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Size-related, seasonal and interdecadal changes in the diet of the Patagonian longfin squid *Doryteuthis gahi* in the South-western Atlantic

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Abstract

The Patagonian longfin squid *Doryteuthis gahi* has an annual life cycle with two seasonal cohorts (autumn and spring spawners). Earlier studies on the Patagonian shelf found a predominance of Euphausiacea in the *D. gahi* diet, but no studies to date have investigated differences between feeding spectra of the two cohorts or decadal diet shifts. The present study investigated differences in diet of *D. gahi* on the Patagonian shelf sampled two decades apart, and differences between seasonal cohorts. Classical stomach content analysis and generalized additive models were used to investigate and model the influence of mantle length, sampling period and spawning cohort on the diet. Results revealed an ontogenetic diet change from ~70% Frequency of Occurrence of Euphausiacea in small squid to more than 60% FO of fish and Cephalopoda at larger sizes. Cannibalism was also frequently observed. Euphausiacea were ingested more frequently and in higher amounts during the austral summer and therefore were consumed more by the autumn spawning cohort, whereas fish was more frequently fed upon during austral winter and also by the spring spawning cohort. Cannibalism was also recorded more in austral winter months but, contrary to feeding on fish, was more prevalent in the autumn spawning cohort. Increased predation of *Munida gregaria* was observed in 2020 compared with 2001. This study is an important step towards improving the knowledge of *D. gahi*'s two seasonal cohorts, providing data that can be used for future ecosystem modelling.

Introduction

Squid play an important role in marine ecosystems, with total standing biomass in the world ocean estimated to attain 375 million tonnes (Boyle & Rodhouse, 2005). Squid occupy intermediate levels in the marine trophic pyramid, being an abundant food resource for top predators such as large predatory fish, seabirds, seals and whales (Clarke, 1996; Smale, 1996). They are voracious predators themselves, with feeding rates between 1–12% body mass per day (Wells & Clarke, 1996; Boyle & Rodhouse, 2005). They are characterized by a high metabolic turnover (Wells & Clarke, 1996; Yatsu *et al.*, 1997), with ingested prey biomass rapidly converted into body growth (Collins & Pierce, 1996). High consumption rates can have an important influence on prey species. For example, the winter-spawning cohort of *Illex argentinus* can consume up to 8.5 million tonnes of their mainly crustacean prey during the feeding period of their annual life cycle on the Patagonian Shelf (Arkhipkin, 2013).

The size of squid prey increases throughout ontogeny, resulting in a diet consisting of a variety of prey species at different trophic levels (Witek & Krajewska-Soltys, 1989). Such ontogenetic diet changes have previously been described in various squid species, such as *I. argentinus* (Rosas-Luis *et al.*, 2017) and *Loligo forbesii* (Collins & Pierce, 1996; Pierce & Santos, 1996). The diet of juveniles usually consists of zooplankton, subsequently changing in adults to fish and Cephalopoda (e.g. at 18–23 cm dorsal mantle length in *I. argentinus*; Ivanovic & Brunetti, 1994), with cannibalism also being widespread (Ibáñez & Keyl, 2010).

Squid of the family Loliginidae are common inhabitants of the coastal and shelf regions of the world oceans. Loliginid squid feed on the outer shelf and transport nutrients to their shallow spawning grounds (Boyle & Rodhouse, 2005; Arkhipkin, 2013). The Patagonian longfin squid *Doryteuthis gahi* (d'Orbigny, 1835), is the most southern loliginid, inhabiting shelves of the South-west Atlantic and South-east Pacific. This medium-sized squid typically attains a dorsal mantle length (DML) of 13–17 cm, with a maximum of 44 cm DML (Jereb & Roper, 2010). It is most abundant in the south-eastern part of the Patagonian Shelf, aggregating in the vicinity of oceanic fronts located around the Falkland Islands and characterized by high productivity (Arkhipkin *et al.*, 2013). The population structure of *D. gahi* consists of two main annual cohorts characterized by different seasons of spawning – the autumn-spawning cohort (ASC) and the spring-spawning cohort (SSC). Hence, the same ontogenetic phases of squid from each cohort live in different seasons and sometimes even in different habitats, experiencing different environmental conditions (Arkhipkin *et al*., 2004*a*, 2004*b*; Jones *et al*., 2018).

Stomach content analysis is one of the main tools used to study the diet of a species and its position in the marine food web (Hyslop, 1980; Buckland *et al*., 2017). Additionally, analysis of time series of stomach samples from key species such as predatory fish or squid can enable us to detect temporal shifts in their diet and reveal possible ecosystem changes (Belleggia *et al*., 2017).

Previous studies of *D. gahi* diet on the Patagonian shelf revealed a predominance of Euphausiacea, pelagic amphipods (mainly *Themisto gaudichaudii*) and Chaetognatha (Guerra *et al*., 1991; Brickle *et al*., 2002). A comparative analysis of stomach contents of three squid, *D. gahi*, *I. argentinus* and *Onykia ingens*, found similar prey items, suggesting overlap in the feeding spectra of these species on the Patagonian Shelf. Additionally, stable isotope analysis revealed pelagic neritic feeding habits in *D. gahi* (Rosas-Luis *et al*., 2014). The diet of *D. gahi* changes during ontogeny, with maturing individuals feeding primarily on small pelagic crustaceans (such as *T. gaudichaudii* and *Euphausia* sp.), and mature individuals feeding on larger crustaceans (*Munida gregaria*), fish and squid (Rosas-Luis *et al*., 2016, 2017). However, the relatively small sample size in these studies prevented analysis of seasonal and inter-cohort variability in the diet of this squid.

The present study aims to expand our knowledge on the diet of *D. gahi* on the Patagonian Shelf by revealing possible differences in feeding spectra of the two main cohorts throughout their ontogenies. Additionally, interannual differences in diet have been analysed to identify the impact of environmental and ecosystem shifts on the Patagonian Shelf on the *D. gahi* diet.

Materials and methods

Data collection

Two sets of samples were examined to compare differences in the diet of *D. gahi* on the Patagonian shelf. Samples of the first set (2001) were collected from August 2000 to June 2001 within the designated fishing area, the 'Loligo Box' (~50–53°S 56–60° W), within the Falkland Island Conservation Zone (FICZ) (Figure 1). A total of 4043 *Doryteuthis gahi* was collected as random samples from 35 different trawls, frozen and brought to the Fisheries Department (FIFD) laboratory for analysis.

Samples of the second set (2020) were collected between October 2019 and November 2020 within the FICZ and also in international waters to the north of the zone (Figure 1). A total of 4023 specimens was collected as random samples from 50 different trawls, frozen and brought to the FIFD laboratory for further analysis.

For both sets of samples, squid were caught by commercial trawlers deploying bottom trawling gear, ~90% of which were conducted during daylight hours with trawling times ranging from 1.5 to ~9 h. In addition, in 2020 squid were also collected from four research cruises carried out within Falkland Islands waters. The complete datasets from both periods were used for analysis. However, due to some differences in the geographic distribution of sampling in 2020 compared with 2001, which could result

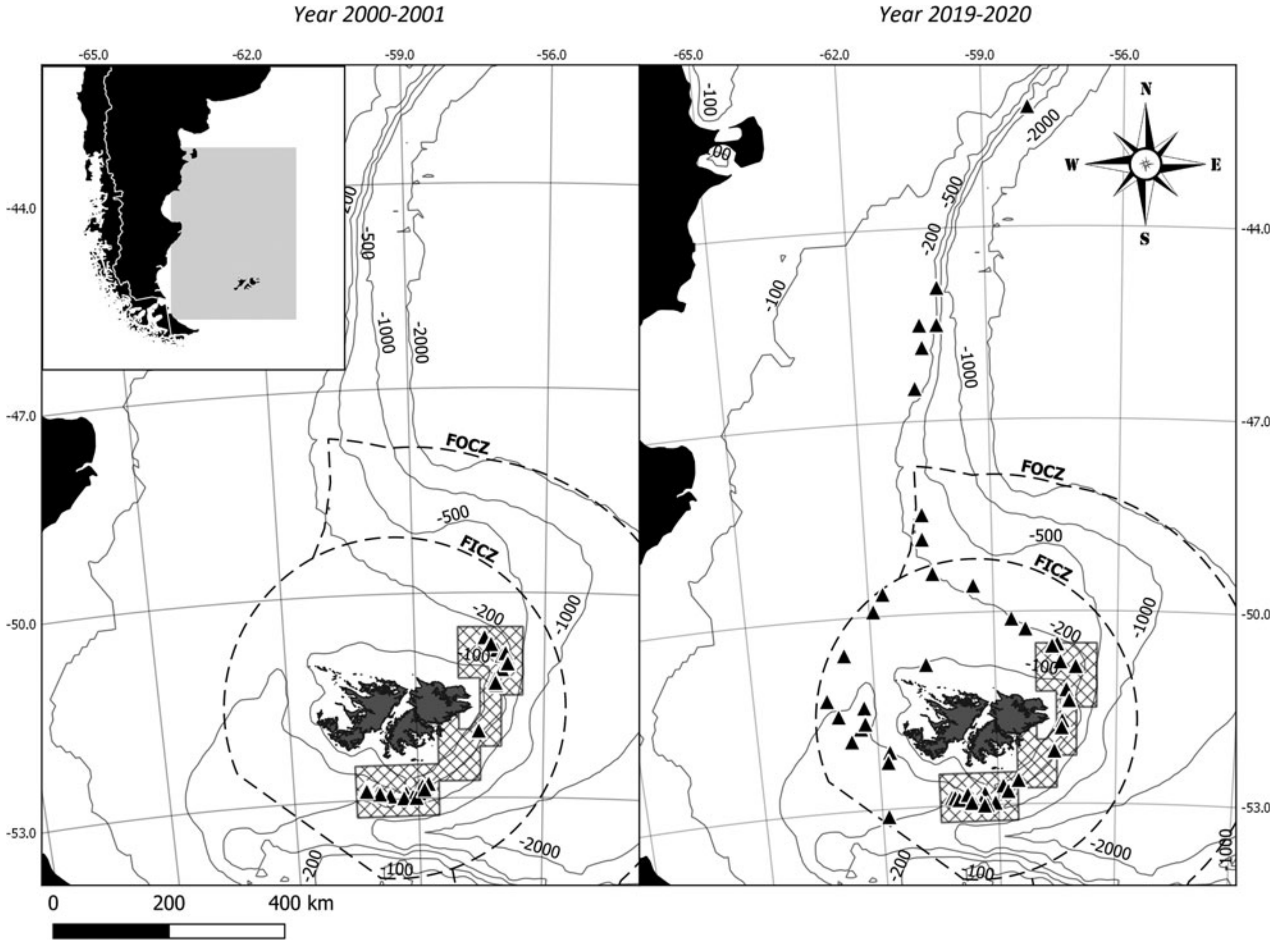


Fig. 1. Sampling sites (triangles) of *D. gahi* from 2001 (left) and from 2020 (right); FICZ & FOCZ = Falkland Inner & Outer Conservation Zone. The designated fishing area, the 'Loligo Box' is represented by the cross-hatched area.

