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PII: S0044-8486(16)30931-0
DOI: doi: 10.1016/j.aquaculture.2017.01.006
Reference: AQUA 632483

To appear in: *aquaculture*

Received date: 9 November 2016
Revised date: 5 January 2017
Accepted date: 6 January 2017

Please cite this article as: Ramona Walsh, Kathy La Fauce, Melanie Crockford, Brian Jones, Leigh Owens , Genomic heterogeneity and prevalence of hepandensovirus in Penaeus esculentus from Western Australia, and P. merguiensis from the Gulf of Carpentaria, Australia. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Aqua(2017), doi: 10.1016/j.aquaculture.2017.01.006

Genomic heterogeneity and prevalence of hepandensovirus in *Penaeus esculentus* from Western Australia, and *P. merguiensis* from the Gulf of Carpentaria, Australia

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Abstract: Decapod Hepandensovirus 1 (HDV), formerly known as hepatopancreatic parvovirus, has been associated with stunting, lowered production and outright mortalities in prawns in aquaculture. Despite the fact that broodstock are sourced and aquaculture farms are planned in the regions of northern and Western Australia, data on these parvoviruses from this region are limited. The prevalence of HDV in *Penaeus esculentus* and *Penaeus merguiensis* is moderate (34-51%) in southern Western Australia, Exmouth Gulf and the Gulf of Carpentaria but statistically higher ($P<0.05$) in Shark Bay (82%). We speculate this is due to the topography of Shark Bay combined with the currents of the Indian Ocean gyre (IOG). Despite an on average 8-12% genomic heterogeneity, the nucleotide sequences of HDV in WA most closely align with HDV in regions associated with the IOG; Thailand, India, Tanzania, Madagascar; eastern Asia, Korea and less commonly, with sequences from the eastern coast of Australia. This potentially changes the paradigm of a single strain of HDV being ubiquitous in Australia and there was little risk in moving broodstock from WA to the eastern states, so there was no testing of broodstock for HDV. There is no strong evidence to clarify whether the strain of HDV in WA *P. esculentus* came from either its' nearest genetic relatives, *P. monodon* or *P. semisulcatus* or from *P. merguiensis* from the Solanderian province of Australia. *P. esculentus* HDV appears to be most related to strains within the IOG. The HDV nucleotide heterogeneity of wild prawns contrasts strongly to studies

undertaken with prawns from aquaculture where genetic selection may have occurred.

Keywords: Decapod Hepandensovirus 1; boodstock; *Penaeus esculentus*; *Penaeus merguiensis*; Indian Ocean; Western Australia; hepatopancreatic parvovirus

1 Introduction

Decapod Hepandensovirus 1 (HDV) (see Cotmore et al. (2013) for the current taxonomy) formerly known as hepatopancreatic parvovirus (HPV) was first reported in Australia from wild caught juveniles of *Penaeus merguiensis* off Townsville, Queensland (Qld) in 1984 (Owens, 1997), and from wild caught *Penaeus esculentus* in Moreton Bay, Qld (Paynter et al., 1985). It was continually reported from different areas of the Qld coast over five subsequent years (Owens, 1997). This strain of HDV is now called Penaeus merguiensis Hepandensovirus (PmeHDV) (Cotmore et al. 2013).

The prevalence of HDV in the Gulf of Carpentaria (13.18° S, 139.13° E) is unknown, which is of concern since white banana prawns *P. merguiensis* and red-legged banana prawns *Penaeus indicus* are major target species of the Northern Prawn Fishery (NPF). More importantly, *P. merguiensis* is used for broodstock in Qld aquaculture farms and PmeHDV has been shown statistically to be responsible for 28% loss of production in farms (Owens et al. 2011). As for presence of HDV in prawn stocks off Western Australia (WA), little had been reported until a survey by Jones (2003) who determined the disease status of commercial prawn stocks in WA. The survey was based upon histology with recognition of the characteristic cytopathic effects of HDV infection. These are well described in the literature (Lightner et al., 1989; Lightner et al., 1993; Mari et al., 1995; Jones, 2003) as large dark basophilic, Cowdrey type A inclusion bodies within hypertrophied nuclei of hepatopancreatic tubule epithelial cells, often causing lateral displacement and compression of the host cell nucleolus, and chromatin margination leading to occasional visible signet ring formation.

