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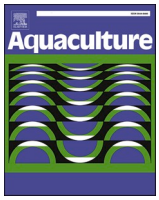

Review

Effect of husbandry on the emergence of pathogens in the aquaculture of redclaw crayfish (*Cherax quadricarinatus*) in Australia

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ABSTRACT

The redclaw crayfish (*Cherax quadricarinatus*) industry has existed for four decades but has failed to thrive. Part of this is due to continued setbacks due to disease. Some of these diseases have been ameliorated by changing husbandry. Herein, we outline the temporal discovery of pathogens and what measures changed the disease landscape. The first change to disease prevalence was when the industry changed to pond-reared broodstock from wild-caught broodstock. Pathogens like microsporidians (*Astathelohania*) and rickettsia (*Paracoxiella cheracis*) seem to need intermediate or alternate hosts and their prevalence plummeted. The next major change was the advent of hatchery produced juveniles that incorporated surface-sterilization of eggs. The prevalence of heirloom viruses (nudivirus, giardiavirus and reovirus) in particular, almost disappeared. Stress events of all kinds often precipitated epizootics. The combination of hatcheries, stress and surface sterilization brought the importance of bacterial disease (*Aeromonas*) to the fore which was solved for many years by bacteriophage intervention. The impact of transport stress allowed cryptic viruses (iflavirus and bunyavirus) to negatively influence production and be discovered. Often, crossing family lines to increase genetic diversity without due attention to the pathogens they carried allowed viruses and sometimes rickettsia to become widespread in the industry. A population of crayfish resistant to iflavirus has been discovered and awaits commercial exploitation. Lastly, the resilience and determination of the crayfish farmers in spite of these challenges must be acknowledged.

1. Introduction

The aquaculture of freshwater crayfish, *Cherax quadricarinatus,* in Australia has been operating for approximately 40 years but has failed to thrive or reach strong commercial outcomes despite considerable promise. Lack of government incentives, relatively low meat to waste ratios relative to marine prawns and poor attention to disease issues have all hampered development. Herein we document and explore the temporal emergence of the different pathogens. We demonstrate how the husbandry practices of the times increased risk and then ameliorated different disease risks with time. This is also an attempt to document this information before the farmers and researchers that gathered this data move to other pursuits or pass away. It is important to capture this corporate knowledge of participants and record hard to locate sources before they are lost and then mistakes are repeated. "Those who cannot remember the past are condemned to repeat it." (George Santayana; The Life of Reason, 1905; Wikipedia, accessed 9 Mar 2025).

We also try to capture the role of the temporal availability of increasing technological tools which had a large impact on the discovery of the aetiological agents of pathogenesis. When thinking about the reporting of the timing of a discovery in the industry, the delays of publishing should be considered. There is usually an investigation, discovery of a pathogen, analysis/confirmation, writing a manuscript, submission to a publishing body, review, acceptance of the manuscript and then publishing. e.g. Owens et al., 1992 gives a superficial discovery date of 1992, but these were peer-reviewed conference proceedings with the conference in Nov 1990 and the submission of abstracts of the research completed at least 6 months before. Therefore, the on-ground discovery occurred early in 1989. Another case in point was the study of Edgerton et al. (2000) where the initial study was in 1997 but not published until 2000. Consequently, all discoveries published, really happened in the industry most often between 3 to 4 years previously. This margin of error should be taken into account when aligning publication dates and husbandry changes.

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2. Approach and methodology

Ninety five percent confidence intervals (CI) were added to the calculated prevalence if the number of individuals examined was known. The website "Interactive Statistical Calculation Pages" (https://statpages.info/#Confidence) was used for the calculations. If the CIs of two samples did not overlap, it was concluded that the two populations from which the samples were drawn were showing statistically, characteristics of different populations which may include husbandry as well as history.

The term "heirloom pathogens" will be used for those pathogens that were inherited from the wild broodstock before any attempted amelioration. Effectively, these are the pathogens discovered in section 1. The Start-Up Years; Wild Caught Broodstock (below). Cherax reovirus (CV) presents a problem as it was clearly an heirloom from wild stock but was only found when the industry had started to raise and manipulate their own broodstock. Therefore, we have termed it an heirloom pathogen but included it in the correct chronological years. The discovery of Gill Parvovirus is similar in time frame but the inability to confirm its identity places it in a category of its own as idiopathic change rather than a proven pathogen.

The epiphytic organisms (*Lagenophrys*, chitrids, saprolegnia, temnocephalids) generally do not affect crayfish health and therefore they are only cursorial dealt with here. However, some maybe egg predators, or perhaps egg scavengers, particularly some temnocephalans (e.g. *Decadidymus valverdi* sp. *nov.*) may possibly affect fecundity and thus production (Brand, 2017; Valverde et al., 2020), although strong evidence for live egg predation is lacking. Others (chitrids, saprolegnia) mostly affect dead, ungroomed eggs. Epiphytic organisms have been left out of Table 1 to make it less cluttered and more understandable.

2.1. The start-up years; wild caught broodstock

The growing of freshwater crustaceans (crayfish and macrobrachium) started as a curiosity driven by fish-keeping hobbyists and societies in the 1970's. Very early, it became apparent there was the possibility of commercial exploitation and almost all the state governments invested in research scientists to aid the development of the industry. The first studies were on wild caught crayfish from particularly the Mitchell River catchment, northern Queensland that could be used as broodstock. The use of gross microscopic examination (Herbert, 1987) found the potential pathogens, *Psorospermium* and microsporidia, probably *Astathelohania* (=*Thelohania*) (Herbert, 1987, 1988; Semple, 1995) c.f. *Astathelohania parastaci* and *Astathelohania montirivulorum* from Australian *Cherax destructor* (Moodie et al., 2003a, 2003b respectively) (Table 1, Figs. 1 and 2). Recently, Stratton et al. (2022) produced

Table 1
Prevalence (%) of potential pathogens & histopathological changes with 95 % confidence limits. % m = percentage mortality.