Table 1. Total number (N), range, median and mean with standard deviation (±) of total weight (TW), dorsal mantle length (DML) of *D. gahi* individuals sampled in 2001 and 2020, respectively

				TW (g)			DML (cm)		
Year	Cohort	Sex	N	Range	Mean	Median	Range	Mean	Median
2001	ASC	F	151	16.6–93.3	43.8 ± 14.9	38.4	8.0–16.0	11.4 ± 1.6	11.0
		M	102	23.4–386.2	75.8 ± 65.2	52.6	8.5–32.5	14.5 ± 5.1	12.7
	SSC	F	632	13.4–89.7	40.6 ± 11.1	39.5	8.0–16.0	11.7 ± 1.5	12.0
		M	658	6.9–366.0	48.2 ± 21.1	44.6	5.5–34.5	12.7 ± 2.2	12.5
2020	ASC	F	158	10.8–316.0	40.2 ± 29.9	34.0	7.0–26.5	10.9 ± 2.3	10.5
		M	142	11.8–338.0	47.6 ± 49.2	33.6	7.0–30.5	11.71 ± 4.2	10.5
	SSC	F	129	3.9–97.5	51.4 ± 24.6	48.4	5.0–17.5	12.7 ± 2.9	13.0
		M	141	5.4–353.0	61.5 ± 44.5	52.3	5.5–35.0	13.8 ± 4.3	13.0

in temporal changes and geographic differences in diet being confounded, most analyses were then repeated using only data from the part of the sampling area that was sampled during both periods.

Squid sampled in 2001 and 2020 were analysed in the laboratory in a similar way, with dorsal mantle length (DML ± 0.5 cm) and total weight (TW ± 0.1 g) measured. A qualitative visual stomach fullness index (SFI; Breiby & Jobling, 1985) was assigned: 1 = empty; 2 = ¼ filled; 3 = ½ filled; 4 = ¾ filled; 5 = completely full. Non-empty stomachs were retained and stored frozen. In 2001 a subsample of 1495 stomachs containing food was collected; in 2020 a subsample of 570 stomachs containing food was collected.

Data on sample numbers (separately by sex) and squid sizes are presented in Table 1. Similar numbers of males and females were collected, and the former were on average larger.

To make inter-annual and inter-seasonal comparisons, data were split into individuals belonging to the ASC and SSC and into three size-classes: small (<13 cm DML), medium (13–19 cm DML) and large (>19 cm DML). Individuals were assigned to each spawning cohort using their DML, maturity, month of collection and depth in which they were caught, based on findings by Arkhipkin *et al.* (2004*a*, 2013). Especially in May–July mixing of cohorts occurs, as adult squid from the ASC were still alive and present in the fishing areas, whereas juvenile squid from the SSC had already hatched (see Figure S1 in supplementary material).

Stomach content analysis

Stomach content examination

Stomach content of each individual squid was transferred into a Petri dish and examined under a dissecting microscope. The stomach weight (SW ± 0.01 g) and the content weight (CW ± 0.01 g) were recorded. An overall digestion state was assigned to each stomach (1 = fresh, 2 = start of digestion, 3 = mostly digested). Food items were identified using a reference collection from the Falkland Islands Fisheries Department (FIFD) and identification keys (Boltovskoy, 1999*a*, 1999*b*). All prey categories were noted as present or absent (PA) and, for each category present, the percentage share of the total stomach contents was determined employing a grid made from graph paper and using a binocular microscope resulting in scores (P) from 1–100 after the Point Method (Wear & Haddon, 1987). This was done because masticated food debris did not allow the use of classical gravimetric or volumetric approaches to obtain the weight and volume of single prey items (Breiby & Jobling, 1985).

The number of otoliths, statoliths, eye lenses (both fish and crustacean) and Euphausiacea mandibles were counted per stomach from samples collected in 2020. Total Euphausiacea mandible length (TML ± 0.05 mm) was measured randomly for up to 12 mandibles in each stomach.

Comparability of datasets

In 2001, several prey items were identified to species level, whereas in 2020 prey items were mostly identified to genus or group level. In order to obtain comparable prey categories for the two datasets, for the various analyses and methods, the prey groups for both datasets were pooled in two different ways. To perform Generalized Additive Models (GAMs), prey groups were pooled into class level or comparable (e.g. unidentified fish). For the standardized niche analysis, they were pooled into genus/family level or comparable (e.g. unidentified Myctophidae). Statistics analysing trends regarding different years were repeated with a subsample of the 2020 dataset including only datapoints with a similar geographic distribution as in 2001.

Calculation of frequency of occurrence and per cent by weight

The scores (P_i) of each prey category $_i$ were divided by the sum of scores to calculate a percentage of prey category $_i$ after Wear & Haddon (1987).

$$P_i\% = \frac{P_i}{\sum^{P}} \tag{1}$$

Percentage of prey category $_i$ was then multiplied by the stomach content weight to obtain the item weight IW.

$$IW = P_i\% \times CW \tag{2}$$

Frequency of occurrence (FO%) was calculated (the percentage of *D. gahi* that fed on a certain prey) as

$$FO(\%) = \frac{n_i}{n} \times 100 \tag{3}$$

where n_i is the occurrence of a specific prey in stomachs and n is total number of stomachs.

Mandible measurements

Nickels *et al.* (2018) found a correlation between the length of a Euphausiacea mandible and the size of the Euphausiacea. To reconstruct the individual total length (TL) of Euphausiacea ingested by *D. gahi*, 16 *Thysanoessa macrura* and 40 *Euphausia lucens* were caught with a Bongo net (350 µm) in January and February 2020 in Falkland Islands waters. Individuals were preserved in ethanol (95%), TL (± 0.01 mm) and TML (± 0.005 mm) of the left and right mandibles were measured and averaged.

The relationship between TL and TML was best described by the linear function:

$$\mathrm{TML} = 0.0652 \times \mathrm{TL} - 0.0229 \tag{4}$$

($R^2 = 0.7273$).

Trophic niche

To compare sampling periods, size classes and cohorts, niche width was used to quantify the degree of foraging specialization (Krebs, 1999). In this study, 'resource states' were based on prey taxa found in the stomachs. To measure uniformity in the utilization of prey items among all prey categories, Levins' Measure of Niche Width and the Shannon Index of Evenness were calculated (Krebs, 1999). Both measures were standardized to a scale from 0 (minimum niche width) to 1 (maximum niche width) according to Krebs (1999).

For Levins' niche width:

$$B = \frac{1}{\sum p_j^2} \tag{5}$$

where B is niche width and p_j is the fraction of items in the diet that are of food category j.

To standardize B:

$$B_A = \frac{B - 1}{n - 1} \tag{6}$$

where n is the number of prey items found in the stomachs of the investigated species or group.

The Shannon Equitability Index (EH) was calculated from the Shannon Diversity Index (H), thus:

$$H = \sum\nolimits_j^p {}^{*}\ln(p_j) \tag{7}$$

$$E_H = \frac{H}{\ln(n)} \tag{8}$$

Both Levins' standardized B and Shannons' EH give an index ranging between 0 and 1, where 1 means dietary generalization or even utilization of resource states and 0 means dietary specialization.

Statistical analysis

All statistical analyses were conducted using R v.4.0.3 (R Core Team, 2020), considering a significance level of $P < 0.05$. All statistical tests were performed on both datasets, except the tests on Euphausiacea size, which could only be done using mandible lengths measured in 2020.

Influential species were detected with a similarity percentages breakdown (SIMPER) procedure (Clarke, 1993). FO% values for each month of the year were investigated and to detect trends a 2nd degree polynomial locally weighted scatter-plot smoother (LOESS) with a span of 0.75 was used.

Generalized Additive Models (GAMs) were used in this study as these generalizations of linear models permit the inclusion of non-linear relationships (Hastie & Tibshirani, 1986). Following the approach advocated by some ecologists (e.g. Whittingham *et al.*, 2006; Mundry & Nunn, 2009) and which is also usual in social sciences, we chose to avoid a stepwise model selection process, preferring to fit full models and determine the significance of each term. However, in GAM, an interaction between a continuous explanatory variable (the effect of which is fitted as a smoother) and a categorical explanatory variable, is represented as multiple smoothers (one per unique value of the categorical variable). As such, the significance of the interaction term is determined by comparing goodness of fit of models with and without the interaction (effectively a stepwise model selection process).

GAMs (with a Gaussian distribution of log transformed IW values) were performed on influential prey groups (groups identified with SIMPER) to investigate the relationship of IW with month (continuous variable), squid size class (3 level factor) and their interaction (equation (9)). Cyclic cubic splines, penalized cubic regression splines whose ends meet up, were fitted to month to avoid discontinuity between December and January. Knots to separate each predictor and specify the dimension of the basis function were used to represent the smoothing term. Number of knots (k) in the smoothers was set to 4. Two models were compared based on their Akaike Information Criterion (AIC) to determine a possible effect of a month~size interaction.

$$\begin{gathered}\mathrm{gam}(\log(\mathrm{IW}) \sim s(\mathrm{month},\ \mathrm{by} = \mathrm{as.factor(sizeclass)},\\ k = 4,\ bs = "cc") + \mathrm{as.factor(sizeclass)},\\ \mathrm{family} = "\mathrm{gaussian}")\end{gathered} \tag{9A}$$

$$\begin{gathered}\mathrm{gam}(\log(\mathrm{IW}) \sim s(\mathrm{month},\\ k = 4,\ bs = "cc") + \mathrm{as.factor(sizeclass)},\\ \mathrm{family} = "\mathrm{gaussian}")\end{gathered} \tag{9B}$$

As Euphausiacea mandible lengths were measured, a Gaussian GAM was performed to model the influence of size and seasonality on the size of Euphausiacea eaten by the squid for the sampling period 2019–2020 (equation (10)). The DML was log-transformed to reduce the influence of high values. The AIC values of models with interaction (equation (10A)) and without the interaction (equation (10B)) of size-class with month were compared. Number of k was set to 4.

$$\begin{gathered}\mathrm{gam}(\mathrm{TML} \sim s(\mathrm{month},\ \log(\mathrm{DML}),\ k = 16),\\ \mathrm{family} = "\mathrm{gaussian}")\end{gathered} \tag{10A}$$

$$\begin{gathered}\mathrm{gam}(\mathrm{TML} \sim s(\mathrm{month},\ k = 4) + s(\log(\mathrm{DML}),\ k = 4),\\ \mathrm{family} = "\mathrm{gaussian}")\end{gathered} \tag{10B}$$

To test for inter-annual and inter-seasonal differences between size-classes, a Permutational Multivariate Analysis of Variance Using Distance Matrices (ADONIS) was performed. 'Bray–Curtis Dissimilarity Distances' and 10,000 permutations on all size-classes of *D. gahi*, based on item weights IW were used. Influential species were detected with a SIMPER procedure.

GAMs were performed on those influential prey groups to model the influence, on the relative abundance of prey, of DML (continuous variable), sampling period (2 level factor) and spawning cohort (2 level factor). In order to test the three-way interaction, we defined a combined categorical variable cohort.year (with 4 levels: ASC-2001, SSC-2001, ASC-2020 and SSC-2020) (equation (11)). Since there are multiple interactions in this model, some between continuous and categorical variables, fitted as multiple smoothers using s(*X1, by as.factor* (*X2*)), in order to determine the significance of some terms it was necessary to compare (based on AIC) different models as follows:

$$\text{gam}(\log(\text{IW}) \sim s(\log(\text{DML}),\ \text{by} = \text{cohort.year},\ k = 4) + \text{year} + \text{cohort} + \text{year:cohort}) \quad (11)$$

$$\text{gam}(\log(\text{IW}) \sim s(\log(\text{DML}),\ \text{by} = \text{year},\ k = 4) + \text{year} + \text{cohort} + \text{year:cohort}) \quad (12)$$

$$\text{gam}(\log(\text{IW}) \sim s(\log(\text{DML}),\ \text{by} = \text{cohort},\ k = 4) + \text{year} + \text{cohort} + \text{year:cohort}) \quad (13)$$

$$\text{gam}(\log(\text{IW}) \sim s(\log(\text{DML}),\ k = 4) + \text{year} + \text{cohort} + \text{year:cohort}) \quad (14)$$

A Gaussian distribution was assumed and IW values were log transformed. The DML was also log transformed to reduce the influence of a small number of high values. Residuals were checked for normality.

