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Feeding Habits of *Scomber japonicus* Inferred by Stable Isotope and Fatty Acid Analyses

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Abstract: *Scomber japonicus* is widely distributed off the coast of Japan and in the northwestern Pacific. It is an important target for fisheries. To reveal the differences in diet shifts and niche changes of *S. japonicus*, we collected samples in the high seas of the northwest Pacific (38°59′ N–43°00′ N, 150°30′ E–161°48′ E) from June to August 2021. We utilized stable isotope and fatty acid analyses to study the differences in body length and sex of *S. japonicus*. The results showed no significant differences in stable isotope values and fatty acid composition between male and female individuals ($p > 0.05$). Differences in $\delta^{13}C$ and $\delta^{15}N$ values among different body length groups were also not significant ($p > 0.05$). Both $\delta^{13}C$ and $\delta^{15}N$ values showed a trend of increasing and then decreasing across different body length groups. The niche width of *S. japonicus* in different body length groups first increased and then decreased, with niche overlap among the groups exceeding 60%. Principal component analysis (PCA) results showed that the main fatty acids in *S. japonicus* were C14:0, C16:0, C18:0, C16:1n-7, C18:1n-9, C18:3n-6, C20:5n-3, C20:4n-6 and 22:6n-3. Except for C18:0 and C20:4n-6, the content of the other fatty acids showed significant differences among different body length groups ($p < 0.05$). The results of the similarity analysis (ANOSIM) indicated that the fatty acid compositions of the 100–130 mm length group were significantly different from those of the 131–160 mm and 161–190 mm length groups ($p < 0.05$). However, there were no significant differences among the other size groups ($p > 0.05$). During the growth and development of *S. japonicus*, the proportion of krill in their diet gradually decreased. Meanwhile, their consumption of zooplankton, diatoms and fish significantly increased. Additionally, *S. japonicus* also consumed crustaceans, but their intakes of planktonic bacteria and green algae were relatively low. We suggested that there were no significant differences between male and female individuals of *S. japonicus*. As they grew and developed, the ecological niche and feeding habits of *S. japonicus* continuously changed.

Keywords: *S. japonicus*; stable isotope analysis; fatty acid analysis; niche; dietary differentiation

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1. Introduction

The northwest Pacific is a region rich in biodiversity and fishery resources, spanning a vast area from the eastern coast of Asia to the Hawaiian Islands [1]. This region is one of the world's most important fishing grounds, encompassing numerous economically significant fish species, with the *Scomber japonicus* holding a prominent position [2]. The *S. japonicus* is a highly migratory pelagic fish widely distributed in temperate and subtropical waters, particularly along the coasts of China, Japan and Korea in the northwest Pacific [3]. This

fish species not only holds significant economic value for local fisheries but also plays a crucial role in the ecosystem as a mid-level predator and a key link in the food chain.

Studying the nutritional ecology of fish is crucial for assessing their behavior, population dynamics, habitat utilization, energy transfer and interspecific interactions within marine food webs [4]. Traditionally, stomach content analysis has been the most commonly used method, which involves directly examining the food remnants in fish stomachs to determine their feeding habits and food web structure [5]. However, this method has some limitations, such as the rapid digestion of stomach contents and the complexity of sample processing. To overcome these limitations, several new methods have emerged in recent years, either used separately or in combination, to provide more comprehensive nutritional ecology information. Stable isotope analysis (SIA) is one important method. By measuring the ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in fish tissues, researchers can infer the long-term food sources and trophic levels of fish [6]. Fatty acid analysis (FAA), on the other hand, examines the composition and proportions of fatty acids in fish tissues to study their food sources and feeding habits [7]. DNA barcoding analysis is an emerging technology that involves extracting and identifying DNA from stomach contents to accurately identify food species. This method can overcome the limitations of traditional stomach content analysis, providing high-resolution dietary information [8].

Stable isotope analysis (SIA) has become an essential tool for studying the nutritional structure of marine food webs. DeNiro et al. (1978) were the first to study and evaluate the distribution of carbon and nitrogen stable isotopes in organisms' diets [9], discovering that $\delta^{13}C$ and $\delta^{15}N$ values reliably reflect the dietary composition of organisms. SIA provides valuable indicators for trophic levels (TL), physiological processes (such as protein and lipid synthesis) and nutritional niches and foraging ranges [10]. By measuring the $\delta^{13}C$ and $\delta^{15}N$ ratios in fish tissues, researchers can reconstruct their long-term food sources and trophic levels. The spatial variability of carbon isotopes reflects the contribution of different carbon sources to consumers, such as the distinction between coastal and offshore environments [11]. Nitrogen isotopes indicate trophic levels and undergo changes during metabolic processes, known as trophic discrimination factors, reflecting physiological fractionation with increasing trophic levels [10]. SIA has been widely applied to various marine organisms, from plankton to top predators (e.g., dolphins), to reveal food web structures and energy flow [12,13].

Fatty acid composition analysis provides a unique methodology, as fatty acids not only serve as biomarkers reflecting the diet and nutritional status of organisms but are also significantly influenced by food sources [14]. Most importantly, fatty acid analysis can reveal the energy flow within food chains and interspecies relationships, offering crucial insights into the function and structure of ecosystems. These advantages make fatty acid analysis an indispensable tool in the fields of ecology and biology. In recent years, fatty acid composition analysis has gained prominence in ecology and has been widely applied to marine ecological studies, including the nutritional ecology of cephalopods [15], bony fishes [16] and cartilaginous fishes [17,18]. *S. japonicus* is not only an important food source, but its fatty acids are often used as sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Certain fatty acids serve as indicators in dietary and nutritional relationship studies, such as C16:1n7 and C20:5n3, which indicate diatom consumption [19], and C20:1 + C22:1, which indicate zooplankton consumption [20].

Combining stable isotope analysis (SIA) with fatty acid analysis (FAA) can provide more comprehensive information on fish nutritional ecology. SIA is suitable for studying long-term food intake and trophic levels [10], while FAA can reveal detailed information on recent food intake [14]. For example, using SIA and FAA together can reveal changes in the diet and nutritional dynamics of *S. japonicus* across different seasons and habitats. The combination of these two methods can compensate for the limitations of each individual method, providing more detailed and accurate information on the food web structure and energy flow [21].

Based on the aforementioned research background on the biodiversity of the northwest Pacific, this study examines the importance of *S. japonicus* and its feeding habits, aiming to contribute further insights into its role in the ecosystem. This study collected the samples of *S. japonicus* from specific areas of the high seas in the northwest Pacific from June to August 2021. This unique sampling location provided detailed regional and temporal context, ensuring the representativeness and reliability of the data. We explored the feeding habits of *S. japonicus* by combining stable isotope analysis (SIA) and fatty acid analysis (FAA). Through this comprehensive analytical approach, we aimed to gain a more thorough understanding of the role of *S. japonicus* in the northwest Pacific ecosystem and its impact on the food web structure and energy flow. These findings will not only provide new insights into the ecology of *S. japonicus* but also offer scientific support for its population management and the development of sustainable fisheries.

