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Bacteriomes associated with white-leg disease in tropical rock lobster (*Panulirus ornatus*) hatcheries

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Abstract

White-leg disease (WLD) is an emerging condition affecting tropical rock lobster, *Panulirus ornatus*, larviculture, characterized by whitening of pereiopods and uropods, followed by rapid mortality. Despite its significant impact on aquaculture productivity, the bacterial drivers and environmental sources of WLD are still poorly understood. This study characterizes the bacteriomes associated with *P. ornatus* larvae and their culture environment across two hatcheries: Ornatas Pty Ltd. in Townsville, Queensland (a commercial hatchery), and the Institute for Marine and Antarctic Studies (IMAS) in Hobart, Tasmania (a research hatchery) to identify bacterial composition and potential sources of pathogens. Using Oxford Nanopore 16s rRNA gene sequencing, we profiled bacterial communities from healthy and diseased phyllosoma, as well as environmental compartments including water, biofilm, fresh and aged feed: Feed 6 h old and feed 20 h old. Diseased larvae across both hatcheries

showed distinct bacterial shifts, with elevated relative abundance of *Aquimarina* spp. and *Vibrio* spp. taxa detected by Nanopore 16S sequencing. Notably, these taxa were also detected in the rearing environment, particularly in fresh feed and biofilm, which suggests possible transmission pathways. The detection of overlapping bacterial communities between larvae and their environment supports the hypothesis that environmental parameters play a critical role in WLD onset and progression. These findings provide new insights into the bacterial ecology of WLD, identifying potential causative agents and their environmental sources. Moreover, this study highlights the importance of integrated microbiome surveillance across the rearing system and informs the future development of microbiome-based health monitoring and disease mitigation strategies in *P. ornatus* hatcheries.

KEYWORDS
bacterial community, phyllosoma, tropical rock lobster, white-leg disease

1 | INTRODUCTION

The global aquaculture industry has experienced significant growth over the past three decades, with its contribution to the global seafood supply increasing from 26% in 2000 to 51% in 2022 (FAO, 2024; Tuynman et al., 2024). Among the high value species cultivated, the tropical rock lobster, *Panulirus ornatus*, is highly prized as a premium seafood product in Australia and is exported globally (Plagányi et al., 2023; Smallwood et al., 2024). In Southeast Asian countries, such as Vietnam and Indonesia, lobster aquaculture predominantly relies on wild-caught seedstock (Jones, 2015a), raising sustainability concerns and complicating long-term disease management (Jones, 2015a, 2015b). Rising global demand for *P. ornatus* has driven the rapid innovation of aquaculture systems, particularly in Australia, to improve production efficiency, larval health, and rearing conditions. However, despite the advancement in onshore lobster farming, the sustainability of the industry continues to face challenges, particularly from emerging bacterial diseases.

One of the most persistent health concerns in *P. ornatus* larviculture is white leg disease (WLD), a recently recognized condition characterized by visible discoloration of the appendages, pereiopods, and antennal gland and associated with reduced larval survival and high mortality (Ooi et al., 2020). The disease has been associated with the Gram-negative bacterium *Aquimarina hainanensis*, a link confirmed through experimental infection studies that also revealed a range of genomic traits implicated in its pathogenic potential (Ooi et al., 2020). Infected phyllosoma often exhibit extensive bacterial colonization of their pereiopods, leading to structural degradation, septicaemia, and mortality.

Within aquaculture settings, the environmental bacterial communities have been shown to correlate with larval microbial succession and health outcomes (Chen et al., 2022). For example, in the gazami crab, *Portunus trituberculatus*, larval-associated bacteria closely resembled the bacterioplankton found in rearing water and diet (Lu et al., 2022). Similarly, in whiteleg shrimp, *Litopenaeus vannamei*, taxa from the family Rhodobacteraceae present in the rearing water have been positively associated with the zoea stage, showing persistent dominance and

contributing to the animal's metabolic potential for organic matter utilization (Wang et al., 2020). Shifts in bacterial communities are often associated with disease outbreaks in aquaculture systems. For example, *Tenacibaculum maritimum*, a bacterium commonly detected in rearing water, has been linked to body lesions and necrosis in farmed turbot, *Scophthalmus maximus*, and Senegalese sole, *Solea senegalensis*, highlighting the importance of understanding environmental bacteria in rearing systems and their interactions with the host (Fernández-Álvarez et al., 2019; Xiao et al., 2021; Xue et al., 2017; Yan et al., 2016).

While recent studies have begun to characterize the gut bacteria of *P. ornatus* (Ooi et al., 2017, 2019), there remains limited understanding of how environmental microbial communities shape the larval bacteriome and contribute to disease processes such as WLD. Investigating these host–environment–bacteria interactions is essential for developing sustainable and health-focused aquaculture practices capable of mitigating disease impacts (Clols-Fuentes et al., 2024). It is important to consider that the bacterial community in an aquaculture system is shaped by both intrinsic factors, such as host genetics, developmental stage, immune competence, and physiology, and extrinsic factors, including water source, diet, temperature, and salinity (Bruno et al., 2023; Chen et al., 2022). While intrinsic factors are largely fixed, extrinsic factors are more easily managed; however, even subtle environmental changes could induce marked shifts in microbial community structure.

In spiny lobster hatchery systems, exposure to bacteria from the rearing environment, particularly those associated with water, biofilm, and feed, can shape the larval microbiome, influencing whether environmental taxa establish as commensals or potential pathogens. Similar patterns have been reported in atlantic cod, *Gadus morhua* larvae, where rearing water, microalgae, and live feed contributed to larval microbial colonization and influenced the establishment of opportunistic taxa (Khadka et al., 2025). Understanding how environmental bacteria influence host-associated microbiota is therefore critical to elucidating disease pathogenesis and timely application of aquaculture health management procedures (Clols-Fuentes et al., 2024).

Environmental conditions are likely to strongly influence bacterial community composition, which in turn affects disease expression in cultured *P. ornatus* larvae. This study examines the bacteriomes associated with the occurrence of WLD in two hatchery systems of *P. ornatus*: Ornatas Pty Ltd. in Townsville, Queensland (a commercial hatchery), and the Institute for Marine and Antarctic Studies (IMAS) in Hobart, Tasmania (a research hatchery). These hatcheries differ in their geographic locations, water source, and genetic background of their cultured animals. Operational variations also result in distinct water quality parameters, all of which can influence phyllosoma quality, and the composition of both environmental and host-associated bacterial communities. By comparing bacteriomes of healthy and diseased phyllosoma, as well as those of the potential environmental factors, including water, feed and biofilms, across the two hatcheries, this study aims to identify bacterial signatures associated with WLD. Furthermore, it assesses how environmental factors contribute to bacterial community structure, and how these, in turn, may influence disease development in *P. ornatus* larvae.

This comparative approach, analyzing bacterial shifts linked to phyllosoma health status, provides insights into hatchery-specific microbial associations with disease expression. These insights can inform targeted and more effective disease management strategies in lobster hatchery systems.

2 | MATERIALS AND METHODS

2.1 | Sample collection

2.1.1 | Animal samples

We collected a total of six symptomatic animals showing clinical signs of WLD, and six asymptomatic animals, identified as the "healthy" group from each hatchery (Figure 1). The larvae were maintained in a low-exchange recirculating aquaculture system (RAS) at both hatcheries. Observations of phyllosoma morphology and

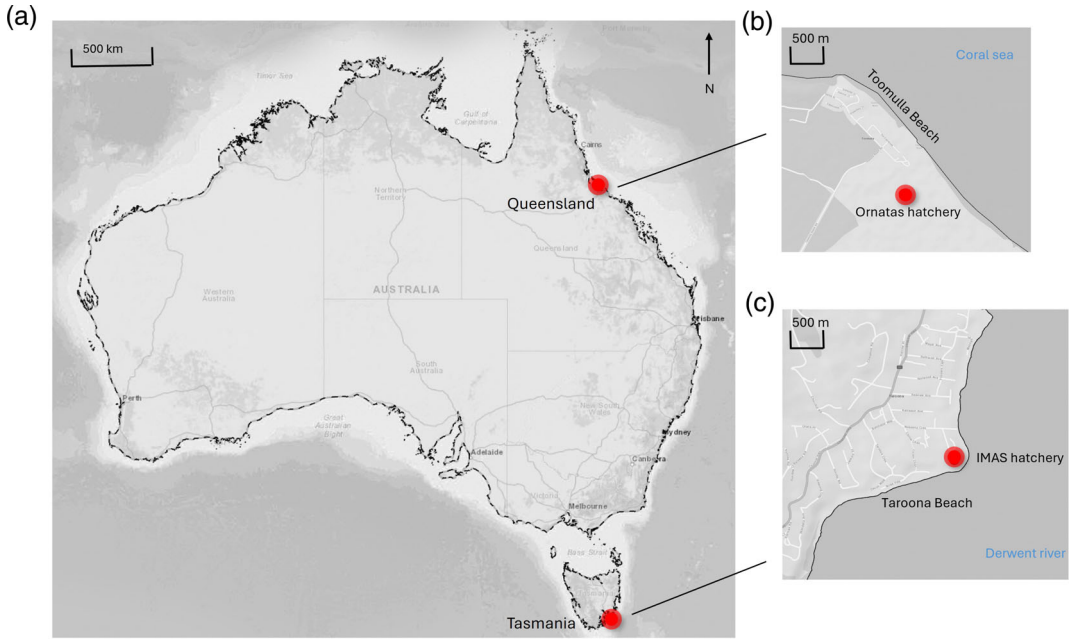

FIGURE 1 (a) Geographic location of study hatcheries marked by red dots. (b) Ornatas Pty Ltd. hatchery Toomulla, Queensland, Australia and (c) Institute for Marine and Antarctic Studies (IMAS) hatchery, Taroona, Tasmania, Australia.

