Aquaculture 499 (2019) 160–166

Contents lists available at ScienceDirect

Aquaculture

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

Reduced growth performance of Black Tiger shrimp (*Penaeus monodon*) infected with infectious hypodermal and hematopoietic necrosis virus

Sellars M.J.[a,*], Cowley J.A.[a], Musson D.[b], Rao M.[a], Menzies M.L.[a], Coman G.J.[b], Murphy B.S.[b]

[a] Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, QLD 4067, Australia
[b] Bribie Island Research Centre, 144 North Street, Woorim, QLD 4507, Australia

ARTICLE INFO

Keywords:
IHHNV
Penaeus stylirostris penstyldensovirus
Shrimp
Virus
Giant Tiger prawn
Leader prawn

ABSTRACT

In Australia, Infectious hypodermal and hematopoietic necrosis virus (IHHNV) occurs endemically in Black tiger shrimp (*Penaeus monodon*) and has been detected at high prevalence in farmed shrimp. Here we examined the role of high IHHNV infection prevalence and loads in reduced growth and survival in *P. monodon* reared under commercial conditions in 0.16 ha research ponds. TaqMan real-time quantitative (q)PCR testing of wild broodstock from the East Coast of Queensland identified lower and higher IHHNV infection loads among females used to generate 2 cohorts of progeny. Tracking of IHHNV loads in eggs and juveniles sampled progressively over grow-out identified these to be substantially higher and to increase more rapidly in 2 ponds stocked with a batch of postlarvae (PL) pooled from 3 females with high-level IHHNV infection at the time they spawned compared to 2 neighbouring ponds stocked with PL pooled from 4 females, of which only 1 possessed IHHNV at a relatively high load. From 120 days of culture (DOC) onwards, the growth performance in the IHHNV-high ponds began to progressively reduce compared to that in the IHHNV-low ponds. When harvested over 2 weeks starting at 155 DOC, the averaged harvest yield of the 2 IHHNV-high ponds was the equivalent of 3.72 t/ha lower than the 2 IHHNV-low ponds, and estimated survival was also markedly lower (79.5–84.5% compared to 95.9–99.8%). As real-time qPCR testing identified no involvement of either Gill-associated virus (GAV) or Yellow head virus genotype 7 (YHV7), other viruses known to cause such production losses in Australia, the data support a sustained high IHHNV infection burden as the primary contributor to the reduced growth and survival. Using a basic extrapolation of yield, stocking low-level IHHNV PL has resulted in an increase of farm gate value of $67,000 per hectare compared to stocking high-level IHHNV PL. Among other problems such as severe shell deformities that acute IHHNV infection has recently been identified to cause in locally-farmed *P. monodon*, the potential impacts on growth and survival identified here reinforce the value in screening-based selection of IHHNV-free/low broodstock for use in hatcheries and in breeding programs employing specific pathogen-free stocks, and strategies to select for IHHNV resistance/tolerance.

1. Introduction

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a small (~20 nm dia.) non-enveloped icosahedral virus that contains a ~4 kb ssDNA genome and has been classified as type species *Penaeus stylirostris* penstyldensovirus 1 (*Pst*PDV) in the *Penstyldensovirus* genus, sub-family *Densovirinae* of the *Parvoviridae* (Lightner, 1999; Shike et al., 2000; Tang et al., 2003; Saksmerprome et al., 2010; Rai et al., 2011; King et al., 2012; Cotmore et al., 2014). It was first identified as the cause of mass mortalities in Pacific Blue shrimp (*Litopenaeus stylirostris*) in 1983 (Brock et al., 1983; Lightner, 1983) and subsequently as the cause of shell deformities and stunted growth described as 'runt deformity syndrome' in Pacific White shrimp (*Litopenaeus vannamei*) (Bell and Lightner, 1984; Kalagayan et al., 1991; Browdy et al., 1993) in shrimp farms in the Americas.

In Australia, IHHNV occurs commonly in wild and farmed Black Tiger shrimp (*Penaeus monodon*) (Krabsetsve et al., 2004; Saksmerprome et al., 2010), and several variants have been detected that cluster phylogenetically among IHHNV Lineage III strains detected elsewhere (Moody et al., 2009). While IHHNV has generally been found as an unapparent benign infection in wild and farmed *P. monodon* (Flegel et al., 2004; Chayaburakul et al., 2005), recently in domesticated lines of *P. monodon* being farmed in Australia, acute IHHNV infection has been associated with severe shell deformities consistent with those reported over 25 years ago in Indonesia also among domesticated lines of this species (Primavera and Quinitio, 2000, G.J. Coman et al., unpubl.).

* Corresponding author.
E-mail address: Melony.Sellars@csiro.au (M.J. Sellars).

https://doi.org/10.1016/j.aquaculture.2018.09.032
Received 5 July 2018; Accepted 15 September 2018
Available online 17 September 2018

The PCR-based detection of strains of IHHNV Lineages I, II and III has been complicated by the presence of non-infectious IHHNV genome forms integrated in the chromosomal DNA of *P. monodon* and detected in populations of *P. monodon* dispersed widely across the natural distribution range of this species (Tang et al., 2003, 2007, Tang and Lightner, 2006, Krabsetsve et al., 2004, Rai et al., 2009, Jaroenram et al., 2015). While a conventional PCR test has been developed to detect IHHNV specifically and exclude detection of genome-integrated IHHNV endogenous viral element (EVE)sequences (Tang et al., 2007), only recently have TaqMan real-time PCR tests been developed specifically to detect and differentiate IHHNV and EVE sequences and to accurately quantify IHHNV infection loads (Cowley et al., 2018).

Due to the common occurrence of IHHNV in regions in northern Australia trawled routinely to supply wild *P. monodon* broodstock to hatcheries, here we had the opportunity to undertake simulated commercial grow-out of 2 cohorts of shrimp that differed in IHHNV prevalence and loads as a result of the females from which they were spawned also differing in IHHNV infection loads. Tracking of IHHNV prevalence and loads progressively over grow-out by TaqMan real-time quantitative (q)PCR test (Cowley et al., 2018) identified a clear association between the early onset of high-level IHHNV infection and substantially reduced growth rates, survival and harvest yields.

