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Review

The Chinese mitten crab (*Eriocheir sinensis*) and its microbiome: A review

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ABSTRACT

The Chinese mitten crab also known as *Eriocheir sinensis,* is an economically valuable species widely farmed in China and South-East Asia. Belonging to the subphylum Crustacea, *E. sinensis* are good examples of catadromous species that inhabit freshwater environment but migrate to saltwater for spawning. These crabs host diverse microbial communities across all major organs including gills, hepatopancreas, gut, and hemolymph. Migration, driven by environmental changes and stress significantly influence the diversity, community compositions, and function of the crab microbiome. These symbiotic microbes play crucial roles in crab health, nutrition, taste and diseases. Despite significant research on the intestinal microbiota of *E. sinensis*, the microbiome associated with other organs remains unexplored. This review, aims to consolidate current knowledge on the *E. sinensis* microbiome, examining the dynamics, functional roles in the host, and the impact of stress on the microbiota composition. Additionally, we identify knowledge gaps and explore possible solutions to enhance and preserve beneficial microorganisms, essential for optimizing aquaculture in the host practices. This review is the first comprehensive analysis of the microbiome associated with *E. sinensis* across various physiological states, providing critical insights for the sustainable management of this aquaculture species.

1. Introduction

The microbiota plays a crucial role in host health and metabolism, including regulating immunity, antagonizing pathogenic bacteria, and participating in nutrient absorption and vitamin synthesis (Bikel et al., 2015). Microbiotas are integral to various host body parts, including the intestine, hemolymph, and hepatopancreas (Fig. 1 and Table 1). The animal intestine is a complex ecosystem of microorganisms that enhance digestive efficiency, nutrient utilization, immune system function, and inhibit the adhesion of opportunistic pathogens (Rungrassamee et al., 2014; Verner-Jeffreys et al., 2003; Gallo and Nakatsuji, 2011). In crustaceans, the intestinal microbiota is vital for maintaining ecological and physiological functions (Zhang et al., 2020a, 2020b) and sustaining homeostasis and immunity (Huang et al., 2018; Qi et al., 2022). Environmental changes can lead to variability in the gut microbiome, affecting development, immunity, nutrition, and disease resistance (Sun et al., 2018). Multiple studies have shown that pollutants can disrupt the intestinal microbiota of aquatic organisms (Pelusio et al., 2020; Qiao et al., 2019), with stressors like ammonia and thermal variations altering bacterial community structures (Duan et al., 2021). For instance, imidacloprid exposure reduces beneficial bacteria and increases pathogens in the gut (Hong et al., 2020).

The hepatopancreas, critical for digestion and nutrient absorption, also plays a key role in innate immunity in invertebrates and hosts pathogens (Ceccaldi, 1989; Leaño et al., 1998; Jiang et al., 2014). Hemolymph is essential in the host immune process, and healthy crustaceans' hemolymph hosts unique microbiota (Potgieter et al., 2015; Wang and Wang, 2015). However, there is limited knowledge about the

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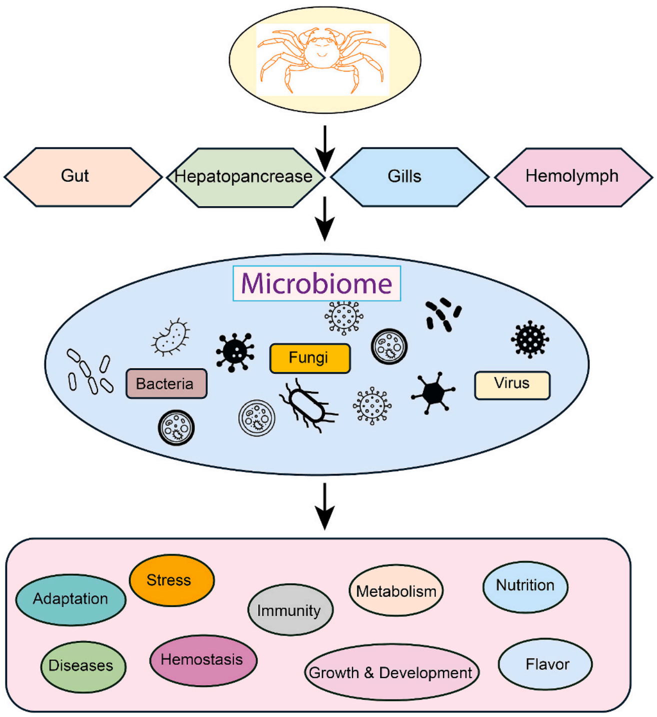

Fig. 1. Overview of the microbiome associated with the Chinese mitten crabs and their involvement in various biological processes of the host crab. It should be noted that most of these functions are mainly linked to bacterial communities because very little information is available on fungal and viral diversities and their roles. The representative orange diagram of *E. sinensis* was retrieved from Dimensions (https://www.dimensions.com/element/chinese-mitten-crab-eriocheir-sinensis).

symbiotic bacteria in the gills of *E. sinensis*, which are crucial for osmotic regulation during salinity changes (Malik and Kim, 2021; An et al., 2022). Salinity is the major factor leading to the alpha diversity and community dissimilarity of microbiome during seawater–freshwater transition (Shao et al., 2022). Recent reports suggest that gill-associated bacteria help the host adapt to dynamic intertidal ecosystems (Fusi et al., 2023). Therefore, the strategic manipulation of microbiota offers a promising avenue for optimizing aquaculture practices and ensuring the long-term sustainability and productivity of this rapidly growing sector. *E. sinensis*, commonly known as the Chinese mitten crab, is a significant component of crustacean farming due to its nutritional value, excellent taste, and cost-effective farming methods (Zhao et al., 2020). Originating from the eastern Pacific Ocean of China and Korea, *E. sinensis* has become invasive in Europe and the USA (Anger, 1991; Dittel and Epifanio, 2009; Xue et al., 2022) and endemic to parts of North America and Europe (Rudnick et al., 2005; Herborg et al., 2003). Chinese mitten crabs are one of the most economically important aquaculture species with an annual production of 808,293 tons in 2021 (FAO, 2024). These crabs are rich in protein, omega-3 fatty acids, riboflavin, and vitamin B12, making them a healthy dietary choice (Chen et al., 2007). The hepatopancreas, gonads, and muscles are the most favored edible tissues, assessed for nutritional quality based on their protein, mineral, free amino acid (FAA), and nucleotide contents (Wu et al., 2007; Nędzarek and Czerniejewski, 2021). Mature female ovaries are nutrient-rich and contain vital amino acids and vitamins, with a higher gonadal index than males (Long et al., 2020). Volatile chemicals such as pentanal, hexanal, and benzaldehyde have been identified in various edible portions (Gu et al., 2013). Crabs cultured in rice fields have shown superior antioxidant enzyme activities, mineral content, and volatile compounds compared to pond-cultured crabs, suggesting enhanced growth and economic viability in the rice-crab system (Mei et al., 2023; Zhang et al., 2017; Hu et al., 2016; Zhang et al., 2022).Chinese mitten crab not only offers significant nutritional benefits but also demonstrates enhanced growth and antioxidant capacities under specific cultivation conditions, highlighting its value in sustainable aquaculture practices (Yuan et al., 2017).

Stress from transportation and cultivation processes, such as thermal, aerial, and alkaline, alters the microbiome. The microbiota significantly contributes to host health by inhibiting pathogenic bacteria, controlling immunity, and participating in vitamin synthesis and nutrient absorption (Bikel et al., 2015). The microbiota's role in growth, health, and survival of cultured organisms has gained interest in aquaculture, including *E. sinensis* (Cornejo-Granados et al., 2017). Studies on the taxonomic and functional characteristics of the hemolymph, hepatopancreas, and gut microbiota of *E. sinensis* have enhanced understanding of symbiotic microbiota and potential health indicators (Yang et al., 2023). Exposure to different pathogens induces structural alterations in the hemolymph microbiota, providing insights into microbial responses to infections (Kong et al., 2023). *E. sinensis* harbors diverse, novel microorganisms that coexist with the host (Chen et al., 2015). Characterizing this microbial diversity is crucial for better management of *E. sinensis* aquaculture. Understanding and managing the microbiome in aquaculture environments is essential for optimizing the health, growth, and survival of cultured organisms like *E. sinensis*. Effective microbial management can mitigate the negative impacts of transportation and cultivation stress, leading to more sustainable and productive aquaculture practices.

