Aquaculture 535 (2021) 736359

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Reduced transmission of IHHNV to *Penaeus monodon* from shrimp pond wastewater filtered through a polychaete-assisted sand filter (PASF) system

Paul J. Palmer [a], Min Rao [b], Jeff A. Cowley [b,*]

[a] Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, 41 Boggo Road, Dutton Park QLD 4102, Australia
[b] Livestock & Aquaculture, CSIRO Agriculture & Food, Queensland Bioscience Precinct, 306 Carmody Road, St. Lucia QLD 4067, Australia

ARTICLE INFO

Keywords:
Infectious hypodermal and hematopoietic necrosis virus
Penaeus stylirostris densovirus (*Pst*DNV)
Black tiger prawn
Virus transmission
Polychaete worms

ABSTRACT

A polychaete-assisted sand filter (PASF) can aid water recirculation systems for shrimp culture ponds by removing large amounts of nutrients and suspended solids and chemically treating wastewater. By ingesting and degrading organic matter, *Perinereis helleri* polychaetes reared in a PASF can accumulate and potentially remove infectious hypodermal and hematopoietic necrosis virus (IHHNV) present in wastewater from ponds rearing virus-infected *Penaeus monodon* shrimp. Reported here are data showing that filtering pond wastewater through a PASF reduces its potential to transmit IHHNV. The trial employed 3 groups of 4 tanks each containing 20 *P. monodon* randomly selected from a pond in which only low-level IHHNV infection was evident at moderate prevalence (IHHNV-low Pond 2). Over a 2 week period, each tank group was supplied with wastewater from either the same pond (Pond 2) or IHHNV-high Pond 1, in which IHHNV was 100% prevalent at ~10^4-fold higher infection loads, either directly or after being filtered through a PASF. IHHNV real-time qPCR data on total nucleic acid (TNA) extracted from pleopods of 35 *P. monodon* selected at random from each group identified an elevated IHHNV infection prevalence (91%) in shrimp tested from tanks supplied directly with wastewater from Pond 1. In comparison, IHHNV was detected at a much reduced prevalence and lower loads among shrimp tested from tanks supplied with wastewater from Pond 2 (33%) or from Pond 1 after it had been filtered through a PASF (31%). The IHHNV prevalence and load data indicate that a PASF can play a useful role in reducing the potential for shrimp pond wastewater to transmit IHHNV infection to naïve *P. monodon*.

1. Introduction

Shrimp farming in Australia remains small in global terms (~5000 t annual production valued at ~AUD$90 million) but supports substantial employment in regional communities and is projected to grow substantially over the next decade (URL1, 2020). Black tiger shrimp (*Penaeus monodon*) is the predominate species farmed. Several pathogens have seriously impacted the culture of this species in Eastern hemisphere countries (Thitamadee et al., 2016) and been a major contributor to a shift over the past 2 decades to the widespread farming of specific pathogen free (SPF) Pacific white shrimp (*P. vannamei*; Newman, 2010). In Australia, however, the import of live shrimp for aquaculture purposes remains prohibited under strict quarantine conditions specified in the Australian Government Environment Protection and Biodiversity Conservation (EPBC) Act 1999 (URL2, 2020).

The endemic pathogens most commonly associated with production losses of *P. monodon* farmed in Australia include (i) gill-associated virus (GAV; Spann et al., 1997; Cowley et al., 1999, 2000) otherwise described as yellow head virus genotype 2 (YHV2), (ii) YHV7 (Mohr et al. 2015; Cowley et al., 2015), (iii) the PirA/B toxin-producing *Vibrio harveyi* strain responsible for causing a hepatopancreatitis and mortality described as *P. monodon* mortality syndrome (*Pm*MS; URL3, 2020; URL4, 2020; Infante-Villamil et al., 2019) somewhat similar to that seen in acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio parahaemolyticus* strains carrying a plasmid expressing the PirA/B toxins (Lightner et al., 2012; Nunan et al., 2014; Gomez-Gil et al., 2014; Tran et al., 2013), and (iv) infectious hypodermal and hematopoietic necrosis virus (IHHNV) that can result in reduced growth performance (Withyachumnarnkul et al., 2006; Sellars et al., 2019) and in some circumstances, severe shell deformities in *P. monodon* (Primavera and Quinitio, 2000; G.J. Coman et al., unpublished data).

Most shrimp pathogens, as like GAV, are transmitted vertically (Cowley et al., 2002). Thus to limit disease impacts occurring during culture of pathogen-carrying shrimp postlarvae, and to overcome a

* Corresponding author.
E-mail address: Jeff.Cowley@outlook.com (J.A. Cowley).

https://doi.org/10.1016/j.aquaculture.2021.736359
Received 2 August 2020; Received in revised form 3 January 2021; Accepted 5 January 2021
Available online 9 January 2021

reliance on wild broodstock often carrying these pathogens as subclinical infections, substantial efforts have been made over the past 2 decades to domesticate and selectively breed SPF or specific pathogen resistant (SPR) lines of *P. monodon* in Australia (Coman and Preston, 2008; Preston et al., 2009; 2010; Norman-López et al., 2016). Methods to address horizontal transmission of pathogens have also been considered increasingly important since the incursion of white spot syndrome virus (WSSV) in the summer 2016–17 grow-out season in Southeast Queensland (Oakey and Smith, 2018; Scott-Orr et al., 2017). While the use of drum filters to remove crustacean larvae that might act as pathogen vectors is being adopted, limiting new seawater inputs through pond wastewater recirculation within biosecure systems is a strategy increasingly being considered. However, methods to disinfect recirculated seawater cost-effectively are not not yet well established.

