

Changes in intestinal microbiota of Pacific white shrimp (*Litopenaeus vannamei*) with *Enterocytozoon hepatopenaei* infection severity

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Introduction

The shrimp *Litopenaeus vannamei* was first introduced in 1988, is widely bred from southern to northern China, and has become more popular in recent years. However, shrimp aquaculture has been hindered by outbreaks of diseases such as *Enterocytozoon hepatopenaei* (EHP) infection, acute hepatopancreatic necrosis syndrome (AHPND), and infectious hypodermal and hematopoietic necrosis virus (IHHNV) infection. Among these pathogens, EHP, as a newly emerging pathogen, has caused tremendous losses for shrimp *P. vannamei* aquaculture. Although EHP was identified in *P. monodon* showing growth retardation in growth out ponds in Thailand in 2009 and was postulated to cause severe growth retardation, it has not attracted enough attention and has spread worldwide in recent years.

The intestinal microbiota is widely considered a “new organ” in animals that has important major systemic effects, playing an important role in digestion and nutrient metabolism and influencing the immune response to disease. In turn, extensive studies have shown that the gut microbiota compositions of aquatic animals are affected by several factors, such as growth stage, health status, rearing water quality and pathogens. However, to our knowledge, there have been no studies of the intestinal microbiota after infection of *L. vannamei* with EHP, although preliminary research was conducted on intestinal microbiota in different-sized shrimp using the DGGE method in our prior study.

Materials and methods

In this study, the severity of EHP infection in *L. vannamei* was confirmed by quantifying EHP copies using RT-PCR and by examining the effects with different body sizes. Second, 16S rRNA gene-based analysis was used to explore the changes in the intestinal microbiota of Pacific white shrimp with different severities of EHP infection. These results could help us to better understand the response of the intestinal microbiota to EHP infection.

Result

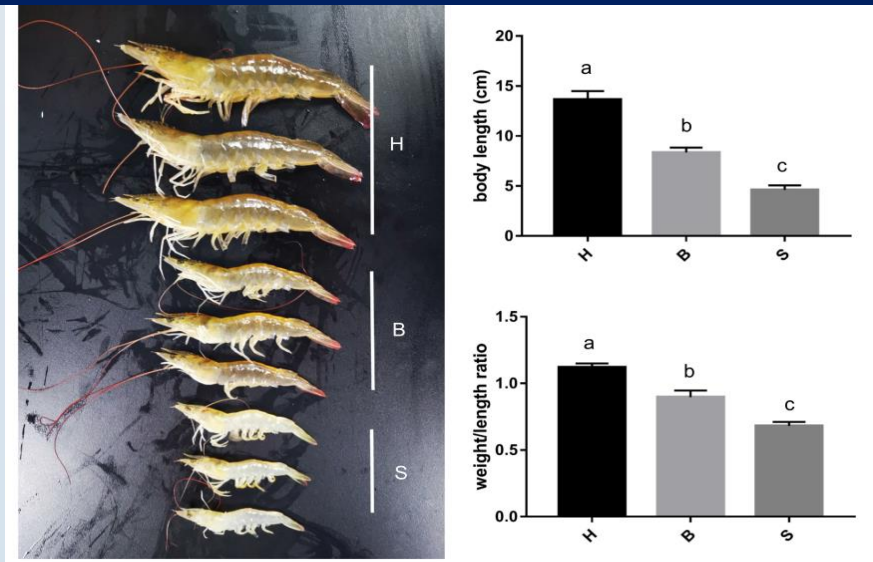


Fig. 1. The individual differences among H, B and S groups. ^{a,b,c} The ranges indicated with another superscript are significantly different from each other (based on average \pm standard deviation) according to a One-way ANOVA ($P < 0.05$).

The results of PCR analysis of pathogens.