All GAMs were performed with the R package 'mgcv' (Wood, 2017). The statistical tests SIMPER and ADONIS were performed with the R package 'vegan' (Oksanen *et al*., 2020).

Results

Feeding spectrum

In 2001, 62% of all sampled stomachs contained food. The mean content weight (CW) was 0.6 g (1.26% of TW) and the median CW was 0.3 g (0.8% of TW), although the distribution of CW was heavily skewed towards higher values. The maximum CW was 32.5 g, measured from a large male individual of 28 cm DML (14.4% of TW). From all the stomachs collected in 2001, a total of 20 prey categories were found (Table 2). In 2020, 63% of all sampled stomachs contained food. Mean CW (0.61 g, 1.28% of TW) and median CW (0.38 g, 0.95% of TW) of 2020 were comparable with 2001. Maximum CW in 2020 was 6.6 g (7.45% of TW). In 2020, a total of 16 prey categories were identified.

The main items within the stomachs were Euphausiacea, Chaetognatha, Amphipoda and squid, identified as *D. gahi* (considered as cannibalism). Crustacean parts were frequently found within the stomachs but were highly macerated. Unidentifiable parts of Crustacea were pooled and assigned to the prey group Crustacea. Of the 24 prey categories, 10 were found in less than 1% of the stomachs.

The amphipods *Themisto gaudichaudii*, *Primno* sp. and *Phronima* sp. were identified. Euphausiacea found in the stomachs were often highly masticated. Intact parts or whole individuals were identified as the species *Euphasia lucens* or *Thysanoessa macrura*. Chaetognatha were identified by the size and shape of hooks found in the stomachs, indicating the presence of species of the genus *Sagitta*. Gastropoda were identified as *Limacina helicana antarctica*, *L. retroversa* and *L.* spp. Fish species identified from otoliths included *Patagonotothen ramsayi*, other species from the genus *Patagonotothen*, *Eleginops maclovinus* and *Sprattus fuegensis* and the myctophid *Gymnoscopelus* spp. The only ctenophore was found in 2001, which was *Mnemiopsis leidyi*.

FO% of all dietary items were calculated for each year, cohort and size class (Table 2). To account for any effects of regionality, FO% were also calculated using data from only the Loligo Box (see Table S1 in supplementary material). Comparing FO% between all areas and only from the Loligo Box, FO% of *D. gahi* and Chaetognatha are higher, FO% of Euphausiacea and *M. gregaria* are lower with fish completely absent looking at data only from the Loligo Box compared with all areas.

Gender-specific differences in the food spectrum were investigated primarily on a graphical basis and did not reveal any differences. The inclusion of 'sex' as a categorical variable to the models did not significantly improve the AIC. For those reasons, an analysis on gender-specific diet variation was not included in this study.

Size-related variation in squid diet

Trends were observed for the diet of *D. gahi* in relation to body size. The most abundant prey group Euphausiacea was consumed by small *D. gahi* (75 FO%), but frequency decreased with increasing DML (Figure 2). Based on FO%, cannibalism and consumption of Cephalopoda and fish increased with increasing DML. Chaetognatha were mainly consumed by squid between 8–15 cm DML and were more frequently consumed than Amphipoda. An increasing trend with DML was observed for the lobster krill *M. gregaria*, remains of which were found in 38 stomachs of squid between 7–18 cm DML.

Table 3 shows the percentage (dietary) similarity values for the different size classes of squid, calculated separately for each of the main prey types. Dietary differences were based on the lowest cumulative sum (cumsum, summed proportions that each prey type contributes to the similarities between different size groups). Differences between large and medium sized squid and between large and small squid were based on differences in IW of cannibalism, followed by Euphausiacea and fish. Differences between medium sized squid and small squid were mainly based differences in IW of Euphausiacea, followed by cannibalism and Chaetognatha (SIMPER, Table 3).

Results from GAM models 11, 12, 13 and 14 indicated that model 11 (which includes the three-way interactions between the effects of DML, year and cohort) was the best model (lowest AIC for all five main prey categories; Table 4).

The GAM (equation (11)) results revealed a significant effect of DML on the importance of prey categories (expressed as IW) in different years and cohorts (Table 5A). Euphausiacea IW peaked in squid between 10–15 cm DML in the SSC 2001 and the ASC 2020 at ~0.5 g (Figure 3). In the SSC 2020, IW of Euphausiacea increased with DML, up to 2 g in squid of ~30 cm DML. The importance of cannibalism increased significantly with DML in all years and cohorts, with highest values (~10 g) for the SSC in 2001. The importance of fish increased with DML in all years and cohorts. The consumption weights of Chaetognatha in the stomachs of *D. gahi* increased significantly with increasing size in ASC 2001.

Using only data from the Loligo Box, most trends remained the same (Table S2A in supplementary material), although uncertainty increased (represented by grey shaded areas as confidence intervals, Figure S2 in supplementary material). IW of Euphausiacea in the SSC 2020 peaked at ~15 cm DML instead of increasing steadily.

Seasonal diet variation

Mean FO% were plotted for each month and trends in FO% were investigated using LOESS smoothers (Figure 4). The average FO% of Chaetognatha increased during winter (except July) and decreased during summer for *D. gahi* individuals smaller than 13 cm DML. LOESS showed for the FO% of cannibalism an

Table 2. Frequency of Occurrence (FO%) of prey items within stomachs of *D. gahi* during 2001 and 2020; summarized separately for the ASC (autumn-spawning cohort) and SSC (spring-spawning cohort)

		FO% ASC						FO% SSC					
		<13 cm		13–19 cm		>19 cm		<13 cm		13–19 cm		>19 cm	
		2001	2020	2001	2020	2001	2020	2001	2020	2001	2020	2001	2020
Cephalopoda		51.43	18.66	38.10	30.56	86.66	58.34	38.41	9.70	47.78	14.87	73.33	33.34
	Doryteuthis gahi	45.14	13.10	38.10	30.56	73.33	41.67	33.33	5.97	43.82	6.61	73.33	26.67
	Octopoda	–	–	–	–	–	–	1.65	–	0.23	–	–	–
	Unidentified Cephalopoda	6.29	5.56	–	–	13.33	16.67	3.43	3.73	3.73	8.26	–	6.67
Chaetognatha		18.86	25.40	23.81	30.56	26.67	8.33	48.94	11.94	40.33	10.74	13.33	13.33
Crustacea		50.2	66.2	54.3	44.8	3.8	20.0	49.4	70.3	45.9	64.8	21.7	33.3
	Amphipoda	26.29	28.57	26.98	36.11	–	16.67	30.50	15.67	26.11	29.75	13.33	6.67
	Copepoda	1.14	–	–	–	6.67	–	8.75	–	1.17	–	–	–
	Euphausiacea	55.43	71.03	69.84	41.67	-	16.67	56.97	82.09	68.53	61.98	20.00	33.33
	Larval decapoda	–	–	–	–	–	–	0.47	–	0.47	–	–	–
	Munida gregaria	–	3.57	–	5.56	–	–	–	2.24	–	19.83	–	–
	Ostracoda	–	–	3.17	–	–	–	0.8	–	–	–	–	–
	Unidentified Crustacea	4.00	11.90	–	–	–	–	6.86	2.24	5.59	4.13	–	6.67
Fishes		8.00	9.93	11.11	30.56	46.67	66.67	11.00	12.30	12.22	28.17	33.00	45.66
	Unspecified fish	5.14	4.37	4.76	5.56	46.67	16.67	2.60	3.73	7.23	7.44	20.00	13.33
	Fish larvae	2.29	–	6.35	–	–	–	5.56	2.6	3.26	4.2	13.0	9.5
	Fish scales	–	5.56	–	25.00	–	33.33	2.6	5.22	1.5	12.40	–	13.33
	Mictophiformes	0.57	–	–	–	–	16.67	0.24	0.75	0.23	4.13	–	9.5
Gastropoda		–	–	1.59	–	–	–	8.87	0.5	7.46	2.3	–	6.67
Other		8.00	10.31	11.11	36.11	6.67	33.33	13.35	17.16	21.44	25.61	13.33	33.33
	Nematoda	–	–	–	–	6.67	–	0.12	11.8	0.23	14.4	8.7	23.8
	Planta	1.14	–	–	–	–	–	0.95	–	3.50	–	–	–
	Polychaeta	0.57	–	–	–	–	–	0.12	–	1.6	–	–	–
	Unidentified	2.86	–	1.59	–	–	–	1.89	–	2.10	–	6.67	–

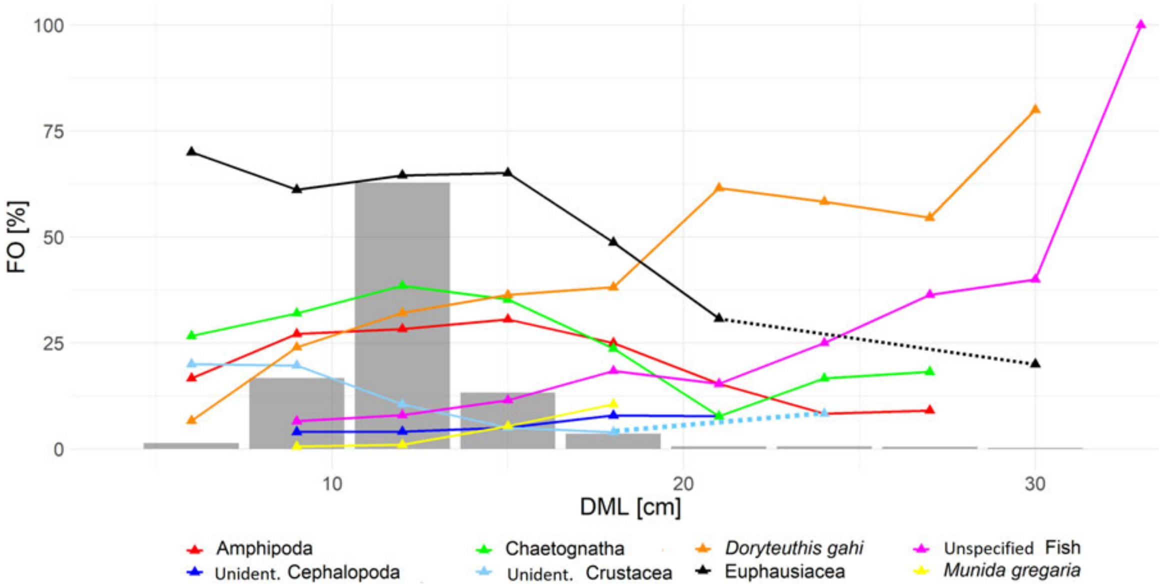


Fig. 2. FO (%) *vs* DML (cm) of *D. gahi* for each prey category. In the background: Histogram of the percentage of total stomachs investigated per 3 cm size range. Unident. = Unidentified.

Table 3. Similarity percentages (CUMSUM) from SIMPER analysis of prey-type IW in stomachs of *D. gahi* for comparisons between size classes

	Chaetognatha	*D. gahi*	Euphausiacea	Fish
Large *vs* medium	–	0.377	0.377	0.808
Large *vs* small	–	0.401	0.631	0.793
Medium *vs* small	0.755	0.656	0.522	–

Table 4. AIC for the model equations (11)–(14) applied for each of the major prey groups with $k = 4$

Species	Model (11)	Model (12)	Model (13)	Model (14)
Amphipoda	1996	2001	2006	2006
Chaetognatha	2570	2595	2588	2591
D. gahi	2130	2133	2135	2131
Euphausiacea	4567	4582	4570	4579
Fish	703	711	720	719

increasing trend in winter months for all size classes. For the FO% of Euphausiacea a decreasing trend in winter months was detected for the size class 13–19 cm. Euphausiacea trends for <13 cm and >19 cm were not distinct.