From histological examination, the overall prevalence of infection with HDV in penaeids from WA, was 28% for *P. merguiensis*, 5% and 4.5% of *P. esculentus* from Exmouth Gulf (22.60° S, 114.25° E) and Shark Bay (25.98° S, 113.78° E) respectively (Jones, 2003). In addition to

histopathology as a survey tool for HDV, the commercial *in situ* hybridisation (ISH) probe for HDV from DiagXotics was used to confirm the presence of the virus, which only reacted positively in *P. merguiensis* (Jones, 2003). The problem with detection of HDV is that if infection is at a low level it can be difficult, if not impossible to detect simply based upon histological changes (Lightner et al., 1993) particularly if newly infected or infected at a low level. To identify presence of the virus including low level infections, sensitive methods such as polymerase chain reaction (PCR) are required.

The aims of this study were to determine the prevalence of HDV infection of one of the main commercially important species in Western Australia, *P. esculentus* and *P. merguiensis* of the Northern Prawn Fishery in the Gulf of Carpentaria. Once HDV was found in *P. esculentus*, it was anticipated that data on viral sequence could solve the question on whether the viral strain in *P. esculentus,* which is endemic and unique to Australia, is from *P. esculentus'* nearest genetic neighbour, *P. monodon* or was acquired from Australian *P. merguiensis* or perhaps from other Indian Ocean species like *P. indicus*. As WA is used for sourcing broodstock for the rest of Australia, and apparently the DiaXotics test was not picking up all strains of HDV, this study was of some critical importance for stock movements and for understanding why the ISH was not reacting with the WA strains.

2 Materials and methods

2.1 Prawn Sample Collection

Frozen *P. esculentus* samples were purchased in 2012 from numerous retail outlets in and around Perth, WA, which had been trawled by the WA fishing industry. Frozen *P. merguiensis* trawled from the Gulf of Carpentaria were also purchased simultaneously. Batches containing 20-50 prawns were selected based upon a variety of different catch dates (frozen 1-3 months) to increase the sample distribution, as prawn trawlers move around and trawl different sites within an area each night. Over a week, the multiple purchases were transported daily in a cooler with ice bricks by car, back to the Department of Agriculture Western Australia for processing.

2.2 Processing of samples

The tissues removed from each individual prawn were a single 3rd pleopod, or the 2nd or 4th if the 3rd was missing, and a section of hepatopancreas. The tissues were each placed into 1.5ml Eppendorf tubes with absolute ethanol (EtOH) (Rome Scientific), and matching labels to record which prawn they were from. The utensils were cleaned between processing each sample, with the bacterial/virucidal disinfectant Virkon® S (Antec® International), then rinsed in water, and placed in absolute ethanol. At the end of processing the sample materials were sent as diagnostic veterinary supplies by road freight to James Cook University, for further processing and analysis.

2.3 Nucleic acid extraction and viral purification

Each sample from an individual prawn was processed using sterile techniques as follows: each sample was patted dry on a tissue to remove excess EtOH, then tissue was cut off the base of the pleopod added to the hepatopancreas, weighed out to 20mg, and placed in a new 1.5 ml microcentrifuge tube. A Wizard® SV Genomic DNA Purification System (Promega, USA) was used to extract and purify DNA following the manufacturer's protocol. After the 275µl of digestion solution mixed with Proteinase K (Promega, USA) was added to each sample, the tissue was crushed into the solution using polypropylene pellet pestles. Samples were incubated overnight in a heat block for 16-18 hours, then processed using the microcentrifuge protocol according to manufacturer's instructions, and stored at -20°C until further use.

2.4 Polymerase Chain Reaction (PCR) amplification

The polymerase chain reaction (PCR) for each sample was a 25µl reaction. Each reaction mixture contained 10.5µl nuclease-free water, 12.5µl Go-Taq Green (Promega, USA), 0.5µl forward primer [10pM] (see below), 0.5µl reverse primer [10pM], and 1µl DNA template. Amplification was performed in an Eppendorf Mastercycler Gradient Thermocycler (Eppendorf, Germany). The PCR cycle consisted of a profile of initial 94°C for 7 min, 35 cycles at 94 °C for 45s denaturation, 55°C annealing for 60s and polymerisation at 72°C for 1 min and an additional 5 min at 72°C following the last cycle (modified from La Fauce et al., 2007). PCR products were visualised on a 1% agarose gel (100ml TAE buffer with 1g agarose) which contained Gel Red Nucleic Acid Stain (Biotium, CA USA) at a concentration of 5.0×10^{-5} ml^{-1}, with a 1kb DNA ladder (Fermentas Gene

Ruler).

2.5 Positive control

A positive control for use during PCR was established from *P. merguiensis* stored frozen at James Cook University, from a farm which had had a high prevalence of HDV infection in 2003 (La Fauce et al., 2007). The 3rd pleopods from five animals were removed and processed as in section 2.2, and DNA extracted as per 2.3. The DNA was then amplified using PCR as described in section 2.4, using primers HPV140F and HPV140R (Table 1) which were designed from the PmeHDV viral capsid protein producing a 140bp amplicon.