This review aims to comprehensively summarize the current knowledge on the microbiota present in different tissues of the *E. sinensis*. By elucidating the microbial compositions and their functional roles in various tissues such as the intestine, hepatopancreas, hemolymph, and gills, this article seeks to highlight the significance of microbiota in the health and aquaculture management of *E. sinensis*. Understanding these microbial communities is essential for improving the cultivation practices and ensuring the sustainable production of this valuable crustacean.

2. Most abundant bacterial phyla in *E. sinensis*

Studying the composition of the symbiotic bacteria in *E. sinensis* and free-living bacteria in water is an important step in aquaculture. Previous studies suggest that water, gills and guts have their own bacterial patterns indicating distinct differences in the structure, composition, and predicted function of the symbiotic microbiota at these sites. Zhang et al., 2016, reported that bacterial diversity in the water in which the crabs live is higher than the diversity in gills or guts of *E. sinensis*, suggesting that aquatic animals specialize their symbiotic microorganisms relative to the environment in which they live. In their study, Proteobacteria, Actinobacteria, Bacteroidetes and Cyanobacteria were the dominant phyla in water as well as in gills (except Cyanobacteria). The OTUs (operational taxonomic units) related to *Limnohabitans* and Sporichthyaceae were dominant in water but almost absent from the gill and gut samples. Considering that Chinese mitten crabs are bottom-dwelling crustacea and *Ilumatobacter* are dominant in sediment or seashore sand, it is not surprising that *Ilumatobacter* are dominant in gills of these crabs. *Albimonas*, which was often detected in the seawater, was present in gills of crabs but almost absent from water. Saqib et al., 2023, show that the dominant phylum is Firmicutes in overall gut bacterial communities, followed by Proteobacteria, Actinobacteria, Campylobacterota and Bacteroidota. Chen et al., 2015, found that *Marinifilum fragile* (Bacteroidetes), which is a marine bacterium, is present in the gut of *E. sinensis*. These studies are consistent with earlier studies in which it was shown that Firmicutes, Bacteroidetes and Proteobacteria are the dominant phyla of the *E. sinensis* gut microbiome. Yang et al., 2023, examined the hemolymph, hepatopancreas, and intestinal microbiota of *E. sinensis*. Their results show that the gut microbiota mainly constitutes Firmicutes, Proteobacteria, and Bacteroidota, where as the hepatopancreas and hemolymph microbiota, in addition to Bacteroidota and

Actinobacteriota shows abundance of Proteobacteria, Firmicutes, and Acidobacteriota, At the genus level, *Candidatus Hepatoplasma*, *Shewanella*, and *Aeromonas* were dominant genera in the hepatopancreas, whereas *Candidatus Bacilloplasma*, *Roseimarinus*, and *Vibrio* were dominant in the intestine. In contrast, the genera *Enterobacter*, *Vicinamibacterales*, and *Pseudomonas* exhibited relatively high abundance in the hemolymph. The *Bacteroidetes* in the gut appear to be persistent within *E. sinensis* population. This group comprises three phylotypes, namely, two uncultured *Bacteroidetes* and the *Bacteroides*. With the differences in water environments, diets, and the temporal and spatial separation, the *E. sinensis* gut contain similar *Bacteroidetes*, which suggest that the *Bacteroidetes* are closely associated with their hosts (Chen et al., 2015). This significant difference in microbiota structures may be due to the different functions of microorganisms at different sites.

There has been a lot of study on migration but the distributional patterns of gut microbiota of *E. sinensis* during the seawater–freshwater migration have been rarely explored (Dehler et al., 2017; Zhang et al., 2020a). During the migration, Firmicutes greatly out numbered Proteobacteria in terms of relative abundance for the dominant phylum. It's interesting to note that during the migration, the relative abundance of Bacteroidetes exhibited a hump-shaped trend. According to Yang et al. (2016), the enrichment of Firmicutes in the intestine of *E. sinensis* may help them consume more calories and absorb more nutrients. Additionally, Bacteroidetes are extremely effective competitors in the gut, displaying significant nutritional adaptability as well as the capacity to react to pressures imposed by the host and the environment of the gut. As a result, the gut microbiome's altered community compositions may help the crab adjust more swiftly during migration.

It was also observed that the *E. sinensis* gut microbiota turnover rate in freshwater was lower than it was during the seawater-to-freshwater transition (1–5 days), and community dissimilarities in terms of bacterial composition significantly increased over time during migration. Species richness and community compositions of gut microbiome is driven by salinity in seawater-freshwater transition, while host selection may become the dominant factor in the freshwater stage. The salinity of water altered more drastically during the seawater-to-freshwater transition, which may be the reason for this disparity (Shao et al., 2022). Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were the phyla that were most prevalent in the freshwater stage. Amino acids, which are important elements for the diet, may promote the development of bacteria and the health of their hosts as well as control the balance of protein and energy. During the seawater-to-freshwater transition (1–5 days), "amino acid metabolism" and "lipid metabolism" made greater contributions, although "carbohydrate metabolism", "enzyme families", and "nucleotide metabolism" significantly increased in the freshwater stage (Dai et al., 2011). In freshwater, gut microbiome of crab has a stronger microbial interaction than during seawater–-freshwater transition. During migration the changes of metabolism-dependent pathways help the host and bacteria themselves to survive in the new environment (Shao et al., 2022). Many more studies are

Table 1
Overview of most abundant bacterial groups associated with different organs of *E. sinensis* under different conditions.

Organ	Condition	Dominant Taxa	Proportions (%)	References
Gut	Aerial Stress	*Acinetobacter*	6.17	Guo et al., 2021
	Thermal Stress	*Rhodococcus* *Morganella*	10 2	Li et al., 2023
	Alkaline Stress	*Firmicutes*	78	Guan et al., 2021
	Glyphosate	*Bacteroidetes* *Proteobacteria*	45 5	Yang et al., 2019a, 2019b
	Ammonia-N group	*Tenericutes* *Proteobacteria*	48 35	Yang et al., 2021a, 2021b
	Migration (Fresh to Sewage)	Fresh water: *Proteobacteria* Sewage water: *Firmicutes*	60.8 55.6	Dehler et al., 2017
	Migration (Sea to Fresh)	Sea water: *Proteobacteria* Fresh water: *Firmicutes*	86.11 29.12	Shao et al., 2022
	White spot virus infection (48 hpi)	*Proteobacteria* *Bacteroidetes*	36 14.6	Ding et al., 2017
	Milky disease	*Methanobacteriales, Acidaminococcales, Rhodocyclales,* and *Leptospirales*	NA	Jiang et al., 2022
	Rice-crab co-culture system	*Ascomycota*	95.12	Xu et al., 2021
	Sampling sites in Jiangsu and Shanghai, China	*Tenericutes* *Bacteroidetes*	(46.62 % to 98.94 %) (0.08 % to 36.11 %)	Zhang et al., 2016
	Beihe and Diaochahu aquaculture farms	*Firmicutes* *Proteobacteria* *Bacteroidota* *Actinobacteria*	41.37 29.50 23.06	Yang et al., 2023
Gills	Sampling sites in Jiangsu and Shanghai, China	*Proteobacteria* *Bacteroidetes*	(2.90 % to 93.82 %) (4.21 % to 57.52 %) (1.06 % to 55.67 %) (0.21 % to 15.69 %)	Zhang et al., 2016
Hemolymph	Beihe and Diaochahu aquaculture farms	*Firmicutes* *Proteobacteria* *Firmicutes* *Acidobacteriota*	34.74 15.10 10.63	Yang et al., 2023
	Staphylococcus aureus infection	*Bacteroidota*	52.48	Kong et al., 2023
	Aeromonas hydrophila infection	*Proteobacteria*	68.05	
	PBS (Control)	*Proteobacteria*	69.18	
Hepatopancreas	Hepatopancreatic necrosis disease (HPND)	*Basidiomycota* and *Tenericutes*	–	Shen et al., 2021
	Beihe and Diaochahu aquaculture farms	*Proteobacteria* *Firmicutes*	34.71 25.73	Yang et al., 2023

needed in host associated systems in the seawater–freshwater transition to get more deeper insight on this concept.

