA and EPA in the white muscle of *D. mawsoni* are characteristic of numerous Antarctic fish. These include the channichthyid *Champsocephalus gunnari* (Nichols et al. 1994a), and the nototheniids *Dolloidraco longedorsalis*, *Trematomus lepidorhinus* (Hagen et al. 2000), *T. bernacchii*, *T. newnesi*, *T. hansonii*, *T. pennellii* (Phleger et al. 1999b), *Notothenia gibberifrons* (Lund and Sidell 1992), *N. coriiceps* and *Lepidonotothen nudifrons* (Kamler et al. 2001). The high levels of DHA and EPA present in these species has largely originated from dietary sources, due to the limited capacity of fish to synthesise long chain omega-3 PUFA

(e.g. Sargent et al. 1993; Ruyter et al. 2000). For instance Lund and Sidell (1992) related the domination of various fatty acids, including DHA and EPA, in the composition of the nototheniids *N. gibberifrons* and *T. newnesi* to similarly high levels of these fatty acids in the diet provided by various euphausiids, amphipods and polychaetes. The elevated DHA and EPA levels of *D. mawsoni* observed in this study suggests a similarly significant dietary source of these components.

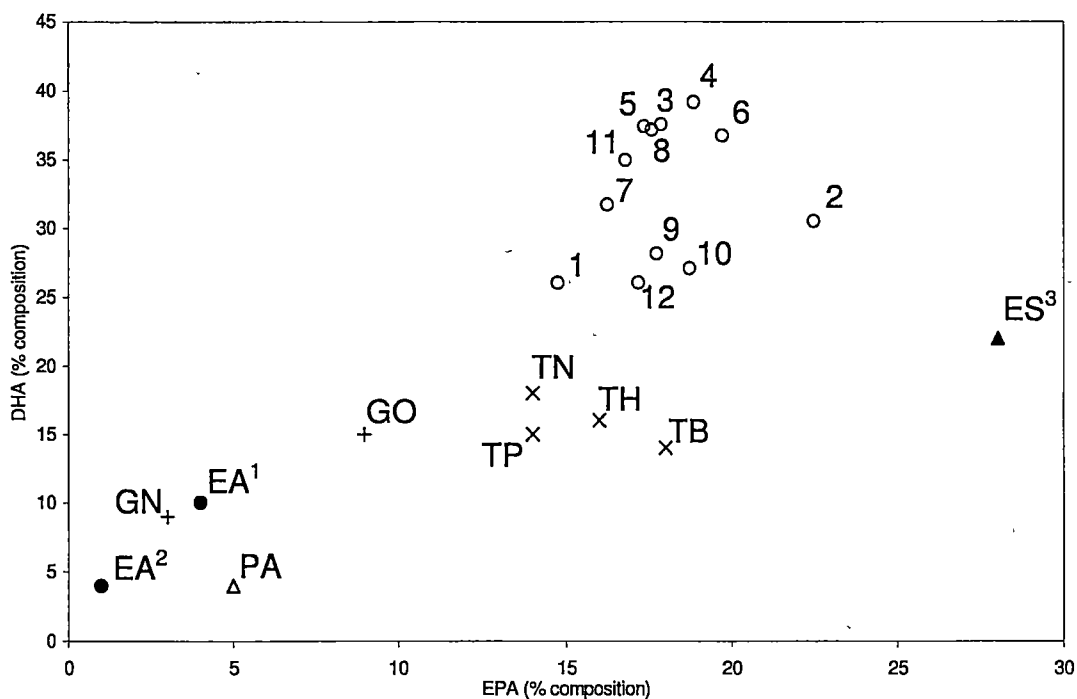
### **Fatty acid composition: comparison with potential prey**

The diet of *D. mawsoni* in the region of Casey Station is currently unknown, but is better understood in regions such as McMurdo Sound (DeVries et al. 1974; Eastman 1985; Eastman 1993). Although the diet of *D. mawsoni* is comprised of a relatively low diversity of prey (Eastman 1993), dietary composition is likely to be heavily dependent upon location and seasonal factors such as the fluctuation in sea ice cover. *D. mawsoni* are thought to follow the advancing sea ice northwards (toward the Antarctic Convergence) during winter, where reliance on prey such as squid may increase (Eastman and DeVries 2000). Similarly, the diet of Antarctic toothfish is heavily influenced by age. In the Commonwealth and Kosmonautov Seas, Antarctica, the diet of juvenile *D. mawsoni* (standard length 80-120mm) was dominated by euphausiids (estimated at 75% of diet, primarily *Euphausia superba* and *Thysanoessa macrura*), contrasting with the complete absence of euphausiids in fish >400mm standard length (Pakhomov 1997).

Based upon the findings of Pakhomov (1997), the diet of *D. mawsoni* within the size range of specimens analysed in this study (TL 240-370mm) is dominated by varying proportions of *Trematomus eulepidotus* (up to 60% of diet), *Pleuragramma antarcticum* (up to 40%), other fish (up to 60%, mainly the myctophids *Electrona antarctica* and *Gymnoscopelus spp.*) and small amounts of *E. superba* (up to 10%).

Comparison with the available fatty acid profiles of potential prey species is difficult due to both a lack of data relating to certain species (*T. eulepidotus*) as well as the potential impact of regional differences in those species for

which lipid compositional data is available. However, for comparative purposes several Antarctic *Trematomus spp.*, previously examined by Phleger et al. (1999b), have been substituted for *T. eulepidotus*, and only Antarctic specimens of the remaining prey species have been included. Whilst many of these prey are high in total PUFA (with the exception of *P. antarcticum*), large differences in fatty acid composition are apparent. Significantly, the relative levels of DHA and EPA, identified as the most important dietary fatty acids, shows great variation between *D. mawsoni* and selected potential prey species (Figure 6.4).



**Figure 6.4** Comparison of the percent composition of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in *Dissostichus mawsoni* from waters adjacent to Casey Station, Antarctica and major prey species identified from stomach contents (Pakhomov 1997) Numbers 1-12 denote individual specimens of *D. mawsoni* (see Table 6.1); EA=*Electrona antarctica*, <sup>1</sup>Eastern Antarctica population, <sup>2</sup>Elephant Island, Antarctica population (Phleger et al. 1997); GN=*Gymnoscopelus nicholsi* and GO=*G. opisthopterus* (Phleger et al. 1999a); PA=*Pleuragramma antarcticum* (Hagen et al. 2000); TP=*Trematomus pennellii*, TN=*T. newnesi*, TH=*T. hansonii* and TB=*T. bernacchii* (Phleger et al. 1999b); ES=*Euphausia superba* <sup>3</sup>average of adult male and females (Phleger et al. 1998); EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid

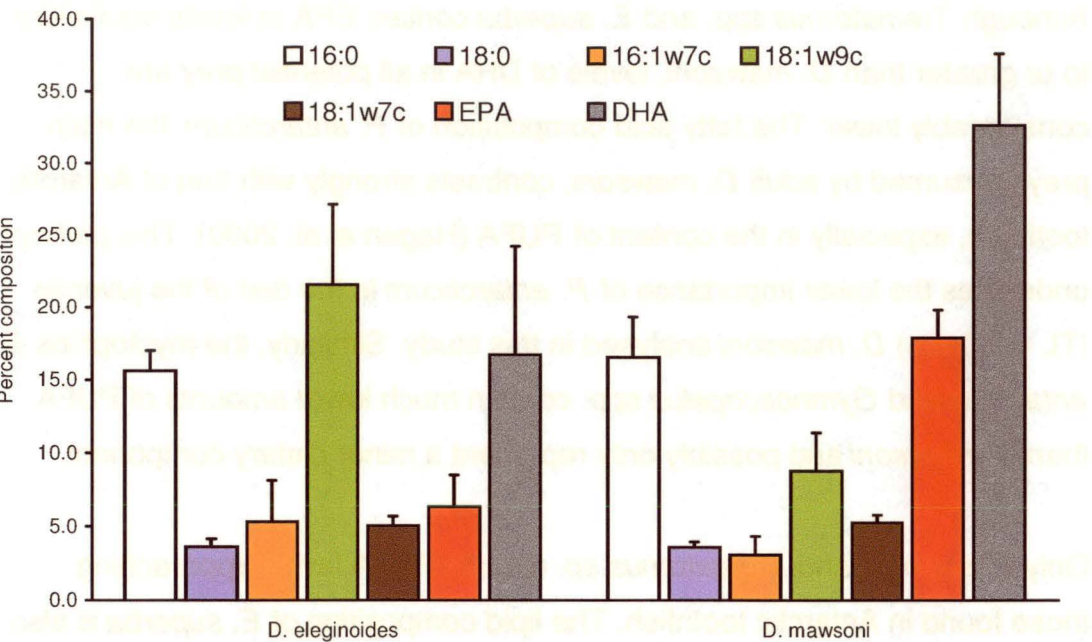
Although *Trematomus* spp. and *E. superba* contain EPA at levels equivalent to or greater than *D. mawsoni*, levels of DHA in all potential prey are considerably lower. The fatty acid composition of *P. antarcticum*, the main prey consumed by adult *D. mawsoni*, contrasts strongly with that of Antarctic toothfish, especially in the content of PUFA (Hagen et al. 2000). This perhaps underlines the lower importance of *P. antarcticum* in the diet of the juvenile (TL <400mm) *D. mawsoni* analysed in this study. Similarly, the myctophids *E. antarctica* and *Gymnoscopelus* spp. contain much lower amounts of PUFA than *D. mawsoni* and possibly only represent a minor dietary component.

Only *E. superba* and *Trematomus* sp. contain PUFA levels approaching those found in Antarctic toothfish. The lipid composition of *E. superba* is also known to vary considerably in relation to factors included sex, time of year and location (e.g. Phleger et al. 1998). DHA and EPA are substantially higher in female than male *E. superba*, and in juveniles compared to adults. Thus, levels of PUFA in *E. superba* could conceivably be much higher in the waters adjacent to Casey Station, accounting for the elevated levels of DHA and EPA in *D. mawsoni* from this region. Without lipid compositional data of prey from the Casey Station region, further exploration of the dietary composition of Antarctic toothfish from this region cannot be determined.

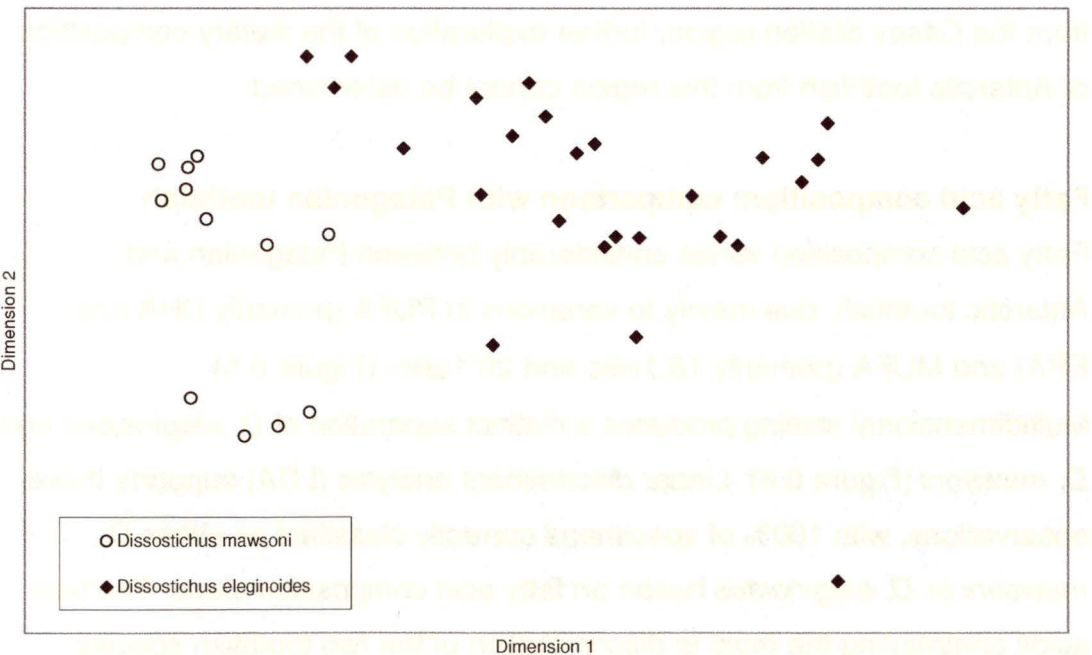
### **Fatty acid composition: comparison with Patagonian toothfish**

Fatty acid composition varies considerably between Patagonian and Antarctic toothfish, due mainly to variations in PUFA (primarily DHA and EPA) and MUFA (primarily 18:1 $\omega$ 9c and 20:1 $\omega$ 9c) (Figure 6.5).

Multidimensional scaling produces a distinct separation of *D. eleginoides* and *D. mawsoni* (Figure 6.6). Linear discriminant analysis (LDA) supports these observations, with 100% of specimens correctly classified as either *D. mawsoni* or *D. eleginoides* based on fatty acid composition alone. The fatty acids contributing the most to discrimination of the two toothfish species include 18:0, 20:0, 16:1 $\omega$ 7c, 17:1, 18:1 $\omega$ 5c, 19:1, 24:1 $\omega$ 9c, 18:4 $\omega$ 3, EPA and 20:3 $\omega$ 6.



**Figure 6.5** Comparison of selected fatty acids (percent composition) of *Dissostichus eleginoides* (n=27) from the Macquarie Island region and *Dissostichus mawsoni* (n=12) from waters adjacent to Casey Station, Antarctica



**Figure 6.6** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of *Dissostichus eleginoides* (n=27) from the Macquarie Island regions and *Dissostichus mawsoni* (n=12) from waters adjacent to Casey Station, Antarctica

Although the major fatty acids (>3% total composition) of both toothfish are similar, the relative proportions of a number of these fatty acids varies significantly, particularly the contents of DHA (*D. mawsoni*  $32.7 \pm 5.0\%$  and *D. eleginoides*  $16.8 \pm 7.5\%$ ), EPA ( $17.9 \pm 1.9\%$  and  $6.4 \pm 2.2\%$ ) and 18:1 $\omega$ 9c ( $8.8 \pm 2.6\%$  and  $21.6 \pm 5.6\%$ ) (Figure 6.6 and Table 6.3).

## 6.5 CONCLUSIONS

The contrasting fatty acid compositions of Antarctic and Patagonian toothfish provides further evidence of the dietary differences, a direct consequence of the immense distance separating Macquarie Island and the Antarctic continent, apparent in these cogenetic species. Even taking into account the effect that cold-adaptation may have on the lipid composition of *D. mawsoni*, it also highlights the dramatic impact that differences in diet can have on the fatty acid composition of two predator species that are, in many regards, identical.

