Understanding the role of the shrimp gut microbiome in health and disease

Corey C. Holt\(^{a,b,c,d,}\), David Bass\(^{a,c}\), Grant D. Stentiford\(^{b,c}\), Mark van der Giezen\(^{b,c,e,}\)

\(^a\) International Centre of Excellence for Aquatic Animal Health Theme, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom
\(^b\) Biosciences, University of Exeter, Stocker Road, Exeter, United Kingdom
\(^c\) Centre for Sustainable Aquaculture Futures, University of Exeter, Stocker Road, Exeter, United Kingdom
\(^d\) Department of Botany, University of British Columbia, Vancouver, Canada
\(^e\) Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, 4021 Stavanger, Norway

**ARTICLE INFO**

**Keywords:**
- Shrimp
- Penaeus monodon
- Litopenaeus vannamei
- Gut microbiota
- Gut microbiome
- Aquaculture

**ABSTRACT**

With rapid increases in the global shrimp aquaculture sector, a focus on animal health during production becomes ever more important. Animal productivity is intimately linked to health, and the gut microbiome is becoming increasingly recognised as an important driver of cultivation success. The microbes that colonise the gut, commonly referred to as the gut microbiota or the gut microbiome, interact with their host and contribute to a number of key host processes, including digestion and immunity. Gut microbiome manipulation therefore represents an attractive proposition for aquaculture and has been suggested as a possible alternative to the use of broad-spectrum antibiotics in the management of disease, which is a major limitation of growth in this sector. Microbiota supplementation has also demonstrated positive effects on growth and survival of several different commercial species, including shrimp. Development of appropriate gut supplements, however, requires prior knowledge of the host microbiome. Little is known about the gut microbiota of the aquatic invertebrates, but penaeid shrimp are perhaps more studied than most. Here, we review current knowledge of information reported on the shrimp gut microbiota, highlighting the most frequently observed taxa and emphasizing the dominance of Proteobacteria within this community. We discuss involvement of the microbiome in the regulation of shrimp health and disease and describe how the gut microbiota changes with the introduction of several economically important shrimp pathogens. Finally, we explore evidence of microbiome supplementation and consider its role in the future of penaeid shrimp production.

1. Introduction

Gut-inhabiting microbes are recognised as important drivers of several metabolic processes in the host. As such, the characterisation and subsequent manipulation of this microscopic community is an attractive proposition for aquaculture research. Penaeid shrimp aquaculture is an important source of economic gain for many Asian and Latin American countries (Hernández-Rodríguez et al., 2001) and shrimp research has subsequently dominated the field of marine-based invertebrate gut microbiomes. However, in comparison with mammals and terrestrial invertebrates, relatively very little is known about the bacteria living in the gut of aquatic invertebrates such as penaeid shrimp.

In this review, we summarise gut microbiome sequence data from currently available penaeid shrimp studies that utilise a high-throughput sequencing (HTS) approach, in order to investigate the diversity of gut-associated bacteria in shrimp grown under a range of conditions across the world. Proteobacteria were the dominant phylum in most studies, the vast majority of which have been carried out in China (Fig. 1A) (Table 1). Proteobacteria are widespread in aquatic invertebrate gut microbiotas and are often a dominant component of this community in other Crustacea (Hakim, 2015; Holt et al., 2020; Huang, 2014; Meziti, 2010; Rungrasamee, 2013, 2014; Zhang, 2014). The phylum Proteobacteria is highly diverse in terms of physiology, morphology, and genetics. They are Gram-negative, and most are facultative or obligate anaerobes (Stackebrandt et al., 1988). Gamma-proteobacteria, the largest class in the phylum, are often described as the most common bacteria in the gut of giant tiger shrimp (Penaeus monodon) (Chaiyapechara et al., 2012; Rungrasamee et al., 2013, 2014, 2016) and Pacific white shrimp (Litopenaeus vannamei) (Tzuc et al., 2014; Rungrasamee et al., 2016; Zheng et al., 2017). This class, mainly comprising Vibrio and Photobacterium spp., has also been...
were more similar to each other despite differences in the community.

Despite this, having expressed virulence in compromised hosts (Manilal et al., 2006). As such, several Vibrio spp. have historically caused large losses to the aquaculture industry, with vibriosis often causing mass mortalities (Lavilla-Pitogo et al., 1998) and seemingly non-pathogenic disease and loose shell syndrome (Liu and Lee, 2002; Jayasree et al., 2013). Although Gammaproteobacteria dominated the gut throughout the different life-stages of P. monodon in Thailand (Fig. 2B), there were shifts from a Photobacterium-based community to a Vibrio-based community between PL and juvenile stages (Rungrassamee et al., 2013). Gammaproteobacteria also dominated the guts of L. vannamei at different life-stages in a holding facility in China, with the exception to 2-month old juveniles which mainly harboured Bacteroidetes (Fig. 2C) (Huang et al., 2014). Aquaculture practices, such as indoor- vs pond-based culture can also impact the composition of the microbiome (Landsman et al., 2020). Overall, the growing wealth of evidence suggests that both environmental and internal, host-associated factors can contribute to the determination of microbial communities and it is often difficult to untangle the direct effects of any one variable.

2. Patterns and processes relating shrimp health to gut microbiota

While the majority of HTS microbiome studies focus on the midgut, or in some cases, an unspecified region of the gut, relatively few describe the community of the foregut and hindgut. The peneaed digestive tract, and the digestive tract of that of all Crustacea, is made up of three sections (Fig. 2A); the foregut, containing the oesophagus and the two chambered stomach; the midgut; which begins at the junction of the hepatopancreas (HP) and traverses the length of the cephalothorax and the majority of the abdomen; and finally the hindgut, containing the rectum and anus. These regions of the gut differ in their cell structure and function (Ceccaldi, 1989). There are few studies describing communities inhabiting the foregut, however Alphaproteobacteria along with Planctomycetes dominate the stomach of healthy Pacific white shrimp (L. vannamei) in a study from Vietnam (Chen et al., 2017). Microbial profiles are likely influenced by the longitudinal axis of the gut itself as different morphologies and functions along the gut will induce differential pressures on selection. These internal pressures are perhaps why wild-caught and domesticated P. monodon shared similar taxa in the gut despite clear differences in their rearing environment (Rungrassamee et al., 2014) and L. vannamei guts from different farms were more similar to each other despite differences in the community structure of their respective rearing waters (Zoqrat et al., 2018). In an earlier study, wild type L. vannamei from Mexico were shown to harbour a more diverse bacterial community compared to healthy cultured animals and, unlike P. monodon, contained substantial proportions of Cyanobacteria (Fig. 2C) (Cornejo-Granados et al., 2017). The availability and diversity of the diet likely impacts spatial comparisons. Mode and location of feeding may determine the abundance of usable substrate and the subsequent proliferation of microbial taxa within the gut. Furthermore, studies tracking gut composition over development stages have implicated changing in feeding to be the cause of bacterial community changes seen at the family-level throughout development. Although Gammaproteobacteria dominated the gut throughout the different life-stages of P. monodon in Thailand (Fig. 2B), there were shifts from a Photobacterium-based community to a Vibrio-based community between PL and juvenile stages (Rungrassamee et al., 2013). Gammaproteobacteria also dominated the guts of L. vannamei at different life-stages in a holding facility in China, with the exception to 2-month old juveniles which mainly harboured Bacteroidetes (Fig. 2C) (Huang et al., 2014). Aquaculture practices, such as indoor- vs pond-based culture can also impact the composition of the microbiome (Landsman et al., 2019a) as can the integration of a multi-trophic aquaculture system, which is also thought to improve productivity (Omont et al., 2020). Overall, the growing wealth of evidence suggests that both environmental and internal, host-associated factors can contribute to the determination of microbial communities and it is often difficult to untangle the direct effects of any one variable.