development were conducted following established methods (Smith et al., 2009). Each phyllosoma and associated environmental samples (in Sections 2.1.2 and 2.1.3 feed and environmental sample collection) were collected on a single day from each hatchery. Sampling occurred when clinical signs of WLD were observed in diseased individuals, ensuring the inclusion of both healthy and WLD-affected phyllosoma during the same day. 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). Then the whole animal was transferred into 500 μL storage buffer (25 mM sodium citrate, 10 mM EDTA, 4 M ammonium sulfate, pH 5.2) and stored at 4°C overnight. The water sources for the Ornatas Pty Ltd. and IMAS hatcheries were Saltwater Creek and the Derwent River, respectively. Both water sources were treated with ozone to achieve sterilization prior to introduction into the respective hatcheries. The details of the water quality parameters routinely collected in two hatchery environments are listed in Table 1, a WTW (Xylem Analytics brand) water quality meter and associated probes were used to measure dissolved oxygen (DO), salinity, temperature and pH. Oxidation–reduction potential (ORP) was measured through the use of both American Marine Pinpoint ORP monitor and DolpHin Series Foxboro probes. The data are collected daily from two hatcheries.

2.1.2 | Feed samples

Phyllosoma were fed the same proprietary diet incorporating live *Artemia* (INVE Sep-art GSL *Artemia* cysts, BMAQUA, Australia) at both hatchery locations. The feeds were made independently at each hatchery. All feed samples were collected in triplicate and consisted of: (1) fresh feed collected from the feed room within an hour of production, (2) uneaten feed that had soaked in tank water for 6 h, and (3) uneaten feed that had turned discolored (white) after 20 h in rearing tank water. These feed samples were representative of

TABLE 1 Water quality parameters and environmental colony count across two tropical rock lobster, *Panulirus ornatus*, hatchery locations: Institute for Marine and Antarctic Studies (IMAS) and Ornatas Pty Ltd.

Parameter	Temperature (°C)		pH		Sat O_2 (%)		ORP (mv)		Bromate (ppb)		ZMA (CFU)		TCBS (CFU)	
Location	Ornatas	IMAS	Ornatas	IMAS	Ornatas	IMAS	Ornatas	IMAS	Ornatas	IMAS	Ornatas	IMAS	Ornatas	IMAS
Week 1	27.77	28.04	8.21	8.24	120.10	104.02	386.86	358.93	292	118	61	31	4	3
Week 2	27.87	28.06	8.20	8.25	119.40	100.94	401.07	333.77	267	240	73	47	1	10
Week 3	27.87	28.02	8.20	8.27	118.90	101.09	366.36	331.78	247	106	136	37	77	1
Week 4	27.87	28.14	8.21	8.25	119.00	106.74	370.00	334.05	190	105	266	15	104	4
Week 5	27.84	28.13	8.22	8.23	120.80	102.81	382.07	349.63	170	160	105	59	55	4
Week 6	27.65	28.11	8.27	8.23	116.66	105.23	390.64	338.07	189	227	205	115	36	12
Week 7	27.72	28.00	8.19	8.28	117.07	105.02	418.57	331.71	165	195	99	370	51	219
Week 8	28.01	27.98	8.21	8.25	120.68	103.33	404.02	328.79	171	161	127	190	55	80
Week 9	28.1	27.90	8.20	8.25	120.57	101.44	369.57	348.65	169	103	104	307	5	117
Week 10	28.05	27.97	8.22	8.22	119.59	101.41	370.85	348.14	138	45	85	187	44	68
Week 11	28.01	28.07	8.22	8.23	118.07	104.39	389.78	348.68	136	78	67	193	4	117
Week 12	27.21	27.9	8.21	8.24	120.15	104.69	386.79	346.64	231	38	127	346	83	124
Week 13	28.06	27.93	8.22	8.22	122.31	105.21	390.25	346.94	263	152	23	682	3	263
p-Value	NS		NS		<0.05		<0.05		<0.05		NS		NS	
Mean	27.85	28.02	8.21	8.24	**119.48**	103.56	**386.62**	341.98	**202**	133	113.69	198.38	40.15	78.62
SD	0.24	0.08	0.02	0.02	1.57	1.88	15.31	9.35	51.74	63.53	63.53	190.21	34.91	87.01
SE	0.02	0.01	0	0	0.12	0.14	1.18	0.72	3.98	4.89	4.89	14.63	2.69	6.69

Note: Significant values are highlighted in bold. $p < 0.05$ as significant difference, highlighted in bold.
Abbreviations: NS, not significant; ORP, oxidation–reduction potential; TCBS, thiosulfate citrate bile salts sucrose agar; ZMA, Zobell Marine Agar.

feed particles to which the lobster phyllosoma were exposed in the culture systems. All feed samples were collected from the same culture tanks from which the phyllosoma were collected. A total of 0.02 g of the feed was collected for each triplicate sample. The fresh feed was collected directly from the feed container and transferred into 500-μL storage buffer (25 mM sodium citrate, 10 mM EDTA, 4 M ammonium sulfate, pH 5.2) using a pipette tip, then stored at 4°C overnight. The aged feed samples (6 and 20 h old) were collected using a sterile Pasteur pipette directly from the culture tank and transferred into a 2-mL tube with 500-μL storage buffer. The tube was centrifuged (12,000 × *g*) to concentrate the feed pellet and remove the residual water. A 0.02 g portion of the pellet was resuspended in 500 μL of storage buffer and stored at 4°C.

2.1.3 | Rearing environment samples

All environmental samples (tank water and biofilm from the tank surface interface and wall) were collected in triplicate for each sample type, and each replicate was stored in 500 μL of storage buffer. Biofilms were sampled by swabbing a 10 cm^2 area, both off the tank walls and at the water–air interface. All swab samples were stored in storage buffer (4°C) until further processing. Tank water samples were collected into 1-L sterile glass bottles and stored on ice, then transferred to the laboratory for immediate filtration. Water samples were filtered through 0.45-μm pore size mesh membrane filter paper (Cytiva, Whatman) (Hasegawa et al., 2003; Wang et al., 2007). The filter was then cut into small pieces using flame-sterilized scissors and transferred into 500-μL storage buffer at 4°C for further extraction processing. The abbreviations of collected samples are listed in Table 2.

2.2 | DNA extraction

Phyllosoma and environmental samples were processed using the same DNA extraction methods. 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*(2-Carboxyethyl)phosphine, 1 M). Samples were then heated at 65°C for 1 h (vortexed every 20 min) and incubated on ice for 5 min. 200 μL Ammonium acetate (7.5 M; Sigma-Aldrich Co., MO, USA) and 100 μL potassium chloride were added to the samples at a final concentration of 2.5 M and 1 M, respectively, 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 with 1.5-μL iso-precipitant 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). The 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), 100 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 with co-precipitant pink and centrifuged at 12,000 × *g* for 15 min. The pellet was rinsed with 70% ethanol twice before resuspension in 50 μL of molecular-grade water (Qiagen, Germany). All resulting DNA samples were quantified by Qubit 4 (Thermo Scientific, USA) using the Qubit 1× dsDNA High Sensitivity Assay Kit (Thermo Scientific, USA) following the manufacturer's instructions.

TABLE 2 The abbreviations of samples collected from two tropical rock lobster, *Panulirus ornatus*, hatchery locations and sample categories.

Abbreviations	Locations	Sample categories
OD	Ornatas	Diseased
OH	Ornatas	Healthy
OW	Ornatas	Water
OBI	Ornatas	Biofilm water interface
OBW	Ornatas	Biofilm wall
OFF	Ornatas	Fresh feed
OF6	Ornatas	Feed 6 h old
OF20	Ornatas	Feed 20 h old
ID	IMAS	Diseased
IH	IMAS	Healthy
IW	IMAS	Water
IBI	IMAS	Biofilm water interface
IBW	IMAS	Biofilm wall
IFF	IMAS	Fresh feed
IF6	IMAS	Feed 6 h old
IF20	IMAS	Feed 20 h old

Abbreviation: IMAS, Institute for Marine and Antarctic Studies.

