frontiers | Frontiers in Marine Science

ORIGINAL RESEARCH
published: 08 April 2022
doi: 10.3389/fmars.2022.884968

Filter-Feeding Bivalve Weakens Food Competition Between Crustaceans (*Portunus trituberculatus*, *Marsupenaeus japonicus*) in Integrated Culture Ponds: Evidence From 18S rDNA Barcoding and Stable Isotope Analysis

Xian Xu[1,2†], Shipeng Dong[1,2†], Dongxu Zhang[3], Liye Yu[1,2], Weijia Pu[1,2], Yicheng Xie[1,2], Hongwei Shan[1,2] and Fang Wang[1,2]*

[1] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, China, [2] Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China, [3] Zhejiang Province Key Laboratory of Mariculture and Enhancement, Zhejiang Marine Fisheries Research Institute, Zhoushan, China

OPEN ACCESS

Edited by:
Min Jin,
Ningbo University, China

Reviewed by:
Ping Liu,
Chinese Academy of Fishery Sciences (CAFS), China
Yunfei Sun,
Shanghai Ocean University, China

***Correspondence:**
Fang Wang
wangfang249@ouc.edu.cn

[†]These authors have contributed equally to the work

Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: 27 February 2022
Accepted: 18 March 2022
Published: 08 April 2022

Citation:
Xu X, Dong S, Zhang D, Yu L, Pu W, Xie Y, Shan H and Wang F (2022) Filter-Feeding Bivalve Weakens Food Competition Between Crustaceans (Portunus trituberculatus, Marsupenaeus japonicus) in Integrated Culture Ponds: Evidence From 18S rDNA Barcoding and Stable Isotope Analysis. Front. Mar. Sci. 9:884968. doi: 10.3389/fmars.2022.884968

In order to investigate the effects of razor clams (*Sinonovacula constricta*) on the food composition and isotopic niches of swimming crabs (*Portunus trituberculatus*) and kuruma shrimp (*Marsupenaeus japonicus*) in polyculture systems, this study analyzed 60 P. *trituberculatus*, 60 *M. japonicus* and 30 *S. constricta* to quantify the food sources, food source contributions, and isotopic niches of cultured organisms using 18S rDNA barcoding and stable isotope techniques. The results were as follows: (1) In the *P. trituberculatus-M. japonicus* (PM) polyculture system, the Sobs and Shannon-Wiener indices of the stomach contents of *P. trituberculatus* and *M. japonicus* were not significantly different ($P > 0.05$). In the *P. trituberculatus-M. japonicus-S. constricta* polyculture (PMS) system, the Sobs and Shannon-Wiener indices of the stomach contents of *P. trituberculatus* and *M. japonicus* were also not significantly different ($P > 0.05$), but the Sobs indices of *P. trituberculatus* in the PMS system were significantly higher than those in the PM system ($P < 0.05$), *M. japonicus* shows a similar pattern. (2) 18S rDNA barcoding analysis showed the dominant taxa in the stomach contents of both *P. trituberculatus* and *M. japonicus* in the PM system were Trebouxiophyceae, Embryophyta and Rotifera, and the food overlap between them was 0.8992, which was significant ($Q > 0.6$). In the PMS system, the dominant taxa in the stomach contents of *P. trituberculatus* were Chrysophyceae, Intramacronucleata, and Embryophyta, and in *M. japonicus* were Chrysophyceae, Embryophyta, and Bacillariophyceae, in this system the food overlap was 0.2061, which was not significant ($Q < 0.6$). (3) Stable isotope analysis suggested, in both systems, the main food sources of *P. trituberculatus* and *M. japonicus* were iced trash fish, zooplankton, phytoplankton, and organic particulate matter (POM).

Iced trash fish accounted 77.67% of food sources for *P. trituberculatus* and 69.42% for *M. japonicus* in the PM system, and 60.82% and 57.60% in the PMS system. (4) The isotopic niche overlap between *P. trituberculatus* and *M. japonicus* was 5.69% in the PM system and 1.21% in the PMS system. These results suggested food competition between *P. trituberculatus* and *M. japonicus*, and *S. constricta* can reduce the competition and isotopic niche overlap, improve the contribution of food sources such as phytoplankton. Razor clams also serve to purify the water and improve the utilization of iced trash fish by filtering phytoplankton (51.10%), POM (32.25%), SOM (7.47%), and iced trash fish (9.18%). Thus, *P. trituberculatus-M. japonicus-S. constricta* is a healthy and sustainable culture model.

Keywords: *Portunus trituberculatus*, integrated pond culture, 18S rDNA barcoding, stable isotope, food composition

INTRODUCTION

Portunus trituberculatus, commonly known as the swimming crab, belongs to Crustacea, Decapoda, and Portunidae, and is widely distributed in the Yellow and Bohai Seas and the East China Sea (Dai et al., 1986). Farm production of *P. trituberculatus* in China reached 113,800 t in 2019, accounting for 41.5% of the farm production of marine crabs (The Ministry of Agriculture Fishery and Fishery Administration, 2021). *P. trituberculatus* is an important seawater pond cultured species, and is usually polycultured with Pacific white shrimp (*Litopenaeus Vannamei*), kuruma shrimp (*Marsupenaeus japonicus*), razor clams (*Sinonovacula constricta*), Manila clams (*Ruditapes philippinarum*), and redlip mullet (*Liza haematocheila*) (Wang et al., 2009; Zhou et al., 2010; Wang, 2011). *P. trituberculatus* and *M. japonicus* can co-exist well in a system due to their different physiological characteristics and food processing methods (Dai et al., 1986; Pérez-Farfante and Kensley, 1997; Buck et al., 2003; Wang et al., 2009; Wang, 2011), improving space utilization and production, iced trash fish are fed in production. Most of the studies about polyculturing *P. trituberculatus* and *M. japonicus* have focused on the effects of environmental factors or microbial communities on the ecosystem (Dong, 2013; Ban, 2015). The food habits and trophic niches of *P. trituberculatus* and *M. japonicus* are similar (Brzeski and Newkirk, 1997; Yang, 2001; Gao et al., 2020; Tao et al., 2020), but there is a lack of quantitative food source analysis between them in polyculture systems.

Filter-feeding bivalves have become the main species in polyculture seawater ponds due to their ability to improve water quality (Dong et al., 1999), increase nutrient utilization efficiency (Vaughn and Hakenkamp, 2010; Guo et al, 2017), improved survival rate of co-cultured animals (Mckindsey et al., 2007; Vaughn and Hakenkamp, 2010), and enhance the stability of culture systems (Wang, 2011). The effects of filter-feeding bivalves on plankton community structure in pond water and nitrogen and phosphorus budgets in culture systems have been reported (Yang, 1998; Dong et al., 1999; Zhang et al., 2015; Guo et al., 2017), but their effects on food resources of cultured organisms in polyculture systems have rarely been addressed.

