Understanding White Spot Syndrome Virus (WSSV) transmission in Moreton Bay

Epidemiological modelling of surveillance data

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We would also like to thank Biosecurity Queensland (BQ) for the provision of data and surveillance methodology. We would like to thank Dr. Stephen Wesche for communicating with us throughout the project.

Abbreviations

Abbreviation	Term
95% CI	95% Confidence Interval
AIMS	The Australian Institute of Marine Science
APFA	Australian Prawn Farmers Association
BoM	Bureau of Meteorology
BQ	Biosecurity Queensland
CCEAD	Consultative Committee on Emergency Animal Disease
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Ct	Cycle Threshold
DAF	Queensland Government Department of Agriculture and Fisheries
DNA	Deoxyribonucleic Acid
GEE	Generalised Estimating Equation
GLM	Generalised Linear Model
IMAS	Institute of Marine and Artic Studies
NSW	New South Wales
OIE	World Organisation of Animal Health

PCR	Polymerase Chain Reaction
Qld	Queensland
UQ	University of Queensland
WSD	White Spot Disease
WSSV	White Spot Syndrome Virus

Executive Summary

Background

White Spot Virus Syndrome (WSSV) is one of the most important disease agents of penaeid prawns in the world. Historically, Australia has been considered free of this disease. In 2016, an outbreak of White Spot Disease (WSD) occurred and spread across several farms along the Logan River, in Queensland, Australia. This caused mass mortality of prawns, mandatory large-scale destocking, and unsustainable economic losses to the local prawn farming industry. Along with the responses to this outbreak, Biosecurity Queensland (BQ) established a surveillance program for WSSV in the Logan River, Brisbane River, and Moreton Bay region over a 4-year period. WSSV was repeatedly found in the Northern Moreton Bay region in 2017, 2018 and 2020.

This project was undertaken to investigate potential mechanisms of spread and transmission of WSSV in the Moreton Bay region and how that may affect zoning and surveillance. There is limited understanding of virus transmission pathways in Australia and whether there is potential for it to spread beyond the current restriction zone. Past surveillance program data and environmental data were analysed to provide further insights on the current dynamics of WSSV in the Moreton Bay region.

Objectives

The objectives for this project were as follows:

- Identify hosts involved in the spread of WSSV, designing surveillance for these
- Describe the most likely distribution of WSSV
- Understand how seasonal factors (i.e., rainfall, temperature changes) impact the spread of WSSV
- Advise whether the boundary of the current zone is likely to change geographically, and if so, establish the likely rate of movement based on current indicators
- Explore other potential risk factors of disease maintenance, transmission and spread.

Methodology

This project is principally a descriptive and risk factor study of WSSV in wild decapods in the Moreton Bay area. The study surveillance area included sites in Moreton Bay, the Logan River and Brisbane River, within the Movement Regulated Area (Figure 1). This study was based on extensive surveillance data provided by BQ and datasets sourced from various government websites to evaluate potential environmental risk factors. The study was limited to the geography and time coverage of the surveillance program conducted by BQ.

