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Microbiome and Pathobiome Characterization in Farmed Barramundi (*Lates calcarifer*) During and Post Scale Drop Disease Outbreaks

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ABSTRACT

Large-scale double-digest RAD sequencing (ddRADseq) datasets generated for genotyping are increasingly available in aquaculture, yet their unmapped reads remain largely unexplored for pathogen surveillance. Here, we evaluated the utility and limitations of repurposing unmapped ddRADseq reads to examine pathogen-associated and disease-associated microbiome-pathobiome patterns during scale drop disease (SDD) outbreaks in farmed barramundi (*Lates calcarifer*). Using fin clips ddRADseq datasets from 4593 barramundi across four commercial sea-cages, we profiled bacterial and viral communities by taxonomic classification of unmapped reads. Fish sampled during active outbreaks consistently exhibited strong enrichment of scale drop disease virus (SDDV-associated signals; 76.3%–80.5%), frequently co-occurrence with infectious spleen and kidney necrosis virus (ISKNV; 0%–13.4%), along with a marked microbial shift towards *Vibrio*-dominated bacterial communities, particularly reads classified within the *Vibrio harveyi* clade. In contrast, clinically healthy post-outbreak fish showed consistently low viral signals and were characterized by distinct, more diverse microbial profiles dominated by *Alphanudivirus*, *Cyvirus*, *Stenotrophomonas* and *Burkholderia*, indicating a comparatively stable microbiome state in the absence of active disease outbreaks. Treating normalized pathogen read counts as proxy traits, exploratory quantitative genetic analyses indicated low overall heritability estimates for SDDV-associated signal ($h^2 = 0.08$), with higher cohort-specific estimates (up to 0.23), consistent with strong environmental or co-infection effects and complex co-infection dynamics in open-sea farming systems. While ddRADseq-based pathogen detection is inherently biased by restriction-enzyme site representation, host-DNA dominance and the lack of absolute quantification, consistent patterns observed across thousands of fish tissues across multiple cohorts and outbreak stages provide biological meaningful population-level insights into farm-associated microbiome and pathobiome dynamics. Together, our results support that the use of unmapped ddRADseq reads as a cost-effective, complementary tool for retrospective pathogen screening and hypothesis generation in aquaculture, alongside targeted surveillance and diagnostic approaches.

Nguyen Thanh Vu and Xueyan Shen contributed equally.

1 | Introduction

Economic losses due to disease outbreaks in aquaculture are significant, estimated at approximately $US 6 billion annually (World-Bank 2014). These outbreaks are driven by multiple pathogens, affecting many economically important aquaculture species (Lafferty et al. 2015; Leung and Bates 2013). Tropical aquaculture, which accounts for 91.4% (119.7 million tons) of total global production in 2022 (FAO 2024), is particularly vulnerable due to accelerated pathogen proliferation in warmer waters and the intensifying impacts of climate change (Leung and Bates 2013; Reid et al. 2019). Effective disease mitigation strategies are therefore essential for securing global food security, especially in open-sea farming systems where preventative operational measures are limited (Aly and Fathi 2024; Bjelland et al. 2024).

Barramundi (*Lates calcarifer*), also known as Asian seabass, is a key species in tropical aquaculture (Jerry 2013; Schipp et al. 2007). In Singapore, barramundi is farmed in nearshore marine systems using both flow-through containment and floating net cages, where fish are exposed to substantial disease pressure and fluctuating environmental conditions (Liang and Neo 2021; Shen et al. 2021; Terence et al. 2021, 2026). Disease outbreaks pose major issues to the barramundi industry in Singapore (Poon et al. 2025; Vij et al. 2024); for instance, one of the largest local producers reported losses of US$ 31.9 million in 2022, likely linked to multiple disease occurrences. The shared use of seawater zones for aquaculture and maritime activities, coupled with unpredictable environmental changes such as harmful algal blooms and oil spills, has further increased disease risk in Singapore's coastal waters (Shen et al. 2021).

Globally, barramundi farming constantly faces health-related challenges including viral infections; for example scale drop disease virus (SDDV) (de Groof et al. 2015; Domingos et al. 2021; Gibson-Kueh et al. 2012; Kiat et al. 2023; Nurliyana et al. 2020), *L. calcarifer* herpes virus (LCHV) (Dang et al. 2023; Gibson-Kueh et al. 2023), infectious spleen and kidney necrosis disease (ISKNV) (Dong, Jitrakorn, et al. 2017), red sea bream iridovirus (Girisha et al. 2020; Sumithra et al. 2022), birnavirus (Chen et al. 2019), red-spotted grouper nervous necrosis virus (Angsujinda et al. 2020; Jaramillo et al. 2017), beta-nodavirus (Binesh 2013); and bacterial infections such as big belly disease (Gibson-Kueh et al. 2021), *Edwardsiella* (Loch et al. 2017), *Streptococcus iniae* (Kayansamruaj et al. 2017; Van Khang et al. 2019), *Tenacibaculum* (Dong, Taengphu, et al. 2017) and *Vibrio harveyi* (Dong, Taengphu, et al. 2017; Ransangan et al. 2012). Importantly, co-infections, rather than a single causative agent, are frequently reported in farmed barramundi (Charoenwai et al. 2021; Domingos et al. 2021; Dong, Taengphu, et al. 2017; Gomes et al. 2019; Kerddee et al. 2020; Terence et al. 2026); however, the interactions among these pathogens remain poorly understood. The complex nature of these co-infections underscores the need for deeper investigation into host–pathogen interactions to improve disease management strategies (Infante-Villamil et al. 2021). Traditionally, disease investigations in aquaculture have focused on identifying single causative agents using targeted diagnostic tools such as PCR or quantitative PCR (qPCR) (Meemetta et al. 2020; Mishra et al. 2023). While these approaches provide high sensitivity and specificity for known pathogens (Yin et al. 2024), they offer limited insight into broader microbial community context or co-infection patterns. Increasing evidence suggests that disease outcomes are shaped not only by individual pathogens but also by interactions among multiple microbes and the host (González and Elena 2021), prompting growing interest in microbiome-based perspectives on aquatic animal health.

Here, we define the 'microbiome' as the collective community of microorganisms, including bacteria, viruses and their genetic material associating with a host tissue or environment, regardless of functional impact. In contrast, the 'pathobiome' refers to the subset of the microbial community whose composition, interactions or activity are associated with disease development or progression, often through synergistic or opportunistic interactions among multiple pathogens. Shifts from a stable microbiome to a disease-associated pathobiome have been implicated in numerous aquatic diseases, where viral infection may predispose hosts to secondary bacterial colonization and dysbiosis (Infante-Villamil et al. 2021). The host-associated microbiome plays a critical role in maintaining fish health, functioning as a first line of defence against pathogens through the gut, gill, mucus, and skin, which harbour diverse and beneficial microbial communities (Ángeles Esteban 2012; Cogen et al. 2008). However, environmental changes, poor water quality or the use of antibiotics can disrupt microbial balance and promote dysbiosis, thereby increasing the risk of disease outbreaks (Infante-Villamil et al. 2021). For instance, changes in the skin microbiome have been identified as early warning signals of disease in salmon (Godoy et al. 2023). In barramundi, fins and mucus have been explored as potential diagnostic samples for detecting SDDV and ectoparasites (Charoenwai et al. 2021). Charoenwai et al. (2021) reported high detection rates of SDDV from fin samples—73.3% in clinically infected fish and 80.0% in subclinical individuals (Charoenwai et al. 2021). Microbiome research has advanced in aquatic species (Rajeev et al. 2021), including gut microbiota (Chen et al. 2022; Perry et al. 2020) and microbial communities in rearing water (Bugten et al. 2022; Zoqratt et al. 2018), and also to external tissues such as the skin, gills and fins (McMurtrie et al. 2025). These surfaces are directly exposed to environmental microbes and may reflect overall fish health, making the fin microbiome a promising, but underexplored area in aquaculture disease research. Notably, microbial genomes can become embedded in host tissues and be detected via next-generation sequencing (NGS), offering novel insights into host–microbe interactions and overall health status.