2. Materials and Methods

2.1. Sample Collection

The samples of *S. japonicus* were collected by the vessel "Song Hang" from June to August 2021 in the northwest Pacific (38°00′ N–43°00′ N, 147°58′ E–163°59′ E, Figure 1). Samples from each station were randomly selected from the catch. After classification and identification at sea, the samples were preserved in a freezer at −20 °C and transported to the laboratory. This study included 158 specimens of *S. japonicus*, comprising 84 males and 74 females.

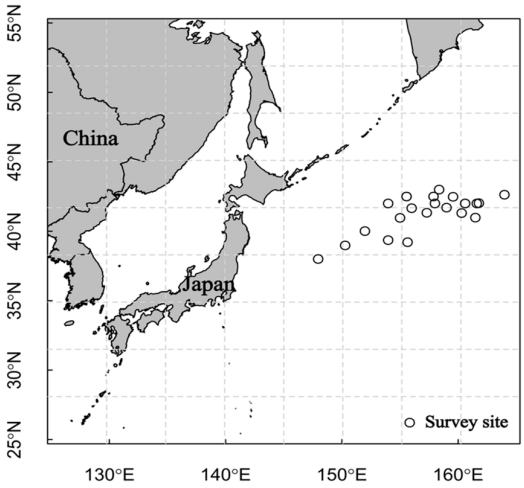

Figure 1. Map of the sampling locations of *S. japonicus*.

2.2. Sample Processing

We measured and recorded the body length, weight and sex of each specimen. Body length was determined using a measuring board, with total length defined as the distance from the anterior-most point of the head to the posterior-most point of the caudal fin, accurate to 1 millimeter (mm). Body weight was assessed using a balance with a high precision of 1 g. Gonad examination entailed a thorough analysis of the body structure, form and appearance, enabling precise sex determination. This facilitated the accurate determination of sex. For tissue analysis, muscle samples measuring approximately 3 cm by 3 cm were excised from the anterior end of the dorsal fin in *S. japonicus*. The samples were immediately frozen at −20 °C to maintain their integrity for subsequent analyses.

2.3. Stable Isotope Analysis

First, muscle tissues were separated from the outer epidermis and subsequently cleaned using ultrapure water. These tissues were subsequently freeze-dried for 24 h at −55 °C and subsequently ground into a fine powder. In the next step, lipids were extracted from the powdered muscle using a chloroform/methanol (2:1) solution for 24 h,

followed by drying at 40 °C for another 4 h. Then, the resulting defatted powder was transferred to 2 mL centrifuge tubes. These tubes were put into an elemental analyzer (Vario ISOTOPE Cube, Elementar Americas Inc., New York, NY, USA) and a stable isotope mass spectrometer (ISOPRIME100, IsoPrime Corporation, Lisle, IL, USA) for stable isotope determination. The outcomes were expressed as $\delta^{13}C$ and $\delta^{15}N$ values, which were computed using the following formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where δX represents ^{13}C or ^{15}N, and R denotes $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $\delta^{13}C$ values were based on PDB values (pee dee belemnite). $\delta^{15}N$ values were based on atmospheric nitrogen. Rstandard was the standard value. For quality control, three laboratory standards (protein $\delta^{13}C = -26.98‰$, $\delta^{15}N = 5.96‰$) were inserted into every ten samples. These calibrated carbon and nitrogen-stable isotopes provided an analytical precision of 0.06‰ during sample determination.

2.4. Fatty Acid Analysis

First, place 200 mg of sample powder into a test tube, add 15 mL of dichloromethane–methanol solution (volume ratio 2:1), and soak for 20 h at room temperature. After soaking, centrifuge to collect the supernatant, and rinse the residue with 10 mL of trichloromethane–methanol solution before centrifuging again to collect the supernatant. Combine the supernatants from both steps, and add 4 mL of 0.9% sodium chloride solution for washing; then, allow to stand for 2 h until the solution separates. Afterward, collect the dichloromethane layer from the round-bottom flask, and use nitrogen gas to remove the organic solvent. Finally, add 4 mL of 0.5 mol/L sodium hydroxide–methanol solution to redissolve, and obtain the crude fat sample from the muscle.

Add 4 mL of 0.5 mol/L potassium hydroxide–methanol solution to a round-bottom flask, and mix thoroughly. Next, connect the flask to a water bath reflux apparatus, and set the temperature to 100 °C, heating for 15–20 min. Then, add 4 mL of boron trifluoride–methanol solution to the flask, and continue boiling for 25–30 min. After completion, add 4 mL of n-hexane for a 2 min reflux extraction. During the experiment, cool the solution, then add 20–30 mL of saturated sodium chloride solution, and shake to homogenize. Pour the mixed solution into a test tube, and let it stand for 1–2 min to allow layer separation. Finally, use a syringe to extract a specific amount of the n-hexane layer for subsequent measurement and analysis.

2.5. Trophic Level Calculation

The nutrient level of each sample was estimated, and a continuous nutrient spectrum was constructed. The formula for estimating nutrient levels is as follows:

$$TL_{consumer} = TL_{base} + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/TEF \quad (2)$$

TLbase represents the trophic level of the primary consumer, and its value was set to 2 [22]. The nitrogen stable isotope values of the consumer and copepod baselines were represented by δ^{15}Nconsumer and δ^{15}Nbase, respectively. In this study, we set $\delta^{15}N$ base to 6‰ [23]. The trophic enrichment factor (TEF) was assumed to be 3.4‰ [24,25].

2.6. Data Analysis

In this study, we utilized Origin 2021 software to generate box plots illustrating $\delta^{13}C$ and $\delta^{15}N$ values for male and female *S. japonicus*. In the box plots used in this study, the box displays the third quartile (Q3) and the first quartile (Q1) at its upper and lower edges, respectively. These boundaries delineate the middle 50% of the data distribution. The horizontal line within the box represents the mean value, providing a clear visual indication of the central position of the distribution. The whiskers, extending from the box, denote the maximum and minimum values of the data but exclude those points

considered outliers. Outliers are defined as points that lie beyond 1.5 times the interquartile range (IQR) from the box. Fatty acid content was quantified as a percentage of total fatty acid content using the internal standard method and expressed as mean ± standard deviation (Mean ± SD). The Shapiro–Wilk test and Levene test were employed to confirm the normality and homogeneity of variances, validating the assumptions for subsequent analyses. Variations in stable isotope values and fatty acid composition across different body length groups were analyzed using one-way ANOVA, while differences in carbon and nitrogen isotopes and fatty acids between sexes were assessed using *t*-tests. The preliminary evaluation of normality and homogeneity of variances confirmed the appropriateness of these tests for isotopic comparisons. Mean distribution plots of $\delta^{13}C$ and $\delta^{15}N$ values across various body length groups were constructed using R 4.2.2, and niche width and overlap were calculated via the standard ellipse area (SEA_B) method, with visualization and analysis facilitated by the "SIBER" package in R 4.2.2 (Bayesian standard ellipse). Fatty acid percentage content data were standardized using the scale function in R, and principal component analysis (PCA) was conducted to identify the primary fatty acids contributing to observed differences based on loading strength. Furthermore, the Bray–Curtis similarity coefficient and similarity analysis (ANOSIM) were applied to compare differences in fatty acid composition among different body length groups of *S. japonicus*, with a significance threshold set at $p < 0.05$.