3. Effect of abiotic stress on *E. sinensis* microbiome

In aquaculture industry, fishes and crustaceans often bear abiotic stress which can reduce their survival rate. Stress such as induced by oxygen, light, temperature, pH and salinity can cause adverse effects on the physiology of these animals (Bowden, 2008). Hence, studying their adaption mechanism can be beneficial to aquaculture industry where some measures can be taken to reduce the harm to these crustaceans.

3.1. *Aerial stress*

Aerial stress is encountered during the transportation of the crabs where they face out-of-water handling process. Aerial stress is one of the most harmful stressors for crustaceans causing imbalance of homeostasis and disruption of immunity (Urbina et al., 2013). Guo et al. (2021) studied the effects of aerial exposure on the immune system and microbial composition in the gut of *E. sinensis*. Two groups were formed: control group (CG), and aerial exposure group (AE) wherein they found increase in LPO (lipid peroxidation) and MDA (malondialdehyde) content in hepatopancreas in response to aerial exposure stress. MDA being the by-product of LPO is the major indicator of oxidative stress. Superoxide dismutase (SOD) activity and T-AOC (total antioxidant capacity) levels also significantly increased after 6–48 h of aerial exposure stress, which shows that antioxidant enzymes can protect cell against the oxidative stress induced by aerial exposure. However, these enzymes got decreased after the 48 h exposure revealing that continuous oxidative stress led to decline in the activity of these enzymes. Bacterial diversity also got significantly altered in aerial exposed crabs (altered Chao and ACE index) as the abundance of Acinetobacter increased while that of Citrobacter decreased in AE crabs as compared to normal crabs. The phylum Acinetobacter are known to be toxic for crustacean population (Bergogne-Bérézin and Towner, 1996) whereas Citrobacter species are helpful for removing pathogens and increasing immunity (Sonnenburg et al., 2006; Buffie and Pamer, 2013).

3.2. *Thermal stress*

The Chinese mitten crab is a significant aquaculture species in China, undergoing a two-year cultivation process from larvae to market size. Thriving in diverse habitats, including estuarine waters and freshwater lakes, this species has become a promising commercial venture, particularly in regions like the Yangtze River. Understanding its response to temperature fluctuations is crucial, especially amidst global climate change. In this regard, Yuan et al. (2017) conducted a study to evaluate the impact of different temperature regimes on molting frequency, growth, and food consumption of *E. sinensis* juveniles. Based on their findings, the growth rate of juvenile *E. sinensis* continues to increase within a temperature range of 18–30 °C. Nevertheless, it's important to acknowledge that higher temperatures can promote precocity in juvenile *E. sinensis*, as indicated by Li et al. (2011). A similar study was conducted by Peng et al. (2019) which revealed that higher temperatures significantly reduced the survival time of juvenile *E. sinensis* crabs.

Though crustaceans are ectothermic organisms but as the water temperature increases from its thermal threshold, it can cause disruption in their metabolic process, immunity and physiological functions and even death (Cook et al., 1998; Martins et al., 2011; Cheng et al., 2017). Li et al. studied enzyme activity of immune genes, metabolism related substances and gut microbiome responses of *E. sinensis* in control (24 °C) and heat stressed groups (32 °C). They observed that the levels of alkaline phosphatase and acid phosphatase enzymes increased significantly in heat stressed group from 12 h to 24 h and then dropped down from 72 to 96 h of heat stress (Li et al., 2023). Gut microbiome analysis revealed significant changes in intestinal microbes of *E. sinensis* induced by heat stress. The abundance of the pathogenic bacteria *Rhodococcus* and *Morganella* increased significantly while that of the beneficial bacteria *Candidatus*, *Hepatoplasma* and *Marinifilum* reduced significantly in heat stressed group compared to control group.

3.3. *Alkaline stress*

A technique of integrating rice and crab farming where in the crab is cultured in rice fields is called crab-rice co-culture technique which has become popular in China during the last decade because of its environmental sustainability (Zhao et al., 2020). North-west China region where crab-rice co-culture is flourishing at a high speed, is marked by high alkalinity. Therefore, Guan et al., investigated the microbes present in the intestines of rice and pond farmed Chinese mitten crabs (Changjiang and Liaohe) in order to assess the impact of co-culture (Guan et al., 2021). They found that the height, length, and width is higher for rice-field farmed crabs than pond-farmed crabs. At the phylum level, Firmicutes constituted approximately 78 % of all analyzed sequences, while Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Patescibacteria each represented more than 1 % of the sequences. The Wilcoxon rank-sum test revealed that among the 15 dominant phyla in the intestinal samples, only Verrucomicrobia showed significant variation between pond-raised and rice field-raised crabs. Further analyses indicated significant differences in Cyanobacteria, Myxococcota, and Fibrobacteres between the two crab groups, though no differences were found between female and male crabs. Spearman correlation analysis results indicated that the dominant phylum, Firmicutes, had a negative correlation with the crabs' body width, weight, and the wet weight of their carapaces.

3.4. *Oxidative stress*

Dissolved oxygen plays a crucial role in aquatic ecosystems, serving as a vital environmental factor that influences the growth and reproductive processes of aquatic organisms. Its levels in water bodies are subject to fluctuations due to a variety of natural and anthropogenic factors, making it one of the most significant limiting factors for the successful development of aquaculture operations. While moderate levels of reactive oxygen species (ROS) can have beneficial effects, such as promoting energy production, facilitating bacterial phagocytosis, stimulating cell growth, and mediating signal transduction pathways, an excessive production of ROS can lead to a cascade of detrimental effects linked to oxidative stress. Oxidative stress results from an imbalance between the production of ROS and the ability of the organism to detoxify these reactive intermediates. Studies have demonstrated that living organisms activate intricate oxidative defense mechanisms as a counter measure against the oxidative stress induced by excessive ROS levels (Gao et al., 2021a). The delicate balance between ROS production and the body's antioxidant defenses is critical for maintaining optimal physiological functions and ensuring the health and well-being of aquatic organisms. Disruptions in this equilibrium can have far-reaching consequences, potentially affecting various aspects of aquatic life, including growth rates, reproductive success, and overall fitness.

In a recent study, the effects of hypoxia and reoxygenation were investigated on the intestinal microbiota of Chinese Mitten Crab (Jiang et al., 2023a, 2023b). The five control groups namely H0, H48, H96, R48 and R96, and post hypoxia groups at 48 h, 96 h and reoxygenation at 48 and 96 h, respectively, were studied for the gut microbiome of *E. sinensis*. Granulicella, Luteibacter, and Mucilaginibacter were significantly less abundant in the H96 group compared to the R96 group ($P < 0.05$), almost reaching zero, while the R96 group showed higher and more stable abundances. Additionally, Pseudoxanthobacter, Acidibacter, and Ancylobacter in the phylum Proteobacteria were more abundant in the H96 group than in the R96 group ($P < 0.05$), and a similar trend was seen for Acidothermus in Actinobacteria, whereas Singulisphaera in Planctomycetes was more abundant in the H0 group.

Significant differences ($P < 0.05$) were found in the relative abundance of most intestinal microbial populations among the H0, H96, and R96 groups. The top seven microorganisms (*Roseimarinus*, *Dysgonomonas*, ZOR006, *Aeromonas*, *Vibrio*, *Paracoccus*, and *Shewanella*) did not differ significantly among the groups. Interestingly, under hypoxic stress, the microbial diversity decreased from 291 species in the H0 group to 60 species in the H96 group. After reoxygenation, the microbial population peaked at 888 species at 48 h (R48) post-hypoxia.

