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Bacterial Community Composition and Succession During Larval Ontogeny of Cultured Tropical Rock Lobster (*Panulirus ornatus*)

Mengjia Jiang[1] | Ivona Mladineo[1,2] | Evan F. Goulden[3] | Gregory G. Smith[1] | Andrew J. Trotter[1]

[1]Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, Hobart, Tasmania, Australia | [2]Institute of Parasitology, Biology Centre Czech Academy of Sciences, Cask Budejovice, Czechia | [3]Queensland Department of Primary Industries, Bribie Island Research Centre, Woorim, Queensland, Australia

Correspondence: Mengjia Jiang (Mengjia.jiang@utas.edu.au)

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ABSTRACT

The dynamics within bacterial communities play a significant role in the health, nutrition and disease resistance of tropical rock lobster (*Panulirus ornatus*) larvae in aquaculture. However, studies on bacterial succession throughout *P. ornatus* larval development remain limited. Therefore, this study investigated bacterial community composition and succession from newly hatched phyllosoma (0-day post-hatching, 0 dph) to the puerulus stage, using Oxford Nanopore 16S rRNA gene sequencing. Alpha and beta diversity metrics were used to assess patterns of diversity and shifts in community composition. Indicator species analysis was applied to identify taxa associated with specific developmental stages. Larval bacterial diversity showed a trend aligned with larvae developing to the puerulus stage. Early phyllosoma stages were enriched in bacterial families, such as Alteromonadaceae and Roseobacteraceae, whereas Pseudoalteromonadaceae dominated later stages. The key bacterial taxa identified as stage-specific indicators were *Alteromonas*, *Ruegeria*, *Shimia*, *Thalassotalea* and *Pseudoalteromonas*. Several intrinsic cues, such as maternal effect, moulting and immune status, were considered factors influencing bacterial composition throughout larval development. This study provides the first comprehensive analysis of bacterial succession during the larval ontogeny of *P. ornatus*, revealing that bacteriome dynamics are closely associated with developmental physiology and environmental inputs. The identification of bacterial indicators specific to lobster developmental stages enhances our understanding of host–bacteria interactions during lobster larval rearing. It also strongly supports the development of strategies to improve larval survival and mitigate disease in aquaculture systems.

1 | Introduction

The tropical rock lobster (*Panulirus ornatus*) is one of the most economically valuable crustaceans in global aquaculture, with demand increasing due to its high market value (FAO 2024). In Southeast Asia, particularly in Vietnam and Indonesia, lobster aquaculture remains heavily reliant on wild-caught seedstock to support grow-out operations (Jones 2015a, 2015b). In contrast, recent technological advances have enabled the successful development of closed-cycle hatchery systems for *P. ornatus*, thereby establishing commercial aquaculture operations in Australia based on hatchery-reared seedstock (Lewis et al. 2024). This progress has driven the rapid innovation of aquaculture systems to enhance production efficiency, improve larval health and optimise rearing conditions.

Larval development in *P. ornatus* comprises a protracted pelagic phase with 11 morphologically distinct phyllosoma stages, followed by a transitional planktonic puerulus stage and subsequent benthic post-puerulus, juvenile and adult stages (Smith et al. 2009; Wu et al. 2012). These stages involve frequent moulting and rapid morphological and tissue development, driven by both environmental exposure and intrinsic host traits. Such factors can influence larval survival and disease susceptibility, collectively driving dynamic shifts in bacteriome composition throughout ontogeny (Lu et al. 2022; Payne et al. 2007; Saqib et al. 2023; Smith et al. 2009; Wu et al. 2012). Additionally, like other crustaceans, *P. ornatus* larvae encounter diverse bacterial communities immediately after hatching, thereby initiating the establishment of bacterial communities that support digestion, metabolism, immunity and stress tolerance (Lu et al. 2022; Wang et al. 2020; Xiong et al. 2015, 2017).

Understanding the bacteriome of *P. ornatus* larvae has been limited by the scarcity of studies spanning the full larval phase. Previous research has primarily focused on specific life stages, most commonly juvenile or adult lobsters, or on healthy wild individuals, identifying dominant bacterial taxa, such as *Vibrio*, *Pseudoalteromonas* and *Ruegeria* (Ooi et al. 2017, 2023). Additionally, several earlier studies relied on culture-based methods or earlier molecular techniques, such as Denaturing Gradient Gel Electrophoresis (Bourne et al. 2007; Goulden, Hall, Bourne, et al. 2012; Payne et al. 2007), which provide only partial insight into bacterial community structure. To our knowledge, bacterial succession throughout the larval development of *P. ornatus* has not been characterised, leaving a substantial gap in our understanding of how bacterial composition changes during ontogeny. Capitalising on a commercial-scale larval rearing facility developed under a validated protocol, we performed metabarcoding to generate comprehensive, stage-resolved profiles of larval bacterial communities. This study addresses this knowledge gap by applying Oxford Nanopore 16S rRNA gene sequencing to examine bacteriome dynamics throughout the early developmental period, providing the first detailed account of bacterial succession in the *P. ornatus* phyllosoma.

In this study, we investigate the succession of larval-associated bacterial communities across the phyllosoma stages of *P. ornatus* reared under controlled, commercial-scale hatchery conditions. Using Oxford Nanopore 16S rRNA sequencing, we aim to characterise bacterial community assemblages during larval development and to explore bacterial taxa that may serve as indicators of the developmental phase. Understanding the dynamics of larval bacteria is essential for optimising hatchery management, enhancing larval survival and preventing disease outbreaks in tropical rock lobster aquaculture.

2 | Materials and Methods

2.1 | Rearing Conditions and Sample Collection

P. ornatus larvae were hatched from broodstock held at the Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, Taroona, Tasmania and reared in a commercial-scale hatchery system. Larvae were maintained in a low-exchange recirculating aquaculture system receiving filtered and ozonated seawater top-ups. Water quality in the larval rearing tanks was closely monitored and maintained within the specific range for each parameter (Table 1). A WTW (Xylem Analytics, Germany) water-quality meter and associated probes were used to measure DO, salinity, temperature and pH. Oxidation-reduction potential (ORP) was measured using both the Pinpoint ORP monitor (American Marine Inc., USA) and Foxboro DolpHin Series probes (Invensys Systems, Inc., USA). These parameters were recorded daily throughout the study period. Due to commercial confidentiality, detailed specifications of the hatchery system cannot be disclosed.

Whole larval samples were collected across major developmental stages (from I through XI) (Smith et al. 2009) at larval hatching (0 days post-hatch, dph) and subsequently at 1, 7, 14, 21, 28, 56, 84, 112 and 140 dph, till the puerulus stage. The phyllosoma were fed a proprietary diet incorporating live *Artemia* (Sep-Art GSL *Artemia* cysts, INVE Aquaculture Inc., USA). More frequent sampling was undertaken during the early period (0–21 dph) to capture the initial establishment of larval-associated bacterial communities, with sampling timed prior to each moult when moulting is highly synchronous. In contrast, the sampling intervals were extended at later stages once moulting became asynchronous within the cohort. Sampling was conducted from a single tank between 25 September 2024 and 5 February 2025. Larvae were maintained at a stocking density of 2.75 animals/L. Each dph sample weighted 0.02 g and included six biological replicates ($n = 6$). Larvae were collected using a sterile siphon and transferred into sterile 15 mL plastic tubes, which were immediately placed on ice for transport to the laboratory. To remove residual water, the samples were first filtered through 1.5 μm pore-size 47 mm Whatman GF/C glass microfiber filters (Cytiva, Whatman). Each replicate was then transferred into a 1.5-mL microcentrifuge tube containing 500 μL of storage buffer and stored at −20°C overnight.

2.2 | DNA Extraction

Extraction was performed by adding 500 μL high-salt lysis buffer (1% SDS, 1.5 M NaCl, 0.1 M EDTA, 1% PVP-10), 5 μL of proteinase K (Bioline Pty. Ltd., NSW, Australia) and 5 μL of TCEP (TRIS phosphine, 1 M). Samples were then heated at 65°C for 1 h (vortexed every 20 min) and incubated on ice for 5 min. Ammonium acetate (7.5 M; Sigma-Aldrich Co., MO, USA) and potassium chloride were added to the samples at final concentrations of 2.5 and 1 M, respectively. The samples were then vortexed for 15 s and centrifuged at 10,000 × *g* for 10 min at 22°C. The supernatant was mixed by inversion with 750 μL of isopropanol and 1.5 μL isoprecipitant (co-precipitant pink, Bioline, London, UK) at room temperature before further centrifugation at 12,000 × *g* for 15 min at 22°C. The visible pellet was rinsed with 500 μL of 70% ethanol twice and resuspended in 100 μL of buffered water (0.05% Triton X-100, 10 mM TRIS pH 7). RNA was removed by digesting the sample with 2 μL of RNase A (10 mg/mL, Thermo Fisher, USA) at 37°C for 30 min. Samples were treated with 200 μL of ammonium acetate (2.5 M), 200 μL 1 M potassium chloride and 400 μL high-salt lysis buffer, vortexed for 30 s, and centrifuged at 10,000 × *g* for 10 min (22°C). The supernatant was mixed with an equal volume of isopropanol containing the co-precipitant, then centrifuged at 12,000 × *g* for 15 min. The pellet was rinsed twice with 70% ethanol before resuspension in 50 μL of molecular-grade water (Qiagen,

TABLE 1 | Water quality parameters in a commercial-scale hatchery system (Institute for Marine and Antarctic Studies, University of Tasmania, Taroona, Tasmania) for the tropical rock lobster (*Panulirus ornatus*) over the study period (from hatching to puerulus stage).

