

Dietary analysis based on 18S rDNA, and stable carbon and nitrogen isotopes in juvenile *Eriocheir sinensis* crabs reared under three feeding modes



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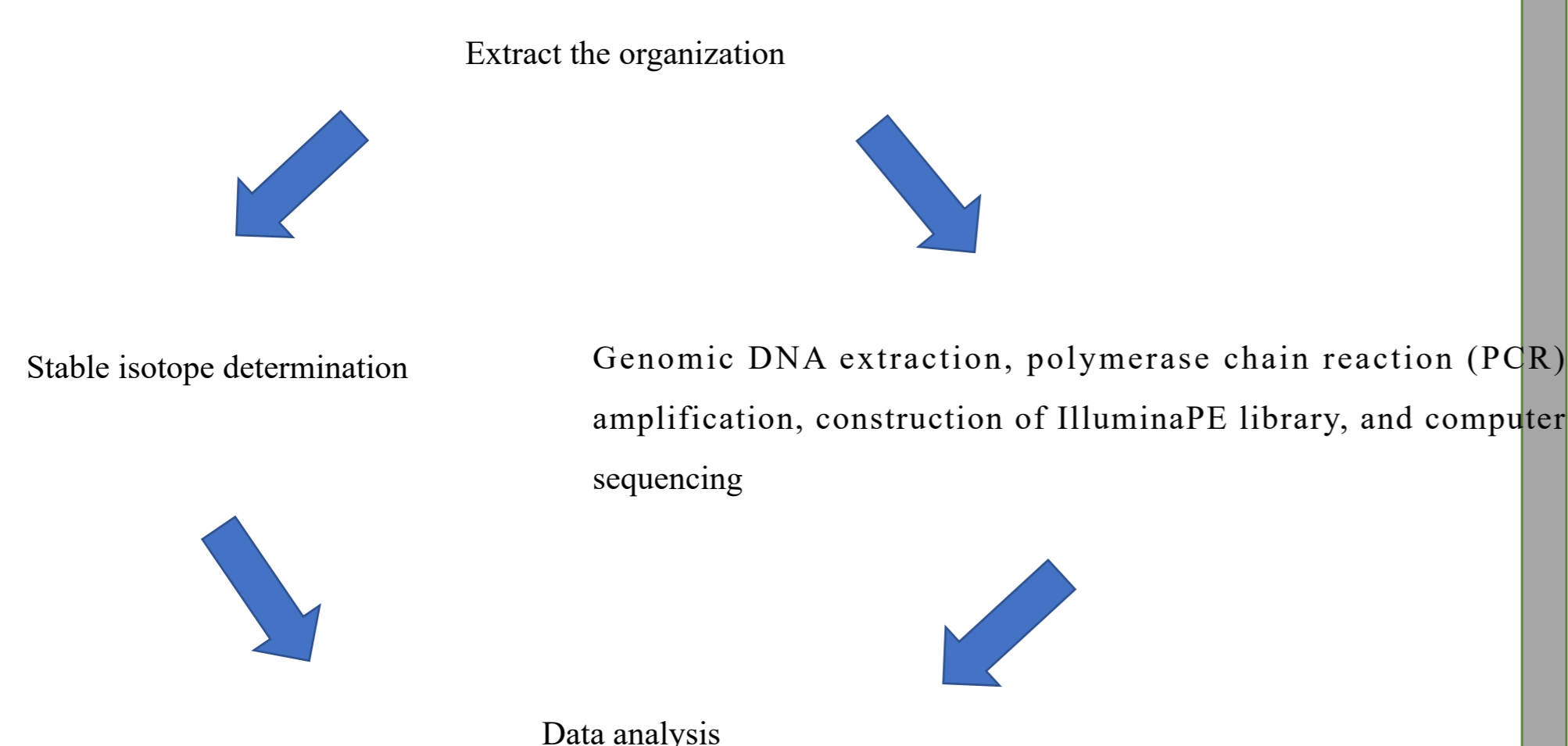
Introduction

- The survival rate, weight gain rate, and first molting time of crabs in the second year could be improved if they were fed with high-quality bait at the latter stage of culture.
- The 18S rDNA can be applied to the analysis of the feeding habits of aquatic animals and stable isotopes can analyze the feed sources of organisms over a long period.
- To investigate the feeding conditions of the Chinese mitten crab, *Eriocheir sinensis*.

