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Environmental adaptation, growth performance and nutrient content of the clam *Cyclina sinensis* from different geographic locations

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The clam *Cyclina sinensis* is an economically important marine species in China. However, overfishing, habitat destruction, and inbreeding have led to the destruction of genetic resources. In this study, five natural populations [Dandong (DD), Dongying (DY), Tianjin (TJ), Wenzhou (WZ), and Yancheng (YC)] from different geographical locations were relocated and cultured homogeneously to study their potential for translocation and environmental adaptation by observing growth performance and nutrient content. There were significant differences in the growth rates of shell length (SL), shell height (SH), and shell width (SW) among the five populations ($P<0.05$). The DD, TJ, and YC populations exhibited the highest SL and SH growth rates, whereas these were lowest in the WZ population. The survival rate was highest in the YC population and lowest in the WZ population, which was significantly lower than the other four populations ($P<0.05$). The DD population exhibited superior plumpness and glycogen content, and the overall glycogen content in male *C. sinensis* was higher compared with that in females ($P<0.05$). All populations displayed a high total amino acid content and the essential amino acid/Total Amino Acid (EAA/TAA) and Non-essential amino acid/Total Amino Acid (NEAA/TAA) ratios in the soft tissue of all five populations aligned with the FAO/WHO ideal protein evaluation standards. Despite the same aquaculture environment, however, the nutrient composition of *C. sinensis* sampled from different populations varied significantly ($P<0.05$).Therefore, ex situ conservation did not eliminate nutritional differences between the different populations of *C. sinensis*. These findings highlight the importance of considering environmental and ecological factors in the nutritional assessment and cultivation of shellfish. In addition, *C. sinensis*

from sampling sites closer to the transplantation sites had higher survival rates and growth rates, and the gonadal development of all populations showed adaption to the local environment, resulting in synchronization of the reproductive period.

KEYWORDS

environmental adaptation, wild populations, growth performance, nutrient analysis, Cyclina sinensis

1 Introduction

Owing to habitat and genetic differences, different geographical groups of the clam *Cyclina sinensis* show significant differences in growth performance (Liu et al., 2002), reproductive indicators (Dong et al., 2011), stress tolerance (Li et al., 2006; Liang et al., 2016), and sex ratio (Dong et al., 2021). The timing of gonadal development and differences in the energy balance of adult *C. sinensis* vary in response to habitat conditions (Liu et al., 2006). Different geographical populations of *C. sinensis* also have a high level of genetic diversity (Shao et al., 2018), with genetic differences between different *C. sinensis* populations being reflected in culture growth, behavioral traits, and reproduction characteristics, as well as significant differences in growth and performance and survival of juvenile clams at different temperatures (Zhao et al., 2016). In addition, the nutrient content of shellfish is affected by a variety of factors—such as shellfish species, the environment in which they grow, the stage of development, and the freshness of the raw material—resulting in large differences in the nutrient value of different groups of shellfish of the same species (Chen et al., 2006; Zhang et al., 2006; Wu and Yan, 2011; Watanabe and Katayama, 2014). However, there are few data available comparing the nutritional differences between groups of *C. sinensis*.

The intricate marine circulation system in China, encompassing warm currents, coastal flows, and localized circulations, combined with salinity and temperature variations between the northern and southern coasts, and the presence of rivers of varying sizes flowing into the sea, has led to geographical isolation and genetic differentiation within the eurythermal, euryhaline *C. sinensis*. Such high genetic diversity is indicative of the environmental adaptability, reproductive capacity, and evolutionary potential of this species and different geographical populations of *C. sinensis* have been reported to have high levels of genetic diversity (Shao et al., 2018).

However, in recent years, factors such as environmental degradation, overfishing, and the increasing scale of aquaculture have severely compromised this genetic diversity, leading to issues including resource degeneration, inbreeding, and suboptimal cultivation outcomes. Consequently, research into the relocation protection of *C. sinensis* has become vital. Given the differences in habitat and genetics, significant variation occurs among geographical populations of *C. sinensis* in terms of growth performance (Liu et al., 2002), reproductive indices (Dong et al., 2011), stress tolerance capabilities (Li et al., 2006; Liang et al., 2016), and gender ratios (Dong et al., 2021). Influenced by their habitats, the timing of gonadal development and energy balance of mature *C. sinensis* vary (Liu et al., 2006).

Thus in the current study, to understand the growth performance and body composition of *C. sinensis* from different geographic locations following *ex situ* conservation, five wild groups of *C. sinensis* of the same size and different geographic locations were intensively reared in the Ganyu Sea area of Lianyungang Port for 1 year. Growth parameters, environmental adaptive ability, nutritional indices, and changes in nutrient composition of the groups were assessed to provide insights into ways to preserve the genetic diversity associated with the growth of this species in the wild.

2 Materials and methods

2.1 Animals and sample processing

Five wild *C. sinensis* populations were sampled from Dandong (DD)(40.1243°N, 124.3947°E), Dongying (DY) (37.4645°N, 118.4914°E), Tianjin (TJ) (39.3434°N, 117.3616°E), Wenzhou (WZ) (27.9938°N, 120.6994°E), and Yancheng (YC) (33.3474°N, 120.1635°E). After disinfection and temporary rearing at the Jiangsu Provincial Shellfish Test Station, individuals of a uniform size and similar specifications were screened within each population.There are 465 clams in each group. Thirty *C. sinensis* were randomly sampled from each population and their body mass, shell length (SL), shell width (SW), and shell height (SH) were recorded (Table 1). They were then temporarily cultured in separate ponds for 7 days at the Jiangsu Province Shellfish System Comprehensive Experimental Station. During this period, food was withheld while oxygen was continuously supplied to facilitate sand expulsion and emptying of internal organs (food residues and feces) to minimize experimental errors caused by impurities within their bodies. Each *C. sinensis* was then thoroughly cleaned using pure water and a brush to remove any attachments from the surface of their body before being wiped dry with a cloth. After being weighed using an electronic balance (accurate to 0.01 g), each *C. sinensis* was dissected with a scalpel and their soft tissues were extracted. Absorbent paper was used to remove any excess moisture from the soft tissues before they were then weighed. The soft tissue from each *C. sinensis* from the same

TABLE 1 Initial body mass, shell length, shell height, shell width; Final body weight, shell length, shell height, and shell width in different wild populations of *C. sinensis*[a].

Indicator	Population				
	Dandong	Dongying	Tianjin	Wenzhou	Yancheng
Initial mass (g)	7.99 ± 0.13	7.92 ± 0.13	7.98 ± 0.17	7.89 ± 0.21	7.97 ± 0.14
Initial shell length (mm)	30.10 ± 0.32	29.31 ± 0.26	30.27 ± 0.26	29.86 ± 0.40	29.36 ± 0.15
Initial shell height (mm)	29.63 ± 0.19^{ab}	29.19 ± 0.24^{b}	29.65 ± 0.29^{ab}	30.65 ± 0.39^{a}	28.94 ± 0.14^{b}
Initial shell width (mm)	18.19 ± 0.20	18.31 ± 0.13	17.94 ± 0.21	16.81 ± 0.13	17.99 ± 0.17
Final weight (g)	12.23 ± 0.27	12.05 ± 0.24	12.12 ± 0.27	11.89 ± 0.24	11.83 ± 0.14
Final shell length (mm)	32.53 ± 0.30^{a}	31.49 ± 0.26^{bc}	32.40 ± 0.32^{ab}	30.86 ± 0.22^{c}	31.92 ± 0.19^{ab}
Final shell height (mm)	32.95 ± 0.28^{a}	31.49 ± 0.26^{c}	32.84 ± 0.28^{a}	31.94 ± 0.22^{bc}	32.43 ± 0.18^{ab}
Final shell width (mm)	19.93 ± 0.16	19.60 ± 0.67	19.91 ± 0.15	20.42 ± 0.17	19.97 ± 0.10

[a]Different lowercase letters between peers indicate significant differences among populations (P<0.05).

geographical population was then crushed and mixed together. A portion of this mixture was stored at –80°C, while the remaining samples underwent drying in a constant temperature drying oven at 105°C to determine the soft tissue moisture content. Once completely dried, these samples were ground into powder for subsequent nutritional analysis.

2.2 Experimental design

The breeding site used for the study was a natural seawater pond located in the Ganyu Sea Area of Lianyungang City in China. On August 1, 2020, five population zones were established evenly across the pond and intensively stocked with *C. sinensis*. The experiment ran for 366 days and concluded on August 1, 2021.

The seine net (3 m long × 3 m wide, 2 m in height, aperture diameter of 0.5 cm) was deployed in the experimental area to ensure the preservation of distinct germplasm among the different *C. sinensis* groups and prevent predation. All net contained a uniform distribution of 150–160 *C. sinensis*. Every day maintenance was conducted to address any potential gaps in the nets. The test site comprised sandy and muddy substrates, with water levels was 1.5 m. Regular water quality assessments by measuring the pond's ammonia, nitrite, dissolved oxygen, and pH on a weekly basis, ensuring timely adjustments to maintain all environmental parameters within normal ranges.

To maximize the restoration of a natural growth environment for *C. sinensis*, no artificial feeding was conducted during the cultivation period, and phytoplankton, sediment, and nutrient salts in natural seawater were utilized as feed sources. During periods of high temperature, an aerator was employed at strategic intervals to ensure adequate oxygenation, keeping oxygen levels above 4 mg/L.

2.3 Data and sample collection

The growth of *C. sinensis* was monitored during the study, with the initial weight (W), SL, SH, and SW of the test clams measured on August 1, 2020 and then again on August 1, 2021. To ensure accurate assessment of reproductive indicators, From April 2021 onwards, 30 random samples were taken each month and subsequently dissected to observe the level of gonadal development. Through visual inspection, microscopy observations, and examination of tissue sections, it was determined that all five groups had entered their reproductive period by August 2021.

Individuals with intact and unspent gonads were selected for dissection. After sex determination, the gonads, hepatopancreas, and other soft tissues were removed using scissors and dissecting forceps. The samples were placed in labeled centrifuge tubes and measured using electronic vernier calipers and an electronic balance to determine SL (to the nearest 0.01 mm) and mass (to the nearest 0.01 g). Results of these measurements were recorded to calculate growth and reproduction indexes for each group of *C. sinensis*.

