

DOI: 10.20098/j.cnki.1001-5132.2023.0343

# The effects of temporary rearing with edetate disodium on physicochemical indices and bacterial communities of shrimp

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**Abstract:** *Litopenaeus vannamei* (*L. vannamei*) is rich in moisture, protein, enzyme activity and astaxanthin. This shrimp, however, has such a feature in that, after death its head turns black rapidly under the action of polyphenol oxidase (PPO), and then its meat gets rot and deteriorate under the action of microorganisms, thus reducing the qualities for commodity sales quickly. At present, researches on the above problems were focused on adding antioxidants and preservatives to keep the dead shrimps fresh through collaborative temperature control, which has the bottleneck of low consumer acceptance. To this end, in this study live *L. vannamei* was selected as the research object, and it was temporarily reared with EDTA-2Na. The effects of temporary rearing with EDTA-2Na on basic physical and chemical indices of *L. vannamei* were explored by analyzing the PPO, total viable count, as well as the color of the shrimps. On this basis, the differences in the bacterial community during temporary rearing of *L. vannamei* were further explored through the microbiome. The specific research results are as follows: the addition amount of EDTA-2Na and the duration of temporary rearing were inversely proportional to PPO activity and total viable count, and the end point of temporary rearing had a better inhibitory effect.  $L^*$  (lightness) value of *L. vannamei* head and abdomen was slightly increased, while  $a^*$  (green to red) and  $b^*$  (blue to yellow) values were decreased. The blackness and redness of *L. vannamei* were effectively inhibited. Besides, EDTA-2Na also exhibited effects on the number and structure of bacteria of *L. vannamei* during temporary rearing. The abundance and diversity of species were higher when the shrimps were temporary reared in an environment in which EDTA was added at 0.1% or 0.3% and the duration of rearing was 24 hours, which were more conducive to the dynamic balance of flora and better quality. The phylum of *L. vannamei* hepatopancreas mainly consisted of Bacteroidota, Proteobacteria, Firmicutes, Actinobacteriota, Desulfobacteriota. Proteobacteria was inhibited in the 0.3%-24 h group, indicating that reproduction of a large number of pathogens was inhibited. The main pathway affecting the *L. vannamei* hepatopancreas was the metabolism.

**Key words:** *Litopenaeus vannamei*; temporary rearing; edetate disodium; blackening; bacterial communities

**CLC number:** TS254

**Document code:** A

**Article ID:** 1001-5132 ( 2024 ) 04-0056-013

## 1 Introduction

*Litopenaeus vannamei* (*L. vannamei*) is currently among the top three cultured shrimps and one of the

most economically important species worldwide. *L. vannamei* contains a variety of nutrients, such as protein, Ca, Mg, Fe, and other mineral elements, and is low in fat and calories. Since *L. vannamei* is adapted to a wide

收稿日期: 2023-03-29.

宁波大学学报(理工版)网址: <http://journal1g.nbu.edu.cn/>

基金项目: 国家重点研发计划(2020YFD0900903).

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range of salinities and temperatures, its economic value is high<sup>[1]</sup>. It has gradually become the main farmed shrimp species in southern China cities because of its strong adaptability and rapid growth rate<sup>[2]</sup>. On the other hand, however, *L. vannamei* contains a wide variety of nutrients and highly energetic enzymes, which actually create a positive environment for microorganisms. This makes shrimps susceptible to microbial damage in the process of breeding, transportation, etc., which may end up causing an outbreak of shrimp disease<sup>[3-6]</sup>. In addition, the tyrosinase present in *L. vannamei* is activated by copper ions to produce melanin, which leads to a blackening of the shrimp body and affects its commercial value<sup>[7]</sup>. Therefore, an antibacterial substance that inhibits blackening must be found.

Current research on the antibacterial and blackening inhibition of aquatic products includes ultrahigh-pressure preservation<sup>[8]</sup>, chitosan coating<sup>[9]</sup>, ozone technology<sup>[10]</sup>, electron beam irradiation preservation<sup>[11]</sup>, plasma-activated water preservation<sup>[12]</sup>, and acidic electrolytic water preservation<sup>[13]</sup>. All these methods have bactericidal and bacteriostatic properties and can inhibit shrimp blackening, thereby extending the shelf life of aquatic product. However, most of the existing research methods on inhibiting shrimp blackening were targeting at the dead state of shrimps, which would bring two disadvantages: low acceptance by consumers and low economic value. Therefore, there is a need to find an efficient method to solve the above problems that are specifically for live shrimps. It has been reported that changing the living environment of aquatic products can inhibit bacterial growth and blackening. Recent studies have shown that ethylenediamine tetraacetic acid (EDTA) and its salts also have this effect. For example, Liang et al<sup>[14]</sup> found that a combination of nisin and EDTA can reduce *Pseudomonas fluorescens*, *Salmonella enterica*, and *Vibrio parahaemolyticus* growth in raw Pacific white shrimp by  $10^{6.77}$ ,  $10^{5.48}$ , and  $10^{6.89}$  CFU·mL<sup>-1</sup>, respectively, compared to the control. Sangcharoen et al<sup>[15]</sup> found that *Salmonella enteritidis* ATCC 13076 was

evidently inhibited by a combination of nisin, ascorbic acid, and EDTA, with a decrease of 2 570 CFU·mL<sup>-1</sup> in total amount. López-Caballero et al<sup>[16]</sup> reported that edetate disodium (EDTA-2Na) was effective in hindering melanosis progression in deep-water pink shrimps. This suggests that EDTA-2Na has great potential as an aquatic product to slow down melanosis development and inhibit the growth of harmful bacteria.