In this study, we combined 18S rDNA barcoding and stable isotope techniques with samples collected at the middle of the culture period (October) as experimental material to quantify the food sources, contributions, and isotopic niches of cultured organisms in a *P. trituberculatus-M. japonicu* (PM) system and a *P. trituberculatus-M. japonicu-S. constricta* (PMS) system, investigate the feasibility of polyculturing *P. trituberculatus* with *M. japonicu* and the effect of *S. constricta* on their food sources and isotopic niches. Our objectives were to (1) describe food competition between *P. trituberculatus* and *M. japonicu*, and (2) determine whether polyculturing *S. constricta* affected food competition between *P. trituberculatus* and *M. japonicu*. The results of this study can provide a scientific basis for rational combination of cultured organisms in integrated ponds with *P. trituberculatus*.

MATERIALS AND METHODS

Pond Management

The experiment was conducted on Changbai Island (30°11′ 15.22″N, 122°2′40.95″E), Zhoushan, Zhejiang Province, China, which has a subtropical monsoon climate with an annual average temperature of 16.1 ~ 16.4 °C and an average precipitation of 1442.9 mm. The experimental pond covered an area of 1.33 ha with an average water depth of 1.2 m. The culture models are *P. trituberculatus-M. japonicu* (PM) and *P. trituberculatus-M. japonicu-S. constricta* (PMS). In July 2020, 50 kg of healthy *S. constricta* (shell length: SL = 1.85 ± 0.14 cm, mean ± SD, n = 53) with uniform size were stocked, 10 kg of vigorous juvenile *P. trituberculatus* (carapace width: CW = 0.41 ± 0.02cm, mean ± SD, n = 82) and 26 kg of energetic *M. japonicus* (body length: BL = 1.02 ± 0.07cm, mean ± SD, n = 64) with sound appendages were stocked after half a month (Dong et al., 2021). 40kg of iced trash fish, including fish (*Nibea albiflora* and *Trachinotus blochii*), shrimp (*Oratosquilla oratoria* and *Solenocera crassicornis*), and crab (*Portunus pelagicus*) were provided daily at 17:00. Water was changed 1-2 times per month, 30% each time. Sediment consists mainly of mud, with very low levels of benthic microalgae and benthos. The experimental period was

from July 2020 to January 2021, and the ranges of water temperature, salinity, dissolved oxygen and transparency during the period were 6.5 ~ 34.0°C, 14.5 ~ 19.0, 6.0 ~ 12.3 mg/L and 40~100 cm, respectively.

Sample Collection and Treatment

Samples of cultured organisms (*P. trituberculatus*, *M. japonicus* and *S. constricta*) and potential food sources (iced trash fish, zooplankton, phytoplankton, POM and SOM) were collected on October 13, 2020. 30 each of *P.trituberculatus* (carapace width: CW = 14.76 ± 1.74 cm, mean ± SD, n = 60) and *M. japonicus* (body length: BL = 11.02 ± 1.13 cm, mean ± SD, n = 60) in the PM system, and 30 each of *P.trituberculatus* (carapace width: CW = 20.07 ± 3.55 cm, mean ± SD, n = 60), *M. japonicus* (body length: BL = 12.05 ± 1.78 cm, mean ± SD, n = 60) and *S. constricta* (shell length: SL = 8.94 ± 1.44 cm, mean ± SD, n = 90) were collected. The pond is divided into 5 points according to the diagonal line to collect mixed water samples of 10 L each, zooplankton and phytoplankton were collected by filtering 50 L water through No. 13 (20 cm mouth diameter, 112 μm mesh size) and No. 25 (20 cm mouth diameter, 64 μm mesh size) plankton nets, respectively, and the filtrate was extracted onto pre-cauterized (500°C, 5 h) Whatman GF/F membranes for POM. SOM were collected from 1–2 cm of the sediment surface with a column collector. All samples were stored on dry ice for rapid transport back to the laboratory.

Stomach contents were collected in 5 mL lyophilized tubes and transferred to -80°C storage for subsequent 18S rDNA analysis. The foot muscles of *S. constricta*, cheliped muscles of *P. trituberculatus*, abdomen muscles of *M. japonicus* (Hill and Mcquaid, 2009), and all muscles of iced trash fish were treated with 1 mol/L hydrochloric acid and then rinsed with distilled water. Muscles and filter membranes containing samples were dried in a 60 °C oven (DGG-9140A) to constant weight. Muscle samples were ground into powder and collected in 1.5 mL centrifuge tubes, and then stored in a desiccator for subsequent stable isotope analysis.

18S rDNA Barcoding Analysis

Genomic DNA extraction from stomach contents was performed using the E.Z.N.A.® soil DNA kit, the quality of extraction was detected using 1% agarose gel electrophoresis, and DNA concentration and purity were evaluated using NanoDrop2000. PCR amplification of the variable V4 region of the 18S rDNA gene was performed using the universal primers TAREF (5′-CCAGCASCYGCGGTAATTCC-3′) and TARER (5′-ACTTTCGTTCTTGATYRA-3′) with the following amplification procedure: 95°C pre-denaturation for 3 min, 27 cycles (95°C denaturation for 30 s, 55°C). The PCR reaction system was as follows: 5 × TransStart FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, upstream primer (5 uM) 0.8 μL, downstream primer (5 uM) 0.8 μL, TransStart FastPfu DNA polymerase 0.4 μL, template DNA 10 ng, made up to 20 μL for three replicates per sample. The PCR products were mixed and recovered on 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen BioPMiences, Union City, CA, USA), and detected using 2% agarose gel electrophoresis. The recovered products were quantified using a Quantus™ Fluorometer (Promega, USA). Libraries were built using NEXTFLEX Rapid DNA-Seq Kit and sequenced using Illumina's Miseq PE300 platform.

Stable Isotope Analysis

All samples were wrapped in aluminum foil and sent to the stable isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher PMientific, Inc.) for analysis. The carbon and nitrogen stable isotope values were based on the international reference materials PDB (Pee Dee Belemnite) and atmospheric N_2, respectively. Stable isotope abundances were expressed in delta (δ) notation as the deviation from the standards in parts per thousand according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000\,‰$$

where X is the isotope (^{13}C or ^{15}N), R_{sample} is the stable isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample, and $R_{standard}$ is the isotope ratio of the standard.

Calculation of the Competition

The competition between cultured organisms can be expressed by food overlap with the following equation:

$$Q_{xy} = \frac{\sum\left(P_{xk} \cdot P_{yk}\right)}{\sqrt{\sum P_{xk}^2 \cdot \sum P_{yk}^2}}$$

where Q_{xy} denotes the food overlap of organisms x and y and values range from 0 (no overlap) to 1 (complete overlap). k is the common food of both organisms, and P_{xk} and P_{yk} are the weight (or volume, quantity) percentages of food k in the food composition of organisms x and y, respectively (all are calculated as quantity percentages in this paper). When Q_{xy} > 0.6, food overlap is significant and there is serious competition (Wallace, 1981). DNA barcoding results are presented in the form of sequences and divided into different OTUs (Operational taxonomic units), then compared OTU representative sequences with the NCBI database to annotate the species classification information. The proportion of each representative OTUs to the total OTUs is the abundance, in accordance with the formula.

