# Microbial community composition associated with early developmental stages of the Indian white shrimp, *Penaeus indicus*

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# Abstract

Gut microbiota is known to influence the physiology, health, nutrient absorption, reproduction, and other metabolic activities of aquatic organisms. Microbial composition can influence intestinal immunity and are considered as health indicators. Information on gut microbial composition provides potential application possibilities to improve shrimp health and production. In the absence of such information for *Penaeus indicus*, the present study reports the microbial community structure associated with its early developmental stages. Bacterial community associated with the early developmental stages (egg, nauplii, zoea, mysis, postlarvae-1, postlarvae-6 and postlarvae-12) from two hatchery cycles were analysed employing 16S rRNA high throughput sequencing. *Proteobacteria* and *Bacteroidetes*, were the two dominant phyla in *P. indicus* development stages. Sequential sampling revealed the constant change in the bacterial composition at genus level. *Alteromonas* was dominant in egg and nauplii stage, whilst *Ascidiaceihabitans* (formerly *Roseobacter*) was the dominant genera in both PL6 and PL12. The bacterial composition was highly dynamic in early stages and our study suggests that the mysis stage is the critical phase in transforming the microbial composition and it gets stabalised by early post larval stages. This is the first report on the composition of microbiota in early developmental stages of *P. indicus*. Based on these results the formation of microbial composition seems to be influenced by feeding at early stages. The study provides valuable information to device intervention strategies for healthy seed production.


# Introduction

Complex symbiotic microbiota inhabits the intestine and is known to play significant role in the host physiology, health, nutrient absorption, reproduction, and other metabolic activities in terrestrial and aquatic animals [1–6]. Diversity of gut bacterial communities shift significantly during early growth stages and stabilizes later [7], and the abundance of certain group of bacteria can be considered as “health or disease indicators” [8]. Studies characterizing the colonization of gut microbiota in aquatic animals [7, 9, 10], and the influence of a bacterial community or an individual bacterial species, on intestinal immunity [1, 2, 11] have been reported recently. However, very few have attempted to describe the dynamics of microbes in the early developmental stages including egg, nauplii, zoea, mysis and early post larvae of shrimp when they are vulnerable to diseases.

Information on microbiota provides potential application possibilities to improve the production of food animals. Recent studies have reported the formation of microbiota, which get stabilized by post larval stages (PL5-PL15), indicating the role of early feeding, food and feeding habits, feed additives in shaping the gut microbiome in penaeid shrimp *P. monodon* [9] and *P. vannamei* [12]. Recently, we have reported the gut microbial composition in adult *P. indicus* obtained from wild and farmed environments [13]. However, information on the formation of microbiota in the early developmental stages of Indian white shrimp, *P. indicus* is not available.

Shrimp farming is a major aquaculture activity in several south-Asian and south American nations including India [14, 15]. *P. indicus* is endemic to Indo-West Pacific and Middle Eastern regions [16] and suggested as an indigenous complimentary species [17] alongside *P. vannamei*, a globally dominant aquaculture species. Infections due to bacterial pathogens is considered as a major challenge in *P. indicus* seed production in hatcheries requiring scientific intervention for economical sustainability. Understanding the dynamics of establishment of species-specific microbiota will help in devising intervention strategies to produce healthy shrimp seeds. The present study reports the investigation of gut microbial communities from early developmental stages (egg to post larva) of *P. indicus* using 16S rRNA high throughput sequencing.

# Materials And Methods

### Shrimp seed production and sampling

Indian white shrimp (*Penaeus indicus*) larvae were produced in two full-sib batches at Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. Healthy brooders were shifted to spawning room and maintained in UV sterilized seawater (35 ppt) in 250L tanks. Post spawning, the eggs were disinfected (Povidone-iodine 100 ppm) and allowed to hatch in 500L tanks. After hatching, the healthy phototactic nauplius was collected and stocked in larval rearing tanks (100 no/L). The first feeding was started from zoea-1 stage with live *Thalassiosira* sp. (40-80,000 cells/ml) till post larvae. The post-larval stages were fed with brine shrimp nauplius (*Artemia*) at 4-5 no/PL. Ten precent water exchange was carried out daily from mysis stage and no antibiotic, probiotic was added throughout the study. Samples from both the batches were collected at egg, nauplii, zoea (Z2), mysis (M2), PL1, PL6 and PL12 stages (Fig. 1). Due to the small size of shrimp larvae, and to avoid individual bias, pooled samples (Egg: 200; Nauplii: 200; Zoea: 100; Mysis: 100; PL1: 50; PL6: 50; PL12: 25) were collected and were washed in sterile NaCl solution for 2-3 minutes and processed immediately for DNA extraction in both the batches.

*DNA extraction, amplification, and sequencing of 16S rRNA (V3-V4 region)*

Whole shrimp pooled larval samples (Approximately: Egg: 200; Nauplii: 200; Zoea: 100; Mysis: 100; PL1: 50; PL6: 50; PL12: 25) were homogenised using sterile pestle for DNA extraction. Genomic DNA was extracted using the QIAamp DNA stool mini kit (Qiagen, Germany) following the manufacturer's protocol. The DNA concentration and purity were determined using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA) and stored at -80 $^{o}$C until used.

The amplicon libraries were prepared using the Nextera XT index kit (Illumina Inc. USA) as per metagenomic sequencing library preparation protocol. Amplification of bacterial 16S rRNA gene was carried out using the primers F GCCTACGGGNGGCWGCAG and R ACTACHVGGGTATCTAATCC. The amplicon libraries were purified by AMPPure XP beads and quantified using Qubit Flurometer. Library QC was checked on Agilent 4200 Tape station system and sequenced using MiSeq platform.