These results were used to calculate the growth and reproduction indexes of each group. The growth indexes included terminal body mass, terminal SL, terminal SW, and terminal SH, and were used to calculate the weight gain rate (RW), specific weight gain rate (SRW), SL growth rate (RSL), SL-specific growth rate (SRSL), SH growth rate (RSH), SH-specific growth rate (SRSH), SW growth rate (RSW), and SW-specific growth rate (SSW). Reproductive indicators included the gonadosomatic index (GSI), the hepatopancreas index (HSI), and the condition factor (CF). To ascertain the survival rate, one must conduct a meticulous exploration of a square meter area of clams, meticulously tallying the survival rates through three random excavations. The survival status was determined by counting the number of survivors and the number of empty shells in each group, and the number of parallel groups was three.

The equations used to determine each index were as follows:

$$\text{Survival rate} = N1/N0 \times 100\,\%$$

$$RW = (W2 - W1)/W1 \times 100\,\%$$

$$SRW = (\ln W2 - \ln W1)/D \times 100\,\%$$

$$RSL = (SL2 - SL1)/SL1 \times 100\,\%$$

$$SRSL = (\ln SL2 - \ln SL1)/D \times 100\,\%$$

$$RSH = (SH2 - SH1)/SH1 \times 100\,\%$$

$$SRSH = (\ln SH2 - \ln SH1)/D \times 100\,\%$$

$$RSW = (SW2 - SW1)/SW1 \times 100\,\%$$

$$SSW = (\ln SW2 - \ln SW1)/D \times 100\,\%$$

$$GSI = \text{gonad/body mass} \times 100\,\%$$

$$HSI = \text{hepatopancreas/body mass} \times 100\,\%$$

$$CF = \text{flesh weight/body mass} \times 100\,\%$$

where N1 and N0 represent the number of survivors and the overall number, respectively; W1 and W2 represent the average body weight (g) of *C. sinensis* at the start and end of the study, respectively; SL1, SW1, and SH1 represent the average SL, SW, and SH (all mm) of *C. sinensis* at the start of the study, respectively; SL2, SW2 and SH2 represent the average SL, SW, and SH (all mm) of *C. sinensis* at the end of the study, respectively; and D is the stocking level.

2.4 Biochemical analysis

2.4.1 Determination of glycogen content

The glycogen content in the mantle,adductor muscle, and gonad-visceral mass of *C. sinensis* was determined using a Glycogen Assay Kit (A043-1-1, Nanjing Jiancheng, China) following the manufacturer's instructions. Five mature male and female *C. sinensis* were selected from each group for dissection, and their tissues were rinsed with saline before being homogenized according to sex and tissue type. For each group, 50 mg of each sample was weighed into a test tube and mixed with 150 μL of alkaline solution before being heated for 20 min at 100°C in a water bath followed by cooling under running water. The samples were then diluted based on weight (mg) with double-distilled water (μL), using a 1:16 ratio of the dilution of the original test solution. Chromogenic powder was added to each tube along with a corresponding volume of chromogenic solution made by adding 25 mL concentrated H_2SO_4 to a final concentration of 98%. After thorough mixing, samples were heated for an additional 5 min at 100°C before measuring OD values using a spectrophotometer; glycogen content was calculated as follows:

$$\text{glycogen content} = \text{assay tube OD value/standard tube OD value} \times \text{standard tube glycogen content} \times 20 \times 100/1.11$$

where 20 reflects the number of times the sample was diluted before carrying out the measurements; and 100 reflects the number of times they were diluted during the measurement.

2.4.2 Nutrient content analysis

Thirty individuals from each population were randomly selected for nutrient content analysis.The various determinants of the nutrient content of the soft tissue of *C. sinensis* were analyzed as follows: moisture content, using the direct drying method according to GB5009.3-2016;Weighing bottles were dried and weighed within 2 mg, and a mixed sample of the mollusc parts of *C. sinensis* (3.0-5.0 g, weighed to 0.0001 g) was added to each bottle, which was then uncapped and placed in a 105°C oven to dry for 16 h. At the end of the drying period, the bottles were placed in a desiccator to dry for 0.5 h, and then weighed and recorded; after that the bottles were placed again in the oven to dry for 6 h, and then placed in a desiccator to dry for 0.5 h, and then weighed for a second time. After that, the weighing bottle with the sample was put into the drying oven again for 6 h, and then put into the desiccator for 0.5 h for the second weighing. If the weighing error is >0.002 g, the drying process is repeated until the two weighing errors are within the specified range. crude protein content, using the Kjeldahl method, following GB5009.5-2016; crude fat content, using the cable extraction method following GB/T 14772-2016; the ash content, determined following GB5009.4-2016; fatty acid content, using the area normalization method, following GB5009.168-2016; and the amino acid content, using oxidative acid hydrolysis GB/T 18246-2019.

2.4.3 Nutritional evaluation of amino acids

Muscle Amino Acid Score (ASS), Muscle Amino Acid Chemistry Score (CS), and Essential Amino Acid Index (EAAI) were used to evaluate the amino acid nutrition of the different groups of *C. sinensis*. ASS was calculated by adopting the model of amino acid scoring criteria (%, dry) jointly recommended by the Food and Agriculture Organization of the United Nations and World Health Organization, using the following equation:

$$ASS = aa/[AA(FAO/WHO)] \times 100\,\%$$

where aa is the corresponding amino acid content (%) in the sample and AA[FAO/WHO] is the corresponding amino acid content (%) in the scoring standard model.

CS was calculated using the amino acid standard model of whole egg protein (%, dry) with the following equation:

$$CS = aa/AA(egg) \times 100\,\% \quad CS = aa/AA(egg) \times 100\,\%$$

where aa is the amino acid content of the protein in the test sample (mg/gN) and AA (egg) is the content of the same amino acid in the egg protein (mg/gN).

EAAI was determined using the following equation:

$$EAAI = \sqrt[n]{\frac{100ThrA}{ThrB} \times \frac{100ValA}{ValB} \times \frac{100LysA}{LysB} \times \ldots\ldots \times \frac{100LeuA}{LeuB}}$$

where *n* denotes the number of essential amino acids for which comparisons were made, A represents the essential amino acid content (%) of the protein of the mollusk portion of *C. sinensis*, and B represents the corresponding essential amino acid content (%) in the protein of the whole egg.

2.5 Statistical analysis

The results were analyzed using SPSS 19.0 (IBM, USA), and one-way ANOVA was used to analyze the differences between groups for data with more than two groups and satisfying the chi-square test for variance. When the variance did not satisfy the chi-square test, significance was analyzed using the Games–Howell test. Differences between two groups were analyzed using the independent samples t-test, with the level of significance set at $P<0.05$. Results are expressed as mean ± standard error (SE).

3 Results

3.1 Annual changes in water temperature and water quality in the study area

The average monthly water temperature in the study area ranged from 4.20°C to 27.91°C (Figure 1), reflecting seasonal changes.Ponds are adjacent to the sea and exchange water with the ocean through tides, and the ambient water temperature is essentially the same as the pond water temperature. The salinity of the water body ranged from 21.2 to 24.3, the pH ranged from 7.63 to 8.03, and the dissolved oxygen ranged from 4.9 mg/L to 6.21 mg/L. The salinity, pH, and dissolved oxygen were relatively stable, which met the conditions required for the normal growth of *C. sinensis*.

3.2 Comparison of *C. sinensis* population survival rates

The survival rates of the five different wild populations of *C. sinensis* after 1-year *ex situ* stocking were as follows: YC (65.86 ± 0.57)%>TJ (64.63 ± 2.72)%>DY (55.50 ± 4.88)%>DD (47.66 ± 3.29)% >WZ (29.90 ± 2.40)% (Table 2). There were significant differences in the survival of some groups after *ex situ* stocking. For example, the survival rate of the WZ group was significantly lower than that of the other four groups ($P<0.05$), whereas that of the DD group was significantly lower than that of the TJ and YC groups ($P<0.05$), but the difference with the DY group was not significant ($P>0.05$).

3.3 Comparison of *C. sinensis* population growth performance

The growth measurements at the start and end of the experimental period are detailed in Table 1, respectively. The average mass of *C. sinensis* among the five groups at the end of the experiment ranged from 11.83g to 12.23g, with no significant difference observed among the groups ($P>0.05$). Significant variations were observed among all five groups in terms of final SL and final SH ($P<0.05$). There was no significant difference in mean initial SL and SW across all five groups. However, by the end of experimental period, the terminal SLs in the DD and TJ groups were similar. These groups demonstrated significantly longer terminal SL compared with the DD and WZ groups ($P<0.05$).

The terminal SH of the five groups of *C. sinensis* ranged from 31.49 mm to 32.95 mm, with the highest being in the DD group (32.95 ± 0.28 mm) and the lowest in the DY group (31.49 ± 0.26 mm). The terminal SH of the DY group were significantly lower than those of the other groups, whereas those of the DD and TJ groups were significantly higher than those of the WZ group ($P<0.05$).

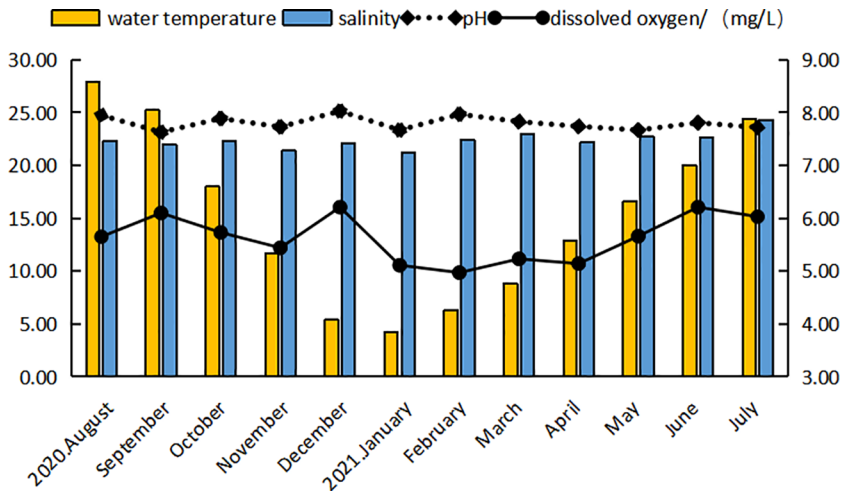

FIGURE 1
Changes in water temperature and quality in the study area across the study period.