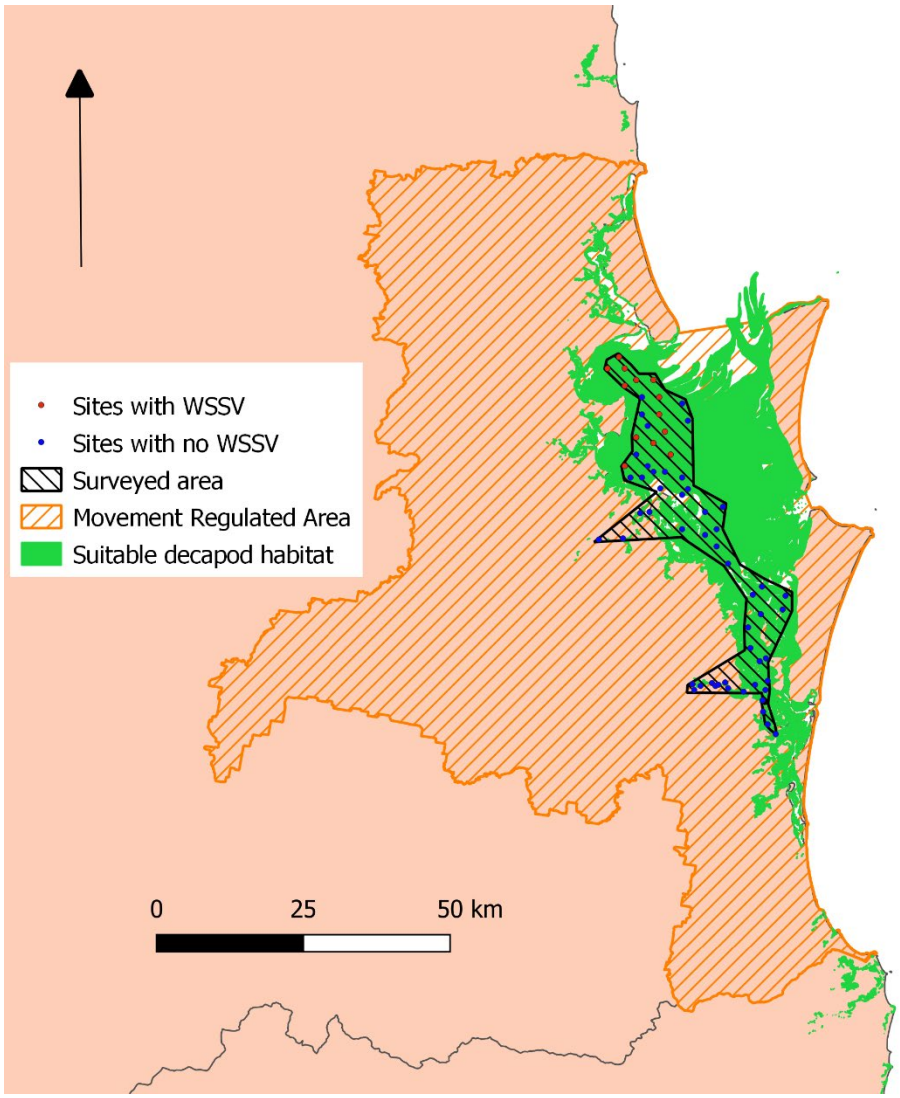

Figure 1 Distribution of presumed decapods suitable habitat (green), current Movement Regulated Area (dashed orange) and study surveillance area (dashed black) with surveillance sites with at least one positive sample (red dot) or without detection (blue dot) for White Spot Syndrome Virus in Moreton Bay region Queensland, Australia (1,2)

A literature review including the history and virology of WSSV, WSD outbreaks in Australia and the biology and ecology of susceptible wild host species was conducted. Peer reviewed literature was searched through Google Scholar and BIOSIS Web of Science. Search terms for WSSV included "White spot virus syndrome" and "White spot virus syndrome + Australia". Each species was used as a search term (e.g., "Greasyback prawns", "Blue swimmer crabs" etc.). Species terms were also searched in grey literature when peer reviewed information was unavailable.

Key topic experts were contacted to gain a better understanding of WSSV, biology of susceptible host species and the outbreak in 2016. Descriptive analysis of BQ surveillance program data and environmental datasets was undertaken. Sea temperature and salinity, undersea habitat, rainfall, and fisheries datasets were compiled and used for regression modelling using generalised estimating equations (GEEs) to account for the longitudinal nature (repeated sampling at the same location) of the detection data.

Results

WSSV was identified in wild crustaceans in the Moreton Bay for several consecutive years. It is possible or perhaps likely that WSSV is currently established and should be considered enzootic in the Moreton Bay region. Positive results were found at sampling sites at the most northern end of the study surveillance area (Figure 1).

Positive samples of WSSV were found in various decapod host species in sites outside of the BQ study surveillance area, but within the Movement Regulated Area, through alternative surveillance activities (3). Therefore, we infer the current geographic distribution of WSSV in Moreton Bay is still unknown, but larger than the BQ study surveillance area in Moreton Bay (3).

We observed that greasyback prawns (*Metapenaeus bennettae)* and mangrove swimming crabs *(Thalamita crenata)* had higher prevalence of disease than other species in the surveillance program we analysed. As well as species, the number of animals per sample was an important factor affecting the result of a sample, larger samples were more likely to detect WSSV.