2. Materials and methods

2.1. Broodstock source, maturation and spawning

Broodstock were caught in coastal waters off Bramston Beach (17.3632° S, 146.0184° E) and Mission Beach (17.8694° S, 146.1069° E) in North Queensland, and were airfreighted (6 per oxygenated broodstock bag, 23.5 ± 1 °C) to the Bribie Island Research Centre (27.0535° S, 153.1942° E) within 48 h. Upon arrival, shrimp in their bags were acclimated for 1–2 h in 120 L tubs supplied with aerated flow-through seawater at 25.0 ± 1 °C. Each broodstock was then sexed, weighed, eye-tagged and had a piece of pleopod tissue (~5 mm in length) removed using sterile scissors and preserved in 1.0 mL RNALater® (Thermo Fisher Scientific). Two groups of 32 females and 32 males were placed in 10,000 L circular maturation tanks employing heated (25.0 ± 1 °C) flow-through (3.5 L/min) seawater, a 3 mm thick layer of sand, a 10 mm twin-walled polycarbonate lid to reduce light and a 24 h dark photoperiod.

Over the next 16 days, broodstock were fed BREED-S Shrimp maturation pellets (INVE) at ~0.5% estimated total bodyweight (ETB) supplemented with chopped raw arrow squid (*Nototodarus gouldi*) at ~6% ETB and chopped raw green-lipped mussels (*Perna canaliculus*) at ~3% ETB. Over the next 22 days, water temperature was increased 1 °C daily until water reached 28 °C, and feeding of BREED-S Shrimp maturation pellets was supplemented with raw squid (~5% ETB), chopped raw mussels (~2.5% ETB), live blood worms (*Chironomus cloacalis*) (~2% ETB) and raw beef liver (~0.5% ETB).

A pleopod tip was then sampled and preserved for each broodstock as described, the ovary stage of each female was recorded (Tan-Fermin and Pudadera, 1989) and an eye-stalk was unilaterally ablated. Females were checked each afternoon for ovary development indicative of imminent spawning (Tan-Fermin and Pudadera, 1989), and if ripe, were placed into a 0.66 m dia. Tank fitted with a 120 μm outlet screen and filled with 80 L flow-through (0.7 L/min) seawater at 28 °C. In the morning following spawning, females were euthanized humanely by submerging in iced water and a pleopod sample preserved as described. Eggs were left in the tank to hatch and the following morning, nauplii were harvested by siphoning through a submerged 120 μm screen and concentrated into 7 L seawater. The total number of nauplii was calculated by counting numbers present in 3 samples of 1.0 mL and extrapolating the average number to 7 L.

2.2. Larval rearing

Nauplii were stocked from 18 to 22 August 2016 at 50–100/L into 6000 L parabolic larval-rearing (LR) tanks covered with black plastic to reduce light intensity and risk of aerosol cross-contamination between tanks and filled with ~4000 L 10 μm filtered and UV-sterilized aerated seawater maintained at 30 °C using submersible heaters. When multiple females spawned within a 48 h period, nauplii families were pooled. From late stage nauplii, the larvae were fed microalgae until they reached postlarvae stage 1 (PL1). The micro-algal diet was supplemented from protozoea stage 2 with Frippak CD#2 larvae diet (Sap International Corp.), from protozoea stage 3 to PL20 with artemia and from mysis stage 1 to PL1 with Frippak CD#3 diet (Sap International Corp.). PL2 to PL4 were fed alternatively on Frippak PL + 150 (Sap International Corp.) and Frippak CD#3. From PL5, Frippak CD#3 was replaced by LANSY® Shrimp PL (Primo Aquaculture) and from PL8, Frippak PL + 150 was replaced by Frippak PL + 300 (Sap International Corp.). From PL10, crushed LANSY® Power Flake (Primo Aquaculture) was used. The various larval feeds were given at standard rates and frequencies used in commercial hatcheries and adjusted based on amounts of unconsumed feed noted prior to each feeding.

2.3. Postlarvae counts and pond stocking

At PL20, batches of < 100,000 postlarvae harvested from each 6000 L LR tank were placed into a tank containing exactly 40 L aerated seawater. Following gentle swirling by hand to distribute PL uniformly, PL in 6 aliquots of water (37 mL) were counted and averaged to estimate numbers of PL20/L and total PL20/tank. PL20 were then transported to pond-sides (~2 min) in 20 L aerated water, acclimated by the addition of 2 × 9 L volumes of pond water over a 5 min period before being gently released into the pond. PL20 were stocked at a density of 44 individuals/m^2.

2.4. Pond grow-out

The fully-lined grow-out ponds were 40 m × 40 m × 2 m deep (0.16 ha) and employed a 100 mm PVC standpipe central drain, a 3 mm oyster mesh screened outlet weir, a 100 mm screened (70% shade cloth) water inlet, a 2 HP paddlewheel placed in one corner and the group of 4 ponds was completely enclosed with bird-netting. Once filled, water was aerated and circulated using the paddlewheel and fertiliser was added twice weekly to encourage algal growth. As no algal blooms had stabilized after 10 days, 500 g Blue Horizon pond dye (Primo Aquaculture) was added to each pond to reduce light penetration and thus benthic algae growth on pond bottoms as well as light stress on the PL20 stocked the following day.

On 16-20th September 2016, two of the 4 grow-out ponds (Ponds 2 and 3) were stocked with PL20 from LR Tank 1 that contained progeny of Females 1, 2, 3 and 4 and Ponds 1 and 4 were stocked with PL20 comprising equal numbers of PL derived from LR Tank 4 containing progeny of Female 5 and LR Tank 6 containing progeny of Females 6 and 7 (Table 2). After 17 days of culture (DOC), blooms dominated by *Coccolithophorid* spp. had established in all ponds, and as this resulted in secchi depth dropping to 20 cm, pond fertilization was ceased. Pond water quality (dissolved oxygen, pH, temperature, salinity and secchi depth) and algal bloom status were assessed daily during the grow-out period in the early morning when dissolved oxygen was lowest. Algal bloom densities and pond water pH and alkalinities in each pond were managed over the grow-out period on a needs basis through controlling seawater flow-through rates and applying dolomite and hydrated lime at rates used typically at commercial farms.

Up to 50 DOC, shrimp were fed 3 times daily (0600, 1200 and 1800 h) with Starter #1 MR pellet feed (Ridley Aqua Feed) at standard rates for early-stage PL (Australian Prawn Farming, Health Management for Profit,

2006). Feed rates were reduced at times over this period to accommodate low water temperatures (~21 °C min, ~23 °C max) that resulted in slower than predicted growth rates. At 44 DOC, 3 belt-feeders were installed on each pond to deliver feed throughout the night. From 53 DOC onwards when the PL73 shrimp began to use feed trays, tray 'scoring' methods were used to set feed amounts (Australian Prawn Farming, Health Management for Profit, 2006). At 100 DOC, an additional belt-feeder was installed on each pond to increase the nightly feed amount. At 110 DOC, when shrimp in all ponds were > 15 g average weight, pond feed rates were capped at 60 kg/day due to ensure nitrogen discharge remained within site licence levels.

