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REVIEW

REVIEWS IN Aquaculture

Towards a 'systems' approach for viral challenge experiments in shrimp: Reporting guidelines for publication

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Abstract

The success of contemporary disease management strategies in shrimp aquaculture, such as the 'systems' approach, is predicated on robust knowledge of the conditions and interactions between the host, pathogen and environment that promote disease. Pathogen challenge experiments (PCEs) are a power tool for investigating these conditions and interactions. However, absence of accurately reported experimental detail in published PCEs limits scientific transparency, reproducibility, and the potential for the research to make progressive advancements contributing to contemporary shrimp disease management strategies. This review identifies and discusses key factors relating to the host (shrimp), pathogen (virus), and environment that should be carefully considered during the design and publication of PCEs. We offer substantial evidence of their impact on viral disease outcomes, drawn from the existing body of literature, to supporting their consideration. The prevalence of reported experimental details for these factors across 186 viral PCEs in shrimp were evaluated. The review highlights a concerning paucity of experimental detail reported in published shrimp PCEs. We propose a checklist for the minimum reportable information in the publication of shrimp viral PCEs, hereafter referred to as the Shrimp PCE Reporting Guidelines (SPERG). The guidelines aim to enhance the transparency and standardisation of reporting in published PCEs, ensuring that key factors pertaining to the shrimp, pathogen, and environment are adequately considered and documented. Adoption of SPERG is envisaged to empower researchers, reviewers, and readers to assess the internal and external validity of PCEs, facilitating critical evaluation and improved utility of PCE findings for contemporary disease management.

KEYWORDS
best practice, bioassay, metadata, transmission trial, virus

1 | INTRODUCTION

Since the initial discovery of viral a pathogen in shrimp aquaculture[1] periodic reduction in global production volume has historically aligned with the emergence of significant pathogens, including infectious hypodermal and haematopoietic necrosis virus (IHHNV, *syn Decapod penstylhamaparvovirus 1*), Monodon baculovirus (MBV, *syn Penaeus monodon* nudivirus), Taura syndrome virus (TSV), yellow head virus (YHV), white spot syndrome virus (WSSV), acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus* (VpAHPND),

Enterocytozoon hepatopenaei, and Decapod iridescent virus 1 (DIV1).[2–4] Viral diseases continue to limit the growth and sustainability of global shrimp aquaculture, with the cost of annual losses estimated to be billions of dollars.[2,5,6] The management of disease in shrimp production is challenged by the limited capacity for conventional disease prevention strategies such as vaccination,[7] which have proven pivotal to the success of other major production species including salmonids.[8] Shrimp aquaculture is further challenged by a limited ability to effectively implement biosecure controls to prevent pathogen entry and disease outbreaks.[9] Primarily contributing to this limited control is the concentration of shrimp production in warm coastal regions in pond-based production systems,[10] which are subject to climatic and environmental fluctuation and high levels of exchange with the external environment.[11] In some production regions, farms are also in close proximity to each other or are connected via water source, increasing the risk of pathogen transmission among farms.[12] As a consequence, disease management in shrimp aquaculture must consider the collective influence of factors within the production system on disease outcomes. Recently, advanced molecular technologies have begun to illustrate how the various factors within aquatic production systems interact to manifest in disease.[13–16] Contemporary strategies to advance disease management in shrimp aquaculture advocate a holistic 'systems' approach, which considers disease as the outcome of host, pathogen, and environmental interactions.[3,15]

Investigation to understand the relationships between the shrimp host, pathogen, and environment and how they interact to yield disease is required for the successful administration of a 'systems' approach to disease management.[3] Generally, disease investigations and pathogen challenge experiments (PCEs) are the primary means for developing knowledge regarding disease and its associated or causal drivers. In some cases, such as infection with WSSV, a correlation-causal conclusion generated via in situ disease investigations or field studies is robust because the presence of WSSV in high copy number is consistently associated with rapid onset of mass mortality.[17] Studying the outcomes of viral disease in situ is relevant to understanding the impact of disease under the complex influence and interactions of factors within the whole system.[18] However, for many other viral pathogens, high copy number detection is not always associated with poor productivity or mortality.[19] In complex cases where the disease outcome does not involve a rapid onset of disease, such as sub-optimal or retarded growth, the ability to define the impact of a specific pathogen within the system is further reduced. Under such conditions, poor control over the conditions in the 'system' limits the confidence and robustness of in situ studies, permitting only associative conclusions that should be cautiously considered.[20,21] For example, reduced or retarded growth in *P. monodon* (so-called *P. monodon* slow growth syndrome) has been associated with a range of viral pathogens including, but not limited to, hepatopancreatic parvovirus,[22–25] MBV,[23–25] Laem Singh virus,[26,27] and IHHNV.[28] In this case, no conclusive causal relationships were able to be demonstrated by these investigative studies due to insufficient control over the conditions of the in situ study system. As a result, the cause of *P. monodon* slow growth syndrome remains elusive, limiting the capacity for the syndrome to be effectively managed.[21]

Considering the difficulty of evaluating the conditions driving disease in situ, due to the complexity of production systems, a complementary or alternative strategy is required for more robust investigations. PCEs (*syn* disease transmission trials or bioassays) involve deliberate, standardised exposure ('challenge') of a study population with a disease agent. PCEs enable the study of host health and disease progression under controlled conditions, allowing for causal relationships to be defined and quantified.[29] PCEs are an important and powerful tool for resolving the impact of pathogens on shrimp health and in the development and elucidation of novel disease prevention technologies, such as immunostimulants,[30,31] and genetic selection.[32] Knowledge gained through PCEs may be used to supplement and support disease investigations, or aid in disease management applying a 'systems' approach. However, for the findings of PCEs to be effectively translated and applied to benefit commercial production, PCEs must be conducted and reported upon accurately and robustly. In the absence of accurate reporting of experimental detail from PCEs, the internal validity, relating to the experimental rigour of the study, and external validity, relating to the capacity for the research findings to be contextualised and integrated into a 'systems' framework, are severely minimised.

Guidelines for standardised reporting of challenge trials are well established within the fields of human medicine (Consolidated Standards of Reporting Trials)[33] and livestock veterinary studies (Reporting Guidelines for Randomised Controlled Trials for Livestock and Food Safety).[34–36] Such standards have been developed to encourage 'best practice' for the design and reporting of experiments, facilitating transparency and reproducibility, and improving the capacity for consumers of the research to evaluate the internal and external validity of the studies. Few guidelines of a similar nature with relevance to research on aquatic species are available,[37–39] for example the MISA guidelines (Minimum Information required to support a Stimulant Assessment experiment) for studies investigating immunostimulants in crustaceans.[40] However, more widespread availability and adoption of such reporting standards is still required. For example, to maximise the value of PCE studies for disease management in shrimp production, guidelines of a similar principle with specificity to shrimp PCEs are needed. With this aim, the current review identifies characteristics pertaining to the shrimp, pathogen (virus), and environment, and provides evidence to support their influence on disease outcomes. Guidelines for reporting in publication of viral PCEs are proposed to incorporate these characteristics and reflect the current evidence to support integration of PCE findings into the multifactorial 'systems' approach for shrimp viral disease management. To underscore the need and significance of these guidelines, the prevalence of relevant reported experimental details from 186 PCE studies of viral diseases in the giant black tiger shrimp, *P. monodon*, published between 1997 and 2023 were evaluated.

