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Feeding Ecology of Common Squid *Todarodes pacificus* in the South Sea of Korea Determined through Stable Isotope and Stomach Content Analyses

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Abstract: The common squid *Todarodes pacificus* is a dominant species within epipelagic communities and an important commercial species in the South and East seas of Korea and in the East China Sea. In this study, to examine the dietary composition, trophic position, and ontogenetic dietary shifts of the common squid, we analyzed stomach contents and stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in muscle tissue across different body sizes (mantle length, ML) and seasons (winter, spring, and summer 2021) in the South Sea of Korea. Regardless of the season, the diet of the common squid predominantly comprised Pisces (37.9–94.0%) and Cephalopoda (6.0–61.0%). However, in the smallest individuals (ML < 10 cm), the stomach contents in August primarily comprised Crustacea (95.5%), thereby indicating a seasonal difference in dietary composition and ontogenetic dietary shifts only during summer. Similarly, our isotope results revealed seasonal isotopic variation among sampling periods and significant positive correlations between ML and both $\delta^{13}C$ and $\delta^{15}N$ values during the summer. These results reveal a seasonal variability in major diet components among the common squid of different size classes and that their feeding strategies are probably adjusted in response to the temporal availability of prey. Collectively, the findings of this study enhance our understanding of the feeding ecology of *T. pacificus* and thereby provide valuable information that will contribute to the ecological fishery management of this commercially important species.

Keywords: Ommastrephidae; stomach composition; stable isotope; feeding strategy; trophic position; ontogenetic dietary shift

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1. Introduction

Squid, which are widely distributed throughout most marine systems worldwide, comprise approximately 300 species characterized by pronounced differences in biological and ecological traits and habitat preferences [1,2]. In general, squid are ecologically important organisms in marine communities, within which they constitute a predominant group, owing to their high biomass, and represent a major prey resource for marine predators, including fishes, seabirds, and mammals [1,3]. Squid are active predators that feed on a diverse range of prey types, including zooplankton, crustaceans, cephalopods, and fishes, which they capture via different predatory behaviors, ambushing, stalking, and pursuit, and can thereby play an important role in marine food webs [4–8]. Most squid are characterized by high feeding rates, which are necessary in maintaining the high metabolic and growth rates associated with their short lifespans of approximately 1 or 2 years [9]. Given their ecological characteristics, squid have attracted considerable attention as potential beneficiaries in the future changing oceans and/or indicators of the impacts of climate change on marine systems [10–12]. Consequently, it is important to understand the ecological roles played by squid populations in marine systems, particularly their feeding ecology, dietary composition, ontogenetic dietary changes, and feeding behaviors.

The common squid *Todarodes pacificus* (Ommastrephidae) is among the predominant species comprising epipelagic communities in the South and East seas of Korea and the East China Sea [13,14]. In Korean waters, the total catch of *T. pacificus* as a warm-water species underwent a rapid increase after the late 1980s, which is assumed to be associated with the general response of marine ecosystems to shifts in the climatic regime [15]. Furthermore, this squid is a commercially important species, accounting for more than 10% of the total catch obtained by the Korean fishery industry [16]. It is a generalist predator that typically feeds on zooplankton and small pelagic fish [17]. Similar to other Ommastrephid squid, *T. pacificus* undergoes ontogenetic dietary changes from crustaceans (e.g., copepods, amphipods, and euphausiids) in juveniles (<50 mm mantle length, ML) to crustaceans and fish in medium-sized individuals (50–99 mm ML), fish in adult-sized individuals (100–150 mm ML), and fish and squid in the largest individuals (>150 mm ML), as assessed via stomach content analyses [14,18]. *T. pacificus* has a wide prey spectrum, including cannibalism, which varies among regions and is dependent on the availability of the prey [14]. Nevertheless, despite the acknowledged ecological importance of *T. pacificus*, studies of its feeding ecology are notably limited. Given its increasing commercial value as a fishery resource, information on the trophic ecology of the common squid is essential for successful fishery management and conservation in rapidly changing oceanic environments.

A stomach content analysis has conventionally been used to assess the feeding ecology and quantify the dietary composition of marine organisms, including cephalopods [8,19,20]. However, given several limitations associated with digested, unidentifiable, or absent dietary items in the stomachs of consumers and differences in the digestibility of dietary components, this approach may present problems with respect to identification, quantification, and interpretation [6,21,22]. To overcome such drawbacks, an analysis of the stable isotopes of carbon and nitrogen has been used as a complementary approach for analyzing the stomach contents to identify assimilated dietary items among available food sources and determine the structure of food webs in marine ecosystems [23–25]. Carbon stable isotope ratios (δ^{13}C) generally increase from the diet to the consumer at an enrichment of approximately 1.0‰ and have been used to infer dietary sources [26,27]. In contrast, nitrogen stable isotope ratios (δ^{15}N) can significantly increase by 2–4‰ from prey to predator, thereby generally providing information on the trophic position (TP) of the consumer species [28,29]. Recently, the combined application of stable isotope and stomach content analyses has been successfully adopted to provide insights into the feeding habits and ontogenetic dietary shifts of various fish species and cephalopods [30–34].

In this study, based on a combined analysis of stable isotopes and stomach contents, we sought to assess the trophic ecology of *T. pacificus* in the South Sea of Korea, which, as the predominant species, may play a significant ecological role in the marine food web of coastal waters. Specifically, to assess the dietary composition, TP, and ontogenetic dietary shifts, we analyzed δ^{13}C and δ^{15}N concentrations in the muscle tissues of common squid and the stomach contents of specimens in different body size classes during different seasons in the South Sea of Korea. As a primary approach for common squid collected in Korean coastal waters and a useful tool for investigating the dietary characteristics of cephalopods, a combination of stable isotope and stomach content analyses can be expected to reflect the trophic role of common squid in the marine food web and its feeding ecology during ontogeny.

