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Research Article

Physicochemical Properties and Bacterial Diversity of Sediment in Sea Cucumber Culture Ponds under High Summer Temperatures

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This study examined the effect of aquaculture management parameters on sea cucumbers in culture ponds during high summer temperatures. The physicochemical properties of 18 sediment samples from six sea cucumber culture ponds were assessed at a high temperature (HT) and the end of the high temperature (EHT) stages of summer. High-throughput sequencing was used to analyze the bacterial community composition and diversity of the sediment in the culture pond. The results showed that total nitrogen (TN), total carbon (TC), total organic carbon (TOC), ammonia nitrogen (NH_4^+), total sulfur (TS), sulfate (SO_4^{2-}), and sulfide (S^{2-}) contents were the lowest in the Geziling sediments at both stages, indicating a favorable sediment environment. The TN, TC, TOC, and NH_4^+ contents decreased significantly in the Wulei Island sediments during the EHT stage, indicating strong self-purification capacity. The TN, TC, TOC, NH_4^+, TS, SO_4^{2-}, and S^{2-} were higher in the Rushankou and Xiaohongcun sediments during both stages, indicating a worse sediment environment. Alpha diversity analysis revealed increased bacterial diversity in the sediments during the HT stage when compared to that during the EHT stage. Correlation analysis between bacterial diversity and the physicochemical properties of the TN sediments had the greatest impact on bacterial diversity, followed by TOC, TC, NH_4^+, TS, S^{2-}, SO_4^{2-}, and NO_2^- (in order). Our results suggest that the physicochemical properties (TC, TOC, TN, and NH_4^+) of the culture pond sediment can significantly influence bacterial community composition and diversity. Management measures for aquaculture, including desilting and monthly water change, could positively affect these physicochemical properties and the bacterial community structure of the sediment. These findings will provide a reference for healthier aquaculture practices of *Apostichopus japonicus* under high summer temperatures.

1. Introduction

Sea cucumbers (*Apostichopus japonicus*) belong to the phylum Echinodermata, class Holothuroidea, order Aspidochirotida, and family Stichopodidae [1]. Approximately 1,500 sea cucumber species are known worldwide, and they have been used throughout history as food supplements and in traditional medicines in Asian countries [2]. *A. japonicus* is one of the most important aquaculture species in China. From 2007 to 2020, the total yield of *A. japonicus* in China increased from 77,500 to 196,600 tons, with the aquaculture area increasing from 6.440×10^8 to $2.428 \times 10^8 \, m^2$ and the total output peaking in 2017 ($1.966 \times 10^7 \, kg$) [3].

The optimum growth temperature for sea cucumbers is approximately 14–15°C [4]. When water temperatures rise above 25°C, *A. japonicus* of different body sizes enter estivation to improve survival [4]. Sea cucumbers die on a mass scale when elevated temperatures persist [5]. This is due to many factors such as alterations in temperature, dissolved oxygen, ammonia nitrogen, sulfide, pathogenic bacteria, and the germplasm of *A. japonicus* [6]. Increased ammonia nitrogen accumulation is lethal for *A. japonicus* as it inhibits the activity of their digestive enzymes [7]. In addition, physiological disorders can occur, which significantly reduce the immunity of *A. japonicus* and increase their susceptibility to pathogenic bacteria. Appropriate ammonia nitrogen concentrations promote
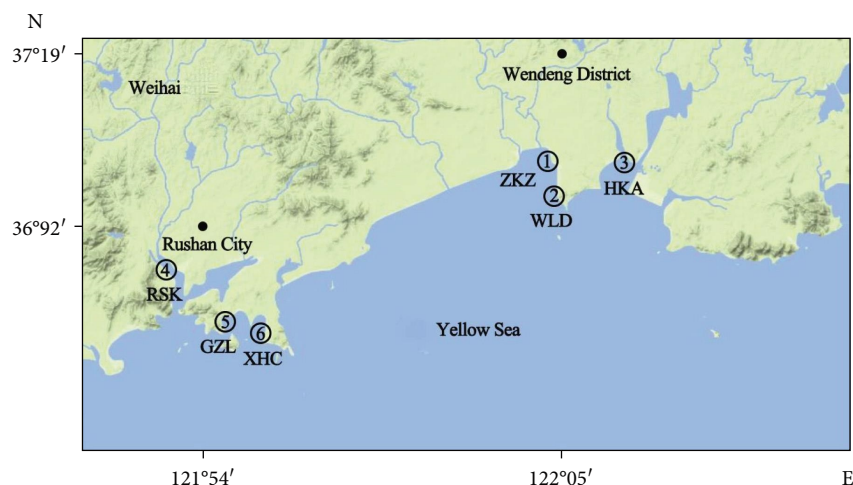
FIGURE 1: Map of the sampling sites of six sea cucumber *A. japonicus* culture ponds off the coast of Weihai, north of the Yellow Sea, China: ①Wulei Island (WLD); ②Zeku Town (ZKZ); ③Haikuan (HKA); ④Rushankou (RSK); ⑤Geziling (GZL); ⑥Xiaohongcun (XHC).

the killing of pathogenic bacteria by *A. japonicus* via the celomic fluid [7]. Compared to other aquaculture organisms, *A. japonicus* has weaker sulfide tolerance, which significantly impacts its survival, wall attachment, and antioxidant defense capacity [8].

Pond culturing is an important production method for *A. japonicus* in northern China [9]. Sea cucumber culture ponds are shallow, semiclosed bodies of water, and they obtain food by filtering seawater and ingesting marine sediment. Studying the microbial community structure and physicochemical properties of aquaculture ecosystems is an effective way to assess the functionality of aquaculture systems [10]. The richness and diversity of bacterial communities were the highest in pond sediment, followed by sea cucumber guts, and the lowest in water [11]. At high temperatures (HTs), the richness and diversity of the bacterial community in water and sediment decreased significantly; however, they significantly increased within *A. japonicus* intestines [12]. During a year-long assessment of *A. japonicus* aquaculture, the water temperature was significantly correlated with species abundance in the core microbiome of pond sediments and negatively regulated sulfur cycling efficiency within sediment-based microbial communities [9]. Sulfide has the greatest influence on microbial community composition in pond sediment in December [13]. The particulate organic carbon, particulate organic nitrogen and total phosphorus contents in the sediment of the ponds increased during sea cucumber estivation (summer) and hibernation (winter), whereas they decreased during feeding periods [14]. During estivation, sea cucumbers stop moving and feeding and the aquaculture environment, especially the sediment, has a significant impact on their growth and survival. Few studies have performed correlation analyses of microbial community structure and the physicochemical properties of the culture pond sediments under high summer temperatures.

In this study, six sea cucumber culture ponds on the coast of Weihai, north of the Yellow Sea, China, were selected, and their aquaculture management measures were investigated.

The physicochemical properties of the sediments of these culture ponds were determined at the HT and end of the high temperature (EHT) stages during summer. The bacterial community composition and diversity of the pond sediments were also assessed. The present findings will provide a reference for healthier aquaculture practices for *A. japonicus* under high summer temperatures.

2. Materials and Methods

2.1. Sample Collection and Preparation. Sediment samples were collected from six *A. japonicus* culture ponds off the coast of Weihai, north of the Yellow Sea, China. Of the six collection sites, Wulei Island (WLD), Zeku Town (ZKZ), and Haikuan (HKA) are located in the Wendeng District of Weihai. Rushankou (RSK), Geziling (GZL), and Xiaohongcun (XHC) are located in the Rushan City of Weihai (Figure 1). The aquaculture management practices of the six culture ponds were investigated, including the pond size, water depth, whether sea cucumbers were cultivated during the summer, yield, use of sea cucumber feed, probiotic use, desilting intervals, and monthly water change.

HT stage culture pond sediment samples were collected from Wendeng District on July 31, 2020, and from Rushan City on August 1, 2020. The seawater temperature in the culture ponds was 30.5–31.5°C during HT sample collection. EHT stage samples were collected from Wendeng District on September 22, 2020, and Rushan City on September 23, 2020. The water temperature of the culture ponds was 25.0–26.0°C during EHT sample collection.

Three sampling points were selected, and surface sediments (top 1–5 cm) were collected from each culture pond using a cylindrical sampler [15]. The sediment samples from the three sampling locations of each culture pond were homogenized in sterile plastic bags. Aliquots of each sediment were dispensed into cryovials and immediately frozen at –80°C until high-throughput sequencing and subsequent physicochemical analysis.

2.2. Physicochemical Analysis. Total carbon (TC), total organic carbon (TOC), total nitrogen (TN), and total sulfur (TS) contents of the sediment samples were measured using Vario Micro Cube Elemental Analyzer (Elementar, Germany) [16–18]. We placed 0.5 g of each sample into a temperature-programmed muffle furnace and heated it to 500°C for 3 hr to remove any sulfide. They were then cooled in a desiccator, and the elemental sulfur–sulfate (S–SO_4^{2-}) content was measured. The sulfide content (S^{2-}) in the sample was determined as follows [18]:

$$w(S - S^{2-}) = w(TS) - w(S - SO_4^{2-}). \tag{1}$$

Lyophilized sediments were leached with KCl (2 M), and the nitrate (NO_3^{-}–N), nitrite (NO_2^{-}–N), and ammonium (NH_4^{+}–N) contents were measured with a nutrient AutoAnalyser (Seal, Germany) [19].

2.3. DNA Extraction and Sequencing. Microbial genomic DNA was extracted from the sediment samples using an E.Z.N.A. Soil DNA Extraction Kit (Omega Bio-Tek, Norcross, GA, USA) and detected via agarose gel electrophoresis. The V4–V5 region of the bacterial 16S ribosomal RNA gene was amplified by polymerase chain reaction (PCR) at 95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min using the following primers: 515 F: 5′-barcode-GTGCCAGCMGCCGCGG-3′ and 907R: 5′-CCGTCAATTCMTTTRAGTTT-3′ [20]. The "barcode" designates an eight-base sequence unique to each sample. Amplicons were extracted from the 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Purified PCR products were quantified using a QuantiFluor™-ST blue fluorescence quantitative system (Promega, Madison, WI, USA) and then mixed in corresponding proportions according to the sequencing quantity requirements of each sample. The pooled DNA product was used to construct an Illumina paired-end (PE) library according to the Illumina genomic DNA library preparation protocol. The amplicon library was PE sequenced (2 × 250) on an Illumina MiSeq platform (Biozeron Biotechnology Co., Ltd., Shanghai, China) according to standard protocols.

The obtained PE reads from sequencing were first spliced according to the overlap relationship. Sequence quality filtering and processing were performed using QIIME software [21]. Sequences were clustered using USEARCH (v.10.0) [22] at a similarity level of 97% to obtain operational taxonomic units (OTUs). The RDP classifier (v.2.2) [23] was used to classify the species. The classification threshold was set at 0.7, and results below this threshold were considered unclassified.