In terms of environmental factors, season had the most profound impact on the probability of a positive sample. All WSSV detection occurred exclusively in March of every year except in 2019, when no positive samples were detected during surveillance. Second to season, rainfall was significantly associated with detection. An elevation in rainfall increased the odds of a positive sample. A rise in sea temperature increased the odds of a positive sample. As the Moreton Bay region has a subtropical climate; hot, wet summers and cool, dry winters; the correlation between the environmental factors and seasonality increasing positive samples cannot be overlooked. All positive samples were collected from mixed soft substrata, a habitat known to have prominent numbers of prawns during summer months. This habitat, and other suitable habitats including mangroves and sand substrate used by susceptible host species throughout their lifecycles, extend beyond the areas of the study surveillance program and the Movement Regulated Area. This should be taken into consideration for future surveillance.

Genetic analysis of isolates was used to retrospectively analyse the relationship between 2016 outbreak variant and overseas variant. The results were inconclusive as the analysis were unable to determine the source of the Australian variant (4).

Implications

This project provides industry and government with surveillance strategies to improve understanding of the distribution and spread of WSSV in SE Qld. The project also identified risk factors to support the implementation of risk-based surveillance for more successful and efficient detection of WSSV. Enhanced surveillance strategies can provide early warning to prawn farmers in other areas of Australia that could be crucial to prevent the devastating production and economic losses seen in the 2016 outbreaks.

Recommendations

It is recommended that future surveillance is undertaken following these principles:

- Sample in warmer months – particularly March
- Target greasyback prawns and mangrove swimming crabs
- Sample from mixed soft substrata and other suitable habitats (i.e., mangroves, bioturbators, sand substrates)
- Store animals individually for sampling if possible, to reduce cross contamination and allow calculation of prevalence
    - Cross contamination between individual animals that were transported together from each site precluded prevalence calculations and hence accurate recommendations about sample sizes required to detect disease reliably at each site. However, based on the sampling methods used, taking large sample sizes are required (a minimum of 20 up to 186 animals). If large samples cannot be taken, the sensitivity of surveillance at a site is reduced and some positive sites may be missed
- Sample after high average rainfall periods (at least 14 days up to 60 days after rainfall event), if possible
- Expand the surveyed area considerably past the last positive site to ensure that there is a chance of detecting the edge of the infected area.

Surveillance zones should be established to allow for a buffer zone between infected and free areas. Although no official buffer zone was established by BQ, targeted surveillance north of the Movement

Regulated Area was conducted concurrently with the surveillance program and has been continued in the last few years (S. Wesche 2022, personal communication, 28 April). Surveillance should continue to be focused on the buffer zone to allow for early detection of potential spread of WSSV out of the infected zone. High resolution molecular epidemiology should be considered to trace transmission pathways or major changes, including new introductions of WSSV. Simulation modelling may be indicated if a deeper understanding of where WSSV may have spread is desired. This modelling would combine tides, water currents, particle transmission, larvae and adult behaviour, time and epidemiology to indicate where disease may have transmitted but would be limited by biological understanding of the system (for example decapod interactions and ecology).

Keywords

White Spot Syndrome Virus (WSSV)
White Spot Disease (WSD)
Biosecurity Queensland (BQ)
Moreton Bay
Greasyback prawns
Mangrove swimming crabs
Surveillance program
Outbreak

Introduction

Background

In 2016, an incursion of White Spot Syndrome Virus (WSSV) caused an outbreak of White Spot Disease (WSD) across all active prawn farms along the Logan River in southeast Queensland. This was the second incursion of WSSV in Australia after the 2000 incursion in the Northern Territory. The outbreak resulted in substantial economic losses, due to disease and depopulation, and prawn farming ceased in this area for several years. WSSV was hypothesised to be introduced into the river by the use of infected imported prawns as bait or burley by recreational fishers (5). This outbreak prompted Biosecurity Queensland (BQ), part of the Queensland Government Department of Agriculture and Fisheries, to implement a surveillance program for WSSV in the Moreton Bay area. This program, as well as a vector surveillance study undertaken by Dr. Ben Diggles, found WSSV in a wide variety of species in the northern Moreton Bay region (3). This report aims to understand potential mechanisms of transmission and spread of WSSV in the Moreton Bay region using a literature review and epidemiological modelling of the extensive BQ surveillance data.