In another study, *Klebsiella pneumoniae* infection also induced oxidative stress in *E. sinensis* and triggered changes in the expression of genes related to immunity, and altered the composition of intestinal microorganisms (Wang et al., 2022). Specifically, the abundance of Prolixibacteraceae, Enterobacterales, and Roseimarinus increased in the infected crabs whereas the abundance of Alphaproteobacteria was decreased.

4. Importance of microbiome in flavor

E. sinensis is widely loved by Chinese and Korean consumers due to its delicious taste and rich nutrition. The habitat of these crabs has significant impact on their flavor. The microalgae and flourishing macrophytes in the ponds directly or indirectly contributed to the faint color and flavor of *E. sinensis* (Kong et al., 2012). In contrast, wild *E. sinensis*, which are widely distributed in the major water systems along the eastern coast of China, encounter more complex environmental factors such as latitude, currents, monsoons, and bait (Nanda et al., 2021). Therefore, differences in taste can be expected. Additionally, *E. sinensis* living in the wild have a complex genetic background, which may result in varied flavors (Tang et al., 2024). The characteristic flavor of *E. sinensis* produced in Yangcheng Lake in the lower reach of the Yangtze River in China is the most preferred by consumers. A comparative study involving wild *E. sinensis* muscles from five rivers was conducted to find the differences in their flavors (Tang et al., 2024). The study revealed that different geographic location produced different flavors in *E. sinensis*. The taste activity value results showed that amino acids Ala, and Arg, and the taste substance GMP contributed to sweetness, bitterness and flavor respectively. They also concluded that *E. sinensis* muscles from Min river was rich in flavor. It has been reported that farming area, environment, and artificial diet can affect the accumulation of nutrients in *E. sinensis* and their flavor (Tao et al., 2018). Many studies are conducted on the differences in the intestinal microbiome and flavor amino acids, nucleotides, and organic acids in these crabs, which showed that *E. sinensis* cultivated in different lakes contained specific microbiota, with significantly different composition and abundance of the dominant microbiota. A study by Tao et al. (2018) shows a remarkable difference in the contents of flavor amino acids, nucleotides, and lactic acid among different tissues and genders of *E. sinensis*. They also interpreted that only some of these indicators varied between crabs of different habitats, with respect to the distinctive flavor of *E. sinensis*. Jiang et al. (2023b) in their study revealed that the fermented diet has positive impact on the meat flavor of crabs, particularly in “sweet” and “umami” tastes. *E. sinensis* has a strong umami taste and high sweetness, which is a unique flavor pursued by consumers. The umami taste and sweetness of edible tissues are largely due to delicious amino acids and nucleotides, such as glutamic acid (umami), glycine (sweet), arginine (bitter/sweet), and adenosine monophosphate (Qiu et al., 2023). Many types of free amino acids have synergistic effects with nucleotides to produce a strong umami taste. The main bacterial strains used for fermentation include *Bacillus*, *Enterococcus*, *Lactobacillus*, *Saccharomyces*, and *Aspergillus*, and using different microorganisms in the fermentation process leads to different effects (Yang et al., 2023). *Bacillus* and *Aspergillus* are the main strains used for the fermentation of protein because they have strong protein and carbohydrate-digesting enzyme-secretion abilities (Suprayogi et al., 2022). Chi and Cho (2016) reported that *Bacillus subtilis* dramatically improved the absorption, digestion, and bioactivity of soybean meal in fermentation. Gao et al. (2021b) reported that the overall flavor quality of edible tissues in female crab was greatly improved by effective microorganisms (EM). They increased umami or sweet taste contributors, that is AMP, GMP and IMP, and decreased off-flavor contributors, that is Hx and HxR. Jiang et al. (2023a, 2023b) confirmed that *Lactobacillus* plays an important role in the sensory, nutritional, and hygiene of various fermented products, and produces a variety of aromatic compounds that impart *E. sinensis* its flavor.

5. Effect of diet on *E. sinensis* microbiome and its consequences

Studies have shown that different feed types affect the growth performance of *E. sinensis* by modifying the gut microbiota and metabolic profile (Table 2). The intestinal microbiota begin colonization in the young hosts and their compositions are directly influenced by the living conditions and growth environment, specifically, the feeding environment (Hansen and Olafsen, 1999; Zoetendal et al., 2001). The hosts supply the environment and nutrients necessary for the growth of intestinal microorganisms (Holben et al., 2002; Nedoluha and Westhoff, 1997). In return, the intestinal microbiome acts as an additional “organ” for the host organism, offering various physiological functions (O’Hara and Shanahan, 2006; Stevens and Hume, 1998) such as vitamin production and the digestion of complex carbohydrates (Hooper et al., 2002; Wexler, 2007; Gilmore and Ferretti, 2003).

Many farmers have tried to improve the protein and fat content of *E. sinensis* diet by feeding small animals such as fish, shrimp and snails during aquaculture. Freshwater snails are a common animal-like food source in *E. sinensis* culture. On the other hand, experienced aquarists know that aquatic plants are essential for the cultivation of healthy crabs. Previous studies have shown many benefits of aquatic plants during culture; For example, aquatic plants regulate pH level, serve as food sources and provide shelter.

Hence, both animal-type and plant-type food resources are required for the culture of *E. sinensis* (Chen et al., 2021). In animal diets, an alternative protein source is black soldier fly (*Hermetia illucens*) due to its high nutritional value (Qiu et al., 2023). In a study, the effects of animal-type and plant-type feed on the gut microbiomes and metabolic processes of *E. sinensis* were compared (Chen et al., 2021). At the phylum level, Tenericutes and Bacteroidetes were the most predominant phyla in animal-only diet. Bacteroidetes was the predominant phyla in plant-only diet, and Proteobacteria was the most predominant phylum in a mixed diet. Different growth performances accompanied with diverse gut microbiome compositions and metabolic profiles, indicating that feed types dramatically affected the growth and development of *E. sinensis*. An optimal daily diet can shape a healthy gut microbiome and

Table 2
Effect of diet on *E. sinensis* microbiome and its consequences.

Diet	Consequences	References
L-tryptophan	Increases survival rate of crabs due to increase in Proteobacteria, Firmicutes, Actinobacteria.	Yang et al., 2019a, 2019b
L-theanine	Improved the total edible yield, muscle yield, and hepatopancreas index. Improved expression of immune-related genes due to increase in the abundance of actinomycetes.	Yang et al., 2024
Mixed group (Plant and animals)	Exhibited superior growth performance because of healthy intestinal microbiome and metabolic homeostasis.	Chen et al., 2021
Plant-type group	Lipid metabolism disorders, which affected the growth of crabs.	
Animal-type group	Increased maleficent Proteobacteria	
Fermented feed	Positively effect the growth performance, antioxidant capacity, digestive enzyme activity, meat flavor, and intestinal microflora of the crabs.	Jiang et al., 2023a, 2023b
Probiotics	Promoted butanoate metabolism	Li et al., 2022

benefit the metabolic processes of the host organism, leading to superior growth performance during aquaculture. Studies indicated that the best fit diet for these crabs includes Tenericutes, Proteobacteria, Bacteroidetes, and Firmicutes as predominant microbiome communities, which received a diet of freshwater snails and waterweed plants (Chen et al., 2015). Though plant proteins are more affordable and more broadly accessible, but their applicability is restricted by characteristics such as high fiber, low protein, imbalanced amino acids, low palatability, and different antinutritional factors (ANFs). Studies have shown that fermentation is useful for breaking down ANFs, crude fiber, and carbs as well as for increasing the amount of crude protein and peptides, and modifying the amino acid pattern in plant sources (Liu et al., 2017; Shi et al., 2017; Wang et al., 2019b; Bueno et al., 2020). In addition to enhancing nutritional value, the fermentation process has been shown to boost the feed's production of antioxidants such as flavonoids, phenolics, and γ-aminobutyric acid.

