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RESEARCH

Stochastic Assembly Increases the Complexity and Stability of Shrimp Gut Microbiota During Aquaculture Progression

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Abstract

The gut microbiota of aquaculture species contributes to their food metabolism and regulates their health, which has been shown to vary during aquaculture progression of their hosts. However, limited research has examined the outcomes and mechanisms of these changes in the gut microbiota of hosts. Here, Kuruma shrimps from the beginning, middle, and late stages of aquaculture progression (about a time duration of 2 months between each stage) were collected and variations in the gut microbiota of Kuruma shrimp during the whole aquaculture process were examined. High-throughput sequencing demonstrated increases in the diversity and richness of the shrimp gut microbiota with aquaculture progression. In addition, the gut microbiota composition differed among cultural stages, with enrichment of Firmicutes, *RF39*, and *Megamonas* and a reduction in Proteobacteria in the mid-stage. Notably, only very few taxa were persistent in the shrimp gut microbiota during the whole aquaculture progression, while the number of taxa that specific to the end of aquaculture was high. Network analysis revealed increasing complexity of the shrimp gut microbiota during aquaculture progression. Moreover, the shrimp gut microbiota became significantly more stable towards the end of aquaculture. According to the results of neutral community model, contribution of stochastic processes for shaping the shrimp gut microbiota was elevated along the aquaculture progression. This study showed substantial variations in shrimp gut microbiota during aquaculture progression and explored the underlying mechanisms regulating these changes.

Keywords Shrimp aquaculture progression · Kuruma shrimp (*Penaeus japonicus*) · Gut microbiota · Functional composition · Co-occurrence network · Community assembly

Introduction

The gut microbiota comprises an extremely intricate microbial aggregate in the intestinal tract of animals, which is critical for immunoregulation, nutrient absorption, physiological responses, and host development (Fan and Pedersen 2021). Homeostasis of the gut microbiota is tightly correlated to the physiology and health of the host (Rinninella et al. 2019). Multiple genetic and physiological factors, including age, sex, and reproductive status, have been reported as the regulators for gut microbiota in diverse animals (Vujkovic-Cvijin et al. 2020). Besides, many non-host factors, including diet, climate, and living conditions, also participate to the variations of gut microbiota among different individuals (Leeming et al. 2019; Tasnim et al. 2017). As an important aquaculture species, associations between shrimp gut microbiota with its growth and health have been investigated previously. For example, the gut microbiota of shrimp with high growth rates showed more interspecies interactions and less phylogenetic clustering (Xiong et al. 2017a). High heterogeneity of the gut microbiota has also been seen in freshwater shrimp, possibly due to both the water-quality factors and surrounding bacterial communities (Zhao et al. 2018). An increase in the complexity of the gut microbiota network was observed in Pacific white shrimp with white feces syndrome, with Rhodobacterales,

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Vibrionales, and Flavobacteriales recognized as the keystone taxa (Dai et al. 2020). Shrimp aquaculture activities take several months from seeding to harvesting. Although the water quality and diet are relatively stable, the shrimp gut microbiota changes, with possible impacts on their growth and health. In this context, characterizing the gut microbiota dynamics of shrimp during aquaculture process may provide a basis for the improvement of aquaculture techniques and management strategies.

Although it is common knowledge that the gut microbiota of shrimp alters during aquaculture, the consequences and mechanisms of these changes remain unclear. Recent network analyses have unraveled the structure of such complex communities by exploring the co-occurrence arrangements of gut microbiota in some animals (Lee et al. 2021; Berry and Widder 2014; Pascoe et al. 2017). Co-occurrence networks, with nodes representing species and links representing species interactions, can be used to characterize the complexity of the gut microbiota (Hirano and Takemoto 2019). Gut microbiota with higher complexity generally possesses high functional redundancy due to the overlapping metabolic activities of different members (Moya and Ferrer 2016). In addition, a healthy gut microbiota tends to exhibit relative stability despite external disturbances (Yuan et al. 2021), which can also be characterized by some features of co-occurrence networks (Coyte et al. 2015). However, although network analysis can reveal the consequences of gut microbiota changes, the underlying mechanisms remain unclear. Microbial ecological theories suggest that microbial communities are determined by a compensate between deterministic and stochastic processes (Zhao et al. 2022). In communities controlled by deterministic processes, taxa take up specific ecological niches, and the occurrence of which can be predicted (Vanwonterghem et al. 2014). The neutral hypothesis, on the other hand, allows for multiple species to occupy similar or overlapping areas, and for the relative abundance of species within the community to change as a result of random fluctuations (Sloan et al. 2006). Variations in the co-occurrence network for shrimp gut microbiota during aquaculture progression have not yet been investigated. Moreover, the assembly mechanisms shaping the gut microbiota network may provide useful information for ecological aquaculture.

In this study, we addressed the following unresolved issues: (i) how does shrimp aquaculture progression affect the gut microbiota at the aspects of diversity, composition, and function? (ii) do the network complexity and stability of the shrimp gut microbiota vary along the aquaculture process? and (iii) do deterministic or stochastic processes dominate the gut microbiota assembly of shrimps? To answer these questions, we followed the temporal dynamics of the gut microbiota in Kuruma shrimp (*Penaeus japonicus*) throughout the whole aquaculture progression using high-throughput sequencing and analyzed changes in the shrimp gut microbiota. We also used co-occurrence network analyses to unveil variations in the complexity and stability of the gut microbiota and determined the underlying assembly mechanisms using a neutral community model (NCM). The findings of our research greatly expanded our knowledge of the ecological mechanism affecting the shrimp gut microbiota during aquaculture progression.