To investigate the seasonality in diet, whilst accounting for ontogenetic change, GAMs were performed on the item weight (IW) values for each prey group with the model formula (equation (9A)), which includes the effects of size, month, and their interaction. To test whether this interaction was important, AIC values were compared between equations (9A) and (9B) (Table 6). The model with equation (9A) showed lower AIC values and was therefore chosen.

The item weights of Euphausiacea had a significant negative trend towards the austral winter months for small squid (Table 7, Figure 5). Amphipoda item weights were higher during winter in squid between 13–19 cm. Chaetognatha IW were significantly higher in summer months for squid larger than 19 cm, even though trends for smaller squid seem to be the contrary (Table 8). Cannibalism was more prevalent during the austral winter months in small and medium sized individuals. Small and medium sized squid consumed more fish during winter months. Trends were not significant for Amphipoda in small and large squid, nor for Euphausiacea in medium sized squid or for fish in large sized squid.

Mandible measurements

Euphausiid mandibles found in the stomachs of *D. gahi* sampled in 2020 ranged from 0.5–2.25 mm.

Using the relationship between TL and TML to recalculate the size of Euphausiacea, it was estimated that Euphausiacea that were preyed upon by *D. gahi* ranged from 10–30 mm TL. Therefore, the predator–prey ratio ranged from 0.28–3.2% (TML/DML). AIC of the model with the interaction equation (10A) was significantly lower (−395 compared with 11) and was therefore chosen to investigate the influence of seasonality with an interaction of DML for samples collected in 2020.

The resulting 3-dimensional gam smoother showed that TML changed significantly in different seasons ($P < 0.01$, $R^2 = 0.529$), with smaller mandibles found in the austral winter months (April–October) and larger mandibles found in the austral summer months (November–March, Figure 6). The interaction of month and DML was significant ($edf = 14.14$, $P < 0.01$) and showed especially in December, that larger Euphausiacea mandibles were found in smaller squid. A 2-dimensional excerpt from this 3-dimensional model for the mean squid length of 11 cm can be found in the supplementary material (Figure S3).

Inter-annual and -cohort diet variation

The ADONIS revealed significant differences in IW between the two sampling periods, the two spawning cohorts and the three size-classes (Table 9). Although the differences were significant, the R^2 values were small, as the 'noise' of the model with many single stomach records was high. ADONIS model based on data only from the Loligo Box can be found in the supplementary material (Table S3).

The SIMPER analysis revealed differences in prey group IW between years and cohorts. Inter-annual differences in the ASC were mainly based on *D. gahi* and secondly on Euphausiacea

Table 5. Summary of GAM results for models of item weights (IW) of the main prey categories *vs* explanatory variables DML, year, cohort and the interactions between them. The three-way interactions are captured by combining year and cohort into a new categorical variable with four possible values and fitting the smoother for DML separately for each value of the variable. (A) Details of smoothers describing the effect of DML for each value of the combined year-cohort variable: expected degrees of freedom (edf) and *P*-values (significance indicated by *); (B) Effects of categorical explanatory variables: Parameter estimate and *P*-value (significance indicated by *). For the variable Year, the coefficients given are for year 2020 (*vs* 2001 as the baseline); thus a positive value indicated greater consumption in 2020. For the variable Cohort, the coefficients given are for the SSC (*vs* ASC as the base); thus a positive value indicates high consumption by the SSC

		Amphipoda		Chaetognatha		*D. gahi*		Euphausiacea		Fish	
		edf	*P*-value	edf	*P*-value	edf	*P*-value	edf	*P*-value	Edf	*P*-value
(A)	2001 ASC	1.000	0.005*	1.962	<0.001*	1	<0.001*	1.000	0.566	1.021	<0.001*
	2001 SSC	1.585	0.288	2.218	0.222	2.796	<0.001*	2.429	<0.001*	2.033	<0.001*
	2020 ASC	1.881	0.028*	1.819	0.071	2.913	<0.001*	2.188	0.002*	1	0.011*
	2020 SSC	1.000	<0.001*	1.000	0.126	1.958	0.001*	1.000	<0.001*	1	0.996
(B)		Estimate	Pr(>|*z*|)	Estimate	Pr(>|*z*|)	Estimate	Pr(>|*z*|)	Estimate	Pr(>|*z*|)	Estimate	Pr(>|*z*|)
	Intercept	−1.741	<0.001*	−3.560	<0.001*	−2.008	<0.001*	−1.135	<0.001*	−3.741	<0.001*
	(Year) 2020	−0.797	0.002*	−0.155	0.559	−0.790	<0.001*	−0.006	0.973	1.205	0.019*
	(Cohort) SSC	−0.715	<0.001*	0.753	<0.001*	−0.360	0.004*	−0.238	0.066	0.260	0.477
	Year-cohort interaction	0.078	0.825	−1.369	<0.001*	−0.018	0.961	0.086	0.683	−0.080	0.902

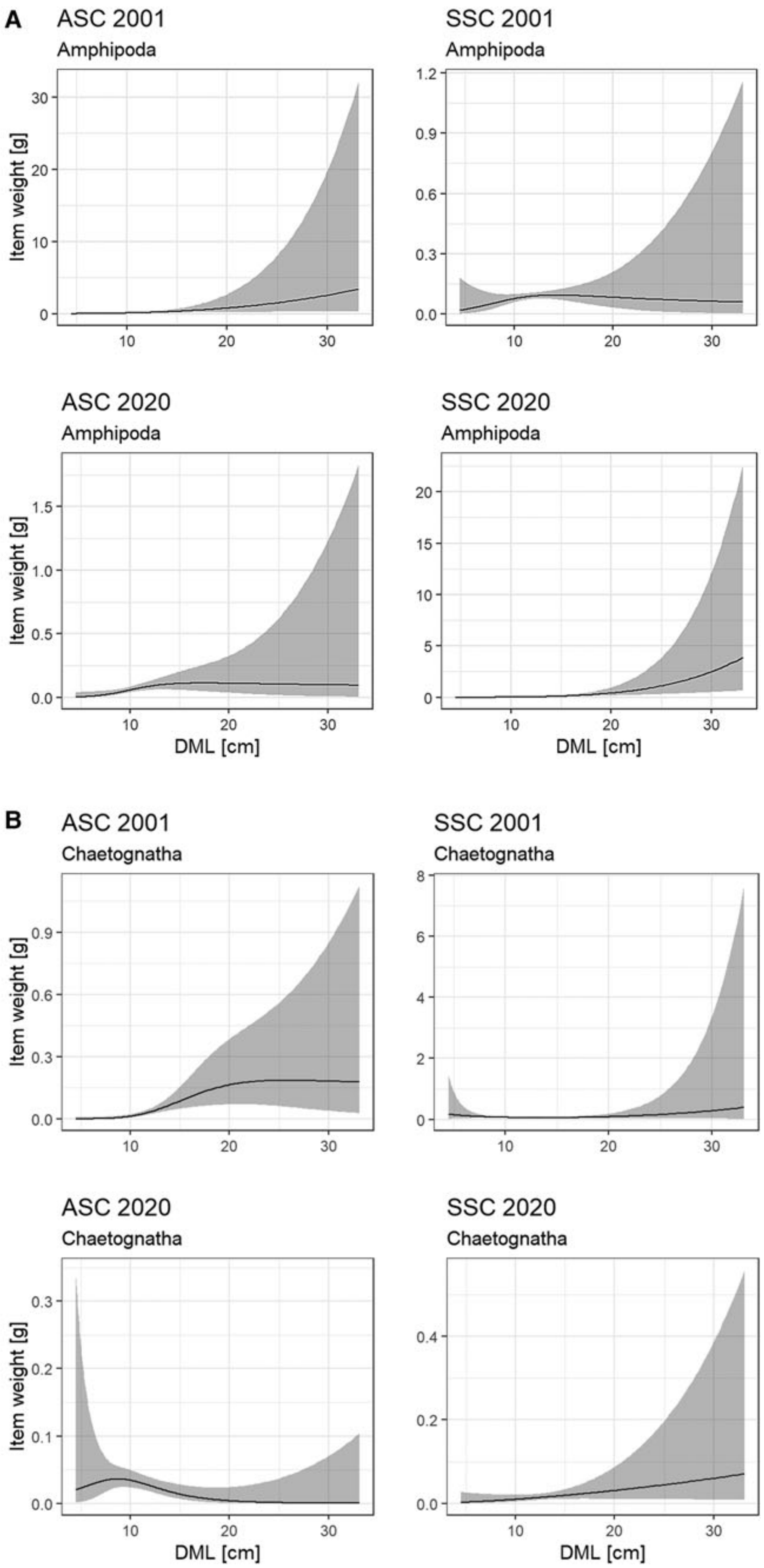


Fig. 3. GAM smoother DML ~ IW for (A) Amphipoda; (B) Chaetognatha; (C) *D. gahi*; (D) Euphausiacea and (E) Fish. Model for both cohorts ASC and SSC and years 2001 and 2020 with 95% confidence intervals (grey shaded area).

(Table 10), inter-annual differences in the SSC were also based on those two prey groups but in reversed order of importance. Similarly, inter-seasonal differences in 2001 were mainly due to Euphausiacea followed by *D. gahi*. In contrast to 2001, differences between cohorts in 2020 were mainly caused by *D. gahi* IW, secondly by Euphausiacea. Differences in IWs of Chaetognatha and Fish also contributed to the dissimilarity between cohorts in 2020. Taking only data from the Loligo Box into account, basic trends remained similar (Table S4 in supplementary material). Differences between years in the ASC were caused by

