

## RESEARCH ARTICLE

# Fatty acid comparison of four sympatric loliginid squids in the northern South China Sea: Indication for their similar feeding strategy

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## Abstract

Feeding strategies of sympatric squid species help to understand their role in marine ecosystems. Four loliginid squids, *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis*, and *Loliolus uyii* are the major cephalopod species in the coastal waters of the northern South China Sea, where they occur together. We investigated their feeding strategies in terms of foraging behavior and habitat use by comparing fatty acid profiles and spatial distributions. There were no significant differences in the proportions of saturated or polyunsaturated fatty acids among species. Similar findings were obtained for most individual fatty acids that made up of an average of more than 84% of total fatty acid content for each species. Substantial overlap and high similarity in the fatty acid composition were observed. However, there were no significant effects of individual size or sampling station on the fatty acid compositions. The spatial overlap analysis demonstrated that there was clear spatial segregation and habitat use among the species. Cumulatively, our results suggest that the four squids are opportunistic carnivores, unselectively foraging on similar prey items, while spatial segregation is likely a major mechanism leading to their coexistence in the northern South China Sea.

## Introduction

Species coexistence depends partly on how organisms utilize their resources and environment [1, 2]. The ability of sympatric species to exploit different parts of a niche space is essential for their coexistence if they have similar ecological requirements [3–5]. Consequently, species

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may adopt different strategies in terms of resource use, for example, through differences in activity patterns (e.g., temporal segregation) and habitat use [3, 6, 7], resource-abundance-mediated foraging behavior such as trophic niche contraction [4, 8, 9], or body size-related resource partitioning [10, 11].

Squids occur in almost all the world's marine environments [12]; and most likely occupy a similar habitat throughout their lives [13, 14] or coexist seasonally [15]. Squids have been identified as among the most important organisms in marine ecosystems, not only because they act as major nutrient vectors but also because they play a key role as "bio-indicators" of environmental conditions [16–19]. Squids are important prey resources for high trophic level predators, such as large predatory fishes, seabirds and marine mammals [19–21]. On the other hand, they feed intensively on a wide spectrum of prey items including crustaceans, micro-nekton and fishes [19–22], and are believed to impose top-down control on low- to mid-trophic level species [19, 23, 24]. They are also cannibalistic, frequently preying on conspecifics and other squid species [15, 25, 26]. These foraging characteristics lead to complex trophic interactions [19, 27].

Investigating the feeding strategy and consequences of resource partitioning of squids is needed for a broader understanding of the dynamics of marine ecosystems. Four sympatric loliginid squids, *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis*, and *Loliolus uyii*, coexist in shelf waters from the western Pacific to the Indian Ocean, competing for available resources [12, 26]. Similar to other squids, these species are characterized by high growth rates and short lifespan (usually 1 year) [28–30]. The four squids play an important role in community structure and population dynamics within the shelf ecosystem where they are found [31, 32]. For example, these squids are the major cephalopod species, and seasonally dominate the regional biomass, in the coastal waters of the northern South China Sea, i.e., *U. duvaucelii* during autumn, and *U. chinensis* in summer [33]. They are also very important for the coastal fisheries on the continental shelf off Thailand, China, and Japan [26, 31, 34, 35]. However, little is known about their feeding strategies, with the exception of *U. chinensis* and *U. duvaucelii* being reported as feeding on crustaceans, fish and cephalopods in southwestern Gulf of Thailand by Islam et al. [26].

Fatty acids are vital for organelle and physiological functions [36, 37]. In cephalopods, fatty acids are essential dietary components, not only playing a critical role in energy sourcing during starvation [38, 39], but more importantly assisting the early stages of development and growth, mostly through maternal allocation to gametes during sexual maturation [40–43]. However, heterotrophic organisms including cephalopods are subject to biochemical limitations in biosynthesis and modification of fatty acids, and assimilate the fatty acids they consume, particularly the polyunsaturated fatty acids (PUFA), in their basic form [44–48]. Fatty acids therefore have potential as dietary tracers in marine systems, providing insight into predator-prey interactions [36, 48–51]. Fatty acid analysis has proved to be a viable way to understand cephalopod diets [52, 53], and is increasingly used as a way to understand their trophic ecology [54–58].

We used fatty acid analysis and spatial analysis to investigate the feeding strategies and spatial distribution of *U. duvaucelii*, *U. edulis*, *U. chinensis* and *L. uyii* in the coastal waters of the northern South China Sea. This area is rich in tropical and subtropical biota, including various groups of phytoplankton, zooplankton, and zoobenthos, as well as pelagic and demersal fishes and invertebrates [59]. Many species, including cephalopods, are highly abundant in the shelf communities of the northern South China Sea [31, 33, 59, 60]. As fatty acids of a heterotrophic organism effectively reflect those of its diet [44–48], our study was designed to determine (1) whether the four sympatric squids adopt an opportunistic foraging strategy by feeding on similar prey items and (2) how they coexist in a coastal area where diets overlap. Our hypothesis

was that there is significant dietary overlap among the squids and spatial segregation in habitat use. Our aim was to understand the potential for trophic interactions and the degree of dietary overlap among the four species, which will assist our understanding of their feeding ecology, and possibly their inclusion in the coastal ecosystem assessments.

## Materials and methods

### Ethics statement

Specimens were collected as dead squids from the small-scale trawl fishery landings, during April 2016. The specimens were analyzed in laboratory using methods that are in line with current Chinese national standards, namely Laboratory Animals—General Requirements for Animal Experiment (GB/T 35823–2018). As all material sampled in this work obtained from commercial fishermen was already dead, there was no requirement for ethical approval of sampling protocols as it did not include live organisms.

### Study area

Data collection was conducted in Guangdong coastal waters, northern South China Sea (Fig 1). This area is characterized by a broad shelf (< 200m depth), and oceanographically involves a complex circulation system that is controlled by monsoons, the Kuroshio intrusion, upwelling in summer and downwelling in winter [61–63]. The Pearl River discharges a large amount of freshwater into Guangdong coastal waters, forming an approximate plume current, which interacts with nearshore circulation due to the warm and saline South China Sea Warm Current [61, 63]. These features lead to a highly complex and dynamic ecosystem, with high nitrate concentrations and enhanced primary production [64, 65].