Microorganisms not only damage the quality of *L. vannamei*, but also cause serious food safety problems<sup>[17-19]</sup>. The development and application of high-throughput sequencing technology has led to many new improvements in food safety and public health<sup>[20]</sup>. Compared to traditional methods, it has distinct advantages, such as a high throughput, low cost, and micro-quantification. This technology has become common in modern life science research, enabling the sequential sequencing of millions of DNA molecules and quickly obtaining all sequences to identify single genes or whole microorganism genomes through bioinformatics analysis<sup>[21]</sup>. It has been widely used in multiple fields, such as disease diagnosis<sup>[22]</sup>, vaccine research<sup>[23]</sup>, ancient wood analysis<sup>[24]</sup>, and exploration of microfloral changes in aquatic products, for example *Procambarus clarkii*<sup>[25]</sup>, *Scylla paramamosain*<sup>[26]</sup>, algal<sup>[27]</sup>, and Spanish mackerel<sup>[28]</sup>. Moreover, *L. vannamei* health is highly correlated with the composition and function of the microbial community in the hepatopancreatic system, and there have been studies on the microbe of *L. vannamei* hepatopancreas<sup>[29-30]</sup>. Therefore, we studied the microbial community in the hepatopancreas of *L. vannamei* using high-throughput sequencing.

In this study, *L. vannamei* was used as the research object, and EDTA-2Na was added during the temporary rearing process. The best groups were selected through physical and chemical sample analysis following different temporary rearing durations and amounts of added EDTA-2Na. Then, high-throughput sequencing was employed to study the hepatopancreatic microfloral composition and change. Further on, the effect of EDTA-2Na was also investigated, so as to provide new

ideas for disease prevention in aquatic products.

## 2 Materials and methods

### 2.1 Sample collection

*L. vannamei* was purchased from the Xiangshan Farm in Ningbo and transported to the laboratory within 2 h using oxygenated seawater. To reduce variability among the samples, we selected uniformly-sized, fresh, and undamaged individuals with a weight of  $(16.5 \pm 1.5)$  g each.

### 2.2 Reagents and instruments

EDTA-2Na was purchased from Dawei Biological Engineering Co., Ltd. (Qingdao, China), and sodium hydroxide from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). MagPure Soil DNA LQ Kit, 1× dsDNA Qubit HS Assay Kit, and Takara Ex Taq were obtained from Magen Biotechnology (Beijing) Co., Ltd., Yeasen Biotechnology (Shanghai) Co., Ltd., and Takara Biotechnology (Beijing) Co., Ltd., China, respectively.

The required equipment included a homogenizer (XHF-DY, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China), centrifuge (5418, Eppendorf, Shanghai, China), colorimeter (CR-400, Konica Minolta, Tokyo, Japan), thermostatic water bath (LTDDK-8D, Shanghai Yiheng Technology Co., Ltd., Shanghai, China), constant-temperature incubator (Saifu Experimental Instrument Factory, Ningbo, China), and vertical autoclaving pot (DGL-50B, Dengguan Medical, Changzhou, China).

### 2.3 Temporary rearing

The environment for the temporary rearing of *L. vannamei* was natural seawater with 1.8‰ salinity, where three different concentrations of EDTA-2Na were added: 0.1% (A group), 0.2% (B group), and 0.3% (C group). The pH of the seawater was adjusted to the same value as that of the control group by using NaOH. We ensured that the water temperature was  $(19 \pm 1)$  °C, with 1:20 shrimp to water ratio. The same number of *L. vannamei* was removed from each tank for quality determination 6, 12, 18, 24 h after temporary rearing. The groups were named as D, A6, A12, A18, A24, B6,

B12, B18, B24, C6, C12, C18, and C24, where the letters sequentially denote the concentration of EDTA-2Na, and the numbers signify the temporary rearing duration, whereas D was the control group.

### 2.4 Polyphenol oxidase (PPO)

PPO was extracted from the hepatopancreas of three *L. vannamei* from each group<sup>[31]</sup>. The samples (0.5 g) were tested, cut, and ground at 0–4 °C, and 5 mL of liquor extract was then added. The mixture was homogenized at  $4\,000\text{ r}\cdot\text{min}^{-1}$  for 1 min at 4 °C and centrifuged at 8 000g for 10 min at 4 °C, after which the supernatant (enzyme solution) was stored at 4 °C. Reagent I (200 µL), Reagent II (50 µL), and 50 µL of the sample were added to the centrifuge tube. The mixture was quickly transferred to a boiling water bath for 10 min, and then placed at 25 °C for 10 min. The obtained samples were centrifuged at 5 000g for 10 min at 25 °C. Next, 200 µL of the supernatant was added to a 96-well plate and the absorbance was measured at 410 nm. Each group consisted of three parallel experimental tubes and one control tube. The crude enzyme solution in the control tube was placed in a boiling water bath for 10 min. One unit of enzymatic activity was defined as a 0.01 change in absorbance at 410 nm. The formula for calculating PPO activity was the following:

$$A_{\text{PPO}} = \frac{\Delta A}{0.01} \frac{V_1 V_2 T}{W V_3},$$

$$\Delta A = A_D - A_C,$$

where  $A_{\text{PPO}}$  is PPO activity,  $\text{U}\cdot\text{g}^{-1}$ ;  $V_1$  is total volume of the reaction system;  $V_2$  is volume of added extract;  $V_3$  is sample volume added to the reaction system;  $T$  is reaction time;  $W$  is sample quality;  $A_D$  and  $A_C$  are the  $A_{\text{PPO}}$  of D group and C group, respectively.

### 2.5 Color properties

The head and abdomen of *L. vannamei* were measured using a colorimeter, during which the light conditions were ensured consistent in general. In accordance with international standards, the  $L^*$  value represents brightness,  $a^*$  value represents red and green, and  $b^*$  value represents yellow and blue. Six shrimps

were collected from each group, and each point was measured 10 times. Data with obvious errors were excluded and the results were determined using the average values.

## 2.6 Total viable count (TVC)

This analysis referred to the protocol used by Zhang et al<sup>[32]</sup>, with minor modifications. Three shrimp hepatopancreas samples were isolated from each group with a scalpel in a sterile environment and mixed. Then, 1 mL of the mixture and 9 mL sterile physiological saline solution (0.085%) were placed into a sterile centrifuge tube and mixed thoroughly for 1 min using a vortex. Three suitable dilution gradients of bacterial suspensions were prepared. 1 mL of bacterial suspension and 15–20 mL of agar medium that had been sterilized and cooled to (46–50) °C were added to a sterile Petri dish, which was shaken lightly to mix. After the agar had solidified, the plates were turned over and incubated at (30±1) °C for (72±3) h. The entire experiment was conducted in a sterile environment. The results were expressed as CFU·g<sup>-1</sup>.