Accompanying these ambitions to reduce disease entry pathways during *P. monodon* culture has been an increased need for successful long-term selective breeding programs employing pathogen-free lines and fresh feeds known to promote broodstock fecundity such as squid, molluscs and polychaete worms (Meunpol et al., 2005; Leelatanawit et al., 2014; Chimsung, 2014). Of these, polychaetes including some sandworms and bloodworms appear to provide a more effective dietary supplement when served to broodstock alive rather than frozen (Meunpol et al., 2005, 2007; Palmer et al., 2014, 2018). With the aim of culturing polychaetes for this purpose while also removing nutrients from shrimp pond wastewater for sustainable recirculation or remediation before discharge, a polychaete-assisted sand filter (PASF) system has been developed (Palmer, 2010). For removing nutrients from wastewater, PASF systems have proved far more effective compared to typical pond-based sediment settlement systems. The reason for this is the polychaetes feed upon and remove nutrients bound in microalgae, detritus and other organic matter, thus converting it to volatile forms also usable as a nutrient source for bacteria and plants (Palmer, 2010; Palmer et al., 2016, 2018).

The PASF system thus offers an attractive proposition for cleansing shrimp pond wastewater whilst reliably suppling consistent-quality live polychaetes at a reasonable cost. However, a potential downside is that pathogens and pathogen-containing matter shed from infected shrimp and discharged in pond wastewater can pose a biosecurity risk if the cultured polychaetes are fed to shrimp broodstock to promote fecundity (Vijayan et al., 2005; Haditomo and Chilmawati, 2012; Desrina et al., 2013; Desrina, 2014; Haryadi et al., 2014). In support of this, the ability of polychaetes to transiently carry virus-infected matter in their digestive tract has recently been demonstrated by supplying PASF systems with wastewater from ponds rearing *P. monodon* with high-load IHHNV infections (Sellars et al., 2019; Liu et al., 2020). Building upon this study, here we report data from real-time challenge trials demonstrating that the use of a PASF to filter/treat such shrimp pond wastewater can dramatically reduce its ability to transmit IHHNV infection to *P. monodon*.

2. Materials and methods

2.1. Polychaetes and shrimp

Juveniles of the omnivorous marine sandworm *Perinereis helleri* (Polychaeta, Nereididae) used to stock PASF Bed 2 were from a line reared over several generations at the Bribie Island Research Centre in Southeast Queensland, Australia (Palmer, 2010; Palmer et al., 2016, 2018). The *Penaeus monodon* postlarvae used to stock the 2 ponds were generated from wild broodstock captured in the vicinity of Innisfail in North Queensland, Australia (Sellars et al., 2019).

2.2. Shrimp pond and polychaete-assisted sand filter (PASF) design and operation

The 2 × 0.16 ha shrimp ponds were maintained under simulated commercial grow-out conditions for *P. monodon* and were stocked with batches of post-larvae (PL15) derived from wild-captured broodstock naturally infected with IHHNV at varying loads as described in detail elsewhere (Sellars et al., 2019). Pond 1 was stocked with a PL15 cohort that rapidly developed high-load IHHNV infections at 100% prevalence. Pond 2 was stocked with a separate PL15 cohort in which infection prevalence only reached 100% toward the end of grow-out, and in which IHHNV loads remained low to moderate (Sellars et al., 2019).

Pond wastewater was pumped twice daily from the monk drains of each pond to a 10-bed PASF system as described previously (Liu et al., 2020), with a total pumping time of 7.5 h d^{-1}. Some wastewater from each pond was also diverted from this pumping system directly to a series of 1700 L experimental shrimp culture tanks (Fig. 1), where a group of 4 tanks received wastewater from Pond 1, a second group of 4 tanks received wastewater from Pond 2, and a third group of 4 tanks received wastewater from Pond 1 that had been filtered through PASF Bed 2. Wastewater began flowing into the experimental tanks on 14 Feb 2017, which was 77 d after stocking PASF Bed 2 with juvenile *P. helleri* and operating the PASF-bed complex in a standardised way (Liu et al., 2020). Upon filling, wastewater flow rates to all tanks were adjusted to ~4.2 L min^{-1}, which provided each tank with ~1890 L (>100% exchange) each day. Clean seawater was also supplied continuously to all tanks at a rate of ~1.2 L min^{-1} (~1728 L d^{-1} ≥100% exchange d^{-1}) to ensure good water quality was maintained during the trial. Flow rates were checked and adjusted every second day of the trial until 28 Feb 2017 when tank water levels were reduced for shrimp assessment and sampling.

The day after filling the 1700 L tanks with the seawater from shrimp ponds as described above, each was stocked randomly with 20 x ~ 32 g *P. monodon* captured from Pond 2 using a cast net. Each group was thus expected to comprise shrimp with a uniform background of low IHHNV prevalence and infection loads. Total weight/shrimp number was used to determine average shrimp weights at the start and end of the trial.

All tanks were provided with gentle aeration, covered with 70% shade cloth to reduce light intensity during the day, and clear plastic to reduce heat loss during the night. Tank water temperatures were monitored daily using max-min thermometers, and ranged from 26 to 32 °C. In addition to having access to nutrients in the pond wastewater, shrimp were supplied with commercial feed pellets (Ridley Grower 1) at ~2% of total shrimp body weight d^{-1}, which equated to 6.5 g $tank^{-1}$ fed twice daily at ~8:00 and 16:00.