TABLE 2 Percentage survival rate of the five populations of *C. sinensis*[a].

Geographical population	Survival rate in each sample (%)			Average survival rate (%)
	1	2	3	
Dandong	39.68	50.67	52.63	47.66 ± 3.29[b]
Dongying	55.79	45.00	65.71	55.50 ± 4.88[ab]
Tianjin	62.79	71.11	60.00	64.63 ± 2.72[a]
Wenzhou	33.96	31.54	24.19	29.90 ± 2.40[c]
Yancheng	64.79	67.19	65.61	65.86 ± 0.57[a]

[a]Different lowercase letters between peers indicate significant differences among populations (P<0.05).

3.3.1 Growth rates of growth indicators

Table 3 details the growth rates calculated using the initial and final values of each growth metric. The WZ group had a significantly slower SL growth rate compared with the other four groups (P<0.05), but a significantly faster growth rate of SH (P<0.05). In terms of specific growth rate, the specific weight gain rate of all five groups exceeded 0.10%, and there was no significant difference between the groups (P>0.05). The highest specific growth rate in SL was observed in the YC group (0.0227 ± 0.0016)%-d^{-1}, whereas the lowest was in the WZ group (0.0088 ± 0.0019)%-d^{-1}, which was significantly lower compared with the other groups (P<0.05).

The SH-specific growth rates of the five groups decreased in order as follows: YC > DD > TJ > DY > WZ. Notably, the WZ group displayed significantly lower growth rates compared with the other four groups, whereas the DY group showed significantly lower growth rates compared with both the DD and YC groups (P<0.05).

3.4 Reproductive indicators of *C. sinensis*

After June 2021, the average monthly water temperature in the study area approached 20°C. The gonads of *C. sinensis* gradually matured, allowing for initial determination of their sex through dissection. However, gamete emission had not yet occurred. From late July to early August, samples were collected from each group of *C. sinensis* and inspected visually. Both male and female *C. sinensis* in each group exhibited fully developed gonads that were visible to the naked eye, with the entire visceral mass filled with mature gametes. Histological examination confirmed that spermatozoa in all groups of clams had reached maturity.

3.4.1 Reproductive indicators of female *C. sinensis*

Reproductive indicators (GSI, HSI), were compared among the five groups of *C. sinensis* (Figure 2). The GIS of female *C. sinensis* ranged from 6.99% to 9.11%, but with no significant difference among the groups from (P>0.05). The plumpness of female *C. sinensis* ranged from 16.78% to 22.03%, being highest in the DD group. One-way ANOVA revealed that females from the DD group were significantly more plump than females from the DY group, and that the environmental adaptations of the wild groups were higher than other group (P<0.05). In terms of HSI, the ratios ranged from 0.58% to 0.69%, but did not differ significantly among the groups (P>0.05).

3.4.2 Reproductive indicators of male *C. sinensis*

There were no statistically significant differences in HSI or GSI among males from the five groups (P>0.05) (Figure 3). In terms of plumpness indices, males in the DD group were most plump, followed by the DG group, whereas YC group were the least plump, with the results being significantly different (P<0.05).

TABLE 3 Percentage growth rates of various indicators of five populations of *C. sinensis*[a].

Indicator	Population				
	Dandong	Dongying	Tianjin	Wenzhou	Yancheng
Weight gain rate (%)	53.04 ± 3.43	52.17 ± 3.05002	51.88 ± 3.37	50.57 ± 3.09	48.49 ± 1.76
Shell length growth rate (%)	8.07 ± 0.98[a]	6.76 ± 0.75[a]	7.03 ± 1.04[a]	3.35 ± 0.72[b]	8.74 ± 0.63[a]
Shell height growth rate (%)	11.22 ± 0.93[a]	7.15 ± 0.75[b]	10.74 ± 0.96[a]	4.20 ± 0.72[b]	12.07 ± 0.65[a]
Shell width growth rate (%)	9.62 ± 0.90[b]	9.43 ± 0.63[b]	12.20 ± 0.85[b]	18.50 ± 0.90[a]	9.95 ± 0.56[b]
Specific weight gain rate (%·d^{-1})	0.1113 ± 0.006	0.1123 ± 0.0054	0.1105 ± 0.0061	0.1100 ± 0.0057	0.1073 ± 0.0032
Shell length specific growth rate (%·d^{-1})	0.0204 ± 0.0024[a]	0.0192 ± 0.0022[a]	0.0179 ± 0.0026[a]	0.0088 ± 0.0019[b]	0.0227 ± 0.0016[a]
Shell height specific growth rate (%·d^{-1})	0.0281 ± 0.0022[a]	0.0204 ± 0.0022[b]	0.0272 ± 0.0023[ab]	0.0110 ± 0.0019[c]	0.031 ± 0.0016[a]
Shell width specific growth rate (%·d^{-1})	0.0242 ± 0.0022[b]	0.0270 ± 0.0019[b]	0.0308 ± 0.0021[b]	0.0461 ± 0.0021[a]	0.0258 ± 0.0014[b]

[a]Different lowercase letters between peers indicate significant differences among populations (P<0.05).

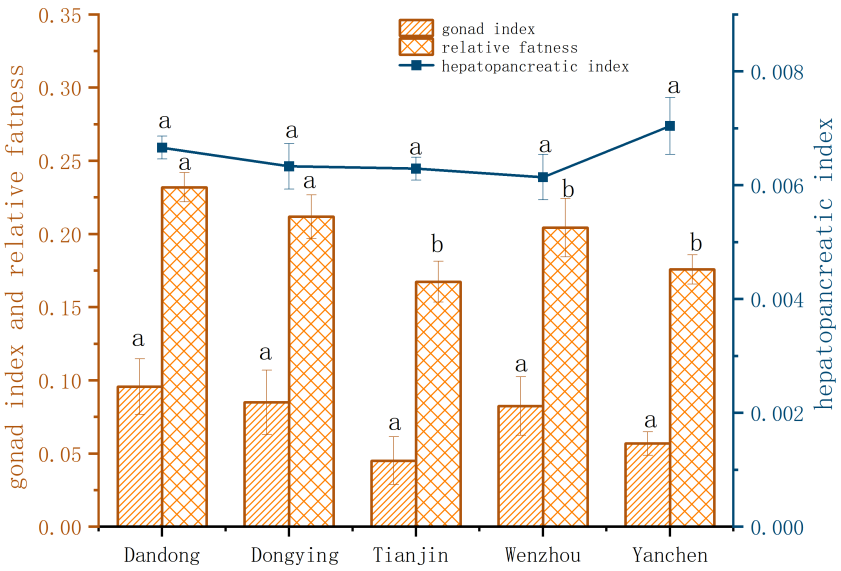

FIGURE 2
Comparison of reproductive indexes of female *C. sinensis* from different populations. Different lowercase letters on the same index indicate significant differences among different populations ($P<0.05$).

3.5 Glycogen content of *C. sinensis* tissues

3.5.1 Glycogen content of tissues of female *C. sinensis*

The glycogen contents of the outer coat membrane, closed-shell muscle, and gonad-visceral mass of female *C. sinensis* from different groups are shown in Figure 4. The glycogen content in the coat membrane of female *C. sinensis* from the YC group was significantly lower than that in the other four groups ($P<0.05$), whereas that of the closed-shell muscle of female *C. sinensis* from different groups did not differ significantly ($P>0.05$). By contrast, the glycogen content of the gonad-visceral mass of female *C. sinensis* from different groups varied considerably, being significantly higher in the DD group compared with all other groups ($P<0.05$) and lowest in the TJ group,.

3.5.2 Glycogen content of tissues of male *C. sinensis*

The glycogen contents of three tissues of male *C. sinensis* are shown in Figure 5. The glycogen content of the coat membrane of

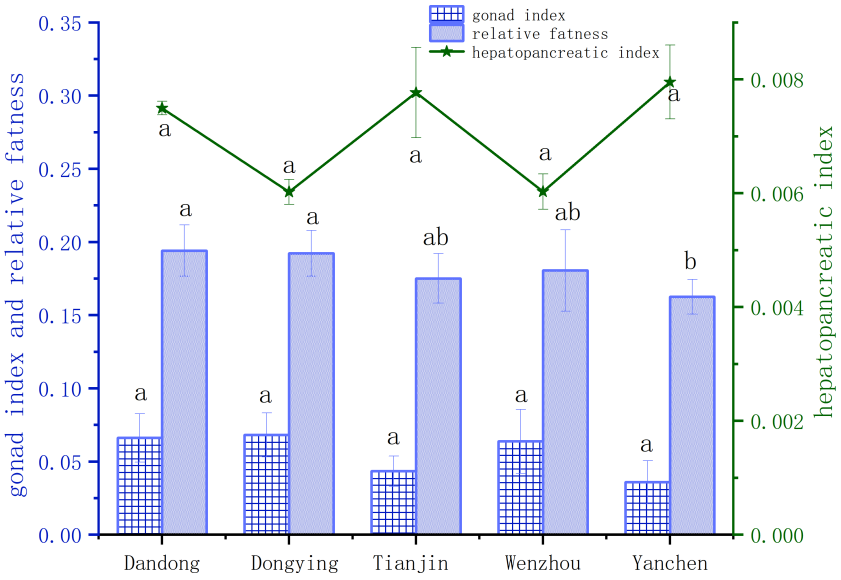

FIGURE 3
Comparison of reproductive indexes of male *C. sinensis* from different populations. Different lowercase letters on the same index indicate significant differences among different populations ($P<0.05$).