The competition between cultured organisms can also be expressed by the overlap of isotopic niches with the following equation:

$$Overlap\ \% = \frac{A_0}{A_x + A_y - A_O} \times 100$$

where *Overlap %* indicates the similarity of resource utilization and potential competition of organisms x and y, A_x and A_y are the isotopic niche area of organisms x and y, and A_O is the overlapping area of isotopic niches of organisms x and y (Ogloff et al., 2019).

Data Analysis

Owing to the presence of interference data, the original data were spliced and filtered to generate more accurate and reliable data for analysis. Sequences were classified as operational taxonomic units (OTUs) at 97% sequence similarity level by UPASE

(version 7.1). The taxonomy of each OTU representative sequence was analyzed using RDP Classifier against the Silva database with a confidence threshold of 0.7.

An SIAR (stable isotope analysis in R) linear mixed model was used to analyze the contribution of different food sources to consumers, and benthos were not considered in the calculation due to their low abundance in the sediment. Aquatic omnivores took muscle tissue, and $\Delta^{13}C$ was taken as 1.3‰ ± 0.3‰ without lipid removal. The value of $\Delta^{13}C$ was taken as 1.5‰ and $\Delta^{15}N$ as 2.5‰ for *S. constricta* (Mccutchan et al., 2003). The stable isotope Bayesian ellipses in R (SIBER) were used to calculate the isotopic niche (standard elliptical area, SEA; convex hull area, TA) of cultured organisms and their overlapping area (O_A) (Jackson et al., 2011). In this paper, we calculate the proportion of the overlap to the niche, and to keep the sample size consistent, the niche is calculated using the standard ellipse area (SEA). The data obtained were analyzed using the MATLAB software. The anova1 function was used for one-way ANOVA to check whether the stable isotope values had the same mean values at a significance level of $P < 0.05$. The plotting software was ORIGIN2020.

RESULTS

α-Diversity of the Stomach Contents of Cultured Organisms

The Sobs index and Shannon-Wiener index reflect the abundance and diversity of species communities, respectively (Beck, 2010). In the PM system, the Sobs indices of the stomach contents of *P. trituberculatus* and *M. japonicus* were 30.5 and 31.0 (**Figure 1A**), and the Shannon-Wiener indices were 2.09 and 1.95 (**Figure 1B**), respectively, none of these differences were statistically significant ($P > 0.05$). In the PMS system, the Sobs indices of the stomach contents of *P. trituberculatus* and *M. japonicus* were 51.6 and 50, and the Shannon-Wiener indices were 2.04 and 1.87, respectively. Likewise, none of these differences were statistically significant ($P > 0.05$). There were, however, significant differences between the Sobs indices of the stomach contents of both *P. trituberculatus* and *M. japonicus* in the two systems ($P < 0.05$). There was no significant difference between the Shannon-Wiener indices ($P > 0.05$). The Sobs index of the stomach contents of *S. constricta* was 48.7 and the Shannon-Wiener index was 0.80.

The Main Eukaryotic Composition of the Stomach Contents of Cultured Organisms

In the PM system, 39 phyla and 68 classes were identified in the stomach contents of *P. trituberculatus*, and the dominant taxa were Trebouxiophyceae, Embryophyta and Rotifera with relative abundances of 30.88%, 19.46% and 11.95%, respectively (**Figure 2A**). A total of 25 phyla and 40 classes were identified in the stomach contents of *M. japonicus*. Trebouxiophyceae, Embryophyta and Rotifera were the dominant taxa, with relative abundances of 47.66%, 18.38% and 13.46%, respectively (**Figure 2B**). In the PMS system, 32 phyla and 58 classes were identified in the stomach contents of *P. trituberculatus*, and the dominant taxa were Chrysophyceae, Intramacronucleata and Embryophyta, with relative abundances of 61.37%, 15.70% and 11.81%, respectively (**Figure 2C**). 36 phyla and 61 classes were identified in the stomach contents of *M. japonicus*. Chrysophyceae, Embryophyta and Bacillariophyceae were the dominant taxa, with relative abundances of 38.99%, 28.32% and 13.30%, respectively (**Figure 2D**). 36 phyla and 70 classes were identified in the stomach contents of *S. constricta*, and the dominant taxa were Dinophyceae, Trebouxiophyceae and Chrysophyceaewith relative abundances of 48.32%, 26.18% and 14.57%, respectively (**Figure 2E**).

In the PM system, the stomach contents of *P. trituberculatus* and *M. japonicus* had a total of 199 OTUs and 38 common OTUs (**Figure 3**), and the food overlap was 0.8992, indicating severe competition ($Q > 0.6$). In the PMS system, the stomach contents of *P. trituberculatus* and *M. japonicus* had a total of 191 OTUs and 19 common OTUs, with a non-significant food overlap of 0.2061 ($Q < 0.6$).

Stable Isotope Characteristics of Cultured Organisms

The $\delta^{13}C$ and $\delta^{15}N$ of *P. trituberculatus* in the PMS system were -16.54 ± 0.86‰ and 11.18 ± 0.59‰, respectively, and were significantly lower than those of *P. trituberculatus* in the PM

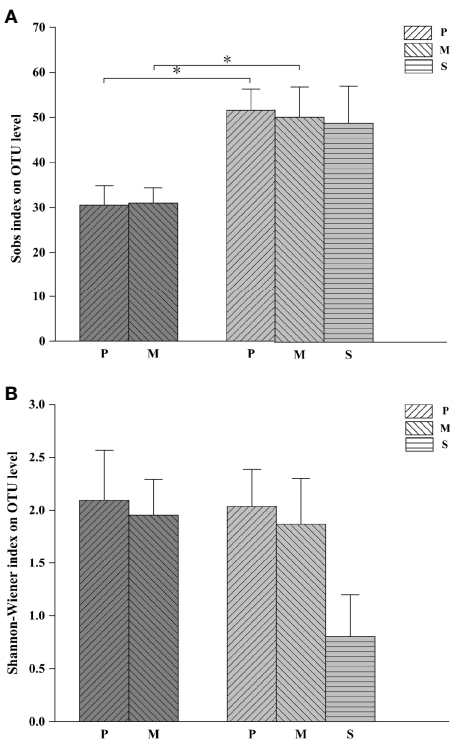

FIGURE 1 | Sobs **(A)** and Shannon-Wiener **(B)** indices of stomach contents of cultured organisms. Dark Gray represents the PM system and light gray represents the PMS system; P represents *P. trituberculatus*; M represents *M. japonicus*; S represents *S. constricta*; * indicates significant difference among groups ($P < 0.05$).