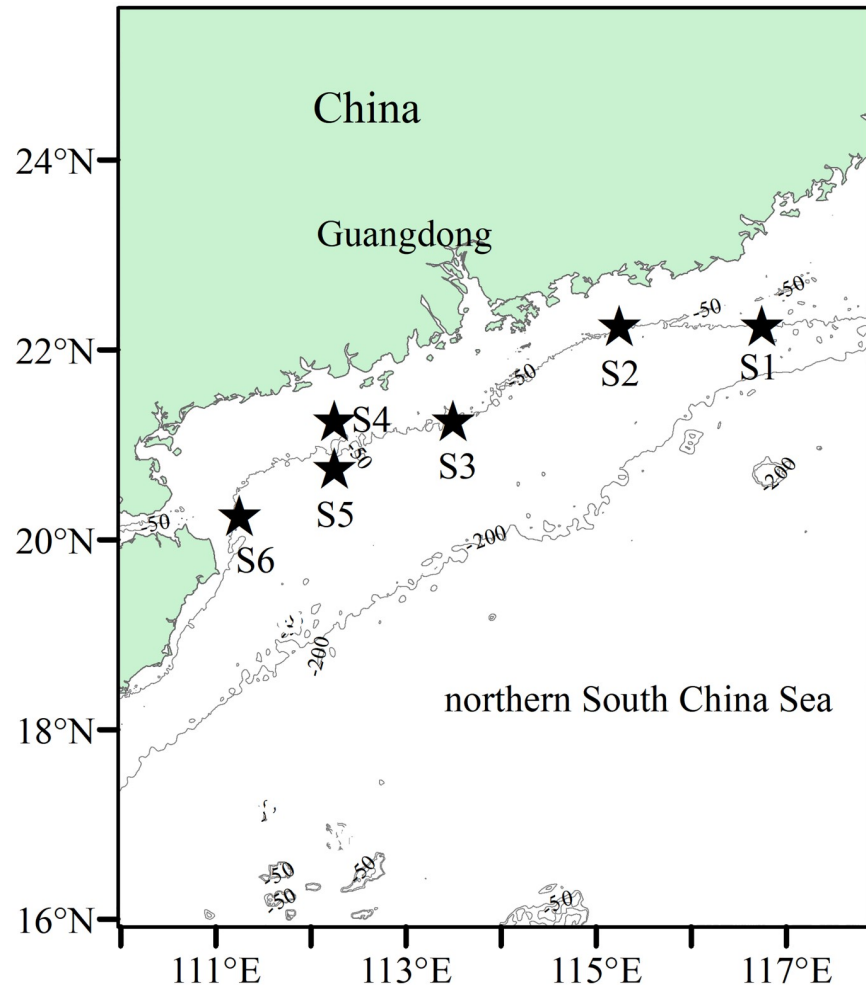
### Sample collection

Squid were randomly collected from the landings of a small-scale trawl fishery from April 2<sup>nd</sup> to April 26<sup>th</sup> 2016, at six stations (Fig 1). Similar to Philips et al. [56] and Pethybridge et al. [58], the whole squid were stored immediately at -30°C after being taken onboard. A total of 709 specimens were randomly sampled and duly labeled, including 286 *U. duvaucelii*, 66 *U. edulis*, 257 *U. chinensis* and 100 *L. uyii* (Table 1).

In the laboratory, a subsample of 62 specimens (3 to 8 specimens of each species per sampling station) was randomly selected from the 709 specimens for fatty acid analyses (Table 1). Before defrosting, muscle tissue (~ 10.0g wet weight) from the ventral mantle of each selected specimen was obtained, and placed immediately in a drying chamber (Crhist Alpha 1-4/LDplus, Germany) to lyophilize it to a constant weight. Each dried sample was then ground into powder, and about 0.2 g of that used for fatty acid analysis. After defrosting at room temperature, measurements of dorsal mantle length (ML, in mm) were taken to the nearest 1 mm (Table 1).

### Lipid and fatty acid analyses

Fatty acid methyl esters (FAME) were analyzed for each tissue sample using a modification of the GAQSIQ [66] method. This modification was to use a mixture of chloroform and methanol 2:1 (v/v) [67] rather than diethyl ether to extract lipids [66]. The extracted lipid of each tissue sample was immediately subject to FAME analysis to avoid contamination and oxidation. This involved the addition of 4 mL 0.5mol/L KOH-MeOH to the lipid extract, incubated at 90°C for 10 minutes. Then 4 mL BF<sub>3</sub>-MeOH were added, and the solution was incubated at 90°C for 30 minutes, followed by the addition of 4 mL n-Hexane for 2 minutes incubation at a



**Fig 1. Study area and sampling stations in the northern South China Sea.** Stars indicate the sampling stations. Grey lines indicate the selected isobaths of -50m and -200m.

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similar temperature. After adding 10 mL saturated NaCl, the solution was stratified at room temperature. Finally, the upper hexane layer was transferred to a vial, evaporated under nitrogen current.

The fatty acid profile for each sample was determined using an Agilent 7890B Gas Chromatograph coupled to a 5977A series Mass Spectrometer Detector (MSD, Agilent Technologies, Inc. USA). Methyl nonadecanoate (19:0) was used as an internal standard. Injection of samples was carried out in splitless mode, using helium as the carrier gas, and a thermal gradient from 125°C to 250°C, with an auxiliary heater at 280°C. Fatty acids were identified by comparison to relative retention times of a known standard [66] and comprise the fatty acid data for this study.

Total content of fatty acids (total FAs) was based on dry tissue weight (mg/g dry weight), and each fatty acid (FA) was reported as a percentage of the total [54–58]. The individual fatty acids were grouped into three main FA classes: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Fatty acids that accounted for <0.5% were excluded from statistical analyses.

**Table 1. Summary of squid specimens (n = 709) collected from the northern South China Sea, and those used in the fatty acid analyses.**

Species	Sampling station	Sampled				Analyzed fatty acid samples			
		n	Mantle length (ML, mm)			n	Mantle length (ML, mm)		
			mean±sd	min	max		mean±sd	min	max
<i>Uroteuthis duvaucelii</i>	S4	13	109.2±23.3	52	158	6	116.5±15.8	92	130
	S6	273	94.2±20.9	50	165	11	98.8±21.3	75	131
	pooled	286	95.2±21.4	50	165	17	105.1±20.9	75	131
<i>Uroteuthis edulis</i>	S1	48	183.2±21.2	143	241	8	181.8±11.3	165	195
	S4	7	130.0±37.2	90	176	3	168.7±7.0	162	176
	S5	11	154.6±29.0	89	186	3	173.7±10.8	166	186
	pooled	66	165.1±36.2	89	241	14	177.2±11.3	162	195
<i>Uroteuthis chinensis</i>	S1	96	208.7±32.4	134	275	6	202.7±34.3	171	246
	S2	39	174.3±37.0	82	226	4	196.0±27.0	166	226
	S3	73	206.7±39.5	148	320	8	188.1±25.5	163	240
	S5	24	179.7±36.1	66	201	3	192.7±8.0	185	201
	S6	25	155.8±23.9	108	199	3	168.3±3.2	166	172
	pooled	257	177.3±53.6	66	320	24	191.2±25.8	163	246
<i>Loliolus uyii</i>	S4	100	69.3±5.8	55	84	7	71.9±7.3	59	79
	pooled	100	69.3±5.8	55	84	7	71.9±7.3	59	79

Sampling station corresponds to the stars in Fig 1.

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## Statistical analysis

The fatty acid data were checked for normality (S1 Table), using a one-sample Kolmogorov-Smirnoff test [68]. The means of each FA profile, main FA class and total FAs by species and sampling station were compared using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test if necessary [68]. The data were analyzed using the Kruskal-Wallis non-parametric test and the Games-Howell post-hoc test when the assumption of normality could not be supported [68].

Permutational multivariate analysis of variance (PERMANOVA) [69], using the Bray-Curtis similarity measure, was used to test whether the FA profiles were related to individual size or sampling station (assessed independently). The analyses were carried out for each species, as well as for the four species combined. The squids were categorized into eight size-classes (<= 80mm, 81-100mm, 101-120mm, 121-140mm, 161-180mm, 181-200mm, 201-220mm and >220mm ML), starting with the smallest individual among the four squids, for the analyses related to size of individuals. *Loliolus uyii* was not tested the influence of individual size or sampling station on the fatty acid composition since it occurred in only one size-class (<= 80 mm ML) and at a single sampling station. Canonical analysis of principal coordinates (CAP) [69] was also used to discriminate between *a priori* groupings based on size-class or sampling station, and to visualize the potential groupings from the PERMANOVA. The sample sizes for each factor level of size-class or sampling station are summarized in Table 2 and S6 Table.

Nonmetric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM), employing the Bray-Curtis similarity measure, were applied to the FA compositions by species to assess whether the species had similar feeding strategy. Morisita's index of overlap [70], calculated using the full data set (709 specimens) from the 6 sampling stations, was used to determine whether the spatial distributions of each species were aggregated or separated in the

**Table 2. Sample size for each factor level of size-class and sampling station for the permutational multivariate analysis of variance (PERMANOVA) and canonical analysis of principal coordinates (CAP).**

Size-class		Sampling station (see Fig 1)	
Bin groups (mm)	n	factor level	n
<80	9	S1	14
81–100	5	S2	4
101–120	6	S3	8
121–140	4	S4	15
161–180	19	S5	6
181–200	12	S6	15
201–220	4		
>220	3		

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coastal waters. The formula for the index is:

$$C_H = \frac{2 \sum_i^n x_{ij} y_{ik}}{\sum_i^n x_{ij}^2 + \sum_i^n y_{ik}^2}$$

Where  $C_H$  is the overlap index between species  $j$  and species  $k$ ,  $x_{ij}$  is the percentage of species  $j$  at station  $i$ ,  $y_{ik}$  is the percentage of species  $k$  at station  $i$ , and  $n$  is the total number of sampling stations.

