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NOTES AND SHORT COMMUNICATIONS

Intestinal Microbiota of White Shrimp *Penaeus vannamei* Under Intensive Cultivation Conditions in Ecuador

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Abstract The goal of the study was to characterize the intestinal tract bacterial microbiota composition of *Penaeus vannamei* in intensive commercial ponds in Ecuador, comparing two shrimp-farming phases: nursery and harvest. Bacterial microbiota was examined by sequencing amplicons V2–V3 of the 16S rRNA using Ion Torrent technology. *Archaea* sequences were detected in both phases. Sequence analyses revealed quantitative and qualitative differences between the nursery phase and the harvest phase in shrimp intestinal microbiota composition. The main differences were observed at the phylum level during the nursery phase, and the prevailing phyla were *CKC4* (37.3%), *Proteobacteria* (29.8%), *Actinobacteria* (11.6%), and *Firmicutes* (10.1%). In the harvest phase, the prevailing phyla were *Proteobacteria* (28.4%), *Chloroflexi* (19.9%), and *Actinobacteria* (15.1%). At the genus level, microbiota from the nursery phase showed greater relative abundances of CKC4 uncultured bacterium (37%) and *Escherichia-Shigella* (18%). On the contrary, in the microbiota of harvested shrimp, the prevailing genera were uncultured *Caldilinea* (19%) and *Alphaproteobacteria* with no other assigned rate (10%). The analysis of similarity ANOSIM test (beta diversity) indicated significant differences between the shrimp microbiota for these two farming phases. Similarly, alfa-diversity analysis (Chao1) indicated that the microbiota at harvest was far more diverse than the microbiota during the nursery phase, which showed a homogeneous composition. These results suggest that shrimp microbiota diversify their composition during intensive farming. The present work offers the most detailed description of the microbiota of *P. vannamei* under commercial production conditions to date.

Keywords *Penaeus vannamei* · Shrimp · Microbiota · Microbiome · OTUs · *Archaea* · *CKC4*

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The "white shrimp" *Penaeus vannamei* is a native species to the west Pacific coast, from Mexico to Peru [1]. Currently, this species represents more than 50% of the cultivated crustaceans worldwide and a global market value of USD 18 billion approximately [2]. The main producing countries are located in the South and Southeast Asia; however, in Latin America, a significant aquaculture industry remains, with significant productions in Ecuador, Mexico, Honduras, and Brazil [1]. Numerous studies have examined the roles of microbiota in nutrition, cell proliferation, and host immunomodulation

[3–6]. The understanding of the microbiota of *P. vannamei* remains insufficient compared to vertebrates, including terrestrial livestock and finfish species [7]. The modulation of microbiota as an alternative approach to maximize production and disease control using prebiotics, probiotics, and synbiotics requires a comprehensive knowledge of the diversity of the microbiota in the host organism. Recently, advances in next-generation sequencing (NGS) have allowed further research of crustacean microbiota [8–12]. These studies focused on modulating the microbiota of *P. vannamei* through dietary interventions at laboratory scale. Therefore, our study was focused to characterize the bacterial microbiota composition in the intestinal tract of *P. vannamei* reared in intensive commercial ponds in Ecuador. This study was performed in the province of El Oro, which corresponds to the area where shrimp commercial cultivation began in 1968 [1]. Understanding the bacterial ecology in the crustacean gut can help to improve both the management of hatcheries for higher productivity and the safety of shrimps as food. Details of the sample site, experimental methodology, and sequence analysis are provided in Online Resource 1. The sequencing of 16S rRNA amplicons using Ion Torrent technology generated a total of 1,005,400 sequences, with an average quality per sequence (Phred score) of 29, equivalent to 99.9% precision in sequence fit and a probability of error of 0.00126. Sequence data have been deposited in the Sequence Read Archive of the National Centre for Biotechnology Information (SRA, NCBI) under the BioProject (PRJNA352369, SAMN05971820, SAMN05971821, SAMN05972042, SAMN05972043, SAMN05972044, SAMN05971813, SAMN05971815, SAMN05971819). After de-replication, quality, chimera, and unique sequence filtering, there were a total of 422,838 high-quality sequences, which were assigned to a total of 1949 operational taxonomic units (OTUs), with 97% grouped by sequence identity. Later, a screening of OTUs assigned to *Archaea* (114 OTUs and 23,057 sequences) yielded a total of 399,781 sequences assigned to 1835 OTUs. Given that the primers used were designed for the V2–V3 region of bacterial 16S RNA, the *Archaea* sequences were filtered after this step to avoid errors in statistical and qualitative inferences. However, during the nursery phase, the *Archaea* domain was represented by the phyla *Thaumarchaeota* (12%) and *Euryarchaeota* (0.05%), while in the harvest stage, the microbiota contained *Thaumarchaeota* (3%) and *Euryarchaeota* (0.2%, Online Resource 1 Fig. 2).