~Year	Source	Cherax	Cherax	Cherax	Gill Signet Ring	*Chequa*	Athtab	IHHNV/EVE	Cherax	*Paracoxiella*	Bacteraemia	*Astathelohania*	Psorospermium	*Tetrahymena*
Reference	W= wild; F= farm	Nudivirus	Giardiavirus	Reovirus	Parvovirus?	*Iflavirus*	Bunyavirus	"SMV"	Aquambidensovirus	*cheracis*				ciliate
Pre 1990	**Wild Collection**													
1986; 20 21	W Mitchell River											7.8 (3.8-14)	present	
1987; 37 52	W + Farm P											problems		
1989; 40	W	present								present				
1989; 26	W + Farm P; 61	**52** (40-65)								22% m				
1992-06	**Pond-reared Broodstock**													
1992-6; 12	Farm A; 44	**6.8** (1.4-19)	**52.3** (37-68)		**6.8** (1.4-19)						0 (0-8)		0 (0-8)	
	Farm B; 44	0 (0-8)	**25** (13-40)		**2.3** (0.06-12)						0 (0-8)		0 (0-8)	
	Farm C; 44	**59.1** (43-74)	**11.4** (4-25)								**61.4** (45-76)		**9.1** (3-22)	
	Farm D; 44	**52.3** (37-68)	**11.4** (4-25)		present						**14** (5-27)		**38.6** (24-55)	
	Farm D; 32	**87.5** (71-96)	**3.1** (0.1-16)							**31.3** (16-50)	**62.5** (44-79)		**84.4** (67- 95)	**9.4** (3.5-29)
	Farm E; 44	**5.9** (0.1-29)	**11.8** (1.5-36)		**13.6** (1.5-36)						**43** (18-71)		0 (0-20)	
	Farm F; 44	**13.6** (5-27)	**6.8** (1.4-19)		**4.2** (0.6-15)						0 (0-8)		**6.8** (1.4-19)	
	Farm G; 44	**11.4** (4-25)	**13.6** (5-27)								0 (0-8)		0 (0-8)	
1997; 13	Farm D; 30+2	present	present	**50** (1-99)	**30** (15-49)									
1998; 39	Farm D; 12		**17** (10-24)					SMV invalid						
1998; 0	W Palmerston; 68	**2.9** (0.4-10)	**8.8** (3-18)										0 (0-5.3)	
	W Adelaide River; 36	**2.8** (.01-15)	**2.8** (0.1-15)										**42** (26-59)	
1999; 51	W Lake Eachum; 5	**20** (3-37)												
1999-0; 5	Farm H								50% m					
2004; 27	Farm D; 45							0 (0-8)						
2005; 30	Farm D; 105			**42** (36-49)						**7** (4-11)	**16** (11-21)			
	Farm I; 105			0 (0-3.5)						0 (0-3.5)	0 (0-3.5)			
2006; 16	Farm H; 44	0 (0-8)	**2.3** (0.06-12)	0 (0-8)							**68.2** (52-81)		0 (0-8)	
	Farm J; 44	**27.3** (15-43)	**2.3** (0.06-12)	**6.8** (1.4-19)							**97.7** (88-100)		0 (0-8)	
	Farm K; 44	0 (0-8)	0 (0-8)	**11.4** (.04-25)							**52.3** (37-68)		**27.3** (15-43)	
	Farm D; 44	0 (0-8)	0 (0-8)	**40.9** (26-57)							**72.7** (57-85)		0 (0-8)	
	Farm L; 44	**13.6** (5-27)	0 (0-8)	**6.8** (1.4-19)							**40.9** (26-57)		0 (0-8)	
2008-12	**Hatchery Craylings**													
2008; 17	Farm J	0	0	0							**17.46**		0	
	Farm K	0	0	0							**31.75**		0	
	Farm D	0	0	0							**12.5**		0	
2012; 45 47	Farm D; 24	0 (0-14)	0 (0-14)	**53** (28-77)	**21** (7-42)			present			**8** (16-55)			
	Farm K; 15	0 (0-22)	0 (0-22)	0 (0-22)				present		0 (0-17)	**27** (8-55)		**33** (12-62)	
	Farm J; 20	0 (0-14)	0 (0-14)	**20** (6-44)				present		**10** (1.2-32)	**20** (6-44)		**25** (9-49)	
	Farm H							present						
	MARFU; 120									**50** (41-59)				
	Farm K; 120										present			
2013-20	**Postgenetic Improvement**													
2014; 48 49	Farm K					20-39% m	20-30% m						high	2
2015; 25	Farm M; 267					65% m	65% m							
	Farm N; 295					30.5% m	30.5% m							
2019; 29	Farm K: 20			**5** (0.1-10)		**50** (39-61)	**55** (44-66)							
	Farm N; 17			**12** (4-20)		**71** (60-82)	**82** (73-91)							
	Farm O; 20			**15** (7-23)		**80** (71-89)	**45** (34-56)							
2020; 25	Farm O; 27	**89** (83-95)		**67** (58-76)							**100** (87-100)			
2021	**SPF Selection**													
2021; 33	Farm I; 10				present	0 (0-31)	**100** (70-100)							

The prevalence of pathogens throughout the years with 95 % confidence limits in brackets. % m = percentage mortality recorded by farmer or researcher. The number sampled n, is recorded in the Source column as Farm A; 44 (e.g. 44 crayfish sampled). Blue boxes are where the prevalence is statistically higher ($P < 0.05$) than minor levels of that pathogen. Pink boxes are the first time a pathogen was recorded. Gold boxes are where a pathogen appears to be removed by a husbandry protocol. The reference number is in the Year column following the semicolon. (e.g. 1989; 39 is reference 39. Owens et al., 1992). 0 = unpublished data.

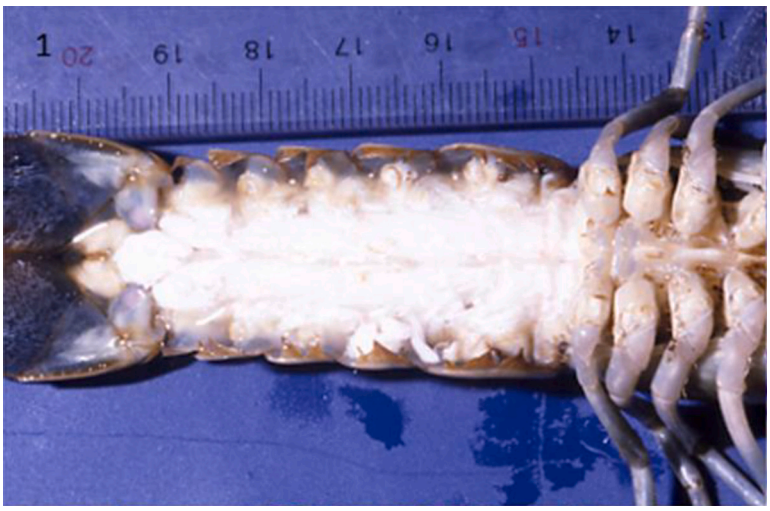

Fig. 1. Gross appearance of porcelain disease (microsporidiosis). (Ian Anderson, Biosecurity Queensland).

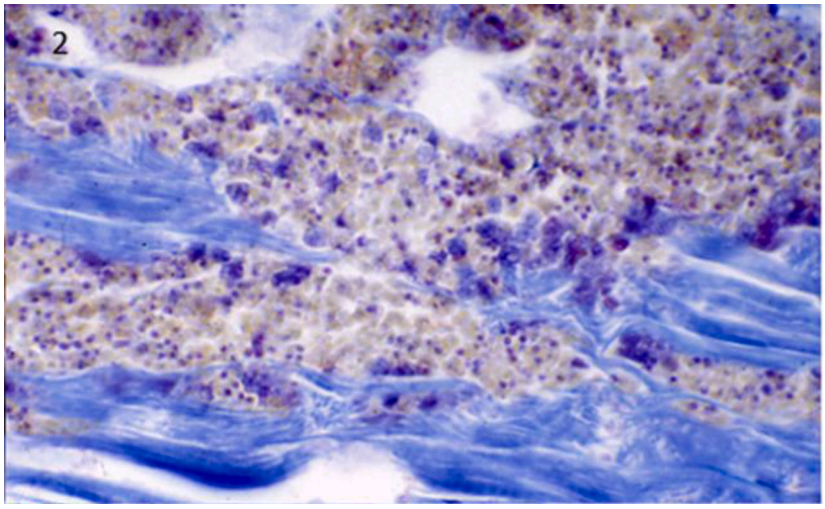

Fig. 2. Muscle infected with microsporidiosis. (Masson's trichrome).

compelling evidence that freshwater microsporidia from crayfish should be placed in the new genus *Astathelohania* including the two species from *C. destructor*. We will follow this line of evidence herein. Given the strong genetic relationship between *C. destructor* and *C. quadricarinatus,* it is highly likely the microsporidian in redclaw will be a member of the *Astathelohania*. After some early recalcitrant infections with microsporidia (Hutchings, 1987 in Owens and Evans, 1989), microsporidia never became an ongoing, major problem. However, exportation of 20,000 crayfish to Alabama, USA from affected farms also moved the *Astathelohania* there (Semple, 1995). Herbert (1988) could not induce any microsporidian infection via injecting infected blood or tissue, feeding infected tissue or from priming spores through a fish host. However, using a much more sensitive PCR assay, Imhoff et al. (2012) showed that *Astathelohania* (= *Thelohania*) *contejeani* could be spread by feeding infected crayfish tissue and water contamination to *Austopotamobius pallipes* and *Pacifiasctcus leniusculus*.

Psorospermium has remained with the industry Edgerton and Owens (1999) pretty much throughout its history becoming only prominent, correlated and therefore pathognomonic for bacteraemia, granulomas and septic conditions. It appears that this protist only sporulates and becomes obvious when the crayfish is immunocompromised or about to die. The preceding amoeboid stages awaits discovery.