All statistical analyses were conducted using OriginPro version 2015 [71] and R version 3.5.0 [72]. The multivariate analyses (PERMANOVA, CAP, nMDS, ANOSIM) and the calculation of Morisita's index of overlap were conducted using the 'vegan' and 'divo' packages in R, respectively. Prior to the multivariate analyses, data were square-root transformed to account for variation in FA abundance. Differences were considered statistically significant when  $P < 0.05$ .

## Results

A total of 33 FAs were identified in the four species, with 18 having relative mean content  $> 0.5\%$  of the total FAs (Table 3). These 18 FAs made up 96% of total FAs in *U. duvaucelii*, 95% in *U. edulis*, 98% in *L. uyii* and 97% in *U. chinensis*.

### Fatty acid profiles

Significant differences in total FAs were found between species ( $F = 30.10$ ,  $P < 0.05$ ), with *L. uyii* having highest total FAs, followed by *U. duvaucelii*, while *U. chinensis* had the lowest total FAs (Table 3, S2 Table). No significant differences in the relative content of the main FA classes were detected, with the exception of MUFA ( $\chi^2 = 8.53$ ,  $P = 0.036$ ), for which the highest amount was found in *U. chinensis* and the lowest in *L. uyii* (Table 3, S2 and S3 Tables).

No significant difference was detected in the relative content of 14:0 among species ( $H = 4.94$ ,  $P = 0.18$ ), and similar results were obtained for 16:0, 17:0, 18:1n9t, 18:1n9c, 18:2n6c, 18:3n6, 20:0, 20:1, 20:2, 20:4n6, 20:5n3 and 22:6n3 (S2 and S3 Tables). These FAs constituted 77–88% (mean $\pm$ SD, 84.91 $\pm$ 3.15) of the total FAs in *U. duvaucelii*, 72–92% (84.77 $\pm$ 5.56) in *U. edulis*, 80–89% (84.72 $\pm$ 3.15) in *L. uyii*, and 76–90% (85.35 $\pm$ 3.25) in *U. chinensis*, respectively. There were significant differences in the relative content of other fatty acids (16:1n7, 18:0, 18:2n6t, 18:3n3 and 22:1n9) among species (S2 and S3 Tables); *U. chinensis* had the highest level of 16:1n7, *L. uyii* the highest level of 18:0, and *U. duvaucelii* the highest levels of 18:2n6t, 18:3n3 and 22:1n9 (Table 2).

**Table 3. Relative abundance of fatty acids for *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Loliolus uyii*, *Uroteuthis chinensis* in northern South China Sea.**

Fatty acid (%TFA)	Species			
	<i>Uroteuthis duvaucelii</i>	<i>Uroteuthis edulis</i>	<i>Loliolus uyii</i>	<i>Uroteuthis chinensis</i>
14:0	2.63±3.10	2.25±2.09	2.66±0.84	2.33±1.46
16:0	19.23±2.66	18.46±2.10	19.01±3.45	19.01±5.24
<b>16:1n7</b>	0.80±0.46 <sup>ab</sup>	0.63±0.45 <sup>a</sup>	0.97±1.20 <sup>ab</sup>	1.79±1.56 <sup>b</sup>
17:0	0.71±0.08	0.70±0.14	0.84±0.10	0.74±0.22
<b>18:0</b>	7.41±2.38 <sup>a</sup>	6.51±3.17 <sup>a</sup>	10.3±2.4 <sup>b</sup>	7.05±2.34 <sup>a</sup>
18:1n9t	0.66±0.40	0.77±0.71	0.69±0.14	0.79±1.13
18:1n9c	2.51±0.47	2.24±0.47	2.79±1.37	3.59±2.27
<b>18:2n6t</b>	1.72±1.10 <sup>b</sup>	1.40±1.33 <sup>ab</sup>	0.44±0.46 <sup>a</sup>	0.93±0.57 <sup>ab</sup>
18:2n6c	0.66±0.22	0.54±0.27	0.50±0.22	0.51±0.19
18:3n6	0.69±0.45	0.55±0.52	0.57±0.15	0.60±0.21
20:0	0.60±0.28	0.52±0.36	0.36±0.13	0.44±0.17
<b>18:3n3</b>	0.97±0.52 <sup>b</sup>	0.72±0.60 <sup>ab</sup>	0.32±0.25 <sup>a</sup>	0.63±0.40 <sup>ab</sup>
20:1	1.85±0.91	2.46±1.05	2.02±0.31	2.04±0.84
20:2	0.72±0.42	0.70±0.53	0.42±0.21	0.47±0.22
20:4n6 (ARA)	2.95±1.32	3.36±1.73	3.32±1.71	3.08±1.60
<b>22:1n9</b>	0.64±0.44 <sup>b</sup>	0.49±0.52 <sup>ab</sup>	0.16±0.11 <sup>a</sup>	0.38±0.22 <sup>ab</sup>
20:5n3 (EPA)	11.15±2.04	12.74±2.38	13.01±2.40	11.55±2.98
22:6n3 (DHA)	41.96±5.45	39.39±7.78	39.22±5.6	40.70±7.48
FAs<0.5%	3.40±1.76	4.09±3.17	1.76±1.14	2.94±1.84
<b>Main FA Classes (%TFA)</b>				
SFA	32.59±4.05	30.70±4.97	34.25±4.78	31.12±5.98
<b>MUFA</b>	7.71±2.02 <sup>ab</sup>	8.28±2.84 <sup>ab</sup>	6.70±2.82 <sup>a</sup>	9.64±3.5 <sup>b</sup>
PUFA	59.71±4.43	61.03±6.12	59.05±4.90	59.24±7.94
<b>Total fatty acids (mg/g dry weight)</b>				
<b>TFA</b>	66.20±9.78 <sup>c</sup>	59.01±3.35 <sup>b</sup>	71.07±4.62 <sup>c</sup>	50.71±4.54 <sup>a</sup>

FAs <0.5% include 11:0, 12:0, 13:0, 14:1n5, 15:0, 15:1n5, 17:1n7, 21:0, 20:3n6, 22:0, 20:3n3, 23:0, 22:2n6, 24:0, 24:1n9. ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids. Values are mean ± standard deviation; TFA is reported as dry tissue weight (mg/g dry weight), other values are reported as percentages of TFA (% TFA). Fatty acids highlighted in bold indicate significant differences ( $P < 0.05$ ) among species. Superscripted letters within rows represent the results of post-hoc test, and different letters indicate significant differences in the relative content of FA between species.

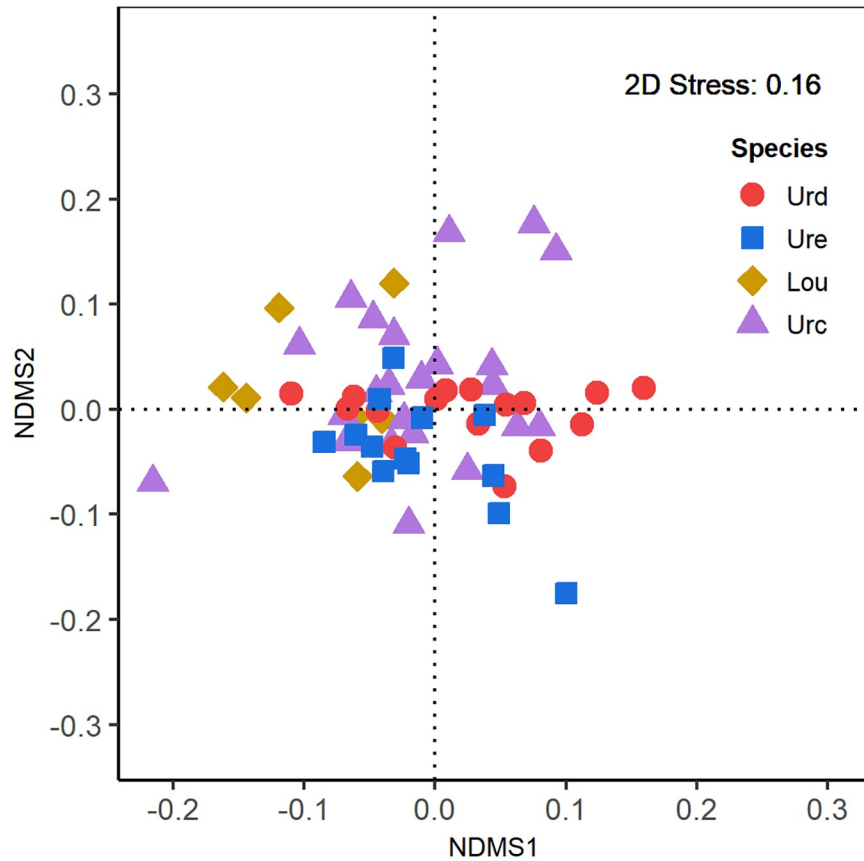
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### Similarity of fatty acid composition among species