2 | INFECTIOUS DISEASE TRIAD

Disease outbreaks are the consequence of exposure of a susceptible host to a virulent agent, under permitting environmental conditions, as described by the infectious disease triad (*syn* epidemiological triad).[41,42] The disease triad concept was translated to the domain of aquatic animal health by Snieszko in the 1970s, in the context of environmental stress influencing the incidence of infectious disease outbreaks in fish.[43] The principles defined by the infectious disease triad underpin the contemporary adoption of a 'systems' approach to disease management.[3,15,44] The 'systems' approach acknowledges the complex interactions of host, pathogen, and environment influencing disease outcomes, and predicates that disease management strategies must therefore be multifactorial. The development of current and effective multifactorial disease management strategies is dependent on the integration of new research findings within the context of the broader system, relating to the host, pathogen and environment of production.[3]

For research findings derived from PCEs, aspects pertaining to the experimental shrimp, pathogenic agent, and study environment must be considered and defined to provide sufficient external validity and background for integration of the findings within the context of the broader system. Characteristics of the experimental shrimp, including genetic and geographical source,[45] life stage,[46,47] and pre-existing pathogen infections[48] may influence the dynamics of disease expression within PCEs and should be considered in the translation of findings to commercial production settings. Addressing the environmental components of the disease triad, factors including experimental replication and system design,[32] environmental conditions,[11] acclimation,[49,50] sampling techniques and scheduling,[51,52] and feed inputs[53] should similarly be considered in the translation of PCE findings to production systems. Pathogen attributes including genetic origin,[54,55] production and processing of the inoculum,[56] method of inoculation (including delivery and dose),[57] and chosen control treatments are important factors for interpretation and integration of study findings within the context of the broader system.

These essential components within the 'system' of shrimp disease (Figure 1), and their influence on disease outcomes in shrimp viral PCEs are discussed herein.

3 | ESSENTIAL COMPONENTS OF VIRAL PATHOGEN CHALLENGE EXPERIMENTS (PCES)

3.1 | Host: The shrimp

Background information on the challenged shrimp is imperative for the interpretation of results of PCEs and to provide external validity to the findings, allowing for appropriate application to shrimp production systems. Characteristics of the experimental shrimp, including species, life stage, genetic and geographical source, stress, and pre-existing pathogen infections can influence the dynamics of disease expression.[45,47,58,59] Detailed reporting of such metadata in published PCEs will also enable progressive knowledge development for larger-scale comparative meta-analyses, such as for understanding species- and population-level responses to pathogen challenges.

3.1.1 | Developmental stage—Size

Differential susceptibility of shrimp to viral infection during ontogenesis has been firmly established.[46,60,61] The operons of the reported differences include morphological and immunological development, immune gene expression, microbiome, and moult-cycle dynamics.[46,47,62,63] During the early stages of shrimp development, progression of nutrient and energy requirements are paralleled by changes in feeding behaviour, shifting from endogenous to various exogenous feed types.[64] Concurrently, morphological features including the branchial complex (gills) and digestive tract become developed,[65] facilitating nutrient and chemical exchange with the surroundings. Behavioural changes and growth of morphological features during the development of shrimp ensues progressive exposure and interaction with the external environment which may impact the capacity for viral entry and replication.[46] For example, in *P. monodon* nauplii, protozoeae, mysis, early post-larvae (PL1-10), late post-larvae (PL11-20) and juveniles challenged with WSSV via immersion under laboratory conditions, significant mortality only resulted for the later stages of post-larvae and juveniles.[46] Increased susceptibility to WSSV at the later stages may have been related to the complete morphological and functional development of the branchial complex (gills). Specifically, because the gills represent a primary site for viral entry and replication, the infection and necrosis of this organ may be a key facilitator for disease and mortality during later development stages.

The immune capacity of shrimp also establishes throughout early development.[47,66] Applying next-generation RNA sequencing, *P. monodon* immune transcripts including those related to pattern-recognition proteins (*c-type lectins*, *ficolins*), the prophenoloxidase system (*PPAF1*, *PPAF2*, and *serpin3*), the immune deficiency pathway (*Relish*), anti-microbial peptides (*crustin Pm1*, *crustin Pm4*, *ALF*, and *antiviral protein*), and heat shock proteins (*HSP70* and *HSP90*) were found to progressively increase during early development.[47] Expression of components from these pathways has been associated with protection against viral infection,[67–69] suggesting that earlier life stages lacking these components may be more susceptible to infection and disease. However, viral hijacking of shrimp immune pathways to facilitate viral replication may conversely be limited in early shrimp developmental stages, in the absence of the established immune pathways.[70–72] Such an effect may have been involved within the aforementioned study,[46] where WSSV disease progression in the later life stages may have been facilitated by both the morphological development of primary infection sites and immunological development enabling viral hijacking. Beyond immunological and morphological development, progressive exposure and interaction with the external environment associated with shrimp development also results in dynamic temporal succession of shrimp microbiota composition.[63,73]