### Amplicon sequence processing and microbiome analysis

The raw sequences were processed using MOTHUR pipeline (v. 1.42) [18] to filter reads, create contigs; reduce noise as per standard MiSeq procedure. Sequences were aligned, clustered, and identified taxonomically with SILVA database (http://arb-silva.de) release 138. Chimera.vsearch option was used to identify and remove chimeras. Sequences with 97% identity threshold were classified into operational taxonomic units (OTUs) at genetic distances of 0.03. Alpha diversity indexes were calculated from rarefied samples using calculators for richness and diversity indices of the bacterial community (*i.e.*, Chao, ACE, and Shannon). Statistically significant correlation with the coordinates was tested for the PCoA plot ($p < 0.05$). Venn diagram was prepared using a web-based tool, interactiVenn [19]. Non-parametric t-test was carried out using linear discriminant analysis (LEfSe) to determine the significantly differing OTU's between the groups. The data output was subjected to detailed statistical and meta-analysis using MicrobiomeAnalyst [20] and Microsoft Excel for visualisation.

*Data availability*

The datasets of 16S rRNA amplicon sequences obtained in this study were submitted to the NCBI BioProject ID (**PRJNA718452**).

## Results

### Overview of sequencing analysis

A total of 2541888 reads were obtained from 14 samples, with mean reads of 181563 per sample. A total of 1221 OTU's were obtained in the study. Good's coverage of >99%, indicated the sufficient sequencing depth to represent all the bacterial communities. Alpha-diversity indices (Ace, Chao 1, and Shannon) of different developmental stages of Indian white shrimp were calculated. Ace and Chao 1 are richness indicators, which measures different kinds of organisms present. The richness index Chao1 varied from 579.86±22.25 to 841.22±16.82 and ACE varied from 564.56±11.72 to 828.70±14.17. Our observations suggested that the richness of microbes steadily increased from egg till mysis and gradually stabalised in post larval stages though there was an increase in PL12 stage. However, Shannon is a diversity indicator, which takes into account the richness of the microbes and how evenly they are distributed. The diversity index Shannon showed that the mysis stage was more diverse and PL12 was less diverse inspite of having increased richness (**Table 1**).

### Bacterial diversity and composition

*P. indicus* developmental stages microbiome contained a diverse community composition characterized into 21, 34, 29, 29, 27, 30 and 33 phyla, which were further classified to 243, 586, 584, 542, 457, 491 and 566 genera for egg, nauplii, zoea, mysis, PL1, PL6 and PL12 respectively (Table S1). The major phyla associated with the early developmental stages of *P. indicus* were *Proteobacteria, Bacteroidetes, Actinobacterota, Firmicutes, Planctomycetes and*

*Verrucomicrobiota*. *Proteobacteria* and *Bacteroidetes* were the top two dominant bacterial phyla in all the developmental stages of the shrimp and the rest varied in the abundance and prevalence (Fig. 2).
The *Proteobacteria* was the most dominating phylum in all the developmental stages studied. Nearly half of the phyla belong to *Proteobacteria* till larvae reach mysis stage, which further increased to more than 60% during the PL stages. *Bacteroidetes* was the second most dominating between 22-35% in the early stages, which further reduced to about 10% during the late post larval stages (Table 2).

## Bacterial community associated with different developmental stages

The most abundant genera from the most abundant phyla (*Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes*) were compared in all the developmental stages (Fig. 3). *Alteromonas* was the most dominant genera in egg (19.67%) and nauplii (12.55%). *Winogradskyella* (12.64%), *Mesoflavibacter* (10.97%), *Haloferula* (5.23 %) and *Rhodobacteraceae*_unclassified (4.61%) were dominant in egg; while *Nautella* (9.02%), *Tenacibaculum* (5.94%), *Vibrio* (5.64%) and *Pseudoalteromonas* (5.42%) were observed in nauplii. The top five bacterial genera in zoea stage were *Roseibacillus* (12.85%), *Saprospiraceae*_unclassified (6.92%), *Rubinisphaeraceae*_unclassified (6.73%), *Rhodobacteraceae*_unclassified (6.44%) and uncultured (5.29%); while uncultured (8.17%), NS3a_marine_group (6.59%), *Pseudoalteromonas* (6.40%), *Rhodobacteraceae*_unclassified (5.90%), *Kordiimonas* (5.24%) were dominant in mysis. *Rhodobacteraceae*_unclassified was found to be common dominant genera between zoea and mysis. In PL1, *Vibrionaceae*_unclassified (14.77%), *Kordia* (11.01%), *Lactobacillus* (3.60%), *Vibrio* (7.35%), *Pseudomonas* (6%) were present. *Pseudomonas* started increasing in postlarvae stages (PL1 (6%), PL6 (13.51%) and PL12 (13.05%); while *Ascidiaceihabitans* was common in PL6 (19.95%) and PL12 (19.05%), *Gammaproteobacteria*_unclassified (PL6: 14.01%, PL12: 11.20%) and *Pseudarthrobacter* (PL6: 4.19%, PL12: 6.01%) were also observed to be common between PL6 and PL12 (Fig. 4, Table 2).

## Comparison of the bacterial community structures

Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was used at the feature level to compare the composition of bacteria associated with *P. indicus* at early developmental stages (Fig. 5). The bacterial communities associated with shrimp were different across all developmental stages. However, the bacterial profiles in PL6 and PL12 were not significantly different as observed in β diversity analysis. The results indicate that the bacterial compositions became stable once they enter post larval stage. PERMANOVA analysis, confirm that bacterial profiles were strongly associated with shrimp developmental stages (F-value: 2.1962; R-squared: 0.65308; p-value $< 0.005$).