Shrimp in each tank were otherwise left undisturbed over the 2 week trial duration. Then, each tank was drained and shrimp remaining alive were counted and weighed to the nearest 0.5 g. A pleopod was then sampled from each shrimp using aseptic techniques that included ethanol washing and flaming of forceps and scissors between uses. Pleopods were preserved in plastic vials containing RNAlater for 2 days at 4 °C before being stored at −20 °C.

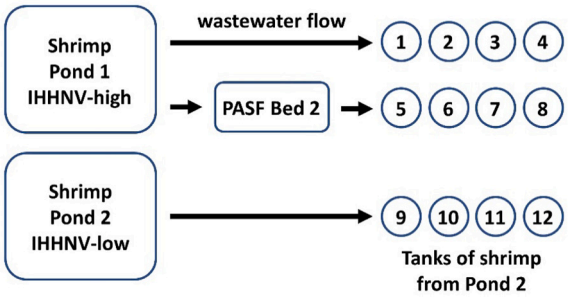

Fig. 1. Schematic diagram showing shrimp pond wastewater flows to groups of 4 tanks (numbered 1–12) each containing 20 *Penaeus monodon* from Pond 2. Flows were directly from Pond 2, or from Pond 1 either directly or after filtration through a functional polychaete-assisted sand filter (PASF Bed 2).

2.3. Total nucleic acid extraction and IHHNV qPCR

Total nucleic acid was extracted from shrimp pleopods preserved in RNAlater using the MagJET RNA kit (Thermo Scientific), with the DNase 1 digestion step omitted, and a KingFisher Flex Magnetic Particle Processor as described in detail elsewhere (Sellars et al., 2019). This TNA extraction procedure was adopted for routine qPCR and RT-qPCR testing of shrimp and polychaete tissues for IHHNV (Cowley et al., 2018; Liu et al., 2020) and other pathogens due to it generating higher purity DNA compared to that extracted using the Proteinase K-based MagJET DNA kit (Thermo Scientific) and dye-binding analyses (Qubit Fluorometer method, Thermo Fisher Scientific) showing the TNA to generally comprise 40%–60% DNA (M. Rao et al., unpublished data). The TNA was eluted from magnetic beads in 50 μL RNase/DNase-free water and a 2 μL aliquot was analysed using a Nanodrop ND8000 UV spectrophotometer (Thermo Scientific) to assess TNA yield and purity. The TNA was stored at −80 °C until used.

IHHNV DNA amounts in each TNA sample were quantified by TaqMan real-time quantitative PCR (qPCR) analysis as described previously (Cowley et al., 2018). Briefly, each PCR (20 μL) comprised 10 μL 2 x SensiFAST™ probe Lo-ROX mastermix (Bioline), 1 μL (0.9 μM) each forward and reverse primer, 1 μL (0.25 μM) TaqMan probe and 4 μL DNA template. Three 5 μL aliquots of each 20 μL PCR were transferred to wells of a 384-well PCR plate as technical replicates and amplified using a ViiA7 qPCR system (Applied Biosystems) employing 40 cycles of the default pPCR thermal cycling profile. Serial 10-fold dilutions of a synthetic linear IHHNV dsDNA template of calculated copy number were also amplified in each plate to generate a standard curve from which a cycle threshold (Ct) value could be converted to IHHNV ssDNA copies 10 ng^{-1} TNA (Cowley et al., 2018). IHHNV DNA copies were expressed as 10 ng^{-1} TNA as this equated to <10 IHHNV DNA copies when the qPCR test limits of detection were being approached, as evidenced by samples giving Ct values >35 in only 1 or 2 of the 3 technical replicates.

3. Results

3.1. Shrimp growth and survival

The average weights of 20 *P. monodon* in each of the groups of 4 tanks determined either before (on 12 Feb 2017) or after (on 28 Feb 2017) exposure for 2 weeks to the 3 different shrimp pond wastewaters were not found to differ significantly ($P > 0.05$, Table 1). While average weight gains over the 2 week trial period were of an expected magnitude, they were marginally but not significantly lower ($P > 0.05$) among shrimp in the tanks exposed to the IHHNV-high Pond 1 wastewater. Average shrimp survival over the period was high (>95%) and not affected significantly by which pond wastewater they were exposed to ($P > 0.05$, Table 1).

3.2. IHHNV infection levels

IHHNV qPCR was used to quantify viral DNA loads in TNA extracted from a pleopod of each of 35 *Penaeus monodon* selected at random from each of the 3 exposure groups (Fig. 2A,B; Table 2). A higher percentage of the 35 shrimp tested from the group exposed to wastewater from IHHNV-high Pond 1 (26% including 1 with a much higher load) were identified to possess >2000 IHHNV DNA copies 10 ng^{-1} TNA, compared to 14% of the 35 shrimp exposed to the same pond filtered through PASF Bed 2, and 20% of the shrimp exposed to wastewater from IHHNV-low Pond 2.