2. Patterns and processes relating shrimp health to gut microbiota

One of the biggest threats to shrimp aquaculture is the onset of disease and subsequent mortality in cultured stocks (Seibert and Pinto, 2012; Stentiford et al., 2012). Even in cases where the clinical signs of disease are well described, little is known about how the presence of a pathogen may impact or interact with the microbial communities in the gut and subsequently influence the metabolic processes within the host. On the other hand, it is unclear whether changes to the gut microbiome may predispose the gut to invasion by (a) pathogen(s). Changes in gut microbiome structure could also facilitate the progression of enteric pathogens that rely on translocation through the gut epithelia to initiate infection in the target tissue. The notion of a ‘one pathogen-one disease’ scenario is being increasingly challenged (Dai et al., 2018; Bass et al.,...
| Reference          | Species            | Location                                      | Growth Stage/Time                          | Hypervariable region/Primer | Sequencing Platform | Data Accession          |
|--------------------|--------------------|------------------------------------------------|---------------------------------------------|------------------------------|---------------------|-------------------------|
| Rungrassamee et al., 2014 | Penaeus monodon | Andaman Sea, Thailand | Wild, Domesticated Wild | V3-V4/338F-518R | 454 | KF329429-KF334451,KF334452-KF344403,KF344404-KF355928 |  |
| Oetama et al., 2016   | Penaeus monodon | Bali, Indonesia | Wild, Aquaculture farm | V4/515F-806R | Illumina | SRP0597721          |
| Cornejo-Granados et al., 2017 | Litopenaeus vannamei | Nayarit coast, Mexico | Wild (healthy and diseased) Cultured (healthy and diseased) Cultured - sediment | V2-4-8 mix, V3-6-7-9 mix/ Unpublished | Ion Torrent | SRR3585664-84. Bioproject: PRJNA387510 |
| Hou et al., 2018a     | Litopenaeus vannamei | Guangdong Province, China | Water Sediment | V4/515F-806R | Illumina | SRR5387734          |
| Huang et al., 2018    | Litopenaeus vannamei | Shandong, China | Middle stage of farming - shrimp gut Middle stage - water Middle stage - sediment Late stage - shrimp gut Late stage - water Late stage - sediment | V4-V5/515F-907R | Illumina | SRP118740          |
| Su et al., 2018*      | Litopenaeus vannamei | Guangdong Province, China | Mud pond Aquaculture farm Intensive pond | V4/515F-806R | Illumina | SRR129489          |
| Zoqrat et al., 2018   | Litopenaeus vannamei | Quang Yen, Quang Ninh, Vietnam | Shrimp gut Water | V3-V4/S-o-Bact-0341-b-S-17(F)-S-o-Bact-07/85-a-A-21(R) | Illumina | SRR126985. Bioproject: PRJNA422950 |
| Deng et al., 2019     | Litopenaeus vannamei | Sitiawan, Perak, Malaysia | Shrimp gut Water | V3-V4/341F-805R | Illumina | SAMN10462254-SARM10462265 |
| Fan et al., 2019b     | Litopenaeus vannamei | Panyu, Guangdong, China | Freshwater cultured - shrimp gut Freshwater cultured - water Marine cultured - shrimp gut Marine cultured - water | V3-V4/338F-806R | Illumina |  |
| Landsman et al., 2019a| Litopenaeus vannamei | Minnesota, USA | Indoor-raised Pond-raised Wild-caught | V1-V3/27F-519R | Illumina | SRR185856.Bioproject PRJNAS22274 |
| He et al., 2020       | Litopenaeus vannamei | Haikou, Hainan province, China | ‘Higher place’ culture ponds - shrimp gut Water | V4-V5/515F-907R | Illumina | SRR9687557-SRR9687559 |
| Huang et al., 2020a   | Litopenaeus vannamei | Wenzhou, Zhejian Province, China | Shrimp gut Small sized biofloc Medium/big sized biofloc Shrimp monoculture Shrimp-oyster co-culture | V4/515F-Y-806RB | Illumina |  |
| Omont et al., 2020    | Litopenaeus vannamei | La Paz, Baja California Sur, Mexico | Shrimp monoculture Shrimp-oyster co-culture | V3/338F-533R | Illumina | PRJNAS94728 |

(continued on next page)
| Reference                        | Species              | Location                           | Comparison          | Hypervariable region/Primers | Sequencing Platform | Data Accession       |
|---------------------------------|----------------------|------------------------------------|---------------------|------------------------------|---------------------|----------------------|
| Rungrasamee et al., 2013         | Penaeus monodon      | Surat Thani province, Thailand     | Postlarvae 15 days  | V3-V6/338F-786R              | 454                 | JX919344-JX926388    |
|                                 |                      |                                    | Juvenile 1 month    |                              |                     | JX919343             |
|                                 |                      |                                    | Juvenile 2 months   |                              |                     | JX926389-JX939518    |
|                                 |                      |                                    | Juvenile 3 months   |                              |                     | JX939519-JX941408    |
| Huang et al., 2014              | Litopenaeus vannamei | Xiamen, Fujian Province, China     | Postlarvae 14 days  | V3-V5/338F-907R              | 454                 | BioProject:PRJNA248359 |
|                                 |                      |                                    | Juvenile 1          |                              |                     |                      |
|                                 |                      |                                    | Juvenile 2          |                              |                     |                      |
|                                 |                      |                                    | Juvenile 3          |                              |                     |                      |
|                                 |                      |                                    | Pond 1              |                              |                     |                      |
|                                 |                      |                                    | Pond 7              |                              |                     |                      |
| Zeng et al., 2017               | Litopenaeus vannamei | Field pond, Zhangzhou, Fujian     | 1 (15 dph)          | V4/515F-806R                 | Illumina            | SRX2946975           |
|                                 |                      | Province, China                   | 2 (30 dph)          |                              |                     |                      |
|                                 |                      |                                    | 3 (45 dph)          |                              |                     |                      |
|                                 |                      |                                    | 4 (60 dph)          |                              |                     |                      |
|                                 |                      |                                    | 5 (75 dph)          |                              |                     |                      |
| Zheng et al., 2017*             | Litopenaeus vannamei | Hainan, China                      | Zoea 1              | V3-V6/341F-1073R             | 454                 | SRP080243            |
|                                 |                      |                                    | Zoea 3              |                              |                     |                      |
|                                 |                      |                                    | Mysis 1             |                              |                     |                      |
|                                 |                      |                                    | Mysis 3             |                              |                     |                      |
|                                 |                      |                                    | Postlarva 1         |                              |                     |                      |
|                                 |                      |                                    | Postlarva 6         |                              |                     |                      |
| Xiong et al., 2017a             | Litopenaeus vannamei | Ningbo, China                     | Larvae              | V3-V4/341F-806R              | Illumina            | DRA005256            |
|                                 |                      |                                    | Postlarvae          |                              |                     |                      |
|                                 |                      |                                    | Juvenile            |                              |                     |                      |
|                                 |                      |                                    | Preadult            |                              |                     |                      |
|                                 |                      |                                    | Adult               |                              |                     |                      |
| Gainza et al., 2018             | Litopenaeus vannamei | El Oro, Ecuador                   | Nursery             | V2-V3/341F-518R              | Ion Torrent         | BioProject:PRJNA352369 |
| Su et al., 2018*                | Litopenaeus vannamei | Guangdong Province, China         | Harvest             | V4/515F-806R                 | Illumina            | SRP129489            |
| Xue et al., 2018*               | Litopenaeus vannamei | Guangdong Province, China         | Nauplii 5           | V4/515F-806R                 | Illumina            | CRA000198            |
|                                 |                      |                                    | Zoea 2              |                              |                     |                      |
|                                 |                      |                                    | Mysis 1             |                              |                     |                      |
| Liu et al., 2019                | Litopenaeus vannamei | Zhejiang Province, China          | ZT strain day 5     | V4/515F_Y-806R_B             | Illumina            | SRP150920            |
|                                 |                      |                                    | ZT strain day 15    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 20    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 40    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 60    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 75    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 90    |                              |                     |                      |
|                                 |                      |                                    | ZT strain day 105   |                              |                     |                      |
|                                 |                      |                                    | PM strain day 5     |                              |                     |                      |
|                                 |                      |                                    | PM strain day 15    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 20    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 40    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 60    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 75    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 90    |                              |                     |                      |
|                                 |                      |                                    | PM strain day 105   |                              |                     |                      |