Using histopathological examination (Owens et al., 1992) on moribund, wild caught broodstock in an experimental facility found the first rickettsia, *Coxiella cheraxi* (Tan and Owens, 2000, Fig. 3). This is now named *Paracoxiella cheracis* after Oren and Garrity (2020); see also https://lpsn.dsmz.d e/species/coxiella-cheraxi-1) corrected the gender of the name and then there is a proposal of a new genus (Ingle et al., 2025, in press). *P. cheracis* was widespread in the connective tissue which caused a major problem (22 % mortality) in one of the pioneering farms (Ketterer et al., 1992). Koch's postulates were proven for this *Paracoxiella* (Tan and Owens, 2000). The *Parcoxiella* has recrudesced in other farms ever since, periodically plaguing the industry (for a mini-review see Elliman and Owens, 2020). Of note is that when DNA from one of the *P. cheracis*-infected crayfish was sequenced by MINIon next-generation sequencing, a contaminating signal of the bacterium *Citrobacter freundi* was recovered (Elliman and Owens, 2020). The role of this bacterium in Australian crayfish farm mortalities is unknown. However, it was not isolated by Hayakijkosol et al. (2017) but was isolated by Valverde et al. (2020). It has been implicated, perhaps falsely, in massive mortalities in Chinese crayfish farms (see Elliman and Owens, 2020).

Also, in the hepatopancreas of some of the experimental crayfish were inclusion bodies of a non-occluded baculovirus (Owens and Evans, 1989, Fig. 4) that became known as Cherax baculovirus, (Anderson and Prior, 1992), then Cherax bacilliform virus (CBV) now recently proposed

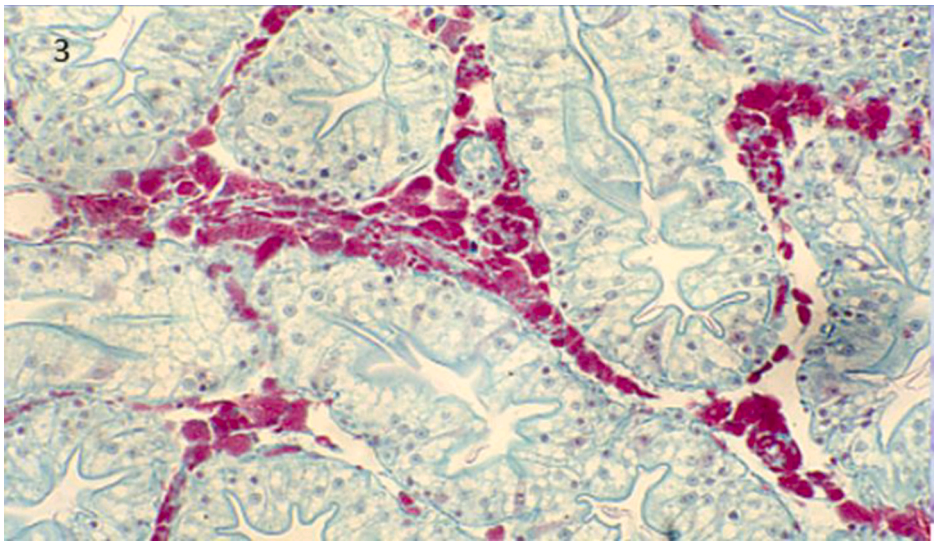

Fig. 3. *Candidatus Paracoxiella cheracis*. Gram Twort.

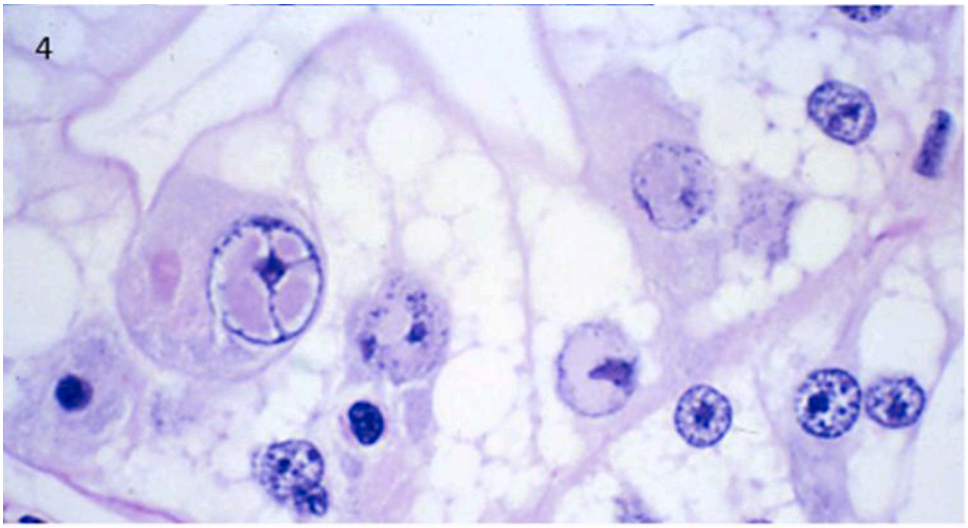

Fig. 4. Intranuclear Cherax Bacilliform Virus (= Cherax Nudivirus). Haematoxylin and eosin. (Brett Edgerton and Leigh Owens).

to be called Cherax Nudivirus (CNV) (Petersen et al., 2024). We will use the abbreviation CNV. A recent paper by Stratton et al. (2024) detailed genomic characteristics of nudiviruses from crayfish in the Americas which will lead the way to appropriate classification of CNV once studies are undertaken. Fifty-two percent of crayfish sampled from SE and NE Queensland farms and research facilities had CNV (Anderson and Prior, 1992) but these crayfish all had come from populations sourced from the Mitchell River, northern Queensland. By the time of the large scale, systematic surveys of Edgerton and others (1992–1996), CNV was widespread in almost all farms (Table 1) using broodstock ultimately derived and dispersed from the Mitchell River.

During the extended disease surveys of 1992–1996 and early heritability studies, another virus, Cherax Giardiavirus (CGV) (Edgerton et al., 1994, Fig. 5) was seen across all farms with prevalence ranging from 2 to 52 % (Edgerton et al., 1995, Edgerton and Owens, 1999). Over time, the prevalence of this virus has dwindled as farmers moved to pond-reared broodstock (Table 1) (see below). Giardiavirus seemed associated with mortalities in craylings in particular, that lessened in severity as the crayfish grew in size (Edgerton and Owens, 1997). The problems with this virus and to a lesser extent, CNV lead to surface sterilizing of eggs in a model hatchery which both proved a vertical faecal/oral route of infection for these viruses and also demonstrated the effectiveness of hatcheries. A population of crayfish initially raised using surface sterilization in 1995 (Edgerton and Owens, 1997) is still free of these two viruses, CNV and CGV in 2023.

In contrast, and of considerable interest is putative Gill Parvovirus (GPV) (Edgerton et al., 2000, Fig. 6) which was discovered and geographically widespread albeit at low prevalence (<30 %); Table 1) during the surveys of Edgerton et al. (1992–1996). It produces a very clear, pathognomonic lesion of a hypertrophied, chromatin-lined nucleus in the gill epithelium. Whilst not clearly associated with mortality, it has been persistent in the lineages of crayfish established free of CNV and CGV from 1995 until 2023. Probably, the known tough nature of parvovirus made it impervious to the surface sterilization protocol that produced eggs free from other viruses. As late as 2022, injections of haemolymph from these infected crayfish have been able to transmit GPV to infection-naïve crayfish (Nambiar et al., 2023). However, an attempt to find its viral genome using suppression subtractive hybridisation was unsuccessful (Rusaini et al., 2013a). This is probably not the best technique to find a novel sequence as it assumes the sequence is in one sample but missing from the subtracting sample (Rusaini et al., 2013a).

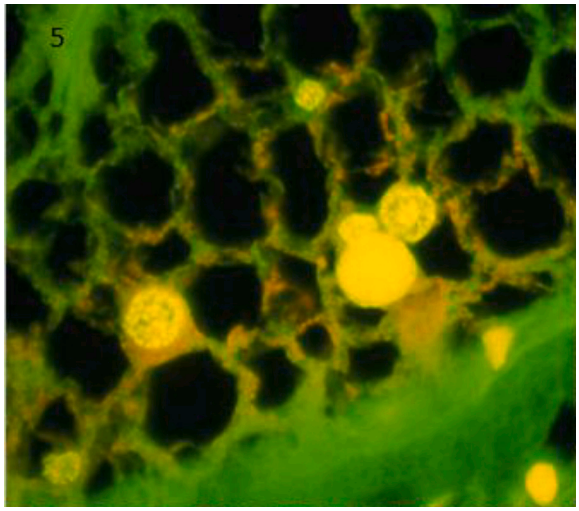

Fig. 5. A single infected nucleus with Cherax Giardiavirus stained by acridine orange demonstrating ds nucleic acid. (Brett Edgerton and Leigh Owens).