# CHAPTER 7

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## DIETARY IMPLICATIONS OF A COMPARATIVE STUDY OF THE FATTY ACID COMPOSITION OF PATAGONIAN TOOTHFISH AND POTENTIAL PREY FROM THE MACQUARIE ISLAND REGION

### 7.1 INTRODUCTION

The sub-Antarctic islands of the Southern Ocean, including Macquarie Island, support a complex assemblage of phytoplankton, zooplankton, cephalopods, fish, marine mammals, and seabirds. Despite the recent encroachment of commercial fishing into many of these regions (e.g. Willock 2002), surprising little is known of the potentially deleterious impacts that these anthropogenic activities may have on ecosystem integrity. Kock (2001) reviewed the impacts of fishing and fishery-related activities in the Southern Ocean, but concentrated primarily on the effects of these activities on marine mammals and seabirds. The effect of fishing on species diversity at the lower end of the food web is not as well described. This is partly because macrofauna such as marine mammals and seabirds are more conspicuous, making it far easier to detect changes in these populations.

Recent development of a fishery for Patagonian toothfish (*Dissostichus eleginoides*) in the Macquarie Island region prompted the Fisheries Research and Development Corporation (FRDC) to commission a report into the ecological sustainability of such a fishery (He and Furlani 2001). A major aim of the FRDC study was the identification of food chain linkages between the fishery, toothfish and the main bird and mammal species, primarily through the examination of stomach and faecal contents (Goldsworthy et al. 2001b; Goldsworthy et al. 2001c; Goldsworthy et al. 2001d; Goldsworthy et al.

2002). A thorough understanding of the dietary interactions that exist between predators and prey is fundamental to the continued conservation of marine ecosystems. These dietary studies have revealed the direct link that toothfish provide between species near the base of the food web (e.g. zooplankton) and those at the top (e.g. marine mammals). In common with populations from other regions (South Georgia, Pilling et al. 2001), Macquarie Island toothfish are opportunistic carnivores that prey upon a wide range of midwater fish, crustaceans and cephalopods. When consumed by predators such as marine mammals, toothfish and other midwater fish are therefore participating in the transfer of energy from lower to higher trophic levels (Kozlov 1995; Pakhomov et al. 1996; Gaskett et al. 2001). Consequentially, total or even partial removal of toothfish from the ecosystem has the potential to severely impede this energy transfer.

Determining diet using the examination of stomach contents, however, has a number of inherent problems (e.g. Antonelis et al. 1987; Harwood and Croxall 1988; Pierce and Boyle 1991). Stomach contents fail to determine, with any certainty, long-term diet and only reflect recent foraging events. Additionally, by relying on the identification of hard body parts (squid beaks, otoliths and statoliths), this technique fails to detect prey lacking these features. The slower rate of passage of squid beaks through the digestive system can also lead to an overestimation in the importance of this prey group in the diet. The low proportion of toothfish stomachs containing identifiable prey from Macquarie Island (Goldsworthy et al. 2002), possibly due to regurgitation during capture (Pilling et al. 2001), also adds to the inherent difficulties of applying this technique to dietary studies.

The use of alternate and complementary methods can overcome the disadvantages associated with the examination of stomach contents. One method is the use of fatty acids. Although aspects of lipid composition contribute valuable insights into the biology of a species, fatty acid composition provides the greatest scope for the exploration of dietary interactions. The ability of vertebrates from higher trophic levels, including marine fish, to either modify or synthesise polyunsaturated fatty acids (PUFA)

*de novo* is particularly limited (e.g. Dunstan et al. 1999). For instance, the production of omega-3 PUFA is limited to primary producers, especially phytoplankton. This necessitates the reliance on dietary sources for these essential fatty acids. Many of these dietary derived fatty acids are readily transferred from predator to prey with little or no modification and therefore represent, to some extent, a temporal integration of diet.

An initial attempt at assessing dietary interactions and the long-term diet of Macquarie Island toothfish, using a comparison of the fatty acid profiles of toothfish and potential fish and squid prey by various statistical methods, was included in the FRDC investigation (Wilson and Nichols 2001). The current comparative study is an extension of this initial research into dietary fatty acids, and is based upon a significantly expanded number of toothfish samples as well as an increase in the number of potential fish and squid species available for comparison.

## **7.2 MATERIALS AND METHODS**

### **Fatty acid compositions**

The fatty acid compositions used in this comparative study are derived from the results of previous chapters. Specifically, 21 species of potential fish prey (Chapter 2) and 6 species of potential squid prey (Chapter 3) were compared to Patagonian toothfish (Chapter 5). All fatty acid data used in comparisons were in percent composition form. For the purposes of comparison, Patagonian toothfish were split into four size (length) groupings based on results previously obtained from multidimensional scaling and linear discriminant analysis (Figure 5.11). Size group 1 consists of toothfish with a mean total length of  $346 \pm 18$  mm (range 323-365 mm), group 2 (mean  $363 \pm 29$  mm; range 310-418 mm), group 3 (mean  $423 \pm 64$  mm; range 363-610 mm) and group 4 (mean  $681 \pm 159$  mm; range 376-1354 mm).

**Statistical analysis**

All statistical analyses were performed using SYSTAT 9 (SYSTAT, Inc, Evanston, Illinois, USA). Non-metric multidimensional scaling (MDS, Kruskal loss function) was used to compare the total fatty acid composition of all Patagonian toothfish (separated into four size groupings) and potential prey species in two dimensions. This allowed for the identification of potential prey that most closely resembled the fatty acid composition of one or more of the various toothfish size groups. A plot of eicosapentaenoic acid (EPA) versus docosahexaenoic acid (DPA) was also used to explore the grouping of prey with toothfish.

Potential prey that clustered close to one or more toothfish size groupings based on either MDS analysis or the relative proportions of EPA and DHA were then compared using linear discriminant analysis (LDA; tolerance=0.01, classification and jackknifed classification matrix, Wilk's lambda, automatic forward stepping [F to enter=4, F to remove=3.9]). In cases where the specimens belonging to a single prey species were spread between more than one toothfish size group, these prey were split into two or more groups and compared with their corresponding toothfish groups individually. Similarly, where a single species of prey clustered closely together, but straddled two toothfish size groups, all specimens of this prey were included in a comparison with both toothfish size groups.

LDA allowed for the identification of those fatty acids (predictors) most responsible for differences or similarities between Patagonian toothfish and potential prey. The jack-knifed classification matrix is included as a means of cross-validating the normal classification matrix. Considerable difference in the percentage of correct classifications between these two matrices would suggest potential difficulties in correctly classifying the data.

LDA was based upon several combinations of fatty acids. To assess the overall similarity of toothfish and prey, total fatty acid composition was compared. Those fatty acids most likely to have originated from the diet were compared by analysis of polyunsaturated fatty acids (PUFA) exclusively.

Finally, the composition of EPA and DHA were compared. EPA and DHA were previously identified as the fatty acids most responsible for the variation in fatty acids composition observed between the various toothfish size groups (Chapter 5).

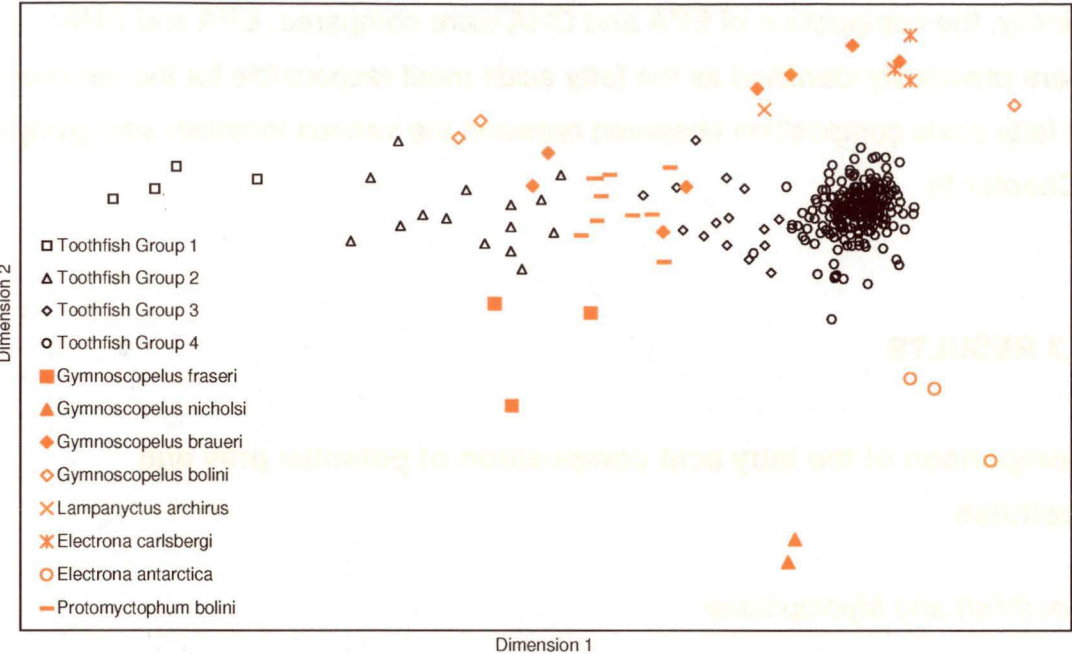
### 7.3 RESULTS

#### Comparison of the fatty acid composition of potential prey and toothfish

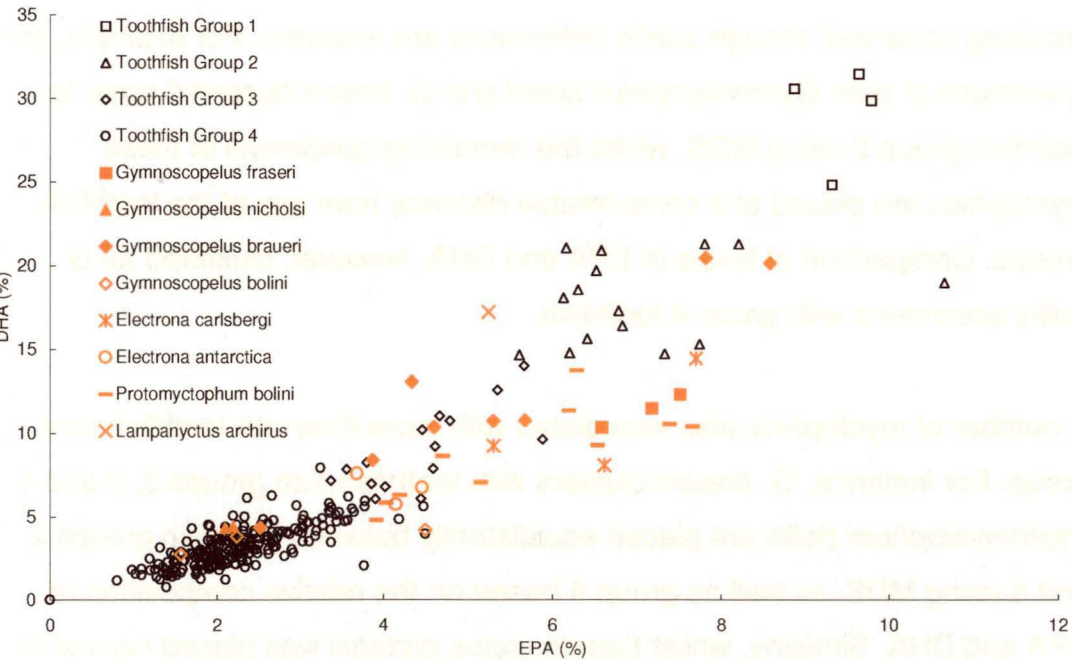
##### *Toothfish and Myctophidae*

A comparison of total fatty acid composition using MDS (Figure 7.1), and the relative proportions of DHA and EPA (Figure 7.2), showed that several myctophid species clustered close to toothfish of all sizes, with the exception of toothfish belonging to size group 1. Each of the comparative methods (i.e. MDS and relative amounts of EPA and DHA) produced similar trends in the grouping observed, though subtle differences are apparent. For example, two specimens of both *Gymnoscopelus bolini* and *G. fraseri* clustered close to toothfish group 2 using MDS, whilst the remaining specimens of these myctophids are placed at a considerable distance from any of the toothfish groups. Comparison of levels of EPA and DHA, however, clustered all *G. bolini* specimens with group 4 toothfish.

A number of myctophids also associated with more than one toothfish size group. For instance, *G. braueri* clusters with toothfish size groups 2, 3 and 4. *Protomyctophum bolini* are placed equidistantly between toothfish groups 2 and 3 using MDS, as well as group 4 based on the relative composition of EPA and DHA. Similarly, whilst *Lampanyctus archirus* was placed closest to toothfish size group 3 using MDS, based upon EPA and DHA levels it clustered with group 2.



**Figure 7.1** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and Myctophidae included in this study from the Macquarie Island region



**Figure 7.2** Relative composition (percentage of total fatty acids) of eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) in *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and Myctophidae included in this study from the Macquarie Island region

The remaining myctophid species, *G. nicholsi*, *Electrona antarctica* and *E. carlsbergi*, clustered closest to group 4 toothfish using MDS, though once again at a considerable distance (Figure 7.1). DHA and EPA composition of these three species placed *G. nicholsi* within toothfish group 4, *E. antarctica* clustered with toothfish group 3, and *E. carlsbergi* split between groups 2 and 3 (Figure 7.2).

LDA reveals vastly different classification matrices using comparisons of the various combinations of fatty acids (Tables 7.1 and 7.2). No statistical similarity in total fatty acid composition was observed between myctophids and toothfish (i.e. 0% overlap in the fatty acid composition of myctophids and closest clustering toothfish size groups). This is especially true for a number of species that differed in composition from toothfish by at least 10 fatty acids. These species include *G. braueri* (compared to toothfish size group 4), *G. nicholsi*, *G. bolini* (toothfish group 4), *L. archirus* (toothfish group 3), *P. bolini* (toothfish group 4), *E. antarctica* (toothfish group 4) and *E. carlsbergi* (toothfish group 4). The fatty acids most responsible for the observed differences (predictors) varied with myctophid species, but consisted of combinations of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA. Amongst these fatty acids components at greater than 1% of total fatty acid composition, including 16:0, 18:0, 16:1 $\omega$ 7c, 18:1 $\omega$ 7c, 20:1 $\omega$ 9c, 22:1 $\omega$ 11c, 18:2 $\omega$ 6, EPA and DHA. The remaining myctophids differed from toothfish due to differences in the composition of a smaller number of fatty acids, the majority of which represent only minor components (less than 1% of total fatty acid composition).

