Stable Isotope Analysis Revealed Ontogenetic Changes in Trophic Ecology and Migration Patterns of *Sepia esculenta* in the Northern Coastal Waters of China

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Golden cuttlefish (*Sepia esculenta*), the most economically important cephalopod in the northern coastal seas of China, had experienced greatly reduced population biomass due to continuous fishing pressure in recent decades. Understanding the trophic ecology and clarifying the spatial distribution of wintering ground can help develop management plans for this species. In this study, the ontogenetic changes in the trophic ecology of *S. esculenta* in the Qingdao coastal water were determined, and the migration patterns were studied using stable isotope analysis. Cluster analysis based on isotopic values divided *S. esculenta* with different lengths into groups: 11–20, 21–100, and 121–200 mm. A significant difference in the δ^{13}C values between the groups 11–20 mm ($-17.10‰$) and 21–100 mm ($-15.89‰$) illustrates an ontogenetic change in the feeding habits. Due to the migratory habits of *S. esculenta*, the δ^{13}C value of the group 121–200 mm ($-16.39‰$) was lower than that of the group 21–100 mm. The δ^{15}N values of *S. esculenta* were found to increase in length, and there was a clear linear relationship between different *S. esculenta* groups, suggesting that the wintering ground may locate in the same latitude as the spawning ground (i.e., the middle Yellow Sea). Furthermore, the trophic relationship between *S. esculenta* and coexisting species was assessed, revealing that the group 11–20 mm of *S. esculenta* has some overlap of carbon isotope space with other species, suggesting that these species may feed on it as prey. Thus, slightly increasing the length to more than 20 mm may reduce the pressure of being the prey of post-release juveniles of *S. esculenta* and improve the effect of release.

Keywords: *Sepia esculenta*, ontogeny, dietary shift, trophic ecology, isotopic analysis, resource management

INTRODUCTION

Sepia esculenta (Hoyle, 1885), also known as golden cuttlefish, is distributed in Chinese, South Korean, and Japanese coastal waters and has fixed migratory habits of pelagic wintering and inshore spawning (Zheng et al., 2004). In China, *S. esculenta* was the most economically important cephalopod in the northern coastal seas and was listed as one of the four major fisheries in the Bohai

Sea and the Yellow Sea until the 1970s (Dong, 1991). Since then, the abundance of this species has been greatly reduced due to continuous fishing pressures and dwindling spawning fields (Hao et al., 2007). Due to this, the northern region of China has adopted a variety of resource conservation measures to restore the stock situation of *S. esculenta*. The release of artificially hatched juveniles has been considered a chief measure to compensate for this decline in *S. esculenta*, and the number of releases is increasing year by year (Zhang et al., 2019). In addition, *S. esculenta* lays sinking eggs, so they wrap their fertilized eggs around the egg-attaching matrix for hatching. Therefore, it is also an important means to protect its resources by placing artificial spawning attachments into the sea during the breeding period of *S. esculenta* (Niu et al., 2017). At present, the catches and resource status of *S. esculenta* are slowly recovering under the joint action of multiple resource conservation measures (Zhang et al., 2019).

Understanding the diets and trophic ecology of species can help to promote ecological knowledge and develop management plans for commercial species (Zhang et al., 2019); however, there have been no such investigations of *S. esculenta*. Unlike most fishes, which swallow prey whole, cephalopods tear food into small pieces, making it more difficult to identify the prey species and hindering the applicability of the traditional, intuitive method of stomach contents (Cherel and Hobson, 2005). This may be the main hindrance to conducting relevant research of *S. esculenta*. In addition, spawning grounds and wintering grounds are indispensable and important areas in the life cycle of migratory species, so it is of great significance to clarify their spatial distribution and strengthen management to maintain the structure and quantity of fishery stocks (Li et al., 2018). The *S. esculenta* spawning off the coast of China can be divided into two geographical populations: one spawn in Zhoushan-Yangtze River estuary and the other spawn in the southeastern coastal waters of Shandong Province (Wang et al., 2019), but the wintering grounds of both populations are not yet clear.

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can reflect the long-term dietary sources, and trophic levels of predators provide powerful tools for researching the trophic ecology of species (Peterson and Fry, 1987; Layman et al., 2012). The carbon isotopic signatures of predators are slightly enriched at a small level compared with those of their prey and are thus used to provide references to the feeding habits and dietary compositions of consumers (McCutchan et al., 2003). In contrast, the nitrogen isotopic signatures of organisms are normally used to estimate trophic levels because there is a greater enrichment in consumers over their prey (Fry, 1988; Zanden and Rasmussen, 2001). Through isotopic analysis, the trophic ecology has been investigated for many cephalopods including *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* (Parry, 2008), *Architeuthis dux* (Guerra et al., 2010), *Sepia officinalis* (Hobson and Cherel, 2006), *Spirula spirula* (Ohkouchi et al., 2013), and *Doryteuthis gahi* (Rosas-Luis et al., 2017). In addition, stable isotope analysis has also been used to study the migratory patterns of cephalopods due to the difference in stable isotopic signatures in different marine ecosystems (e.g., the relative abundance of ^{13}C in coastal food webs is typically higher

than that in oceanic food webs), such as *Dosidicus gigas* (Argüelles et al., 2012; Trasviña-Carrillo et al., 2018), *S. officinalis* (Rooker et al., 2020), and *Illex argentinus* (Rosas-Luis et al., 2017; Queirós et al., 2019).

Qingdao is one of the main spawning grounds of the *S. esculenta* population along the southeastern coastal waters of Shandong Province and is also one of the main areas of the *S. esculenta* proliferation and conservation in northern China (Zhang et al., 2019). In this study, the ontogenetic changes in the trophic ecology of *S. esculenta* in the coastal waters of Qingdao were determined using carbon and nitrogen stable isotope analysis, and the trophic relationships between *S. esculenta* and other species were further clarified. In addition, the migration pattern of the *S. esculenta* population in this area was studied, and the spatial distribution of its wintering ground was speculated. The purpose of this study is to enrich the basic biological knowledge of *S. esculenta* and to provide references for resource management.