Internal *Tetrahymena* ciliates have been discovered only twice in moribund crayfish in 1992–96 (Edgerton et al., 1995) and 2021 (Nambiar et al., 2023). It is believed they are saprophytic attacking only when there is a major breach of the cuticle and the crayfish is so close to death it cannot fight off the ciliates. Therefore, they will not be dealt with further herein.

In 1998, the hepatopancreata wild *Cherax quadricarinatus* collected from Palmerston Golf Club (n = 68), Darwin and Adelaide River, Northern Territory (n = 36) were examined by histopathology (Owens unpublished 1998). Low levels of CNV and CGV, 2.9 % and 8.8 % at Palmerston; and 2.8 % and 2.8 % at Adelaide River; and zero and 41.7 % of *Psorospermium* respectively were found. This demonstrates that crayfish across their natural range are carrying similar heirloom pathogens.

2.2. The first attempt at genetic outbreeding and subsequent pond-raised broodstock

The crayfish industry was very aware of the fact that almost all farms had stock originally from the Mitchell River catchment and the possibility that inbreeding depression might occur. Phenotypically, crayfish from the Gilbert River had narrower chelicerae, so there was an observable genetic variability between the crayfish from different river systems. By the mid-1990s, crayfish were sourced from the Gilbert and Flinders Rivers to broaden the gene pool of farmed crayfish. Unfortunately, screening for pathogens was not undertaken at the same time and this led to the introduction of Cherax reovirus (CRV) from the Flinders River (unpublished data) into recipient populations at least by 1997 (Edgerton et al., 2000, Fig. 7) which later became widespread in farms. There was a centralised, crayfish receiving operation at Cardwell that was organised to fill retail crayfish orders that unfortunately supplied broodstock back to farms which is likely how CRV became so widespread. Via natural routes of infection (feeding), CRV produced 5 % mortality and 13 % stunting in juvenile crayfish. Injection of purified CRV caused 20 % mortality and 41 % stunting in crayfish (Hayakijkosol and Owens, 2011).

In the mid to late 1990s, crayfish were introduced into the wild in the isolated, ex-volcanic Lake Eachum on the Atherton Tablelands. When tested, these pest crayfish had about 20 % CNV (Sciliano and Owens, 1999). This crayfish population is of interest as it was proposed to export some as a founder population to Italy, and whilst they were known to be collected, it is unknown if exportation occurred. However, it should be noted that *C. quadricarinatus* was recorded to be only on-farm in Italy in 2010 (Aquiloni et al., 2010). It would be of interest to examine this Italian population of redclaw and see if any pathogens were transshipped especially CNV and *P. cheracis*.

At the end of this decade (1999), on an isolated farm close to the coast near Mission Beach, the first and only known outbreak of the parvovirus, *Cherax Aquambidensovirus* (CADV) occurred (Bowater et al., 2002, Bochow et al., 2015, Fig. 8). Approximately, 50 % of the farm's crayfish died with one pond's mortality reaching 96 %. CADV never spread to any other farms demonstrating high levels of effective biosecurity and isolation imposed by the Queensland Government. Destocking, drying out and chemical sterilization apparently killed the virus as the farm was successfully restocked. CADV is most closely related to sea star wasting Aquambidensovirus. So, the working hypothesis is that seagulls dropped viral infected starfish material into the crayfish pond in much the same way seagulls may spread Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV; now named Penstylhamaparvovirus, PSHP) and Taura Syndrome Virus in the Americas (Van Patten et al., 2004). Indeed, seagulls were seen commonly resting on the side of the crayfish ponds where this CADV outbreak took place.

Spawner-isolated Mortality Virus (SMV) was purported to be present in crayfish (Owens and Mc, 2000), but the conclusions were based on a flawed methodology and were incorrect (Owens, 2023). This virus has been abolished as an entity. However, the description of the transport/

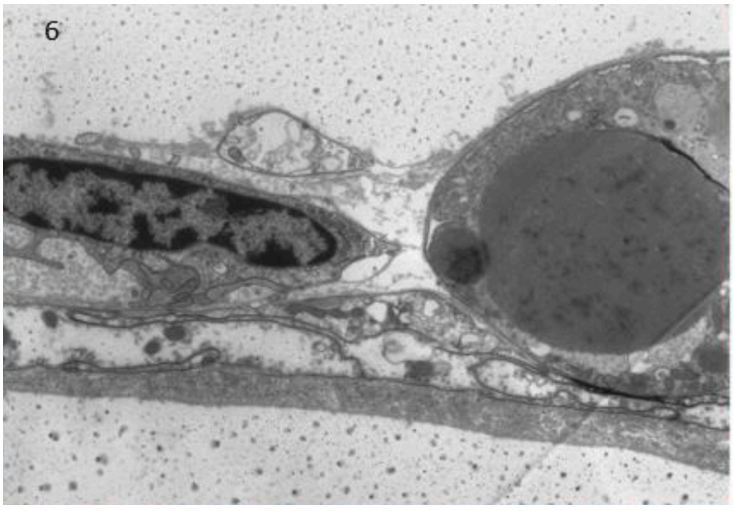

Fig. 6. A single nuclear inclusion body (right) with remains of chromatin in gills of crayfish with gill parvovirus. (Rusaini and Leigh Owens). Transmission Electron Microscope.

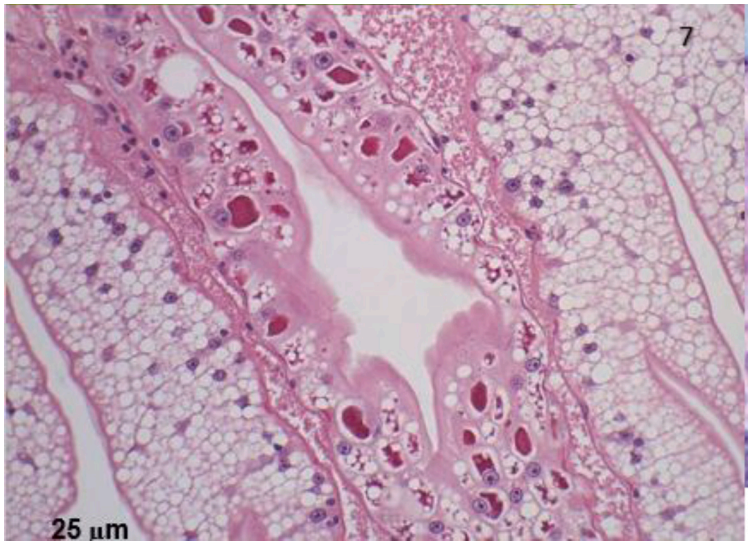

Fig. 7. Cytoplasmic Cherax Reovirus, Haematoxylin and eosin.

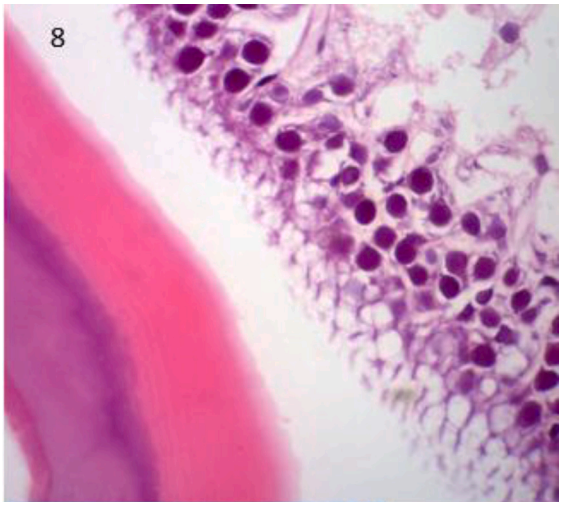

Fig. 8. Intranuclear Cherax Aquambidensovirus. (Shaun Bochow).

stress related deaths and the observations of low CGV in these crayfish are still valid. The SMV electron micrographs are now attributed to IHHNV = Penstylhamaparvovirus (see below). The DNA in-situ hybridization was due to non-specific binding with moth (arthropod)-like elements in the nuclei of rapidly dividing crustacean cells and the PCRs are attributed to probiotic *Carnobacterium divergens* bacteria in the gut of the hosts (Owens, 2023).