3. Results

3.1. Differences between Male and Female Individuals

The analysis revealed no significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between male and female individuals of *S. japonicus* (Table 1 and Figure 2). The results of fatty acid analysis (Table 2) showed a total of 28 fatty acids detected, including 10 saturated fatty acids (SFAs), 8 monounsaturated fatty acids (MUFAs) and 10 polyunsaturated fatty acids (PUFAs). Among them, MUFA had the highest content, followed by SFA, while PUFA had the lowest content. There were no significant differences in fatty acid composition between male and female individuals ($p > 0.05$).

Table 1. Stable isotope analyses of muscles for different body length groups and male and female individuals.

Species	Sex/BL	Number	$\delta^{15}N$ (‰)			$\delta^{15}C$ (‰)		
			Range	Mean ± SD	*p*	Range	Mean ± SD	*p*
S. japonicus	Male	70	7.58~11.69	10 ± 0.79	0.684	−20.50~−18.69	−19.65 ± 0.45	0.331
	Female	49	8.03~11.98	10.02 ± 0.76		−20.71~−18.43	−19.62 ± 0.42	
	100–130	29	8.75~11.52	9.83 ± 0.65	>0.05	−20.46~−18.74	−19.76 ± 0.39	>0.05
	131–160	27	8.03~11.69	10.14 ± 0.9		−20.71~−18.98	−19.73 ± 0.49	
	161–190	24	7.58~11.66	10.05 ± 0.9		−20.50~−18.69	−19.65 ± 0.51	
	191–220	19	8.23~10.8	9.89 ± 0.68		−20.19~−18.80	−19.53 ± 0.42	
	221–250	20	8.28~11.53	9.8 ± 0.74		−20.48~−19.08	−19.77 ± 0.40	

Table 2. Fatty acid profiles of females and males.

Fatty Acid	Female/%	Male/%	*p*
C14:0	1.92 ± 1.52	1.71 ± 1.41	0.39
C15:0	0.46 ± 0.21	0.42 ± 0.17	0.27
C16:0	25.30 ± 8.36	22.45 ± 7.84	0.13
C17:0	0.76 ± 0.27	0.74 ± 0.25	0.61
C18:0	4.53 ± 0.94	4.63 ± 1.44	0.63
C20:0	0.08 ± 0.05	0.08 ± 0.08	0.87
C21:0	0.05 ± 0.13	0.03 ± 0.02	0.14
C22:0	0.03 ± 0.05	0.03 ± 0.07	0.97

Table 2. *Cont.*

Fatty Acid	Female/%	Male/%	*p*
C23:0	0.04 ± 0.10	0.02 ± 0.03	0.20
C24:0	0.03 ± 0.04	0.03 ± 0.06	0.66
ΣSFA	33.20 ± 11.67	30.14 ± 11.37	0.35
C14:1n-5	0.05 ± 0.10	0.03 ± 0.04	0.10
C15:1n-5	0.29 ± 0.27	0.30 ± 0.26	0.07
C16:1n-7	3.39 ± 2.10	2.78 ± 1.97	0.09
C17:1n-7	0.45 ± 0.60	0.33 ± 0.20	0.07
C18:1n-9	6.13 ± 4.12	4.72 ± 2.85	0.20
C22:1n-9	0.27 ± 0.34	0.20 ± 0.26	0.13
C24:1n-9	0.39 ± 0.38	0.33 ± 0.31	0.30
C20:1	0.85 ± 0.60	0.72 ± 0.60	0.12
ΣMUFA	11.82 ± 8.51	9.41 ± 6.49	0.41
C18:2n-6	1.00 ± 0.35	1.05 ± 0.31	0.33
C18:3n-3	0.26 ± 0.14	0.31 ± 0.24	0.22
C18:3n-6	6.11 ± 1.86	5.38 ± 2.00	0.17
C20:2	0.34 ± 0.29	0.28 ± 0.13	0.18
C20:3n-3	0.56 ± 0.62	0.54 ± 0.33	0.66
C20:3n-6	0.46 ± 0.33	0.45 ± 0.18	0.71
ARAC20:4n-6	7.61 ± 2.95	8.17 ± 3.04	0.26
EPAC20:5n-3	2.42 ± 2.47	2.80 ± 3.03	0.41
C22:2n-6	0.26 ± 0.37	0.20 ± 0.26	0.24
DHAC22:6n-3	35.33 ± 12.90	40.68 ± 12.79	0.10
ΣPUFA	54.35 ± 22.28	59.86 ± 22.31	0.08

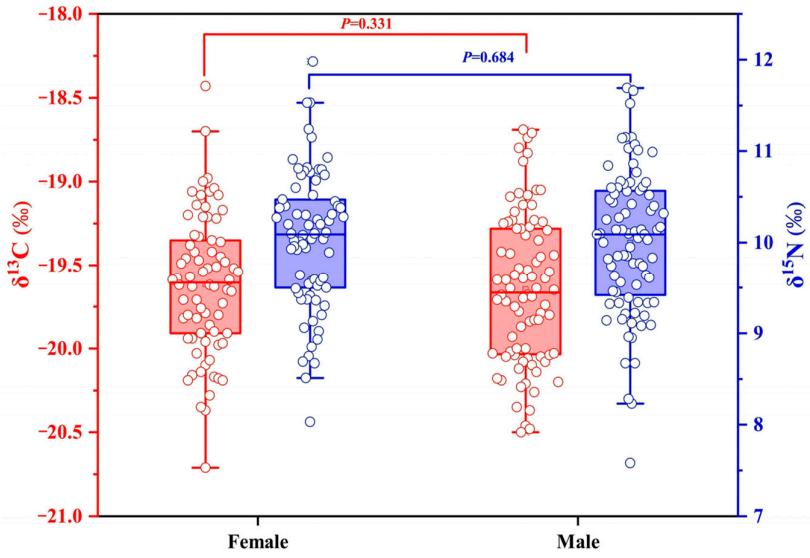

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ trend graph for females and males.

3.2. Differences among Different Size Groups

3.2.1. Differences in Stable Isotope Values among Different Size Groups

The results showed that there were no significant differences in $\delta^{15}N$ and $\delta^{13}C$ values between the different length groups ($p > 0.05$, Figure 3 and Table 3). The $\delta^{13}C$ and $\delta^{15}N$ values exhibited a trend of first increasing and then decreasing among the different length groups. The length groups 131–160 mm and 161–190 mm had higher average $\delta^{15}N$ values, at 10.14 ± 0.90‰ and 10.05 ± 0.90‰, respectively. The length groups 100–130 mm and 221–250 mm had very similar $\delta^{13}C$ and $\delta^{15}N$ values. By calculating the mean trophic levels of different length groups, the average trophic level of *S. japonicus* was found to

be 3.19. The average trophic levels of the five length groups were 3.13, 3.22, 3.19, 3.14 and 3.12, respectively.