## 2.7 Bacterial community analysis

Shrimp hepatopancreas samples were collected and frozen at -80 °C. DNA was extracted using a MagPure Soil DNA LQ Kit. The amount of DNA was determined using NanoDrop 2000 (Thermo Fisher Scientific, USA) and agarose gel. The primers 343F(5'-TACGGRAGGC AGCAG-3') and 798R(5'-AGGGTATCTAATCCT-3') were used for PCR amplification of the V3–V4 (343F/798R) region of the 16S rRNA gene. PCR reactions were performed with the Takara Ex Taq high-fidelity DNA polymerase under the following conditions: initial pre-denaturation (5 min, 94 °C), followed by 26 cycles of denaturation (30 s, 94 °C), annealing (30 s, 56 °C), and extension (20 s, 72 °C), ending with a final extension period (5 min, 72 °C).

Amplicon quality was determined using gel electrophoresis, purified with AMPure XP beads. After two rounds of PCR amplification, the final amplicon was quantified using a Qubit dsDNA assay kit. Sequencing was performed on an Illumina NovaSeq

6000 (Illumina Inc., USA) with 250 bp paired-end reads. The operation was performed by the Shanghai Personalbio Biotechnology Company.

## 2.8 Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) and significant difference analysis ( $P<0.05$ ) of Duncan multiple comparisons using the SPSS 22 software. The results were expressed as the mean±standard deviation. Origin 2021 was used for data plotting.

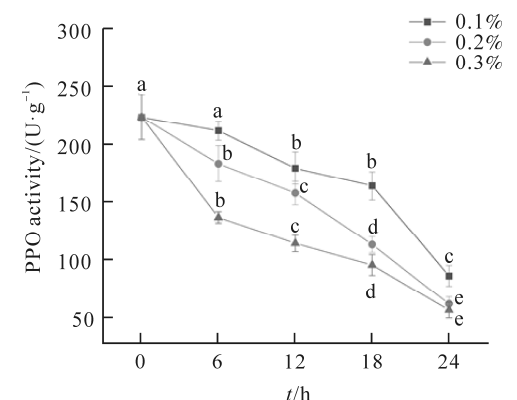
For the bacterial 16S rDNA genes, QIIME2 (2020.11) was used for quality control analysis to filter out representative sequences along with the ASV (Amplicon Sequence Variant) abundance table<sup>[33]</sup>. All representative sequences were annotated against the Silva (version 138) database using the default parameters of the q2-feature-classifier software. The QIIME2 software was used for alpha and beta diversity analyses. The R package was used for differential analysis.

# 3 Results and discussion

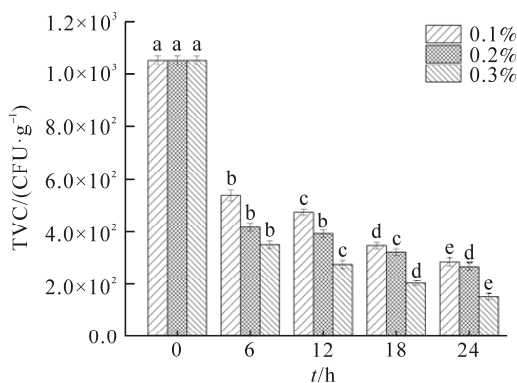
## 3.1 Effect of EDTA-2Na on PPO

The effect of EDTA-2Na on PPO activity in the hepatopancreas of *L. vannamei* was shown in Fig. 1(a). PPO oxidase activity decreased gradually with an increase in EDTA-2Na and temporary rearing duration ( $P<0.05$ ), and it was 230.91 U·g<sup>-3</sup> at the beginning and decreased to the lowest value of 53.58 U·g<sup>-3</sup> at the end of temporary rearing. Similar results were observed by Zheng et al<sup>[34]</sup>, where EDTA-2Na-mediated inhibition of PPO activity increased from 10.9% to 31.6% with increasing EDTA. This result was attributed to two functions of EDTA-2Na. As an antioxidant, it inhibited the formation of free tyrosine and phenylalanine, which were PPO substrates. As a metalloproteinase, PPO activity was inhibited by the chelation of the copper repair group presented at its active site or the reduction of copper available for incorporation<sup>[35]</sup>. Enzymatic browning caused by PPO was one of the main mechanisms underlying blackening in *L. vannamei*.

Under aerobic conditions, tyrosine in organisms was catalyzed to L-3,4-dihydroxyphenylalanine (L-DOPA) by PPO. Then, L-DOPA was converted to quinones and further oxidized to dark brown melanin or was involved in the polymerization of functional protein groups to form cross-linked polymers, which eventually appeared as dark melanin<sup>[36]</sup>. EDTA-2Na can inhibit multiple reactions during the entire process and effectively inhibit shrimp blackening.



(a) Polyphenol oxidase activity



(b) Total viable count

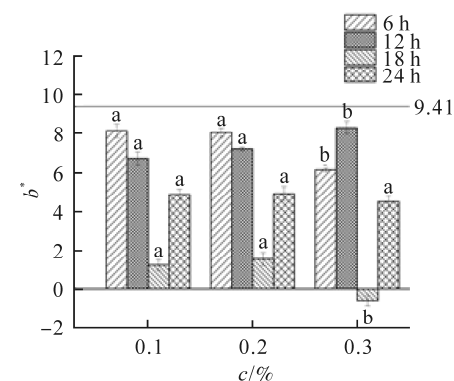
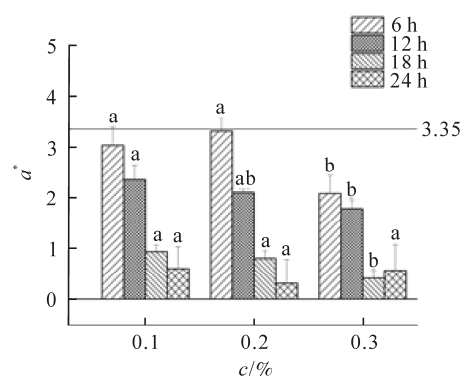
Different letters (a, b, c, d, and e) represent significant differences between different groups,  $P < 0.05$ .