At mid-range infection levels (Fig. 2B) the percentage of shrimp in which IHHNV was detected unequivocally at between 20 and 200 IHHNV DNA copies 10 ng^{-1} TNA was relatively high (48%) in prawns exposed to the unfiltered high-load wastewater, compared to those that received wastewater from this same pond filtered through PASF Bed 2 (6%) or directly from Pond 2 (3%) (Table 2). Accordingly, the percentage of shrimp in which IHHNV DNA was either not detected, or detected equivocally or unequivocally at low loads (ie. <20 IHHNV DNA copies 10 ng^{-1} TNA) was much higher in the groups exposed to PASF-filtered (80%) or low-load (77%) wastewater than the group exposed directly to high-load wastewater from Pond 1 (26%). Consistently with this, the percentages of shrimp in which IHHNV was either detected unequivocally in only 1 or 2 of the 3 replicate tests or not at all were also substantially higher. Significantly, IHHNV DNA was not detected in substantial proportions of 35 shrimp tested from the groups exposed to wastewater from Pond 2 (40%) or from Pond 1 after being filtered through PASF Bed 2 (46%). In comparison, IHHNV DNA was detected in all (100%) of the 35 shrimp tested from the group exposed directly to unfiltered wastewater from Pond 1 (Table 2).

Table 1
Average weights and survivals (± se) of *Penaeus monodon* (20 per tank; $n = 4$) exposed for 2 weeks to wastewater from IHHNV-low Pond 2 or from IHHNV-high Pond 1 either bypassing or using PASF filtration. Across each data type (row) for the 3 wastewater exposure sources, numbers with similar superscripts are not statistically different ($P > 0.05$).

Average shrimp weight (g) and survival (%)	Shrimp pond wastewater exposure		
	IHHNV-high Pond 1	IHHNV-high Pond 1 + PASF	IHHNV-low Pond 2
Before trial (g)	32.3 ± 1.1[a]	31.6 ± 0.4[a]	32.5 ± 0.7[a]
After trial (g)	34.6 ± 0.7[a]	34.4 ± 0.8[a]	35.6 ± 1.2[a]
Weight gained (g)	2.3 ± 0.7[a]	2.8 ± 0.6[a]	3.1 ± 1.3[a]
Survival (%)	97.5 ± 2.5[a]	97.5 ± 1.4[a]	96.3 ± 3.8[a]

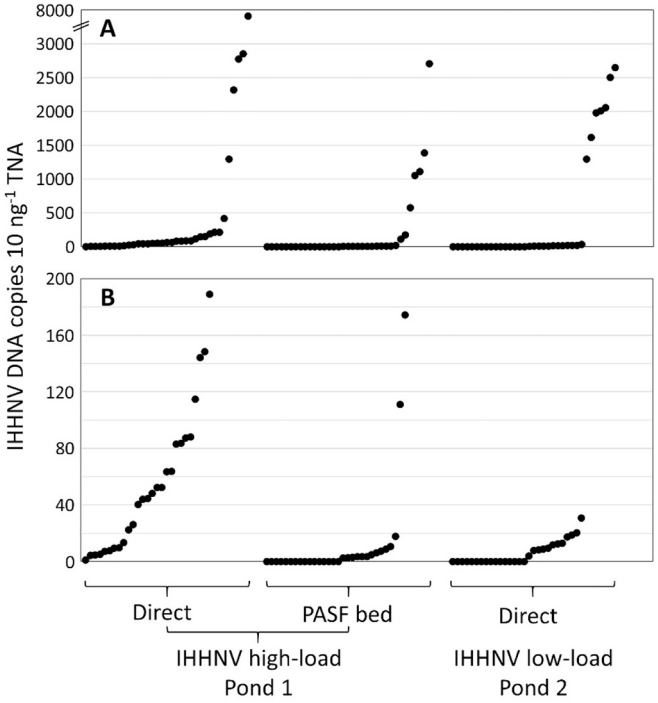

Fig. 2. IHHNV DNA loads quantified by real-time qPCR in pleopod TNA from 35 *Penaeus monodon* selected at random from each of 3 groups of 4 tanks of 20 shrimp from IHHNV-low Pond 2 exposed to IHHNV-high Pond 1 wastewater either directly or after filtration through a PASF or directly with IHHNV-low Pond 2 wastewater. IHHNV DNA load ranges are shown from (A) undetected to 8000 IHHNV DNA copies 10 ng^{-1} TNA to highlight shrimp with higher IHHNV loads and (B) undetected to 200 IHHNV DNA copies 10 ng^{-1} TNA to better resolve differences among shrimp in which IHHNV DNA was either not detected or detected at lower loads. Data on the 35 shrimp from each of the 3 exposure groups are ordered from lowest to highest IHHNV loads to more easily visualize similarities and differences.

Table 2
Summary of IHHNV DNA loads quantified by real-time qPCR in pleopod TNA from groups of 35 *Penaeus monodon* from IHHNV-low Pond 2 selected at random from the groups of 4 tanks exposed to wastewater from the same pond or from IHHNV-high Pond 1 either directly or after being filtered through PASF Bed 2.

IHHNV qPCR DNA copies 10 ng^{-1} TNA	Shrimp number					
	IHHNV-high Pond 1 wastewater				IHHNV-low Pond 2 wastewater	
	Direct	%	+PASF	%	Direct	%
>200	9	26	5	14	7	20
20 to 200	17	48	2	6	1	3
<20	9	26	28	80	27	77
Shrimp no. tested =	35		35		35	
Limit of detection[a]	3	9	8	23	9	26
Undetected[b]	0	0	16	46	14	40

[a] Ct value obtained in only 1 or 2 of the 3 replicate tests.
[b] No Ct value obtained in any of the 3 replicate tests.

4. Discussion

Shrimp species such as *P. stylirostris*, *P. vannamei* and *P. monodon* can vertically transmit IHHNV to progeny, which during pond culture can result in high-load acute infections that compromise their health and growth (Motte et al., 2003; Sellars et al., 2019). In *P. stylirostris*, such acute IHHNV infections can result in high mortality rates (Lightner et al., 1983). In *P. vannamei* and *P. monodon*, they more typically result in retarded growth sometimes associated with severe shell deformities (Bell and Lightner, 1984; Lightner et al., 1983; Primavera and Quinitio, 2000).