Materials and Methods

Aquaculture Progression and Sampling

Kuruma shrimps were obtained from a cultural pond in the coastal area of Dalian, China. The surface area of the pond was about 7 hm^2 with a 1.5–2.0 m water depth, with twice water-exchanges per month. Kuruma shrimp seedlings were put into the pond in late May and harvested in late September. The animal concentrations and diets are shown in Table S1. The use of therapeutic or growth-promoting compounds, such as antibiotics, was strictly prohibited during the aquaculture process. Ten healthy individuals were randomly selected from the aquaculture pond on June 3 (initial, several days after releasing seedlings into the ponds), July 27 (Mid), and September 27 (end, the time of harvest) 2021, respectively, and dissected to collect the intestinal tracts. The intestinal contents were gently squeezed out from the intestinal tracts and kept at – 80 °C for further analyses.

DNA Extraction and High-Throughput Sequencing

DNA of gut microbiota was obtained from the contents of the intestinal samples using a QIAamp Power Fecal DNA Kit (Qiagen, CA, USA). Successful DNA extraction was verified by agarose gel electrophoresis (1% concentration). Subsequently, Nanodrop 2000 (ThermoFisher, CA, USA) was applied to estimate the purity and concentration of successfully extracted DNA. And then, all successfully extracted DNA were stored at −20 °C for further application. Gut microbiota of Kuruma shrimps were investigated using the amplicon sequencing based on bacterial 16S rRNA gene using the primers (341F–806R) for V3–V4 regions (Berg et al. 2012). PCR amplification, gel extraction of PCR products, and sequencing library construction were according to a previous study (Hou et al. 2021). Finally, the library of each sample was sequenced at BIOZERON Biotech. Co., Ltd. (Shanghai, China) using an Illumina Novaseq6000 platform based on the 250 bp paired-end strategy.

Sequence Data Processing

The raw reads were assigned to each sample on the basis of their unique barcodes and then the barcode sequences were truncated. Low-quality reads (Phred scores lower than 20, mismatches in the primers, homopolymer runs high than 8, containing ambiguous bases, or read length lower than 250 bp) were eliminated (Bokulich et al. 2013). Then, paired reads were assembled, chimeras were eliminated, and the clean data were aggregated to the amplicon sequence variants (ASVs) using the QIIME2 software with the DADA2 plugin unit (Bokulich et al. 2018). All ASVs were assigned to a taxonomy according to the SILVA database (Release 138) (Yilmaz et al. 2014), and then, singletons (ASVs with only one read) were dropped. Finally, the ASV abundance table was normalized based on the lowest read number among all samples (22,758).

Statistical Analysis

Four alpha diversity indices related to different facets, including richness (Chao1), diversity (Shannon), evenness (Pielou's evenness index, Pielou_J), and evolution (Faith's phylogenetic diversity, Faith_pd) were estimated. Tukey's honest significant difference (HSD) test was applied to confirm the variations in alpha diversity indices, major gut bacteria abundances, and functional pathways of shrimp gut microbiota during aquaculture progression ("multcomp" package in R). In addition, according to the Bray–Curtis distance, adonis test and principal coordinate analysis (PCoA) were executed ("vegan" package in R) to evaluate the impacts of shrimp aquaculture progression on the gut microbiota compositions. The specificity and occupancy of each ASV in samples from each aquaculture stage were calculated and projected onto a SPEC-OCCU plot (Gweon et al. 2021) to explore specialists present in different culture stages. Venn diagram analysis ("ggplot2" package in R) was executed to recognize the ASVs shared by all studied shrimps, and their composition was exhibited using a Sankey diagram ("d3Network" package in R). Tukey's HSD test was also performed to determine if the sum abundances of these ASVs in individuals from different aquaculture stages were significantly different.

Spearman's rank correlations among all ASVs of samples from each aquaculture stage were respectively calculated using the "WGCNA" package in R to visualize the co-occurrence network. Only ASVs detected in at least six out of 10 samples in each aquaculture stage were used for correlation analysis. Correlations between two ASVs with the $|\text{correlation coefficient}| > 0.8$ and p value adjusted by the Benjamini–Hochberg method < 0.05 were retained. Network graphs were visualized using the Gephi software (Bastian and Jacomy 2009) and the network topological parameters were calculated using the "igraph" package in R. To assess the gut microbiota, stability, robustness, vulnerability, and cohesion were estimated for the co-occurrence networks of samples from each aquaculture stage (Yuan et al. 2021). Changes in these stability indices during shrimp aquaculture progression were analyzed by Tukey's HSD test. In addition, a NCM was applied to estimate the relative importance of stochastic processes shaping the shrimp gut microbiota (Sloan et al. 2006). The ASVs of samples from each aquaculture stage were then divided into three partitions according to whether they occurred more frequently (above partition), less frequently (below partition), or within (neutral partition) the 95% confidence interval of the NCM.

Results

Shifts in Diversity of Shrimp Gut Microbiota During Aquaculture Progression

The gut microbiota from each of 10 shrimp from the initial, mid, and end aquaculture stages were analyzed, and a total of 2,533,629 high-quality reads were obtained in total (Table S2). These reads were clustered into 7319 ASVs, which were annotated into 58 phyla, 138 classes, 334 orders, 527 families, 1125 genera, and 635 species (Table S3). All the ASVs were annotated at the phylum level, 75.71% were assigned to a genus, but only 10.75% were annotated at the species level (Fig. S1). These results suggested that analyses at the bacterial genus level enabled efficient assessment of relative intact shrimp gut microbiota.