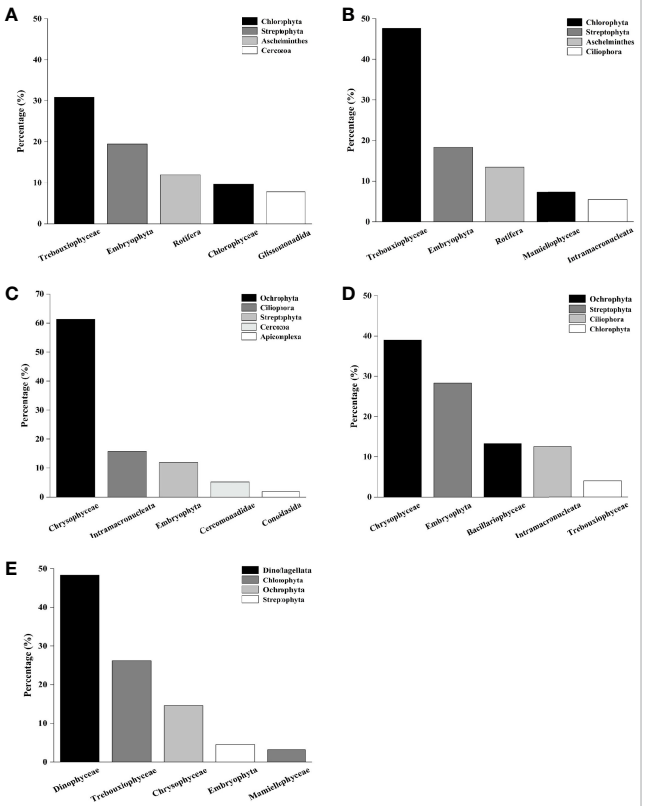

FIGURE 2 | Main eukaryote compositions of stomach contents of cultured organisms. **(A)** Main eukaryote compositions of stomach contents of *P. trituberculatus* in the PM system; **(B)** Main eukaryote compositions of stomach contents of *M. japonicus* in the PM system; **(C)** Main eukaryote compositions of stomach contents of *P. trituberculatus* in the PMS system; **(D)** Main eukaryote compositions of stomach contents of *M. japonicus* in the PMS system; **(E)** Main eukaryote compositions of stomach contents of *S. constricta* in the PMS system.

system ($\delta^{13}C$ = -15.78 ± 0.36‰ and $\delta^{15}N$ = 12.05 ± 0.62‰) ($P < 0.05$, **Table 1**). The $\delta^{13}C$ and $\delta^{15}N$ of *M. japonicus* in the two systems were not significantly different ($P > 0.05$), and the $\delta^{13}C$ and $\delta^{15}N$ of *S. constricta* were -26.85 ± 0.75‰ and 4.07 ± 0.38‰, respectively.

Contributions of Different Food Sources to Cultured Organisms

An SIAR linear mixed model was used to analyze the contributions of different food sources to the cultured organisms (**Figure 4**) and showed that the main food sources of *P. trituberculatus* and *M. japonicus* included iced trash fish, zooplankton, phytoplankton, and POM. Their contributions to *P. trituberculatus* in the PM system were 77.67%, 3.78%, 8.22%, and 10.34%, respectively, and their contributions to *M. japonicus* in the PM system were 69.42%, 1.86%, 6.76%, and 21.97%, respectively. Their contributions to *P. trituberculatus* in the PMS system were 60.82%, 2.40%, 17.54%, and 19.24%, respectively, and their contributions to *M. japonicus* in the PMS system were 57.60%, 2.03%, 18.62%, and 21.74%, respectively. The main food sources of *S. constricta* included phytoplankton (51.10%), POM (32.25%), SOM (7.47%), and iced trash fish (9.18%).

Isotopic Niches of Cultured Organisms

In both culture systems, each cultured organism occupied a unique niche space (**Figure 5**). In the PM system, SEA and Overlap for *P. trituberculatus* and *M. japonicus* were 0.687, 0.197, and 0.046, with a niche overlap of 5.69%. In the PMS system, SEA and Overlap for *P. trituberculatus* and *M. japonicus* were 1.658, 0.343, and 0.024, with a niche overlap of 1.21%. The SEA of *S. constricta* was 0.888, and did not overlap with either *P. trituberculatus* or *M. japonicus*. It is noteworthy that the width of the isotopic niches of both *P. trituberculatus* and *M. japonicus* in the PMS system expanded relative to the PM system, and the isotopic niche of *P. trituberculatus* shifted down obviously.

DISCUSSION

Food Sources of *P. trituberculatus* and *M. japonicus*

Currently, DNA barcoding technologies such as COI (mitochondrial cytochrome oxidase subunit I gene), ITS (internal transcribed spacer region within ribosomal rRNA gene) and 18S rDNA (DNA encoding the small subunit RNA of eukaryotic ribosomes) are widely used to analyze the diets of aquatic animals. 18S rDNA is the most commonly used technology due to its more complete database and better classification ability (Bachy et al., 2013; Leray et al., 2013). 18S rDNA can solve the problem of indistinguishable food fragments from morphological identification, leading to more comprehensive identification of the diets of study subjects (Redmond et al., 2013) and the discovery of easily-overlooked

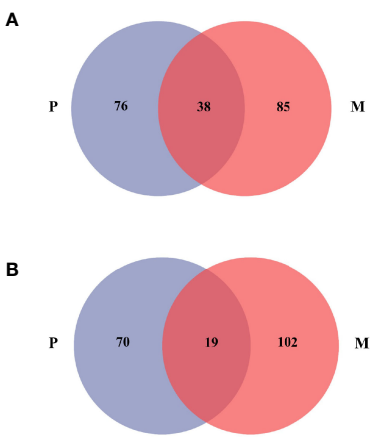

FIGURE 3 | Distribution of OTUs of the stomach contents of *P.trituberculatus* and *M.japonicus* in the PM **(A)** and PMS **(B)** systems. Blue circle represents *P.trituberculatus* and red circle represents *M.japonicus*.

TABLE 1 | $\delta^{13}C$ and $\delta^{15}N$ of cultured organisms (‰).

Cultured organisms	Stable isotopes	Culture systems	
		PM	PMS
P.trituberculatus	$\delta^{13}C$	-15.78 ± 0.36[a]	-16.54 ± 0.86[b]
	$\delta^{15}N$	12.05 ± 0.62[a]	11.18 ± 0.59[b]
M. japonicus	$\delta^{13}C$	-16.57 ± 0.40	-16.68 ± 0.41
	$\delta^{15}N$	11.65 ± 0.16	11.57 ± 0.27
S. constricta	$\delta^{13}C$		-26.85 ± 0.75
	$\delta^{15}N$		4.07 ± 0.38

PM represents P. trituberculatus-M. japonicus system; PMS represents P. trituberculatus-M. japonicus-S. constricta system. Significant differences (P < 0.05) between PM and PMS are indicated by different letters.