Materials and Methods



- The experiment was carried out Dongtan Aquaculture Base at Shanghai Daohong Aquaculture Technology Co., Ltd (31.62° N, 121.40° E) from July to November 2018. Alternanthera was transplanted evenly the circular groove of the pond and account for 75% of experiment pond. The crabs were fed under three different feeding modes: traditional (consisting of wheat, bran, and soybean meal), formulated (Zhejiang Aohua Feed Co., Ltd., Jiaying, China), and mixed (1:1 mixture of traditional and formulated feeds) feeds.
- 1–2 g of formulated feed, POM, and *A. philoxeroides* was collected, and *E. sinensis* samples were collected in October and November 2018.

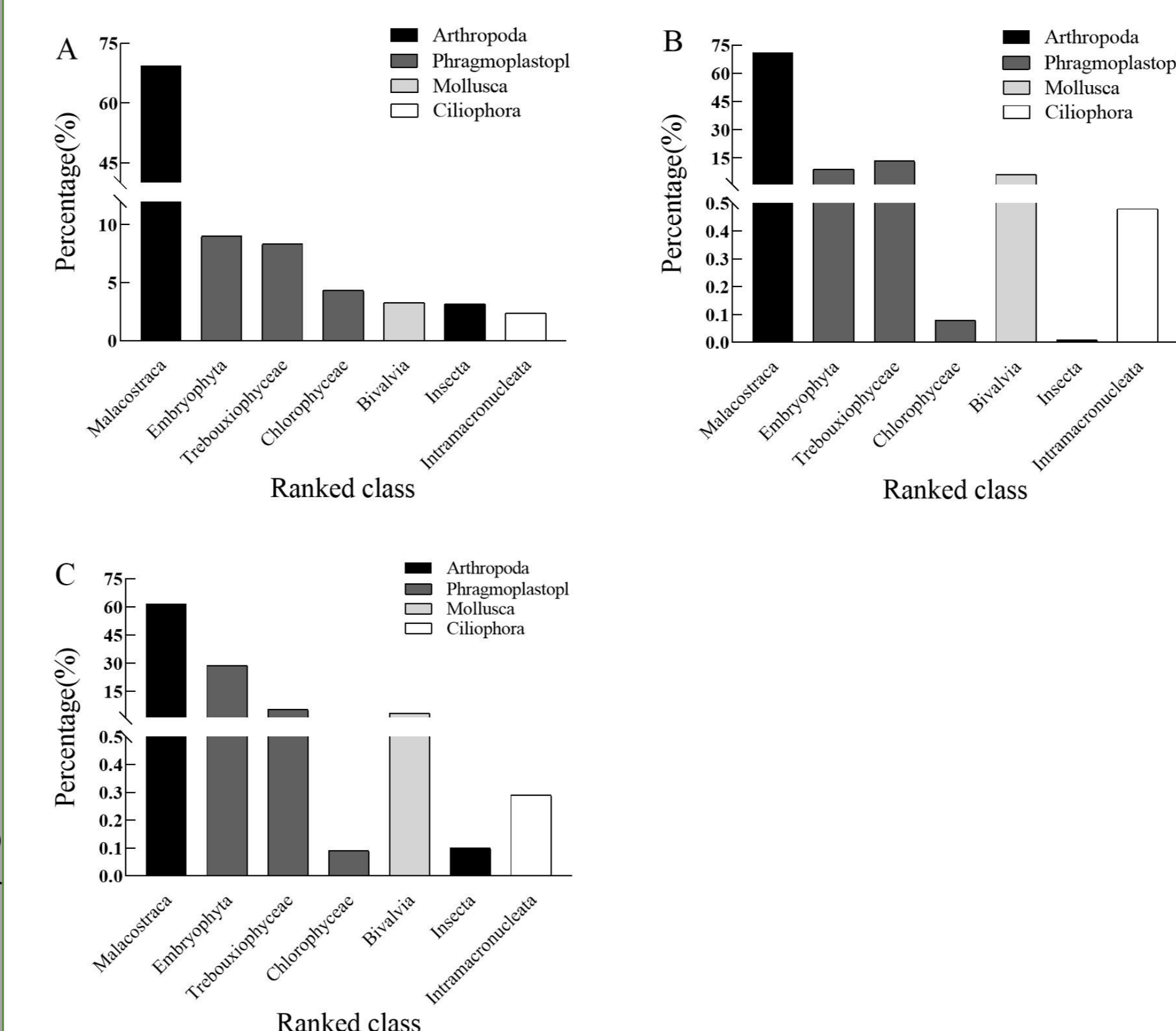


Results

3. Analysis of the main potential eukaryotic components in the stomach of *E. sinensis* under three feeding modes

Phylum	Gender	Traditional feed mode	mode	
			Formulated feed mode	Mixture feed mode
Arthropoda	Female	51.48 ± 0.12	50.87 ± 0.11	40.93 ± 0.11
	Male	45.68 ± 0.14	30.25 ± 0.08	36.76 ± 0.27
Phragmoplastophyta	Female	16.90 ± 0.13	23.20 ± 0.15	24.74 ± 0.11
	Male	6.80 ± 0.01	18.99 ± 0.16	5.08 ± 0.04
Diatomea	Female	3.72 ± 0.03	8.2 ± 0.04	8.43 ± 0.02
	Male	4.35 ± 0.02	3.13 ± 0.03	6.96 ± 0.02