To harvest shrimp, larger individuals were captured from each pond in small quantities (500–800 kg) using 'wing traps' on 3 to 4 occasions, depending on numbers of larger shrimp, between 150 and 170 DOC. The remaining shrimp were harvested by draining ponds completely and collecting shrimp in seine nets and by hand.

2.5. Shrimp samples

Upon their arrival, 1 pleopod tip was sampled as described and again on the morning after each female had spawned, at which time a sample of ~50 eggs from each spawn were concentrated on a mesh screen and preserved in 1.0 mL RNALater, noting that they had been 'washed' extensively by the tank flow-through water system. Batches of ~50 nauplii were harvested then preserved similarly the following morning. When postlarvae reached PL20 ready for pond stocking, ~200 were harvested then preserved similarly and preserved in 10 mL RNALater. At 51, 86, 120, 133 and 155 DOC, 142 to 144 shrimp were generally collected at random from each pond by cast-netting. Each shrimp was sexed, weighed and had a pleopod preserved in 1.0 mL RNALater. A total of 144 shrimp were sampled to allow virus to be detected by qPCR down to close to a 2% prevalence threshold (Lightner 1996). Initially, pleopods from only random groups of 48 shrimp from each sampling time point of each pond were processed into TNA. At 140 DOC, 30 shrimp were collected and sampled similarly from each pond except that in addition to pleopod, lymphoid organ (LO) tissue from each shrimp was preserved in RNALater. Testing of 30 shrimp would allow virus to be detected by PCR down to 10% prevalence threshold (Lightner 1996) and LO tissue was collected to maximize the sensitivity of detecting low infection loads of Gill-associated virus (GAV) and Yellow-head virus genotype 7 (YHV7), another virus endemic to *P. monodon* inhabiting northern Australia (Cowley et al., 2015; Mohr et al., 2015) and for confirming relative IHHNV infection prevalence and loads (OIE, 2017).

2.6. TNA extraction, cDNA synthesis and TaqMan real-time qPCR

Larval stages and tissues of juvenile shrimp in RNALater were removed from 96-well deep-well plates or tubes using either sterile forceps or by pouring onto absorbent paper towel to remove excess liquid. With the PL20, cephalothorax cut from the abdomen with a scalpel blade was pooled in lots of 10 PL for extraction. Tissue was placed in 600 µL RLT lysis buffer (QIAGEN), homogenized using a bead beater and TNA was extracted using a MagJET RNA Kit (Thermo Scientific) and a KingFisher Flex 96 (Thermo Scientific) extraction robot following the manufacturer's protocols, except that the DNase 1 digestion step was omitted and an additional Wash Buffer 2 step was included. TNA quantity and purity was assessed by examining a 1.5 µL aliquot of each extract using a ND-8000 UV spectrophotometer (ThermoFisher Scientific). To detect GAV and YHV7 by real-time qPCR, cDNA was synthesized in a 10 µL reaction containing 500 ng TNA and prepared using SensiFAST™ cDNA Synthesis Kit (Bioline) reagent containing an optimized mix of random hexamers and anchored oligo-dT primers as described in the manufacturer's protocol.

Primer and probe sequences used in the qPCR tests to detect IHHNV (Cowley et al., 2018) as well as GAV (de la Vega et al., 2004) and YHV7 (Cowley et al., 2015) are described in detail elsewhere. For each test, reactions (20 µL) were prepared to contain 1 x SensiFAST™ Probe Lo-ROX mastermix (Bioline), 0.9 µM each primer, 0.25 µM probe and a normalized amount (100 ng) of either TNA or cDNA. Reaction aliquots (5 µL containing 25 TNA/cDNA) were dispensed into each of 3 wells of a 384-well real-time PCR plate as technical replicates using an EpMotion 5075 liquid handling robot (Eppendorf). DNA was amplified using a ViiA 7 Real-Time PCR System (Applied Biosystems) and a thermal cycling profile (95 °C/2 min for polymerase activation, 40 cycles of 95 °C/15 s, 60 °C/30 s) within the parameters recommended in the SensiFAST™ Probe Lo-ROX Kit instructions (Bioline).

To quantify IHHNV DNA and GAV/YHV7 RNA copy numbers accurately in clinical samples, 10-fold dilution series of IHHNV plasmid DNA, GAV cDNA or YHV7 cDNA of predetermined copy number was amplified in the same plate to determine a linear regression plot of mean cycle threshold (Ct) values vs. copy number (Sellars et al., 2014).

2.7. Statistical analyses

Individual shrimp weights and IHHNV DNA loadings determined progressively over pond rearing were assessed by factorial ANOVA with model factors including treatment, pond nested within treatment, and sex (from 86 DOC onwards when the sex of shrimp could be determined visually) (SAS Institute Software, 1999). IHHNV DNA copy number was log-transformed prior to analysis of IHHNV loads.

3. Results

3.1. IHHNV and GAV loads in female broodstock used to produce families

Of the 4 wild-captured female *P. monodon* broodstock contributing to PL20 reared in LR Tank 1, qPCR analysis of TNA extracted from a piece of pleopod tissue at the time they were received identified IHHNV loads to be extremely low in 3 (Females 1, 2 and 3; 16–50 IHHNV DNA copies/µg TNA) and moderate in the other (Female 4, 5.44×10^4) (Table 1). Of the 3 female broodstock contributing to PL20 reared in LR Tanks 4 (Female 5) and 6 (Females 6 and 7), qPCR identified IHHNV loads to be extremely low in 2 (Females 5 and 6: ~11 IHHNV DNA copies/µg TNA) but moderately-high in the other (Female 7; 6.71×10^5). However, testing of TNA extracted from pleopod tissues sampled at the time they spawned after being matured in tanks for ~6 weeks, IHHNV loads remained quite low among the IHHNV-low group, Females 1 and 2 and moderate in Female 3, but were increased to 9.51×10^6 in Female 4 (Table 1). Among the IHHNV-high group females, IHHNV loads were increased to 4.65×10^8 in Female 4, 2.25×10^7 in female 5 and 1.1×10^9 in Female 7. IHHNV loads in batches of eggs spawned from the 7 females were highest in those from Females 5 and 7 (Table 1).