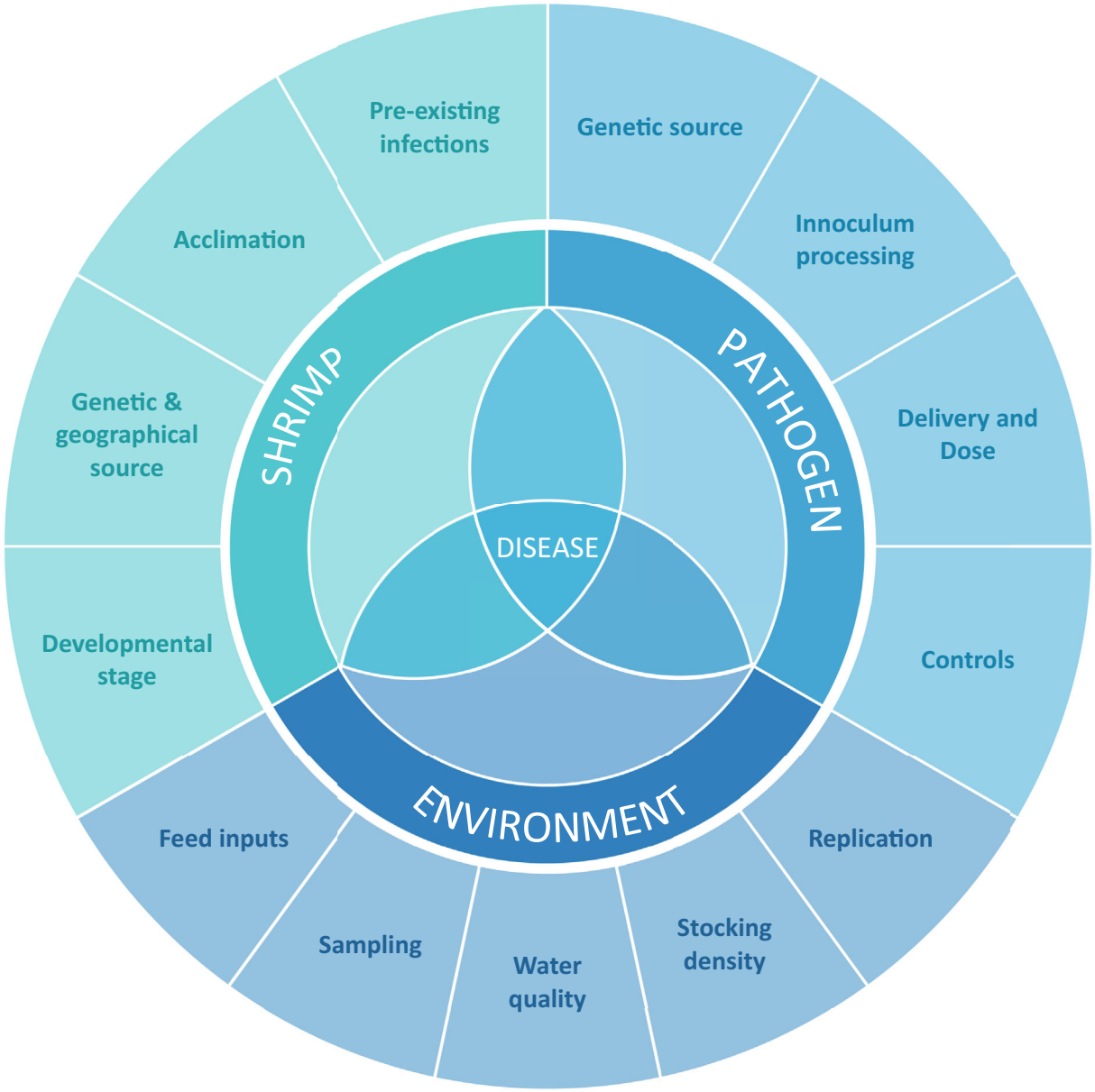

FIGURE 1 Disease outbreaks are the consequence of interactions between the susceptible host (shrimp), a virulent pathogen and conducive environmental conditions of the production system. Essential factors of each component within a viral pathogen challenge experiment on shrimp include those listed around the perimeter of the disease triad. Adapted from Snieszko (1974).

The dynamics of the microbiome are associated with shrimp health and immune capacity,[74,75] thus, changes in its composition and diversity during ontogenesis may further influence disease susceptibility.[37,76,77] Characterisation and analysis of the microbiome is yet to become a standard practice in PCE studies and currently remains cost-prohibitive in many instances. However, as research interest in the microbiome continues to expand, it is anticipated that the accessibility of this analysis will increase. In future, integrating microbiome analysis into PCE studies would enhance our understanding of disease outcomes and shrimp health from a 'systems' perspective.

The developmental progression of shrimp also drives changes in moult frequency and duration.[62,78] The dynamics of the moult cycle modulate large physiological changes which can result in increased susceptibility to environmental stressors and disease.[79,80] Throughout pre- and post-moulting the euryhaline osmoregulatory capacity of shrimp is largely reduced, and conformation to environmental osmolyte facilitates water uptake, increasing shrimp water content by up to 70%. Reduction in osmoregulatory capacity lessens shrimp resilience to fluctuating environmental stressors including low dissolved oxygen (DO), sudden salinity reduction, or increased ammonia, and may considerably alter the composition of shrimp haemolymph including the presence and concentration of immune factors.[79] The large volumes of water incorporated into the body and changes to the infrastructure of the exoskeleton during moulting increase the opportunity for viral acquirement, resulting in variable susceptibility to pathogen infection and disease as moult dynamics shift throughout shrimp development.[80,81]

Consistent reporting of shrimp age (days of culture [DOC] or days post hatch [dph]) as a standardised and quantitative measure for developmental stage should be practiced for publication of PCEs. Alternatively, depending on the purpose of the PCE, or if exact age is not known, it may also be appropriate to limit the description of size to a production group such as hatchery, grow-out, or broodstock. Where possible, when the cycle of synchronous moulting within the experimental population is known, or individual shrimp are assessed, reporting of moult stage should also be included. This information is essential for contextualising PCE results and enabling appropriate and specific application of PCE findings to commercial production systems.

3.1.2 | Source

Variation between shrimp from different sources can strongly influence their susceptibility to pathogen challenge.[45,82] Genetic variation and pathogen pre-exposure are critical factors that may influence disease susceptibility and should be evaluated when considering the relevance of PCE findings to other shrimp stocks. Genetic variation between shrimp may arise naturally, for example, through random mutation or local adaption of geographically distanced stocks,[83,84] or anthropogenically through the course of domestication and selective breeding.[85,86] Genetic variation associated with increased pathogen resistance and tolerance has been identified and targeted in studies for application in selective breeding programs to yield more robust production stocks.[32,85,87–91] In the context of disease resistance, such genetic variation resulting in stock improvement can be slight. For example, a single nucleotide polymorphism (SNP) (g.1186A > G) in the anti-lipopolysaccharide factor 3 (ALFPm3) gene of *P. monodon* has been associated with higher rates of survival during WSSV challenge.[92] This SNP was found to be variably present among three independent sources of farmed shrimp within the same province in Thailand, highlighting the importance of specific reporting of shrimp source for contextualisation of PCE findings.

Whilst rigorous genetic management and breeding strategy can effectively select for variants conferring reduced susceptibility to viral infection, poor genetic management of breeding populations can conversely result in genetic erosion.[93] Considering over 50% of globally cultured shrimp originate from 'copied breeding stocks' (i.e., shrimp intended for grow-out production that are matured and used for breeding without appropriate consideration of genetic relatedness),[94] considerable genetic erosion is expected in a large portion of farmed shrimp populations. Differences in the extent of genetic erosion between farmed shrimp sources can result in varying impacts on disease incidence, susceptibility, prevalence, and related mortality, further highlighting the importance of shrimp source for contextualisation of PCE findings.

Variable historic exposures to viral pathogens[95,96] and origin-specific microbiome[97] may further diverge the viral susceptibility of shrimp between different sources. Viral pathogens in both wild and commercial systems are relatively ubiquitous, and pathobiome composition between geographical sources is diverse.[19,24,98] Excluding specific pathogen-free (SPF) lines, depending on the pathogen under investigation and the country or region the experimental shrimp are sourced from, the potential for historic exposure of shrimp stocks to the studied pathogen, or other endemic pathogens, is considerable. Such exposures are also likely unique to each stock. Historic and contemporary pathogen exposure of experimental animals, including their ancestral genetic lines, prior to PCEs may yield variable susceptibility or resilience to pathogen infection between stocks, giving rise to variation in clinical outcomes of PCEs. Accordingly, the source of experimental shrimp used in PCEs should be accurately reported in publication, including their geographical, temporal, and genetic origin where possible. Beyond exposure to known pathogens, the potential for experimental shrimp to be exposed to, or infected with unidentified but consequential pathogens should also be considered. In this case, while technologies such as next-generation sequencing remain cost-prohibitive, and targeted pathogen screening (see Section 3.1.3) is unable to detect unidentified pathogens, accurate reporting of shrimp source may allow for retrospective contextualisation of PCE findings if a pathogen of consequence is identified and confirmed to be present within the source region or population.

3.1.3 | Screening experimental animals for additional pathogens

Pre-existing viral infections in overtly 'healthy' shrimp are commonly reported from both wild and cultured stocks.[19,25,46,98] For example, in a large-scale investigation of pathogen presence in Australian *P. monodon* production systems, in the absence of overt disease events 76% of shrimp analysed were found to be simultaneously positive for the detection of multiple (2–5) pathogen targets by qPCR analysis.[19] Such persistent, tolerated, or chronic infections can be amplified by external stressors[52] and can influence host susceptibility to other co-infecting pathogens in a protective[48,99] or exacerbating[100] manner. Bacterial co-infection and microbiome dysbiosis can further influence viral pathogenesis via immune overstimulation and annexing of the upregulated anti-bacterial shrimp immune pathways by viruses.[101–103]

Commonly, the observed clinical symptoms during co-infection are attributed to the more virulent pathogen,[58,104] despite limited understanding about the outcomes of the interactions between co-infecting pathogens and the host.[105] In the absence of additional analysis to exclude potential co-infections from differential diagnoses, attribution of clinical symptoms to a pathogen on the sole basis of its detection provides limited grounds for drawing robust experimental conclusions.[106] Deficient eliminatory analysis of possible co-infecting pathogens diminishes the rigour and validity of the research conclusions. In PCEs where the presence of co-infections is not considered, ambiguity arises in attributing the observed experimental outcomes to the specific study pathogen, particularly when the disease outcomes may be sub-clinical.[107]

Given the frequency and significance of multiple pathogen infections in production shrimp,[19,98] pre-screening experimental shrimp prior to introduction into the PCE system is critical to the validity of subsequent PCE results. Particularly, screening for a broad range of potential pathogens with focus on those that are endemic to the source of the shrimp, prior to and, at the conclusion of PCEs is necessitated. Pre-screening experimental shrimp would enable the preferential selection of shrimp free of specific pathogens, or at a minimum, would allow for the consideration of viral burden in the interpretation of the experimental results.[40] Reporting of the screening conducted, and relevant pathogen detection in PCE publication would improve scientific transparency and thoroughness of PCEs.