Comparison of PUFA by LDA also produced little similarity between toothfish and myctophids, once again due to differences in the composition of major fatty acids such as 18:2 $\omega$ 6, EPA and DHA. However, the jack-knifed classification of several species, specifically *G. braueri* (compared to toothfish size groups 4 and 2), *G. bolini* (toothfish size group 4) and *L. archirus* (toothfish size group 2), varied considerably from normal

**Table 7.1** Comparison of the percentage of correctly classified Myctophidae (genus *Gymnoscopelus*) with size groupings of *Dissostichus eleginoides* by linear discriminant analysis, relative to different combinations of fatty acids

Myctophidae species	Toothfish size group <sup>1</sup>	Correct classification (%) of potential Myctophidae prey (genus <i>Gymnoscopelus</i> )								
		All fatty acids			PUFA only			EPA and DHA only		
		LDA	JK	Predictors	LDA	JK	Predictors	LDA	JK	Predictors
<i>Gymnoscopelus fraseri</i>	3 <sup>^</sup>	0	0	22:1ω11c, 22:1ω7c, 24:1ω11c, 24:1ω9c, 18:3ω6	0	0	18:3ω6, 18:2ω6, 20:3ω6, 20:4ω3, DPA	0	100	EPA
	2	0	0	16:1ω7t, 18:3ω6, 18:4ω3, 18:2ω6	0	0	18:3ω6, 18:4ω3, C <sup>21</sup> PUFA	13	0	DHA
<i>Gymnoscopelus braueri</i>	4 <sup>^</sup>	0	0	16:0, 17:0, 16:1ω9c, 18:1, 20:1ω7c, 22:1ω11c, 22:1ω7c, 24:1ω11c, 24:1ω9c, AA, 20:4ω3, DHA, DPA	0	33	18:4ω3, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, DHA, DPA	0	33	
	3 <sup>^</sup>	0	0	18:1, 19:1, 18:4ω3, AA	0	0	18:4ω3	100	100	
	2 <sup>^</sup>	0	0	18:0, 14:1ω5c, 16:1ω7t, 18:1ω9c, 18:1, 20:1ω7c, 22:1ω9c, EPA	0	50	18:3ω6, 18:4ω3, 18:2ω6, 20:3ω6	0	0	DHA
<i>Gymnoscopelus nicholsi</i>	4	0	0	14:0, 18:0, 20:0, 16:1ω9c, 20:1ω9c, 20:1ω7c, 18:2ω6, 20:3ω6, 20:4ω3, DHA	0	0	18:3ω6, 18:4ω3, 18:2ω6, 20:3ω6, 20:4ω3, DPA	100	100	
<i>Gymnoscopelus bolini</i>	4 <sup>*</sup>	0	0	16:0, 17:0, 14:1ω5c, 16:1ω9c, 16:1ω7t, 18:1, 19:1, 20:1ω9c, 18:3ω6, EPA, 20:3ω6, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, DHA, DPA	0	0	18:3ω6, 18:4ω3, EPA, 20:3ω6, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, DHA, DPA	100	100	
	4 <sup>^</sup>	0	0	16:0, 17:0, 16:1ω7t, 18:1ω7c, 18:1, 19:1, 22:1ω7c, 18:3ω6, EPA, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, DHA, 22:4ω6, DPA	0	100	18:3ω6, EPA, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, DHA, 22:4ω6, DPA	100	100	
	2 <sup>^</sup>	0	0	20:0, 22:0, 22:1ω9c, 20:2ω6	0	0	18:3ω6, 20:2ω6	0	0	DHA

<sup>1</sup>closest clustering toothfish size group (refer to text for details on toothfish sizing) determined from multidimensional scaling of entire fatty acid profile (Figure 7.1) except for \*determined from a plot of DHA versus EPA (Figure 7.2); <sup>^</sup>comparison based upon the separation of myctophid specimens into two or more groups, each corresponding with a different toothfish size group; PUFA=polyunsaturated fatty acid; AA=arachidonic acid (20:4ω6); EPA=eicosapentaenoic acid (20:5ω3); DHA=docosahexaenoic acid (22:6ω3); DPA=docosapentaenoic acid (22:5ω3); LDA=linear discriminant analysis classification (0%=no similarity in fatty acid composition of toothfish and prey); JK=jack-knifed LDA classification

**Table 7.2** Comparison of the percentage of correctly classified Myctophidae with size groupings of *Dissostichus eleginoides* by linear discriminant analysis, relative to different combinations of fatty acids

Myctophidae species	Toothfish Size Group <sup>1</sup>	Correct classification (%) of potential Myctophidae prey											
		All fatty acids						PUFA only			EPA and DHA only		
		LDA	JK	Predictors	LDA	JK	Predictors	LDA	JK	Predictors			
<i>Lampanyctus archirus</i>	3	0	0	16:1ω7c, 18:1ω9c, 19:1, 20:1ω11c, 22:1ω11c, 24:1ω11c, 24:1ω9c, 18:3ω6, 18:2ω6, 20:4ω3	0	100	18:3ω6, 18:4ω3, C <sup>21</sup> PUFA	0	100	DHA			
	2*	0	0	19:1, 22:1ω11c, 22:1ω9c, 18:3ω6, 18:2ω6	0	0	18:3ω6, 18:4ω3, 18:2ω6	100	100				
<i>Protomyctophum bolini</i>	4*^	0	0	16:0, 16:1ω9c, 16:1ω7t, 16:1ω5c, 17:1, 24:1ω11c, 24:1ω9c, 18:4ω3, EPA, 20:3ω6, 20:4ω3, DHA, 22:4ω6	0	0	18:4ω3, AA, EPA, 20:4ω3, 22:4ω6	0	0	EPA			
	3	0	0	17:0, 14:1ω5c, 16:1ω9c, 16:1ω7t, 16:1ω5c, 18:1ω9c, 18:1ω7c, 20:1ω7c	0	0	18:4ω3, AA, EPA, 20:4ω3, 22:5ω6	40	50	EPA, DHA			
	2	0	0	17:0, 18:0, 16:1ω7t, 18:1ω5c, 19:1, 22:1ω7c, 18:4ω3, C <sup>21</sup> PUFA	0	0	20:2ω6, C <sup>21</sup> PUFA, DHA, DPA	10	10	DHA			
<i>Electrona antarctica</i>	4	0	0	14:0, 16:0, 17:0, 16:1ω7c, 16:1ω7t, 18:1, 20:1ω7c, 22:1ω7c, 24:1ω9c, 18:3ω6, AA, 20:3ω6, 22:5ω6, DHA, 22:4ω6, DPA	0	0	18:4ω3, 20:3ω6, C <sup>21</sup> PUFA	0	0	EPA, DHA			
	3*	0	0	17:0, 16:1ω7c, 18:1ω7c	0	0	18:4ω3, 18:3ω6, EPA	100	100				
<i>Electrona carlsbergi</i>	4	0	0	18:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, 18:1ω7c, 19:1, 22:1ω11c, 24:1ω9c, 18:3ω6, 18:4ω3, 20:3ω6, 20:2ω6, C <sup>21</sup> PUFA, 22:5ω6	0	0	18:3ω6, 18:4ω3, 18:2ω6, EPA, 20:3ω6, 20:2ω6, C <sup>21</sup> PUFA	0	0	EPA, DHA			
	3*^	0	0	19:1, 20:1ω11c, 18:3ω6, 20:3ω6	0	0	18:3ω6, 20:3ω6, 20:2ω6	50	50	EPA, DHA			
	2*^	0	0	18:1ω7c, 18:1ω5c, 19:1, 20:1ω7c, 24:1ω11c, 24:1ω9c, 18:3ω6, 18:2ω6	0	0	18:3ω6, 18:4ω3, 18:2ω6	100	100				

<sup>1</sup>closest clustering toothfish size group (refer to text for details on toothfish sizing) determined from multidimensional scaling of entire fatty acid profile (Figure 7.1) except for \*determined from a plot of DHA versus EPA (Figure 7.2); <sup>^</sup>comparison based upon the separation of myctophid specimens into two or more groups, each corresponding with a different toothfish size group; PUFA=polyunsaturated fatty acid; AA=arachidonic acid (20:4ω6); EPA=eicosapentaenoic acid (20:5ω3); DHA=docosahexaenoic acid (22:6ω3); DPA=docosapentaenoic acid (22:5ω3); LDA=linear discriminant analysis classification (0%=no similarity in fatty acid composition of toothfish and prey); JK=jack-knifed LDA classification

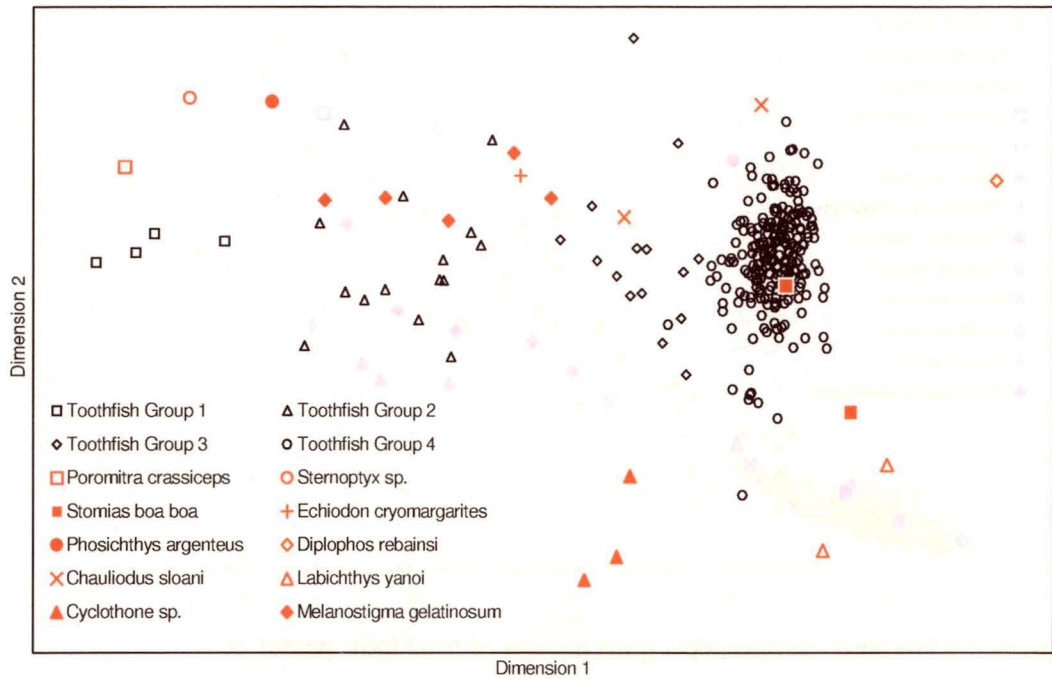
classification. The disparity in the percentage of correctly classified suggests difficulty in comparing these species.

Contrasting with the low similarity of both total fatty acid and PUFA profiles between myctophids and toothfish, comparison of only EPA and DHA revealed a greater degree of similarity. Statistical difference in the relative composition of EPA and DHA could not be distinguished in several specimens of *G. braueri* (compared with toothfish size group 3), *G. nicholsi* (toothfish group 4), *G. bolini* (toothfish group 4), *L. archirus* (toothfish group 2), *E. antarctica* (toothfish group 3) and *E. carlsbergi* (toothfish group 2). Additionally a degree of overlap with toothfish was observed for *P. bolini* (toothfish group 3) and *E. carlsbergi* (toothfish group 3).

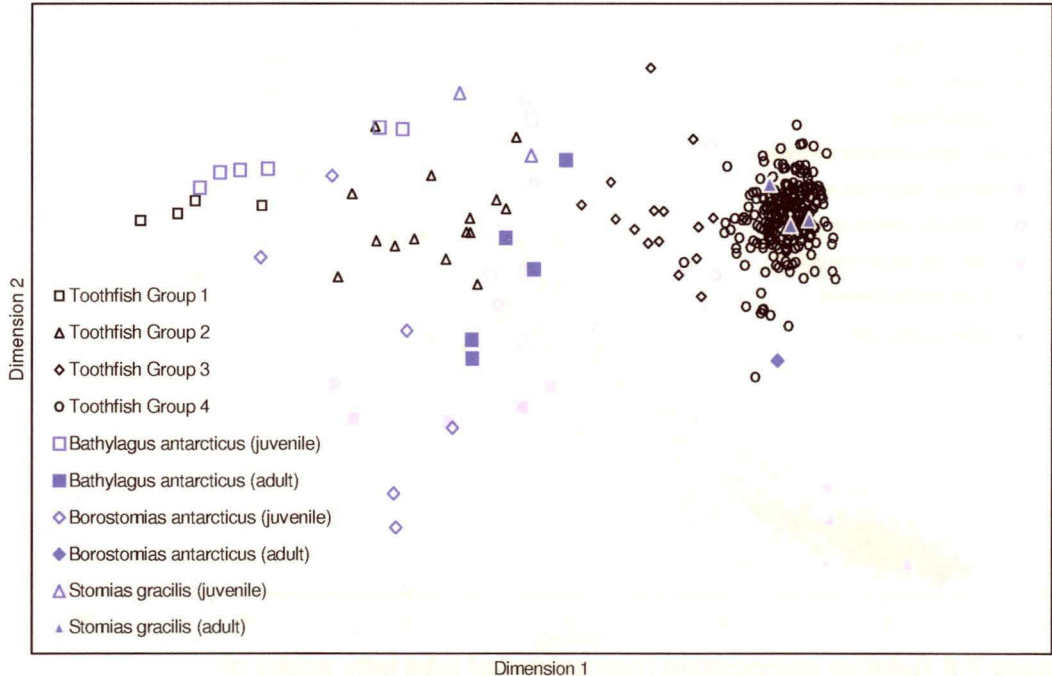
Notwithstanding, the composition of EPA and DHA in several myctophids showed little or no statistical similarity to that of toothfish, including *G. fraseri* (compared to toothfish groups 2 and 3), *G. braueri* (toothfish groups 2 and 4), *G. bolini* (toothfish group 2), *P. bolini* (toothfish groups 2 and 4), *E. antarctica* (toothfish group 4) and *E. carlsbergi* (toothfish group 4).