## Shared bacterial community associated with shrimp at early life stages

Venn diagram showed 70 shared genera that were associated with all the developmental stages (Fig. 6). The shared genera were classified to phyla *Proteobacteria, Bacteroidetes*, *Planctomycetes, Actinobacteria,* *Firmicutes, Cyanobacteria, Chloroflexi* and *Bdellovibrionota* mainly dominated by *Proteobacteria.* Further, unique genera specific to egg (6), nauplii (65), zoea (44), mysis (30), PL1 (17) and PL12 (57) were observed in the study. Linear discriminant analysis effect size (LEfSe) was performed to identify the specific taxa significantly varied in abundance in early developmental stages. In total, 34 genera, varying significantly were identified with LDA scores >4 (Fig. S1).

# Discussion

It is increasingly evident that host-microbiota interaction plays a major role in growth and developmental stages of both terrestrial and aquatic animals.  Co-evolution of host and microbes has always been overlooked during the evolution of aquatic organisms [10]. Further, it was always considered that the embryo/ egg is sterile and has no microbiota. However, with the advancement of technology, the presence of bacteria in the eggs of Cod and Halibut was reported previously [21] and it is well understood that the microbiota is transferred vertically and horizontally. Recent studies in *P. monodon* [9], *P. vannamei* [8, 12], *Macrobrachium rosenbergii* [10]; *Procambarus clarkii* [22], *Panulirus ornatus* [23] have shown the presence of microbiota in the eggs and early developmental stages of these aquatic crustaceans. Understanding these host-microbial community relationship will help in devising intervention strategies for healthy shrimp production. Here, we report the microbiota associated with the early developmental stages of *P. indicus* for the first time employing high throughput sequence analysis.

Analysis of 16S rRNA remains the standard culture-independent approach to investigate microbial diversity [24]. Alpha diversity is a measure of compositional complexity within an ecosystem. Ace and Chao 1 are richness indicators, which measures different kinds of organisms present. While, Shannon is diversity indicator, which takes into account the richness of the microbes and how evenly they are distributed. Our observations suggested that the richness of microbes steadily increased from egg till mysis and gradually stabalised in post larval stages though there was an increase in PL12 stage. The diversity index Shannon showed that the mysis stage was more diverse and PL12 was less diverse inspite of having increased richness. The observed richness in PL12 might be due to the presence of some rare microbes which are not evenly distributed. While, the zoea and mysis stages was highly diverse, which might be due to the initiation of feeding during these stages. Several studies tracking gut microbial composition during developmental stages have implicated the feeding to be the cause of bacterial community changes [1, 9, 12]. PCoA plot showed that samples belonging to PL6 and PL12 from full sib families clustered together indicating similar diversity. Interestingly, the bacterial profile at each developmental stage varied between the families as the growth progress. Further studies with large number of full-sib batches and multiple replicates could explain the influence of family and individual on bacterial profile.

The present study identified 70 shared genera, classified to phyla *Proteobacteria, Bacteroidetes*, *Planctomycetes, Actinobacteria, Firmicutes, Cyanobacteria, Chloroflexi* and *Bdellovibrionota,* during the developmental stages of *P. indicus*. *Proteobacteria* and *Bacteroidetes* were the top two dominant bacterial phyla associated with all the developmental stages, while *Actinobacterota, Firmicutes,*

*Planctomycetes and Verrucomicrobiota* varied in prevalence and abundance.
*Proteobacteria* and *Bacteroidetes* are considered as resident microbiota of several aquatic organisms such as *Neocaridina denticulate* [25], *P. chinensis* [26], *P. vannamei* [12], *P. monodon* [27-29]. Angthong et al. [9], Liu et al. [10] and Wang et al. [12] reported that *Proteobacteria* and *Bacteroidetes* are the dominant phyla in the early developmental stages in in *P. monodon, M. rosenbergii* and *P. vannamei* respectively in agreement with the present study.

Each developmental stages showed the dominance of different microflora indicating the role of host factors in shaping the microbiota of an organism. *Alteromonas* (19.67% and 12.55%) were the major bacterial genera in egg and nauplii, which diminished in later stages. The symbiotic association of *Alteromonas* sp with the embryo of marine crustacean *Palaemon macrodactylus*, protecting from fungal infection by producing antifungal metabolite was reported [30]. The dominance of genus *Alteromonas* in the egg and nauplii of *P. indicus* suggest similar role during the developmental stage. Further, *Vibrio* was observed in all the stages, but was abundant in PL1 stage. Studies have shown that *Vibrio* is commonly associated with several aquatic organisms and our observations agree with the observations of Angthong et al. [9] in the developmental stages of *P. monodon*. Initiation of feeding during the zoea and mysis stages may be the reason for the observed colonisation by several group of microbes without being dominated by any specific group. *Ascidiaceihabitans* (formerly *Roseobacter*) was the dominant genera in both PL6 and PL12. *Roseobacter* are reported to be associated with the gut of several aquatic organisms and have essential metabolic capabilities to utilize proteins [31, 32], which help in growth of an organism. Recently, several species of *Roseobacter* are reported to have probiotic activity [33].