3.2 | Environment: The culture system

Aquaculture production systems are diverse in structure, composition and, administration and represent complex, multi-trophic environments.[108] With the majority of global shrimp production occurring in tropical coastal regions, volatile climatic conditions can result in large dynamic ranges of environmental culture conditions. The productivity of aquaculture is inherently dependent on abiotic and biotic factors within the aquatic environment, and the interactions of these factors with the cultured organism.[11] Similarly, for PCEs, the design and maintenance of the experimental system can have considerable impacts on the outcome of the trials, and if conducted poorly or with limited foresight, can result in confounding effects and biases in PCE results. Factors such as experimental replication, system design, maintenance of environmental conditions throughout the PCE, sampling, and feed inputs are all significant for critical interpretation of results and contextualisation within the 'systems' framework.

3.2.1 | Replication and sample size

To derive reliable and accurate results, PCE data must be generated in a manner that is robust, repeatable, and reproducible. Replication serves to measure and isolate sources of variation, limit the impact of spurious variation on effect estimates and hypothesis testing, and establish reproducibility.[109,110] Clarity in reporting of sample or treatment replicates and independent repeats of PCEs are important for the transparency and interpretation of findings, as replication has an applied effect on inferencing errors and the power of subsequent analysis.[109] In PCEs, especially those investigating the genetic parameters influencing disease progression, families or groups are often reared or challenged in separate tanks, for example, to physically facilitate maintenance of pedigrees.[111] Under such conditions, nongenetic effects arising from separated rearing before or during experiments, including inconsistent water quality conditions, stocking density, or exposure to infected conspecifics, can significantly impact response to challenge.[112] In the absence of replicated tanks for each family or group, the combined effects of environmental and methodological variation become confounded with that of the treatment and/or genetic effect.[32] PCE details including the number of shrimp stocked per tank, the number of tank replicates per treatment group, the definition of the treatments groups, and if the PCE was independently replicated should be clearly reported to enable critical evaluation of study robustness and scope.

3.2.2 | Density

Stocking density can significantly influence disease susceptibility in shrimp.[113] Negative health effects associated with increased stocking density can be primarily attributed to elevated stress, reduced water quality, and greater opportunity for horizontal viral transmission through cannibalism and exposure to water-borne viral particles shed by infected conspecifics.[114–116] For example, 14 days post-infection (dpi) with WSSV, increased cumulative mortality in *M. japonicus* (~1.1 g) was associated with increased stocking density, yielding 18% mortality at ~0.26 kg m^{-3}, 46% mortality at ~0.48 kg m^{-3} and, ~72% mortality at ~0.92 kg m^{-3}.[116] A similar trend was observed in WSSV infected *P. monodon*, *P. indicus* and *Litopenaeus vannamei* (~5 g), where mortality rates were highest for all three species when stocked at ~1.0 kg m^{-3}, followed by ~0.50 kg m^{-3}, and were lowest at the stocking density of 0.25 kg m^{-3}, demonstrating a clear positive association with stocking density and mortality arising during PCEs.[115] Reduced antioxidant capability and stress resistance associated with increased stocking density has also been reported in *L. vannamei* (~1.9 g), where shrimp stocked at higher density in cages within the same pond had reduced feed utilisation, growth, and survival during the 60 day experiment.[114] Direct reporting of stocking density, or parameters used to determine stocking density including the number of shrimp stocked per experimental unit, the weight of the shrimp stocked, and the volume of the experimental unit, should be included for context in publication of PCEs.

3.2.3 | Environmental conditions

Abiotic environmental conditions including temperature, salinity, DO, and pH are operative upon both host and pathogen function, and their interactions. Dynamic fluctuation or sustained sub-optimal environmental conditions can lead to physiological stress and reduced ability for the host to resist disease.[11] Reporting of abiotic environmental conditions in published PCEs is critical to the transparency and external validity of the associated findings.

Temperature

Temperature directly influences many processes, including shrimp metabolic and developmental processes, pathogen replication, and the solubility of other abiotic factors in the system.[117–119] Penaeid shrimp can tolerate a wide range of temperatures, with optimal temperatures for growth and survival dependent on size, but generally ranging between 28 and 32°C.[118–120] Despite this wide tolerance range, numerous PCEs report an influence of variable or sub-optimal water

temperature on infection dynamics in shrimp, via experimental manipulation of water temperature or as a coincidental finding. For example, comparing the replication rate of IHHNV in *L. vannamei* at different temperature treatments, Montgomery-Brock et al.[121] demonstrated the replication of IHHNV in shrimp held at 32.8 ± 1.0°C (1.20×10^5 virus copies/50 ng DNA at 17dpi) was reduced compared to shrimp held at 24.4 ± 0.5°C (4.48×10^6 virus/50 ng DNA at 17 dpi).[121] Similarly, in studies investigating the effect of water temperature on WSSV infection in *L. vannamei*, maintenance of shrimp at higher temperatures of 32–33°C was demonstrated to significantly reduce mortality compared to maintenance of shrimp at temperatures of ~26–30°C after WSSV challenge[50,122] Manipulation of water temperature was also determined to significantly influence mortality rates in *P. monodon* challenged with YHV-7 by various methods, including injection, cohabitation and feeding of infected tissue.[123] Experimental shrimp held at 30°C experienced significantly reduced mortality due to YHV-7 infection compared to shrimp held at 25°C.[123] Given the critical influence of temperature on PCE outcomes via interaction with host, pathogen, and other environmental parameters, consistent reporting of temperature in publication of PCEs is required. Specifically, the experimental temperature maintained throughout the PCE, with inclusion of a measure of variation (e.g., a minimum to maximum range or mean with standard deviation of temperature) should be reported to enable contextualisation of experimental findings within a 'systems' framework.

