Diet study of Antarctic toothfish caught in the east Antarctic based on stomach content, fatty acid and stable isotope analyses

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Abstract

To identify the major prey items for Antarctic toothfish (*Dissostichus mawsoni*) in the small-scale research unit (SSRU) 5841C in the east Antarctic, their stomach contents, fatty acid (FA) compositions and stable carbon and nitrogen isotope ratios were determined and compared with those of species caught as by-catch and collected from toothfish stomachs. Stomach content analyses showed that Antarctic toothfish fed primarily on fish and to a lesser extent squid. FA profiles in muscle tissues of Antarctic toothfish were

very similar to those of Channichthyidae caught as by-catch and several species collected from toothfish stomachs, including unidentified icefish, *Arctozenus risso*, *Macrourus* spp., and *Gymnodraco acuticeps*, indicating a trophic connection between them. $\delta^{15}N$ values of Antarctic toothfish were higher than for the other species collected, indicating a higher trophic position. This is the first study to provide information on the diet of Antarctic toothfish and the trophic relationship between the toothfish and other species in the east Antarctic using these methods. Further studies on the trophic relationship between Antarctic toothfish and other species and a regional comparison of their dietary composition by the collection and subsequent biomarker analyses of more species is needed to understand better the carbon flow through Antarctic ecosystems.

Introduction

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) monitors fluctuations in the availability of fishery resources and biodiversity in the Southern Ocean. The changes in the number of Antarctic (*Dissostichus mawsoni*) and Patagonian (*D. eleginoides*) toothfish with their high commercial value are being closely watched to assess the size of the resources and the ecological risk of overfishing. Antarctic toothfish has been known to occupy a higher trophic position than other Antarctic fish as revealed by chemical tracers, and thus large decreases or increases in its population size could be a crucial cascading force in the food web of the Southern Ocean (Jo et al., 2013; Ainley and Pauly, 2014; Pinkerton and Bradford-Grieve, 2014). In addition, longline fisheries for toothfish can weaken lower trophic levels by removals of fish such as grenadiers and skates as by-catch (Kock, 2001; Pinkerton and Bradford-Grieve, 2014). For a sustainable fisheries management in the region, a comprehensive understanding of the trophic ecology of Antarctic toothfish is required in terms of their major prey, predators and competitors.

Several techniques can be used to investigate the trophic ecology of fish communities. The traditional method for assessing fish diets is stomach content analysis, which only offers information on the recent food items of an organism. In contrast, fatty acid (FA) and stable isotope analyses can indicate the assimilated diets of consumer species over a longer period of time. FAs have been used as biomarkers to identify the dietary information of marine organisms at higher trophic levels through multivariate analyses. This is because the FA profiles in the tissues of consumers are derivatives of those of potential prey items (Kelly and Scheibling, 2012). Stable isotope analyses of carbon and nitrogen have been used to identify organic matter pathways and trophic interactions within food webs. This is based on the assumption that the isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of consumers reflect those of their assimilated diets (Peterson and Fry, 1987; Michener and Schell, 1994). $\delta^{13}C$ in an animal usually occurs within 1‰ compared with that of its diet (DeNiro and Epstein, 1978; Fry and Sherr, 1984), whereas $\delta^{15}N$ is enriched by 2–4‰ with each trophic level (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002). Recently, the combination of stomach content, FA and stable isotope analyses has proven to be a powerful approach for identifying the diets of consumers by considering the advantages and limitations of each technique (Alfaro, 2006; Jo et al., 2013; Kolts et al., 2013).

Antarctic toothfish is abundant on the continental slope of east Antarctica in the Indian Ocean and Atlantic Ocean sectors. The characterised distribution with different size classes and clear migration patterns of the Antarctic toothfish among the research units shows the difference of their trophodynamics in the fishing grounds (Petrov and Tatarnikov, 2010). However, most studies on the diet of *D. mawsoni* have focused on the population in the Ross Sea region (Fenaughty et al., 2003; Hanchet et al., 2012; Pinkerton et al., 2012; Jo et al., 2013; Stevens et al., 2014). Studies on the feeding strategy of *D. mawsoni* in other parts of the Antarctic can provide important information for comparing the trophic structure between Antarctic regions as well as providing a better understanding of the ecological effects of fishing in these areas.

In the present investigation, stomach contents, lipid compositions and stable carbon and nitrogen isotope ratios of the Antarctic toothfish and their potential prey species from the east Antarctic (SSRU 5841C) were analysed to identify their feeding relationship in the region. To our knowledge, this is the first study on the trophic ecology of *D. mawsoni* that has been conducted in the east Antarctic.

Materials and methods

Sampling and sample treatment

Sample collection for this survey was carried out to the south of the SSRU in research blocks in SSRU 5841C (Figure 1). Sample collection started on 25 February 2013 and finished on 4 March. A total of six trotlines and five Spanish lines were set and 86 *D. mawsoni* retained for sampling (Table 1). The total length (TL) and wet body weight of the sampled toothfish were measured on board to the nearest centimetre and gram respectively. The samples for muscle tissue were collected from Antarctic toothfish and individual fish specimens that were taken as by-catch. Stomach content samples were also collected from the sampled toothfish, these were preserved by freezing immediately after being extracted, and taken to the laboratory. All fish samples for FA and stable isotope analyses were dissected and muscle tissues were only collected from the dorsal part.