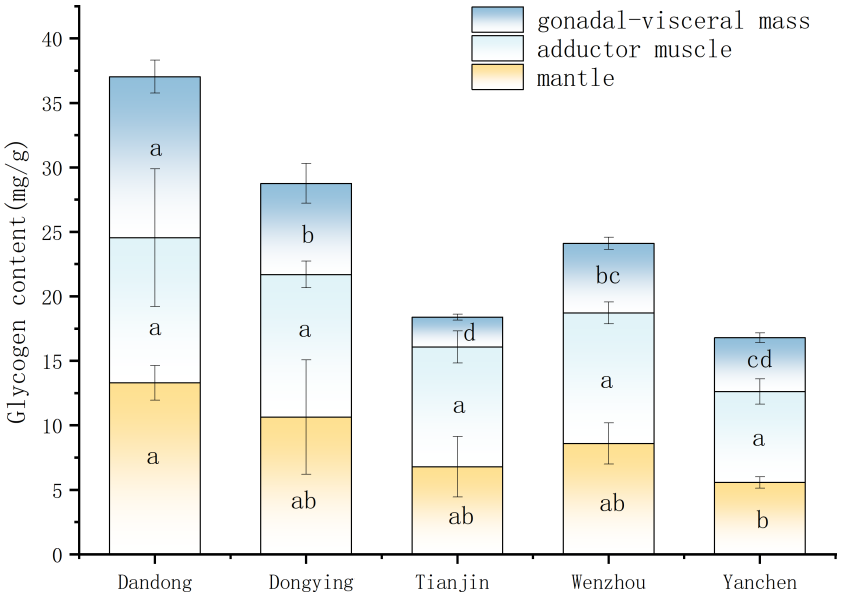

FIGURE 4
Comparison of glycogen content of female *C. sinensis* from different populations. Different lowercase letters on the same index indicate significant differences among different populations ($P<0.05$).

male *C. sinensis* from the DD group was significantly higher than that of the other groups ($P<0.05$), with no significant differences found among the other groups ($P>0.05$). In addition, the glycogen content of the closed-shell muscle of male *C. sinensis* was significantly higher in the DD group compared with all other groups ($P<0.05$), and lowest in the DY group. The glycogen content of the gonads of male *C. sinensis* from different groups ranged from 4.96 to 10.68 mg/g, being highest in the DY group and lowest in the WZ group, the latter being significantly lower compared with all other groups ($P<0.05$).

3.6 Comparison of the nutritional value of *C. sinensis*

The general nutritional composition of the soft tissues of five wild populations of *C. sinensis* were determined at the start of the

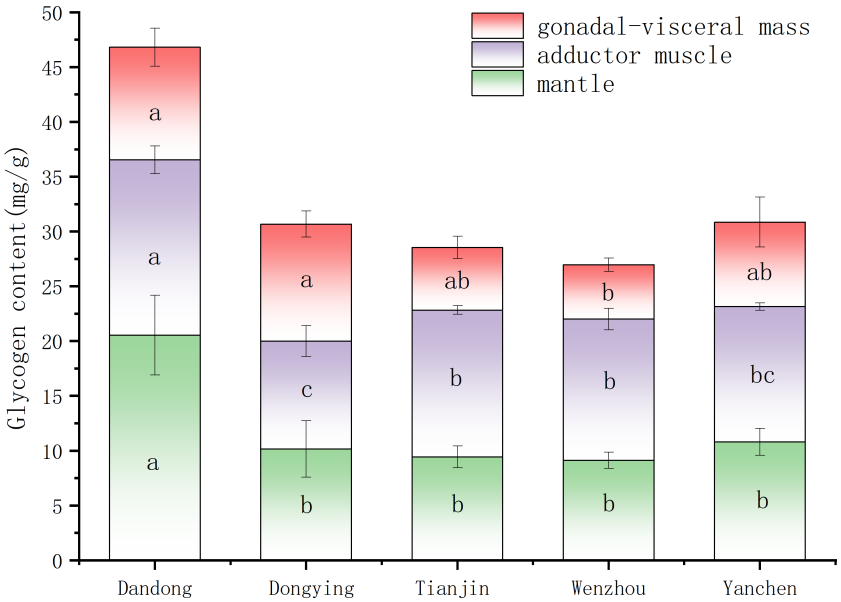

FIGURE 5
Comparison of glycogen content of male *C. sinensis* from different populations. Different lowercase letters on the same index indicate significant differences among different populations ($P<0.05$).

study (Group A from each location) and then 1 year after ex situ conservation in the same environment (Group B from each original location) (Table 4). The moisture content was highest in the DY A and TJ A groups (82.21 ± 0.29% and 82.04 ± 0.05%, respectively) and lowest in DD A, which was significantly lower than that of the other four groups (P<0.05). The crude protein contents of DD A, WZ A, and DY A were significantly different from each other, with that of DY A being significantly lower than that of the other four groups (P<0.05).

The crude fat content ranged from 3.44% to 8.60%, being highest in WZ A and YC A (8.60 ± 0.57% and 8.27 ± 0.13%, respectively), which were significantly higher than in DD A, DY A, and TJ A (P<0.05). There was no significant difference in the ash content among the five groups (P>0.05).

The moisture content of the five populations of *C. sinensis* after 1 year in the same environment ranged from 80.62% to 82.62%, being highest in DY B and lowest in WZ B; there was a significant difference in moisture content only between these two populations (P<0.05). The crude protein content ranged from 55.88% to 62.03%, being highest in YC B, and lowest in WZ B, with a significant difference between these two groups (P<0.05). There was a significant difference in average crude fat content between TJ B and WZ B (P<0.05) and between WZ B and DY B in terms of ash content (P<0.05).

The results also showed that, except for the ash content, the biochemical composition of the different populations of *C. sinensis* changed significantly after *ex situ* conservation in terms of comparisons between the same geographic groups. Among the five populations, the moisture content of DD B was significantly higher than that of DD A, whereas that of the TJ B population showed a significant decrease (P<0.05). There was a significant increase in crude protein in DY B compared with DY A, but a significant decrease in TJ B compared with TJ A, and WZ B compared with WZ A (P<0.05). The crude fat content of the soft tissues of two groups, DD B and DY B, were significantly increased (P<0.05) compared with that of DD A and DY A, respectively.

3.7 Comparison of fatty acid composition

Five saturated fatty acids (ΣSFA), five monounsaturated fatty acids (ΣPUFA), and seven polyunsaturated fatty acids (ΣPUFA) were detected in the soft tissues of the five populations of *C. sinensis* (Table 4). The EPA content of TJ A was significantly higher than that of DY A, WZ A, and YC A, whereas the DHA content of WZ A was significantly higher than that of DY A and YCA (P<0.05).

After transplantation, the fatty acid species measured in the soft parts of the *C. sinensis* of the five groups were the same as those 1 year previously. The saturated fatty acid content of DY B was significantly higher than that of the other four groups (P<0.05), whereas there were no significant differences in the PUFA content among the five groups (P>0.05). However, the long-chain PUFA content was highest in YC B and lowest in DD B, with a significant difference between the two groups (P<0.05). Moreover, there were no significant differences among the five populations after transplantation in terms of the EPA, ΣSFA, ΣPUFA, and ΣMUFA + PUFA content (P>0.05).

By contrast, the total amount of saturated fatty acids in the transplanted DY, TJ, and YC populations was significantly lower than at the start of the study (P<0.05). The total amount of monounsaturated fatty acids in the DD and DY populations was significantly higher than before transplantation, whereas that in the TJ population was significantly lower (P<0.05). The PUFA content in the DY and YC populations was significantly higher, whereas that in the WZ population was significantly lower (P<0.05). However, there were no significant differences in the long-chain PUFA content before and after transplantation, although it increased significantly in the YC group compared with the other groups, but decreased significantly in the DD, DY, and WZ groups (P<0.05). The total amount of unsaturated fatty acids in the DY and YC groups was significantly increased, and ΣPUFA/(ΣMUFA + ΣPUFA) was elevated significantly in the TJ and YC groups, and significantly elevated in the DD, DY and WZ groups significantly decreased (P<0.05).

3.8 Comparison of amino acid composition

In total, 17 amino acids were detected in the soft tissues of the five populations of *C. sinensis*, including seven essential amino acids, two semi-essential amino acids, and eight non-essential amino acids (Table 5).

The proportion of essential amino acids in YC A was significantly higher than that in the other four groups (P<0.05), whereas that of non-essential amino acids in DY A was significantly higher than that in the other four groups (P<0.05). There was no significant difference in the proportion of non-essential amino acids between DD A and WZ A (P>0.05), but there was a significant difference in the proportion of fresh flavor (Glutamic acid,Glycine,Alanine and Aspartic acid) amino acids between the five groups (P<0.05).

After 1 year of *ex situ* conservation, there was no change in the amino acid profile of the groups, although the specific contents differed significantly. The essential amino acid contents of DD B and WZ B were significantly lower than those of the other three groups (P<0.05), whereas the difference between YC B, TJ B, and DY B was not significant (P>0.05). The non-essential amino acid content of YC B was significantly higher than that of the other four groups (P<0.05). The total amount of amino acids of DD B and WZ B was significantly lower than that of the other three groups (P<0.05), and the fresh flavor amino acid content of YC B was significantly higher than that of the other four groups (P<0.05). The proportion of essential amino acids in DY B was significantly higher than that in DD B, TJ B, and YC B (P<0.05). The proportion of essential amino acids to non-essential amino acids in DY B was significantly higher than the other four groups (P<0.05). The proportion of non-essential amino acids and of fresh flavor amino acids was highest in YC B. The proportion of essential amino acids compared with non-essential amino acids and fresh flavor amino acids of DD B and WZ B was significantly higher than that of TJ B and WZ B (P>0.05).

3.9 Amino acid scores for soft tissues

The crude protein content of each group (Table 6) was converted into milligrams of amino acids per gram of protein,

TABLE 4 Percentage fatty acid composition of five populations of *C. sinensis* before (A) and after (B) transplantation[a].