Significantly higher Shannon and Chao1 indices (Tukey's HSD test, $p < 0.05$) were found in samples from the end stage than those from the initial and mid-stages (Fig. 1a). In contrast, the Pd_faith indices of samples from the mid and end stages were significantly lower (Tukey's HSD test, $p < 0.05$) compared with individuals from the initial stage (Fig. 1). Moreover, the Pielou_J index of shrimp gut microbiota continued to increase during aquaculture progression and was significantly different (Tukey's HSD test, $p < 0.05$) among the different aquaculture stages (Fig. 1). These results suggested that the richness, diversity, and evenness of the shrimp gut microbiota increased but the phylogenetic diversity decreased during aquaculture progression. PCoA revealed that the gut microbiota of shrimp from different aquaculture stages clustered separately (Fig. 1b). The adonis test also proved the significant effects ($p < 0.05$) of aquaculture progression on the shrimp gut microbiota, which could explain 34.2% of the gut microbiota variations (Fig. 1b).

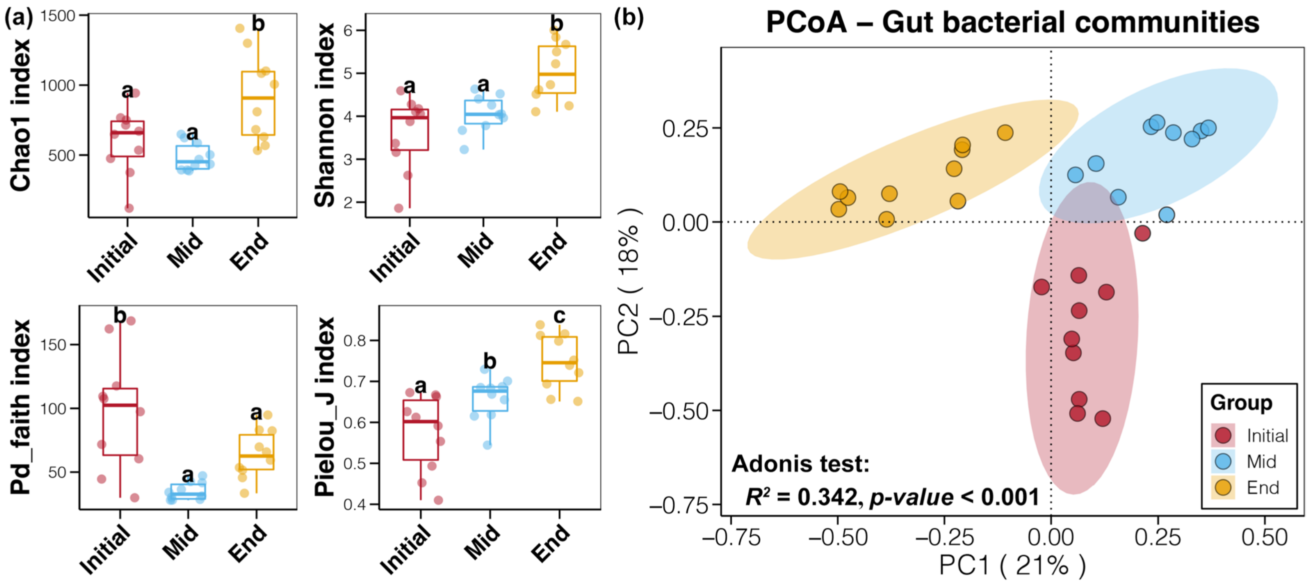

Fig. 1 **a** Variations in alpha diversity of shrimp gut microbiota among different aquaculture stages. Different lowercases letters in the same sub-figure represent significant differences among samples from different aquaculture stages (Tukey's HSD test, $p < 0.05$). **b** PCoA and adonis test based on the Bray–Curtis distance for shrimp gut microbiota samples from different aquaculture stages

Changes in Composition of Shrimp Gut Microbiota During Aquaculture Progression

Firmicutes (25.57%) and Proteobacteria (25.18%) were the major bacterial phyla in the shrimp gut microbiota, followed by Desulfobacterota (8.77%), Bacteroidota (7.96%), Chloroflexi (6.80%), and Planctomycetota (5.57%) (Fig. S2). Firmicutes increased while Proteobacteria decreased in shrimps at the mid-stage in contrast to individuals at the initial and end stages (Fig. 2a). Additionally, Acidobacteriota and Verrucomicrobiota increased but Chloroflexi, Desulfobacterota, and Planctomycetota decreased in shrimp gut microbiota in line with aquaculture progression (Fig. 2a). In terms of bacterial genera, *RF39* (9.70%) was the most abundant genus in the shrimp gut microbiota, followed by *Megamonas* (4.95%), *OM190* (4.05%), *Roseibacterium* (3.84%), *SBR1031* (2.34%), and *Lactobacillus* (Fig. S3). Among these, *RF39* and *Megamonas* were significantly enriched (Tukey's HSD test, $p < 0.05$) in shrimp from the mid-stage compared to individuals from the initial and end stages (Fig. 2b).