Group	PCR Results					
	EHP	WSSV	IHHNV	AHPND	TSV	YHV
H	-	-	-	-	-	-
B	+	-	-	-	-	-
S	+	-	-	-	-	-
Water	+	-	-	-	-	-
Faeces	+	-	-	-	-	-
Over-Sed	+	-	-	-	-	-

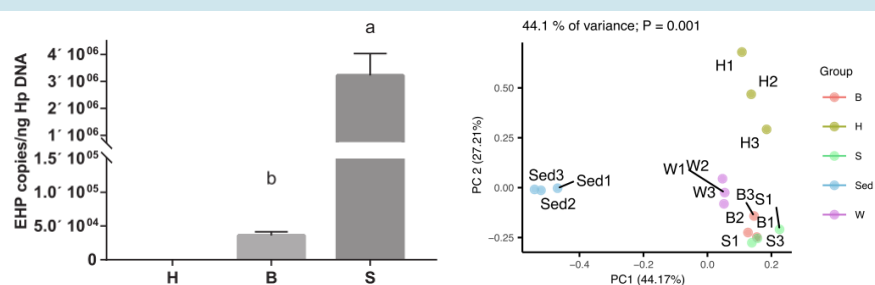


Fig. 2. The number of EHP copy in hepatopancreatic tissue of H, B and S groups. ^{a,b} indicated significant difference according to a One-way ANOVA ($P < 0.05$).

Fig. 3. Beta-diversity analysis of the microbiota in H, B, S, Sed and W groups using the PCoA analysis.

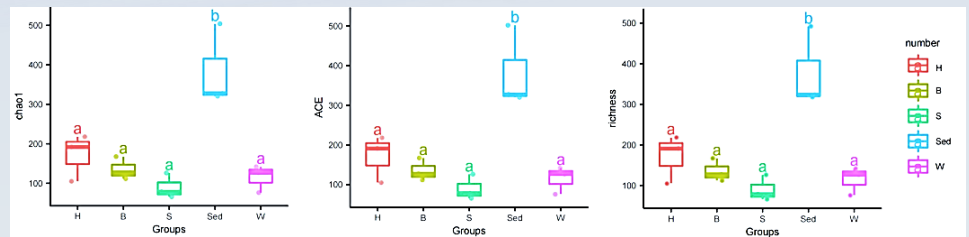


Fig. 4. Alpha-diversity analysis of the microbiota of *L.vannamei* in H, B, S, Sed and W groups. ^{a,b} indicated significant difference according to a One-way ANOVA ($P < 0.05$).

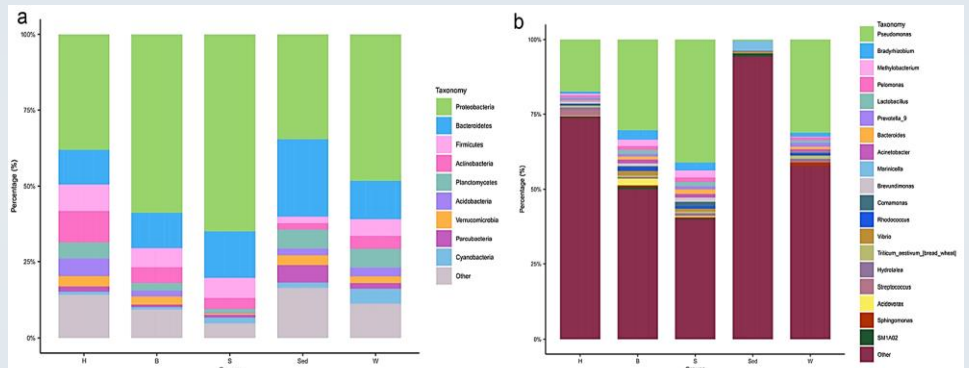


Fig. 5. Taxonomic composition distribution bar plot of top ten most abundant phyla (a) and top twenty most abundant genera (b) of microbiota in H, B, S, Sed and W groups.