The nMDS indicated considerable overlap in fatty acid profiles when the data for the four species were combined (Fig 2), and in paired species comparisons (S1 Fig). The overlap between *U. duvaucelii* and *L. uyii* and between *U. edulis* and *L. uyii* appeared to be relatively smaller than the other overlaps (S1 Fig). These findings were confirmed using ANOSIM ( $R = 0.08$ ; “pooled” in Table 4). There was considerable similarity in the fatty acid profiles among species, except between *U. duvaucelii* and *L. uyii* (ANOSIM  $R = 0.36$ ) (Table 3).

### Effects of individual size and sampling station on the fatty acid composition

PERMANOVA found no effects of individual size on fatty acid composition for any of the species (*U. duvaucelii*,  $F = 0.66$ ,  $p = 0.69$ ; *U. edulis*,  $F = 1.64$ ,  $p = 0.11$ ; *U. chinensis*,  $F = 0.91$ ,



**Fig 2. Non-metric multidimensional scaling (nMDS) ordination of fatty acid composition among *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis* and *Loliolus uyii* in the northern South China Sea.** Urd, *Uroteuthis duvaucelii*; Ure, *Uroteuthis edulis*; Lou, *Loliolus uyii*; Urc, *Uroteuthis chinensis*.

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$p = 0.57$ ) and when the data were aggregated over species ( $F = 1.45, p = 0.07$ ). No distinct groupings of fatty acid profiles were found when the data were grouped by size-class (CAP  $p = 0.07$ , Fig 3a).

There were no significant differences in the relative content of each fatty acid profile and the main FA classes (SFA, MUFA, PUFA) between sampling stations within *U. duvaucelii*, *U.*

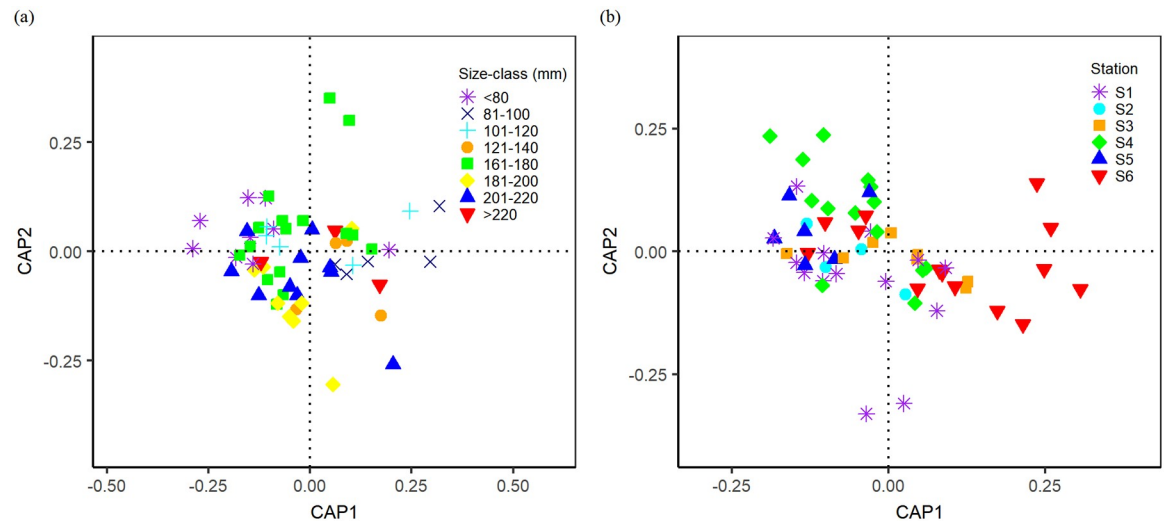
**Table 4. Results of the analysis of similarities (ANOSIM) for the fatty acid composition among *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis* and *Loliolus uyii* in the northern South China Sea.**

Terms	R	P
Pooled	0.08	0.04
<i>U. duvaucelii</i> vs. <i>U. edulis</i>	0.13	0.05
<i>U. duvaucelii</i> vs. <i>L. uyii</i>	0.36	0.01
<i>U. duvaucelii</i> vs. <i>U. chinensis</i>	0.06	0.11
<i>U. edulis</i> vs. <i>L. uyii</i>	0.12	0.14
<i>U. edulis</i> vs. <i>U. chinensis</i>	0.003	0.43
<i>L. uyii</i> vs. <i>U. chinensis</i>	0.05	0.33

R ranges from -1 to 1, with values close to 0 indicating high similarity.

<https://doi.org/10.1371/journal.pone.0234250.t004>





**Fig 3. Canonical analysis of principal coordinates (CAP) based on size-class (a) and sampling station (b) of the fatty acid profiles among *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Loliolus uyii* and *Uroteuthis chinensis* in northern South China Sea. Station in (b) corresponds to the stars in Fig 1.**

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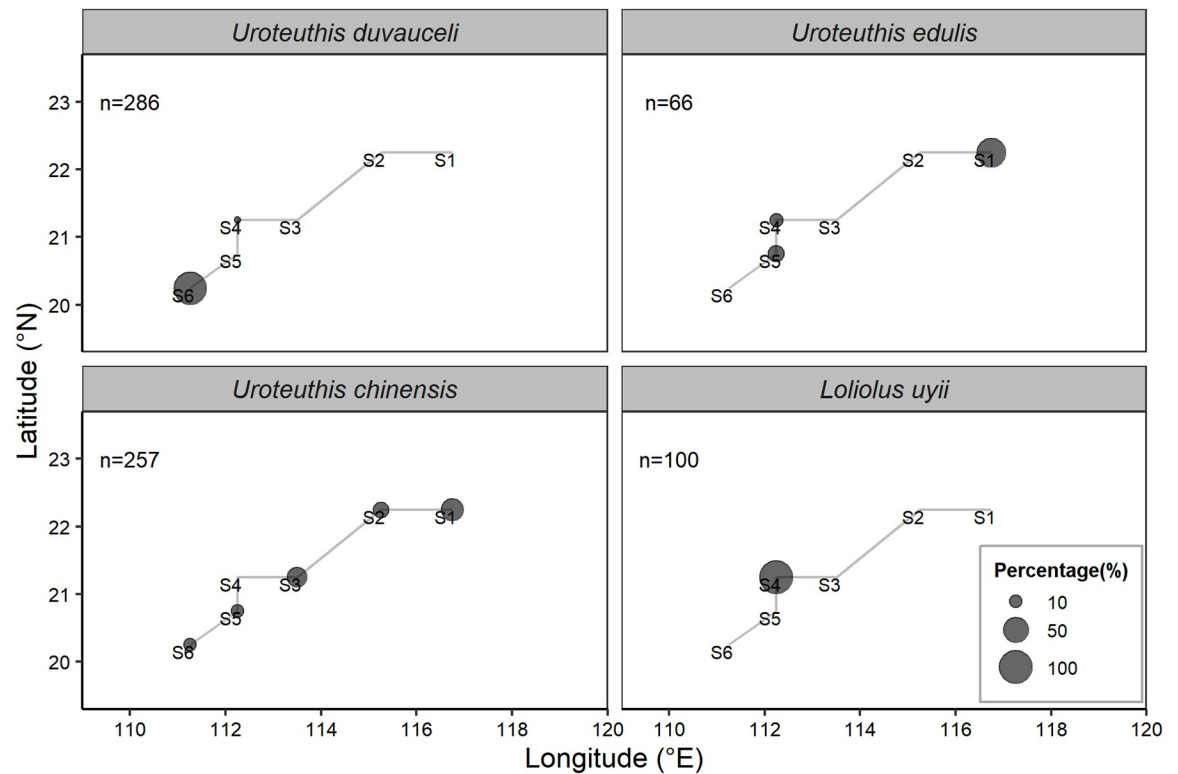
*edulis* and *U. chinensis* (S4 and S5 Tables). Similar results were obtained for the analysis of the effect of sampling station on fatty acid composition using PERMANOVA for *U. duvaucelii* ( $F = -0.001$ ,  $p = 0.99$ ), *U. edulis* ( $F = 1.05$ ,  $p = 0.42$ ), *U. chinensis* ( $F = 0.95$ ,  $p = 0.54$ ), and when the data were aggregated over species ( $F = 1.14$ ,  $p = 0.29$ ). Obvious overlap in fatty acid profiles among the sampling stations was observed in the CAP ordination ( $p = 0.25$ , Fig 3b).