2. Materials and Methods

2.1. Study Area and Sample Collection

For the study, we collected samples at four sites in the subtidal zone off the southern part of the Korean Peninsula (104: 34°25′ N, 127°75′ E; 106: 34°25′ N, 128°75′ E; 111: 33°75′ N, 128°75′ E; and 224: 33°75′ N, 127°25′ E) in February, May, and August 2021

(Figure 1). The water depth at the sampling sites ranged from 83 m to 123 m, with a low tidal amplitude less than 30 cm. Samples of all fish and zooplankton species were collected from the Tamgu 20 (885 t) and Tamgu 22 (1458 t) research vessels of the Fisheries Resources Research Center of the National Institute of Fisheries Science (NIFS). Fish samples were collected by trawl surveys (12 cm mesh in the main body, 8 cm mesh in the intermediate part, and 6 cm mesh in the cod end with a 2 cm cod end net liner), and zooplankton samples were collected using a Bongo net (2.0 m^2 mouth opening, 500 µm mesh). For the fish samples, biological parameters, including total length (to the nearest 0.1 cm) and biomass (to the nearest 0.1 g) of each individual were gauged onboard. All collected fish and zooplankton samples were initially stored in a freezer (−20 °C) and then transported to the laboratory for processing.

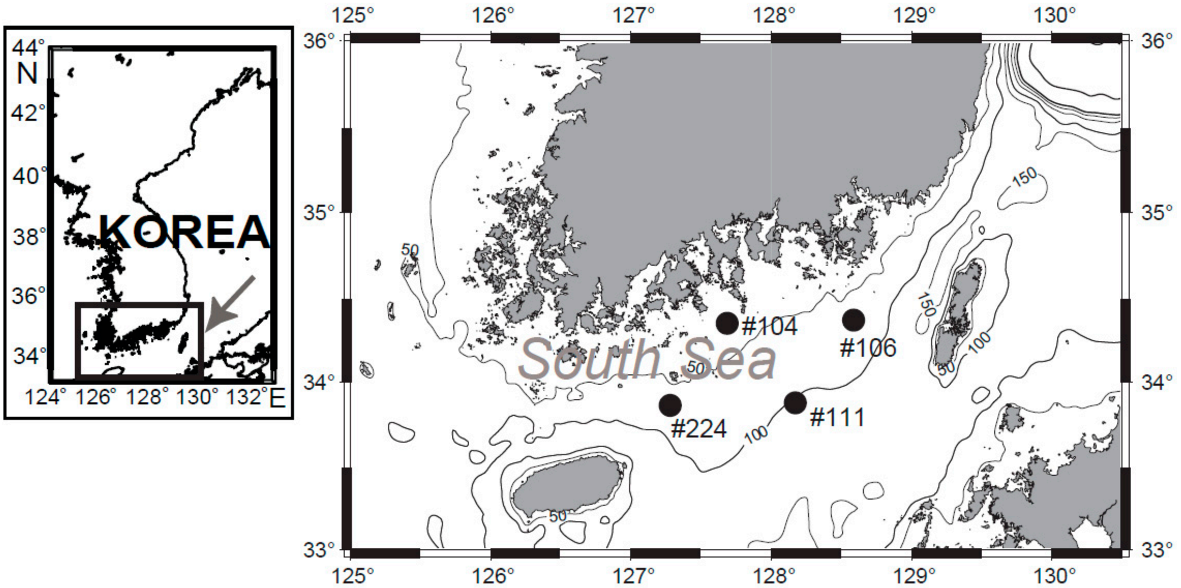

Figure 1. A map showing the location of the sampling areas in the South Sea of Korea. Filled circles indicate the four sampling sites (#104, #106, #111, and #224) at which zooplankton, common squid *Todarodes pacificus*, and other fish consumers were collected.

2.2. Sample Processing

In the laboratory, all sampled fish and common squid were dissected, and muscle tissues were collected from the anterior dorsal parts for the stable isotope analysis. Whole-body samples were prepared for the stable isotope analysis of dominant zooplankton, including copepods and euphausiids. All prepared samples were freeze-dried, ground to a homogenous powder using a ball mill, and stored in a vacuum desiccator until used for further analysis. Common squid stomach samples were collected and individually preserved in 10% formalin for further analyses, as described below.

2.3. Stomach Content Analysis

During the sampling period, a total of 701 *T. pacificus* individuals were collected; among which, the stomachs of 233 were sampled for content analysis. Each stomach sample was examined using a LEICA L2 stereomicroscope (Leica Microsystems, Wetzlar, Germany), and prey items were counted and categorized to the lowest possible taxonomic level. The wet weight of each item was measured to the nearest 0.0001 g using an analytical balance (ME204TE/00; Mettler Toledo, Greifensee, Switzerland). Given the uncertainty and biases associated with the estimates of cephalopod dietary compositions obtained using the index of relative importance (IRI), we applied a ranking index (RI) in this study [6,8]. Food items in the stomachs of common squid were assessed based on the percentage frequency of occurrence (%*F*), which is the number of stomachs in which a particular food item occurs (as a percentage of the total number of examined stomachs), and percentage wet weight (%*W*) of each food item to the total wet weight of the identifiable food items. RI values were estimated for all food items of each prey type [*RI* = %*W* × %*F*] and expressed as a percentage (%*RI*) as follows: $\%RI = RIi/\sum_{i=1}^{n} RIi \times 100$ [35], where n is the total number of food items categorized as class levels. Size-related ontogenetic dietary changes in common squid were examined using the three size classes <10, 10–20, and ≥20 cm ML.

