



Intestinal microbiome in crayfish: Its role upon growth and disease presentation

Ariadne Hernández-Pérez^{a,*}, Irene Söderhäll^{b,**}

^a Departamento de Medicina y Zootecnia de Abejas, Conejos y Organismos Acuáticos. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito de la Investigación Científica s/n, 04510, Ciudad Universitaria, México

^b Department of Organismal Biology, Uppsala University, Norbyvägen 18A, 752 36, Uppsala, Sweden

ARTICLE INFO

Keywords:

Microbiome
Intestinal microbiota
Disease
Growth
Environmental
Crayfish
Crustaceans

ABSTRACT

The intestine-associated microbiota in crustaceans are considered a key element for maintaining homeostasis and health within the organisms. Recently, efforts have been made to characterize bacterial communities of freshwater crustaceans, including crayfish, and their interplay with the host's physiology and the aquatic environments. As a result, it has become evident that crayfish intestinal microbial communities display high plasticity, which is strongly influenced by both the diet, especially in aquaculture, and the environment. Moreover, studies regarding the characterization and distribution of the microbiota along the gut portions led to the discovery of bacteria with probiotic potential. The addition of these microorganisms to their food has shown a limited positive correlation with the growth and development of crayfish freshwater species. Finally, there is evidence that infections, particularly those from viral etiology, lead to low diversity and abundance of the intestinal microbial communities. In the present article, we have reviewed data on the crayfish' intestinal microbiota, highlighting the most frequently observed taxa and emphasizing the dominance of phylum within this community. In addition, we have also searched for evidence of microbiome manipulation and its potential impact on productive parameters, and discussed the role of the microbiome in the regulation of diseases presentation, and environmental perturbations.

1. Introduction

The intestinal microbiota of aquatic organisms is a complex and dynamic biological system with a central role in physiological processes such as digestion, nutrition, ion osmoregulation, intestinal development, and water uptake. Moreover, interactions between commensal, symbiotic and pathogenic microbial communities play a vital role in the immunity of aquatic species (Sehna et al., 2021). Host-associated microbiome interactions in aquatic organisms are strongly influenced by environmental conditions, where the water microbiomes are key elements, however, other numerous environmental and biological selection pressures are involved in such relations (Sehna et al., 2021).

In the case of crayfish, studies regarding the role of intestinal microbiota upon health and growth have been conducted in a few numbers of species, the majority of them relevant for aquaculture, *Cherax cainii*, *Cherax quadricarinatus*, and *Procambarus clarkii*, as well as the invasive species *Pacifastacus leniusculus*. These studies are

summarized in Table 1.

In the last decade, the production of crustaceans has increased rapidly, with freshwater crustaceans representing 30% of the total (FAO, 2022). Despite the productive increase in this sector, the main challenge for freshwater aquaculture development and management in terms of biosecurity continues to be the health of the organisms and disease prevention (FAO, 2017). In this regard, gut microbiota manipulation represents an attractive opportunity for aquaculture to develop and has been suggested as a possible alternative to using broad-spectrum antibiotics in managing disease outbreaks. However, the development of appropriate gut supplements requires prior knowledge of the host microbiome, and little is still known about the gut microbiome of the aquatic invertebrates, and marine shrimp being the most studied (Holt et al., 2021).

In addition, microbiome studies can be applied to understand the processes behind successful alien species establishment in new habitats, as well as host fitness response to adaptation during pollution of aquatic

* Corresponding author.

** Corresponding author.

E-mail address: ariadne.hernandez@comunidad.unam.mx (A. Hernández-Pérez).

Table 1

Studies of the intestinal microbiome in crayfish. *Weight at the time of the beginning of the experiments. OTUs: Operational Taxonomic Units; ASVs: Amplicon Sequence Variant; RDP: Ribosomal Database Project; Empty: unclear information or not stated.

Crayfish species	Reference	Condition	Weight (grams)	Diet	Intestine portion analyzed	Type of sample	Hypervariable region	Sequencing platform	ASV/OUT; % similarity; threshold; database	Data accession
<i>Cherax cainii</i>	Tulsankar et al. (2022b)	Trace elements supplementation for plankton	11.1 ± 1.10	Formulated food	Hindgut	Pool of 2 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva	PRJNA749331
	Foysal et al. (2022a)	Different sources of protein	71.2 ± 0.4*	Fishmeal	Gut with mucosa	Individual	V3–V4	Illumina 16S MiSeq platform	OTUs; 99% similarity; Silva 1.32	
	Foysal et al. (2022b)	Zeolite impact in rearing environments	70 ± 5.8*	Fishmeal	Midgut and hindgut	Pool	V3–V4	Illumina MiSeq platform	OTUs; 99% similarity; Silva 132	PRJNA797514
	Foysal et al. (2021)	Different sources of protein and probiotic bacteria <i>Lactobacillus plantarum</i>	6.21 ± 0.38	Fishmeal, black soldier fly, black soldier fly with probiotic bacteria	Hindgut	Individual	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva	PRJNA609769
	Foysal et al. (2020a)	Probiotic bacteria <i>Bacillus mycoides</i>	68.8 ± 0.5*	Commercial food and probiotic bacteria	Hindgut	Pool of 2 individuals	V4–V5	Illumina MiSeq platform	OTUs; 97% similarity	PRJNA558040
	Foysal et al. (2020b)	Probiotic bacteria <i>Lactobacillus acidophilus</i> and <i>Lactobacillus plantarum</i>	70.2 ± 0.6*	Fishmeal with probiotic	Distal gut	Pool of 12 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva	PRJNA579035
	Foysal et al. (2020c)	Biological water filters	71.8 ± 0.7	Fishmeal	Hindgut	Pool of 2 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva 1.32	PRJNA549032
	Siddik et al. (2020)	Dietary poultry by-product meal fermented by <i>Lactobacillus casei</i> and <i>Saccharomyces cerevisiae</i>	105.3 ± 3.39		Distal gut	Pool of 3 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva 1.32	PRJNA521663
<i>Cherax quadricarinatus</i>	Foysal et al. (2019b)	<i>Clostridium butyricum</i> as a dietary probiotic	69.65 ± 1.04*	Fishmeal and probiotic bacteria	Hindgut	Pool of 6 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva 132	PRJNA515886
	Foysal et al. (2019a)	Different sources of protein	65.01 ± 5.09*	Fishmeal	Hindgut	Pool of 3 individuals	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; Silva 1.32	PRJNA505066
	Liu et al. (2020b)	Reared at different salinities	18.28 ± 0.03*	Commercial food	Midgut	Pool of 5 individuals	V4	Illumina MiSeq PE300 platform	OTUs; 97% similarity; Greengenes database (release 13.8)	PRJNA717320
	Zhang et al. (2021)	Tail blister disease (<i>Aeromonas hydrophila</i> and <i>Aeromonas veronii</i> natural infection)			Midgut and hindgut	Individual		Illumina MiSeq platform		
<i>Pacifastacus leniusculus</i>	Hernández-Pérez et al. (2022)	Exposition to environmentally-relevant concentrations of sulfamethoxazole	37 ± 2	Carrots and potatoes	Midgut and hindgut	Individual	V3–V4	Illumina MiSeq platform	ASVs; Silva 138	PRJNA717320
<i>Procambarus clarkii</i>	Dragičević et al. (2021)	Invasion range		Natural environment	Midgut and hindgut	Pool of 44 individuals	V3–V4	Illumina MiSeq platform	ASVs; 99%	PRJEB43749
	Zhu et al. (2023)	<i>Bacillus amyloliquefaciens</i> and <i>Lactococcus lactis</i> as dietary probiotics	22.37 ± 0.57*	Commercial food	Intestinal content scraped from the intestine		V3–V4	Illumina MiSeq platform	OTUs; 70% similarity; SILVA and RDP	

(continued on next page)

Table 1 (continued)