### Spatial overlap

Spatial distribution analyses indicated that there is spatial segregation among *Uroteuthis duvaucelii*, *U. edulis* and *L. uyii* (Fig 4). *Uroteuthis duvaucelii* was found predominantly in the southwest of the study area, with *U. edulis* primarily in the northeast, and *L. uyii* at only one of center stations. A broader distribution was observed for *Uroteuthis chinensis*, which was found at the five out of the six sampling stations, with higher abundance in the northeast (Fig 4). Consequently, there appears to be considerable spatial distribution niche overlap between *U. chinensis* and *U. edulis* (Moristita's index of 76.1%; Table 5). In contrast, lesser spatial overlap was observed between *U. duvaucelii* and *U. chinensis*, and between *U. edulis* and *L. uyii*, (Moristita's indices of 14.6% and 12.0%, respectively). There was complete spatial segregation between *U. chinensis* and *L. uyii* (Moristita's index = 0).

### Discussion

The coastal waters of the northern South China Sea are characterized by high nitrate concentrations and enhanced primary production, which is responsible for the variety and abundance of tropical and subtropical biota [32, 59, 60, 64, 65]. These features could affect the feeding ecology of predators, especially species such as squids that are voracious and opportunistic predators [12, 19–20, 26]. Here, we demonstrate that the four sympatric squids, *U. duvaucelii*, *U. edulis*, *U. chinensis* and *L. uyii*, appear to be opportunistic carnivores, unselectively foraging on common prey items in the coastal waters of the northern South China Sea. There is also a clear spatial segregation among the four squids, which arises from niche differences [3–5, 73].



**Fig 4. Percentage of specimens collected by sampling station for *Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Loliolus uyii*, *Uroteuthis chinensis*.** S1, S2, ...S6 correspond to the stations in Fig 1. The size of grey circle represents the percentage of specimens collected at the station.

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The spatial segregation appears to be a mechanism to reduce competition in resource use for these sympatric species in the coastal water of the northern South China Sea.

There were significant differences in the total content of fatty acid profiles among *U. duvaucelii*, *U. edulis*, *U. chinensis* and *L. uyii* probably due to variation in their lipid contents. Fatty acids form an essential and integral part of living organism’s lipids, the content of which in turn is responsible for the total amount of fatty acids [36, 48, 52]. Although squids are well documented for low lipid content (usually 2% on a wet weight basis [40, 74, 75]), there appear to be species-specific differences in the lipid content. For example, it has been reported that the lipid content of muscle tissue on a wet weight basis is around 0.8% for *Todarodes filippovae* [58] compared to 2% for *Onykia ingens* [56]. Thus, although we have not determined the lipid

**Table 5. Spatial niche overlap (%) among pairs of squid species—*Uroteuthis duvaucelii*, *Uroteuthis edulis*, *Loliolus uyii*, *Uroteuthis chinensis* in northern South China Sea.**

Species	Urd	Ure	Urc	Lou
<i>Uroteuthis duvaucelii</i> (Urd)	-			
<i>Uroteuthis edulis</i> (Ure)	0.6%	-		
<i>Uroteuthis chinensis</i> (Urc)	14.6%	76.1%	-	
<i>Loliolus uyii</i> (Lou)	4.6%	12.0%	0	-

Percentages were calculated based on Morisita’s index.

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content for the four squid in this study, it would be expected that the difference in the total FA content is the result of different lipid content due to phylogenetic differences [43, 47].

The revealed little variation in the relative content of the main FA classes (i.e., SFA, PUFA) among the four squids is mainly contributed by the insignificant differences in the relative content of most individual FAs used as trophic markers in aquatic systems. These individual FAs include 16:0, 20:4n6, 20:5n3 and 22:6n3 [36, 76–78]. 20:5n3 indicates diatom-based food web and is identified as tracer for first-order carnivores [36, 78], 16:0 and 22:6n3 are respectively important tracers for omnivorous copepods and dinoflagellates, and are used as tracers for second-order carnivores [36, 78, 79], and 20:4n6 is a recognized indicator for benthic markers and top predators [76, 78]. Thus, the similarity in elevated levels of these FAs among the four squids implies that these species display first-order and second-order carnivore benthic feeding habit, an indication of opportunistic carnivore foraging strategy in the study area. Additional evidence can be provided by the little variation in 16:1n7 between *U. duvaucelii*, *U. edulis* and *L. uyii*, and the insignificant difference in 18:0 among the three *Uroteuthis* species, in which 16:1n7 and 18:0 are recognized respectively as indicators for first-order carnivores and second-order carnivores [78].

The similarity in the relative content of fatty acid profiles among the squids indicates species to prey on similar prey items, given heterotrophes generally exhibit parallel patterns of change in their FAs as they change their diets [36, 40, 46, 47, 50]. This is statistically confirmed by the multivariate analyses, in which there was substantial overlap and high similarity in the fatty acid compositions among and between pairs of species (Fig 2, S1 Fig). Although there is no definitive way to determine and quantify the prey items on which these squids fed, the high overlap of the fatty acid profiles of these species which justify sharing similar prey items, corresponds with the results of stomach contents by Islam et al. [26] who reported that *U. chinensis* and *U. duvaucelii* from the southwestern Gulf of Thailand displayed dietary similarity by feeding on three major diet groups representing crustaceans, fish and molluscs. It is worthy to note that each pair of the three *Uroteuthis* species consistently showed high similarity in fatty acid composition compared to the high dissimilarity in the fatty acid composition between *L. uyii* and *U. duvaucelii* and the significantly low levels of 18:2n6t, 18:3n3 and 22:1n9 in *L. uyii*, possibly suggesting phylogenetic differences in the bioaccumulation of fatty acids among these species [43, 47]. Further research, however, are needed to address the effects of phylogeny, as which is increasingly less important in higher trophic groups [78].

We also found that the fatty acid composition among the squids did not change with increasing body size. This feature suggests that these squids may not shift diet ontogenetically, but instead adopt a strategy that focuses on the amount and not quality of food as their voracious feeding habits [12, 19–20, 26]. The abundant food resources in the coastal waters of the northern South China Sea [32, 59, 60] may be a possible reason and meet their requirements with ontogeny. Islam et al. [26] also reported that *U. chinensis* and *U. duvaucelii* in the southwest of the Gulf of Thailand of different size-classes had high overlap in their food items and minimal dietary shift with ontogeny. Preying on the common prey items with increasing size may be an optimal foraging strategy for squid to maximize energy intake, enhance their growth rate and minimize predation risk [80, 81]. We also found non-significant effects of sampling station on the fatty acid composition for either species and among the four squids considered simultaneously. This observation may suggest that the squids unselectively exploit common prey items, presumably owing to the fairly stable species diversity of the northern shelf of South China Sea [33].