Sequence analysis revealed that the most abundant phyla in the microbiota of shrimp during the nursery phase were *CKC4* (37.3%), *Proteobacteria* (29.8%), *Actinobacteria* (11.6%), and *Firmicutes* (10.1%), while the intestinal microbiota of harvested shrimp comprised mostly *Proteobacteria* (28.4%), *Chloroflexi* (19.9%), and *Actinobacteria* (15.1%, see Fig. 1a). In total, 32 phyla were detected, with 23 phyla in the nursery phase and 32 phyla in the harvest phase. The taxonomic analysis identified a total of 520 genera (306 in nursery; 454 in harvest). Among them, the OTUs with greater relative abundances in the microbiota of nursery phase shrimp were *CKC4 uncultured bacterium* (37%) and *Escherichia-Shigella* (18%). Among the microbiota in harvested shrimp, the most abundant genera were *uncultured Caldilinea* (19%) and *Alphaproteobacteria uncultured bacterium* (10%, see Fig. 1b). The Chao1 and Robbins indexes indicated that the alpha diversity was higher in the harvested shrimp compared to the microbiota of shrimp in the nursery stage (Figs. 1 and 2a and Online Resource 1). Non-directional beta diversity is a measure of the variation in community structure in response to some environmental/experimental factors [13]. The beta diversity of the bacterial communities associated with *P. vannamei*, comparing nursery and harvest stages, was investigated through a PCoA showed in Fig. 2b. The first two components explain a total of 58.39% of the variation (first component, 40.88%; second component, 17.51%). This figure also showed that the composition of the nursery's microbiota is uniform, as the dots are grouped in the graph. In contrast, harvested shrimp's microbiota showed wider dispersion, indicating differences in their beta diversity. The ANOSIM test [14] results, with $R = 0.98 \approx 1$ ($p = 0.0219$), indicated significant differences between the shrimp microbiota for these two farming phases. Microbiota was compared with linear discriminant analysis effect size (LEfSe) [15] to identify characteristic taxa associated with each phase. The graphical results of the LEfSe (Fig. 3) indicate that the taxa associated with the nursery phase were, in descending order, the genera *Staphylococcus*, the *Staphyloccaceae* family, the *Pseudomonadales* and *uncultured Cyanobacteria* orders, the *Pseudomonadaceae* family, and the *Frankiales* order. In contrast, in the microbiota of the harvested shrimp, the taxa represented were the phylum *Verrucomicrobia*, class *Spartobacteria*, *Chthoniobacteria* family *LD29*, the *Chthoniobacteria* order, phylum *Chloroflexi*, the *Caldilinea* order, and the *Caldilineae* family. The present work offers the most detailed description of the microbiota of *P. vannamei* under commercial production conditions to date. Despite the economic importance of the species, the examination of the intestinal microbiota of the white shrimp has been limited to probiotic effects and laboratory scale assays. Few studies have explored the microbiota composition during the growth of this crustacean [16–19]. Furthermore, several reports about lab scale *P. vannamei* microbiota have observed that *Proteobacteria* is the most abundant phylum, with relative abundance between 68 and 97% [8–12, 17, 18, 20, 21]. As described in Online Resource 1 Table 1, our results showed differences with the composition described in those reports. The differences in microbiota during growth were reflected in our study, where we observed an increase in the diversity of the microbiota of the harvested shrimp compared to that of the nursery shrimp. Huang et al. [17] used NGS to describe the microbiota during lab scale growth (14, 30, 60, and 90 days; Online Resource 1 Table 1) and found that the most prevalent phylum was