Crayfish species	Reference	Condition	Weight (grams)	Diet	Intestine portion analyzed	Type of sample	Hypervariable region	Sequencing platform	ASV/OUT; % similarity threshold; database	Data accession
	Li et al. (2022)	<i>Citrobacter freundii</i> experimental infection	16.18 ± 1.10	Commercial food and dried <i>Tenebrio molitor</i>	Complete intestine	Pool of 3–5 organisms	V3– V4	Illumina MiSeq PE300 platform/ NovaSeq PE250 platform	OTUs; 97% similarity; Silva	PRJNA755431
	Han et al. (2022a)	Polystyrene nano plastic exposure	18 ± 2	Commercial food	Complete intestine	Pool of 3 organisms	V3–V4	Illumina MiSeq platform	OTUs	
	Han et al. (2022b)	<i>Limosilactobacillus fermentum</i> GR-3 strain as feed additive vs. arsenic accumulation	6.48 to 9.35*	Commercial food	Complete intestine		V3–V4	Illumina MiSeq platform		
	Xue et al. (2022)	White spot syndrome virus natural infection			Posterior intestine	Pool of 3 organisms	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; RDP	
	Chen et al. (2021)	Reared in aquaculture ponds and rice paddy field		Commercial food	Complete intestine	Pool of 40 and 30 individuals		Illumina HiSeq 2500 sequencing platform		PRJNA671745
	Feng et al. (2021)	Healthy and diseased (apparently <i>Citrobacter freundii</i> experimental infection)		Commercial food	Complete intestine	Pool of 3 individuals	V3–V4	MiSeq system	OTUs; 97% similarity; Silva and RDP	
	Huang et al. (2021)	Insecticide imidacloprid exposition	25.7 ± 3.3*	Commercial food	Complete intestine		V3–V4	Illumina MiSeq platform	OTUs; 97% similarity	
	Xie et al. (2021)	Different growth stages		Commercial food	Complete intestine	Pool	V3–V4	Illumina HiSeq 2500 platform	OTUs; 97% similarity; RDP	PRJNA723682
	Xu et al. (2021)	<i>Saccharomyces cerevisiae</i> YFI-SC2 as a dietary probiotic	13.71 ± 0.58*	Commercial food	Complete intestine	Pool of 8 individuals	V3–V4	Illumina MiSeq PE300 platform	OTUs; 97% similarity	RJNA740135
	Guo et al. (2020)	Acute nitrite and sulfide stress	4.5 ± 0.2	Food-deprived during experiment	Complete intestine	Pool of 3 individuals	V4	Illumina	OTUs; 97% similarity	
	Liu et al. (2020a)	Pond and rice fields during different seasons	28–35	Pond productivity	Intestinal content	Pool of 10 individuals	V3–V4	Ion S5 TM XL platform	OTUs	PRJNA607348
	Shui et al. (2020)	Integrated crayfish-rice cultivation model	15–25	Pond productivity	Complete intestine	Individual	V3–V4	Illumina MiSeq platform	OTUs; 97% similarity; RDP	PRJNA557576
	Zhang et al. (2020a)	Heavy metal cadmium exposition	20.96 ± 3.05	Food-deprived during experiment	Complete intestine	Pool of 3 individuals	V3–V4	Illumina MiSeq platform	OTUs	
	Zhang et al. (2020b)	Hepatotoxin microcystin-LR exposition	20.28 ± 2.98	Food-deprived during experiment	Intestinal content	Pool of 3 individuals	V3–V4	Illumina MiSeq platform	OTUs	SRR 9671444
	Zhang et al. (2020c)	Developmental stage, diet, and geographic origin	20.54 ± 0.36, 41.25 ± 0.29 and 42.57 ± 0.51	Fermented and non-fermented food	Complete intestine	Pool of 6 individuals	V3–V4	Illumina HiSeq 2500 platform	OTUs; 97% similarity	PRJNA609648

environments episodes. In the case of freshwater crayfish, these are important issues since, due to anthropogenic effects, 32% of the freshwater crayfish species in the world are threatened with extinction (Richman et al., 2015), and one major cause is the introduction of invasive crayfish species such as *P. clarkii* and *P. leniusculus*. Also, the translocation and dispersal of alien crayfish species with aquaculture relevance are considered to be a threat to natural populations all over the world (Hernández et al., 2008).

Detailed studies regarding crustaceans' intestinal microbiome have become possible to perform and provide substantial knowledge, thanks to the 16S rRNA gene amplicon sequencing approach development. Despite that, differences in experimental planning, sampling, sequencing methodologies, and databases used, make it difficult to draw conclusions, therefore, the implementation of standard experimental practices is highly recommended (Evariste et al., 2019). Furthermore, a comprehensive summary of the intestinal microbiome sequence data in crustaceans, and how this is affected by various environmental factors could provide guidance to improve productivity and biosecurity in aquaculture, as well as in the study of alien-species invasion.

In the present review, we summarise the most relevant phyla across four freshwater crayfish species, emphasize the state of the knowledge regarding the involvement of the intestinal microbiome during growth, disease presentation, and ecological disturbances, and discuss future challenges.

2. Towards crayfish intestinal core microbiome

In the last years, the concept of core microbiome has become increasingly important as a result, in part, of the increment of high-throughput sequencing research. The core microbiome is commonly used to identify and quantify the microbial communities or genes shared by organisms in a given condition, usually referred to as a normal state. This term, however, encompasses complex interactions from genomic to host and environmental levels (Risely, 2020), and given the broad spectrum of crayfish species, its wide distribution, and the scarce information regarding its intestinal microbial communities' functionality, a definition of a core microbiome in these organisms cannot be drawn yet. Despite this, identification of the microbial composition in the intestine of freshwater crustaceans will contribute to a better understanding of host-microbe-environment interactions. Therefore, in the present review, available relative abundances of different bacterial phyla in crayfish' intestinal microbiome, were obtained from published studies (Supplementary Table S1), in order to identify a crayfish common core microbiome, defined as the microbial taxa that most frequently occurs in a host or populations of hosts (Neu et al., 2021; Risely, 2020). Figs. 1–4 show the relative abundance of the most common core phylum only in control treatments, in four crayfish species, *C. cainii*, *C. quadricarinatus*, *P. leniusculus*, and *P. clarkii*, to facilitate the identification of a common core microbiome in these

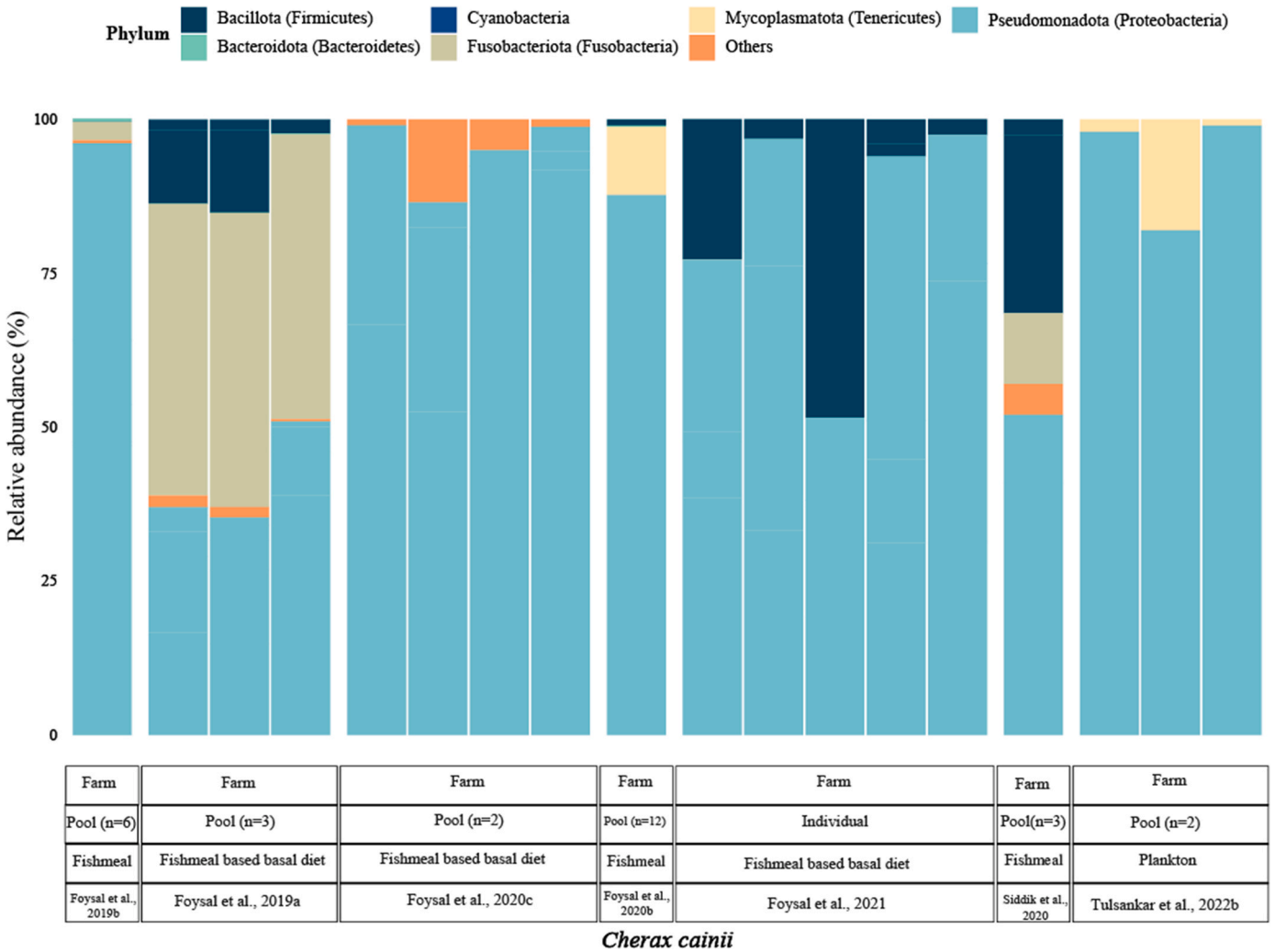


Fig. 1. Overview of the common core microbiome of freshwater crayfish *Cherax cainii* in relation to diet, culture environment, and processing of samples (individual or pooled samples). Major bacterial phyla associated with the intestine were included. Relative abundance values were taken from studies cited in Table 1. When relative abundances were not stated in the manuscript, corresponding bars in original figures were measured as a percentage of the axis scale.