It is known that overlap in dietary resources between sympatric species may lead to spatial segregation, which appears to be the essential for coexistence [2, 73, 81]. The four squids exhibit the characteristics of demersal predators, evidenced by the non-significant differences

and high levels in the relative content of 16:0, 20:5n3 and 20:4n6, which are indicators for demersal habitat [76, 78]. However, we found a clear spatial separation of the four squids in the coastal waters, leading to low niche overlap among them except for *U. chinensis* and *U. edulis* (Fig 3; Table 4). Regarding the spatial overlap between *U. chinensis* and *U. edulis*, we found that the former distributed broadly (five out of the six sample stations) and evenly whereas the latter was found predominantly in the northeast of the survey area. *L. uyii* was only found at one sampling station inside the 50m isobath (Figs 1 and 4), and appears to occupy a more brackish area as evidenced by the significantly higher level of 18:0, an indicator for brackish habitats [78]. These observations suggest that the four squids likely exploit different parts of the coastal waters. Similar patterns of spatial partitioning have been reported for the sympatric squids *I. argentinus*, *D. gahi*, and *O. ingens* off the Patagonian Shelf, where they exploit similar prey resources, with evidence for spatial segregation between mature *D. gahi* and other squids [15]. Thus, spatial segregation may be one of the coexistence mechanisms for these sympatric squids to reduce competition in resource use such as dietary sources.

## Conclusion

In conclusion, our findings indicate that the four sympatric squids *U. dawaucelii*, *U. edulis*, *U. chinensis* and *L. uyii* are opportunistic carnivores, adopting a similar foraging strategy by unselectively preying on common prey items in the coastal waters of the northern South China Sea. Spatial segregation is likely a major mechanism that promotes their coexistence by reducing competition for food resources, as well as possibly buffering their trophic interactions. The abundant prey resources of the shelf waters of the northern South China Sea may allow them to adopt the similar feeding strategy and also enhance the likelihood of coexistence within the studied area. This is the first study to use fatty acid profiles to study the feeding ecology of coastal squids in the northern South China Sea. The findings advance our understanding of the feeding ecology of these sympatric squids. More importantly, our results provide a new perspective on their ecology and illustrate how fatty acids can be used to understand feeding strategy in terms of food resource use and species coexistence.

## Supporting information

**S1 Table. The results of the one-sample Kolmogorov-Smirnoff test for each fatty acid content among *Uroteuthis dawaucelii*, *Uroteuthis edulis*, *Loliolus uyii*, *Uroteuthis chinensis* in northern South China Sea.**

(DOCX)

**S2 Table. Results of one-way analysis of variance (ANOVA) by species for those fatty acids that meet the requirements of normality among *Uroteuthis dawaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis*, *Loliolus uyii* in northern South China Sea.**

(DOCX)

**S3 Table. Results of Kruskal-Wallis nonparametric test by species for those fatty acids that do not meet the requirements of normality among *Uroteuthis dawaucelii*, *Uroteuthis edulis*, *Uroteuthis chinensis*, *Loliolus uyii* in northern South China Sea.**

(DOCX)

**S4 Table. Results of one-way analysis of variance (ANOVA) by sampling stations for those fatty acids that meet the requirements of normality for *Uroteuthis dawaucelii*, *Uroteuthis edulis*, and *Uroteuthis chinensis* in northern South China Sea.**

(DOCX)

**S5 Table. Results of Kruskal-Wallis nonparametric test by species for those fatty acids that do not meet the requirements of normality for *Uroteuthis duvaucelii*, *Uroteuthis edulis*, and *Uroteuthis chinensis* in northern South China Sea.**

(DOCX)

**S6 Table. Sample size within each factor level of size-classes and sampling stations for permutational multivariate analysis of variance (PERMANOVA) and canonical analysis of principal coordinates (CAP) by species.**

(DOCX)

**S7 Table. The dataset of fatty acid profiles (% relative content >0.5% of total FAs) determined for each squid specimen, including the sampling station and mantle length.**

(DOCX)

**S1 Fig. Non-metric multidimensional scaling (nMDS) ordination of fatty acid composition between each species pairing: Urd, *Uroteuthis duvaucelii*; Ure, *Uroteuthis edulis*; Lou, *Lololus uyii*; Urc, *Uroteuthis chinensis*.**

(DOCX)

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## References

1. Schoener TW. Resource Partitioning in Ecological Communities. *Science*. 1974; 185(4145):27–39. <https://doi.org/10.1126/science.185.4145.27> PMID: 17779277
2. Kuhnen VV, Romero GQ, Linhares AX, Vizontin-Bugoni J, Porto EAC, Setz EZF. Diet overlap and spatial segregation between two neotropical marsupials revealed by multiple analytical approaches. *PLOS ONE*. 2017; 12(7):e0181188. <https://doi.org/10.1371/journal.pone.0181188> PMID: 28704561
3. Brown JS, Kotler BP, Mitchell WA. Foraging Theory, Patch Use, and the Structure of a Negev Desert Granivore Community. *Ecology*. 1994; 75(8):2286–300. <https://doi.org/10.2307/1940884>
4. Wauters LA, Gurnell J, Martinoli A, Tosi G. Interspecific competition between native Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? *Behav Ecol Sociobiol*. 2002; 52(4):332–41. <https://doi.org/10.1007/s00265-002-0516-9>
5. Carrete M, Sánchez-Zapata JA, Calvo JF, Lande R. Demography and habitat availability in territorial occupancy of two competing species. *Oikos*. 2005; 108(1):125–36. <https://doi.org/10.1111/j.0030-1299.2005.12942.x>
6. Macandza VA, Owen-Smith N, Cain I, James W. Dynamic spatial partitioning and coexistence among tall grass grazers in an African savanna. *Oikos*. 2012; 121(6):891–8. <https://doi.org/10.1111/j.1600-0706.2012.20250.x>
7. de Lima RC, Franco-Trecu V, Vales DG, Inchausti P, Secchi ER, Botta S. Individual foraging specialization and sexual niche segregation in South American fur seals. *Mar Biol*. 2019; 166(3):32. <https://doi.org/10.1007/s00227-019-3480-x>
8. Correa SB, Winemiller KO. Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. *Ecology*. 2014; 95(1):210–24. <https://doi.org/10.1890/13-0393.1> PMID: 24649660
9. Wickman J, Diehl S, Brännström Å. Evolution of resource specialisation in competitive metacommunities. *Ecol Lett*. 2019; 22(11):1746–56. <https://doi.org/10.1111/ele.13338> PMID: 31389134
10. Woodward G, Hildrew AG. Body-size determinants of niche overlap and intraguild predation within a complex food web. *J Anim Ecol*. 2002; 71(6):1063–74. <https://doi.org/10.1046/j.1365-2656.2002.00669.x>
11. Woodward G, Ebenman B, Emmerson M, Montoya JM, Olesen JM, Valido A, et al. Body size in ecological networks. *Trends Ecol Evol*. 2005; 20(7):402–9. <https://doi.org/10.1016/j.tree.2005.04.005> PMID: 16701403
12. Jereb P, Roper CFE. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 2. Myopsid and Oegopsid Squids. Rome: FAO; 2010.
13. Nesis KN. Cephalopods of seamounts in the western Indian Ocean. *Oceanology*. 1986; 26(1):91–6.
14. Laptikhovskiy V, Boersch-Supan P, Bolstad K, Kemp K, Letessier T, Rogers AD. Cephalopods of the Southwest Indian Ocean Ridge: A hotspot of biological diversity and absence of endemism. *Deep-Sea Research II*. 2017; 136:98–107. <http://dx.doi.org/10.1016/j.dsr2.2015.07.002>.
15. Rosas-Luis R, Navarro J, Sánchez P, Del Río JL. Assessing the trophic ecology of three sympatric squid in the marine ecosystem off the Patagonian Shelf by combining stomach content and stable isotopic analyses. *Mar Biol Res*. 2016; 12(4):402–11. <https://doi.org/10.1080/17451000.2016.1142094>
16. Pecl GT, Moltschanivskiy NA, Tracey SR, Jordan AR. Inter-annual plasticity of squid life history and population structure: ecological and management implications. *Oecologia*. 2004; 139(4):515–24. <https://doi.org/10.1007/s00442-004-1537-z> PMID: 15054657
17. Forsythe JW. Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. *Mar Freshwater Res*. 2004; 55(4):331–9. <http://dx.doi.org/10.1071/MF03146>.
18. Arkhipkin AI. Squid as nutrient vectors linking Southwest Atlantic marine ecosystems. *Deep-Sea Research II*. 2013; 95:7–20. <http://dx.doi.org/10.1016/j.dsr2.2012.07.003>.
19. Navarro J, Coll M, Somes CJ, Olson RJ. Trophic niche of squids: Insights from isotopic data in marine systems worldwide. *Deep-Sea Research II*. 2013; 95:93–102. <http://dx.doi.org/10.1016/j.dsr2.2013.01.031>.
20. Boyle P, Rodhouse P. Cephalopods: ecology and fisheries. Oxford, UK: Wiley-Blackwell; 2005.
21. Young JW, Olson RJ, Rodhouse PGK. The role of squids in pelagic ecosystems: An overview. *Deep-Sea Research II*. 2013; 95:3–6. <https://doi.org/10.1016/j.dsr2.2013.05.008>.
22. Cheral Y, Ducatez S, Fontaine C, Richard P, Guinet C. Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. *Mar Ecol Prog Ser*. 2008; 370:239–47.
23. Rodhouse PG, Nigmatullin CM. Role as Consumers. *Philos Trans R Soc Lond B Biol Sci*. 1996; 351(1343):1003–22. <https://doi.org/10.1098/rstb.1996.0090>