In 2004, 45 crayfish from a single farm were tested by polymerase chain reaction (PCR) for the presence of and susceptibility to IHHNV = Penstylhamaparvovirus, (Krabsetsve, 2004). The PCR used the F/R 392 primers (Krabsetsve et al., 2004) which have subsequently been shown to also amplify endogenous viral elements (EVE) of IHHNV incorporated into crustacean genomes. No crayfish were PCR positive. However, when crayfish from the same farm and two other farms were tested in 2012 (Rusaini et al., 2013b) using many different PCR primers for IHHNV, these crayfish all had EVEs from IHHNV. So, with the caveat of the power of the number of crayfish tested (45), the prevalence could have been as high as 8 % and been undetected in 2004. This data suggests IHHNV became widespread in the crayfish farms sometime after 2004. It is noteworthy that 2004 was the start of poor growth and stamina ascribed to inbreeding suppression that was recorded on two farms (Stevenson et al., 2012).

Around 2005, crayfish were again collected from the Flinders River and introduced into farms as part of attempted genetic outbreeding (see the first paragraph of this section). Unfortunately, these crayfish were not screened for pathogens before being released to farms and they were subsequently found to be heavily infected with Cherax reovirus (CRV). One farm (D) with the index case, became statistically ($P < 0.05$) more heavily infected than most farms with reovirus at greater than 40 % prevalence (Table 1) and farm O followed. This trend continued from 2005 to at least 2012. Farm D used to allow the broodstock to cohabit at high densities for feeding of special maturation diets before spawning, thus ensuring the easy spread of reovirus throughout the female crayfish brooders. Crayfish from this farm were moved to many farms also spreading the reovirus.

In 2006, the last detailed survey of multiple farms for disease and potential genetic gain was undertaken by Ghosh (2006) before the widespread use of hatchery-raised crayfish called craylings (stage 3 juveniles, S3Js) by the industry. The three heirloom viruses, CNV, CGV and CRV, were at low, sporadic levels across the industry except for the original farm (D) that received CRV which had statistically ($P < 0.05$) higher levels of CRV (as mentioned above). *Psorospermium* was moderate in one farm. The most worrying trend was the high (>50 %) to very high levels (98 %) of bacterial granulomas across all farms suggesting an underlying, undiagnosed problem across the industry.

In summary, the industry's move to not using wild-caught broodstock gave sustained reduction in the pathogens. Microsporidia apparently disappeared altogether, CGV was dwindling in prevalence and *P. cheracis* became more intermittent and arguably linked to exposure to wild crayfish.

2.3. The Hatchery Era (2006 Onwards)

An extremely informative account of the lead-up to the hatchery era is provided by Stevenson et al. (2012). The hatchery era, (approximately after 2006) was characterized by the removal of fertilized eggs from females kept in large tanks, surface sterilization of the eggs and placing them in small mesh containers which were mechanically agitated for water flow; a Finnish designed system called the Heputin incubator with the first incubator acquired in 2005. A second incubator and later a third incubator were manufactured based on the first incubator. The long-term strategy was that craylings would be sent to farms for stocking and grow out. The initial samples from three participating farms were astounding with none of the heirloom viruses (CNV, CGV, CRV) nor *Psoropermium* present and prevalence of granulomas in crayfish dropped

to lower levels below 32 % (Hayakijsokol and Owens, 2008).

A later follow-up investigation to look at hypertrophied nuclei (GPV) in the gills of crayfish (Rusaini et al., 2013a, 2013b), showed that CRV had returned to historic, significantly high levels in the original, point-source farm (D) that was not continuing to follow the hatchery technology but was using pond-reared, berried broodstock.

Using PCR, Rusaini et al. (2013b) demonstrated the presence of endogenous viral elements (EVEs) probably derived from exposure to IHHNV (=PSHP). The pattern of the EVEs showed the farmed crayfish populations were not homogenous, suggesting a relatively recent exposure to IHHNV with not enough time for crayfish movement to homogenise the populations. The Group 1 crayfish sampled around 2000 (Bowater et al., 2002) had the most incomplete EVE structure, followed by a population isolated since 1995 (Group 2 crayfish). The other sampled farms (Group 3) were homogeneous and identical containing all EVEs in the NS1/NS2 area of IHHNV but not in the area downstream of these genes which would have been detected by the QPF1/QFP2 primers. All these Group 3 farms had obtained crayfish from the central hatchery. Interestingly, crayfish from one of these farms were tested in 2004 by PCR (F/R 392) for IHHNV which would have found the EVEs (Krabsetsve, 2004) and they were test-negative. Taken all together, this information suggests some crayfish populations were exposed to IHHNV individually in the 1990s, perhaps by using *Penaeus monodon* bodies as a maturation diet supplement, but the use of hatchery-raised craylings for stocking spread the EVEs of Group 3 through the industry after 2008.

Late in this period, March 2012, large mortalities were recorded at a research facility receiving crayfish from three farms (Powell, 2013; Rusaini et al., 2013b). Whilst not likely to be the only aetiological agent, *Paracoxiella cheracis* (GenBank Accession no. PP818860) was traced back to crayfish from one farm on the Atherton Tableland (GenBank Accession no. PP818859). The identity of *P. cheracis* was confirmed by 16SrRNA sequencing where any bi-nucleotide ambiguities (about 14 %) were resolved by comparison against the original sequence of *P. cheracis* (Tan and Owens, 2000). The sequences were identical with none of the >1000 unambiguous nucleotides called by the sequencing being incorrect. This was the first recrudescence of *P. cheracis* since 2004 (La Fauce and Owens, 2007). Crayfish from this farm had low genetic similarity to other farms and low genetic diversity in some genetic microsatellites (Stevenson et al., 2012) suggesting high stock isolation from other farms and from wild stocks. The crayfish from this farm would have had the highest genetic gain from introduction of wild stock which may have been the source either accidentally or deliberately of *P. cheracis* to the farm.

As the hatchery procedure removed the females which would have groomed the eggs and removed infertile and rotting eggs, bacterial diseases and to a lesser extent, fungal diseases (chytrids and *Pythium*) became problematic (Owens and Evans, 1989; Payne and Owens, 2008). A number of in-house investigations were reported that identified antibiotic-resistant *Aeromonas hydrophila* as the main culprit (Payne and Owens, 2008, Cooper and Owens, 2012, Hayakijkosol et al., 2017, Fig. 9). This led to successful investigations in using 11 strains of bacteriophage to combat *A. hydrophila* in eggs and/or the S3J craylings (Elliott, 2014; Valverde et al., 2020). A successful business was run for many years suppling these bacteriophage-treated eggs and/or craylings to Australian farms and around the world. The success of this protocol allowed reviews of male fertility (Aquino et al., 2021) and investigations into reproduction of male-influenced, separated females (Nesa et al., 2023). Unfortunately, the business ultimately closed because of the failure to control cryptic viruses (see below) that took time to identify and affected survival (see following section).

2.4. After identified genetic gain (2013 Onwards); the discovery of cryptic viruses

Pre-release and after the release of the report of Stevenson et al., 2012), it was clear genetic gain and some disease mitigation was possible by using cross-breeding and hatchery technology to stock craylings. However, chronic low-grade mortalities in craylings, particularly after transport stress, were widespread in the industry. Reported in 2015, histological examination of broodstock and craylings from day 1 after hatching to day 6 showed novel lesions. The broodstock had fragmentation and haemocytic infiltration of the striated muscle reminiscent of either vitamin E/selenium deficiency seen in penaeid prawns (Owens and Hall-Mendelin, 1990) or viral-caused lesions similar to those caused by *Macrobrachium rosenbergii* Nodavirus of which a strain was endemic in Australia (Owens et al., 2009). Also, *Macrobrachium* spp. cohabit most for the crayfish farms, so cross-species exposure was highly probable.