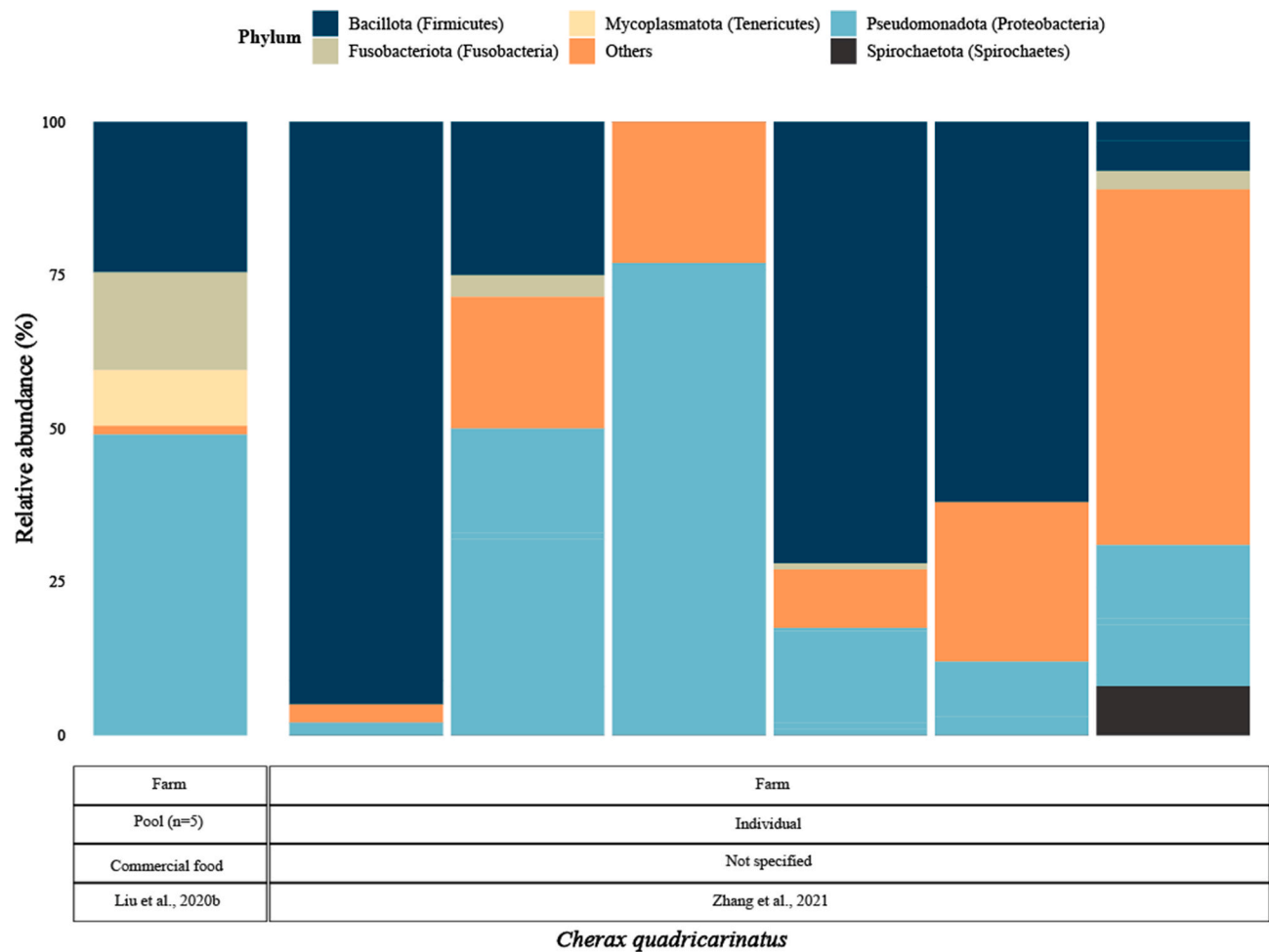


Fig. 2. Overview of the common core microbiome of freshwater crayfish *Cherax quadricarinatus* in relation to diet, culture environment, and processing of samples (individual or pooled samples). Major bacterial phyla associated with the intestine were included. Relative abundance values were taken from studies cited in Table 1. When relative abundances were not stated in the manuscript, corresponding bars in original figures were measured as a percentage of the axis scale.

taxonomic groups. Noticeably, the Pseudomonadota (Proteobacteria) phylum is, with some exceptions, the most well-represented across the four crayfish species with a relative abundance of up to 95% in *C. cainii* for instance (Foysal et al., 2019b). One specific exception was in *P. leniusculus* isolated from rivers in Croatia (Dragičević et al., 2021). Pseudomonadota (Proteobacteria) is ubiquitous in aquatic ecosystems, including freshwater (Battistuzzi and Hedges, 2009) and in aquatic hosts (Dragičević et al., 2021). This bacterial group comprises Gram-negative bacteria and includes the Gammaproteobacteria class, a vast group of enteric bacteria such as opportunistic pathogenic genera like *Vibrio* and *Pseudomonas*, which cause disease in different species including crayfish (Bradley and Pollard, 2017; Dong et al., 2016; Jiravanichpaisal et al., 2009). Other major phyla in crayfish intestinal microbiome are Bacillota (Firmicutes), a group of Gram-positive bacteria that comprises important species commonly used as probiotics in crayfish and shrimp from *Bacillus*, *Clostridium*, and *Lactobacillus* genera (Chomwong et al., 2018; Foysal et al., 2019b, 2021), Fusobacteriota (Fusobacteria), Gram-negative fusiform bacteria (Gupta and Sethi, 2014), and Mycoplasmatota (Tenericutes), a widely distributed phyla that can be found in plants, arthropods, vertebrates, and marine waters (Wang et al., 2020). Interactions of these phyla have been documented to participate in the maintenance of intestinal homeostasis in terrestrial vertebrates (reviewed by Stojanov et al., 2020), including humans (Bradley and Pollard, 2017). However, the dynamics and function upon freshwater species, particularly crayfish, require further investigation.

3. Probiotics modulating crayfish intestinal microbiome and growth

Cherax cainii is the most studied crayfish species regarding the use of probiotic supplementation. *C. cainii*, belonging to the Parastacidae family, is a commercially important freshwater crayfish endemic from Western Australia known as marron. Although marron’s aquaculture has remained stagnant, efforts have been made to improve its commercial status since farming development of this species is considered a strategy for sustainable aquaculture due especially to its disease-free status. Indeed, no disease outbreaks have been reported in the aquaculture of this species, and information regarding *C. cainii* susceptibility to diseases is limited to experimental infections with *Vibrio* spp. (Foysal et al., 2019b; Machin et al., 2008; Nugroho and Fotedar, 2013; Siddik et al., 2020). One of the main problems to solve for *C. cainii* aquaculture is to find a diet to improve weight gain performance since growth in this species is considerably low compared to other commercial crayfish. Freshwater crayfish are omnivorous animals therefore, the protein requirement is considered moderate (approximately 30% of the dry weight). This characteristic facilitates nutrition since the source of protein can be diversified from fish to insects, plants, and animal by-products. Research about, *C. cainii* microbiome has mainly focused on dietary supplementation, especially in the search for ingredients to substitute fish protein in the food. Such studies include, for example, testing plants and insects as protein sources for feeding (Foysal et al., 2019a, 2021, 2022a; Siddik et al., 2020), the inclusion of probiotics in