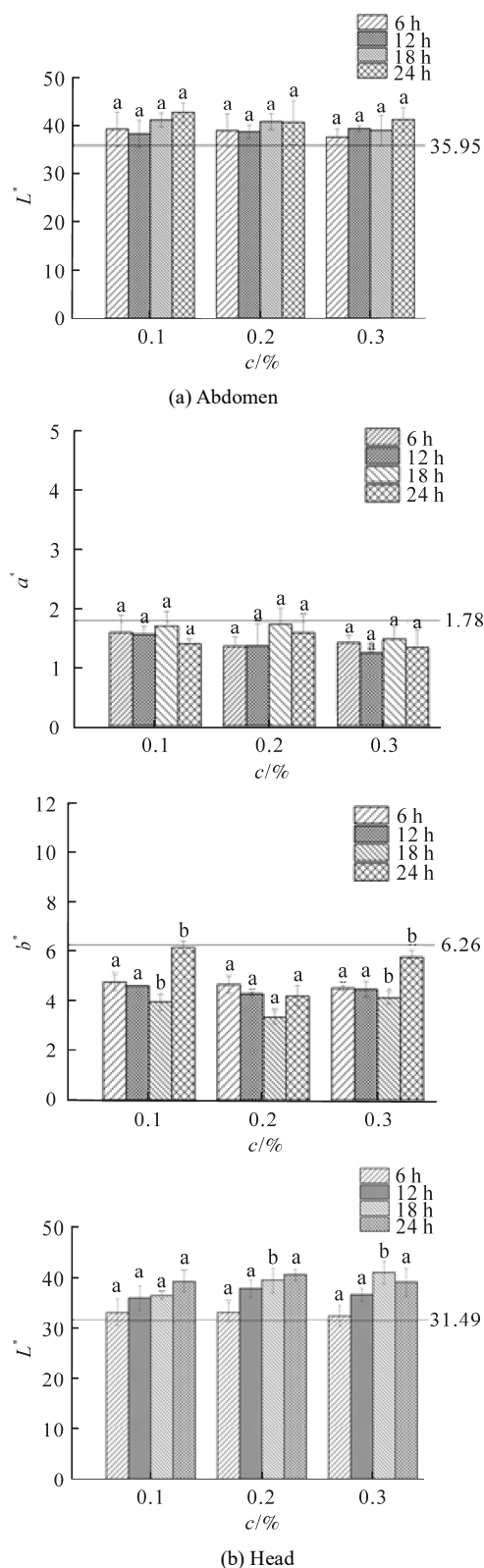
**Fig. 1 Effect of temporary rearing with EDTA-2Na on PPO and TVC**

### 3.2 Effect of EDTA-2Na on color

The effect of EDTA-2Na on the color of the head and abdomen of *L. vannamei* was shown in Fig. 2. The color changes of the shrimp abdomens were presented in Fig. 2(a), with initial values of  $a^*$ ,  $b^*$ , and  $L^*$  measured at 3.35, 9.41, and 35.95 respectively. On the other hand, Fig. 2(b) displayed the color changes of the shrimp heads, with initial values of  $a^*$ ,  $b^*$ , and  $L^*$  measured at

1.78, 6.26, and 31.49 respectively. The  $L^*$  value demonstrated a slight increase, while the  $a^*$  and  $b^*$  values exhibited a gradual decrease as the quantity of EDTA-2Na and temporary rearing duration increased, regardless of whether it was the head or abdomen of the shrimp. The increase in the  $L^*$  value can be attributed to two main factors. Firstly, the temporary rearing process involved washing the shrimp shells with seawater. Secondly, when the shrimps were not fed, excretion occurred, which expelled the contents of the hepatopancreas and intestinal line, resulting in enhanced brightness of the shrimp color. The changes in the  $a^*$  and  $b^*$  values were attributed to the effect of EDTA-2Na on astaxanthin. The conformational changes that occurred when astaxanthin bound to proteins influenced the color of the shrimp<sup>[37]</sup>. The bluish-gray appearance of the shrimp was due to the combination of astaxanthin and various types of proteins. The addition of EDTA-2Na enhanced the chemical stability of astaxanthin and effectively suppressed its degradation<sup>[38]</sup>. Consequently, EDTA-2Na strengthened the bond between astaxanthin and proteins, leading to a reduction in the redness and yellowness of the shrimp.





Different letters (a and b) represent significant differences between different groups,  $P < 0.05$ .

**Fig. 2** Effect of temporary rearing with EDTA-2Na on the color of the abdomen and the head

### 3.3 Effect of EDTA-2Na on TVC

As shown in Fig. 1(b), the initial total number of colonies in the hepatopancreas of fresh shrimp was  $1\,073\text{ CFU}\cdot\text{g}^{-1}$ , which was consistent with the results obtained in previous studies<sup>[16,39]</sup>. With an increase in the concentration of EDTA-2Na and temporary rearing duration, the total number of colonies continued to decrease, which was similar to the results obtained by Liang et al<sup>[14]</sup>. In comparison to the control group, the most successful group demonstrated a reduction in bacterial number by an order of magnitude, indicating the strong bacteriostatic property of EDTA-2Na<sup>[40-41]</sup>. The inhibitory effect of EDTA-2Na on bacteria was found to be enhanced by increased acidity, while its inhibitory effect on mycobacteria was not dependent on pH. EDTA-2Na blocked the process of o-quinone formation by inhibiting the stimulation of PPO activity through microbial metabolites<sup>[42]</sup>.

