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How the intestinal microbiota of black tiger shrimp and the microbial community of polyculture pond water interact

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Results demonstrated that the intestinal microbiota of *P. monodon* and the bacterial communities in polyculture pond water are closely linked yet distinct



Study investigated the relationship between the intestinal microbiota of black tiger shrimp and the microbial community of polyculture pond water. Results demonstrated that the intestinal microbiota of *P. monodon* and the bacterial communities in polyculture pond water are closely linked yet distinct. These findings highlight a close link between water quality and microbial composition, stressing the importance of pond management to maintain ecological stability and shrimp health. Photo by Darryl Jory.

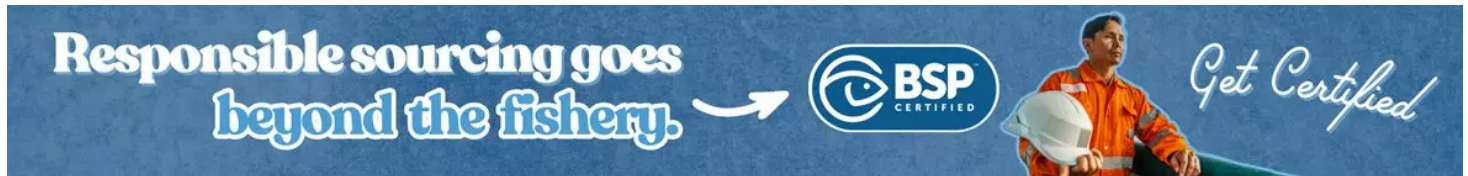
The shrimp gut microbiota is a critical determinant of host nutrition, immunity, and overall performance. These microbiota communities aid digestion, produce vitamins and enzymes, and enhance immune function. **Dysbiosis** (<https://doi.org/10.1007/s00253-024-13213-3>), or imbalance of the gut microbiota, is associated with major shrimp diseases including acute hepatopancreatic necrosis disease (AHPND), white feces syndrome (WFS) and white spot disease (WSSV). Reports from several researchers indicate that gut microbial balance is closely linked to shrimp disease resistance and production outcomes.

Environmental factors strongly influence shrimp gut microbiota. Salinity, temperature, diet, and stocking density have all been reported to shape gut bacterial communities. Developmental stage is another driver. Host genetic factors also contribute, with selective breeding lines of shrimp showing distinct gut microbial profiles. Beyond the host, interactions between gut and environmental microbiota are increasingly recognized as critical to shrimp aquaculture, and many studies underscore the need to consider host-environment microbial interactions in aquaculture management.

Reports from several researchers highlight the plasticity of the shrimp gut microbiome and the potential to harness it for health management. Manipulating shrimp microbiomes has become an emerging strategy for improving culture outcomes. **Probiotics, prebiotics and synbiotics** (<https://doi.org/10.1128/aem.02420-24>) introduced via feed or water have been shown to modulate

gut communities, enhance immunity, and improve feed efficiency. The microbiome itself has been proposed as a **determinant of aquaculture productivity** (<https://doi.org/10.1186/s40168-020-00802-3>), with specific bacterial taxa correlating with growth performance.

Despite many advances, little is known about the gut-water microbiota relationship in black tiger shrimp (*Penaeus monodon*), particularly under polyculture pond systems. Polyculture practices, where shrimp are co-reared with fish or other aquatic organisms, create complex microbial milieus that may affect both water quality and host microbiota. To date, few studies have explicitly examined gut and water microbial communities concurrently in *P. monodon* under polyculture. Addressing this gap is essential for understanding microbial ecology in traditional pond systems and for developing microbiome-based strategies to improve shrimp health and sustainability.



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This article – **summarized** (<https://creativecommons.org/licenses/by/4.0/>) from the **original publication** (<https://doi.org/10.3390/w17223194>) (Sun, X. et al. 2025. Effects of Polyculture Patterns in Ponds on Water Quality and Intestinal Flora of *Penaeus monodon*. *Water* 2025, 17(22), 3194) – discusses a study that investigated the relationship between the intestinal microbiota of *P. monodon* and the microbial community of polyculture pond water.

Study setup

Water and shrimp samples were collected from polyculture ponds located in Jingwu Town, Xiqing District, Tianjin, China. The ponds were stocked with the commercial *P. monodon* line “Nanhai No. 1” and with koi carps (*Cyprinus carpio*). Each pond had a surface area of approximately 800 square meters and an average water depth of 1.8 meters, with a 10 percent weekly water exchange rate and continuous aeration to maintain water quality.

Shrimp were stocked at a density of 20 individuals per square meter, and *C. carpio* were stocked at 1.5 individuals per square meter. Shrimp were fed a commercial pelleted diet containing 38 percent crude protein at a rate of 5 percent of body weight per day. All ponds were managed under standard intensive aquaculture practices used in the Tianjin region.