In the experimental *P. monodon* culture ponds used as a source of wastewater in this study, 2 possessed shrimp in which extremely high-load IHHNV infection developed at high prevalence and 2 possessed shrimp in which only low-load IHHNV infection developed at low prevalence (Sellars et al., 2019). In the ponds in which high-load IHHNV infection developed, there was a pronounced slowing of shrimp growth rates from midway through grow-out and survival rates and yields at harvest were markedly lower (Sellars et al., 2019). In a trial run in conjunction with that described here, when used as a nutrient source for polychaete-assisted sand filter (PASF) systems, wastewater from high-load IHHNV ponds was shown to result in IHHNV accumulating in the *Perinereis helleri* sand worms used to maintain their filtering capacity (Palmer, 2010; Liu et al., 2020). This alerted to potential biosecurity risks of the direct use of such polychaetes, for example, as a maturation diet supplement for shrimp broodstock (Chimsung, 2014; Meunpol et al., 2005, 2007). However, as a potential positive, it also suggested that the polychaetes used as an integral component of the PASF system might play a significant role in its capacity to sanitize wastewater discharged from ponds rearing shrimp heavily laden with IHHNV.

The transmission trial described here was thus designed to evaluate whether the PASF system might reduce or mitigate the potential for shrimp pond wastewater to transmit IHHNV infection to *P. monodon* by the well-documented per os exposure mechanisms (Lightner et al., 1983; Bell and Lightner, 1984; Lightner, 2011). The trial employed groups of 4 tanks each stocked with 20 *P. monodon* from a pond in which IHHNV infections were known to exist at low-loads in some shrimp (Sellars et al., 2019; Liu et al., 2020). Compared to an experimental design using pre-screened IHHNV-free or specific pathogen free (SPF) shrimp, not knowing the IHHNV status of individual shrimp selected at random for use in the trial had the potential to complicate comparisons of IHHNV qPCR data between the experimental groups, particularly if differences were not patently obvious. However, in the group of 35 shrimp selected from IHHNV-low Pond 2 that were exposed to wastewater from the same pond, IHHNV was either not detectable or detected at the qPCR test sensitivity limits in the majority (66%) of shrimp. Thus in the other groups of 35 Pond 2 shrimp exposed to wastewater from the IHHNV-high Pond 1, qPCR data on IHHNV prevalence and loads were able to unequivocally demonstrate IHHNV transmission to the shrimp directly from the wastewater but not after it being sanitized though a PASF system.

With regard to the mechanisms by which infectious IHHNV was removed from the shrimp pond wastewater by the PASF system, more elaborate investigations would be needed to establish the relative contributions of the polychaetes in ingesting IHHNV contaminated organic matter compared to the size-based filtering capacity or chemical actions (see below) of the sand substrate. In the context of IHHNV, important factors include its small (~22 nm dia.) icosahedral-shaped particles being structurally-robust like other viruses classified in the *Parvoviridae* (Lightner, 2011; Mathews, 1982). For example, when expressed in *Escherichia coli*, the IHHNV capsid protein can self-assemble spontaneously into virus-like particles (VLPs) that are indistinguishable in structure from native particles and that are highly stable in basic or acidic environments and in the presence of proteolytic enzymes (Hou et al., 2009; Kiatmetha, P. et al., 2018). While IHHNV DNA has been detected by qPCR in head, mid and tail sections of *P. helleri* reared in PASF systems supplied wastewater from ponds rearing IHHNV-infected *P. monodon* (Liu et al., 2020), this study did not specifically investigate the infectivity of IHHNV material in these digestive tract regions. The potential capacities of the polychaete digestive system and/or the chemical attributes of PASF discharge water to chemically or enzymatically inactivate IHHNV particles as they pass through a PASF thus remains to be determined.

There is growing evidence that neither IHHNV nor WSSV replicate in polychaetes in the genus *Perinereis* (e.g. *P. helleri*: Liu et al., 2020; *P. nuntia*: Laoaroon et al., 2005; *P. cultifera*: Shalini et al., 2016) and that they simply act as a passive carrier. However, it remains to be determined whether this holds true for all Nereidids (e.g. *Dendronereis*: Desrina et al., 2013; Desrina, 2014). As applied here to effectively reduce virus transmission risks of wastewater from ponds rearing *P. monodon* heavily infected with IHHNV, the primary role of the polychaetes in the PASF system is to ingest particulate organic matter that would otherwise rapidly clog the sand matrix (Palmer, 2010). As *P. helleri* feed upon organic matter settled on the surface of a PASF bed, what they ingest and accumulate in their gut might thus also prevent potentially infectious IHHNV particles from being dispersed into the sand substrate as it becomes degraded through other biological processes.

In a PASF system, the sand matrix provides a filtering capacity itself as a function of its pore size and the percolation rates induced by suction pressure at the discharge point. This can extend to particulate matter as small as 1–2 μm in diameter as evidenced by it trapping many species of phytoplankton in this size range including small chlorophytes (Palmer, 2010; Palmer et al., 2018). Such filtering would thus likely include tissue fragments shed, as an example, during the cannibalism of moribund IHHNV-infected shrimp. Based on the standard PASF operating system including a daytime drying period, potential exists for particulate matter settled on the surface of the sand bed matrix to be desiccated and sterilised though air and heat drying and exposure to solar UV irradiation. Subsurface layers have upper aerobic areas and lower anaerobic areas that support a wide variety of protozoa and bacteria as found in other sand filter systems (Campos et al., 2002), and the capacity of these microbes to decompose organic matter trapped within these layers appears to continue both during and between water percolation cycles (Palmer, 2011).