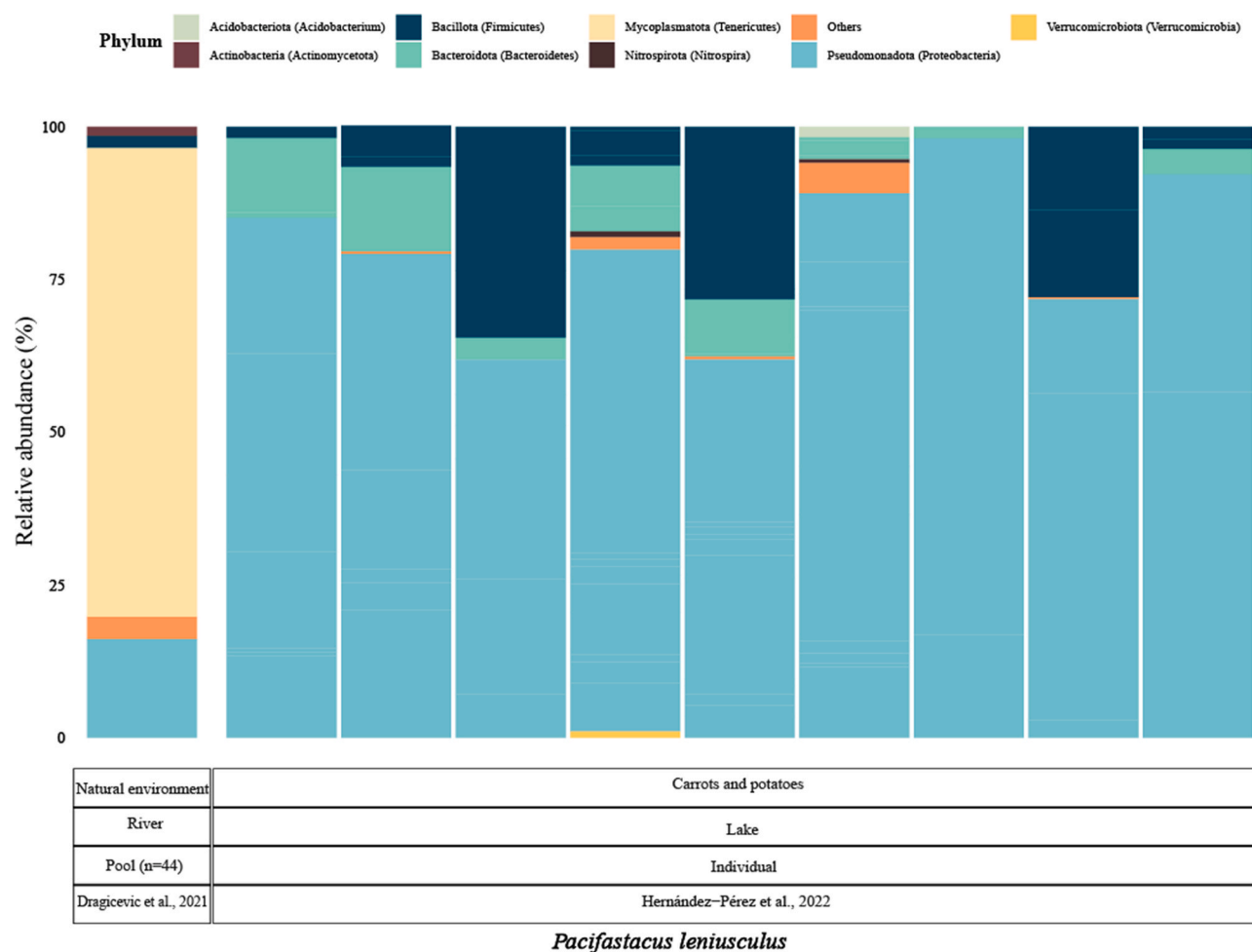


Fig. 3. Overview of the common core microbiome of freshwater crayfish *Pacifastacus leniusculus* in relation to diet, culture environment, and processing of samples (individual or pooled samples). Major bacterial phyla associated with the intestine were included. Relative abundance values were taken from studies cited in Table 1. When relative abundances were not stated in the manuscript, corresponding bars in original figures were measured as a percentage of the axis scale.

the diet (Foysal et al., 2019b, 2020b, 2021) and the addition of trace elements to the ponds to improve plankton diversity and intestinal microbiome (Tulsankar et al., 2022b).

Regarding different protein-source in *C. cainii* feed, the study conducted by Foysal et al., (2022a), showed that among different dietary groups, only 2.8% of the total intestinal microbiome taxa were shared, showing the high plasticity of the microbiota depending on diet, in agreement with studies in another crayfish species like *P. clarkii* (Chen et al., 2021; Liu et al., 2020a; Shui et al., 2020). The genera *Aeromonas* sp. was the most predominant bacteria in the gut followed by *Vibrio*, *Hafnia-Obesumbacterium*, *Candidatus Bacilloplasma*, and *Shewanella*. Both *Aeromonas* sp. and *Vibrio* sp. are considered important pathogenic bacteria for crayfish (Hernández-Pérez et al., 2021; Jiravanichpaisal et al., 2009), and noticeably, their abundance was influenced depending on the source of protein. *Aeromonas* sp. abundance was higher during soy meal-based feeding and was less abundant in marrons fed with fishmeal. On the contrary, *Vibrio* sp. was more abundant after fishmeal and less abundant during soy meal-based diet feeding. Also, *Aeromonas* sp. was predominant in water samples and the richness and alpha diversity were significantly higher in water than in the intestine.

More recently, Tulsankar et al., (2022b) found that in *C. cainii* cultured with plankton, its microbiome at the phylum level was dominated by Pseudomonadota (Proteobacteria), followed by Mycoplasmatota (Tenericutes), and at the genus level, *Vibrio* was the most abundant bacteria followed by *Aeromonas*, *C. bacilloplasma*, and *Candidatus hepatoplasma*. Moreover, when supplementation of trace elements was

performed in plankton at high concentrations, the later genera *C. hepatoplasma* were replaced by *Citrobacter*, *Acinetobacter*, and *Pseudomonas*. However, health parameters such as total and differential hemocyte count were not altered when trace elements were added to plankton, and therefore its importance is difficult to estimate. A later study from the same research group showed that live plankton is an important food source and the inclusion improves the growth, survival, and health of juvenile marron (Tulsankar et al., 2022a). Results from these studies indicate that manipulation of primary productivity, including organic matter in the ponds and trace elements, represents an attractive strategy to improve growth in crayfish, especially since the crayfish's other physiological response wasn't affected.

Probiotic supplementation of *Clostridium butyricum* tested by Foysal et al., (2019b) has been so far the only probiotic that resulted in a significant weight gain in *C. cainii*, and it was attributed to an increase in the number of molts. It is worth noting that this probiotic-feed group showed higher species diversity in their intestinal microbiota, with Fusobacteriota (Fusobacteria) and Pseudomonadota (Proteobacteria) predominantly at the phylum level. At the immunological level, the probiotic inclusion also enhanced significantly the total hemocyte count and lysozyme activity in the hemolymph. However, after challenging the probiotic-feed marrons with *Vibrio mimicus*, no direct effect upon infection was possible to observe, since neither mortalities nor clinical signs of disease were observed in either control or probiotic groups. Another successful example of the inclusion of a probiotic for crayfish productivity is *Saccharomyces cerevisiae* YFI-SC2 (Xu et al., 2021), where