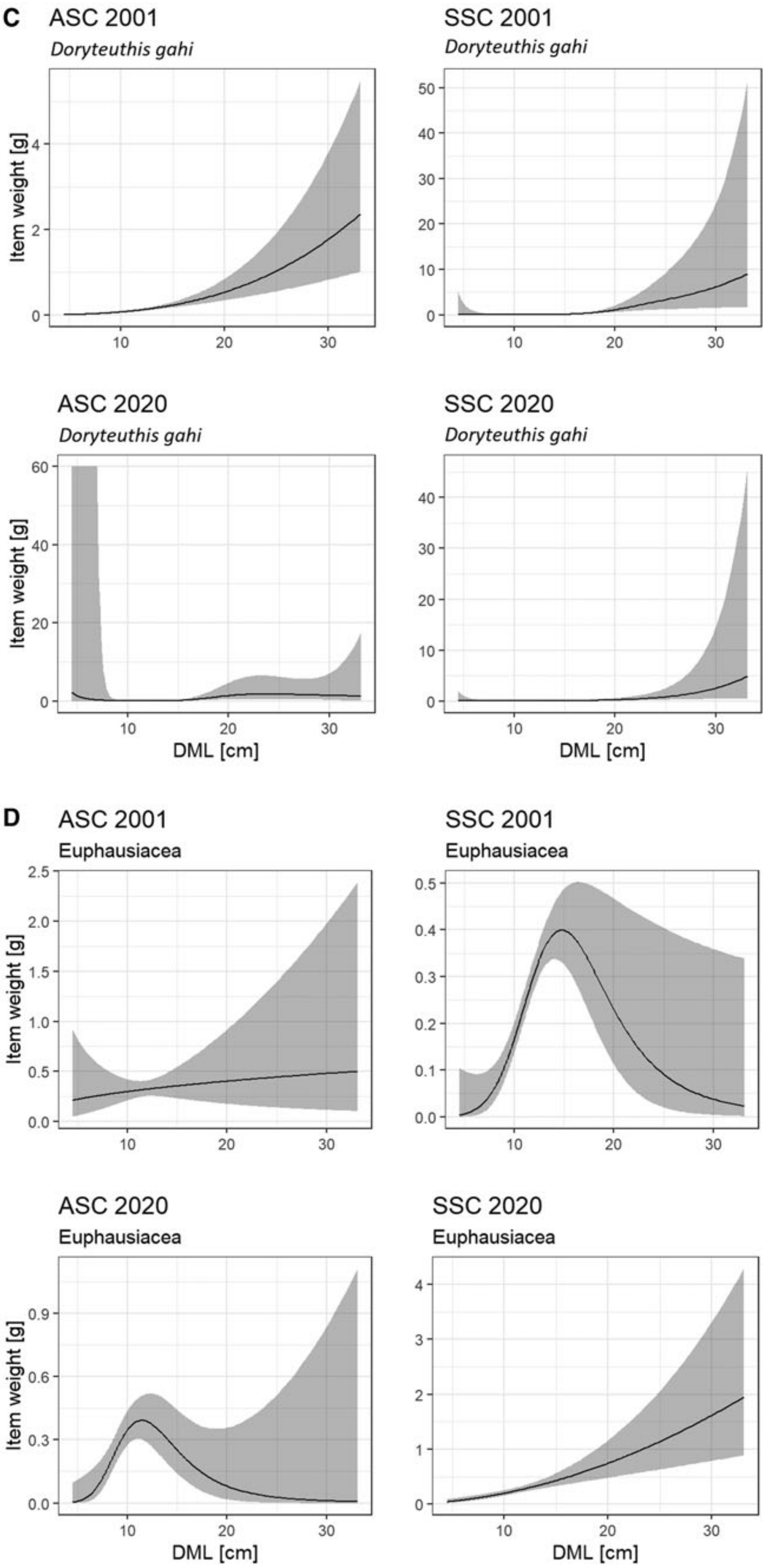
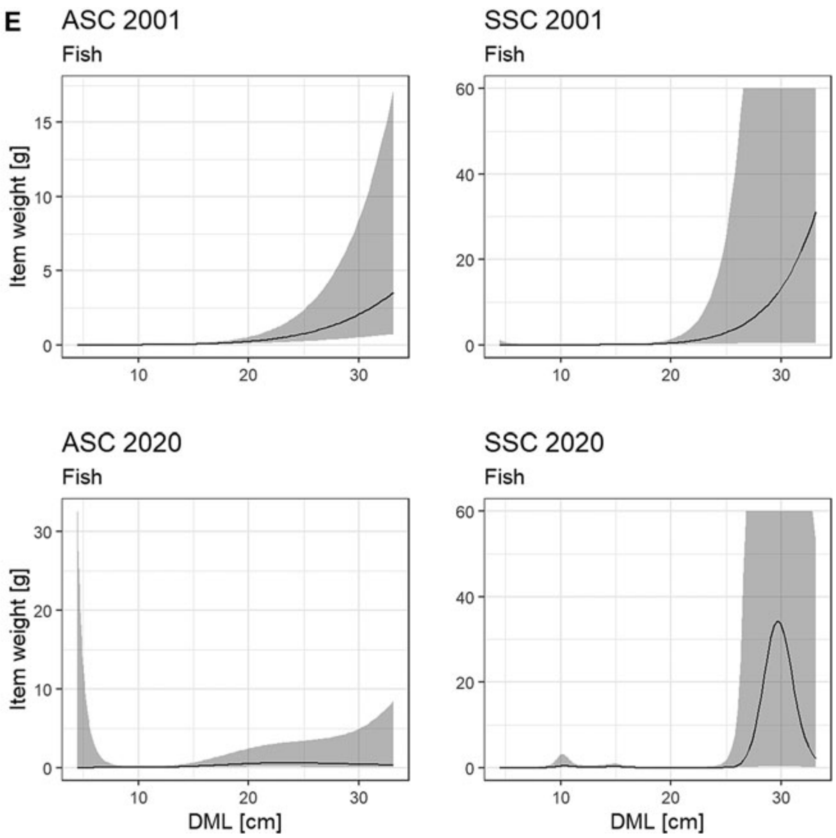


Fig. 3. Continued.

D. gahi and fish. Differences between cohorts in 2020 were caused by *D. gahi*, Euphausiacea and Amphipoda rather than Chaetognatha.

The GAM (equation (11)) showed that importance of Amphipoda as prey was significantly different between years and cohorts, with higher item weights in 2001 compared with 2020 and in the ASC compared with the SSC (Table 5B). Chaetognatha IW had a significant interaction between year and cohort and higher values in the SSC compared with the ASC (Figure 3). Less cannibalism occurred in 2020 compared with 2001 and in the SSC compared with the ASC. Differences in IW between years or cohorts were non-significant at the 5%



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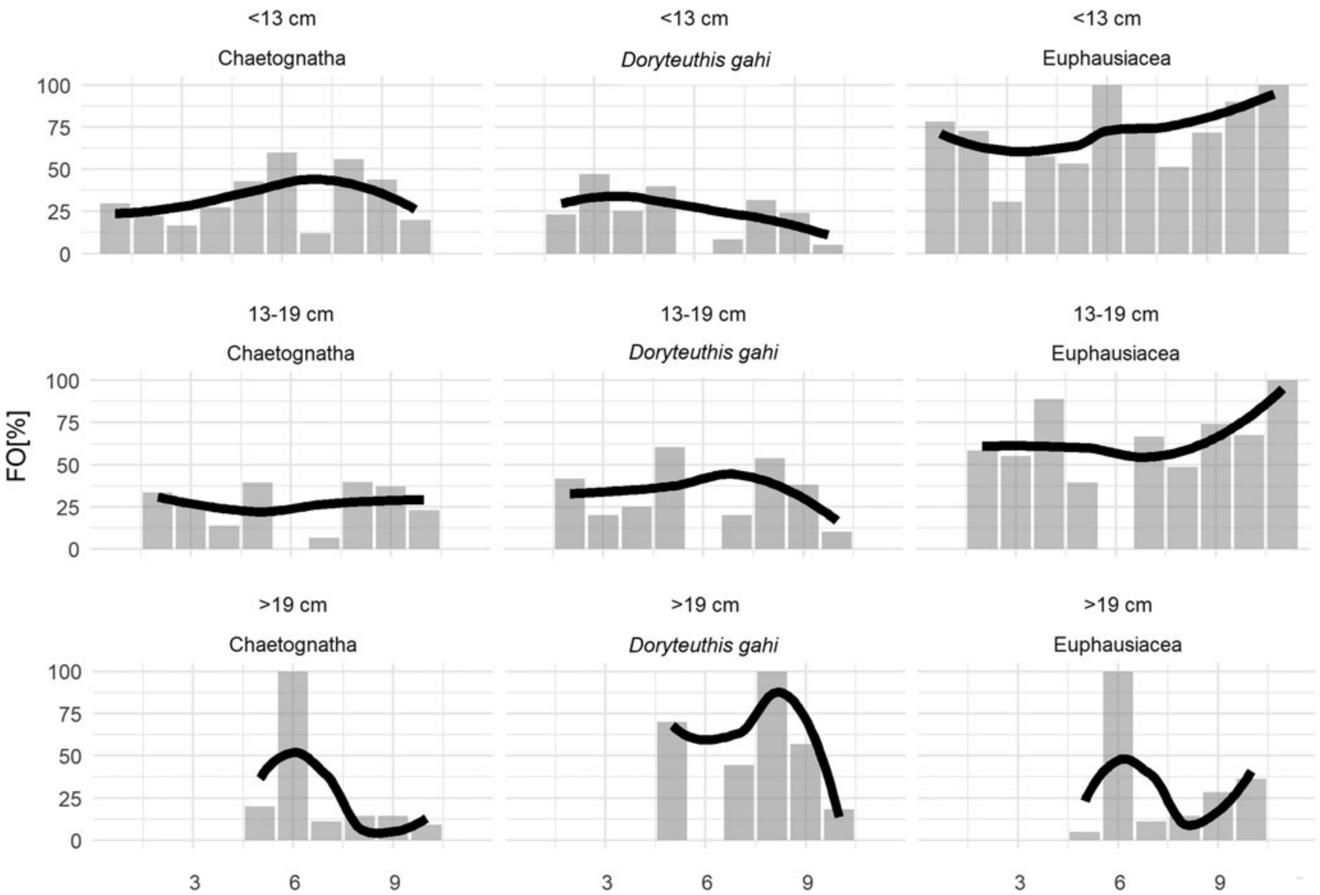

Fig. 4. Mean FO% per month plotted as bars for each size class of squid (<13 cm, 13–19 cm and >19 cm) for Chaetognatha, *D. gahi* (cannibalism) and Euphausiacea. Black line = 'loess' smoother with span = 1.

Table 6. AIC for the models equations (9A) and (9B) applied for each of the major prey groups with $k = 4$

	Model 9A (with interaction)	Model 9B (without interaction)
Amphipoda	2024	2031
Chaetognatha	2632	2636
D. gahi	2171	2178
Euphausiacea	4582	4583
Fish	716	718

level for Euphausiacea, but there was a trend of lower IW in the SSC compared with the ASC. Fish was significantly more consumed in 2020 compared with 2001.

However, these observations could be a result of underlying irregularities due to the different geographic distribution of the samples collected in 2001 compared with 2020. For this reason, the same model was run with data sampled only within the Loligo Box from both years. Associated tables and plots can be found in the supplementary material. Number of knots k was set to 4.

A comparison of the two models, one containing all data and one containing only data from the Loligo Box, revealed similar trends with some important differences (Table IIB supplementary material). In contrast to the first model, IW of Chaetognatha and Euphausiacea in the Loligo Box model were higher in 2020 compared with 2001. Excluding data from the areas resulted in the exclusion of data of fish as a prey item for the ASC 2020. Smoother terms of all species for all years and cohorts remained significant except for Amphipoda and Fish in the SSC 2020.

Niche width

Levins' niche width and the Shannon Equitability Index showed similar trends (Table 11). Overall, the niche width increased with size for both years and cohorts. Niche width was wider in 2020 than in 2001. In 2020, the ASC had a wider niche than the SSC, given the same amount of utilized prey categories. The use of these prey categories also seems to have been evenly distributed, represented by a higher Shannon Equitability Index, but increased with DML. Smaller squid fed on a higher variety of prey categories but used them in a less balanced manner, focusing mainly on Euphausiacea. Therefore, the Shannon Equitability Index was smaller for smaller squid. It was also smaller in 2001 compared with 2020, when a higher variety of prey categories was identified, most of them occurring rarely.

Similar to the series of GAMs, Levins' niche width and the Shannon Equitability Index were also calculated for data obtained only from the Loligo Box (see (Table 5 in supplementary material). Trends did not change due to exclusion of data. Overall, the niche width and the even use of resources indicated by the Shannon Index increased for 2020 except for small squid. Naturally they did not change for 2001 as the same data were used.

Discussion

This study used extensive datasets on stomach contents of *D. gahi* collected throughout the year and during two sampling periods almost 20 years apart. Despite each cohort experiencing different environmental factors at the same ontogenetic phase, their feeding spectra appear to be broadly similar although with variations in the detailed dietary composition. Observed interannual changes in the *D. gahi* diet may reflect decadal changes in plankton composition within the Patagonian Shelf ecosystem.