(continued on next page)
| Reference          | Species                  | Location               | Comparison               | Hypervariable region/Primers | Sequencing Platform | Data Accession |
|--------------------|--------------------------|------------------------|--------------------------|-----------------------------|---------------------|-----------------|
| Xiong et al., 2019 | Litopenaeus vannamei      | Xianhshan, Ningbo, China | Larvae, Juvenile, Adult   | V3-V4/341F-806R              | Illumina            | DRA007714       |
|                    |                          | Zhannqi, Ningbo, China |                          |                             |                     |                 |
| Garibay-Valdez et al., 2020 | Litopenaeus vannamei | Sonora, Mexico          | Farm, Larvae, Juvenile, Adult | V4/515F-806R              | Illumina            |                 |
| Zhang et al., 2014 | Litopenaeus vannamei      | Shenzhen, China         | Soybean oil (Diet), Beef tallow, Limed Oil, Fish Oil, SBF | V4-V5/515F-907R          | Illumina            | PRJNA253075     |
| Qiao et al., 2017  | Litopenaeus vannamei      | Shenzhen, China         | Glucose, Sucrose, Corn starch | V4-V5/515F-907R          | Illumina            | PRJNA291010     |
| Niu et al., 2018   | Litopenaeus vannamei      | Quanzhou, China         | Control diet, 1% *Porphyra haitanensis* 2% 3% 4% 5% 6% | V4/515F-806R            | Illumina            |                 |
| Cheng et al., 2019 | Litopenaeus vannamei      | Pingtung, Taiwan        | Control diet *Bacillus subtilis* E20-fermented soybean meal, Antimicrobial peptide isolated from *B. subtilis* E20-PSBM | V3-V4/S17-A21           | Illumina            |                 |
| Fan et al., 2019a  | Litopenaeus vannamei      | Shan-Wei, China         | Fishmeal (Week 1), Fishmeal (Week 2), Fishmeal (Week 3), Fishmeal (Week 4), Fishmeal (Week 5), Fishmeal (Week 6), Fishmeal (Week 7), Fishmeal (Week 8), Krill meal (Week 1), Krill meal (Week 2), Krill meal (Week 3), Krill meal (Week 4), Krill meal (Week 5), Krill meal (Week 6), Krill meal (Week 7), Krill meal (Week 8) | V3-V4/                     | Illumina            | SRP136220       |
| Reference          | Species                          | Location                       | Comparison                                      | Hypervariable region/ Primers | Sequencing Platform | Data Accession |
|--------------------|----------------------------------|--------------------------------|------------------------------------------------|-------------------------------|----------------------|-----------------|
| Shao et al., 2019  | Litopenaeus vannamei             | Qingdao, China                 | Control fishmeal diet                           | V3-V4/338F-806R              | Illumina             | SRP128484       |
|                    |                                  |                                | 10% fermented soybean meal (FSM)                 |                               |                      |                 |
|                    |                                  |                                | 20% FSM                                         |                               |                      |                 |
|                    |                                  |                                | 30% FSM                                         |                               |                      |                 |
|                    |                                  |                                | 40% FSM                                         |                               |                      |                 |
| Yuan et al. 2019   | Litopenaeus vannamei             | China                          | No supplemented copper                          | V3-V4/338F-806R              | Illumina             | PRJNA417739     |
|                    |                                  |                                | Copper sulphate                                 |                               |                      |                 |
|                    |                                  |                                | Copper amino acid complex from Availa*Cu100     |                               |                      |                 |
|                    |                                  |                                | 1:1 copper sulphate + copper amino acid complex  |                               |                      |                 |
| Eliando-Gomáñez et al., 2020 | Penaeus vannamei syn. Litopenaeus vannamei | La Paz, Baja California Sur, Mexico | Control diet (P)                                | V4/515F-806R                 | Illumina             | PRJNA417739     |
|                    |                                  |                                | P + Ulva lactuca                                |                               |                      |                 |
|                    |                                  |                                | P + Ulva clathrata                              |                               |                      |                 |
|                    |                                  |                                | Ulva lactuca                                    |                               |                      |                 |
|                    |                                  |                                | Ulva clathrata                                  |                               |                      |                 |
| Schleder et al., 2020 | Litopenaeus vannamei             | Santa Catarina, Brazil          | Control diet                                    | V3-V4/314F-806R              | Illumina             |                 |
|                    |                                  |                                | 0.5% Sargassum filipendula: 1%                  |                               |                      |                 |
|                    |                                  |                                | Undaria pinnatifida: 0.5S:2U                     |                               |                      |                 |
|                    |                                  |                                | 0.5S:4U                                         |                               |                      |                 |
| Simon et al., 2020 | Litopenaeus vannamei             | Australia                      | Control diet (Fishmeal)                         | V1-V3/27F-519R               | Illumina             |                 |
|                    |                                  |                                | Novacq*                                        |                               |                      |                 |
|                    |                                  |                                | Krill meal                                      |                               |                      |                 |
|                    |                                  |                                | Krill hydrolysate                               |                               |                      |                 |
|                    |                                  |                                | Whole squid                                     |                               |                      |                 |
| Xiong et al., 2015 | Litopenaeus vannamei             | Zhanqum Ningbo, China          | Black intestine (Healthy)                       | V4/515F-816R                 | Illumina             | DRA002398       |
|                    |                                  |                                | Red intestine (Sub-healthy)                     |                               |                      |                 |
|                    |                                  |                                | Empty intestine (Diseased)                      |                               |                      |                 |
| Runggrassamee et al., 2016 | Penaeus monodon                | Shrimp Biotechnology Business Unit (SBRU), Thailand | 0 h post exposure                              | V3-V4/338F-786R              | 454                  | KP944208-KP944681 |
|                    |                                  |                                | 6HPE                                           |                               |                      | KP949836-KP949529 |
|                    |                                  |                                | 12HPE                                          |                               |                      | KP944682-KP945671 |
|                    |                                  |                                | 24HPE                                          |                               |                      | KP946572-KP946691 |
|                    |                                  |                                | 48HPE                                          |                               |                      | KP946692-KP948363 |
|                    |                                  |                                | 72HPE                                          |                               |                      | KP948530-KP948831 |
|                    |                                  |                                | 0HPE                                           |                               |                      | KP948802-KP951735 |
|                    |                                  |                                | 6HPE                                           |                               |                      | KP955329-KP953763 |
|                    |                                  |                                | 12HPE                                          |                               |                      | KP951736-KP952247 |
|                    |                                  |                                | 24HPE                                          |                               |                      | KP952248-KP952978 |
|                    |                                  |                                | 48HPE                                          |                               |                      | KP952979-KP953298 |
|                    |                                  |                                | 72HPE                                          |                               |                      | KP953764-KP953903 |
| Chen et al., 2017  | Litopenaeus vannamei             | Ben Tre Province, Vietnam       | AHPND - HP                                      | V3-V4/S17-A21                | Illumina             | SRP102384       |
| Dai et al., 2017   | Litopenaeus vannamei             | Xiangshan, Ningbo, China       | AHPND + HP                                      | V2-V3/18FJ82-Iuk_F5316       | Illumina             | DRA005322.      |
| Xiong et al., 2017b| Litopenaeus vannamei             | Xiangshan, Ningbo, China       | Normal                                          | V3-V4/338F-806R              | Illumina             | DRA005153       |
|                    |                                  |                                | Retarded Overgrown                              |                               |                      |                 |
|                    |                                  |                                | Overgrown Water                                 |                               |                      |                 |
| Zheng et al., 2017*| Litopenaeus vannamei             | Hainan, China                   | Healthy                                         | V3-V6/341F1073R              | 454                  | SRF080243       |
|                    |                                  |                                | Diseased                                        |                               |                      |                 |

(continued on next page)
| Reference          | Species                         | Location                | Comparison                                                           | Hypervariable region/Primers                  | Sequencing Platform | Data Accession   |
|--------------------|---------------------------------|-------------------------|                                                                     |                                            |                    |                |
| Dai et al., 2018   | Litopenaeus vannamei            | Ningbo, China           | Healthy – Disease emergence (July 1st)                               | V3-V4/341F-806R                             | Illumina            | DRA005997       |
|                    |                                 |                         | Diseased – Disease emergence (July 1st)                             |                                              |                    |                |
|                    |                                 |                         | Healthy – Disease exacerbation (July 4th)                            |                                              |                    |                |
|                    |                                 |                         | Diseased – Disease exacerbation (July 10th)                          |                                              |                    |                |
| Hou et al., 2018b  | Litopenaeus vannamei            | Guangzhou, China        | WFS + Healthy Asymptomatic gut                                      | V4/S1F-806R                                 | Illumina            | SRR6286523      |
| Le et al., 2018    | Penaeus monodon                 | Dong Hai district, Bac Lieu province, Vietnam |                                                                     |                                            |                    |                |
| Pilotto et al., 2018 | Litopenaeus vannamei           | Florianópolis, Brazil   | Healthy Biofloc Healthy Clear seawater WSSV + Biofloc WSSV - Clear seawater | V3-V4/341F-806R                            | Illumina            |                |
| Xiong et al., 2018a| Litopenaeus vannamei            | Ningbo, China           | Healthy larvae Healthy juveniles Healthy adults Diseased adults Healthy postlarvae Healthy juveniles Healthy adults Disease emergence Disease exacerbation | V3-V4/341F-806R                            | Illumina            | DRA005792       |
| Xiong et al., 2018b| Litopenaeus vannamei            | Zhanqi, Ningo, China    |                                                                     |                                            |                    |                |
| Yao et al., 2018   | Litopenaeus vannamei            | Ningbo, China           | Healthy (sampled at 70 days) Healthy 80 days Healthy 85 days Diseased 70 days Diseased 80 days Diseased 85 days | V3-V4/338F-806R                            | Illumina            | SRP131736       |
| Dai et al., 2019   | Litopenaeus vannamei            | Ningbo, China           | Pre-WFS Pre-Healthy WFS Healthy                                      | V4/3NDf-V4_Euk_R2                           | Illumina            |                |
| (Li et al., 2019)  | Litopenaeus vannamei            | Guangdong, China        | White faeces Black gill Retarded growth Healthy                     | ITS1/ITS1F-ITS2                             | Illumina            | PRJNA495902     |
| Wang et al., 2019  | Litopenaeus vannamei            | Maoming, Guangdong Province, China | Control                                                              | V4/S1F-806R                                 | Illumina            | SRP145560       |
| Zhou et al., 2019  | Litopenaeus vannamei            | Wenchang, Hainan, China | WSSV + Healthy Diseased                                             | V3-V4/338F-806R                            | Illumina            | SRP192810       |
| Dai et al., 2020   | Litopenaeus vannamei            | Ningbo, China           | Healthy 84 days Diseased 84 days Healthy 87 days Diseased 87 days Healthy 93 days Diseased 93 days | V3-V4/341F-806R                            | Illumina            | DRA005256       |