While GAV loads detected by RT-qPCR in the 7 female *P. monodon* at the time of receipt were very low in general, none escalated like IHHNV over the ~6 week maturation period leading up to spawning (Table 1). Moreover, unlike IHHNV, no GAV was reliably detected in the egg batches spawned from any of the females.

3.2. IHHNV and GAV loads and prevalence of PL20 batches

The IHHNV qPCR test was used to quantify IHHNV DNA loads in TNA extracted from 16 pools of 10 x PL20 sampled from LR Tank 1 (progeny of Females 1, 2, 3 and 4) and LR Tanks 4 (progeny of Female 5) and 6 (progeny of Females 6 and 7) on the day grow-out ponds were stocked (Table 2). While these varied markedly among the 16 pools of PL20 sampled from each tank (as evidenced by a very high standard deviation), loads detected in PL20 pools from IHHNV-high LR Tanks were in the order of 45- and 865-fold higher, respectively, than among PL20 pools sampled from the IHHNV-low LR Tank. GAV was only detected by RT-qPCR in 4 of the 16 PL20 pools from IHHNV-low LR Tank

Table 1
GAV RNA and IHHNV DNA copy numbers quantified by TaqMan real-time qPCR in pleopod tips sampled from female *P. monodon* and from eggs and nauplii contributing the PL20 families used to stock ponds.

PL20 Group	Larval-rearing tank	Female	Mean GAV RNA copies/μg TNA[a]				Mean IHHNV DNA copies/μg TNA[a]			
			On arrival	Injection/eye stalk ablation	At spawning	Egg pool	On arrival	Injection/eye stalk ablation	At spawning	Egg pool
IHHNV-low	1	1	14	2190	85	UD	16	4270	4070	50
	1	2	83	370	19	34[b]	25	17[b]	790	30
	1	3	4.26×10^3	3680	57	19[c]	5.44×10^4	7.39×10^4	1.83×10^5	255
	1	4	577	3810	28	UD	50[c]	35	9.51×10^6	193
IHHNV-high	4	5	24	223	504	19[c]	11[c]	709	4.65×10^8	1030
	6	6	828	2070	1080	11[c]	11	2450	2.25×10^7	225
	6	7	177		81	23[b]	6.71×10^5	3.56×10^7	1.10×10^9	2.80×10^5

UD = all 3 technical replicates undetermined, LR tank = Larval-rearing tank.
[a] Means were determined from 3 technical replicates and low RNA/DNA copy numbers were rounded up/down to a whole number/μg TNA.
[b] 1 of 3 technical replicates UD.
[c] 2 of 3 technical replicates UD.

1, and these detections were marginal at the test detection sensitivity limits (Table 2).

3.3. IHHNV, GAV and YHV7 prevalence and loads during pond grow-out

Pleopod or LO TNA from between 30 and 48 shrimp sampled from each of the 4 ponds at 51, 86, 120, 140 and 155 DOC were tested by RT-qPCR for GAV and YHV7 and by qPCR for IHHNV to determine virus prevalence and infection loads (Fig. 1, Supplementary Table 1). In Ponds 1 and 4 stocked with IHHNV-high group PL20, IHHNV prevalence rose from 67%–83% at 51 DOC to 100% by the next sampling time point (86 DOC), after which it remained at 100%. In Ponds 2 and 3 stocked with IHHNV-low group PL20, IHHNV prevalence was relatively lower (31%–56%) at 51 DOC and increased more slowly, reaching 97% among the batches of 30 LO tissue samples collected at 140 DOC and only reach 100% among the pleopod batches collected at harvest (155 DOC).

While IHHNV loads varied markedly among individual shrimp (as evidenced by a very high stdandard deviation), mean IHHNV loads increased rapidly from 0.97–1.86 × 10^4 IHHNV DNA copies/μg TNA at 51 DOC to 3.37–3.66 × 10^8 at next sampling time (86 DOC) in IHHNV-high group shrimp (Ponds 1 and 4) after which they stayed around these levels until reaching 1.94–2.24 × 10^9 at time shrimp being harvested (155 DOC) (Fig. 1B, Supplementary Table 1). In contrast, mean IHHNV DNA loads in shrimp from IHHNV-low group shrimp in Ponds 2 and 3 were in the order of 27-fold to 69-fold lower at 51 DOC and generally stayed between approx. 10,000-fold and 70,000-fold lower when tested at all subsequent sampling times during grow-out.

GAV RNA was not detected by RT-qPCR in any shrimp up until 140 DOC, at which time it was only detected at the test detection sensitivity limit in LO tissue from 1 of the 30 shrimp sampled from Pond 4 (Supplementary Table 1). At harvest (155 DOC), GAV was detected at very low loads in pleopods of 71% of the 48 shrimp sampled from Pond 3 and a single shrimp sampled from Pond 4. YHV7 was not detected by RT-qPCR in LO of shrimp sampled at 140 DOC from any of the 4 ponds (Supplementary Table 1).

3.4. Shrimp weight increases over grow-out and pond production metrics

Although some difficulties were experienced in establishing and maintaining algal blooms across the 0.16 ha research ponds, these were remediated using measures typical of those employed in 1 ha commercial ponds and were not localised to Ponds 2/3 or Ponds 1/4. At 51 DOC mean weights of 144 shrimp sampled at random from either Pond 1 (2.05 ± 0.85) or Pond 4 (1.76 ± 0.77) were significantly higher ($P < .001$) compared to that of 144 shrimp sampled from Pond 3 (1.43 ± 0.67) but not Pond 2 (1.73 ± 0.67) (Supplementary Table 1 for data resolution; Fig. 1C). At the next sampling time (86 DOC), mean weights (7.27 ± 4.06 to 7.83 ± 4.01) were similar across all 4 ponds. However, at sampling times thereafter, mean weights of shrimp from either Pond 1 or Pond 4 (IHHNV-high) were progressively and significantly lower compared to those of shrimp from either Pond 2 or Pond 3 (IHHNV-low) (Fig. 1C). Weight differences between males (lighter) and females (heavier) became evident among shrimp from all 4 ponds among batches sampled from 133 DOC onwards (data not shown). However, differences in mean weights of shrimp sampled from either Ponds 2 and 3 or Ponds 1 and 4 at any time point after which their sex could be determined reliably (i.e. 86 DOC) were not influenced by imbalances in male:female ratios (Supplementary Table 1).