The dominant association of genera *Winogradskyella and Mesoflavibacter* under phylum *Bacteroidetes* with egg stage was observed, however their role in the development of larvae is not known. *Tenacibaculum* is normally associated with the marine algae [34] and the presence of these genera in various stages of shrimp development may be associated with the planktonic feed provided in the hatchery. *Kordia* was dominant during PL1 stage, and it is known to utilize polysaccharides originated by marine phytoplankton or algae [35] and it is reported to increase in the presence of algal bloom and utilize the organic matter. Further, the genus under *Actinobacterota, Firmicutes, Planctomycetes and Verrucomicrobiota* were also reported. In a similar study in *M. rosenbergii,* strong association of *Proteobacteria, Bacteroidetes and Actinobacterota* was observed [10], while *Planctomycetes and Verrucomicrobiota* were present in relation to feeding and environment. Similarly, the shift in the microbial dynamics at different life stages has been reported from other invertebrates like butterfly, silkworm [36, 37]. Our study suggests that the mysis stage is the critical phase in transforming the microbial composition and gets stabalised by post larval stages. During the early stage, shrimp nauplii develop using yolk. The possible entry of microbial community associated with early feeding in addition to other metabolic activities in the gut might be the reason for the observed changes in the bacterial community. Similar observation in *P. monodon* was suggested to be due to the influence of host physiology and diets [9]. Further studies including a greater number of replicates are required for enhanced resolution of the bacterial diversity specific to each developmental stage of the species.

To our knowledge this is the first report on the characterization of microbiota associated with early developmental stages of *P. indicus*. The bacterial composition was highly dynamic, and stage specific dominance of genera was observed. Modulation of gut microbial composition through supplementation of beneficial microbes at the mysis stage might improve survival and help in healthy and disease-free shrimp seed production. Further studies including multiple families is required to confirm the influence of external feeding on the microbial diversity.

# Declarations

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### Author contributions:

C.P.B, P.K.P, and V.T.N: planning of the study, P.K.P: critical evaluation and fund mobility. V.T.N, A.R, and S.A.P.S: Shrimp seed production and larval rearing, V.T.N and V.B: Sampling and Sequencing. V.T.N: Metagenomic analysis and data interpretation. P.K.P and V.T.N wrote the manuscript, C.P.B: reviewed and provided valuable inputs to the manuscript. All authors have read and approved the final manuscript.

**Availability of Data and Material:** The original contributions presented in the study are included in the article/Supplementary Information; further inquiries can be directed to the corresponding author.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Tables

Table 1. Alpha diversity indices for the sequence libraries of *P. indicus* larval stages

| Developmental stage | Chao | ACE | Shannon |
|---|---|---|---|
| **Egg** | 579.86±22.25 | 564.56±11.72 | 3.81±0.02 |
| **Nauplii** | 662.61±20.19 | 789.84±11.93 | 4.29±0.12 |
| **Zoea** | 830.5±16.87 | 816.72±14.01 | 4.17±0.21 |
| **Mysis** | 841.22±16.82 | 828.70±14.17 | 4.37±0.17 |
| **PL1** | 627.08±26.06 | 594.20±11.51 | 3.56±0.42 |
| **PL6** | 603.49±25.50 | 586.23±11.14 | 3.38±0.47 |
| **PL12** | 706.53±33.85 | 816.72±12.44 | 3.35±0.32 |

Table 2. Most abundant microbiota at early developmental stages of *P. indicus.*

| Shrimp developmental stage | Phylum | Relative abundance (%) | Genus | Relative abundance (%) |
|---|---|---|---|---|
| **Egg** | *Proteobacteria* | 51.20 | *Alteromonas* | 19.67 |
| | | | *Alteromonadaceae_unclassified* | 7.65 |
| | | | *Rhodobacteraceae_unclassified* | 4.62 |
| | | | *Nautella* | 4.60 |
| | | | *Kordiimonas* | 3.03 |
| | | | *Pseudoalteromonas* | 2.92 |
| | | | *Shimia* | 2.49 |
| | | | *Vibrio* | 1.36 |
| | *Bacteroidota* | 35.15 | *Winogradskyella* | 12.64 |
| | | | *Mesoflavibacter* | 10.97 |
| | | | *NS11-12_marine_group_ge* | 3.74 |
| | | | *Flavobacteriaceae_unclassified* | 2.76 |
| | | | *Muricauda* | 2.46 |
| | *Verrucomicrobiota* | 5.52 | *Haloferula* | 5.23 |
| | *Firmicutes* | 2.51 | * | |
| | *Myxococcota* | 1.99 | * | |
| **Nauplii** | *Proteobacteria* | 71.234 | *Alteromonas* | 12.55 |
| | | | *Nautella* | 9.02 |
| | | | *Vibrio* | 5.64 |
| | | | *Pseudoalteromonas* | 5.42 |
| | | | *Vibrionaceae_unclassified* | 3.53 |
| | | | *Rhodobacteraceae_unclassified* | 3.42 |
| | | | *Thalassotalea* | 3.20 |
| | | | *Hyphomonadaceae_unclassified* | 3.17 |
| | | | *Pseudomonas* | 3.00 |
| | | | *Kordiimonas* | 2.56 |
| | | | *Shimia* | 2.50 |
| | *Bacteroidota* | 21.81 | *Tenacibaculum* | 5.94 |
| | | | *Flavobacteriaceae_unclassified* | 2.75 |
| | | | *Mesoflavibacter* | 2.53 |
| | *Firmicutes* | 1.59 | * | |
| | *Actinobacteriota* | 1.08 | * | |
| | *Desulfobacterota* | 0.83 | * | |
| **Zoea** | *Proteobacteria* | 49.38 | *Rhodobacteraceae_unclassified* | 6.44 |
| | | | *Gammaproteobacteria_unclassified* | 4.90 |
| | | | *Shimia* | 4.06 |
| | | | *Alteromonas* | 3.88 |
| | | | *Pseudoalteromonas* | 3.29 |
| | | | *Neptuniibacter* | 2.45 |
| | | | *Marivita* | 2.35 |
| | | | *Nautella* | 2.34 |
| | *Bacteroidota* | 22.33 | *Saprospiraceae_unclassified* | 6.92 |
| | | | *Tenacibaculum* | 4.02 |
| | | | *Flavobacteriaceae_unclassified* | 1.51 |
| | *Verrucomicrobiota* | 14.06 | *Roseibacillus* | 12.85 |
| | *Planctomycetota* | 8.73 | *Rubinisphaeraceae_unclassified* | 6.74 |
| | *Margulisbacteria* | 1.67 | *Margulisbacteria_ge* | 1.68 |
| **Mysis** | *Proteobacteria* | 51.62 | *Pseudoalteromonas* | 6.40 |
| | | | *Rhodobacteraceae_unclassified* | 5.90 |
| | | | *Kordiimonas* | 5.24 |