2.4. Microbial Diversity Analysis. Mothur (version 1.30.1) [24] was used to calculate alpha diversity indices, including Good's coverage and Chao, Simpson, and Shannon diversity indices. Good's coverage index was used to characterize the sequencing depth. The Chao index was used to identify community richness. The Shannon and Simpson indices were used to identify community diversity. Principal coordinate analysis (PCoA) was used to study the similarity of the

bacterial community structure among the sediment samples from the six *A. japonicus* culture ponds. R [25] was used for statistical analyses and to graph the PCoA results. Venn diagrams were drawn using R to analyze overlapping and unique OTUs at the HT and EHT stages. An OTU sample table with a similarity level of 97% was used for analysis. Data on the species' community composition and taxonomic relationships were obtained. R was used for statistics and to visualize the bacterial species and relative abundances in the sediment samples.

2.5. Statistical Analysis. Canonical correlation analysis (CCA) is a multivariate statistical method used to study the correlations between two groups of variables. CCA can reveal the intrinsic relationship between two groups of variables. To investigate the relationship between bacterial diversity and physicochemical properties, CCA was performed in R using the "vegan" package. In addition, R was used to analyze the correlation between bacterial abundance and physicochemical properties at the phylum level. All statistical analyses were conducted using SPSS v. 26.0 (SPSS Inc., Chicago, IL, USA) and one-way analysis of variance.

3. Results

3.1. Summary of Sea Cucumber Culture Ponds. Table 1 presents data on the pond size, water depth, whether sea cucumber cultivation occurred in the summer, yield, use of sea cucumber feed, use of probiotics, the time between desilting events, and monthly water change. The WLD (3.33×10^4 m^2), ZKZ (5.33×10^4 m^2), and GZL (6.67×10^4 m^2) culture ponds were small, and relatively standard culture pond management practices were used. Feed and probiotics were used (with the exception of GZL). Desilting and monthly water change practices were better in the WLD and GZL ponds. The HKA (2.67×10^5 m^2) and XHC (1.67×10^5 m^2) culture ponds were relatively large and did not use feed or probiotics. They employed fairly simple culture pond management practices, and sea cucumbers grew at close-to-natural rates, so yields were lower. The RSK culture pond is located inside an estuary with poor water exchange. This pond was readily affected by the surrounding farmland during the summer. During sampling, little organic matter was found in the RSK sediment, the sediment surface was black, and the culture environment had deteriorated significantly. Sea cucumbers are vulnerable to diseases and moss overgrowth during the HT stage; thus, they were not cultivated in the RSK pond during the summer.

3.2. Physicochemical Properties of Sediments from Sea Cucumber Culture Ponds. The physicochemical properties of the sediment from the sea cucumber culture ponds at the HT (WLD, ZKZ, HKA, RSK, GZL, and XHC) and EHT (WLDb, ZKZb, HKAb, RSKb, GZLb, and XHCb) stages are shown in Table 2. TC determines the productivity of aquaculture ponds. Carbon content and changes in marine sediments are closely related to the marine environment. Among the six culture ponds (Table 2), the TC content of the GZL sediment was the lowest during the HT and EHT

TABLE 1: Summary of six sea cucumber culture ponds.

Location	Culture pond	Pond size (m^2)	Water depth (m)	Whether sea cucumber cultivation occurred in the summer	Yield per m^2 (kg)	Use of sea cucumber feed	Use of pro-biotics	Time since the last desilting (year)	Monthly water change to total water (%)
Wendeng District	WLD	3.33×10^4	2	Y	0.15–0.19	Y	Y	3	120–150
	ZKZ	5.33×10^4	2	Y	0.15–0.19	Y	Y	4	80–100
	HKA	2.67×10^5	2	Y	0.11–0.15	N	N	>6	50–60
Rushan City	RSK	5.33×10^4	2	N	0.11–0.15	N	N	4	30–50
	GZL	6.67×10^4	2	Y	0.13–0.17	Y	N	2	80–100
	XHC	1.67×10^5	2	Y	0.11–0.15	N	N	5	60–80

Note. Y, yes; N, no.

TABLE 2: The physicochemical properties of the sediments in sea cucumber culture ponds at HT and EHT stages.

Location	Culture pond	TC (‰)	TC change (%)	TOC (‰)	TOC change (%)	TN (‰)	TN change (%)	NH_4^+ (mg/kg)	NH_4^+ change (%)
Wendeng District	WLD	10.987 ± 1.182^a	−22.64	6.764 ± 1.182^a	−24.48	0.741 ± 0.135^{bc}	−54.12	11.1733 ± 1.1197^{de}	−30.40
	WLDb	8.499 ± 0.591^a		5.108 ± 1.043^{bc}		0.340 ± 0.071^{cd}		7.7767 ± 1.5201^{de}	
	ZKZ	9.771 ± 0.741^a	+3.23	7.068 ± 0.834^a	−4.06	0.731 ± 0.170^{bc}	+0.55	11.44 ± 2.1439^{de}	−0.47
	ZKZb	10.087 ± 0.847^a		6.781 ± 0.887^a		0.735 ± 0.119^{bc}		11.3867 ± 1.8115^{de}	
	HKA	12.036 ± 0.440^a	−15.45	7.635 ± 0.190^a	−18.36	0.724 ± 0.093^{bc}	−28.45	12.2567 ± 0.9846^{de}	−12.54
	HKAb	10.176 ± 0.065^a		6.233 ± 0.185^a		0.518 ± 0.032^{cd}		10.72 ± 0.8225^{de}	
Rushan City	RSK	10.277 ± 1.295^a	−13.72	8.993 ± 1.055^a	−14.32	1.247 ± 0.187^a	−16.20	24.3233 ± 7.2921^a	−16.82
	RSKb	8.867 ± 1.529^a		7.705 ± 1.490^a		1.045 ± 0.194^{ab}		20.2333 ± 2.0855^{ab}	
	GZL	3.732 ± 0.441^b	+10.83	1.839 ± 0.429^c	−7.88	0.178 ± 0.075^d	−11.24	7.2 ± 1.8831^e	−17.68
	GZLb	4.136 ± 0.236^b		1.694 ± 0.308^c		0.158 ± 0.024^d		5.9267 ± 0.3598^e	
	XHC	11.482 ± 4.870^a	−15.46	10.019 ± 3.945^a	−21.64	1.193 ± 0.513^{ab}	−9.39	17.33 ± 2.1104^{bc}	−18.93
	XHCb	9.707 ± 2.534^a		7.851 ± 2.285^a		1.081 ± 0.318^{ab}		14.05 ± 3.4254^{cd}	

Location	Culture pond	NO_2^- (µg/kg)	NO_2^- change (%)	TS (‰)	TS change (%)	SO_4^{2-} (‰)	SO_4^{2-} change (%)	S^{2-} (‰)	S^{2-} change (%)
Wendeng District	WLD	157.8433 ± 6.560^{bc}	−45.55	1.127 ± 0.008^{cd}	−16.24	0.461 ± 0.100^c	+3.25	0.667 ± 0.093^{cd}	−29.84
	WLDb	85.9456 ± 7.191^f		0.944 ± 0.163^{cd}		0.476 ± 0.090^c		0.468 ± 0.124^{cd}	
	ZKZ	162.38 ± 2.153^{bc}	−62.27	1.457 ± 0.075^{cd}	+28.14	0.56 ± 0.364^c	+41.25	0.896 ± 0.337^{cd}	+20.09
	ZKZb	61.2659 ± 0.660^g		1.867 ± 0.396^{bc}		0.791 ± 0.214^{bc}		1.076 ± 0.315^{cd}	
	HKA	141.482 ± 0.385^d	−16.69	1.665 ± 0.081^{bc}	−21.86	0.503 ± 0.133^c	+1.99	1.162 ± 0.076^{cd}	−32.19
	HKAb	117.8686 ± 0.144^e		1.301 ± 0.105^{cd}		0.513 ± 0.117^c		0.788 ± 0.012^{cd}	
Rushan City	RSK	135.898 ± 0.261^d	+39.79	2.249 ± 0.664^{ab}	−27.79	0.7777 ± 0.659^{bc}	−74.65	1.472 ± 0.321^{bc}	−3.13
	RSKb	189.9733 ± 7.066^a		1.624 ± 0.199^{cd}		0.197 ± 0.024^c		1.426 ± 0.198^{bc}	
	GZL	153.179 ± 1.602^c	−41.78	0.667 ± 0.024^{cd}	−61.47	0.236 ± 0.053^c	−44.92	0.431 ± 0.030^{cd}	−70.53
	GZLb	89.1867 ± 6.200^f		0.257 ± 0.050^d		0.130 ± 0.027^c		0.127 ± 0.034^d	
	XHC	124.3093 ± 1.969^e	+34.87	3.363 ± 1.559^a	+3.39	1.613 ± 0.581^a	−13.08	1.75 ± 1.075^{ab}	+18.57
	XHCb	167.6533 ± 2.671^b		3.477 ± 0.0817^a		1.402 ± 0.0199^{ab}		2.075 ± 0.809^a	

Note. TC, total carbon; TOC, total organic carbon; TN, total nitrogen; NH_4^+, ammonia nitrogen; NO_2^-, nitrous nitrogen; TS, total sulfur; SO_4^{2-}, total sulfate; S^{2-}, sulfide; Change (%) = ($Data_{EHT}$ − $Data_{HT}$)/$Data_{HT}$ × 100%. "−", the decrease of each value at the EHT stage compared with the HT stage; "+", the increase of each value at the EHT stage compared with the HT stage. The data are the mean ± standard deviation (SD) ($n = 3$). Superscripts represent significant differences between the sediment data of six culture ponds under the same physicochemical properties ($p < 0.05$).

stages (3.732 and 4.136‰, respectively). The TC content of the HKA sediment was the highest during the two stages (12.036 and 10.176‰, respectively). The TC content of the WLD, HKA, RSK, and XHC sediments decreased at the EHT stage. Of these changes, the TC content of the WLD and RSK sediments decreased the most (22.64%) and least (13.72%), respectively. The TC content of the ZKZ and GZL sediments increased at the EHT stage.

Among the six culture ponds, the TOC content of the GZL and XHC sediments was the lowest and highest at both stages, respectively. XHC had high organic contamination and was heavily affected by farming relative to the other five ponds. The TOC content in the sediment of all six culture ponds decreased at the EHT stage, with the greatest decrease in the WLD sediment (24.48%), indicating a markedly improved sediment environment in the WLD pond. The TOC content of the ZKZ sediment decreased the least (4.06%), indicating that the self-repair capacity of the ZKZ pond was relatively weak, which may pose concerns for cultivation.

Among the six culture ponds, the TN content of the GZL sediment was the lowest at the HT and EHT stages (0.178 and 0.158‰, respectively). At the HT stage, the TN content of the RSK sediment was the highest (1.247‰), followed by the XHC sediment (1.193‰). At the EHT stage, the TN content of the XHC sediment was the highest (1.081‰), followed by the RSK sediment (1.045‰), indicating that the sediment environment of these two ponds had worsened. In terms of changes in TN, only the TN content of the ZKZ sediment increased (from 0.731 to 0.735‰) at the EHT stage. The TN content of the other five culture ponds decreased at the EHT stage, with the greatest decrease (from 0.741 to 0.340‰) observed in the WLD sediment, indicating that the self-purification capacity of this culture pond was high.