MATERIALS AND METHODS

Sample Collection

The study area (water depth range: 25–30 m) is located in the coastal waters of Qingdao, in the western Yellow Sea (**Figure 1**). Juvenile and immature individuals (mantle length: 11–100 mm) of *S. esculenta* were collected using a trawl net of a commercial fishing vessel in September 2017. Other coexisting species were also extensively caught by the trawl net and were identified to the lowest taxonomic level. These species contain crustaceans, cephalopods, and teleosteans. Considering the diversity of teleosteans species, they were further subdivided into mesodemersal and demersal fishes based on their ecological habits such as the distribution of the water layer (excerpted from Fishbase[1]). Zooplankton was collected by oblique towing with the net mesh size of 300 μm at the same station to represent baseline species in this study. Adult *S. esculenta* (mantle length: 120–200 mm) were collected in June 2018, which fell into the closed fishing period in the Yellow Sea. Therefore, *S. esculenta* adults were captured using cages. The mantle length of the *S. esculenta* specimens ranged from 11 to 200 mm, which essentially covered the size range observed throughout its life cycle, with the maximum mantle length of about 200 mm (Natsukari and Tashiro, 1991). Due to the mesh size limitations of the sample nets, we did not capture *S. esculenta* larvae less than 10 mm in mantle length. The mantle length of newborn *S. esculenta* was 4–5 mm, with egg yolk carried in the mouth, which was the main nutritional source before starting prey (Wang et al., 2018). In the earliest stages of a species, they initially carry stable isotopic traits from their mothers for some time, especially those that feed on the yolk after hatching (Rudolf, 2020). Therefore, the introduction of *S. esculenta* larvae less than 10 mm will not help to increase the integrity of this study. All the samples were carefully stored at −20°C until laboratory processing.
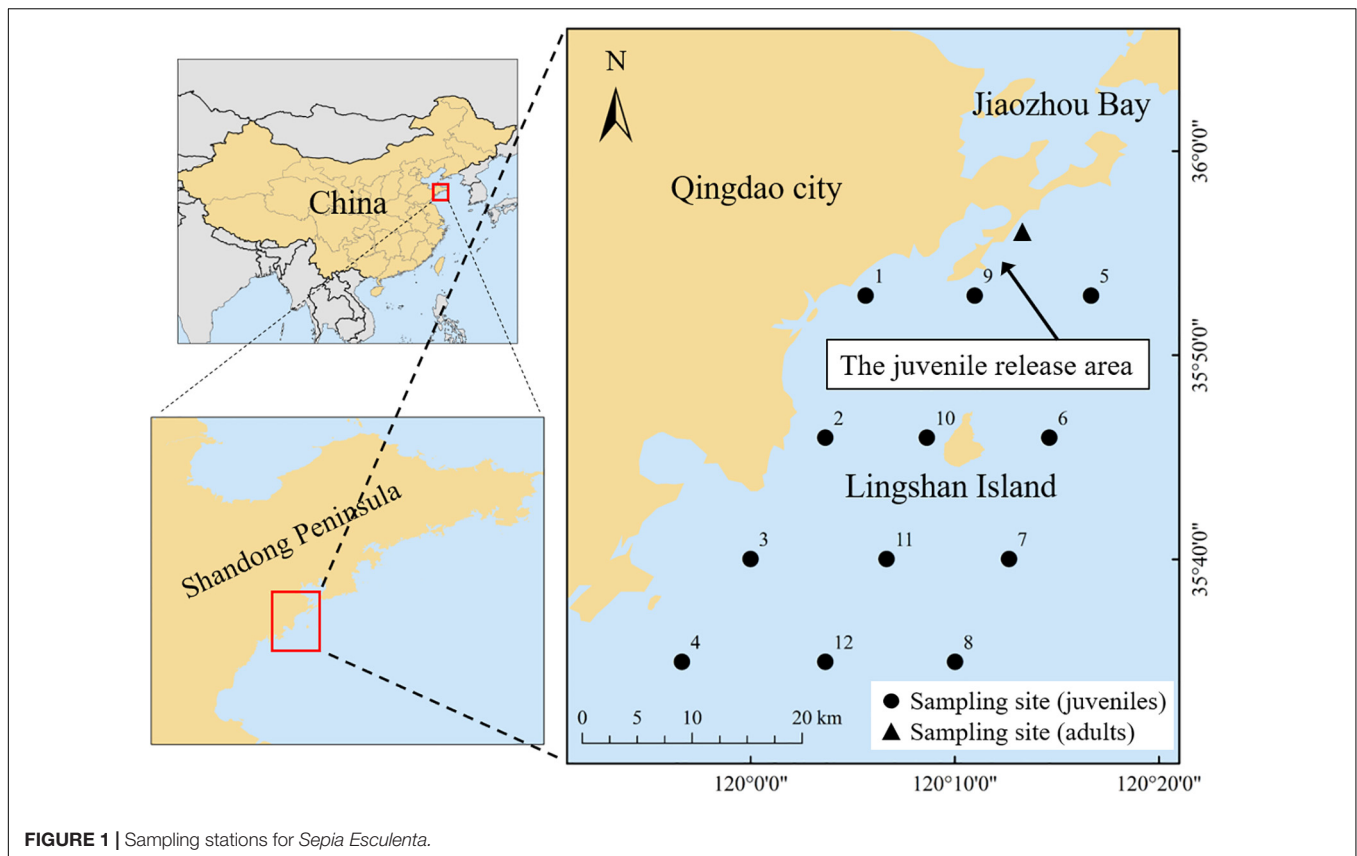
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[1]www.fishbase.org

FIGURE 1 | Sampling stations for *Sepia Esculenta*.