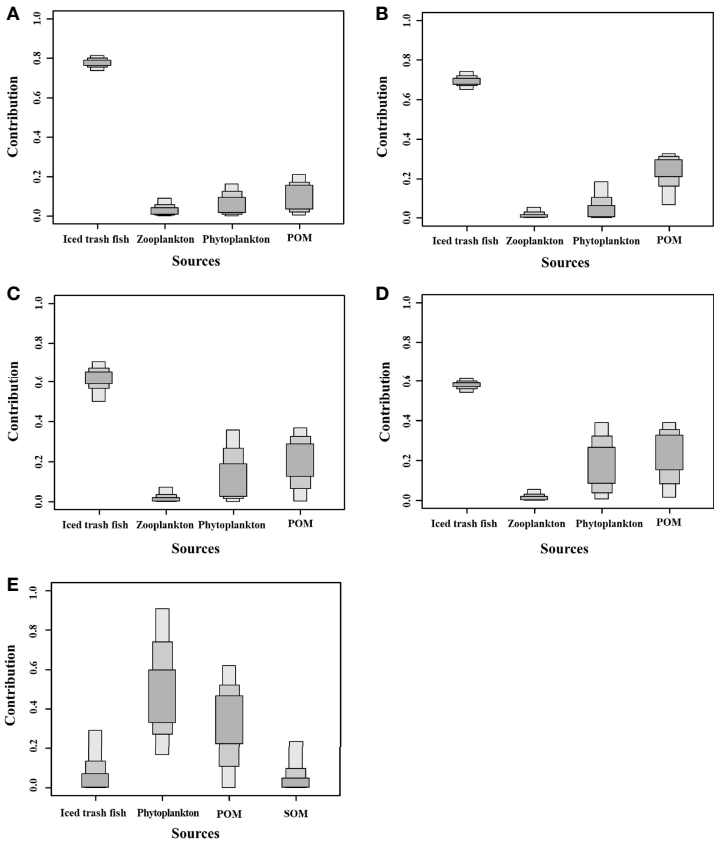

FIGURE 4 | Contributions of different food sources to cultured organisms. **(A)** Contributions of different food sources to *P. trituberculatus* in the PM system; **(B)** Contributions of different food sources to *M. japonicus* in the PM system; **(C)** Contributions of different food sources to *P. trituberculatus* in the PMS system; **(D)** Contributions of different food sources to *M. japonicus* in the PMS system; **(E)** Contributions of different food sources to *S. constricta* in the PMS system.

food sources (Rorke et al., 2012), with obvious advantages in terms of data volume, sensitivity, and resolution (Liu et al., 2018). This technique has been applied to food composition studies in sea cucumber (*Apostichopus japonicus*) and red rock lobster (*Jasus edwardsii*) (Rorke et al., 2012; Zhang et al., 2016). In this study, we found that the dominant taxa in the stomach contents of both *P. trituberculatus* and *M. japonicus* in the PM system included Trebouxiophyceae, Embryophyta, and Rotifera, and their total abundances were 62.29% and 79.50% (**Figure 2**), respectively. The dominant taxa in the stomach contents of *P. trituberculatus* and *M. japonicus* in the PMS system included Chrysophyceae, Intramacronucleata, and Embryophyta, and their total abundances were 88.88% and 79.84%, respectively. That result differed from the stomach contents of shrimp and crabs observed by Yang (2001) may be related to the feeding of iced trash fish and the low abundance of benthos (not collected in this experiment) in this experimental pond. There were no significant differences ($P > 0.05$) in either the Sobs or Shannon indices of stomach contents of *P. trituberculatus* or *M. japonicus* between the two systems (**Figure 1**), indicating that

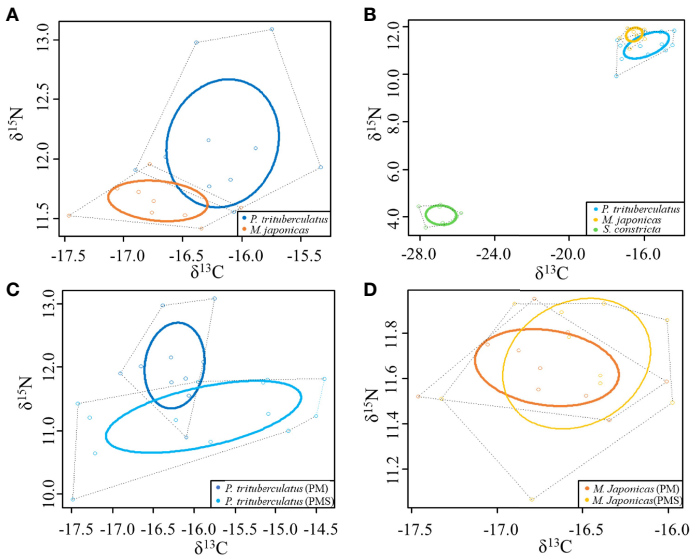

FIGURE 5 | Stable isotopic niches of cultured organisms. **(A)** Stable isotopic niches of cultured organisms in the PM system; **(B)** Stable isotopic niches of cultured organisms in the PMS system; **(C)** Stable isotopic niches of *P. trituberculatus* in the PM and PMS systems; **(D)** Stable isotopic niches of *M. japonicus* in the PM and PMS systems.

their food sources are quite similar, likely due to their omnivorous and carnivorous nature (Dai et al., 1986; Pérez-Farfante and Kensley, 1997).

Although the 18S rDNA barcoding technique has clear advantages in diet analysis of farmed animals, the degree of digestion of food by predators can limit its detection intensity (Albaina et al., 2010; Traugott et al., 2020). Stable isotope techniques can reveal trophic relationships between consumers and prey based on their isotope ratio relationships, and can reveal the diets of organisms over a longer period of time, serving as both a complement and correction to 18S rDNA barcoding (Post, 2002; Boecklen et al., 2011; Nielsen et al., 2018). Nelson et al. (2017) used DNA barcoding and stable isotope techniques to compare the feeding habits of different fish. Georgina et al. (2022) used both techniques, founding that the main contribution to the diet of the green crab (*Carcinus maenas*) came from phytoplankton. Combining these two methods not only identifies the prey being ingested, but also provides information on what is being absorbed.