The NH_4^+ content of the GZL sediment was the lowest, while that of the RSK sediment was highest at both stages. The NH_4^+ content of all six culture ponds decreased at the EHT stage, with the greatest decrease observed in the WLD sediment (30.40%) and the smallest in the ZKZ sediment (0.47%).

In this study, the NO_2^- content in the XHC sediment was lowest during the HT stage, whereas that in the ZKZ sediment was highest. The NO_2^- content of the WLD, ZKZ, HKA, and GZL sediments decreased at the EHT stage. This decrease was greatest in the ZKZ sediment. The NO_2^- content of the RSK and XHC sediments increased at the EHT stage, with a greater increase seen in the RSK sediment.

Among the six culture ponds, the S^{2-} content of the GZL sediment was the lowest during the two stages, while that of the XHC sediment was the highest. In terms of the change of S^{2-}, the S^{2-} the content of the WLD, HKA, RSK, and GZL sediments decreased at the EHT stage, with the greatest decrease in the GZL sediment (70.53%) and the smallest in the RSK sediment (3.13%). The S^{2-} content of the XHC and ZKZ sediments increased. Under anoxic conditions, sulfate-reducing bacteria convert SO_4^{2-} into reduced S^{2-} or S using sulfur-reducing enzymes [26]. In this study, the TS content of the XHC sediment was highest during the HT and EHT

stages, while that of the GZL sediment was the lowest. The TS content in the WLD, HKA, RSK, and GZL sediments decreased at the EHT stage. This decrease was greatest in the GZL sediment (61.47%). In contrast, the TS content in the ZKZ and XHC sediments increased at the EHT stage. The SO_4^{2-} content at both stages was highest for the XHC sediment and lowest for the GZL sediment. The SO_4^{2-} content of the ZKZ, WLD, and HKA sediments increased at the EHT stage, with the increase being greatest in the ZKZ sediment (41.25%). In contrast, the SO_4^{2-} of the RSK, GZL, and XHC sediments decreased at the EHT stage, and this decrease was most pronounced in the RSK sediment (74.65%).

From the HT to EHT stage, all physicochemical contents of the sediment samples from the WLD ponds showed a downward trend, with the exception of SO_4^{2-}. The TN, TC, TOC, and NH_4^+ contents decreased the most across all six ponds. The WLD ponds displayed a pronounced self-recovery capacity, with the sediment environment improving to a certain extent. All physicochemical contents of the ZKZ sediment samples decreased, except for TOC, NH_4^+, and NO_2^-. The physicochemical contents of the HKA sediment showed a downward trend, except for SO_4^{2-}. The physicochemical contents of the RSK sediment were the highest of all six ponds, indicating that this sediment environment was inferior and posed significant risks to successful cultivation. All physicochemical contents in the GZL sediment samples showed a downward trend, except for TC. The TN, TC, TOC, NH_4^+, TS, SO_4^{2-}, and S^{2-} contents were the lowest in the GZL samples, indicating that the sediment environment of GZL was the best for successful cultivation among all six ponds. The XHC sediment displayed high levels of all physicochemical compounds except NO_2^-, indicating a poor sediment environment.

3.3. Bacterial Diversity of Sediments from Sea Cucumber Culture Ponds.

The coverage of the 12 sediment samples from the six culture ponds at the HT (WLD, ZKZ, HKA, RSK, GZL, and XHC) and EHT (WLDb, ZKZb, HKAb, RSKb, GZLb, and XHCb) stages exceeded 95% (Figure 2(a)). This indicates that the sequencing depth was sufficient to capture the microbial diversity of all samples. The Chao index was used to assess the richness of alpha diversity in the sediments, and it found that diversity was higher at the HT stage than at the EHT stage, indicating the presence of more species at the HT stage (Figure 2(b)). The Shannon and Simpson indices were used to measure the diversity of the bacterial community, and the indices differed significantly between the two stages (Figures 2(c) and 2(d)). The Shannon index values were generally higher at the HT stage than at the EHT stage, indicating greater bacterial community diversity at the HT stage. The Simpson index values were generally lower at the HT stage than at the EHT stage, also indicating greater diversity of the bacterial communities in the sediments at the HT stage.

Beta diversity analysis was used to assess the similarities and differences in bacterial communities among the sediment samples from the six culture ponds at the HT and EHT stages (Figure 3). PCoA finding of the close distance
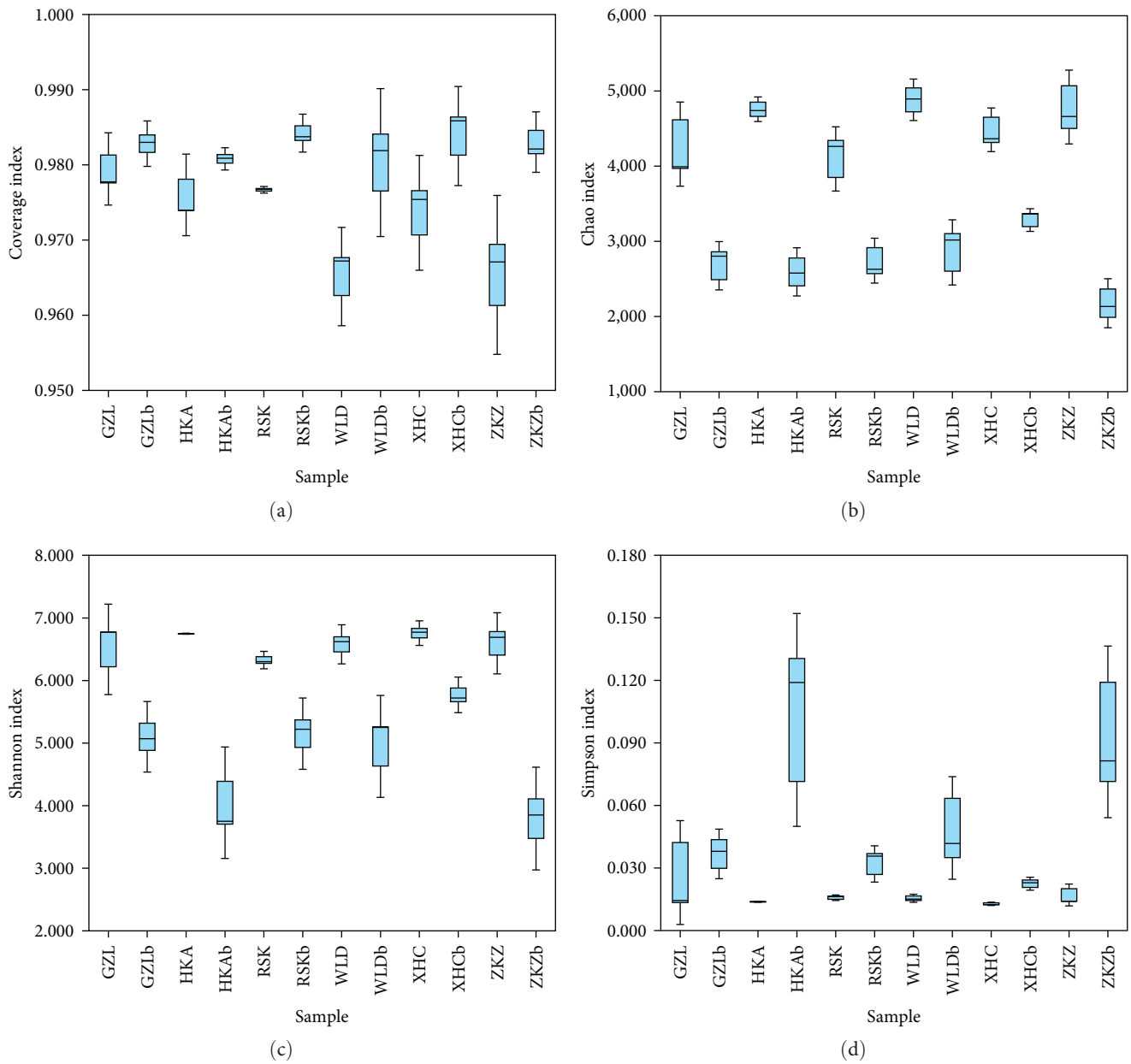
FIGURE 2: Bacterial alpha diversity indices in the sediment from six sea cucumber culture ponds: (a) Coverage index; (b) Chao index; (c) Shannon index; (d) Simpson index. WLD, ZKZ, HKA, RSK, GZL, and XHC at the HT stage and WLDb, ZKZb, HKAb, RSKb, GZLb, and XHCb at the EHT stage.

between the sample points in Figure 3 indicated the similarity of the sample bacterial community compositions. The bacterial community structures of each sediment sample differed at the HT and EHT stages. At the HT stage, the six ponds were divided into two groups based on the similarity of the bacterial community structures; one group included the WLD, ZKZ, HKA, and GZL culture ponds, while the other included the RSK and XHC culture ponds. At the EHT stage, there were differences in the bacterial community structures in the sediments of the six ponds.

The Venn diagram of the bacterial OTU distribution in the sediment of the culture ponds is shown in Figure 4. The 12 samples shared 466 OTUs. The unique OTUs in the six culture ponds during the two stages were quite different. Higher OTU specificity was observed in GZL (549 OTUs at the HT stage), followed by XHC (330 at the HT stage). In contrast, ZKZb and HKAb had OTU specificities of 39 and 47 at the EHT stage, respectively. In addition, the number of unique OTUs in the WLD sediment at the HT stage was less than that at the EHT stage, whereas the number of unique OTUs in the sediment of the other five culture ponds was higher at the HT stage than at the EHT stage.