Investigations into vitamin E/selenium (Se) deficiency showed that the soils of the Atherton Tablelands, northern Queensland are considered selenium deficient (Pirozzi et al., 2016). A number of direct measures of the muscle lesion frequency and production related to selenium in a good producing pond and a poorer producing pond showed significant differences (all $P < 0.01$–0.003). However, there appeared to be enough Se in the pelletised diet for adequate production. Experimentally, higher levels of Se significantly increased Food Conversion Ratio by 12 % and decreased muscle lesions by 7 % (Pirozzi et al., 2016). However, chronic mortalities continued.

The histopathology of the craylings showed pyknotic nuclear lesions

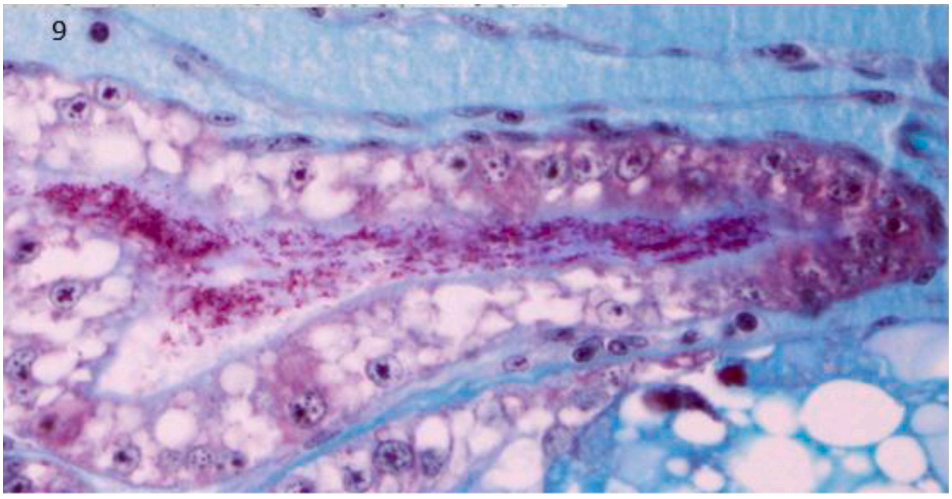

Fig. 9. *Aeromonas hydrophila* in the lumen and tissue of day 4, s3j craylings. Gram.

in nerve tissue in day 1 craylings suggesting transovarian spread of a virus. After the embryonic meconium membrane broke down on day 5 allowing the first feed of the craylings, Gram-negative bacteria, presumptive *Aeromonas hydrophila,* secondarily were able to enter the gut and invade other tissues (Owens unpublished 2015; Fig. 9).

As there appeared to be virus in both the broodstock and craylings, an intense whole transcriptome approach was used to try and find all viruses in the crayfish (Sakuna et al., 2017). Ten million, 125 base-pair reads of one moribund and one healthy crayfish were assembled by via 10 high performance computers in time-share over 3 weeks. Five hundred thousand contigs were produced with and average depth of 18 replicates. Within the contigs, two new RNA viruses were found, *Chequa Iflavirus* (CIV) (Sakuna et al., 2017) which was only 36 % similar to other members of the genus and Athtab Bunyavirus (ABV) which was ~43 % similar to other bunyaviruses (Sakuna et al., 2018a). Due to tissue tropism and lesions produced by similar viruses, it was hypothesized that the Iflavirus was in the muscle and the bunyavirus was likely in the nerve tissue. Development of diagnostic RT-qPCRs allowed investigators to demonstrate both viruses were widespread across five farms, two crayfish research populations and one wild crayfish population.

2.5. Diet optimisation, stress and the effect on viruses

Concurrent with the genetic gain and disease studies were investigations into protein substitution and optimizing of the micronutrients in experimental diets (Joyce, 2020). One diet using soya bean protein significantly ($P < 0.05$) increased the amount of CNV but no significant ($P > 0.05$) change in CRV, granulomas or the size of the hepatopancreas occurred in crayfish. Soya beans are renowned for having anti-nutritional factors that have been genetically selected for removal for human consumption but perhaps not in all animal feeds. It is hypothesized that the stress associated with the anti-nutritional factors partially suppressed the immune system and allowed the virus to proliferate in tubules. Indeed, stress caused by hyper-salinity (12 ppt) has been used experimentally to increase the amount of CNV in crayfish by over 6 % ($P < 0.01$) (Claydon et al., 2004). Transport stress-related mortalities also lead to the discovery of the cryptic CIV and ABV (above) (Sakuna et al., 2017, 2018a). The inclusion in the diet of the immunomodulator quercetin dropped CIV levels significantly ($P < 0.018$) with a 90 % drop in viral copies (Sakuna et al., 2018b). There is anecdotal evidence that rapid cold shock/stress affected the survival of S3Js. Conversely, higher water temperatures at 32 °C significantly ($P < 0.05$) decreased CIV by 82 % (Sakuna et al., 2018b). However, temperature stress above 32 °C killed almost all stages of crayfish associated with *Astathelohania* studies but the role of *Astathelohania* vs heat stress in these mortalities was not clarified (Semple, 1995).

2.6. Development of specific pathogen-free crayfish (Specified Pathogen Chequa Iflavirus) (2017 onwards)

Observations that lesions were present in the day 1 craylings suggested that virus might be transmitted vertically via ova. This was significant as it meant that surface sterilizing eggs would not reduce or eliminate virus. Eggs from three crayfish farms had viral copies measured by RT-qPCR after surface sterilization (Jaroenram et al., 2021). Copies of *Chequa Iflavirus* and Athtab bunyavirus did not change from traditional tissue loads after sterilization but Cherax reovirus was extremely low between 3 and 200 copies. This suggested iflavirus and bunyavirus were internal in the eggs (transovarian transmission) whilst CRV was external and largely removed by surface sterilization.

As surface sterilization would not reduce the viruses of concern, another avenue was sought. It had been noticed in the intervention trial of different therapeutics by Sakuna et al. (2018b) that 5 out of 140 crayfish tested multiple times remained free of *Chequa Iflavirus* despite being cohabited with infected crayfish. These crayfish were bred together to form a nuclear family of potentially Iflavirus-free crayfish. In a pilot study, some of these progeny crayfish were injected with purified Iflavirus/ bunyavirus and later also injected with haemolymph from viral infected crayfish (Nambiar et al., 2023). The Iflavirus was shown to be cleared within 24 h whilst the load of bunyavirus was constant. Furthermore, transport-stressed, virus-donor crayfish had all died whilst only one of the heavily manipulated crayfish (transported, weighed, injected twice) died. This suggested that the Iflavirus was a strong contributing factor to the transport-related mortalities seen on crayfish farms. There was strong melanisation in viral injected crayfish but not in the control crayfish suggesting specific pattern-recognition, phagocytosis and melanisation was involved in clearing the Iflavirus. It was concluded these were indeed Specific Pathogen Free crayfish with the specific pathogen being Iflavirus. Unfortunately to date, this small, nuclear SPF crayfish family has not been taken up by industry to expand to a full SPF program.

3. Conclusions

Firstly, the perseverance and resilience of the crayfish farmers through the years of decreased production caused by pathogens should be strongly complimented and admired. Most of the farmers have a love for the crayfish that kept them going when the economics were poor. Their unceasing co-operation with researchers has been outstanding.

Secondly, the low quantities of funds for research in all aspects of farming has meant that almost everything was done on shoe-string budgets which slowed all advancement that ultimately meant the farmers suffered through low production more than they should have.

Thirdly, with the industry moving away from wild-caught broodstock to pond-reared broodstock, pathogen lifecycles were broken. For the first time, some pathogens reduced in prevalence. Microsporidia were not reported again on farms. The lack of microsporidia probably had wide, unintentional surveillance as one farmer reported eating a microsporidia-infected animal and it was “like eating a mouthful of sand”. Such a distasteful experience would have been commented on and therefore the presence of microsporidia noted. *P. cheracis* seems to have only recrudesced when a husbanded population was probably exposed to wild crayfish. CGV seems to have disappeared suggesting crayfish were poor alternate hosts, not the main host.