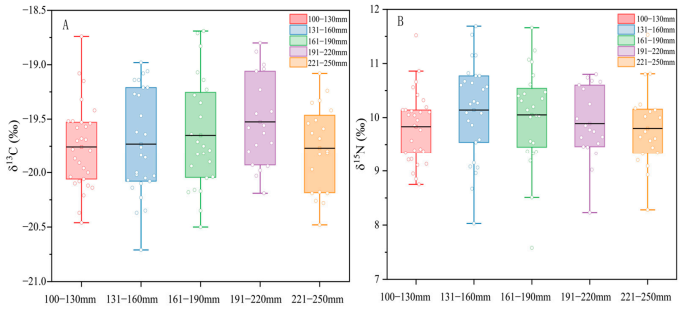

Figure 3. Mean distribution of different body length groups in $\delta^{13}C$ (**A**) and $\delta^{15}N$ (**B**) dimensions.

Table 3. Statistical analysis of the influence of body length on $\delta^{13}C$ and $\delta^{15}N$ isotopes.

Species	Isotope	Variable	Degree of Freedom	F	*p*	DE%
S. japonicus	$\delta^{13}C$	BL	4	10.09	0.33	37.6%
	$\delta^{15}N$	BL	4	1.902	0.844	58.4%

3.2.2. Niche Differences among Different Size Groups

The niche widths of *S. japonicus* in different length groups were 0.653‰^{2}, 0.895‰^{2}, 0.901‰^{2}, 0.735‰^{2} and 0.680‰^{2}, respectively (Table 4). The niche widths of the different length groups showed a trend of initially increasing and then decreasing (Figure 4), with the niche widths of the 100–130 mm and 221–250 mm length groups being similar. As the length increased, the niche overlap between adjacent groups first increased and then decreased, and the niche area overlap between all length groups was greater than 60%. The 161–190 mm length group of *S. japonicus* had the highest niche area overlap with other length groups and the largest niche width.

Table 4. Ecological niche width (‰^{2}) and ecological niche overlap (%) for different body length groups.

Body Length (mm)	100–130	131–160	161–190	191–220	221–250
100–130	0.653‰^{2}				
131–160	0.63	0.895‰^{2}			
161–190	0.63	0.79	0.901‰^{2}		
191–220	0.72	0.55	0.69	0.735‰^{2}	
221–250	0.89	0.71	0.70	0.66	0.680‰^{2}

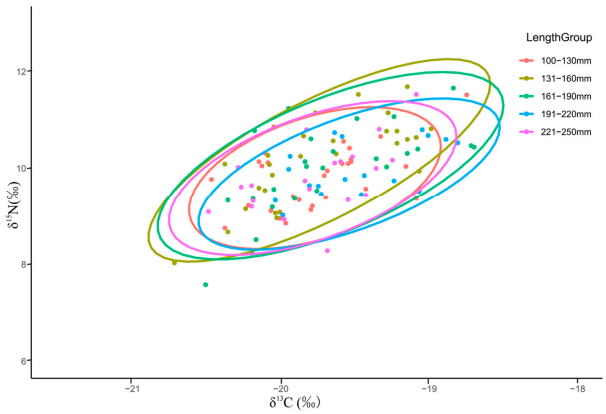

Figure 4. Isotopic niches of different body length groups.

3.3. Differences in Fatty Acids among Different Body Length Groups

3.3.1. Fatty Acid Composition among Different Body Length Groups

The analysis results presented in Table 5 indicate that the content of saturated fatty acids (SFAs) ranged from 26.07% to 33.36%. Among them, palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0) were predominant, accounting for 77.80%–81.03% of total SFAs. The monounsaturated fatty acids (MUFAs) content varied from 5.60% to 13.03%, with palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9) being the major constituents, representing 75.36%–80.42% of all MUFAs. Polyunsaturated fatty acids (PUFAs) ranged from 53.22% to 67.47%, with docosahexaenoic acid (DHA, C22:6n-3) constituting more than 65.69% of the total PUFAs.

Table 5. Fatty acid composition of muscles in different body length groups.