2.6 Statistics

The means and confidence intervals for the prevalence of each population of prawns were calculated using the Interactive Statistical Calculator (Interactive Statistical Calculator, StatPages.org 2012).

2.7 Polymerase Chain Reaction (PCR) amplification

A multilocus sequence analysis (MLSA) approach was used to optimise the use of resources. This is where portions of many viral genomes were sequenced rather than fully sequencing fewer genomes. Five sets of primers designed from the genome of Australian PmeHDV (Table 1) were used to amplify segments of the HDV. Eight sets of primers were designed using Oligo Primer Analysis Software 7 (Molecular Biology Insights Inc., CO USA) from the full genome of Fenneropenaeus chinensis HDV (FcHDV Korea) (JN082231) (Table 1), to overlap our partial sequence obtained from *P. esculentus*. The primer nucleotide sequences were confirmed to match FcHDV in a BLAST search, then synthesised by Sigma-Genosys Australia. Another nine sets of primers designed from Penaeus monodon Hepandensovirus 4 (NC_011545, India) were also manufactured to cover most of that genome and were synthesised as described above.

Table 1**:** Sequences of the primers used to amplify segments of the HDV genome found in *P. esculentus* from Western Australia and *P. merguiensis* from the Gulf of Carpentaria. Primers were designed from the genome of PmeHDV (DQ458781), from *P. merguiensis* from the east-coast of Australia (La Fauce et al. 2007); from the genome of FcHDV (JN082231) from South Korea and

from PmoHDV4 from India (NC_011545). They are labelled HDV, FcHDV and PmoHDV respectively.

Forward Primer	Nucleotide sequence 5' – 3'	Reverse Primer	Nucleotide sequence 5' – 3'
HPV140F	GTGGCGTTGGAAGGCACTTC	HPV140R	CTACTCCAATGGAAACTTCTGAGC
HDV347F	GGATACCGTATATACAAGGTT	HDV1683R	TTATTGGCTAAGTCTTTACAAC
HDV1363F	ATGGTATAGAAGCCTGAACACA	HDV2128R	GCCCTAGAACTGCTTAACCTT
HDV1916F	TTCAGAGATAGAAAGTATGCCAA	HDV2735R	AATGTTCCACCATCACCGAC
HDV4933F	GGGGCACATATATTATCCAG	HDV5377R	CTGTTGTTAGCCATACCGTTC
HDV5151F	ATTCAACTAACCCGACAAGGA	HDV5892R	CTCTGTCCATACGGCCTCT
FcHDV453F	GGACTTTGTGACATCTTTCCG	FcHDV 1235R	CCATAACATTACCCTGCTCGT
FcHDV 1055F	GGAAGTTCTCGCTTACTAACACA	FcHDV 1871R	TCCCATAGACTCATCGACGAA
FcHDV 1778F	TAGCAAGAGGGTTTTATTCCG	FcHDV 2656R	TTTCCACTGTTACTATTTCCGT
FcHDV 2545F	CAAGGCCAAAGACAATACCG	FcHDV 3439R	CCGCCAACAAAGTAATCGAC
FcHDV 3219F	GTTCAAGTTTTCCCGGCAT	FcHDV 4047R	ACTGTCCGAAAATCCTGATGC
FcHDV 3876F	CAAAGACAACTAGGACGTGA	FcHDV 4773R	TGTTGTCAATACCCCATGC
FcHDV 4521F	CGCAATCAACCTATCGATCTCA	FcHDV 5240R	TACAGTTACTTGGCATGACT
FcHDV 5103F	ACAGACTTAAGAGATGCACCA	FcHDV 5761R	TCTCAGTAGCTCCAAATGTCC
PmoHDV851F	GGTATTAGGTGGAGTGGTAAC	PmoHDV1688R	GCAGTTCATGACAGATGTAAAG
PmoHDV1375F	TAGTATGAAACCAGAACACAAT	PmoHDV2189R	GAAAATTTACAAGTGTTGGAGAA
PmoHDV1842F	GCAACTATGGCAAGACTTGGG	PmoHDV2716R	GTTAATTGAGGCTTTAACTGGTC
PmoHDV2372F	GAGAAGACCTATGGAACACTAAC	PmoHDV3206R	GCTTTATGACATGCTGTTTGAGG
PmoHDV2863F	GAGAATGTAACAATGCCTATG	PmoHDV3707R	GCGAAAGAATAAACTAGCGAAG
PmoHDV3339F	TCGGCAGTAGCTGCAGTCATA	PmoHDV4202R	GCAGTGTAGCACTACAATTGG
PmoHDV3841F	GAGAAACACATTTGCAGGATTAC	PmoHDV4708R	GCAACAGTAACACAGGAGATATC
PmoHDV4376F	AACACTGAAGCACCTGTCCC	PmoHDV5193R	CCCCCTAAAATAAACGGTATAGG
PmoHDV4852F	CCACTTGCAGGCATTTGAGAT	PmoHDV5715R	GGCATGTATACAATGATTTCTAC