| | | | | |
|---|---|---|---|---|
| | | | *Gammaproteobacteria_unclassified* | 3.92 |
| | | | *Grimontia* | 3.90 |
| | | | *Alphaproteobacteria_unclassified* | 3.18 |
| | | | *Vibrio* | 2.55 |
| | *Bacteroidota* | 31.89 | *NS3a_marine_group* | 6.59 |
| | | | *Gaetbulibacter* | 4.19 |
| | | | *Balneola* | 2.90 |
| | | | *Spongiimonas* | 2.37 |
| | | | *Fluviicola* | 2.00 |
| | *Actinobacteriota* | 4.98 | *Ilumatobacteraceae_unclassified* | 3.35 |
| | *Planctomycetota* | 4.66 | *Pirellula* | 1.85 |
| | *Verrucomicrobiota* | 3.03 | * | |
| **PL1** | *Proteobacteria* | 58.31 | *Vibrionaceae_unclassified* | 14.77 |
| | | | *Vibrio* | 7.35 |
| | | | *Pseudomonas* | 6.01 |
| | | | *Rhodobacteraceae_unclassified* | 5.21 |
| | | | *Marinicella* | 4.30 |
| | | | *Ascidiaceihabitans* | 1.97 |
| | | | *Pseudoalteromonas* | 1.83 |
| | | | *Acetobacter* | 1.59 |
| | | | *Gammaproteobacteria_unclassified* | 1.59 |
| | | | *Shimia* | 1.57 |
| | | | *Hyphomonadaceae_unclassified* | 1.48 |
| | *Bacteroidota* | 16.27 | *Kordia* | 11.01 |
| | *Firmicutes* | 12.19 | *Lactobacillus* | 9.60 |
| | *Actinobacteriota* | 4.90 | * | |
| | *Planctomycetota* | 4.40 | *Planctomycetota_unclassified* | 3.68 |
| **PL6** | *Proteobacteria* | 66.54 | *Ascidiaceihabitans* | 19.95 |
| | | | *Gammaproteobacteria_unclassified* | 14.01 |
| | | | *Pseudomonas* | 13.52 |
| | | | *Acinetobacter* | 3.53 |
| | | | *Rhodobacteraceae_unclassified* | 1.72 |
| | | | *Pseudoalteromonas* | 1.69 |
| | | | *Vibrio* | 1.45 |
| | *Bacteroidota* | 9.69 | *Tenacibaculum* | 2.34 |
| | | | *Prevotella* | 1.21 |
| | *Actinobacteriota* | 8.48 | *Pseudarthrobacter* | 4.19 |
| | | | *Rhodococcus* | 1.99 |
| | *Firmicutes* | 6.99 | *Lactobacillus* | 1.10 |
| | | | *Staphylococcus* | 1.08 |
| | *Planctomycetota* | 1.68 | * | |
| **PL12** | *Proteobacteria* | 63.51 | *Ascidiaceihabitans* | 19.06 |
| | | | *Pseudomonas* | 13.05 |
| | | | *Gammaproteobacteria_unclassified* | 11.20 |
| | | | *Vibrio* | 6.70 |
| | | | *Rhodobacteraceae_unclassified* | 1.77 |
| | | | *Vibrionaceae_unclassified* | 1.52 |
| | | | *Pseudoalteromonas* | 1.26 |
| | *Bacteroidota* | 10.39 | *Tenacibaculum* | 2.47 |
| | | | *Proteiniphilum* | 1.41 |
| | | | *Prevotella* | 1.01 |
| | *Actinobacteriota* | 10.27 | *Pseudarthrobacter* | 6.02 |
| | | | *Rhodococcus* | 1.80 |

| | | | |
|---|---|---|---|
| *Firmicutes* | 7.00 | *Lactobacillus* | 0.92 |
| *Planctomycetota* | 1.89 | * | |

Note: (*); Represented by many genera in lower abundance.

# Figures

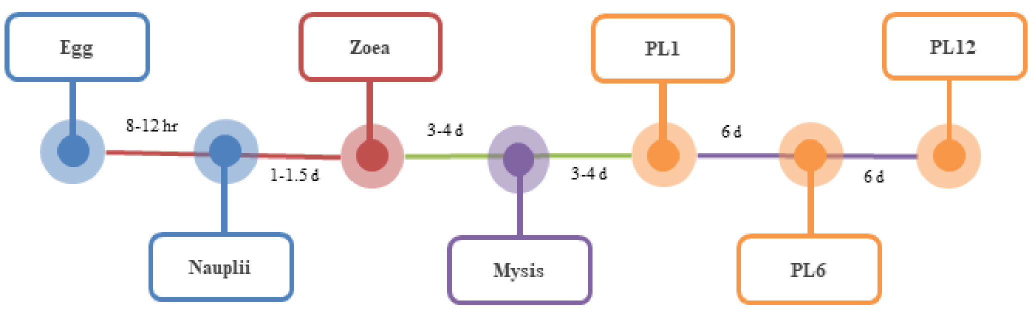


Fig 1

## Figure 1

Schematic diagram showing the sampling points during *P. indicus* hatchery cycle.