Fatty Acid	100–130 mm	131–160 mm	161–190 mm	191–220 mm	221–250 mm	*DF*	*F*	*p*
C14:0	1.26 ± 0.78	2.34 ± 1.97	1.82 ± 1.25	1.34 ± 1.37	1.26 ± 1.03	4	2.95	<0.05 *
C15:0	0.39 ± 0.17	0.51 ± 0.16	0.40 ± 0.13	0.35 ± 0.17	0.47 ± 0.23	4	3.30	<0.05 *
C16:0	19.02 ± 4.89	25.00 ± 4.26	18.92 ± 7.14	22.55 ± 5.90	24.32 ± 9.28	4	5.23	<0.01 **
C17:0	0.78 ± 0.19	0.93 ± 0.27	0.77 ± 0.19	0.62 ± 0.16	0.68 ± 0.24	4	6.62	<0.01 **
C18:0	4.36 ± 1.02	4.93 ± 0.84	4.69 ± 1.83	4.35 ± 0.69	4.75 ± 1.05	4	1.17	0.33
C20:0	0.10 ± 0.05	0.08 ± 0.03	0.09 ± 0.14	0.06 ± 0.05	0.08 ± 0.05	4	0.80	0.53
C21:0	0.06 ± 0.17	0.02 ± 0.01	0.03 ± 0.04	0.04 ± 0.06	0.06 ± 0.08	4	0.68	0.61
C22:0	0.03 ± 0.06	0.02 ± 0.01	0.04 ± 0.13	0.02 ± 0.01	0.04 ± 0.04	4	0.87	0.49
C23:0	0.04 ± 0.14	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.05 ± 0.05	4	0.85	0.50
C24:0	0.03 ± 0.05	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.03	0.11 ± 0.14	4	0.82	0.51
ΣSFA	26.07 ± 7.52	33.36 ± 6.85	26.27 ± 10.65	29.37 ± 8.45	32.90 ± 13.13	4	5.34	<0.05 *
C14:1n-5	0.04 ± 0.11	0.02 ± 0.01	0.03 ± 0.03	0.03 ± 0.03	0.11 ± 0.14	4	3.44	<0.05 *
C15:1n-5	0.16 ± 0.23	0.28 ± 0.19	0.49 ± 0.33	0.36 ± 0.19	0.44 ± 0.31	4	6.51	<0.01 **
C16:1n-7	1.66 ± 1.37	3.22 ± 1.96	2.30 ± 1.36	2.62 ± 0.99	3.58 ± 2.12	4	5.39	<0.01 **
C17:1n-7	0.26 ± 0.29	0.34 ± 0.17	0.34 ± 0.22	0.30 ± 0.16	0.69 ± 1.05	4	2.80	<0.05 *
C18:1n-9	2.56 ± 1.62	4.71 ± 2.78	4.37 ± 2.19	4.98 ± 2.18	6.12 ± 3.03	4	7.43	<0.01 **
C22:1n-9	0.23 ± 0.40	0.24 ± 0.31	0.31 ± 0.43	0.15 ± 0.08	0.32 ± 0.28	4	6.89	0.49
C24:1n-9	0.30 ± 0.33	0.28 ± 0.19	0.32 ± 0.39	0.27 ± 0.23	0.64 ± 0.61	4	3.84	<0.01 **
C20:1	0.39 ± 0.32	0.77 ± 0.50	0.79 ± 0.54	0.74 ± 0.50	1.13 ± 0.59	4	6.89	<0.01 **
ΣMUFA	5.60 ± 4.67	9.86 ± 6.11	8.95 ± 5.49	9.45 ± 4.36	13.03 ± 8.13	4	2.34	<0.01 **
C18:2n-6	0.94 ± 0.26	1.06 ± 0.44	1.12 ± 0.47	1.02 ± 0.23	1.11 ± 0.20	4	1.12	0.35
C18:3n-3	0.33 ± 0.33	0.25 ± 0.18	0.22 ± 0.14	0.27 ± 0.15	0.29 ± 0.15	4	0.91	0.46
C18:3n-6	3.45 ± 1.57	6.66 ± 1.20	6.26 ± 2.10	5.81 ± 1.96	5.60 ± 1.34	4	15.26	<0.01 **
C20:2	0.28 ± 0.20	0.30 ± 0.13	0.36 ± 0.28	0.30 ± 0.14	0.39 ± 0.38	4	0.89	0.47
C20:3n-3	0.84 ± 0.76	0.48 ± 0.21	0.65 ± 0.48	0.52 ± 0.48	0.50 ± 0.33	4	2.34	0.06
C20:3n-6	0.57 ± 0.48	0.38 ± 0.07	0.45 ± 0.19	0.48 ± 0.26	0.45 ± 0.15	4	1.68	0.16
ARAC20:4n-6	7.65 ± 3.60	8.52 ± 2.15	8.92 ± 3.45	9.14 ± 2.06	7.37 ± 2.77	4	1.53	0.2
EPAC20:5n-3	5.10 ± 4.73	1.90 ± 0.60	3.40 ± 2.88	1.96 ± 0.52	1.58 ± 0.57	4	7.50	<0.01 **
C22:2n-6	0.27 ± 0.45	0.17 ± 0.18	0.22 ± 0.27	0.22 ± 0.41	0.31 ± 0.33	4	0.59	0.67
DHAC22:6n-3	34.96 ± 16.14	36.98 ± 8.92	42.97 ± 10.95	41.18 ± 9.97	48.04 ± 8.44	4	5.63	<0.01 **
ΣPUFA	54.39 ± 28.53	56.70 ± 14.08	64.12 ± 21.07	60.69 ± 15.56	65.64 ± 14.66	4	4.59	<0.05 *

Notes: * indicates significant difference ($p < 0.05$), ** indicates highly significant difference ($p < 0.01$).

3.3.2. Differences in Fatty Acid Composition among Different Body Length Groups

The results of the one-way ANOVA (Table 5) showed that the content of saturated fatty acids and polyunsaturated fatty acids differed significantly among the different length groups ($p < 0.05$), while the content of monounsaturated fatty acids differed highly significantly ($p < 0.01$). Among the 28 detected fatty acids, 14 showed highly significant differences in content, namely C16:0, C17:0, C15:1n-5, C16:1n-7, C18:1n-9, C22:1n-9, C20:1, C20:4n-6 and DHA (C22:6n-3) ($p < 0.01$). Additionally, C14:0, C15:0, C20:2, C14:1n-5 and C17:1n-7 showed significant differences in content among different length groups ($p < 0.05$). According to the similarity analysis results (Table 6), the fatty acid compositions of the 100–130 mm length group were significantly different from those of the 131–160 mm and

161–190 mm length groups ($p < 0.05$). However, there were no significant differences among the other length groups ($p > 0.05$).

Table 6. ANOSIM analysis of fatty acid composition among different length groups.

Body Length	Similarity Coefficients	R	*p*
GroupA/GroupB	Bray–Curtis	−0.04	0.03
GroupA/GroupC	Bray–Curtis	0.18	0.04
GroupA/GroupD	Bray–Curtis	0.21	0.32
GroupA/GroupE	Bray–Curtis	0.33	0.23
GroupB/GroupC	Bray–Curtis	0.17	0.22
GroupB/GroupD	Bray–Curtis	0.16	0.76
GroupB/GroupE	Bray–Curtis	0.28	0.48
GroupC/GroupD	Bray–Curtis	0.44	0.35
GroupC/GroupE	Bray–Curtis	0.49	0.01
GroupD/GroupE	Bray–Curtis	0.06	0.22

Notes: GroupA represents 100–130 mm, GroupB represents 131–160 mm, GroupC represents 161–190 mm, GroupD represents 191–220 mm, and GroupE represents 221–250 mm; R > 0 indicates that the between-group difference is greater than the within-group difference, and R < 0 indicates that the between-group difference is less than the within-group difference; $p < 0.05$ indicates significant differences between or within groups.

We selected fatty acids from different length groups for principal component analysis (PCA), ensuring that each fatty acid content exceeded 1%. The results (Figure 5 and Table 7) showed that there were three principal components with initial eigenvalues greater than 1, with variance contributions of 51.57%, 14.99% and 11.46%. This indicated that the results reflect the information of fatty acid content in the muscle of *S. japonicus*. Principal components 1 and 2 were able to better distinguish the samples from different length groups. Combined with the component matrix results and the relative loading intensity and direction of the main vectors, it could be seen that C14:0, C16:0, C18:0, C16:1n-7, C18:1n-9, C18:3n-6, C20:5n-3, C20:4n-6 and 22:6n-3 were the main fatty acids causing the differences among the different length groups.

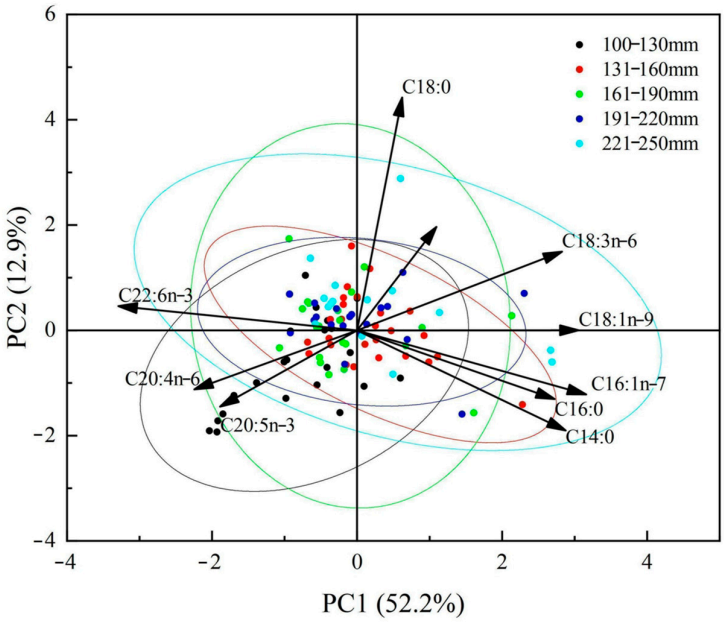

Figure 5. Principal component analysis of the fatty acid compositions among different body length groups. Note: Vectors indicate the relative load intensity and direction of major fatty acids.