ddRADseq is widely used in aquaculture genetics to generate genome-wide single nucleotide polymorphism (SNP) data for population genetics, quantitative trait analysis, and selective breeding (Aguirre et al. 2019; Konar et al. 2017; Lavretsky et al. 2019). Although ddRADseq libraries are designed to enrich host genomic regions, a substantial fraction of sequencing reads typically remains unmapped to the host reference genome. These unmapped reads may include microbial sequences derived from host-associated tissues, representing an untapped resource for retrospective pathogen screening and microbial community profiling. Recent studies have suggested that analysing unmapped reads from genomic datasets can provide

biologically meaningful insights into pathogen presence, albeit with inherent biases related to library preparation and restriction enzyme representation (Liu et al. 2017). Despite this potential, the feasibility and limitations of using unmapped ddRADseq reads for pathogen and pathobiome surveillance in aquaculture remain poorly explored. For example, using large-scale ddRADseq datasets from commercial barramundi cohorts, Terence et al. (2026) demonstrated that unmapped reads could be repurposed as a sensitive, population-level surveillance tool for SDDV. By aligning raw ddRADseq reads to the SDDV genome, the study showed a striking contrast between moribund and asymptomatic fish, with viral prevalence exceeding 85% in diseased individuals but remaining negligible in healthy cohorts. Importantly, ddRADseq-derived viral read counts were strongly correlated with quantitative PCR estimates of viral load from the same fin tissues (84% concordance or Spearman's ρ: 0.55–0.84), validating ddRADseq as a reliable proxy for pathogen detection despite its lower sensitivity relative to targeted diagnostics. These findings establish that routine genotyping datasets can simultaneously support host genomic analyses and retrospective pathogen monitoring, providing a practical framework for integrating disease surveillance into aquaculture breeding programmes.

The aim of this study was therefore to evaluate the utility of unmapped reads from ddRADseq data for population-level detection of pathogens and characterization of microbiome–pathobiome patterns during SDD outbreaks in farmed barramundi. Using fin-clip ddRADseq data from 4593 fish across four commercial sea-cage cohorts, we profiled bacterial and viral communities associated with diseased and healthy individuals, examined co-infection patterns, and explored the heritability of pathogen-associated read-count proxies. By explicitly addressing methodological biases and limitations, this study assesses the value of repurposing ddRADseq datasets as a cost-effective, complementary tool for pathogen surveillance and hypothesis generation in aquaculture systems.

2 | Material and Methods

2.1 | Ethics Declaration

This research was done under James Cook University Singapore Institutional Animal Care and Use Commission (IACUC) approval number A2018. All procedures involving animals were performed in accordance with the relevant guidelines and regulations and complied with the requirements of the approving institutional animal ethics committee. This study is reported in accordance with the ARRIVE guidelines (ARRIVE 2.0).

2.2 | Experimental Design, Fish Origin, Farming System, Disease Outbreak, and Sampling

Barramundi used in this study originated as fingerlings from a commercial hatchery in Australia and were produced in four independently reared cohorts between June 2018 and February 2019. Upon arrival in Singapore, fish were transferred to commercial farming facilities operated by Barramundi Group, where all subsequent nursery and grow-out phases were conducted. Fish were reared through a two-stage land-based nursery system located at Semakau Island, Singapore. The first nursery stage operated under a recirculating aquaculture system (RAS) at low salinity (5 ppt) until fish reached approximately 20 g. Fish were then transferred to a second nursery stage using a flow-through seawater system and reared until reaching approximately 40–50 g (70–80 days post-hatch). Juveniles were subsequently transferred to sea cages for grow-out under standard commercial conditions. Fish were initially stocked in 8 × 8 m square sea cages and later transferred to larger circular sea cages (25–26 m in diameter and approximately 10 m deep) located at a commercial marine farm in the southern waters of Singapore, adjacent to Semakau Island. Seawater salinity during grow-out ranged from 26 to 31 ppt. Each cohort was stocked at commercial scale, with approximately 100,000 individuals per sea cage, and reared to harvest size following routine industry practices.

Fish health was monitored daily throughout the grow-out period, with scuba divers conducting routine inspections of sea cages and the farm veterinarian examining moribund or newly deceased fish to investigate disease outbreaks and identify causative agents. All four cohorts experienced naturally occurring outbreaks of SDD between November 2018 and October 2019. Affected fish displayed clinical signs including scale loss, skin lesions, haemorrhagic spots on the fins, and surface erosions. PCR diagnostics conducted by the company's diagnostic laboratory confirmed SDDV as the causative agent (Terence et al. 2026).

It is important to note that diseased and clinically healthy fish were not sampled contemporaneously within each cohort. Healthy fish were collected weeks to months after disease outbreaks had subsided, resulting in differences in age, size, environmental conditions, and production stage between comparison groups. Consequently, observed differences in microbial profiles may reflect a combination of disease status, temporal dynamics, host development, and environmental variation rather than disease alone. Specifically, sampling was conducted at two distinct stages relative to the SDD outbreaks. First sampling occurred during active SDD outbreaks, typically 3–8 weeks after transfer from land-based nursery systems to sea cages, when mortalities peaked within the first 1–2 weeks and persisted for a further 2–4 weeks. During this stage, moribund fish (hereafter termed diseased) were retrieved directly from sea cages by scuba divers, euthanized in 50 ppm Aqui-S, measured for total length (nearest mm) and body weight (nearest g), and sampled for caudal fin tissue (0.5–1.0 cm^2), which was preserved in 95% ethanol. Second sampling was conducted after disease outbreaks had subsided and mortalities had completely ceased, as confirmed by the farm's technical manager and veterinarian. Clinically healthy, post-outbreak fish; these are likely survivors, not naïve healthy fish (hereafter termed post-outbreak) were collected from sea cages using a seine net and scoop net operated from a harvest vessel equipped with a crane. Fish were sedated with 20 ppm Aqui-S prior to length and weight measurements and caudal fin tissue collection. Following sampling, fish were placed in recovery tanks and returned to their original sea cages. Due to the large population sizes within each sea cage, only a subset of individuals was sampled from each cohort. Sample sizes ranged from 1058 to 1223 fish per cohort, comprising both diseased and healthy individuals. In total, 2305 diseased and 2288 healthy fish were sampled across all four cohorts (Table 1).

TABLE 1 | Growth characteristics of farmed barramundi in open sea cages across four spawning cohorts.