Extrapolating shrimp harvest yields realized in the 0.16 ha experimental ponds to commercial ponds 1 ha in size, yields from IHHNV-low Ponds 2 and 3 (12.3 and 14.2 t/ha, respectively) were 22% to 58% higher than yields from IHHNV-high Ponds 1 and 4 (9.0 and 10.1 t/ha, respectively) (Table 3). Estimated shrimp survival in IHHNV-low Ponds 2 and 3 (95.9% and 99.8%, respectively) was also 13% to 25% higher than survival in IHHNV-high Ponds 1 and 4 (79.9% and 84.5%, respectively). As expected due to the higher growth rates, survival and

Table 2
Mean GAV RNA and IHHNV DNA copy numbers quantified by TaqMan real-time qPCR in 16 pools of 10 x PL20 sampled from each larval-rearing tank on the day ponds were stocked.

PL20 Group	Female	Larval-rearing tank	Ponds stocked	Mean RNA or DNA copies/μg TNA ± SD	
				GAV RT-qPCR	IHHNV qPCR
IHHNV-low	1, 2, 3, 4	1	2 and 3	22.4 ± 88.2*	$4.81 \pm 8.36 \times 10^4$
IHHNV-high	5	4	1 and 4	UD	$2.14 \pm 8.49 \times 10^6$
IHHNV-high	6, 7	6	1 and 4	UD	$4.16 \pm 3.99 \times 10^7$

UD = undetermined in all tests and all technical replicates.
* 12 of the 16 pools were undetermined in all 3 RT-qPCR technical replicates with the other 4 GAV-positive in 1, 2 or 3 technical replicates at the detection sensitivity limits of the test, SD = standard deviation.

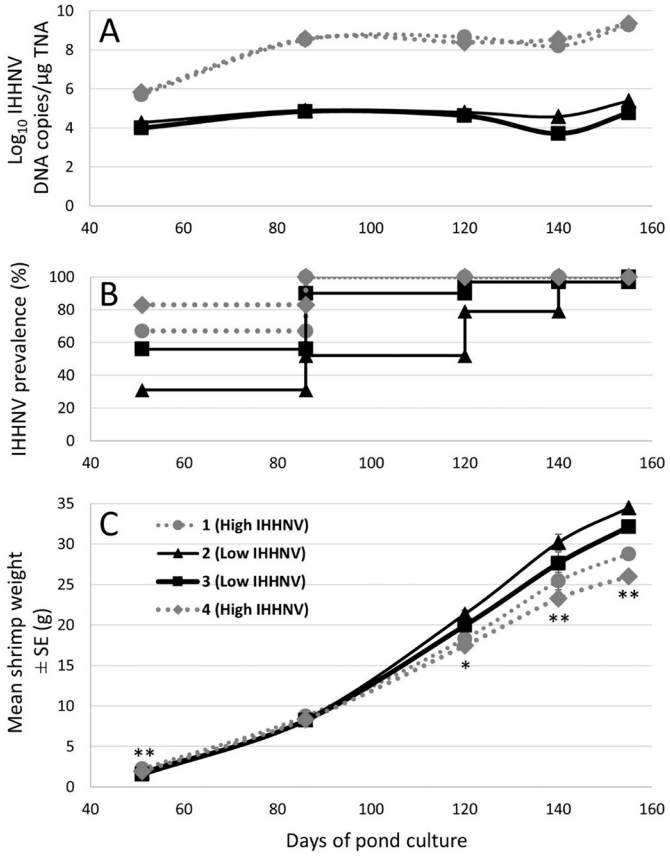

Fig. 1. (A) Log_{10} mean IHHNV DNA copies/µg TNA as determined by real-time qPCR using pleopod tissue from 48 of the 144 shrimp sampled from each of the 4 ponds at each time point, except at 140 DOC when lymphoid organ tissue from 30 shrimp was tested. (B) Prevalence (%) at which IHHNV was detected by real-time qPCR in the 48 shrimp examined from each pond at each time point, except at 140 DOC when prevalence was assessed for only 30 shrimp. (C) Mean shrimp weight ± SE (g) of 144 shrimp/pond sampled progressively throughout grow-out except at 140 DOC when only 30 shrimp/pond were weighed. Levels of statistical significance (* $P < .05$; ** $P < .001$) were determined using logarithmic-transformed mean weights of shrimp from IHHNV-high Ponds 1 and 4 combined and IHHNV-low Ponds 2 and 3 combined at each sampling time point. Pond codes are indicated, Pond 1 (●), Pond 4 (♦), Pond 2 (▲) and Pond 3 (■).

harvest yields, shrimp reared in IHHNV-low Ponds 2 and 3 collectively consumed ~36% more feed than the shrimp reared in IHHNV-high Ponds 1 and 4 (Table 3).

Using total shrimp harvest weights extrapolated from the 0.16 ha research ponds to 1 ha commercial ponds, IHHNV-low ponds collectively yielded an additional 7.44 t (ave. 3.72 t/ha) compared to IHHNV high ponds. Based on shrimp having mean weight at harvest meeting a 35–38 pieces/kg size class with a typical cooked wholesale value in Australia of ~AUD$18/kg, this 3.72 t/ha average increase in pond yield increased gross value by AUD$67 K/ha pond.

4. Discussion

Reported here are data associating the sustained presence of high-level IHHNV infection with reduced growth performance and survival of *Penaeus monodon* reared under simulated commercial conditions in 0.16 ha research ponds. The role of IHHNV in reduced growth performance was investigated due to the absence of both GAV, which has been associated most commonly over that past 2 decades with reduced survival and harvest yields of *P. monodon* farmed in eastern Australia (Spann et al., 1997; Callinan et al., 2003; Callinan and Jiang, 2003; Munro et al., 2011), and YHV7, a genotypic variant of GAV which has also been associated with the similar stock losses since being introduced into Queensland more recently through the use of wild broodstock sourced from remote locations in northern Australia (Cowley et al., 2015, Mohr et al., 2015; Moody et al., 2017; J.A. Cowley et al. unpublished). Pivotal to examining IHHNV infection severity as the cause of reduced pond productivity was its detection by TaqMan real-time qPCR (Cowley et al., 2018) at higher loads in 3 females that contributed postlarvae (PL) stocked into 2 research ponds in which growth performance and survival was markedly compromised compared to that detected in 4 females contributing PL stocked into 2 neighbouring research ponds.