(continued on next page)
| Reference            | Species | Location                        | Comparison                                                                 | Hypervariable region/Primers | Sequencing Platform | Data Accession    |
|----------------------|---------|---------------------------------|---------------------------------------------------------------------------|-----------------------------|---------------------|-------------------|
| Huang et al., 2020b  | *P. vannamei* | Zhuhai, Guangdong, China        | Healthy WFS, Healthy + PBS transplant, Healthy + WFS transplant, Healthy + PBS transplant 2 | V4/S515F-806R               | Illumina            | PRJN542015        |
| Liang et al., 2020   | *P. vannamei* syn. *L. vannamei* | Guangdong Province, China       | V3-V4/341F-806R                                                          | Illumina                    |                     |                   |
| Supplement           | Sha et al., 2016 | Qingdao, China                | V1-V2/38F-338R                                                            | Illumina                    |                     | SRP071046         |
| Duan et al., 2017    | *P. vannamei* | Shenzhen, China                 | V4/515F-806R                                                              | Illumina                    |                     |                   |
| He et al., 2017      | *P. vannamei* | Xiamen, China                  | V3-V4/341F-805R                                                           | Illumina                    |                     |                   |
| Vargas-Albores et al., 2017 | *P. vannamei* | Empalme, Sonora, Mexico        | V1-V3/27F-519R                                                            | Illumina                    |                     |                   |
| Liu et al., 2018b    | *P. vannamei* | Zhanqi, Ningbo, China          | V4/515F-806R                                                              | Illumina                    |                     |                   |
| Landsman et al., 2019b | *P. vannamei* | Minnesota, USA                 | V3/338F-533R                                                              | Illumina                    |                     |                   |
| Mazón-Suátegui et al., 2019 | *P. vannamei* | Rl8 (Strep)                    | V4/515F-806R                                                              | Illumina                    |                     |                   |
| Xie et al., 2019     | *P. vannamei* | Sanya, Hainan province, China   | V2-V3/341F-519R                                                           | Illumina                    |                     |                   |
| Gainza and Romero, 2020 | *P. vannamei* | Huaquillas, El Oro Province, Ecuador | V1-V3/27F-519R                                                            | Illumina                    |                     |                   |

(continued on next page)
| Reference        | Species                  | Location                     | Comparison                        | Hypervariable region/Primers | Sequencing Platform | Data Accession |
|------------------|--------------------------|------------------------------|-----------------------------------|------------------------------|---------------------|-----------------|
| Stress           |                          |                              |                                   |                              |                     |                 |
| Su et al., 2017  | *Litopenaeus vannamei*   | Shenzhen, China              | 0 µg/L sulphide 425.5 µg/L sulphide 851 µg/L sulphide | V4-V5/                       | Illumina            | SRP091598       |
| Duan et al., 2018| *Litopenaeus vannamei*   | China                        | Control group Ammonia stress      | V4/515F-806R                 | Illumina            |                 |
| Duan et al., 2019a| *Litopenaeus vannamei*   | Shenzhen, China              | Control group Nitrile stress      | V4/515F-806R                 | Illumina            |                 |
| Duan et al., 2019b| *Litopenaeus vannamei*   | Shenzhen, China              | Control group Low pH stress       | V4/515F-806R                 | Illumina            |                 |
| Duan et al., 2019b| *Litopenaeus vannamei*   | Shenzhen, China              | Control group High pH stress      | V4/515F-806R                 | Illumina            |                 |
| Jiang et al., 2019| *Litopenaeus vannamei*   | Yangjiang, Guangdong Province, China | Control group Sulfide stress group | V3-V4/341F-806R              | Illumina            |                 |
| Yu et al., 2020  | *Litopenaeus vannamei*   | Hainan, China               | pH 6.5 pH 8.0 pH 9.5              | V3-V4/339F-806R              | Illumina            | PRJNAs8594      |
| Qian et al., 2020| *Litopenaeus vannamei*   | Wenchang, Hainan, China     | 0 mg/L Cu exposure 0.1 mg/L Cu exposure 1 mg/L Cu exposure | V3-V4/338F-806R              | Illumina            | PRJNAs86585     |
| Library preparation | *Litopenaeus vannamei*   | Guangdong Province, China   | Bacterial DNA kit Mollusc DNA kit Stool DNA kit Tissue DNA kit | V4/515F-806R                 | Illumina            | CRA00198        |
| Xue et al., 2018* | *Litopenaeus vannamei*   |                              |                                   |                              |                     |                 |
| Garcia-Lopez et al., 2020 | *Litopenaeus vannamei*   | Sinaloa, Mexico             | V3 16S rRNA hypervariable region V3 V4 16S rRNA hypervariable region V4 16S rRNA hypervariable region | V3/338F-533R V3-V4/341F-806R V4/515F-805r | Illumina |                 |
The ‘pathobiome’ concept argues that the interactions between free-living microbes in the environment, host-associated symbionts (including the gut microbiota) and the host itself likely drive both beneficial and detrimental impacts on host health (Bass et al., 2019). In humans, changes to the gut microbiota have been implicated in a wide range of health conditions. Characterisation of the interplay between the microbiota and the host immune system is becoming increasingly well-defined (Sekirov et al., 2010). Pattern recognition receptors (PRRs) such as Toll-like receptors on the surface of the gut epithelia are in close proximity to microbial associated molecular patterns (MAMPs) of the microbiota such as lipopolysaccharides (Chu and Mazmanian, 2014). Although there are key differences between the vertebrate and invertebrate immune system, the gut microbiota likely has an important role to play in maintaining the health of the shrimp. The presence alone of symbiotic microbiota could itself provide a kind of immunity. A general theory true of all hosts is that space and resources within the gut are ultimately finite and colonisation resistance may...
limit the proliferation of pathogenic organisms through competitive exclusion (Lawley and Walker, 2013). Furthermore, colonization resistance may be further supported through microbiota-derived antimicrobial compounds, which may limit the establishment and proliferation of transient microbes in the digestive tract (Kobayashi and Ishibashi, 1993). A more species-diverse microbiota in the gut may facilitate resistance to a greater degree of potentially pathogenic colonizers, as there is consequently a larger set of species-species antagonisms. Reducing the abundance of certain bacterial classes within the microbiota can allow previously symbiotic species to become pathogenic (Blumberg and Powrie, 2016).

Because of the links between the gut microbiota and the host immune system, it is often suggested that a reduction in bacterial diversity within the gut or the differential abundance of particular microbial taxa may be responsible for the onset of pathogenesis. However, without follow-up studies involving gut supplementation and/or gnotobiotic organisms (germ free animals and/or organisms that harbour a defined microbial community) it is often impossible to discern between cause and effect. Nevertheless, these correlations should not be dismissed without merit and several studies have described such correlations in shrimp under the affliction of important pathogens which cause massive economic loss (Table 1, Fig. 2D).

3. Changes to the gut microbiome can correlate with the incidence of disease

The following section summarises what is known of the microbiome in relation to key diseases which can impact production. Although we have collated these studies in Fig. 2, it is important to recognise that these samples were analysed independently of each other within their original studies. Therefore, differences in methodologies and/or analyses (for example in the DNA extraction method, the region of the genes sequenced and the bioinformatics approaches used) may in turn bias comparisons between microbiomes associated with different disease studies (Cornejo-Granados et al., 2018; Xue et al. 2018; García-López et al., 2020). The majority of studies sequencing the bacterial gut microbiota utilise the V3-V4 amplicon (Fig. 1B). The V3-V4 amplicon targeting the shrimp gut microbiota produced a larger number of operational taxonomic units (OTUs) compared to V3 and V4 alone (García-López et al., 2020). However, the V4 region, which is the second most used 16S region, is much less variable in sequence length (García-López et al., 2020) and is sometimes preferred on this basis to V3 or both regions together. Unfortunately, short read data were not accessible for all studies shown in Fig. 2 when we attempted a meta-analysis to directly compare the results of all health studies.

3.1. Clinical signs of disease in penaeids

In ‘diseased’ Pacific white shrimp raised in a commercial hatchery in Hainan, China, and characterised by poor growth, inactivity, lack of appetite, empty digestive tracts and/or low survival rate, there were no significant differences in the microbiota when compared to healthy individuals up to and including 18 days post-larvae (Fig. 2D) (Zheng et al., 2017). However, Linear Discriminate Analysis (LDA) Effect Size (LEFSe) highlighted several taxa that were indicative of the disease state (Zheng et al., 2017). Species of the Neustella genus (Rhodobacteraceae), which can be pathogenic to algae and brine shrimp (Gardiner, Thomas and Egan, 2015; Zheng et al., 2016) showed the greatest association with diseased individuals and the water in which they were reared. Unlike the shrimp samples themselves, water from healthy and diseased ponds formed distinct clusters when ordinated with non-metric multidimensional scaling (NMDS), therefore environmental DNA (eDNA) assessment of the microbiome within the rearing environment may be a useful indicator of disease in the cultivar. Due to size restrictions, these data were based on whole-body homogenates, however external tissues were cleaned prior to extraction in a bid to remove adherent microorganisms (Zheng et al. 2017).

3.2. Acute hepatopancreatic necrosis disease (AHPND)

Sometimes referred to as Early Mortality Syndrome (EMS), AHPND has been responsible for large production losses of cultured shrimp. The disease results in atrophy of the HP and ultimately necrosis of the HP tubules, and is caused by plasmid-born toxin-producing genes carried by several species of Vibrio, resulting in the production of Photobacterium insect-related (Pir) binary toxins (Lee et al., 2015; Liu et al., 2018b; Restrepo et al., 2018). The incidence of AHPND in L. vannamei corresponded to a significant reduction in bacterial diversity of the HP compared to that of healthy individuals (Fig. 2D), with those infected with AHPND showing a reduction in diversity of over 53% within 7 days. Several Vibrio clusters were associated with AHPND positive individuals, along with a high abundance of ‘Candidatus Bacilloplasma’-like sequences. By analysing interaction networks within the community, it is suggested that different commensal ‘Candidatus Bacilloplasma’ OTUs, which are found in several aquatic invertebrates, interact with the pathogenic Vibrio strains and either enhance or inhibit infection (Chen et al., 2017).