Month/Year	Weeks covered	Day post-hatch (dph)	Dissolved oxygen (%)	Temperature (°C)	pH	Oxidation reduction potential (mV)
Sep-24	Week 1[a]	0	102.55	27.17	8.19	324
Oct-24	Weeks 2–6	1, 7, 14, 21 and 28	104.00 ± 1.96	27.91 ± 0.13	8.24 ± 0.03	377.6 ± 8.82
Nov-24	Weeks 7–10	58	102.37 ± 1.14	28.10 ± 0.20	8.22 ± 0.02	373.75 ± 10.28
Dec-24	Weeks 11–14	84	101.56 ± 1.21	28.19 ± 0.09	8.23 ± 0.01	373.5 ± 14.91
Jan-25	Weeks 15–18	112	103.63 ± 2.55	28.32 ± 0.11	8.22 ± 0.01	364.25 ± 8.81
Feb-26	Weeks 19–20	150, puerulus	104.50 ± 0.59	28.25 ± 0.22	8.23 ± 0.01	371.0 ± 1.41

[a]In Week 1, a single measurement was recorded for each water quality parameter. Values are presented as mean ± standard deviation.

Germany). All samples were quantified by Qubit 4 (Thermo Scientific, USA) using the Qubit 1x dsDNA BR Assay Kit (Thermo Scientific, USA) following the manufacturer's instructions.

2.3 | Quantitative PCR, Library Preparation and 16S rRNA Sequencing on the MinION Platform

2.3.1 | Pre-Amplification

All the samples were pre-amplified by qPCR targeting the 16S rRNA locus using a high fidelity polymerase and amplification cycles to minimise amplification bias (Matsuo 2023; Ooi et al. 2023). The number of pre-amplification cycles was standardised to 35 cycles across all samples to ensure sufficient amplification yield from low-biomass samples for sequencing (Matsuo 2023; Ooi et al. 2023; Wong et al. 2023). The primary SYBR Green qPCR mixture consisted of 5 μL of 2x MyTaq Hot Start mix (Bioline, Meridian Bioscience, USA), 100 nM each of 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492 R (5′ GGTTACCTTGTTACGACTT 3′) 16S rRNA gene primers (melting temperatures between 86°C and 88°C) (Ooi et al. 2023), 2 μL of 1:10 diluted DNA, and 2 μL of molecular-grade water. The qPCR was conducted using a thermal cycler (Bio-Rad Laboratories Inc., USA) with the following thermal cycling program: initial denaturation for 3 min at 95°C; denaturation for 10 s at 95°C, annealing for 30 s at 55°C, extension for 30 s at 72°C and a final extension for 3 min at 72°C. Amplified DNA was purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) with a 1:1.8 wash ratio, following the manufacturer's instructions (AMPure XP DNA Cleanup Workflow). The final cleaned DNA was quantified using a Qubit 4. The 'No template controls' were not included in the pre-amplification and sequencing stages, given the present study's context of longitudinal bacteriome development (Hornung et al. 2019; Williamson et al. 2025).

2.3.2 | Library Preparation

The library preparation mixture consisted of 10 ng of input DNA, adjusted to a total of 15 μL with molecular-grade water, 25 μL Long Amp Hot Start Taq 2x Master Mix (New England Biolabs, Ipswich, MA, USA) and 10 μL of 16S barcode (16S Barcoding kit, SQK 16S 114.24, Oxford Nanopore Company, UK). The qPCR was conducted using a thermal cycler (Bio-Rad Laboratories Inc., USA) with the following thermal cycling program: initial denaturation for 1 min at 95°C; 25 cycles of denaturation for 25 s at 95°C, annealing for 25 s at 55°C, extension for 25 s at 65°C and a final extension for 5 min at 65°C. EDTA (1 μL) was added to each sample to stop the reaction. Amplified DNA was purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) with a 1:1.8 wash ratio, following the manufacturer's instructions (AMPure XP DNA Cleanup Workflow). The final cleaned DNA was quantified using a Qubit 4.

2.3.3 | Priming and Sequencing

After library preparation, a total of 50 ng of DNA was pooled from different samples and then incubated with 1 μL of a mixture of Rapid Adapter (RA) and Adapter Buffer (AB) at room

temperature for 5 min. The prepared DNA pool library was mixed with 34 μL of Sequencing Buffer (SQB), 25.5 μL of Loading Beads (LB) and 4.5 μL of molecular-grade water and then loaded onto the SpotON R10.4.1 flow cell (Oxford Nanopore Technologies, UK) for 48 h. The library was sequenced on the MinION Mk 1C. The generated sequencing data were monitored in real-time using the MinKNOW software (version 4.0.20). A total of three flow cells have been used, each containing 24, 24 or 15 samples. As a control, the DNA of a microbial community (Zymo Biomics Microbial Community Standard) was included in the sequencing runs (Table S3, representative result).

2.4 | Bioinformatics and Data Analysis

Dorado software ver. 0.7.1 (Oxford Nanopore Technologies, https://github.com/nanoporetech/dorado) with the sup-basecalling model was used for basecalling the sequencing data (POD5 files) to generate FASTQ files with a mean quality score >10, and for demultiplexing and adapter trimming of the reads. Reads were filtered using NanoFilt ver. 2.8.0 (De Coster et al. 2018) on the basis of length, retaining reads between 1200 and 1700 bp. Taxonomic assignment was performed using EMU (Curry et al. 2022), which implements an expectation-maximisation algorithm to probabilistically assign reads to taxa using the full-length 16S rRNA gene and the NCBI 16S database. This approach helps to reduce misclassification caused by sequencing errors and improves reliability at the genus level. To visualise changes in bacterial community composition across larval stages, bacterial data aggregated at the family and genus levels were first imported and reshaped in R (version 4.4.3). Alluvial plots were generated using ggalluvial (Brunson and Read 2020), which displays the relative abundance flows of the top 10 families and genera across larval developmental stages.

Alpha diversity of bacterial communities was assessed using Observed, Shannon and Simpson indices to evaluate species richness and evenness across experimental groups. All analyses were conducted in R (version 4.4.3) using the vegan package (Oksanen et al. 2025), and alpha diversity was visualised using bar plots with ggplot2. Differences across developmental stages were assessed using the Kruskal–Wallis test.

Beta diversity was assessed using Bray–Curtis dissimilarities to evaluate differences in bacterial community composition among sample points. Homogeneity of multivariate dispersion among groups was tested with PERMDISP (Anderson 2006), which showed no significant differences ($p = 0.27$), indicating homogeneity of variance. Global differences in bacterial community composition among developmental stages were assessed using Permutational multivariate analysis of variance (PERMANOVA). Pairwise PERMANOVA comparisons were performed to examine differences between all stage pairs. Resulting p values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure. Beta diversity patterns were visualised using principal coordinates analysis (PCoA) on the basis of the Bray–Curtis distance matrix.

To identify stage-specific bacterial genera, Indicator Species Analysis (IndVal) (Podani and Csányi 2010) was performed using the multipatt() function with the duleg = TRUE from the indicspecies package (De Caceres et al. 2016). Significant indicator genera were identified at $p \leq 0.05$. Indicator values (statistics from multipatt()) were plotted across larval stages in a bubble plot using ggplot2, with bubble size reflecting the strength of association with each stage, and colour corresponding to the larval stage. To examine correlations in bacterial community composition across larval stages, the normality of the data was first assessed, and Spearman's rank correlation was selected. Correlations between larval stages were computed using the corrplot package (Wei and Simko 2021) in R 4.4.3.

3 | Results

After quality filtering (mean $Q > 10$) and length selection (1200–1700 bp), a total of 1,831,757 reads across all 60 samples were retained. The Good's coverage estimates ranged data available from Table S8, indicating that the majority of dominant bacterial taxa were captured. Even in samples with relatively low sequencing depth, coverage remained high, supporting robust genus-level analysis.

3.1 | Bacterial Composition Across Larval Development

At the genus level, *Vibrio* dominated most larval stages, showing very high abundance at hatching (79.5% at 0 dph), a brief decline at 1 dph (2.6%), and then rising sharply again from 7 dph onward. From 28 to 140 dph, *Vibrio* remained consistently elevated (33.3%–81.9%), peaking at 112 dph (82.3%) and maintaining high levels until 140 dph (81.9%). In contrast, *Pseudoalteromonas* displayed a different pattern: It was moderately abundant during the early and mid-larval stages (14.3%–60.9% at 0, 14 and 56 dph) but became the dominant genus at the puerulus stage (99.6%). *Alteromonas* exhibited pronounced early enrichment, peaking at 1 dph (69.1%), with additional increases at 21 dph (34.2%) and 84 dph (22.2%), but remained lower at most other time points (3.9%–9.1%). Several genera appeared intermittently at lower abundances: *Ruegeria* increased gradually from early stages (<1%) to moderate levels at 7 dph (7.9%) and 56 dph (2.8%); *Shimia* peaked sharply at 7 dph (23.4%) but remained <1% elsewhere. Minor genera, such as *Alkalimarinus, Maritalea, Neptunomonas* and *Oceanospirillum*, consistently remained below 5% throughout lobster development (Figure 1a, Tables S2 and S7).

The taxonomic composition of bacterial communities associated with *P. ornatus* larvae changed measurably over time. At the family level, Vibrionaceae were consistently present at all stages (6.3%), showing a fluctuating trend; a high abundance immediately at hatching (68.9% at 0 dph), then a reduction at 1 dph, and subsequently increasing again from 7 dph onward, being highly present at 112 dph (75.1%) and 140 dph (71.2%). Interestingly, only very few Vibrionaceae were present at the puerulus stage (0.3%). Pseudoalteromonadaceae (mean 23.4%) followed the opposite pattern, being dominant at the puerulus stage (99.6%) and elevated early in development (58.7% at 14 dph), then oscillating across the remaining time points. In contrast, Alteromonadaceae peaked transiently at 1 dph (50.3%) and 21 dph (32.1%) before remaining at low levels thereafter (Figure 1b, Tables S1 and S6).