Fatty acid	Population									
	Dandong A	Dongying A	Tianjin A	Wenzhou A	Yancheng A	Dandong B	Dongying B	TianjinB	Wenzhou B	Yancheng B
C14:0	11.65 ± 0.33b	9.78 ± 0.08c	9.76 ± 0.21c	7.08 ± 0.10b	13.78 ± 0.04a	12.56 ± 0.08	12.36 ± 0.16^{+}	7.74 ± 1.04	12.24 ± 0.10^{+}	10.03 ± 0.58^{-}
C14:1	0.13 ± 0.01bc	0.07 ± 0.00d	0.15 ± 0.01b	0.12 ± 0.01c	0.22 ± 0.00a	0.22 ± 0.01B^{+}	0.30 ± 0.01A^{+}	0.12 ± 0.02B	0.22 ± 0.00B^{+}	0.16 ± 0.01B^{-}
C15:0	1.06 ± 0.01c	1.40 ± 0.01b	1.09 ± 0.02c	1.58 ± 0.02a	1.04 ± 0.01c	1.34 ± 0.00C^{+}	1.32 ± 0.01C^{-}	1.60 ± 0.04AC^{+}	1.38 ± 0.00C^{-}	1.51 ± 0.01B^{+}
C16:0	49.9 ± 0.21a	48.62 ± 0.15	46.39 ± 0.67b	49.8 ± 0.08	49.17 ± 0.2	47.05 ± 0.58A^{-}	43.88 ± 0.39^{-}	45.57 ± 0.82B	44.68 ± 0.15B^{-}	44.68 ± 0.36B^{-}
C16:1	5.48 ± 0.13c	5.06 ± 0.08d	6.33 ± 0.04b	5.13 ± 0.02d	6.85 ± 0.06a	6.80 ± 0.12B^{+}	8.03 ± 0.10A^{+}	4.54 ± 0.18D^{+}	6.88 ± 0.02B^{+}	5.18 ± 0.22C^{-}
C17:0	1.58 ± 0.02e	2.04 ± 0.03c	1.93 ± 0.01d	2.97 ± 0.01a	2.27 ± 0.01b	2.10 ± 0.01D^{+}	2.18 ± 0.03D^{+}	3.11 ± 0.08AC^{-}	2.47 ± 0.01C^{-}	2.93 ± 0.02AB^{+}
C18:0	9.27 ± 0.18	10.51 ± 0.16a	10.36 ± 0.06a	7.71 ± 0.04b	7.79 ± 0.02b	8.55 ± 0.08	8.66 ± 0.10^{-}	9.46 ± 0.16^{-}	8.57 ± 0.04^{+}	10.22 ± 0.40^{+}
C18:1n-9c	1.67 ± 0.04b	2.34 ± 0.02a	2.39 ± 0.08a	2.44 ± 0.01a	2.42 ± 0.03a	2.76 ± 0.04C^{+}	3.22 ± 0.07B^{+}	3.46 ± 0.05A^{+}	3.19 ± 0.01B^{+}	3.24 ± 0.08AB^{+}
C18:1n-9t	2.59 ± 0.08c	3.09 ± 0.05b	4.25 ± 0.15a	3.28 ± 0.07b	2.43 ± 0.03c	2.67 ± 0.10	3.02 ± 0.06	3.42 ± 0.28	2.98 ± 0.04^{-}	2.77 ± 0.01^{+}
C18:2n-6c	0.53 ± 0.01d	0.68 ± 0.00b	0.60 ± 0.03c	0.82 ± 0.00a	0.86 ± 0.01b	1.26 ± 0.02B^{+}	1.36 ± 0.04B^{+}	1.96 ± 0.06A^{+}	1.26 ± 0.02B^{+}	1.31 ± 0.02B^{+}
C18:3n-3	1.34 ± 0.01c	1.70 ± 0.02b	1.02 ± 0.03d	2.38 ± 0.05a	2.37 ± 0.03a	4.11 ± 0.10^{+}	4.47 ± 0.11^{+}	5.22 ± 0.40^{+}	4.14 ± 0.05^{+}	4.15 ± 0.08^{+}
C20:1n-9	1.45 ± 0.08b	1.87 ± 0.06ab	2.19 ± 0.200a	1.47 ± 0.06b	0.96 ± 0.04c	0.83 ± 0.04^{-}	0.96 ± 0.02^{-}	1.13 ± 0.10^{-}	0.92 ± 0.00^{-}	1.15 ± 0.06
C20:2	0.47 ± 0.01d	0.64 ± 0.02b	0.58 ± 0.02bc	0.88 ± 0.02a	0.54 ± 0.01c	0.71 ± 0.03C^{+}	0.77 ± 0.02BC^{+}	0.92 ± 0.06A^{+}	0.80 ± 0.02	0.89 ± 0.03AB^{+}
C20:4n-6	1.40 ± 0.02b	2.53 ± 0.08a	1.48 ± 0.05b	1.44 ± 0.02b	0.91 ± 0.02c	0.58 ± 0.03^{-}	0.53 ± 0.02B^{-}	0.80 ± 0.04A^{-}	0.68 ± 0.01A^{-}	1.09 ± 0.08^{+}
C20:3n-3	0.40 ± 0.05b	0.24 ± 0.03c	0.42 ± 0.06b	0.67 ± 0.02a	0.36 ± 0.01bc	0.99 ± 0.05^{+}	1.09 ± 0.04^{+}	1.11 ± 0.12^{+}	1.00 ± 0.04^{+}	0.87 ± 0.04^{-}
C20:5n-3(EPA)	6.48 ± 0.01a	6.03 ± 0.05b	6.72 ± 0.09a	5.89 ± 0.04b	4.79 ± 0.10c	3.60 ± 0.14^{-}	3.64 ± 0.08^{-}	4.52 ± 0.43^{-}	3.70 ± 0.02^{-}	4.38 ± 0.17
C22:6n-3(DHA)	4.60 ± 0.23	3.41 ± 0.14b	4.34 ± 0.25	6.36 ± 0.03a	3.24 ± 0.04c	3.87 ± 0.09B	4.21 ± 0.13^{+}	5.34 ± 0.47	4.89 ± 0.05B^{-}	5.43 ± 0.27^{+}
ΣSFA	73.45 ± 0.32a	72.34 ± 0.15b	69.52 ± 0.85b	69.13 ± 0.16c	74.05 ± 0.21a	71.60 ± 0.62	68.40 ± 0.39^{-}	67.47 ± 1.59^{-}	69.35 ± 0.22	69.37 ± 0.40^{-}
ΣMUFA	11.34 ± 0.15c	12.43 ± 0.15b	15.31 ± 0.43a	12.43 ± 0.09b	12.87 ± 0.11b	13.28 ± 0.25C^{+}	15.53 ± 0.20A^{+}	12.67 ± 0.14CD^{-}	14.20 ± 0.03B	12.51 ± 0.13D
ΣPUFA	15.22 ± 0.22b	15.23 ± 0.11b	15.17 ± 0.43b	18.44 ± 0.12a	13.07 ± 0.13c	15.12 ± 0.41	16.07 ± 0.21^{+}	19.86 ± 1.45	16.46 ± 0.19^{-}	18.12 ± 0.50^{+}
ΣLC-PUFA	12.48 ± 0.22b	11.98 ± 0.14b	12.55 ± 0.37b	13.69 ± 0.06a	8.94 ± 0.13c	8.05 ± 0.25^{-}	8.38 ± 0.11B^{-}	10.66 ± 0.94	9.27 ± 0.06B^{-}	10.90 ± 0.51A^{+}
ΣMUFA^{+}ΣPUFA	26.55 ± 0.32bc	27.66 ± 0.15b	30.48 ± 0.85	30.87 ± 0.16a	25.95 ± 0.21c	28.40 ± 0.62	31.60 ± 0.39^{+}	32.53 ± 1.59	30.65 ± 0.22	30.63 ± 0.40^{+}
ΣPUFA/(ΣMUFA^{+}ΣPUFA)	57.31 ± 0.36b	55.06 ± 0.41c	49.78 ± 0.15d	59.73 ± 0.21a	50.38 ± 0.23d	53.24 ± 0.46^{-}	50.86 ± 0.15B^{-}	60.82 ± 1.60^{+}	53.69 ± 0.23A^{-}	59.14 ± 0.88A^{+}

[a]Different lowercase letters between peers indicate significant differences among populations (P<0.05).

The five wild populations of *C. sinensis* were set for nutrient determination in 2020 and 2021, as groups A and B, respectively. In the results, those with different lowercase letters indicate that there were significant differences between those in group A (P<0.05); those with different capital letters indicate that there were significant differences between those in group B (P<0.05). A "+" in the superscript of group B indicates that there is a significant increase in the same nutritional indicators between this group and the corresponding group in group A (P < 0.05); a "-" in group B indicates that there is a significant decrease in the nutritional indicators between this group and the corresponding group in group A (P < 0.05).

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TABLE 5 Percentage amino acid composition of five populations of *C. sinensis* before (A) and after (B) transplantation[a].