Sample Treatment

Details on sample treatment and analysis were provided in a study by Newsome et al. (2004). The length and weight of all the specimens were measured to the nearest 0.1 mm and 0.1 g, respectively, and the *S. esculenta* samples were classified at mantle length intervals of 10 mm. For the stable isotope analyses, chitinous beaks and soft tissues, such as dorsal muscle and wrist, have typically been applied as research materials in trophic ecology studies of cephalopods (Parry, 2008; Guerra et al., 2010; Ohkouchi et al., 2013; Guerreiro et al., 2015; Rosas-Luis et al., 2017; Trasviña-Carrillo et al., 2018; Golikov et al., 2019; Urrutia-Olvera et al., 2021), but there were significant differences in isotopic ratios among various tissues (Cherel et al., 2009a). These tiny differentiae could bias the results of experiments (McCutchan et al., 2003), especially in dietary research of consumers based on the $\delta^{13}C$ values. Consequently, pairwise comparisons of the stable isotopic values between tissues (muscle tissue of the wrists and wings of the later beaks) from the same *S. esculenta* individuals were performed before the formal experiment in this study. Then, a small section of muscle tissue was sampled from the wrist of the *S. esculenta* and other cephalopods, together with dorsal muscles, abdominal muscles, and claw muscles for fishes, shrimps, and crabs, respectively. When available, six individuals from each species were randomly selected for isotopic determination. Before the analyses, the samples were fully dried to a constant weight in a drying oven at 65°C and then ground to a homogeneous powder with a quartz mortar, after which lipids were extracted from the powdered samples with a mixture of 2:1 chloroform:methanol (Folch et al., 1957). The processed samples were subsequently dried and ground again and preserved in a low temperature and dry environment for stable isotope determination.

Stable Isotope Analysis

The stable isotopic ratios of aquatic species were analyzed using a Flash EA 1112 Elemental analyzer coupled with a Thermo Finnigan MAT 253 stable isotope ratio mass Spectrometers (EA-IRMS) (Thermo Finnigan Scientific, Inc., United States) at the Qingdao Institute of Marine Geology, and the obtained results were presented in the conventional δ notation relative to international standard materials (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen), which is calculated as follows: $X (\%) = \left(R_{sample}/R_{standard} - 1\right) \times 10^3$, where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The quality control for the samples was performed after measuring each ten samples by interspacing the standard reference materials with known elemental composition and isotope ratios (UREA, $\delta^{13}C$: −8.02 ± 0.05%, $\delta^{15}N$: 20.17 ± 0.06%; B2151, $\delta^{13}C$: −26.27 ± 0.15%, $\delta^{15}N$: 4.42 ± 0.29%; EDTA, $\delta^{13}C$: −40.23 ± 0.01%, $\delta^{15}N$: −0.73 ± 0.09%). All the samples were analyzed in a continuous flow mode.

The trophic levels of *S. esculenta* were estimated using the method described by Post (2002): $TL = (\delta^{15}N_{sample} -$

$\delta^{15}N_{\text{baseline}})/\Delta\delta^{15}N + TL_{\text{baseline}}$, where $\delta^{15}N$ is the stable isotopic ratio ($^{15}N/^{14}N$) of the specimens and baseline organisms, which were measured directly using the mass spectrometer, and $\Delta^{15}N$ is the enrichment in $\delta^{15}N$ per trophic level, which is obtained from published data (3.4‰, DeNiro and Epstein, 1981; Post, 2002). TL_{baseline} is the trophic level of the baseline organism (zooplankton in this study) and was given a value of 2 in this study.

Statistical Analysis

The *S. esculenta* samples with different measurements were grouped using cluster analysis. Their $\delta^{13}C$ and $\delta^{15}N$ values were used to distinguish the feeding habits of different developmental stages. Since all the *S. esculenta* samples were presented in the form of $\delta^{13}C - \delta^{15}N$ biplots based on the cluster analysis results, the size-corrected Bayesian standardized ellipse areas (SEAc) were plotted using SIBER (Jackson et al., 2011) to represent the isotopic niche and trophic breadth of different *S. esculenta* groups. The isotopic niche size of *S. esculenta* groups was calculated using the metrics of total areas encompassing all data points (TA), Bayesian standardized ellipse areas (SEA), and SEAc (Jackson et al., 2011). The overlap proportion between two SEAc was used as a proxy for trophic similarity and was calculated by estimating the extent of niche overlap between different *S. esculenta* groups. The percentage of overlap between *S. esculenta* groups was calculated by dividing the area of overlap (‰²) by the total combined SEAc (‰²) of the two groups being compared using SIBER (Jackson et al., 2011). After the normality and homogeneity of variance were examined using the Shapiro–Wilk test and Levene's test, the assumption of correlations between $\delta^{13}C$ and $\delta^{15}N$ and *S. esculenta* mantle length were checked using a linear regression model. All the data were analyzed using R 4.1.1 software (R Development Core Team, 2020).