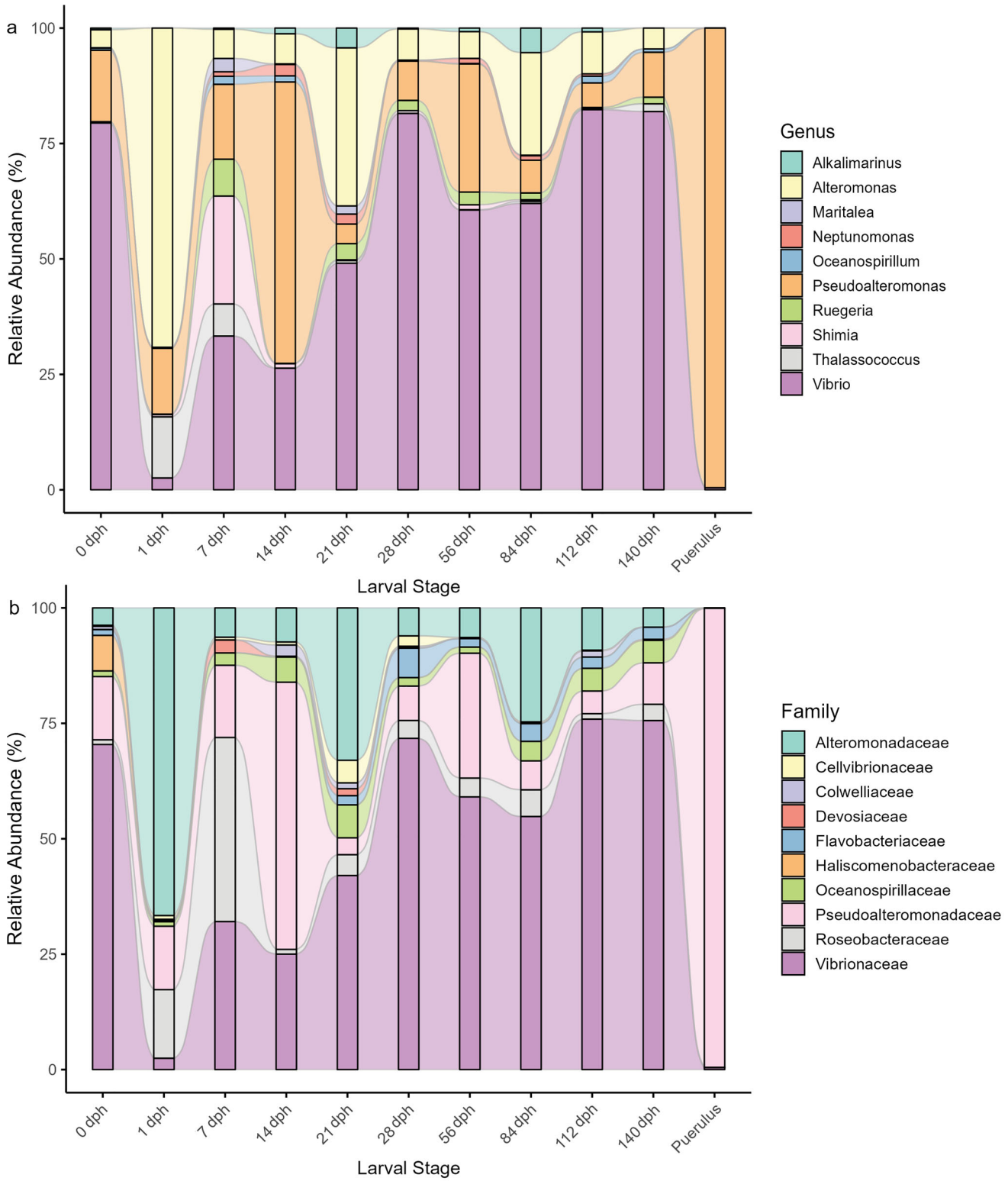

FIGURE 1 | Alluvial plot showing relative abundance flows of top 10 bacterial taxa at (a) genus level and (b) family level associated with *Panulirus ornatus* larvae (0 dph-puerulus).

3.2 | Alpha and Beta Diversity Across Larval Development

Bacterial alpha diversity fluctuated throughout larval development. Observed richness showed no significant change across the developmental stages, and the index remained stable throughout larval development (Kruskal–Wallis $H = 38.57$, df = 11, $p = 0.06$) (Figure 2). Shannon and Simpson indices showed a general decrease at later larval development stages. Diversity remained stable from 7 through 140 dph and then declined sharply during the puerulus stage ($p = 0.0041$ for Shannon diversity; $p = 0.0042$ for Simpson diversity).

Beta diversity visualised using PCoA showed that the first two axes explained 22.5% and 16.1% of the total variation in bacterial community composition, respectively (Figure 3). Bacterial communities differed significantly across the larval developmental sequence. Clear separation was observed between 0 dph and all subsequent stages, with particularly strong differences compared with 112 and 140 dph ($p = 0.001$ and $p = 0.005$, respectively). In contrast, bacterial communities at 28 dph were only marginally different from those at 84 dph ($p = 0.076$). The most similar communities were observed between 112 and 140 dph ($p = 0.312$) (Figure 3, Table S4). PERMANOVA based on Bray–Curtis dissimilarity confirmed that bacterial community composition varied significantly among developmental stages (PERMANOVA: $F_{10,49} = 9.54$, $R^2 = 0.661$, $p = 0.001$; 999 permutations).

3.3 | Indicator Bacterial Taxa Across Larval Development

Indicator taxa varied across larval development (Figure 4). At 0 dph, indicator genera included *Portibacter*, *Nitratireductor* and *Flavilitoribacter*. At 7 dph, the highest number of indicator bacteria was detected, comprising *Thalassococcus*, *Shimia*, *Ruegeria*, *Phaeobacter* and *Maritimonas*. At later stages, *Dokdonia* and *Bradyrhizobium* were identified as indicators at 28 dph, whereas *Malaciobacter* was the primary indicator at 56 dph. In the puerulus stage, *Pseudoalteromonas* was the dominant genus indicator. No indicator taxa were identified at 21, 84, 112 or 140 dph. Indicator values (IndVal statistics) for all taxa are provided in Table S5.

Spearman correlation analysis showed variable similarity in bacterial community composition across larval stages (0 dph to puerulus) (Figure 5). The strongest correlations were observed between 56 and 112 dph (0.57), 140 and 112 dph (0.55) and between 1 and 7 dph (0.52). In contrast, 0 dph consistently showed the lowest correlations with later stages. Most comparisons among mid-stages (i.e., 7–84 dph) yielded moderate correlations, ranging from 0.30 to 0.50 (Figure 5).

4 | Discussion

Understanding bacterial dynamics in *P. ornatus* larvae is essential for optimising hatchery management and improving health and survival. In this study, we examined the composition and succession of bacterial communities throughout larval development, from 0 dph to the puerulus stage. Our findings demonstrate that bacterial communities of *P. ornatus* larvae undergo periods of directional shifts and stabilisation, and that specific developmental stages may be characterised by key indicator bacteria.

4.1 | Larval Bacterial Alpha Diversity and Host Development

The alpha diversity of *P. ornatus* larvae displayed a fluctuating trend, consistent with previous observations in other aquatic larvae, including whiteleg shrimp (*Litopenaeus vannamei*) (Wang et al. 2020) and Prussian carp (*Carassius auratus gibelio*) (Li et al. 2017). In this study, larval alpha diversity indices decreased at the puerulus stage. Newly hatched larvae lack fully developed features, and their associated bacteria can be derived primarily from within fertilised eggs or the egg epidermal surface (Lu et al. 2022). By 1 dph, when larvae are transferred to rearing tanks and begin exogenous feeding, intestinal colonisation commences, leading to reassembly of the larval bacterial community. Subsequently, larval alpha diversity remained relatively stable, coinciding with developmental stages in which the intestinal tract and associated microhabitats are relatively simple and provide limited ecological niches (Li et al. 2017; Yan et al. 2012). At the puerulus stage, both Shannon and Simpson indices indicated a further decline in diversity. Puerulus is a secondarily lecithotrophic stage that metamorphoses from the final planktotrophic phyllosoma stage (Barret 2019; Guerao et al. 2006). The dramatic changes in feeding activity, anatomy and physiology during this transition period likely contribute to the purging and resetting of bacterial communities, resulting in low bacterial diversity.

4.2 | Larval Bacterial Community Composition

The bacterial communities associated with *P. ornatus* larvae undergo succession across the phyllosoma and puerulus stages. As the intestinal tract and digestive capacity mature, early bacterial colonisers are reorganised into larval stage-specific communities, a pattern consistent with bacterial succession reported in other crustaceans, such as mud crab (*Scylla paramamosain*), Pacific whiteleg shrimp and European lobster (*Homarus gammarus*) (Angthong et al. 2023; Bateman et al. 2012; Sun et al. 2025; Zhang et al. 2021). At the family level, Flavobacteriaceae, Alteromonadaceae and Pseudoalteromonadaceae exhibited dynamic shifts throughout development. Pseudoalteromonadaceae and Vibrionaceae are commonly dominant in juvenile lobster gut bacteria, whereas Alteromonadaceae are frequently abundant in the hepatopancreas (Ooi et al. 2017, 2023). At the genus level, *Alteromonas*, *Pseudoalteromonas*, *Ruegeria* and *Vibrio* were consistently abundant throughout larval development. These shifts likely reflect the evolving morphology and physiology of the digestive tract, including increased surface area and substrate availability, as well as consequent host-mediated selection pressures (Attramadal et al. 2014; Tzeng et al. 2015).