#### *Toothfish and other fish*

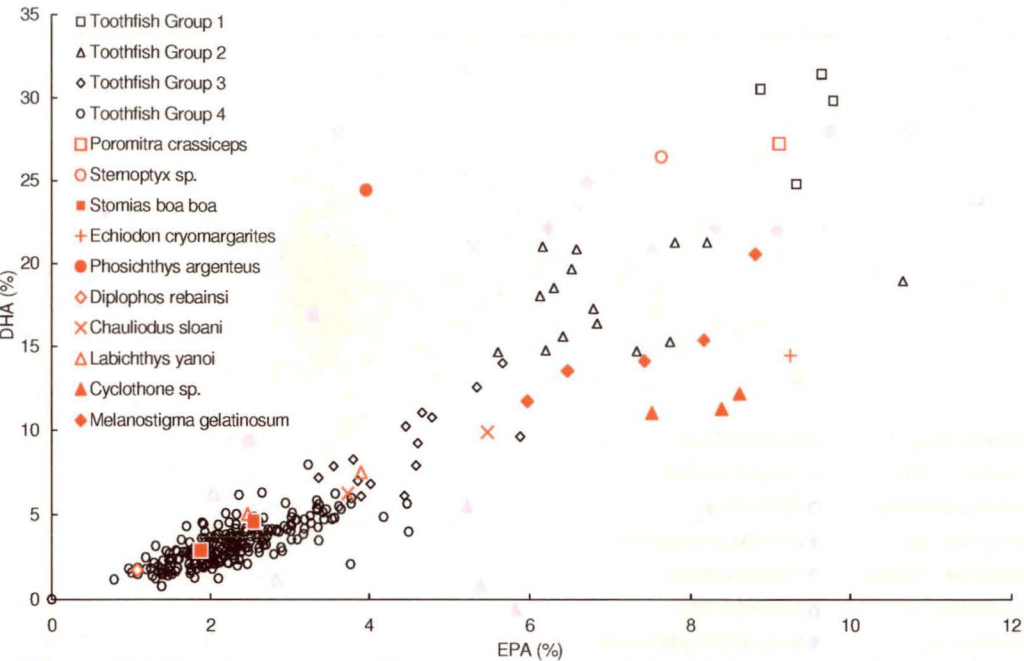
MDS analysis (Figures 7.3 and 7.4) and comparison of EPA and DHA composition (Figures 7.5 and 7.6) suggest a large degree of overlap in the fatty acid composition of many fish species and all four of the toothfish size groups. In contrast to myctophids, a number of other fish species group with the smallest sized toothfish (comprising group 1), including *Poromitra crassiceps*, and *Sternoptyx* sp. The single specimen of *Phosichthys argenteus* is also placed equidistantly between toothfish size groups 1 and 2 by MDS analysis (Figure 7.3), though comparison of EPA and DHA levels reveals little similarity with any toothfish sizes (Figure 7.5). Juveniles of both *Bathylagus antarcticus* and *Borostomias antarcticus* (MDS analysis only) clustered close to group 1 toothfish, though several juvenile specimens also cluster with group 2 toothfish, especially on the basis of EPA and DHA composition (Figures 7.4 and 7.6). In addition, adults and juveniles of these



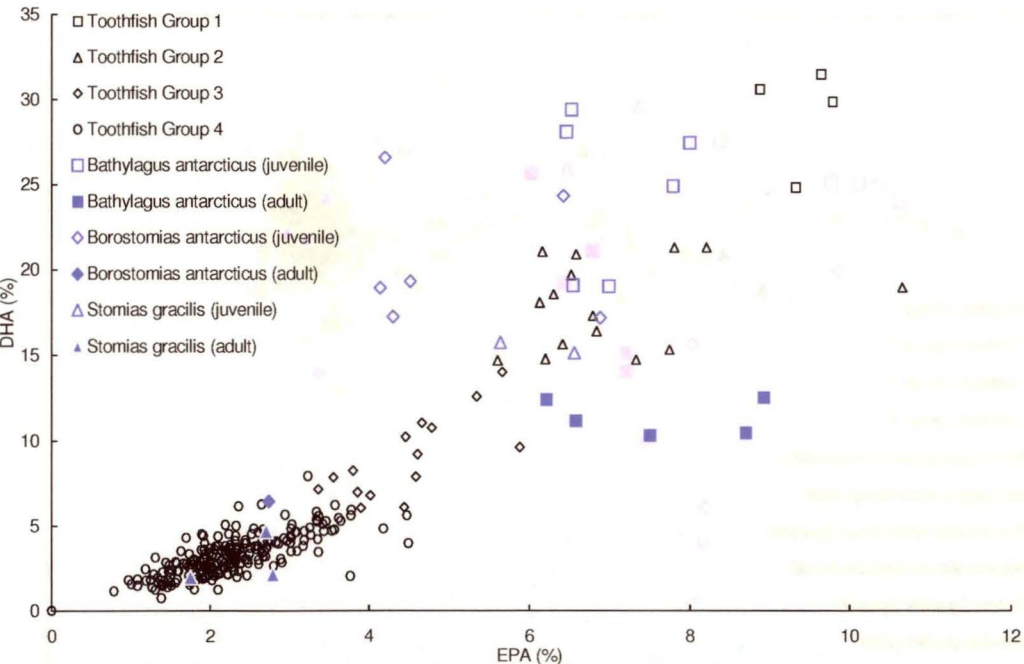
**Figure 7.3** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and various fish species included in this study from the Macquarie Island region



**Figure 7.4** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and adults and juveniles of various fish species (refer to text for details of sizing) included in this study from the Macquarie Island region



**Figure 7.5** Relative composition (percentage of total fatty acids) of eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) in *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and various fish species included in this study from the Macquarie Island region



**Figure 7.6** Relative composition (percentage of total fatty acids) of eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) in *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and adults and juveniles of various fish species (refer to text for details of sizing) included in this study from the Macquarie Island region

two species, as well as *Stomias gracilis*, cluster with larger and smaller sized toothfish respectively.

None of the remaining fish species clustered exclusively with toothfish from size groups 2 or 3. Specimens of *Melanostigma gelatinosum* are split between these two toothfish sizes, based on both MDS analysis and EPA and DHA levels. The single specimen of *Echiodon cryomargarites* is equidistantly placed between toothfish groups 2 and 3 using MDS analysis, though is closer to group 2 when only EPA and DHA levels are compared. The two specimens of *Chauliodus sloani* are split between toothfish size groups 3 and 4 using both MDS analysis and a comparison of EPA and DHA levels.

Several fish species, *Labichthys yanoi*, *Diplophos rebainsi* and *Stomias boa*, associated exclusively with the largest sized toothfish (group 4), based on both MDS analysis and, especially, the relative levels of EPA and DHA.

All three specimens of *Cyclothone* sp. grouped together tightly, though at some distance from toothfish. They clustered closest to toothfish size group 4 using MDS analysis and group 2 toothfish when EPA and DHA levels were compared.

Continuing the trend observed for comparisons with the myctophid species, LDA revealed little similarity in either total fatty acid or PUFA composition between the various fish species and their corresponding toothfish size groups (Table 7.3). A number of species, particularly *L. yanoi* (compared to toothfish size group 4), *E. cryomargarites* (toothfish group 3), adult *S. gracilis* and *Cyclothone* sp (toothfish group 4), differ from their corresponding toothfish size groups by large differences in the relative composition of 8 or more fatty acids (based on a comparison of total fatty acid profiles).

Differences are due to various combinations of SFA, MUFA and PUFA including a number of major fatty acids such as 16:0, 16:1 $\omega$ 7c, 18:1 $\omega$ 9c, 18:1 $\omega$ 7c, 20:1 $\omega$ 9c and EPA.

**Table 7.3** Comparison of the percentage of correctly classified various fish species with size groupings of *Dissostichus eleginoides* by linear discriminant analysis, relative to different combinations of fatty acids

Fish species	Toothfish size group <sup>1</sup>	Correct classification (%) of potential fish prey								
		All fatty acids			PUFA only			EPA and DHA only		
		LDA	JK	Predictors	LDA	JK	Predictors	LDA	JK	Predictors
<i>Poromitra. crassiceps</i>	1	0	0	19:1, DPA	0	0	AA, 22:5ω6	100	100	
<i>Sternopyx sp.</i>	1	0	0	18:3ω6, 20:4ω3	0	0	18:3ω6, 20:4ω3	0	0	EPA
<i>Phosichthys argenteus</i>	2	0	0	17:0, 22:0, EPA	0	100	EPA, 20:2ω6, 22:5ω6	0	100	EPA, DHA
	1	0	0	20:0, DPA	0	0	18:2ω6, EPA	0	0	EPA
<i>Labichthys yanoi</i>	4	0	0	16:0, 17:0, 18:0, 18:1ω7c, 18:1, 20:1ω9c, 20:1ω7c, 22:1ω11c, 22:1ω9c, 24:1ω9c, 18:3ω6, 18:4ω3, 20:4ω3, C <sup>21</sup> PUFA, 22:4ω6	0	0	18:3ω6, EPA, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, DHA	0	50	DHA
<i>Melanostigma gelatinosum</i>	3 <sup>^</sup>	0	0	22:0, 14:1ω5c, 18:1ω5c, 20:1ω7c, 20:3ω6, 20:2ω6, C <sup>21</sup> PUFA	0	0	18:4ω3, 20:3ω6, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA	100	100	
	2 <sup>^</sup>	0	0	15:0, 17:0, 18:0, 16:1ω9c, 22:1ω7c, 22:4ω6	0	0	18:3ω6, 18:4ω3	100	100	
<i>Echiodon cryomargarites</i>	3	0	0	16:0, 17:0, 16:1ω7c, 16:1ω5c, 18:1ω9c, 18:1, 20:1ω11c, 18:2ω6, AA, 22:5ω6	0	100	EPA	0	100	EPA
	2	0	0	17:0, 18:0, 22:0, 18:1ω5c, 19:1, 20:1ω11c, 24:1ω11c	0	0	18:4ω3, 18:2ω6, AA, 20:3ω6, 20:2ω6, C <sup>21</sup> PUFA, 22:5ω6, DHA	100	100	
<i>Diplophos rebainsi</i>	4	0	0	16:0, 17:0, 18:0, 14:1ω5c, 16:1ω9c, 16:1ω7c, 16:1ω7t, 17:1, 18:1ω9c, 18:1ω7c, 18:1, 18:3ω6	0	100	EPA, 20:4ω3, C <sup>21</sup> PUFA, 22:4ω6	100	100	
<i>Cyclothone sp.</i>	4	0	0	15:0, 16:0, 17:0, 18:0, 20:0, 14:1ω5c, 16:1ω9c, 16:1ω7c, 18:1, 19:1, 22:1ω11c, 18:4ω3, 18:2ω6, AA, EPA, 20:3ω6, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, 22:4ω6, DPA	0	0	18:3ω6, 18:4ω3, 18:2ω6, AA, EPA, 20:3ω6, 22:4ω6	0	0	EPA
	2 <sup>*</sup>	0	0	16:0, 20:0, 18:1, 20:2ω6, 22:5ω6	0	0	EPA, 20:4ω3	0	0	EPA, DHA

Table 7.3. continued next page

Table 7.3 Continued from previous page

Fish species	Toothfish size group <sup>1</sup>	Correct classification (%) of potential fish prey								
		All fatty acids			PUFA only			EPA and DHA only		
		LDA	JK	Predictors	LDA	JK	Predictors	LDA	JK	Predictors
<i>Chauliodus sloani</i>	4 <sup>^</sup>	0	100	16:0, 16:1ω9c, 16:1ω7c, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, DPA	0	100	18:4ω3, EPA, 20:3ω6, 20:4ω6, C <sup>21</sup> PUFA	0	100	DHA
	3 <sup>^</sup>	0	0	17:0, 18:1, 18:4ω3, 22:4ω6	0	100	20:4ω3, 20:2ω6	100	100	
<i>Stomias boa boa</i>	4	0	50	16:0, 16:1ω9c, 16:1ω5c, 18:1, 18:3ω6, 18:4ω3, 20:3ω6, 22:4ω6	0	0	20:3ω6, 22:4ω6	100	100	
<i>Stomias gracilis</i> <sup>J</sup>	2	0	0	16:0, 22:1ω7c, 24:1ω11c	0	50	18:3ω6, 18:4ω3	100	100	
<i>Stomias. gracilis</i> <sup>A</sup>	4	0	0	17:1, 18:1ω9c, 24:1ω11c, 18:2ω6, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, 22:4ω6	0	0	20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, 22:4ω6	100	100	
<i>Bathylagus antarcticus</i> <sup>J</sup>	2 <sup>^</sup>	0	0	17:0, 14:1ω5c, 24:1ω9c, 18:3ω6, 22:5ω6	0	0	18:3ω6, 18:4ω3, C <sup>21</sup> PUFA, 22:5ω6, DHA	100	100	
	1 <sup>^</sup>	0	0	16:1ω9c, 18:2ω6	0	0	18:2ω6	0	0	EPA
<i>Bathylagus antarcticus</i> <sup>A</sup>	2	0	0	16:1ω7t, 20:1ω7c, 20:3ω6, 20:2ω6	0	0	18:3ω6, 18:4ω3, C <sup>21</sup> PUFA, 22:5ω6, DHA	0	0	DHA
<i>Borostomias antarcticus</i> <sup>J</sup>	2 <sup>*</sup>	0	0	20:0, 14:1ω5c, 16:1ω9c, 18:1, 20:1ω9c, 22:1ω7c, 24:1ω11c, 18:3ω6, 20:4ω3	0	0	18:4ω3, 20:3ω6, 22:5ω6	33	33	EPA
	2 <sup>^</sup>	0	0	20:0, 16:1ω9c, 17:1, 18:1ω7c, 20:1ω9c, 22:1ω11c, 22:1ω7c, 18:3ω6, 18:4ω3	0	0	18:3ω6, 18:4ω3, 20:4ω3, DHA	40	40	EPA
	1 <sup>^</sup>	0	0	20:0, DPA	0	0	20:3ω6, DPA	0	0	EPA
<i>Borostomias antarcticus</i> <sup>A</sup>	4	0	100	17:0, 18:0, 17:1, 18:1ω9c, 18:1ω7c, 20:1ω9c, 20:1ω7c, C <sup>21</sup> PUFA, DPA	0	100	EPA, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, DHA, DPA	0	100	DHA