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 in a quantitative polymerase chain reaction (qPCR) at 16S rRNA locus to increase DNA quantity (Matsuo, 2023; Ooi et al., 2023). The primary SYBR Green qPCR mixture consisted of 5 μL of 2 × MyTaq HS mix (Bioline, Meridian Bioscience, USA), 100 NM each of 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTACGACTT-3′) 16S rRNA gene primers (melt temperature between 86 and 88°C) (Ooi et al., 2023), 2 μL of 1:10 diluted DNA and 2 μL of molecular water. The qPCR was conducted using a Thermal Cycler (Bio-Rad Laboratories Inc., USA) with the following thermal cycling program: initial melting for 3 min at 95°C; 35 cycles of 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 wash ratio 1:1.8 (AMPure XP DNA Cleanup Workflow). The final cleaned DNA was additionally quantified by Qubit 4.

2.3.2 | Library preparation

The library preparation mixture consisted of 10 ng of input DNA, adjusted in the total of 15 μL with molecular water, 25-μL Long Amp Hot Start Taq 2X Master Mix (New England Biolabs, Ipswich, MA, USA) and 10 μL of unique 16S barcode (16S Barcoding Kit SQK-114.24, Oxford Nanopore Technologies, Littlemore, Oxford, 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 wash ratio 1:1.8. The final cleaned DNA was additionally quantified by Qubit 4.

2.3.3 | Priming and sequencing

After library preparation, a total of 50 ng of DNA was pooled together from different samples and then incubated with 1 μL of mixture of Rapid Adapter (RA) and Adapter Buffer (AB) at room temperature for 5 min. The prepared DNA pool library (11 μL) was mixed with 34 μL of Sequencing Buffer (SQB), 25.5 μL of Loading Beads (LB), and 4.5 μL of molecular-grade water, loaded onto the SpotON R10.4.1 flow cell (Oxford Nanopore Technologies) for 48 h, and sequenced on the MinION™ Mk 1C. The generated sequencing data were monitored in real-time using the MinKNOW software (version 4.0.20).

As a control, the DNA of a microbial community (Zymo Biomics Microbial Community Standard) was included in the sequencing runs and processed and analyzed in the same way as the samples. Use of such standard communities in microbial community studies for calibration of relative abundances and detection of bias. The whole laboratory workflow presented in Figure 2 below.

2.4 | Bioinformatic pipeline

Dorado software ver. 0.7.1 (Oxford Nanopore Technologies, https://github.com/nanoporetech/dorado) with the SUP model was used for basecalling the sequencing data (POD5 files) to generate FASTQ files with a mean quality score >10, demultiplexing and adapter trimming. Data quality and read length were assessed using NanoFilt ver. 2.8.0 (De Coster et al., 2018), retaining reads between 1200 and 1700 bp. The resulting reads were formatted using TaxonKit (Shen & Ren, 2021), and taxonomic profiling was performed using EMU with the NCBI 16S database (Curry et al., 2022). After taxonomic assignment, unidentified reads were removed from the dataset.

2.5 | Calculation of diversity indices of bacterial communities and statistical analyses

Water quality parameters routinely collected from the two hatcheries were compared using a two-sample *t*-test. A table containing assigned reads of each sample was uploaded to Microbiome Analyst (Lu et al., 2023) relative abundance and core microbiota across a range of detection thresholds (0.01%–0.85% relative abundance). Data were filtered to a minimum count of 4 with 20% prevalence in the sample and normalized by rarefaction to the minimum library size and total sum scaling (Ooi et al., 2023). A total of 1170 low abundance features were removed.

Alpha diversity of bacterial communities was assessed using Observed, Shannon, and Simpson indices to evaluate species richness and evenness across experimental groups. For each alpha diversity metric, summary statistics (mean ± standard deviation) were calculated per group. Pairwise comparisons between groups within each pair were performed using Mann–Whitney *U* tests (Mann & Whitney, 1947; Wilcoxon, 1945). To account for comparisons, *p*-values were adjusted using the Benjamini–Hochberg (BH) method (Benjamini & Hochberg, 1995), and significance was defined as follows: * for $p < 0.05$.

For beta diversity, dispersion among groups was assessed using permutational multivariate analysis of dispersion (PERMDISP) (Anderson, 2006), which showed no significant differences ($p = 0.091$), indicating homogeneity of variance and justifying the use of permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) to test for compositional differences. PERMANOVA was subsequently performed to

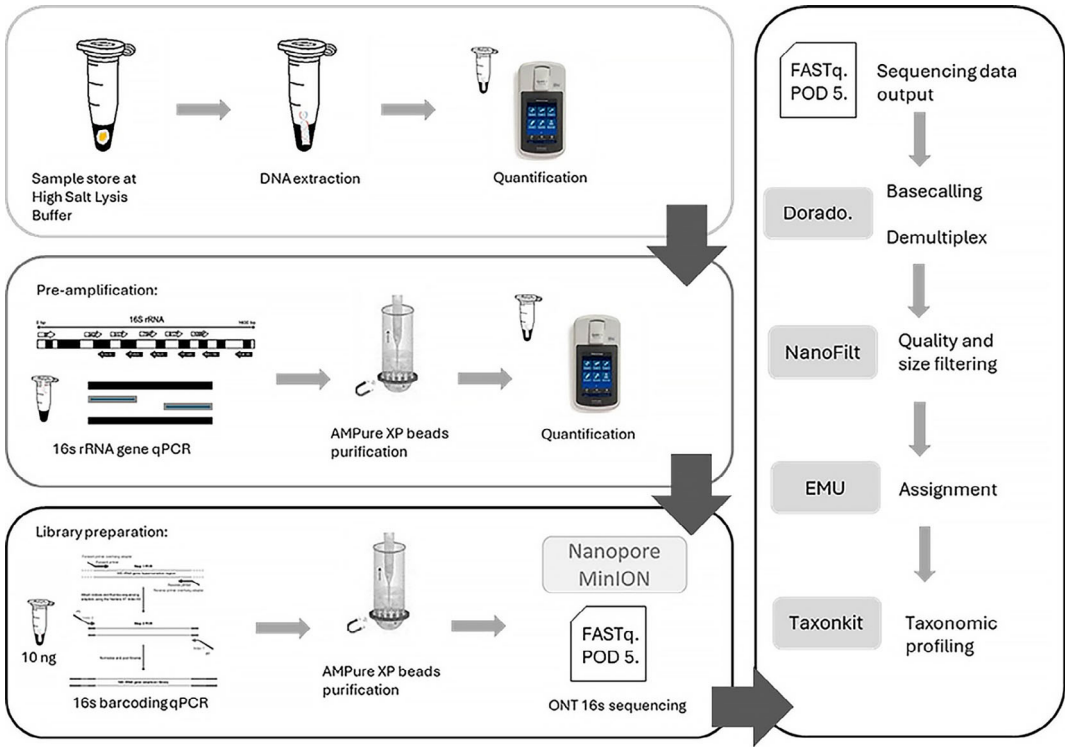

FIGURE 2 Workflow diagram used for characterization of bacteriomes associated with phyllosoma and environment samples collected from two tropical rock lobster, *Panulirus ornatus* hatcheries, Ornatas Pty Ltd. and Institute for Marine and Antarctic Studies (IMAS). qPCR, quantitative polymerase chain reaction.

evaluate differences in microbial community composition between groups using pairwise multilevel comparison. and *p*-values adjusted for multiple testing using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995). Microbial community differences were visualized using principal coordinates analysis (PCoA) based on Bray–Curtis distances (Shi et al., 2020), with samples colored according to experimental group.

The shared and unique bacterial genera among groups of samples were visualized in Venn diagrams, produced by InteraciVenn (Heberle et al., 2015). To compare the bacterial composition in healthy and diseased phyllosoma at two hatcheries, sample counts were aggregated at the top 15 genera level to generate an OTU count table. Differential abundance of the top 15 genera across experimental groups (Ornatas diseased [OD], Ornatas healthy [OH], IMAS diseased [ID], IMAS healthy [IH]) was assessed using DESeq2 for modeling counts with a negative binomial GLM (~ Group) and considering adjusted $p < 0.05$ as significant. Counts were variance-stabilized with rlog transformation for visualization. Heatmaps were separated (Kolde, 2025) for OD versus OH and ID versus IH, with columns annotated by group and rows annotated to indicate DESeq2 significance via a colored side bar. Hierarchical clustering (Euclidean distance and complete linkage) was applied to rows and columns.

All statistical analysis and graphing were conducted in R version 4.4.2 (R Core Team, 2024) and RStudio version 2024.09.1 using the stats (Bolar, 2019), vegan (Oksanen et al., 2025), phyloseq (McMurdie & Holmes, 2013), dplyr (Wickham et al., 2023), tidyr (Wickham & Vaughan, 2023), ggpubr (Kassambara, 2025), DESeq2 (Love et al., 2014) pheatmap (Kolde, 2025), and ggplot2 (Wickham et al., 2025) packages.