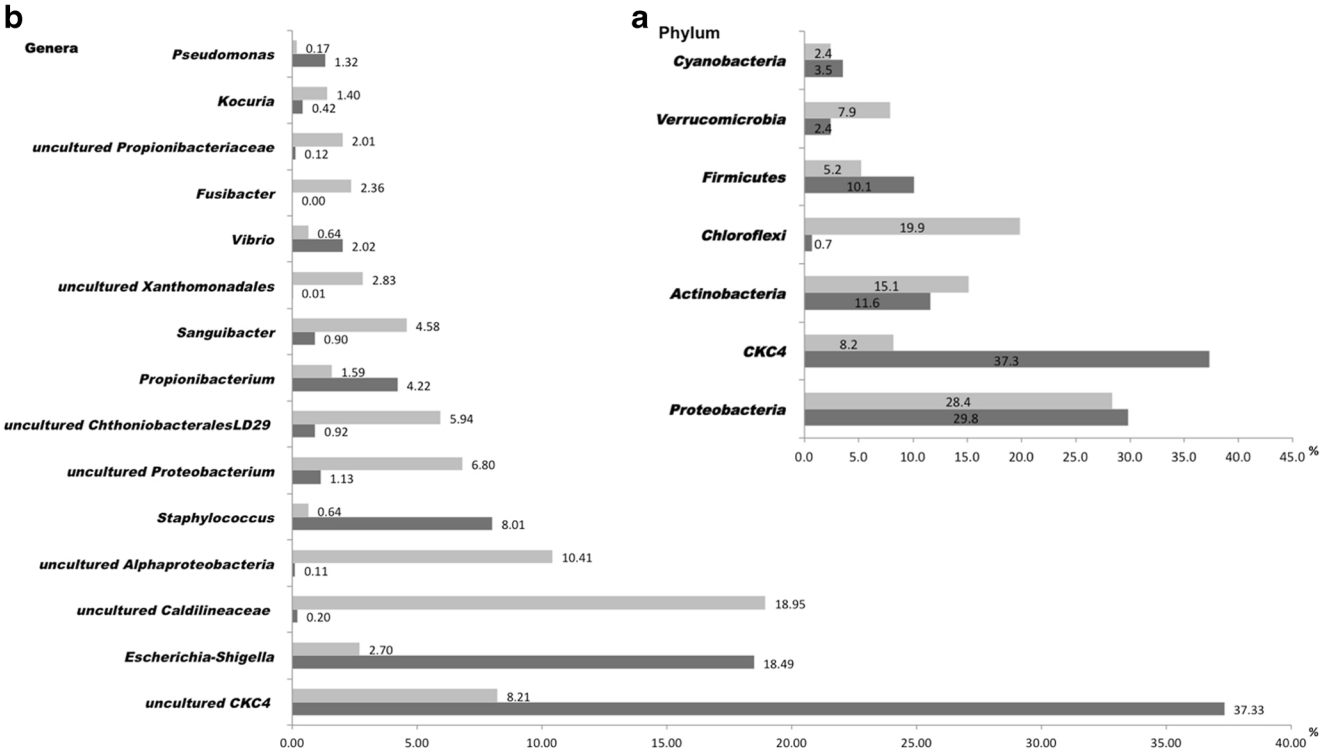

Fig. 1 Comparison of relative abundance. **a** Shrimp microbiota composition (relative to OTUs composition) at phylum level, including seven phyla showing the highest abundance. **b** Shrimp microbiota composition (relative to OTUs composition) at genus level, including 15 genera showing the highest abundance between nursery (■) and (■) harvested shrimp microbiota

Proteobacteria, (averaging 43.3%), corresponding to > 70% of the relative abundance in the early phases. This contrasts with our results that showed that the nursery phase microbiota was dominated by the CKC4 phylum (37%). This finding is of interest because little information is available regarding this bacterial group, and this leads to new questions regarding the basis for this association and the consequences (beneficial or detrimental) for the host. It has been suggested that changes in intestinal microbiota may be associated with the severity of shrimp diseases [20, 21]. However, in our study, under intensive commercial farming