<sup>1</sup>closest clustering toothfish size group (refer to text for details on toothfish sizing) determined from multidimensional scaling of entire fatty acid profile (Figures 7.3 and 7.5) except for \*determined from a plot of DHA versus EPA (Figures 7.4 and 7.6.); <sup>^</sup>comparison based upon the separation of fish specimens into two or more groups, each corresponding with a different toothfish size group; PUFA=polyunsaturated fatty acid; AA=arachidonic acid (20:4ω6); EPA=eicosapentaenoic acid (20:5ω3); DHA=docosahexaenoic acid (22:6ω3); DPA=docosapentaenoic acid (22:5ω3); LDA=linear discriminant analysis classification (0%=no similarity in fatty acid composition of toothfish and prey); JK=jack-knifed LDA classification; J=juvenile specimens; A=adult specimens (refer to main body of text for details on sizing)

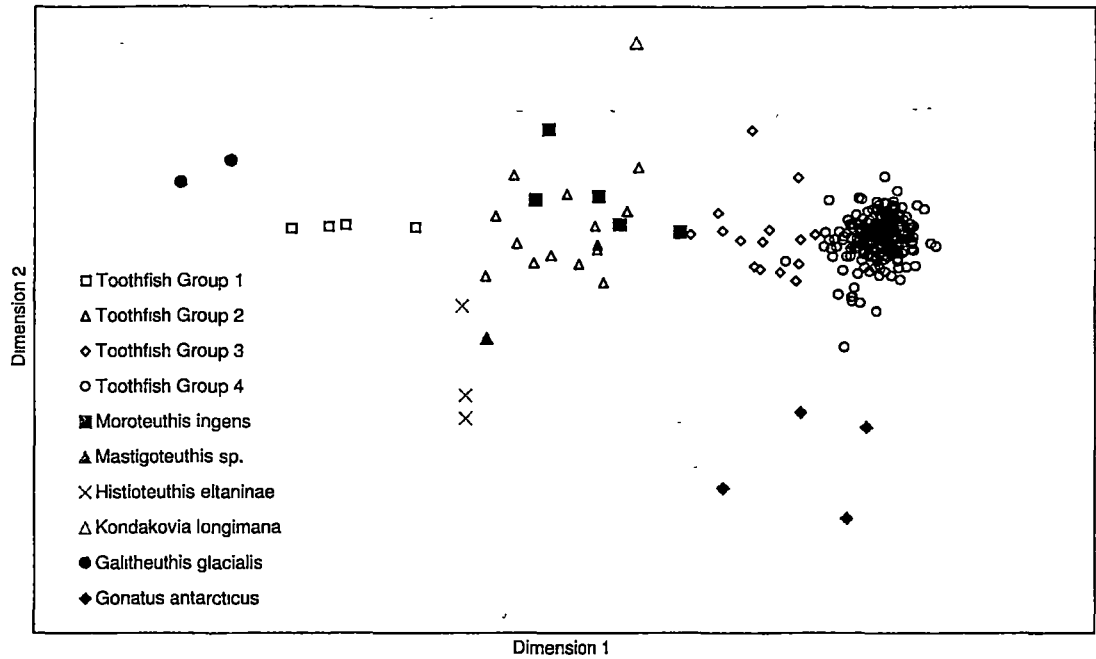
Although total fatty acid and PUFA composition of the remaining fish species and toothfish showed little statistical similarity, the differences observed were due to a much smaller number of fatty acids that, in the main, constituted relatively minor components. These include *P. crassiceps*, *Sternoptyx* sp., *P. argenteus* (toothfish size group 1), *M. gelatinosum* (toothfish groups 2 and 3), juvenile *S. gracilis* and *Bathylagus antarcticus* (adults and juveniles).

Similarly, whilst the total fatty acid composition of *D. rebaini* differed substantially from toothfish, the disparity of normal and jack-knifed classification suggests difficulty in comparing the fatty acid composition of these species. This holds for several other species including *C. sloani* (compared to toothfish size group 4), *S. boa boa* and adult *Borostomias antarcticus*. Comparison using only PUFA also produced large differences in normal and jack-knifed classification in the aforementioned species (excluding *S. boa boa*) in addition to *P. argenteus* (in comparison to toothfish size group 2), *E. cryomargarites* (toothfish group 3), *C. sloani* (toothfish groups 3 and 4), and juvenile *S. gracilis*.

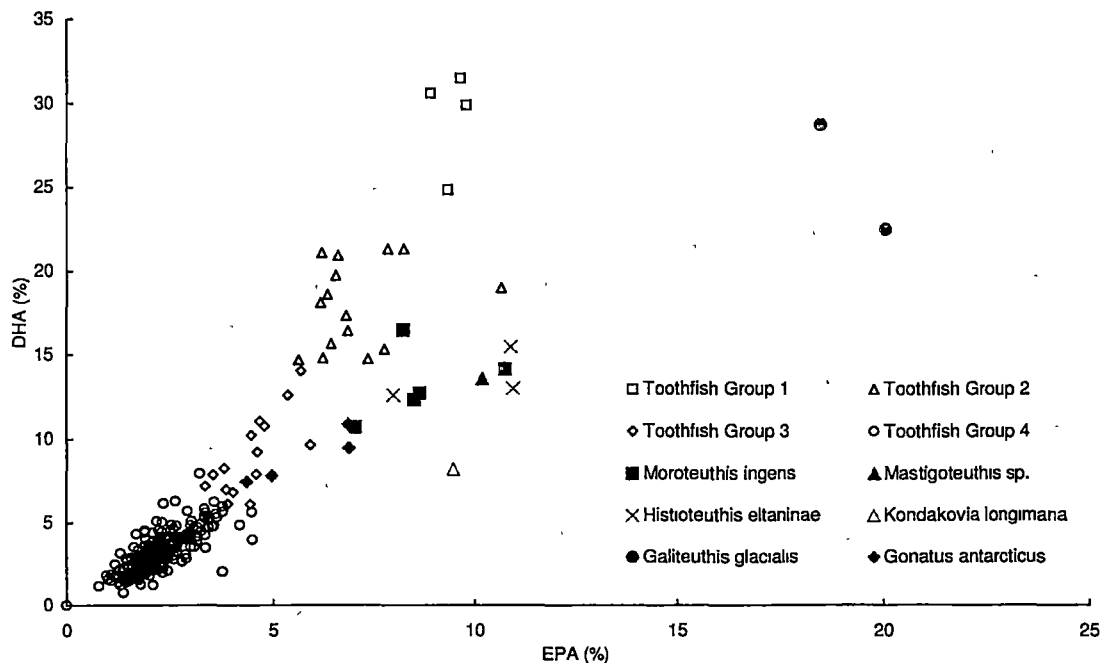
The relative composition of EPA and DHA in several fish did, as was observed with a number of myctophids, concur with the levels of various sized toothfish using LDA. These species included *P. crassiceps*, *M. gelatinosum* (toothfish size groups 2 and 3), *Echiodon cryomargarites* (toothfish group 2), *D. rebaini*, *C. sloani* (toothfish group 3), *S. boa boa*, *S. gracilis* (adult and juvenile), and juvenile *Bathylagus antarcticus*. A degree of statistical similarity in EPA and DHA composition was also observed between juvenile *Borostomias antarcticus* and toothfish of size group 2. Disparity was, once again, observed in the normal and jack-knifed classification of *P. argenteus* (toothfish group 2), *L. yanoi*, *E. cryomargarites* (toothfish group 3), *C. sloani* (toothfish group 4) and adult *Borostomias antarcticus*.

#### *Toothfish and squid*

A comparison of the total fatty acid composition of squid and toothfish by MDS (Figure 7.7) and the relative composition of EPA and DHA (Figure 7.8)



**Figure 7.7** Scatterplot of multidimensional scaling (MDS) based upon the total fatty acid composition of *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and various squid species included in this study from the Macquarie Island region



**Figure 7.8** Relative composition (percentage of total fatty acids) of eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) in *Dissostichus eleginoides* (refer to text for details of toothfish size groups) and various squid species included in this study from the Macquarie Island region

reveals clustering of several potential prey and the predator. Of the six squid species, only *Moroteuthis ingens* and *Gonatus antarcticus* clustered close to toothfish. The five specimens of *M. ingens* were split between toothfish size groups 2 and 3 by both MDS and the levels of EPA and DHA. All four specimens of *G. antarcticus* were distanced from toothfish size group 4 by MDS analysis, but the relative compositions of EPA and DHA placed them at closer proximity to toothfish size group 3.

The remaining squid clustered at a greater distance from toothfish. Both specimens of *Galiteuthis glacialis* are separated, by a large distance, from toothfish size group 1 using both MDS and levels of EPA and DHA. The single specimen of *Mastigoteuthis sp.* clustered closest to toothfish size group 2 using both comparative methods. MDS analysis placed the single specimen of *Kondakovia longimana* equidistantly from toothfish size groups 2 and 3, whilst the relative proportions of EPA and DHA cluster *K. longimana* closer to toothfish group 3. All three specimens of *Histioteuthis eltaninae* are placed equidistantly from toothfish size groups 1 and 2 by MDS and closer to toothfish group 2 based on levels of EPA and DHA.

LDA of the total fatty acid and PUFA profiles, as well as EPA and DHA levels, suggests little statistical similarity in the fatty acid compositions of squid and toothfish (Table 7.4). For instance, based upon a comparison of total fatty acids, *G. antarcticus* (compared to toothfish size group 4) and *M. ingens* (toothfish group 2) differ from their respective toothfish groups in the composition of a total of 17 and 14 fatty acid respectively. Additionally, many of these differences are due to varying combinations of major fatty acids such as 16:0, 18:1 $\omega$ 9c, 18:1 $\omega$ 7c, 22:1 $\omega$ 11c, 22:1 $\omega$ 9c and 18:2 $\omega$ 6. Similarly, differences in PUFA composition between *M. ingens* and *G. antarcticus* and toothfish size groups 2 and 4 respectively are due to several major components including 18:2 $\omega$ 6, EPA and DHA. Comparison using only EPA and DHA also revealed little statistical similarity.

Although little similarity existed in the total fatty acid composition of toothfish and remaining squid, the differences observed were not as great as those for

**Table 7.4** Comparison of the percentage of correctly classified squid species with size groupings of *Dissostichus eleginoides* by linear discriminant analysis, relative to different combinations of fatty acids (100%=no similarity between prey and toothfish)

Squid species	Toothfish size group <sup>1</sup>	Correct classification (%) of potential squid prey								
		All fatty acids			PUFA only			EPA and DHA only		
		LDA	JK	Predictors	LDA	JK	Predictors	LDA	JK	Predictors
<i>Mastigoteuthis</i> sp.	2	0	0	16:1ω9c, 22:1ω9c, 22:1ω7c	0	0	18:3ω6, 18:4ω3, 18:2ω6	0	0	EPA
<i>Kondakovia longimana</i>	2	0	0	16:1ω5c, 18:1ω5c, 20:1ω11c, 20:1ω9c, 22:1ω7c, C <sup>21</sup> PUFA	0	0	18:4ω3, C <sup>21</sup> PUFA	0	0	EPA, DHA
	3									
<i>Gonatus antarcticus</i>	4	25	25	14:0, 16:0, 17:0, 18:0, 18:1ω9c, 19:0, 20:1ω11c, 22:1ω11c, 22:1ω9c, 22:1ω7c, 24:1ω11c, 18:3ω6, 20:4ω3, 20:2ω6, C <sup>21</sup> PUFA, 22:4ω6, DPA	25	25	18:3ω6, EPA, 20:3ω6, 20:4ω3, C <sup>21</sup> PUFA, DHA, 22:4ω6, DPA	0	0	EPA, DHA
	3*	0	0	14:1ω5c, 18:1, 24:1ω9c	0	0	18:4ω3, 20:2ω6	25	25	EPA, DHA
<i>Moroteuthis ingens</i>	3^	0	0	16:1ω7t, 17:1, 24:1ω11c, 24:1ω9c	0	0	20:2ω6	0	0	EPA, DHA
	2^	0	0	16:0, 16:1ω9c, 16:1ω7t, 17:1, 18:1ω9c, 18:1ω7c, 18:1ω5c, 20:1ω11c, 22:1ω9c, 18:3ω6, 18:2ω6, 20:3ω6, 20:2ω6, DPA	0	0	18:2ω6, 20:2ω6, C <sup>21</sup> PUFA	20	20	EPA, DHA
<i>Histioteuthis eltaninae</i>	2	0	0	16:1ω9c, 20:1ω7c, 22:1ω9c, 22:1ω7c, 18:3ω6, 20:2ω6	0	0	18:3ω6, 20:2ω6	0	0	EPA, DHA
	1	0	0	16:1ω9c, 18:1ω9c, 20:1ω11c	0	0	20:4ω3, 20:2ω6, DHA, DPA	0	0	DHA
<i>Galiteuthis glacialis</i>	1	0	0	15:0, 18:1ω5c, DPA	0	0	18:3ω6, AA, DPA	0	0	EPA

<sup>1</sup>closest clustering toothfish size group (refer to text for details on toothfish sizing) determined from multidimensional scaling (Figure 7.7) except for \*determined from a plot of DHA versus EPA (Figure 7.8); ^comparison based upon the separation of squid specimens into two or more groups, each corresponding with a different toothfish size group; PUFA=polyunsaturated fatty acid; AA=arachidonic acid (20:4ω6); EPA=eicosapentaenoic acid (20:5ω3); DHA=docosahexaenoic acid (22:6ω3); DPA=docosapentaenoic acid (22:5ω3); LDA=linear discriminant analysis classification (0%=no similarity in fatty acid composition of toothfish and prey); JK=jack-knifed LDA classification

*M. ingens* (compared to toothfish group 2) and *G. antarcticus* (toothfish group 4). Comparison of *G. antarcticus* with toothfish size group 3 rather than group 4 reveals greater statistical dissimilarity, but separation is based on a smaller number of fatty acids, of which only 24:1 $\omega$ 9c represented a major component (>1%). Differences between toothfish and squid based on small numbers of relatively minor fatty acids were also observed for *Mastigoteuthis sp.*

(16:1 $\omega$ 9c, 22:1 $\omega$ 9c, 22:1 $\omega$ 7c), *M. ingens* (one specimen only compared to toothfish size group 3, 16:1 $\omega$ 7t, 17:1, 24:1 $\omega$ 11c, 24:1 $\omega$ 9c), and *G. glacialis* (15:0, 18:1 $\omega$ 5c, DPA). *H. eltaninae* varied from toothfish size group 2 with respect to mainly minor fatty acids (16:1 $\omega$ 9c, 20:1 $\omega$ 7c, 22:1 $\omega$ 7c, 18:3 $\omega$ 6, 20:2 $\omega$ 6), but also with regards to one major fatty acid (22:1 $\omega$ 9c). Similarly, whilst *H. eltaninae* differed from toothfish group 1 in the composition of only 3 fatty acids, these included the major fatty acid 18:1 $\omega$ 9c.