3 | RESULTS

3.1 | Relative abundance of bacteria in two hatcheries

After quality and length trimming, there were 5,847,603 reads available for analysis. The most abundant bacterial genus, *Aquimarina*, was present in 82.4% of the diseased phyllosoma from Ornatas and 81.9% from IMAS diseased phyllosoma (Figure 3). The genus *Vibrio* accounted for 17.2% of the genera in diseased phyllosoma from IMAS, while the second most abundant genus in diseased phyllosoma at Ornatas Pty Ltd. hatchery was *Shimia*, representing 15.3% as an average.

Notably, the genus *Shimia* was dominant in healthy Ornatas phyllosoma (76.8%), but rare in IMAS healthy phyllosoma (9.7%). The genera *Tateyamaria* and *Leisingera* were unique to healthy (4.1% and 2.4%) phyllosoma recovered from the Ornatas hatchery while the IMAS hatchery had high *Vibrio* (17.1%) presence in healthy samples.

The main taxa identified in feed samples were *Vibrio* and *Aquimarina* in fresh feed. *Vibrio* represented 11.1% and 29.1% at Ornatas and IMAS hatchery, respectively, while *Aquimarina* accounted for 14.0% of the reads at Ornatas and 9.8% at the IMAS hatchery. The genus *Halomonas* was present in fresh feed samples (IMAS: 9.4%; Ornatas: 3.5%) from both hatcheries and highly detected in 6-h-old feed (28.1%) in IMAS as well. The genus *Pseudoalteromonas* was present in Ornatas 6 h feed (5.3%) and 20-h-old feed (16.2%). It was also highly abundant in IMAS 6-h-old feed (17.4%) but not in IMAS 20-h-old feed.

In environmental samples, at the IMAS hatchery, *Vibrio* and *Oceanococcus* were the most abundant genera in the interface biofilms, with average relative abundances of 21.5% and 44.0%, respectively. In contrast, at the Ornatas

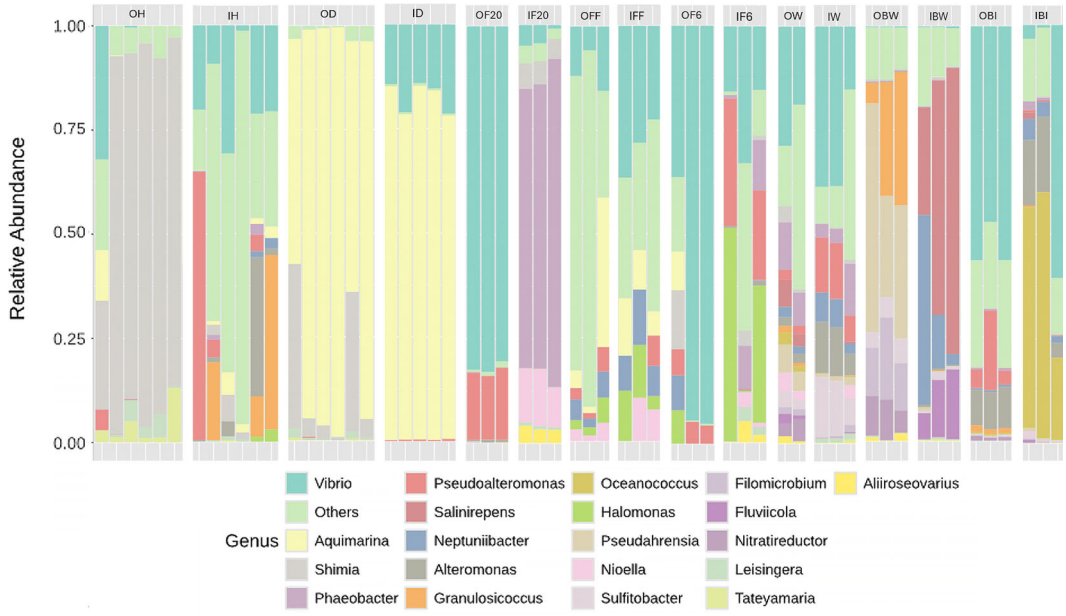

FIGURE 3 Relative abundance (0.00–1.00) of reads for bacterial genera present in samples collected at two tropical rock lobster, *Panulirus ornatus*, hatcheries: Ornatas Pty Ltd. and Institute for Marine and Antarctic Studies (IMAS). IBI, IMAS biofilm interface; IBW, IMAS biofilm wall; ID, IMAS diseased; IF6, IMAS feed 6 h old; IF20, IMAS feed 20 h old; IFF, IMAS fresh feed; IH, IMAS healthy; IW, IMAS water; OBI, Ornatas biofilm interface; OBW, Ornatas biofilm wall; OD, Ornatas diseased; OF6, Ornatas feed 6 h old; OF20, Ornatas feed 20 h old; OFF, Ornatas fresh feed; OH, Ornatas healthy; OW, Ornatas water.

hatchery, interface biofilm was dominated by *Vibrio* (53.6%) and *Alteromonas* (9.2%), while *Pseudahrensia* (37.0%) was higher in wall biofilm rather than interface biofilm. Data available at Table S1.

3.2 | Core bacterial communities and shared bacteria at two hatcheries

The core bacteriome in Ornatas Pty Ltd. hatchery consisted of *Vibrio*, *Shimia*, *Aquimarina* and *Pseudoalteromonas* (Figure 4a). At the Ornatas Pty Ltd. hatchery, the interface biofilm presented the highest number of unique species ($n = 376$), with 4 species shared between OD, biofilms and fresh feed groups (Figure 4b, Table S2). The core bacteriome at the IMAS hatchery consisted of *Vibrio*, *Aquimarina*, *Phaeobacter*, and *Neptuniibacter*, which were the most dominant genera (Figure 5a). At the IMAS hatchery, the interface and wall biofilm samples contained

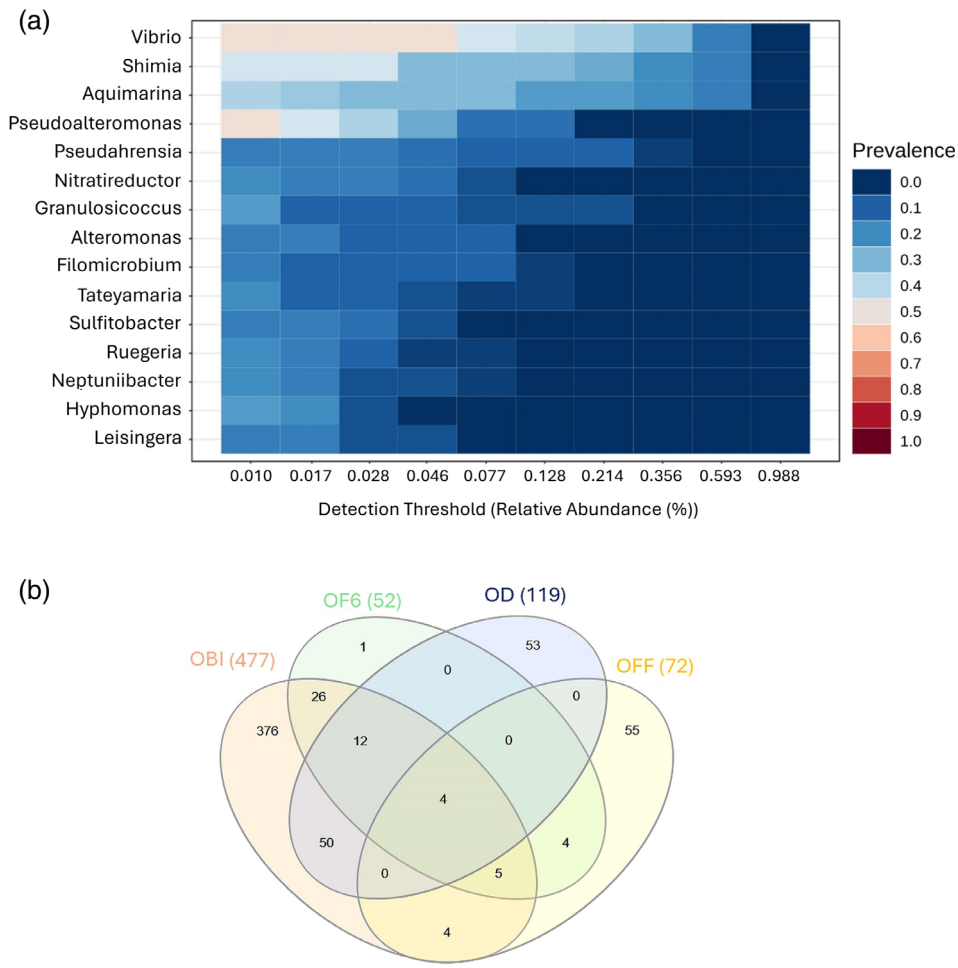

FIGURE 4 Core microbiome analysis of two tropical rock lobster, *Panulirus ornatus*, hatcheries based on relative abundance (0.00–1.00) and fraction of samples (prevalence) of bacteria at the genus level. (a) Ornatas Pty Ltd. hatchery, color key legend denotes the sample prevalence, ranging from dark blue being the lowest (0.00) to dark red being the highest (1.00) sample prevalence, and (b) Venn diagram showing shared and unique species level in diseased phyllosoma, fresh feed, wall biofilm and interface biofilm from the Ornatas Pty Ltd. hatchery. OBI, Ornatas biofilm interface; OBW, Ornatas biofilm wall; OD, Ornatas diseased; OFF, Ornatas fresh feed; OW: Ornatas water.

the highest number of shared bacterial species ($n = 197$), while the interface biofilm samples had the highest number of unique species ($n = 255$), followed by the wall biofilm samples ($n = 246$) (Figure 5b, Table S2).