Salinity

Penaeid shrimp are euryhaline, beginning as osmoconformers in larvae and transitioning to osmoregulators as adults.[124,125] *P. monodon* are tolerant to salinities ranging from 1 to 57 ppt, but are optimally reared at 10–25 ppt.[126–128] Despite the wide tolerance range, rapid fluctuation in salinity is associated with increased susceptibility to pathogens and increased incidence of disease outbreaks.[129–132] Beyond the physiological stress and energetic cost to maintain osmotic homeostasis resulting in reduced capacity for immune response,[124,133] fluctuating salinity may also be a key mechanism of viral entry into shrimp.[81] During conditions causing increased frequency of urination (i.e., osmotic stress following a sudden drop in environmental salinity), the recurrent opening of the nephropore (antennal gland) for urination to regulate haemocoel volume may increase the opportunity for pathogen exposure to the nephrocomplex.[81] Following coordinated WSSV immersion challenge and hyposalinity exposure (35 g L^{-1} to 5 g L^{-1} over 5 h) in *P. vannamei*, cumulative mortality resulting from WSSV infection reached 100% within 48 h of exposure, while all WSSV-exposed shrimp not subjected to the hyposalinity conditions remained WSSV negative and survived.[81] Higher salinity culture conditions can also result in the dominance of opportunistic pathogen communities in the microbiota of shrimp,[134] potentially increasing disease susceptibility. In PCEs, fluctuation in salinity levels (i.e., during pre-experiment acclimation) may result in significant changes to the immune capacity of the shrimp or dynamics of pathogen transmission, confounding subsequent results of pathogen challenge. As such, consistent reporting of salinity levels and their maintenance throughout the experiment (i.e., range or mean with standard deviation) in publication of PCEs is critical.

Dissolved oxygen

DO fluctuates in systems due to temperature, photosynthetic activity, decomposition of organic matter, and biotic respiration. Penaeids have osmoregulatory capacity, allowing for maintenance of internal O_2 levels, independent of environmental partial pressure (PO_2), up to a critical threshold. Below this threshold, O_2 consumption becomes limiting for metabolic activity.[135] For *P. monodon* the critical threshold to support normal function is approximately 3.7 ppm,[136] with optimum conditions for growth at DO levels greater than 4–5 ppm.[128] Shrimp exposed to conditions below the optimal range (<4 ppm) become energetically stressed and thus have increased susceptibility to multiple other stressors, including pathogen infection.[117,137,138] Given the fundamental impact of DO on shrimp health and associated disease outcomes, inclusion of DO conditions in publication of PCEs is likely to improve the robustness and reliability of experimental findings.

Beyond the parameters of temperature, salinity, and DO, there are many other consequential environmental parameters including pH, nitrogenous compounds, and contaminants. These factors all contribute to the stasis of the organism, the interactions between the host and the pathogen, and hence the dynamics and outcomes of infection.[139–143] As such, their inclusion as reported parameters of PCEs is recommended, where possible.

Acclimation

Acclimation of experimental animals to the study system prior to the commencement of experimental activities (e.g., pathogen challenge) is important to enable return to homeostasis after stress induction from transport, handling, and fluctuations in environmental conditions.[144] The acclimation period may also serve to quarantine stock and enable pathogen screening prior to the experiment.[145] Acclimation herein will be discussed with reference to variations in environmental conditions. Stress-related to handling is discussed below in Section 3.2.4 'Sampling and handling'.

Rapid changes in environmental conditions occurring during transfer of animals from source tanks or ponds into the artificial 'captive' conditions of an experimental system can reduce shrimp health and immunocompetence, leading to increased susceptibility to pathogens or acute disease states from chronic pre-infections.[11,49,146] For example, the transfer of overtly healthy *M. japonicus* from ponds to controlled-environment tank-rearing systems induced elevated Mourilyan virus loads and increased associated mortality to 89%.[147] Additionally, when *L. vannamei* chronically infected with WSSV were cooled from 32.3 ± 0.8°C to 25.8 ± 0.7°C, acute disease was induced resulting in 100% mortality within 8 days of the temperature reduction.[50] Currently, there is limited evidence to support best practices for acclimation of shrimp when transferred into experimental systems. However, some studies have investigated return to homeostasis following salinity and thermal stressors.[119,148] For example, SPF juvenile

(~0.2 g) *P. monodon* maintained at 20‰ were subjected to six different salinity levels (0, 2.5, 5, 10, 20, or 30‰) and monitored for 60 days.[148] Physiological, biochemical, and gene expression parameters were measured at 0, 12, and 24 h, and 2, 3, 4, 5, 15, 30, and 60 days after reaching the target salinities by adjustment of 2.5‰ per day. By the 5th day, oxygen consumption, haemolymph serotonin and glucose levels, and expression of osmoregulatory genes had stabilised across all treatments. However, total haemocyte count and the expression of growth and immune-related genes only stabilised by the 20th day.[148] In a similar study, SPF *P. monodon* post-larvae (~25 dph) maintained at 28°C were subjected to six different temperature levels (24, 26, 28, 30, 32, or 34°C) and monitored for 60 days.[119] Thermal stress (heat shock protein gene *HSP70)* was measured at 0, 12, and 24 h, and 2, 3, 4, 5, 15, 30, and 60 days after reaching the target temperatures by adjustment of 1°C per 6 h. *HSP70* levels increased in all treatment groups up to 24 h, then declined until levels stabilised by day 3, although no treatments reached expression levels similar to the control group (28°C).[119] While such findings provide an indication of physiological and biochemical responses of *P. monodon* to gradual change of a single environmental parameter, the effects of more sudden changes, or simultaneous change of multiple parameters are yet to be elucidated. In the absence of adequate acclimation, rapid changes in multiple environmental conditions could result in confounded disease outcomes within a PCE. Adequate duration and consistent conditions during acclimation of experimental shrimp prior to challenge are required to ensure that the observed outcomes of the PCE are not related to stress caused by prior exposure to environmental fluctuation, or differential acclimation conditions between treatment groups. Although there is limited evidence to currently support best practice for acclimation of shrimp to experimental systems, consistent reporting of the adopted acclimation practices and conditions for publication of PCEs is important to provide context of animal condition and may be of increased value and further relevance as more knowledge in this area is developed.

3.2.4 | Sampling and handling

Beyond the critical importance of animal welfare maintenance when working with live animals, stress-induced from handling and sampling throughout an experiment can have significant impacts on shrimp physiology and the susceptibility of shrimp to disease.[52,149,150] These impacts may become additive with the experimental treatment or pathogen challenge, confounding experimental findings.[151] Stress due to handling or sampling of crustaceans can result in significant shifts in physiological, metabolic, and immune responses[144,149,150] and can yield significant differences in mortality during long-term experiments involving repeated handling.[152] Despite the lack of well-defined stress tolerance in shrimp related to handling and sampling, subtle changes in the captive environment or treatment of the shrimp, such as handling shrimp between tanks, may be sufficient to induce a stress response[144] or elevate disease expression.[52] In some studies, stress due to handling and sampling of experimental animals has been attributed to the triggering of acute disease states in *P. monodon* from chronic infection with gill-associated virus[52] and WSSV.[153] Post-stress settlement periods, based on return to normal physiological state after a stress event, would reduce the impact of handling/sampling stress on experimental outcomes and have been advised for multiple crustacean species.[144,151,154] However, in the case of PCEs where animals are pathogen-challenged and immediately stocked at the commencement of the experiment, or when sampled animals are returned to the experimental system during repeat/time-series based sampling, such settlement periods are not possible. As such, it is important to include handling and sampling protocols in publication of PCEs, so that the conditions and potential impact can be considered when evaluating the reported disease outcomes and interpretations.