Table 7. Results of principal component analysis for different fatty acids.

Fatty Acid	PC1	PC2
C14:0	0.35	−0.31
C16:0	0.34	−0.21
C18:0	0.07	0.74
C16:1n-7	0.39	−0.20
C18:1n-9	0.38	0.13
C18:3n-6	0.35	0.25
EPAC20:5n-3	−0.24	−0.24
ARAC20:4n-6	−0.28	−0.19
DHAC22:6n-3	−0.41	0.08

3.3.3. Characteristic Fatty Acids and Dietary Differences

By comparing the percentage changes in characteristic fatty acid content among different length groups (Table 8), the results showed that C18:1n-9 could indicate zooplankton, and C16:1n-7 could indicate diatoms, with both fatty acids increasing in content with body length. DHA (22:6n-3) was the primary fatty acid in all length groups, suggesting that fish might be the main food source for these groups. ARA (20:4n-6) remained relatively stable across all groups and could indicate crustaceans, but its content slightly decreased with increasing body length. EPA (20:5n-3) could indicate krill, with its content decreasing as the body length increased. C18:3n-3 and C18:2n-6 could indicate green algae, while C15:0 and C17:0 could indicate planktonic bacteria, but the content of these food sources was low in all length groups.

Table 8. Content and dietary indicators of signature fatty acids across different body length groups.

Signature Fatty Acid	Corresponding Sources	100–130 mm	131–160 mm	161–190 mm	191–220 mm	221–250 mm
C18:1n-9	Zooplankton [19]	2.56 ± 1.62	4.71 ± 2.78	4.37 ± 2.19	4.98 ± 2.18	6.12 ± 3.03
C16:1n-7	Diatoms [20]	1.66 ± 1.37	3.22 ± 1.96	2.30 ± 1.36	2.62 ± 0.99	3.58 ± 2.12
DHAC22:6n-3	Fish [20]	34.96 ± 16.14	36.98 ± 8.92	42.97 ± 10.95	41.18 ± 9.97	48.04 ± 8.44
ARAC20:4n-6	Crustacea [7]	7.65 ± 3.60	8.52 ± 2.15	8.92 ± 3.45	9.14 ± 2.06	7.37 ± 2.77
EPAC20:5n-3	Krill [26]	5.10 ± 4.73	1.90 ± 0.60	3.40 ± 2.88	1.96 ± 0.52	1.58 ± 0.57
C18:3n-3 C18:2n-6	Green Algae [27]	0.94 ± 0.26/ 0.94 ± 0.26	1.06 ± 0.44/ 0.25 ± 0.18	1.12 ± 0.47/ 0.22 ± 0.14	1.02 ± 0.23/ 0.27 ± 0.15	1.11 ± 0.20/ 0.29 ± 0.15
C15:0 C17:0	Planktonic Bacteria [28]	0.39 ± 0.17/ 0.78 ± 0.19	0.51 ± 0.16/ 0.93 ± 0.27	0.40 ± 0.13/ 0.77 ± 0.19	0.35 ± 0.17/ 0.62 ± 0.16	0.47 ± 0.23/ 0.62 ± 0.16

4. Discussion

4.1. Differences between Males and Females

Stable isotope analysis has been widely applied to the study of marine fish, providing valuable information about their nutritional ecology, habitat use and migration behavior [24]. We assessed the feeding habits of male and female *S. japonicus* individuals using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis. The results showed no significant differences in the carbon and nitrogen stable isotope ratios between males and females, indicating similar feeding habits. This finding differs from other studies on marine fish. For example, a study on sockeye salmon (*Oncorhynchus nerka*) [29] showed no significant difference in $\delta^{13}C$ values between males and females but a significant difference in $\delta^{15}N$ values. Another study found that three-spined sticklebacks (*Gasterosteus aculeatus*) showed no significant difference in $\delta^{15}N$ values between sexes, but females had significantly higher $\delta^{13}C$ values than males, indicating possible differences in food sources [30]. Unlike these studies, *S. japonicus* showed no significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values between males and females, suggesting they feed on similar primary food sources within the same habitat and have no significant differences in energy utilization. This highly consistent feeding habit may be related to the ecological characteristics and habitat of *S. japonicus*.

Additionally, *S. japonicus* may have no significant sexual differentiation in life history strategies, leading to high consistency in trophic levels and food chain positions [31].

In studying ecosystems and biological behavior, fatty acid composition analysis provides a unique methodology in addition to stable isotope analysis. Fatty acids serve as biomarkers, reflecting the diet and nutritional status of organisms, and their composition is significantly influenced by food sources [15]. In this study, both male and female *S. japonicus* individuals contained the same types of fatty acids, with 28 fatty acids detected (Table 2). There were no significant differences in fatty acid composition between males and females ($p > 0.05$), indicating that gender is not a major factor affecting the fatty acid composition of *S. japonicus*. A study by Muro-Torres et al. (2023) found no significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between male and female yellowtail snapper (Ocyurus chrysurus) [32]; the differences were mainly related to body length, with no significant differences in the types and quantities of their diet. Although this study used stable isotope methods, the results are similar to those of the present study. Baranek et al. (2024) analyzed the fatty acid composition of different genders of rainbow trout (Oncorhynchus mykiss) and found no significant differences in major fatty acids, such as DHA and EPA, between males and females [33]. Additionally, Usydus et al. (2012) studied the fatty acid composition of Baltic herring (Sprattus sprattus balticus) and found no significant differences between males and females under similar environmental and feeding conditions [34]. These findings suggest that there are no significant differences in fatty acid composition between male and female fish, indirectly indicating that their feeding habits are not substantially different.

4.2. Differences among Different Size Groups

4.2.1. Stable Isotope Differences

Stable isotope analysis of carbon and nitrogen is widely used in ecology to study the food web structure and trophic position of animals. The carbon isotope ratio ($\delta^{13}C$) typically reflects food sources and habitats [35], while the nitrogen isotope ratio ($\delta^{15}N$) indicates the trophic level [36]. In this study, stable isotope analysis was conducted on different length groups of *S. japonicus*. There were no significant differences in $\delta^{15}N$ and $\delta^{13}C$ values between the different length groups ($p > 0.05$). Notably, both $\delta^{13}C$ and $\delta^{15}N$ values exhibited a significant inverted U-shaped trend among the different length groups. For $\delta^{15}N$ values, the length groups 131–160 mm and 161–190 mm had slightly higher values, suggesting that individuals in these groups might be at higher trophic levels. This phenomenon may reflect changes in the feeding habits within these length ranges, or the sample size and within-group variability may mask more subtle feeding differences. Similar phenomena have been observed in other studies. For example, Ben-Aderet et al. (2020) found that yellowtail kingfish showed minimal individual differences in $\delta^{15}N$, but $\delta^{13}C$ had a strong correlation with fork length and body weight [37]. The similarity in $\delta^{15}N$ was attributed to high trophic level feeding, while differences in $\delta^{13}C$ among individuals reflected spatial variability in resource utilization.