Specialists and Generalists in Shrimp Gut Microbiota During Aquaculture Progression

SPEC-OCCU plots were applied to explore potential specialists in the shrimp gut microbiota from different aquaculture stages. ASVs with specificity and occupancy ≥ 0.7 were identified as specialists, indicating that they were specific to an aquaculture stage and common in samples from this stage. The number of these specialists showed an increasing trend during aquaculture progression, representing 43.35%, 23.33%, and 46.72% of the total taxa in the initial, mid, and end stages, respectively (Fig. 3a). Proteobacteria were specialists in all three aquaculture stages, while notable specialists at the end stage included ASVs from multiple rare bacterial phyla (Others) (Fig. 3b). In contrast, only seven ASVs were shared by all the studied samples (generalists) from different stages of aquaculture progression (Fig. 3c). These generalists belonged to six bacterial phyla, including Firmicutes, Desulfobacterota, Proteobacteria, Actinobacteriota, Gemmatimonadota, and Latescibacterota (Fig. 3d). Among these, ASVs annotated to *RF39*, *Megamonas*, *Desulfosarcinaceae*, and *Roseibacterium* were more abundant (Fig. 3d). These generalists made up a significant proportion of the total gut microbiota, averaging about 10% in samples from the initial and end stages but > 40% in individuals from the mid-stage (Fig. 3e).

Co-occurrence Networks of Shrimp Gut Microbiota

Co-occurrence networks of the shrimp gut microbiota from the different aquaculture stages were constructed (Fig. 4a–c), and the topological parameters are given in Table 1. Networks for all aquaculture stages obeyed a power-law distribution, suggesting a non-random distribution pattern. In addition, the small-world coefficient was > 1 for all three networks, indicating the small-world characteristic. The networks for the initial and mid-stages had 49 and 47 nodes, 203 and 230 edges, and average degrees of 8.286 and 9.787, respectively. In contrast, the network for samples from the

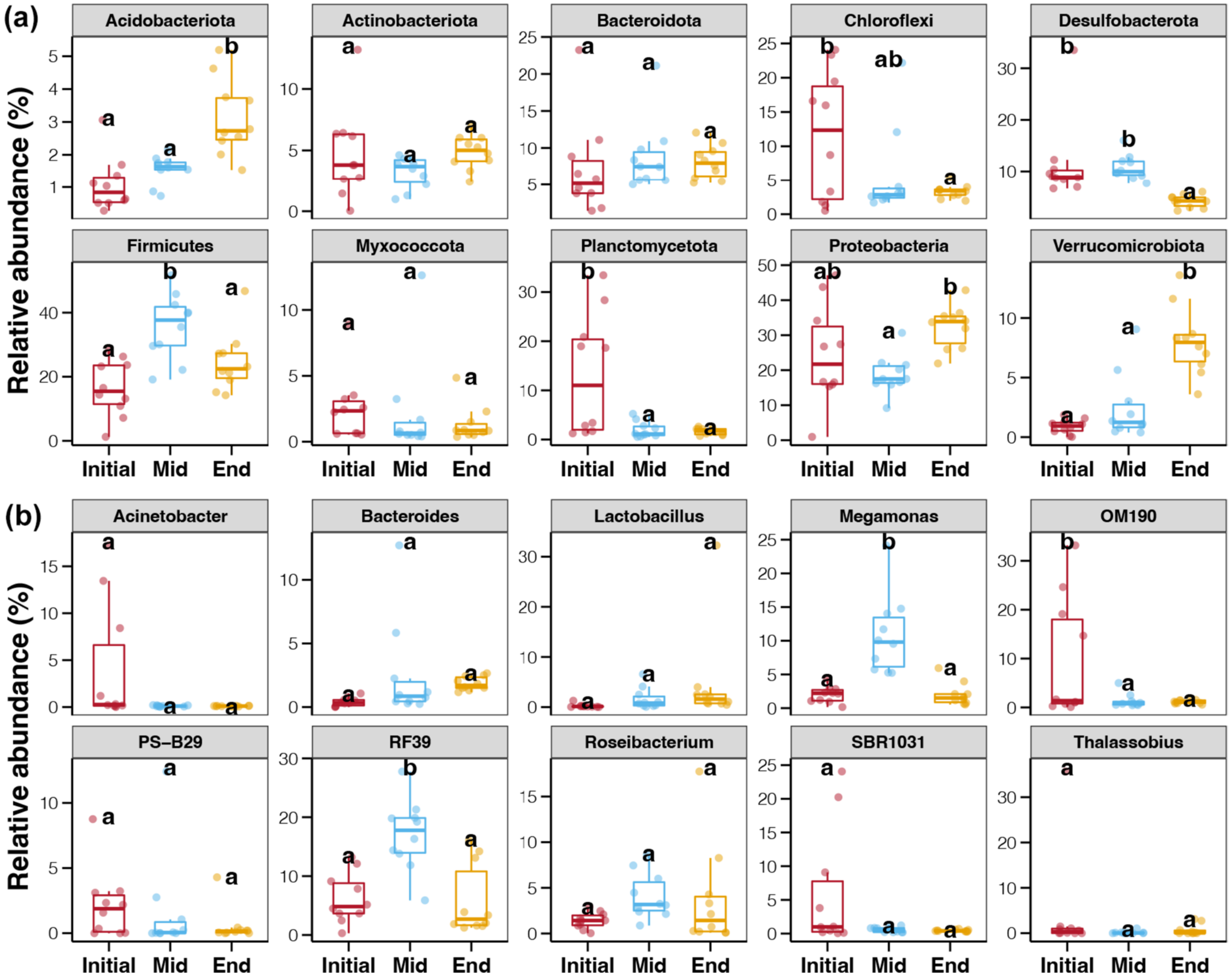

Fig. 2 Variations in relative abundances of top 10 bacterial phyla (**a**) and genera (**b**) in shrimp gut microbiota during aquaculture progression. Different lowercase letters in the same sub-figure represent significant differences among samples from different aquaculture stages (Tukey's HSD test, $p < 0.05$)

end stage presented 112 nodes and 1367 edges, with average degree of 24.411. Moreover, the modularity of the networks for the samples from the initial, mid, and end stages were 0.455, 0.249, and 0.259, respectively. These results revealed a more complex co-occurrence pattern in the shrimp gut microbiota at the end of aquaculture activities.