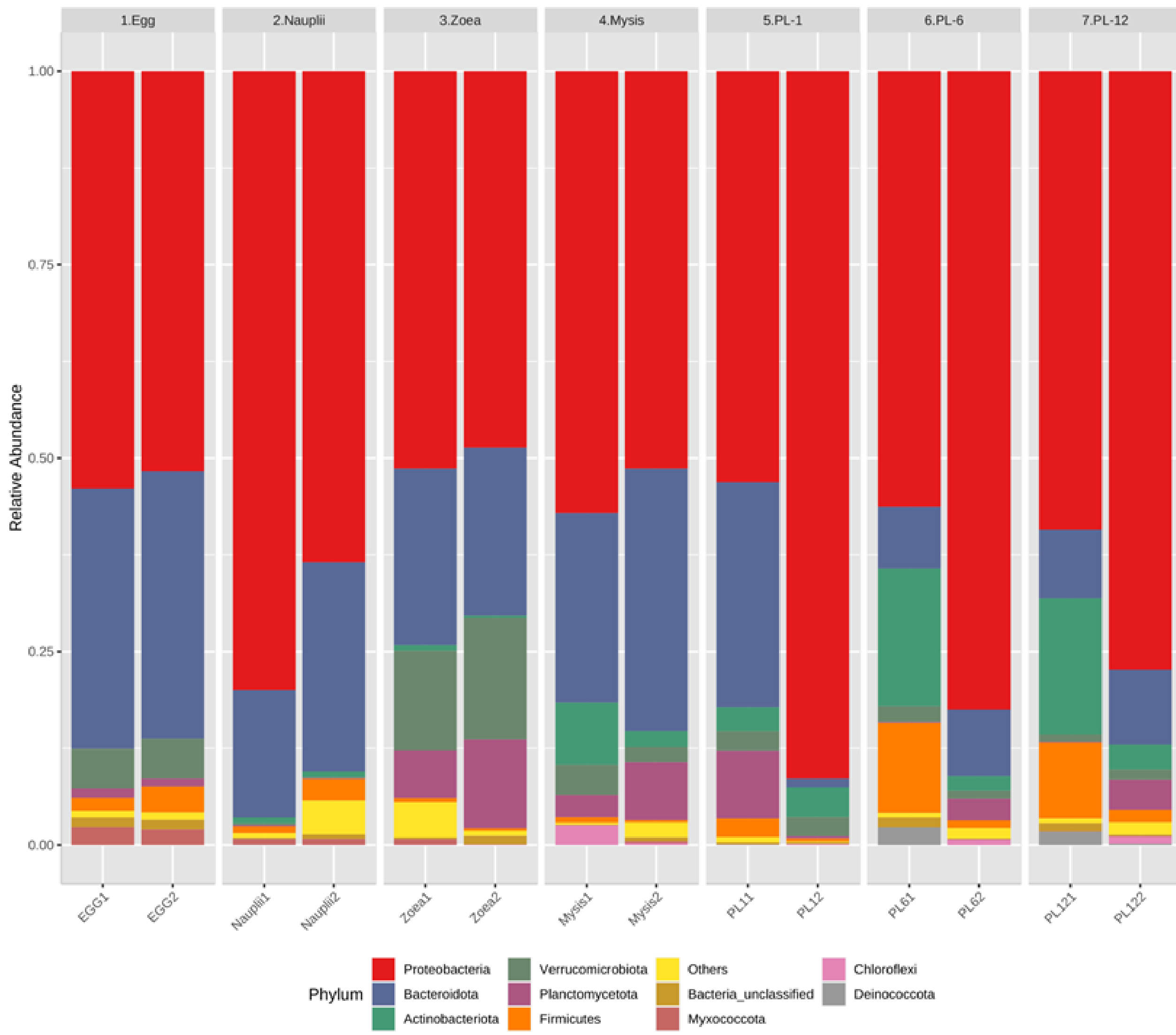


Fig 2

## Figure 2

Distribution of bacterial phyla (Relative abundance) in various developmental stages of *P. indicus.*