2.4. Stable Isotope Analyses

Small quantities (0.5–1.0 mg) of the powered samples were weighed into tin combustion capsules. All encapsulated samples were combusted at a high temperature (1020 °C) using a CNSOH elemental analyzer (EA Isolink, Bremen, Germany), and the resulting gas was analyzed for carbon and nitrogen stable isotope ratios using a continuous-flow isotope ratio mass spectrometer (CF-IRMS: DELTA V PLUS; Bremen, Germany). The carbon and nitrogen isotope ratios are expressed in delta (δ) notation as a difference from the respective conventional standards (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen) as follows: $\delta X\,(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 10^3$, where X is ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. To calibrate the analyzed isotope values, we obtained the international standard values of sucrose (ANU $C_{12}H_{22}O_{11}$; NIST, Gaithersburg, MD, USA) and ammonium sulfate ($[NH_4]_2SO_4$; NIST) after measuring each group of 10 samples. The analytical reproducibility based on 20 urea replicates was within 0.11‰ and 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Lipids are generally highly abundant in fish, which can lead to a bias in $\delta^{13}C$ value estimates. If the mass ratio of carbon to nitrogen (C/N) for fish species exceeds 3.5, it is considered to be attributable to a reduction in the $\delta^{13}C$ value owing to ^{12}C enrichment, and in this case, we applied lipid correction (non-lipid extracted) according to Post et al. [36]. The lipid correction formula used for non-lipid extracted samples was as follows: $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:N\ (ratios)$, where $\delta^{13}C_{untreated}$ and $\delta^{13}C_{normalized}$ are the measured and lipid-corrected $\delta^{13}C$ values of the non-lipid extracted sample, respectively.

The TP values of consumer species were calculated according to the following formula: $TP_i = (\delta^{15}N_i - \delta^{15}N_{baseline})/\Delta^{15}N + 2$, where $\delta^{15}N_i$ represents the $\delta^{15}N$ value of each target consumer, $\delta^{15}N_{baseline}$ is the mean $\delta^{15}N$ of baseline organisms (copepod group) selected in this study, $\Delta^{15}N$ is the nitrogen enrichment factor of 3.4‰ in $\delta^{15}N$, and 2 represents the baseline TP [28,29].

2.5. Data Analyses

All data were initially assessed for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) prior to further statistical analyses using the R software (R Core Team, Vienna, Austria, 2016). A permutational multivariate analysis of variance (PERMANOVA) using PRIMER version 6 + PRIMER add-on (PRIMER-e, Auckland, New Zealand) was used to compare seasonal differences in the $\delta^{13}C$ and $\delta^{15}N$ values of common squid and other consumer species (zooplankton and fish). A one-way analysis of variance (ANOVA, San Francisco, CA, USA) with Tukey's honest significant difference (HSD) multiple-comparison *post hoc* test in R software was performed to compare the stable isotope and TP values of consumer species and the MLs of common squid. A linear regression analysis was performed using the R software to assess the size-based trophic relationship between ML and stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in common squid. Moreover, dietary compositions were assessed based on a detrended correspondence analysis (DCA) using the R program, which visually depicts the relationship between samples and species in a reduced space [8,37].

3. Results

3.1. Dietary Composition: Relative Importance

Among the stomachs of the 701 common squid examined, 468 stomachs were found to be empty, whereas the contents of the remaining stomachs comprised prey from eight groups, as examined based on class (Figure 2). Regardless of season, Pisces (37.9% in August to 94.0% in February) and Cephalopoda (6.0% in February to 61.0% in August) contributed the largest proportions of the stomach contents of common squid. An analysis of size-related stomach contents based on ML (<10, 10–20, and >20 cm) also revealed Pisces and Cephalopoda to be the major constituents. However, among squid in the ML <10 cm group, the stomach contents of specimens collected in August comprised primarily Crustacea (95.5%), whereas Pisces and Cephalopoda were found to be minor components (2.3% and 2.2%, respectively).