In this study, the diets of *P. trituberculatus* and *M. japonicus* in both systems consisted primarily of iced trash fish, zooplankton, phytoplankton, and POM, with iced trash fish contributing the most (57.60%-77.67%) (**Figure 4**). Iced trash fish is nutritious, palatable, and easily available. The crustaceans provide calcium for *P. trituberculatus* and *M. japonicus* to form new shells during the molting period (Mykles and Skinner, 1982; Chang et al., 1993), and the fish provide protein and essential micronutrients (Gasco et al., 2018), therefore iced trash fish can meet essential growth needs. POM was the second most important food source, accounting for 10.34%-21.97% of *P. trituberculatus* and *M. japonicus* diets. POM is an organic particulate matter formed *via* microbial fermentation of plankton, feces, and residual bait (Liu, 1999). experimental ponds were likely too large to utilize iced trash fish fully, and POM were formed through biological action and ingested by *P. trituberculatus* and *M. japonicus*. In cases of excess animal-based bait, consumers will choose plant-based bait, which is lacking in the main diet, for nutritional supplementation, thus increasing the palatability of food and promoting nutritional balance and growth (Buck et al., 2003). This is likely the reason that phytoplankton acted as the third food source for *P. trituberculatus* and *M. japonicus*, accounting for 6.76%-18.62% of their diets. The smallest contribution of food sources in this study was zooplankton, perhaps due to its lower nutritional value relative to iced trash fish and its swimming nature (Genin et al., 2005). Isotopic niche is a means of describing trophic niche that reflects the trophic positions of organisms and the degree of competition for resources among populations (Abrams, 1980; Layman et al., 2007a; Post et al., 2007). In this experiment, the isotopic niches of *P. trituberculatus* and *M. japonicus* in both systems overlapped, indicating competition for resource utilization between them (Layman et al., 2007a). Therefore, 18S rDNA and stable isotope techniques analyses revealed competition for food resources between *P. trituberculatus* and *M. japonicus* in the ponds. Both *P. trituberculatus* and *M. japonicus* are basically living at the bottom of the pond, and they have similar foraging times (Dai et al., 1986; Pérez-Farfante and Kensley, 1997). However, because their feeding methods differ (*P. trituberculatus* use chelipeds to process food and then ingest it, and some food scraps can be used by *M. japonicus*) and they use the same resources in different ways (Kassen, 2002), there is less competition for food resources, and the bait utilization rate is improved. Thus, it is feasible to polyculture *M. japonicus* in *P. trituberculatus* ponds.

Effect of *S. constricta* on Food Composition and Isotopic Niche of *P. trituberculatus* and *M. japonicus*

In the PM system, the dominant taxa in the stomach contents of *P. trituberculatus* and *M. japonicus* were mainly Trebouxiophyceae, Embryophyta, and Rotifera, and the food overlap was obvious (**Figure 3**, Q > 0.6). After polyculturing with *S. constricta*, the dominant taxa in the stomach contents of *P. trituberculatus* were Chrysophyceae, Intramacronucleata, and Embryophyta. The abundance of Chrysophyceae in the stomach contents of *M. japonicus* increased significantly, and the abundance of Embryophyta and Bacillariophyceae decreased significantly, resulting in a decrease in food overlap (Q < 0.6), and indicating that adding *S. constricta* in the PM system could change the food composition of *P. trituberculatus* and *M. japonicus* and reduce food competition. This change may be related to the growth and metabolism of *S. constricta*, which changed the phytoplankton community structure in the culture water and increased phytoplankton diversity (Yang, 1998; Dong et al., 1999).

The $\delta^{13}C$ and $\delta^{15}N$ values of *P. trituberculatus* were significantly different in the two systems (**Table 1**, $P < 0.05$), indicating that *P. trituberculatus* food sources differed significantly (Deniro and Epstein, 1981). The higher $\delta^{15}N$ in the PM system was likely a result of the consumption of more high - $\delta^{15}N$ food (iced trash fish), while the lower $\delta^{13}C$ in the PMS system was likely a result of the consumption of more low - $\delta^{13}C$ food (phytoplankton). This indicates that polyculturing with *S. constricta* can increase the contribution of phytoplankton to *P. trituberculatus*' diet. Salman et al. (2008) found that plant - based sources facilitated the increase of bioflocs in the environment, which mainly consisted of residual bait, zooplankton, phytoplankton, and POM, and this corroborated the explanation for increased overall contributions of zooplankton, phytoplankton, and POM in PMS systems (Li et al., 2018). This may be related to the fact that individuals with higher $\delta^{15}N$ tend to be larger within the same consumer population (Wilsona et al., 2009).

Species with wider niches are more adaptable to the environment, and expanding niche width can improve the risk resistance of cultured organisms and make their systems more stable (Layman et al., 2007b; Rossi et al., 2015). In this experiment, the contributions of different food sources to *P. trituberculatus* in the two systems differed significantly; the contribution of iced trash fish to *P. trituberculatus* in the PMS system decreased by about 18%, while the total contribution of phytoplankton and POM increased by about 18%, indicating significant reduction and expansion of the isotopic niche of *P. trituberculatus*. In addition, the isotopic niche overlap of *P. trituberculatus* and *M. japonicus* decreased from 5.69% to 1.21% (**Figure 5**, **Table 2**), indicating that polyculturing *S. constricta* in the PM system can weaken food competition. *S. constricta* not only reduced food competition between *P. trituberculatus* and *M. japonicus*, but also filtered a total of 48.90% of POM, SOM, and iced trash fish, which had a significant water purification effect. Therefore, the polyculture of *P. trituberculatus*, *M. japonicus* and *S. constricta* is a healthy and sustainable culture model.

TABLE 2 | SEA, TA and Overlap of isotopic niches of cultured organisms.

Culture systems	Cultured organisms	SEA	TA	O_A
PM	*P. trituberculatus*	0.687	1.396	0.046
	M. japonicas	0.197	0.404	
PMS	*P. trituberculatus*	1.658	3.461	0.024
	M. japonicus	0.343	0.765	
	S. constricta	0.888	1.387	

PM represents P. trituberculatus-M. japonicus system; PMS represents P. trituberculatus-M. japonicus-S. constricta system. SEA represents standard elliptical area; TA represents convex hull area; O_A represents overlapping area.

CONCLUSION

The food composition of *P. trituberculatus* and *M. japonicus* in two systems did not differ much, and both main food items were iced trash fish, phytoplankton, zooplankton and POM. Polyculturing *S. constricta* improved the contribution of plant - based sources, reduced the food competition and isotopic niche overlap between *P. trituberculatus* and *M. japonicus*. And *S. constricta* filtered phytoplankton, POM, SOM and iced trash fish to purify water and improve the utilization of iced trash fish. From the food composition and isotopic niche analysis, *P. trituberculatus-M. japonicus-S. constricta* integrated culture model is healthier and more feasible.

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 below: https://www.ncbi.nlm.nih.gov/, PRJNA809854.

ETHICS STATEMENT

All procedures were performed under the Regulations of the Administration of Affairs Concerning Experimental Animals of China, as well as the Regulations of the Administration of Affairs Concerning Experimental Animals of Shandong Province.

AUTHOR CONTRIBUTIONS

XX and SD gathered, analyzed and interpreted data, discussed the results and co-wrote the manuscript. DZ, LY, WP and YX done animal collection and maintenance. FW was the major instructor. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Key Research and Development Plan Blue Granary Scientific Innovation (No. 2019YFD0900402) and Yellow River Delta Industry Leading Talents Project.

REFERENCES

Abrams, P. (1980). Some Comments on Measuring Niche Overlap. *Ecology* 61, 44–49. doi: 10.2307/1937153

Albaina, A., Fox, C. J., Taylor, N., Hunter, E., Maillard, M., Taylor, M. I. (2010). A TaqMan Real-Time PCR Based Assay Targeting Plaice (*Pleuronectes Platessa* L.) DNA to Detect Predation by the Brown Shrimp (*Crangon Crangon* L.) and the Shore Crab (*Carcinus Maenas* L.)-Assay Development and Validation. *J. Exp. Marine Biol. Ecol.* 391, 178–189. doi: 10.1016/j.jembe.2010.06.029

Bachy, C., Dolan, J., López-García, P., Deschamps, P., Moreira, D. (2013). Accuracy of Protist Diversity Assessments: Morphology Compared With Cloning and Direct Pyrosequencing of 18S rRNA Genes and ITS Regions Using the Conspicuous Tintinnid Ciliates as a Case Study. *Isme J.* 7, 244–255. doi: 10.1038/ismej.2012.106

Ban, W. B. (2015). *Structure Optimization, Nitrogen and Phosphorus Budget and Emergy Analysis of Polyculture Systems in Different Portunus Trituberculatus Ponds [D]* (Qingdao: Ocean University of China (in Chinese).