For the stable isotope analysis, muscle tissues underwent lipid extraction two times in a mixed solution of methanol, chloroform and water (2:1:0.8) according to the method of Bligh and Dyer (1959). The lipid extraction was performed to prevent effects of variation in the $\delta^{13}C$ values because of interspecific differences in the concentration of ^{13}C-depleted lipids compared with other biochemical components (Focken and Becker, 1998). Lipid extraction may affect the loss of some non-lipid compounds that may alter $\delta^{15}N$ values (Sweeting et al., 2006; Logan et al., 2008). In the present study, lipid extraction significantly increased average 0.7‰ for tissue $\delta^{15}N$ of toothfish and other fish, which were corrected. All samples were freeze-dried and ground into a homogeneous powder with a mortar and pestle.

Stomach content analysis

The stomach contents transported to the laboratory were identified under a dissecting microscope. Evidence of regurgitation was not observed in any fish samples. The number and wet weight (g) of each food item were counted and measured. As far as possible, stomach contents were identified to the lowest taxonomic level. Only fresh prey items were considered to reduce the bias of stomach content analysis. Partially digested fish and cephalopods were identified from sagittal otoliths and beaks respectively. Diet was quantified by frequency of occurrence (%*F*), numerical percentage (%*N*) and wet weight percentage (%*W*), which were calculated by the following equations:

$$\%F = A_i/A_{total} \times 100,$$
$$\%N = N_i/N_{total} \times 100,$$
$$\%W = W_i/W_{total} \times 100,$$

where F represents occurrence frequency, A_i is the number of fish preying on species i, A_{total} is the total number of fish examined (excluding individuals with empty stomachs), N_i (W_i) are the numbers (wet weight) of prey individual i and N_{total} (W_{total}) are the total numbers (wet weight) of prey individuals. Then, the index of relative importance (*IRI*; Pinkas et al., 1971) was calculated for each prey item as follows:

$$IRI = (\%N + \%W) \times \%F,$$

and expressed as a percentage (%*IRI*),

$$\%IRI = IRI_i \Big/ \sum_{i=1}^{n} IRI \times 100,$$

where n is the total number of food categories considered at a given taxonomic level. To assess the precision of the index values of prey importance, bootstrap methods which consist of 1 000 replicates of independent random samples with replacement were used to estimate means and 95% confidence intervals for the dietary statistics (Tirasin and Jørgensen, 1999).

FA analysis

Muscle tissues were collected from each individual to analyse its FA compositions. Lipid extraction was performed with a solution of methanol and chloroform (2:1, v/v) from freeze-dried samples according to the procedure of Bligh and Dyer (1959). The FA compositions of all the samples were analysed as FA methyl esters (FAMEs) with a methylation method as described by Metcalfe et al. (1966). The extracted lipids were saponified at 100°C for 2 h with 1.5 ml of 0.5 N NaOH–methanol. FAMEs were obtained by transesterification with a 2 ml solution of BF_3–methanol (14%). The mixture was shaken, sealed under nitrogen and then heated on a hot block at 100°C for 30 min. After cooling, 1 ml of hexane was added to the mixture. Vortexing was undertaken after capping under nitrogen and then the upper hexane phases containing FAMEs

were isolated using a Pasteur pipette. The hexane phases were mixed with sodium sulfate (Na_2SO_4: hexane phase = 1.5:1, v/v) and concentrated under nitrogen. The upper layer was transferred into a vial and kept frozen at −20°C until further analysis. FAMEs were analysed by a gas chromatograph (GC; Agilent Technologies, USA) equipped with a flame ionisation detector. A flexible fused silica capillary column (bonded carbowax, 30 m × 0.25 mm internal diameter and 0.25 μm film thickness) was used to separate the FAME classes. Nitrogen was used as a carrier gas. The GC temperature was programmed from 50°C for 1 min, ramping to 150°C at 30°C min^{-1}, and holding at 250°C for 10 min after ramping at 2°C min^{-1}. The FAMEs were identified by comparing the retention times with standard mixtures (Supelco Co. 37 Component FAME Mix, 18919-1AMP, USA).

Stable isotope analysis

Carbon and nitrogen stable isotope ratios were measured on a continuous flow isotope ratio-mass spectrometer (CF-IRMS; Isoprime 100, GV Instruments, Manchester, UK) coupled with an elemental analyser (vario MICRO cube, Elementar, Hanau, Germany). Powdered samples were weighed (about 1.0 mg), wrapped in tin capsules and placed into the elemental analyser to oxidise at high temperature (1 030°C). The resultant gases of CO_2 and N_2 were introduced into the CF-IRMS using a He carrier. Data are expressed as the relative difference between isotopic ratios of the sample and conventional standard gases (i.e. Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). The delta (δ) notation was used to express these relative differences according to the following equation:

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3,$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. A secondary standard of known relation to the international standard (USGS-24 for carbon and IAEA-N1 for nitrogen) was used as reference material. The analytical precision for 20 replicates of urea was approximately 0.1‰ and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

Data analysis

Statistical analyses were performed by using IBM SPSS Statistics (version 12.0; IBM Corp., Armonk, NY, USA). Prior to the statistical analysis, all data were tested for normality with the Shapiro–Wilk normality test. Homogeneity of variances was then tested using Levene's test. FAs from each sample were expressed as the percentage of total FAs. FAs that contributed a mean of less than 1.0% (of total FAs) to the profile were omitted from statistical analyses. FA data were arcsine square root transformed for multivariate normality before all statistical analyses (Kelly and Scheibling, 2012). A nonparametric procedure (Kruskal–Wallis test) was used to compare the FA values of samples. To distinguish among the FA profiles of Antarctic toothfish, by-catch taxa and prey items contained in stomachs, and to identify the relationship among the animals, principal component analysis (PCA) was conducted with PRIMER software (version 6; PRIMER-E, Ltd, Luton, UK). PCA analysis was used to separate observed groups by reducing variables from the large number of FA datasets and identifying FAs that were highly correlated. A hierarchical cluster analysis based on Bray–Curtis similarity was also conducted on a matrix of FA profiles from all samples. The results from cluster analysis on the PCA plots were used to visualise trophic relationships among samples. One-way ANOVA was used to test differences in isotope data ($\delta^{13}C$ and $\delta^{15}N$) among groups of Antarctic toothfish, by-catch taxa and prey items contained in stomachs. A Tukey honest difference significant test was subsequently used to distinguish significant differences among variables.