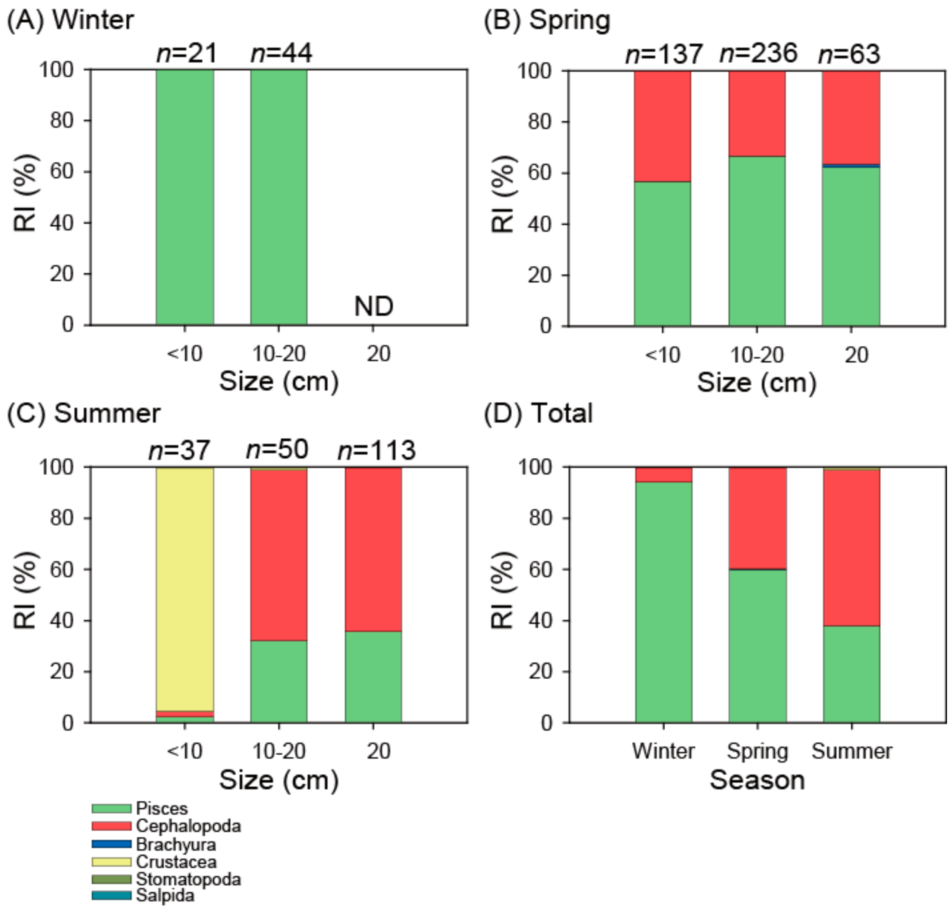

Figure 2. Size-related (mantle length, ML; three size classes, <10 cm, 10–20 cm, and >20 cm) and total (**D**) compositions of the stomach contents of common squid *Todarodes pacificus* based on the ranking index (%RI) expressed as a percentage of the sum of the RI values in the South Sea of Korea during February (winter, (**A**)), May (spring, (**B**)), and August (summer, (**C**)) 2021.

The arrangement of *T. pacificus* and its dietary items based on DCA ordination yielded eigenvalues of $\lambda_1 = 0.928$, $\lambda_2 = 0.221$, and $\lambda_3 = 0.138$ for the first composition gradients (Figure 3). The smallest individuals of common squid collected in August were associated with Crustacea, whereas the other groups were plotted in the vicinity of Pisces in February, that of Stromatopoda and Salpida in May, and that of Brachyura in August.

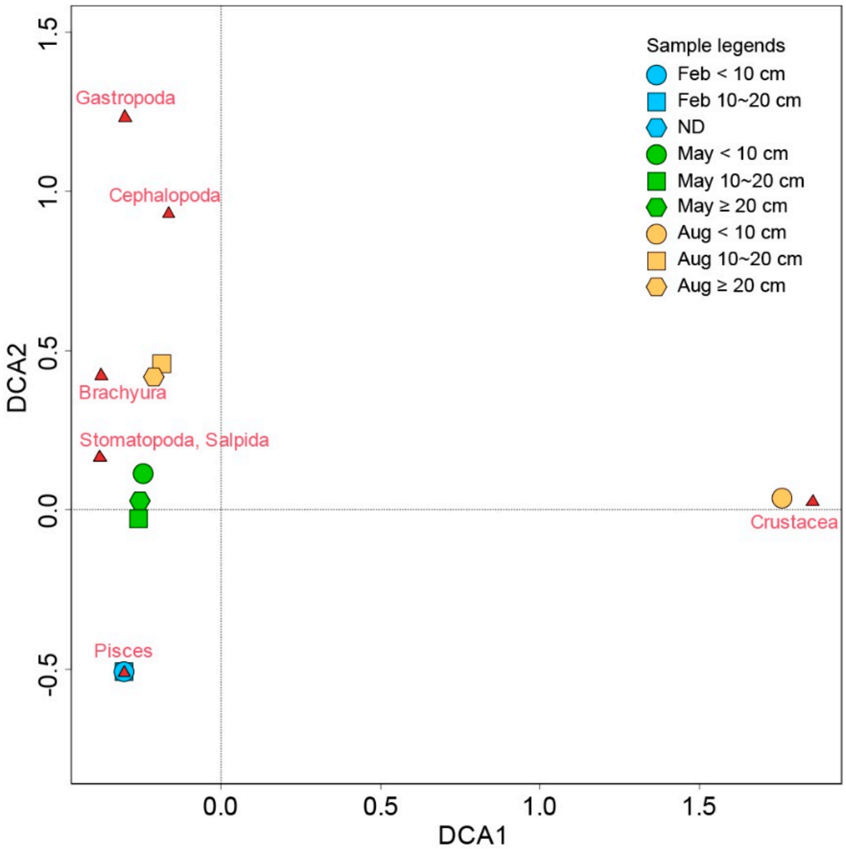

Figure 3. Bi-plot of detrended correspondence analysis (DCA) derived from consumer species and their dietary compositions in a reduced space. Red triangles represent prey items. Circles, squares, and hexagons represent common squid *Todarodes pacificus* in the South Sea of Korea during February (blue), May (green), and August (yellow) 2021.

3.2. Stable Isotope Ratios

3.2.1. Common Squid

The $\delta^{13}C$ and $\delta^{15}N$ values of common squid were found to differ significantly among the three assessed seasons (PERMANOVA: pseudo-$F_{2,95}$ = 8.01, p = 0.002), with $\delta^{13}C$ values being higher in August (−18.9‰ ± 0.6‰) than in February (−19.3‰ ± 0.8‰) and May (−19.5‰ ± 0.7‰) (Tukey's HSD test: each p < 0.01), whereas $\delta^{15}N$ values were higher in February (11.3‰ ± 0.6‰) than in May (10.4‰ ± 0.7‰) and August (10.8‰ ± 1.0‰) (Tukey's HSD test: each p < 0.01). The TP values of common squid were higher in February than in May and August (Tukey's HSD test: each p < 0.01), ranging from 2.9 ± 0.3 (August) to 3.4 ± 0.2 (February).

In contrast, we detected no significant differences in the ML of common squid collected in the three seasons (one-way ANOVA: p = 0.232), ranging from 16.8 ± 4.0 cm (6.9–23.7 cm in May) to 18.6 ± 4.6 cm (8.6–23.5 cm in February). Furthermore, in February and May, no significant correlations were found between the MLs and isotopic values ($\delta^{13}C$: r = 0.096, p = 0.689, and r = 0.260, p = 0.060, respectively; $\delta^{15}N$: r = 0.399, p = 0.081, and r = 0.086, p = 0.540, respectively) (Figure 4). However, in August, ML was significantly correlated with both isotopic values ($\delta^{13}C$: r = 0.556, p = 0.006; $\delta^{15}N$: r = 0.839, p < 0.001).