3.3. White spot syndrome virus (WSSV)

White spot syndrome virus is the biggest threat to shrimp health worldwide (Stentiford et al., 2009). The double-stranded DNA (dsDNA) virus infects nuclei of mesodermal- and/or ectodermal-derived tissues and results in lethargy of the infected host and a reduction in food intake (Pradeep and Rai, 2012). Although predominantly infecting shrimp, its severe pathogenesis results in a reduction in growth and ultimately high mortality rates in a wide range of cultured species (Stentiford et al., 2009; Bateman et al., 2012). The gut microbiota of L. vannamei, obtained from a farm in Maoming, China, was recently shown to be significantly altered in association with WSSV infection (Wang et al., 2019). Individuals infected with WSSV saw a significant increase in Proteobacteria and Fusobacteria in the gut, including potentially pathogenic bacteria belonging to the Arcobacter genus, together with a reduction in Bacteroidetes and Tenericutes (Fig. 2D). Despite changes in relative abundances of particular phyla, there was no change in overall bacterial OTU richness and/or diversity of the gut reported in animals infected with WSSV (Wang et al., 2019). It would seem that compositional changes in response to WSSV infection are also impacted by environmental factors in relation to culture environment, which might obscure microbiome changes specifically associated with the disease and/or presence of the virus. When comparing clear seawater and biofloc systems before and after WSSV infection, there were inconsistent changes in phyla abundance and diversity (Pilotto et al., 2018). Furthermore, although Proteobacteria did increase after WSSV challenge in the biofloc system, a decrease in Bacteroidetes was not observed in either culture condition, indicating a degree of disparity between both studies. Evidence suggests that the gut microbiota of shrimp raised in a variably-sized biofloc system have similar bacterial communities to those of only medium-large sized bioflocs (Huang et al., 2020a) therefore, the presence of a biofloc could alter any microbiome-mediated resistance to WSSV infection.

3.4. White faeces syndrome (WFS)

White faeces syndrome, characterised by white-golden gut contents and white faecal strings, is a syndromic condition of unknown aetiology. WFS was initially thought to be linked to the presence of the microsporidian Enterocytozoon hepatopancreatis. Although PCR and in situ hybridization has since demonstrated that ponds with high levels of environmental (i.e. host-independent) E. hepatopancreatis signal often lack characteristic symptoms of the disease in the corresponding stocks (Tangprasittipap et al., 2013), it is also true that white faeces can
contain densely packed E. hepatopenaei spores (Tang et al., 2016). Gregarine-like vermiform bodies are also associated with characteristic signs of WFS, through the transformation, sloughing and aggregation of microvilli within the hepatopancreas (Sriruriratana et al., 2014). The cause of this phenomenon is unknown, however it would seem that white faeces is a common characteristic of multiple health conditions, and that EHP may be a necessary but insufficient cause of WFS, at least in some manifestations. When comparing bacterial gut profiles of WFS infected shrimp and asymptomatic individuals, there was an increase in ‘Candidatus Bacillicola’ (Tenericutes) and Phascolarctobacterium (Firmicutes) along with a decrease in Paracoccus (Proteobacteria) and Lactococcus spp. (Firmicutes), which correlated with a significant reduction in overall diversity of the bacterial community (Fig. 2D) (Hou et al., 2018b). ‘Candidatus Bacillicola’ is commonly found in the shrimp gut. Considering how well adapted this genus is for living in the gut environment (Kostanjšek et al., 2007), its increased relative abundance in diseased individuals is likely a consequence of the reduction in other taxa, and overall diversity of the gut microbiota. An increase in ‘Candidatus Bacillicola’ and a reduction in overall richness and diversity in WFS-infected guts has also been confirmed elsewhere, where the probability of disease could be estimated with 99.4% diagnostic accuracy using disease-discriminatory taxa in the gut (Huang et al., 2020b). Furthermore, this study demonstrated that 36.7% of healthy shrimp that received intestinal microbiota transplants (IMTs) from WFS-infected donors eventually became infected with the disease. Conversely, WFS-infected shrimp receiving IMTs from healthy donors recovered from the disease (Huang et al., 2020b).

White faeces was also associated with changes to the eukaryotic gut community but with somewhat contradicting results. Li et al. (2019) noted Ascomycota and Basidiomycota were abundant in healthy and diseased individuals with an increase in pathogenic Candida spp. in individuals exhibiting clinical signs of WFS. Dai et al. (2019) reported an overrepresentation of Ascomycota and Basidiomycota in WFS-infected individuals. Both studies also reported significant differences in non-host eukaryotic (Shannon) diversity associated with WFS.

3.5. ‘Cotton shrimp-like’ disease (CSL)

Despite no differences when comparing bacterial diversity, estimates of species richness were significantly increased in individuals suffering with a disease referred to as cotton shrimp-like disease, herein referred to as CSL (Zhou et al., 2019). The clinical signs of this disease include reduced growth, associated with atrophy of the HP and an asexual process (a definitive characteristic of cotton shrimp disease) (Zhou et al., 2019). The authors note that an increase in Tenacibaculum was associated with CSL, along with the presence of Rickettsiaceae, however at very low abundance (~0.03%) (Zhou et al., 2019). Despite a shared clinical sign (white, opaque muscle) with cotton-shrimp disease, CSL is of unknown aetiology, unlike cotton shrimp disease which is primary associated with the presence of several microsporidian genera: Pleistophora, Thelohania, Perezia, Agamasoma and Amonos (Sprague and Couch, 1971; Overtstreet, 1973; Lightner 1996; Ramasamy et al., 2001; Sokolva et al., 2015; Han et al., 2016). The gut microbiota at the phylum level was reported to be very similar when comparing healthy and CSL-infected individuals (Fig. 2D) (Zhou et al., 2019). However, interspecies interaction was substantially reduced in gut bacterial community networks associated with the disease (Zhou et al., 2019).

3.6. Blue body syndrome (BBS)

Characterised by a blue colouration of the body and internal tissues, ‘blue body syndrome’ (BBS) or ‘blue body disease’, reportedly occurs accompanied with slow growth, reduced or no feed intake and thin bodies Liang et al., 2020). The blue shell is the result of low levels of carotenoid astaxanthin, a reddish pigment found in several animals (Baticados, 1990), and therefore a microbiota-dependent dietary deficiency is a valid mechanism to explore. Healthy shrimp express more penaein, lectin and defensins1 compared to those with BBS. However, no significant and/or substantial differences in gut community composition or alpha diversity were observed when comparing healthy and BBS-positive individuals (Fig. 2D) (Liang et al., 2020). On the contrary, NMDS indicated a significant dissimilarity between the gut microbiota of healthy and BBS individuals, which were more similar to bacterial communities in the water (Liang et al., 2020), perhaps indicating a reduction in the environmental filtering capacity of the infected host.

3.7. Nutritional acquisition and slow growth

The bacterial gut microbiome can impact the growth of the shrimp through the modification of digestive enzyme activity. After rearing larval L. vannamei for 70 days in ponds located in Xiangshan, China, body size and weight significantly and positively correlated with amylase, pepsin and lipase activity (Xiong et al., 2017b). Structural equation modelling (SEM) demonstrated how gut community composition of both bacteria and eukaryotes accounted for significant positive effects on enzymatic activity (Dai et al., 2017; Xiong et al., 2017b). Bacterial diversity was significantly reduced in retarded shrimp as the relative abundance of Gammaproteobacteria dramatically increased (Fig. 2D) (Xiong et al., 2017b). Retarded shrimp also harboured less phylogenetically clustered gut communities compared to normal individuals, indicating a reduction in host determinism in the assemblage of bacterial gut communities (Xiong et al., 2017b).

Gut microbiota are repeatedly noted to be distinct from the bacterial communities of their rearing waters (Harris 1993; Meziti et al., 2012; Xiong et al., 2015; Zhang et al., 2016). This may be explained by deterministic processes, such as environmental filtering, in the colonisation of the shrimp gut during early life stages (Xiong et al., 2017a; Xiong et al., 2018a). The onset of disease, however, can cause compositional shifts to atypical microbiota, often referred to as dysbiosis (Xiong et al., 2015; Zhu et al., 2016; Xiong et al., 2017a; Xiong et al., 2018a). The emergence of disease may also correlate with a reduction in deterministic processes that influence microbiota composition and a more stochastic assembly of gut colonisers (Zhu et al., 2016; Xiong et al., 2017a). Therefore a dysbiosis may indicate (or precede) the presence of a disease (Zhu et al., 2016). Furthermore, considering healthy, sub-healthy, and diseased L. vannamei, based on characteristic gross pathology of the gut, the severity of disease correlated with the degree of dysbiosis, and the onset of disease can be modelled based on the composition of the gut microbiota (Xiong et al., 2015; Xiong et al., 2017a). Specifically, the shift in foregut microbiota associated with AHPND was hypothesised to be a result of increased inability of the shrimp to select gut bacteria (a deterministic process) thus increasing the role of stochastic processes shaping gut microbiota assembly (Chen et al., 2017). Furthermore, gut profiles of shrimp challenged with Vibrio harveyi showed a lower degree of similarity (20–40% similar DGGE profiles) compared to the uninfected, control group (80% similarity) (Rungrassamee et al., 2016), which we suggest may be the result of a shift to more stochastic determination of the gut flora post-challenge. This shift in ecological processes is not only limited to bacterial community assembly; the eukaryotic microbiota of WFS-infected shrimp showed more stochastic assembly compared to healthy individuals (Dai et al. 2019). We hypothesise that early stochastic outcomes could result in variation in microbiotas between members of a shrimp population which then predispose certain individuals to pathogenesis; a phenomenon which could help explain variations in disease susceptibilities within a population.