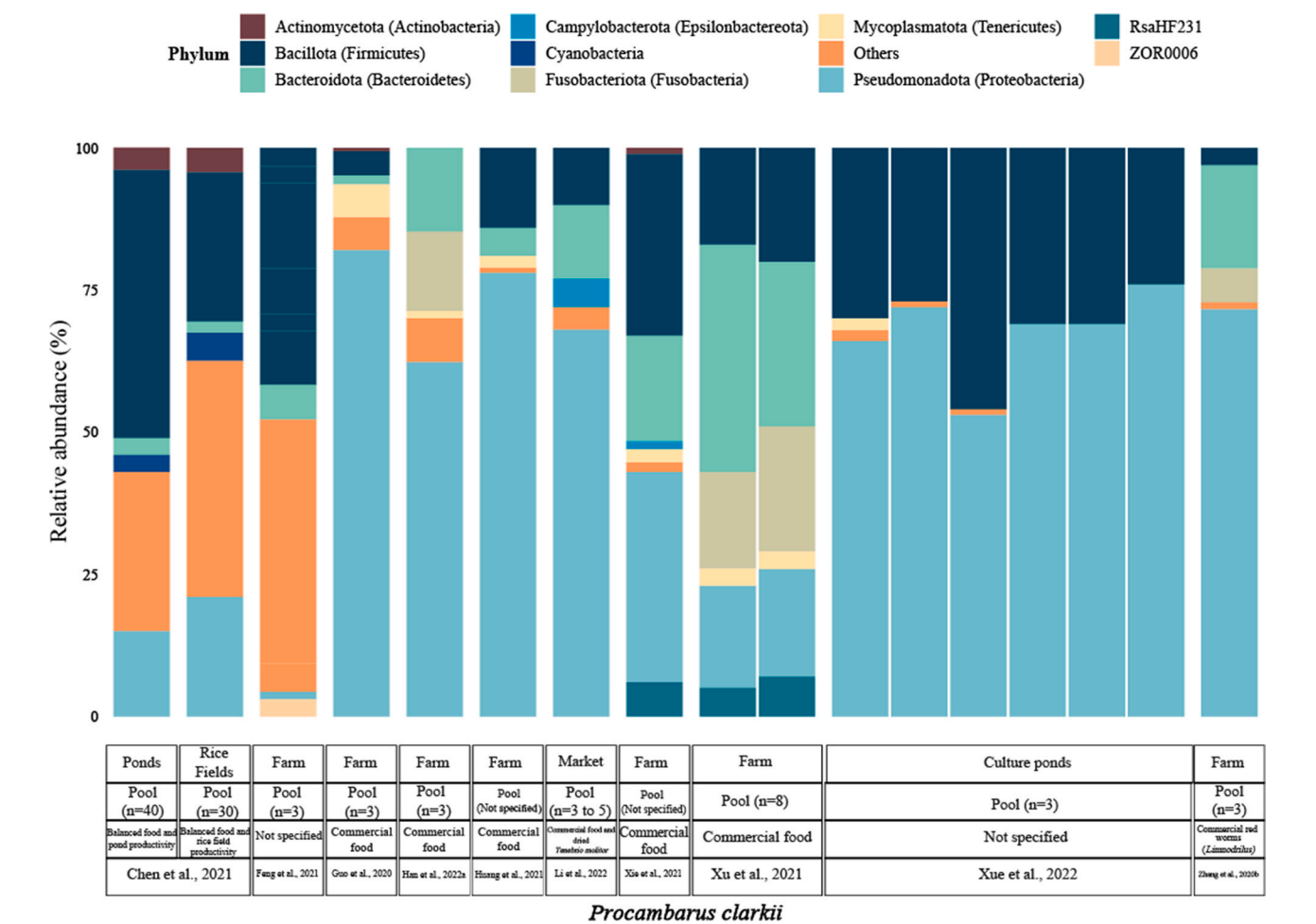


Fig. 4. Overview of the common core microbiome of freshwater crayfish *Procambarus clarkii* in relation to diet, culture environment, and processing of samples (individual or pooled samples). Major bacterial phyla associated with the intestine were included. Relative abundance values were taken from studies cited in Table 1. When relative abundances were not stated in the manuscript, corresponding bars in original figures were measured as a percentage of the axis scale.

P. clarkii presented a better weight gain after 28 days of feeding. These two probiotics should be further studied as plausible candidates to apply in crayfish aquaculture.

Additionally, Foysal et al., (2020b) tested the inclusion of well-known probiotic bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum* in the diet of *C. cainii* and reported that these probiotics shifted the microbial communities, increasing the already present Pseudomonadota (Proteobacteria), Mycoplasmatota (Tenericutes), Bacteroidota (Bacteroidetes), and Bacillota (Firmicutes) phyla. No significant differences were obtained in weight gain, however, total hemocyte count was enhanced, as well as phenoloxidase that showed an upregulated expression. Since no infection challenge was performed in this last study, it is difficult to estimate if the up-regulation of immune parameters could have a positive effect on crayfish. Likewise, in a recent study, probiotic bacteria (*Bacillus amyloliquefaciens* and *Lactococcus lactis*) derived from fish were administrated as feed additives to *P. clarkii* (Zhu et al., 2023), and resulted in a more diversified intestinal microbiota, as well as in an increased expression of some immune-related genes. Interestingly, mortalities after challenges with viral and bacterial pathogens were delayed in probiotic-supplementation organisms, showing a potential for these probiotics to be used in crustaceans.

Regarding *C. cainii*, Foysal et al. (2021) compared fishmeal (FM), black soldier fly (BSF), and BSF with probiotic bacteria *Lactobacillus plantarum* (BSFLP) diets for feeding of *C. cainii*, finding no significant differences upon growth. However, they found that microbiomes were dominated by Pseudomonadota (Proteobacteria), Mycoplasmatota

(Tenericutes), and Bacillota (Firmicutes) depending on the diet, confirming that the composition of the intestinal microbiome is determined by the composition of the diet. The inclusion of *L. plantarum* in the diet reduced potentially harmful genera, and increased parameters such as total and differential hemocyte counts, as well as lysozyme activity when combined with insects as a source of protein. Contrary to probiotic *C. butyricum* addition, *L. plantarum* had a positive effect against *V. mimicus* infection, since the *L. plantarum* feed group presented significantly fewer mortalities. Notably, the authors used the same *V. mimicus* doses in both *C. butyricum* (Foysal et al., 2019b) and *L. plantarum* studies, obtaining different effects of susceptibility to *V. mimicus* infection. This shows the relevance of standardizing doses of pathogens during experimental infections, especially during non-well-characterized pathogens for testing such treatments as probiotics, in order to improve experimental reproducibility.

Regarding *marron's* intestinal development, the addition of *Lactobacillus* probiotics produced higher microvilli counts in the hindgut. This has according to Foysal et al., (2020b) been associated with better utilization of nutrients in other aquatic animals like rainbow trout (Dimitroglou et al., 2009).

Finally, is it worth mentioning that in other crayfish species like *P. clarkii*, probiotics inclusion in the diet have been tested in order to study the restoration of the gut microbiome during aquatic pollution. The inclusion of the probiotic *Limosilactobacillus fermentum* GR-3 strain in the food of *P. clarkii* exposed to arsenic, resulted in the restoration of the gut microbiome (Han et al., 2022b), showing probiotics may have

the potential to reduce food toxicity.

Taking into consideration the substantial growth of aquaculture worldwide, studies focused on the role of intestinal microbiota to improve productivity are of utmost importance. According to the studies reviewed in the present article, it is observed that manipulation of the intestinal microbiota of crayfish could scarcely improve weight gain or productive parameters in the short term. However, the lack of long-term accurate studies, makes it difficult to draw conclusions. Proof of interaction between this manipulation (i.e. natural pond productivity, food source of protein and administrated probiotic bacteria) should be prioritized and undertaken to generate stronger evidence of the real potential of probiotics for aquaculture improvement and prosperity.

4. Intestinal microbiome during disease progression

The effect of diseases on crayfish intestinal microbiome has been scarcely studied and involves infections with aquaculture-relevant pathogens such as White spot syndrome virus (WSSV), *Citrobacter freundii* and *Aeromonas* sp.

The 16S rRNA high-throughput sequencing studies are valuable tools to characterize the microbiome changes during pathogenesis. In this regard, viral infections are a clear cause of intestinal microbiota dysbiosis. In naturally WSSV infected *P. clarkii*, a significant decrease in diversity and richness was observed during the infection, and the relative abundance of pathogenic bacteria such as *Aeromonas* increased also significantly (Xue et al., 2022).

Regarding bacterial infections, research conducted by Zhang et al. (2021) combined 16S rRNA high-throughput sequencing and conventional bacteriological methods to identify the causal agent of mass mortalities (up to 80%) in a commercial farm of the crayfish *C. quadricarinatus*. Bacteria isolated from blisters and hemolymph were identified as *A. hydrophila* and *Aeromonas veronii*, although some inconsistencies were found between biochemical and molecular identification. Accordingly, results obtained from 16S sequencing showed that the enriched bacteria in the hemolymph of the diseased organisms were *Aeromonas*. Conversely, the most predominant genera in the intestine of diseased organisms were *Vibrio* and *Enterobacter*. Moreover, the richness of microbial communities in the intestine of diseased organisms compared with the healthy group was not different, showing that pathogenic bacteria didn't colonize the intestine and no shift occurred in the microbial communities due to the disease. Likewise, experimental infections of *P. clarkii* with *C. freundii* did not result in any statistical difference in the diversity and richness between control and infected crayfish (Li et al., 2022). These are interesting results since it is widely accepted that the crayfish's natural route of infection is mainly through the intestine, and due to the open circulatory system in crustaceans the pathogens are easily transferred from the intestine to the hemolymph. Results obtained by these authors show the relevance of incorporating intestinal microbiome studies to help characterize different etiologies affecting freshwater crayfish, especially since many of the causative agents and the pathogenesis of the diseases are still unknown.

5. The role of the environment in modulating intestinal microbiomes

Crayfish microbiomes are shaped to a great extent by the abiotic environment (Skelton et al., 2017). Therefore, dynamics in microbial communities are key factors to adapt to environmental changes including pollution, translocation to a new habitat, and water quality parameters.

In this regard, intestinal microbiome studies in the alien-species crayfish *P. leniusculus* have been conducted in order to understand such interactions. *P. leniusculus* is a freshwater crustacean that belongs to the Astacidae family. It is native to North America although, due to its introduction in the 1960s by humans, it is now found in almost all countries in Europe, largely due to its high adaptability to a wide range

of environments (CABI, 2022; Kouba et al., 2014). It is considered one of the top three most invasive crayfish species in the world.