Fourthly, the advent of hatchery technology and the concomitant use of surface sterilization of eggs was the biggest game changer for the industry. The levels of the heirloom pathogens dropped to insignificant levels and largely ceased to be problems. Consistent health and production of S3J allowed continued optimism in the industry. However, almost immediately, previously cryptic viruses became widespread due to attempts to increase genetic diversity (below).

Fifthly, the many attempts for genetic improvement and the hype around the industry have been too often accompanied by poor attention to the presence of pathogens. This has allowed pathogens to be widely spread, even overseas (*Astathelohania*, CNV, *P. cheracis*, CRV; ABV was not recorded to be transshipped, but it is impossible for this not to have occurred as all populations of crayfish tested in Australia have this virus), before mortalities and low production have triggered disease investigations. The latest attempt at genetic improvement allowed the cryptic viruses to become widespread before they were discovered, thus causing a major down turn in the crayfish industry.

Lastly, the role of stress of multiple types (transport, diet, salinity, cold, heat) have been scientifically shown to be significantly linked to higher viral loads and mortality. Some efforts to ameliorate the stressors have been successful with quercetin in the diet and higher water temperature not above 32 °C having beneficial effects.

With the advent of cost-effective, whole transcriptome sequencing it is possible to prevent spread of unknown pathogens to other geographical areas and farms. It is even possible to examine crayfish via transcriptome analysis on a statistical scale using confidence limits to limit pathogens. Despite the lessons that COVID-19 should have taught us, we believe the hype of a good crayfish story coupled with the

ignorance of pathogens and health in general, more pathogens will emerge and be translocated to hinder crayfish farmers in the future. Hopefully, this paper will alert some farmers enough to slow the spread of pathogens through better attention to biosecurity.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Anderson, I.G., Prior, H.C., 1992. Baculovirus infections in the mud crab, *Scylla serrata*, and a freshwater crayfish, *Cherax quadricarinatus*, from Australia. J. Invertebr. Pathol. 60 (3), 265–273.

Aquiloni, L., Tricarcio, E., Gherardi, F., 2010. Crayfish in Italy: distribution, threats and management. International Aquatic Research 2, 1–14.

Aquino, J.I.L., Elliott, L., Zeng, C., Paris, D.B.B.P., 2021. Recent developments in male fertility evaluation, sperm cryopreservation and artificial fertilisation, and their potential application to decapod crustacean aquaculture. Rev. Aquac. 14, 848–889. https://doi.org/10.1111/raq.12627.

Bochow, S.R., Condon, K.M.L., Rusaini, Elliman J.R., Owens, L., 2015. First complete genome of an Ambidensovirus; Cherax quadricarinatus densovirus, from freshwater crayfish *Cherax quadricarinatus*. Mar. Genomics 24, 305–312.

Bowater, R.O., Wingfield, M., Andrew, Fisk A., Condon, K.M.L., Reid, A., Prior, H., Kulpa, E.C., 2002. A parvo-like virus in cultured redclaw crayfish *Cherax quadricarinatus* from Queensland, Australia. Dis. Aquatic Org 50, 79–86. https://doi.org/10.3354/dao050079.

Brand, J., 2017. The Association of a Novel *Decadidymus* Species (Temnocephalida) with Australian Redclaw Crayfish (*Cherax quadricarinatus*); Impacts and Management. Master of Science. Marine Biology SA, University of Southampton, United Kingdom.

Claydon, K., Cullen, B., Owens, L., 2004. Methods to enhance the intensity of intranuclear bacilliform virus in *Cherax quadricarinatus*. Dis aquatic Org. 60, 173–178.

Cooper, A., Owens, L., 2012. Identification and elimination of OTC-resistant bacteria in a redclaw crayfish (*Cherax quadricarinatus*) hatchery. Internal Report to RIRDC Project No PRJ 000327.

Edgerton, B.F., Owens, L., 1997. Age at first infection of *Cherax quadricarinatus* by Cherax bacilliform virus (=Cherax baculovirus) and Cherax giardiavirus-like virus; and production of virus-free crayfish. Aquaculture 152, 1–12.

Edgerton, B.F., Owens, L., 1999. Histopathological surveys of the redclaw freshwater crayfish, *Cherax quadricarinatus,* in Australia. Aquaculture 180, 23–40.

Edgerton, B., Owens, L., Glasson, B., De Beer, S., 1994. A small double stranded RNA virus from *Cherax quadricarinatus*. Dis. Aquatic Org. 18, 63–69.

Edgerton, B., Owens, L., Harris, L., Thomas, A., Wingfield, M., 1995. A health survey of farmed redclaw crayfish, *Cherax quadricarinatus* (von martens) in tropical Australia. Proc. Astacology Soc. 10, 322–338.

Edgerton, B.F., Webb, R., Anderson, I.G., Kulpa, E.C., 2000. Description of a presumptive hepatopancreatic reovirus, and a putative gill parvovirus, in the freshwater crayfish *Cherax quadricarinatus*. Dis. Aquat. Organ. 41, 83–90. https://doi.org/10.3354/dao041083.

Elliman, J., Owens, L., 2020. Confirmation that candidatus *Coxiella cheraxi* from redclaw crayfish (*Cherax quadricarinatus*) is a close relative of *Coxiella burnetii*, the agent of Q-fever. Lett. Appl. Microbiol. https://doi.org/10.1111/lam.13336.

Elliott, L., 2014. Bacteriophage Therapy in Aquaculture-Friend or Foe? World Nutrition Forum: Munich, Germany, 2014. ResearchGate.net. https://www.researchgate.net/.

Ghosh, B., 2006. Predicting genetic gains and disease risk in a selective-breeding program involving farmed populations of the redclaw crayfish *Cherax quadricarinatus* (von Martens). Batchelor of Science Honours Thesis, James Cook University, 75pp.

Hayakijkosol, O., Owens, L., 2011. Investigation into the pathogenicity of reovirus to juvenile *Cherax quadricarinatus*. Aquaculture 316, 1–5.

Hayakijkosol, O., Owens, L., Picard, J., 2017. Case report of bacterial infections in a redclaw crayfish (*Cherax quadricarinatus*) hatchery. Aquaculture 475, 1–7.

Hayakijsokol, O., Owens, L., 2008. Preliminary histopathology from hatchery reared juvenile *Cherax quadricarinatus*. In: Internal Report to RIRDC Project No PRJ 000327.

Herbert, B., 1987. Notes on diseases and epibionts of *Cherax quadricarinatus* and *C. Tenuimanus* (Decapoda: Parastacidae). Aquaculture 64, 165–173. https://doi.org/10.1016/0044-8486(87)90322-X.

Herbert, B., 1988. Infection of *Cherax quadricarinatus* (Decapoda: Parastacidae) by the microsporidium *Thelohania* sp. (microsporidia: Nosematidae). J. Fish Dis. 11, 301–308.

Hutchings, R., 1987. Exciting new crayfish. Austasia Aquaculture 1 (12), 12–13.

Imhoff, E.M., Mortimer, R.J.G., Christmas, M., Dunn, A.M., 2012. Horizontal transmission of *Thelohania contejeani* in the endangered white-clawed (*Austropotamobius pallipes)* and the invasive signal crayfish (*Pacifastacus leniusculus*). Parasitology 139 (11), 1471–1477. https://doi.org/10.1017/S0031182012000777.

Ingle, D.J., Walsh, C.J., Samuel, G.R., Wick, R.R., Davidovich, N., Fiocchi, E., Judd, L.M., Elliman, J.R., Owens, L., Stinear, T.P., Basso, A., Pretto, T., Newton, H.J., 2025. The complete genome sequence of the crayfish pathogen Candidatus *Paracoxiella cheracis* n.g. n.sp. provides insight into pathogenesis and the phylogeny of the Coxiellaceae family. bioRxiv. https://doi.org/10.1101/2024.12.02.626477.

Jaroenram, W., Owens, L., Hayakijkosol, O., Elliman, J., 2021. Natural, *in ovo*, vertical transmission of the RNA viruses, *Chequa iflavirus* and Athtab bunyavirus, but not Cherax reovirus in redclaw crayfish (*Cherax quadricarinatus*). Aquaculture 534, 736285.