Amino acid	Population									
	Dandong A	Dongying A	Tianjin A	Wenzhou A	Yancheng A	Dandong B	Dongying B	Tianjin B	Wenzhou B	Yancheng B
Aspartic acid#	7.71 ± 0.03d	8.13 ± 0.08c	8.00 ± 0.07c	8.96 ± 0.02b	9.35 ± 0.04a	7.59 ± 0.02C	7.86 ± 0.09C	8.00 ± 0.07AC	7.61 ± 0.04C-	8.21 ± 0.03A$^-$
Threonine*	3.80 ± 0.02d	3.93 ± 0.04c	3.81 ± 0.03d	4.14 ± 0.01b	4.40 ± 0.02a	3.56 ± 0.01B$^-$	3.66 ± 0.04A$^-$	3.65 ± 0.03$^-$	3.57 ± 0.02B$^-$	3.76 ± 0.01A$^-$
Serine#	3.41 ± 0.01c	3.44 ± 0.04c	3.88 ± 0.03b	3.90 ± 0.01b	4.16 ± 0.02a	3.58 ± 0.01B$^+$	3.77 ± 0.03A$^+$	3.56 ± 0.03B$^-$	3.43 ± 0.02C$^-$	3.63 ± 0.02B$^-$
Glutamic acid#	10.94 ± 0.03c	12.03 ± 0.12b	11.21 ± 0.11c	11.91 ± 0.03b	13.12 ± 0.04a	10.22 ± 0.04C$^-$	10.47 ± 0.12C$^-$	11.00 ± 0.11B	10.18 ± 0.05C$^-$	11.65 ± 0.09A$^-$
Glycine#	5.54 ± 0.01a	5.47 ± 0.05a	5.46 ± 0.04a	4.96 ± 0.01b	4.85 ± 0.03b	4.89 ± 0.02C$^-$	4.87 ± 0.05C$^-$	5.04 ± 0.03B$^-$	4.53 ± 0.03D$^-$	5.19 ± 0.01A$^+$
Alanine#	6.20 ± 0.02a	5.94 ± 0.06b	5.32 ± 0.06d	5.18 ± 0.02d	5.52 ± 0.02b	4.78 ± 0.02C$^-$	4.88 ± 0.06BC$^-$	5.00 ± 0.04B$^-$	4.59 ± 0.02D$^-$	5.36 ± 0.02A$^-$
Cysteine#	6.12 ± 0.03b	6.11 ± 0.07b	5.94 ± 0.04b	6.10 ± 0.02b	6.75 ± 0.06a	5.76 ± 0.03C$^-$	5.79 ± 0.04BC$^-$	5.93 ± 0.03B	5.73 ± 0.06C$^-$	6.13 ± 0.01A$^-$
Valine*	3.91 ± 0.03c	4.01 ± 0.05b	3.83 ± 0.03c	4.07 ± 0.01b	4.52 ± 0.02a	3.65 ± 0.01D$^-$	3.77 ± 0.04BC$^-$	3.88 ± 0.02B	3.72 ± 0.02CD$^-$	3.99 ± 0.01A$^-$
Methionine*	3.27 ± 0.00c	3.26 ± 0.04c	3.28 ± 0.03c	3.81 ± 0.04b	4.27 ± 0.03a	3.42 ± 0.04B	3.56 ± 0.04$^+$	3.69 ± 0.02A$^+$	3.55 ± 0.04-	3.68 ± 0.01A$^-$
Isoleucine*	3.52 ± 0.01d	3.66 ± 0.05c	3.44 ± 0.03d	3.90 ± 0.01b	4.18 ± 0.02a	3.33 ± 0.01D$^-$	3.47 ± 0.04C	3.58 ± 0.02B$^+$	3.43 ± 0.02C$^-$	3.70 ± 0.01A$^-$
Leucine*	5.76 ± 0.02d	6.02 ± 0.07c	5.68 ± 0.06d	6.33 ± 0.01b	6.83 ± 0.03a	5.43 ± 0.02C$^-$	5.64 ± 0.06B$^-$	5.78 ± 0.05B	5.45 ± 0.03C$^-$	6.01 ± 0.03A$^-$
Lorine#	0.46 ± 0.04a	0.39 ± 0.03ab	0.35 ± 0.02b	0.35 ± 0.02b	0.20 ± 0.01c	0.12 ± 0.01C$^-$	0.16 ± 0.04$^-$	0.28 ± 0.01A$^-$	0.12 ± 0.01C$^-$	0.20 ± 0.00B
Phenylalanine*	2.96 ± 0.03	3.03 ± 0.01	2.93 ± 0.02	3.05 ± 0.12	2.92 ± 0.03	2.41 ± 0.05$^-$	2.41 ± 0.11$^-$	2.42 ± 0.03$^-$	2.42 ± 0.08$^-$	2.54 ± 0.04$^-$
Lysine*	5.94 ± 0.02a	5.57 ± 0.06b	6.03 ± 0.07a	4.99 ± 0.01c	5.99 ± 0.02a	5.27 ± 0.01B$^-$	5.85 ± 0.05A$^+$	5.25 ± 0.04B$^-$	4.67 ± 0.03C$^-$	5.38 ± 0.03B$^-$
Histidine &	0.39 ± 0.03ab	0.55 ± 0.01a	0.56 ± 0.05a	0.34 ± 0.06bc	0.22 ± 0.01c	0.16 ± 0.00B$^-$	0.18 ± 0.01B$^-$	0.16 ± 0.00B$^-$	0.17 ± 0.00B	0.20 ± 0.00A
Arginine &	6.11 ± 0.07c	6.00 ± 0.09c	6.35 ± 0.08b	5.91 ± 0.00c	6.67 ± 0.02a	5.79 ± 0.03B$^-$	6.06 ± 0.06A	6.08 ± 0.04A	5.24 ± 0.02C$^-$	6.03 ± 0.04A$^-$
Proline#	2.46 ± 0.01c	2.61 ± 0.02b	2.40 ± 0.01d	2.61 ± 0.01b	2.84 ± 0.01a	2.32 ± 0.02C$^-$	2.32 ± 0.04C$^-$	2.55 ± 0.02B$^+$	2.41 ± 0.01C$^-$	2.67 ± 0.02A$^-$
Total essential amino acids	29.17 ± 0.08c	29.48 ± 0.30c	29.00 ± 0.22c	30.28 ± 0.17b	33.1 ± 0.15a	27.08 ± 0.14B$^-$	28.37 ± 0.26A	28.26 ± 0.18A	26.8 ± 0.20B$^-$	29.05 ± 0.12A$^-$
Total non-essential amino acids	42.86 ± 0.08c	44.13 ± 0.45b	42.55 ± 0.37c	43.96 ± 0.09b	46.79 ± 0.19a	39.26 ± 0.10CD$^-$	40.11 ± 0.46C$^-$	41.36 ± 0.33B	38.61 ± 0.22D$^-$	43.04 ± 0.15A$^-$
Total amino acids	78.53 ± 0.23b	80.15 ± 0.83b	78.46 ± 0.72b	80.49 ± 0.29b	86.77 ± 0.35a	72.30 ± 0.26C$^-$	74.73 ± 0.74B$^-$	75.86 ± 0.55B	70.82 ± 0.44C$^-$	78.33 ± 0.31A$^-$
Total flavor amino acids	33.83 ± 0.09cd	34.99 ± 0.33b	33.27 ± 0.27d	34.40 ± 0.18bc	35.96 ± 0.15a	30.01 ± 0.13CD$^-$	30.65 ± 0.35C$^-$	31.73 ± 0.25B$^-$	29.46 ± 0.18D$^-$	33.16 ± 0.18A$^-$
Essential amino acids (%)	37.15 ± 0.02c	36.77 ± 0.05d	36.97 ± 0.06cd	37.62 ± 0.08b	38.15 ± 0.02a	37.46 ± 0.06B$^+$	37.97 ± 0.17A$^+$	37.25 ± 0.04B$^+$	37.84 ± 0.08A$^+$	37.09 ± 0.03B$^-$
Non-essential amino acids (%)	54.58 ± 0.05b	55.05 ± 0.03a	54.23 ± 0.02c	54.62 ± 0.09b	53.92 ± 0.03d	54.31 ± 0.07B	53.68 ± 0.15C$^-$	54.51 ± 0.04B$^+$	54.52 ± 0.07B$^-$	54.95 ± 0.02A$^+$
Flavor amino acid ratio	43.08 ± 0.01b	43.66 ± 0.03a	42.40 ± 0.04d	42.73 ± 0.07c	41.45 ± 0.06e	41.50 ± 0.04C$^-$	41.02 ± 0.07D$^-$	41.83 ± 0.03B$^-$	41.60 ± 0.04C$^-$	42.33 ± 0.06A$^+$
Required/non-required	68.06 ± 0.07b	66.80 ± 0.05c	68.17 ± 0.10b	68.88 ± 0.23ab	70.75 ± 0.07a	68.97 ± 0.21B$^+$	70.75 ± 0.51A$^+$	68.34 ± 0.12C$^+$	69.41 ± 0.22B$^+$	67.49 ± 0.06C$^-$

[a]Different lowercase letters between peers indicate significant differences among populations (P<0.05).

The five wild populations of *C. sinensis* were set for nutrient determination in 2020 and 2021, as groups A and B, respectively. In the results, those with different lowercase letters indicate that there were significant differences between those in group A (P<0.05); those with different capital letters indicate that there were significant differences between those in group B (P<0.05). A "+" in the superscript of group B indicates that there is a significant increase in the same nutritional indicators between this group and the corresponding group in group A (P < 0.05); a "-" in group B indicates that there is a significant decrease in the nutritional indicators between this group and the corresponding group in group A (P < 0.05).

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TABLE 6 Proximate composition of soft tissues of five populations of *C. sinensis*[a].

Population	Composition			
	Protein	Crude protein	Crude fat	Ash
Dandong A	79.15 ± 0.04^{c}	60.46 ± 0.12^{b}	4.52 ± 0.76^{b}	16.48 ± 0.55
Dongying A	82.21 ± 0.29^{ab}	57.74 ± 0.19^{d}	4.11 ± 0.16^{b}	17.05 ± 0.84
Tianjin A	82.04 ± 0.05^{b}	61.87 ± 0.08^{a}	3.44 ± 0.44^{b}	17.41 ± 0.73
Wenzhou A	81.47 ± 0.06^{a}	59.55 ± 0.18^{c}	8.60 ± 0.57^{a}	14.78 ± 1.03
Yancheng A	81.45 ± 0.06^{a}	61.79 ± 0.25^{a}	8.27 ± 0.13^{a}	16.37 ± 0.93
Dandong B	80.97 ± 0.07^{+}	59.31 ± 0.67^{A}	7.83 ± 0.86^{+}	16.64 ± 0.55
Dongying B	82.62 ± 0.01^{A}	59.52 ± 0.34^{B+}	9.53 ± 1.21^{+}	14.60 ± 0.25^{B}
Tianjin B	80.71 ± 0.02^{-}	58.91 ± 0.21^{B-}	4.14 ± 0.61^{B}	16.16 ± 0.72
Wenzhou B	80.62 ± 0.02^{B}	55.88 ± 0.15^{C-}	11.60 ± 1.25^{A}	17.88 ± 1.19^{A}
Yancheng B	81.55 ± 0.12	62.03 ± 0.35^{A}	9.34 ± 2.16	15.48 ± 0.95

[a]'A' and 'B' represent values before and after transplantation (%, dry matter). Different superscript lowercase letters indicate significant differences between the A groups ($P<0.05$); different superscript capital letters indicate significant differences between the B groups ($P<0.05$).
$^{+}$ indicates that this B population showed significant improvement compared with group A in the same nutritional indicator ($P<0.05$); $^{-}$ indicates that this B population showed significant improvement compared with the corresponding group A in the same nutritional indicators ($P<0.05$).

and the essential amino acid content, AAS, CS, and EAAI of the soft-bodied tissues of each group were calculated according to the FAO/WHO standard model and the amino acid standard model of the protein of the whole egg. Whether the limiting amino acids in the soft-bodied fraction changed or not, the first limiting amino acid of the populations of *C. sinensis* in the AAS model was phenylamino acid + complex amino acid, and the second limiting amino acid was valine. There was no change in the first and second limiting amino acids of the five populations after 1 year of *ex situ* conservation (Table 7). After transplantation of the wild populations of *C. sinensis*, the EAAI of all populations decreased, apart from that of TJ B, which was slightly higher than that of TJ A (Table 8).