Regarding the invasion range of *P. leniusculus*, Dragičević et al. (2021) analyzed microbiomes from wild populations of this crustacean which were established at four different locations along a river in Croatia. They obtained the microbial descriptions from the environment (water and sediment) and from crayfish tissues (exoskeleton, hemolymph, hepatopancreas, and intestine) to study if microbial communities affect or are affected by crayfish range expansion. As a result, they found that the intestine was among the tissues with the lowest richness and the tissue with the lowest evenness (measured as Pielou's evenness index), in agreement with the small core and high heterogeneity reported in *P. leniusculus* (Hernández-Pérez et al., 2022). Moreover, higher crayfish population density increases microbial variation in the intestine, and crayfish density was determined by location. These observations support the idea that the composition of available food in the sediments from different locations, crayfish detritivores habits, and resource competition driven by high-density populations determine microbial communities in the intestine in natural communities.

Hernández-Pérez et al. (2022) analyzed the composition of intestine-associated microbiota after chronic exposure to environmentally relevant concentrations of the antibiotic sulfamethoxazole. Exposure to the antibiotic didn't affect microbiota diversity or richness, in agreement with exposure to other contaminants like insecticides (Huang et al., 2021). However high heterogeneity of microbial communities between individuals was observed. This high variation between individuals from the same population has been observed during environmental degradation episodes and has been considered a stress sign in aquatic animals (Clever et al., 2022). Moreover, when antibiotic-exposed and non-antibiotic-exposed crayfish were challenged with pathogenic bacteria, the antibiotic-exposed groups survived longer. Interestingly, in a previous study conducted by the same group, exposure to the same SMX concentrations lead to negative regulation of antimicrobial peptide Crustin3 (a recognized antiviral factor), and a higher susceptibility to white spot syndrome virus infection (Hernández-Pérez et al., 2020). Taken together, these results indicate that crayfish depends more importantly on its immune system to maintain homeostasis and overcome diseases during environmental challenges, rather than interactions with microbial populations in the intestine.

In regards to water quality adaptation, research conducted by Liu et al. (2020b) in the crayfish *C. quadricarinatus* reared at different salinities, showed an increment in the abundance of Mycoplasmatota (Tenericutes), particularly regarding the genus *Candidatus Bacilloplasma*. On the contrary, the phylum Bacillota (Firmicutes) decreased significantly compared with the control. Interestingly, none of these changes in the microbiome were manifested through productivity parameters such as weight gain, specific growth rate, feed conversion ratio, or survival, and were not affected by salinity. These findings suggest that crayfish can adapt to different levels of salinity without affecting microbiome composition, and adaptation depends more on other physiological processes.

6. Future challenges and conclusions

The available information regarding crayfish microbiome and its relevance for host fitness is limited partially by aquaculture bias since studies have been mostly focused on growth parameters. The most important thing going forward, however, is to reveal properly what causes changes in the gut microbiome in crayfish, and what impact such changes will have upon crayfish fitness. Perhaps, the next step to improve productivity parameters and disease control should be taking into consideration the design of commercial products according to each farm's characteristics, for example, using the microbiome-based precision medicine approach. This method consists of using microbiome as a tool for creating and developing personalized diagnostics and therapeutics to improve health and productivity.

An important consideration for the study of the crayfish microbiome involves morphological characteristics of the gastrointestinal tract. For example, It is accepted that the complexity of intestinal microbial communities increases as the complexity of the gastrointestinal tract increases (Kostic et al., 2013), which strengthen the development of specialized symbiosis. In this sense, crayfish have a relatively simple gastrointestinal tract, without specialized organs to support symbiotic interactions. This could indicate that crayfish poorly rely in specialized interactions with the intestinal microbial communities. Moreover, it's important to specify which gut sections (midgut or/and hindgut) are used for microbiome studies because the distribution of microbial species could be different along the intestine. In addition, colonization of probiotics in the crayfish intestine should be conducted through visual and conventional bacteriology techniques, since 16S only detects the presence of the bacteria but no viability.

Another factor to consider is crayfish' developmental stage since even though there are few studies covering different biological life stages, the richness and diversity from early to mature stages are diminished (Zhang et al., 2020c), and some microbial communities have shown stage-specific signatures (Xie et al., 2021). Definitions of different developmental stages should be clarified during such studies, because crayfish life cycle doesn't include larval stage in contrast to marine shrimp. Crayfish embryos hatch to juvenile and then they mature into adults, and depending on the species, these stages have different duration.

Regarding reproducibility, one example is the research by Zhang et al. (2021), who referred to two different studies by Wu et al. (2020, 2019), in order to report the methods for sequencing and bioinformatics used in their study. However, the referred articles used different approaches and regions of amplification, making it difficult to follow or reproduce the methodology. Technical advances in microbiome research in the last years require setting consistent standards, which increase a more realistic comparison of data between the information generated. This standardization includes experimental planning, sampling of the intestine, DNA extraction procedures, hypervariable region amplified, bioinformatics analysis, the use of ASVs instead of OTUs since the first offer higher resolution, and setting values for filtering (Berg et al., 2020). This last is a relevant issue since it is accepted that the most relevant phyla in the intestinal microbiome have the potential to mask an important number of less abundant microbial communities, therefore information regarding their functional importance could be underestimated.

In summary, it is clear that the main factors determining crayfish microbiome are developmental stage, food intake, season, geographic point, and diseases. Elucidation of the microbiome's role in crayfish physiology will help not only to establish more sustainable aquaculture practices but to comprehend and hopefully restore the natural communities.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution statement

Conceptualization AHP and IS, Writing - original draft AHP, Writing - review & editing AHP and IS. Both authors approved the final version.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dci.2023.104703>.

References

- Battistuzzi, F.U., Hedges, S.B., 2009. A major clade of prokaryotes with ancient adaptations to life on land. *Mol. Biol. Evol.* 26, 335–343. <https://doi.org/10.1093/molbev/msn247>.
- Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C.C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G.H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J.A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelke, B., Roume, H., Kiran, G.S., Selvin, J., Souza, R.S.C. de, van Overbeek, L., Singh, B.K., Wagner, M., Walsh, A., Sessitsch, A., Schloter, M., 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8, 103. <https://doi.org/10.1186/s40168-020-00875-0>.
- Bradley, P.H., Pollard, K.S., 2017. Proteobacteria explain significant functional variability in the human gut microbiome. *Microbiome* 5, 1–23. <https://doi.org/10.1186/s40168-017-0244-z>.
- CABI, 2022. Invasive Species Compendium [WWW Document]. CAB Int, Wallingford, UK. www.cabi.org/isc, 7.22.22.
- Chen, X., Fan, L., Qiu, L., Dong, X., Wang, Q., Hu, G., Meng, S., Li, D., Chen, J., 2021. Metagenomics analysis reveals compositional and functional differences in the gut microbiota of red swamp crayfish, *Procambarus clarkii*, grown on two different culture environments. *Front. Microbiol.* 12, 1–10. <https://doi.org/10.3389/fmicb.2021.735190>.
- Chomwong, S., Charoensapsri, W., Amparyup, P., Tassanakajon, A., 2018. Two host derived lactic acid bacteria activate the proPO system and increase resistance to an AHPND-causing strain of *Vibrio parahaemolyticus* in the shrimp *Litopenaeus vannamei*. *Dev. Comp. Immunol.* 89, 54–65. <https://doi.org/10.1016/j.dci.2018.08.002>.
- Clever, F., Sourisse, J.M., Preziosi, R.F., Eisen, J.A., Guerra, E.C.R., Scott, J.J., Wilkins, L.G.E., Altieri, A.H., McMillan, W.O., Leray, M., 2022. The gut microbiome variability of a butterflyfish increases on severely degraded Caribbean reefs. *Commun. Biol.* 5, 770. <https://doi.org/10.1038/s42003-022-03679-0>.
- Dimitroglou, A., Merrifield, D.L., Moate, R., Davies, S.J., Spring, P., Sweetman, J., Bradley, G., 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Anim. Sci.* 87, 3226–3234. <https://doi.org/10.2527/jas.2008-1428>.
- Dong, X., Li, Z., Wang, X., Zhou, M., Lin, L., Zhou, Y., Li, J., 2016. Characteristics of *Vibrio parahaemolyticus* isolates obtained from crayfish (*Procambarus clarkii*) in freshwater. *Int. J. Food Microbiol.* 238, 132–138. <https://doi.org/10.1016/j.jfoodmicro.2016.09.004>.
- Dragičević, P., Bielen, A., Petrić, I., Vuk, M., Žucko, J., Hudina, S., 2021. Microbiome of the successful freshwater invader, the signal crayfish, and its changes along the invasion range. *Microbiol. Spectr.* 9. <https://doi.org/10.1128/spectrum.00389-21>.
- Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., Pinelli, E., 2019. Gut microbiota of aquatic organisms: a key endpoint for ecotoxicological studies. *Environ. Pollut.* 248, 989–999. <https://doi.org/10.1016/j.envpol.2019.02.101>.
- FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation (Rome).
- FAO, 2017. The 2030 Agenda and the Sustainable Development Goals: the Challenge for Aquaculture Development and Management, Management. John Hambrey. FAO Fisheries and Aquaculture Circular No. 1141, Rome, Italy.
- Feng, Y., Li, M., Duan, H., Li, L., Ouyang, P., Chen, D., Geng, Y., Huang, X., Yang, S., Yin, L., Jiang, J., Zhang, X., 2021. Microbial analysis reveals the potential colonization of pathogens in the intestine of crayfish (*Procambarus clarkii*) in traditional aquaculture environments. *Ecotoxicol. Environ. Saf.* 224, 112705. <https://doi.org/10.1016/j.ecoenv.2021.112705>.
- Foysal, M.J., Dao, T.T.T., Fotedar, R., Gupta, S.K., Tay, A., Chaklader, M.R., 2022a. Sources of protein diet differentially stimulate the gut and water microbiota under freshwater crayfish, marron (*Cherax cainii*, Austin 2002) culture. *Environ. Microbiol. Rep.* 14, 286–298. <https://doi.org/10.1111/1758-2229.13049>.
- Foysal, M.J., Nguyen, T.T.T., Sialumano, M., Phiri, S., Chaklader, M.R., Fotedar, R., Gagnon, M.M., Tay, A., 2022b. Zeolite mediated processing of nitrogenous waste in the rearing environment influences gut and sediment microbial community in freshwater crayfish (*Cherax cainii*) culture. *Chemosphere* 298, 134276. <https://doi.org/10.1016/j.chemosphere.2022.134276>.
- Foysal, M.J., Fotedar, R., Siddik, M.A.B., Chaklader, M.R., Tay, A., 2021. *Lactobacillus plantarum* in black soldier fly (*Hermetia illucens*) meal modulates gut health and immunity of freshwater crayfish (*Cherax cainii*). *Fish Shellfish Immunol.* 108, 42–52. <https://doi.org/10.1016/j.fsi.2020.11.020>.
- Foysal, M.J., Chua, E.G., Gupta, S.K., Lamichhane, B., Tay, C.Y., Fotedar, R., 2020a. *Bacillus mycoides* supplemented diet modulates the health status, gut microbiota and innate immune response of freshwater crayfish marron (*Cherax cainii*). *Anim. Feed Sci. Technol.* 262, 114408. <https://doi.org/10.1016/j.anifeedsci.2020.114408>.
- Foysal, M.J., Fotedar, R., Siddik, M.A.B., Tay, A., 2020b. *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). *Sci. Rep.* 10, 1–13. <https://doi.org/10.1038/s41598-020-62655-y>.
- Foysal, M.J., Fotedar, R., Tay, C.Y., Gupta, S.K., 2020c. Biological filters regulate water quality, modulate health status, immune indices and gut microbiota of freshwater