Jiang et al. (2023a, 2023b) investigated the effects of fermented food on the intestinal microbiome of *E. sinensis*. The experimental fermented diet (FD) had higher crude protein, lactic acid, and essential amino acid content while having lower crude fiber and antinutritional factors compared to the non-fermented diet (NFD). FD also showed increased protein breakdown into lower molecular weight proteins. No significant differences were observed in crude lipid, ash, or total essential amino acids between the feeds. Crabs fed with fermented diet showed significantly higher final weight, weight gain rate, and specific growth rate, along with lower feed conversion rates and higher protein efficiency ratios, compared to those on the NFD. The analysis of alpha diversity, using Shannon, Simpson, and Chao indices, revealed that the FD group had a significantly higher Shannon index and a lower Simpson index, indicating higher microbial diversity ($P < 0.05$). The Chao index showed no significant difference between the two groups ($P > 0.05$). Beta diversity analyses, including principal coordinate analysis (PCoA) and nonmetric multidimensional scaling analysis (NMDS), demonstrated distinct separation of microbial communities between the FD and NFD groups. At the phylum level, Proteobacteria and Firmicutes were the most abundant, with Firmicutes being more prevalent in the FD group (Feng et al., 2021). At the class level, Gammaproteobacteria was dominant in the NFD group, while *Bacilli* became the most abundant class in the FD group. Additionally, the FD group saw a noticeable increase in *Bacteroidia* compared to the NFD group.

At the genus level, the FD group had a significantly lower abundance of *Morganella* and higher abundances of *Candidatus_Hepatoplasma*, *Candidatus_Bacilloplasma*, and *Shewanella* ($P < 0.05$). Studies have demonstrated that *Candidatus_Hepatoplasma* is a dominant genus in the intestines of *E. sinensis* (Feng et al., 2021). Functional predictions based on KEGG pathways showed that the FD group had significant enrichment in pathways related to amino acid metabolism, biosynthesis of secondary metabolites, and metabolism of terpenoids and polyketides ($P < 0.05$) (Shao et al., 2019). Conversely, energy metabolism and xenobiotics biodegradation and metabolism were significantly lower in the FD group compared to the NFD group. The overall trends of unigenes affiliated with other KEGG functions were inconsistent between the groups.

Studies have also explored the microbial communities in the juvenile male and female Chinese mitten crab intestine using three different feeding modes (traditional (A), formulated (B), and mixture diet (C)) (Sun et al., 2020a). The relative enrichment of dominant bacteria in the intestines of male and female crabs varied across different feeding modes. The findings showed that the feeding status had no effect on bacterial diversity, dominant bacterial communities, or similarities in bacterial communities. There were no notable differences in bacterial diversities across the intestines of juvenile crabs fed traditional, formulated, or combined diets. The study indicated that the microbial communities in crab intestines exhibited a consistently high average similarity among the three feeding modes. Sun et al. (2020b) also investigated the relationship between the gut microbial communities of Chinese mitten crabs and the bacterial communities in the surrounding water and sediment. They compared the bacterial communities by using two different feeds: a) formulated diet mode, and b) iced fresh trash fish mode. They observed that *E. sinensis* gut showed lowest OTU richness and bacterial community diversity than the surrounding water and sediment. Specifically, the results show that microbial compositions are different in Chinese mitten crab gut, water, and sediment, and exhibit different relative abundances of same bacterial communities. This study highlights the relationship between bacterial communities in the crab gut and their surroundings (Sun et al. 2020b). Additionally, it also offers detailed information on how different feeding modes influence the intestinal microbiota of crabs.

Other diets improve the gut microbiota, immune response, and survival rate of *E. sinensis.* Yang et al. (2019a) identified the influence of L-tryptophan (L-trp) diet significantly increased the survival rate of crabs by increasing the richness of Proteobacteria, Firmicutes, and Actinobacteria in the intestine. This signifies the importance of L-trp diet supplement in *E. sinensis*. L-theanine is another amino acid known for its various physiological benefits, including potential impacts on the gut microbiota and immune function. Yang et al. explored the effects of L-theanine on the gut microbiota diversity and immune responses in *E. sinensis* (Yang et al., 2024). In this study, different concentrations of L-theanine were supplemented in the diet of *E. sinensis*, and their effects on gut microbiota were evaluated. The Chao1 index, which measures microbial abundance, increased significantly in the group supplemented with 1.0 % L-theanine. The Shannon and Simpson diversity indices, which measure microbial diversity, increased significantly in the group supplemented with 0.5 % L-theanine. However, these indices did not differ significantly between the 1.0 % and 1.5 % L-theanine supplementation groups and the control group. These findings indicate that 1.0 % L-theanine supplementation enhances the abundance of gut microbiota, while 0.5 % supplementation improves microbial diversity. The study identified Proteobacteria, Firmicutes, and Bacteroidota as the primary phyla in the gut microbiota of *E. sinensis*, consistent with previous studies. L-theanine supplementation influenced the relative abundance of these key phyla. Firmicutes are known for their role in nutrient absorption and immune modulation. L-theanine significantly increased the abundance of Firmicutes in the gut, suggesting its potential to enhance nutrient metabolism and immune response. The study also found a significant increase in the abundance of Actinobacteriota with 1.0 % L-theanine supplementation compared to controls. Actinobacteriota are beneficial bacteria known for enhancing host defense against pathogens by forming a defense barrier and producing antibiotics. With respect to the development, different diets also contribute to the growth of Chinese mitten crabs at the larval stage by changing its protease activity (Pan et al., 2005). Diet contents are important elements that determine the diet effects in animal's microbiota. For example, in an experiment using highly unsaturated fatty acid (HUFA) diet supplement, a significant correlation was observed between the dietary HUFA level and the HUFA content in various organs such as hepatopancreas, ovary, and egg (Sui et al., 2011). Another important feed additive which has shown promise in preserving the health of Chinese mitten crabs are Probiotics. Although, their effectiveness in *E. sinensis* culture habitats is still unknown. A recent study (Li et al., 2022) accessed the effects of commercial probiotics on *E. sinensis* microbiome and found that probiotic application significantly influenced the microbial community structure (beta-diversity) in the intestines of these crabs, without affecting the overall composition (alpha-diversity). Notably, the abundance of Gammaproteobacteria increased, while Mollicutes decreased following probiotic treatment, indicating that these bacteria respond differently to probiotic intervention. This shift suggests that Gammaproteobacteria might have a synergistic relationship with probiotics, which is supported by similar observations in other aquatic species. Furthermore, probiotics promoted butanoate metabolism, leading to an increase in beneficial metabolites like butyric acid (Berni Canani et al., 2017), which improves intestinal health and supports growth performance in *E. sinensis*. The application of probiotics also enhanced microbial interactions within the *E. sinensis* intestine, as evidenced by a more connected and cooperative microbial network. The increase in positive correlations and decrease in negative interactions suggest that probiotics foster a more synergistic and less competitive environment among the intestinal microbiota (Yang et al., 2017). This cooperative dynamic may help the intestinal ecosystem respond more effectively to external perturbations. Overall, these changes in microbial community composition and interactions, coupled with enhanced metabolic functions, underscore the potential of probiotics to create a healthier intestinal environment in *E. sinensis*.

6. Microbiome and *E. sinensis* metabolism

Metabolomic profiles quantify the complete set of metabolites in a cell, tissue, or organ of a species and can be applied for the assessment of the physiological condition of an organism in response to diverse genetic and environmental factors. Recent research reported that gut microorganisms affect various aspects of the host, such as metabolism, growth, development, immunology, nutrition, and behavior. The optimal diet and nutritious food resources, results in healthy gut microbiome and benefit the metabolic processes, leading to superior growth and development during aquaculture (Chen et al., 2021). A comprehensive analysis of the composition and dynamics of the gut microbiome will offer important insights into microbially mediated metabolic processes and help improve the efficiency and effectiveness of *E. sinensis*. The composition and diversity of the core gut microbiota (Proteobacteria, Bacteroidetes, Tenericutes, and Firmicutes) of *E. sinensis* varied among the different environments while the metabolites associated with amino acids, fatty acids, and terpene compounds displayed significantly different concentration levels. Jiang et al. (2023a, 2023b), confirmed that the intestinal microorganisms of crabs are rich in metabolism functions, especially the amino acid and carbohydrate metabolic pathways.