The bacterial community structure in the sediment samples differed between the ponds and stages (Figure 5). The bacterial composition of the six culture pond samples at the HT stage differed slightly at the phylum level. Among the
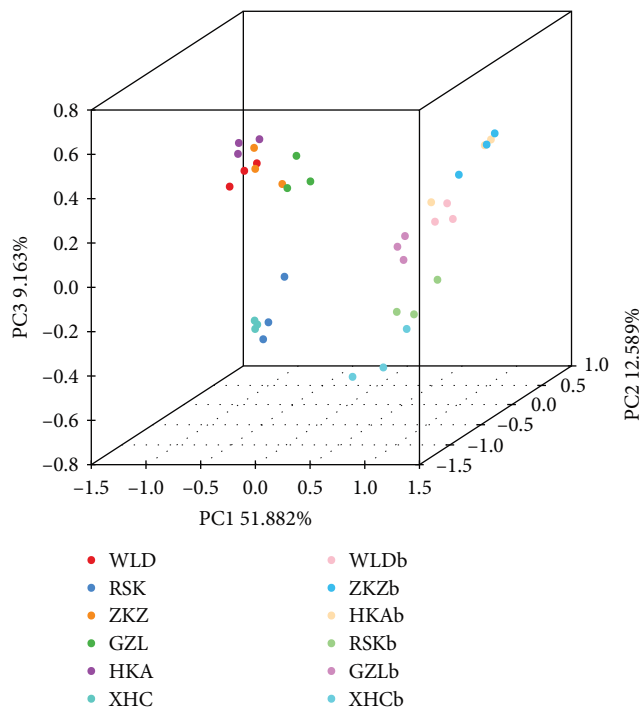
FIGURE 3: PCoA analysis of bacteria in the sediment of *A. japonicus* culture ponds. Different colors represent different groups. The closer samples mean the more similar bacterial composition of the sediment samples. Percent variation expected were PC1 51.882%, PC2 12.589%, and PC3 9.163%.
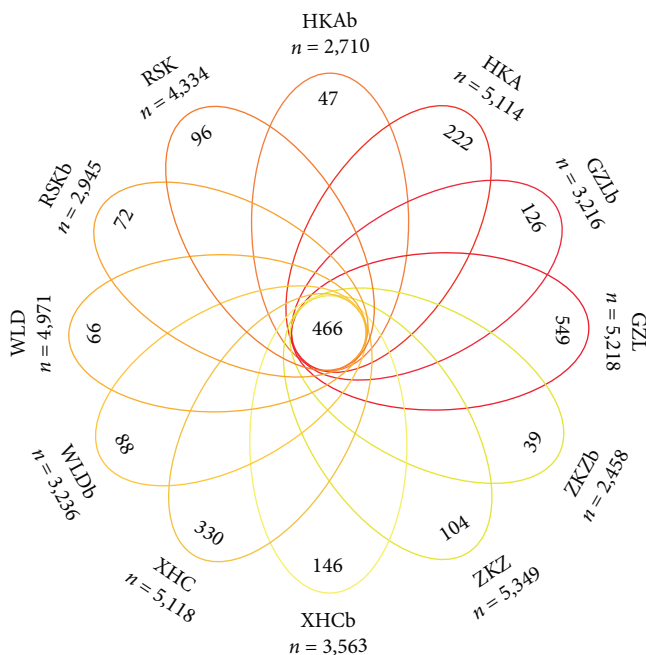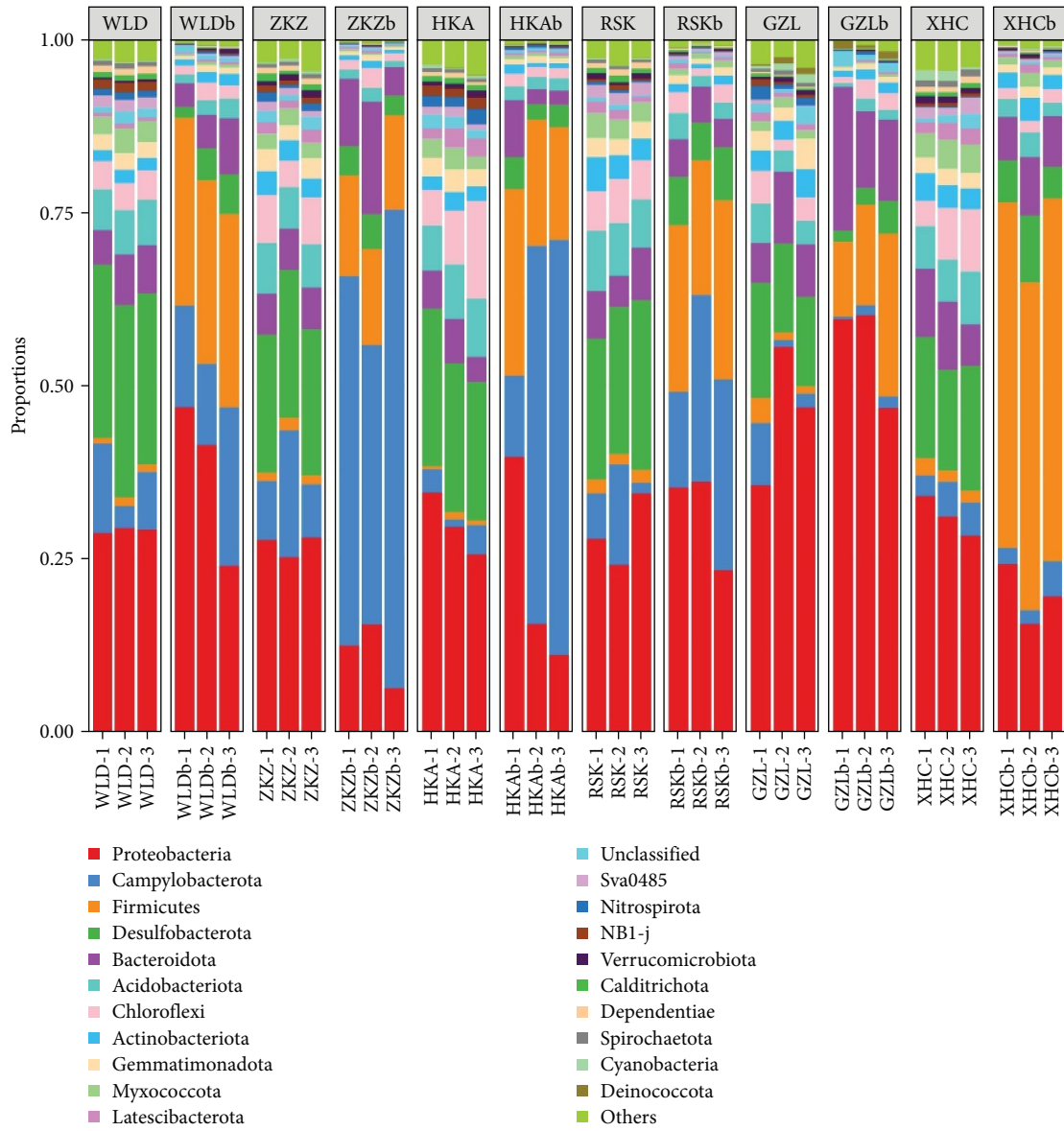

FIGURE 4: Venn diagram of OTU distribution of bacteria in the sediment of *A. japonicus* culture ponds. The diagram shows unique and shared OTU numbers in the 12 samples. *n* is the total number of OTUs for different samples. Different colors represent different samples, and the marked numbers in the overlapping areas of the circles with different colors indicate that different samples have sequences that are classified into the same OTU, and the number of shared OTUs is the marked number.

prokaryotic phyla detected in the sediments of the six culture ponds, Desulfobacterota, Campylobacterota, Bacteroidetes, and Proteobacteria were predominant during the HT and EHT stages. Proteobacteria was the most prominent among all phyla (45.97%, 29.10%, 28.80%, 26.99%, 29.90%, and 31.14% for GZL, WLD, RSK, ZKZ, HKA, and XHC, respectively). This was followed by the phyla Desulfobacterota (25.77%, 21.99%, 20.76%, 14.11%, 21.38%, and 16.65% abundance for the WLD, RSK, ZKZ, GZL, HKA, and XHC sediments, respectively). In addition, the phyla Campylobacterota, Bacteroidetes, Acidobacteria, Chloroflexi, Myxococcus, Gemmatimonadota, and Actinobacteria were identified. Proteobacteria was increased from the HT to EHT stages in the WLD, RSK, and GZL sediments and decreased in the ZKZ, HKA, and XHC sediments. Campylobacterota increased in the WLD, ZKZ, HKA, and RSK sediments. This increase was greatest in the ZKZ sediment (from 11.45% to 54.10%), with less marked change observed in the GZL and XHC sediments. Firmicutes increased significantly in all the samples, with the greatest change observed in the XHC sediment. In the WLD, RSK, ZKZ, GZL, HKA, and XHC sediments, the proportion of Firmicutes increased from 1.04% to 27.13%, 1.79% to 23.07%, 1.44% to 14.00%, 1.92% to 16.24%, 0.72% to 20.44%, and 1.96% to 49.77%, respectively. Finally, the phylum Desulfobacterota decreased significantly in the sediments from all six ponds: from 25.77% to 3.97% in WLD, 21.99% to 6.68% in RSK, 20.76% to 4.03% in ZKZ, 14.11% to 2.92% in GZL, 21.38% to 3.36% in HKA, and 16.65% to 6.75% in XHC.

The bacterial diversity compositions of all the samples were similar at the HT stage at the genus level. The predominant genera were Sva1033_norank, *Woeseia*, an unknown genus in Desulfobulbaceae, an unknown genus in Syntrophobacterales, Subgroup 23, *Halioglobus*, an unknown genus in the class of γ-Proteobacteria, and others. From the HT to the EHT stage, the abundance of *Sulfurovum* in the WLD, ZKZ, HKA, and RSK sediment samples increased significantly, with increases of 43.07% and 39.35% in the ZKZ and HKA sediment samples, respectively. *Sulfurovum* decreased in the GZL and XHC sediment samples. The abundance of *Bacillus* significantly increased in the sediment samples from all six culture ponds, with the greatest change seen in the WLD sediment (17.69% increase). *Woeseia* decreased significantly in the sediments of all six culture ponds, with the most prominent decrease seen in the HKA sediment (10.02% decrease).

3.4. Correlation Analysis of Bacterial Diversity and Physicochemical Properties. The results of the correlation analysis between bacterial diversity and physicochemical properties are shown in Figure 6. The distance between the two points is evidence of the similarity in community composition between the two sediment samples. Thirty-six sample points were distributed across four quadrants, indicating that different physicochemical properties affected the structure of the sediment community in each culture pond. The bacterial community compositions of RSK, RSKb, XHC, and XHCb were similar. Arrows from the origin in Figure 6 represent different physicochemical properties, and the length

(a)

FIGURE 5: Continued.