Results

All samples (i.e. Antarctic toothfish, by-catch species and identified and unidentified prey items contained in toothfish stomachs) collected in SSRU 5841C are listed in Table 2. A total of 36 individuals of *D. mawsoni* (TL, 104–176 cm) were sampled in SSRU 5841C during the study period. Among the 36 stomachs collected, three were empty. The cumulative prey curves for *D. mawsoni* started to level off after 32 stomachs (Figure 2). Fish were the most common prey item for the toothfish, comprising 87.9% of the occurrence frequency of the diet, 65.0% of the number, 88.7% of the weight and 90.2% of the IRI (Table 3). It was assumed at the time that the main species of grenadier was *Macrourus whitsoni*. However, a new cryptic species of *Macrourus* (*M. caml*) has recently been described by McMillan et al. (2012), which is morphometrically very similar to *M. whitsoni*. Therefore, as it was originally identified as

M. whitsoni, it is referred to in this document as *M. whitsoni*, but note that it is likely to comprise both species. *Macrourus whitsoni* was the most common prey item, comprising up to 14.7% of the weight of the stomach contents. Molluscs (mainly squids) were the second most common dietary component, comprising 39.4% of the occurrence, 21.3% of the number, 10.9% of the weight, and 8.5% of the IRI in the diet of the toothfish. The dietary composition of the Antarctic toothfish, classified into two size classes (Class I, 104–140 cm; Class II, 140–176 cm), did not significantly differ between the groups ($\chi^2 = 1.800$, $df = 4$, $p > 0.05$). This indicated that fish were the dominant prey item, comprising 84.5% for Class I and 90.5% for Class II of the weight of the diet.

The total FA compositions of Antarctic toothfish, by-catch species and prey items contained in toothfish stomachs are presented in Table 4. Monounsaturated FAs (MUFAs) comprised approximately 50% of the FAs in muscle tissues of Antarctic toothfish, while polyunsaturated FAs (PUFAs) comprised the lowest amount of FAs in the muscle tissues. FAs in the muscle tissues of two by-catch species (*Macrourus* spp. and *Pogonophryne* spp.) showed significantly higher PUFAs than MUFAs. The stomach samples varied considerably in FA compositions. The FA compositions of Antarctic toothfish were similar irrespective of size and area (Table 5). The MUFA C18:1n9c was the most abundant FA in muscle tissues of Antarctic toothfish. Two FAs, decosahexaenoic acid (C22:6n3) and eicosapentaenoic acid (C20:5n3), were the most abundant PUFAs in Antarctic toothfish. The FA profiles of specimens collected from by-catch and stomach contents of Antarctic toothfish showed that C16:0 and C18:1n9c were the most abundant FAs, ranging from 15.1% (*Pogonophryne* spp.) and 10.6% (unidentified squid) to 26.4% (unidentified skate) and 29.8% (unidentified icefish) respectively (Table 4). The FAs C22:6n3 and C20:5n3 occurred in considerable amounts in muscle tissues of all by-catch and stomach content samples, ranging from 6.8% (unidentified icefish) and 8.7% (unidentified squid) to 18.3% (*Cygnodraco mawsoni*) and 31.9% (*Macrourus* spp.) respectively.

The FA profiles of Antarctic toothfish were compared with those of specimens collected from bycatch and their stomachs using PCA analysis based on the 11 FAs that comprised more than 1.0% of the total FAs in all animals (Figure 3). PC1 accounted for 64.3% of the total variance, and 22:6n3, 18:1n9c, 16:1 and 14:0 FAs contributed to the separation of Antarctic toothfish and two by-catch specimens (*Macrourus* spp. and *Pogonophryne* spp.) along this axis. PC2 accounted for 15.6% of the total variance and the major FAs contributing to this were 18:0, 16:1, 22:6n3, 20:3n3 and 16:0. PCA analyses showed that Antarctic toothfish were closer to the Channichthyidae of the by-catch specimens. PCA analyses of the stomach contents of Antarctic toothfish showed they were closer to unidentified icefish, *Arctozenus risso*, *M. whitsoni* and *Gymnodraco acuticeps* than other species.

$\delta^{13}C$ and $\delta^{15}N$ values of Antarctic toothfish and species that were taken as by-catch and from prey items contained in toothfish stomachs collected in SSRU 5841C are presented in Table 6. The $\delta^{13}C$ values of all the specimens analysed ranged from –24.9‰ (*A. risso*) to –20.3‰ (*Bathyraja eatonii*). Antarctic toothfish (on average –22.2 ± 0.2‰) had similar $\delta^{13}C$ values among size groups (one-way ANOVA, $p = 0.403$). In contrast, $\delta^{15}N$ values of Antarctic toothfish differed among size groups, showing that groups A and B had lower $\delta^{15}N$ values than groups C and D (one-way ANOVA, Tukey post hoc test, $p < 0.05$). $\delta^{15}N$ values of all the specimens ranged from 10.3‰ (*A. risso*) to 16.4 ± 0.7‰ (group D of Antarctic toothfish). $\delta^{15}N$ values of Antarctic toothfish (on average 15.5 ± 0.9‰) were placed at the highest level with the by-catch species, *Pogonophryne* spp., on average 14.7 ± 0.6‰ (Figure 4).