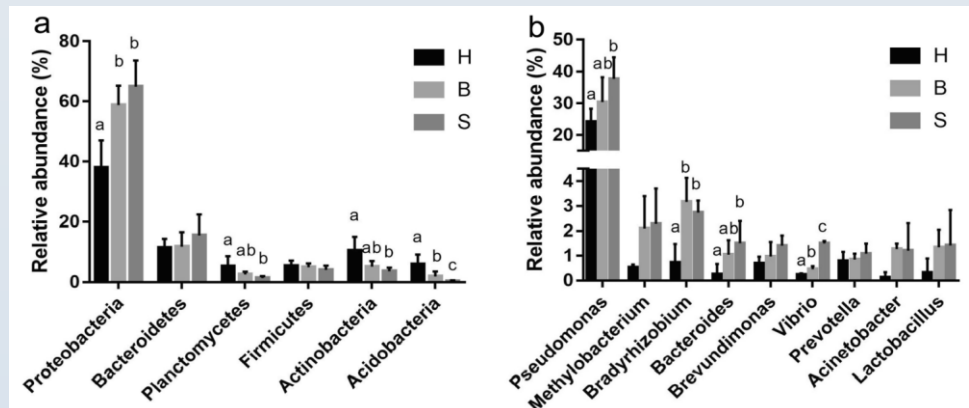


Fig. 6. Significant difference analysis of the top six most abundant phyla (a) and top nine most abundant genera (b) among H, B and S groups. ^{a,b,c} indicated significant difference according to a One-way ANOVA ($P < 0.05$).

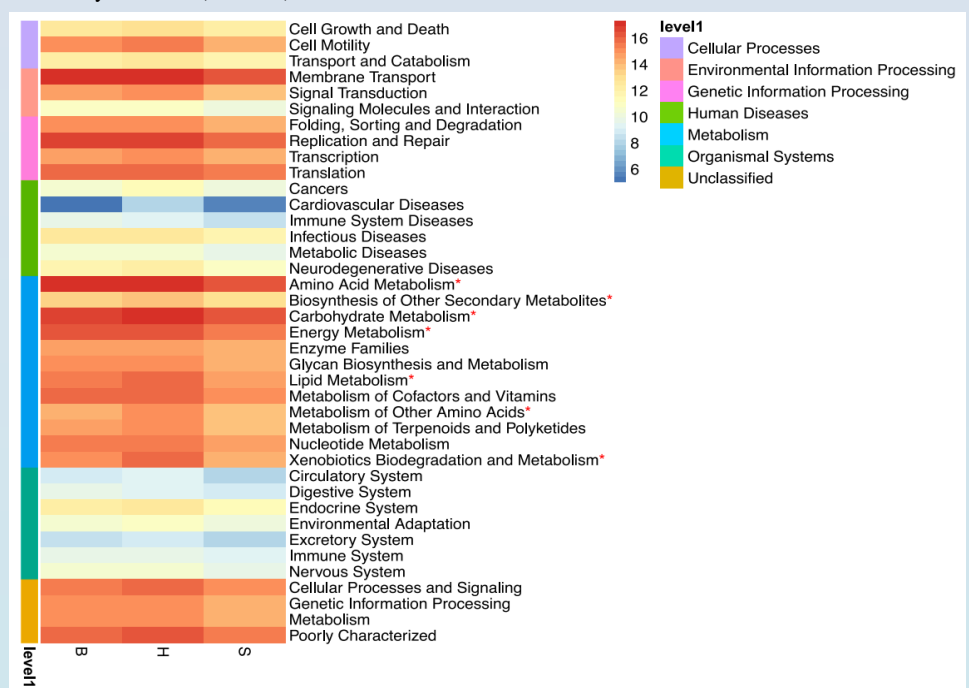


Fig. 7. Heatmap and hierarchical clustering of the significantly different KEGG functions (level 2) of intestinal microbiota in shrimp with different EHP infection severity. Red asterisks shows several markedly different categories belonging to metabolism among H, B and S groups.

Conclusions

The results showed that bacterial diversity in the intestine of *L. vannamei* was high, but it decreased with increasing severity of EHP infection. The relative abundances of the phyla Planctomycetes, Actinobacteria and Acidobacteria decreased significantly with a decrease in body size or EHP infection severity ($P < 0.05$). The most abundant genera were *Pseudomonas*, *Methylobacterium*, *Bradyrhizobium*, *Bacteroides*, *Vibrio*, *Prevotella* and so on. In addition, the relative abundances of some bacteria, such as *Pseudomonas*, *Bradyrhizobium*, *Bacteroides* and *Vibrio*, increased significantly with a decrease in body size or EHP infection severity ($P < 0.05$). These findings suggest that changes in the intestinal microbiota occur depending on the severity of EHP infection.