Legend:

- *Sulfurovum*
- *Bacillus*
- *Woeseia*
- *Halomonas*
- *Marinobacter*
- Sva1033_norank
- Desulfobulbaceae_unculture
- Gammaproteobacteria incertae sedis_uncultured
- Syntrophobacterales_norank
- Subgroup 23
- *Halioglobus*
- Sva0081 sediment group
- Anaerolineaceae_uncultured
- *Pseudomonas*
- *Clostridiisalibacter*
- *Guyparkeria*
- *Salinimicrobium*
- Actinomarinales_norank
- B2M28_norank
- *Psychrobacter*

- Desulfocapsaceae_uncultured
- Unclassified
- BD2-11 terrestrial group_norank
- Sva0485_norank
- Sandaracinaceae_uncultured
- PAUC43f marine benthic group_norank
- Anaerolineae_norank
- Subgroup 10
- Latescibacterota_norank
- Subgroup 22_norank
- SBR1031_norank
- *Anaerobacillus*
- *Actibacter*
- Bacteroidetes BD2-2_norank
- Cyclobacteriaceae_uncultured
- *Brassicibacter*
- *Salegentibacter*
- SEEP-SRB1
- Thermodesulfovibrionia_norank
- NB1-j_norank

- Gammaproteobacteria_norank
- OM60 (NOR5) clade
- *Tepidibacillus*
- Thioalkalispira–Sulfurivermis
- Izemoplasmatales_norank
- *Ilumatobacter*
- *Haloplasma*
- *Microbulbifer*
- Subgroup 21_norank
- *Lysinibacillus*
- *Lutimonas*
- *Leisingera*
- Desulfosarcinaceae_uncultured
- 1013-28-CG33_norank
- *Tepidibacter*
- *Erythrobacter*
- Clostridiaceae_uncultured
- Thiotrichaceae_uncultured
- *Thiogranum*
- Aminicenantales_norank

- *Paraclostridium*
- Rhodobacteraceae_unclassified
- *Alkaliphilus*
- Flavobacteriaceae_unclassified
- SZB85
- *Vibrionimonas*
- Paracoccus
- Chloroplast_norank
- Kiloniellaceae_uncultured
- *Candidatus Thiobios*
- *Fusibacter*
- Rhodobacteraceae_uncultured
- *Desulfosarcina*
- *Psychromonas*
- Caldilineaceae_uncultured
- Balneolaceae_uncultured
- *Paenisporosarcina*
- *Oceanirhabdus*
- *Ralstonia*
- *Thiohalophilus*

- *Geothermobacter*
- *Sulfitobacter*
- *Truepera*
- *Methylophaga*
- *Pseudoalteromonas*
- *Crassaminicella*
- *Limimaricola*
- *Fictibacillus*
- Desulfuromonadales_norank
- *Thiomicrorhabdus*
- *Vibrio*
- Clostridium sensu stricto 1
- *Limibacillus*
- *Nautella*
- *Gramella*
- *Ferrimonas*
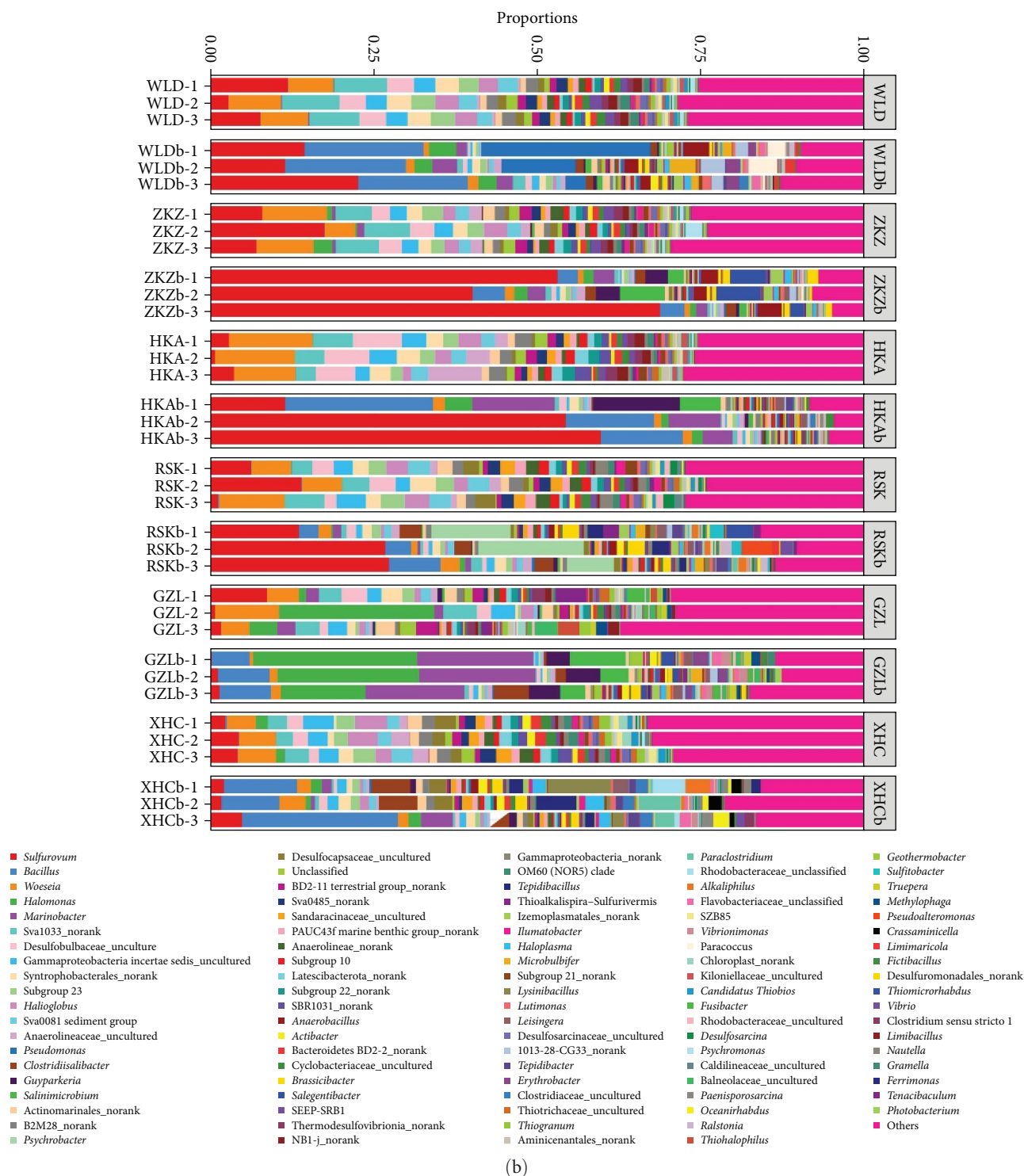- *Tenacibaculum*
- *Photobacterium*
- Others

(b)

FIGURE 5: The composition of the bacterial community in the sediment of *A. japonicus* culture ponds: (a) the composition of the bacterial community at the phylum level; (b) the composition of the bacterial community at the genus level. Stacked bar plots showing the average relative abundance of each taxon at the phylum and genus level. Different colored bars represent different phylum and genus levels in the sediment of sea cucumber culture ponds.

of the arrow represents the influence of that property on the bacterial community structure. The intensity of the influence of each physicochemical property on the bacterial community structure was as follows (from largest to smallest): TN, TOC, TC, NH_4^+, TS, S^{2-}, SO_4^{2-}, and NO_2^-. The vertical

distance from the sample point to the physicochemical properties' arrow and its extension line indicates the influence of the physicochemical properties on the sediment samples. The closer the distance between the sample point and the arrow, the stronger the influence of the physicochemical
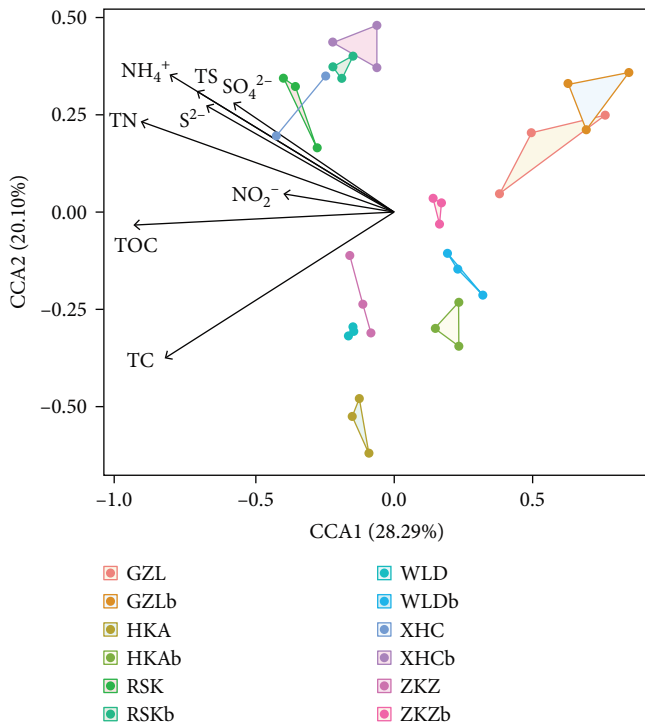
FIGURE 6: Correlation analysis of bacterial diversity and physicochemical properties in the sediment of sea cucumber culture ponds.

property on the sediment sample. All the environmental factors had a significant impact on the ZKZb sediment.

The results of the correlation analysis between bacterial abundance and the physicochemical properties of various phyla are shown in Figure 7. Proteobacteria was highly negatively correlated with TS and SO_4^{2-} (both $p < 0.001$) and with TOC, TN, TC, and S^{2-} (all $p < 0.01$). Desulfobacterota was negatively correlated with NO_2^-. Acidobacteria was positively correlated with TC, TN, and TOC (all $p < 0.01$), while Chloroflexi was positively correlated with TOC ($p < 0.01$). Myxococcota was positively correlated with NH_4^+, TC, TN, TOC, and NO_2^- (all $p < 0.01$). Gemmatimonadota was positively correlated with NO_2^- ($p < 0.01$), whereas Latescibacterota was positively correlated with TC and TOC (both $p < 0.01$). Deinococcota was negatively correlated with NH_4^+ and TN (both $p < 0.01$).

4. Discussion

4.1. Factors Influencing Physicochemical Properties in the Sediment of Sea Cucumber Culture Ponds. Physicochemical properties can significantly impact the success of aquacultural ecosystems. TOC is the total amount of organic carbon content. Organic carbon in sediment is a substrate for many microorganisms, and the TOC can provide a quick and accurate assessment of the degree of organic contamination [27, 28]. A high TOC indicates increased pollution, and a decreasing trend in TOC indicates strong self-purification capacity [29]. TN represents the total amount of various forms of inorganic and organic nitrogen, including organic nitrogen, ammonia, nitrate, and nitrite [30]. The TC/TN

ratio in the sediment can be used to evaluate the extent of pollution and self-purification of the sediment [29]. TOC and TN accumulation can reflect the functionality of the sediment environment, and low TOC and TN contents are indicative of low organic matter and high sediment stability [31]. High nitrogen and carbon concentrations in the sediment indicate that the biodegradation of carbonaceous and nitrogenous substances is slow, with low nitrification and denitrification rates in the seabed [32].