Discussion

Antarctic toothfish has been consistently managed by the CCAMLR as a key component in the food web dynamics of the Antarctic ecosystem (CAMLR Convention text, Article II principle b) and is generally known to feed on a wide range of prey items, but it is primarily a piscivorous fish (Fenaughty et al., 2003; Jo et al., 2013). As a top predator, Antarctic toothfish may have various effects on other species lower down the food chain through trophic cascades (Pinkerton and Bradford-Grieve, 2014). Despite the ecological importance of *D. mawsoni*, there have been few quantitative investigations of their diet, and these have been mostly from the Ross Sea region (Fenaughty et al., 2003; Hanchet et al., 2012; Jo et al., 2013; Stevens et al., 2014). In this study, the results from the

combination of stomach contents and stable isotope ratios were in close agreement with previous studies in the Ross Sea region (Bury et al., 2008; Jo et al., 2013). The stomach content analysis showed that *M. whitsoni* was the dominant prey item for toothfish. FA analysis showed that the FA compositions in muscle tissues of Antarctic toothfish were very similar to those of by-catch Channichthyidae. From their stomach contents, FA compositions in muscle tissues were very similar to unidentified icefish, *A. risso*, *M. whitsoni* and *G. acuticeps* (see Table 2), indicating a trophic connection between them. Moreover, considering the trophic fractionation effect of 2–4‰ in $\delta^{15}N$ (McCutchan et al., 2003), Antarctic toothfish showed much higher $\delta^{15}N$ values than those of four species (unidentified icefish, *A. risso*, *M. whitsoni* and *G. acuticeps*). While *G. acuticeps* had very similar $\delta^{15}N$ to those of size groups A and B (TOA, <100 cm TL), the values were about 2‰ lower than those of groups C and D (>100 cm TL), suggesting that the species may only be a prey item for Antarctic toothfish over 100 cm in TL.

In the present study, stomach contents showed that Antarctic toothfish consumed fish as principal prey items and molluscs as secondary prey items. These results indicate that *D. mawsoni* is piscivorous, as shown by earlier studies from other regions. Fenaughty et al. (2003) reported that fish rank as the most important food category in Antarctic toothfish stomachs collected in the Ross Sea, Subarea 88.1 (range 77–86%). This especially includes icefish (Channichthyidae) and Whitson's grenadier (*M. whitsoni*). Stevens et al. (2014) reported that Whitson's grenadier (*M. whitsoni*), icefish (*Chionobathyscus dewitti*), eel cods (*Muraenolepis* spp.) and cephalopods represent the major dietary items of Antarctic toothfish in the continental slope of the Ross Sea. They also reported that *M. whitsoni*, violet cods (*Antimora rostrata*) and cephalopods represent the major dietary items of Antarctic toothfish in the oceanic seamount of the Ross Sea area. Recently, Hanchet et al. (2012) reported that fish occur in about 85% of the Antarctic toothfish stomachs sampled in the southern Ross Sea. The majority identified were rock cods (mainly *T. loennbergii*) and icefish (mainly *Neopagetopsis ionah*, *Chionodraco hamatus* and *C. myersi*), which occurred in 17.6% and 10.4% of stomachs respectively. Antarctic toothfish from several other locations, including McMurdo Sound (Calhaem and Christoffel, 1969; Eastman, 1985), open oceanic waters of the Pacific sector of Antarctica (Yukhov, 1971), Cosmonaut Sea (Pakhomov and Tseytlin, 1992) and South Shetland Islands (Takahashi and Iwami, 1997), also had similar dietary compositions to that shown by the stomach content analysis of this study.

The $\delta^{15}N$ values of Antarctic toothfish were higher than those of most of the other species collected, indicating their high trophic position. The high $\delta^{15}N$ values show that toothfish occupies a high trophic position as in other ecosystems (Jo et al., 2013). As shown in the present study, previous studies based on stomach content analyses have reported that fish are the most dominant prey items of Antarctic toothfish in the Ross Sea continental shelf and slope (Fenaughty et al., 2003; La Mesa et al., 2004). From this and other diet studies (e.g. Petrov et al., 2014) it is evident that the main dietary item of the Antarctic toothfish is fish, but that the species composition will depend on prey availability and geographic prey abundance (Pihl, 1985; Gkenas et al., 2012).

The diet composition of Antarctic toothfish changes with size, with larger individuals feeding more on fish than other prey items (Takahashi and Iwami, 1997; Fenaughty et al., 2003; Stevens et al., 2014). Gröhsler (1992) found that mysids and amphipods were the most important food items for small *D. mawsoni* (11–19 cm TL) around Elephant Island. Near et al. (2003) reported that ontogenetic changes in buoyancy and habitat of Antarctic toothfish might have a significant effect on the diet composition of juveniles and adults. In the present study, no significant differences in toothfish stomach contents were found between the size groups (104–140 cm vs. 140–176 cm). However, there were significant differences in $\delta^{15}N$ values among the four size groups, suggesting an ontogenetic shift in their dietary composition. In addition, FA analyses displayed a tendency of slightly increasing MUFAs and decreasing saturated FAs and PUFAs with toothfish size, consistent with the results of Jo et al. (2013) in the Ross Sea. These differences may be associated with the optimisation of energy acquisition.