Cohort	Sampling date	Health status	Sample size	Age difference (day)	Weight (g) (± SD)	CV (%)	Length (cm) (± SD)	CV (%)
Cohort 1	11/2018	Diseased	622	167	n.r.	n.r.	n.r.	n.r.
	12/2018	Post-outbreak	556		642.4 ± 142.8	22.2	34.6 ± 2.5	7.3
Cohort 2	12/2018	Diseased	496	70	71.6 ± 24.5	34.2	16.4 ± 1.8	10.9
	02/2019	Post-outbreak	638		169.2 ± 43.3	25.5	22.9 ± 1.9	8.3
Cohort 3	02/2019	Diseased	587	148	79.3 ± 24.6	31.0	17.9 ± 2.1	11.7
	03/2019	Post-outbreak	471		675.1 ± 193.1	28.6	33.6 ± 3.5	10.4
Cohort 4	09/2019	Diseased	600	30	189.0 ± 50.1	26.5	22.0 ± 2.3	10.5
	10/2019	Post-outbreak	623		279.0 ± 59.5	21.3	26.5 ± 2.2	8.30

Abbreviations: Age difference, number of days between diseased and healthy sampling; CV, coefficient of variation; n.r., not recorded; SD, standard deviation.

2.3 | Genotyping and Quality Control

Here, ddRADseq libraries were generated exclusively for host genotyping while microbial detection was not a design objective of the original sequencing effort. Briefly, genotyping by sequencing was performed following the protocol of Elshire et al. (2011) with modifications as described by Dodds et al. (2015). Construction of libraries used a double digestion based on *PstI* and *MspI* restriction enzymes, followed by fragment size selection (220–340 bp, including 148 bp of adapter sequence) using a Pippin Prep system. Single-end sequencing (101 bp) was performed on a NovaSeq6000 platform utilizing v1.5 chemistry. Raw reads underwent quality control using FastQC v0.12.1 and trimming with Trim Galore v0.6.10 (Krueger 2015). Bases with Phred-score below 20 (error rates > 1%) were removed and only reads longer than 60 bp were retained. Cleaned reads were then processed using the Stacks v2.6 pipeline (Catchen et al. 2011). Pooled short reads were demultiplexed into the individual samples with no barcode mismatches allowed (barcodes 7–9 bases in length); and only samples with more than 800,000 reads were retained for downstream analyses. High-quality reads were aligned to the barramundi genome (Accession: GCF_001640805.2, accessed September 2024) (Vij et al. 2014) using BWA v0.7.17 (Li 2013). Unmapped reads (UR) and mapped reads (MR) were then separated using SAMtools v1.19.2 (Danecek et al. 2021) for microbiota profiling and genomic relationship matrix construction, respectively.

2.4 | Microbiota Profiling

To examine microbial abundance, unmapped reads from ddRADseq were aligned to the global 'Standard Kraken2 (Wood et al. 2019) database' (https://genome-idx.s3.amazonaws.com/kraken/k2_standard_20240605.tar.gz, accessed on 28/05/2025), which includes a 55 Gb collection of reference genomes spanning a broad range of taxa—from humans and animals to fungi, parasites, bacteria and viruses. Kraken2 was executed using flags '--report-minimize-data', '--report-zero-counts' and '--minimum-hit-group 1' to retain all possible taxonomic assignments, including those with low abundance. This database is absent of some important taxonomy known to be pathogens infecting barramundi farming, thus their genomes were built an additional database. These included a range of viruses: barramundi-associated picorna-like virus 2 (Isolate BarrFG5-6, GenBank: PP767961.1), barramundi calicivirus 1 (GenBank: PP767962.1), barramundi parvovirus (GenBank: BK064846.1), *L. calcarifer* herpesvirus—LCHV (Isolate V511, GeneBank: PP098466.1), Latid herpesvirus 1 (GenBank: BK064844.1), Lymphocystis disease virus 1 (Isolate LCDV1-4/23ZH, GenBank: PP918970.1), Red seabream iridovirus (GenBank: MK689686.1), turbot reddish body iridovirus (GenBank: GQ273492.1) and a bacterium: *S. iniae* (Strain YSFST01-82, RefSeq: GCF000831485.1). Because the custom database included partial or full genomes of pathogens, whereas the standard Kraken2 database classifies taxa using only a subset of diagnostic k-mers, the read counts generated from each database reflect different structures and therefore cannot compare directly. The custom pathogen database was designed to screen for a predefined set of known barramundi-associated pathogens and should not be interpreted as an unbiased survey of microbial diversity.

Given the reduced representation nature of ddRADseq and limited microbial sequencing depth, species- and strain-level taxonomic assignments should be considered putative and interpreted with caution, particularly for closely related taxa such as Vibrio species. Thus, the results of species-level inference will be interpreted as putative or classified within species-level clades only.

Raw read counts were normalized by counts-per-million (CPM) (i.e., read count/total reads $\times 10^6$) to account for differences in sequencing depth. The microbiota communities with the highest relative abundances were characterized at both the genus and species level, focusing on the topmost abundant bacterial and viral taxa across cohorts. To estimate the proportion of reads mapping to bacteria and viruses, we pooled all sequences within each cohort by health status (disease vs. healthy) and

aligned them to the standard database, then visualized the taxonomic composition using Krona (Ondov et al. 2015).

2.5 | SNP Discovery in Barramundi and Pathogen Normalized Read Counts Genetic Analyses

Quantitative genetic analyses were conducted using data from diseased cohorts only, as pathogen-associated read counts identified via Kraken2 in healthy cohorts were negligible. Variant discovery was performed using the Stacks pipeline (Catchen et al. 2011) in reference-based mode, which yielded a total of 269,500 raw variants. Quality control was performed using BCFtools (Danecek et al. 2021), which excluded INDELs, multiallelic SNPs, low-quality mapping raw reads (Phred-score < 30), total mapped reads (< 0.82 million), read depths (< 10 or > 100; i.e., to minimize technical artefacts, PCR duplicates, mitochondrial DNA regions), minor allele frequency (< 0.0001) and missingness for both SNP and individual (> 10%). After filtering, the final dataset comprised of 2078 individuals and 5089 high-quality SNPs for building the genomic relationship matrix of diseased cohorts using the VanRaden (2008) method.

Genetic variance components for pathogen read counts abundance were estimated using a genomic best linear unbiased prediction model by the BLUPF90+ package of the BLUPF90 family programme (Misztal et al. 2002). The first two eigenvectors (i.e., principal components—PCs) were fitted as continuous covariates aiming to account for stratification of relatedness (e.g., family structure evidenced by Figure S2), as proposed by Price et al. (2006). For variance estimation, two univariate models were used for full or cohort data, which include or omit the cohort effect, respectively.

2.6 | Methodological Context and Validation

Although matched qPCR data were not available for all individuals analysed in this study, the cohorts investigated here overlap in origin, production system, disease status, and sampling period with those analysed in a recently published ddRADseq-qPCR validation study on barramundi SDD outbreaks (Terence et al. 2026). In that study, ddRADseq reads derived from fin clips were directly compared with qPCR measurements of SDDV load in both fin and spleen tissues from a nested subset of fish sampled from the same commercial production system. Because individual-level identifiers were not shared between datasets, direct cross-matching was not possible; however, the published study provides an independent methodological validation of the ddRADseq-based pathogen detection framework applied here.