### 3.4 ASV cluster analysis

ASVs are based on OUT (Operational Taxonomic Units) sequences and were de-duplicated using DADA2 in QIIME2 (2020.11) after noise reduction and chimera removal. Each de-duplicated sequence is called an ASV. Fig. 3 showed the results of the analysis for all groups of samples, visually depicting the common and unique ASVs in the *L. vannamei* hepatopancreas at different concentrations of EDTA-2Na and temporary rearing duration. Fig. 3(a) showed that on the time gradient, 70 ASVs were common to D, C6, C12, C18 and C24, whereas 797, 836, 862, 549, and 1 185 ASVs, respectively, were unique to each group. Fig. 3(b) showed that on the concentration gradient, 84 ASVs were shared by 4 sample groups, whereas 807, 1 275, 588, and 1 206 ASVs were unique to D, A24, B24, and C24, respectively. Furthermore, the presence of EDTA-2Na had an impact on the bacterial community in the shrimp hepatopancreas. Specifically, the ASVs of B24 and C18 were significantly lower as compared to the control group. Additionally, it is noteworthy that the samples on the time gradient exhibited less variation compared to the samples on the concentration gradient. Hence, it can be concluded that the bacterial community

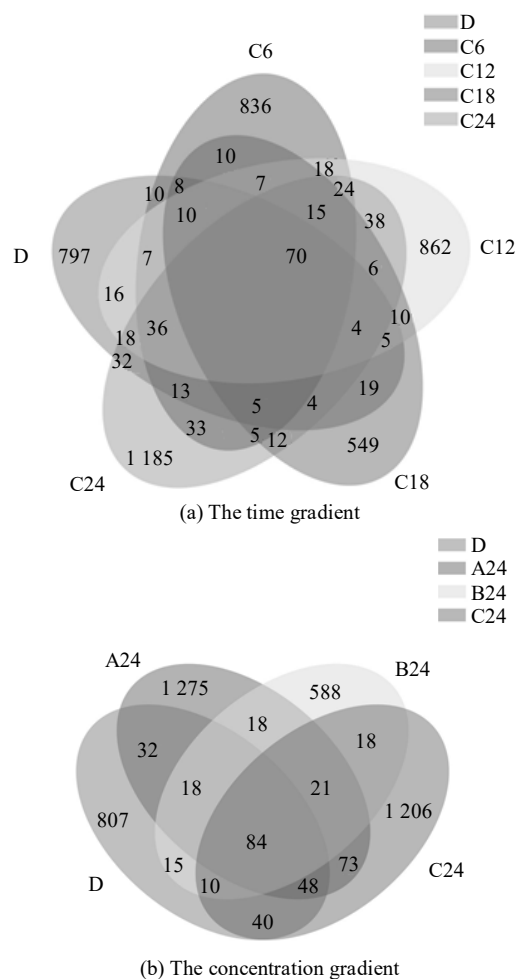
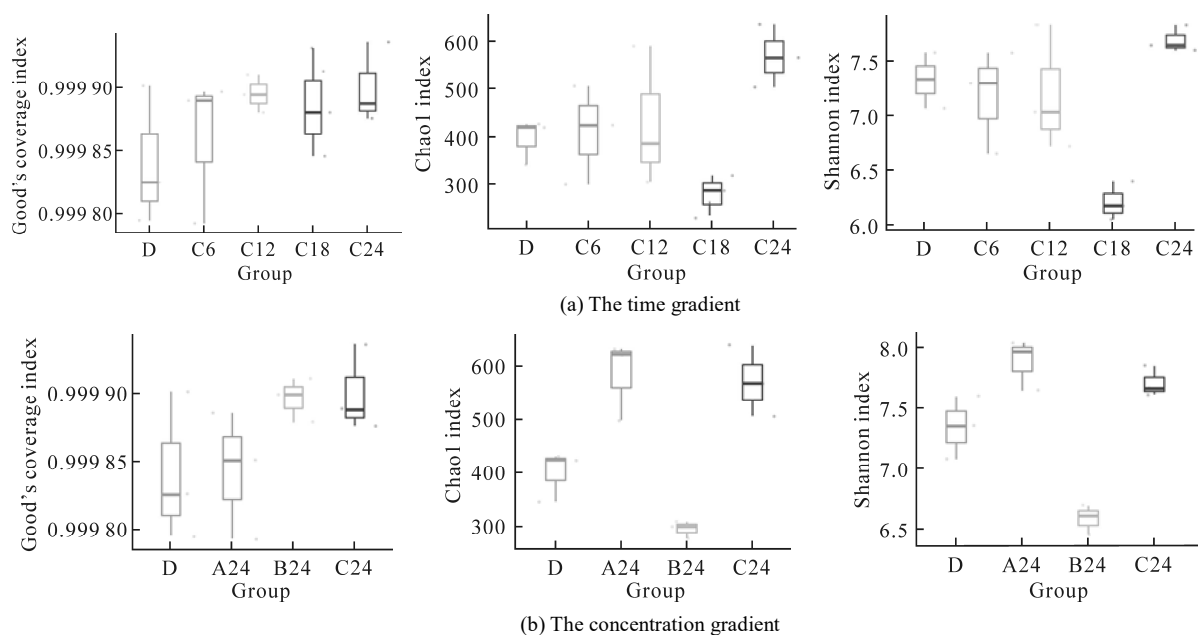


Fig. 3 Effect of temporary rearing with EDTA-2Na on the ASVs



Good's coverage, Chao1, Shannon represent the sequencing depth, strain richness, sample diversity, respectively.

Fig. 4 Alpha diversity index analysis

in the *L. vannamei* hepatopancreas was influenced by both the concentration and time gradients of EDTA-2Na.

### 3.5 Alpha diversity analysis

In this study, a set of alpha diversity indices including Good's coverage, Chao1, and Shannon indices were selected to analyze the degree of species diversity within the biological environment. Fig. 4 showed the results of time gradient and concentration gradient respectively. As shown in Fig. 4, all samples exhibited sequencing depths that met the specified requirements (Good's coverage > 99%), successfully encompassing all the species present in the samples. Comparatively, regarding the actual number of species in the control group, C6 and C12 displayed similarity, A24 and C24 demonstrated higher values, while B24 and C18 exhibited lower values, all approaching 3 000. By considering the Shannon index, it can be concluded that B24 and C18 witnessed a significant reduction in species richness and evenness, whereas C6 showed comparable outcomes to the control group, C12 experienced a slight reduction, and C24 and A24 demonstrated an increase in comparison to the control group. These changes in species richness and evenness were influenced by the number of species, with A24 and

C24 displaying higher richness and diversity, thereby classifying them as better-quality groups<sup>[43]</sup>.