- A total of 34 phyla were identified in the stomach contents of crabs. The number of phyla identified in the stomach contents of crabs reared under traditional, formulated, and mixed feeding modes were 24, 24, and 29, respectively.
- Arthropoda showing the highest overall phylum abundance in the stomach contents of crabs reared under the three feeding modes.



- Malacostraca has the highest overall abundance in the stomach contents of crabs under the three feeding modes, with the lowest being 71.02% (formulated feeding mode), the highest 61.84% (mixed feeding mode), and an average of 67.44%.

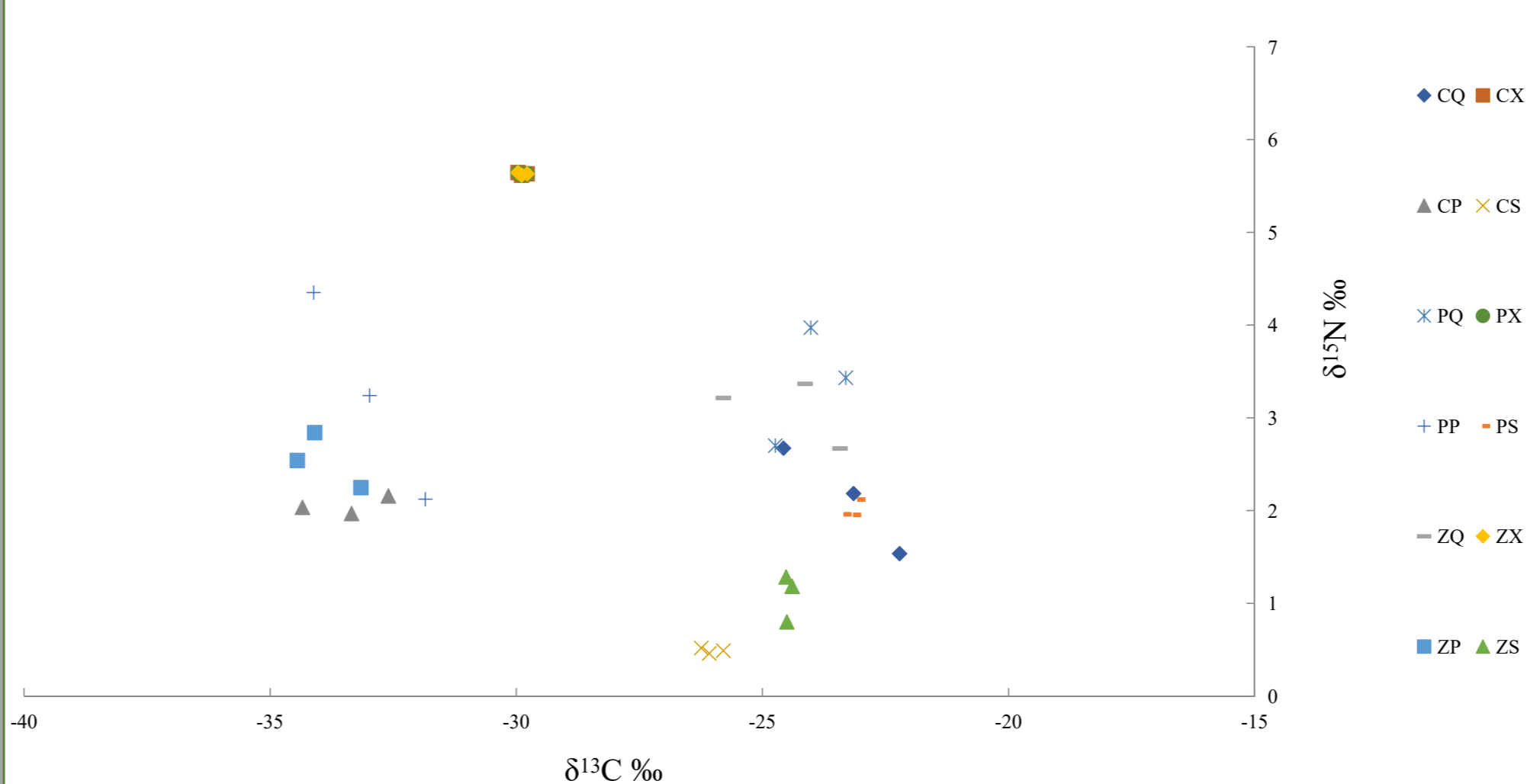
4. Analysis of variance of ¹³C and ¹⁵N isotope contents in muscle of Chinese mitten crab in different feeding modes (%)