4. Improving shrimp production with gut supplementation

In light of the disease-associated compositional changes described in...
the studies cited above, it is perhaps unsurprising that manipulating the gut microbiota has been shown to produce a number of positive effects on the shrimp host. The addition of live, beneficial microorganisms (probiotics) have been explored in a range of farmed animals for decades and is now becoming commonplace in shrimp aquaculture. Probiotic supplementation can increase competition in the gut, potentially supporting colonisation resistance against pathogenic microbes (Farzanfar, 2006). Furthermore, supplemental bacteria can directly affect and antagonise pathogens. Streptomycetes spp., for example, have demonstrated a protective effect in Artemia, P. monodon and L. vannamei when challenged with pathogenic Vibrio strains, with an increase in survival reported for all three shrimp species (Das et al., 2010; Augustine et al., 2016; Garcia Bernal et al., 2017; Mazón-Suástegui et al., 2019). Notably, the addition of Streptomycetes sp. RL8 alone and a combination of Streptomycetes and Bacillus spp. led to an increase in bacterial diversity in the guts of L. vannamei and also increased the abundance of antimicrobial-producing gut bacteria (Mazón-Suástegui et al., 2019). Isolation of lactic acid bacteria from wild shrimp guts enabled experiments showing that application of Lactobacillus plantarum MRO3.12 can also cause a reduction of V. harveyi, a common cause of shrimp mortalities. Shrimp supplemented with L. plantarum in their diet showed a significant increase in growth and survival rates, along with an increased abundance of haemocytes and a reduction of V. harveyi in the haemolymph (Kongnum and Hongpattarakere, 2012). Infection with V. harveyi has also shown to alter the intestinal bacterial profiles of both P. monodon and L. vannamei. Interestingly, the altered profiles of infected L. vannamei reverted back to that of a healthy animal after 72 h post infection. This was not observed with infected P. monodon. The ability to regain intestinal normality was noted as a possible explanation for the greater survival rate of L. vannamei infected with V. harveyi (Rungrassamee et al., 2016).

There is now a range of probiotic complexes that are marketed to the farming industry, however application of general combinations may not be beneficial to the host (Liu et al., 2018b; Landsman et al., 2019b). Firstly, probiotics must be able to survive passage through the gut. Common probiotic mixtures used in shrimp aquaculture often contain bacterial species that are not indigenous to the marine environment and subsequently have limited proliferation potential (Vargas-Albores et al., 2017). Identifying candidate probiotics from shrimp guts themselves, much as in the case of Lactobacillus plantarum MRO3.12 above, reduces the uncertainty about survivability in the host environment. Host genetics, however, is an important consideration and constraint on the ability of probiotics to illicit change in the gut microbiome (Landsman et al., 2019b; Liu et al., 2019). Despite probiotics being an attractive alternative to the use of broad-spectrum antibiotics, their use should be tightly monitored. For example, antibiotic resistant genes have been identified in probiotic supplements (Wong et al., 2015), including those often applied to shrimp culture (Uddin et al., 2015). However, the latter study did not identify any genetic elements associated with horizontal gene transfer.

Probiotic supplementation (inert sources of bacterial nutrition) offers an alternative to using probiotic strains and may also offer benefit to the microbiome by encouraging the proliferation of beneficial microbes within the gut. In an eight-week feeding trial using juvenile L. vannamei, mannan oligosaccharide (MOS), one of the most common prebiotics, significantly improved weight gain and growth rate. The prebiotic also significantly increased the length of the microvilli in the intestine which could account for increased surface area for nutrient absorption, subsequently improving growth (Zhang et al., 2012). Although MOS did not significantly improve survival, its addition did significantly increase the activity of phenoloxidase and superoxide dismutase – both important pathways in the invertebrate immune system. In contrast, the application of MOS to an intensive commercial culture of L. vannamei did not correspond to increases in growth parameters but did improve survival (Gainza and Romero, 2020). Inulin, a prebiotic oligosaccharide isolated from grain, fruits and vegetables, has also demonstrated positive effects on the gut microbiota. An inulin-enriched diet significantly increased the abundance of lactic-acid bacteria (LAB), which are recognised as beneficial to host health, and correlated to a significant increase in survival of Indian white shrimp post-larvae, Fenneropenaeus indicus (Hoseinifar et al., 2015).

Co-application of both pre- and probiotics, termed symbiotics, can stimulate an immune response in L. vannamei infected with WSSV, subsequently increasing survival (Li et al., 2009), and could offer a potential alternative to the traditional yet ineffective use of antibiotics to treat the viral infection. Twenty-seven per cent (15/56) of shrimp farmers interviewed in Thailand incorrectly used antibiotics as antiviral preventions and treatments (Holmström et al., 2003) therefore gut supplementation may serve as a more effective means to manage (particularly viral) disease in aquaculture production and prevent unnecessary antibiotic pressures on the environment. Dietary supplementation of the probiotic Bacillus PC465, isolated from Chinese white shrimp (Fenneropenaeus chinensis) also reduced cumulative mortalities of L. vannamei infected with WSSV (Chai et al., 2016) and recent evidence suggests a diet of brown seaweeds impacts the composition of the shrimp gut microbiota and subsequently improves resistance to WSSV infection (Schleder, 2020). As the seaweed was not sterilised, however, it is unclear whether this effect was due to the addition of seaweed itself or the microorganisms that were associated with the seaweed. The addition of the macroalgae Porphyra haitanensis was previously associated with improving survival after WSSV challenges (3 and 4% supplement) and increased growth, as a result of increased feed intake (Niu et al., 2018). It is difficult to separate any health/growth benefits arising from ameliorisation of the gut microbiome from simply increased nutritional resources provided by the addition of dietary supplements.

As well as being ineffective in the treatment of several of the above-mentioned diseases, antibiotics can have a direct impact on the gut microbiome which may be detrimental to the host. Antibiotic application can decrease colonisation resistance within the gut, alter its microbial composition, and facilitate the emergence of disease (Jernberg et al., 2019). Zeng et al. (2019) showed that the addition of ciprofloxacin and sulphonamide, which are commonly used to treat bacterial diseases in aquaculture, caused a short-term reduction in bacterial richness and diversity of the gut along with a significant increase in antibiotic resistant genes in healthy L. vannamei. Antibiotic resistant genes have been detected in aquaculture facilities throughout the world and can persist in bacterial reservoirs even after the initial pressure for their selection (Tamminen et al., 2011). Furthermore, antibiotic resistance genes have been found to be more abundant in adult shrimp compared to juveniles (Su et al., 2018). Phylogenetic analysis suggests resistance genes are transferred from intestinal bacteria to those in the culture environment (Zeng et al., 2019) and horizontal gene transfer can spread resistance between microbes in the environment, including those that are serious human pathogens (Tomova et al., 2015).

5. Recommendations for future microbiome studies

The investigation into the gut microbiome of the aquatic invertebrates is a relatively new discipline. Therefore, any attempts to guide the field into a more consistent and reliable consensus, in terms of the information required for accurate reporting, should be encouraged. Given the increasing number of available sample preparations and bioinformatic tools, it is unrealistic to limit all future studies to one methodology or analytical pipeline. However, that is not to say that these same studies should not include the same level of detail, samples sizes and availability of data that we expect from other, more established fields. For example, the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines have established a strong precedent for publishing reliable gene expression datasets by encouraging best experimental practices through a set of
standardised guidelines (Bustin et al., 2009). The Minimum Information required to support a Stimulant Assessment experiment (MISA) guidelines aimed to provide the same for immunostimulant work (Hauton et al., 2015). There are several extensive ‘best practice’ papers which offer useful instruction for the design and implementation of marker gene microbiome studies (Goodrich et al., 2014; Pollock et al., 2018; Hornung et al., 2019), along with standards for the minimum information about a marker gene sequences (MIMARKS) (Yilmaz et al., 2011). When considering implementing marker gene analysis for microbial profiling (metabarcoding), we offer the following suggestions:

(1) Always include adequate sample sizes in the experimental design. Low sample sizes are unlikely to capture the individual variation often associated with microbiome surveys. Furthermore, without an accurate description of the distribution of data, robust statistical testing may not be possible. Multiplexing with dual indexes and custom library preparations (Kozich et al., 2013) now allow for the inclusion of hundreds of samples per run. Therefore, the cost of sequencing is now less of a barrier to adequate sample sizes.

(2) Consider the limitations of the gene/region of interest. The hypervariable regions of the ribosomal small subunit RNA (SSU rRNA) gene can differ in their ability to detect specific taxa (Kim et al., 2011) and can impact the richness and diversity inferred from a community, including those isolated from shrimp (Garcia-Lopez et al., 2020).