Using single pleopod tips as a source of TNA for qPCR analysis, IHHNV loads in the 7 wild female broodstock were noted to increase significantly, in some cases by up to 10^7-fold between when they were tested upon receipt compared to when each female spawned ~6 weeks later. While factors contributing to these increased IHHNV infection loads might have included stresses induced by capture, tank rearing, dietary changes and handling (de la Vega et al., 2004), variability in virus loads in different pleopods (Noble et al., 2018) and/or virus horizontal transmission occurring over the maturation period (Walker et al., 2001), the exact causes are unknown. The presence of IHHNV in the 7 female broodstock resulted in it also being detected in pools of washed eggs spawned from each, confirming the well-recognized propensity for IHHNV to be transmitted vertically from infected female L. *vannamei* (Lotz, 1997; Lightner, 1999; Motte et al., 2003) and *P. monodon* (Withyachumnarnkul et al., 2006).

Data on nauplii produced from a large number of female L. *vannamei* determined to be either nested PCR-negative or positive for IHHNV identified a likely association between IHHNV infection loads in broodstock and compromised egg hatch rates and larval survival/growth performance (Motte et al., 2003). No substantial differences in egg hatch rates or larval growth were evident in this study among progeny the 7 female *P. monodon* broodstock examined. Despite not knowing the IHHNV infection status of males that inseminated the females, the role of males in IHHNV transmission has been discounted previously based on the rarity of detecting IHHNV DNA in L. *vannamei* spermatophores by nested PCR (Motte et al., 2003). As reported previously (Motte et al., 2003), the qPCR data in this study highlight the potential value in undertaking testing in hatcheries to identify and select for either IHHNV-free or IHHNV-low broodstock, when sufficient numbers are available to entertain this option, as is recommended commercial practice to preclude virus from being transmitted to seedstock at high efficiency. The data also highlight potential value in pursuing RNA interference (RNAi) approaches, as examined more

Table 3
Penaeus monodon pond production metrics.

Pond No.	IHHNV Group	Feed consumed (kg)	Harvest weight (kg)	Feed conversion ratio (FCR)	Yield (tonne/ha[a])	Survival (%)
2	IHHNV-low	3245	2273	1.43	14.2	95.9
3		3337	1966	1.70	12.3	99.8
1	IHHNV-high	2434	1613	1.51	10.1	84.5
4		2388	1436	1.66	9.0	79.9

[a] Extrapolated from the 0.16 ha pond (i.e. total weight/0.16).

extensively with other viruses (Sellars et al., 2011, 2014, 2016, 2018; Rao et al., 2018; Escobedo-Bonilla, 2013; Itsathitphaisarn et al., 2017), as a means of reducing or more ideally clearing viral infections prior to broodstock spawning, thus further reducing the potential for IHHNV transmission.

At PL20 when grow-out ponds were stocked, mean IHHNV loads were 45-fold to 865-fold higher in IHHNV-high PL20 from LR Tanks 4 and 6, respectively, in comparison to the PL20 from IHHNV-low LR Tank 1. However, these were tested using TNA extracted from 16 pools of 10 PL (total = 160) from each tank for logistical reasons of testing of > 150, and IHHNV loads varied substantially among the pools. While this might have been due to each of the 3 different females transmitting IHHNV at variable levels to the PL reared in 2 of the 3 LR tanks, significant variability was also evident among the 16 PL pools from LR Tank 4 that contained progeny of a single female (5). While both vertical and horizontal transmission likely contributed to variability in IHHNV loads, the findings that Female 7 was (i) infected with IHHNV at moderate loads upon receipt, (ii) maintaining this infection through to spawning and (iii) spawning eggs in which IHHNV was detected at loads 280-fold to 9333-fold higher than eggs spawned from any of the other 6 females suggested a clear role in it contributing to the higher IHHNV loads in the PL20 reared in LR Tank 6. While associations between the duration of IHHNV infection and the propensity of a female to spawns eggs carrying IHHNV at substantial loads will need to be demonstrated, our findings suggest that wild broodstock identified upon receipt to be infected at moderate to high loads with IHHNV present an elevated risk of transmitting infection to progeny. Thus early PCR screening to identify and cull such broodstock before being matured and spawned is recommended. To further reduce production impacts, and as IHHNV can be detected reliably by PCR at earlier PL life stages (i.e. PL8, Lightner, 1983; M.J. Sellars et al. unpubl.), opportunities also exist in hatcheries to select at the PL rearing stage for IHHNV-free/low seedstock to either culture or sell at a premium price.

At 51 DOC, mean IHHNV loads in juveniles from each of the 4 ponds remained comparable to those detected in hatchery tanks at the time PL20 were stocked into ponds. However, between then and 86 DOC, IHHNV loads increased by ~300-fold among the shrimp in Ponds 1 and 4 compared to only ~10-fold among the shrimp in Ponds 2 and 3. As IHHNV prevalence in Ponds 1 or 4 also reached 100% at this time, infection severity likely reached levels sufficient to be transmitted horizontally at high efficiency. These findings are consistent with transmission cycle dynamics expected in ponds as infection loads increase to acute levels capable of compromising shrimp health (Walker et al., 2001).

From 120 DOC onwards, mean weights of juveniles being reared in Pond 1 and particularly Pond 4 began to lag significantly behind those in Ponds 2 and 3. As there was no evidence of either GAV or YHV7 occurring in shrimp from any of the ponds at 133 DOC, their role in reduced growth, which was clearly evident at this time, could be discounted. While IHHNV infection has generally been observed to be benign in both wild and farmed *P. monodon* (Flegel et al., 2004; Chayaburakul et al., 2005; Withyachumnarnkul et al., 2006), such associations between reduced pond growth performance and viral infection burden have been made in farmed L. *vannamei* derived from IHHNV-infected compared to IHHNV-free broodstock (Castille et al., 1993). The data obtained here indicate that growth and general health impacts might be influenced not only by infection loads but also by the duration over grow-out that shrimp have had to withstand a high infection burden. Such circumstances are not that dissimilar to observed reductions in survival and harvest yields of *P. monodon* farmed in north-eastern Australia resulting from a high prevalence of high-level GAV infection (Callinan and Jiang, 2003; Munro et al., 2011).