3.2.5 | Feed

The influence of nutrition on shrimp immune capacity and disease susceptibility is well-established, for example, through studies examining the protective effects of feed additives against viral infections.[30,155,156] Nevertheless, it is essential to note that feed can also serve as a potential pathway for pathogens to enter experimental and commercial rearing systems.[53] Commonly used unprocessed feeds, including polychaete worms, shrimp, squid, contaminated Artemia, and unpasteurised pelleted feeds, may introduce viable pathogens to the shrimp culture systems. Positive detection of pathogens including, but not limited to WSSV, IHHNV, and other bacterial and parasitic pathogens, have been extensively reported from the aforementioned feed types.[53] The transmission of pathogens from the feed source to the shrimp has also been demonstrated in multiple studies,[157–159] suggesting a plausible route for pathogen introduction into rearing and experimental systems. For pelleted feeds, extrusion at high heat or autoclaving of formulated pellets renders contaminant pathogens nonviable, removing the risk of viable pathogen introduction into the system.[160] However, in cases where studies or production processes rely on PCR-based analysis for pathogen detection and determination of infection status, remnant nonviable pathogen template introduced to the system and shrimp from feed may yield positive detection results, in the absence of infection.[161]

While in many cases the content of the feed used in PCEs, such as commercial pelleted feed types, is not controlled directly within the study, the potential for pathogen introduction via this route should be considered. The use of gamma irradiated shrimp[162] originating from the experimental source population, or a population of known health status, as feed within a PCE, would eliminate the risk of introducing nonviable pathogen templates from commercial feed types, and additional, potentially viable pathogens via unprocessed feed types, into the experimental system.[163] Nonetheless, where possible, pathogen screening analysis should be conducted for the experimental feed as with the experimental animals. Pathogen screening of feed would improve PCE robustness by allowing the risk of introduction of viable pathogens into the experimental system via unprocessed feeds to be considered and managed, or to enable the reductive comparison of

non-viable viral template detected in the feed to be considered against the pathogens detected in the experimental animals prior to, and throughout the PCE.

3.3 | Pathogen: The viral inoculum

The viral inoculum assumes fundamental importance in PCEs, with several key factors pertaining to the inoculum exerting significant influence on the outcomes and dynamics of the experimental disease. Understanding and accurately reporting on these factors, including the origin (genetic, geographical, temporal, and host), processing, delivery, and dosage of the viral inoculum, is vital for proper interpretation and contextualization of the results in the broader context of shrimp disease research and management.

3.3.1 | Genetic strain

The genetic composition of the virus used in a PCE is distinctively related to its genetic potential for pathogenicity and level of virulence.[164,165] Genetic variation yielding differences in pathogenicity has been widely reported for viruses of shrimp, including IHHNV,[166] TSV,[54,167,168] infectious myonecrosis virus,[55] and WSSV.[164,169–171] In these instances, subtle genetic variation can yield significant alteration of infection dynamics. For example, synonymous SNPs within wsv151 and wsv226 genes of the Chinese mainland WSSV strain (WSSV-CN, GenBank accession no. AF332093.1) were demonstrated to facilitate the avoidance of host siRNA-mediated RNAi immunity in *M. japonicus*, demonstrating that mutation of a single base can attenuate antiviral immune response in shrimp.[172] Regular genetic monitoring is required to aid management of associated changes in virulence and transmissibility, given high rates of genetic diversity within viral genotypes, rapid evolutionary rate, and significant regional subdivision of viral strains arising from biogeographic drivers.[173] In the absence of indicative details for viral source, encompassing strain variation related to geographical, temporal, and host species' origin, reproducibility of PCEs and contextualisation of PCE results within a relevant global perspective is limited. Hence, it is crucial to clearly document the origin of the viral inoculum, encompassing geographical, temporal, and host species details, and provide the genetic sequence whenever possible when publishing PCEs.

3.3.2 | Inoculum processing

Given the prevalence of multiple viral infections in both farmed and wild shrimp populations,[19,98,174] co-infections in tissue samples used to generate a viral inoculum for PCEs should be considered. Such co-infections may influence the clinical results of the PCE (also see Section 3.1.3 'Screening experimental animals for additional pathogens'). The possibility of multiple viral infections within the same shrimp or homogenate sample raises doubts about the reliability of using crude inoculums, such as unprocessed infected tissue or tissue homogenates, to assess the pathogenicity and virulence of a virus in a specific shrimp species.[56] The presence of multiple viruses within the challenge inoculum can lead to issues in attributing clinical outcomes and interpreting experimental results accurately, affecting the assessment of the true pathogenicity and virulence of the studied pathogen. PCEs that neglect consideration of co-infections within the inoculum, along with any assumptions derived from the study, lack proper substantiation and should be interpreted with caution. Future PCEs should aim to analyse and disclose the presence of co-infecting pathogens within the study inoculum. In studies employing inoculation methods such as intramuscular injection, the generation and use of purified viral inoculum should be targeted whenever possible. This approach would significantly minimise potential confounding factors associated with less-processed viral inoculums.

3.3.3 | Inoculation method

Inoculation method can influence the quantity of viral particles delivered to the experimental animals resulting in varying infection consistency.[57] Studies employing immersion-based inoculation may experience inconsistencies in viral delivery and infection efficacy due to fluctuations in salinity,[81] differences in susceptibility to viral entry at different moult stages, or the presence of cuticular damage (e.g., excised pleopod).[80] Similarly, inoculation via feeding of infected tissue (per os), may be influenced considerably by inconsistent viral distribution within the fed infected tissue[51,175] and variable feeding behaviour of the experimental shrimp.[176] Intramuscular and intrabladder injection yield high consistency of viral delivery and infection, compared to oral inoculation and immersion.[57,81,115] However, injection of virus directly into the target organ artificially avoids natural defence barriers to produce rapid infection of susceptible cells and is misrepresentative of natural infection pathways probable to occur during production.[17,177] Although less consistent and amenable to standardisation, oral inoculation, and immersion are more demonstrative of natural infection routes, including horizontal transmission of virus through cannibalism of infected conspecifics and viral shedding into the water.[116] While the inoculation method used is dependent on each specific study aims and will not be standard across all PCE studies, reporting of inoculation method in publication should nonetheless be clear and detailed to enable critical evaluation. Secondary or ongoing exposure of experimental animals to infected material should also be considered in the design and implementation of PCEs. For example, in paired-contact cohabitation studies investigating the horizontal transmission dynamics of WSSV the prevalence of WSSV infection and resultant mortality was higher in non-inoculated ('healthy') shrimp when the cohabitating inoculated shrimp were not removed after they died, compared to those where dead inoculated shrimp were removed.[178] The removal or retention of dead or moribund conspecifics from the challenge system should be detailed in the publication of PCEs to allow for consideration of secondary or ongoing re-infection via cannibalism.

3.3.4 | Inoculum concentration/dose

The quantity of viral particles delivered to an experimental shrimp, herein referred to as dose, is related to the speed and severity of disease onset within a PCE.[179] Critically, the quantified viral dose must be confirmed as viable. In the absence of crustacean cell lines to confirm inoculum viability and infectivity,[180] pilot PCEs or LD50 trials (median lethal dose) may be alternatively conducted. Herein, discussion related to dose assumes viability of the viral particles of the inoculum has been confirmed.

Standardisation and reporting of dosage in PCEs are dependent on the method of inoculum delivery. For inoculation via intramuscular injection, dose can be simply and clearly standardised to the total number of viral copies injected (e.g., X µL g^{-1} shrimp weight of inoculum at X viral copies μL^{-1}).[32,181] However, for inoculation via immersion, dose calculation must consider exposure as a product of viral particle concentration in the immersion bath and the length of time the shrimp is in contact with the particles.[57] Precise dose calculation and normalisation for per os inoculation can be further challenged by inconsistent viral particle distribution within the tissue and variable feeding behaviour of the experimental shrimp (also see Section 3.3.3 'Inoculation method'). Maceration or homogenisation of tissue samples for per os challenge can minimise the influence of heterogeneous viral particle distribution on dosage.[182–184] Additionally, implementing a starving period for experimental animals before per os challenge can increase shrimp receptivity to the inoculum feed, improving dose consistency.[57,159,185]

Regardless of inoculation method, and the approach used and reported for normalisation of dosage, standardisation and reporting of dosage necessitates accurate quantification of viral copy number within the inoculum. Quantification of viral copy number is commonly achieved using qPCR detection and standard curves generated using quantification standards. However, the use of diverse quantification standards, such as nucleic acid from specific biological samples, circular plasmid DNA constructs, or synthetic RNA or DNA oligonucleotides may yield variable quantification for the same sample.[39] For example, heterogeneity of plasmid stock, where plasmids may carry variable copies of the target sequence insert, or issues with target sequence amplification from circular plasmids due to the secondary helical structure of the plasmid, compared to linearised forms,[186] can yield highly variable quantification within the standard materials. Given these variabilities, clear and consistent reporting of inoculum dose and how the dose was quantified (see MIQE guidelines)[39] is essential to the internal and external validity of PCE results.