3 | Results

3.1 | Growth Characteristics of Barramundi During Active SDD Outbreaks and Post-Outbreak Periods Across Cohorts

Table 1 presents the growth metrics of farmed barramundi exhibiting clinical signs of SDD and their post-outbreak counterparts across four spawning cohorts reared in open sea cages. For each cohort, diseased and post-outbreak fish were sampled at different time points, with age differences ranging from 30 to 167 days (total fish ages ranged from 235 to 355 day-post-hatch). In Cohort 1, growth data for diseased fish sampled were not recorded. Post-outbreak fish sampled had an average body weight of 642.4 ± 142.8 g and length of 34.6 ± 2.5 cm, respectively. In Cohort 2, diseased fish had a mean weight of 71.6 ± 24.5 g and a length of 16.4 ± 1.8 cm, while post-outbreak fish sampled 70 days later were considerably heavier, averaging 169.2 ± 43.3 g and 22.9 ± 1.9 cm in weight and length, respectively. A similar pattern was observed in Cohort 3. In Cohort 4, which had the shortest age gap between samplings (30 days), diseased fish averaged 189.0 ± 50.1 g and 22.0 ± 2.3 cm, while post-outbreak fish reached 279.0 ± 59.5 g and 26.5 ± 2.2 cm. Across all cohorts, post-outbreak fish consistently exhibited greater body weights and lengths and lower CVs than their diseased counterparts.

3.2 | Descriptive Statistics of Unmapped Reads From ddRADseq and SDD-Associated Pathogens

Table 2 summarizes the statistics of unmapped reads from ddRADseq reads that failed to align to the barramundi genome and were subsequently taxonomically classified using Kraken2 across four spawning cohorts. The average of these unaligned reads accounted for approximately 3.4% (standard deviation of 1.4%) of total ddRADseq data (96.8 of 2546 Gb) and were analysed to characterize the microbial community composition, focusing on bacterial and viral taxa, including known key pathogens related to SDD such as *V. harveyi*, SDDV and ISKNV.

On average, 19 Mb per sample (SD = 9.3, Min = 3.4, Max = 286.0 Mb) (averaged 110,000 reads; Figure S1) were unmapped reads. Across cohorts, diseased fish consistently exhibited a higher proportion of read counts classified by Kraken2 compared to post-outbreak fish (7.99% vs. 5.07%). Diseased fish ($n = 2307$) yielded over 19.4 million read counts, with 7.99% classified by Kraken2, of which 7.39% were bacterial and 0.06% were viral. In contrast, post-outbreak fish ($n = 2288$) produced 10.7 million read counts, with 5.07% classified (4.51% bacterial and 0.002% viral).

Because post-outbreak fish were sampled weeks to months after diseased fish, observed differences may include temporal shifts rather than purely disease-driven effects.

3.3 | Abundance of Viral and Bacterial Communities at Species and Genus Levels

Viral community profiles differed markedly between post-outbreak and diseased fish across cohorts at both the genus and species levels (Figure 1). At the species level (Figure 1A), SDDV was the most dominant virus in all diseased fish, accounting for over 77.9% of the total viral community. ISKNV was also more prevalent in diseased fish compared to post-outbreak fish, particularly in Cohort 2 (6.3%) and Cohort 3 (13.4%). In contrast, post-outbreak fish displayed elevated higher proportions of *Alphanudivirus quartudromelanogasteris* (17.4%–55.5%), *Cyvirus anguillidallo2* (23.7%–51.6%), and *Stenotrophomonas* phage S1 (9.6%–54.7%, except Cohort 3 with only 0.63%), all of which were minimally detected in diseased individuals. At the genus level (Figure 1B),

Megalocytivirus dominated the viral community in all diseased cohorts (77.9%–90.4%), whereas post-outbreak fish showed higher relative abundances of *Alphanudivirus* (17.4%–55.5%) and *Cyvirus* (23.7%–51.8%). Additionally, the customized pathogen genome database revealed relatively low average read counts and prevalence for *L. calcarifer* herpesvirus (LCHV) and Latid herpesvirus 1, with

TABLE 2 | Statistics of unmapped reads from ddRADseq data (UR) and percentage of alignment for bacteria and viruses across four barramundi cohorts for diseased and post-outbreak fish using the standard Kraken2 database.

Cohort	Status	Unmapped ddRADseq (UR)	Mapped to standard database (%)	Bacteria (%)	Virus (%)
Cohort 1	Post-outbreak	1,544,199	3.14	2.53	0.0009
	Diseased	3,623,682	6.09	5.51	0.0400
Cohort 2	Post-outbreak	7,652,996	11.62	11.14	0.0050
	Diseased	3,589,895	6.55	5.91	0.0500
Cohort 3	Post-outbreak	452,139	1.17	0.50	0.0002
	Diseased	3,170,564	5.05	4.91	0.0700
Cohort 4	Post-outbreak	1058,801	1.84	1.31	0.0008
	Diseased	9,106,080	12.66	12.08	0.0600
Healthy	Post-outbreak	10,708,135	5.07	4.51	0.0020
Diseased	Diseased	19,490,221	7.99	7.39	0.0600

Note: % were extracted from Krona plots.
Abbreviations: Bacteria (%) and Virus (%), proportions relative to total unmapped reads from ddRADseq data; Mapped to standard database (%), proportion of UR reads taxonomically classified; UR, total number of reads unaligned to the barramundi genome.