Vibrio was consistently detected across all stages, and its ecological role in larval health and development is likely to be context dependent. Commensal *Vibrio* species inhibit colonisation by other bacteria through the production of antimicrobial compounds and assist in chitin digestion by facilitating digestion

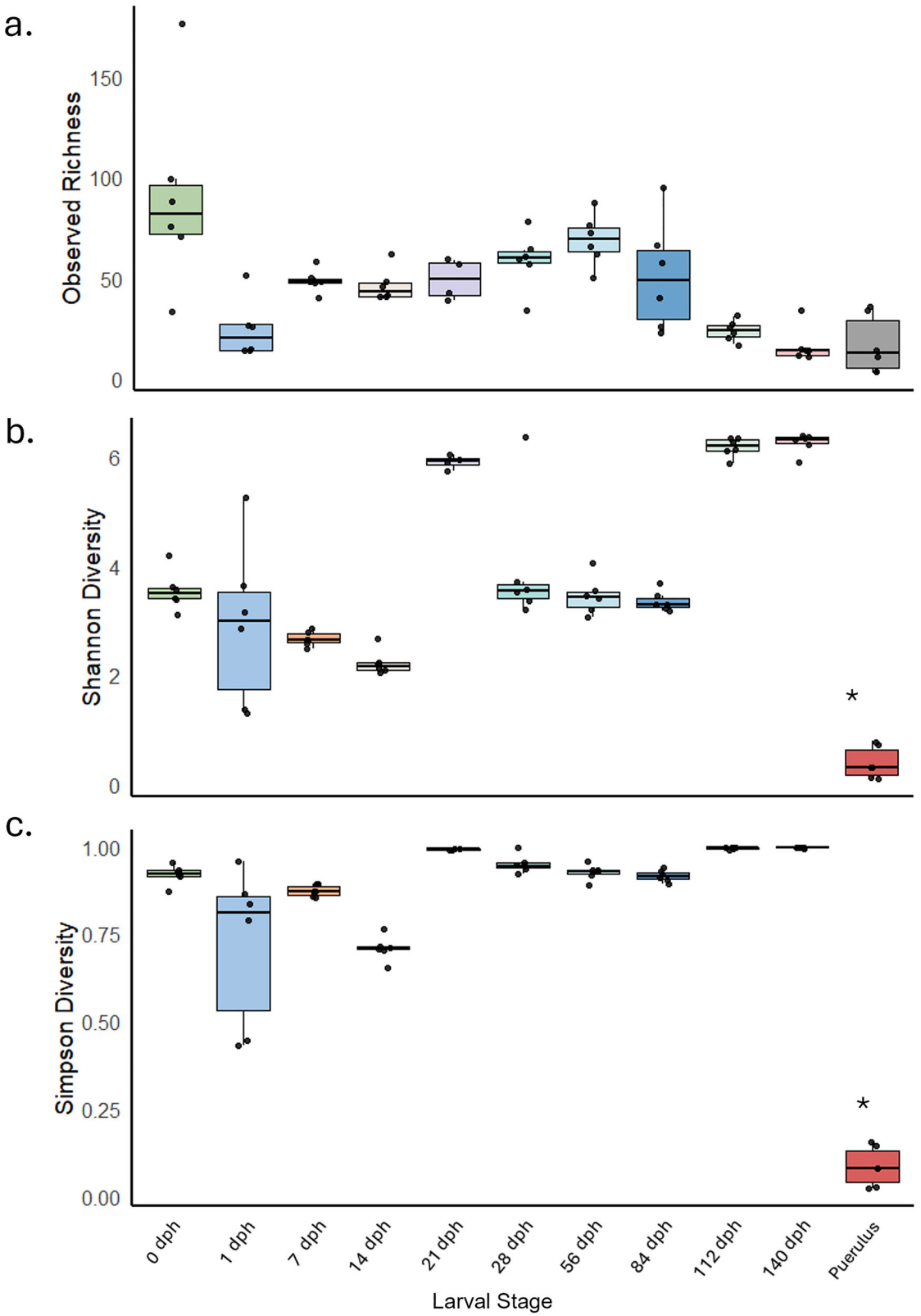

FIGURE 2 | Boxplot showing comparisons of alpha diversity indices of bacterial communities associated with *Panulirus ornatus* larvae (0 dph-puerulus). (a) Observed OTUs richness, (b) Shannon diversity index as an estimate for species abundance and (c) Simpson diversity. Boxes represent the interquartile range, the horizontal line within the box indicates the median, whiskers show the minimum and maximum values, and individual points represent single samples. Asterisks (*) denote significant differences between puerulus and other days post-hatch at $p < 0.05$.

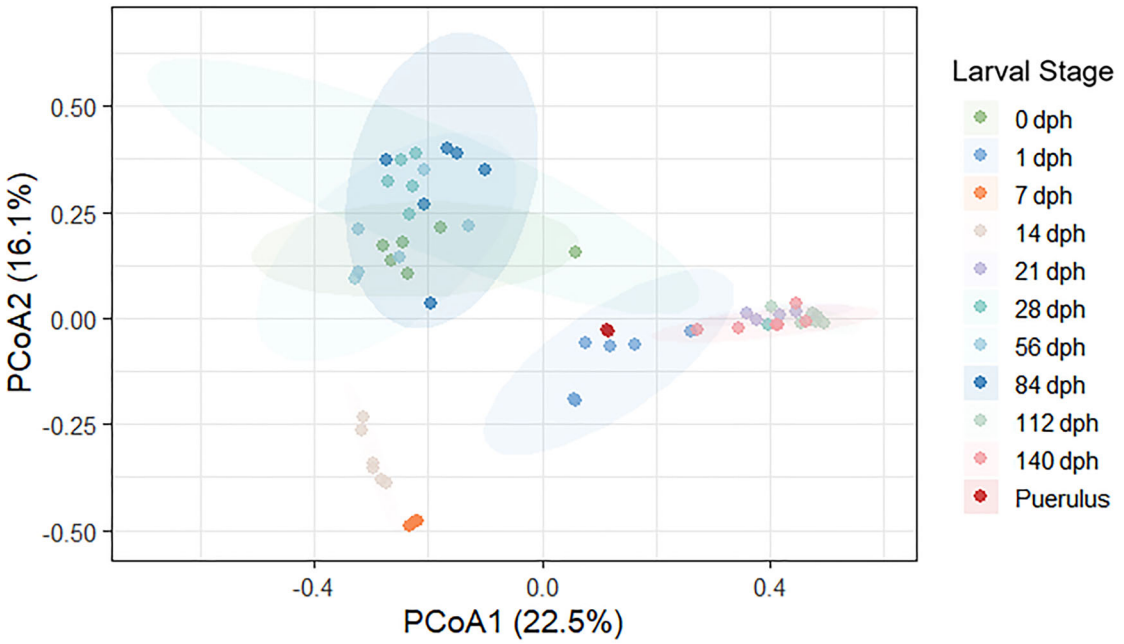

FIGURE 3 | Principal coordinate analysis showing similarity of bacterial communities at the genus level in *Panulirus ornatus* larvae (0 dph-puerulus).

of feed and exoskeletal material from moulting (Goulden, Hall, Pereg, et al. 2012; Perera and Simon 2015; Thompson et al. 2004; Wietz et al. 2010). *Vibrio* spp., together with *Pseudoalteromonas* spp., may also degrade algal polysaccharides, aiding the digestion of natural diets (Egan et al. 2013; Martin et al. 2014). However, many pathogenic strains within the *Vibrio* genus pose significant disease risks to aquatic animals and may emerge under perturbed environmental conditions and immunocompromised states, for example, during white leg disease outbreaks (Ooi et al. 2020; Shields 2011).

4.3 | Indicator Bacteria Present During *P. ornatus* Larvae Development

During the first week post-hatch (0–7 dph), the larval bacterial community was dominated by Alteromonadaceae and Roseobacteraceae, including *Phaeobacter*, *Portibacter*, *Mariniblastus*, *Alteromonas*, *Shimia* and *Ruegeria*. Members of the Alteromonadaceae, particularly *Alteromonas*, are fast-growing copiotrophic bacteria that thrive in nutrient-rich marine microhabitats and are frequently detected in aquaculture environments (Ivars-Martinez et al. 2008; Pedler et al. 2014). Their prevalence during early phyllosoma stages likely reflects transient colonisation from the rearing environment, where they degrade dissolved organic matter and help establish physicochemical conditions conducive to subsequent bacterial community development (Goto et al. 2017; Henríquez-Castillo et al. 2022). In parallel, members of the Roseobacteraceae, including *Shimia* and *Ruegeria*, are widely distributed in coastal ecosystems and participate in sulphur cycling. They are also recognised for producing secondary metabolites with antibacterial activity, which exert antagonistic effects against opportunistic *Vibrio* species, such as *V. anguillarum*, in farmed turbot, *Scophthalmus maximus* (Buchan et al. 2005; Porsby et al. 2008). The genus *Shimia* has been implicated in promoting bacterial community stability, biofilm formation, osmoregulation and protein metabolism, potentially supporting early larval nutrition and resilience (Hyun et al. 2013; Zhang and Sun 2022). Additional early indicators, such as *Portibacter* and *Mariniblastus*, also play critical ecological roles by degrading complex polysaccharides derived from algal or detrital material, likely contributing to bacterial food web dynamics and nutrient recycling (Lage et al. 2017; Peng et al. 2023; Yoon et al. 2012).

The bacterial taxa detected in newly hatched larvae (0 dph) may have been acquired via maternal provisioning through eggs or maternal tissues (hepatopancreas, reproductive tract and pleopods), establishing the first core bacteriome (Liu et al. 2021; Sun et al. 2025). Similar mechanisms have been proposed for larvae of the giant freshwater prawn *Macrobrachium rosenbergii* (Liu et al. 2021). In subsequent stages of larval development (>7 dph), gradually increasing digestive capacity, immunocompetence and repeated moulting that generate cycles of gut and cuticle restructuring (Ciaramella et al. 2014; Gorissen and Sandeman 2022; Lewis et al. 2024) create new niches and attachment sites for a range of bacterial taxa. For example, the transition from Stage I to II larvae at 7 dph is associated with elongation of the cephalic shield and eyestalk segmentation (Smith et al. 2009), making new surfaces available for rapid colonisers like members of the Roseobacteraceae (Dang and Lovell 2016) that were shown to dominate the community at this stage.