4 Discussion

4.1 Differences in survival rates of different populations after *ex situ* conservation

Bivalve shellfish are susceptible to pathogens and environmental fluctuations and, in response to such threats, *C. sinensis* activates resistance genes (Yao et al., 2018; Hou et al., 2019). The survival or mortality of transplanted individuals not only reflects natural selection, but also indicates the adaptability of different populations to their habitats. In terms of the survival rates during the study, both the YC and TJ groups had a survival rate exceeding 60%, whereas the WZ group had the lowest survival rate, which did not even reach 30%. Geographically, the origin of the YC group was closest to the study area and exhibited the most similar environmental conditions. Conversely, the origins of the TJ and DY groups were relatively close to each other, and no significant difference was observed in their post-transplantation survival rates. Different populations of *C. sinensis* display varying capacities for temperature adaptation because of genetic differentiation (Jin, 2005; Chen et al., 2016). The collection sites of the DD and WZ populations were farthest from the study area; DD was from the northernmost location, whereas WZ was from the southernmost location. Variations in the annual seawater temperature at the collection sites of the DD and WZ populations differed significantly from those observed at the experimental area, which could explain the lower survival rates of these groups at the study site. Therefore, temperature could be one of the primary factors contributing to lower survival rates in these two groups compared with the other wild populations.

As a benthic shellfish, *C. sinensis* is highly susceptible to humus and bacterial infections in their environment, which could have a detrimental impact on their survival rate (Chen et al., 2016). To facilitate daily management and environmental monitoring, this study used offshore ponds and simulated natural environments for stocking different groups of *C. sinensis*. However, smaller water bodies have limited self-purification capacity, leading to the production of harmful substances through organic matter decomposition and subsequent substrate deterioration (Zhou, 2018). Therefore, sowing different groups in the self-purifying environment of Lianyungang beach or using alternative stocking methods, such as net-pen or hanging-bag culture, could enhance the survival rate of *C. sinensis* in aquaculture systems (Wang et al., 2003).

4.2 Variability in growth performance and reproductive indicators

Growth-related traits and disease resistance of shellfish can be influenced by parental inheritance, and the growth performance of different populations has a crucial role in determining optimal shellfish selection. The study results revealed that the DG *C. sinensis* population exhibited superior overall performance in terms of body mass, SL, and SH increases. Conversely, the WZ population

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TABLE 7 AAS scores of essential amino acids in the soft tissue of five wild populations of *C. sinensis* before (A) and after (B) transplantation.

Essential amino acids AAS score	Dandong A	Dongying A	Tianjin A	Wenzhou A	Yancheng A	Dandong B	Dongying B	Tianjin B	Wenzhou B	Yancheng B	FAO/WHO
Threonine	0.98	1.06	0.95	1.09	1.12	0.94	0.96	0.97	1.00	0.95	40
Valine	0.81	0.87	0.77	0.86	0.92	0.77	0.79	0.82	0.83	0.80	50
Isoleucine	0.91	0.99	0.86	1.03	1.06	0.88	0.91	0.95	0.96	0.93	40
Leucine	0.85	0.93	0.81	0.95	1.00	0.82	0.85	0.88	0.87	0.86	70
Lysine	1.12	1.10	1.09	0.96	1.11	1.01	1.12	1.01	0.95	0.99	55
Methionine + cystine	2.78	2.90	2.63	3.01	3.21	2.77	2.81	2.92	2.96	2.82	35
Phenylalanine + complexine	0.59	0.62	0.55	0.60	0.54	0.44	0.45	0.48	0.47	0.46	60

displayed lower increments in SL and SH after transplantation, but exceptional growth in terms of SW increases.

The findings also suggest that establishment of hybrid populations of *C. sinensis* should be based on the degree to which morphological traits influence body mass. Different populations of *C. sinensis* are influenced by local water temperature and environmental factors, and their reproductive periods have a specific duration, thus imposing limitations on the hybrid breeding of *C. sinensis* (Li et al., 2005a). Common methods for catalysis include shade drying, running water stimulation, and temperature regulation, among others (Li et al., 2005b). It was observed that 1 year of environmental transplantation enabled different wild populations of *C. sinensis* to achieve overall synchronization in their reproductive period.

Higher reproductive indexes can, to a certain extent, reflect the adaptability of the population to the local environment. Conversely, higher index coefficients for good growth and development indicate limitations imposed by environmental factors on development. Dong et al. observed significant differences in three reproductive indicators between two wild populations of *C. sinensis*, whereas this study found no significant differences in GSI and HSI 1 year after transplantation, suggesting that the environment has a crucial role in influencing the reproductive indicators of *C. sinensis* (Dong et al., 2011).

The groups with higher GSI exhibited superior fecundity, indicating a strong correlation between fecundity and GSI of *C. sinensis*. Previous studies also highlighted the close relationship between fecundity and gonadal development in shellfish (Kim et al., 2005; Ke et al., 2010). Zhao et al. observed variations in predevelopmental rates among females of different Chinese mitten crab species under identical culture conditions, whereas, by the end of October, there were no significant differences in GSI between the populations (Zhao et al., 2017). These studies demonstrated that samples originating from different locations gradually acclimatize to local environmental conditions after 1 year or more of cultivation, leading to a convergence in growth patterns and reproductive indices over time (Ahn et al., 2016).

4.3 Glycogen differences

Glycogen phosphorylase is the rate-limiting enzyme for glycogen degradation, which directly replenishes blood glucose through glycogenolysis. Additionally, glycogen has a crucial role in germ cell synthesis. In shellfish, the synthesis of yolk proteins heavily relies on lipid production from glycogen (Bayne et al., 1982). The stored glycogen in shellfish also serves as an energy source to defend against external stimuli and is vital for germ cell development. Consequently, measurement of tissue-specific glycogen content is used as an indicator of environmental stress tolerance (Ansell, 1974). Yan revealed that the lowest levels of glycogen are observed during gonad development and maturation in male and female *C. sinensis*. However, after spawning, glycogen content increased. Furthermore, Yan suggested that the higher body glycogen content in *C. sinensis* represents energy accumulation to support gamete development and overwintering post discharge (Yan, 2009).

The current results revealed that male *C. sinensis* had a higher overall glycogen content at maturity compared with females, suggesting

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TABLE 8 CS scores and essential amino acids index EAAI in the soft tissue of *C. sinensis* before (A) and after (B) transplantation.

Essential amino acids	Population CS score										Egg white patterns
	Dandong A	Dongying A	Tianjin A	Wenzhou A	Yancheng A	Dandong B	Dongying B	Tianjin B	Wenzhou B	Yancheng B	
Threonine	0.87	0.95	0.85	0.97	0.99	0.83	0.85	0.86	0.89	0.84	45
Valine	0.75	0.8	0.71	0.79	0.85	0.71	0.73	0.76	0.77	0.74	54
Isoleucine	0.74	0.81	0.7	0.84	0.87	0.72	0.74	0.78	0.78	0.76	49
Leucine	0.9	0.99	0.86	1.01	1.06	0.87	0.9	0.93	0.92	0.92	66
Lysine	0.93	0.86	0.86	0.75	0.87	0.79	0.88	0.8	0.75	0.77	66
Methionine + cystine	2.07	2.16	1.96	2.24	2.39	2.06	2.09	2.17	2.21	2.1	47
Phenylalanine + complexine	0.41	0.43	0.38	0.42	0.38	0.31	0.31	0.33	0.33	0.32	86
EAAI	78.94	83.52	75.21	82.88	86.24	72.88	75.56	76.90	76.74	74.99	

their superior energy storage capacity and enhanced adaptability to the environment. Additionally, the DD group displayed significantly advantageous glycogen levels in the outer coat membrane, closed shell muscle, and gonad-visceral mass compared with the other four groups. This observation led to the hypothesis that this particular group relies on elevated glycogen reserves during autumn and winter for improved metabolic functionality.

4.4 Comparison of nutritional quality

4.4.1 Factors affecting nutritional levels

The nutritional composition of shellfish is influenced by the growth environment, developmental cycle, and meat freshness (Yang et al., 2016). Results relating to the environmental conditions of the areas where each *C. sinensis* group originated revealed a significant variation in their geographical locations, with obvious differences in air temperature, air pressure, and soil.

Previous studies have reported significant variations in the species composition and biomass of phytoplankton across the sampling sites. Diatoms were dominant in terms of cell abundance and species richness within the phytoplankton communities at all five sampling sites. Specifically, the shallow waters at DY contained 51 diatom species, with linear cirripedes and radiolarian algae being the dominant species (Fan et al., 2020). Yueqing Bay in WZ revealed 57 diatom species, with *Sphaerophora* predominantly represented by round sieve algae followed by horned *Trichoderma* (Song et al., 2010). The nearshore area of TJ contained 49 diatom species, primarily comprising horned *Trichoderma* and *Sphaerophora*, but the latter were dominant in terms of number of species (Yin et al., 2015). Offshore YC exhibited middle ribbed stripe algae and short-horned curved horn algae (Mao et al., 2018). Given its proximity to the Yalu River estuary and freshwater influences, the DD sampling site demonstrated lower diversity and evenness among phytoplankton communities in its marine area. This resulted in an absolute dominance in terms of individual species abundance and led to reduced diversity of edible shellfish species compared with other marine areas (Liu et al., 2013). Thus, various factors contribute significantly toward variations in nutritional quality among different groups of *C. sinensis*.