The PCR reaction mixture and profile were the same as in 2.4, with an adjusted lower annealing temperature of 50°C instead of 55°C. PCR products (bands) of/or around the expected amplicon size were cut out of the agarose gels using sterile surgical blades under UV light, then placed in a sterile 1.5ml microcentrifuge tube. DNA was purified from the gels using a Wizard® SV Gel and PCR Clean-up System (Promega, USA) according to manufacturer's instructions.

2.8 Cloning and sequencing

The fragments of viral DNA which were cut out of the gel and purified, were then cloned in *Escherichia coli* JM 109 cells, using the pGEM-T Easy Vector System (Promega, USA) according

to manufacturer's instructions. Three white colonies of the recombinant plasmids from each Luria Bertoni (LB) agar plate were selected for isolation, which were individually inoculated into 10ml LB medium with ampicillin [1.0x 10^{-3} ml^{-1}], and incubated overnight (18-20 hours) in a shaking incubator at 150 rpm at 37°C. Plasmid DNA extractions were performed on each sample using the Wizard *Plus* SV Minipreps DNA Purification System (Promega, USA) following the manufacturer's protocol. Spectrophotometry was performed on each sample of purified DNA for yield and quality, and then samples were sent to Macrogen Inc. (Seoul, Korea) for both forward and reverse sequencing.

2.9 Nucleotide sequence analysis and phylogenetic comparison

Nucleotide sequences returned from Macrogen were analysed using Sequencher v5.0 sequence analysis software (Gene Codes Corporation, Ann Harbour, MI USA), and Geneious v7.1.3 (Biomatters Ltd, Auckland, New Zealand) to clean up and remove all primer nucleotides, analyse and align overlapping sequences. Chromatograph read qualities below 95% were generally discarded. Sequence information from HDV in this study was entered in a Basic Local Alignment Search Tool (BLAST) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi.) to compare with other HDV strains. Sequencher and Geneious software was used to align all the partial sequences obtained from this study with the same sequences from entire genomes of other HDV strains including PmeHDV from Australia (DQ458781), PchHDV South Korea (JN082231), PindHDV (KR492908, KR492912) imported into Egypt, PsemHDV (KR492908, KR492912) from the Mediterranean Sea, Egypt, PmoHDV1 from Thailand (DQ002783), PmoHDV2 from Madagascar (AY008257), PmoHDV3 from Tanzania (EU588991), and PmoHDV4 from India (FJ410797) (Table 2).

When it became clear that prawns were carrying multiple HDV genotypes (see results below) then if "replicate" clones were not identical, they were analysed separately.

Table 2. List of Hepandensvovirus strain abbreviations, host species, location, and Genbank accession numbers.

Virus abbr.	Host Species	Geographic location	Genbank

			Accession #
PchHDV	*Penaeus chinensis*	Korea	JN082231
PescHDV	*P. esculentus*	Western Australia	Not submitted
PindHDV	*P. indicus*	imported into Egypt	KR492908 KR492912
PmeHDV	*P. merguiensis*	Australia (east coast)	DQ458781
PmeHDV	*P. merguiensis*	Gulf of Carpentaria	Not submitted
PmoHDV1	*P. monodon*	Thailand	DQ002873
PmoHDV2	*P. monodon*	Madagascar	EU247528
PmoHDV3	*P. monodon*	Tanzania	EU588991
PmoHDV4	*P. monodon*	India	FJ410797
PsemHDV	*P. semisulcatus*	Mediterranean Sea	KT316242 KT316245

3 Results

3.1 Confirmation of decapod hepandensovirus 1

The HDV140F and HDV140R primers used to initially screen purified DNA from *P. esculentus* from WA, gave the expected 140 bp amplicon. The 140bp bands were cut out and the nucleotide sequence isolated gave a 100% match to PmeHDV (DQ458781), and 93% match to structural protein (viral capsid) of HDV from New Caledonia in a BLAST search. Bands of differing size which were associated with samples positive for HDV at the lower annealing temperatures were also cut out and sequenced, but did not match any nucleotide sequences in the BLAST database.