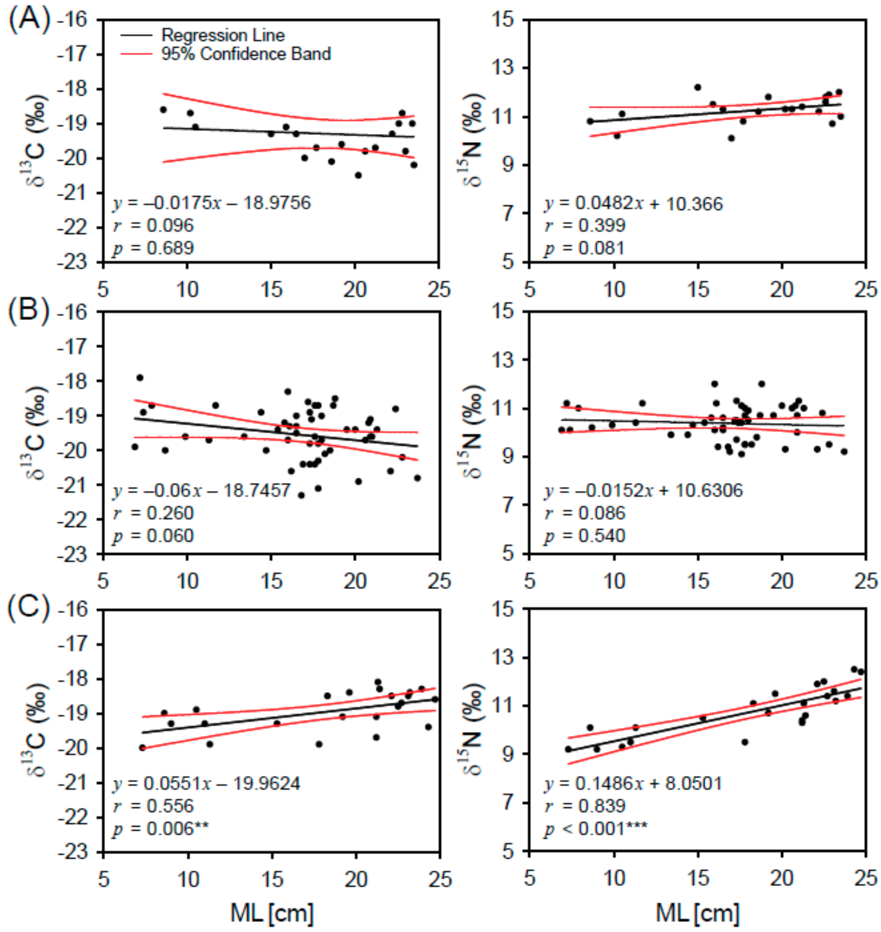

Figure 4. Regression relationships between δ13C—mantle length (cm, left) and δ15N—mantle length (cm, right) of common squid *Todarodes pacificus* collected in the South Sea of Korea during February (winter, (**A**)), May (spring, (**B**)), and August (summer, (**C**)) 2021. ** $p < 0.01$, *** $p < 0.001$.

3.2.2. Zooplankton and Other Fish Consumers

Significant differences were detected among the sampling periods with respect to the δ13C and δ15N values of the two zooplankton groups copepods and euphausiids (PERMANOVA: pseudo-$F_{2,29}$ = 13.22, p = 0.001 and pseudo-$F_{2,19}$ = 5.43, p = 0.003, respectively) (Table 1). For copepods, we obtained δ13C and δ15N values of −22.2‰ ± 0.9‰ (May) to −20.1‰ ± 1.0‰ (February) and 6.5‰ ± 0.4‰ (February) to 8.2‰ ± 0.3‰ (August), respectively. Comparatively, the δ13C and δ15N values of euphausiids ranged from −21.3‰ ± 1.1‰ (August) to −20.2‰ ± 1.0‰ (February) and from 6.9‰ ± 0.9‰ (February) to 7.8‰ ± 0.7‰ (August), respectively.

Table 1. Mean δ13C and δ15N values of the zooplankton groups calanoid copepods and euphausiids, collected during February, May, and August 2021 in the South Sea of Korea (#104, #106, #111, and #224). PERMANOVA test of δ13C and δ15N values for each zooplankton among seasons. Bold-faced font indicates significance at p < 0.05. Data represent means ± 1 SD.

	February					May					August					PERMANOVA	
Zooplankton group	*n*	δ13C Mean	SD	δ15N Mean	SD	*n*	δ13C Mean	SD	δ15N Mean	SD	*n*	δ13C Mean	SD	δ15N Mean	SD	pseudo-*F*	*p*
Copepods	10	−20.1	1.0	6.5	0.4	12	−22.2	0.9	7.3	0.4	7	−20.2	0.5	8.2	0.3	13.22	0.001
Euphausiids	5	−20.2	1.0	6.9	0.9	10	−21.1	0.8	6.9	0.4	4	−21.3	1.1	7.8	0.7	5.43	0.003

With the exception of those of common squid, the δ^{13}C and δ^{15}N values of all fish consumers differed significantly among the sampling periods (PERMANOVA: pseudo-$F_{2, 87}$ = 8.87, p = 0.001). The mean δ^{13}C values of fish were relatively high in February (−19.4‰ ± 0.5‰ to −16.4‰ ± 0.1‰) and August (−19.1‰ ± 0.8‰ to −17.9‰) compared with those in May (−20.1‰ ± 0.8‰ to −17.1‰ ± 0.8‰) (Figure 5 and Table 2). In contrast, fish had lower δ^{15}N values in August (8.9–11.9‰) than in February (9.3–14.9‰ ± 0.5‰) and May (8.5–13.1‰ ± 0.6‰). Moreover, the mean TP values of fish were significantly higher in February (3.72 ± 0.50) than in May (3.10 ± 0.33) and August (2.84 ± 0.27; Tukey's HSD test: each p < 0.01).