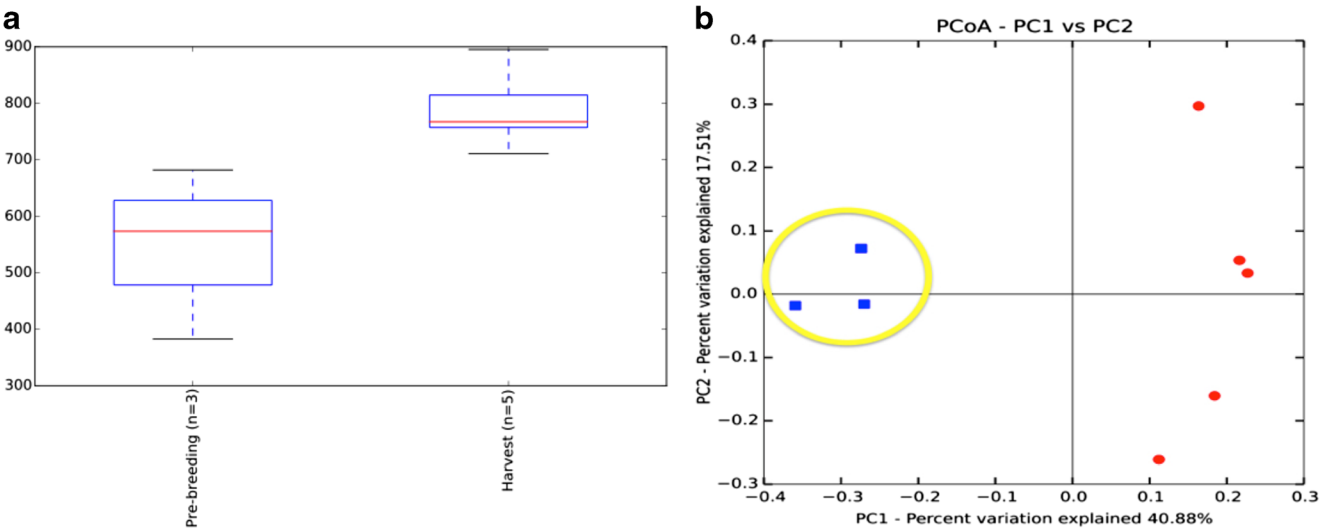

Fig. 2 Diversity analysis. **a** Alpha diversity index Chao1. OTU richness indices are shown for the nursery and harvest intestinal microbiota of shrimps (t stat p value = 0.018 < 0.05). The line in each box plot indicates the median, the box delimits the 25th and 75th percentile, and the whisker is the range. **b** Principal component analysis (PCoA) normalized distribution plot (ANOSIM test statistic ≈ 0.989, p value = 0.0219 < 0.05, number of permutations 999). ■ corresponds to microbiota of nursery shrimps. ○ corresponds to microbiota of harvest shrimps

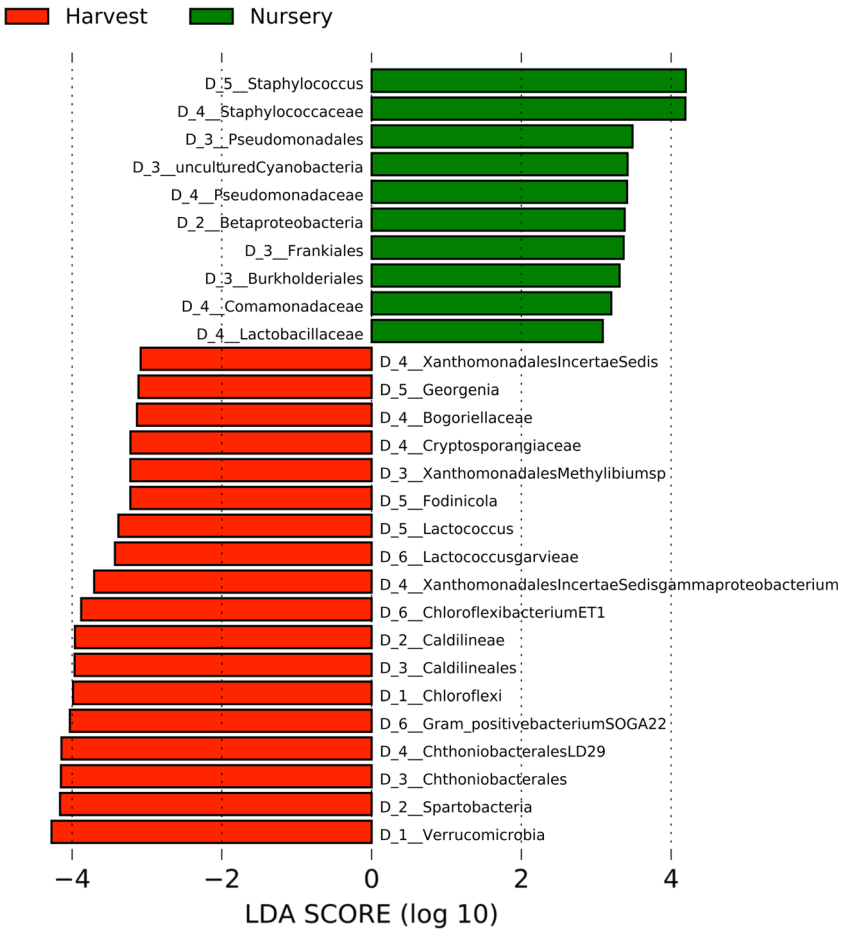

Fig. 3 Graphical summary of LEfSe (Linear discriminant analysis effect size) results. The histogram shows the LDA scores computed for differentially abundant taxa between nursery and harvest shrimps microbiota. The histogram identifies which taxa among all those detected as statistically and biologically differential explain the differences between microbial communities