3.2 Prevalence of Hepandensovirus

In Gulf of Carpentaria, the prevalence of HDV was 51% (27/53) between samples of the combined banana prawns *P. merguiensis/ P. indicus*. Phenotypically, it is difficult to separate *P. merguiensis* from *P. indicus*, so the name *P. merguiensis* will be used throughout this manuscript even though it is acknowledged that the population of banana prawns in northern Australia contains both species. Of the *P. esculentus* screened from Exmouth Gulf in WA, 34% (43/127) were infected with HDV

(Table 3). *P. esculentus* from the south-western WA (32.00° S, 115.51° E) were 40% infected with HDV (8/20). The population of *P. esculentus* from Shark Bay, WA however, had higher prevalence of HDV at 82% (101/123). Confidence intervals calculated from each population of tiger prawns (*P. esculentus*) overlapped for the populations from south-west WA, Exmouth Gulf and with the banana prawns (*P. merguiensis*) from the Gulf of Carpentaria. The prevalence of HDV in the *P. esculentus* population in Shark Bay was around double and statistically significantly ($P<0.05$) higher than that of the other geographic areas (Table 3).

Table 3. Mean and 95% confidence intervals (CI) for prevalence of infection with Hepandensovirus in *Penaeus esculentus* from south-west Western Australia, Exmouth Gulf, Shark Bay and *P. merguiensis* from the Gulf of Carpentaria. Those prevalences with the same superscript are statistically similar ($P>0.05$).

Area	Species	Prevalence	95% CI
Gulf of Carpentaria	*P. merguiensis*	51[a]	37 - 65
Exmouth Gulf	*P. esculentus*	34[a]	26 - 48
South Western Australia	*P. esculentus*	40[a]	19 - 64
Shark Bay	*P. esculentus*	82[b]	74 - 88

3.3 Sequence Analysis

Over 130 sequencing PCRs of HDV-positive prawns where conducted with three clones of each sent for sequencing. The results of sequencing were surprising. Rather than getting single viral genomes that would form one contig, different amplicons in "replicate" clones aligned more closely with various widespread geographical HDV strains even from the one individual prawn. E.g. Prawn 3, from Shark Bay had amplicons 95% identical to PmeHDV from the Qld eastern coast, 86% identical to PmoHDV4 from India and other amplicons were 84% similar with FcHDV from Korea (Table 4). Generally translating nucleotide sequence to amino acids increased the similarity (identity plus positivity (substitutions by similarly functioning amino acid)) by a few percent as would be expected given the silent nature of many nucleotide substitutions. To keep the scope of this paper reasonable, the protein data will be reported in a separate publication. Despite considerable genetic heterogeneity of ~10%, on average (Table 4), with the furthest being 77% for a PmeHDV, all sequences remained within the strain variation boundaries (approximately >67%

identity, Fauquet et al. 2005) of Decapod hepandensovirus 1 (Cotmore et al. 2013).

All banana prawns from the Gulf of Carpentaria had sequence most similar to PmeHDV at 77-99% identity, mean 93.4% (Table 4). Exmouth Gulf had the most diverse and dissimilar sequences (overall 88.4%) with the sequences from Australian PmeHDV (99%); Korean *P. chinensis* (84-92%) and from *P. monodon* (85-87%) from India and Thailand which are very similar genomes; and the more distant PmoHDV3 Tanzania (84-85%). *P. esculentus* from Shark Bay had slightly stronger identity to known HDV strains (90.9%) with the most dissimilar sequence being FchDV (84%). The HDV viral sequences from south-western Australia were also quite diverse with one PmoHDV2 being most closely aligned to the Madagascan strain.

Table 4. The nucleotide similarity of hepandensovirus sequenced from various clones from individual prawns from Western Australia and the Gulf of Carpentaria.

Area/Prawn Species	Individual	Viral Region	Primer Coverage	nt	Clones	Closest HDV Strain	% nt Identity
Gulf of Carpentaria *Penaeus merguiensis* /*Penaeus indicus*	57	NS2/NS1	815-1688	753	36-2	PmeDV Aust	89
	57	NS2/NS1	1363-2128	786	7-3	PmeDV Aust	99
	57	NS1	2372-3206	753	62-2	PmeDV Aust	91
	57	VP	4933-5377	465	3-1,2	PmeDV Aust	92
	57	VP	4933-5377	465	3-3	PmeDV Aust	77
	71	VP	5530-6044	515	71-1	PmeDV Aust	99
	77	NS2/NS1	1363-2128	786	8-2,3	PmeDV Aust	97
	77	VP	4933-5377	465	4-1,2,3	PmeDV Aust	94
	99	VP	4933-5377	465	1-3	PmeDV Aust	99
	107	VP	4933-5377	465	2-1	PmeDV Aust	94
						Mean Similarity	**93.4**
Exmouth Gulf *Penaeus esculentus*	31	VP	4933-5397	465	31-1	PmoHDV3 Tanzania	84
	32	VP	4933-5397	465	32-1	FchDV Korea	84
	34	VP	4933-5397	465	34-1	PmoHDV3 Tanzania	85
	35	VP	4933-5397	465	35-1	FchDV Korea	84