For network indices, significantly higher robustness (Tukey's HSD test, $p < 0.05$) was observed in the network from the mid and end stage samples compared with the initial stage (Fig. 4d). In contrast, vulnerability was obviously lower in the network for samples from the end stage than those from the initial and mid-stages (Fig. 4e). Moreover, there was an increasing trend in cohesion in networks in line with aquaculture progression, with a significant difference (Tukey's HSD test, $p < 0.05$) between the initial and end stages (Fig. 4f). Taken together, all the network indices demonstrated increasing stability of the network pattern for shrimp gut microbiota in line with aquaculture progression.

Assembly Mechanism of Shrimp Gut Microbiota

Based on the results of NCM, 28.1%, 42.9%, and 55.4% of the ASVs conformed to the neutral-based theory (Fig. 5). These results indicated that the assembly of shrimp gut microbiota at the beginning and ending of the aquaculture activities were managed by the deterministic and stochastic processes, respectively. The ratio of ASVs revealed that the neutral fraction occupied for most of the richness in all three aquaculture stages (Fig. 5). In contrast, the abundance proportions of the above and below partitions occupied more than the richness proportions, with the above fraction

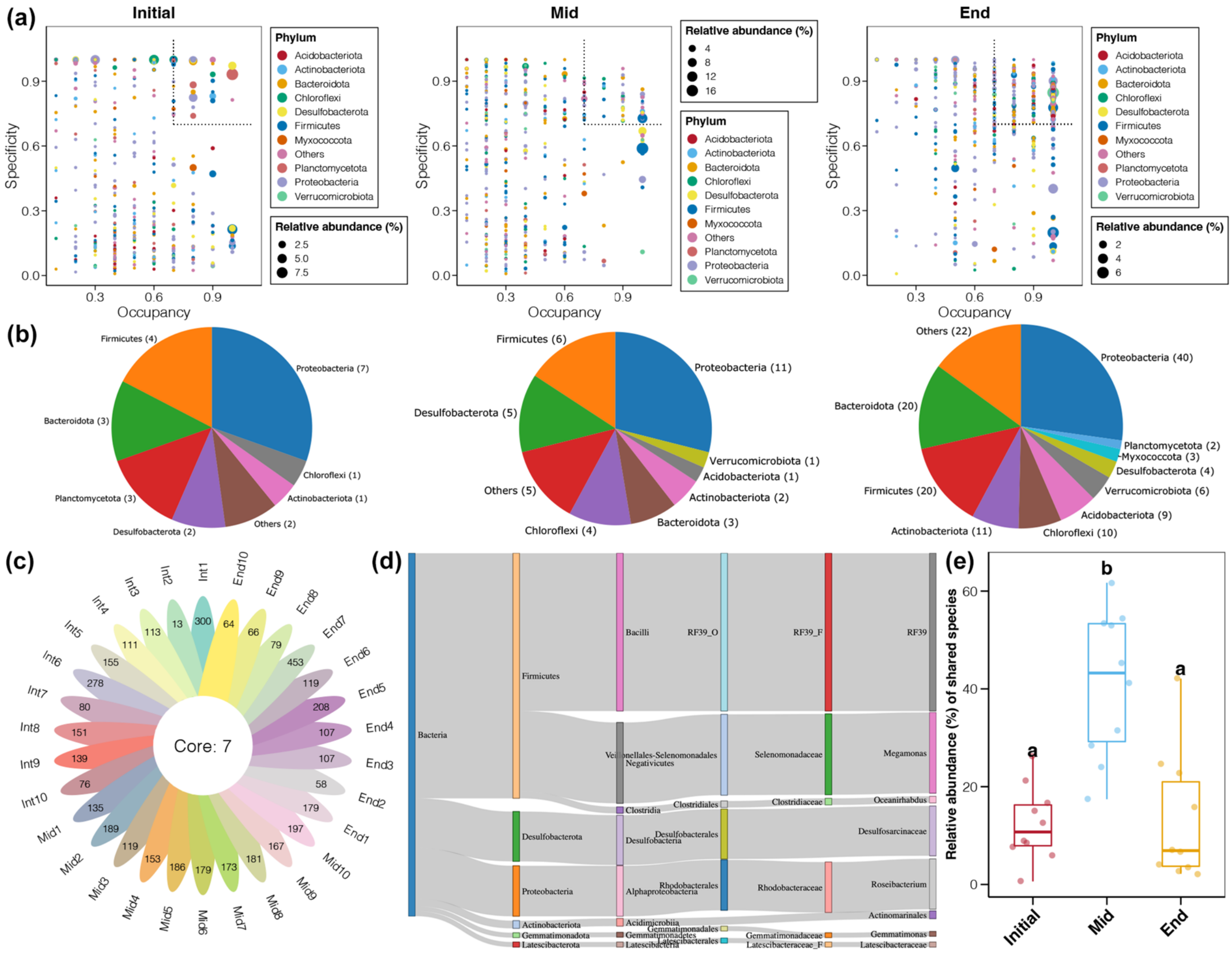

Fig. 3 **a** SPEC-OCCU plot showing amplicon sequence variants (ASVs) in gut microbiota of shrimp from different aquaculture stages. **b** Pie charts showing the composition of specialists in each aquaculture stage. **c** Venn diagram exhibiting shared ASVs in shrimp gut microbiota during aquaculture progression. **d** Average relative abundance and taxonomy of generalists in all samples. **e** Total proportion of shared ASVs in gut microbiota of samples from different aquaculture stages. Different lowercase letters represent significant differences among samples from different aquaculture stages (Tukey's HSD test, $p < 0.05$)

dominant in the shrimp gut microbiota in the mid and end stages (Fig. 5).