In the present study, the TOC content of the sediments in the six culture ponds was higher in the HT stage than in the EHT stage. Sediments from five of the culture ponds (WLD, HKA, RSK, GZL, and XHC) had higher TN contents at the HT stage than at the EHT stage. During the HT stage, microorganism reproduction and metabolism in the sediments were enhanced, and the degradation of organic matter was accelerated. When organic matter decomposition by microorganisms exceeds the natural rate of supplementation, the TOC and TN contents decreased (i.e., at the EHT stage), which could improve the sediment environment of the culture pond. Among the six culture ponds, only the ZKZ sediment displayed increased TN content at the EHT stage. This may be due to low bacterial decomposition efficiency, less degradable material in the sediment, and poor sediment recovery capacity. The TOC and TN contents in the WLD sediment decreased the most at the EHT stage, indicating strong self-purification capacity. The WLD pond follows relatively good management procedures, with a high monthly water change to total water (120%–150%). The WLD pond was last desilted 3 years ago. Increasing the frequency of water changes in the summer can reduce the contents of organic carbon, organic nitrogen, TN, NO_3^-, NH_4^+, total phosphorus, and phosphate in the sediment [33]. The physicochemical properties of the RSK sediment were high relative to the other culture ponds, indicating a poor sediment environment. The TN (1.247 and 1.045‰), NO_2^- (135.898 and 189.973 μg/kg), and NH_4^+ (24.323 and 20.233 mg/kg) contents of this sediment were the highest at both the HT and EHT stages. The TOC (8.993 and 7.705‰), TS (2.249 and 1.624‰), SO_4^{2+} (0.777 and 0.197‰), and S^{2-} (1.472 and 1.426‰) contents were also relatively high at both stages. This may be because sea cucumbers are not cultured in the RSK pond during the summer. Sea cucumber cultivation can effectively stop nutrient accumulation at the bottom of the culture ponds [14]. The organic matter accumulation rate and the POC, PON, TP, Chl *a*, and pheophytin contents in the sediment were even lower than those found in ponds without sea cucumbers ($p < 0.05$) [14].

Nitrite is a toxic intermediate produced during ammonia nitrification and is also a product of bacterial denitrification of nitrate during nitrogen cycling [34]. Nitrite is the most common toxic nitrogenous compound in aquaculture systems, and it accumulates during intensive aquaculture [35]. NO_2^- affects the normal growth and survival of aquatic animals and is one of the main environmental stress factors affecting their health [36]. The NO_2^- content in the ZKZ sediment at the HT stage was 162.38 μg/kg. This may have reflected increased bacterial denitrification activity in the
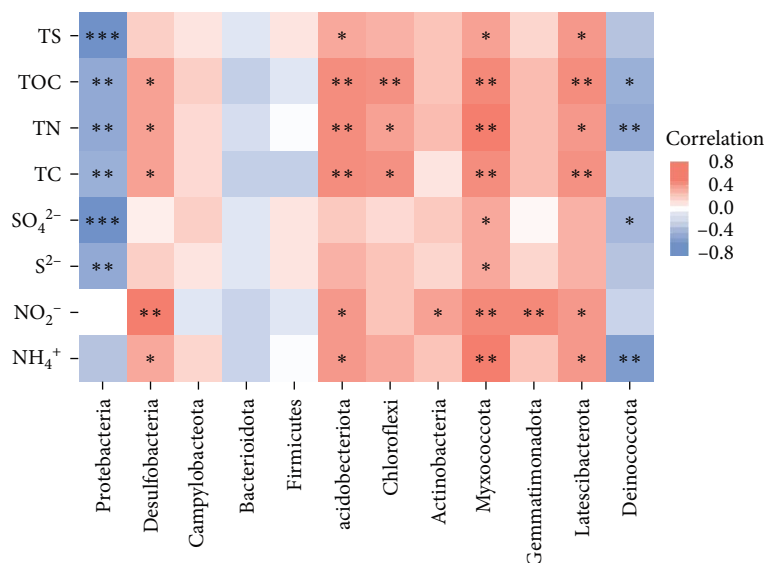
FIGURE 7: Correlation between bacterial abundance and physicochemical properties at the phylum level in the sediment of the culture ponds. *indicates the p-value of the correlation, ***indicates $p < 0.001$, **indicates $p < 0.01$, and *indicates $p < 0.05$.

ZKZ sediment at the HT stage, which converted organic matter to NO_2^-. The higher NO_2^- content in the RSK sediment at the EHT stage may also be due to this phenomenon. Most denitrifying bacteria (DB) are facultative anaerobic heterotrophs, and organic carbon can act as an electron donor to reduce nitrate-nitrogen to NO_2^- and finally to nitrogen [37]. When there is insufficient dissolved oxygen in the culture environment, the growth and reproduction of aerobic nitrifying bacteria are inhibited. Inhibited nitrification leads to the accumulation of ammonia nitrogen and nitrite produced through denitrification. Appropriate dissolved oxygen supplementation may decrease the high NO_2^- content in the RSK culture pond sediment.

Ammonia nitrogen is an important environmental indicator in aquaculture, and NH_4^+ toxicity directly affects the survival of aquatic animals [38]. One of the most important challenges in aquaculture is to keep ammonia levels as low as possible [39] to maintain normal sea cucumber growth. At the EHT stage, NH_4^+ levels were reduced in all six culture ponds, possibly due to the increased abundance of nitrifying and DB, which eventually oxidized ammonia to nitrate and N_2 [40].

The inorganic sulfur in the culture environment mainly consisted of sulfide (S^{2-}), elemental sulfur (S), and sulfate (SO_4^{2-}) [41]. Sulfide is a significant pollutant in aquaculture. Long-term sulfide exposure can cause detrimental changes in metabolism and protein synthesis, thereby affecting the health of aquatic organisms and reducing their survival rates [42]. In sediments, sulfates, sulfites, and inorganic sulfur may be reduced to compounds such as H_2S [43]. HTs in the summer inhibit the growth of sulfur-metabolizing bacteria [9]. Subsequently, excess H_2S in sea cucumber culture pond sediments cannot be effectively removed by sulfur-oxidizing bacteria, increasing the risk of cultivation failure and leading to an increase in pathogenic bacteria and disease. The RSK

culture pond undergoes potassium bisulfate disinfection in the summer, which may be related to the high TS and SO_4^{2-} levels found in its sediments at the HT stage. The decrease in S^{2-} in the RSK sediment during the EHT stage may have been due to water exchange, which decontaminated the sulfur in the sediment. During the HT stage, the S^{2-} content in the ZKZ and XHC sediments increased, likely due to the increased number of sulfate-reducing bacteria and increased bacterial metabolism and reproduction, which led to an increase in the content of sulfides, such as H_2S [13].

4.2. Bacterial Community Structure in the Sediments of Sea Cucumber Culture Ponds. Among the 74 prokaryotic phyla detected in the sediments of the six culture ponds, four phyla (Acidobacteria, Chloroflexi, Euryarchaeota, and Proteobacteria) were predominant at both stages. Proteobacteria was the most predominant phylum in all six ponds at the HT stage and in the WLD, RSK, and GZL ponds at the EHT stage. Bacterial communities in the surface water and sediments of *A. japonicus* culture ponds differ throughout the year [9]. Although the relative abundance of Proteobacteria from May to October was reportedly not as high as that from November to April, it remained the most abundant phyla in all samples. Proteobacteria are related to organic matter degradation [44], which supports the negative correlation between Proteobacteria, TC, and TOC (Figure 7). Proteobacteria are a major contributor to organics, nitrogen, and phosphorus removal [45]. At the HT and EHT stages, the GZL sediment had the highest abundance of Proteobacteria. This sediment also had the lowest TN, TC, and TOC contents and the best sediment environment among all six culture ponds. The increased abundance of Proteobacteria in the sediment during the EHT stage was conducive to organic matter degradation. However, some Proteobacteria may pose risks to the sediment environment. For example, we observed that the

abundance of Proteobacteria and *Halomonas* in the GZL sediment increased during the EHT stage. The optimal growth temperature of *Marinobacter* is 30°C, and lower temperatures can reduce NO_3^- to NO_2^- [46]. *Halomonas* can survive at 30°C and reduce NO_3^- [47]. At the EHT stage, the high abundances of *Marinobacter* and *Halomonas* in the GZL sediment were likely to affect the NO_2^- content, which can pose risks to animal health. *Woeseia* was significantly higher in samples from the HT stage than in samples from the EHT stage. *Woeseia* can perform various ecological functions, such as dissimilatory sulfur oxidation and denitrification [48].

The phylum Campylobacterota includes a group of Gram-negative bacteria, including *Sulfurospirillum* spp., *Sulfurovum* spp., and *Nitratiruptor* spp. [49]. In contrast to those observed in the HT stage, changes in abundance of this phylum in the EHT stage were mainly attributed to changes in the genus *Sulfurovum*. Members of this genus use sulfur, sulfide, and thiosulfate as electron donors and oxygen as an electron acceptor to oxidize thiosulfate to sulfate [50]. In addition, *Sulfurovum* can grow at 15–37°C, with an optimum growth temperature of 33°C [51]. Thus, they could multiply rapidly in the HT stage, and their abundance increased greatly at the EHT stage, especially in the ZKZ and HKA ponds.

In this study, the phylum Firmicutes was significantly more abundant in the EHT stage than in the HT stage. This likely reflected a significant increase of bacteria in the genus *Bacillus*. These bacteria are beneficial in removing waste, maintaining water quality, reducing stress in aquaculture environments, improving the immune physiological balance of target aquatic animals, promoting their growth, and improving their survival rates [52]. The increased prevalence of *Bacillus* in the six culture ponds at the EHT stage may be closely related to the ecological maintenance of the sediments in the culture ponds, enhanced immunity, and improved survival rate of the cultured sea cucumbers.

Nitrifying and DB have obvious effects on the purification of both the pond water and sediment [53]. Nitrification oxidizes ammonia nitrogen to nitrite nitrogen via ammonia-oxidizing bacteria (AOB) and eventually converts the latter into nitrate nitrogen via nitrite-oxidizing bacteria (NOB). However, nitrification does not directly affect the N stock in the system [54]. Denitrification is a gradual reduction of nitrate nitrogen into nitrite nitrogen via DB and eventually into gaseous nitrogen, thus reducing the N stock in the system [37]. *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, and *Nitrosolobus* are all known to be common AOB [55]. NOB such as *Nitrococcus*, *Nitrobacter*, and *Nitrospira* are needed for efficient utilization of nitrogenous compounds [55]. DB includes several facultative or strict anaerobes, and most belong to Proteobacteria, including the genera *Paracoccus* (Alpha-proteobacteria), *Ralstonia* (Beta-proteobacteria), *Desulfosarcina* (Delta-proteobacteria), and *Pseudomonas* (Gamma-proteobacteria). These genera are all capable of reducing nitrates and nitrites [40]. In contrast to autotrophic nitrifying bacteria and anoxic DB, a special class of microorganisms called aerobic DB (i.e., *Acinetobacter*, *Bacillus*, *Cuprobacter*, *Halomonas*, *Pseudomonas*, and *Photobacterium*) can simultaneously accomplish nitrification and denitrification under aerobic conditions [56, 57]. In our study, bacteria belonging to the genera *Bacillus*, *Desulfosarcina*, *Erythrobacter*, *Ferrimonas*, *Halomonas*, *Limibacillus*, *Marinobacter*, *Microbulbifer*, *Paracoccus*, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, and *Ralstonia* may be associated to anoxic denitrification and aerobic denitrification [56].