RESULTS

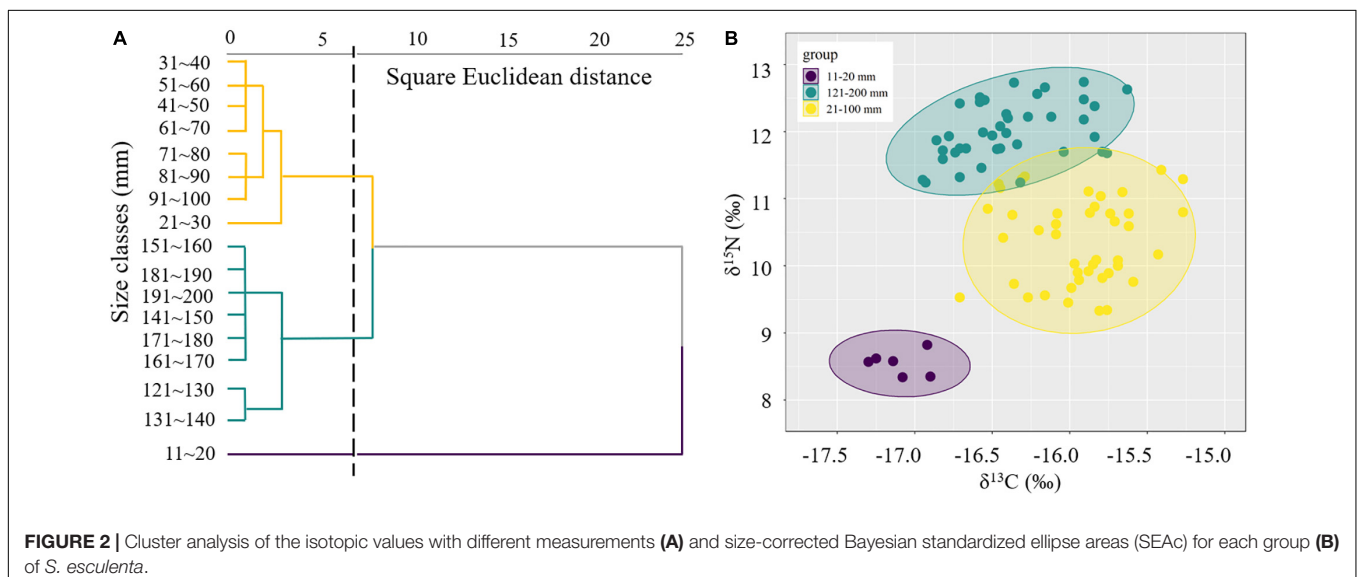## Isotopic Values in Wrists and Beaks of *Sepia esculenta*

No significant variations were observed in the mean $\delta^{13}C$ values (Mann–Whitney U-test, $n = 7$, $P > 0.05$) between muscle (-16.41 ± 0.29) and beaks (-16.26 ± 0.46), but the $\delta^{15}N$ values of the muscle (9.64 ± 0.17) were significantly higher than those of beaks (7.11 ± 0.33) (Mann–Whitney U-test, $P < 0.05$).

Ontogenetic Changes in the Trophic Ecology of *Sepia esculenta*

The isotope values of the juvenile and immature *S. esculenta* (11–100 mm) ranged from -17.30 to -15.27‰ for $\delta^{13}C$ and from 8.34 to 11.43‰ for $\delta^{15}N$. For adults (121–200 mm), the ranges of $\delta^{13}C$ and $\delta^{15}N$ were from -16.95 to -15.63 and 11.24 to 12.74‰, respectively (**Supplementary Table 1**). Cluster analysis was used to measure the square Euclidean distance of the $\delta^{13}C$ and $\delta^{15}N$ values of *S. esculenta* and to distinguish the feeding habits of individuals with different measurements, and the results illustrated that the *S. esculenta* samples were sorted into three distinct groups: group 11–20 mm, group 21–100 mm, and group 121–200 mm (**Figure 2A**). Isotopic niche size, represented by SEAc, was largest for group 21–100 mm (0.690), followed by group 121–200 mm (0.455) and group 11–20 mm (0.117) (**Table 1**). There was no niche overlap between group 11–20 mm and the other two groups, while a little niche overlap (7.3%) was found between the groups 21–100 and 121–200 mm (**Figure 2B**).

Correlations Between the Isotopic Values and Mantle Length of *Sepia esculenta*

The changes in the $\delta^{13}C$ and $\delta^{15}N$ values associated with increasing *S. esculenta* mantle length were examined using regression analysis and are illustrated in **Figure 3**. There was no trend for the $\delta^{13}C$ values with mantle length ($P > 0.05$).

FIGURE 2 | Cluster analysis of the isotopic values with different measurements **(A)** and size-corrected Bayesian standardized ellipse areas (SEAc) for each group **(B)** of *S. esculenta*.

TABLE 1 | Metrics for estimating isotopic niche size in three *Sepia esculenta* groups.

Metrics	11–20 mm	21–100 mm	121–200 mm
TA	0.117	2.279	1.468
SEA	0.094	0.670	0.443
SEAc	0.117	0.690	0.455

TA, the total area contained all data points of each S. esculenta group; SEA, Bayesian standardized ellipse area for each S. esculenta group; SEAc, size-corrected Bayesian standardized ellipse area for each S. esculenta group. All the metrics were expressed in ‰².

Thus, we fit the linear regression within the groups, respectively (**Figure 3A**). In the groups 11–20 and 121–200 mm, the δ^{13}C values were increased with mantle length, while for group 21–100 mm, the δ^{13}C values hardly changed with mantle length. In contrast, there was an increasing tendency in the δ^{15}N values related to mantle length ($P < 0.001$, $R^2 = 0.87$, **Figure 3B**).

Isotopic Information of Coexisting Species

In terms of the coexisting species, the δ^{13}C values ranged from -19.24 ± 0.78‰ [red tongue sole (*Cynoglossus joyneri*) (Günther, 1878)] to -16.83 ± 0.15‰ [small yellow croaker (*Larimichthys polyactis*) (Bleeker, 1877)]. Forceps snapping shrimp (*Alpheus distinguendus*) (De Haan, 1844) had the lowest δ^{15}N (9.75 $\pm$ 0.34‰) values, while Japanese sillago (*Sillago japonica*) (Temminck and Schlegel, 1843) had the highest (13.23‰) (**Supplementary Table 2**). The coexisting species were divided into four logical groups (mesodemersal fishes, demersal fishes, crustaceans, and cephalopods) based on their habitat relevance. Overall, most mesodemersal fishes had the δ^{13}C values of approximately -17.5‰, most demersal fishes had the δ^{13}C values of approximately -19.0‰, and most crustaceans had intermediate δ^{13}C values, falling between those of the demersal and mesodemersal fishes, at approximately -18.5‰ (**Figure 4**).