Extrapolating harvest yields from the 0.16 ha research ponds to 1 ha commercial ponds typical of those used in Australia, the 2 ponds in which shrimp possessed and maintained IHHNV infections at lower prevalence and lower loads produced on average 3.72 t more shrimp than the 2 ponds in which all shrimp rapidly developed high-load IHHNV infections. With the average weight of these ponds being in the 35–38 pieces / kg range, they are worth around AU $18 /kg on the current market, which is approximately a $67 K increased farm gate value per hectare as a result of stocking low IHHNV shrimp. The ability of IHHNV to reduce pond yields by this magnitude together with sustained acute infection also being found recently to cause severe shell deformities in farmed progeny of domesticated *P. monodon* (G.J. Coman et al., unpubl.) clearly suggest value in (i) hatchery-based PCR screening of individual broodstock and/or suitably-sized pools of postlarvae to ensure that only IHHNV-free or IHHNV-low seedstock are farmed, (ii) investing further in RNA interference (RNAi) strategies aimed at reducing IHHNV loads in broodstock prior to them spawning and (iii) excluding IHHNV from domesticated *P. monodon* breeding lines, and ultimately within such breeding program, selecting for lines with enhanced IHHNV resistance/tolerance.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2018.09.032.

Acknowledgements

This project (2015–240 APFA IPA) was funded through the Fisheries Research and Development Corporation and included ancial contributions from the Australian Prawn Farmers Association (APFA), the Science and Industry Endowment Fund (SIEF) and CSIRO, FRDC Final Report ISBN978–0–646-98,999-0. We thank Sara Wedgewood and Andrew Chalmers for assisting with the project application, Roger Green for catching and supplying wild broodstock, Cheryl Tan, Greg Coman, Isaak Kadel, Tansyn Noble, Chris Stratford, Erika Giardina, Paul Palmer, Kadisha Wormald, Cam Cotterell, David Gould, Joel Slinger, David Mann, Michael Cosgrove and Lou Ricci for technical assistance with shrimp husbandry, sampling and maintaining aquaculture systems and Ridley Aqua Feed for contributing to shrimp diet costs.

References

Australian Prawn Farming, Health Management for Profit, 2006. The State of Queensland, Department of Primary Industries and Fisheries. ISSN 0727–6273. Printed by Queensland Complete Printing Services, Nambour, Queensland, Australia.

Bell, T.A., Lightner, D.V., 1984. IHHN virus: Infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture 38, 185–194.

Brock, J.A., Lightner, D.V., Bell, T.A., 1983. A review of four virus (BP, MBV, BMN, and IHHNV) diseases of penaeid shrimp with particular reference to clinical significance, diagnosis and control in shrimp aquaculture. In: Proceedings of the 71st International, Council for the Exploration of the Sea, C.M. 1983/Gen, 10/1–18.

Browdy, C.L., Holloway, J.D., King, C.O., Stokes, A.D., Hopkins, J.S., Sandifer, P.A., 1993. IHHN virus and intensive culture of *Penaeus vannamei*: effects of stocking density and water exchange rates. J. Crustacean Biol. 13, 87–94.

Callinan, R.B., Jiang, L., 2003. Fatal, virus-associated peripheral neuropathy and retinopathy in farmed *Penaeus monodon* in eastern Australia. II. Outbreak descriptions. Dis. Aquat. Org. 53, 195–202.

Callinan, R.B., Jiang, L., Smith, P.T., Soowannayan, C., 2003. Fatal, virus-associated peripheral neuropathy and retinopathy in farmed *Penaeus monodon* in eastern Australia. I. Pathology. Dis. Aquat. Org. 53, 181–193.

Castille, F.L., Samocha, T.M., Lawrence, A.L., He, H., Frelier, P., Jaenike, F., 1993. Variability in growth and survival of early postlarval shrimp (*Penaeus vannamei* Boone 1931). Aquaculture 113, 65–81.

Chayaburakul, K., Lightner, D.V., Sriurairattana, S., Nelson, K.T., Withyachumnarnkul, B., 2005. Different responses to infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Penaeus monodon* and *P. vannamei*. Dis. Aquat. Org. 67, 191–200.

Cotmore, S.F., Agbandje-McKenna, M., Chiorini, J.A., Mukha, D.V., Pintel, D.J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D., Davison, A.J., 2014. The family *Parvoviridae*. Arch. Virol. 159, 1239–1247.

Cowley, J.A., Moody, N.J.G., Mohr, P.G., Rao, M., Williams, L.M., Sellars, M.J., Crane, M.StJ., 2015. Tactical Research Fund: Aquatic Animal Health Subprogram: Viral presence, prevalence and disease management in wild populations of the Australian Black Tiger prawn (*Penaeus monodon*). In: FRDC al Report Project No (2013-036 EP158864 ISBN 978-1-4863-0631-2).

Cowley, J.A., Rao, M., Coman, G.J., 2018. TaqMan real-time PCR tests to specifically detect Infectious hypodermal and haemopoietic necrosis virus (IHHNV) lineages and an IHHNV endogenous viral element (EVE) integrated in the genome of Black Tiger shrimp (*Penaeus monodon*). Dis. Aquat. Org. https://doi.org/10.3354/dao03243.

de la Vega, E., Degnan, B.M., Hall, M.R., Cowley, J.A., Wilson, K.J., 2004. Quantitative real-time RT-PCR demonstrates that handling stress can lead to rapid increases of gill-associated virus (GAV) infection levels in *Penaeus monodon*. Dis. Aquat. Org. 59,