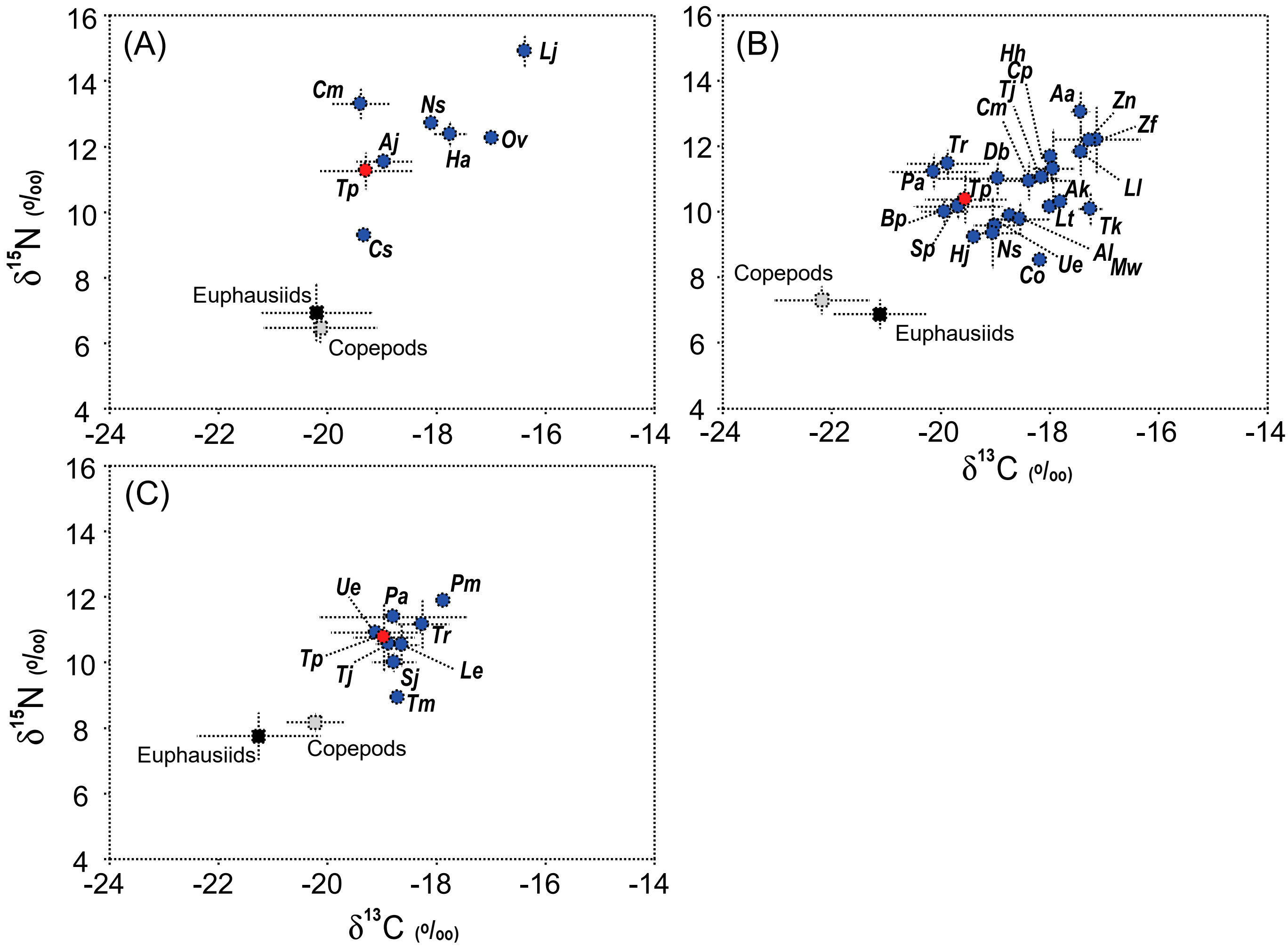

Figure 5. Dual isotope plots of δ^{13}C and δ^{15}N values of zooplankton (black squares, euphausiids; gray squares, copepods), common squid *Trichiurus japonicus* (red circles), and other fishes (blue circles) in the South Sea of Korea during February (winter, (**A**)), May (spring, (**B**)), and August (summer, (**C**)) 2021. Values are presented as mean δ^{13}C and δ^{15}N (‰ ± 1 SD). Species codes are shown in Table 2.

Table 2. Mean $\delta^{13}C$ and $\delta^{15}N$ values and trophic position (TP) of common squid *Todarodes pacificus* and other fish consumers collected during February, May, and August 2021 in the South Sea of Korea (#104, #106, #111, and #224). Data represent means ± 1 SD.