Discussion

Numerous studies have examined the gut microbiota associated with the Pacific white shrimp (*Litopenaeus vannamei*) due to its extensive aquaculture and high production (Holt et al. 2021). The relationships of shrimp diseases and its gut microbiota are important issues, and the gut microbiota has become as an important driver of successful aquaculture (Sha et al. 2022). Kuruma shrimp is a commercial important species cultured in China, but high mortality and slow growth being the main barriers to the prosperity of the Kuruma shrimp industry (Dong et al. 2014). Limited studies of the gut microbiota of Kuruma shrimp investigated the influences of host immune factors or probiotic supplementation (Duan et al. 2017; Yang et al. 2016). To our knowledge, variations in gut microbiota of Kuruma shrimp throughout the whole aquaculture progression have been investigated for the first time in the present study. We sequenced the gut microbiota of Kuruma shrimp and revealed significant variations in its diversity and composition during the whole aquaculture process from putting the seedlings into the pond to harvest.

A broad spectrum of health conditions has been linked to changes in the gut microbiota of animals. Reduction in the richness and diversity of the gut microbiota has been shown to be responsible for the poor health rank of hosts with weaker resistance to potentially harmful factors (Lawley and Walker 2013). The currently observed increasing

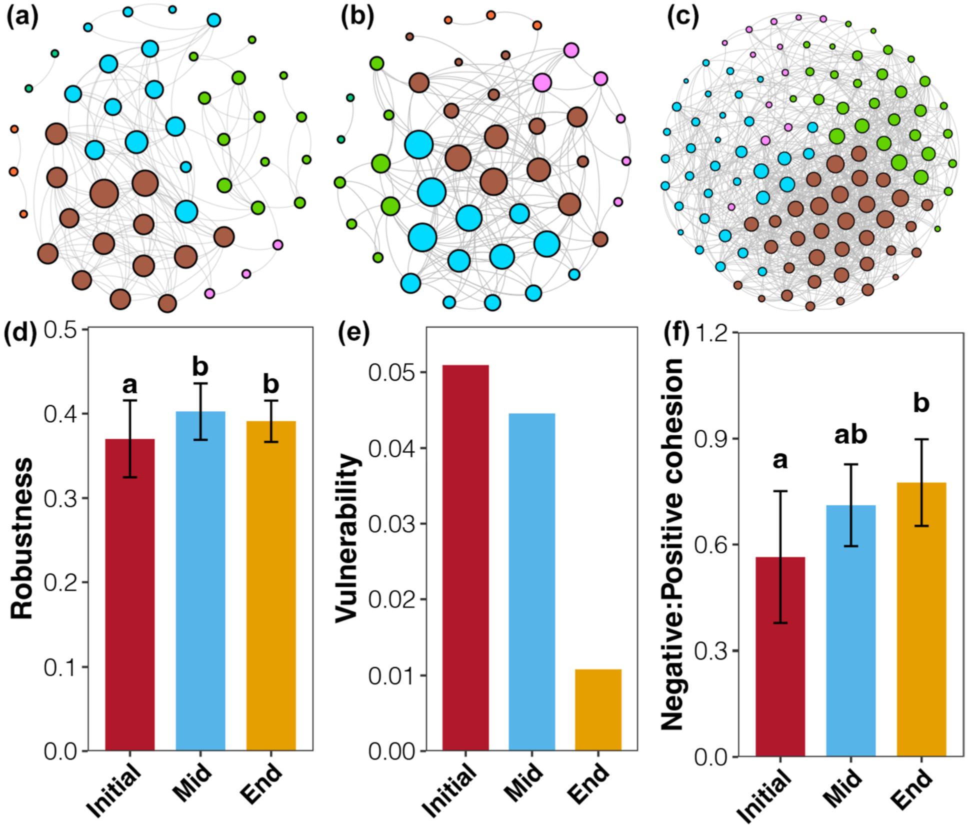

Fig. 4 Co-occurrence networks of shrimp gut microbiota at the initial (**a**), mid (**b**), and end (**c**) aquaculture stages. Nodes belonging to different modules labeled in different colors. Differences in robustness (**d**), vulnerability (**e**), and negative:positive cohesion (**f**) among networks of different aquaculture stages, respectively. Different lowercase letters in the same sub-figure represent significant differences among samples from different aquaculture stages (Tukey's HSD test, $p < 0.05$)