	181	VP	4933-5397	465	181-1	PmeDV Aust	99
	182	VP	4933-5397	465	181-2	PmeDV Aust	99
	190	NS1/nc	2863-3707	788	67-1,2,3 68-3	PmoHDV4 India	87
	198	NS2/NS1	815-1688	757	36-3, 38-1,2	FchDV Korea	89
	198	NS2/NS1	1055-1871	674	32-1,2,3	FchDV Korea	92
	198	NS1	1778-2656	821	34-1,2,3	PchDV Korea	90
	198	NS1/nc	2863-3707	772	69-1,2,3 70-1,2	PmoHDV4 India	87
	198	VP	4933-5377	785	102-1,2,3	PmoHDV1 Thailand	85
						Mean Similarity	**88.4**
Shark Bay *Penaeus esculentus*	1	NS2/NS1	1363-2128	786	9-2	PmeDV Aust	100
	1	NS2/NS1	1361-2146	786	1-1	FchDV Korea	91
	1	NS2/NS1	1361-2146	786	1-2,3	FchDV Korea	91
	1	VP	4933-5377	465	5-1,2,3	PmeDV Aust	95
	2	NS2/NS1	1361-2146	786	2-10	FchDV Korea	91
	3	VP	4933-5377	465	6-1	PmeDV Aust	95
	3	VP	4933-5377	465	6-2,3	FchDV Korea	84
	3	VP	4933-5378	465	3-1,2,3	PmoHDV4 India	86
	4	VP	4933-5379	465	4-2	PmoHDV4 India	86
	11	NS1/nc	2709-2407	699	11-1 12-1	PmeDV Aust	99
						Mean Similarity	**90.9**
sth Western Australia *Penaeus esculentus*	233	NS1	1778-2656	794	33-1,2	PchDV Korea	92
	241	NS2/NS1	1055-1871	697	27-1,2,3	PmoHDV2 Madagascar	92
	241	NS2/NS1	815-1688	753	40-3	PmeDV Aust	90
						Mean Similarity	**91.7**
Shark Bay? (label lost) *Penaeus esculentus*		NS2/NS1	1363-2128	786	1-1,2,3 2-10	FchDV=PmoHDV 1	91

4 Discussion

4.1 Differences in Prevalence

The statistical difference between the prevalences of hepatopandensovirus in populations of *P. esculentus* from areas of Western Australia was unexpected. From histological examination of the penaeids by Jones (2003), the overall prevalence of HDV from WA was 28% for *P. merguiensis*, 5% and 4.5 % of *P. esculentus* from Exmouth Gulf, and Shark Bay respectively. Traditional methods such as histology are far less sensitive than molecular based techniques such as PCR, dot blotting and ISH (Kahn, 1998). This study used polymerase chain reaction (PCR) as a sensitive detection tool to identify individuals from a population infected with HDV, and revealed much higher levels of viral presence. These higher levels of infection diagnosed by PCR instead of histology are likely due to the sensitivity of the diagnostic tools used.

The reason for almost double the levels of HDV infection in the Shark Bay population is unknown. We speculate that the underlying oceanic topography, the elongated bay and contained nature of Shark Bay allowed for less movement of water and animals leading to a higher density compared to that of the free coastlines of south-west WA, or the wide open Exmouth Gulf, where the virus can be more easily tidally flushed and diluted and prawns can easily migrate away. Indeed, Hetzel et al. (2013) describe Shark Bay as a reverse estuary where evaporation drives water from the ocean into Shark Bay with only quasi-periodic outflows driven by hypersalinity. This would most often trap penaeid larvae in the bay.