(3) Avoid restricting taxonomic analyses to the phylum level. Phyla are high-level, diverse assemblages of taxa and the differential abundance of a phylum is often too ambiguous to infer specific mechanistic action or interaction with the host and/or other taxa in the microbiome. This is particularly true of Proteobacteria, which often dominate the gut of aquatic invertebrates.

(4) Avoid using marker genes to infer functional potential of aquatic invertebrate gut microbiomes. The lack of annotated genomes from marine microbes creates an analytical bias that may significantly impact gene inference and the assessment of differential abundance of functional gene profiles associated with aquatic invertebrate gut microbiomes (Sun, Jones and Fodor, 2020).

(5) Consider the use of exact sequence variants as opposed to operational taxonomic units (OTUs). There is often valid reason to cluster sequences according to percentage identity, such as accounting for error and taxonomically uninformative variation (e.g. intragenomic polymorphism of multi-copy genes). However, clustering overlooks the high sequencing accuracy possible with modern-day sequencing technologies, and can also obscure meaningful, biological variation (Callahan et al., 2016). Analysis of ESVs allows the generation of sequence clusters that are not dependent on the dataset itself and are therefore comparable across other datasets.

(6) Do not refer to amplicon sequencing data as ‘metagenomics’. This is particularly misleading in titles and abstracts of publications. Metagenomics refers to shotgun (not amplicon) sequencing of all DNA in a sample, (sub)sampling genomes of eukaryotes, viruses, and prokaryotes. Amplicon or marker-gene sequencing, by definition and design, targets a very specific region of those genomes and, more often, a very specific region of a single gene (e.g. hypervariable regions of the SSU rRNA gene).

6. Conclusions

The gut microbiomes of penaeid shrimp are becoming increasingly well characterised in comparison to other aquatic invertebrates. There are, however, still substantial gaps in the literature across all the penaeid species, and from the range of farming systems utilised in their culture. In support of the ‘pathobiome’ concept (Bass et al., 2019), pathogenesis may not be directly linked to the relative abundance of a particular taxon but rather the change in interactions between multiple taxa and the host (Chen et al., 2017; Zhou et al., 2019; Huang et al., 2020; Dai et al., 2020). However, we currently lack enough data to make generalisations about the gut microbiome of different shrimp species in regard to growth conditions and health status. We propose that a concerted global effort to increase our understanding of microbial complexity in these systems is needed. Inferences made from small datasets may not be representative of a true change or general patterns in terms of differential compositions in relation to disease, and provide little to go on for the development of positive interventions. The contexts in which different microbiome states arise (shrimp species, development stage, culture conditions, treatments, pond ecology, etc.) are very varied and their own influences on shrimp microbiomes are largely unknown. What is ‘normal’ in a wide range of situations needs to be known before abnormal conditions, for example associated with or predisposing to disease, can be reliably identified. Furthermore, the ability for the global scientific community to access raw sequencing data and experimental information (metadata) needs to improve in order to undertake meta-analyses and generalise across studies. This information is vital as demand for aquatic-based protein increases and shrimp aquaculture becomes more intensive. Better characterisation of the microbiota across the entire length of the gut, and across growth and development cycles will likely facilitate the improvement of shrimp probiotics to aid in improving growth and reducing the susceptibility towards disease, which will ultimately maximise the sustainable production of these key species.

Acknowledgements

This work was conducted within the Centre for Sustainable Aquaculture Futures (SAF), a joint initiative between the University of Exeter and the Centre for Environment, Fisheries and Aquaculture Science (Cefas) in the United Kingdom and funded by a Cefas-Exeter University Alliance PhD Studentship to CH, in collaboration with the National Lobster Hatchery (Padstow, UK). DB was supported by Defra (UK) Research Project C7277C (FC1214) and BBSRC/Newton Fund project BB/N00504X/1. GDS was supported by Defra (UK) under grants FB002 and F3001. We thank the original authors of the work reviewed in this paper.

References

Augustine, D., Jacob, J.C., Philip, R., 2016. Exclusion of Vibrio spp. by an antagonistic marine actinomycete Streptomyces rubrolavendulae M56. Aquac. Res. 47 (9), 2951–2960.
Bass, D., et al., 2019. The pathobiome in animal and plant diseases. Trends Ecol. Evol. 34 (11), 996–1008.
Bateman, K.S., Tew, I., French, C., Hicks, R.J., Martin, P., Munro, J., Stentiford, G.D., 2012. Susceptibility to infection and pathogenicity of White Spot Disease (WSD) in non-model crustacean host taxa from temperate regions. J. Invertebr. Pathol. 110 (3), 340–351. https://doi.org/10.1016/j.jip.2012.03.022.
Baticados, M.C.L., 1990. Diseases of penaeid shrimps in the Philippines. Tigbauan, Iloilo. Blumberg, R. and Powrie, F., Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. ‘Microbiota, disease, and back to health: a metastable journey’, Pediatr. Neurol. 52(6), 2016, pp. 566–584.
Bustin, S.A., et al., 2009. The MiQQE Guidelines: M inimum I nformation for Publication of Q uantitative Real-Time PCR Experiments. Clin. Chem. 55 (4), 611–622.
Callahan, B.J., et al., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581.
Ceccaldi, H. J. (1989) ‘Anatomy and physiology of digestive tract of Crustaceans Decapods reared in aquaculture’, pp. 243–259.
Chai, P.C., et al., 2016. Dietary supplementation of probiotic Bacillus PC465 isolated from the gut of Fenneropenaeus chinensis improves the health status and resistance of Litopenaeus vannamei against white spot syndrome virus. Fish Shellfish Immunol. 54, 602–611.
Chattopadhyaya, S., et al., 2012. Bacterial community associated with the intestinal tract of P. monodon in commercial farms. Microb. Ecol. 63 (4), 938–953.
Chen, W.Y., et al., 2017. Microbiome dynamics in a shrimp grow-out pond with possible outbreak of Acute Hepatopancreatic Necrosis Disease. Sci. Rep. Springer, US 7 (1), 1–12.
Cheng, A., et al., 2019. Intestinal microbiota of white shrimp, Litopenaeus vannamei, fed diets containing Bacillus subtilis E20-fermented soybean meal (FSBM) or an anti-microbial peptide derived from B. subtilis E20-FSBM. Aquac. Res. 51 (1), 41–50.
Chu, H., and Mazmanian, S. K. Innate immune recognition of the microbiota promotes hostmicrobial symbiosis, 14(7), 2014, pp. 668–675.
Cornejo-Granados, F., et al., 2017. Microbiome of Pacific Whiteleg shrimp reveals different bacterial community composition between Wild, Aquacultured and AHPND/EHS outbreak conditions. Sci. Rep. 7 (1), 1–15.

Cornejo-Granados, F., et al., 2018. A meta-analysis reveals the environmental and host factors shaping the shrimp microbiota. PLoS ONE, 13 (5), e0196047.

Dai, W., et al., 2017. The gut eukaryotic microbiota influences the growth performance among cohabitating shrimp. Appl. Microbiol. Biotechnol. 101 (16), 6447-6457.

Dai, W., et al., 2018. Integrating molecular and ecological approaches to identify potential polymicrobial pathogens over a shrimp disease progression. Appl. Microbiol. Biotechnol. 102, 3755-3764.

Dai, W., et al., 2019. Gut eukaryotic disease-discriminatory taxa are indicative of Pacific white shrimp (Litopenaeus vannamei) white税费 syndrome. Aquat. Microb. Biotechnol. 103, 4241–4252.

Duan, Y., et al., 2017. Effects of dietary poly-β-hydroxybutyrate (PHB) on microbiota composition and the mTOR signaling pathway in the intestines of Litopenaeus vannamei. J. Microbiol. 55 (12), 946-954.

Duan, Y., et al., 2018. Impairment of the intestine barrier function in Litopenaeus vannamei exposed to ammonia and nitrite stress. Fish Shellfish Immunol. 78, 279–288.

Duan, Y., et al., 2019a. Changes in the intestine barrier function of Litopenaeus vannamei in response to pH stress. Fish Shellfish Immunol. 88, 142–149.

Duan, Y., et al., 2019b. Effects of dietary and microbial communities on Litopenaeus vannamei intestine subjected to acute sulfdioxide exposure. Fish Shellfish Immunol. 88, 335-343.

Elzondo-González, R., et al., 2020. Changes in the intestinal bacterial communities of white shrimp Litopenaeus vannamei fed with green seaweeds. J. Appl. Phycol.

Fan, J., et al., 2019a. Microbial communities of the gut mycobiota of Litopenaeus vannamei reveal its association with body weight. Sci. Rep. 9 (1), 2–11.

Fan, L., et al., 2019b. Microbiota comparison of Pacific white shrimp intestine and sediment at freshwater and marine cultured environment. Sci. Total Environ. 657 (20), 1194–1204.

Farzanfar, A., 2006. The use of probiotics in shrimp aquaculture. FEMS Immunol. Med. Microbiol. 48 (2), 149–158.

Gainza, O., Romero, J., 2020. Effect of mannan oligosaccharides on the microbiota and their association with environmental factors and shrimp physiological health. Appl. Microbiol. Biotechnol. 102, 8585-8598.

García-López, R., et al., 2020. Doing More with Less: A Comparison of 16S Hypervariable.

Hauton, C., Hudspith, M., Gunton, L., 2015. Future prospects for prophylactic immune system complexity. Dev. Comp. Immunol. 48 (2), 360–368.