conditions, the harvest shrimp microbiota showed no more than 28% *Proteobacteria*. There is a lack of detailed information about the relative abundance at the genus level in shrimp microbiota. Sha et al. [11] studied *P. vannamei* microbiota in China at the lab scale and reported that the relative abundance of the top 10 genera ranged 0.36–0.81%, whereas the top three genera were *Octadecabacter* (2.7%), *Acinetobacter* (2.0%), and *Demequina* (1.1%). In contrast, we found that harvest shrimp showed high levels of *uncultured Caldilinea* (19%) and *Alphaproteobacteria uncultured bacterium* (10%). It is well known that the colonization of microorganisms in host intestine is influenced by factors relative to host and non-host interactions [5, 22]. Such factors include the already present intestinal assemblage [23, 24], host physiological condition [25], growth stage of the host [17, 26, 27], and environmental conditions [20]. Previous investigation developed in laboratory conditions over other crustacean's species like *Homarus gammarus* [28], *Neocaridina denticulata* [29], and *Penaeus monodon* [30] shows that microbiota highly dissimilar to water/fed microbiota have been developed by time, suggesting that host selection structures the gut microbiota influenced by environmental, physiological, and nutritional factors. A stable microbial community is important to the host's health but it can be constantly influenced by various environmental factors [31]. For example, salinity is one of these factors, since recent reports have revealed the influence of hyposaline/hypersaline environments on shrimp microbiota composition [12]. Furthermore, other authors have reported the important influence of water and biofloc on shrimp intestinal microbiota [31]. It is worthy to notice that this study was performed at commercial scale, and we tried to homogenize the environmental factors (water, diet, temperature, oxygen, salinity). In agreement with our results, Rungrassame et al. [30] suggest that there is an increasing diversity in the microbiota of *P. monodon* between 30 and 90 days of culture. Furthermore, Cheung et al. [29] associated this increase in diversity with the sexual maturation of the shrimp *Neocaridina denticulata*. The published results on the composition of the microbiota in *P. vannamei* argue that major bacterial groups shifted at different growth stages. The molting process of the host, the differences in diet, and development of the host digestive and reproductive systems might be responsible for the variance of bacterial composition [17, 18]. Therefore, the differences observed between intestinal microbiota of nursery and harvest shrimp could be attributed to the growth stage of the host [17, 26, 27] rather than environmental conditions [20]. The marked difference observed between the microbiota reported in Asia and our results could be explained by the non-host factor interaction, but host factors associated with the culture of shrimp genetic lines may influence

this marked differentiation. *P. vannamei* was introduced in Asia experimentally from 1978 to 1979 but commercially only since 1996 in mainland China and Taiwan, followed by other Asian countries in 2000 [32]. Most Asian countries have legislated against the importation of *P. vannamei* due to risk of introducing new pathogenic bacterial diseases, keeping isolated shrimp genetic lines. Potential pathogenic *Vibrio* species are halophilic bacteria that are ubiquitous in marine and coastal environments. Some of them are pathogenic to human and marine animals [33] and have been identified as serious disease problem in shrimp culture ponds all over the world [33–35]. In most cases, however, *Vibrio* genus is considered opportunistic pathogens for shrimps; however, vibrio-related emerging infectious diseases have recently expanded in geographic range, increasing the risk of economic losses in the future [36]. In the present study, the genus *Vibrio* was detected in the nursery and harvest stages, 2.02 and 0.64% of relative abundance, respectively, whereas the salinity of the ponds was $5^{0}/_{00}$, very close to the lower tolerance limits for *V. parahaemolyticus* and *V. vulnificus* [37, 38]. This result suggests that risk of contamination with potentially pathogenic *Vibrio* still remains in inland intensive culture facility. Previous studies have reported the presence of coliforms in *P. vannamei* microbiota. Those studies include data from different geographic locations such as Texas, USA [39], China [40], Brazil [41, 42], Thailand [33], and India [43]. Those reports agree with our observation about the presence of the *Escherichia/Shigella* genus in shrimp microbiota. Moreover, Rungrassamee et al. [44] reported variations in relative abundance of the *Escherichia/Shigella* genus during the growth the *P. monodon*. On the other hand, several publications have been focused in dietary modulation of the microbiota of shrimp and reported some minor changes when specific cultivable strains have been evaluated, mostly *Vibrio* [45–52]. However, probiotic-induced microbiota changes may enhance the immune responses of shrimp [47], and enhancing immune responses could require an unnecessary expenditure of energy [19]. Non-indigenous probiotics may lead to environmental imbalances, particularly because effluent treatments may have reduced effect on probiotic removal prior to discharge [19]. Our results suggest that the microbiota of *P. vannamei* under conditions of intensive cultivation in Ecuador diversifies during the farming process. The increased number of phyla and genera at harvest may be important to the host in terms of increased metabolic capabilities and production of valuable substances, such as amino acids, vitamins, enzymes, and specific growth factors [27].