Joyce, M., 2020. Understanding the Nutritional Requirements of Redclaw (*Cherax quadricarinatus*): Determining the Apparent Digestibility of Raw Materials and Quantifying Dietary Lysine Requirement. Master of Philosophy thesis. College of Science and Engineering, James Cook University, 115pp.

Ketterer, P.J., Taylor, D.J., Prior, H.C., 1992. Systemic rickettsia-like infection in farmed freshwater crayfish *Cherax quadricarinatus*. In: Shariff, M., Subasinghe, R.P., Arthur, J.R. (Eds.), Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 173–179.

Krabsetsve, K., 2004. Investigation and Verification of Infectious Hypodermal and Haematopoietic Necrosis Virus in Australian Penaeid Prawns. Honours/Master of Science Thesis, James Cook University, 242pp.

Krabsetsve, K., Cullen, B.R., Owens, L., 2004. Rediscovery of the Australian strain of infectious hypodermal and Haematopoietic necrosis virus (IHHNV). Dis. aquatic Org 61, 153–158.

La Fauce, K., Owens, L., 2007. Investigation into the pathogenicity of *Penaeus merguiensis* densovirus (*Pmerg*DNV) to juvenile *Cherax quadricarinatus*. Aquaculture 27, 31–38.

Moodie, E.G., Le Jambre, L.F., Katz, M.E., 2003a. *Thelohania parastaci* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae). Parasitol. Res. 91, 151–165. https://doi.org/10.1007/s00436-003-0941-3.

Moodie, E.G., Le Jambre, L.F., Katz, M.E., 2003b. *Thelohania montirivulorum* sp. nov. (Microspora: Thelohaniidae), a parasite of the Australian freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae): fine ultrastructure, molecular characteristics and phylogenetic relationships. Parasitol. Res. 91, 215–228. https://doi.org/10.1007/s00436-003-0948-9.

Nambiar, G., Owens, L., Elliman, J., 2023. *Cherax quadricarinatus* resistant to Chequa iflavirus: a pilot study. Microorganisms 11, 578. https://doi.org/10.3390/microorganisms11030578.

Nesa, N.U., Elliott, L., Zeng, C., Jones, R., Cavalieri, J., 2023. Redclaw, *Cherax quadricarinatus* sex-separated rearing strategy enhances reproduction in females. Aquaculture 573, 739592. https://doi.org/10.1016/j.aquaculture.2023.739592.

Oren, A., Garrity, G.M., 2020. Candidatus list no. 3. Lists of names of prokaryotic *Candidatus* taxa. Int. J. Syst. Evol. Microbiol. 72, 005186. https://doi.org/10.1099/ijsem.0.005186.

Owens, L., 2023. Abolishment of spawner-isolated mortality virus and where the remaining science leads. J. Gen. Virol. 104, 001878. https://doi.org/10.1099/jgv.0.001878.

Owens, L., Evans, L.H., 1989. Common diseases of freshwater prawns (*Macrobrachium*) and crayfish (marron and yabbies) relevant to Australia. In: Invertebr. Aquacult. Proc. 117 Post Grad. Comm. Veterin. Sc., Univ. Sydney, pp. 227–240. ISSN 0814–6829.

Owens, L., Hall-Mendelin, S., 1990. Diseases relevant to penaeid mariculture in tropical Australia. In: Perkins, F.O., Cheng, T.C. (Eds.), Pathology in Marine Science, pp. 421–432.

Owens, L., Mc, Elnea C., 2000. Natural infection of the redclaw crayfish *Cherax quadricarinatus* with presumptive spawner-isolated mortality virus. Dis. aquatic Org 40, 219–223.

Owens, L., Muir, P., Sutton, D., Wingfield, M., 1992. The pathology of microbial diseases in tropical Australian crustacea. In: Shariff, M., Subasinghe, R.P., Arthur, J.R. (Eds.), Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp. 165–172.

Owens, L., La Fauce, K., Juntunen, K., Hayakijkosol, O., Zeng, C., 2009. *Macrobrachium rosenbergii* nodavirus disease (white tail disease) in Australia. Diseases in Aquatic Organisms 85, 175–180.

Payne, M., Owens, L., 2008. Report on the investigation of mortalities in the *Cherax* egg incubator. In: Internal Report to RIRDC Project No PRJ 000327.

Petersen, J.M., Burgess, A.L., van Oers, M.M., Herniou, E.A., Bojko, J., 2024. Nudiviruses in free-living and parasitic arthropods: evolutionary taxonomy. Trends Parasitol. https://doi.org/10.1016/j.pt.2024.06.009.

Pirozzi, I., Joyce, M., Clay, S., Owens, L., Conrado, D.V., Le, H.H., Nguyen, M.C., Pichette, A., Stright, T., 2016. Boosting redclaw industry productivity with improved nutrition and feed management. RIRDC publication no 13/. Project no PRJ-008536 (ISSN1440-6845).

Powell, S., 2013. Comparative Study of Two Australian *Coxiella* Species: *Coxiella Burnetii* and *Candidatus* Coxiella Cheraxi *Nov.* sp. (TO-98). MSc Microbiology and Immunology, James Cook University.

Rusaini, Ariel E., Burgess, G.W., Owens, L., 2013a. Investigation of an idiopathic lesion in redclaw crayfish *Cherax quadricarinatus* using suppression subtractive hybridization. J. Virol. Microbiol. https://doi.org/10.5171/2013.569032 article ID 569032. 15pp.

Rusaini, Elliman J., La Fauce, K., Bowater, R., Owens, L., 2013b. Endogenous Brevidensovirus-like elements in *Cherax quadricarinatus*: friend or foe? Aquaculture 396-399, 136–145.

Sakuna, K., Elliman, J., Owens, L., 2017. Discovery of a novel *Picornavirales*, Chequa iflavirus, from stressed redclaw crayfish (*Cherax quadricarinatus*) from farms in northern Queensland, Australia. Virus Res. 238, 148–155.

Sakuna, K., Elliman, J., Tzamouzaki, A., Owens, L., 2018a. A novel virus (order Bunyavirales) from stressed redclaw crayfish (*Cherax quadricarinatus*) from farms in northern Australia. Virus Res. 250, 7–12.

Sakuna, K., Elliman, J., Owens, L., 2018b. Therapeutic trials against pre-existing Chequa iflavirus in redclaw crayfish (*Cherax quadricarinatus*). Aquaculture 492, 9–14.

Sciliano, L., Owens, L., 1999. Histopathology of *Cherax quadricarinatus* from Lake Eachum, Atherton Tablelands. Internal report to RIRDC Project No PRJ 000327.

Semple, G.P., 1995. Occurrence of the pathogenic *Thelohania* (Misrosporida: Nosematidae) in the Australian freshwater crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). Proceeding of the Linnaean Society of New South Wales 115, 225–231.

Stevenson, J., Jerry, D., Owens, L., 2012. Redclaw selective breeding project. In: RIRDC Project No PRJ 000327 (ISSN 1440-6845).

Stratton, C.E., Reisinger, L.S., Behringer, D.C., Bojko, J., 2022. Revising the freshwater *Thelohania* to *Astathelohania* gen. Et comb. nov., and description of two new species. Microorganisms 10 (3), 636. https://doi.org/10.3390/microorganisms 10030636.

Stratton, C.E., Reisinger, L.S., Behringer, D.C., Gray, S.N., Larson, E.R., Bojko, J., 2024. North American crayfish harbour diverse members of the *Nudiviridae*. Virology 598, 110183. https://doi.org/10.1016/j.virol.2024.110183.

Tan, C.K., Owens, L., 2000. The infectivity, transmission and 16S rRNA sequencing of a rickettsia, *Coxiella cheraxi sp. nov.* from the freshwater crayfish, *Cherax quadricarinatus*. Dis aquatic Org. 41, 115–122.

Valverde, C., Jones, C., Rigg, D., Elliott, E., Owens, L., Elliman, J.R., 2020. Eliminate factors inhibiting redclaw farming from reaching its full potential. In: Agrifutures Australia Publication No 20–095 (ISBN 978-1-76063-122-5; ISSN1440-685).

Van Patten, K.A., Nunan, L.M., Lightner, D.V., 2004. Seabirds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. Aquaculture 26, 31–46. https://doi.org/10.1016/j.aquaculture.2004.08.012.