4.2 Sequence Analyses

The heterologous nature of the viral genomes is probably a true indication of the viruses in a natural ecosystem. This is compared to the genetic bottleneck caused by large populations of siblings selected by breeding in an aquaculture hatchery and kept in high concentrations in aquaculture ponds. This is where most listed sequences of HDVs have come from. Animals in aquaculture are renowned for being mostly genetic monocultures with the consequence of near homogeneous genetic diversity. Indeed, there can be as few as two female broodstock used to fill one entire pond. This would allow for evolutionary selection of a virus that grows well in that restricted, genetic host mix, thus limiting the sequences obtained for the virus. In addition, HDV are single stranded DNA viruses with no proof-reading ability as the DNA-replicase does not have

a complemental strand to compare against. This leads to higher mutation rates and higher rates of evolution similar to RNA viruses. Indeed Jaroenram et al. (2015) showed that the similar ssDNA brevidensovirus, Penaeus stylirostris densovirus had high mutation rates (1.55×10^{-3} substitutions/site/year) like an RNA virus. Without the bottleneck of genetic selection for aquaculture, the HDV sequences in the wild fishery prawns have been relatively unrestrained allowing increased genetic drift and diversity.

During the summer Asian monsoon, the northern part of the Indian Ocean forms a clockwise gyre (IOG) (Figure 1) which sweeps the waters from around Indonesia, the Indian sub-continent and further afield from Tanzania and even Madagascar on to the Western Australian coast down to Exmouth Gulf and beyond (Public Domain: https://en.wikipedia.org/wiki/Indian_Ocean_Gyre: accessed 30 Sep 2016). The IOG reverses direction outside of the monsoon season. The gyre would allow planktonic larvae from further west to be swept into Exmouth Gulf in particular and to a lesser extent further down the coast with varying strength from year to year. Indeed, the southerly Leeuwin current can even sweep around the southern tip of WA into the Great Australian Bight (Maxwell et al. 1981). This probably explains the diversity of HDV sequences from prawns of the WA coast and their similarity to those sequences from the Indian Ocean. These diverse WA HDV sequences support the concept of a Damperian zoogeographical province (Owens 1990) as part of the Indian Ocean fauna. The diversity of sequences probably explains why the ISH reported by Jones (2003) was not successful.

Of interest is the high representation of sequences from *Penaeus* (*Fenneropenaeus*) *chinensis* from Korea and the Yellow Sea. This would suggest the members of the *Penaeus* (*Fenneropenaeus*) subgenus; *merguiensis* and *indicus* in Indonesia as an interoceanic connecting zone called the Indonesian Throughflow (de Dekker et al. 2012) are likely to have these same HDV sequences. Indeed, the IOG is fed by currents moving south through the Indonesian archipelago as a part of the Great Ocean Conveyor Belt.

The Gulf of Carpentaria was dry land during the last glaciation and is now a link area between the Indian Ocean (Australia's Damperian province) and the Pacific Ocean (Australia's Solanderian province) with the Torres Strait as a zoogeographical barrier (Owens 1990), so the sequences from