The PUFA composition of all of the aforementioned squid also differed from their corresponding toothfish size groups with respect to PUFA. However, for the most part, these differences were due to limited numbers of minor constituent PUFA. Exceptions included *Mastigoteuthis sp.* and *H. eltaninae* that differed from toothfish with respect to the major PUFA 18:2 $\omega$ 6 and DHA respectively. The normal and jack-knifed classification of *M. ingens* (compared to toothfish size group 3) also varied considerably.

Large differences in the composition of EPA and DHA between toothfish and squid are also apparent. *G. antarcticus*, *M. ingens* (compared to toothfish size group 2 only), *H. eltaninae* and *G. glacialis* show little statistical similarity (normal and jackknifed classification) to their corresponding toothfish in the composition of EPA and DHA. A large discrepancy, however, in the normal and jack-knifed classification of *Mastigoteuthis sp.*, *K. longimana* and *M. ingens* (compared to toothfish size group 3 only) suggests difficulty in the comparison of these squid species.

## 7.4 DISCUSSION

Determining diet necessitates the amalgamation of various factors, including species habitat and general biology, to reach a conclusion based on all aspects of foraging ecology. For example, despite the limitations of stomach content analysis this technique provides a base from which to speculate on the diet of toothfish. Assessing diet using fatty acids requires more than the comparison of predators and potential prey profiles and searching for similarities. It also requires careful consideration of the interaction of species within the biological constraints of the marine environment. In particular, understanding the relative distribution of species to each other, both geographically and within the water column, is an essential part of predicting predator-prey interactions. Equally relevant is the influence that environmental adaptation and numerous physiological processes can have on fatty acid composition, adding to the complexities involved in determining predator-prey interactions using only lipid compositional data.

### **Toothfish diet determined from stomach content analysis**

#### *Potential fish prey*

Fish represent the major prey group consumed by toothfish in the Macquarie Island region both by frequency of occurrence (65%) and prey biomass (58%), based upon the analysis of stomach contents (Goldsworthy et al. 2002). These findings are directly applicable to the current study. The mean total length of toothfish investigated by Goldsworthy et al. (2002) (600-700mm) is comparable to samples examined for fatty acids in this study (642mm). Toothfish from both studies were also collected during the same seasons (excluding season 1995/1996) from the same grounds, and were caught at a similar range of fishing depths. Potential differences in the diet of toothfish examined in this study and that of Goldsworthy et al. (2002), due to any of these factors, can accordingly be excluded.

Eleven of the twenty-one species of midwater fish examined for fatty acids in this study are consumed by toothfish at Macquarie Island according to stomach content analysis (Goldsworthy et al. 2002). In particular, *Bathylagus* sp. (represented by *Bathylagus antarcticus*) is the group of fish most commonly consumed by toothfish in terms of frequency of occurrence (14%) and biomass (14%). Although the total proportion of fish in the diet is independent of toothfish size, the relative proportions of individual fish species do vary. This is particularly evident in relation to the dietary importance of Myctophidae. In two of the three fishing seasons (1995/1996 and 1997/1998) examined by Goldsworthy et al. (2002) myctophids account for a small proportion of toothfish diet in terms of frequency of occurrence (10%), and biomass (3%). Additionally, the proportion of myctophids in the diet of toothfish from these two seasons is independent of toothfish size and fishing ground. However, in the third season examined (analogous to the 1998/1999 fishing season of this study), the diet of small toothfish (<450mm total length) from the southern ground was dominated by myctophids (>65% prey biomass). This holds considerable significance for the current study. Of those toothfish measuring <450mm that were analysed for fatty acid composition (n=42), the majority of specimens (n=27, 64%) were collected from the southern fishing ground during the 1998/1999 season. Five of the myctophid species analysed for fatty acid composition in this study are represented in the stomach contents of Macquarie Island toothfish; *Electrona carlsbergi*, *Gymnoscopelus braueri*, *G. fraseri*, *G. nicholsi* and *Lampanyctus archirus*. The remaining myctophids examined, *G. bolini*, *Protomyctophum bolini* and *E. antarctica*, were not detected in toothfish stomachs.

A host of other fish represent minor contributors to toothfish diet in both frequency of occurrence (combined contribution 4%) and biomass (5%). Of those fish analysed for fatty acids in this study, *Labichthys yanoi*, *Stomias* sp. (represented by *S. gracilis* and *S. boa boa*), *Borostomias* sp. (represented by *Borostomias antarcticus*) and *Poromitra* sp. (represented by *P. crassiceps*) are included in this category.

Although not amongst those species identified in the stomach contents of toothfish, the remaining fish species for which fatty acid compositions were determined are likely to be included in the category of 'unidentified fish'. Unidentified fish potentially includes the following species; *Chauliodus sloani*, *Sternoptyx sp.*, *Echiodon cryomargarites*, *Phosichthys argenteus*, *Cyclothone sp.*, *Diplophos rebainsi*, and *Melanostigma gelatinosum*. Unidentified fish form a major component of toothfish diet, both in terms of frequency of occurrence (36%) and biomass (15%).

### *Squid*

Squid represent, on the basis of stomach contents, the second most important prey group (after fish) for toothfish in the Macquarie Island region (Goldsworthy et al. 2002). Squid occur in 35% of toothfish stomachs and account for 32% of the prey biomass. All six squid species included in this study are consumed by toothfish. *Gonatus antarcticus* is the single most important prey species consumed by toothfish, representing 16% of the total prey biomass (frequency of occurrence 8%). The remaining five squid species are less commonly consumed by toothfish. *Mastigoteuthis sp.* (frequency of occurrence 5%; biomass 2%) and *M. ingens* (frequency of occurrence 2%; biomass 4%) are minor prey items. *Galiteuthis glacialis* and *Histioteuthis eltaninae* are grouped together as part of 'other cephalopods' (frequency of occurrence 3.6%; biomass 2.2%). The importance of squid in the diet increases with toothfish size, their presence being uncommon in toothfish <550mm, but accounting for ~40% of biomass in toothfish >550mm.

### **Fatty acid composition of toothfish as evidence of dietary variation**

The application of fatty acid tracers to a dietary study of a voracious and seemingly opportunistic predator as toothfish would initially appear to be limited. Toothfish from many populations consume a range of prey from several different trophic levels, including fish, cephalopods and invertebrates. The complex combination of fatty acids provided by these prey species means that tracking the source of fatty acids through the food web becomes more difficult as the trophic level at which toothfish forage similarly increases.

Determining which fatty acids are provided by exactly which prey in a near top-level predator such as toothfish could prove a difficult task.

Against this background though, the diet of toothfish from regions such as Crozet and Kerguelen Islands (Duhamel and Hureau 1985), South Georgia Island (García de la Rosa et al. 1997; Pilling et al. 2001) and the Argentinian slope (García de la Rosa et al. 1997) varies considerably with size, as well as fishing depth and location. The partitioning of diet between different sizes is a common feature of fish and is most likely an adaptation aimed at minimising intra-species competition for resources. The consequence of this partitioning is that each developmental stage of toothfish can, for dietary purposes, be treated as separate predatory entities. That is, small and large toothfish are most likely foraging (with a certain amount of overlap) within different trophic niches. This, in turn, limits the range of prey species that small and large toothfish consume, narrowing the potential source of dietary fatty acids. Separating toothfish into dietary groups based upon size therefore simplifies the process of trying to identify trophic links solely using fatty acid composition.

This shift in diet with maturation may be reflected in the considerable variation of fatty acid composition in relation to size (analogous to age) of Macquarie Island toothfish, especially in the composition of EPA and DHA (see Chapter 5). However, the diet of Macquarie Island toothfish appears to be more homogeneous than other populations. Variations in toothfish diet are typically observed between years and location but not age, fishing depth or time of day (Goldsworthy et al. 2001c; Goldsworthy et al. 2002). This would seem to largely discount the influence of diet as a major factor leading to the disparate fatty acid composition of small and large toothfish. Significantly though, the majority of small (<450mm) toothfish analysed in this study were collected from southern fishing ground during the 1998/1999 fishing season, a ground and season that corresponds with the atypical predominance of myctophid in the diet of this size class. Combined with the virtual absence of squid, the diet of the majority of small toothfish analysed in this study is considerably different to that of larger toothfish (>450mm). This size-related

partitioning of diet may account for the variation in fatty acid observed. To test whether this variation is reflected in the range of fatty acids provided by the diet requires examination of potential prey species.

The ability to separate toothfish in a number of dietary groups based upon size presents certain advantages when attempting to simplify the determination of foraging ecology. However, complications still arise. Although the diet of toothfish shifts with age, the likelihood that toothfish forage upon a wide range of prey at all stages of their life history is high. The larger the range of prey consumed by toothfish, the more difficult it becomes to recognise the signatures of prey in the fatty acid composition of toothfish. Each of the fatty acids contributing to the composition of toothfish could have been supplied by a countless number of prey, or unlimited combinations of prey. Therefore, similarity in the fatty acid composition of toothfish and prey may not necessarily indicate a direct dietary relationship. Different combinations of prey can produce the same fatty acid compositions in predators. For instance, the reliance upon one or many different prey species that share a common fatty acid composition (by virtue of a shared diet) will produce the same combination of fatty acids in the predator. In the same way, even if the majority of toothfish diet is composed of just one or two species, the influence of minor, oil-rich prey species may lead to the 'corruption' of the fatty acid signatures of these major prey such that they are no longer recognisable.

### **Comparison of the fatty acid composition of toothfish and potential prey species**

#### *Limitations of the data set*

A major restriction of this comparative study is the absence of fatty acid data pertaining to a number of important species identified in the stomach contents of toothfish (see Goldsworthy et al. 2002). In particular, data is lacking for fish species belonging to the families Macrouridae (*Coryphaenoides* sp. and *Cynomacrus piri*), Moridae (*Halargyreus*

*johnsonii* and *Lepidion* sp.) and Nototheniidae (*Lepidonotothen squamifrons* and *Paranotothenia magellanica*). The combined dietary significance of these species amounts to ~20% of prey biomass. The Macquarie Island region also supports a range of prawn-like crustaceans, including euphausiids and mysids, for which no fatty acid data exists. Crustaceans can contribute up to 9% of the prey biomass in the diet of toothfish. The lack of available fatty acid data for species that contribute close to 30% of the prey biomass of toothfish represents a potentially large oversight.

A similar problem applies to the range of toothfish samples analysed. Although the toothfish examined in this study are representative of the overall size-distribution of the Macquarie Island population, fatty acid compositional data for toothfish TL <300mm and >1300mm is lacking. In addition, most toothfish (77%) examined in this study are >500mm TL, meaning that the lipid composition of small fish (i.e. <500mm TL) may not properly represent the true variation in fatty acid composition that exists.

The use of arbitrary toothfish size groups, for comparative purposes, also presents difficulties. The large and abrupt change in PUFA levels in toothfish <500mm TL means that separating the fatty acid compositions of these toothfish into a number of clusters inevitably leads to the artificial 'smoothing' of variations within the data set. This can accentuate differences in fatty acid composition between toothfish and prey, leading to statistical errors. This is especially the case when the fatty acid composition of prey is intermediate to two toothfish size groups (i.e. where a prey straddles two toothfish size groups).

#### *Total fatty acid composition*

In general, the total fatty acid composition of toothfish and potential prey are statistically dissimilar. Much of this dissimilarity is, however, due to fatty acids that are easily created by the modification of related fatty acids, or those readily synthesised from non-lipoidal sources (e.g. protein and carbohydrate). Many MUFA belong to one or both categories (e.g. 16:1 $\omega$ 7c, 18:1 $\omega$ 9c,

18:ω7c), whilst most saturates belong to the latter (e.g. Allen 1976; Arts et al. 2001). Several factors affect the rate of fatty acid synthesis and modification. For instance, the synthesis of fatty acids in fish generally decreases with increasing dietary lipid intake, due to the suppression of lipogenic enzymes such as fatty acid synthetase, malic enzyme and glucose-6-phosphate dehydrogenase (e.g. Regost et al. 2001). Thus, when the dietary intake of lipids is sufficient to meet physiological demands, the synthesis of fatty acids is repressed. Whether or not lipid intake is a limiting factor in the diet of toothfish is currently unknown. Similarly, the rate of elongation and desaturation of C<sub>18</sub> fatty acids in the liver hepatocytes of Atlantic salmon (*Salmo salar*) is regulated by the presence or absence of certain fatty acids in the diet (Ruyter et al. 2000).

Whilst elevated levels of long-chained MUFA (20:1 and 22:1) in Northern hemisphere marine organisms are often cited as evidence of a diet high in wax ester-rich copepods (e.g. Hagen et al. 2000), it is difficult to apply this observation to the present study. The lipid composition of copepods from the sub-Antarctic generally and Macquarie Island specifically are poorly described, making it impossible to test the validity of this assumption.

Although saturates and MUFA are unquestionably consumed and integrated into storage tissue by fish, biosynthetic activity can elevate the levels of these fatty acids above those of the diet or, where modification occurs, substantially lower them. In spite of the fact that LDA analysis separates toothfish and potential prey (i.e. fish and squid) into different classification groups, in the majority of cases this separation is due primarily to fatty acids that are not strictly of dietary origin.

#### *Polyunsaturated fatty acid composition*

Due to the various changes that may affect SFA and MUFA, as described above, the fatty acid components that hold the greatest potential for use as dietary indicators are PUFA. Whilst PUFA are essential to the proper functioning of a range of biochemical activities and mechanisms, many fish

are either unable to synthesise PUFA or can only do so in extremely small quantities (e.g. Dunstan et al. 1999). This restriction to biosynthesis means that the vast majority of fish obtain PUFA almost exclusively from the diet. However, the differences in PUFA composition observed in toothfish and potential prey are still great enough that they are separated by LDA (Tables 7.1-7.4), making it difficult to assign predator-prey relationships based only on PUFA composition.

### *EPA and DHA composition*

Any examination of differences also has to take into account what proportion of the total composition these fatty acids represent. In fact, most of the PUFA responsible for differences amongst toothfish and potential prey are minor components, representing less than 1% of total fatty acids (e.g. 18:3 $\omega$ 6, 18:4 $\omega$ 3, 20:4 $\omega$ 3). Relatively small differences in the compositions of these minor PUFA can produce statistically significant results. They are also more sensitive to metabolic pressures. The relative proportions of minor fatty acid components are more rapidly decreased, in comparison to major components, by metabolic processes.