Stable isotopes	Traditional feed mode	Formulated feed mode	Mixture feed mode
C	-23.31 ± 1.19	-24.02 ± 0.72	-24.45 ± 1.22
N	2.13 ± 0.57	3.37 ± 0.64	3.08 ± 0.37

Note: values with different small letters mean significant differences in the same row (P < 0.05).

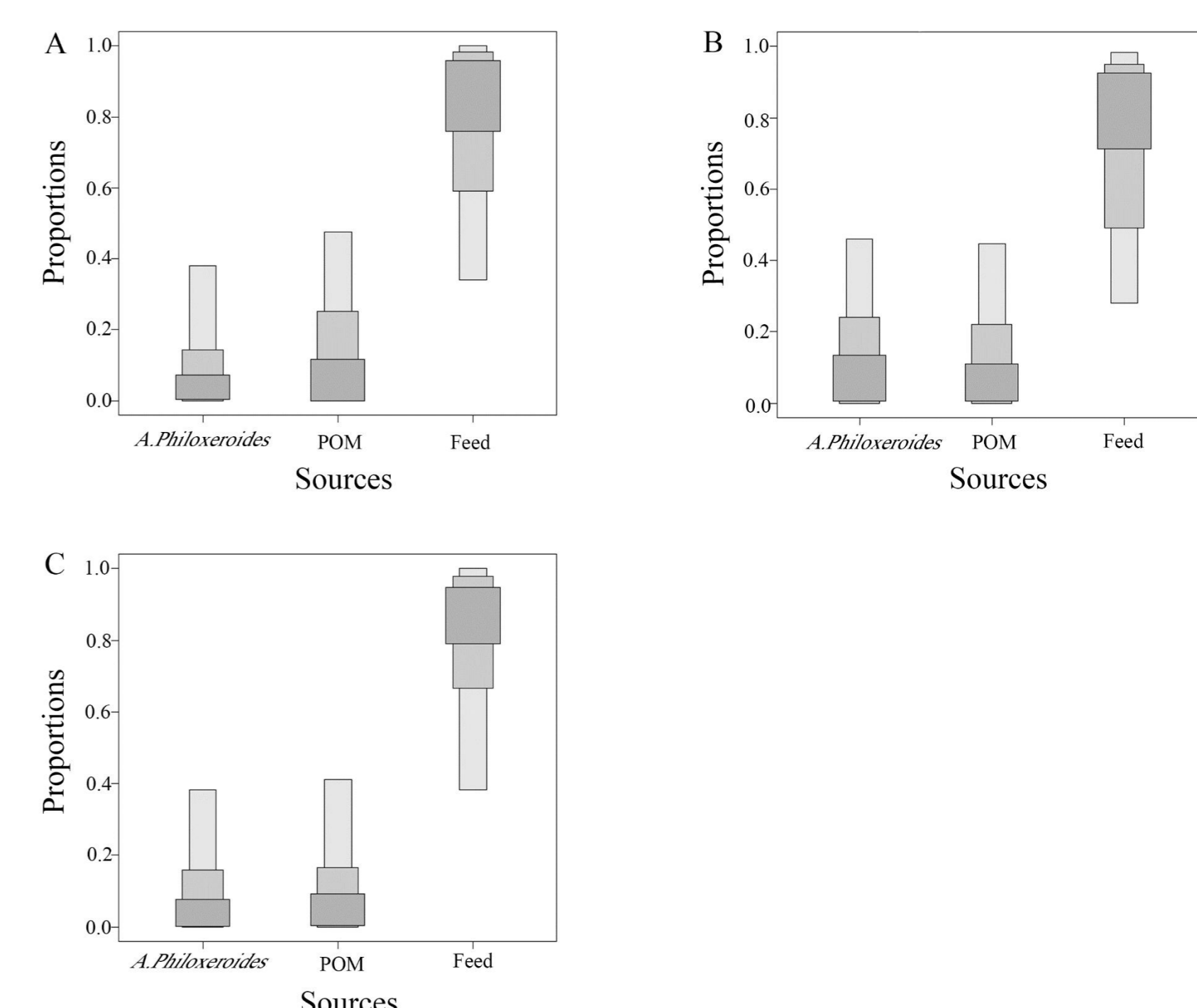
The data in the table are expressed as mean ± standard deviation ($\bar{X} \pm SD$).