4.4.2 Comparison of nutritional quality

The results of the present analysis demonstrated significant variations in conventional nutrient content among different populations of *C. sinensis*. Compared with Gu et al.'s results (41.02% and 38.18%) (Gu et al., 2006) for cultured populations from Shooting Yang in June and December respectively, the five wild populations examined in this study demonstrated a substantial higher crude protein content before and after transplantation. However, the crude fat content was much lower than that observed for cultured populations from Sheyang in Jiangsu Province during June and December (24.08% and 22.34%, respectively). This suggests that wild populations of *C. sinensis* have a significantly higher value of crude protein compared with their cultured counterparts, resulting in enhanced nutritional value.

The results of nutritional studies on farmed and wild turbot demonstrated a significantly higher crude protein content in wild populations compared with farmed populations, whereas the crude fat content was significantly lower in the wild population (Han et al., 2015). Similarly, for Masu salmon, the crude protein content was significantly higher in the wild population than in the farmed population, with a corresponding significant decrease in crude fat content (Wei et al., 2020). These findings align with, and support, the results presented in this study.

4.4.3 Comparison of the nutritional quality of fatty acids

Comparison of the fatty acid composition of different groups of *C. sinensis* showed similar profiles in terms of fatty acid content. Fatty acid composition can serve as an indicator of nutritional quality and, although saturated fatty acids are a source of energy, excessive intake can contribute to cardiovascular diseases, such as hypertension and hyperlipidemia (Zhang et al., 2007).

The results indicated that WZ A exhibited the highest nutritional value, with long-chain PUFAs, including ARA (C20:4n-6), EPA (C20:5n-3), and DHA (C22:6n-3). Adequate intake of these fatty acids can reduce the risk of cardiovascular and neurological diseases in humans (Fernandes and Venkatraman, 1993; Sun, 1993; You et al., 2019). In the current study, the five *C. sinensis* groups exhibited varying fatty acid profiles because of differences in their original growth environments and food sources. YC A and DD A had higher levels of saturated fatty acids, whereas WZ A had the highest content of PUFAs and long-chain PFUAs. Therefore, we recommend using these three groups as parents for improving the fatty acid nutritional quality of *C. sinensis*.

4.4.4 Comparison of amino acid nutritional quality

Amino acids are fundamental building blocks of proteins. Thus, assessments of the nutritional quality of the soft tissues of *C. sinensis* populations should consider protein content, amino acid composition, and abundance (Zheng, 2013). Experimental results revealed that glutamic acid was the most abundant amino acid in both pre- and post-measurements across all five populations, which aligns with other nutritional research on *C. sinensis* (Wei et al., 2022). According to the FAO/WHO ideal protein model, a food source can be deemed high quality if it has an essential amino acid content-to-total amino acid content ratio of ~40%, along with an essential-to-non-essential amino acid ratio exceeding 60%.

According to the current results, the essential amino acid ratio of the five populations of *C. sinensis* before and after intensive stocking ranged from 36.77% to 38.15%. Additionally, the ratio of essential amino acids to non-essential amino acids ranged from 66.80% to 70.75%, which aligns with the ideal protein model, indicating that both before and after transplantation, all five populations of *C. sinensis* exhibited a well-balanced profile of amino acids and would be excellent sources of high-quality proteins. In terms of the amino acid scores, two measurements revealed consistent findings: phenylamino acid + complex amino acid were identified as the first limiting amino acid in the populations of *C. sinensis* according to AAS model analysis; valine emerged as the second limiting amino acid across these populations. Gu et al. also reported methionine + cystine as the first limiting amino acid in *C. sinensis* based on ASS scoring, while valine remained as the second limiting amino acid (Gu et al., 2006). By contrast, Li et al. identified valine as the first limiting amino acid in *C. sinensis* whereas phenylamino acid + complexine ranked second (Li et al., 2010).

The EAAI is a protein evaluation method, a higher ratio of which indicates closer proximity to the standard of egg white, thereby indicating superior nutritional value (Bing and Zhang, 2006; Shi et al., 2020). Based on the results obtained, the initial EAAI values for the five groups ranged from 75.21 to 86.24 (YC A > DY A > WZ A > DD A > TJ A), suggesting that proteins from the YC group could be more efficiently absorbed and utilized by the human body.

4.5 Nutritional changes in different populations after transplantation

The results of the study revealed that, even after 1 year of stock cultivation in the same environment, the nutritional variability among the five populations of *C. sinensis* could not be eliminated, as evidenced by substantial differences in conventional nutrients, fatty acid content, and amino acid content. Basic indicators, such as crude protein, crude fat, moisture, and ash content, are commonly used to assess nutritional quality but often vary with environmental conditions (Yang et al., 2018; Lü et al., 2022). Comparing the pre- and post-transplantation differences in conventional nutrients among populations, the YC population maintained its advantage in terms of crude protein content, while DY B exhibited a significant increase compared with DY A. Moreover, there were notable differences in crude protein content between the DY B and DY A populations. No significant variations were found in saturated fatty acids and PFUAs among all five groups following transplantation.

Meanwhile, the total essential amino acid levels were significantly lower in the DD, WZ, and YC groups 1 year after transplantation. Additionally, the total non-essential amino acid and overall amino acid contents were significantly reduced in the DD, DY, WZ, and YC groups compared with pre-transplantation. Despite this decrease, the YC group still exhibited the highest concentration of flavor-related amino acids among all five groups.

In summary, the transplantation of various wild populations of *C. sinensis* to local sea areas had a positive impact on the content of a limited number of amino acid species in their soft tissues, while adversely affecting the flavor profiles of geographically distinct wild populations. In terms of the amino acid index, changes were observed in the indices (72.88–76.90) and alterations in their order from highest to lowest were TJ B > WZ B > DY B > YC B > DD B. The essential amino acid index increased for TJ B (76.90) compared with TJ A (75.21), whereas protein quality significantly decreased in the other four populations. These findings suggest that most *C. sinensis* populations experienced reduced protein quality following transplantation into a Lianyungang Sea habitat. After 1 year of transplantation, several nutrient indices showed significant decreases compared with pre-transplantation levels, indicating that bait conditions in this region were less favorable compared with the original environments.

Furthermore, the persistence of significant differences in nutrient content among groups post transplantation suggests that 1 year of cohabitation in the same environment was insufficient to eliminate inherent variations in nutrient composition between *C. sinensis* populations.

5 Conclusion

Our comparisons of the environmental adaptability of five populations of *C. sinensis* showed that the survival rates of different populations varied greatly, with those from sampling sites closer to the transplantation sites having higher survival rates after transplantation. After 1 year of transplantation, the gonadal development of all populations had adapted to the local environment, resulting in overall synchronization of the reproduction period, which would facilitate cross-breeding. The results showed that the gonadal development of all groups also adapted to the local environment after 1 year of transplantation. Meanwhile, the GSI and HSI of *C. sinensis* in the reproductive stage after transplantation were no longer significantly different from each other. There was a strong correlation between the plumpness and GSI of different groups of *C. sinensis*, and the changes in the two tended to be consistent. Combined with the plumpness and the glycogen content of different parts of *C. sinensis*, the DD group increased energy storage, suggesting that this group might be more able to survive the following autumn and winter because of its superior metabolism. Meanwhile, the overall glycogen content of male *C. sinensis* was higher than that of female *C. sinensis*, suggesting that male *C. sinensis* have a better energy storage capacity and are more adaptable to the environment.

In the nutrient analysis and quality evaluation of the five groups of *C. sinensis*, the levels of fatty acids and amino acids were consistent across groups, but differed in terms of their composition. The soft body parts of different groups of *C. sinensis* were rich in saturated fatty acids, mainly C16:0. The contents of EPA and DHA in PUFAs were higher, indicating that *C. sinensis* from different sources were all of high nutritional value. The large proportion of fresh flavor amino acids in the soft tissue of *C. sinensis* and the ratio of EAA/TAA and NEAA/TAA in accordance with the FAO/WHO evaluation criteria for the ideal protein pattern also indicate that *C. sinensis* have good nutritional flavor and are a high-quality protein source.

AAS evaluation showed that the first limiting amino acid of the populations of *C. sinensis* was phenylamino acid + complex amino acid, and the second was valine, whereas the CS scoring pattern was slightly different, showing that the second limiting amino acid was isoleucine in the DD population and the TJ population before transplantation. The essential amino acids index was highest in the YC group and lowest in the TJ group before transplantation, but highest in the TJ group after transplantation.

Comparison of the nutritional indices before and after transplantation showed that the nutritional complexity and variability of different populations remained, suggesting that 1 year of stocking in the same environment did not eliminate the nutritional differences between different populations of *C. sinensis*. Despite transplantation into a common environment, significant nutritional differences persist among populations, indicating that inherent variations in nutrient composition are not easily homogenized. These findings highlight the importance of considering environmental and ecological factors in the nutritional assessment and cultivation of shellfish.

The complexity and diversity observed in the nutritional quality of *C. sinensis* from various groups after transplantation indicate that a singular centralized transplantation approach failed to maintain the distinct nutrient advantages for each group. Consequently, the successful *ex situ* conservation of *C. sinensis* in the Lianyungang Sea requires not only meticulous selection of superior breeding stock, but also an emphasis on enhancing bait quality and diversifying bait species to improve the overall nutritional profile.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

SY: Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft. QX: Investigation, Writing – review & editing. ZC: Investigation, Writing – review & editing. GR: Investigation, Writing – review & editing. XL: Investigation, Writing – review & editing. HG: Investigation, Writing – review & editing. ML: Investigation, Writing – review & editing. ZD: Conceptualization, Funding acquisition, Writing – review & editing.

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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

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