White Spot Syndrome Virus (WSSV)

WSSV is a viral pathogen affecting a wide range of decapods around the world. It was first discovered in China in 1992 and rapidly spread to all major prawn-farming countries (6). The spread of WSSV between countries can be facilitated by the trade of uncooked live or frozen prawns and the trade of broodstock (7). WSSV is the causative agent of WSD in penaeid prawns, a disease which may cause mass mortality events in farmed prawns. Infected animals show characteristic white spots on the exoskeleton, as well as lethargy, reduction in food intake, reddish to pink body discolouration and loose cuticles (6). The main mechanisms of spread of WSSV include horizontal transmission through the ingestion of dead infected prawns, and contact with free virus particles in water (6). It is theorized that vertical transmission may occur when the virus is passed to offspring via oocytes; however, virus particles have not been reported in mature eggs (6,7). Environmental factors including sudden changes in salinity, temperature and pH are known stressors in farmed prawns and have been shown to influence outbreaks and transmission by increasing disease expression (8).

WSSV affects crab species differently to prawns. In experimental studies, crabs survived up to 45 days with no gross signs of disease or mortality, and rapidly transferred infection to prawns (9). Crabs may act as a reservoir for WSSV in wild populations (3). The role of seabirds in the transmission of WSSV is largely unknown. A study found that WSSV DNA was detected by PCR in faeces of seagulls for up to three days after consuming infected prawns. Virus was found to be non-infectious via bioassay and infection was not observed when prawns were fed extracts from the infected faeces. Therefore, transmission via faecal matter from birds is unlikely, however, the possibility of birds as mechanical vectors cannot be dismissed (10). There was no data on wild bird movements associated with the BQ surveillance data.

Outbreaks in Australia

Australia has experienced two incursions of WSSV. The first occurred in Darwin, in the Northern Territory, in 2000 when imported prawns with WSSV were fed to crabs and fish at an aquaculture centre. Prawns and crabs captured near the outfall pipe tested positive to WSSV. Approximately a month later, crabs found near the outfall pipe and around Darwin Harbour all tested negative for WSSV (11). Genomic sequencing of virus from this incident closely aligned with strains from Indonesia, confirming the prawns were imported (4). Approximately six months from the incursion, the region was declared free of WSSV. As a result of this incursion, a national survey was conducted in 2001 by the Consultative Committee on Emergency Animal Disease (CCEAD) (11). No positive samples were found in over 3000 samples from 64 locations of both wild and farmed crustaceans tested via polymerase chain reaction (PCR) (11). In May 2002, Australia was declared free from WSD.

In November 2016, a prawn farmer on the Logan River noticed a small amount of deaths in one of his ponds (5). BQ confirmed the presence of WSD through genetic testing of *Peneaus monodon* (black/giant tiger prawns) sampled from this farm. The outbreak affected seven prawn farms on the Logan River between November 2016 and February 2017. Economic losses were estimated at more than $35 million AUD (5,12,13). An investigation by Dr Ben Diggles of DigsFish Services concluded that the spread of WSD between farms was associated with the intake of water from the Logan River or Moreton Bay (5).

During the outbreak, WSSV positive *P. monodon*, *Metapaenus sp* and *Acetes sibogae australis* (Jelly prawns) were sampled from the Logan River in front of farms. Dr Ben Diggles hypothesised that *P. monodon* were likely escapees from farms, as natural stocks are rare in Moreton Bay, however, without genetic testing, distinguishing between wild and farmed prawns was not possible (5). Compliance activities conducted by Federal government compliance officers in 2016 found several groups of recreational fishers using WSSV positive green prawns, purchased from supermarkets and meant for human consumption, as bait in the Logan River (5).

A study occurred examining the distribution of WSSV genotypes and included samples from the Logan River farms and Moreton Bay during the outbreak in 2016. Two distinct, but closely related, clusters were identified. For both clusters, there were no clear linkages to other geographical regions; strains from 2016 were unrelated to the Darwin incursion in 2000 (4). Due to the paucity of international WSSV sequences, the source of the 2016 Queensland WSSV incursion/s was not able to be determined. The use of genomics in the surveillance of WSSV is important and further research in this area is required to use this approach effectively.