3.3 | Alpha diversity of feed, phyllosoma and biofilm at two hatcheries

For the Observed richness index, IMAS feed at 6 h old and 20 h old showed significantly higher values than those from the Ornatas hatchery. However, no significant differences in richness were observed between healthy and diseased phyllosoma, nor among any of the interface biofilm samples from the two hatcheries (Figure 6a). Healthy phyllosoma from Ornatas had a lower Shannon index than IMAS, whereas interface biofilm at Ornatas showed higher

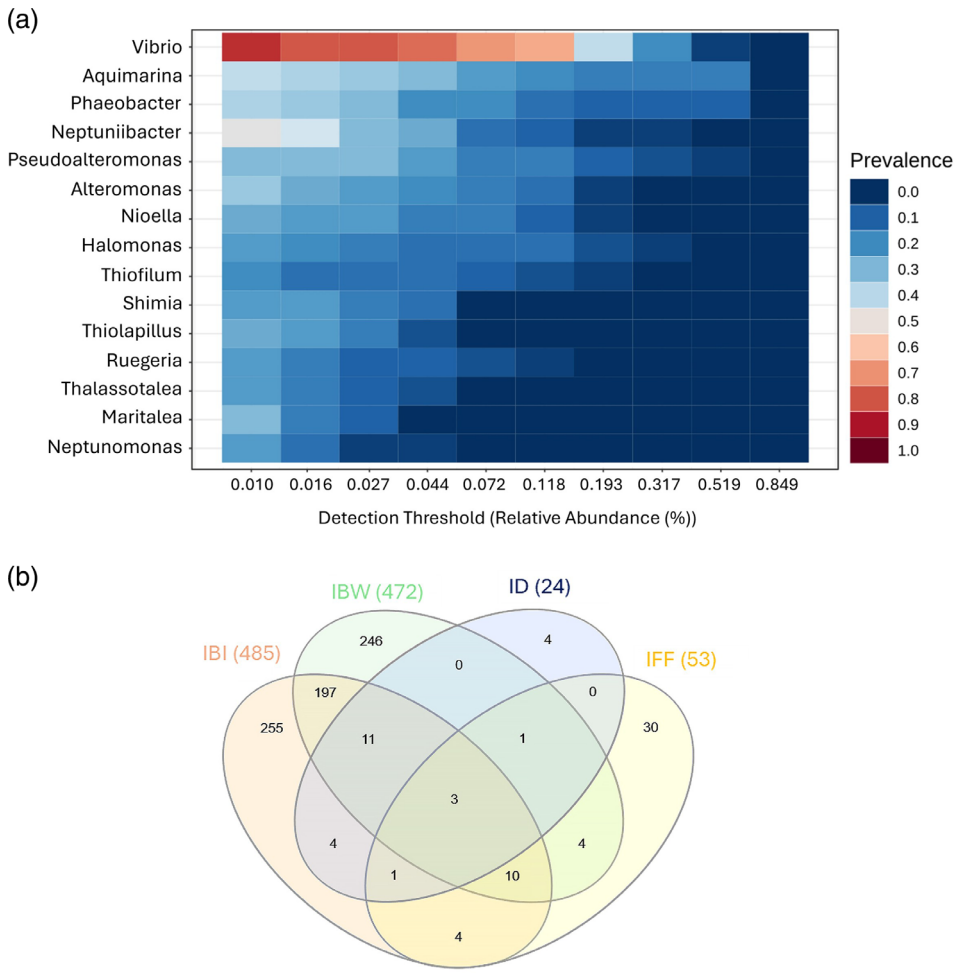

FIGURE 5 Core microbiome analysis of two tropical rock lobster, *Panulirus ornatus*, hatcheries based on relative abundance (0.00–1.00) and fraction of samples (prevalence) of bacteria at the genus level. (a) Institute for Marine and Antarctic Studies (IMAS) hatchery. Color key legend denotes the sample prevalence, ranging from dark blue being the lowest (0.00) to dark red being the highest (1.00) sample prevalence, and (b) Venn diagram showing shared and unique species level in diseased phyllosoma, fresh feed, wall biofilm and interface biofilm from IMAS hatchery. IBI, IMAS biofilm interface; IBW, IMAS biofilm wall; ID, IMAS diseased; IFF, IMAS fresh feed; IW, IMAS water.

Shannon diversity than at IMAS (Figure 6b). For the Simpson index, healthy phyllosoma from IMAS hatchery were more diverse than their Ornatas Pty Ltd. counterparts (Figure 6c).

3.4 | Bacteria beta diversity within hatchery and healthy, diseased phyllosoma

In the Ornatas Pty Ltd. hatchery, beta diversity visualized in two dimensions (PCoA) showed that PCoA1 (23.5%) and PCoA 2 (14.6%) explained 38.1% of the total variation in bacteriome composition (Figure 7a). From the pairwise PERMANOVA results, significant pairwise dissimilarity was detected between several larval and sample groups ($R^2 = 0.46–0.73$, adjusted $p < 0.05$), including OD and OH versus OBI, Ornatas feed 6 h old, Ornatas Fresh Feed. No significant differences were found between OH and OBW or OW ($p = 0.06$), OD similar with Ornatas Water

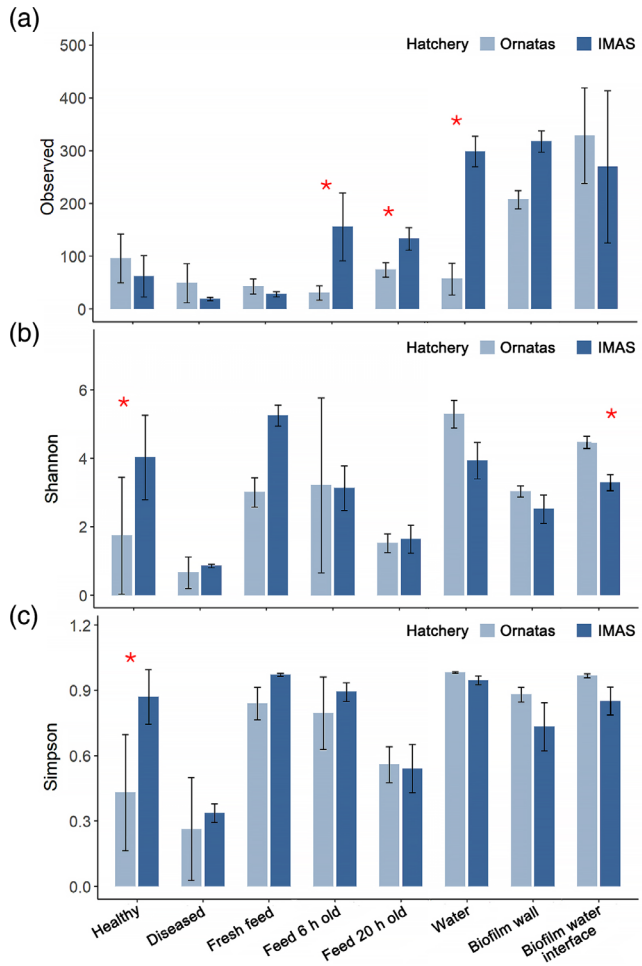

FIGURE 6 Barplots of alpha diversity indices of the bacteriome of two tropical rock lobster, *Panulirus ornatus*, hatcheries, Ornatas Pty Ltd. and Institute for Marine and Antarctic Studies (IMAS). (a) Observed diversity, (b) Shannon diversity, and (c) Simpson diversity. Barplots show the interquartile range and bars the range of values. Bar heights represent the mean values, with error bars indicating standard deviation. Asterisks (*) denote statistically significant differences between respective groups from the two hatcheries.

($p = 0.08$) (Figure 7a). The first two axes (PCoA1 and PCoA2) explained 54.3% and 22.6% of the variation for Ornatas; diseased and healthy phyllosoma formed distinct, well-separated clusters ($p = 0.004$, Figure 7b).

In the IMAS hatchery, beta diversity visualized in PCoA showed that PCoA 1 accounts for 17.7% of the total variation, while PCoA 2 explains 12.4% (Figure 8a). Pairwise PERMANOVA analysis supported these observations, showing multiple significant differences in beta diversity between IMAS Diseased and environmental groups, for instance like: IMAS Diseased versus IMAS Biofilm Interface, IMAS Biofilm Wall, IMAS Water ($p = 0.05$), but IMAS Healthy similar with IMAS Fresh Feed, IMAS Feed 6 h old. The first two axes (PCoA1 and PCoA2) explained 47.2% and 16.2% for IMAS ($p = 0.005$, Figure 8b).