Shrimp and water samples were collected from polyculture ponds at four time points during the rearing period. Water-quality parameters were measured, and microbial community structures were analyzed by high-throughput 16S rRNA sequencing, a method to identify and compare bacterial diversity from complex microbiomes or environments that are difficult to study.

For detailed information on the experimental design, animal husbandry and data collection and analysis, refer to the original publication.

Analysis of water quality data

From July to September, nutrient accumulation in the ponds became increasingly pronounced, despite the pH remaining within 7.5–8.1, a range considered acceptable for shrimp culture. The elevated concentrations of nitrogen and phosphorus suggest an increasing risk of eutrophication. Previous studies have established thresholds of total phosphorus (TP) for eutrophication control, for example, proposed target values of soluble reactive phosphorus under 0.05 mg per liter, and others have reported TP thresholds around 0.03–0.05 mg per liter. In the present study, the measured TP in the late stage exceeded these benchmarks, reinforcing concerns about eutrophic onset. High nitrogen and phosphorus levels can exacerbate dissolved oxygen (DO) fluctuations through enhanced primary productivity followed by increased organic matter decomposition.

In pond systems, DO depletion may stress *P. monodon*, impair growth, and favor pathogens like *Vibrio* spp. Elevated nitrogen can also alter microbial communities, as seen in coastal aquaculture with high N loading shifting bacterial responses. Our results match prior intensive shrimp culture studies showing nutrient buildup worsens water quality and disease risk later in the cycle. Recommended mitigations include sediment remediation, probiotics, and optimized feeding to improve self-purification and ecosystem stability.

Operational taxonomic unit (OUT) cluster analysis

An operational taxonomic unit (OTU) is a definition used to classify groups of closely related individuals. At the OTU level, study results showed that intestinal and water-associated microbial communities of *P. monodon* exhibited distinct clustering – a data analysis technique aimed at partitioning a set of objects into groups such that objects within the same group, called a cluster, exhibit greater similarity to one another than to those in other groups or clusters. Prior studies show shrimp gut microbiota differs markedly from pond water assemblages, forming separate clusters. Rearing water typically hosts greater microbial complexity and diversity, while the gut undergoes host-selective colonization, yielding lower diversity.

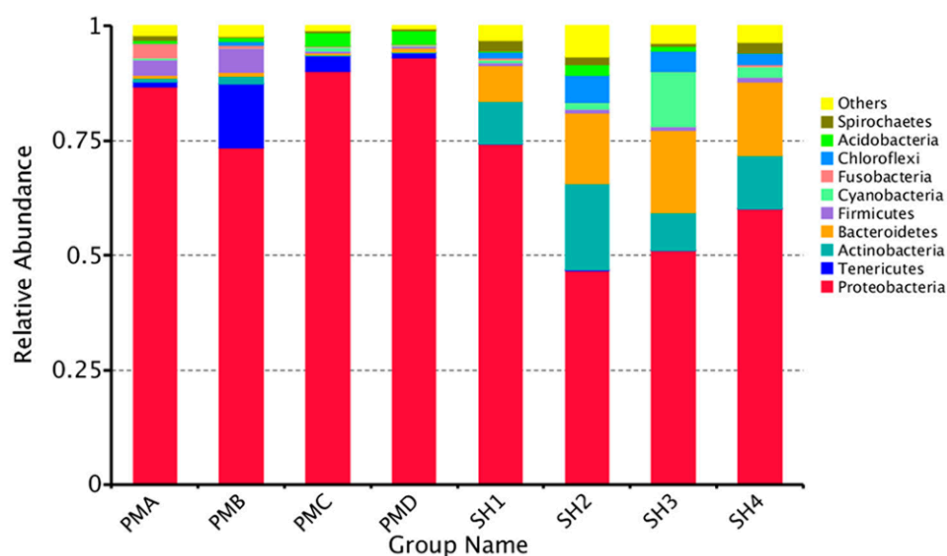


Fig. 1: Histogram of the relative abundance of top 10 species at the phylum level.

Our observed differences in OTU counts across stages likely stem from both intestinal microbial shifts and environmental nutrient dynamics. Similar nutrient-gut diversity links appear in greenhouse systems, where $\text{NH}_3\text{-N}^+$, total nitrogen and TP shape microbial composition. Shrimp under N/P stress also show oxidative stress and immune changes, indicating **indirect nutrient impacts on host-microbe interactions** (<https://doi.org/10.1111/are.14770>).

Gut and water microbiota, though separate, share common OTUs. Dominant genera persist in both, implying water serves as a colonist source. Results of the present study showed that early *P. monodon* stages shared major genera over time, with some unique to each habitat. Thus, pond environment molds gut composition, but host filtering limits successful colonists. Despite OTU-level separation, shared OTUs underscore host-environment microbial exchange. This interplay highlights the **need for nutrient and microbial balance** (<https://doi.org/10.1016/j.aqrep.2021.100948>): A healthy and balanced pond microbiome provides beneficial microbial sources for shrimp, whereas eutrophication or environmental dysbiosis can destabilize the intestinal community and impair shrimp health.