Given the influence of these factors on the interpretation of lipid data, the approach taken in this study was to focus attention on PUFA that are both major fatty acid components and vary to an extent that suggests a significant dietary influence. Previous examination reveals that the size-related variation in toothfish fatty acid composition can be attributed to just two PUFA, EPA and DHA (Chapter 5). Because DHA and EPA mainly originate from the diet, coupled with variation in the composition of these fatty acids of around 10% of total fatty acids, comparable levels of these PUFA in toothfish and potential prey species could indicate a trophic link. Diminishing levels of EPA and DHA are associated with increasing dietary distance between the source of these PUFA (primary producers) and the predator species (Dunstan et al. 1999). The shift in EPA and DHA observed therefore adds further support to the assumption that larger toothfish occupy a higher trophic level than that of smaller toothfish.

A range of potential fish and squid contain similar proportions of EPA and DHA to toothfish (separated into various size classes). In general, the compositions of EPA and DHA detected in fish correlate more closely with toothfish than those of squid. Fish also associate with all four toothfish size groups, though the make-up of species that associate with each size group differs markedly. This equates with the consistent presence of fish in the diet of toothfish (regardless of toothfish size) detected by stomach content analysis, as well as the shift in the species composition of fish consumed by toothfish as they mature. In particular, the Myctophidae, *L. archirus*, *E. antarctica* and *E. carlsbergi* contain levels of EPA and DHA comparable to that of small to medium toothfish (size groups 2 and 3) using LDA. *G. braueri* also associates with toothfish from size group 3, however, the large variation in fatty acid composition (including EPA and DHA) of this species meant that this association was limited to only 3 of 8 specimens. The closeness of EPA and DHA levels is consistent with the predominance of myctophids in the diet of the majority of smaller toothfish included in this study. This contrasts sharply with the secondary importance of myctophids in the diet of larger toothfish (>450mm, corresponding to toothfish size group 4). The similarity of two myctophids, *G. nicholsi* and *G. bolini*, to larger toothfish therefore seemingly contradicts the findings of stomach content analysis. This closeness raises the possibility that myctophids are of greater dietary importance to larger Macquarie Island toothfish than previously thought, or that they share (at least in part) a common diet.

Although *Bathylagus antarcticus* was identified by stomach content analysis as being of major dietary importance across all sizes of toothfish, EPA and DHA levels are closest to intermediately-sized toothfish (group 2). The range of sizes and fatty acid compositions necessitated the separation of *Bathylagus antarcticus* into juveniles and adults. Juveniles straddled toothfish size groups 1 and 2, though only those *Bathylagus antarcticus* clustering with group 2 were identified by LDA having comparable levels of EPA and DHA to toothfish. Adult *Bathylagus antarcticus* and toothfish are separated using LDA by differences in DHA composition.

In concurrence with the close association observed between several myctophid species and toothfish, the majority of the remaining fish contain EPA and DHA at levels comparable to intermediate sized toothfish (toothfish size groups 2 and 3). These species include *E. cryomargarites*, juvenile *S. gracilis*, juvenile *Borostomias antarcticus* (toothfish size group 2) and *C. sloani* (size group 3). In addition, *M. gelatinosum* straddles both toothfish size groups 2 and 3. The high levels of DHA and EPA present in the smallest toothfish analysed in this study (size group 1) are only matched by *P. crassiceps*, although levels in *Sternoptyx sp.* are approaching those of toothfish from size group 1 (though not confirmed by LDA). The largest toothfish (size group 4) associates with three species, adult *S. gracilis*, *S. boa* and *D. rebainsi*. Whilst *S. gracilis*, *S. boa*, *P. crassiceps* and *Borostomias antarcticus* have been identified in the stomach contents of Macquarie Island toothfish (as minor constituents of the diet), the same does not hold for the latter species. It is likely that they are amongst the large proportion of unidentified fish remains, the dietary importance of which may have previously underestimated by stomach content analysis.

Several prey species differ considerably in fatty acid composition to toothfish. Although *L. yanoi*, *P. bolini* and *G. fraseri* cluster with medium-large toothfish based on MDS and EPA and DHA levels, they show great dissimilarity based on LDA. Both *L. yanoi* and *G. fraseri* have been identified in the stomach contents of toothfish, however, the dietary importance of these species is small based on stomach content analysis. LDA also reveals a substantial difference in the fatty acid composition of all six squid species examined and toothfish. In general, the EPA levels of all squid exceeded that of corresponding toothfish, especially in *G. glacialis* (approximately 10% higher than small toothfish). Differences in the relative levels of a number of other major fatty acid were also identified. The absence of a strong correlation between squid and toothfish is surprising, though it could support certain dietary observations. For instance, the dissimilarity of small-medium toothfish (size groups 1,2,3) and squid fatty acid compositions may partly reflect that squid are rarely consumed by toothfish <550mm. However, given that based on stomach content analysis the dietary importance of squid rises to ~40% of

prey biomass in toothfish >550mm, a greater degree of similarity in the fatty acid composition of large toothfish and squid (especially *G. antarcticus*) would be expected. Although a comparison of EPA and DHA levels and, to a lesser extent, MDS do cluster *G. antarcticus* with larger size toothfish (straddling size groups 3 and 4), LDA does not support these observations. The importance of squid in the diet of large toothfish from Macquarie Island could be well below that suggested by Goldsworthy et al. (2002), more in line with toothfish populations from other regions (e.g. García de la Rosa et al. 1997; Pilling et al. 2001). However, the small number of squid samples available for analysis means that the extent of within-species variation in fatty acid composition (due to age and seasonal factors) presently cannot be properly ascertained. Additionally, the simultaneous consumption of MUFA-rich fish (e.g. myctophids) or other prey could account for the reduction in PUFA (especially EPA) observed in toothfish, even if squid are a large portion of the diet.

### **Physiological factors influencing the fatty acid composition of toothfish**

A number of fundamental difficulties arise when attempting to use biologically active compounds, such as fatty acids, as dietary tracers. The processes governing the biosynthesis and modification of fatty acids previously outlined are generally well understood. In contrast, the manifold physiological processes responsible for regulating the function and utilisation of fatty acids are poorly understood within wild fish populations (e.g. Sidell 1991). The cumulative influence of these processes on the fatty acid composition of toothfish holds great relevance to their use as dietary tracers. Current understanding of these processes is based upon the investigation of species principally reared in artificial environments (e.g. Navas et al. 1998; Cahu et al. 2000; Ruyter et al. 2000; Gapasin and Duray 2001; Koven et al. 2001; Regost et al. 2001). Whilst the findings of these studies are important, the level to which they can be applied to wild fish populations is unknown.

*Rate of fatty acid turnover*

Although dietary fatty acids are likely to be deposited into storage with little or no modification, the relatively low lipid content of small toothfish suggests that the capacity to store lipid on a long-term basis is limited to more mature toothfish only. Ongoing physiological processes, including metabolism, modification and incorporation into structural components (e.g. cell membranes, visual and neural organs) mean that the turnover of fatty acids in fish may be as little as three weeks (Kirsch et al. 1998). Turnover of lipid in small toothfish would also be expected to exceed that of larger fish. The higher lipid content of larger toothfish means that a greater proportion of dietary lipid is initially directed into storage rather than physiological activities.

The constituent fatty acids of fish are consequently a temporal integration of diet spanning weeks rather than the months observed in, for example, marine mammals, though this still represents a significant advancement over stomach content analysis. This is of little importance if dietary intake is consistent as the fatty acid composition will be the same regardless of when fish are sampled. In situations where the composition of prey consumed is in flux though, for example during seasonal shifts in diet from one prey to another, constituent fatty acids reflect a transitional state rather than a clear integration of diet. The 'blurring' of fatty acid signatures creates obvious difficulties in applying this information to the identification of dietary links. Seasonal shifts in diet are apparent in the diet of toothfish from Macquarie Island (Goldsworthy et al. 2002). However, detection of any shift in fatty acid composition associated with these seasonal differences was obscured by the overriding influence of size-related dietary variation within this population (Chapter 5). Regardless, variation in diet over the relatively short period in which the toothfish analysed in this study were collected (approximately 2 months) is likely to be minimal.

*Influence of total lipid content of potential prey*

The impact of the total lipid content of a prey organism on the fatty acid composition of toothfish also needs to be considered. Given two prey of equal dietary importance to toothfish (in terms of biomass), the lipid-rich prey will exert disproportionately greater influence on fatty composition compared to the lipid-poor prey species. The dietary importance (in terms of biomass) of many of the lipid-rich species (>10% lipid content), including adult *S. gracilis* (14.2%), *S. boa boa* (10.2%), *D. rebaini* (17.0%), *E. antarctica* (17.5%) and *G. bolini* (14.5%) and the squid *G. antarcticus* (17.5%) and *K. longimana* (13.5%) may therefore be overestimated as they have a masking effect on low lipid containing species. Conversely, the importance of the lipid-poor species such as *P. argenteus* (1.0%), *P. crassiceps* (1.3%), *E. cryomargarites* (2.0%), and juvenile *Bathylagus antarcticus* (2.2%), may be underestimated.

The unique lipid storage mechanism of squid also adds a level of complexity. Phillips et al. (2002) observed that the fatty acids stored in the prominent, lipid-rich digestive glands of some squid, including *M. ingens* (26.8% lipid content, Phillips et al. 2001) and *K. longimana* (38.0%) and *G. antarcticus* (47.7%) from the current study, is a more direct measure of diet than the lipid stores of fish. As most squid depend more on amino acids rather than fatty acids for metabolic energy, the vast majority of lipid consumed by squid is directed into the digestive gland for storage, with little or no modification. This 'warehouseing' of lipid makes it more difficult to distinguish between the fatty acid composition of the prey species of squid and the composition of squid mantle tissue.

*Size-related and seasonal variation in fatty acid composition of potential prey*

The size-related variation in fatty acid composition of toothfish (i.e. increase in PUFA in larger fish) is also apparent in several fish species analysed in the current study, namely *Bathylagus antarcticus*, *Borostomias antarcticus* and *S. gracilis*. The ability of a prey species to express a range of fatty acid

profiles at different ages complicates the identification of predator-prey interactions. Determining the likelihood of a predator-prey interaction is only partially possible in the absence of details on the extent of fatty acid variation within a prey species. Many species may also experience seasonal changes in fatty acid composition. It is currently not possible to speculate on whether substantial shifts in the fatty acid composition of potential prey are likely in the Macquarie Island region. Detection of these potential fluxes, by sampling from different times of the year, has not been undertaken.

### *Selective metabolism, mobilisation and partitioning of fatty acids*

Added to the factors noted above are other physiological influences on the fatty acid composition of toothfish. The potential for the selective mobilisation of certain fatty acids, for either metabolic or physiological needs, is an important consideration. The ability of fish to selectively deposit lipid and fatty acids into different tissues is well-acknowledged (Navarro et al. 1995; Phleger et al. 1997; Phleger et al. 1999a; Phleger et al. 1999b; Kamler et al. 2001), including for toothfish (Chapter 4). The type of tissue in which lipids are stored also influences the rate at which fatty acids are metabolised. When energy is required, fatty acids contained within oxidative muscle, adipose tissue and organs including the liver are depleted at a far greater rate (e.g. Lund and Sidell 1992; Friedrich and Hagen 1994) than in tissues located in organs such as the brain and eyes (e.g. Navarro et al. 1995). In addition, MUFA such as 18:1 $\omega$ 9c and 16:1 $\omega$ 7c may be metabolised in preference to PUFA (Sidell 1991; Lund and Sidell 1992). However, Crockett and Sidell (1992) question the preferential metabolism of MUFA over PUFA, and suggest that fatty acids such as EPA and DHA should be considered as equally important substrates for energy metabolism.

The combination of this disparate, tissue-specific and fatty acid-specific metabolism is that fatty acids present in metabolically resistant tissues can impart a disproportionately greater influence on overall fatty acid composition. However, the vast majority of lipid in toothfish is stored within

tissues readily exposed to metabolic processes (Chapter 4), mitigating the influence of these processes on overall fatty acid composition.

#### *Influence of environmental adaptation on fatty acid composition*

Environmental adaptation also plays a role in the pattern of fatty acid distribution within fish. The best described is the tendency for fish from colder environments to accumulate PUFA, especially in the PL fraction, in order to maintain membrane fluidity (e.g. Dunstan et al. 1999; Cahu et al. 2000). The deposition of PUFA (particularly EPA and DHA) into PL rather TAG stores (e.g. Medina et al. 1995; Guerden et al. 1997; Montgomery et al. 1999; Hagen et al. 2000) in part explains the domination of EPA and DHA observed in the smaller, more PL-rich toothfish analysed in this study. The effect of this process is exacerbated by the coincidental increase in total lipid content in the form of TAG (and associated decrease in the relative proportion of PL) as toothfish mature.

#### **Relevant biological and environmental factors**

Assessing the likelihood of predator-prey interactions also requires consideration of environmental and biological factors that can exert significant influence on diet. Similarity in the fatty acid composition of toothfish and potential prey does not necessarily imply the presence of a simple predator-prey relationship. Overlapping fatty acid compositions could imply a similar diet or that the predator-prey relationship is reversed, such that toothfish are in fact consumed by potential prey. Thus, knowing the size of prey is essential in order to determine the likelihood of predation by toothfish and information regarding prey diet is required to assess the likelihood of dietary overlap. A further consideration is how prey are distributed within the water column; do the habitats of toothfish and potential prey overlap to an extent suggesting that foraging is a real possibility?

*Distribution within the water column*

The distribution of a potential prey species within the water column provides an indication as to the probability that they are available for consumption by toothfish. Assuming the size related stratification of toothfish into different depth zones is associated with minimal vertical movement of toothfish within the water column, only those prey within the distribution range of toothfish are likely to be consumed. Such a situation is complicated by diurnally migrating fish (e.g. myctophidae), that may be consumed by toothfish of differing sizes at different times of the day. Figure 7.9 presents the approximate depth distribution pattern, at Macquarie Island, of toothfish (determined from trawl depth) and potential fish prey (determined from Williams 1985; Gon and Heemstra 1990; Oven et al. 1990; Kock 1992; Miller 1993; Williams et al. 2001) included in this study. As the distribution of fish within the water column is heavily influenced by factors including time of year (related to depth of primary production) and water temperature, the patterns presented in Figure 7.9 can only be used as an approximate guide. The majority of fish included in the current study are meso-bathypelagic in distribution, although most myctophids are also found in the upper water column (epi-mesopelagic), and are thus well within the reach of toothfish. Significantly, the peak distribution of most myctophids does not overlap significantly with that of toothfish >500mm TL, supporting dietary and fatty acid observations. Of those fish that group with toothfish <500mm TL based on DHA and EPA levels, most are distributed at depths that overlap with toothfish of this size. These species include *P. crassiceps*, *M. gelatinosum*, *S. gracilis*, *Bathylagus antarcticus*, *E. cryomargarites*, *C. sloani* and the myctophids *L. archirus*, *E. carlsbergi*, and *G. braueri*. An exception is *E. antarctica*, a myctophid not present in the stomach contents of toothfish, which is mainly found at shallower depths. In the same way, those fish that group with toothfish >500mm TL, *S. gracilis*, *S. boa boa* and *D. rebainsi*, all reside in water at depths that coincide with large toothfish. The lower depth limit of the myctophids *G. nicholsi* and *G. bolini* at Macquarie Island is not known. The ability of toothfish >500mm to consume these species is therefore unknown.