He, W., et al., 2017. Effects of organic acids and essential oils blend on growth, gut microbiota, immune response and disease resistance of Pacific white shrimp Litopenaeus vannamei. Aquaculture 503, 357–368.

Hoseinifar, S.H., Zare, P., Kolangi Miandare, H., 2015. ‘The effects of different routes of inulin administration on gut microbiota and survival rate of shrimp Litopenaeus vannamei’. Aquac. Res. 47 (6), 1377-1376.

Huang, F., et al., 2018. Microbiota assemblages of water, sediment, and intestine and their associations with environmental factors and shrimp physiological health. Appl. Microbiol. Biotechnol. 102, 6817–6830.

Huang, L., et al., 2020a. The bacteria from large-sized bioflocs are more associated with the shrimp gut microbiota in culture system. Aquaculture 523, 735159.

Jernberg, C., et al., 2020b. Microecological Koch’s postulates reveal that intestinal microbiota dysbiosis contributes to shrimp white税费 syndrome. Microbiome 8 (1), 1–15.

Jayaree, L., Janakiram, P., Madhavi, R., 2006. Characterization of Vibrio spp. associated with diseased shrimp from pond conditions of Andhra Pradesh (India). J. World Aquacult. Soc. 37 (4), 521–529.

Jiang, L., et al., 2019. Individual and combined effects of ammonia-N and sulfide on the immune function and intestinal microbiota of Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 92, 230–240.

Kim, M., Morrison, M., Yu, Z., 2011. Evaluation of different partial 16S RNA gene sequences for regional phylogenetic analysis of microorganisms. J. Microbiol. Methods 84 (1), 81–87.

Kobyayshi, Y., Ishibashi, M., 1993. Bioactive metabolites of symbiotic marine microorganisms. Chem. Rev. 93 (5), 1753–1769.

Kongnum, K., Hongpattarakere, T., 2012. Effect of Lactobacillus plantarum isolated from digesta tract of wild shrimp on growth and survival of white shrimp (Litopenaeus vannamei) challenged with Vibrio harveyi. Fish Shellfish Immunol. 32 (1), 170–177.

Kostanjek, R., Strus, J., Avguštin, G., 2007. “Candidatus bacilloplasma”, a novel lineae of Mollicutes associated with the hindgut wall of the terrestrial isopod Porcellio scaber (Crustacea: Isopoda). Appl. Environ. Microbiol. 73 (3), 1006–1012.

Kozich, J.J., et al., 2013. Development of a dual-index sequencing strategy and curat pipeline for analysing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79 (17), 5112–5120.

Landsman, A., et al., 2019a. Impact of aquaculture practices on intestinal bacterial profiles of Pacific whiteleg shrimp Litopenaeus vannamei. Microorganisms 7 (4), 93.

Landsman, A., et al., 2019b. Investigation of the potential hosts of genitic and probiotic treatment on the gut bacterial community composition of aquaculture-reared Pacific whiteleg shrimp Litopenaeus vannamei. Microorganisms 7 (8), 217.

Le, D.H., et al., 2018. Characterization of bacterial community in the gut of Litopenaeus monodon and its culture water in shrimp ponds. Turk. J. Fish. Aquat. Sci 19 (11), 977-986.

Lee, C., et al., 2015. The opportunistic marine pathogen Vibrio parahaemolyticus becomes virulent by acquiring a plasmid that expresses a deadly toxin. PNAS 112 (34), 1–6.

Li, J., et al., 2019. The effect of female season and season to hepatopancreas and intestinal mycobiota of Litopenaeus vannamei. Front. Microbiol. 10 (APR), 1–13.

Li, J., Tan, B., Mai, K., 2009. Probiotic bacillus Bacillus OJ and isomaltolooligosaccharides influence the intestinal microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (Litopenaeus vannamei). Aquaculture 291 (1–2), 355–359.

Liu, P.C., Lee, K.K., 2002. Cysteine protease is a major exotoxin of pathogenic luminous Vibrio harveyi. Fish Shellfish Immunol. 15 (4), 164–173.

Liu, J., et al., 2019. Strain-specific changes in the gut microbiota profiles of the white shrimp Litopenaeus vannamei isolated from cohabiting species. Appl. Environ. Microbiol. 85 (13), 5184–5195.

Liu, L., et al., 2018. A Vibrio owensii strain as the causative agent of AHPND in cultured shrimp, Litopenaeus vannamei. J. Invertebr. Pathol. Elsevier 153, 156–164 (October 2018).

Liu, L., et al., 2018b. Effects of a commercial microbial agent on the bacterial communities in shrimp culture system. Front. Microbiol. 9 (OCT), 1–10.

Liu, J., et al., 2019. Strain-specific changes in the gut microbiota profiles of the white shrimp Litopenaeus vannamei isolated from cold stress. Aquaculture 503, 357-366.

Manílal, A., et al., 2010. Virulence of Vibrio isolated from diseased black tiger shrimp, Penaeus monodon. Fabricius. J. World Aquacult. Soc 41 (3), 332–343.

Manzio-Suástegui, J.M., et al., 2019. Effect of Streptomyces probioticus on the gut microbiota of Litopenaeus vannamei challenged with Vibrio parahaemolyticus. Microbiol. Open 8 (2), e967.

Mezit, A., et al., 2010. Temporal shifts of the Norway lobster (Nephrops norvegicus) gut bacterial community during the growth of white shrimp Litopenaeus vannamei. Aquaculture 291 (1–2), 572–577.

Mezit, A., Mente, E., Kormas, K.A., 2012. Gut bacteria associated with different diets in reared Nephrops norvegicus. Syst. Appl. Microbiol. 35 (7), 473–482.

Niu, J., et al., 2018. Dietary values of macroalgae Porphyra haitanensis in Litopenaeus vannamei under normal rearing and WSSV challenge conditions: Effect on growth, immune response and intestinal microbiota. Fish Shellfish Immunol. 81, 135–149.

Oetama, V.S.P., et al., 2016. Microbiome analysis and detection of pathogenic bacteria of Penaeus monodon from Jakarta Bay and Bali. Mar. Pollut. Bull. 110 (2), 718–725.

Omont, A., et al., 2020. Digestive microbiota of shrimp Litopenaeus vannamei and oyster
C.C. Holt, et al.

Agmasomapenaei Shao, J., et al., 2019. Replacement of fishmeal by fermented soybean meal could enhance...n. J. Invert. Biol. 23 (1), 77–81.

Qian, D., et al., 2020. Toxic effect of chronic waterborne copper exposure on growth, immunity, anti-oxidative capacity and gut microbiota of Pacific white shrimp Litopenaeus vannamei. Immunol. 100, 445–455.

Pilotto, M., et al., 2018. Exploring the Impact of the Biofloc rearing system and an oral different probiotics or probiotic supernatant. J. Microbiol. Biotechnol. 26 (10), 1736–1745.

Seibert, C.H., Pinto, A.R., 2012. Challenges in shrimp aquaculture due to viral diseases: distribution and biology of the five major penaeid viruses and interventionsto avoid viral incidence and dispersion. Brazilian J. Microbiol. 43 (3), 857–864.

Shu, Y., et al., 2016. Bacterial population in intestines of Litopenaeus vannamei that is not...

Zhang, J., et al., 2012. Effects of dietary mannooligosaccharide on growth performance, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 45, 451–463.

Zhu, J., et al., 2016. Contrasting ecological processes and functional compositions between intestinal bacterial community in healthy and diseased shrimp. Microb. Ecol. 1968–1977.

Zeng, S., et al., 2019. Antibiotic supplement in feed can perturb the intestinal microbial community of Pacific whiteshrimp (Litopenaeus vannamei). Appl. Microbiol. Biotechnol. 103 (7), 3111–3122.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2017. Composition, diversity and function of intestinal microbiota in Pacific white shrimp (Litopenaeus vannamei) at different culture stages. PeerJ 5, e3980.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2019. Antibiotic supplement in feed can perturb the intestinal microbial composition and function in Pacific white shrimp. Appl. Microbiol. Biotechnol. 103 (7), 3111–3122.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2017. Composition, diversity and function of intestinal microbiota in Pacific white shrimp (Litopenaeus vannamei) at different culture stages. PeerJ 5, e3980.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2019. Antibiotic supplement in feed can perturb the intestinal microbial composition and function in Pacific white shrimp. Appl. Microbiol. Biotechnol. 103 (7), 3111–3122.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2017. Composition, diversity and function of intestinal microbiota in Pacific white shrimp (Litopenaeus vannamei) at different culture stages. PeerJ 5, e3980.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.

Zhang, M., et al., 2014. Characterization of the intestinal microbiota in Pacific white shrimp, Litopenaeus vannamei, fed diets with different lipid sources. Aquaculture 434, 449–455.

Zhang, M., et al., 2016. Symbiotic bacteria in gills and guts of Chinese mitten crab (Eriothocus sinensis) differ from the free-living bacteria in water. PLoS ONE 11 (1), e0148135.

Zeng, S., et al., 2017. Composition, diversity and function of intestinal microbiota in Pacific white shrimp (Litopenaeus vannamei) at different culture stages. PeerJ 5, e3980.

Zhang, J., et al., 2012. Effects of dietary mannoligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 33 (4), 1027–1032.