Between 14 and 56 dph, the indicator bacterial genera shifted from early, rapid colonisers to more specialised marine heterotrophs, including *Litoribacillus*, *Thalassotalea*, *Dokdonia* and *Malaciobacter*. Genus *Thalassotalea* includes marine heterotrophs that produce extracellular enzymes degrading proteins and polysaccharides, engage in dissolved organic carbon turnover and nutrient cycling, and interact with resident bacteria (Hou et al. 2015;

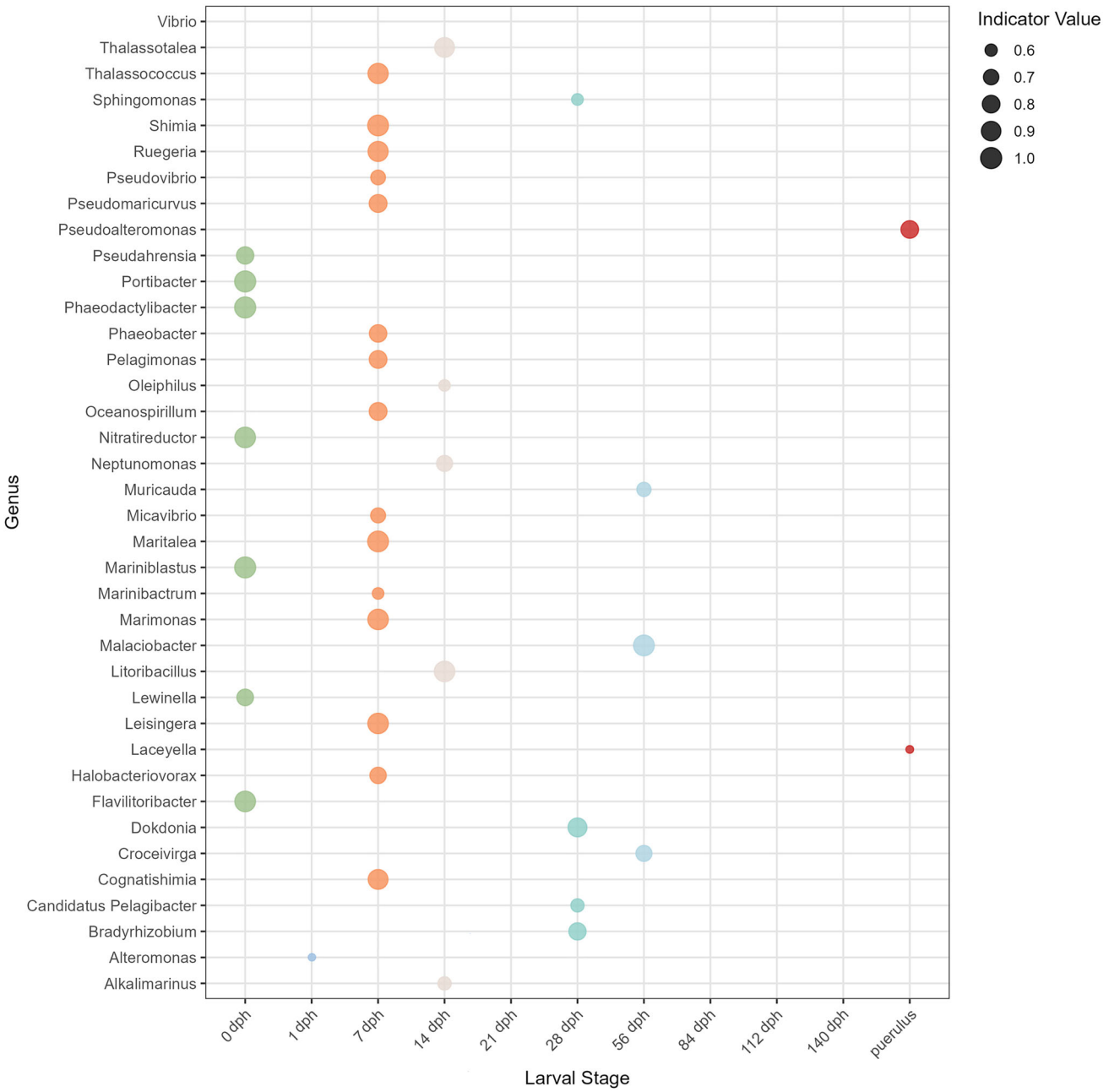

FIGURE 4 | Taxonomic bubble plot of bacterial relative abundance at the genus level present in larval *Panulirus ornatus* larvae (0 dph-puerulus). Each bubble represents a genus-stage association identified by indicator species analysis. Bubble size is proportional to the IndVal statistic, reflecting the strength of association of each genus with the corresponding larval stage. Bubble colour denotes the larval stage.

Kim et al. 2020; Lee et al. 2024). *Dokdonia* is a photoheterotrophic genus that combines peptide and protein degradation with light-driven energy capture, enabling persistence in nutrient-limited environments and functional stability during periods of low feeding (Bogachev et al. 2016; Gómez-Consarnau et al. 2016; González et al. 2011). *Malaciobacter*, frequently associated with shellfish and surrounding waters, contributes to biofilm formation and bacteria stability and also serves as an indicator of water quality in aquaculture systems (Rahman 2023; Salas-Massó et al. 2024). The presence of such indicator bacterial taxa associated with nutrient cycling at 14–56 dph may reflect increased availability of organic substrates, including proteins, polysaccharides and fatty acids (Genodepa et al. 2022; Johnston 2006), linked to expanding digestive capacity and dietary change during larval rearing. Moreover, the upregulation of cellular and humoral immune function through crustacean larval development (Angthong et al. 2021; Bowden 2017; Koiwai et al. 2021; Ren et al. 2022) may also shape bacterial community structure. Therefore, the 14–56 dph period could represent a window in which feeding, moulting and immune maturation interact to shape bacterial succession, establishing a more specialised and stable community that enhances digestion, nutrient assimilation and resistance to opportunistic pathogens. Between 84 and 140 dph, no new indicator bacteria were identified, except for the genus *Pseudoalteromonas*, which was detected in the puerulus stage and emerged as a dominant and functionally significant taxon. *Pseudoalteromonas* are widely

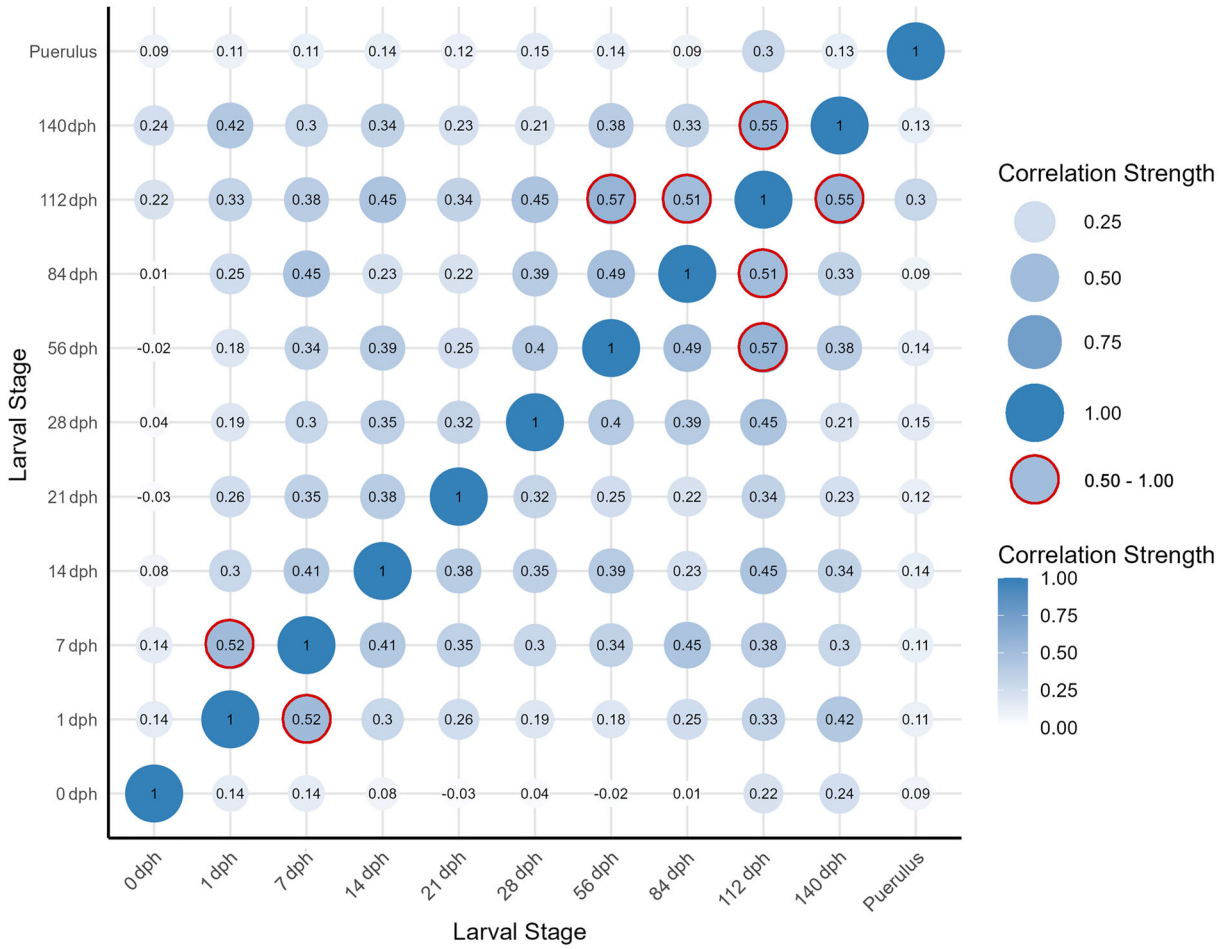

FIGURE 5 | Spearman correlation bubble plot of similarity in bacterial community composition across *Panulirus ornatus* larval stages (0 dph-puerulus).