24. de la Chesnais T, Fulton EA, Tracey SR, Pecl GT. The ecological role of cephalopods and their representation in ecosystem models. *Rev Fish Biol Fisheries*. 2019. <https://doi.org/10.1007/s11160-019-09554-2>
25. Ruiz-Cooley RI, Markaida U, Gendron D, Aguíñiga S. Stable isotopes in jumbo squid (*Dosidicus gigas*) beaks to estimate its trophic position: comparison between stomach contents and stable isotopes. *J Mar Biol Assoc UK*. 2006; 86(2):437–45. Epub 2006/03/01. <https://doi.org/10.1017/S0025315406013324>
26. Islam R, Hajisamae S, Pradit S, Perngmk P, Paul M. Feeding habits of two sympatric loliginid squids, *Uroteuthis (Photololigo) chinensis* (Gray, 1849) and *Uroteuthis (Photololigo) duvaucelii* (d'Orbigny, 1835), in the lower part of the South China Sea. *Molluscan Res*. 2018; 38(3):155–62. <https://doi.org/10.1080/13235818.2017.1409066>
27. Rosa R, Pissarra V, Borges FO, Xavier J, Gleadall IG, Golikov A, et al. Global Patterns of Species Richness in Coastal Cephalopods. *Front Mar Sci*. 2019; 6(469). <https://doi.org/10.3389/fmars.2019.00469>
28. Bhosale B, Nitin P, Vivek N, Metar S, Bhosale B.P, et al. Age, Growth and Mortality Studies of Indian Squid, *Uroteuthis (Photololigo) duvaucelii* (d' Orbigny) along Ratnagiri Coast of Maharashtra, India. *Indian J Geo-Mar Sci Vol*. 4. 2015; 4:93–6.
29. Yamaguchi T, Kawakami Y, Matsuyama M. Migratory routes of the swordtip squid *Uroteuthis edulis* inferred from statolith analysis. *Aquat Biol*. 2015; 24(1):53–60.
30. Jin Y, Li N, Chen X, Liu B, Li J. Comparative age and growth of *Uroteuthis chinensis* and *Uroteuthis edulis* from China Seas based on statolith. *Aquacult Fish*. 2019; 4(4):166–72. <https://doi.org/10.1016/j.aaf.2019.02.002>.
31. Huang Z. Species composition and resources density of Cephalopoda in the continental shelf of northern South China Sea. *South China Fish Sci*. 2008; 4(5):1–7.
32. Yuan M, Chen Z, Zhang J, Jiang Ye, Tang Y, Xu S. Community structure of mesoplegagic fish species in the northern slope of South China Sea. *South China Fish Sci*. 2018; 14(1):85–91.
33. Ye Jiang, Lin Z, Huang Z. Biodiversity of fishery resources in the continental shelf of northern South China Sea. *South China Fish Sci*. 2009; 5(5):32–7.
34. Voss GL, Williamson GR. *Cephalopods of Hong Kong*. Hong Kong: Hong Kong Government Press; 1971.
35. Yan Y, Li Y, Yang S, Wu G, Tao Y, Feng Q, et al. Biological Characteristics and Spatial: Temporal Distribution of Mitre Squid, *Uroteuthis Chinensis*, in the Beibu Gulf, South China Sea. *J Shellfish Res*. 2013; 32(3):835–44. <https://doi.org/10.2983/035.032.0327>
36. Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W. Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol*. 2003; 46:225–340. [http://dx.doi.org/10.1016/S0065-2881\(03\)46005-7](http://dx.doi.org/10.1016/S0065-2881(03)46005-7) PMID: 14601414
37. Tocher DR. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Rev Fish Sci*. 2003; 11(2):107–84. <https://doi.org/10.1080/713610925>
38. García-Garrido S, Hachero-Cruzado I, Garrido D, Rosas C, Domingues P. Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. *Aquacult Int*. 2010; 18(6):1223–41. <https://doi.org/10.1007/s10499-010-9335-6>
39. García S, Domingues P, Navarro JC, Hachero I, Garrido D, Rosas C. Growth, partial energy balance, mantle and digestive gland lipid composition of *Octopus vulgaris* (Cuvier, 1797) fed with two artificial diets. *Aquacult Nutr*. 2011; 17(2):e174–e87. <https://doi.org/10.1111/j.1365-2095.2009.00746.x>
40. Navarro JC, Villanueva R. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture*. 2000; 183(1–2):161–77. [http://dx.doi.org/10.1016/S0044-8486\(99\)00290-2](http://dx.doi.org/10.1016/S0044-8486(99)00290-2).
41. Navarro JC, Villanueva R. The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. *Aquaculture*. 2003; 219(1–4):613–31. [http://dx.doi.org/10.1016/S0044-8486\(02\)00311-3](http://dx.doi.org/10.1016/S0044-8486(02)00311-3).
42. Bachan MM, Fleming IA, Trippel EA. Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin. *Mar Biol*. 2012; 159(10):2281–97. <https://doi.org/10.1007/s00227-012-2014-6>
43. Fuiman LA, Faulk CK. Batch spawning facilitates transfer of an essential nutrient from diet to eggs in a marine fish. *Biology letters*. 2013; 9(5):20130593. <https://doi.org/10.1098/rsbl.2013.0593> PMID: 23985349
44. Sargent JR, Bell JG, Bell MV, Henderson RJ, Tocher DR. Requirement criteria for essential fatty acids. *J Appl Ichthyol*. 1995; 11(3–4):183–98. <https://doi.org/10.1111/j.1439-0426.1995.tb00018.x>
45. Ackman RG. Comparison of Lipids in Marine and Freshwater Organisms. In: Arts MT, Wainman BC, editors. *Lipids in Freshwater Ecosystems*. New York, NY: Springer New York; 1999. p. 263–98.