Performance of fishmeal replacement with rice protein meal in experimental diets for juvenile Pacific white shrimp



Replacing 10 percent of fishmeal with rice protein meal significantly improves digestibility, protein synthesis, antioxidant capacity and disease.



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Flora structure of shrimp intestinal and pond water

Results of the high-throughput 16S rRNA sequencing characterized the microbial communities in the intestines of *P. monodon* and in its rearing water, and revealed a clear ecological linkage between the intestinal microbiota and the surrounding pond environment. Nutrient enrichment, reflected by elevated nitrogen and phosphorus concentrations, not only increased the risk of algal blooms but also triggered cascading effects on microbial community dynamics. The accumulation of organic matter and eutrophic conditions likely enhanced bacterial proliferation and altered the taxonomic structure of both waterborne and intestinal communities, facilitating microbial exchange between habitats and reshaping their ecological interactions.

Common genera in intestinal and water samples included *Vibrio*, *Shewanella*, *Aeromonas*, *Pseudomonas* and *Actinobacteria* among the top 10 by abundance, plus shared taxa like *Hydrogenophaga*, *Photobacterium*, and *Brevinema*. *Vibrio* stood out as the most abundant and ecologically important.

Fig. 2: Histogram of relative abundance of top 30 species at the genus level.

Vibrio plays dual roles in shrimp gut: commensal under normal conditions, often enriched due to host selective pressure from nutritional landscapes, immune tolerance, or other causes. Pond water supplies the microbial pool, but host factors sculpt final structure; similar selection occurs in other crustaceans. But under stress or immune compromise, commensal *Vibrio* turns pathogenic, and is linked to AHPND and WSSV with over 90 percent dominance during dysbiosis. Stressors like low salinity, nutrient buildup, and hypoxia promote *Vibrio* growth and virulence. Conversely, beneficial commensals and immune mechanisms suppress pathogenicity. *Vibrio* is thus conditionally pathogenic: beneficial in balanced states, but dominant and disease-driving when stability falters. Therefore, sustaining water quality and intestinal homeostasis is key to curbing *Vibrio* risks in aquaculture operations for shrimp and other farmed seafood species.

Microbial diversity

Alpha diversity measures the variation of species within a particular ecosystem. The alpha diversity of shrimp gut microbiota was heavily shaped by host developmental stage and environmental factors. Various studies by other researchers have shown dynamic gut community establishment in early stages, then gradual richness and stability gains as shrimp matured. For instance, adult black tiger shrimp gut microbiota displayed higher richness and diversity than juveniles, **reflecting a more resilient ecosystem** (<https://doi.org/10.1016/j.micpath.2025.107636>). Conversely, stressors or disease can sharply curtail diversity, and **low diversity** (<https://doi.org/10.1111/1462-2920.13701>) will undermine ecological stability, functional redundancy and host resilience to pathogens and fluctuations.

A **diverse microbiota** (<https://doi.org/10.1128/spectrum.02465-21>) provides functional breadth, stability, opportunistic bacteria suppression and robust metabolic host support. Thus, high gut diversity is key to shrimp health and disease resistance. Avoiding abrupt environmental changes and using probiotics/prebiotics to preserve diversity can help **stabilize gut ecosystems** (<https://doi.org/10.1080/23308249.2018.1440530>). Overall, results showed that intestinal alpha diversity in *P. monodon* increases with development but falls under stress or disease; therefore, it follows that sustaining diversity is vital for resilience and aquaculture sustainability.

Perspectives

This study demonstrated that the intestinal microbiota of *P. monodon* and the bacterial communities in polyculture pond water are closely linked yet distinct. Water quality parameters showed gradual changes across the culture cycle, with increasing nitrogen and phosphorus compounds indicating a higher risk of eutrophication in later stages. High-throughput sequencing revealed more than 350 OTUs in both gut and water samples, with 166 OTUs shared, reflecting both environmental seeding and host selection.

Water samples exhibited higher richness and complexity, while shrimp intestines were dominated by specific taxa such as *Vibrio*, which play both commensal and opportunistic roles depending on environmental and host conditions. Alpha diversity patterns indicated that gut microbial diversity peaked during active growth stages and declined under stress or potential dysbiosis.

Together, these findings underscore the ecological importance of gut–water microbial interactions in aquaculture systems. Future research should extend sampling across seasons, employ metagenomics (the study of all genetic material from all organisms in a particular environment) or metabolomics (the study of chemical processes involving metabolites) to quantify functional pathways, and experimentally test host-microbiota-environment interactions under controlled nutrient and stress gradients. This will help elucidate microbial resilience mechanisms and guide ecological regulation for sustainable shrimp aquaculture.

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