Trophic Levels Estimates

Zooplankton was used as primary consumers to set the baseline, and their mean δ^{15}N values (6.26 $\pm$ 1.28‰) were used to estimate the trophic levels of each species (**Figure 5**). The trophic levels of the main species in the Qingdao coastal food web could reach up to fourth Trophic level (*TL*), and most fishes, crustaceans, and cephalopods fell within the third and fourth *TL*s. In fishes, the *TL* was largest for *S. japonica* and lowest for red barracuda (*Sphyraena pinguis*) (Günther, 1874); Japanese squillid mantis shrimp (*Oratosquilla oratoria*) (De Haan, 1844) exhibited the highest *TL* in crustaceans and followed by Shiba shrimp (*Metapenaeus joyneri*) (Miers, 1880); The highest *TL* of cephalopod was found in group 121–200 mm of *S. esculenta*, with the highest *TL* of individuals within the group reaching 3.97 (**Supplementary Table 2**). Group 11–20 mm of *S. esculenta* was the lowest *TL* consumer other than zooplankton, with the trophic levels between the second and third *TL*s. With the ontogeny of *S. esculenta*, approximately 1.3 trophic levels experienced the difference between small and large individuals (**Figure 5**).

DISCUSSION

The Use of Muscle in Isotopic Analysis for *Sepia esculenta*

In this study, the δ^{15}N values of the muscle were significantly higher than those of beaks in *S. esculenta*, which is in accordance with the nitrogen signatures of the tissues in the Antarctic flying squid (*Todarodes filippovae*) (Adam, 1975), caused by the depletion of ^{15}N compared with protein during chitin formation (Cherel and Hobson, 2005; Cherel et al., 2009a). Hence, the wrist muscle was used for the isotopic analyses in the subsequent process.

The Characteristics of Isotopic Changes With Ontogeny

For a more intuitive view of the feeding conversion process, cluster analysis based on isotopic signatures was used to interpret ontogenetic transition patterns in trophic niches of *S. esculenta* and divided the individuals into three groups (11–20, 21–100, and 121–200 mm) (**Figure 2A**). There was a significant difference (**Supplementary Table 1**) in the δ^{13}C values between the groups 11–20 mm (δ^{13}C: -17.10 ± 0.15‰) and 21–100 mm (δ^{13}C: -15.89 ± 0.31‰), illustrating an ontogenetic change in resource use and feeding habits. This is consistent with other research assessing the ontogenetic shift in the diet of cephalopods and fishes. For example, stable isotope analysis revealed a positive relationship between isotope ratios and body size of *D. gigas*, which reflect the changes in the diet of the species (Trasviña-Carrillo et al., 2018). Also, based on stable isotope and stomach content analyses, the walleye pollock (*Theragra chalcogramma*) (Pallas, 1814) was found to exhibit a dietary shift with growth from consuming mesozooplankton to cephalopods and shrimps as grew (Park et al., 2018). This may be explained by the improvement in predation capacity and a change in the relative abundance of prey. The dietary shift in fish species is almost exclusive due to their improvement in predation ability, gape size, locomotor skills, and speed (Grey, 2001; Borrell et al., 2011). Similarly, for *S. esculenta*, the predatory ability also increases rapidly with body growth in early life (Lei et al., 2016).

Within group 21–100 mm, the δ^{13}C values hardly changed with mantle length, suggesting a relatively stable feeding orientation or feeding habit, which may indicate that the predation ability of *S. esculenta* has been equipped at this stage. However, as the mantle length increased, the points representing the δ^{13}C values became more dispersed (**Figure 3A**). For this, this *S. esculenta* group was divided into two parts according to the length interval including part 21–60 and part 61–100 mm, and the SEAc of the two parts were calculated, respectively. It was found that part 61–100 mm (SEAc = 0.521) had a larger trophic niche/isotopic niche breadth than part 21–60 mm (SEAc = 0.352), indicating that the *S. esculenta* within this group expanded its food source range with an increase in mantle length. The ontogenetic increase in the niche width was also noted for other cephalopods. For example, with the increase in the number and abundance of prey species, the SEAc of two generalist feeders