3.5 | Heatmap of differentially abundant bacterial genera in healthy and diseased phyllosoma at two hatcheries

Heatmaps revealed clear patterns of differential bacterial abundance between healthy and diseased phyllosoma in both hatcheries. In the Ornatas hatchery, genera such as *Aquimarina*, *Laceyella*, *Shimia*, *Kordia*, and *Ruegeria* were significantly present between healthy and diseased groups, *Aquimarina* more proportionately abundant in diseased phyllosoma, *Shimia* and *Kordia* more abundant in the healthy group, with several taxa (e.g., *Pseudoalteromonas*)

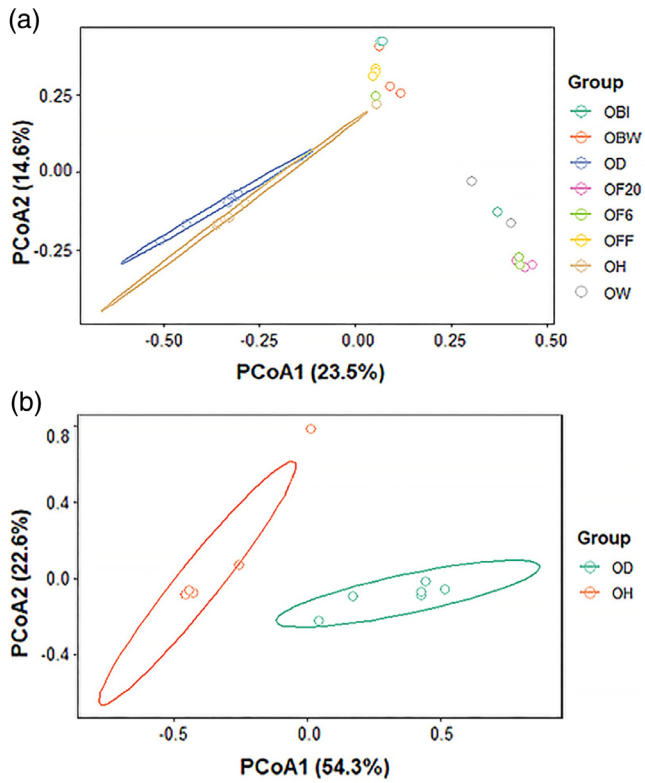

FIGURE 7 Difference in bacterial community structure in tropical rock lobster, *Panulirus ornatus*, from Ornatas Pty Ltd. hatcheries visualized in principal coordinates analysis (PCoA) as a beta diversity in (a) all samples, and (b) diseased and healthy phyllosoma. OBI, Ornatas biofilm interface; OBW, Ornatas biofilm wall; OD, Ornatas diseased; OF6, Ornatas feed 6 h old; OF20, Ornatas feed 20 h old; OFF, Ornatas fresh feed; OH, Ornatas healthy; OW, Ornatas water.

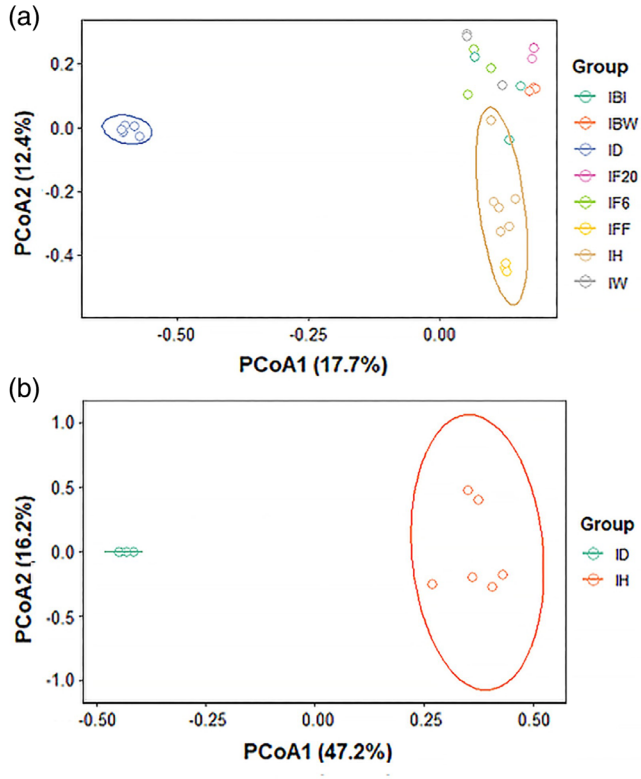

FIGURE 8 Difference in bacterial community structure in tropical rock lobster, *Panulirus ornatus*, from Institute for Marine and Antarctic Studies (IMAS) hatcheries visualized in principal coordinates analysis (PCoA) as a beta diversity in (a) all samples and (b) diseased and healthy phyllosoma. IBI, IMAS biofilm interface; IBW, IMAS biofilm wall; ID, IMAS diseased; IF6, IMAS feed 6 h old; IF20, IMAS feed 20 h old; IFF, IMAS fresh feed; IH, IMAS healthy; IW, IMAS water.

showing no significant differences. Similarly, in the IMAS hatchery, *Aquimarina* was more prevalent in diseased phyllosoma. *Shimia*, *Pseudoalteromonas*, *Ruegeria*, and *Thalassotalea* were much higher abundant in healthy phyllosoma (Figure 9).

4 | DISCUSSION

This study compared bacterial communities between healthy and diseased *P. ornatus* larvae between two hatcheries and their associated environments. Differences in bacterial composition were observed, suggesting that both larval health status and rearing conditions significantly influence microbial community structure.

4.1 | Environmental shape bacterial communities in *P. ornatus* larval culture

At both hatcheries, *Vibrio* and *Aquimarina* were consistently dominant and formed part of the core microbiome in the *P. ornatus* aquaculture system. Other key genera included *Pseudoalteromonas*, *Shimia* at Ornatas Pty Ltd., and *Phaeobacter*, *Neptuniibacter* at IMAS. These site-specific variations likely reflect differences in environmental