The various feed type (plant and animal types) shapes the gut microbiota and metabolic processes. Chen et al., (2021) divided Chinese mitten crabs into three study groups: Group 1 was fed with waterweed plants (*Elodea canadensis*) and freshwater snails (*Sinotaia quadrata*); Group 2 was fed with plant type (waterweed plants); and Group 3 was fed with animal type (freshwater snails). Results indicated that at the phylum level, Tenericutes and Bacteroidetes were observed to be the predominant phyla in Group 1. Bacteroidetes and Proteobacteria were the predominant phyla in Group 2 and Group 3, respectively. The abundance of Bacteroidetes was highest and Firmicutes was lowest in Group 2 compared with Group 1 and 3. The abundances of Proteobacteria, Actinobacteria, and Verrucomicrobia were highest in Group 3. The microbial communities were predominately associated with carbohydrate metabolism and glycan biosynthesis in Group 2 (Table 3). The main functions of Group 3 microbial communities were lipid metabolism, replication and repair, and infectious disease. Several studies indicated that Bacteroidetes can produce carbohydrate metabolism-related enzymes to promote food digestion and provide nutrients to the host. Firmicutes promote energy acquisition by improving lipid metabolism. Moreover, the proportion of Bacteroidetes and Firmicutes can affect the ability of intestinal bacteria to metabolize nutrients, thus affect growth. Chen et al. (2021) identified several metabolites associated with flavor, such as ethyl 4-methylphenoxyacetate and ganoderic acid. These metabolites might have contributed to the special taste of Chinese mitten crabs collected from the Yangtze River. Their findings also indicate the high phenotypic plasticity of gut microbiome and muscle metabolome of *E. sinensis* when it faces environmental changes, which would also facilitate its acclimation and adaptation to diverse and even introduced environments.

Table 3
Dominant CMC microbiota and its role in metabolism.

Microbiome	CMC metabolism
Proteobacteria, Actinobacteria, Verrucomicrobiota	Carbohydrate metabolism, glycan biosynthesis
Bacteroides	Carbohydrate metabolism-related enzymes
Firmicutes	Improving lipid metabolism
Effective microorganisms	Increase cellular energy source

Other environmental challenges such as water salinity and biological migration are part of environmental changes and key drivers that determine the diversity and community compositions of gut microbiome. In an experiment using *E. sinensis*, the turnover rate of the gut microbiome community was higher during the seawater-freshwater transition (Shao et al., 2022). Added to that, the metabolic pathways become altered with a higher amino acid, and lipid metabolism during seawater-freshwater transition than in only freshwater. This study advanced our understanding of gut ecology in different aquatic environments, which can be extended to non-crustacean aquatic animals as well.

Effective microorganisms (EMs) are a form of compounds widely used in aquaculture to improve water quality. Using *E. sinensis* as an aquatic organism, EMs supplementation increased cellular energy sources while protein consumption and oxidative stress were decreased (Li et al., 2024). Unlike other diet supplements, EMs supplement however, does not affect the abundance of the main intestinal microbes, therefore Proteobacteria, Tenericutes, Firmicutes, Bacteroidetes, and Actinobacteria remain the same in both control and EMs groups, with varying bacterial biomarkers (Li et al., 2024). Specifically, Fusobacteriaceae, Desulfovibrio, and Morganella were observed as biomarkers in the control group, and Exiguobacterium and Rhodobacteraceae represented biomarkers of the EM group.

7. *E. sinensis* microbiome and diseases

Due to high nutritional content and pleasant flavor of Chinese mitten crabs, its culturing is flourishing at a high pace. However, this has led to increase in number of diseases (Shen et al., 2015) generating economic losses in crab aquaculture industry. For example, recent reports on infection of *E. sinensis* by a parasitic barnacle, *Polyascus gregaria*, has gained some interest of the research community (Yang et al., 2021b; Zhao et al., 2023). However, crab breeders are mostly unaware of the disease and lack any standardized methods to prevent or treat the outbreaks, resulting in huge economic losses to *E. sinensis* aquaculture. With the development of technologies such as metagenomics which confers direct sequencing of the microbiome from disease and healthy samples has led to the identification of disease markers. The gut microbiota has become a crucial part of metagenomics becoming an attractive target for researchers due to its ability to protect against various pathogens and infections (Jin et al., 2017; Milan et al., 2018). In crustaceans, several studies have linked the intestinal microbiota to host disease and metabolism (Chaiyapechara et al., 2012). Variability in bacterial microbiota may lead to host diseases and the converse of this is also true i.e., onset of aquatic animal diseases is often accompanied by changes in the intestinal microbiome. Many studies have shown the connection between gut microbiome and aquatic host disease such as impairment of gut microbiota accompanied with shrimp disease (Xiong et al., 2015; Zhu et al., 2016a; Xiong, 2018). One of the studies has also demonstrated a change in gut microbiome followed by the *Vibrio alginolyticus* infection in *Portunus trituberculatus* (Xia et al., 2018). Similar results have been shown in the Chinese mitten crabs infected by the fungus *Metschnikowia bicuspidata,* an opportunistic pathogen known to cause milky disease in aquatic organisms and thus harms the aquaculture industry (Zhang et al., 2021). The infected crabs suffer with poor feeding and movement with the end stage marked with total hemolymph emulsification (Jiang et al., 2022). The study reflected a significant increase in anaerobic bacteria in the infected crab gut as compared to healthy crabs. The anaerobic bacteria included Methanobacteriales, Acidaminococcales, Rhodocyclales, and Leptospirales whereas Methanobacteriales being the strict anaerobes. Ascomycota is a significant part of *E. sinensis* (Xu et al., 2021) gut but was significantly reduced in the infected crabs. In addition to the change in bacterial communities, the infected crabs also exhibited decrease in the levels of metabolites related to amino acid biosynthesis, the tricarboxylic acid (TCA) cycle, as well as lysine, histidine, linolenic, arachidonic, and linoleic acid metabolism (Jiang et al., 2022). These results suggested a significant effect of the *M. bicuspidata* infection on the growth, energy metabolism and immunity of the *E. sinensis*, and highlights the role of gut microbiota in regulating the metabolite production.

One of the important diseases in *E. sinensis* which gained a lot of attention due its mortality rate (40 to 50 %) in crabs is hepatopancreatic necrosis disease (HPND) (Ding et al., 2016). The disease is marked by hepatopancreatic atrophy and degeneration of gastrointestinal tract. Though the diseased crab can live for long, but it is not beneficial for farming because of lack of protein and lipid content and reduced growth rate (Shen et al., 2021). A causative agent of the HPND is not yet identified. Earlier, it was believed that microsporidian parasites caused HPND (Ding et al., 2016), however, the following studies did not identify the microsporidians or viruses in diseased crabs (Pan et al., 2017; Shen et al., 2017). It is speculated that abiotic factors such as pesticides, acidity, alkalinity, or water hypoxia may be related to HPND (Yang et al., 2018; Zhu et al., 2016b; Gu et al., 2017). Recently, Shen et al. carried out metatranscriptomic study to access the hepatopancreatic microbiota of the HPND-infected crabs as compared to healthy crabs (Shen et al., 2021). The results showed the decrease in the relative abundance of *Proteobacteria* and *Mucoromycota* while an increase in the phylum *Basidiomycota* in infected crabs as compared to healthy crabs. The study also identified a marked increase in the relative abundance of phylum *Tenericutes* (106 times) in the severe cases. Therefore, they concluded that a change in hepatopancreas microbiome is associated with the HPND disease, and no correlation was found between the disease and the virus. Another study also confirmed the imbalance in gut microbiota associated with HPND infected using 16S rRNA gene analysis (Zhan et al., 2022a, 2022b). There was significant increase in abundance of Bacteroidota whereas a marked decrease in the Firmicutes in the HPND group as compared to healthy crabs. There was also a significant change in the abundance of various genra such as *Bacteroides*, *Aeromonas*, *Candidatus Bacilloplasma* and *Desulfovibrio* among the two groups. Recently, a study showed that a long term exposure of a herbicide 'prometryn' altered the morphological characteristics of *E. sinensis* intestine and hepatopancreas (Huang et al., 2023). Additionally, the prolonged use of prometryn altered the species composition of their gut microbiome. Specifically, prometryn treated crabs exhibited an increase in the abundance of *Zoogloea* and a firmicutes bacterium ZOR0006, and a decrease in the abundance of *Shewanella*. Interestingly, the study also highlighted the correlation between the differential gut microbiota and hepatopancreatic disorder, and suggested that gut microbiome was responsible for the intestinal organ's resistance towards stress and increased the possibility for opportunistic infection.