### 3.6 Beta diversity analysis

Beta diversity is used to calculate the distance between samples based on evolutionary relationships and abundance information. This analysis allows us to describe the structural composition of each community under different environmental conditions and determine the turnover rate of species composition caused by environmental factors. Therefore, beta diversity analysis can help us determine if there are significant differences in microbial communities among sample groups. Fig. 5 presents the results of the time gradient and concentration gradient, respectively. In the non-metric multidimensional scale (NMDS) analysis, the stress of both sets was less than 0.1, which were 0.083 and 0.065 respectively, indicating that both can reflect the true distribution of the sorted data. Likewise, the unrestricted principal axis analysis (PCoA) based on Bray-Curtis distances represented the proximity relationship based on the distance between each sample group. As can be seen from the figures, in the time gradient, the first and

second principal coordinates accounted for 9.18% and 7.52% of the differences between the groups, respectively. In the concentration gradient, the first and second principal coordinates accounted for 10.68% and 9.51% of the differences, respectively. Moreover, the overlapping area of the various time gradients was larger than that of the concentration gradients. These results indicate significant differences among D, A24, B24, and C24, but small differences among C6, C12, and C18. This suggests that EDTA-2Na concentration has a greater impact on microbial inhibition in the *L. vannamei* hepatopancreas than temporary rearing duration. Additionally, sample similarity clustering analysis (UPGMA) confirmed the systematic classification between sample groups, aligning with the results of the previous analyses. There was a significant difference in the concentration gradient between the sample groups at the same time.

### 3.7 Sample bacterial community composition and abundance analysis

Community structure analysis assesses the proportion of species in a sample at the phylum, class,

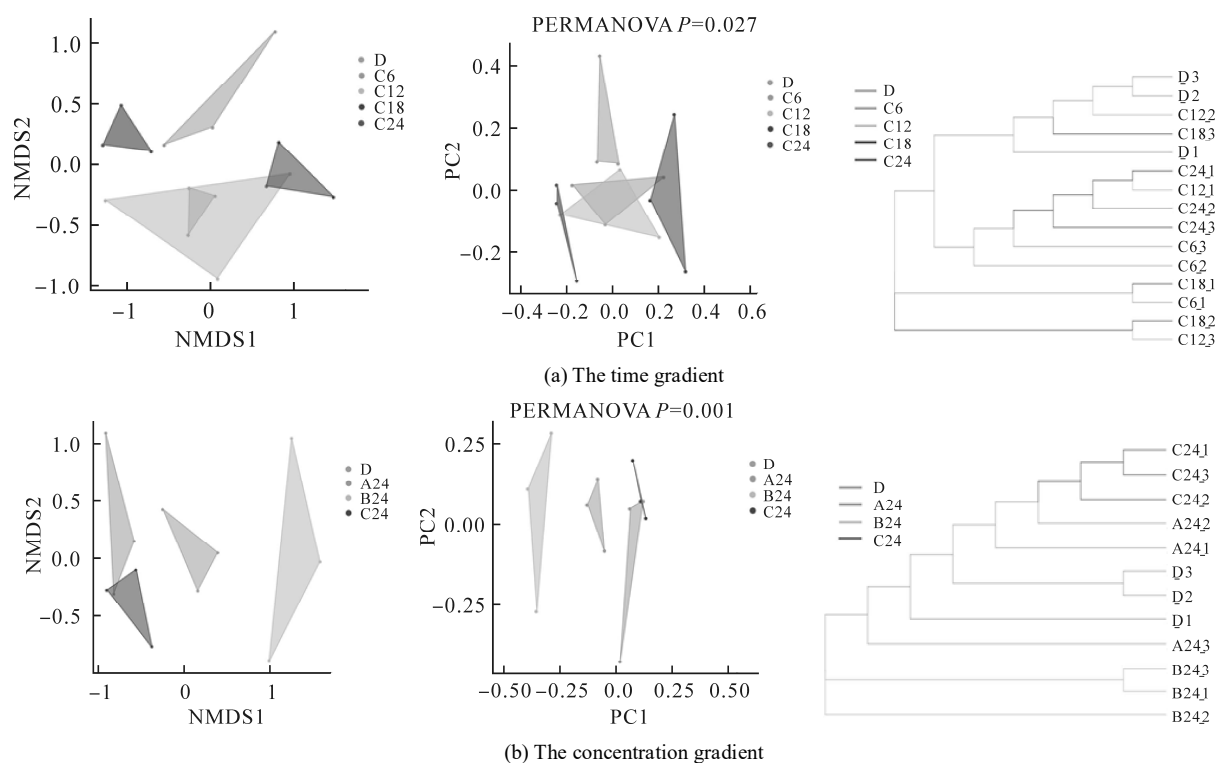


Fig. 5 Beta diversity analysis

order, family, and genus levels, including the results of absolute and relative abundances. In this study, the species composition of the *L. vannamei* hepatopancreas was studied at the phylum and genus levels, and the results were shown in Fig. 6.

The results of the phylum level were shown in Fig. 6(a) under the time gradient, Proteobacteria (31.94%, 35.68%, 34.38%, 51.47%, 22.74%), Bacteroidota (34.60%, 31.82%, 31.27%, 26.53%, 34.39%), Firmicutes (27.08%, 26.70%, 28.22%, 15.55%, 36.80%), Actinobacteriota (2.64%, 2.24%, 1.83%, 3.13%, 1.02%), and Desulfobacterota (1.01%, 1.06%, 1.48%, 0.76%, 2.55%) were also the dominant phyla in the *L. vannamei* hepatopancreas in the D, C6, C12, C18, and C24 groups, respectively; for those under the concentration gradient, Bacteroidota (34.60%, 36.79%, 29.47%, 34.39%), Proteobacteria (31.94%, 24.12%, 43.73%, 22.74%), Firmicutes (27.08%, 33.36%, 20.72%, 36.80%), Actinobacteriota (2.64%, 1.25%, 2.77%, 1.02%), and Desulfobacterota (1.01%, 1.76%, 0.6%, 2.55%) were the dominant phyla in the hepatopancreas of *L. vannamei* in the D, A24, B24 and C24 groups, respectively, which

was consistent with previous results<sup>[44]</sup>. The community structures of the B24 and C18 sample groups were similar, but there were significant differences among the other sample groups. These results indicated that EDTA-2Na affected the hepatopancreatic community structure of *L. vannamei* to a certain extent. At the end of the temporary rearing (C24), Proteobacteria was effectively inhibited. Proteobacteria was inhibited because it mainly consisted of Gram-negative bacteria, and EDTA had antibiofilm activity against Gram-negative bacteria<sup>[45]</sup>. Proteobacteria was the largest phylum of bacteria, and included a variety of pathogens, such as *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Helicobacter pylori*, and other well-known genera. Therefore, Proteobacteria reduction was beneficial to white shrimp health.