Beck, J. (2010). Comparing Measures of Species Diversity From Incomplete Inventories: An Update. *Methods Ecol. Evol.* 1 (1), 38–44. doi: 10.1111/j.2041-210X.2009.00003.x

Boecklen, W. J., Yarnes, C. T., Cook, B. A., James, A. C. (2011). On the Use of Stable Isotopes in Trophic Ecology. *Annu. Rev. Ecol. Evol. Syst.* 42, 411–440. doi: 10.1146/annurev-ecolsys-102209-144726

Brzeski, V., Newkirk, G. (1997). Integrated Coastal Food Production Systems - a Review of Current Literature. *Ocean Coastal Manage.* 34 (1), 55–71. doi: 10.1016/S0964-5691(97)82690-7

Buck, T. L., Breed, G. A., Pennings, S. C., Chase, M. E., Zimmer, M., Carefoot, T. H. (2003). Diet Choice in an Omnivorous Salt-Marsh Crab: Different Food Types, Body Size, and Habitat Complexity. *J. Exp. Marine Biol. Ecol.* 292, 103–116. doi: 10.1016/S0022-0981(03)00146-1

Chang, E. S., Bruce, M. J., Tamone, S. L. (1993). Regulation of Crustacean Molting: A Multi-Hormonal System. *Am. Zool.* 33, 324–329. doi: 10.1093/icb/33.3.324

Crab, R., Defoirdt, T., Bossier, P., Verstraete, W. (2012). Biofloc Technology in Aquaculture: Beneficial Effects and Future Challenges. *Aquaculture* 356–357, 351–357. doi: 10.1016/j.aquaculture.2012.04.046

Dai, A., Yang, S., Song, Y. (1986). *Marine Crabs in China Sea* (Beijing: Marine Publishing Company).

Deniro, M. J., Epstein, S. (1981). Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. *Geochimica Cosmochim. Acta* 45 (3), 341–351. doi: 10.1016/0016-7037(81)90244-1.

Dong, J. (2013). *Experimental Study on Structure Optimization and Nitrogen and Phosphorus Budget of Polyculture System in Portunus Trituberculatus Ponds [D]* (Ocean University of China (in Chinese).

Dong, S. L., Wang, F., Wang, J., Qi, Z. X., Lu, J. (1999). Effects of Bay Scallop on Plankton and Water Quality of Marineculture Pond. *Acta Oceanologica Sinica.* 21, 138–144. doi: 10.1017/S0266078400010713

Dong, S. P., Wang, F., Zhang, D. X., Yu, L. Y., Pu, W. J., Xu, X., et al. (2021). Assessment of the Carrying Capacity of Integrated Pond Aquaculture of *Portunus Trituberculatus* at the Ecosystem Level. *Front. Marine Sci.* 8, 747891. doi: 10.3389/fmars.2021.747891

Gao, S. K., Yu, W. W., Zhang, S. (2020). The Potential Carbon Source and Trophic Level Analysis of Main Organisms in Coastal Water of Lüsi Fishing Ground, Based on Carbon and Nitrogen Stable Isotope Analysis. *Chin. J. Appl. Ecol.* 31, 301–308. doi: 10.3969/J.ISSN.2095-4972.2021.03.006

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., et al. (2018). "Fishmeal Alternative Protein Sources for Aquaculture Feeds," in *Feeds for the Aquaculture Sector. Springer Briefs in Molecular Science* (Cham: Springer), 1–28.

Genin, A., Jaffe, J. S., Reef, R., Richter, C., Franks, P. J. (2005). Swimming Against the Flow: A Mechanism of Zooplankton Aggregation. *Science* 308, 860–862. doi: 10.1126/science.1107834

Georgina, C., Mariana, L., Elisabet, V., Bettina, T., Gregorio, B., Dario, A.L., et al. (2022). Metabarcoding, Direct Stomach Observation and Stable Isotope Analysis Reveal a Highly Diverse Diet for the Invasive Green Crab in Atlantic Patagonia. *Biol. Invasions* 24 (2), 505–526. doi: 10.1101/2020.08.13.249896

Guo, K., Zhao, W., Jiang, Z., Dong, S. L. (2017). A Study of Organic Carbon, Nitrogen and Phosphorus Budget in Jellyfish-Shellfish-Fish-Prawn Polyculture Ponds. *Aquac. Res.* 48, 68–76. doi: 10.1111/are.12861

Hill, J. M., Mcquaid, C. D. (2009). Effects of Food Quality on Tissue-Specific Isotope Ratios in the Mussel *Perna Perna*. *Hydrobiologia* 635, 81–94. doi: 10.1007/s10750-009-9865-y

Jackson, A. L., Inger, R., Parnell, A. C., Bearhop, S. (2011). Comparing Isotopic Niche Widths Among and Within Communities: SIBER Stable Isotope Bayesian Ellipses in R. *J. Anim. Ecol.* 80, 595–602. doi: 10.1111/j.1365-2656.2011.01806.x

Kassen, R. (2002). The Experimental Evolution of Specialists, Generalists, and the Maintenance of Diversity. *J. Evol. Biol.* 15, 173–190. doi: 10.1046/j.1420-9101.2002.00377.x

Layman, C. A., Arrington, D. A., Montana, C. G. , Post, D. M. (2007a). Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure? *Ecology* 88, 42–48. doi: 10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Layman, C. A., Quattrochi, J. P., Peyer, C. M., Allgeier, J. E. (2007b). Niche Width Collapse in a Resilient Top Predator Following Ecosystem Fragmentation. *Ecol. Letters.* 10, 937–944. doi: 10.1111/j.1461-0248.2007.01087.x

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., et al. (2013). A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial COI Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents. *Front. Zool.* 10, 1–14. doi: 10.1186/1742-9994-10-34

Li, C., Cheng, Y. X., Guan, Q. Z., Xi, Y. W., Li, J. Y. (2018). Using Stable Isotopes to Estimate the Effects of Different Carbon/Nitrogen Feeds on Feeding Habit of *Procambarus Clarkii* in the Rice-Crayfish Co-Culture System. *J. Fish. China.* 42, 1778–1786. doi: 10.11964/jfc.20171211115

Liu, J. K. (1999). *Advanced Aquatic Biology* (Beijing: Science Press).