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Schwarz L. (2005). Visión general del sector acuícola nacional, Ecuador. FAO Fisheries and Aquaculture Department [online]. Rome http://www.fao.org/fishery/countrysector/naso_ecuador/es. Accessed 12 June 2016
2. FAO. (2017) Fisheries. Aquaculture Department. Statistical Collections. Online Query Panels. FAO-FIGIS. Global Aquaculture Production. FAO-Fisheries and Aquaculture Information and Statistics Service [online query]. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/es. Accessed 02 May 2017
3. Izvekova GI (2006) Hydrolytic activity of enzymes produced by symbiotic microflora and its role in digestion processes of bream and its intestinal parasite *Caryophyllaeus laticeps* (*Cestoda*, *Caryophyllidea*). Biol. Bull. 33(3):287–292. https://doi.org/10.1134/S1062359006030125
4. Roeselers G, Mittge EK, Stephens WZ, Parichy DM, Cavanaugh CM, Guillemin K, Rawls JF (2011) Evidence for a core gut microbiota in the zebrafish. The ISME Journal 5(10):1595–1160. https://doi.org/10.1038/ismej.2011.38
5. Sullam KE, Essinger SD, Lozupone CA, O'Connor MP, Rosen GL, Knight RO, Russell JA (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol. Ecol. 21(13):3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x
6. Torrecillas S, Montero D, Izquierdo M (2014) Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish and Shellfish Immunology 36(2):525–544. https://doi.org/10.1016/j.fsi.2013.12.029
7. Colston TJ, Jackson CR (2016) Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. Mol. Ecol. 25(16):3776–3800. https://doi.org/10.1111/mec.13730
8. Zhang M, Sun Y, Chen K, Yu N, Zhou Z, Chen L, Li E (2014) Characterization of the intestinal microbiota in Pacific white shrimp, *Litopenaeus vannamei*, fed diets with different lipid sources. Aquaculture 434:449–455. https://doi.org/10.1016/j.aquaculture.2014.09.008
9. Qiao F, Liu YK, Sun YH, Wang XD, Chen K, Li TY, Zhang ML (2016) Influence of different dietary carbohydrate sources on the growth and intestinal microbiota of *Litopenaeus vannamei* at low salinity. Aquac. Nutr. https://doi.org/10.1111/anu.12412
10. Zheng Y, Yu M, Liu Y, Su Y, Xu T, Yu M, Zhang XH (2016) Comparison of cultivable bacterial communities associated with Pacific white shrimp (*Litopenaeus vannamei*) larvae at different health statuses and growth stages. Aquaculture 451:163–169. https://doi.org/10.1016/j.aquaculture.2015.09.020
11. Sha Y, Wang B, Qi C (2016) Bacterial population in intestines of *Litopenaeus vannamei* fed different probiotics or probiotic supernatant. J. Microbiol. Biotechnol. 26(10):1736–1745
12. Zhang M, Sun Y, Liu Y, Qiao F, Chen L, Liu WT, Li E (2016) Response of gut microbiota to salinity change in two euryhaline aquatic animals with reverse salinity preference. Aquaculture 454:72–80. https://doi.org/10.1016/j.aquaculture.2015.12.014
13. Anderson M et al. (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol. Lett. 14(1):19–28. https://doi.org/10.1111/j.1461-0248.2010.01552.x