The genus-related analysis results were shown in Fig. 6(b) under the time gradient, *Burkholderia* (10.65%, 13.32%, 13.00%, 19.94%, 9.30%), *Muribaculaceae* (9.57%, 7.94%, 9.08%, 5.82%, 10.55%), *Sphingomonas* (8.59%, 10.61%, 10.14%, 15.40%, 7.14%), *Alloprevotella* (7.07%, 7.53%, 6.22%, 2.67%, 9.97%), and *Bacteroides* (7.08%, 6.19%, 6.82%, 9.57%, 4.61%) were also

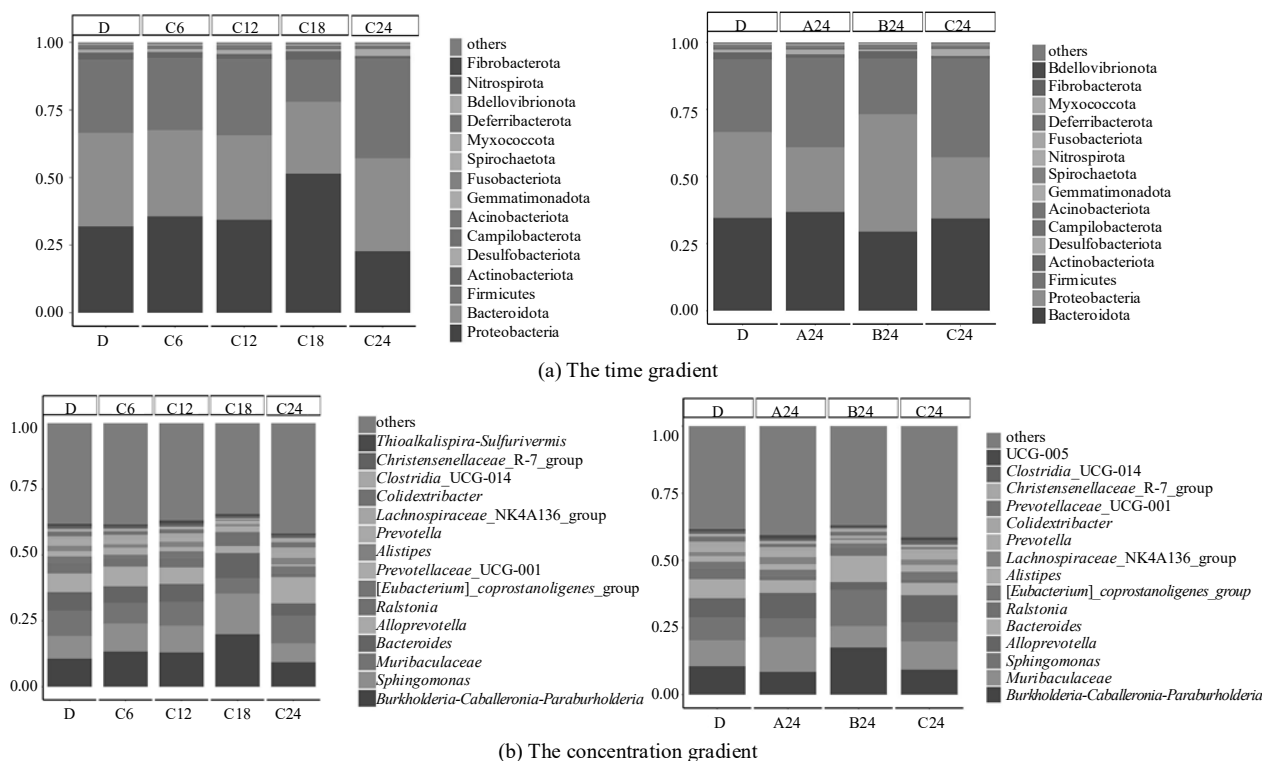


Fig. 6 Bacterial composition

dominant in the *L. vannamei* hepatopancreas in the D, C6, C12, C18, C24 groups, respectively; under the concentration gradient, *Burkholderia* (10.65%, 8.50%, 17.60%, 9.30%), *Muribaculaceae* (9.57%, 12.92%, 7.93%, 10.55%), *Sphingomonas* (8.59%, 7.08%, 13.44%, 7.14%), *Alloprevotella* (7.07%, 9.35%, 2.94%, 9.97%), and *Bacteroides* (7.08%, 4.75%, 9.75%, 4.61%) were the dominant bacterial genera in the *L. vannamei* hepatopancreas in the D, A24, B24, C24 groups, respectively. Under both gradients, the changes in community structure were similar. For example, *Burkholderia* and *Sphingomonas* first increased and then decreased, *Muribaculaceae* first decreased and then increased, with a more obvious change under the concentration gradient.

### 3.8 Community structure analysis

In community structure analysis, phylogenetic trees are a means to describe the evolutionary relationships either within or across bacterial species. Fig. 7 showed the circular phylogenetic tree of the top 50 OUTs of all samples with the highest relative abundance at the phylum level. The most abundant OUT in the sample was divided into two branches: one composed of Bacteroidota, which occupied the largest proportion; the other of Proteobacteria, Firmicutes, and Campilobacterota. These results indicated that these phyla played an important role in the structure of *L. vannamei* microflora.