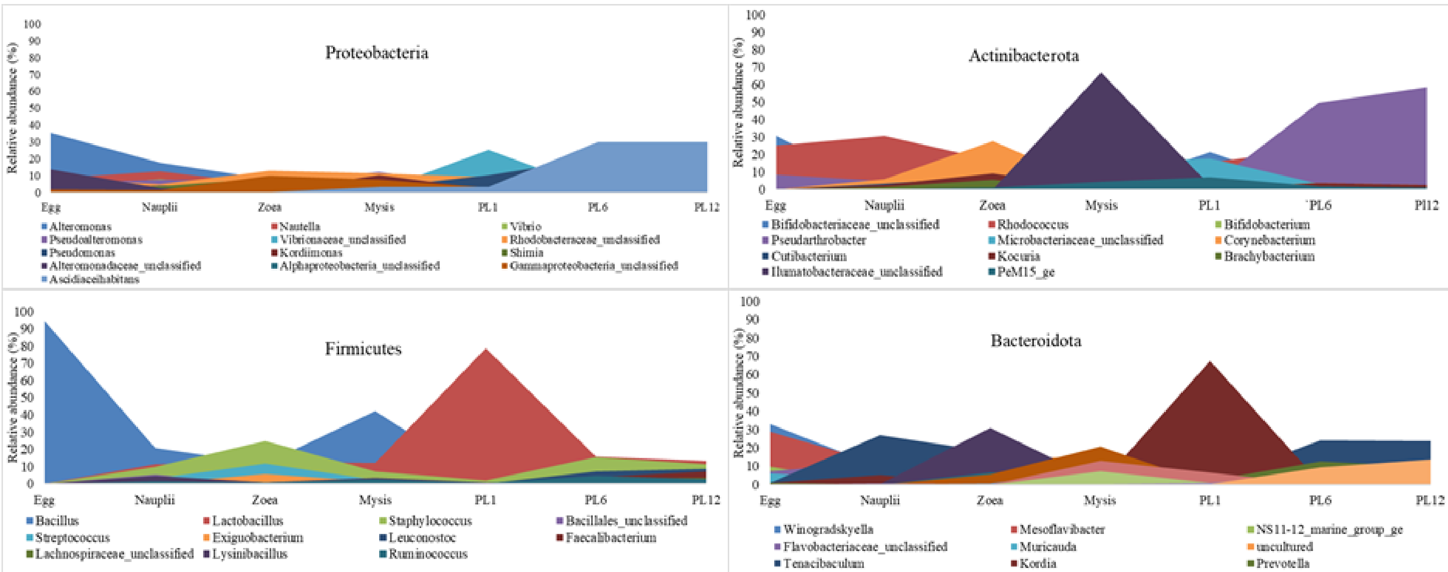


Fig 3

## Figure 3

Relative abundance of dominant genus under major phylum in various developmental stages of *P. indicus.*