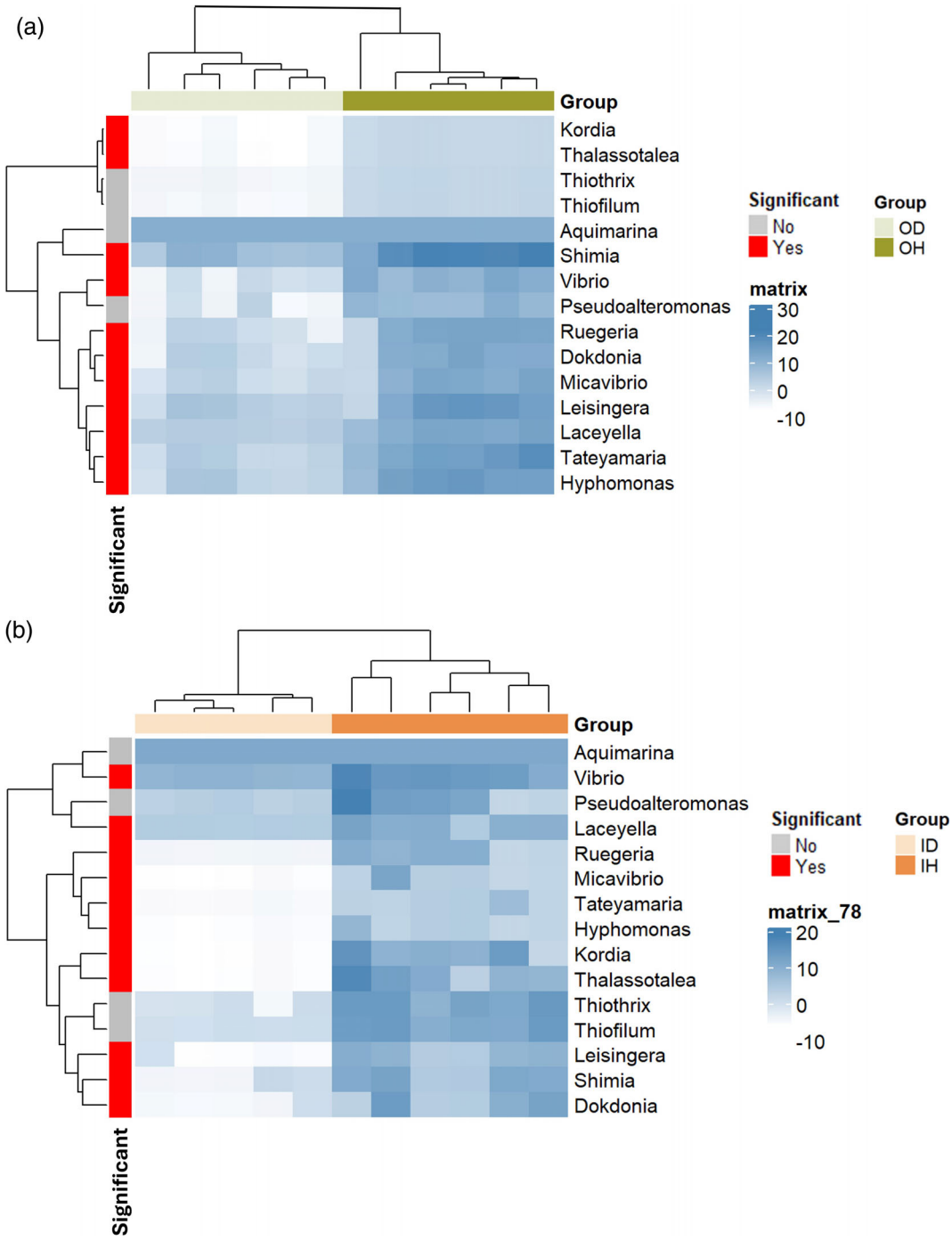

FIGURE 9 Heatmap of differentially abundant bacterial genera in healthy and diseased phyllosoma from two topical rock lobster, *Panulirus ornatus*, hatcheries, Ornatas Pty Ltd. and Institute for Marine and Antarctic Studies (IMAS). Hierarchical clustering was applied to both rows (bacterial taxa) and columns (samples). Color intensity indicates relative abundance (matrix values), and red bars on the left indicate genera showing significant differences between healthy and diseased phyllosoma. Sample groups are annotated at the top. (a) Ornatas Pty Ltd. hatchery, and (b) IMAS hatchery. ID, IMAS diseased; IH, IMAS healthy; OD, Ornatas diseased; OH, Ornatas healthy.

conditions between the hatcheries. For instance, the Ornatas Pty Ltd. hatchery sources its water from a saltwater creek near Toomulla Beach, on the Great Barrier Reef coastline, an area influenced by terrestrial runoff and nearshore inputs. In contrast, IMAS draws water from the Derwent River, an estuarine system characterized by fluctuating nutrient loads and a unique microbial profile (Abell et al., 2013).

Both hatcheries treat incoming water with ozone, to reduce microbial load and enhance overall water quality. However, differences in ozonation treatments will result in variations to DO and oxidation–reduction potential (ORP), which can significantly influence microbial communities (Chatziantoniou et al., 2022; Gonçalves & Gagnon, 2011; Lindholm-Lehto, 2023; Xu, Wang, et al., 2023). For instance, the Ornatas Pty Ltd. hatchery generally operated at a higher ORP, resulting in elevated DO and bromate contents in the hatchery water. These are conditions that are known to suppress anaerobic and opportunistic bacterial taxa such as *Vibrio* and *Arcobacter* that pose a threat to animal health (Guo et al., 2022). Conversely, the IMAS system, with lower DO and ORP, may favor colonization by facultative anaerobes like *Vibrio*, which is known to thrive in low-oxygen and hypoxic environments (Bhattacharyya et al., 2019; Bueno et al., 2020). Although oxidation conditions have been linked to microbial shifts in other aquatic diseases (Guo et al., 2022), a direct association between DO, ORP and WLD has yet to be established.

Both hatcheries showed similar bacterial diversity across environmental compartments (water, feed, and biofilm), yet the phyllosoma larvae hosted distinct bacterial communities. Several genera, *Vibrio*, *Pseudoalteromonas*, *Pseudaghrensia* and *Alteromonas*, were shared between environmental sources and larval bacteriomes. Interface and tank wall biofilms exhibited the highest bacterial richness, consistent with previous findings that biofilms represent key reservoirs of microbial diversity in aquatic systems (Tasneem et al., 2018). Notably, *Vibrio* species are frequently identified in biofilms and can act as reservoirs for disease and antibiotic resistance (Acosta et al., 2021; Cai et al., 2013). Biofilm formation also facilitates quorum sensing and the expression of virulence factors, such as hemolysins and proteases, which have been linked to ulceration and septicaemia in species such as zebrafish, *Danio rerio* (Juszczuk-Kubiak, 2024; Sharma & Gandhi, 2024). The presence of *Vibrio* in biofilms suggest a potential pathogen reservoir for secondary pathogen in WLD at IMAS. To assess bacterial succession during larval rearing, three stages of feed: fresh, 6 h old, and 20 h old were analyzed. Fresh feed samples exhibited the lowest alpha diversity, indicating limited microbial colonization. This microbial simplicity likely reflects minimal environmental exposure, with bacterial composition largely shaped by feed preparation and storage conditions. At the Ornatas Pty Ltd. hatchery, fresh feed was dominated by *Acidibacter* (phylum Acidobacteria), a heterotrophic genus commonly associated with soils, sediments, and aquatic environments, and its presence here likely reflects feed-driven enrichment and a role in nutrient cycling (Duarte et al., 2019; Ifon et al., 2023; Prayogo et al., 2018). However, within 6 h of exposure to the rearing environment feed samples showed increased bacterial diversity and a distinct community shift, including a marked increase in *Vibrio*. This rapid colonization suggest that *Vibrio* is an opportunistic environmental bacterium and may contribute to elevated disease risk as feeding progresses (Frans et al., 2011). Similar patterns of *Vibrio* enrichment have been associated with disease outbreaks in Atlantic salmon, *Salmo salar*, rainbow trout, *Oncorhynchus mykiss*, and black tiger prawn, *Penaeus monodon* (Diwan et al., 2023; Haifa-Haryani et al., 2022; Reyes et al., 2022).

At IMAS, both fresh and 6-h-old feed samples showed increased levels of *Halomonas*, a halotolerant genus adapted to nutrient-rich, saline environments such as those created by uneaten feed in tanks (Kudo et al., 2023). *Halomonas* is known to produce digestive enzymes like amylases, proteases, and lipases that enhance nutrient absorption and metabolism processes in *S. maximus* (Xu, Zhao, et al., 2023). Their presence may reflect early-stage microbial colonization facilitated by nutrient leaching and physical degradation of the feed surface (Dang & Lovell, 2016; Gao et al., 2012). By 20 h, the feed at the IMAS hatchery exhibited a high relative abundance of *Phaeobacter*, a genus commonly found in marine biofilms and known for producing antimicrobial compounds, such as tropodithietic acid (TDA), which inhibits pathogens like *Vibrio* (Droumpali et al., 2023; Porsby & Gram, 2016; Sonnenschein et al., 2021). This may suggest a late-stage competitive exclusion dynamic within the feed-associated microbiota; similar mechanisms are reported in black tiger prawn, *P. monodon*, penaeid larvae aquaculture, where probiotic Bacillus spp. (administered via feed) can suppress opportunistic pathogens through competition for nutrients and production of antimicrobial compounds (Kolanchinathan et al., 2022), although direct evidence for such interactions on live

aquafeeds remains limited. *Aquimarina* was detected in fresh feed from both hatcheries, 6-h-old feed at Ornatas Pty Ltd., and in diseased phyllosoma, as well as interface and wall biofilms, indicating multiple transmission pathways. These could include direct exposure via contaminated feed or water, attached to organic particles, or persistence via biofilm formation on tank surfaces (Kudo et al., 2023). While *Aquimarina* pathogenicity in aquaculture remains underexplored, it has been implicated as a causative agent in epizootic shell disease (ESD) in American lobsters, *Homarus americanus* (Quinn et al., 2017), potentially transmitted through currents or ingestion of infected molts (Ishaq et al., 2022). This study indicates that *Aquimarina* can actively colonize feed and other environmental compartments and may represent a stable contamination source.

The detection of overlapping bacterial taxa between environmental samples and phyllosoma bacteriomes suggests that environmental reservoirs may serve as ecological bridges between external and host-associated communities (Chen et al., 2022). These shared bacterial reservoirs may influence phyllosoma health and potentially increase their vulnerability to diseases such as WLD.

4.2 | Bacterial communities differ in healthy and diseased phyllosoma

This study found there was no significant difference in alpha diversity between healthy and diseased larvae in the Ornatas Pty Ltd. hatchery. This may suggest that WLD in this population may not be driven by a general loss of bacterial richness. It is also possible that diseased larvae were collected at an earlier stage of infection, before a detectable collapse in microbial diversity had occurred. This aligns with previous findings that some diseases, particularly those driven by opportunistic pathogens, may not result in reduced diversity but instead manifest through shifts in microbial composition or dominance of pathogenic taxa (O'Brien et al., 2019). Furthermore, diseases caused by opportunistic pathogens can often occur without a loss of microbial diversity, but rather due to the dominance of certain pathogenic taxa that outcompete other microorganisms (O'Brien et al., 2019). This could explain the lack of significant changes in overall diversity at the Ornatas Pty Ltd. hatchery, where pathogenic bacteria may play a more central role in disease onset while leaving the broader bacterial community relatively unchanged.