3.3.5 | Control treatment

The control treatment of a PCE should constitute an absence of treatment, or a standard treatment of known effect, from which the challenge treatment can be compared against. In studies where a standardised solution can be used as a control treatment, there is certainty that the control group is unchallenged and the effects of inoculation and handling stress in the challenged group are accounted for in the control group conditions. For example, inoculation of the challenge group with a purified viral suspension in tris-buffered saline, and inoculation of the control group with tris-buffered saline only.[27] However, in studies where a non-standardised control treatment is used, such as PCEs with per os inoculation which uses tissue from overtly 'healthy' shrimp as the control treatment, there is less certainty in the standardisation of the treatment. As discussed in both Sections 3.1.3 and 3.3.2, unintentional introduction of viruses other than the principal virus being studied may influence the clinical results of the PCE. Absence of viral screening of the control tissue to rule out sub-clinical infections is an ongoing and critical issue that requires addressing before conclusive results from such challenge experiments can be justifiably drawn. Careful selection and standardisation of control treatments are essential to ensure the validity and reliability of PCE findings and should be detailed accordingly within publication of PCEs to enable critical evaluation of study findings.

4 | REPORTED INFORMATION IN SHRIMP PCES

Considering the substantial evidence supporting the multifactorial influence of host, pathogen, and environmental components on disease outcomes, it is important to examine the existing literature on shrimp PCEs within this context. We gathered data from 186 peer-reviewed publications of viral PCEs involving *P. monodon* related to the host, environment, and pathogen components of the disease triad, with reference to a 'systems' approach. This subset of studies was identified via a literature search of PubMed, conducted on 1 August, 2023, using the search terms 'virus' and 'monodon'. The publications identified from this search were further refined to include only PCEs of *P. monodon*. Reported details related to the components of the disease triad (Figure 1), were obtained from each study where available (Supplementary Table 1). The prevalence of these reported details within the literature is displayed in Figure 2. The results of the literature survey highlighted a concerning paucity of reported experimental detail for viral PCEs in *P. monodon*.

Experimental details were most consistently reported for the host component, followed by pathogen and environment. Most studies reported the size of the experimental *P. monodon* (weight in grams; 91%, 169/186), gave reference to shrimp age or developmental stage (45%, 84/186), and provided shrimp source with respect to habitat (82%, 152/186) and origin country (85%, 159/186) or region (59%, 109/186). The use of shrimp declared as SPF was reported in a small portion of the studies assessed (8%, 14/186). Of the remaining studies that did not report the use of SPF shrimp (172 of 186), reports of pathogen screening (e.g., using PCR or qPCR) were alarmingly limited (43%, 75/172). Adding to this concern, very few of these studies (12%, 20/172) reported screening for possible co-infections.

Factors related to the challenge system (environment) were variably reported. The number of shrimp per replicate tank (83%, 155/186) was reported in most studies, however, tank volume (46%,

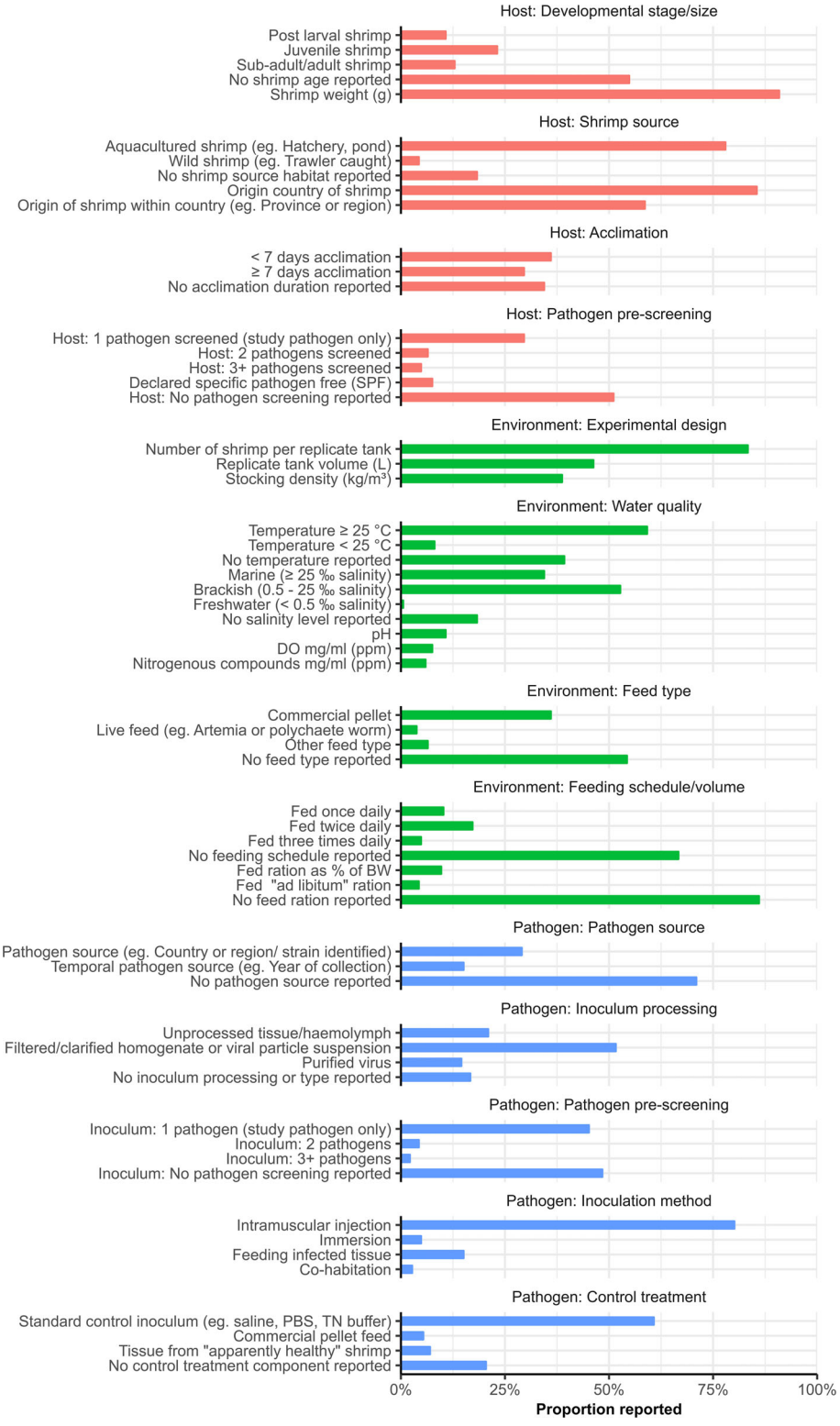

FIGURE 2 Prevalence of reported details from 186 peer-reviewed publications including viral pathogen challenge experiments on *Penaeus monodon*, published between 1997 and 2023.