In the present study, slight differences in the main diets of Antarctic toothfish were found between the results from stomach content and chemical tracer (i.e. FAs and stable isotopes) analyses. Although stomach contents provide important data for

establishing prey taxonomy and size distribution, the analyses do not offer information on long-term diets and actual assimilation because of differences in detectability, quantifiability and digestibility of prey items; they only represent a snapshot of the diet (Reñones et al., 2002). In particular, because opportunistic predators such as Antarctic toothfish usually undergo spatial and temporal variation in food availability, a snapshot from stomach content analysis may give rise to biased information when identifying the actual assimilated food. Conversely, as shown in the present study, the combination of stable isotope ratios and FA profiles can provide long-term integrated information on the diet of a species.

In conclusion, the present study provides ecological information on the diet of Antarctic toothfish and the trophic relationship between the toothfish and other species in SSRU 5841C using the combined methods of FAs, stable isotopes and stomach contents. These findings are consistent with previous studies, suggesting that icefish, ribbon barracudina (*A. risso*), Whitson's grenadier (*M. whitsoni*), and naked dragonfish (*G. acuticeps*, only for >100 cm TL) may be the main prey items for Antarctic toothfish. Their ontogenetic dietary change was also shown by $\delta^{15}N$ differences between the size groups of greater or less than 100 cm TL. Further studies on the trophic relationship between Antarctic toothfish and other species and a regional comparison of their dietary composition by collection and subsequent biomarker analyses for more organisms are needed to understand better the carbon flow through Antarctic ecosystems.

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Table 1: Information on fishing type, number of hook, fish weight of *Dissostichus mawsoni* (TOA), *Macrourus* spp. (GRV), Channichthyidae (ICX), and *Pogonophryne* spp. (POG), and CPUE for TOA during this survey in the research blocks in SSRU 5841C.

Fishing type	Number of sets	Number of hooks	TOA (kg/number)	CPUE (kg/hook)	GRV (kg/number)	ICX (kg/number)	POG (kg/number)
Spanish line	5	12 000	919.9/30	0.077	3.2/5	0.8/1	0.3/1
Trot line	6	14 040	2 027.1/56	0.144	5.6/3	3.7/7	-

Table 2: Species codes for Antarctic toothfish, by-catch and prey items contained in stomachs collected during this survey.

Species codes	Species	Common name
ARC[2]	*Arctozenus risso*	Ribbon barracudina
BAM[2]	*Bathyraja maccaini*	McCain's skate
BEA[2]	*Bathyraja eatonii*	Eaton's skate
CYM[2]	*Cygnodraco mawsoni*	Mawson's dragonfish
GYA[2]	*Gymnodraco acuticeps*	Naked dragonfish
WGR[2]	*Macrourus whitsoni*	Grenadier
ICX[1]	Channichthyidae	Icefish
GRV[1]	*Macrourus* spp.	Grenadier
POG[1]	*Pogonophryne* spp.	Plunderfish
TOA	*Dissostichus mawsoni*	Antarctic toothfish
UIf[2]		Unidentified icefish
USq[2]		Unidentified squid
UFh[2]		Unidentified flathead
USk[2]		Unidentified skate

[1] The species were taken as by-catch.
[2] The species were collected from Antarctic toothfish stomachs.

Table 3: Composition of the stomach contents of Antarctic toothfish (*Dissostichus mawsoni*) by percentage frequency of occurrence (%*F*), percentage by number (%*N*), percentage by weight (%*W*) and the index of relative importance (%*IRI*) expressed as a percentage of the sum of the *IRI* values in research blocks in SSRU 5841C on March 2013. Species codes are presented in parenthesis.

Prey organisms	%*F*	%*N*	%*W*	%*IRI*
Crustacea	3.0	2.5	0.0	0.1
Decapoda				
Macrura	3.0		0.0	0.0
Mollusca	39.4	21.3	10.9	8.5
Gastropoda	3.0	1.3	0.1	0.0
Cephalopoda				
Squid	39.4	20.0	10.8	8.1
Pisces	87.9	65.0	88.7	90.2
Macrouidae				
Macrourus whitsoni (WGR)	18.2	7.5	14.7	2.7
Rajidae				
Bathyraja maccaini (BAM)	3.0	1.3	1.1	0.1
Bathyraja eatonii (BEA)	3.0	1.3	4.0	0.1
Unidentified Rajidae	3.0	1.3	1.1	0.1
Paralepididae				
Arctozenus risso (ARC)	6.1	2.5	5.7	0.3
Bathydraconidae				
Cygnodraco mawsoni (CYM)	3.0	1.3	3.7	0.1
Gymnodraco acuticeps (GYA)	3.0	1.3	5.1	0.1
Unidentified Bathydraconidae	3.0	1.3	3.1	0.1
Nototheniidae	6.1	3.8	12.3	0.7
Unidentified Pisces	63.6	43.8	37.8	34.7
Coral	3.0	1.3	0.2	0.0
Other	18.2	10.0	0.2	1.2
Rocks	18.2	10.0	0.2	1.2
Total		100.00	100.00	100.0

Table 4: Fatty acid (FA) composition (% of total fatty acids) of by-catch species (marked by *) and stomach samples during the collection of Antarctic toothfish in SSRU 5841C of the east Antarctic. Species codes are represented in Table 2. Data are means ± SD.