trends in the diversity and richness of the shrimp gut microbiota during aquaculture progression indicated that the shrimp in the study ponds were in a healthy growth state. This inference consistent to the weighted increase trend of Kuruma shrimps during the aquaculture progression, which from the 0.1 g weight at the initial stage increased to about 15 g at the middle stage and finally reached to 20 g at the end stage. The gut microbiota can also affect the growth of the host by regulating the proportion of functional bacteria (Magne et al. 2020). The enrichment of Firmicutes in the intestines of animals can induce a more capable assimilation of energy from the diet (Estrada-Velasco et al. 2015). In contrast, Proteobacteria were dramatically increased in the shrimp gut microbiota with retarded growth compared with normal individuals (Xiong et al. 2017a). Enriched Firmicutes and deleted Proteobacteria in the gut microbiota of mid-stage shrimp in this study indicated the potential for a period of fast growth of shrimp during the aquaculture progression. In addition, the *RF39* genus was the most dominant bacterial genus in the shrimp gut microbiota and significantly enriched in samples from the mid-stage. Previous phylogenomic analysis revealed that *RF39* was received with novel clades in *Bacilli* and probably with acetate and hydrogen producers in the animal gut environment (Wang et al. 2020). *Megamonas*, as another bacterial genus that was significantly enriched in shrimp at the mid-stage, has been reported to include major propionate producers in the gut microbiota of chickens (Polansky et al. 2016). Propionate and acetate are important short-chain fatty acids (SCFAs) formed by the gut microbiota by breakdown of complex carbohydrates (Macfarlane and Macfarlane 2003). SCFAs play a crucial role in the maintenance of host health through regulating the homeostasis of gut microbiota and immunity (Tan et al. 2014). The high abundance of SCFA producers in the gut microbiota reflected the favorable health status of the shrimp during the aquaculture progression.

Examining the dynamics of the shrimp gut microbiota during aquaculture progression provided key insights into the roles of aquaculture activities in mediating the stability and complexity of the gut microbial network. The richness, specialists, and co-occurrence network results indicated that the aquaculture activities improved the complexity of the shrimp gut microbiota over time, possibly because shrimp growth acts as a deterministic screening agent for the selection of specific intestinal bacteria (Xiong et al. 2017b). Consequently, less responsive neutral taxa could prevail in the shrimp gut microbiota at the beginning of aquaculture (Burns et al. 2016), resulting in less complex network structures. This theory was agreed with the findings of the NCM in this study, indicating that a small number of non-neutral taxa occupied for most of the gut microbiota. In contrast to the complexity, gut microbiota stability could be more critical for host health and well-being by ensuring that beneficial symbionts and the functions associated with them are maintained in a long term (Lozupone et al. 2012). However, the relationship between the complexity and stability of microbial communities is still hotly debated, with controversial research results (Landi et al. 2018). A highly complex network often means that the gut microbiota is more robust to external perturbations, suggesting high stability (Santolini and Barabási 2018). A similar phenomenon was found in the gut microbiota of healthy pigs in relation to age (Ke et al. 2019), fish gut microbiota after exposure to silver nanoparticles (Chen et al. 2021), and shrimp gut microbiota in line with disease progression (Dai et al. 2020). The current results provided clear proof that network stability increased in line with network complexity in the shrimp gut microbiota during aquaculture progression. Cooperating networks of microbes can be efficient but are often unstable, while the introduction of species to enhance competition can stabilize the cooperating network (Coyte et al. 2015). The richness of the shrimp gut microbiota continued to increase with aquaculture progression. Most of the increased richness was previously shown to come from the living environment of the shrimp (Zhang et al. 2021), with microbes colonizing the shrimp gut leading to increased competition.

Previous research showed that larvae often have a stochastic dominated gut microbiota because of their unripe intestine (Vallès et al. 2012), while deterministic processes governed more in adults, due to stronger selection exerted by the host as their age (Stephens et al. 2016). However, the current NCM results showed that the higher contribution of stochastic processes in terms of determining the shrimp gut microbiota during aquaculture progression compared to deterministic processes. In addition to an unstable state in larvae, the stochastic assembly of gut microbiota could also result in a more balanced state in adults, which may enhance the resistance of the microbiota to external stresses (Zhao et al. 2022). The increased stability of the gut microbiota of shrimp at the end of the aquaculture process could be due to the contribution of stochastic processes to gut microbiota assembly (Chase 2010). Moreover, studies of shrimp diseases indicated more-stochastic assembly of the gut microbiota in diseased shrimp in contrast to healthy individuals (Chen et al. 2017; Dai et al. 2019; Zhu et al. 2016). Notably, the gut microbial network in healthy shrimp has been reported to be better connected and more complex than the networks in diseased counterparts (Sha et al. 2022). The occurrence of shrimp diseases was closely related to the gut microbiota dysbiosis, with less complexity and weak stability (Xiong et al. 2017b), while the current study suggested that healthy shrimp showed a more complex and stable gut microbiota. Therefore, in addition to the roles of stochastic

Table 1 Topological parameters of co-occurrence networks of gut microbiota for *Penaeus japonicus* during the aquaculture process

	Empirical network									Random network			
	Noses	Edges	Modularity	Average degree	Diameter	Density	Average path length	Clustering coefficient	Power-law model	Modularity (SD)	Average path length (SD)	Clustering coefficient (SD)	Small-world coefficient (SD)
Initial	49	203	0.455	8.286	6	0.183	2.502	0.699	0.917	0.251 (0.290)	2.231 (0.035)	0.220 (0.024)	2.860 (0.255)
Mid	47	230	0.249	9.787	7	0.213	2.421	0.589	0.913	0.124 (0.176)	2.171 (0.037)	0.296 (0.022)	1.796 (0.127)
End	112	1367	0.259	24.411	5	0.220	2.049	0.593	0.821	0.133 (0.179)	1.902 (0.008)	0.270 (0.012)	2.037 (0.056)