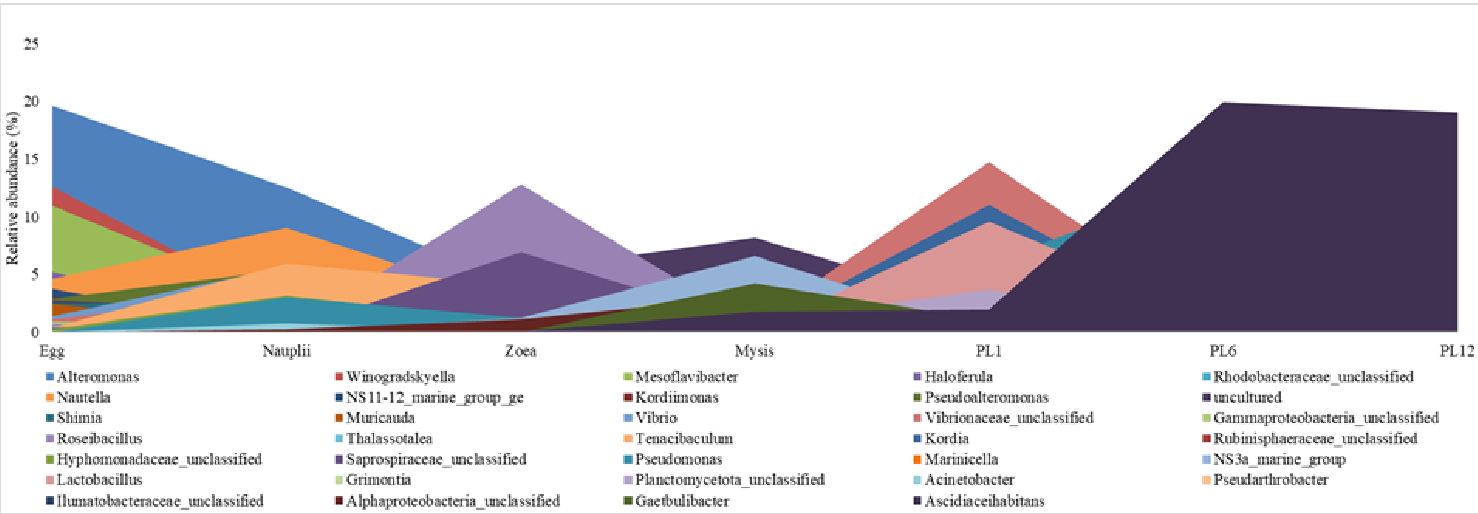


Fig 4

## Figure 4

Dynamics of dominant genera in in various developmental stages of *P. indicus.*

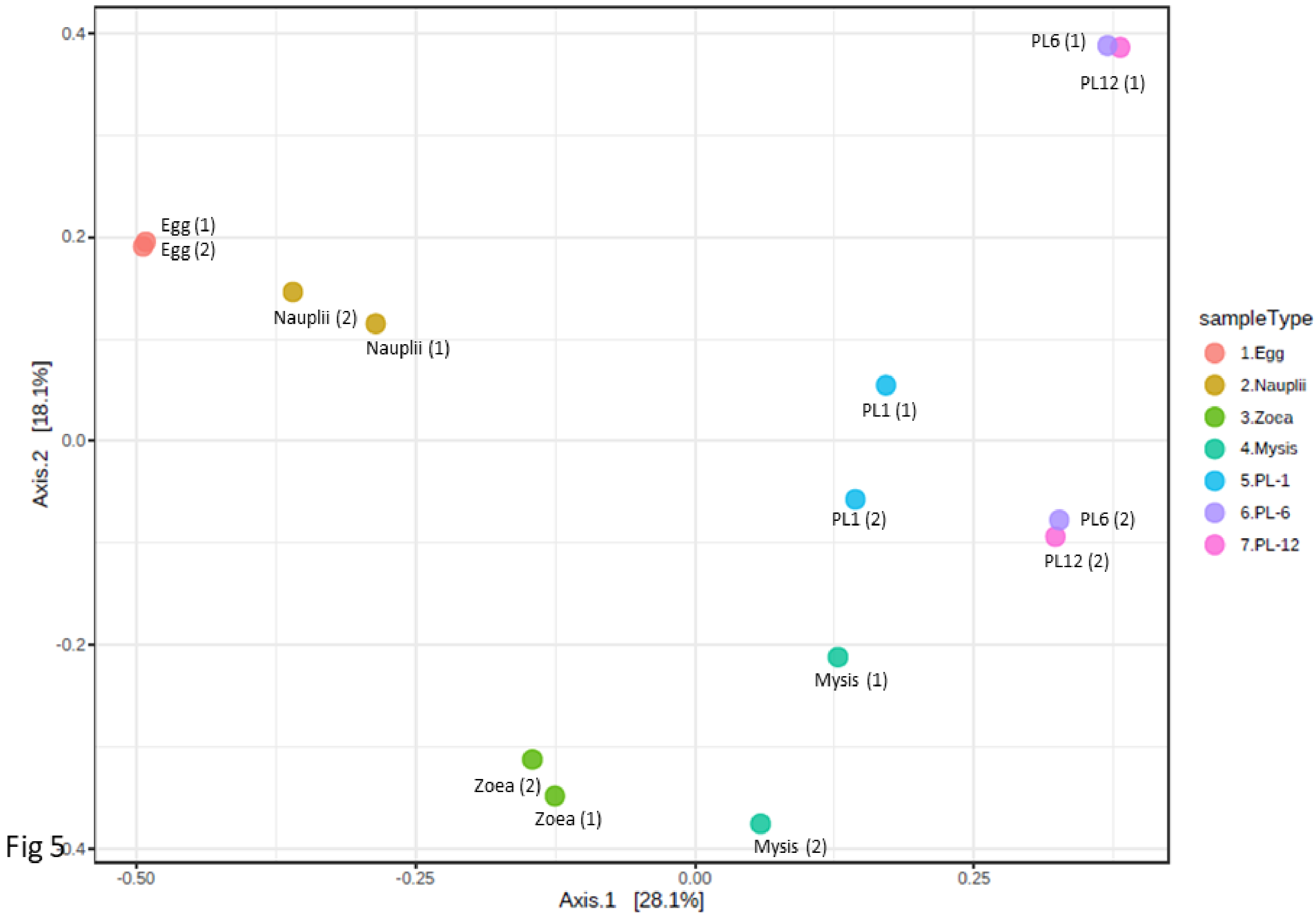


Fig 5

## Figure 5

Principal coordinates analysis (PCoA) of microbial communities associated with various developmental stages of *P. indicus* in two full sib families (Egg, Nauplii, Zoea, Mysis, PL1, PL6, PL12).

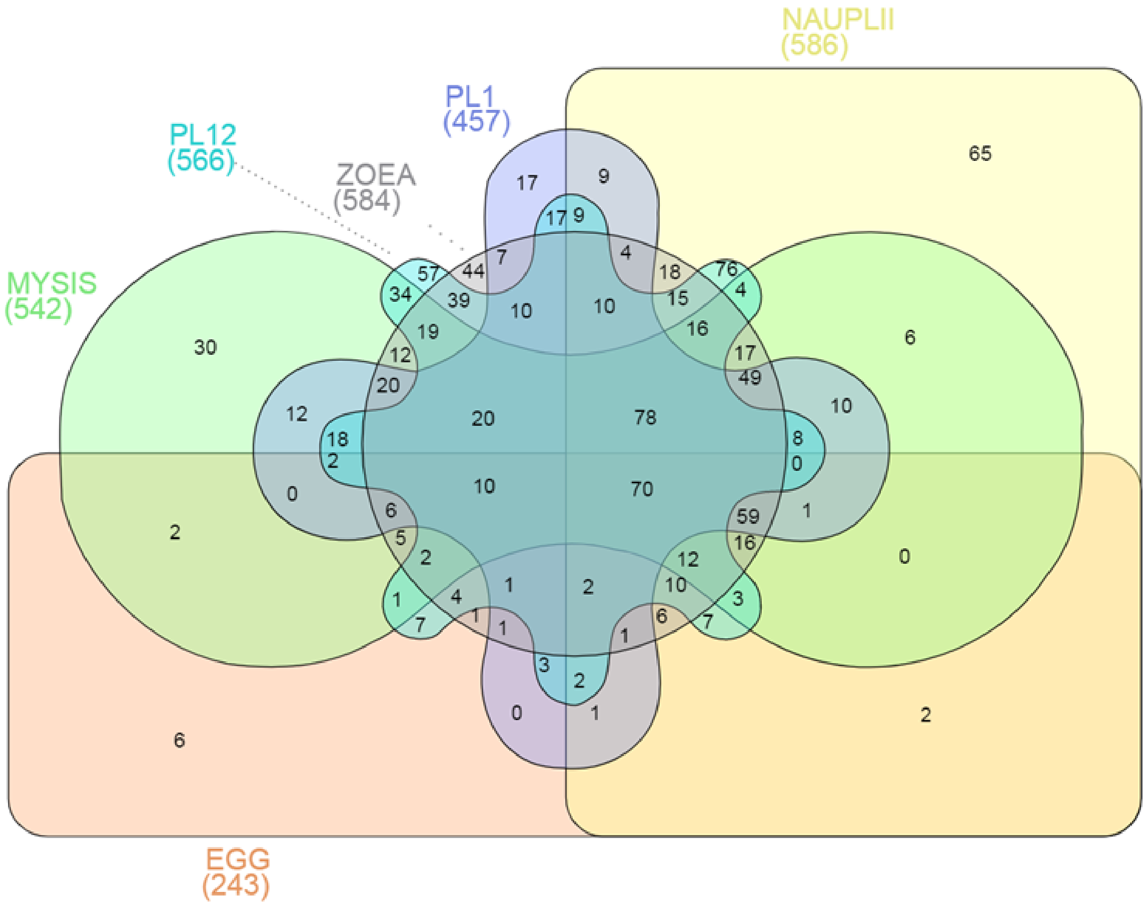


Fig 6

**Figure 6**

Venn diagram depicting the shared and unique genera among various developmental stages of *P. indicus.*

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigS1.pdf
- SupplementaryTaxonomy.xlsx