- crayfish, marron (*Cherax cainii*, Austin, 2002). Chemosphere 247, 125821. <https://doi.org/10.1016/j.chemosphere.2020.125821>.
- Foyals, M.J., Fotedar, R., Tay, C.Y., Gupta, S.K., 2019a. Dietary supplementation of black soldier fly (*Hermetia illucens*) meal modulates gut microbiota, innate immune response and health status of marron (*Cherax cainii*, Austin 2002) fed poultry-by-product and fishmeal based diets. PeerJ. <https://doi.org/10.7717/peerj.6891>, 2019.
- Foyals, M.J., Nguyen, T.T.T., Chaklader, M.R., Siddik, M.A.B., Tay, C.Y., Fotedar, R., Gupta, S.K., 2019b. Marked variations in gut microbiota and some innate immune responses of freshwater crayfish, marron (*Cherax cainii*, Austin 2002) fed dietary supplementation of *Clostridium butyricum*. PeerJ. <https://doi.org/10.7717/peerj.7553>, 2019.
- Guo, K., Ruan, G., Fan, W., Fang, L., Wang, Q., Luo, M., Yi, T., 2020. The effect of nitrite and sulfide on the antioxidant capacity and microbial composition of the intestines of red swamp crayfish, *Procambarus clarkii*. Fish Shellfish Immunol. 96, 290–296. <https://doi.org/10.1016/j.fsi.2019.11.052>.
- Gupta, M.S., Sethi, M., 2014. Phylogeny and molecular signatures for the phylum Fusobacteria and its distinct subclades. Anaerobe 28, 182–198. <https://doi.org/10.1016/j.anaerobe.2014.06.007>.
- Han, M., Gao, T., Liu, G., Zhu, C., Zhang, T., Sun, M., Li, J., Ji, F., Si, Q., Jiang, Q., 2022a. The effect of a polystyrene nanoplastic on the intestinal microbes and oxidative stress defense of the freshwater crayfish. *Procambarus clarkii*. Sci. Total Environ. 833, 155722. <https://doi.org/10.1016/j.scitotenv.2022.155722>.
- Han, R., Khan, A., Ling, Z., Wu, Y., Feng, P., Zhou, T., Salama, E.S., El-Dalatony, M.M., Tian, X., Liu, P., Li, X., 2022b. Feed-additive *Limosilactobacillus fermentum* GR-3 reduces arsenic accumulation in *Procambarus clarkii*. Ecotoxicol. Environ. Saf. 231, 113216. <https://doi.org/10.1016/j.ecoenv.2022.113216>.
- Hernández-Pérez, A., Noonin, C., Söderhäll, K., Söderhäll, I., 2020. Environmental concentrations of sulfamethoxazole increase crayfish *Pacifastacus leniusculus* susceptibility to White Spot Syndrome Virus. Fish Shellfish Immunol. 102, 177–184. <https://doi.org/10.1016/j.fsi.2020.04.022>.
- Hernández-Pérez, A., Söderhäll, K., Sirikharnin, R., Jiravanichpaisal, P., Söderhäll, I., 2021. *Vibrio areninigræ* as a pathogenic bacterium in a crustacean. J. Invertebr. Pathol. 178. <https://doi.org/10.1016/j.jip.2020.107517>.
- Hernández-Pérez, A., Zamora-Briseño, J.A., Söderhäll, K., Söderhäll, I., 2022. Gut microbiome alterations in the crustacean *Pacifastacus leniusculus* exposed to environmental concentrations of antibiotics and effects on susceptibility to bacteria challenges. Dev. Comp. Immunol. 126. <https://doi.org/10.1016/j.dci.2021.104181>.
- Hernández, L., Maeda-Martínez, A.M., Ruiz-Campos, G., Rodríguez-Almaraz, G., Alonzo-Rojas, F., Sainz, J.C., 2008. Geographic expansion of the invasive red crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea: Decapoda) in Mexico. Biol. Invasions 10, 977–984. <https://doi.org/10.1007/s10530-007-9175-0>.
- Holt, C.C., Bass, D., Stentiford, G.D., van der Giezen, M., 2021. Understanding the role of the shrimp gut microbiome in health and disease. J. Invertebr. Pathol. 186, 107387. <https://doi.org/10.1016/j.jip.2020.107387>.
- Huang, Y., Hong, Y., Yin, H., Yan, G., Huang, Q., Li, Z., Huang, Z., 2021. Imidacloprid induces locomotion impairment of the freshwater crayfish, *Procambarus clarkii* via neurotoxicity and oxidative stress in digestive system. Aquat. Toxicol. 238, 105913. <https://doi.org/10.1016/j.aquatox.2021.105913>.
- Jiravanichpaisal, P., Roos, S., Edsman, L., Liu, H., Söderhäll, K., 2009. A highly virulent pathogen, *Aeromonas hydrophila*, from the freshwater crayfish *Pacifastacus leniusculus*. J. Invertebr. Pathol. 101, 56–66. <https://doi.org/10.1016/j.jip.2009.02.002>.
- Kostic, A.D., Howitt, M.R., Garrett, W.S., 2013. Exploring host-microbiota interactions in animal models and humans. Genes Dev. 27, 701–718. <https://doi.org/10.1101/gad.212522.112>.
- Kouba, A., Petrussek, A., Kozák, P., 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowl. Manag. Aquat. Ecosyst. <https://doi.org/10.1051/kmae/2014007>.
- Li, M., Wang, J., Deng, H., Li, L., Huang, X., 2022. The damage of the crayfish (*Procambarus clarkii*) digestive organs caused by *Citrobacter freundii* is associated with the disturbance of intestinal microbiota and disruption of intestinal-liver Axis homeostasis. Front. Cell. Infect. Microbiol. 12, 1–11. <https://doi.org/10.3389/fcimb.2022.940576>.
- Liu, Q., Long, Y., Li, B., Zhao, L., Luo, J., Xu, L., Luo, W., Du, Z., Zhou, J., Yang, S., 2020a. Rice-shrimp culture: a better intestinal microbiota, immune enzymatic activities, and muscle relish of crayfish (*Procambarus clarkii*) in Sichuan Province. Appl. Microbiol. Biotechnol. 104, 9413–9420. <https://doi.org/10.1007/s00253-020-10797-4>.
- Liu, S., Qi, C., Jia, Y., Gu, Z., Li, E., 2020b. Growth and intestinal health of the red claw crayfish, *Cherax quadricarinatus*, reared under different salinities. Aquaculture 524, 735256. <https://doi.org/10.1016/j.aquaculture.2020.735256>.
- Machin, D., Dearden, M., Lacey, P., 2008. Marron aquaculture strategic extension campaign. An interim report [WWW Document]. Reg. Inst. 1–14. URL: http://www.regional.org.au/au/apen/2003/non_refereed/080machind.htm, 7.20.22.
- Neu, A.T., Allen, E.E., Roy, K., 2021. Defining and quantifying the core microbiome: challenges and prospects. Proc. Natl. Acad. Sci. USA 118, 1–10. <https://doi.org/10.1073/pnas.2104429118/-DCSupplemental.Published>.
- Nugroho, R.A., Fotedar, R., 2013. Dietary organic selenium improves growth, survival and resistance to *Vibrio* infections in cultured marron, *Cherax cainii* (Austin, 2002). Fish Shellfish Immunol. 35, 79–85. <https://doi.org/10.1016/j.fsi.2013.04.011>.