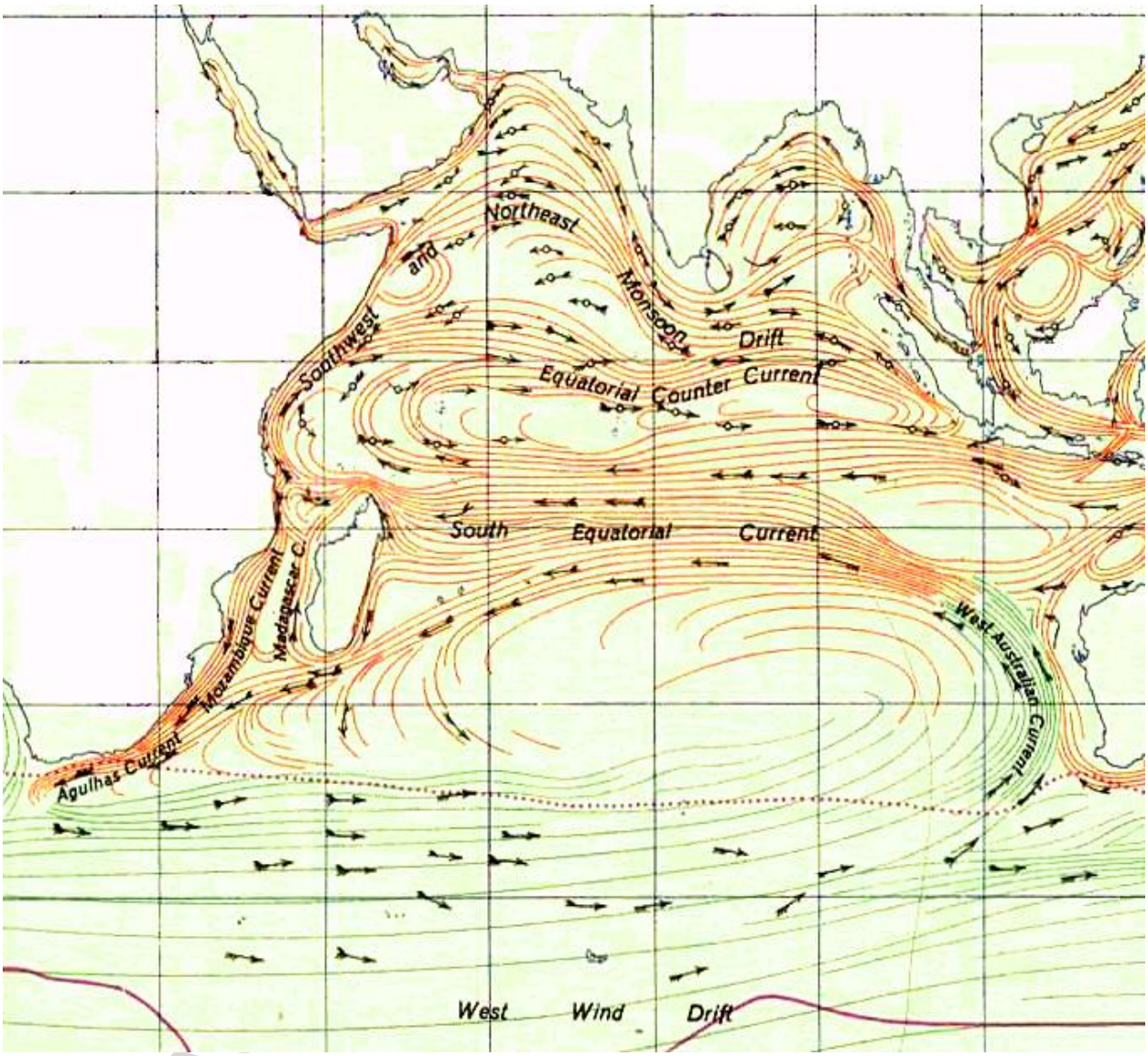
this area are of considerable curiosity. Despite ~7% genetic heterogeneity, Gulf of Carpentaria VP sequences from banana prawns align with the east coast PmeHDV exclusively. This supports the idea of historic closer ties with the east coast banana prawns rather than postlarvae brought in with the IOG. Sequencing the HDVs from *P. esculentus* and *P. semisulcatus* from the Gulf of Carpentaria would be most enlightening. Would they align with the east coast PmeHDV strain or the Damperian HDV mix of strains?

One further outcome from this work is that movement of broodstock prawns from WA to QLD and NSW risks spreading Indian Ocean derived viral strains into naive Pacific prawn stocks. There is no testing for HDV prior to the movement of broodstock prawns. Previous superficial risk assessments would have determined that HDV is ubiquitous in Australia and that translocation confers no change in risk. However, we now know we have 8-12% genetic drift in WA relative to the Pacific coast viruses which could change infection dynamics considerably.

5 Conclusions

The prevalence of HDV across western northern Australia is moderate (34-51%) in south Western Australia, Exmouth Gulf and the Gulf of Carpentaria but high in Shark Bay (82%). The reason for this is unknown. There is no strong evidence to clarify whether the strain of HDV in *P. esculentus* from WA came from either its' nearest genetic relatives, *P. monodon* or *P. semisulcatus* or from *P. merguiensis* from the Solanderian Provence, Qld. WA's *P. esculentus* HDV appears to be most related to strains within the IOG. The higher than expected heterogeneity of HDV sequences changes the paradigm that one strain of HDV was ubiquitous in Australia and there was little risk in moving broodstock from WA to the eastern states. The HDV sequence heterogeneity of wild prawns contrasts strongly to studies undertaken with prawns from aquaculture where genetic selection and bottlenecks may have occurred.

Figure 1. The Indian Ocean Gyre during the South Asian monsoon season (Public Domain: https://en.wikipedia.org/wiki/Indian_Ocean_Gyre: accessed 30 Sep 2016).



Acknowledgements

This study was partially funded by the Indo-Australian Biotechnology Grant BF50090 and RW's personal funds.

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Highlights

- Decapod Hepandensovirus 1 (HDV) causes stunting, lowered production and mortalities in prawns in aquaculture.
- There is not a single strain of HDV ubiquitous in Australia; therefore there is risk in moving WA broodstock to the eastern states.
- WA HDV aligns with viruses from the Indian Ocean gyre; Thailand, India, Tanzania, Madagascar, and Korea and less commonly with the eastern coast of Australia.
- HDV nucleotide heterogeneity (8-12% genomic drift) in wild prawns contrasts strongly to prawns from aquaculture where genetic bottlenecks have occurred.
- The prevalence of HDV in wild *Penaeus esculentus* and *Penaeus merguiensis* is 34-51% in SW Australia, Exmouth Gulf and Gulf of Carpentaria but higher is in Shark Bay (82%).