FA	GRV*	ICX*	POG*	ARC	BAM	BEA	CYM	GYA	GRV	UFh	UIf1	UIf2	USk	Usq	WGR
C10:0	-	0.1 ± 0.0	0.3	0.2	0.5	0.3	0.3	0.2	0.3	0.2	0.3 ± 0.1	0.2	0.5	0.4	0.3 ± 0.0
C11:0	0.2	0.2 ± 0.0	0.3	0.2	0.7	0.3	0.4	0.3	0.4	0.2	0.4 ± 0.2	0.3	0.6	0.7	0.4 ± 0.1
C12:0	0.2	0.1 ± 0.0	0.3	0.2	0.5	0.2	0.3	0.2	0.3	0.2	0.3 ± 0.1	0.2	0.4	0.4	0.3 ± 0.1
C14:0	2.0	10.7 ± 1.6	3.0	9.2	3.4	1.9	2.2	5.8	2.5	4.1	4.0 ± 1.7	9.0	2.2	1.9	4.3 ± 1.1
C14:1	-	0.7 ± 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
C15:0	-	0.4 ± 0.1	-	0.4	-	0.3	-	-	0.4	0.3	0.3 ± 0.1	0.4	-	-	0.4 ± 0.2
C16:0	19.7	18.2 ± 2.3	15.1	22.2	23.6	24.1	21.1	17.6	25.1	20.1	19.0 ± 0.8	20.4	26.4	21.8	20.4 ± 2.6
C16:1	4.1	14.1 ± 1.1	9.4	6.1	5.3	3.9	4.1	9.0	4.1	6.1	7.5 ± 1.4	6.4	2.8		9.3 ± 0.5
C17:0	-	-	-	0.3	-	-	-	-	-	0.2	-	0.3			0.3 ± 0.1
C18:0	3.4	1.4 ± 0.2	3.6	8.3	10.3	8.8	7.1	6.4	11.3	4.9	8.1 ± 0.5	9.1	14.3	12.7	8.2 ± 1.9
C18:1n9	14.1	20.4 ± 6.2	21.9	27.2	21.5	17.1	13.2	21.4	19.0	13.3	28.0 ± 5.4	29.8	19.1	10.6	24.7 ± 9.2
C18:2n6c	1.1	2.1 ± 0.1	2.2	1.9	2.2	1.7	1.9	2.0	1.5	1.9	1.8 ± 0.3	1.9	1.6	0.5	1.6 ± 0.2
C18:3n6	-	0.3 ± 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
C18:3n3	-	0.5 ± 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
C20:0	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-
C20:1n9	1.9	3.8 ± 1.9	3.8	2.9	5.5	3.7	4.9	8.9	2.4	6.7	6.1 ± 0.5	2.9	5.0	7.8	5.9 ± 0.9
C20:2	-	-	-	0.4	-	0.4	-	-	-	0.3	-	-	0.6	-	-
C20:3n3	2.1	0.8 ± 0.3	3.7	0.4	2.3	3.8	4.0	-	1.4	1.2	2.0 ± 0.1	-	1.8	-	1.0 ± 0.4
C20:5n3	18.1	15.7 ± 5.2	16.6	12.0	10.2	11.7	18.3	12.5	11.7	17.9	11.4 ± 2.9	11.4	8.7	15.9	10.2 ± 3.9
C22:1n9	0.8	1.4 ± 0.6	1.2	0.7	1.4	0.6	1.4	3.3	0.4	1.7	1.3 ± 0.3	0.8	0.6	0.9	1.7 ± 0.4
C22:6n3	31.9	9.5 ± 3.5	18.6	7.5	12.6	21.2	20.9	12.3	19.2	20.8	10.7 ± 6.7	6.8	14.8	26.3	11.4 ± 4.3

Table 5: Fatty acid (FA) composition (% of total fatty acids) of different size classes of Antarctic toothfish (*Dissostichus mawsoni*) from SSRU 5841C in the east Antarctic: 60–79 cm length (A), 80–99 cm (B), 100–139 cm (C), and >140 cm (D). Data are means ± SD. Replicates (n) are presented in parenthesis.

FA	A (5)	B (4)	C (3)	D (4)
C10:0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.2	0.3 ± 0.0
C11:0	0.5 ± 0.3	0.3 ± 0.0	0.4 ± 0.2	0.3 ± 0.1
C12:0	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
C14:0	6.4 ± 2.1	6.8 ± 0.3	8.4 ± 2.1	7.6 ± 1.1
C14:1	-	0.5± 0.1	0.3 ± 0.1	-
C15:0	-	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
C16:0	17.2 ± 2.1	17.8 ±1.1	14.9 ± 2.3	13.4 ± 1.4
C16:1	10.0 ± 4.0	12.0 ± 1.2	13.9 ± 4.0	12.6 ± 0.9
C17:0	-	-	0.7 ± 0.3	-
C18:0	6.9 ± 2.9	4.2 ± 3.0	2.3 ± 0.9	2.2 ± 0.9
C18:1n9c	27.0 ± 4.3	27.8 ± 1.3	30.9 ± 5.3	36.4 ± 1.3
C18:1n9t	-	-	-	-
C18:2n6c	1.7 ± 0.5	2.0 ± 0.2	2.1 ± 0.5	1.9 ± 0.5
C18:3n6	-	-	-	-
C18:3n3	-	-	-	-
C20:0	-	-	-	-
C20:1n9	5.8 ± 0.1	7.6 ± 0.7	6.5 ± 0.1	10.4 ± 1.1
C20:2	-	-	-	-
C20:3n3	1.7 ± 0.1	-	0.6 ± 0.1	-
C20:5n3	11.4 ± 0.4	11.4 ± 1.5	8.2 ± 0.5	4.9 ± 0.4
C22:1n9	2.2 ± 0.3	2.3 ± 0.1	3.4 ± 0.4	5.0 ± 0.3
C22:6n3	9.3 ± 1.0	8.8 ± 1.2	8.6 ± 1.5	7.3 ± 1.0