4.3. Correlations between Bacterial Diversity and Physicochemical Properties in the Sediment of Culture Ponds. A correlation between structural changes in the bacterial composition and the environmental factors of the sediment of culture ponds has been described previously [13]. Nitrate-nitrogen (N), nitrite-N, ammonium-N, and phosphate-phosphorous (P) are key factors influencing bacterial communities in pond environments [58, 59]. Sulfide has the greatest influence on the microbial community composition of pond sediments in the winter [13]. In this study, the bacterial community structures varied between the six culture ponds as well as between the HT and EHT stages; these changes may be caused by various environmental factors. The CCA results indicated that among the studied physicochemical properties, the TC, TOC, TN, and NH_4^+ had the greatest impact on sediment bacteria. The TS, S^{2-}, SO_4^{2-}, and NO_2^- also contributed to the community composition of sediment bacteria. The correlation between phosphorus and bacterial community composition in *A. japonicus* culture pond sediment needs to be explored. Our observations on the correlations between bacterial community structures and culture pond physicochemical properties may provide a reference for future efforts to improve and regulate aquaculture management.

4.4. Measures to Improve the Management of Sea Cucumber Culture Ponds. In this study, the physicochemical properties of the sediment of six culture ponds were evaluated in the HT and EHT stages. Organic matter in the sediment was decomposed by bacteria throughout the study stage, and most of the physicochemical properties showed a downward trend. Thus, when sea cucumber estivation ends after the HT stage, interventions are needed in ponds with poor/deteriorated culture environments to suitably adjust the pond ecology. Our study findings have helped us develop several management strategy suggestions for the six aquaculture ponds, as described below.

Among the six culture ponds, the physicochemical contents of the WLD sediment were lower, with significant reductions in these contents at the EHT stage, indicating high self-purification capacity. This is likely closely related to the small pond size (3.33×10^4 m^2) and relatively rigorous aquaculture management, such as a high monthly water change to total water (120%–150%) and a relatively short time between desilting events. These findings indicate that superior culture management measures in the early stages of culturing may have positive effects. Because of these practices, this pond will not require any intervention after the HT stage ends.

The physicochemical contents of the ZKZ sediment were relatively intermediate among the six culture ponds, but the increase in sulfate at the EHT stage needs to be considered. The genus *Sulfurovum*, with an increase in abundance from

11.03% to 54.10% (from the HT to EHT stage), resulted in a 41.25% increase in sulfate levels. Probiotics should be added to the culture pond at the EHT stage to improve water quality, and the pond should also be properly oxygenated when temperatures are high to reduce sulfate levels. In addition, there is a long period (4 years) between desilting events; harmful substances can accumulate to a greater extent. Therefore, the desilting interval should be reduced.

The physicochemical contents of the HKA sediment were moderate among the six culture ponds, and management measures before the HT stage did not cause sediment deterioration at the EHT stage. The abundance of the genus *Sulfurovum* increased from 2.63% at the HT stage to 41.98% at the EHT stage. This led to a decrease in total sulfur and sulfide (by 21.86% and 32.19%, respectively) and an increase in sulfate (by 1.99%) at the EHT stage. Probiotics should be added to the culture pond at the EHT stage to improve water quality, and the pond should also be properly oxygenated when temperatures are high to reduce sulfate levels. This was the largest culture pond ($2.67 \times 10^5 \, \text{m}^2$), but it had the lowest output (0.11–$0.15 \, \text{kg/m}^2$). Therefore, sea cucumber feed may be used to increase this pond's yield in the future.

The physicochemical contents of the RSK sediment were high relative to the other six ponds, and moss growth was exacerbated due to the high summer temperatures. Aquaculture ponds should be thoroughly desilted to purify the cultural environment. Culture ponds adjacent to farmland may be affected by farmland drainage. The RSK culture pond is located in an estuary with poor water exchange. Thus, more rigorous monitoring of the water quality and sediment environment of this pond is required.

The physicochemical contents (except NO_2^-) of the GZL sediment were the lowest among the six culture ponds, indicating that the sediment of this pond was in good condition. This is likely related to the small pond size and relatively rigorous aquaculture management, such as the monthly water change to total water (80%–100%) and the desilting event interval (2 years since the last desilting). However, *Seabacter* and *Halomonas*, which can generate NO_2^- at the EHT stage, both increased in abundance, which poses a threat to culturing success. The water exchange frequency and amount of water exchange should be appropriately increased to improve the NO_2^- content. Probiotics such as *Bacillus* can be added to the pond to adjust the bacterial community structure of the sediment, which may improve the survival of sea cucumbers at the EHT stage.

The physicochemical contents of the XHC sediment were relatively high among the six culture ponds, indicating a relatively poor sediment environment. The daily management practices of this pond were fairly simple, including no feed or probiotic use. The monthly water change to total water (60%–80%) was low, and the last desilting was performed 5 years ago; these factors likely all contributed to the very low yield per m^2. The sediment environment needs to be improved at the EHT stage, and probiotic use is strongly recommended. The use of feed and desilting at the end of the breeding cycle is recommended to increase production.

Our study findings support the following seven suggestions for the daily management of sea cucumber aquaculture ponds. First, sediment quality should be monitored regularly, and desilting intervals should be minimized. Second, water quality should be controlled and monitored, and excessive organic matter accumulation at the bottom of the pond must be avoided. Third, timely discharge of water, sediment organic matter, and harmful substances is required. Fourth, culturing density should be controlled to maximize the conversion of organic matter. Fifth, feeding should be performed conservatively to avoid an accumulation of excess bait at the bottom of the culture pond. Sixth, the addition of an immunity enhancer can prevent environmental stress in sea cucumbers. Finally, the use of a mixed breeding model can help transform organic matter and establish a diverse breeding system.

The study findings also support the following suggestions for sea cucumber culture ponds to cope with the HT stage. Forecast and trend analyses of the HT stage should be strengthened. An early warning mechanism should be established. Daily management measures in sea cucumber culture areas should be strengthened. Finally, new sea cucumber varieties with high tolerance should be screened.

5. Conclusion

In this study, informed management measures for aquaculture (desilting and monthly water changes) have a positive effect on the physicochemical properties and bacterial community structure of the sediments. Most of the physicochemical contents and bacterial community diversity in the sediment showed a decreasing trend from the HT to EHT stage, indicating that the self-purification capacity of the sediment at the HT stage was greater than natural organic matter accumulation. The physicochemical contents (TN, TC, TOC, and NH_4^+) of the culture pond sediments significantly influenced the bacterial community composition and diversity. In addition, daily management methods and improvement measures for each sea cucumber pond culture were suggested. These findings will provide a reference for effective *A. japonicus* aquaculture at high summer temperatures.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] X. S. Ru, L. B. Zhang, X. N. Li, S. L. Liu, and H. S. Yang, "Development strategies for the sea cucumber industry in China," *Journal of Oceanology and Limnology*, vol. 37, no. 1, pp. 300–312, 2019.

[2] R. Pangestuti and Z. Arifin, "Medicinal and health benefit effects of functional sea cucumbers," *Journal of Traditional and Complementary Medicine*, vol. 8, no. 3, pp. 341–351, 2018.

[3] Bureau of Fisheries, "Ministry of Agriculture and Rural Affairs of the People's Republic of China, National Fisheries Technology Extension Center, China Society of Fisheries," in *China Fisheries Statistics Yearbook of 2022 (in Chinese)*, China Agricultural Press, Beijing, 2022.

[4] H. S. Yang, X. T. Yuan, Y. Zhou, Y. Z. Mao, T. Zhang, and Y. Liu, "Effects of body size and water temperature on food consumption and growth in the sea cucumber *Apostichopus japonicus* (Selenka) with special reference to aestivation," *Aquaculture Research*, vol. 36, no. 11, pp. 1085–1092, 2005.

[5] Z. Zheng, H. Sun, J. Wang, and Z. Zhou, "Expression analysis of genes involved in TLR signalling pathway under temperature stress in sea cucumber *Apostichopus japonicus*," *Aquaculture Research*, vol. 50, no. 9, pp. 2724–2728, 2019.

[6] Z. Yu, Y. Zhou, H. Yang, and C. Hu, "Bottom culture of the sea cucumber *Apostichopus japonicus* Selenka (Echinodermata: Holothuroidea) in a fish farm, southern China," *Aquaculture Research*, vol. 45, no. 9, pp. 1434–1441, 2014.

[7] S. Xia, H. Yang, Y. Li, S. Liu, Y. Zhou, and L. Zhang, "Effects of different seaweed diets on growth, digestibility, and ammonia-nitrogen production of the sea cucumber *Apostichopus japonicus* (Selenka)," *Aquaculture*, vol. 338, pp. 304–308, 2012.

[8] X. Wang, W. Tao, and H. Lv, "Effects of sulfide stress on acute toxicity and antioxidant defense system of *Apostichopus japonicas*," *Journal of Yantai University (Natural Science and Engineering Edition) (in Chinese)*, vol. 33, no. 3, p. 6, 2020.

[9] Z. Zhao, J. Jiang, Y. Pan et al., "Temporal dynamics of bacterial communities in the water and sediments of sea cucumber (*Apostichopus japonicus*) culture ponds," *Aquaculture*, vol. 528, Article ID 735498, 2020.

[10] F. Sun, C. Wang, L. Chen, G. Weng, and Z. Zheng, "The intestinal bacterial community of healthy and diseased animals and its association with the aquaculture environment," *Applied Microbiology and Biotechnology*, vol. 104, no. 2, pp. 775–783, 2020.

[11] Y. Zhou, J. Zhang, L. Wang et al., "Characterization of the bacterial community in the ecosystem of sea cucumber (*Apostichopus japonicus*) culture ponds: correlation and specificity in multiple media," *Water*, vol. 14, no. 9, Article ID 1386, 2022.

[12] L. Wang, C. Wei, Y. Chang, and J. Ding, "Response of bacterial community in sea cucumber *Apostichopus japonicus* intestine, surrounding water and sediment subjected to high-temperature stress," *Aquaculture*, vol. 535, Article ID 736353, 2021.

[13] H. Xu, L. Wang, X. Bao et al., "Microbial communities in sea cucumber (*Apostichopus japonicus*) culture pond and the effects of environmental factors," *Aquaculture Research*, vol. 50, no. 4, pp. 1257–1268, 2019.

[14] Y. Ren, S. Dong, F. Wang, Q. Gao, X. Tian, and F. Liu, "Sedimentation and sediment characteristics in sea cucumber *Apostichopus japonicus* (Selenka) culture ponds," *Aquaculture Research*, vol. 42, no. 1, pp. 14–21, 2010.

[15] L. M. Fan, K. Barry, L. L. Shi et al., "Archaeal community compositions in tilapia pond systems and their influencing factors," *World Journal of Microbiology & Biotechnology*, vol. 34, no. 3, Article ID 43, 2018.

[16] X. Zhang, Q. Zhang, A. Yang et al., "Incorporation of microbial functional traits in biogeochemistry models provides better estimations of benthic denitrification and anammox rates in coastal oceans," *Journal of Geophysical Research-Biogeosciences*, vol. 123, no. 10, pp. 3331–3352, 2018.

[17] J. W. T. Tung and P. A. Tanner, "Instrumental determination of organic carbon in marine sediments," *Marine Chemistry*, vol. 80, no. 2-3, pp. 161–170, 2003.