Surveillance of WSSV

Targeted surveillance along the Queensland coast from Brisbane to Cairns began shortly after detection of WSSV in 2016, samples were collected yearly along the east coast from 2017 to 2020; no positive samples were found beyond the northern Moreton Bay region (14). In January 2017, a surveillance program was implemented by BQ to monitor compliance with requirements for prohibited or restricted matter, confirm presence or absence of WSSV and monitor levels of WSSV in the Moreton Bay region (13). The study surveillance area included sites within Moreton Bay, the Logan River and Brisbane River, within the Movement Regulated Area (Figure 1). The surveillance program, in the study surveillance area, ended in March 2020. Surveillance was conducted just north of the Movement Regulated Area to the east of Bribie Island concurrently with the surveillance program and was continued further north in the following years (S. Wesche 2022, personal communication, 28 April). Published and unpublished data show no positive samples have been detected at the time of writing this report. Data from these surveillance activities was not available for this analysis.

In March 2020, WSD outbreaks occurred in the two remaining prawn farms on the Logan River (3). In April and May 2020, Dr Ben Diggles conducted surveillance of potential wild decapod hosts of WSSV, targeting non-commercial species and zooplankton (3). Samples testing positive for WSSV were obtained from decapods, zooplankton and amphipods in the Caboolture River, Beachmere and Logan River (Figure 2). Samples taken from Bribie Island were negative, however only zooplankton and jelly prawns were taken from this site (3).

Figure 2 **Map of Movement Regulated Area within White Spot Biosecurity Area 1. Red circles show surveillance sites sampled by Dr. Ben Diggles surveillance (Adapted from Queensland Department of Agriculture and fisheries (1,3))**

Moreton Bay

The Moreton Bay region covers more than 3,400km^2 and hosts an array of habitats, plants and animals (15). It is home to whales, dugongs, shorebirds and turtles, and the mangroves and seagrass provide habitat and nursery to many decapods (15). This region is also a popular commercial and recreational fishing area. A recent survey conducted by the Queensland Government Department of Agriculture and Fisheries (DAF) estimated around 400,000 people participated in recreational fishing in the Moreton Bay/Brisbane area (16).

Genetic studies and repeated positive samples support the hypothesis that WSSV is now enzootic in the Moreton Bay region (4). A Movement Regulated Area was established in 2017 to prevent uncooked crustacean being moved from this zone (17). No WSSV has been detected outside the Moreton Bay area despite passive surveillance of prawn farms since the outbreaks, active surveillance along the east coast of Australia between 2017 and 2019 (14). Sampling along the east coast occurred from March to June during these years. A wider variety of species were caught due to variations in sampling methodology

and changing habitat and population structure along the east coast. Sampling was fisheries dependent, relying on commercial fishing vessels for sampling along the east coast. Therefore, sampling technique, time of sampling, number of samples and species were highly varied between sampling sites (14).

To establish future surveillance and risk management measures for WSSV in Australia, it is important to understand factors that could lead to spread and transmission in wild populations. These include, identifying potential host species and their movement and migration patterns, the geographic distribution of potential host species and viable habitats and environmental effects such as rainfall, sea temperature and salinity. This report will investigate these factors through a literature review and epidemiological modelling of surveillance data. In addition, it is important to assess whether the current structure of surveillance is adequate or may need tweaking.

Objectives

The objectives/hypotheses are as follows, with notes covering any changes from the original project agreement:

1. **Identify hosts involved in the spread of WSSV, designing surveillance for these**
    a. the terms ‘vector’ and ‘sentinel’ have been replaced by the term ‘host’, which more accurately describes the epidemiological role of wild decapods in the transmission and maintenance of WSSV.

2. **Describe the most likely distribution of WSSV**
    a. The original objective was to describe the rate at which WSSV is spreading by defining the current zone. It was not feasible to estimate the *rate* at which WSSV spread, because the spatial and temporal coverage of available data are limited, and the project focused on analysing available data (rather than modelling spatio-temporal spread as originally intended but revised through FRDC recommendations). It was also not possible to ‘define the current zone’ [of WSSV in wild decapods], given that positive samples have been reported at the edge of the current surveillance zone, but there were no longitudinal data available to us outside the defined surveillance zone.

3. **Understand how seasonal factors (i.e., rainfall, temperature changes) impact the spread of WSSV**

4. **Advise whether the boundary of the current zone is likely to change geographically, and if so, establish the likely rate of movement based on current indicators**

5. **Explore other potential risk factors of disease maintenance, transmission and spread**
    a. addition of the term ‘maintenance’, to reflect the importance of possible reservoir of WSSV in wild decapod population.