Ding et al. (2017) also correlated the severity of white spot syndrome virus (WSSV) infection with gut microbiome of *E. sinensis* in order to identify the indicator taxa which can predict the health status of the crab. They observed the dominance of Firmicutes, Proteobacteria, Tenericutes and Bacteroidetes in the intestines of *E. sinensis* irrespective of the WSSV infection stage. They also found 12 phyla representing 44 orders and 68 families with significantly different abundance rates at various stages of the infection. Moreover, bacteria belonging to class *Mollicutes* were dominant at Lake Gucheng, in Nanjing city of China. However, no *Mollicutes* were found in the intestinal microorganisms of *E. sinensis* from other locations such as the Chongming Islands and Yangtze River estuary (Chen et al., 2015; Li et al., 2011).

In addition to the changes in the gut and hepatopancreas microbiome, alterations in the microbiota of hemolymph of *E. sinensis* infected with *Staphylococcus aureus* or *Aeromonas hydrophila* have also been reported (Kong et al., 2023). Specifically, a significant increase in the abundance of *Flavobacterium* and *Aeromonas* was found in the infected crabs. Moreover, the functional profile analysis of crabs in response to these two pathogens exhibited the predominance of different pathways. For example, crabs infected with *S. aureus* displayed the enrichment of pathways such as translation, metabolic diseases, and cellular processes and signaling. In contrast, crabs in response to *A. hydrophila* showed abundance of pathways related to cell growth and death, metabolism, cancer and neurodegenerative diseases.

8. *E. sinensis* microbiome and immune response

The effects of glyphosate, a widely used herbicide, were studied on the *E. sinensis* (Yang et al., 2019a, 2019b), and it was found that exposure to glyphosate significantly reduced the survival rate of the crabs. The researchers also observed changes in the antioxidant capacity, digestive enzyme activities, and gut microbiota of the crabs after exposure to glyphosate. The results showed that glyphosate decreased the total antioxidant capacity and increased the malondialdehyde content in the midgut of the crabs. The activities of digestive enzymes in the intestinal tract were also affected, with lipase and amylase activities decreasing and trypsin activity increasing. In the hepatopancreas, the activities of three digestive enzymes increased after glyphosate exposure. The gut microbiota revealed that glyphosate decreased the diversity of the crab's gut microbiota and increased the taxonomic richness of Bacteroidetes and Proteobacteria. Dominant phyla in the intestine were Proteobacteria, Bacteroidetes, Tenericutes, and Firmicutes. The study suggested that the changes in gut microbiota may be involved in the effects of glyphosate on survival by affecting immune and digestive functions. The researchers also highlighted the potential threat of glyphosate to crabs bred in rice-shrimp co-culture systems due to its long half-life in water and repeated application. Overall, the study demonstrated that glyphosate has adverse effects on the survival, immune response, digestive activities, and gut microbiota of *E. sinensis*. These findings contribute to the understanding of the potential risks of glyphosate exposure in aquaculture systems and underscore the need for further research on the effects of herbicides on aquatic organisms.

Yang et al., 2021a, also investigated the effects of ammonia-N stress on various parameters in *E. sinensis,* including immune parameters, antioxidant capacity, digestive function, intestinal microbiota. Ammonia-N stress refers to the exposure of the crabs to elevated levels of ammonia in their environment, which can occur in aquatic systems due to pollution or improper management. They found increased THC (total haemocyte count) in ammonia stressed crabs as compared to normal crabs. THC is a major indicator of innate immune response. ACP (phosphatase) and AKP (alkaline phosphatase) are considered two important parameters to gauze the overall health status of crustaceans (Yang et al., 2021a). Though ACP showed no significant differences among the groups, interestingly AKP levels were significantly decreased in ammonia-stressed crabs. Regarding antioxidant activities, there was a decrease in total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) index in haemolymph of ammonia stressed crabs. The total T-AOC, T-SOD, GSH-Px, and lipid peroxidation products malondialdehyde (MDA) are regarded as biological indicators of stress in aquatic organisms (Draper and Hadley, 1990; Shan et al., 2019). Furthermore, they also found the significant difference in intestinal microbiome between control and ammonia stress groups. Among phyla, Dysgonomonas and Rubellimicrobiu were significantly hiked in the control group than the ammonia-N group. They suggested Dysgonomonas's role in development of the hindgut system on account of previous published report of related termites (Bridges and Gage, 2021). Rubellimicrobiu are capable of hydrolyzing starch and absorbing cellulose, histidine, leucine, and fructose and can aid in digestive functions (Riedel et al., 2014). The order Lactobacillus was significantly higher in the control group which can disrupt the bacterial cell wall and thus increasing the antibacterial activity which is therefore decreased in ammonia stress group. There was also an increase in Citrobacter abundance in ammonia-N group, which causes infections in impaired-immune organisms.

9. Techniques and challenges in investigating *Eriocheir sinensis* microbiome

Investigating the microbiome of the *E. sinensis* encompasses various techniques that collectively offer insights into the microbial structure, composition, diversity, and functional potential of microbiota associated with the host (Fig. 2). There are two commonly used methods for microbiome analysis namely marker gene analysis and shotgun genome analysis. Among these, the former method is mainly used for the *E. sinensis* microbiome analysis. Irrespective of the method used, one of the sources of potential bias may be the DNA extraction methods. In general, different studies use different DNA extraction methods (Tao et al., 2018; Guo et al., 2021; An et al., 2024) which in turn can have varying results and may introduce bias in the representation of microbial communities. For example, mechanical disruption, lysis due to enzymatic or chemical reactions can differentially affect the recovery of microbial DNA from various taxa, leading to potential biases in downstream analyses.

Marker gene analysis is primarily used to sequence specific genes like the 16S rRNA gene for bacteria and the ITS region for fungi, which serve as barcodes for microbial identification due to their conserved and hypervariable regions (Antil et al., 2022). Current sequencing often employs the Illumina MiSeq platform, targeting regions such as V1-V3 or V4 (Sinclair et al., 2015), with a trend towards full-length sequencing using PacBio or Oxford Nanopore technologies, though database support for these is still evolving. A key challenge is defining unique sequences due to hypervariable regions and potential identical sequences. PCR amplification and sequencing errors further complicate accurate identification. Advanced error correction approaches and chimera detection methods are necessary to rectify these issues. The selection of primers for amplifying target regions of microbial marker genes such as 16 s rRNA can add bias by preferentially amplifying some bacterial taxonomic groups over other taxa. PCR amplification can also give rise to chimeric sequences or other artifacts which could aggravate downstream analysis. To mitigate these issues, sequences are binned into OTUs based on similarity thresholds (typically 97 % or 99 %) (Callahan et al., 2017), which helps control for biological and technical variations. Taxonomic assignment can be achieved using machine learning methods like the RDP classifier (Wang and Cole, 2024) or by mapping sequences to databases such as Greengene (DeSantis et al., 2006) and SILVA (Glöckner et al., 2017). One of the limitations of such databases is that they may lack comprehensive representation of all microbial organisms due to the quality or completeness of these databases. This may significantly affect the accuracy of taxonomic classification. Tools like Mothur (Schloss et al., 2009), QIIME (Caporaso et al., 2010), and DADA2 (Callahan et al., 2016) are used for taxonomic assignment and subsequent analysis. However, the underlying algorithm for each of these pipelines is different and can result in inconsistent outcomes affecting the interpretation of microbial community structure and composition.