[18] S. H. Cheng, D. H. Chen, and Z. S. Lei, "Determination of sulfide in marine sediments by elemental analyzer," *Rock & Mineral Analysis (in Chinese)*, vol. 30, no. 1, pp. 63–66, 2011.

[19] X. Zhang, H. Agogue, C. Dupuy, and J. Gong, "Relative abundance of ammonia oxidizers, denitrifiers, and anammox bacteria in sediments of hyper-nutrified estuarine tidal flats and in relation to environmental conditions," *Clean-Soil Air Water*, vol. 42, no. 6, pp. 815–823, 2014.

[20] J. Xiong, Y. Liu, X. Lin et al., "Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan Plateau," *Environmental Microbiology*, vol. 14, no. 9, pp. 2457–2466, 2012.

[21] J. G. Caporaso, J. Kuczynski, J. Stombaugh et al., "QIIME allows analysis of high-throughput community sequencing data," *Nature Methods*, vol. 7, no. 5, pp. 335-336, 2010.

[22] R. C. Edgar, "Search and clustering orders of magnitude faster than BLAST," *Bioinformatics*, vol. 26, no. 19, pp. 2460-2461, 2010.

[23] Q. Wang, G. M. Garrity, J. M. Tiedje, and J. R. Cole, "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy," *Applied and Environmental Microbiology*, vol. 73, no. 16, pp. 5261–5267, 2007.

[24] P. D. Schloss, S. L. Westcott, T. Ryabin et al., "Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities," *Applied and Environmental Microbiology*, vol. 75, no. 23, pp. 7537–7541, 2009.

[25] R Core Team, "R: a language and environment for statistical computing., R Foundation for Statistical Computing," Vienna, Austria, 2020, https://www.R-project.org/.

[26] T. Treude, L. J. Hamdan, S. Lemieux, A. W. Dale, and S. Sommer, "Rapid sulfur cycling in sediments from the Peruvian oxygen minimum zone featuring simultaneous sulfate reduction and sulfide oxidation," *Limnology and Oceanography*, vol. 66, no. 7, pp. 2661–2671, 2021.

[27] H. Shao, H. Dong, Y. Liu, G. Zhou, and X. Guan, "Chemiluminescence quenching capacity as a surrogate for total organic carbon in wastewater," *Journal of Hazardous Materials*, vol. 440, Article ID 129765, 2022.

[28] K. Zhang, X. Tian, S. Dong, J. Feng, and R. He, "An experimental study on the budget of organic carbon in polyculture systems of swimming crab with white shrimp and short-necked clam," *Aquaculture*, vol. 451, pp. 58–64, 2016.

[29] M. Y. Semenov, I. I. Marinaite, N. A. Zhuchenko, A. V. Silaev, K. E. Vershinin, and Y. M. Semenov, "Revealing the factors affecting occurrence and distribution of polycyclic aromatic hydrocarbons in water and sediments of Lake Baikal and its tributaries," *Chemistry and Ecology*, vol. 34, no. 10, pp. 925–940, 2018.

[30] Y. Kuwayama, S. M. Olmstead, D. C. Wietelman, and J. Zheng, "Trends in nutrient-related pollution as a source of potential water quality damages: a case study of Texas, USA," *Science of the Total Environment*, vol. 724, Article ID 137962, 2020.

[31] Z. Zheng, S. Dong, X. Tian, F. Wang, O. Gao, and P. Bai, "Sediment-water fluxes of nutrients and dissolved organic

carbon in extensive sea cucumber culture ponds," *Clean-Soil Air Water*, vol. 37, no. 3, pp. 218–224, 2009.

[32] A. R. Dincer, S. Yumun, M. Once, and Z. U. Yumun, "Investigation of inland pollution using total carbon (TC), total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN) and TOC/TN rations," *Applied Ecology and Environmental Research*, vol. 17, no. 6, pp. 14751–14765, 2019.

[33] C. Lu, Y. Sun, Q. Gao, S. Dong, and B. Wen, "Effects of different water exchange frequencies on the environments of the sediments in sea cucumber culture ponds," *Transactions of Oceanology and Limnology*, no. 4, Article ID 8, 2014.

[34] Z. S. Li, S. Ma, H. W. Shan, T. Wang, and W. Xiao, "Responses of hemocyanin and energy metabolism to acute nitrite stress in juveniles of the shrimp *Litopenaeus vannamei*," *Ecotoxicology and Environmental Safety*, vol. 186, Article ID 109753, 2019.

[35] Z. Yao, X. Zhang, Q. Lai, K. Zhou, and P. Gao, "Acute exposure to key aquaculture environmental stressors impaired the aerobic metabolism of *Carassius auratus gibelio*," *Biology-Basel*, vol. 9, no. 2, Article ID 27, 2020.

[36] Z. Li, S. Ma, and H. Shan, "Accumulation of nitrite and responses of energy metabolism exposed to nitrite stress in *Litopenaeus vannamei*," *Journal of Fisheries of China*, vol. 45, no. 11, pp. 1825–1834, 2021.

[37] Z. Xia, Q. Wang, Z. She et al., "Nitrogen removal pathway and dynamics of microbial community with the increase of salinity in simultaneous nitrification and denitrification process," *Science of the Total Environment*, vol. 697, Article ID 134047, 2019.

[38] Z. Xu, J. Cao, X. Qin, W. Qiu, J. Mei, and J. Xie, "Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and tissue structure in fish exposed to ammonia nitrogen: a review," *Animals*, vol. 11, no. 11, Article ID 3304, 2021.

[39] G. Luo, J. Xu, and H. Meng, "Nitrate accumulation in biofloc aquaculture systems," *Aquaculture*, vol. 520, Article ID 734675, 2020.

[40] C. Wang, Y. Liu, W. Lv et al., "Enhancement of nitrogen removal by supplementing fluidized-carriers into the aerobic tank in a full-scale A(2)/O system," *Science of the Total Environment*, vol. 660, pp. 817–825, 2019.

[41] B. P. Han, "The variation of sulfide in culture ponds of Binhai and the relationship between sulfide and other factors," *Transactions of Oceanology and Limnology*, vol. 3, Article ID 6, 2008.

[42] X. Wang, J. Wang, Y.-G. Zhao et al., "Control of toxic sulfide in mariculture environment by iron-coated ceramsite and immobilized sulfur oxidizing bacteria," *Science of the Total Environment*, vol. 793, Article ID 148658, 2021.

[43] C. Chen, Y. Shen, W. Shen, J. He, Z. Lin, and Y. Dong, "Defense responses of sulfur dioxygenase to sulfide stress in the razor clam *Sinonovacula constricta*," *Genes & Genomics*, vol. 43, no. 5, pp. 513–522, 2021.

[44] N. K. Bereded, G. B. Abebe, S. W. Fanta et al., "The impact of sampling season and catching site (wild and aquaculture) on gut microbiota composition and diversity of nile tilapia (*Oreochromis niloticus*)," *Biology-Basel*, vol. 10, no. 3, Article ID 180, 2021.

[45] B. Ji, X. Zhang, S. Zhang, H. Song, and Z. Kong, "Insights into the bacterial species and communities of a full-scale anaerobic/anoxic/oxic wastewater treatment plant by using third-generation sequencing," *Journal of Bioscience and Bioengineering*, vol. 128, no. 6, pp. 744–750, 2019.

[46] Z. Zhong, Y. Liu, H. Liu, F. Wang, Y. Zhou, and Z. Liu, "*Marinobacter halophilus* sp nov., a halophilic bacterium isolated from a salt lake," *International Journal of Systematic and Evolutionary Microbiology*, vol. 65, pp. 2838–2845, 2015.

[47] A. L. Dieguez, S. Balboa, and J. L. Romalde, "*Halomonas borealis* sp. nov. and *Halomonas niordiana* sp. nov., two new species isolated from seawater," *Systematic and Applied Microbiology*, vol. 43, no. 1, Article ID 126040, 2020.

[48] X. Zhang, Z. Ji, Y. Shao et al., "Seasonal variations of soil bacterial communities in Suaeda wetland of Shuangtaizi River estuary, Northeast China," *Journal of Environmental Sciences*, vol. 97, pp. 45–53, 2020.

[49] A.-X. van der Stel and M. M. S. M. Wosten, "Regulation of respiratory pathways in campylobacterota: a review," *Frontiers in Microbiology*, vol. 10, Article ID 1719, 2019.

[50] F. Inagaki, K. Takai, K. H. Nealson, and K. Horikoshi, "*Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing ε-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough," *International Journal of Systematic & Evolutionary Microbiology*, vol. 54, no. Pt 5, Article ID 1477, 2004.

[51] S. Mino, H. Kudo, T. Arai, T. Sawabe, K. Takai, and S. Nakagawa, "*Sulfurovum aggregans* sp nov., a hydrogenoxidizing, thiosulfate-reducing chemolithoautotroph within the epsilonproteobacteria isolated from a deep-sea hydrothermal vent chimney, and an emended description of the genus *Sulfurovum*," *International Journal of Systematic and Evolutionary Microbiology*, vol. 64, pp. 3195–3201, 2014.

[52] M. Soltani, K. Ghosh, S. H. Hoseinifar et al., "Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish," *Reviews in Fisheries Science & Aquaculture*, vol. 27, no. 3, pp. 331–379, 2019.

[53] D. Han, Z. Hu, D. Li, and R. Tang, "Nitrogen removal of water and sediment in grass carp aquaculture ponds by mixed nitrifying and denitrifying bacteria and its effects on bacterial community," *Water*, vol. 14, no. 12, Article ID 1855, 2022.

[54] J. M. Ebeling, M. B. Timmons, and J. J. Bisogni, "Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems," *Aquaculture*, vol. 257, no. 1–4, pp. 346–358, 2006.

[55] S. Ge, S. Wang, X. Yang, S. Qiu, B. Li, and Y. Peng, "Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: a review," *Chemosphere*, vol. 140, pp. 85–98, 2015.

[56] J. Chen, S. Gu, H. Hao, and J. Chen, "Characteristics and metabolic pathway of *Alcaligenes* sp. TB for simultaneous heterotrophic nitrification-aerobic denitrification," *Applied Microbiology and Biotechnology*, vol. 100, no. 22, pp. 9787–9794, 2016.

[57] T. Song, X. Zhang, J. Li, X. Wu, H. Feng, and W. Dong, "A review of research progress of heterotrophic nitrification and aerobic denitrification microorganisms (HNADMs)," *Science of the Total Environment*, vol. 801, Article ID 149319, 2021.

[58] L. Dai, C. Liu, L. Yu et al., "Organic matter regulates ammonia-oxidizing bacterial and archaeal communities in the surface sediments of *Ctenopharyngodon idellus* aquaculture ponds," *Frontiers in Microbiology*, vol. 9, Article ID 2290, 2018.

[59] F. Sun, C. Wang, and H. Yang, "Physicochemical factors drive bacterial communities in an aquaculture environment," *Frontiers in Environmental Science*, vol. 9, Article ID 709541, 2021.