distributed in marine environments and are commonly associated with marine host, where they can exhibit probiotic properties, including the production of bioactive compounds that inhibit pathogens, such as *Vibrio* (Newaj-Fyzul et al. 2014; Ringø 2020). Their ability to form biofilms and produce protective metabolites likely confers significant advantages for the host, particularly during the extensive morphological and energetic transitions at the puerulus stage (Amin et al. 2024; Ringø 2020; Zeng, Cai, et al. 2017). During this stage, larvae undergo substantial morphological changes, transitioning from phyllosoma to puerulus. These changes include increased body size, elongation of appendages, retraction of the digestive gland and preparation for benthic settlement, which together increase surface area for bacterial colonisation and elevate metabolic and energy demands (Smith et al. 2009). The high adaptability of *Pseudoalteromonas*, including stress tolerance conferred by pyomelanin production, may facilitate persistence under fluctuating environmental conditions (Zeng, Huang, et al. 2017). Members of this genus have also been documented to stimulate animal metamorphosis. For example, *Pseudoalteromonas luteoviolacea* produces a contractile injection system that forms metamorphosis-associated contractile structures (MACs), which deliver protein effectors and induce metamorphosis in the tubeworm *Hydroides elegans* (Huang et al. 2012; Shikuma et al. 2014). Other *Pseudoalteromonas* species can induce larval settlement through biofilm formation and stimulate metamorphosis in diverse marine invertebrates, including annelids (*Phragmatopoma californica*) and mussels (*Mytilus coruscus*) (Liang et al. 2018). Although this study provides no direct evidence that *Pseudoalteromonas* actively influences metamorphosis in *P. ornatus*, its consistent presence at the puerulus stage is notable and aligns with known bacteria-mediated larval transition phenomena. No significant indicator taxa were detected at 21, 84, 112 and 140 dph. This absence may reflect limited statistical power or lower sequencing depth at these stages rather than a true lack of stage-specific microbial associations. The increased relative abundance of *Vibrio* observed at 112–140 dph may reflect environmental influences that support colonisation by bacterial communities present in rearing water, feed, or tank-associated biofilms, in addition to potential host-associated processes (Nankervis and Jones 2022; Zoqratt et al. 2018). Previous studies have shown that larval bacteria in aquaculture systems are strongly shaped by concomitant environmental conditions and husbandry practices (Rajeev et al. 2021). Therefore, the elevated abundance of *Vibrio* at later larval stages may not necessarily indicate host selection alone but could also reflect changes in the rearing environment during larval development.

During the larval–puerulus transition, immune functions in crustaceans are reported to become increasingly developed, including

enhanced humoral and cellular responses (Bowden 2017; Ventura et al. 2015). The bacterial succession observed at the puerulus stage is therefore consistent with a period of physiological transition in which host–bacteria associations may become more stable, although direct links between immune maturation and bacteria structure were not examined in this study.

Under aquaculture conditions and with the introduction of feed, such as *Artemia*, a range of organic substrates for bacterial proliferation is provided (Xu et al. 2024). Waterborne bacteria, biofilms on tank surfaces and microbes associated with live feed can all colonise the host gut, particularly during early developmental stages when the immune system is immature (Lu et al. 2022; Paralika and Makridis 2025). Similar patterns have been observed in shrimp and fish larvae, where the composition of the rearing water strongly influences early bacterial communities, promoting the establishment of taxa, such as Vibrionaceae and Alteromonadaceae (Paralika and Makridis 2025). Meanwhile, environmental parameters, such as temperature, salinity, stocking density and feed composition, further modulate bacterial colonisation and succession, influencing community diversity and stability (Castejón et al. 2015; Freitas et al. 2021). These interactions underscore the dynamic interplay between environmental exposure and host development in driving stage-specific bacteria assembly, which ultimately supports nutrient assimilation, growth and disease resilience in cultured *P. ornatus* larvae.

Although this study provides new insights into the bacterial community of larval *P. ornatus*, some limitations should be considered. Functional metagenomic analyses and environmental bacteria sampling should be applied in future to further elucidate bacterial-host interactions, whereas an alternative to pre-amplification, which may have influenced relative abundance estimates, should be addressed. Additionally, the study is based on a single-hatchery system, which may have limited the broader interpretation of the findings. Despite these constraints, the results offer valuable baseline data and complement previous studies that incorporate environmental sources, providing a foundation for future investigations into bacterial–host interactions in larval rearing systems.

5 | Conclusion

This study provides the first comprehensive overview of bacterial dynamics during the larval development of *P. ornatus*. Bacterial alpha diversity was stable during larval development but decreased at the puerulus stage, indicating a gradual diversification and stabilisation of the bacterial community as larvae matured. Community composition shifted significantly across developmental stages, reflecting the combined influence of host physiological changes. Distinct indicator taxa were identified at different developmental stages, from *Alteromonas*, *Shimia* at 0–7 dph to *Thalassotalea, Dokdonia* and *Pseudoalteromonas* at the later to puerulus stage. This suggests potential functional roles in animal physiological changes and host–microbe interactions. In summary, these findings reveal a clear pattern of stage-associated bacterial structure that aligns with larval development, emphasising the integral role of bacterial assembly in supporting larval health and developmental progression in *P. ornatus* aquaculture.

Author Contributions

Gregory G. Smith: supervision, project administration. **Mengjia Jiang**: writing – original draft, methodology. **Ivona Mladineo**: writing – review and editing, supervision. **Evan F. Goulden**: supervision, writing – review and editing. **Andrew J. Trotter**: project administration, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and analysed during the study are available from the corresponding author on request.

References

Amin, M., H. Taha, L. Musdalifah, et al. 2024. "Structure and Diversity of Microbiomes Associated With the Gastrointestinal Tracts of Wild Spiny Lobsters and Profiling Their Potential Probiotic Properties Using eDNA Metabarcoding." *Fishes* 9: 264.

Anderson, M. J. 2006. "Distance-Based Tests for Homogeneity of Multivariate Dispersions." *Biometrics* 62: 245–253.

Angthong, P., S. Chaiyapechara, and W. Rungrassamee. 2023. "Shrimp Microbiome and Immune Development in the Early Life Stages." *Developmental & Comparative Immunology* 147: 104765.

Angthong, P., T. Uengwetwanit, S. Arayamethakorn, and W. Rungrassamee. 2021. "Transcriptomic Analysis of the Black Tiger Shrimp (Penaeus monodon) Reveals Insights Into Immune Development in Their Early Life Stages." *Scientific Reports* 11: 13881.

Attramadal, K. J., T. M. H. Truong, I. Bakke, J. Skjermo, Y. Olsen, and O. Vadstein. 2014. "RAS and Microbial Maturation as Tools for K-Selection of Microbial Communities Improve Survival in Cod Larvae." *Aquaculture* 432: 483–490.

Barret, L. 2019. "Spiny Lobster (*Panulirus* sp.) Life Cycle–A Review and Fisheries Management Implications." *Seychelles Research Journal* 1: 180–188.

Bateman, K., J. Munro, B. Uglow, H. J. Small, and G. Stentiford. 2012. "Susceptibility of Juvenile European Lobster *Homarus gammarus* to Shrimp Products Infected With High and Low Doses of White Spot Syndrome Virus." *Diseases of Aquatic Organisms* 100: 169–184.

Bogachev, A. V., Y. V. Bertsova, M. L. Verkhovskaya, M. D. Mamedov, and V. P. Skulachev. 2016. "Real-Time Kinetics of Electrogenic Na+ Transport by Rhodopsin From the Marine Flavobacterium *Dokdonia* sp. PRO95." *Scientific Reports* 6: 21397.

Bourne, D., L. Høj, N. Webster, et al. 2007. "Microbiological Aspects of Phyllosoma Rearing of the Ornate Rock Lobster *Panulirus ornatus*." *Aquaculture* 268: 274–287.

Bowden, T. J. 2017. "The Humoral Immune Systems of the American Lobster (*Homarus americanus*) and the European Lobster (*Homarus gammarus*)." *Fisheries Research* 186: 367–371.

Brunson, J. C., and Q. D. Read. 2020. *ggalluvial: Alluvial Plots in'ggplot2'*. R Package Version 0.12. Github.

Buchan, A., J. M. González, and M. A. Moran. 2005. "Overview of the Marine Roseobacter Lineage." *Applied and Environmental Microbiology* 71: 5665–5677.

Castejón, D., G. Rotllant, L. Giménez, G. Torres, and G. Guerao. 2015. "The Effects of Temperature and Salinity on the Survival, Growth and Duration of the Larval Development of the Common Spider Crab *Maja brachydactyla* (Balss, 1922) (Brachyura: Majidae)." *Journal of Shellfish Research* 34: 1073–1083.

Ciaramella, M. A., A. L. Battison, and B. Horney. 2014. "Measurement of Tissue Lipid Reserves in the American Lobster (*Homarus americanus*): Hemolymph Metabolites as Potential Biomarkers of Nutritional Status." *Journal of Crustacean Biology* 34: 629–638.

Curry, K. D., Q. Wang, M. G. Nute, et al. 2022. "Emu: Species-level Microbial Community Profiling of Full-length 16S rRNA Oxford Nanopore Sequencing Data." *Nature Method* 19: 845–853.

Dang, H., and C. R. Lovell. 2016. "Microbial Surface Colonization and Biofilm Development in Marine Environments." *Microbiology and Molecular Biology Reviews* 80: 91–138.

De Caceres, M., F. Jansen, and N. Dell. 2016. *Package 'Indicspecies'. Relationship Between Species and Groups of Sites*. R Package Version (1.7.6).