Month	Species Name	Code	*n*	$\delta^{13}C$		$\delta^{15}N$		TP
				Mean	S.D.	Mean	S.D.	
February	*Todarodes pacificus*	Tp	20	−19.3	0.8	11.3	0.6	3.41
	Acropoma japonicum	Aj	3	−19.0	0.5	11.5	0.2	3.48
	Champsodon snyderi	Cs	1	−19.3		9.3		2.82
	Conger myriaster	Cm	2	−19.4	0.5	13.3	0.4	4.00
	Hoplobrotula armata	Ha	3	−17.8	0.3	12.4	0.4	3.73
	Lateolabrax japonicus	Lj	3	−16.4	0.1	14.9	0.5	4.47
	Niphon spinosus	Ns	1	−18.1		12.7		3.82
	Octopus vulgaris	Ov	1	−17.0		12.2		3.69
May	*Todarodes pacificus*	Tp	53	−19.5	0.7	10.4	0.7	3.03
	Apogon lineatus	Al	3	−18.7	0.2	9.9	0.1	2.87
	Argentina kagoshimae	Ak	2	−17.8	0.0	10.3	0.1	3.00
	Argyrosomus argentatus	Aa	2	−17.4	0.2	13.0	0.6	3.81
	Benthosema pterotum	Bp	3	−19.9	0.4	10.0	0.4	2.91
	Cleisthenes pinetorum	Cp	3	−17.9	0.4	11.3	1.2	3.30
	Coelorinchus multispinulosus	Co	1	−18.2		8.5		2.47
	Conger myriaster	Cm	3	−18.4	0.8	10.9	0.6	3.19
	Doederleinia berycoides	Db	3	−19.0	1.1	11.0	0.5	3.21
	Helicolenus hilgendorfi	Hh	1	−18.0		11.7		3.40
	Hyperoglyphe japonica	Hj	1	−19.4		9.2		2.68
	Liparis tanakae	Lt	1	−18.0		10.1		2.96
	Lophius litulon	Ll	3	−17.4	0.2	11.8	0.7	3.45
	Malakichthys wakiyae	Mw	3	−18.5	0.5	9.8	0.5	2.84
	Neobythites sivicola	Ns	3	−19.0	0.5	9.3	1.1	2.72
	Psenopsis anomala	Pa	3	−20.1	0.8	11.2	0.5	3.27
	Synagrops philippinensis	Sp	3	−19.7	0.8	10.2	0.4	2.96
	Tanakius kitaharae	Tk	3	−17.3	0.2	10.1	0.5	2.94
	Trachurus japonicus	Tj	3	−18.1	0.2	11.0	0.2	3.22
	Trichiurus japonicus	Tr	3	−19.9	0.7	11.5	0.4	3.34
	Uroteuthis edulis	Ue	3	−19.0	0.4	9.6	0.2	2.79
	Zenopsis nebulosa	Zn	1	−17.3		12.2		3.55
	Zeus faber	Zf	3	−17.1	0.8	12.2	1.0	3.56
August	*Todarodes pacificus*	Tp	23	−19.0	0.6	10.8	1.0	2.89
	Loligo edulis budo	Le	3	−18.6	0.3	10.5	0.6	2.80
	Pagrus major	Pm	1	−17.9		11.9		3.20
	Psenopsis anomala	Pa	2	−18.8	1.3	11.4	0.1	3.05
	Scomber japonicus	Sj	4	−18.8	0.4	10.0	0.3	2.65
	Thamnaconus modestus	Tm	1	−18.7		8.9		2.33
	Trachurus japonicus	Tj	3	−18.9	0.2	10.6	0.4	2.81
	Trichiurus japonicus	Tr	3	−18.3	0.5	11.2	0.7	2.99
	Uroteuthis edulis	Ue	3	−19.1	0.8	10.9	0.1	2.91

4. Discussion

The feeding ecology of marine species is generally influenced by environmental conditions, food availability, trophic interactions, and species competition [2,27,38]. In the present study, we adopted the combined application of stomach content and stable isotope analyses to examine the feeding ecology of the common squid *T. pacificus* along the southern coast of Korea, from seasonal and size-related ontogenetic feeding perspectives. Collectively, the findings of the study revealed temporal and size-related patterns among common squid, indicating that these cephalopods are opportunistic carnivores based on seasonal differences in their dietary composition and ontogenetic dietary shifts during the sampling periods. Such information on seasonal dietary changes and ontogenetic variation in the common squid provides further insight into how changes in environmental conditions and prey availability may affect the trophic ecology of this commercially important species.

The results of the present study revealed seasonal shifts in the diets of common squid in terms of prey composition, of which the main groups, based on stomach content analyses, were Pisces (37.9–94.0%) and Cephalopoda (6.0–61.0%) throughout the three sampling periods, with a gradual increase in the proportion of dietary items from Pisces to Cephalopoda from winter to summer). Similarly, the DCA results indicated a seasonal variability in the dietary composition of common squid and dietary changes relative to ML. Although the potential effects of body size were considered, we observed that dietary trends were similar across the sampling periods. Such seasonal shifts in the dietary items consumed by common squid may be associated with changes in regional food availability, as indicated by the wide regional and seasonal variation in the feeding spectrum [14]. In general, most omnivorous and carnivorous consumers in marine ecosystems tend to be characterized by dietary plasticity, that is, feeding on a variety of prey, depending on food availability and environmental conditions [25,39–41]. Many squid species, including *T. pacificus*, actively feed on zooplankton, cephalopods, and small fish, and may also engage in cannibalism [6–8]. However, despite the dietary opportunism of *T. pacificus*, the stomach contents observed in the present study showed relatively little seasonal variation and low prey diversity compared with the results obtained in previous studies [14,18], which we suspect could be due to the geographical differences in the abundance and availability of potential prey for *T. pacificus*.

A stomach content analysis revealed very similar patterns in dietary prey composition among common squid of the three different size classes in February and May, which indicates high intraspecific feeding competition with no size-related dietary shifts. Although marine fishes typically undergo ontogenetic changes that entail the partitioning of dietary resources, our results indicate an absence of ontogenetic size-related dietary shifts in prey composition during winter and spring. Compared with those of the other two size classes, the common squid of size class ML <10 cm showed a rather obvious distinction in dietary composition in August with respect to the high proportion of Crustacea (95.5%) in the diet. In this regard, numerous studies have reported that marine fishes and cephalopods undergo ontogenetic dietary shifts that are characterized by changes in size-specific prey–predator relationships [22,33,38]. Consistent with the findings of the present study, Uchikawa and Kidoroko [18] detected a clear size-related dietary change in *T. pacificus*, in which small individuals fed mainly on crustaceans, whereas larger individuals shifted to a more fish-based diet. Such ontogenetic shifts in the diets of marine consumers are assumed to be associated with their higher predatory ability and improved swimming capacity with increasing growth [42,43]. Growth and morphological changes in *T. pacificus* may result in higher swimming performance, and thus a higher proportion of fish in their diets [18,44]. However, the feeding characteristics of *T. pacificus* observed during only one specific season (i.e., summer) may be an effect of seasonal changes in prey availability, as opportunistic consumers generally tend to achieve energy optimization by reducing intra- and/or interspecific food competition [45,46]. Thus, size-related changes in common squid diets that occur only during summer may be closely associated with prey availability and environmental conditions in the foraging area, rather than with an enhancement in predation capacity.