5. ¹³C and ¹⁵N stable isotopic characteristics of potential feed sources of *E. sinensis*



- The $\delta^{13}C$ values ranged from -34.45‰ to -22.21‰, and the $\delta^{15}N$ values ranged from 0.27‰ to 5.66‰.
- Among them, the $\delta^{13}C$ variation ranges of the potential feed sources of crabs under the traditional, formulated, and mixed feeding modes were -33.43‰ to -23.31‰, -32.98 to -23.19‰, and -33.90 to -23.19‰, which changes substantially, like the corresponding $\delta^{15}N$ variation under the three feeding modes (0.49–5.66‰, 1.93–5.66‰, and 0.97–5.66‰, respectively).

6. Potential feed source composition of *E. sinensis* under three feeding modes based on stable isotope analysis



- Under the traditional feeding mode, the main diet of *E. sinensis* was a traditional feed, with an average contribution of 71.89%, followed successively by POM and *A. philoxeroides*, with average contributions of 16.66% and 11.46%, respectively.
- Under the formulated feeding mode, the main diet of crabs was formulated feed, with an average contribution of 66.48%, followed successively by *A. philoxeroides* and POM, with average contributions of 17.50% and 16.03%, respectively.
- Under the mixed feeding mode, the main diet of crabs was a mixed feed, with an average contribution of 76.84%, followed by POM (11.92%) and *A. philoxeroides* (11.24%), whose average contribution rates were not significantly different.

Discussion

1. Differences in the dietary composition of *E. sinensis* under the three feeding modes

The male and female *E. sinensis* samples collected from the study site at Shanghai displayed a broad omnivorous diet, consuming the majority of phyla present at the study area. The most representative phyla reported for the sampling site (Arthropoda, Phragmoplastophyta, and Diatomea) also showed the highest occurrence in the dietary samples analyzed.

Under the traditional feeding mode, crabs mainly consumed POM as a feed supplement in addition to the traditional feed, which is consistent with the results of Paning (1939) and Rudnick et al. (2000), who reported that the stomach contents of *E. sinensis* contain a large amount of POM. POM is the substrate of complex biological communities, formed by the decomposition and mineralization of dead animals and plants, humus, and feces by microorganisms, and some of its components are the main feed sources for benthic invertebrates.

Under the formulated feeding mode, apart from the formulated feed, the proportion of *A. philoxeroides* was the highest. In the presence of sufficient animal feed, crabs will still choose *A. philoxeroides* as a feed supplement. This is because *A. philoxeroides* is rich in cellulase, which helps to improve the cellulase activity and digestive ability of crabs.

The mixed feed containing plant- and animal-based feeds had a balanced nutritional ratio and high palatability, and the proportion of mixed feed consumed by *E. sinensis* was the highest, resulting in the lowest feeding proportion of POM and *A. philoxeroides* between the three feeding modes. The results of the stable isotope technique were consistent with those of the 18S rDNA analysis. Juvenile crabs feed on both plant- and animal-based feeds in an aquaculture pond, but they are not complete predators and selectively feed on animal or plant feeds as supplements of that which is deficient, in addition to their main feed.

2. Difference in stomach content composition between male and female *E. sinensis*

Various studies have shown that there are no differences in feed composition between male and female crabs, and they are, therefore, ecological equivalents. In this study, the diversity and abundance of female crabs were generally higher than that of male crabs, female crabs have a higher feeding rate on available feed sources in the pond, which may be because female crabs increase their feeding rate during October to November every year in order to meet their own growth and development, while promoting gonad maturation and transformation.

However, no significant differences were observed in Simpson's diversity and Chao's abundance indices of male and female crabs under the same feeding mode as well as in the abundance of the three main feeding sources, Arthropoda, Phragmoplastophyta, and Diatomea, indicating that male and female crabs showed similar feed composition under the same feeding mode. Cordone et al. studied the feeding habits of *C. maenas* and found that different living water depths and a wide range of available feed sources lead to differences in feed composition between male and female crabs, which is different from the results of this study. This may be because the experiment was conducted in a shallow pond with few feeding sources, and there was no significant difference in feeding habits between male and female crabs.

Acknowledgements

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The richness of feed source species and the amount of sequencing data of the crab stomach contents under different feeding modes were reasonable.

The sequencing data were reasonable and relatively comprehensive, covering the feeding source species of juvenile crab under the three feeding modes