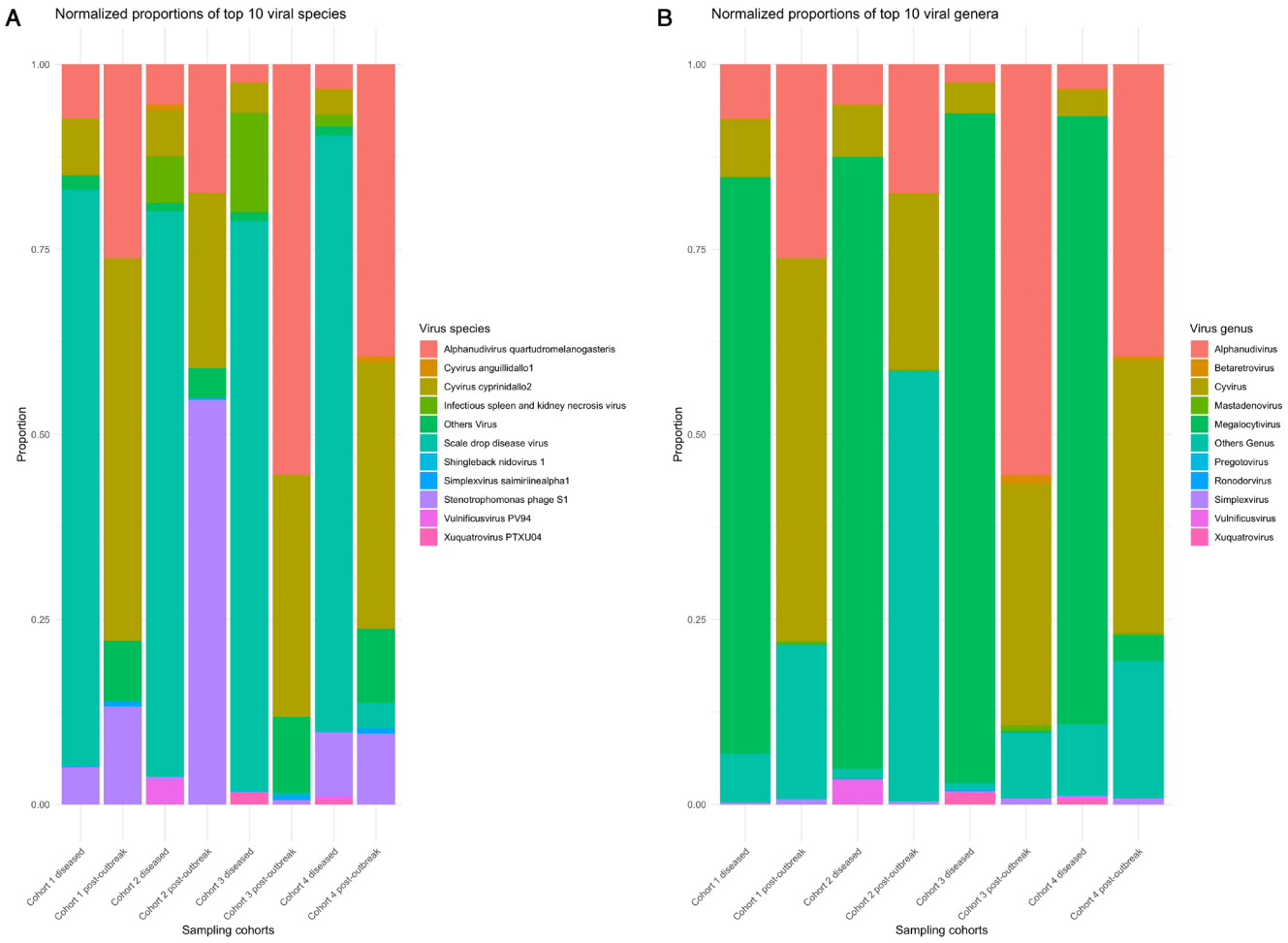

FIGURE 1 | Viral community composition in barramundi fin tissues as relative abundance of the top 10 viral abundances at the species (A) and genus (B) levels across four cohorts.

mean read counts of 0.17 (prevalence: 17.8%) and 0.13 (prevalence: 16.1%), respectively (Table 3). No reads were detected for other viral pathogens of interest, including barramundi calicivirus, barramundi parvovirus, lymphocystis disease virus, red seabream iridovirus, and turbot reddish body iridovirus.

The composition of bacterial and viral communities at both genus and putative species levels is shown in Figure 2. The bacterial community composition in post-outbreak and diseased fish revealed that *Stenotrophomonas* and *Burkholderia* were the most dominant genera across cohorts (Figure 2A). However, their relative abundances markedly declined in the diseased fish from Cohorts 2 and 3, which corresponded with a sharp increase in *Vibrio* spp. (85.4% in Cohort 2 and 86.8% in Cohort 3). At the species level (Figure 2B), *Stenotrophomonas maltophilia* and *Burkholderia contaminans* were the most prevalent taxa in both post-outbreak and diseased fish across most cohorts, except for Cohorts 2 and 3. In these diseased cohorts, *Vibrio* species collectively represented 68.9% (Cohort 2) and 64.0% (Cohort 3) of the bacterial community, with *V. harveyi* being the most dominant species, comprising 39.3% in Cohort 2 and 42.6% in Cohort 3 (Figure 2B,C). Other enriched *Vibrio* species/strains included *V. campbellii*, *V. parahaemolyticus*, *V. owensii*, *V. rotiferianus*, *V. tubiashii*, *V. panuliri* and *Vibrio* spp. (Figure 2C). Additionally, *Tenacibaculum maritimum* was detected in diseased fish at relative abundances of 1.3% (Cohort 2) and 4.7% (Cohort 3) (Figure 2C). All detected *Vibrio* spp. and *T. maritimum* are well-documented as pathogenic or opportunistic bacteria in aquatic animals. In Cohorts 1 and 4, bacterial community profiles were more similar between diseased and post-outbreak fish; both displayed an increased relative abundance of *V. harveyi* in the diseased individuals.

3.4 | More Putative Pathogens Were Detected Using Custom Database

Mapping against partial and complete pathogen genomes did not detect Barramundi-associated picorna-like virus 2, Barramundi calicivirus 1, Barramundi parvovirus, Lymphocystis disease virus 1, or Turbot reddish body iridovirus (Table 3). Other viral pathogens, including red seabream iridovirus, *L. calcarifer* herpesvirus, and Latid herpesvirus 1, were detected at low normalized read counts (0.04–0.11) and low prevalence (2.4%–17.8%). In contrast, *S. iniae* was identified as the predominant pathogen, with a high prevalence (90.7%) and an average normalized read count of 1.19 across samples. It should be noted that these findings were based on mapping to a custom pathogen database and may reflect limitations in reference genome completeness and taxonomic resolution inherent to k-mer-based classification approaches.

3.5 | Concurrent Infection Between SDDV, LCHV, ISKNV and *V. harveyi*

Across all cohorts, diseased fish exhibited substantially higher rates of co-infection compared to post-outbreak individuals (Table 4). The majority of diseased fish carried two or more pathogens, with 68.1% and 66.5% of fish in Cohorts 3 and 4, respectively, showing dual infections. In contrast, over 70%–85% of post-outbreak fish carried no detectable pathogens, and co-infections involving three or four agents were rare. Notably, co-infections with three pathogens occurred in up to 18.7% of diseased fish (Cohort 3), while no post-outbreak fish showed evidence of four-pathogen infection (Table 4). These findings underscore a microbial shift towards a pathobiome, in which multiple infectious agents are concurrently present during disease expression, supporting the concept that polymicrobial interactions may play a central role in SDD outbreaks.

3.6 | Estimated Genetic Variances of Pathogen Normalized Read Counts Associated With SDD

The genetic and phenotypic variances for pathogen read counts abundances within individual cohorts and across the combined dataset are summarized in Table 5. Among viral pathogens detected, SDDV exhibited the relatively highest prevalence (92.9%) with low heritability of 8%. The results revealed notable

TABLE 3 | Detection summary of potential putative pathogens in farmed barramundi, including genome sizes, normalized read counts and prevalence across sampled individuals using custom database.

Pathogen	Genome size (base)	Normalized read counts[b]	Prevalence (%)
Barramundi associated picorna-like virus 2	8486[a]	0	0
Barramundi calicivirus 1	7128[a]	0	0
Barramundi parvovirus	1126[a]	0	0
Lates calcarifer herpesvirus[c]	153,008	0.11	17.8
Latid herpesvirus 1[c]	104,963	0.11	16.1
Lymphocystis disease virus 1	102,661	0	0
Red seabream iridovirus	112,710	0.04	2.4
Turbot reddish body iridovirus	110,104	0	0
Streptococcus iniae	5,488,947	1.19	90.7

Note: [a]Partial genomes, [b]read counts were normalized by sequencing depth for 100k base of genome. [c]Latid herpesvirus 1 and *Lates calcarifer* herpesvirus (LCHV) are closely related viral strains infecting barramundi, sharing high genomic similarity and likely representing variants of the same species within the *Alloherpesviridae* family.