The stable isotope data obtained in this study revealed isotopic variation over the sampling periods, thereby indicating a seasonal shift in dietary sources, and thus a change in TP values, as observed based on the stomach content analysis. As discussed above, most squid can rapidly adjust their feeding strategies in response to changes in seasonal and annual prey availability [22,47]. This may be supported by seasonal isotopic variability, which reflects the temporal changes in the feeding ecology of *T. pacificus*. In the present study, we established that the relatively higher $\delta^{15}N$ values of *T. pacificus* in February compared to those obtained in other seasons may be associated with the high proportion of fish in their diets, with respect to the generally high TP of fish species. In August, despite a reduction in the contribution of dietary fish to the composition of common squid tissue, the relatively high $\delta^{13}C$ values compared to those in other months may be associated with seasonal variability in the trophic baseline (i.e., phytoplankton-derived organic matter) in sampling regions with different prey composition [48]. In marine ecosystems, the $\delta^{13}C$ values of phytoplankton-derived organic matter generally reflect spatial and temporal variations in marine particles, including phytoplankton, under different ambient conditions, and thereby contribute to the $\delta^{13}C$ values of higher TP consumers along food chains [27]. The $\delta^{13}C$ values of common squid (−19.5‰ to 18.9‰) obtained in the present study may reflect a tendency of trophic connections of the general pelagic system with two zooplankton groups, indicating a linkage between *T. pacificus* and phytoplankton-derived organic matter [25]. Accordingly, seasonal changes in the isotopic composition of the common squid are considered to be indicative of the variability of dietary items and isotopic baseline sources corresponding to changes in seasonal environmental conditions.

The $\delta^{13}C$–$\delta^{15}N$ dual-isotope plots shown in Figure 5 indicate discrimination between pelagic and benthic feeders along the $\delta^{13}C$ axis. Compared with the benthic-feeding demersal fish, pelagic feeders, such as the common squid, are generally characterized by relatively low $\delta^{13}C$ and $\delta^{15}N$ values [25,33]. Consistently, our findings in the present study revealed the relatively low $\delta^{13}C$ and $\delta^{15}N$ values of common squid, indicating that the isotopic niche of this species is closer to that of the zooplankton group's copepods and euphausiids regardless of the season. Moreover, the distribution and migration of *T. pacificus* are significantly influenced by physical conditions (e.g., water temperature), which can play an important role in altering the seasonal patterns in marine fish food webs [14,25].

The TP estimates obtained for common squid and other consumers based on the $\delta^{15}N$ values of zooplankton have revealed temporal variation resulting from a seasonal difference in the $\delta^{15}N$ values of food web baselines [25]. The relatively high $\delta^{15}N$ values of zooplankton recorded in August compared with those obtained in February may have contributed to the low TP estimates obtained for consumers during summer. The high $\delta^{15}N$ values of phytoplankton-derived organic matter as an isotopic baseline during summer can probably be attributed to the proliferation of phytoplankton influenced by excessive nutrient input associated with heavy monsoonal rainfall, which is consistent with previous observations in the southern coastal waters of Korea [48]. Similar to the temporal variability of common squid $\delta^{13}C$, seasonal differences in the TP values of these squid may result from the variability of zooplankton $\delta^{15}N$ influenced by phytoplankton dynamics associated with regional oceanographic conditions.

In the present study, significant positive correlations between ML and both the $\delta^{13}C$ and $\delta^{15}N$ values of common squid were detected only in summer, thereby indicating clear size-dependent shifts in ontogenetic dietary composition. which is consistent with our observations of seasonal dietary changes based on the stomach content analysis (Figure 2). The high contribution of Crustacea (more than 95%) to the diet of the smallest common squids (ML < 10 cm) in August may have led to lower $\delta^{13}C$ and $\delta^{15}N$ values compared with those of larger individuals, and thereby to positive isotopic gradients with increasing body size. Such relationships between size and isotopic values of cephalopods and fish species have been reported in several studies and may be indicative of a general size-related pattern in ontogenetic dietary shifts [32,33,49]. Similarly, an increase in the $\delta^{15}N$ values of common squid with increasing ML is assumed to reflect an increase in the consumption of larger and trophically higher prey items (e.g., fish and cephalopods) during summer. In contrast, the lack of size-related isotopic relationships in common squid during winter and spring indicates a high dietary overlap among the specimens in the three assessed size classes associated with the consumption of similar diets. This lack of size-related patterns in resource use by common squid may result in heightened intraspecific competition, as indicated by the composition of stomach contents. Overall, the observed seasonal patterns in size-related isotopic distributions of common squid may be explained in terms of the temporal variability of the major dietary components for specific size classes associated with the seasonally changing availability of food in the region.

In conclusion, our combined application of stomach content and stable isotope analyses in this study revealed seasonal differences in the dietary composition and ontogenetic dietary patterns of the common squid in the South Sea of Korea. Our observations indicate that the feeding strategy adopted by these squid changes with the seasonal availability of prey in this region. In particular, although we detected no apparent size-related dietary changes during winter or spring, there was a clear ontogenetic dietary shift from crustaceans to cephalopods and fishes during summer, thereby indicating temporal variability in feeding strategies in response to the availability of prey. Collectively, the findings of this study advance our understanding of the feeding ecology of *T. pacificus*, thereby providing valuable information that will contribute to refining the ecological-based fishery management of this commercially important species.

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