The most frequent prey items of *D. gahi* found in the present study were Euphausiacea and Chaetognatha. Cannibalism was also reported frequently. These results confirm findings from previous studies in this area (Guerra *et al.*, 1991; Rosas-Luis *et al.*, 2014). Cannibalism is also reported in other loliginid squid (Pierce *et al.*, 1994; Collins & Pierce, 1996). Cannibalistic net feeding may have occurred but was never reported on research cruises or by fisheries observers. Excluding data and focusing on the Loligo Box showed higher FO% of *D. gahi*, probably due to the

Table 7. GAM results (equation (9A)): Intercept and size classes of each prey category found in the stomachs of *D. gahi* (estimate, standard error, *t*-value and *P*-value)

		Estimate	Std. Error	*t* value	Pr(>|*t*|)
Amphipoda	(Intercept)	−2.51	0.08	−33.16	<0.001*
Amphipoda	13–19 cm	0.38	0.15	2.58	0.010*
Amphipoda	>19 cm	0.44	0.66	0.67	0.505
Chaetognatha	(Intercept)	−3.00	0.06	−48.89	<0.001*
Chaetognatha	13–19 cm	0.05	0.12	0.38	0.702
Chaetognatha	>19 cm	1.09	0.56	1.97	0.050*
D. gahi	(Intercept)	−2.71	0.06	−43.20	<0.01*
D. gahi	13–19 cm	0.55	0.11	4.98	<0.001*
D. gahi	>19 cm	3.26	0.27	12.18	<0.001*
Euphausiacea	(Intercept)	−1.35	0.05	−27.80	<0.001*
Euphausiacea	13–19 cm	0.44	0.08	5.30	<0.001*
Euphausiacea	>19 cm	−0.30	0.46	−0.66	0.511
Fish	(Intercept)	−3.92	0.17	−23.21	<0.001*
Fish	13–19 cm	0.65	0.28	2.29	0.023
Fish	>19 cm	3.71	0.46	8.06	<0.001*

Significant results indicated by *.

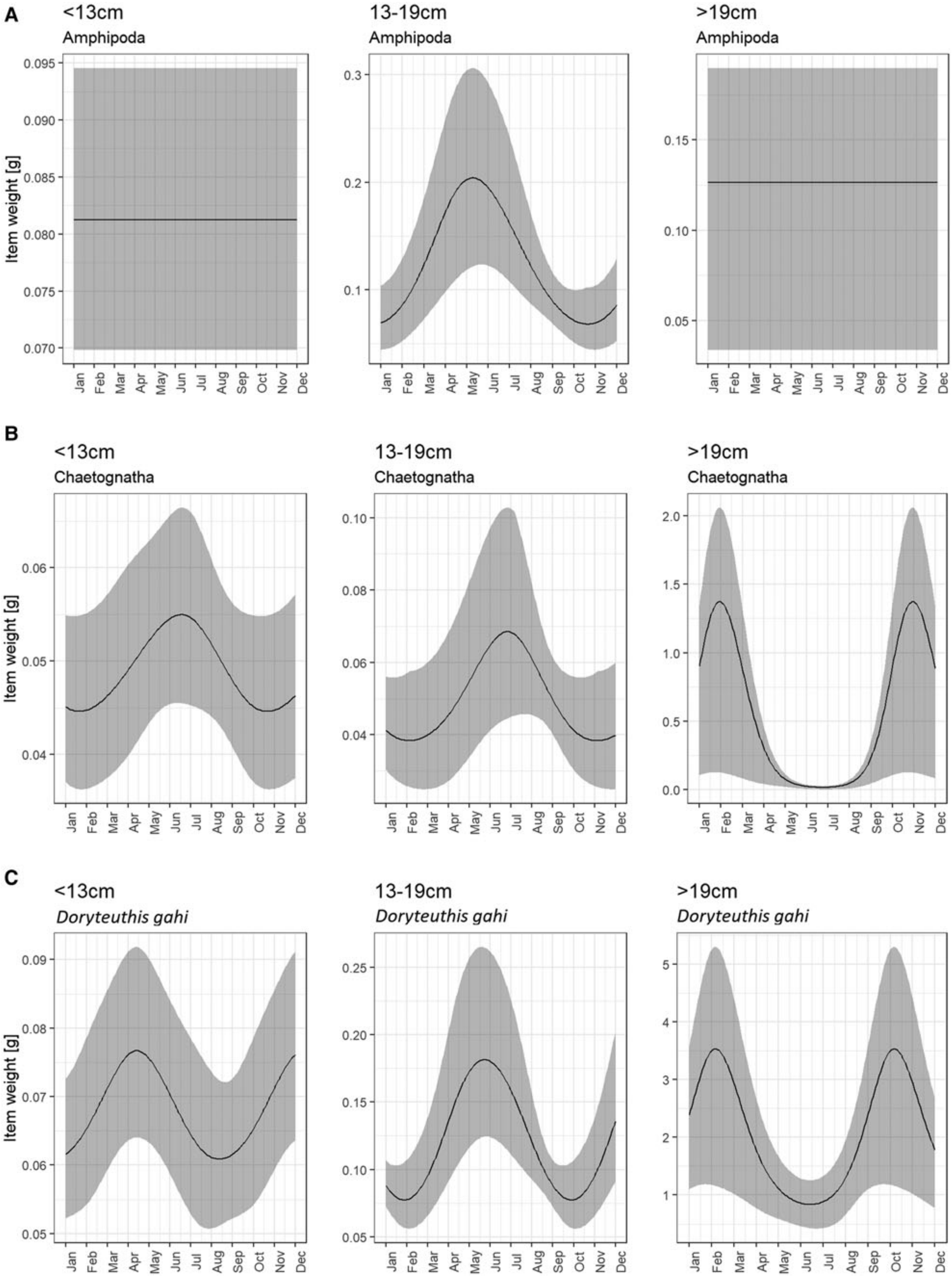

Fig. 5. GAM smoothing curves: Seasonal variation and 95% confidence interval (grey shaded area) in the abundance of (A) Amphipoda, (B) Chaetognatha, (C) *D. gahi*, (D) Euphausiacea and (E) Fish in the stomachs of *D. gahi* over the months.

higher concentration of animals in this area and in lower FO% of Euphausiacea, *M. gregaria* and fish.

The diet composition of *D. gahi* changed with increasing DML. Smaller squids fed mainly on Euphausiacea, whereas larger squid fed mainly on fish and squid, including cannibalism. Similar ontogenetic changes in diet were described previously in other loliginid squid, such as *Loligo forbesii* (Collins & Pierce, 1996) and *Doryteuthis pealeii* (Macy, 1982; Vovk, 1985). This

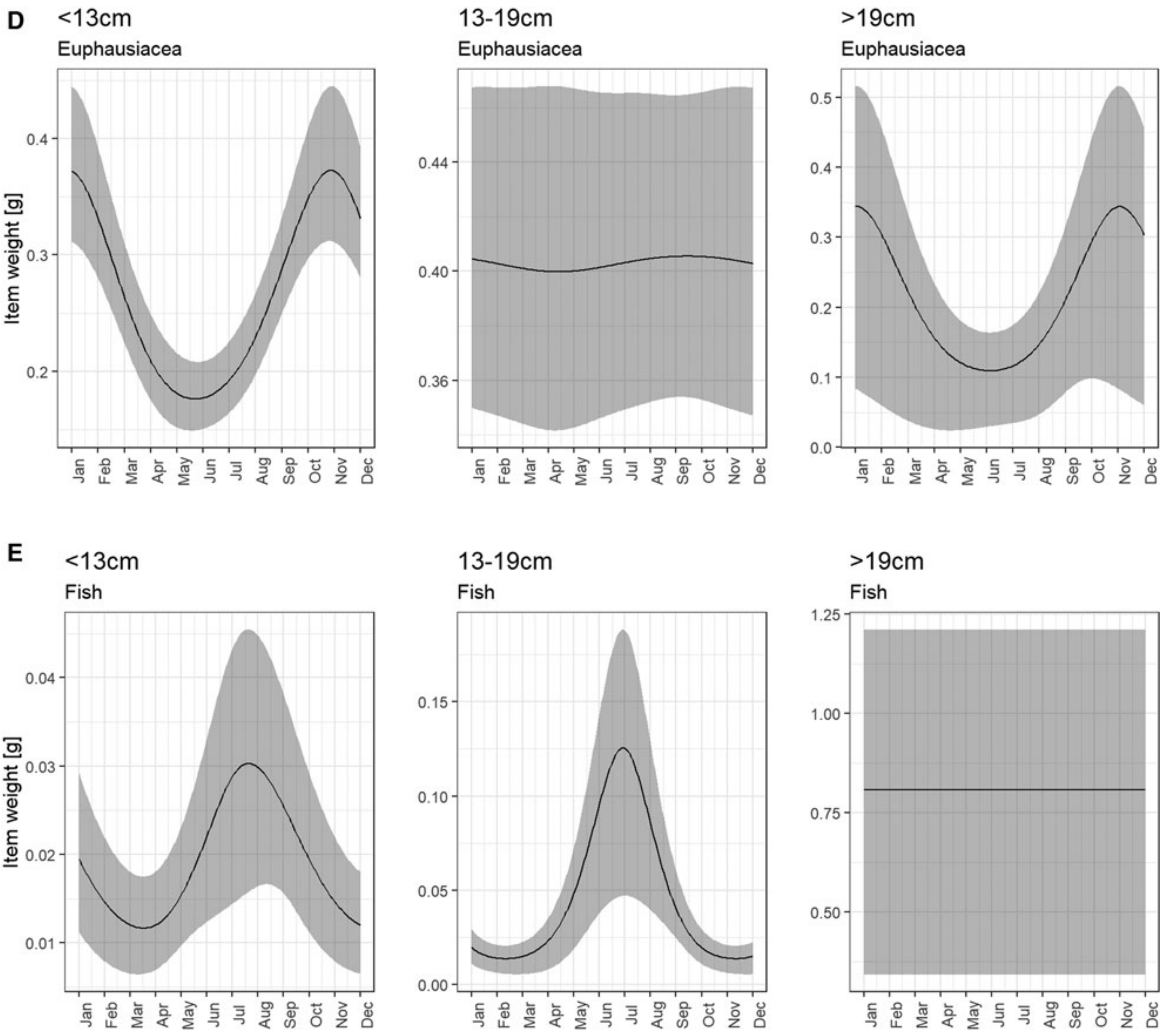

Fig. 5. Continued.

Table 8. Model output for the smoother of month by size class. All factors of size class with 2 reference degrees of freedom

Prey category	s(month): size class	Df	*F*	*P*-value
Amphipoda	<13 cm	0.000	0.000	0.415
Amphipoda	13–19 cm	1.668	5.040	0.003*
Amphipoda	>19 cm	0.000	0.000	0.807
Chaetognatha	<13 cm	1.134	0.903	0.203
Chaetognatha	13–19 cm	1.360	1.469	0.116
Chaetognatha	>19 cm	1.704	3.360	0.020*
D. gahi	<13 cm	1.100	1.772	0.042*
D. gahi	13–19 cm	1.699	5.256	0.002*
D. gahi	>19 cm	1.570	1.959	0.084
Euphausiacea	<13 cm	1.895	14.035	<0.001*
Euphausiacea	13–19 cm	0.080	0.042	0.333
Euphausiacea	>19 cm	0.774	0.825	0.121
Fish	<13 cm	1.456	2.022	0.066
Fish	13–19 cm	1.744	4.116	0.010*
Fish	>19 cm	0.000	0.000	0.919

variation reflects the development of morphological features related to feeding, e.g. growth of the beak and tentacles, thus enabling the squid to feed on larger prey (Boucher-Rodoni *et al.*, 1987). In the present study, diet did not seem to be dependent on sex.