De Coster, W., S. D'hert, D. T. Schultz, M. Cruts, and C. Van Broeckhoven. 2018. "NanoPack: Visualizing and Processing Long-read Sequencing Data." *Bioinformatics* 34: 2666–2669.

Egan, S., T. Harder, C. Burke, P. Steinberg, S. Kjelleberg, and T. Thomas. 2013. "The Seaweed Holobiont: Understanding Seaweed–Bacteria Interactions." *FEMS Microbiology Reviews* 37: 462–476.

FAO. 2024. "The State of Food and Agriculture 2024." In *Value-Driven Transformation of Agrifood Systems*. Food and Agriculture Organization of the United Nations (FAO).

Freitas, E., L. Hayd, and R. Calado. 2021. "Effects of Salinity, Stocking Density and Feeding in *Macrobrachium pantanalense* Larviculture." *Aquaculture Reports* 20: 100706.

Gómez-Consarnau, L., J. M. González, T. Riedel, et al. 2016. "Proteorhodopsin Light-Enhanced Growth Linked to Vitamin-B1 Acquisition in Marine Flavobacteria." *ISME Journal* 10: 1102–1112.

Genodepa, J., C. Zeng, T. A. Militz, and P. C. Southgate. 2022. "Ontogenetic Variation in Digestive Enzyme Activities Within Embryos and Newly-hatched Larvae of the Tropical Spiny Lobster." *Panulirus ornatus Aquaculture* 548: 737595.

González, J. M., J. Pinhassi, B. Fernández-Gómez, et al. 2011. "Genomics of the Proteorhodopsin-Containing Marine Flavobacterium *Dokdonia* sp. Strain MED134." *Applied and Environmental Microbiology* 77: 8676–8686.

Gorissen, S., and D. Sandeman. 2022. "Moult Cycle Staging in Decapod Crustaceans (Pleocyemata) and the Australian Crayfish, *Cherax destructor* Clark, 1936 (Decapoda, Parastacidae)." *Crustaceana* 95: 165–217.

Goto, S., Y. Tada, K. Suzuki, and Y. Yamashita. 2017. "Production and Reutilization of Fluorescent Dissolved Organic Matter by a Marine Bacterial Strain, *Alteromonas macleodii*." *Frontiers in Microbiology* 8: 507.

Goulden, E. F., M. R. Hall, D. G. Bourne, L. L. Pereg, and L. Høj. 2012. "Pathogenicity and Infection Cycle of *Vibrio owensii* in Larviculture of the Ornate Spiny Lobster (*Panulirus ornatus)*." *Applied and Environmental Microbiology* 78: 2841–2849.

Goulden, E. F., M. R. Hall, L. L. Pereg, and L. Høj. 2012. "Identification of an Antagonistic Probiotic Combination Protecting Ornate Spiny Lobster (*Panulirus ornatus*) Larvae Against *Vibrio owensii* Infection." *PLoS ONE* 7: e39667.

Guerao, G., D. Díaz, and P. Abelló. 2006. "Morphology of Puerulus and Early Juvenile Stages of the Spiny Lobster *Palinurus mauritanicus* (Decapoda: Palinuridae)." *Journal of Crustacean Biology* 26: 480–494.

Henríquez-Castillo, C., A. M. Plominsky, S. Ramírez-Flandes, A. D. Bertagnolli, F. J. Stewart, and O. Ulloa. 2022. "Metaomics Unveils the Contribution of Alteromonas Bacteria to Carbon Cycling in Marine Oxygen Minimum Zones." *Frontiers in Marine Science* 9: 993667.

Hornung, B. V., R. D. Zwittink, and E. J. Kuijper. 2019. "Issues and Current Standards of Controls in Microbiome Research." *FEMS Microbiology Ecology* 95: fiz045.

Hou, T.-T., Y. Liu, Z.-P. Zhong, H.-C. Liu, and Z.-P. Liu. 2015. "*Thalassotalea marina* sp. nov., Isolated From a Marine Recirculating Aquaculture System, Reclassification of *Thalassomonas eurytherma* as *Thalassotalea eurytherma* Comb. nov. and Emended Description of the Genus *Thalassotalea*." *International Journal of Systematic and Evolutionary Microbiology* 65: 4710–4715.

Huang, Y., S. Callahan, and M. G. Hadfield. 2012. "Recruitment in the Sea: Bacterial Genes Required for Inducing Larval Settlement in a Polychaete Worm." *Scientific Reports* 2: 228.

Hyun, D.-W., M.-S. Kim, N.-R. Shin, et al. 2013. "*Shimia haliotis* sp. nov., a Bacterium Isolated From the Gut of an Abalone, *Haliotis discus hannai*." *International Journal of Systematic and Evolutionary Microbiology* 63: 4248–4253.

Ivars-Martinez, E., A.-B. Martin-Cuadrado, G. D'auria, et al. 2008. "Comparative Genomics of Two Ecotypes of the Marine Planktonic Copiotroph Alteromonas Macleodii Suggests Alternative Lifestyles Associated With Different Kinds of Particulate Organic Matter." *ISME Journal* 2: 1194–1212.

Johnston, M. D. 2006. Feeding and Digestion in the Phyllosoma Larvae of Ornate Spiny Lobster, Panulirus ornatus and the Implications for their Culture. U.o.W. Australia.

Jones, C. 2015a. "Spiny Lobster Aquaculture Development in Indonesia, Vietnam and Australia." In *Proceedings of the International Lobster Aquaculture Symposium, Lombok, Indonesia, 22–25 April 2014. ACIAR Proceedings Series*. Australian Centre for International Agricultural Research (ACIAR).

Jones, C. 2015b. "Summary of Disease Status Affecting Tropical Spiny Lobster Aquaculture in Vietnam and Indonesia." In *Proceedings of the International Lobster Aquaculture Symposium, Lombok, Indonesia, 22–25 April 2014. ACIAR Proceedings Series*. Australian Centre for International Agricultural Research (ACIAR).

Kim, M., I.-T. Cha, K.-E. Lee, E.-Y. Lee, and S.-J. Park. 2020. "Genomics Reveals the Metabolic Potential and Functions in the Redistribution of Dissolved Organic Matter in Marine Environments of the Genus *Thalassotalea*." *Microorganisms* 8: 1412.

Koiwai, K., T. Koyama, S. Tsuda, et al. 2021. "Single-cell RNA-seq Analysis Reveals Penaeid Shrimp Hemocyte Subpopulations and Cell Differentiation Process." *Elife* 10: e66954.

Lage, O. M., L. Albuquerque, A. Lobo-da Cunha, and M. S. da Costa. 2017. "*Mariniblastus fucicola* gen. nov., sp. nov. a Novel Planctomycete Associated With Macroalgae." *International Journal of Systematic and Evolutionary Microbiology* 67: 1571–1576.

Lee, J., S.-H. Song, K. Moon, et al. 2024. "*Thalassotalea aquiviva* sp. nov., and *Thalassotalea maritima* sp. nov., Isolated From Seawater of the Coast in South Korea." *Journal of Microbiology* 62: 1099–1111.

Lewis, C. L., S. Glendinning, B. M. Codabaccus, et al. 2024. "Changes in the Physiology and Chitin Metabolism in Cultured Juvenile *Panulirus ornatus* Across the Moult Cycle." *Aquaculture Reports* 38: 102308.

Li, X., L. Zhou, Y. Yu, J. Ni, W. Xu, and Q. Yan. 2017. "Composition of Gut Microbiota in the Gibel Carp (*Carassius auratus gibelio*) Varies With Host Development." *Microbial Ecology* 74: 239–249.

Liang, X., Y.-R. Chen, W. Gao, et al. 2018. "Effects on Larval Metamorphosis in the Mussel *Mytilus coruscus* of Compounds That Act on Downstream Effectors of G-Protein-Coupled Receptors." *Journal of the Marine Biological Association of the United Kingdom* 98: 333–339.

Liu, B., C. Song, Q. Gao, et al. 2021. "Maternal and Environmental Microbes Dominate Offspring Microbial Colonization in the Giant Freshwater Prawn *Macrobrachium rosenbergii*." *Science of the Total Environment* 790: 148062.

Lu, Z., Z. Ren, W. Lin, et al. 2022. "Succession, Sources, and Assembly of Bacterial Community in the Developing Crab Larval Microbiome." *Aquaculture* 548: 737600.

Martin, M., D. Portetelle, G. Michel, and M. Vandenbol. 2014. "Microorganisms Living on Macroalgae: Diversity, Interactions, and Biotechnological Applications." *Applied Microbiology and Biotechnology* 98: 2917–2935.

Matsuo, Y. 2023. "Full-Length 16S rRNA Gene Analysis Using Long-Read Nanopore Sequencing for Rapid Identification of Bacteria From Clinical Specimens." In *Nanopore Sequencing: Methods and Protocols*. Springer.

Nankervis, L., and C. Jones. 2022. "Recent Advances and Future Directions in Practical Diet Formulation and Adoption in Tropical Palinurid Lobster Aquaculture." *Reviews in Aquaculture* 14: 1830–1842.

Newaj-Fyzul, A., A. Al-Harbi, and B. Austin. 2014. "Developments in the Use of Probiotics for Disease Control in Aquaculture." *Aquaculture* 431: 1–11.

Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2025. *Vegan: Community Ecology Package*. https://github.com/vegandevs/vegan.

Ooi, M. C., E. F. Goulden, G. G. Smith, B. F. Nowak, and A. R. Bridle. 2017. "Developmental and Gut-Related Changes to Microbiomes of the Cultured Juvenile Spiny Lobster *Panulirus ornatus*." *FEMS Microbiology Ecology* 93: fix159.