I. argentinus and *D. gahi* also increased with ontogeny (Rosas-Luis et al., 2017). Similar results were also found for the greater hooked squid (*Onykia ingens*) (Smith, 1881; Rosas-Luis et al., 2014). In this study, more food sources provide more options for *S. esculenta* and help meet their energy storage needs for overwintering and migration.
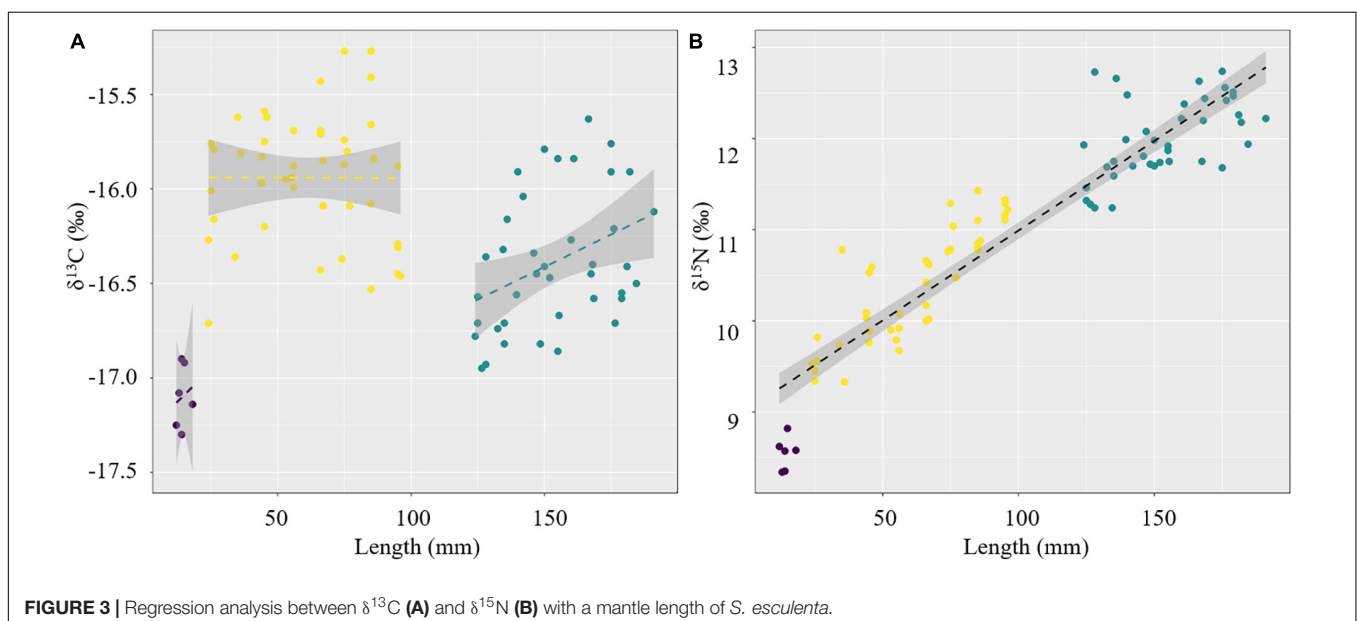
For group 121–200 mm, the δ^{13}C values ($-16.39 \pm 0.35‰$) were characterized by a depletion compared with that in group 21–100 mm ($-15.89 \pm 0.31‰$) (**Figure 3A**), which was mainly related to the migratory habits of *S. esculenta*. According to previous surveys in the coastal waters of Qingdao, the population abundance of *S. esculenta* was gradually decreased in September–October and almost completely left the area by November (Wang et al., 2019; Zhang et al., 2019). Next spring, adult *S. esculenta* returned from wintering ground to spawning ground, so that we were able to collect individuals with a size of more than 120 mm during this period. Compared with oceanic waters, the nutrient flow through relatively long and complex energy pathways in coastal food webs may lead to more enrichment of ^{13}C in species and lead to larger δ^{13}C values of the ecosystem (McCutchan et al., 2003; Cherel et al., 2008; Loc'H et al., 2008). Stable isotopes in food usually take several weeks to reflect in the muscle tissue of the predator (Taylor and Mazumder, 2010), so the lower δ^{13}C value of the *S. esculenta* 121–200 mm group may partly reflect the stable isotopic characteristics of the wintering ground ecosystems. This is consistent with the isotopic signatures of *D. gigas* in Peruvian waters, whose δ^{13}C values showed a decreasing trend with increasing distance to the shelf breaks (Argüelles et al., 2012). Similar conditions were also found in fishes (Wu et al., 2019) and seabirds (Hobson et al., 1994; Cherel et al., 2000). The δ^{13}C values within group 121–200 mm increased with mantle length, suggesting that *S. esculenta* were more inclined to capture prey with higher δ^{13}C values and trophic levels to meet higher energy requirements during the reproductive period. This was different from the way *S. esculenta* (group 21–100 mm) stored energy by expanding its food source range before overwintering, which may be due to the stronger locomotion and predation ability of the adult *S. esculenta* (group 121–200 mm), making them more active and selective in predation.

The δ^{15}N values of *S. esculenta* were found to increase with mantle length, suggesting that the larger an *S. esculenta* is, the higher its trophic level, indicating a preferred change in dietary items from lower δ^{15}N values to higher δ^{15}N values (**Figure 3B**). There was a clear and significant linear relationship ($P < 0.001$, $R^2 = 0.87$) between different *S. esculenta* groups, with no difference in slope, suggesting that their spawning ground and wintering ground may have similar nitrogen stable isotope characteristics. The stable isotope values (especially for δ^{15}N) at the base (baseline species) of the food webs showed a latitudinal (north–south) gradient change under the influence of CO_2 concentration, sea surface temperature, and specific growth rates (Argüelles et al., 2012; Troina et al., 2020), and these latitudinal differences also have been observed in the northern Pacific (Ruiz-Cooley et al., 2010). Previous studies have reported that the wintering grounds of *S. esculenta* in the coastal waters of China are in the middle and southern Yellow Sea and the northern and middle East China Sea, with large and ambiguous latitudinal spans (Zheng et al., 2003; Wang et al., 2019). Given the variation of δ^{15}N with latitude, we speculate that the wintering ground of the *S. esculenta* population in Qingdao may be located in the middle of the Yellow Sea, at the same latitude as the spawning ground.

Trophic Relationship and Trophic Levels of *Sepia esculenta* and Other Organisms

Except for cephalopods, most species in each logical group have similar δ^{13}C values, which mainly reflects the feeding habits of different logical groups. There was one outlier in each logical