14. Clarke K (1993) Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18(1):117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x
15. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol. https://doi.org/10.1186/gb-2011-12-6-r60
16. Tzuc JT, Escalante DR, Herrera RR, Cortés GG, Ortiz ML (2014) Microbiota from *Litopenaeus vannamei:* digestive tract microbial community of Pacific white shrimp (*Litopenaeus vannamei*). SpringerPlus 3(1):280. https://doi.org/10.1186/2193-1801-3-280
17. Huang Z, Li X, Wang L, Shao Z (2014) Changes in the intestinal bacterial community during the growth of white shrimp, *Litopenaeus vannamei*. Aquac. Res. 6(47):1737–1746. https://doi.org/10.1111/are.12628
18. Zheng Y, Yu M, Liu Y, Su Y, Xu T, Yu M, Zhang XH (2016) Comparison of cultivable bacterial communities associated with Pacific white shrimp (*Litopenaeus vannamei*) larvae at different health statuses and growth stages. Aquaculture 451:163–169. https://doi.org/10.1016/j.aquaculture.2015.09.020
19. Vargas-Albores F, Porchas-Cornejo MA, Martínez-Porchas M, Villalpando-Canchola E, Gollas-Galván T, Martínez-Córdova LR (2017) Bacterial biota of shrimp intestine is significantly modified by the use of a probiotic mixture: a high throughput sequencing approach. Helgol. Mar. Res. 71(1):5. https://doi.org/10.1186/s10152-017-0485-z
20. Xiong J, Wang K, Wu J, Qiuqian L, Yang K, Qian Y, Zhang D (2015) Changes in intestinal bacterial communities are closely associated with shrimp disease severity. Appl. Microbiol. Biotechnol. 99(16):6911–6919. https://doi.org/10.1007/s00253-015-6632-z
21. Rungrassamee W, Klanchui A, Maibunkaew S, Karoonuthaisiri N (2016) Bacterial dynamics in intestines of the black tiger shrimp and the Pacific white shrimp during *Vibrio harveyi* exposure. J. Invertebr. Pathol. 133:12–19. https://doi.org/10.1016/j.jip.2015.11.004
22. Pérez T, Balcázar JL, Ruiz-Zarzuela I, Halaihel N, Vendrell D, De Blas I, Múzquiz JL (2010) Host–microbiota interactions within the fish intestinal ecosystem. Mucosal Immunol. 3(4):355–360. https://doi.org/10.1038/mi.2010.12
23. Stecher B et al. (2010) Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog. 6(1): e1000711. https://doi.org/10.1371/journal.ppat.1000711
24. Robinson CJ, Schloss P, Ramos Y, Raffa K, Handelsman J (2010) Robustness of the bacterial community in the cabbage white butterfly larval midgut. Microb. Ecol. 59(2):199–211. https://doi.org/10.1007/s00248-009-9595-8
25. Li CC, Chen JC (2008) The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under low and high pH stress. Fish & shellfish immunology 25(6): 701–709. https://doi.org/10.1016/j.fsi.2008.01.007
26. Burns AR, Stephens WZ, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJ (2016) Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. The ISME journal 10(3):655. https://doi.org/10.1038/ismej.2015.142
27. Yan Q et al. (2016) Environmental filtering decreases with fish development for the assembly of gut microbiota. Environ. Microbiol. 18(12):4739–4754. https://doi.org/10.1111/1462-2920.13365
28. Kristensen E. (2015). Temporal development of the gut microbiota in European lobster (*Homarus gammarus*) juveniles exposed to two different water treatment systems. NTNU Norwegian University of Science and Technology, Department of Biotechnology. Open BIBSYS Web [online]: https://brage.bibsys.no/xmlui/handle/11250/2351631. Accessed 15 August 2017.
29. Cheung MK et al. (2015) Rapid change of microbiota diversity in the gut but not the hepatopancreas during gonadal development of the new shrimp model *Neocaridina denticulata*. Mar. Biotechnol. 17(6):811–819. https://doi.org/10.1007/s10126-015-9662-8
30. Rungrassamee W et al. (2013) Bacterial population in intestines of the black tiger shrimp (Penaeus monodon) under different growth stages. PLoS One 8(4):e60802. https://doi.org/10.1371/journal.pone.0060802
31. Cardona E, Gueguen Y, Magré K, Lorgeoux B, Piquemal D, Pierrat F, Saulnier D (2016) Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. BMC Microbiol. 16(1):157. https://doi.org/10.1186/s12866-016-0770-z
32. Briggs M, Funge-Smith S, Subasinghe R, Phillips M (2004) Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. RAP Publication 10:92 http://www.fao.org/docrep/007/ad505e/ad505e00.htm. Accessed 02 May 2017
33. Yano Y, Hamano K, Satomi M, Tsutsui I, Ban M, Aue-Umneoy D (2014) Prevalence and antimicrobial susceptibility of Vibrio species related to food safety isolated from shrimp cultured at inland ponds in Thailand. Food Control 38:30–36. https://doi.org/10.1016/j.foodcont.2013.09.019
34. Jayasree L, Janakiram P, Madhavi R (2006) Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). J. World Aquacult. Soc. 37(4):523–532. https://doi.org/10.1111/j.1749-7345.2006.00066.x
35. Elmahdi S, DaSilva LV, Parveen S (2016) Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. Food Microbiol. 57:128–134. https://doi.org/10.1016/j.fm.2016.02.008
36. Soto-Rodriguez SA, Gomez-Gil B, Lozano-Olvera R, Betancourt-Lozano M, Morales-Covarrubias MS (2015) Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (Litopenaeus vannamei) in Northwestern Mexico. Appl. Environ. Microbiol. 81(5):1689–1699. https://doi.org/10.1128/AEM.03610-14
37. Parvathi A, Kumar HS, Karunasagar I, Karunasagar I (2004) Detection and enumeration of Vibrio vulnificus in oysters from two estuaries along the southwest coast of India, using molecular methods. Appl. Environ. Microbiol. 70(11):6909–6913. https://doi.org/10.1128/AEM.70.11.6909-6913.2004
38. Leyton Y, Riquelme C (2008) Vibrios en los sistemas marinos costeros. Rev. Biol. Mar. Oceanogr. 43(3):441–456. https://doi.org/10.4067/S0718-19572008000300004
39. Christopher FM, Vanderzant C, Parker JD, Conte FS (1978) Microbial flora of pond-reared shrimp (Penaeus stylirostris, Penaeus vannamei, and Penaeus setiferus). J. Food Prot. 41(1): 20–23. https://doi.org/10.4315/0362-028X-41.1.20
40. Chen QS, Yan YL, Yang XQ, Ma Y (2008) Effects of different temperatures on polyphenoloxidase activity, microbiological index and freshness of Metapenaeus ensis. J Food Sci. 10:151
41. Vieira RHDF, Carvalho EM, Carvalho FC, Silva CM, Sousa OV, Rodrigues DP (2010) Antimicrobial susceptibility of Escherichia coli isolated from shrimp (Litopenaeus vannamei) and pond environment in northeastern Brazil. J. Environ. Sci. Health B 45(3): 198–203. https://doi.org/10.1080/03601231003613526.
42. Parente LS, Costa RA, Vieira GHF, dos REIS EMF, Hofer E, Fonteles AA, dos Fernandes Vieira RHS (2011) Bactérias entéricas presentes em amostras de água e camarão marinho *Litopenaeus vannamei* oriundos de fazendas de cultivo no Estado do Ceará, Brasil. Braz. J. Vet. Res. Anim. Sci. 48(1):46–53 http://www.revistas.usp.br/bjvras/article/view/34375. Accessed 02 May 2017