While in the IMAS hatchery, there was a significant difference in bacterial alpha diversity between healthy and diseased *P. ornatus* phyllosoma, with diseased individuals showing reduced microbial diversity. The diseased phyllosoma at IMAS hatchery exhibited a marked reduction in bacterial richness compared to healthy individuals. Alpha diversity is a widely accepted indicator of host health, as higher bacterial diversity shows stable bacteria structures that promote resilience (Williams et al., 2024). In contrast, reduced diversity is commonly associated with disease onset, and has been reported in various aquatic species (Kriss et al., 2018). For example, healthy largemouth bronze gudgeon, *Coreius guichenoti*, exhibits higher gut microbial diversity than diseased counterparts, suggesting that a diverse microbiome improves metabolic function and limits opportunity for pathogen colonization (Xiong et al., 2018). Reduced alpha diversity is often associated with microbial dysbiosis, an imbalance in the microbiome leading to instability in the bacterial community and the loss of beneficial taxa (Kriss et al., 2018; Levy et al., 2017). In diseased phyllosoma, this likely reflects a dysbiosis shift that compromises the host's ability to regulate its microbiome, increasing susceptibility to opportunistic pathogens. Similar patterns have been reported in other aquaculture species; for example, in *S. salar*, larval gut dysbiosis with reduced bacterial diversity was linked to increased susceptibility to *Vibrio* and *Aeromonas* infections (Dehler et al., 2017), and reduced bacterial diversity and a shift toward dominance of specific taxa (e.g., Proteobacteria) indicate early dysbiosis and a loss of microbiome stability associated with declining cultivated yellowtail kingfish, *Seriola lalandi*, gut health (Legrand et al., 2018).

Across both hatcheries, microbial composition differed significantly between healthy and diseased phyllosoma. *Aquimarina*, a known opportunistic pathogen and causative agent of WLD, was consistently and significantly enriched in diseased individuals (Midorikawa et al., 2020; Ooi et al., 2020). Members of the genus *Aquimarina* are yellow-pigmented, non-flagellated bacteria previously isolated from diseased phyllosoma and during WLD (Ooi et al., 2020). *Aquimarina* sp. TRL1 was detected in experimentally infected phyllosoma affected by WLD but was

absent in healthy control animals (Ooi et al., 2020). Several *Aquimarina* species have been implicated in shell diseases and the mortality of larval and juvenile crustaceans (Dan & Hamasaki, 2015; Midorikawa et al., 2020; Yu et al., 2014). During disease events, pathogenic bacteria like *Aquimarina* can outcompete other members of the microbial community, dominating the host microbiome and suppressing beneficial bacteria (Williams et al., 2024), resulting in the onset of disease clinical signs (Wynne et al., 2020).

Healthy phyllosoma from the Ornatas Pty Ltd. hatchery exhibited higher relative abundances of *Shimia* and *Tateyamaria*. *Shimia* is commonly found in marine environments, including surface seawater, cold-seep sediments, corals, and the gut of abalone, and is one of the most abundant genera in healthy *L. vannamei* (Hyun et al., 2013; Nogi et al., 2015; Sutanti et al., 2024; Zhu et al., 2021). It may contribute to bacterial community stability through biofilm formation and the production of antimicrobial compounds that inhibit opportunistic pathogens (Zheng et al., 2022). In pacific abalone, *Haliotis discus*, aquaculture, *Shimia* spp. may facilitate early gut microbial colonization, helping maintain microbial balance during larval development (Huang et al., 2020; Hyun et al., 2013). Additionally, some *Shimia* species participate in sulfur and nitrogen cycling, influencing microbial ecosystem function by regulating nutrient availability and supporting a balanced community structure through metabolic interactions with other bacteria (Zhang & Sun, 2022). Genus *Tateyamaria* are marine heterotrophs found in seawater and sediment, capable of degrading complex organic compounds and participating in nutrient cycling, including sulfur metabolism in kelp, *Laminaria hyperborea* (Bengtsson et al., 2012) Further, *Tateyamaria* spp. are associated with aquaculture hosts such as red abalone, *Haliotis rufescens*, and in greater abundance in healthy individuals which likely suggests a role in maintaining microbiome resilience and community balance (Sass et al., 2010; Villasante et al., 2020; Yoon, 2019). However, still relatively high abundance of *Vibrio* in healthy phyllosoma at both hatcheries may indicate a potentially unstable microbiome with some *Vibrio* species opportunistic pathogens able to trigger dysbiosis when under environmental stress (Reyes et al., 2022; Silva et al., 2022). In this study the ORP in both systems was consistently maintained between 330 and 420 mV, due to continuous ozonation. While ozonation reduces overall microbial load, it can also suppress sensitive taxa and reduce community diversity. Similar effects have been reported in aquaculture and water treatment systems; for example, ozonation decreased the abundance of Betaproteobacteria and Planctomycetales in RASs (Wietz et al., 2009), and moderate ozonation in *G. morhua* larval RAS altered microbial composition and stability, suggesting that stronger disinfection reduces bacterial numbers and destabilizes the community (Attramadal et al., 2012). This may create a selection pressure that favors ORP-tolerant bacteria, including some *Vibrio* strains. Elevated *Vibrio* levels have been observed in pre-diseased stages of *L. vannamei* hatcheries, where their biofilm-forming ability and hepatopancreas colonization contributed to the development of Acute Hepatopancreatic Necrosis Disease (AHPND) (Reyes et al., 2022). Following acute infection, other opportunistic bacteria such as *Shewanella* or *Aquimarina*, as observed in our study, may outcompete *Vibrio*, colonizing damaged tissues and further disrupting the microbial community (Reyes et al., 2022). However, the dynamics of this progression remain poorly understood and warrant further investigation.

4.3 | Feed-associated risk factor and management strategies for WLD

Effective management of host–microbiome interactions is crucial in aquaculture, where environmental bacteria can support larval development but may also lead to dysbiosis or disease under suboptimal conditions (Bentzon-Tilia et al., 2016). In *L. vannamei* aquaculture systems, microbiome modulation through probiotics, prebiotics, and biofloc systems has improved animal health and productivity, while supporting sustainability (Rajeev et al., 2021).

Feed is a critical control point. Uneaten or residual feed can persist in the rearing environment, providing a nutrient-rich substrate that supports the growth of opportunistic bacteria (Diyie et al., 2024). In this study, *Aquimarina* was present in fresh and aged feed as well as in environmental samples, indicating multiple potential transmission routes. Similar findings in catfish, *Ictalurus punctatus*, and tilapia, *Oreochromis niloticus*, systems show that contaminated feed can introduce pathogens such as *Streptococcus*, leading to widespread disease outbreaks

(Diyie et al., 2024). Residual feed often contains limited nutritional value but contributes as a substrate for bacterial growth and biofilm formation (Pandey & Kumar, 2022). Feed-risk control strategies such as adjusting feed conversion ratio, precision feeding, using microbial floc technology, adding sponge, screen, sand, gravel to mechanical filtration can all reduce pathogen growth the proliferation (Munguti et al., 2021; Potnis et al., 2022; Yakupitiyage, 2013). Moreover, computer vision based artificial intelligence technology has been applied in aquaculture for enhancing efficacy, this approach uses AI estimate feeding requirement and feeding schedules, combine with image analysis to track cultured animal behaviors and appetite (Pradhan, 2023).

Biofilm and water quality are critical components in microbial regulation. Management strategies in RAS are essential for controlling bacterial loads and reducing pathogen risks (Infante-Villamil et al., 2021). A number of hygiene strategies to reduce the organic loads in systems are available and include a biofilters mix (submerged, trickling, rotating, bead), foam fractionation to remove dissolved protein, chemical disinfection (ozone), and thermal shock (raise 5–10°C above normal for minutes or hours) to help maintain the water quality and promote the stability of the bacterial community (Areerachakul, 2018; Renaud et al., 2025).

Maintaining optimal and stable water parameters, including temperature, salinity, pH, and available DO helps ensure environmental consistency and supports a balanced microbial population in both the aquaculture system and the cultured larvae (Ghosh et al., 2022; Lukassen et al., 2022; Tumwesigye et al., 2022). These integrated environmental control strategies decrease the risk of disease while promoting beneficial microbial communities, improved larval survival and productivity.

5 | CONCLUSION

This study investigated the bacterial communities associated with WLD outbreaks in *P. ornatus* phyllosoma and their surrounding aquaculture environments, specifically water, biofilms, and feed, across two hatcheries. The genus *Aquimarina*, previously identified as the causative agent of WLD, was consistently detected not only in diseased phyllosoma larvae but also across multiple environmental compartments. This suggests potential transmission pathways via shared environmental reservoirs.

The findings underscore the complex and dynamic interactions between host-associated and environmental microbiota in aquaculture systems. Shared bacteria between the environment and host may serve as reservoirs or vectors of infection, influencing larval health and increasing susceptibility to opportunistic pathogens.

Future studies should focus on the role of feed, particularly fresh and residual feed, as a potential vector for pathogenic bacteria involved in WLD outbreaks. Additionally, implementing microbiome monitoring and targeted interventions may improve larval health outcomes. Whole genome sequencing and functional studies could further elucidate host-pathogen interactions, transmission pathways, and the molecular mechanisms underlying WLD pathogenesis. These insights will support the development of more sustainable disease-resilient lobster hatchery practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of the present study are available within the article and its Supporting Information. Data will be made available on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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