Primary producers in coastal areas typically have higher $\delta^{13}C$ values, while primary producers in pelagic areas have lower $\delta^{13}C$ values [38]. This suggests that the higher $\delta^{13}C$ values of the length groups 131–160 mm, 161–190 mm and 191–220 mm of *S. japonicus* may indicate a greater reliance on coastal or benthic food sources. In contrast, the length groups 100–130 mm and 221–250 mm may predominantly feed on pelagic prey. Additionally, we observed that the $\delta^{13}C$ and $\delta^{15}N$ values of the 100–130 mm and 221–250 mm length groups were very similar. The similar $\delta^{13}C$ values suggest that these two length groups may feed on similar food sources or within similar ecosystems, and the similar $\delta^{15}N$ values indicate that these length groups occupy similar positions in the food chain [39]. This phenomenon may reflect that the foraging behavior and ecological niche of the fish in these two length ranges have not significantly changed. This could be because fish in these length groups occupy similar ecological niches, utilize similar resources or face similar ecological pressures in the environment [40]. Moreover, the observed similarity in food sources among different length groups of *S. japonicus* may be attributable to their migratory behavior. *S. japonicus*

exhibits distinct migratory patterns and habitat use that can significantly influence their feeding ecology. According to previous research, *S. japonicus* in the East China Sea migrates between different spawning and feeding grounds throughout the year [41]. This seasonal migration is often driven by changes in environmental conditions, such as temperature and food availability. Similarly, studies have shown that *S. japonicus* in the western north Pacific demonstrate seasonal movements and habitat shifts, adapting to varying ecological conditions [42]. During migration, *S. japonicus* adjusts its foraging strategies based on varying environmental conditions, likely leading to a convergence in food sources among different length groups [43]. This adaptability allows individuals of varying sizes to maintain similar diets despite potentially differing life stages. By calculating the average trophic levels for different length groups, the average trophic levels for the 100–130 mm and 221–250 mm length groups were found to be 3.13 and 3.12, respectively, further supporting the similarity between these two length groups.

4.2.2. Trophic Ecology of *S. japonicus*

The nutritional niche reflects a species' function and position within an ecosystem, influenced not only by its biological characteristics but also by interactions with other species and environmental factors [44]. Variations within a species' nutritional niche highlight the diversity in resource utilization and environmental adaptation among individuals or groups, which is crucial for adapting to environmental changes and maintaining population stability [45]. The concept of the nutritional niche plays a pivotal role in exploring the complexity and diversity of ecosystems [46,47].

This study used the Bayesian standard ellipse area (SEA) method to calculate the niche width and overlap among different length groups. The results showed that as *S. japonicus* grows, its niche width initially increases and then decreases, reaching its maximum in the 191–220 mm length group. This may be related to the diversified feeding behaviors within this length group. Similar studies have found that fish may exhibit a broader range of food sources at certain growth stages, leading to an increase in niche width [37]. As the body length of *S. japonicus* increases, the number of prey types initially increases and then decreases [48]. This phenomenon may be due to the development of feeding structures, such as the mouthparts [49], which gradually enhance feeding capability, leading to a dietary shift from zooplankton to fish and shrimp. This observation is consistent with the results of this study. The niche width of the 100–130 mm and 221–250 mm length groups is similar, indicating significant overlap in food resources and habitat selection between these two length groups. They may prey on similar types of food, utilize similar habitats and exhibit similar behavioral patterns [39].

The niche areas of different length groups of *S. japonicus* exhibit a high degree of overlap, indicating significant intraspecific competition. The 161–190 mm length group of *S. japonicus* shows the highest niche area overlap with other groups and also has the widest niche width. This suggests that individuals in this length group utilize a broader range of resources, possibly due to being in a rapid growth phase and having higher demands for diverse food resources [50]. Additionally, resource competition is most intense at this stage, and individuals may converge on similar food resources and habitats to secure enough resources [11]. This phenomenon aligns with the theory of intraspecific competition, which suggests that in environments with limited resources, individuals tend to utilize similar resources, leading to increased niche overlap [51].

In summary, *S. japonicus* exhibits high adaptability in resource utilization, resource competition and response to environmental changes at different growth stages. This adaptability allows different length groups to survive and reproduce under the same environmental conditions [52]. These findings are significant for understanding the ecological characteristics of *S. japonicus* and for developing effective conservation and management strategies.

4.3. Fatty Acid Analysis

4.3.1. Differences in Fatty Acid Composition among Different Body Length Groups

According to the results of one-way ANOVA (Table 5), there are significant differences in the fatty acid composition among different length groups of *S. japonicus*. Among the 28 fatty acids detected in the muscles of *S. japonicus*, 16 showed significant differences in content among different length groups, including saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, such as C14:0, C16:1n-7, C20:5n-3 and C22:6n-3. These variations in fatty acids may reflect changes in the energy demands of fish of different lengths during physiological metabolism or the influence of environmental conditions, such as food sources and temperature, on fatty acid composition [53]. Combining the results of the principal component analysis, the main fatty acids characterizing *S. japonicus* include C14:0, C16:0, C18:0, C16:1n-7, C18:1n-9, C18:3n-6, C20:5n-3 and C22:6n-3. This indicates that the demand for energy and nutrients may change with increasing body length, which in turn affects physiological processes, such as growth and development, the immune system and the nervous system.

According to the similarity analysis results (Table 6), the fatty acid compositions of the 100–130 mm length group were significantly different from those of the 131–160 mm and 161–190 mm length groups ($p < 0.05$). This indicated significant differences in metabolic demands and environmental adaptability between smaller and medium-sized *S. japonicus*. This might be because smaller fish were in a rapid growth stage and had higher metabolic demands, requiring different fatty acids to support cell membrane growth and energy metabolism [54]. Additionally, there were significant differences in fatty acid composition between the 161–190 mm and 221–250 mm length groups, indicating that further increases in body length might involve new metabolic adjustments or environmental adaptations [55]. For example, larger fish might require more energy to maintain physiological functions, or their food sources might change, affecting fatty acid composition [56].