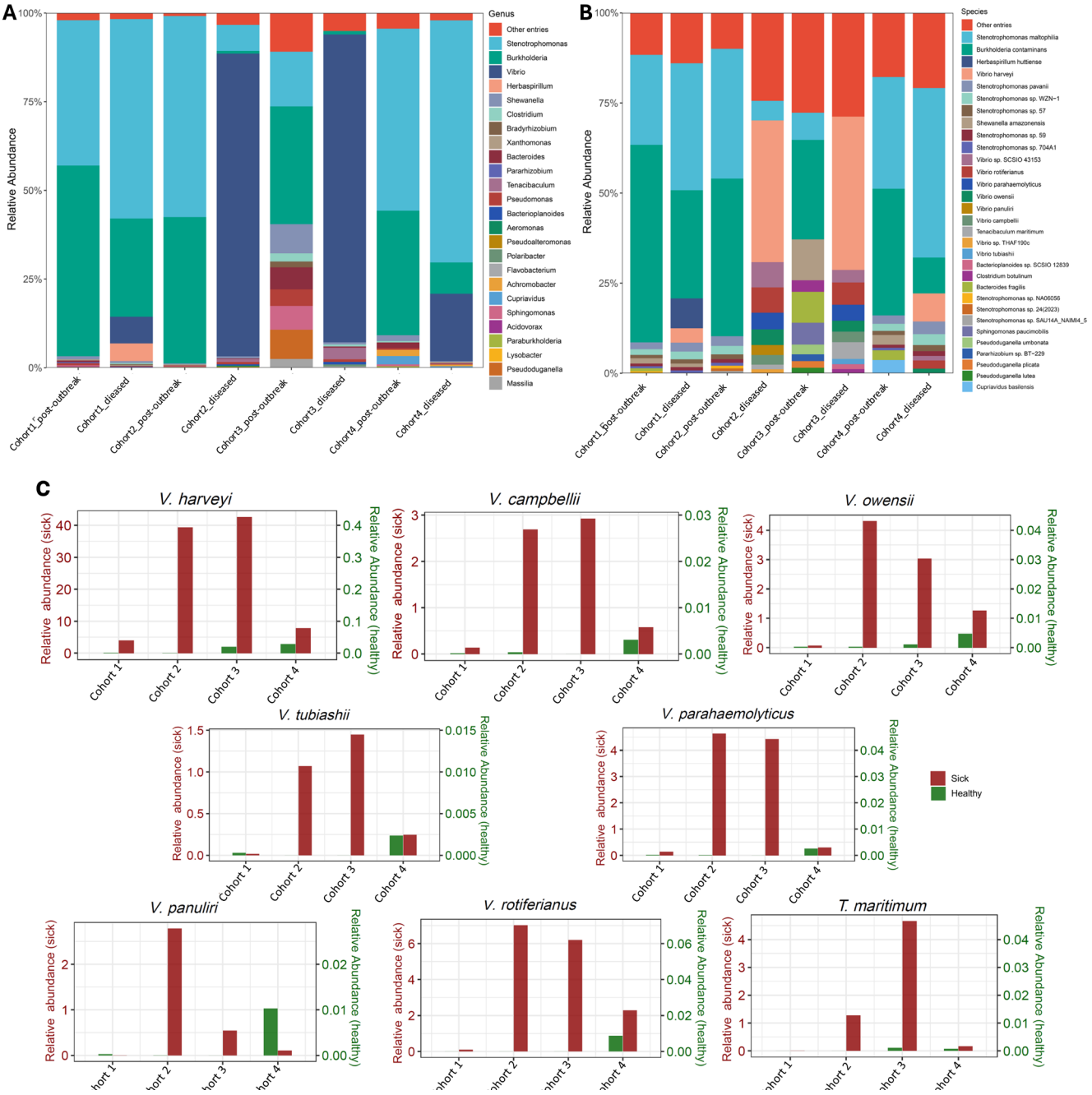

FIGURE 2 | Composition and relative abundance of bacterial communities in fin tissues of barramundi. (A, B) Relative abundance of the top 10 taxa at the genus and putative species levels across four cohorts. (C) Comparison of pathogenic and opportunistic bacterial genera between diseased and post-outbreak fish across cohorts with relative abundance (%, y-axis).

cohort-specific variability, evidenced by proportions of additive genetic variance ranging from 0.08 ± 0.06 to 0.23 ± 0.08, with the highest heritability observed in Cohort 2 (0.23 ± 0.08) followed by Cohort 4 (0.14 ± 0.06), suggesting a higher contribution of additive genetic influence on levels of SDDV abundance in these cohorts. Conversely, ISKNV and LCHV showed much lower prevalence (2.4% and 17.8%) with minimal read counts abundance (mean 0.04 and 0.11 read counts/sample), revealing no heritable component. *V. harveyi* read counts were comparable to SDDV, but small and insignificant heritability values were estimated. The same trend was observed for *S. iniae*, with prevalence of 90.7 but showed no heritable component of read counts.

4 | Discussion

Disease remains a major constraint to sustainable aquaculture, including the farming of barramundi. Improving disease management requires deeper understanding of pathogen prevalence and how host-associated microbial communities shift from a 'microbiome' towards a disease-associated 'pathobiome'. Using unmapped reads from a large ddRADseq dataset spanning four commercial sea-cage cohorts, we observed pronounced differences in bacterial and viral community compositions between clinically diseased and post-outbreak fish, providing insights into microbial dynamics and interactions

TABLE 4 | Concurrent infections of four pathogens (SDDV, LCHV, ISKNV and *Vibrio harveyi*) in diseased and healthy fish across cohorts.

Cohort	Status	No pathogen (%)	One pathogen (%)	Two pathogens (%)	Three pathogens (%)	Four pathogens (%)
Cohort 1	Disease	10.5	43.9	42.6	3.1	0.0
	Post-outbreak	85.1	11.9	2.7	0.4	0.0
Cohort 2	Disease	0.2	17.3	65.1	17.3	0.0
	Post-outbreak	70.4	22.1	7.2	0.3	0.0
Cohort 3	Disease	0.2	12.1	68.1	18.7	0.9
	Post-outbreak	53.1	42.0	2.8	2.1	0.0
Cohort 4	Disease	0.2	30.8	66.5	2.5	0.0
	Post-outbreak	82.7	14.9	1.9	0.5	0.0
All		38.2	24.2	32.1	5.4	0.1

Note: Values represent the percentage of fish in each health group and cohort with 0 to 4 pathogens detected.

TABLE 5 | Genetic variance (V_a), residual variances (V_e) and heritability (h^2) of three estimates for scale drop disease virus (SDDV), *Lates calcarifer* herpesvirus (LCHV), infected spleen and kidney virus (ISKNV), *Vibrio harveyi* and *Streptococcus iniae* read counts abundance across four diseased cohorts and combined data. Heritability (h^2) reflects the proportion of total phenotypic variance explained by additive genetic effects.

Cohort	Pathogen	Normalized read counts	Prevalence (%)	Genetic variance (V_a)	Phenotypic variance (V_p)	$h^2 \pm$ SE
Cohort 1	SDDV	6.48	86.5	25.2	349.3	0.07 ± 0.07^{ns}
Cohort 2	SDDV	10.86	95.4	66.5	284	$\mathbf{0.23} \pm 0.09$
Cohort 3	SDDV	15.98	90.8	35.9	443.3	0.08 ± 0.06^{ns}
Cohort 4	SDDV	9.87	98.7	52.1	369.6	$\mathbf{0.14} \pm 0.06$
All	SDDV	10.70	92.9	28.0	359.5	$\mathbf{0.08} \pm 0.03$
All	LCHV	0.11	17.8	0.001	0.24	0.01 ± 0.01^{ns}
All	ISKNV	0.04	2.4	0.00	28.74	0.00 ± 0.00^{ns}
All	*V. harveyi*	6.59	88.4	25.3	349.4	0.07 ± 0.07^{ns}
All	*S. iniae*	1.19	90.7	61.63	847.2	0.01 ± 0.01^{ns}

Note: ns, not significant estimate; heritability without ns is significant estimate ($p < 0.05$).

among pathogen burden, microbiota composition and host health status.