86/186) or stocking density (39%, 72/186; including studies that provided metadata from which density could be calculated) were reported in less than half of all PCEs evaluated. None of the studies assessed reported PCEs conditions for the core water quality parameters including salinity, temperature, pH, DO, and nitrogenous wastes in combination. Salinity (82%, 152/186) and temperature (61%, 113/186) were reported most frequently, while pH (11%, 20/186), DO (8%, 14/186), and nitrogenous wastes (6%, 11/186)

TABLE 1 Shrimp PCE Reporting Guideline (SPERG) checklist.

Factor	Experimental detail
Host	
Age	Stage, including days post hatch (dph) or days of culture (DOC)
Size	Weight (g) and/or length (mm)
Source	Habitat (farmed or wild, hatchery, pond, etc.)
	Genetic/geographic/temporal source (country, region, date collected)
Acclimation	Duration (days)
	Conditions (see System: environmental conditions)
Pathogen pre-screening	Sample size screened (proportion of experimental cohort)
	Pathogens screened
	Method of screening (PCR, qPCR, etc., See MIQE)
	Screening results (pathogen load and prevalence)
Environment	
Experimental design	Definition of experimental and control groups
	Duration of the experiment (days post infection—dpi)
Replication	Shrimp per tank
	Tank size (L)
	Stocking density
	Tanks per treatment
	Treatments within the experiment
	Repetition of the experiment
Sampling and analysis	Sample tissue type
	Sampling technique
	Sample storage conditions
	Sampling schedule (hours post infection—hpi or dpi)
	Shrimp replacement or sacrifice (during sampling and for mortalities/moribund shrimp)
	Details of laboratory and statistical analysis performed (see MIQE)
Environmental conditions/ water quality	Temperature
	Salinity
	pH
	DO
	Nitrogenous compounds (e.g., NH_3/NH_4, NO_3, NO_2)
	Aeration provisions
	Filtration and water treatment provisions
Feeding	Feed source and pathogen screening results
	Feed treatment provisions
	Feeding schedule (BW% per feeding event)
Pathogen	
Source (strain)	Genetic/geographic/temporal source (country, region, date collected)
	Genetic sequence and strain identification (if available)
Processing method	Tissue sampling
	Homogenisation, filtration, clarification, purification, and so forth
Pathogen pre-screened	Pathogens screened
	Method of screening (PCR, qPCR, etc., See MIQE)
	Screening results (pathogen load)
Volume and concentration	Volume of inoculum used
	Viral copies per unit volume
Inoculation method and conditions	Intramuscular injection: injection site
	Immersion: bath duration and concentration, washing post bath
	Per os: starvation period, feeding period, volume of tissue (%BW)
	Co-habitation: exposure duration, removal of mortalities, holding configuration
Control inoculum	Control treatment used
	If nonstandard control is used; pathogens screened, method of screening, screening results (pathogen load)

were rarely reported. Studies should aim to report a range or mean ± SD for water quality parameters, as it provides information as to the consistency of the conditions maintained throughout the experiment. For example, in PCEs where a temperature range is reported, the impacts of temperature fluctuation can be considered with respect to the outcomes of the study, especially of those that report temperature ranges across a large gradient (~4–6°C variation).[27,187–192] Shrimp acclimation period was reported for more than half of the studies analysed (66%, 122/186). Feeding during the challenge experiments was uncommonly reported, with details of feed type, schedule, and ration reported in combination for only 12% (23/186) of PCEs.

Of concern was the apparent absence of reported viral (inoculum) data within the PCEs assessed. Most of the studies did not report the source of the study virus (71%, 132/186), with only 29% (54/186) directly reporting the geographic or genetic source, and 15% (28/186) reporting the temporal origin of the virus. The type of inoculum used (e.g., filtered homogenate or purified viral suspension) was more consistently reported (83%, 155/186) jointly with the inoculation method (100%, 186/186). The limited reporting of pre-screening experimental shrimp was mirrored in the screening of the viral inoculums. Screening

for multiple viruses in non-purified viral inoculums or un-processed inoculum types was very rarely reported (4%, 8/186).

The authors of this review acknowledge that the absence of reported experimental detail within published PCEs does not necessarily mean that the factors were not managed or measured within the PCE. However, the utility of research and its value to directing successive studies is reliant on the information provided within the publication to assess the internal and external validity of the experiment and its findings.[36] Consequently, the lack of reported detail in published PCEs severely limits the potential for the research to make progressive advancements and contribute to contemporary shrimp disease management strategies applying a 'systems' approach.

5 | MINIMUM REPORTABLE INFORMATION FOR PUBLICATION

Considering the evidence to support a 'systems' approach to PCEs, and the scarcity of reported details from the existing body of literature (Figure 2), a more rigorous and repeatable strategy to improve the utility and translation of experimental findings from PCEs must be developed. We propose guidelines in the form of a checklist for the minimum reportable information in the publication of shrimp viral PCEs, hereafter referred to as the Shrimp PCE Reporting Guidelines (SPERG) (Table 1). The proposed guidelines include a core set of characteristics pertaining to the shrimp host, viral pathogen, and environment, and reflect current evidence supporting the multifactorial influence of components on disease outcomes. Given the complexity of disease as a manifestation of interactions within the 'system', the checklist does not account for all parameters which may impact the outcome of a PCE. As such, we encourage reporting of additional parameters beyond those listed in the checklist, where possible. However, we also acknowledge that in some instances, specific information included in the checklist may not easily be obtained by the researcher. Nonetheless, it is expected that reasonable adoption of the reporting checklist will improve the utility and transparency of future PCE-based studies, ultimately improving understanding of viral diseases of shrimp within a 'systems' framework.

6 | CONCLUSION

Successful contemporary disease management strategies applying a 'systems' approach must be predicated on current and robust knowledge of the host, pathogen, and environment. PCEs are a powerful research tool for resolving the impact of pathogens on shrimp health and identifying solutions to the pervasive threat of viral disease in shrimp production. While defined experimental hypotheses can be robustly tested using well-designed PCEs, the high levels of control and 'artificial' nature of PCEs pose inherent limitations. As such, for these findings to provide realised value to global shrimp production, they must be contextualised to the broader 'system' framework. To achieve this effectively, the conditions of the experiment, including but not limited to the items described within the SPERG checklist, must be accurately and thoroughly detailed. In the absence of thorough reporting of experimental detail, the evaluation of both internal experimental validity and external validity to a 'systems' framework is limited, and the potential for the research to contribute to contemporary disease management is reduced. The SPERG checklist is proposed to improve the utility and transparency of future shrimp viral PCEs. We further envisage that adoption of SPERG will not only aid individual studies but also enable more robust and insightful meta-analyses from future shrimp PCEs.

While we targeted our review of reported experimental details in PCEs to viral challenge of *P. monodon* shrimp, the wider concepts posed are applicable to shrimp, and perhaps crustacean PCEs more generally, and may be adaptable to other pathogens beyond viruses. Ultimately, the SPERG checklist is aimed at improving the accessibility and application of PCE findings to viral disease management in commercial production for the benefit of animal health and the productivity of shrimp aquaculture.

AUTHOR CONTRIBUTIONS

P. M. Arbon: Conceptualization; investigation; writing – original draft; writing – review and editing; visualization. **K. Condon:** Conceptualization; writing – review and editing; funding acquisition. **M. Andrade Martinez:** Writing – review and editing. **D. R. Jerry:** Conceptualization; writing – review and editing.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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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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