The diet of *D. gahi* was found to vary throughout the year, with higher item weights of Euphausiacea being ingested during austral summer months (November–March) and higher item weights of cannibalism in small and medium sized squid during austral winter months (April–October), although large sized squid had a less cannibalistic diet in winter. The importance of seasonality for the diet composition of squid was described in *L. forbesii* by Wangvoralak *et al.* (2011), with peak copepod consumption coinciding with peak copepod biomass off the eastern coast of Scotland. Likewise, predation of *D. gahi* on Euphausiacea seem to be dependent on the Euphausiacea biomass peak of the Southern Ocean, where higher abundances of zooplankton can be found between January and March (Sabatini & Colombo, 2001; Sabatini *et al.*, 2004). The increased amount of ingested fish and the prevalence of cannibalism in *D. gahi* during the austral winter months could be due to the decreased availability of plankton such as Euphausiacea and Chaetognatha.

The present study showed that the diet of *D. gahi* is dependent on squid growth and seasonal availability of prey. Very few studies

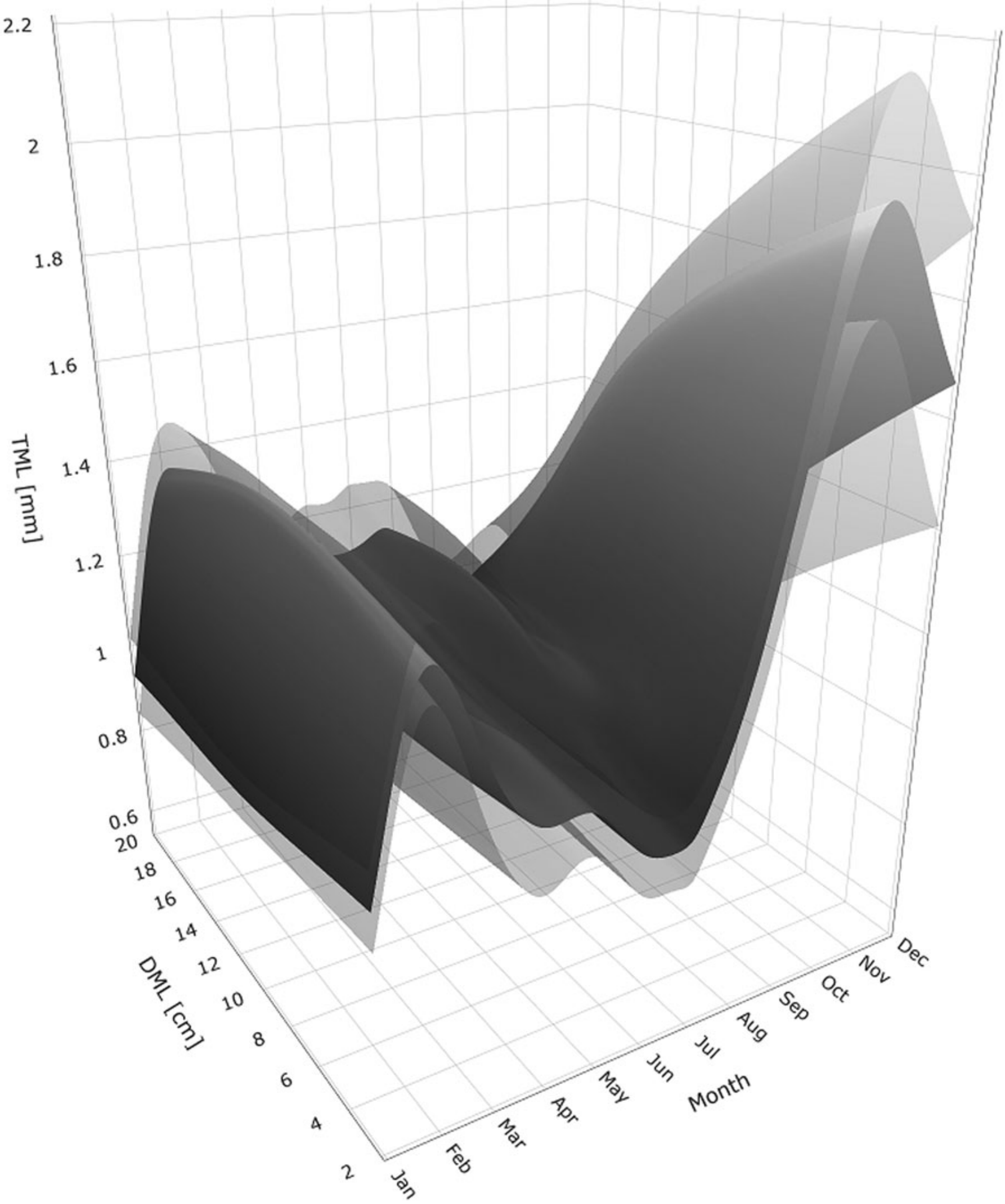

Fig. 6. Three-dimensional GAM: Euphausiacea TML *vs* month × log(DML) (solid surface) and 95% confidence interval (semi-translucent surface). Month and DML on *x*- and *y*-axes, TML on *z*-axis.

have investigated dietary differences between squid spawning cohorts. These differences may be related to seasonal differences in environmental conditions and prey availability experienced by the two cohorts. The two spawning cohorts of *D. gahi* had similar feeding spectra but different diet compositions, mainly due to the variable role of Euphausiacea in their diet, probably due to seasonality. *Doryteuthis gahi* individuals undergo complex horizontal and vertical migrations during their ontogeny and are affected by different environmental conditions (Arkhipkin *et al*., 2004*a*, 2004*b*). Individuals of the ASC have their feeding period during the austral summer, remaining in warm shallow waters of the Transient Zone of the Patagonian shelf (100–200 m). SSC individuals have their feeding period during winter, residing in the warmest available water layer almost exclusively between 150–250 m depths (Arkhipkin *et al*., 2013). This coincides with the main depth range of *Euphausia lucens* (Gibbons *et al*., 1991). Furthermore, *D. gahi* and Euphausiacea show the same diel vertical migration pattern (Nocera *et al*., 2021). The SSC of *D. gahi* in 2020 fed on smaller amounts of Euphausiacea, due to lower abundances of Euphausiacea during winter (Sabatini & Colombo, 2001). TML of Euphausiacea was smaller in winter months compared with summer months, suggesting smaller Euphausiacea prey size in winter. SSC individuals seem to feed more on fish when taking all sampled areas into account. This coincides with the higher amounts of fish being ingested during winter. However, in the GAM models, both of which used all areas and only data from the Loligo Box, slightly less cannibalism was observed in the SSC. This seems to contradict the results from

Table 9. Results of the ADONIS (Permutational Multivariate Analysis of Variance Using Distance Matrices); Df (Degrees of freedom) based on the IW (item weights) of *D. gahi* prey items for different years, cohorts and size-classes

	Df	Sum Of Sqs	R^2	F	Pr(>F)
Year	1	11.372	0.015	32.871	<0.001*
Cohort	1	2.347	0.003	6.786	<0.001*
Size class	2	12.998	0.017	18.786	<0.001*
Residual	2108	729.264	0.965		
Total	2112	755.982	1.000		

Sum Of Sqs (Sum of Squares), R^2, F statistic and P-value of the F-statistic.

Table 10. CUMSUM from SIMPER analysis of prey-type IW in stomachs of *D. gahi* for inter-annual and inter-seasonal comparisons

	Chaetognatha	*Doryteuthis gahi*	Euphausiacea	Fish
Interannual				
ASC	–	0.44	0.76	–
SSC	–	0.79	0.59	–
Inter-seasonal				
2001	–	0.79	0.67	–
2020	0.65	0.32	0.51	0.76

the seasonal model which showed more cannibalism in winter. But less cannibalism in the SSC revealed from the inter-annual and inter-cohort model could be due to seasonal overlap, as individuals of each cohort are known to live up to ∼300 days, or due to a mixing of cohorts. Especially in the autumn and winter months March–April squid cohorts often cannot be distinguished without age determination, for example by statolith readings (Jones *et al.*, 2018).

Studies on decadal diet shifts are scarce, especially in squid. The ecosystem of the Patagonian shelf underwent some significant changes within the last two decades. For example, stocks of the planktivorous southern blue whiting, *Micromesistius australis*, collapsed at the end of the 2000s, leading to a temporary explosion of another planktivorous fish, rock cod (*Patagonotothen ramsayi*). These two species are considered as important planktivorous species, occupying intermediate trophic levels and therefore competing for the same resources as *D. gahi* (Laptikhovsky *et al.*, 2013). Changes in the prevalence of planktivorous fish stocks may have restructured the ecosystem of the Patagonian shelf, especially as cold-water ecosystems are particularly vulnerable to trophic cascades (Frank *et al.*, 2005, 2007). Inter-annual effects in the present study revealed fewer Amphipoda, more fish, less cannibalism and the appearance of *Munida gregaria* in the diet of *D. gahi* in 2020 compared with 2001 considering all sampled areas. Focusing only on the Loligo Box, also slightly less cannibalism occurred in 2020 compared with 2001. It is important to mention that Brickle *et al.* (2002) also found *M. gregaria* in the stomachs of *D. gahi*, but its prominence within the last decade seems to have increased (Rosas-Luis *et al.*, 2016). *Munida gregaria* became one of the most important bycatch species in Patagonian fisheries in Argentine waters (Varisco *et al.*, 2015) and in the last decade an increased abundance of *M. gregaria* was observed on the Patagonian shelf (Diez *et al.*, 2016). A study by Diez *et al.*

Table 11. Levins' niche width, Shannon Equitability Index and number of prey categories found per year, cohort and size class of *D. gahi*; Small: <13 cm, medium: 13–19 cm, large: >19 cm

Year	Cohort	Size class	Levins' standardized	Shannon Equitability	N (prey categories)
2001	ASC	Small	0.267	0.675	14
		Medium	0.375	0.741	9
		Large	0.450	0.804	7
2001	SSC	Small	0.248	0.685	20
		Medium	0.239	0.662	17
		large	0.500	0.834	5
2020	ASC	Small	0.360	0.765	9
		Medium	0.767	0.922	6
		Large	0.761	0.944	7
2020	SSC	Small	0.156	0.603	10
		Medium	0.415	0.821	10
		Large	0.654	0.899	7
2001	ASC	All size-classes	0.223	0.644	17
	SSC		0.234	0.674	20
2020	ASC	All size-classes	0.352	0.755	10
	SSC		0.259	0.731	10
Both years	ASC	All size-classes	0.209	0.639	18
	SSC		0.217	0.670	21
2001	Both cohorts	All size-classes	0.231	0.670	20
2020			0.315	0.757	10

(2016) indicated a population expansion of *M. gregaria* over the whole Patagonian shelf due to the shelf's increased productivity. As squid are opportunistic, the increased abundance of *M. gregaria* would explain their higher frequency in stomachs of *D. gahi*.

The diet of squid can differ regionally, such as in *Onykia ingens* (Phillips *et al*., 2003). Although geographic variation in diet composition seems to be important for *D. gahi*, it was not statistically evaluated in this study due to the focus on seasonal and decadal diet shifts. However, major trends remained the same when investigating only the Loligo Box, the area of highest density of *D. gahi* within catches and investigating the entire FICZ and international waters. In this case, excluding data would maintain comparability and rule out the effects of regionality but would weaken the resolution of the study. For the application of future ecosystem models, diet observations over the whole habitat would be necessary.

Wangvoralak *et al*. (2011) found a change in the diet composition of *L. forbesii* over a period of ~15 years. Due to fishing pressure, abundance of small, non-commercially targeted fish increased in Scottish waters alongside their proportion in the diet of *L. forbesii*. Nothing like this was observed in *D. gahi*, although the species assemblage around the Falkland Islands may have changed due to fishing activities within the last 20 years. As fish in the diet of *D. gahi* was only important for the minority of squid over ~20 cm DML, these effects may be difficult to identify.