The decomposition of organic matter within PASF subsurface water flows also generates a particularly hostile chemical environment (Palmer, 2010). Water discharged from the PASF system can often possess up to 5 mg L^{-1} total ammonia nitrogen and 11 mg L^{-1} total sulphide (Palmer et al., 2016). Moreover, microbial respiration depletes most oxygen at lower substrate layers, leading to very little if any oxygen in the discharge water. This chemical environment greatly limits what organisms can passage through a PASF without being degraded. While

thus likely to inactivate infectivity of IHHNV and other less robust viral pathogens of shrimp, this remains to be assessed.

The data reported here suggest that there is value in using the PASF system to sanitize shrimp pond wastewater contaminated with IHHNV. They also suggest value in investigating its generic ability to reduce the biosecurity risks of shrimp pond wastewater laden with other viral and bacterial pathogens of concern to shrimp aquaculture. Where the PASF system is deployed in recirculated shrimp rearing systems to reduce wastewater nutrient and sediment loads, it might also provide an integrated and cost-effective means of controlling pathogen spread between ponds and/or the escalation of pathogen infection loads within a pond.

Organisational contributions

The work was equally funded through labour and operating funds provided by the CSIRO, and the Queensland Department of Agriculture and Fisheries, and all authors made significant contributions to the intellectual outcomes of the work.

Declaration of Competing Interest

None.

Acknowledgements

We thank Queensland Department of Agriculture and Fisheries and CSIRO technical staff at the Bribie Island Research Centre for assisting in shrimp sampling and maintaining the experimental shrimp ponds/tanks and PASF systems.

References

Bell, T.A., Lightner, D.V., 1984. IHHN virus: infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture 38, 185–194. https://doi.org/10.1016/0044-8486(84)90142-X.

Campos, L.C., Su, M.F.J., Graham, N.J.D., Smith, S.R., 2002. Biomass development in slow sand filters. Water Res. 36, 4543–4551. https://doi.org/10.1016/S0043-1354(02)00167-7.

Chimsung, N., 2014. Maturation diets for black tiger shrimp (*Penaeus monodon*) broodstock: a review. Songklanakarin J. Sci. Technol. 36, 265–273.

Coman, G., Preston, N., 2008. Black tiger domestication, selective breeding advance in Australia. In: Global Aquaculture Advocate. Jan/Feb 2008. https://www.aquaculturealliance.org/advocate/black-tiger-domestication-selective-breeding-advance-australia/.

Cowley, J.A., Dimmock, C.M., Wongteerasupaya, C., Boonsaeng, V., Panyim, S., Walker, P.J., 1999. Yellow head virus from Thailand and gill-associated virus from Australia are closely related but distinct prawn viruses. Dis. Aquat. Org. 36, 153–157. https://doi.org/10.3354/dao036153.

Cowley, J.A., Dimmock, C.M., Spann, K.M., Walker, P.J., 2000. Detection of Australian gill-associated virus (GAV) and lymphoid organ virus (LOV) of *Penaeus monodon* by RT-nested PCR. Dis. Aquat. Org. 39, 159–167. https://doi.org/10.3354/dao039159.

Cowley, J.A., Hall, M.R., Cadogan, LC., Spann, K.M., Walker, P.J., 2002. Vertical transmission of gill-associated virus (GAV) in the black tiger prawn Penaeus monodon. Dis. Aquat. Org. 50 (2), 95–104. https://doi.org/10.3354/dao050095.

Cowley, J.A., Moody, N.J.G., Mohr, P.G., Rao, M., Williams, L.M., Sellars, M.J., Crane, M. St J., 2015. Viral presence, prevalence and disease management in wild populations of the Australian black Tiger prawn (*Penaeus monodon*). In: Final Report - 2013-036-DLD, pp. 1–61. ISBN 978-1-4863-0631-2.

Cowley, J.A., Rao, M., Coman, G.J., 2018. Real-time PCR tests to specifically detect IHHNV lineages and an IHHNV EVE integrated in the genome of *Penaeus monodon*. Dis. Aquat. Org. 129, 145–158. https://doi.org/10.3354/dao03243.

Desrina, H., 2014. On the Role of the Polychaete *Dendronereis* spp. in the Transmission of White Spot Syndrome Virus in Shrimp Ponds. Wageningen University.

Desrina, J.A.J., Verreth, S.B., Prayitno, J.H., Rombout, W.M., Vlak, J.M., Verdegem, M.C. J., 2013. Replication of white spot syndrome virus (WSSV) in the polychaete *Dendronereis* spp. J. Invertebr. Pathol. 114, 7–10. https://doi.org/10.1016/j.jip.2013.05.002.

Gomez-Gil, B., Soto-Rodriguez, S., Lozano, R., Betancourt-Lozano, M., 2014. Draft genome sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of shrimps in Mexico. Genome Announc. 2, e00055-14 https://doi.org/10.1128/genomeA.00055-14.

Haditomo, A.H.C., Chilmawati, D., 2012. The white spot syndrome virus (WSSV) load in *Dendronereis* spp. J. Coastal Dev. 15 (3), 270–275.