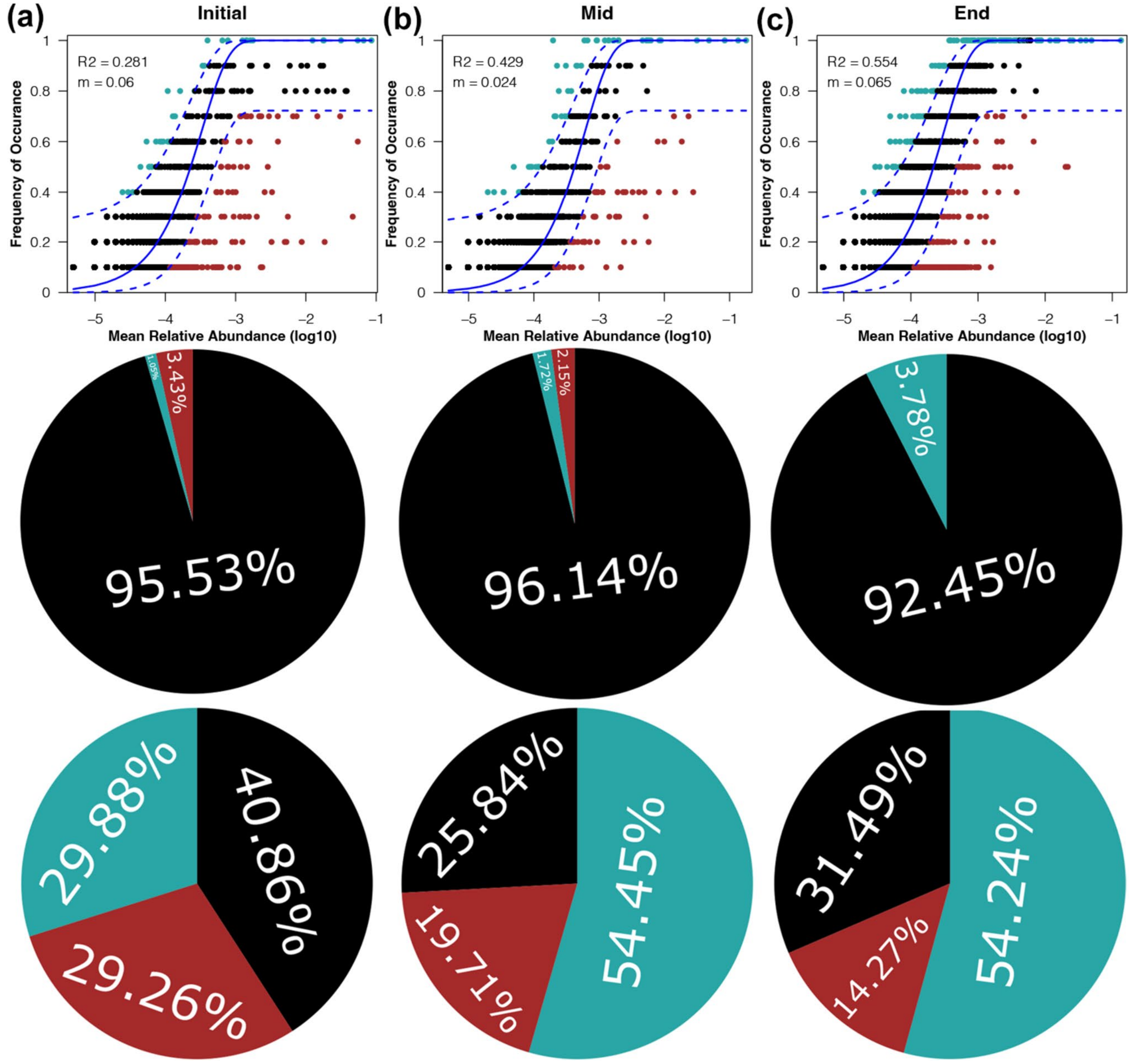

Fig. 5 Distinct fitting distribution, richness, and abundance of shrimp gut microbiota at the initial (**a**), mid (**b**), and end (**c**) stages by neutral community models. The middle and bottom pie charts represent the percentage of the ASVs belong to different parts based on the number and abundance, respectively

and deterministic processes, more information is needed to determine if the gut microbiota is in balance or dysbiosis in order to evaluate the health status of hosts.

Conclusions

The present study provided the first molecular perspective of the roles of aquaculture progression on the gut microbiota of Kuruma shrimp. The richness and diversity of the shrimp gut microbiota increased significantly in line with aquaculture progression. The gut microbiota composition also varied significantly during the aquaculture process, with enrichment of bacteria involved in energy absorption and SCFA production in the mid-stage. Notably, these results revealed increases in the stability and complexity of the shrimp gut microbiota with aquaculture progression. Although deterministic processes dominated the shrimp gut microbiota at the beginning of aquaculture, stochastic assembly had a greater contribution at the end of the process. These findings contributed valuable insights into our knowledge of the shrimp gut microbiota and provided information to help improve management strategies in relation to shrimp health and productivity in aquaculture.

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Author Contribution Saisai Zhang: methodology, formal analysis, and writing original draft; Shuang Liu, formal analysis; Hongwei Li: resources; Hui Li: formal analysis; Jun Luo: methodology; Yinpeng Ding, sources; Tongjun Ren: conceptualization, and writing—review and editing; Wenbo Chen: conceptualization, project administration, and writing—review and editing.

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Data Availability The data that support the findings of this study will be made available when needed and on request.

Declarations

Competing Interest The authors declare no competing interests.

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