FIGURE 3 | Regression analysis between δ^{13}C **(A)** and δ^{15}N **(B)** with a mantle length of *S. esculenta*.
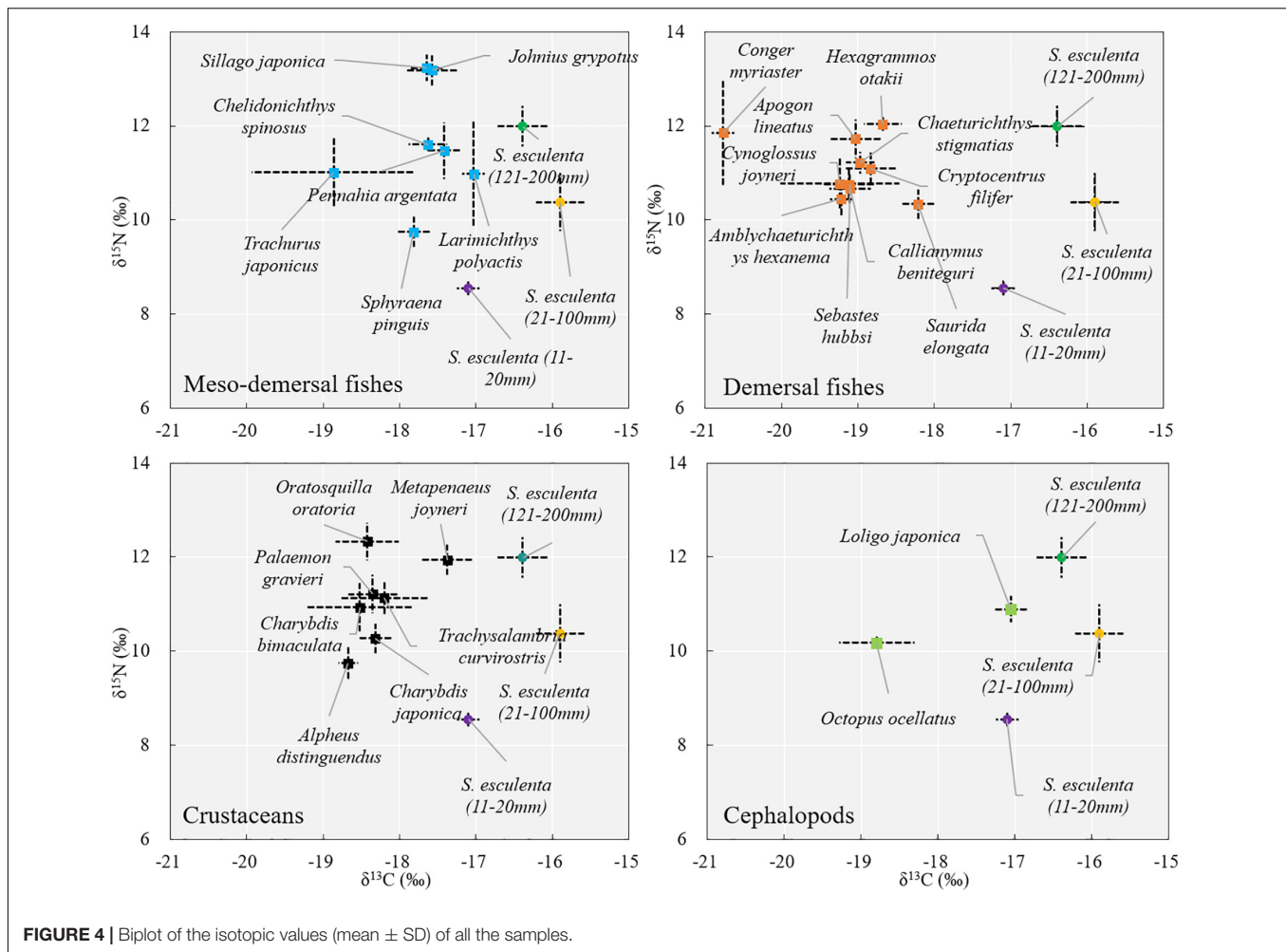
FIGURE 4 | Biplot of the isotopic values (mean ± SD) of all the samples.

group (mesodemersal fishes, demersal fishes, and crustaceans), which may be because the species we collected were still too small to form a fixed life habit. For example, the maximum total length of whitespotted conger (*Conger myriaster*) (Brevoort, 1856) in demersal fishes can reach 1,000 mm (Fishbase), while the sample lengths in this study were only about 220 mm.

Larimichthys polyactis, silver croaker (*Pennahia argentata*) (Houttuyn, 1782), *Johnius grypotus* (Richardson, 1846) of mesodemersal fishes, *M. joyneri* of crustaceans, and Japanese squid (*Loligo japonica*) (Hoyle, 1885) of cephalopods had some overlaps with the group 11–20 mm of *S. esculenta* in δ^{13}C values, and higher δ^{15}N values (2–4‰) than that of *S. esculenta*, which suggests that these species may feed on group 11–20 mm of *S. esculenta* as one of their food sources. Other studies based on stomach content analysis have also shown that some fishes, crustaceans, and cephalopods in the Yellow Sea feed on *S. esculenta* (Cheng and Zhu, 1997; Zhang et al., 2011). The stable isotope space (area bounded in the stable isotope biplot) of *S. esculenta* in the groups 21–100 and 121–200 mm did not overlap with other species after correcting for trophic enrichment. It has been reported that *S. esculenta* feed on small fishes and crustaceans (Natsukari and Tashiro, 1991), but

this relationship was not observed in this study, which may be due to the other species collected did not include their larva/juvenile stage.

There is quite a large range in the δ^{15}N values (4.4‰, **Supplementary Table 1**), corresponding to 1.3 trophic level (**Figure 5**) throughout the 1-year life cycle of *S. esculenta*. This is a shared feature of cephalopods. For example, isotope analysis revealed that *T. filippovae* with the length of 250–450 mm had δ^{15}N values between 10 and 13‰, corresponding to one trophic grade level (Cherel et al., 2009a). The δ^{15}N value range of *S. oualaniensis* with a length of 0–300 mm was also about 3%, and *O. bartramii* with a length of 0–600 mm had a larger range of δ^{15}N value of about 7% (Parry, 2008). The δ^{15}N range of the *S. esculenta* is relatively large when the size of these cephalopods is taken into account. Such large ranges were also found in both *I. argentinus* and *D. gahi* (Rosas-Luis et al., 2017), which reflects the characteristics of trophic plasticity and fast growth of cephalopods (Rodhouse and Nigmatullin, 1996; Parry, 2008; Cherel et al., 2009b). The increase in trophic position indicates the importance of *S. esculenta* as consumers at various trophic levels throughout their life cycle (Cherel et al., 2009b).