Haryadi, D., Verreth, J.A.J., Verdegem, M.C.J., Vlak, J.M., 2014. Transmission of white spot syndrome virus (WSSV) from *Dendronereis* spp. (Peters) (*Nereididae*) to penaeid shrimp. J. Fish Dis. 38 (5), 419–428. https://doi.org/10.1111/jfd.12247.

Hou, L., Wu, H., Xu, L., Yang, F., 2009. Expression and self-assembly of virus-like particles of infectious hypodermal and hematopoietic necrosis virus in *Escherichia coli*. Arch. Virol. 154, 547–553. https://doi.org/10.1007/s00705-009-0336-6.

Infante-Villamil, S., Huerlimann, R., Condon, K., Maes, G.E., Jerry, D.R., 2019. Bacterial signatures of productivity decay in *Penaeus monodon* ponds infected with PirA toxin. Aquaculture 511, 734202. https://doi.org/10.1016/j.aquaculture.2019.734202.

Kiatmetha, P., Chotwiwatthanakun, C., Jariyapong, P., Santimanawong, W., Ounjai, P., Weerachatyanukul, W., 2018. Nanocontainer designed from an infectious hypodermal and hematopoietic necrosis virus (IHHNV) has excellent physical stability and ability to deliver shrimp tissues. PeerJ 6, e6079. https://doi.org/10.7717/peerj.6079.

Laoaroon, S., Boonnat, A., Poltana, P., Kanchanaphum, P., Gangnonngiw, W., Nash, G., Withyachumnarnkul, B., 2005. Infectivity of white spot syndrome virus (WSSV) to the polychaete *Pereneis nuntia* and a possibility of WSSV transmission from the polychaete to the black tiger shrimp *Penaeus monodon*. Diseas. Asian Aquac. V, 353–361.

Leelatanawit, R., Uawisetwathana, U., Khudet, J., Klanchui, A., Phomklad, S., Wongtripop, S., Angthoung, P., Jiravanichpaisal, P., Karoonuthaisiri, N., 2014. Effects of polychaetes (*Perinereis nuntia*) on sperm performance of the domesticated black tiger shrimp (*Penaeus monodon*). Aquaculture 433, 266–275. https://doi.org/10.1016/j.aquaculture.2014.06.034.

Lightner, D.V., 2011. Virus diseases of farmed shrimp in the Western hemisphere (the Americas): a review. J. Invertebr. Pathol. 106, 110–130. https://doi.org/10.1016/j.jip.2010.09.012.

Lightner, D.V., Redman, R.M., Bell, T.A., 1983. Infectious hypodermal and hematopoietic necrosis a newly recognized virus disease of penaeid shrimp. J. Invertebr. Pathol. 42, 62–70. https://doi.org/10.1016/0022-2011(83)90202-1.

Lightner, D.V., Redman, R.M., Pantoja, C.R., Noble, B.L., Tran, L., 2012. Early mortality syndrome affects shrimp in Asia. Glob. Aquac. Advoc. 15, 40. https://www.aquaculturealliance.org/advocate/early-mortality-syndrome-affects-shrimp-in-asia/.

Liu, S., Rao, M., Cowley, J.A., Morgan, J.A.T., Barnes, A.C., Palmer, P.J., 2020. *Perinereis helleri* reared in sand beds filtering nutrients from *Penaeus monodon* culture ponds can transiently carry IHHNV. Aquaculture 526, 735560. https://doi.org/10.1016/j.aquaculture.2020.735560.

Mathews, R.E.F., 1982. Classification and nomenclature of viruses. 4th report of the international committee on taxonomy of viruses. Intervirology 1, 1–199. https://doi.org/10.1159/000149278.

Meunpol, O., Meejing, P., Piyatiratitivorakul, S., 2005. Maturation diet based on fatty acid content for male *Penaeus monodon* (Fabricus) broodstock. Aquac. Res. 36, 1216–1225. https://doi.org/10.1111/j.1365-2109.2005.01342.x.

Meunpol, O., Iam-Pai, S., Suthikrai, W., Piyatiratitivorakul, S., 2007. Identification of progesterone and 17α-hydroxyprogesterone in polychaetes (*Perinereis* sp.) and the effects of hormone extracts on penaeid oocyte development *in vitro*. Aquaculture 270, 485–492. https://doi.org/10.1016/j.aquaculture.2007.05.031.

Mohr, P.G., Moody, N.J., Hoad, J., Williams, L.M., Bowater, R.O., Cummins, D.M., Cowley, J.A., St.J Crane,M., 2015. New yellow head virus genotype (YHV7) in giant tiger shrimp Penaeus monodon indigenous to northern Australia. Dis. Aquat. Org. 115 (3), 263–268. https://doi.org/10.3354/dao02894.

Motte, E., Yugcha, E., Luzardo, J., Castro, F., Leclercq, G., Rodriguez, J., Miranda, P., Borja, O., Serrano, J., Terreros, M., Montalvo, K., Narvaez, A., Tenorio, N., Cedeno, V., Mialhe, E., Boulo, V., 2003. Prevention of IHHNV vertical transmission in the white shrimp *Litopenaeus vannamei*. Aquaculture 219, 57–70. https://doi.org/10.1016/S0044-8486(02)00631-2.

Newman, S.G., 2010. Paradigm shifts in shrimp farming. In: Global Aquaculture Advocate Jan/Feb 2010. https://www.aquaculturealliance.org/advocate/paradigm-shifts-shrimp-farming/.

Norman-López, A., Sellars, M.J., Pascoe, S., Coman, G.J., Murphy, B., Moore, N., Preston, N., 2016. Productivity benefits of selectively breeding black Tiger shrimp (*Penaeus monodon*) in Australia. Aquac. Res. 47, 3287–3296. https://doi.org/10.1111/are.12782.