It is noteworthy that there are no significant differences in the fatty acid composition between the 100–130 mm and 221–250 mm length groups, and there are also no significant differences in their stable isotope values. While it is true that both stable isotopes and fatty acids can indicate diet, they each provide different layers of information. $\delta^{13}C$ can suggest the primary productivity source (i.e., whether the food source is more terrestrial or marine, or benthic vs. pelagic) [10,11], whereas fatty acids can give more detailed information about the types of fats the fish are consuming, which can be traced back to specific types of prey like different kinds of plankton or fish [14]. First, there are no significant differences between the two length groups, suggesting they may inhabit similar environmental conditions. This similarity could extend to accessing the same types of prey within their respective ecosystems [57]. Second, the migratory behavior of *S. japonicus* might also contribute to the similarity in dietary profiles between different length groups. If these fish undertake migrations that traverse similar feeding grounds, or if their migratory routes overlap during crucial feeding times of the year, this could result in similar dietary intakes, regardless of size or age [42]. This behavior would ensure that both juvenile and adult stages of *S. japonicus* exploit similar food resources, leading to a convergence in their fatty acid profiles. Stable isotope analysis, particularly $\delta^{13}C$, could indeed corroborate the first two points regarding the stable environment and migratory patterns affecting the diet of *S. japonicus*. The final aspect to consider is the physiological or developmental needs of *S. japonicus* across different stages of growth. Fatty acids play critical roles in energy storage, membrane structure and as precursors to important signaling molecules [7]. Similar fatty acid profiles among the groups might indicate that these different length groups have similar nutritional requirements, which are met through the consumption of similar types of fatty acids [58]. The similarity in dietary needs could thus reflect an evolutionary adaptation to ensure developmental stability across various life stages.

In marine organisms, saturated fatty acids (SFAs) primarily served as an energy source. They were ubiquitously present in the body and could be synthesized endogenously. Especially during physical activity, SFAs were preferentially utilized to meet essential energy

needs [59]. Fatty acids such as C14:0, C16:0 and C18:0 provided energy for fish growth and development, regulated immune cell activity and contributed to the construction and maintenance of cell membrane structure and function, playing vital roles in fish metabolism [53]. Monounsaturated fatty acids (MUFAs) had numerous vital physiological and biochemical functions in fish. These functions ranged from energy provision to the maintenance of cellular structures and support for environmental adaptability. Oleic acid C18:1n-9, stored abundantly as a vital energy source, ensured the normal development of ovaries [60]. Palmitoleic acid C16:1n-7 played various roles in fish, from maintaining cellular structures and functions to participating in energy metabolism and physiological regulation. Organisms supplemented fatty acids mainly through biosynthesis and dietary intake. Fish, as higher level aquatic organisms, generally could not synthesize polyunsaturated fatty acids (PUFAs) on their own and had to rely on consuming lower trophic level plankton to obtain these essential fatty acids [61]. Arachidonic acid C20:4n-6 regulated fish functions, helping fish resist adverse environmental effects [62] and enhancing the quality of juvenile fish [63]. DHA was an essential fatty acid for fish growth and development [64], with significant amounts required for the development of visual and brain nerves in juvenile fish [65]. Studies showed [66] that EPA played a crucial role in tissue biosynthesis and was vital for the survival and development of larvae [67]. Furthermore, like DHA, EPA maintained the integrity of biomembrane structures and functions, especially under cold conditions [68].

4.3.2. Dietary Differences

The types of fatty acids in organisms were primarily determined by the fatty acids present in their diet, and these specific fatty acids could serve as indicators of food sources [69]. For SFAs, they were ubiquitously present in the body and could be synthesized endogenously, thus not providing accurate information as indicators. The characteristic fatty acids corresponding to planktonic bacteria in the food web were C15:0 and C17:0 [28], indicating that planktonic bacteria contributed to the energy sources of *S. japonicus*, possibly from suspended particles or detritus attached to bacteria. Meanwhile, C14:0 and C16:0 played important roles in providing energy and maintaining cell structure and function. The content of these fatty acids showed a trend of first increasing and then decreasing with body length. The study by GONÇALVES et al. (2012) showed that phytoplankton in marine ecosystems affected the fatty acid composition of organisms, and each type of phytoplankton could be indicated by certain characteristic fatty acids [70].

For MUFAs, C16:1n-7 indicated diatoms [20], with C16:1n-7 comprising 30% of MUFAs, serving as an important food source for *S. japonicus*. Fatty acid C18:1n-9 indicated zooplankton [19]. Diatoms, the most efficient photosynthetic organisms in the world, were also a preferred food source for zooplankton, fish and shrimp. *S. japonicus* likely ingested these by feeding on planktonic diatoms or zooplankton. The overall rising content of C16:1n-7 and C18:1n-9 suggested an increasing proportion of zooplankton and diatom consumption by *S. japonicus*.

Organisms primarily relied on biosynthesis and dietary intake to replenish their fatty acid content. In terms of fatty acid synthesis, lower organisms had a greater capacity than higher organisms; most polyunsaturated fatty acids (PUFAs) could not be synthesized by the body and had to be obtained through the consumption of lower trophic level planktonic flora and fauna [71]. EPA indicated krill, and its content generally decreased as the body length increased. DHA indicated small fish species [20], with its content trending upwards, suggesting that as the body length increased, fish gradually became the primary diet of *S. japonicus*. C18:3n-6 and ARA C20:4n-6 showed an initial increase followed by a decrease. ARA C20:4n-6 indicated crustaceans [7], and its content showed an increasing and then decreasing trend, with no significant differences between different body length groups, indicating a consistent intake of crustaceans by *S. japonicus*. To meet its growth needs, *S. japonicus* changed its feeding strategies. This strategy was also reflected in individual development processes; generally, the DHA/EPA ratio increased with the trophic level [72]. In this study, as the body length increased, the DHA/EPA ratio of *S. japonicus* showed an

increasing trend, indicating a dietary shift during growth and development. As the body length increased, they shifted from consuming food sources with low DHA/EPA ratios to those with high DHA/EPA ratios [73].

5. Conclusions

This study analyzed the dietary habits of *S. japonicus* in the open waters of the northwest Pacific by measuring the stable isotope values and fatty acid compositions of their muscles across different sexes and body size groups. The results showed no significant differences in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios or fatty acid compositions between male and female individuals. *S. japonicus* individuals in the length group 131–160 mm were at a higher trophic level. There were considerable niche overlaps among different length groups, indicating strong intraspecific competition. As growth proceeded, the niche breadth initially increased and then decreased. Increased body length enhanced their range of movement and predatory capabilities, allowing them to utilize a broader variety of food resources. However, further growth led to increased dependence on and preference for specific resources, narrowing the niche breadth and intensifying intraspecific competition. Changes in fatty acid content reflected shifts in the energy and nutritional demands of *S. japonicus*, gradually transitioning from feeding on krill to diatoms, zooplankton, fish and crustaceans. This study reveals the dietary habits and ecological niche shifts of *S. japonicus* across different sexes and body lengths in the open waters of the northwest Pacific, providing a crucial foundation for further understanding their ecological traits. Future research could employ genomics and metabolomics to explore the metabolic pathways and gene expressions of *S. japonicus* at different growth stages and under various environmental conditions for a more precise understanding of their energy utilization and nutritional demands.

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