4.1 | Viral Patterns and Co-Infections

Viral community analysis revealed a substantial enrichment of SDDV and low prevalence of ISKNV, both members of the *Megalocytivirus* genus in the *Iridoviridae* family, and both recognized as major viral threats in barramundi aquaculture across Southeast Asia (de Groof et al. 2015; Nurliyana et al. 2020; Senapin et al. 2019; Shen et al. 2026). SDDV primarily affects barramundi juveniles (~50 to 500 g) reared in sea cages, causing scale loss, darkened bodies, fin and tail erosion, and mortality rates of 40%–50% of cultured stock (de Groof et al. 2015; Domingos et al. 2021; Senapin et al. 2019). ISKNV is associated with similar or even higher mortality rates, posing significant risks to barramundi aquaculture operations (Kerddee et al. 2021; Sun et al. 2024; Zhu et al. 2021). LCHV was also detected at low prevalence, consistent with previous documentation of SDDV–LCHV co-infection in barramundi under commercial conditions (Domingos et al. 2021). In contrast, post-outbreak fish exhibited higher proportions of *A. quartudromelanogasteris*, *C. anguillidallo2* and *Stenotrophomonas* phage S1, although their roles in aquatic animal health remain poorly defined. It is hypothesized that some viruses, including bacteriophages, may contribute to microbial homeostasis by suppressing opportunistic pathogens or modulating immune responses. For instance, *Stenotrophomonas* phage S1, which targets *Stenotrophomonas* species, including *S. maltophilia*, may play a regulatory role in maintaining microbial balance (McCutcheon and Dennis 2021).

4.2 | Bacterial Dysbiosis and Opportunists

Across cohorts, *S. maltophilia* and *B. contaminans* were abundant across both post-outbreak and diseased fish, although their pathogenicity in barramundi remains uncertain. While

these bacteria are opportunistic pathogens known to associate with respiratory infections in humans (Brooke 2021; Tavares et al. 2020), their presence here may reflect environmental exposure or colonization rather than direct causation of disease. Notably, a marked decline in their relative abundance in the diseased fish from Cohort 2 and Cohort 3, coupled by an enrichment of *Vibrio* species, suggested a shift towards *Vibrio*-dominated dysbiosis during disease progression. While many *Vibrio* species are non-pathogenic and naturally present in healthy aquatic animals, certain species and strains are pathogenic, causing a variety of vibriosis diseases (Lan et al. 2024; Novriadi 2016; Sheikh et al. 2022). The mortality rate associated with *Vibrio* infections in barramundi can range from 40% to 100% depending on the outbreak contexts (Dong, Jitrakorn, et al. 2017; Gibson-Kueh et al. 2012). In this study, *V. harveyi* was the most abundant species in diseased fish (39.2% in Cohort 2 and 42.3% in Cohort 3), consistent with its established role in vibriosis of barramundi, characterized by tissue necrosis and rapid transmission in sea-cage farming systems (Chew et al. 2023; Chin et al. 2020; Dong, Jitrakorn, et al. 2017; Ransangan et al. 2012). Other detected *Vibrio* species, including *V. campbellii*, *V. owensii*, *V. parahaemolyticus* and *V. rotiferianus*, are also known as causative agents of vibriosis in fish, shrimp and decapod crustaceans, leading to significant mortality rates and economic losses (Aly et al. 2024; de Souza Valente and Wan 2021; Haifa-Haryani et al. 2022, 2023; Harrison et al. 2022; Lan et al. 2024). Additionally, *T. maritimum*—the agent of tenacibaculosis—was detected in diseased fish from Cohorts 2 and aligning with reports of ulcerative disease and high cumulative losses in marine fish, including barramundi (Miyake et al. 2020). Detection of bacterial taxa such as *Vibrio* spp. must therefore be interpreted in a pathobiome context rather than as evidence of systemic infection, particularly given the ubiquity of Vibrio in marine environments. Nevertheless, the consistent enrichment of SDDV, *V. harveyi* and *T. maritimum* in fish sampled during active outbreaks across multiple cohorts suggests biologically meaningful disease-associated microbial shifts rather than random environmental contamination.

4.3 | Host Genetics and Pathogen Proxy Traits

This study also explored the heritability of pathogen-associated read-count traits (SDDV, LCHV, ISKNV, *S. iniae* and *V. harveyi*), treating normalized read counts as proxy phenotypes. Host genetic effects on pathogen burden have been documented in aquaculture species, including moderate heritability for viral loads in banana shrimp (*Fenneropenaeus merguiensis*) (e.g., hepatopancreatic parvovirus and gill-associated virus, $h^2 = 0.41 \pm 0.08$) (Phuthaworn et al. 2016) and gill-associated virus (GAV) ($h^2 = 0.21 \pm 0.07$) in *Penaeus monodon* (Noble et al. 2020). In this study, SDDV normalized read counts showed low heritability across the entire dataset ($h^2 = 0.08$ or 8%), with higher values in some cohorts (up to 23%), consistent with strong environmental influences, and/or complex co-infection dynamics typical of open-sea farming system (Defoirdt 2014; Poon et al. 2026). Resolving these processes likely requires richer longitudinal designs and higher dimensional datasets, for example host transcriptomics, meta-transcriptomics, functional profiling (Canellas et al. 2022; Chai et al. 2025; Natnan et al. 2021; Nguyen et al. 2025).

4.4 | Compare Fin-Clip ddRADseq Versus Shortgun Metagenomic Sequencing and Targeted Screening Methods

Shotgun metagenomic sequencing provides far greater taxonomic resolution and functional insight, often enabling species- or strain-level classification and reconstruction of metabolic pathways and virulence factors (Quince et al. 2017). Recent metagenomic studies in aquaculture have demonstrated the power of this approach for pathogen discovery and functional profiling (Macedo et al. 2024). Nevertheless, shotgun metagenomics typically requires deep sequencing, host DNA depletion, and specialized library preparation, making it costly and logistically challenging to apply at the scale of thousands of commercial fish across multiple cohorts (Liu et al. 2017). As a result, most metagenomic studies in aquatic animals remain limited to relatively small sample sizes or controlled experimental settings.

The ddRADseq approach used here occupies an intermediate methodological space. Reduced-representation sequencing captures fragmented microbial DNA incidentally, resulting in lower sequencing depth and strong biases linked to restriction enzyme site distribution and fragment size selection (Liu et al. 2017). Consequently, unmapped ddRADseq reads should be interpreted as semi-quantitative proxies of relative microbial and pathogen abundance rather than absolute measures of infection intensity. Species- and strain-level assignments, particularly among closely related taxa such as *Vibrio* spp., are therefore best viewed as putative classifications rather than definitive identifications.

Despite these limitations, several features distinguish ddRADseq-based surveillance from both shotgun metagenomics and targeted diagnostics. First, the ability to repurpose existing genotyping datasets enables retrospective pathogen screening at minimal additional cost, a critical advantage in large-scale breeding and production contexts. Second, when applied consistently across large cohorts, ddRADseq-derived microbial signals can robustly capture dominant pathogens and major disease-associated community shifts, as demonstrated here for SDDV, *V. harveyi* and *T. maritimum*. Notably, similar ddRADseq-derived viral signals from fin tissue have been independently validated against qPCR measurements in barramundi, showing strong concordance despite reduced sensitivity at low pathogen loads (Terence et al. 2026).

Importantly, our findings align with previous qPCR-, histopathology- and sequencing-based studies of SDD outbreaks, which consistently report SDDV as the dominant viral agent alongside frequent bacterial co-infections, particularly involving *Vibrio* spp. (Domingos et al. 2021; Dong, Jitrakorn, et al. 2017; Dong, Taengphu, et al. 2017; Nurliyana et al. 2020). The concordance between ddRADseq-derived patterns and established diagnostic approaches supports the biological relevance of the detected

signals, while underscoring the complementary nature of this method.