Table 6: Isotope ($\delta^{13}C$ and $\delta^{15}N$) values (mean ± SD) for individual fish specimens which were taken as by-catch and from toothfish stomachs, and Antarctic toothfish (*Dissostichus mawsoni*).

Species codes	Species	Common name	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n
By-catch					
ICX	Channichthyidae	Icefish	−24.2 ± 0.2	10.4 ± 0.6	3
GRV	*Macrourus* spp.	Grenadier	−23.2 ± 0.1	12.6 ± 0.3	3
POG	*Pogonophryne* spp.	Plunderfish	−21.3 ± 0.2	14.7 ± 0.6	3
Stomachs					
ARC	*Arctozenus risso*	Ribbon barracudina	−24.9	10.3	1
BAM	*Bathyraja maccaini*	McCain's skate	−20.8	12.0	1
BEA	*Bathyraja eatonii*	Eaton's skate	−20.3	12.6	1
CYM	*Cygnodraco mawsoni*	Mawson's dragonfish	−21.8	15.1	1
GYA	*Gymnodraco acuticeps*	Naked dragonfish	−22.8	14.2	1
GRV	*Macrourus* sp.	Grenadier	−24.9	12.5	1
WGR	*Macrourus whitsoni*	Grenadier	−22.9 ± 0.2	12.2 ± 0.3	3
UIx		Unidentified icefish	−23.6 ± 1.3	11.5 ± 1.2	3
USq		Unidentified squid	−24.4 ± 0.2	11.3 ± 0.4	2
UFh		Unidentified flathead	−22.2 ± 0.1	13.2 ± 0.3	2
USk		Unidentified skate	−21.7 ± 0.2	12.0 ± 0.3	3
TOA	*Dissostichus mawsoni*	Antarctic toothfish			
		Size group	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n
		A	−22.0 ± 0.2	14.6 ± 0.5	4
		B	−22.3 ± 0.3	15.1 ± 0.3	5
		C	−22.2 ± 0.2	16.1 ± 0.5	6
		D	−22.0 ± 0.2	16.4 ± 0.7	6

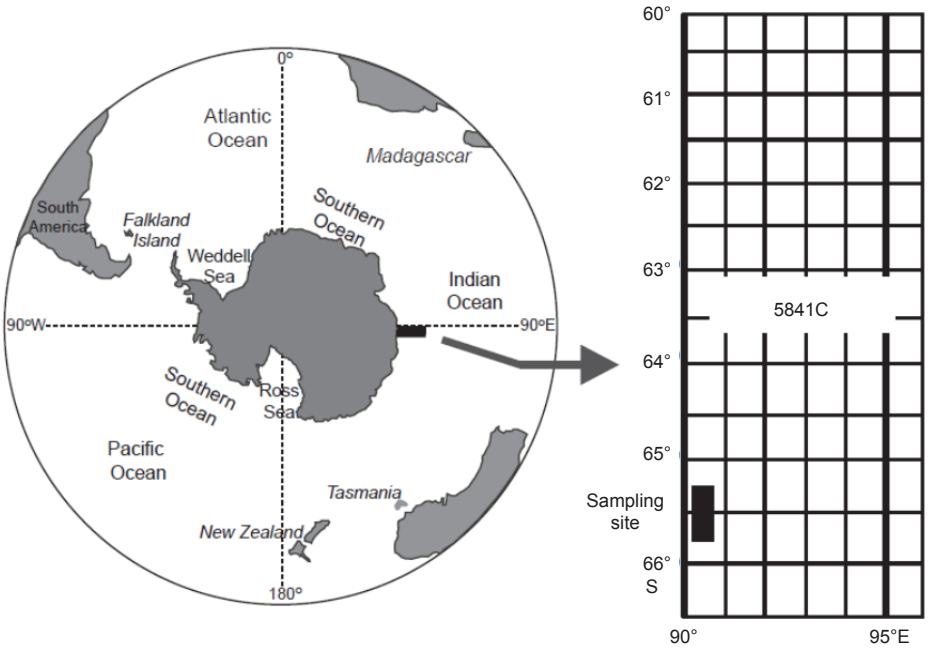

Figure 1: Sampling area of Antarctic toothfish (*Dissostichus mawsoni*) caught by bottom longline (trotline and Spanish line) in SSRU 5841C in March 2013.