43. Reddy P, Hareesh K, Suneetha Y, Reddy S. (2016). Efficacy of Animal and Plant Feed Ingredients in the Formulation of feeds for induction of growth potentials in penaeid prawn *Litopenaeus vannamei*. International Journal of Fisheries and Aquatic Studies. 4(2):07–14. http://www.fisheriesjournal.com/archives/2016/vol4issue2/PartA/4-1-30.pdf. Accessed 02 May 2017.
44. Rungrassamee W, Klanchui A, Chaiyapechara S, Maibunkaew S, Tangphatsornruang S, Jiravanichpaisal P, Karoonuthaisiri N (2013) Bacterial population in intestines of the black tiger shrimp (Penaeus monodon) under different growth stages. PLoS One 8(4):e60802. https://doi.org/10.1371/journal.pone.0060802
45. Vieira F, Jatobá A, Mouriño J, Buglione Neto C, Silva J, Seiffert W, et al. (2016) Use of probiotic-supplemented diet on a Pacific white shrimp farm. Rev. Bras. Zootec. 45(5):203–207. https://doi.org/10.1590/S1806-92902016000500001
46. Bernal M, Marrero R, Campa-Córdova Á, Mazón-Suástegui J (2017) Probiotic effect of Streptomyces strains alone or in combination with Bacillus and Lactobacillus in juveniles of the white shrimp *Litopenaeus vannamei*. Aquac. Int. 25(2):927–939. https://doi.org/10.1007/s10499-016-0085-y
47. Vargas-Albores F, Martínez-Porchas M, Arvayo M, Villalpando-Canchola E, Gollas-Galván T, Porchas-Cornejo M (2016) Immunophysiological response of Pacific white shrimp exposed to a probiotic mixture of Proteobacteria and Firmicutes in farm conditions. N. Am. J. Aquac. 78(3):193–202. https://doi.org/10.1080/15222055.2016.1167797
48. Chumpol S, Kantachote D, Nitoda T, Kanzaki H (2017) The roles of probiotic purple nonsulfur bacteria to control water quality and prevent acute hepatopancreatic necrosis disease (AHPND) for enhancement growth with higher survival in white shrimp (*Litopenaeus vannamei*) during cultivation. Aquaculture 473:327–336. https://doi.org/10.1016/j.aquaculture.2017.02.033
49. Haryanti H, Sugama K, Tsumura S, Nishijima R (2017) Enhance production of black tiger shrimp Penaeus monodon postlarvae by probiotic bacterium Alteromonas sp. Indonesian Fisheries Research Journal 7(1):1–6. 10.15578/ifrj.7.1.2001.1-6
50. Duan Y, Zhang Y, Dong H, Wang Y, Zhang J (2017) Effect of the dietary probiotic Clostridium butyricum on growth, intestine antioxidant capacity and resistance to high temperature stress in kuruma shrimp Marsupenaeus japonicus. J. Therm. Biol. 66:93–100. https://doi.org/10.1016/j.jtherbio.2017.04.004
51. Javadi A, Khatibi S (2017) Effect of commercial probiotic (Protexin®) on growth, survival and microbial quality of shrimp (*Litopenaeus vannamei*). Nutrition & Food Science 47(2):204–216. https://doi.org/10.1108/NFS-07-2016-0085
52. Xia Z, Zhu M, Zhang Y (2014) Effects of the probiotic Arthrobacter sp. CW9 on the survival and immune status of white shrimp (*Penaeus vannamei*). Lett. Appl. Microbiol. 58(1):60–64. https://doi.org/10.1111/lam.12156