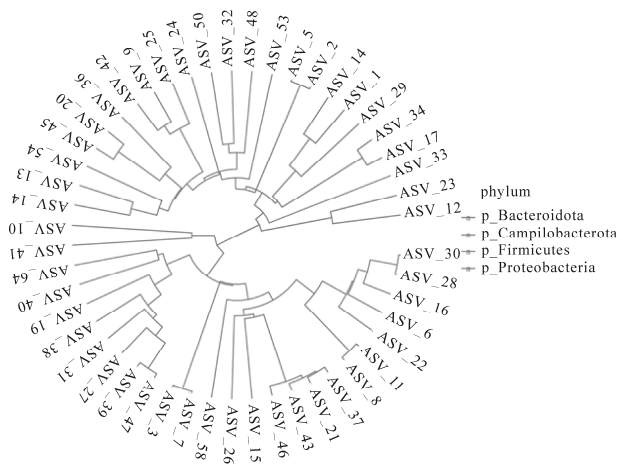
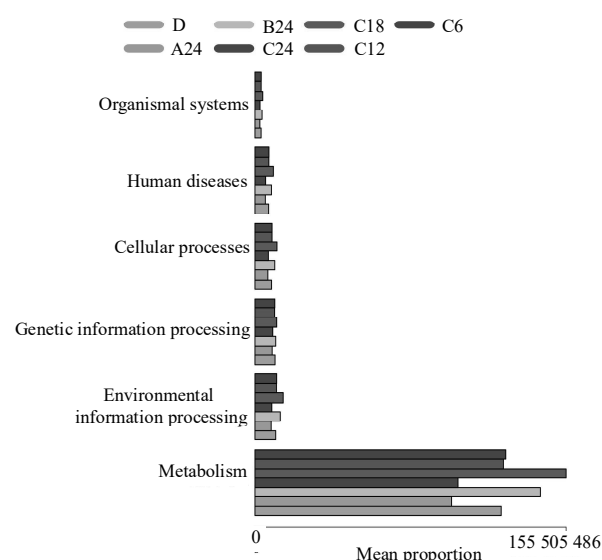


Fig. 7 Community structure analysis

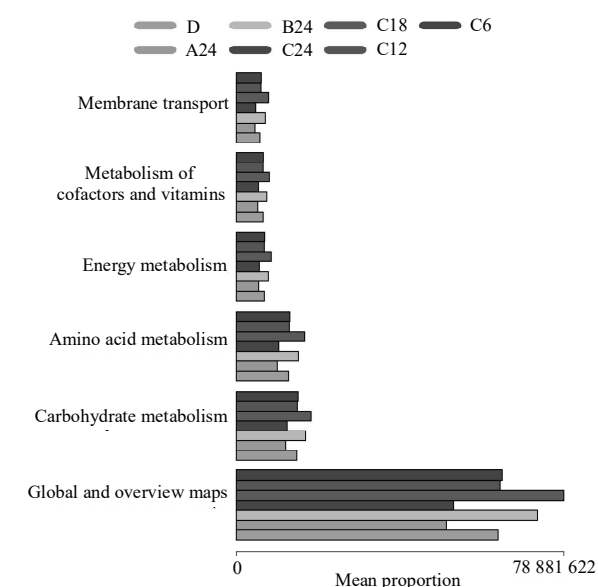
### 3.9 Functional pathways analysis

Based on PICRUST analysis, the relative abundance

of operational taxonomic units (OTUs) in the top 50 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways at the gate level was explored to predict the potential functional profiles of *L. vannamei* hepatopancreas quality. In line with expectations, the six major KEGG pathways at level 1 (Fig. 8(a)) were found in all seven groups of samples and included metabolism, such as global and overview maps, carbohydrate environmental information processing, genetic information processing, cellular processes, human diseases, and organismal systems. Notably, metabolism was the most abundant



(a) The level 1



(b) The level 2

Fig. 8 Functional pathways analysis

pathway across all groups. Moreover, at level 2 (Fig. 8(b)), the hepatopancreas microbiome of *L. vannamei* exhibited enrichment in functional categories, metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, and membrane transport.

## 4 Conclusion

In conclusion, the temporary rearing with EDTA-2Na of *L. vannamei* effectively reduced PPO activity in the hepatopancreas, with increased  $L^*$  values of body surface, reduced yellowness and redness, and decreased hepatopancreatic total viable count. The results showed that the end point of temporary rearing was the optimum temporary rearing condition. EDTA-2Na affected species diversity and community structure of the hepatopancreas under certain conditions, with concentration and time gradients being similar in terms of fluctuation, but the effect of concentration was more obvious than that of time. A24 and C24 had richer and more diverse species, which was conducive to regulating the imbalance of species. Proteobacteria of C24 was effectively inhibited. According to KEGG function prediction, metabolic pathways were mostly responsible for the quality of *L. vannamei*.

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## 乙二胺四乙酸二钠胁迫暂养对南美白对虾理化指标和细菌群落的影响

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**摘要:** 南美白对虾具有高水分、高蛋白、高酶活、高虾青素等特点, 死后在多酚氧化酶作用下虾头会快速黑变, 接着在微生物作用下腐败变质. 针对上述问题现有的研究都集中在南美白对虾死后添加抗氧化剂、防腐剂等协同温控进行保鲜, 造成消费者接受度低. 本研究以活鲜南美白对虾为研究对象, 采用添加 EDTA-2Na 水体胁迫暂养, 通过分析多酚氧化酶活力、菌落总数、色泽等探究 EDTA-2Na 对南美白对虾基础理化指标的影响; 在此基础上, 利用微生物组技术, 进一步探究南美白对虾在暂养过程中细菌群落的差异. 结果表明: EDTA-2Na 的添加量、暂养时长与多酚氧化酶(PPO)活性、菌落总数成反比, 暂养终点有较好的抑制作用. 南美白对虾头部、腹部的  $L^*$ (明暗度)值略有升高,  $a^*$ (红绿色)和  $b^*$ (黄蓝色)值均下降, 黑变和红变被有效抑制. EDTA-2Na 在暂养过程中对南美白对虾肝胰腺部位的菌群数量和菌落结构均有影响, 0.1%-24 h 和 0.3%-24 h 两组的菌群多样性和丰富度较高, 更利于菌群动态平衡. 南美白对虾肝胰腺的菌门主要有拟杆菌门、变形菌门、厚壁菌门和放线菌门. 0.3%-24 h 组的变形菌门被抑制, 表明大量病原体的繁殖被抑制. 主要影响南美白对虾肝胰腺的途径为代谢.

**关键词:** 南美白对虾; 暂养; 乙二胺四乙酸二钠; 黑变; 细菌群落

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