46. Berge JP, Barnathan G. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Adv Biochem Eng Biot.* 2005; 96:49–125. <https://doi.org/10.1007/b135782> PMID: 16566089
47. Li Y-y, Chen W-z, Sun Z-w, Chen J-h, Wu K-g. Effects of n-3 HUFA content in broodstock diet on spawning performance and fatty acid composition of eggs and larvae in *Plectorhynchus cinctus*. *Aquaculture.* 2005; 245(1):263–72. <http://dx.doi.org/10.1016/j.aquaculture.2004.12.016>.
48. Iverson SJ. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Kainz M, Brett MT, Arts MT, editors. *Lipids in Aquatic Ecosystems.* New York, NY: Springer New York; 2009. p. 281–308.
49. Lee RF, Nevenzel JC, Paffenhöfer G-A. Importance of wax esters and other lipids in the marine food chain: Phytoplankton and copepods. *Mar Biol.* 1971; 9(2):99–108. <https://doi.org/10.1007/bf00348249>
50. Graeve M, Kattner G, Wiencke C, Karsten U. Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. *Mar Ecol Prog Ser.* 2002; 231:67–74. <https://doi.org/10.3354/meps231067>
51. Every SL, Pethybridge HR, Crook DA, Kyne PM, Fulton CJ. Comparison of fin and muscle tissues for analysis of signature fatty acids in tropical euryhaline sharks. *J Exp Mar Bio Ecol.* 2016; 479:46–53. <https://doi.org/10.1016/j.jembe.2016.02.011>.
52. Stowasser G, Pierce GJ, Moffat CF, Collins MA, Forsythe JW. Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. *J Exp Mar Bio Ecol.* 2006; 333(1):97–114. <https://doi.org/10.1016/j.jembe.2005.12.008>.
53. Fluckiger M, Jackson G, Nichols P, Virtue P, Daw A, Wotherspoon S. An experimental study of the effect of diet on the fatty acid profiles of the European Cuttlefish (*Sepia officinalis*). *Mar Biol.* 2008; 154(2):363–72. <https://doi.org/10.1007/s00227-008-0932-0>
54. Phillips KL, Jackson GD, Nichols PD. Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. *Mar Ecol Prog Ser.* 2001; 215:179–89.
55. Phillips KL, Nichols PD, Jackson GD. Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: implications for food-web studies. *Antarct Sci.* 2002; 14(3):212–20.
56. Phillips KL, Nichols PD, Jackson GD. Size-related dietary changes observed in the squid *Moroteuthis ingens* at the Falkland Islands: stomach contents and fatty-acid analyses. *Polar Biol.* 2003; 26(7):474–85. <https://doi.org/10.1007/s00300-003-0509-9>
57. Pethybridge H, Virtue P, Casper R, Yoshida T, Green CP, Jackson G, et al. Seasonal variations in diet of arrow squid (*Nototodarus gouldi*): stomach content and signature fatty acid analysis. *J Mar Biol Assoc UK.* 2012; 92(1):187–96. Epub 08/03. <https://doi.org/10.1017/S0025315411000841>
58. Pethybridge HR, Nichols PD, Virtue P, Jackson GD. The foraging ecology of an oceanic squid, *Todarodes filippovae*: The use of signature lipid profiling to monitor ecosystem change. *Deep-Sea Research II.* 2013; 95:119–28. <http://dx.doi.org/10.1016/j.dsr2.2012.07.025>.
59. Liu JY. Status of Marine Biodiversity of the China Seas. *PLOS ONE.* 2013; 8(1):e50719. <https://doi.org/10.1371/journal.pone.0050719> PMID: 23320065
60. Ma C, You K, Zhang M, Li F, Chen D. A preliminary study on the diversity of fish species and marine fish faunas of the South China Sea. *J Ocean Univ China.* 2008; 7(2):210–4. <https://doi.org/10.1007/s11802-008-0210-2>
61. Ji X, Sheng J, Zheng J, Zhang W. Numerical study of seasonal circulation and variability over the inner shelf of the northern South China Sea. *Ocean Dynam.* 2015; 65(8):1103–20. <https://doi.org/10.1007/s10236-015-0862-6>
62. Ding Y, Yao Z, Zhou L, Bao M, Zang Z. Numerical modeling of the seasonal circulation in the coastal ocean of the Northern South China Sea. *Front Earth Sci.* 2018. <https://doi.org/10.1007/s11707-018-0741-9>
63. Shu Y, Wang Q, Zu T. Progress on shelf and slope circulation in the northern South China Sea. *Sci China Earth Sci.* 2018; 61(5):560–71. <https://doi.org/10.1007/s11430-017-9152-y>
64. Dong Y, Li QP, Liu Z, Wu Z, Zhou W. Size-dependent phytoplankton growth and grazing in the northern South China Sea. *Mar Ecol Prog Ser.* 2018; 599:35–47.
65. Chen F, Zhou X, Lao Q, Wang S, Jin G, Chen C, et al. Dual isotopic evidence for nitrate sources and active biological transformation in the Northern South China Sea in summer. *PLOS ONE.* 2019; 14(1):e0209287. <https://doi.org/10.1371/journal.pone.0209287> PMID: 30601849
66. GAQSIQ. Determination of total fat, saturated fat, and unsaturated fat in foods: Hydrolytic extraction-Gas chromatography. Beijing: Standards Press of China; 2008.



67. Parrish CC. Determination of Total Lipid, Lipid Classes, and Fatty Acids in Aquatic Samples. In: Arts MT, Wainman BC, editors. *Lipids in Freshwater Ecosystems*. New York, NY: Springer New York; 1999. p. 4–20.
68. Zar JH. *Biostatistical Analysis*, fourth Edition. New Jersey: Prentice Hall; 1999.
69. Anderson MJ. Permutational Multivariate Analysis of Variance (PERMANOVA). In: Balakrishnan N, Colton T, Everitt B, Piegorisch W, Ruggeri F, Teugels JL, editors. *Wiley StatsRef: Statistics Reference Online*: John Wiley & Sons, Ltd.; 2017. <https://doi.org/10.1002/9781118445112.stat07841>
70. Krebs CJ. *Ecological methodology*. Second ed. Boston: Addison Wesley Longman; 1999.
71. OriginLab Corporation. *OriginPro 2015*. Northampton, MA, USA2015.
72. R Core Team. *R: A language and environment for statistical computing*. 3.5.0 ed. Vienna, Austria: R Foundation for Statistical Computing; 2018.
73. Hutchinson GE. Homage to Santa Rosalia or Why Are There So Many Kinds of Animals? *Am Nat*. 1959; 93(870):145–59. <https://doi.org/10.1086/282070>
74. O'Dor RK, Mangold K, Boucher-Rodoni R, Wells MJ, Wells J. Nutrient absorption, storage and remobilization in *Octopus vulgaris*. *Mar Behav Physiol*. 1984; 11(3):239–58. <https://doi.org/10.1080/10236248409387049>
75. Lee PG. Metabolic substrates in cephalopods. In: ortner HO, O'Dor RK, MacMillan DL, editors. *Physiology of Cephalopod Mollusc Lifestyle and Performance Adaptations*. Basel, Switzerland: Gordon and Breach; 1994. p. 35–51.
76. Sargent JR, Tocher DR, Bell JG. The Lipids. In: Halver JE, editor. *Fish Nutrition (Third Edition)*. San Diego, CA: Academic Press; 2003. p. 181–257.
77. Kelly JR, Scheibling RE. Fatty acids as dietary tracers in benthic food webs. *Mar Ecol Prog Ser*. 2012; 446:1–22.
78. Meyer L, Pethybridge H, Nichols PD, Beckmann C, Huveneers C. Abiotic and biotic drivers of fatty acid tracers in ecology: A global analysis of chondrichthyan profiles. *Funct Ecol*. 2019; 33: 1243–55. <https://doi.org/10.1111/1365-2435.13328>
79. Kattner G, Hagen W. Lipids in marine copepods: latitudinal characteristics and perspective to global warming. In: Kainz M, Brett MT, Arts MT, editors. *Lipids in Aquatic Ecosystems*. New York, NY: Springer New York; 2009. p. 257–80.
80. Brown JA. The adaptive significance of behavioural ontogeny in some centrarchid fishes. *Environ Biol Fish*. 1985; 13(1):25–34. <https://doi.org/10.1007/bf00004853>
81. Paul M, Hajjsamae S, Pradit S, Perngmark P, Islam R. Trophic Ecology of Eight Sympatric Nemipterid Fishes (Nemipteridae) in the Lower Part of the South China Sea. *Turk J Fish Aquat Sci*. 2018; 18:277–87. [https://doi.org/10.4194/1303-2712-v18\\_2\\_07](https://doi.org/10.4194/1303-2712-v18_2_07)