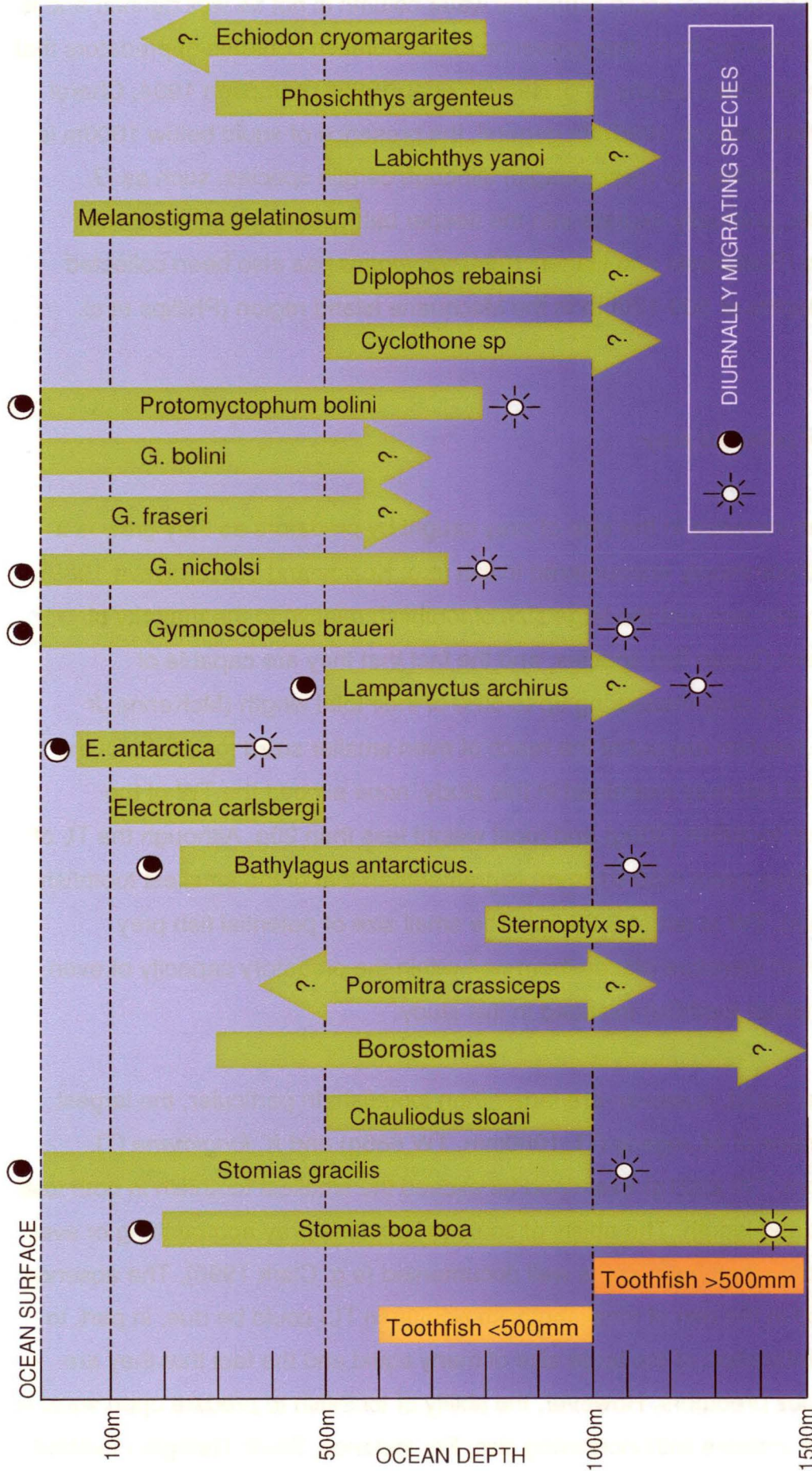


Figure 7.9 Comparison of the approximate depth distribution of toothfish (*Dissostichus eleginoides*) included in this study (separated by size) with the peak distribution of potential fish prey from Macquarie Island

The distribution of squid within the water column is not as well defined and is usually inferred from their presence in the stomach contents of predators that forage at known depths (e.g. Nemoto et al. 1985; Thompson 1994; Cherel and Weimerskirch 1999). In general, the presence of squid below 1000m is rare (i.e. mainly epi-mesopelagic) although certain species, such as *G. glacialis*, gradually migrate into the deeper bathypelagic depths as they mature (Piatkowski and Hagen 1994). *M. ingens* has also been collected from depths of 500-1200m in the Macquarie Island region (Phillips et al. 2001).

#### *Size of potential prey*

A mean increase in the size of prey caught by predators as they grow is a feature commonly encountered in fish (e.g. Kozlov and Tarverdiyeva 1989). Taking into account the large size of toothfish relative to the majority of Southern Ocean fish species, and the fact that they are capable of consuming prey measuring up to 50% of their total length (McKenna Jr 1991), few fish are out of the reach of even smaller sized toothfish. Of the potential fish prey examined in this study, none exceed the TW of the smallest toothfish (300g) and most weight less than 20g. Although the TL of *Labichthys yanoi* (460-610mm) is greater than that of the smallest toothfish (310mm), TW is not (4.9-23.5g). The small size of potential fish prey examined therefore places them well within the predatory capacity of even the smallest toothfish included in this study.

Several squid, however, are larger than toothfish. In particular, the largest specimens of *M. ingens* (TL 1000mm, TW 686g) and *K. longimana* (TL 1050mm, TW 2321g) examined far exceed the smallest toothfish in both total weight and length. The ability of squid to consume prey approaching or even exceeding their own size is well documented (e.g. Clark 1996). The absence of squid in the diet of small toothfish (<550mm TL) could be due, in part, to the combination of the large size of many squid and the fact that they are voracious predators. However, the ability of toothfish to predate upon squid is likely to improve with increasing size. For instance, South Georgian toothfish

consume *K. longimana* with a mean mass of over 4000g, including the consumption of a 13.4kg squid by a 5.5kg toothfish (Xavier et al. 2002).

### *Dietary overlap*

In the same way, similarities in the diet of toothfish and potential prey may be reflected by similarities in fatty acid composition. A certain degree of overlap is likely between toothfish and the larger squid species such as *M. ingens*, *G. antarcticus* and *K. longimana*, although dietary information is scarce. The diet of *M. ingens* in the Macquarie Island region is similar to that of small-medium toothfish, and includes the myctophids *E. carlsbergi*, *E. subaspera*, *Krefflichthys anderssoni*, *G. nicholsi* and *G. fraseri*, and the bathylagid *Bathylagus antarcticus* (Phillips et al. 2001). Although not supported by LDA, both EPA and DHA levels and MDS of fatty acid profiles cluster *M. ingens* and small to medium toothfish together. This clustering may indicate the existence of a complex dietary interaction between *M. ingens* and toothfish, involving a combination of a shared diet as well as the mutual predation of each species on the other. Limited information regarding the diet of other squid species is available. The diets of *K. longimana* (Nemoto et al. 1985) and *G. glacialis* (Lu and Williams 1994a) are apparently dominated by *Euphausia surperba*, although these studies only apply to squid from Antarctic waters. If the diets of these squid are similarly dominated by euphausiids in the Macquarie Island region, overlap would be considered minimal as crustaceans only contribute ~10% of prey biomass in the diet of toothfish (Goldsworthy et al. 2002).

Although the diet of the vast majority of fish species included in this are unknown, a substantial overlap in diet between toothfish and many of these species can immediately be discounted because of the large size gap previously mentioned. Of the mesopelagic and bathypelagic fish from the Macquarie Island for which diet has been determined (Gaskett et al. 2001), several may potentially share elements of their diet with toothfish. An overlap in the diets of toothfish and piscivorous fish such as *E. cryomargarites*, *G. bolini*, *Borostomias antarcticus*, *P. argenteus* and *S. gracilis* is possible.

However, the species composition of the fish that form the diet of these species could not be ascertained, due in part to the advanced state of digestion of much of the stomach contents. In addition, copepods and euphausiids dominate the diet of many of the myctophids included in this study, namely *E. antarctica*, *E. carslbergi*, *G. braueri*, *G. fraseri*, *G. nicholsi* and *P. bolini*. Although crustaceans are acknowledged prey of toothfish in the Macquarie Island region, the minimal dietary overlap between toothfish and myctophids would not be expected to lead to a great similarity in fatty acid composition. Dietary overlap between toothfish and *Bathylagus antarcticus*, *L. archirus* and *M. gelatinosum* is also unlikely as these species principally consume amphipods (Gaskett et al. 2001), a crustacean group contributing <0.1% of the prey biomass of toothfish (Goldsworthy et al. 2002).

Significant overlaps in diet of toothfish and potential prey, leading to the observed similarity in EPA and DHA composition, are in most cases unlikely. Squid are a possible exception; their large size (even relative to toothfish), voracious predatory ability and reliance on many of the same fish consumed by toothfish (e.g. *M. ingens* and myctophids) could point to a considerable dietary overlap. In contrast, the diets of most fish do not coincide with that of toothfish. Overlap is possible with the larger piscivorous fish species such as *S. gracilis*, *E. cryomargarites*, *G. bolini*, *P. argenteus* and *B. antarcticus*, however the exact species composition of their diet is currently unknown.

## **7.5 CONCLUSIONS**

Although many differences are apparent in the fatty acid composition of toothfish and potential prey, correspondence in the composition of PUFA such as DHA and EPA may point to the existence of a number of authentic dietary interactions. These are particularly evident in relation to small-medium toothfish that group with many species of fish and myctophids. The fatty acid composition of squid and toothfish in comparison show a number of differences, contrasting with the dietary importance of squid suggested by stomach content analysis.

However, the limitations associated with the use of fatty acids as dietary indicators must be acknowledged. Various physiological processes, including selective metabolism and biosynthesis of fatty acids, and complicating factors such as vast differences in total lipid content of prey complicate the origins of the fatty acids contributing to the overall lipid composition of toothfish. The decrease in PUFA with increasing toothfish size can also be partly attributed to the concomitant decrease in PL levels. Finally, this study also highlights the limitations of current statistical methods. Interpretation of the results of statistical methods such as LDA and MDA require careful consideration of the various factors that contribute to fatty acid composition.

Nonetheless, the significant impact of toothfish size on fatty acid composition, especially the large variations in PUFA such as DHA and EPA, most likely represents an underlying dietary influence. This contrasts to some extent with the generally constant diet of toothfish, independent of size, inferred from stomach content analysis. Significantly though, the variations in fatty acid composition coincides with the atypical disparity in diet, determined from stomach contents, that was observed between large and small fish in the season from which the majority of the specimens analysed in this study were collected.

# CONCLUSIONS

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Emphasis on the diet of top-level mammalian predators in regions such as Macquarie Island has meant that the ecological importance of those species at lower trophic levels, including midwater fish, squid and invertebrates, is often overlooked. This includes information related to chemical composition, including lipids. Lipids perform a range of important biological functions in marine organisms, including the regulation of buoyancy. Lipids also facilitate the transfer of metabolic energy from lower to higher trophic levels through the complex dietary interactions of the food-web. It is this transfer of lipids, particularly the constituent fatty acids, from prey to predators that allows for the tracking of certain aspects of dietary interaction.

This study represents the first significant study of the lipid composition of Macquarie Island fish, particularly Patagonian toothfish (*Dissostichus eleginoides*), and also expands current understanding of the composition of squid in the region. The wide-ranging lipid class compositions and contents observed across the species examined highlights several aspects of biological function. For instance, the vast quantities of WE (*Electrona antarctica*, *Gymnoscopelus bolini*, *G. braueri*, *Labichthys yanoi*, *Cyclothone* sp., *Diplophos rebainsi*) and DAGE (*Gonatus antarcticus*) stored in a number of species most likely to contribute to the maintenance of buoyancy. The TAG-rich stores of other species (e.g. *Kondakovia longimana*, *Protomyctophum bolini*, *Stomias gracilis*) may also jointly function as both buoyancy regulators as well as long-term stores of energy. The increase in lipid content observed for toothfish in relation to both increasing size and fishing depth suggests that, in the absence of a swimbladder, lipids may also play a role in maintaining buoyancy.

The variation in fatty acid composition observed generally reflects the varying diet of fish and squid in the Macquarie Island region. However, large differences in the fatty acid composition of small and large Patagonian toothfish point to a more extensive shift in diet with age than was inferred from stomach contents. This disparity may be a consequence of the acknowledged biases of stomach content analysis, biases possibly avoided by the examination of dietary fatty acids.

However, limitations on the use of fatty acids as dietary indicators also exist. Biologically active compounds such as fatty acids are exposed to a multitude of physiological processes of modification and biosynthesis, all of which alter the original composition (or signature) of fatty acids obtained from the diet. These biases can be partly overcome by placing particular importance on the use of fatty acids that are of strictly dietary origin and more likely to be incorporated directly into storage with minimal modification (such as EPA and DHA). The limitations of this technique are therefore potentially significant and need to be acknowledged.

The application of fatty acids to dietary studies is therefore at its most effective when used in combination with traditional techniques such as stomach content analysis. In the context of the present study stomach contents suggest that, in general, the diet of toothfish in the Macquarie Island population is broad but is not significantly influenced by variations in factors such as size. Fatty acid analysis appears to detect a much more pronounced shift in diet as toothfish age. This shift perhaps reflects an increasing dietary importance of larger, MUFA-rich fish accompanied by a decrease in the dietary importance of PUFA-rich prey such as squid. The ecological implications of such a shift in diet are potentially substantial, underscoring the importance of toothfish as an important marine predator at all levels of the Macquarie Island foodweb.

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