Ooi, M. C., E. F. Goulden, A. J. Trotter, G. G. Smith, and A. R. Bridle. 2020. "*Aquimarina* sp. Associated With a Cuticular Disease of Cultured Larval Palinurid and Scyllarid Lobsters." *Frontiers in Microbiology* 11: 573588.

Ooi, M. C., A. J. Trotter, G. G. Smith, and A. R. Bridle. 2023. "Characterisation of the Gut Bacteria of Cultured and Wild Spiny Lobster." *Applied Microbiology* 3: 241–253.

Paralika, V., and P. Makridis. 2025. "Microbial Interactions in Rearing Systems for Marine Fish Larvae." *Microorganisms* 13: 539.

Payne, M. S., M. R. Hall, L. Sly, and D. G. Bourne. 2007. "Microbial Diversity Within Early-Stage Cultured *Panulirus ornatus* Phyllosomas." *Applied and Environmental Microbiology* 73: 1940–1951.

Pedler, B. E., L. I. Aluwihare, and F. Azam. 2014. "Single Bacterial Strain Capable of Significant Contribution to Carbon Cycling in the Surface Ocean." *Proceedings of the National Academy of Sciences* 111: 7202–7207.

Peng, S., L. Ye, Y. Li, et al. 2023. "Microbiota Regulates Life-Cycle Transition and Nematocyte Dynamics in Jellyfish." *Iscience* 26: 108444.

Perera, E., and C. Simon. 2015. "Digestive Physiology of Spiny Lobsters: Implications for Formulated Diet Development." *Reviews in Aquaculture* 7: 243–261.

Podani, J., and B. Csányi. 2010. "Detecting Indicator Species: Some Extensions of the IndVal Measure." *Ecological Indicators* 10: 1119–1124.

Porsby, C. H., K. F. Nielsen, and L. Gram. 2008. "Phaeobacter and Ruegeria Species of the Roseobacter Clade Colonize Separate Niches in a Danish Turbot (*Scophthalmus maximus*)-Rearing Farm and Antagonize *Vibrio anguillarum* Under Different Growth Conditions." *Applied and Environmental Microbiology* 74: 7356–7364.

Rahman, F. U. 2023. "Potential Impact of Emerging Bacterial Pathogens on Shellfish Production." Doctoral thesis, University of Rovira i Vigili.

Rajeev, R., K. Adithya, G. S. Kiran, and J. Selvin. 2021. "Healthy Microbiome: A Key to Successful and Sustainable Shrimp Aquaculture." *Reviews in Aquaculture* 13: 238–258.

Ren, X., S. Jia, B. Gao, et al. 2022. "Application of Proteomics and Metabolomics to Assess Ammonia Stress Response and Tolerance Mechanisms of Juvenile Ornate Rock Lobster Panulirus Ornatus." *Science of The Total Environment* 837: 155751.

Ringø, E. 2020. "Probiotics in Shellfish Aquaculture." *Aquaculture and Fisheries* 5: 1–27.

Salas-Massó, N., A. Fernández-Bravo, E. Bertomeu, K. B. Andree, M. J. Figueras, and D. Furones. 2024. "Depuration of *Aliarcobacter butzleri* and *Malaciobacter molluscorum* in Comparison With *Escherichia coli* in Mussels (*Mytilus galloprovincialis*) and Oysters (*Crassostrea gigas*)." *Pathogens* 13: 973.

Saqib, H. S. A., Y. Yuan, S. S. U. H. Kazmi, et al. 2023. "Salinity Gradients Drove the Gut and Stomach Microbial Assemblages of Mud Crabs (*Scylla paramamosain*) in Marine Environments." *Ecological Indicators* 151: 110315.

Shields, J. 2011. "Diseases of Spiny Lobsters: A Review." *Journal of Invertebrate Pathology* 106: 79–91.

Shikuma, N. J., M. Pilhofer, G. L. Weiss, M. G. Hadfield, G. J. Jensen, and D. K. Newman. 2014. "Marine Tubeworm Metamorphosis Induced by Arrays of Bacterial Phage Tail–Like Structures." *Science* 343: 529–533.

Smith, G., M. Salmon, M. Kenway, and M. Hall. 2009. "Description of the Larval Morphology of Captive Reared *Panulirus ornatus* Spiny Lobsters, Benchmarked Against Wild-Caught Specimens." *Aquaculture* 295: 76–88.

Sun, Y., W. Lin, Q. Wu, C. Shi, C. Wang, and Y. Ye. 2025. "Bacterial Dynamics and Biotic Sources in the Developing Swimming Crab Embryos." *Aquaculture* 595: 741523.

Thompson, F. L., T. Iida, and J. Swings. 2004. "Biodiversity of Vibrios." *Microbiology and Molecular Biology Reviews* 68: 403–431.

Tzeng, T.-D., Y.-Y. Pao, P.-C. Chen, F. C.-H. Weng, W. D. Jean, and D. Wang. 2015. "Effects of Host Phylogeny and Habitats on Gut Microbiomes of Oriental River Prawn (*Macrobrachium nipponense*)." *PLoS ONE* 10: e0132860.

Ventura, T., Q. P. Fitzgibbon, S. C. Battaglene, and A. Elizur. 2015. "Redefining Metamorphosis in Spiny Lobsters: Molecular Analysis of the Phyllosoma to Puerulus Transition in *Sagmariasus verreauxi*." *Scientific Reports* 5: 13537.

Wang, Y., K. Wang, L. Huang, et al. 2020. "Fine-Scale Succession Patterns and Assembly Mechanisms of Bacterial Community of *Litopenaeus vannamei* Larvae Across the Developmental Cycle." *Microbiome* 8: 1–16.

Wei, T., and V. R Simko. 2021. *"Corrplot": Visualization of a Correlation Matrix*. R Package Version Version 0.92.

Wietz, M., M. Mansson, C. H. Gotfredsen, T. O. Larsen, and L. Gram. 2010. "Antibacterial Compounds From Marine *Vibrionaceae* Isolated on a Global Expedition." *Marine Drugs* 8: 2946–2960.

Williamson, E. M., T. J. Hammer, K. Hogendoorn, and R. Eisenhofer. 2025. "Blanking on Blanks: Few Insect Microbiota Studies Control for Contaminants." *MBio* 16: e02658–02624.

Wong, L. L., Z. M. Deris, M. Asaduzzaman, et al. 2023. "Gut Microbiome Variation Based on 16s rRNA Gene Amplicon Sequencing of the Wild and Domesticated Broodstock Populations of Black Tiger Shrimp (*Penaeus monodon*) in the Indo-Pacific Region." *Ecological Genetics and Genomics* 29: 100204.

Wu, X., G. Smith, and M. Hall. 2012. "Patterns of Larval Growth, Lipid Composition and Fatty Acid Deposition During Early to Mid Stages of Development in *Panulirus ornatus* Phyllosoma." *Aquaculture* 330: 63–73.

Xiong, J., K. Wang, J. Wu, et al. 2015. "Changes in Intestinal Bacterial Communities are Closely Associated With Shrimp Disease Severity." *Applied Microbiology and Biotechnology* 99: 6911–6919.

Xiong, J., J. Zhu, W. Dai, C. Dong, Q. Qiu, and C. Li. 2017. "Integrating Gut Microbiota Immaturity and Disease-Discriminatory Taxa to Diagnose the Initiation and Severity of Shrimp Disease." *Environmental Microbiology* 19: 1490–1501.

Xu, Y., D. Hu, H. Cheng, F. Peng, and Q. Gao. 2024. "Research on the Artemia Nauplii Microorganism Compositions Based on Metagenomics Next-Generation Sequencing." *New Microbiologica* 46: 332–339.

Yan, Q., C. J. van der Gast, and Y. Yu. 2012. "Bacterial Community Assembly and Turnover Within the Intestines of Developing Zebrafish." *PLoS ONE* 7: e30603.

Yoon, J., Y. Matsuo, H. Kasai, and A. Yokota. 2012. "*Portibacter lacus* gen. nov., sp. nov., a New Member of the Family *Saprospiraceae* Isolated From a Saline Lake." *Journal of General and Applied Microbiology* 58: 191–197.

Zeng, S., Z. Huang, D. Hou, J. Liu, S. Weng, and J. He. 2017. "Composition, Diversity and Function of Intestinal Microbiota in Pacific White Shrimp (*Litopenaeus vannamei*) at Different Culture Stages." *PeerJ* 5: e3986.

Zeng, Z., X. Cai, P. Wang, et al. 2017. "Biofilm Formation and Heat Stress Induce Pyomelanin Production in Deep-Sea *Pseudoalteromonas* sp. SM9913." *Frontiers in Microbiology* 8: 1822.

Zhang, M., X. Zhang, N. T. Tran, et al. 2021. "Molting Alters the Microbiome, Immune Response, and Digestive Enzyme Activity in Mud Crab (*Scylla paramamosain*)." *Msystems* 6: e0091721.

Zhang, S., and X. Sun. 2022. "Core Gut Microbiota of Shrimp Function as a Regulator to Maintain Immune Homeostasis in Response to WSSV Infection." *Microbiology Spectrum* 10: e02465–02421.

Zoqratt, M. Z. H. M., W. W. H. Eng, B. T. Thai, C. M. Austin, and H. M. Gan. 2018. "Microbiome Analysis of Pacific White Shrimp Gut and Rearing Water From Malaysia and Vietnam: Implications for Aquaculture Research and Management." *PeerJ* 6: e5826.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Table: aff270271-sup-0001-TableS1.csv **Supplementary Table**: aff270271-sup-0002-TableS2.csv **Supplementary Table**: aff270271-sup-0003-TableS3.xlsx **Supplementary Table**: aff270271-sup-0004-TableS4.xlsx **Supplementary Table**: aff270271-sup-0005-TableS5.csv **Supplementary Table**: aff270271-sup-0006-TableS6.csv **Supplementary Table**: aff270271-sup-0007-TableS7.csv **Supplementary Table**: aff270271-sup-0008-TableS8.xlsx