FIGURE 5 | Trophic position estimates were calculated using δ^{15}N data of each species.
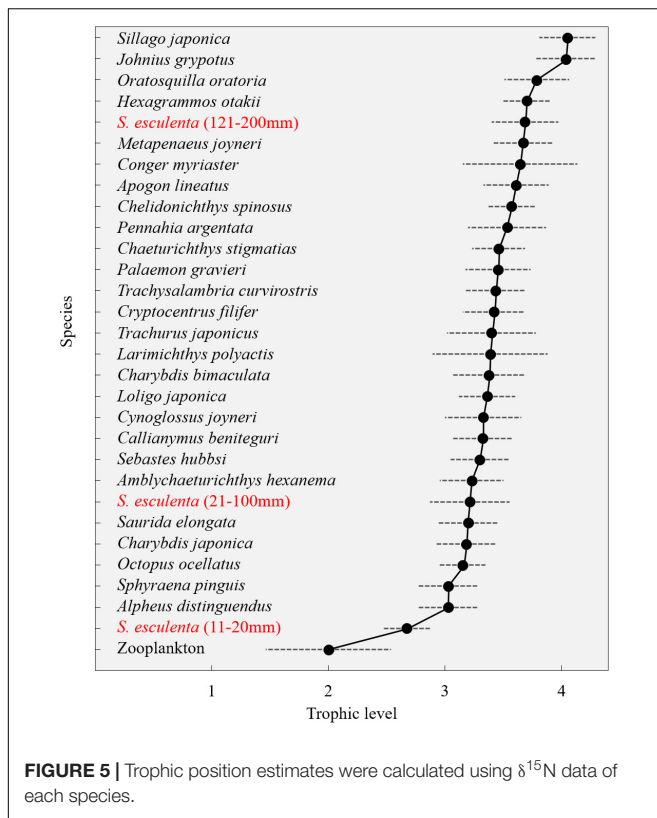
Implications for Resource Proliferation and Management

In recent years, the release intensity of *S. esculenta* juveniles in Shandong Province has increased year by year, with the release number of more than 1.3 million per year, which has become the most important measure for resource conservation (Oceanic Administration of Shandong Province, 2016). Of these, 0.6 million were released into the coastal waters of Qingdao, accounting for 46% (People, 2018). The low survival rate of juveniles was an important factor affecting the effect of releasing (Hervas et al., 2010). *S. esculenta* has a 1-year life cycle (Yamada et al., 1986); however, the phenomenon of growth dispersion within the population in natural water is very prominent. According to our previous resource survey (in October 2017) in coastal Qingdao, the mantle length of different individuals captured at the same period could vary by up to six times (16–100 mm). Based on the trophic shifts (the difference in feeding habits between the groups 11–20 and 21–100 mm), we speculate that this may be a life history strategy adopted by *S. esculenta*, through food conversion and resources partitioning, to reduce the intraspecies predatory competition (Mablouké et al., 2013) and increase the survival rates of juveniles. Therefore, juvenile releases in different periods should be considered to improve the survival rate of the larvae during large-scale juvenile release events.

It is suggested that the survival rates of post-release juveniles were significantly affected by the length (Hervas et al., 2010). At present, the mantle length of *S. esculenta* releasing juveniles

is mostly between 12 and 15 mm (Oceanic Administration of Shandong Province, 2016). *S. esculenta* in this period may be in the prey range of fishes and crustaceans, such as *L. polyactis* and *M. joyneri*, as one of their prey organisms (**Figure 4**). However, the δ^{13}C and δ^{15}N values of *S. esculenta* increased significantly (**Supplementary Table 1**) when they grew to 21–30 mm (**Supplementary Table 1**), with the overlap of carbon isotope space with other species decreased obviously, indicating that they had been greatly reduced the proportion in the diet composition of other species. *S. esculenta* grows fast (Zhang et al., 2019), so if the artificial culture time of prerelease can be slightly extended to increase the length to more than 20 mm, the pressure of being prey may be reduced significantly and the effect of release can be improved.

CONCLUSION

In this study, the ontogenetic trophic ecology and migration patterns of *S. esculenta* in Chinese northern coastal areas were investigated using the isotope analysis. *S. esculenta* were divided into three groups (11–20, 21–100, and 121–200 mm) based on isotopic values, with a significant difference in the δ^{13}C values between the groups 11–20 and 21–100 mm, illustrating an ontogenetic change in resource use and feeding habits. The δ^{13}C value of group 121–200 mm was lower than that of group 21–100 mm, which was mainly related to the migratory habits of *S. esculenta* and partly reflected the stable isotopic characteristics of the wintering ground ecosystems. The δ^{15}N values of *S. esculenta* were found to increase in mantle length, and there was a clear and significant linear relationship between different groups. It is suggested that the wintering ground of *S. esculenta* in Qingdao coastal waters may have similar nitrogen stable isotope characteristics with spawning ground. In addition, it suggested that some species may feed on group 11–20 mm of *S. esculenta* as one of their food sources. Thus, slightly increasing the length to more than 20 mm may significantly reduce the pressure of being the prey of post-release juveniles and improve the effect of release.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Ethics Committee of the Ocean University of China.

AUTHOR CONTRIBUTIONS

LW contributed to conceptualization, methodology, investigation, resources, writing-original draft, and

writing-review and editing. YL contributed to data curation and investigation. XZ contributed to supervision, project administration, conceptualization, methodology, resources, and writing-review and editing. All authors contributed to the article and approved the submitted version.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.818088/full#supplementary-material

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