Processing microbiome data produces a feature abundance matrix that links taxa or genes to samples, requiring careful statistical analysis due to high dimensionality and sparsity. Alpha diversity measures feature diversity within samples, using metrics like species richness and the Shannon index, while beta diversity examines dissimilarity between samples with metrics such as Bray–Curtis and UniFrac. Tools like Mothur (Schloss et al., 2009), QIIME (Caporaso et al., 2010), and the R package vegan facilitate these calculations, with statistical tests like PERMANOVA and ANOSIM evaluating significant clustering (Anderson and Walsh, 2013). Ordination techniques like PCoA and PCA visualize beta diversity data, often using tools like EMPeror for interactive manipulation (Vázquez-Baeza et al., 2013). It should be noted that most of the sequencing approaches provide data in the form of relative abundance, which can be impacted by factors including differential amplification efficiency and sequencing depth, making it challenging to precisely quantify the absolute abundance of microbial taxa (Barlow et al., 2020). Therefore, methods like quantitative PCR (qPCR) or flow cytometry are sometimes used to supplement sequencing data for absolute quantification. Additionally, the microbial composition can differ significantly over various time points across different body sites or environmental factors. This necessitates meticulous experimental design and replication. Moreover, differences among individual crabs including gender, age, health and diet can also impact the underlying microbiota and add can add potential variability to the data. Nevertheless, in order to better understand the ecological roles and interactions of microbial communities within this economically important crab, it is indispensable to integrate various approaches besides their limitations.

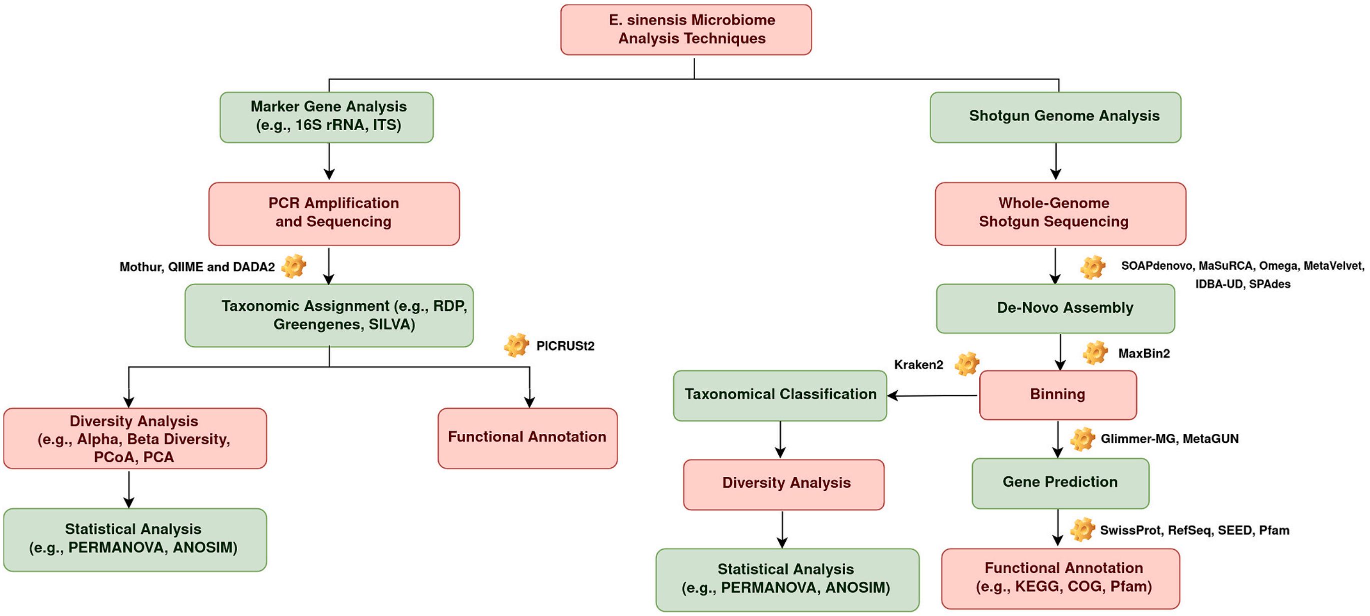

Fig. 2. Schematic representation of various steps involved in the *E. sinensis* microbiome data analysis.

10. Conclusion and future directions

The Chinese Mitten Crab is an economically significant crustacean species found in China and the Korean peninsula. The microbiota of these crab plays a crucial role in their health and metabolism by antagonizing pathogenic bacteria, regulating host immunity, and participating in nutrient absorption and vitamin synthesis. Despite this, current microbiome-based studies have predominantly focused on bacterial communities, leaving significant gaps in our understanding of fungal and viral diversity.

Recent research has demonstrated that the gut of *E. sinensis* harbors a significant number of filamentous fungi, including ascomycetous and basidiomycetous yeasts (Xu et al., 2021). Notably, female Chinese mitten crabs exhibit a higher fungal diversity compared to males, suggesting gender-related differences in gut mycobiota that warrant further investigation to understand their triggers and consequences on the host.

Additionally, studies on the viral diversity in *E. sinensis* are sparse. The only comprehensive report to date analyzed the RNA viromes of asymptomatic, milky disease (MD)-affected, and hepatopancreatic necrosis syndrome (HPNS)-affected Chinese mitten crabs from three different locations in China, revealing high regional variation and numerous novel viruses (Guo et al., 2023). These findings underscore the need for multi-region sampling and further studies targeting fungal and viral diversity, particularly in different organs of *E. sinensis*.

Identifying the microbial composition and structure in various organs of these crabs can provide valuable insights into their relationship with the environment. Future research should leverage multi-omics approaches, such as metagenomics, metatranscriptomics, proteomics, and metabolomics, to comprehensively characterize the microbiota under different biological conditions. These advanced methodologies will be instrumental in elucidating the complex interactions between *E. sinensis* and their associated microbiota, ultimately aiding in the development of improved aquaculture strategies and reducing economic losses.

Moreover, understanding the microbiota's influence on different organs can enhance our knowledge of *E. sinensis* physiology and health. The microbiota's impact on the taste of these crabs is particularly relevant for marketability and consumer preference, as microbial communities in the gut, hepatopancreas, and other tissues are likely involved in nutrient absorption and metabolism, affecting the taste and texture of the crabs. Additionally, the modulation of immune responses by the microbiota may influence disease resilience and overall health.

In conclusion, a comprehensive understanding of the microbiota in different organs of *E. sinensis* and their effects on aspects such as taste, immune response, and overall health is essential. This requires a concerted effort utilizing advanced multi-omics approaches to unravel the complex interactions between *E. sinensis* and their microbiota. These insights will not only advance our knowledge of *E. sinensis* biology but also help optimize aquaculture practices, enhancing the quality and yield of this valuable species.

CRediT authorship contribution statement

Arpita Ghosh: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Ankita Rathore:** Writing – original draft, Resources, Methodology, Formal analysis. **Sonam Gaba:** Methodology, Formal analysis, Data curation. **Majid Rasool Kamli:** Writing – original draft, Validation, Formal analysis. **Abdulkadir Yusif Maigoro:** Validation, Investigation, Data curation. **Hyung Wook Kwon:** Writing – review & editing, Validation. **Nitin Mahajan:** Writing – review & editing, Methodology, Validation, Visualization. **Chang-Bae Kim:** Writing – review & editing, Supervision, Resources, Conceptualization. **Adeel Malik:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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