195–203.
Escobedo-Bonilla, C.M., 2013. Application of RNA interference (RNAi) against viral infection in shrimp: a review. Journal of Antivirals and Antiretrovirals 5, 1–12.
Flegel, T.W., Nielsen, L., Thamavit, V., Kongtim, S., Pasharawipas, T., 2004. Presence of multiple viruses in non-diseased, cultivated shrimp at harvest. Aquaculture 240, 55–68.
Itsathitphaisarn, O., Thitamadee, S., Weerachatyanukul, W., Sritunyalucksana, K., 2017. Potential of RNAi applications to control viral diseases of farmed shrimp. J. Invertebr. Pathol. 147, 76–85.
Jaroenram, W., Chaivisuthangkura, P., Owens, L., 2015. One base pair deletion and high rate of evolution: Keys to viral accommodation of Australian *Penaeus stylirostris* densovirus. Aquaculture 443, 40–48.
Kalagayan, G., Godin, D., Kanna, R., Hagino, G., Sweeney, J., Wyban, J., Brock, J., 1991. IHHN virus as an etiological factor in Runt-Deformity Syndrome of juvenile *Penaeus vannamei* cultured in Hawaii. J. World Aquacult. Soc. 22, 235–243.
King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J., 2012. Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press London United Kingdom.
Krabsetsve, K., Cullen, B.R., Owens, L., 2004. Rediscovery of the Australian strain of infectious hypodermal and haematopoietic necrosis virus. Dis. Aquat. Org. 61, 153–158.
Lightner, D.V., 1983. Diseases of Cultured Penaeid Shrimp. In: McVey, J.P. (Ed.), CRC Handbook of Mariculture. Crustacean Aquaculture Vol. 1. CRC Press, Boca Raton, FL, USA, pp. 289–320.
Lightner, D.V., 1999. The Penaeid shrimp viruses TSV, IHHNV, WSSV and YHV. Research Gate. https://doi.org/10.1300/J028v09n02_03.
Lotz, J.M., 1997. Special topic review: viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. World J. Microbiol. Biotechnol. 13, 405–413.
Mohr, P.G., Moody, N.J., Hoad, J., Williams, L.M., Bowater, R.O., Cummins, D.M., Cowley, J.A., Crane, M.StJ., 2015. New yellow head virus genotype (YHV7) in giant tiger shrimp *Penaeus monodon* indigenous to northern Australia. Dis. Aquat. Org. 115, 263–268.
Moody, N., Williams, L.M., Crane, M.St.J., Davies, K., Carlile, G., Young, J., White, J., Pye, T., Hansson, E., McDonald, T., Foord, A., 2009. Molecular detection of infectious hypodermal and haematopoietic necrosis virus (IHHNV) in farmed *Penaeus monodon* in Queensland. In: Abstract, 4th FRDC National Aquatic Animal Health Scientific Conference; 22–24 July, 2009; Cairns, QLD, Australia.
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.
Munro, J., Callinan, R., Owens, L., 2011. Gill-associated virus and its association with decreased production of *Penaeus monodon* in Australian prawn farms. J. Fish Dis. 34, 13–20.
Noble, T.H., Stratford, C.N., Wade, N.M., Cowley, J.A., Sellars, M.J., Coman, G.J., Jerry, D.R., 2018. PCR testing of single tissue samples can result in misleading data on gill-associated virus infection loads in shrimp. Aquaculture 492, 91–96.
OIE, 2017. Manual of Diagnostic Tests for Aquatic Animals. http://wwwoieint/international-standard-setting/aquatic-manual/access-online/.
Primavera, J.H., Quinitio, E.T., 2000. Runt-Deformity Syndrome in cultured giant tiger prawn *Penaeus monodon*. J. Crustac. Biol. 20, 796–802.
Rai, P., Safeena, M.P., Karunasagar, I., Karunasagar, I., 2011. Complete nucleic acid sequence of *Penaeus stylirostris* densovirus (PstDNV) from India. Virus Res. 158, 37–45.
Saksmerprome, V., Puiprom, O., Noonin, C., Flegel, T.W., 2010. Detection of infectious hypodermal and haematopoietic necrosis virus (IHHNV) in farmed Australian *Penaeus monodon* by PCR analysis and DNA sequencing. Aquaculture 298, 190–193.
SAS Institute Software, 1999. SAS/STAT Software, Version. SAS Institute, Cary, NC, USA, pp. 7.
Sellars, M.J., Rao, M., Arnold, S.J., Wade, N.M., Cowley, J.A., 2011. Penaeus monodon is protected against gill-associated virus by muscle injection but not oral delivery of bacterially-expressed dsRNAs. Dis. Aquat. Org. 95, 19–30.
Sellars, M.J., Rao, M., O'Leary, Z., Wood, A., Degnan, B.M., Cowley, J.A., 2014. Reduced loads of pre-existing Gill-associated virus (GAV) infection in juvenile *Penaeus monodon* injected with single or multiple GAV-specific dsRNAs. Aquaculture 434, 272–276.
Sellars, M.J., Rao, M., Cowley, J.A., 2016. Spawning of female Black tiger shrimp (*Penaeus monodon*) is not impacted by muscle injection of dsRNA targeted to Gill-associated virus. Aquac. Res. https://doi.org/10.1111/are.13123.
Sellars, M.J., Rao, M., Murphy, B.S., Cowley, J.A., 2018. RNAi treatment of broodstock to reduce disease impacts in farmed prawns. In: FRDC Final Report; (2015-240. ISBN 978-0-646-98999-0).
Shike, H., Dhar, A.K., Burns, J.C., Shimizu, C., Jousset, F.X., Klimpel, K.R., Bergoin, M., 2000. Infectious hypodermal and hematopoietic necrosis virus of shrimp is related to mosquito Brevidensoviruses. Virology 277, 167–177.
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.
Tan-Fermin, D.D., Pudadera, R.A., 1989. Ovarian maturation stages of the wild giant tiger prawn *Penaeus monodon* Fabricius. Aquaculture 77, 229–242.
Tang, K.F.J., Lightner, D.V., 2006. Infectious hypodermal and hematopoietic necrosis virus (IHHNV)-related sequences in the genome of the Black Tiger prawn *Penaeus monodon* from Africa and Australia. Virus Res. 118, 185–191.
Tang, K.F.J., Poulos, B.T., Wang, J., Redman, R.M., Shih, H.H., Lightner, D.V., 2003. Geographic variations among infectious hypodermal and hematopoietic necrosis virus (IHHNV) isolates and characteristics of their infection. Dis. Aquat. Org. 53, 91–99.
Tang, K.F.J., Navarro, S.A., Lightner, D.V., 2007. PCR assay for discriminating between infectious hypodermal and hematopoietic necrosis virus (IHHNV) and virus-related sequences in the genome of *Penaeus monodon*. Dis. Aquat. Org. 74, 165–170.
Walker, P.J., Cowley, J.A., Spann, K.M., Hodgson, R.A.J., Hall, M.R., Withyachumnarnkul, B., 2001. Yellow head complex viruses: transmission cycles and topographical distribution in the Asia-Pacific region. In: Browdy, C.L., Jory, D.E. (Eds.), The New Wave: Proceedings of the Special Session on Sustainable Shrimp Culture. Aquaculture 2001 The World Aquaculture Society, Baton Rouge, LA, pp. 227–237.
Withyachumnarnkul, B., Chayaburakul, K., Lao-Aroon, S., 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.