Nunan, L., Lightner, D., Pantoja, C., Gomez-Jimenez, S., 2014. Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. Dis. Aquat. Org. 111, 81–86. https://doi.org/10.3354/dao02776.

Oakey, H.J., Smith, C.S., 2018. Complete genome sequence of a white spot syndrome virus associated with a disease incursion in Australia. Aquaculture 484, 152–159. https://doi.org/10.1016/j.aquaculture.2017.11.009.

Palmer, P.J., 2010. Polychaete-assisted sand filters. Aquaculture 306, 369–377. https://doi.org/10.1016/j.aquaculture.2010.06.011.

Palmer, P.J., 2011. Commercial application of polychaete sand filters for wastewater remediation and broodstock feeds. In: Landcare Sustainable Practices Grant No. SEQC1418. Technical Report, p. 34. http://era.daf.qld.gov.au/id/eprint/2038/.

Palmer, P.J., Wang, S., Nash, W.J., 2016. Polybridge technical report. In: National Landcare Programme innovation Grant no. 041. Technical report, p. 130. http://era.daf.qld.gov.au/id/eprint/5132/.

Palmer, P.J., Wang, S., Houlihan, A., Brock, I., 2014. Nutritional status of a nereidid polychaete cultured in sand filters of mariculture wastewater. Aquaculture Nutrition 20 (6), 675–691. https://doi.org/10.1111/anu.12129.

Palmer, P.J., Wang, S., Mann, D., Nash, W.J., 2018. Polybridge season 3: ecosystem effects of polychaete-assisted sand filters. In: National Landcare Programme innovation Grant no. 041 Legacy Project. Technical Report, p. 44. http://era.daf.qld.gov.au/id/eprint/6150/.

Preston, N., Coman, G., Cowley, J., Moore, N., Murphy, B., 2010. Black tiger breeding program yields record shrimp harvests in Australia. Global Aquaculture Advocate 95. https://www.aquaculturealliance.org/advocate/black-tiger-breeding-program-yields-record-shrimp-harvests-in-australia/.

Preston, N.P., Coman, G.J., Sellars, M.J., Cowley, J.A., Dixon, T.J., Li, Y., Murphy, B.S., 2009. Advances in Penaeus monodon breeding and genetics. In: The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp farming, Aquaculture 2009 (ed. by C.L. Browdy & D.E. Jory). The World Aquaculture Society, Baton Rouge, LA, USA.

Primavera, J.H., Quinitio, E.T., 2000. Runt-deformity syndrome in cultured giant tiger prawn *Penaeus monodon*. J. Crustac. Biol. 20, 796–802. https://doi.org/10.1163/20021975-99990101.

Scott-Orr, H., Jones, J.B., Bhatia, N., 2017. Uncooked prawn imports: effectiveness of biosecurity controls. In: Australian Government Inspector-General of Biosecurity Review Report No. 2017-18/01, p. 180.

Sellars, M.J., Cowley, J.A., Musson, D., Rao, M., Menzies, M.L., Coman, G.J., Murphy, B. S., 2019. Reduced growth performance of black Tiger shrimp (*Penaeus monodon*) infected with infectious hypodermal and hematopoietic necrosis virus. Aquaculture 499, 160–166. https://doi.org/10.1016/j.aquaculture.2018.09.032.

Shalini, R., Haq, M.A.B., Ahamed, A.S., Kumar, P., Sedhuraman, V., Banu, M.N., 2016. WSSV transmission studies on polychaete *Pereneris cultifera* to Pacific white shrimp SPF *Litopenaeus vannamei* in captivity. J. Pure Appl. Biosci. 4 (6), 59–75. https://doi.org/10.18782/2320-7051.2366.

Spann, K.M., Cowley, J.A., Walker, P.J., Lester, R.J.G., 1997. A yellow-head-like virus from Penaeus monodon cultured in Australia. Dis. Aquat. Org. 31, 169–179. https://doi.org/10.3354/dao031169.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W., Itsathitphaisarn, O., 2016. Review of current disease threats for cultivated penaeid shrimp in Asia. Aquaculture 452, 69–87. https://doi.org/10.1016/j.aquaculture.2015.10.028.

Tran, L., Nunan, L., Redman, R., Mohney, L., Pantoja, C., Fitzsimmons, K., Lightner, D., 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Org. 105, 45–55. https://doi.org/10.3354/dao02621.

URL1, 2020. http://apfa.com.au/prawn-farming/.

URL2, 2020. https://www.environment.gov.au/epbc/about.

URL3, 2020. https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=19665.

URL4, 2020. http://apfa.com.au/wp-content/uploads/2015/01/10.1-Penaeus-monodon-mortality-syndrome-M-Landos-K-Condon.pptx.

Vijayan, K.K., Raj, V.S., Balasubramanian, C.P., Alavandi, S.V., Sekhar, V.T., Santiago, T. C., 2005. Polychaete worms - a vector for white spot syndrome virus (WSSV). Dis. Aquat. Org. 63, 107–111. https://doi.org/10.3354/dao063107.

Withyachumnarnkul, B., Chayaburakul, K., Supak, L.A., Plodpai, P., Sritunyalucksana, K., Nash, G., 2006. Low impact of infectious hypodermal and hematopoietic necrosis virus (IHHNV) on growth and reproductive performance of *Penaeus monodon*. Dis. Aquat. Org. 69, 129–136. https://doi.org/10.3354/dao069129.