Liu, G., Ning, Y., Xia, X. F., Gong, M. (2018). The Application of High-Throughput Sequencing Technologies to Wildlife Diet Analysis. *Acta Ecologica Sinica.* 38, 3347–3356. doi: 10.5846/stxb201706151092

Mccutchan, J. H., Lewis, W. M., Kendall, C., Mcgrath, C. C. (2003). Variation in Trophic Shift for Stable Isotope Ratios of Carbon, Nitrogen, and Sulfur. *Oikos* 102, 378–390. doi: 10.1034/j.1600-0706.2003.12098.x

Mckindsey, C. W., Landry, T., Obeirn, F. X., Davies, I. M. (2007). Bivalve Aquaculture and Exotic Species: A Review of Ecological Considerations and Management Issues. *J. Shellfish Res.* 26 (2), 281–294. doi: 10.2983/0730-8000(2007)26[281:BAAESA]2.0.CO;2

Mykles, D. L., Skinner, D. M. (1982). Crustacean Muscles: Atrophy and Regeneration During Molting. *Soc. Gen. Physiol.* 37, 337–357. doi: 10.1371/journal.pone.0181914

Nelson, E. J. H., Holden, J., Eves, R., Tufts, B. (2017). Comparison of Diets for Largemouth and Smallmouth Bass in Eastern Lake Ontario Using DNA Barcoding and Stable Isotope Analysis. *PloS One* 12, e0181914. doi: 10.1371/journal.pone.0181914

Nielsen, J. M., Clare, E. L., Hayden, B., Brett, M. T., Kratina, P. (2018). Diet Tracing in Ecology: Method Comparison and Selection. *Methods Ecol. Evol.* 9, 278–291. doi: 10.1111/2041-210X.12869

Ogloff, W. R., Yurkowski, D. J., Davoren, G. K., Ferguson, S. H. (2019). Diet and Isotopic Niche Overlap Elucidate Competition Potential Between Seasonally Sympatric Phocids in the Canadian Arctic. *Marine Biol.* 106, 1–12. doi: 10.1007/s00227-019-3549-6

Pérez-Farfante, I., Kensley, B. (1997). "Penaeoid and Sergestoid Shrimps and Prawns of the World," in *Keys and Diagnoses for the Families and Genera*, vol. 175. , 1–233.

Post, D. M. (2002). Using Stable Isotopes to Estimate Trophic Position: Systems, Methods, and Assumptions. *Ecology* 83, 703–718. doi: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., Montana, C. G. (2007). Getting to the Fat of the Matter: Systems, Methods and Assumptions for Dealing With Lipids in Stable Isotope Analyses. *Oecologia* 152, 179–189. doi: 10.1007/s00442-006-0630-x

Redmond, N. E., Morrow, C. C., Thacker, R. W., Diaz, M. C., Esnault, N., Boury, C. P., et al. (2013). Phylogeny and Systematics of Demospongiae in Light of New Small-Subunit Ribosomal DNA (18S) Sequences. *Integr. Comp. Biol.* 53, 388–415. doi: 10.1093/icb/ict078

Rorke, R. O., Lavery, S., Chow, S., Takeyama, H., Tsai, P., Beckley, L. E., et al. (2012). Determining the Diet of Larvae of Western Rock Lobster (*Panulirus Cygnus*) Using High-Throughput DNA Sequencing Techniques. *PloS One* 42, e42757. doi: 10.1371/journal.pone.0042757

Rossi, L., Lascio, A. D., Carlino, P., Calizza, E., Costantini, M. L. (2015). Predator and Detritivore Niche Width Helps to Explain Biocomplexity of Experimental Detritus-Based Food Webs in Four Aquatic and Terrestrial Ecosystems. *Ecol. Complexity.* 23, 14–24. doi: 10.1016/j.ecocom.2015.04.005

Tao, L. S. R., Lau, D. C. P., Perkins, M. J., Hui, T. T. Y., Yau, J. K. C., Mak, Y. K. Y., et al. (2020). Stable-Isotope Based Trophic Metrics Reveal Early Recovery of Tropical Crustacean Assemblages Following a Trawl Ban. *Ecol. Indic.* 117, 106610. doi: 10.1016/j.ecolind.2020.106610

The Ministry of Agriculture Fishery and Fishery Administration (2021). *China Fishery Statistical Yearbook* (Beijing: China Agriculture Press).

Traugott, M., Thalinger, B., Wallinger, C., Sint, D. (2020). Fish as Predators and Prey: DNAbased Assessment of Their Role in Food Webs. *J. Fish Biol.* 98, 367–382. doi: 10.1111/jfb.14400

Vaughn, C. C., Hakenkamp, C. C. (2010). The Functional Role of Burrowing Bivalves in Freshwater Ecosystems. *Freshwater Biol.* 46, 1431–1446. doi: 10.1046/j.1365-2427.2001.00771.x

Wallace, R. (1981). An Assessment of Diet-Overlap Indexes. *Trans. Am. Fisheries Soc.* 110, 72–76. doi: 10.1577/1548-8659(1981)110<72:AAODI>2.0.CO;2

Wang, Y. Q. (2011). *Integrated Mariculture With Multi-Trophic Levels* (Beijing: Ocean Press).

Wang, X. Q., Cao, M., Yan, B. L., Zhang, Q. Q. (2009). Integrated Culture of Swimming Crab *Portunus Trituberculatus*. *Fisheries Sci.* 2, 105–108. doi: 10.16378/j.cnki.1003-1111.2009.02.013

Wilsona, R. M., Chantona, J., Lewisb, G., Nowaceka, D. (2009). Isotopic Variation (δ^{15}n, δ^{13}c, and δ^{34}s) With Body Size in Post-Larval Estuarine Consumers. *Estuarine Coastal Shelf Sci.* 83, 307–312. doi: 10.1016/j.ecss.2009.04.006

Yang, H. S. (1998). Research Progress on the Environmental Impact of Filter-Feeding Shellfish on Mariculture Area. *Marine Sci.* 22, 42–44. doi: 10.1017/S0266078400010713

Yang, J. M. (2001). A Study on Food and Trophic Levels of Bohai Sea Invertebrates. *Modern Fisheries Inf.* 16, 8–16.

Zhang, K., Tian, X. L., Dong, S. L., Dong, J., Yan, F. J. (2015). *Nitrogen and Phosphorus Budgets of Polyculture System of Portunus Trituberculatus, Litopenaeus Vannamei and Ruditapes Philippinarum* Vol. 45 (Qingdao: Periodical of Ocean University of China, 44–53.

Zhang, H., Xu, Q., Zhao, Y., Yang, H. (2016). Sea Cucumber (*Apostichopus Japonicus*) Eukaryotic Food Source Composition Determined by 18s rDNA Barcoding. *Marine Biol.* 163, 1–11. doi: 10.1007/s00227-016-2931-x

Zhou, Y. G., Ma, S., Su, Y. P., Yan, B. L., Wang, X. Q., Liu, Y. K. (2010). *Studies on the Effects of Polyculture of Litopenaeus Vannamei With Portunus Trituberculatus* Vol. 40 (Qingdao: Periodical of Ocean University of China), 11–16.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.