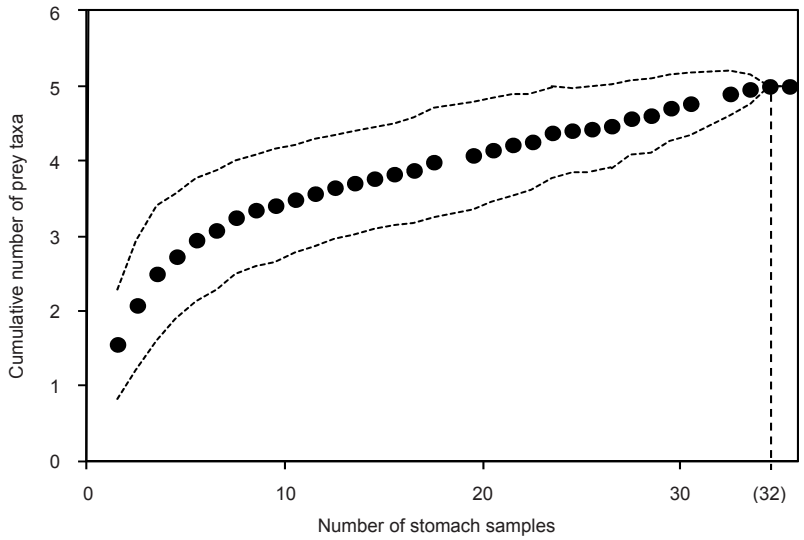

Figure 2: Cumulative prey curves of prey taxa per stomach of *Dissostichus mawsoni* in SSRU 5841C in March 2013. Dashed lines represent standard deviations after 100 permutations.

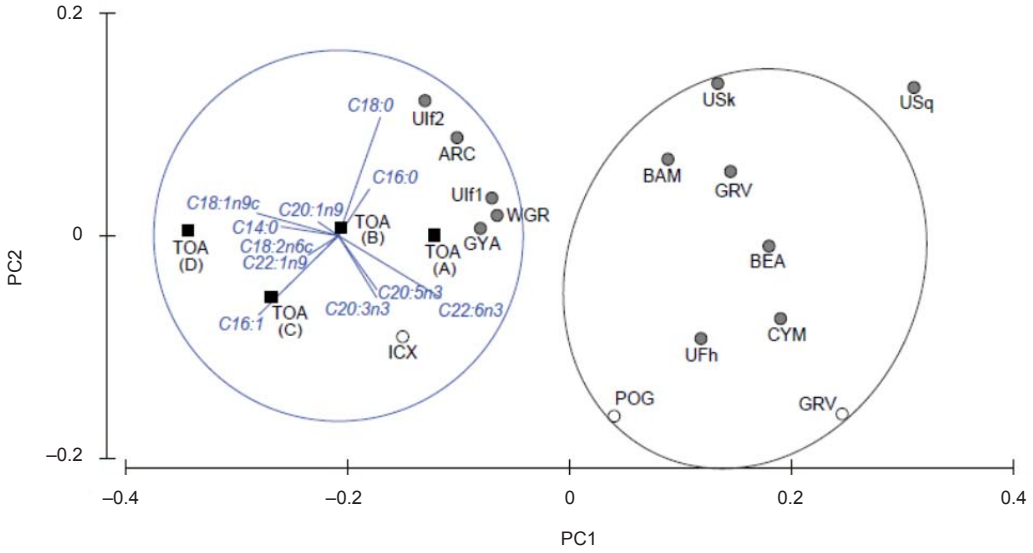

Figure 3: Principal component analysis (PCA) derived from fatty acid composition (each comprising >1% of the total fatty acids in all animals) of Antarctic toothfish and species collected from by-catch and prey items contained in stomachs. Antarctic toothfish (TOA, black square) include four size groups of 60–80 cm length (A), 80–99 cm (B), 100–119 cm (C) and 120–135 (D). Species codes are represented in Table 2 (white circle, by-catch species; gray circle: stomachs samples). Ellipses around samples represent hierarchical clustering based on Bray-Curtis similarity (—, > 85%).

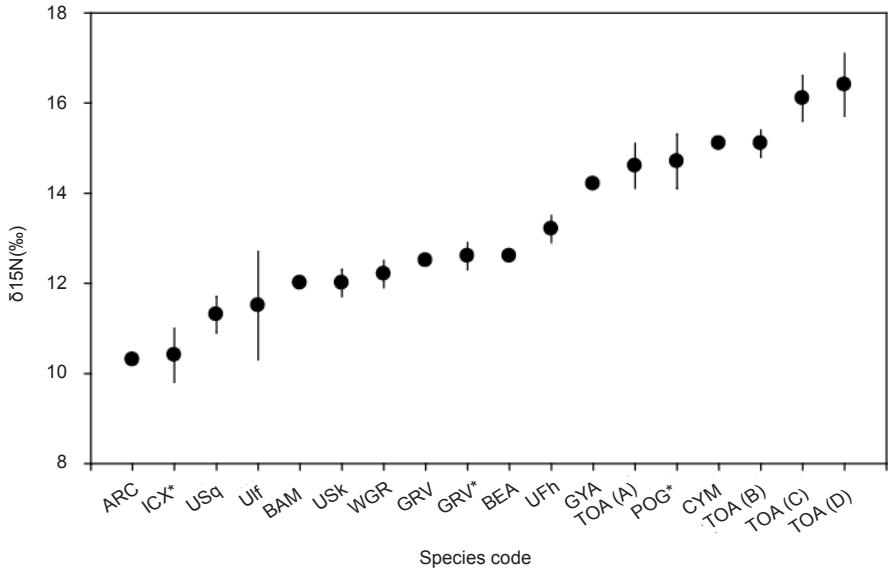

Figure 4: $\delta^{15}N$ values of Antarctic toothfish and species collected from by-catch and prey items contained in stomachs. Species codes are represented in Table 2. Antarctic toothfish (TOA) are classified by four size groups of 60–79 cm length (A), 80–99 cm (B), 100–139 cm (C) and > 140 cm (D). * Species were taken as by-catch.