Overall, the niche width of *D. gahi* indicated a moderate specialization in foraging behaviour. Niche width increased with growth, similar to *D. pealeii* (Hanlon *et al*., 2013). The niche of the ASC in 2020 was wider than the SSC. As the ASC stay in shallower waters compared with the SSC squid, prey may be more diverse, resulting in a wider niche width. The presence of *M. gregaria* might explain the wider niche in 2020 compared with 2001. Overall, the niche width was similar to other squid species like *Ommastrephes bartramii* (0.22–0.56), whereas *Sthenoteuthis oualaniensis* showed a higher degree of specialization (0.086–0.097; Parry, 2006).

Conclusions

The Patagonian long-finned squid *Doryteuthis gahi* appears to have the typical ontogenetic diet change found in many other squid species. Seasonal differences in Euphausiacea consumption were found, which coincide with Euphausiacea biomass peaks on the Patagonian shelf. A decadal shift to higher frequencies of the planktonic stage of lobster krill *Munida gregaria* within the diet is probably due to increased abundances of *M. gregaria* within the ecosystem itself. Differences in diet composition between spawning cohorts seem to be related to the opportunistic behaviour of *D. gahi*, comparable with many other cephalopod species. Effects of regionality on the diet of this study species seem to be likely and should be addressed in future studies. Niche width and evenness of prey category utilization appear to be similar between spawning cohorts and consistent over decades, but both increase with size.

This study provided the basis for future studies on ecosystem dynamics of the Patagonian shelf focusing on the commercially important squid species *D. gahi* and its seasonal spawning cohorts. With these findings and in combination with powerful tools such as stable isotope analysis, more detailed information on the trophic position of *D. gahi* could be revealed, helping the management of this important resource as well as the overall management of the ecosystem.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315422000194.

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References

Arkhipkin A (2013) Squid as nutrient vectors linking Southwest Atlantic marine ecosystems. *Deep-Sea Research Part II: Topical Studies in Oceanography* **95**, 7–20.

Arkhipkin AI, Campana SE, FitzGerald J and Thorrold SR (2004*a*) Spatial and temporal variation in elemental signatures of statoliths from the Patagonian longfin squid (*Loligo gahi*). *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1212–1224.

Arkhipkin AI, Grzebielec R, Sirota AM, Remeslo AV, Polishchuk IA and Middleton DAJ (2004*b*) The influence of seasonal environmental changes on ontogenetic migrations of the squid *Loligo gahi* on the Falkland shelf. *Fisheries Oceanography* **13**, 1–9.

Arkhipkin AI, Hatfield EMC and Rodhouse PGK (2013) *Doryteuthis gahi*, Patagonian long-finned squid. In Rosa R, O'Dor R and Pierce G (eds), *Advances in Squid Biology, Ecology and Fisheries*. Part 1. New York, NY: Nova Science, pp. 123–157.

Belleggia M, Giberto D and Bremec C (2017) Adaptation of diet in a changed environment: increased consumption of lobster krill *Munida gregaria* (Fabricius, 1793) by Argentine hake. *Marine Ecology* **38**, 1–9.

Boltovskoy D (1999*a*) *South Atlantic Zooplankton, Volume 1*. Leiden: Backhuys.

Boltovskoy D (1999*b*) *South Atlantic Zooplankton, Volume 2*. Leiden: Backhuys.

Boucher-Rodoni R, Boucaud-Camou E and Mangold K (1987) Feeding and digestion. In Boyle PR (ed.), *Cephalopod Life Cycles. Comparative Reviews Vol. 2*. London: Academic Press, pp. 85–108.

Boyle P and Rodhouse PGK (2005) *Ecology and Fisheries*. Oxford: Blackwell Science.

Breiby A and Jobling M (1985) Predatory role of the flying squid (*Todarodes sagittatus*) in North Norwegian waters. *NAFO Scientific Council Studies* **9**, 125–132.

Brickle P, Olson PD, Littlewood DTJ, Bishop A and Arkhipkin AI (2002) Parasites of *Loligo gahi* from waters off the Falkland Islands, with a phylogenetically based identification of their cestode larvae. *Canadian Journal of Zoology* **79**, 2289–2296.

Buckland A, Baker R, Loneragan N and Sheaves M (2017) Standardising fish stomach content analysis: the importance of prey condition. *Fisheries Research* **196**, 126–140.

Clarke RK (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**, 117–143.

Clarke MR (1996) Cephalopods as prey. III. Cetaceans. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**, 1053–1065.

Collins MA and Pierce GJ (1996) Size selectivity in the diet of *Loligo forbesi* (Cephalopoda: Loliginidae). *Journal of the Marine Biological Association of the United Kingdom* **76**, 1081–1090.

Diez MJ, Cabreira AG, Madirolas A and Lovrich GA (2016) Hydroacoustical evidence of the expansion of pelagic swarms of *Munida gregaria* (Decapoda, Munididae) in the Beagle Channel and the Argentine Patagonian Shelf, and its relationship with habitat features. *Journal of Sea Research* **114**, 1–12.

Frank KT, Petrie B, Choi JS and Leggett WC (2005) Trophic cascades in a formerly cod-dominated ecosystem. *Science* **208**, 1621–1623.

Frank KT, Petrie B and Shackell NL (2007) The ups and downs of trophic control in continental shelf ecosystems. *Trends in Ecology & Evolution* **22**, 236–242.

Gibbons MJ, Barange M and Pillar SC (1991) Vertical migration and feeding of *Euphausia lucens* (Euphausiacea) in the Southern Benguela. *Journal of Plankton Research* **13**, 473–486.

Guerra A, Castro BG and Nixon M (1991) Preliminary study on the feeding by *Loligo gahi* (Cephalopoda: Loliginidae). *Bulletin of Marine Science* **49**, 309–311.

Hanlon R, Buresch K, Moustahfid H and Staudinger M (2013) *Doryteuthis pealeii*, longfin inshore squid. In Rosa R, O'Dor R and Pierce G (eds), *Advances in Squid Biology, Ecology and Fisheries. Part I*. New York, NY: Nova Science, pp. 205–240.

Hastie T and Tibshirani R (1986) Generalized additive models. *Statistical Science* **1**, 297–318.

Hyslop EJ (1980) Stomach contents analysis – a review of methods and their application. *Fish Biology* **17**, 411–429.

Ibáñez CM and Keyl F (2010) Cannibalism in cephalopods. *Reviews in Fish Biology and Fisheries* **20**, 123–136.

Ivanovic ML and Brunetti NE (1994) Food and feeding of *Illex argentinus*. *Antarctic Science* **6**, 185–193.

Jereb P and Roper CFE (2010) *Cephalopods of the World. An Annotated and Illustrated Catalogue of Cephalopod species Known to Date. Volume 2. Myopsid and Oegopsid Squids*. FAO Species Catalogue for Fishery Purposes 4(2). Rome: FAO.

Jones JB, Arkhipkin AI, Marriott AL and Pierce GJ (2018) Using statolith elemental signatures to confirm ontogenetic migrations of the squid *Doryteuthis gahi* around the Falkland Islands (Southwest Atlantic). *Chemical Geology* **481**, 85–94.

Krebs CJ (1999) Niche measures and resource preferences. *Ecological Methodology*, 2nd Edn. Menlo Park, CA: Benjamin Cummings, pp. 597–653.

Laptikhovsky V, Arkhipkin A and Brickle P (2013) From small bycatch to main commercial species: explosion of stocks of rock cod *Patagonotothen ramsayi* (Regan) in the Southwest Atlantic. *Fisheries Research* **147**, 399–403.

Macy WK (1982) Feeding patterns of the long-finned squid, *Loligo pealei*, in New England waters. *Biological Bulletin* **162**, 28–38.

Mundry R and Nunn CL (2009) Stepwise model fitting and statistical inference: turning noise into signal pollution. *American Naturalist* **173**, 119–123.

Nickels CF, Sala LM and Ohman MD (2018) The morphology of euphausiid mandibles used to assess selective predation by blue whales in the southern sector of the California current system. *Journal of Crustacean Biology* **38**, 563–573.

Nocera AC, Gimenez EM, Diez MJ, Retana MV and Winkler G (2021) Krill diel vertical migration in Southern Patagonia. *Journal of Plankton Research* **43**, 610–623.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E and Wagner H (2020) *vegan: Community Ecology Package*. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Parry M (2006) Feeding behavior of two ommastrephid squids *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* off Hawaii. *Marine Ecology Progress Series* **318**, 229–235.

Phillips KL, Nichols PD and Jackson GD (2003) Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. *Journal of the Marine Biological Association of the United Kingdom* **83**, 523–534.

Pierce GJ, Boyle PR, Hastie LC and Santos MB (1994) Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. *Fisheries Research* **21**, 149–163.

Pierce GJ and Santos MB (1996) Trophic interactions of squid *Loligo forbesi* in Scottish waters. In Greenstreet SPR and Tasker ML (eds), *Aquatic Predators and their Prey*. Chichester: Wiley, pp. 58–64.

R Core Team (2020) *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rosas-Luis R, Navarro J, Martínez-Baena F and Sánchez P (2017) Differences in the trophic niche along the gladius of the squids *Illex argentinus* and *Doryteuthis gahi* based on their isotopic values. *Regional Studies in Marine Science* **11**, 17–22.

Rosas-Luis R, Navarro J, Sánchez P and Del Río JL (2016) Assessing the trophic ecology of three sympatric squid in the marine ecosystem off the Patagonian shelf by combining stomach content and stable isotopic analyses. *Marine Biology Research* **12**, 402–411.

Rosas-Luis R, Sánchez P, Portela JM and Del Rio JL (2014) Feeding habits and trophic interactions of *Doryteuthis gahi*, *Illex argentinus* and *Onykia ingens* in the marine ecosystem off the Patagonian shelf. *Fisheries Research* **152**, 37–44.

Sabatini ME and Colombo GLÁ (2001) Seasonal pattern of zooplankton biomass in the Argentinian shelf off Southern Patagonia (45°–55°S). *Scientia Marina* **65**, 21–31.

Sabatini ME, Reta R and Matano R (2004) Circulation and zooplankton biomass distribution over the southern Patagonian shelf during late summer. *Continental Shelf Research* **24**, 1359–1373.

Smale MJ (1996) Cephalopods as prey. IV. Fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**, 1067–1081.

Varisco M, Vinuesa JH and Góngora ME (2015) Bycatch of the squat lobster *Munida gregaria* in bottom trawl fisheries in San Jorge Gulf, Argentina. *Revista de Biología Marina y Oceanografía* **50**, 249–259.

Vovk AN (1985) Feeding spectrum of longfin squid (*Loligo pealei*) in the Northwest Atlantic and its position in the ecosystem. *NAFO Scientific Council Studies* **8**, 33–38.

Wangvoralak S, Hastie LC and Pierce GJ (2011) Temporal and ontogenetic variation in the diet of squid (*Loligo forbesii* Streenstrup) in Scottish waters. *Hydrobiologia* **670**, 223–240.

Wear RG and Haddon M (1987) Natural diet of the crab *Ovalipes catharus* (Crustacea, Portunidae) around central and northern New Zealand. *Marine Ecology Progress Series* **35**, 39–49.

Wells MJ and Clarke A (1996) Energetics: the costs of living and reproducing for an individual cephalopod. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**, 1083–1104.

Whittingham MJ, Stephens PA, Bradbury RB and Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology* **75**, 1182–1189.

Witek Z and Krajewska-Soltys A (1989) Some examples of the epipelagic plankton size structure in high latitude oceans. *Journal of Plankton Research* **11**, 1143–1155.

Wood SN (2017) *Generalized Additive Models: An Introduction with R*, 2nd Edn. New York: Chapman and Hall/CRC.

Yatsu A, Midorikawa S, Shimada T and Uozumi Y (1997) Age and growth of the neon flying squid, *Ommastrephes bartrami*, in the North Pacific Ocean. *Fisheries Research* **29**, 257–270.