- Richman, N.I., Bohm, M., Adams, S.B., Alvarez, F., Bergey, E.A., Bunn, J.J.S., Burnham, Q., Cordeiro, J., Coughran, J., Crandall, K.A., Dawkins, K.L., DiStefano, R., Doran, N.E., Edsman, L., Eversole, A.G., Fureder, L., Furse, J.M., Gherardi, F., Hamr, P., Holdich, D.M., Horwitz, P., Johnston, K., Jones, C.M., Jones, J.P.G., Jones, R.L., Jones, T.G., Kawai, T., Lawler, S., Lopez-Mejia, M., Miller, R.M., Pedraza-Lara, C., Reynolds, J.D., Richardson, A.M.M., Schultz, M.B., Schuster, G.A., Sibley, P.J., Souty-Grosset, C., Taylor, C.A., Thoma, R.F., Walls, J., Walsh, T.S., Collen, B., 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: astacidea). Philos. Trans. R. Soc. B Biol. Sci. 370. <https://doi.org/10.1098/rstb.2014.0060>, 20140060–20140060.
- Risely, A., 2020. Applying the core microbiome to understand host-microbe systems. J. Anim. Ecol. 89, 1549–1558. <https://doi.org/10.1111/1365-2656.13229>.
- Sehnal, L., Brammer-Robbins, E., Wormington, A.M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C.J., Simonin, M., Adamovsky, O., 2021. Microbiome composition and function in aquatic vertebrates: small organisms making big impacts on aquatic animal health. Front. Microbiol. 12. <https://doi.org/10.3389/fmicb.2021.567408>.
- Shui, Y., Guan, Z.B., Liu, G.F., Fan, L.M., 2020. Gut microbiota of red swamp crayfish *Procambarus clarkii* in integrated crayfish-rice cultivation model. Amb. Express 10. <https://doi.org/10.1186/s13568-019-0944-9>.
- Siddik, M.A.B., Fotedar, R., Chaklader, M.R., Foyals, M.J., Nahar, A., Howieson, J., 2020. Fermented animal source protein as substitution of fishmeal on intestinal microbiota, immune-related cytokines and resistance to *Vibrio mimicus* in freshwater crayfish (*Cherax cainii*). Front. Physiol. 10. <https://doi.org/10.3389/fphys.2019.01635>.
- Skelton, J., Geyer, K.M., Lennon, J.T., Creed, R.P., Brown, B.L., 2017. Multi-scale ecological filters shape the crayfish microbiome. Symbiosis 72, 159–170. <https://doi.org/10.1007/s13199-016-0469-9>.
- Stojanov, S., Berlec, A., Strukelj, B., 2020. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms 8, 1–16. <https://doi.org/10.3390/microorganisms8111715>.
- Tulsankar, S.S., Fotedar, R., Cole, A.J., Gagnon, M.M., 2022a. Live plankton supplementation improves growth and health status of marron (*Cherax cainii* Austin 2002). Aquaculture 558, 738327. <https://doi.org/10.1016/j.aquaculture.2022.738327>.
- Tulsankar, S.S., Foyals, M.J., Cole, A.J., Gagnon, M.M., Fotedar, R., 2022b. A mixture of manganese, silica and phosphorus supplementation alters the plankton density, species diversity, gut microbiota and improved the health status of cultured marron (*Cherax cainii*, austin and ryan, 2002). Biol. Trace Elem. Res. 200, 1383–1394. <https://doi.org/10.1007/s12011-021-02721-2>.
- Wang, Y., Huang, J.M., Zhou, Y.L., Almeida, A., Finn, R.D., Danchin, A., He, L.S., 2020. Phylogenomics of expanding uncultured environmental Tenericutes provides insights into their pathogenicity and evolutionary relationship with *Bacilli*. BMC Genom. 21, 1–12. <https://doi.org/10.1186/s12864-020-06807-4>.
- Wu, Z., Gatesoupe, F.J., Zhang, Q., Wang, X., Feng, Y., Wang, S., Feng, D., Li, A., 2019. High-throughput sequencing reveals the gut and lung prokaryotic community profiles of the Chinese giant salamander (*Andrias davidianus*). Mol. Biol. Rep. 46, 5143–5154. <https://doi.org/10.1007/s11033-019-04972-8>.
- Wu, Z., Wang, S., Zhang, Q., Hao, J., Lin, Y., Zhang, J., Li, A., 2020. Assessing the intestinal bacterial community of farmed Nile tilapia (*Oreochromis niloticus*) by high-throughput absolute abundance quantification. Aquaculture 529, 735688. <https://doi.org/10.1016/j.aquaculture.2020.735688>.
- Xie, M., Zhang, S., Xu, L., Wu, Z., Yuan, J., Chen, X., 2021. Comparison of the intestinal microbiota during the different growth stages of red swamp crayfish (*Procambarus clarkii*). Front. Microbiol. 12, 1–16. <https://doi.org/10.3389/fmicb.2021.696281>.
- Xu, Y., Li, Y., Xue, M., Yang, T., Luo, X., Fan, Y., Meng, Y., Liu, W., Lin, G., Li, B., Zeng, L., Zhou, Y., 2021. Effects of Dietary *Saccharomyces cerevisiae* Yfi-Sc2 on the Growth Performance, Intestinal Morphology, Immune Parameters, Intestinal Microbiota, and Disease Resistance of Crayfish (*Procambarus Clarkii*). <https://doi.org/10.3390/ani11071963>. Animals 11.
- Xue, M., Jiang, N., Fan, Y., Yang, T., Li, M., Liu, W., Li, Y., Li, B., Zeng, L., Zhou, Y., 2022. White spot syndrome virus (WSSV) infection alters gut histopathology and microbiota composition in crayfish (*Procambarus clarkii*). Aquac. Reports 22. <https://doi.org/10.1016/j.aqrep.2022.101006>.
- Zhang, Q., Lin, Y., Zhang, T., Wu, Y., Fang, P., Wang, S., Wu, Z., Hao, J., Li, A., 2021. Etiological characteristics of “tail blister disease” of Australian redclaw crayfish (*Cherax quadricarinatus*). J. Invertebr. Pathol. 184, 107643. <https://doi.org/10.1016/j.jip.2021.107643>.
- Zhang, Y., Li, Z., Kholodkevich, S., Sharov, A., Chen, C., Feng, Y., Ren, N., Sun, K., 2020a. Effects of cadmium on intestinal histology and microbiota in freshwater crayfish (*Procambarus clarkii*). Chemosphere 242, 125105. <https://doi.org/10.1016/j.chemosphere.2019.125105>.
- Zhang, Y., Li, Z., Kholodkevich, S., Sharov, A., Feng, Y., Ren, N., Sun, K., 2020b. Microcystin-LR-induced changes of hepatopancreatic transcriptome, intestinal microbiota, and histopathology of freshwater crayfish (*Procambarus clarkii*). Sci. Total Environ. 711, 134549. <https://doi.org/10.1016/j.scitotenv.2019.134549>.
- Zhang, Z., Liu, J., Jin, X., Liu, C., Fan, C., Guo, L., Liang, Y., Zheng, J., Peng, N., 2020c. Developmental, dietary, and geographical impacts on gut microbiota of red swamp crayfish (*Procambarus clarkii*). Microorganisms 8, 1–15. <https://doi.org/10.3390/microorganisms8091376>.
- Zhu, L., Kong, Y., Chang, X., Feng, J., Wang, X., Hou, L., Zhao, X., Pei, C., Kong, X., 2023. Effects of two fish-derived probiotics on growth performance, innate immune response, intestinal health, and disease resistance of *Procambarus clarkii*. Aquaculture 562, 738765. <https://doi.org/10.1016/j.aquaculture.2022.738765>.