Taken together, ddRADseq-derived microbial profiling should not be viewed as a replacement for targeted diagnostics or shotgun metagenomics. Rather, it represents a low-resolution, population-scale surveillance and hypothesis-generation tool that can guide targeted follow-up using qPCR, histopathology, or metagenomic sequencing. In the context of commercial aquaculture, where large genotyping datasets are increasingly generated for selective breeding, the integration of ddRADseq-based pathogen screening offers a pragmatic framework for linking host genetics, disease outbreaks, and pathobiome dynamics at an unprecedented scale.

4.5 | Methodological Limitations and Interpretation

Our approach differs fundamentally from standard shotgun metagenomic or targeted qPCR workflows in that the sequencing data were generated for host genotyping rather than microbiome profiling. Consequently, both DNA extraction and library preparation were optimized to enrich host genomic DNA from fin tissue, with microbial DNA captured only incidentally. In contrast, shotgun metagenomics typically minimizes host DNA to maximize recovery of microbial genomes (Quince et al. 2017), while qPCR targets specific pathogen loci with high analytical sensitivity and specificity (Sriisan et al. 2020; Yin et al. 2024). As a result, unmapped ddRADseq reads should be interpreted as semi-quantitative proxies of relative pathogen and microbiome abundance, rather than absolute measures of pathogen load. This interpretation is further shaped by technical biases inherent to the ddRADseq protocol, which employs *PstI* and *MspI* restriction enzymes and size selection of 220–340 bp fragments. Only genomic regions flanked by compatible restriction sites are represented, meaning that pathogens with lacking suitable recognition motifs may be under-represented or entirely missed. In silico digestion indicated that 127 fragments from the SDDV genome and 8374 fragments from the *V. harveyi* genome fall within the targeted RAD fragment size range, supporting their detectability with this protocol. Nevertheless, the dominance of host DNA and the absence of microbial-specific extraction steps reduce effective sequencing depth for microbes and limit sensitivity for low-abundance taxa relative to dedicated metagenomic libraries.

A further limitation of this study is the absence of individual-level qPCR or shotgun metagenomic validation for the same samples analysed. However, the methodological robustness of ddRADseq-based pathogen detection has recently been independently validated in barramundi using fin-clip DNA, where ddRADseq viral read counts were strongly correlated with qPCR-measured SDDV loads in both fin and spleen tissues (Spearman's $\rho = 0.76–0.84$; Terence et al. (2026)). That study reported no false positives from ddRADseq relative to qPCR, although reduced sensitivity was observed at low viral loads—an expected outcome given restriction-enzyme-based library construction and the absence of target amplification. Consistent with these findings, the strong enrichment of SDDV and *V. harveyi* in clinically diseased fish and their near absence in post-outbreak individuals observed here aligns with previous qPCR, 16S sequencing and histopathological studies of SDD outbreaks in barramundi (Domingos et al. 2021; Dong, Taengphu, et al. 2017; Nurliyana et al. 2020). Together, these observations support the use of ddRADseq unmapped reads as a proxy indicator of relative pathogen presence and abundance at the population level, while emphasizing that absolute quantification and detection of low-level infections require targeted validation. This caveat applies particularly to the quantitative genetic analyses, where heritability estimates reflect variance in sequence-derived proxy traits rather than direct measures of pathogen burden.

Tissue tropism represents an additional limitation of this study. Fin tissue is externally exposed to seawater environments and may harbour environmental or surface-associated microbes, potentially inflating signals from waterborne taxa. However, non-destructive tissues such as fins and skin have been shown to reliably detect several key viral pathogens in barramundi, including SDDV, ISKNV and LCHV, with high concordance to internal-organ diagnostics (Charoenwai et al. 2021; Terence et al. 2026). Additionally, custom reference databases inherently reflect a priori knowledge and may bias detection towards included taxa while under-representing uncharacterized or divergent pathogens. Consequently, results derived from the custom database are best interpreted as targeted surveillance signals rather than comprehensive pathogen discovery.

Finally, sampling timing differed between diseased and post-outbreak fish, which may confound disease-associated effects with temporal/environmental variation (Huang et al. 2018). Future studies should implement longitudinal sampling across disease progression and integrate targeted diagnostics (PCR/qPCR), histopathology and/or meta-transcriptomics to improve causal inference and functional interpretation (Macedo et al. 2024; Zhao et al. 2024). Despite these limitations, repurposing unmapped reads from ddRADseq datasets offers an exploratory, practical, cost-effective framework for retrospective screening and broad microbial profiling in aquaculture populations (Goldberg et al. 2015). While unmapped-read approaches have been proposed for pathogen discovery (Richard and Poulicard 2025), their application to ddRADseq in aquaculture has been little explored. Here, we demonstrated that this strategy could recover signals consistent with key pathogens (SDDV, LCHV, ISKNV, *S. iniae*) and disease-associated community shifts, supporting its value as part of an integrated surveillance toolbox.

5 | Conclusion

This study showed that unmapped reads from ddRADseq fin-clip datasets can be repurposed as retrospective, population-level resources for screening SDDV and for exploring disease-associated microbiome-pathobiome patterns in farmed barramundi. Across multiple cohorts, fish sampled during active diseased outbreaks consistently showed strong enrichment of SDDV-associated signals and shifts towards *Vibrio*-dominated bacterial communities, particularly reads classified within the *V. harveyi* clade, alongside the detection of other clinically relevant agents such as LCHV, ISKNV and *T. maritimum*. Treating normalized pathogen read counts as proxy traits suggested

low overall heritability for the SDDV-associated signal, with cohort-specific variation indicating strong environmental effects and complex co-infection dynamics typical of open-sea farming systems. Taken together, these findings supported the use of ddRADseq-derived microbial profiling as a cost-effective, complementary strategy to targeted diagnostics for broad-scale pathogen surveillance and hypothesis generation, while emphasizing that confirmatory diagnostics remain essential for clinical interpretation and disease management in barramundi aquaculture.

Author Contributions

Nguyen Thanh Vu: conceptualization, methodology, writing – original draft, software, writing – review and editing, formal analysis, visualization, validation, data curation. **Jiun-Yan Loh:** conceptualization, investigation, writing – review and editing. **Dean R. Jerry:** conceptualization, funding acquisition, project administration, supervision. **Jose A. Domingos:** conceptualization, funding acquisition, supervision, project administration. **Celestine Terence:** conceptualization, writing – review and editing, resources. **Susan Gibson-Kueh:** investigation, writing – review and editing. **Xueyan Shen:** conceptualization, methodology, software, writing – review and editing, writing – original draft, visualization, formal analysis, data curation, validation. **Zhi Weng Josiah Poon:** conceptualization, writing – review and editing, resources. **Maura Carrai:** conceptualization, writing – review and editing. **Sarah Priyanka Nelson:** conceptualization, writing – review and editing, resources. **Ha Thanh Dong:** conceptualization, investigation, writing – review and editing. **Saengchan Senapin:** conceptualization, writing – review and editing, investigation.

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Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw ddRADseq sequencing data have been deposited in the NCBI SRA under BioProject accession PRJNA1393218. Filtered SNP datasets and associated metadata are available via Figshare (https://doi.org/10.6084/m9.figshare.30929762).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Data distribution of unmapped reads in Mb across individuals. **Figure S2:** Principal component analysis for the first two components inferred from genomic relationship matrix of the full data. The first two PCs may indicate the family structures in each cohort.