Method

This project is principally a descriptive and risk factor study of WSSV in wild decapods in the Moreton Bay area. This study was based on surveillance data provided by BQ and datasets sourced to evaluate potential environmental risk factors. The study was geographically and temporally limited by the surveillance program conducted by BQ (Figure 9).

Information synopsis and refinement of hypotheses

A literature review including the history and virology of WSSV, WSD outbreaks in Australia and the biology and ecology of susceptible host species was conducted. Peer-reviewed literature was searched through Google Scholar and the BIOSIS Web of Science. Search terms for WSSV included "White spot virus syndrome" and "White spot virus syndrome + Australia". Each species was used as a search term (e.g. "Greasyback prawns", "Blue swimmer crabs" etc.). Species terms were also searched in grey literature when peer reviewed information was unavailable. Government websites including BQ, DAF, Parliament of Australia and New South Wales Department of Primary Industries were used to search "White spot virus syndrome", fisheries data and species terms.

Key experts were contacted to gain a better understanding of WSSV, biology of susceptible host species and the outbreak in 2016. Dr Ben Diggles (DigsFish Services) provided details about his surveillance report and likely mechanisms of transmission of WSSV. Dr. Stephen Wesche from BQ provided detail about the surveillance program as well as some information about biology and habitat in the Moreton Bay region. Monthly meetings were conducted with representatives from the APFA and involved discussions about the hypotheses.

Minor refinements were made to the project objectives in consultation with APFA representatives (see Objectives section).

Data management and integration

Datasets sourced for the project are described below.

Biosecurity Queensland Surveillance Data

- This dataset was a result of a Surveillance Program by the Queensland Government Department of Agriculture and Fisheries, it was provided by BQ
- The program started in February 2017 and concluded in March 2020
- The surveillance sites in Moreton Bay extended from Deception Bay in the north, to the Gold Coast Broadwater in the south and included Brisbane River and Logan River.
- Sixty-four sampling sites were initially selected at random; a small number of additional sites were added to ensure coverage across the area (Appendix 3).
- A minimum depth of 5 meters was used to exclude areas inaccessible for the research vessel to trawl (Appendix 3).
- Samples were taken in summer (February – March) and spring (August – November) to allow a six-month period between samplings
- Initially, samples up to 150 animals of each species were separated into sampling bags at each site, excess animals were discarded. Due to new information on diagnostic sensitivity of the test used, sample size was revised to be up to 186 animals for each species by the end of 2020.
- The following species were targeted because they were known to be susceptible to WSSV and considered abundant across the sampling area:
    - Greasyback prawn *Metapenaeus bennettae*
    - Banana prawn - *Fenneropenaeus merguiensis*
    - Blue swimmer crab - *Portunus armatus*

    - Mangrove swimming crab - *Thalamita crenata*
    - Brown tiger prawn - *Penaeus esculentus* (Appendix 3)
- Individual animals were tested via PCR at an Australian laboratory by in house methods and as per the World Organisation of Animal Health (OIE) standards (18)
- All animals in a sample were tested, however due to potential contamination (i.e., pooling of animals, trawl, and sorting methods on boat), prevalence could not be assessed. Results were reported per sample at each trawl site. Each trawl was divided by species and up to 186 animals from each species retained in a single bag.
- Results were categorised based on cycle threshold (Ct) value and a sample (a bag of up to 186 animals) was deemed positive if one or more animals tested positive. PCR results were:
    - Positive: Ct value ≤ 36
    - Suspect: $36 >$ Ct value ≤ 45
    - Negative: Ct value > 45
- If any sample at a site tested positive, the site was deemed "WSSV positive" for that period

Bureau of Meteorology (BoM) rainfall data

- Daily rainfall values (mm) were sourced from publicly available weather station (BoM)
- Longitude and latitude of each trawling site was used to identify the nearest weather station
- Daily rainfall (mm) and leading average daily rainfall (mm) values were recorded against each trawl
- Leading average daily rainfall values were calculated by averaging daily rainfall over a 60-, 30- and 14-day period prior to trawling date
- Some rainfall observations were missing or excluded. This occurred when the recorder was unavailable for manual observations, failure in equipment or when suspect data was produced (19)

eReefs Data Extraction tool – sea surface salinity and temperature

- Data were sourced from the eReefs Data Extraction tool, a collaboration between the Great Barrier Reef Foundation, BoM, Commonwealth Scientific and Industrial Research Organisation (CSIRO), the Australian Institute for Marine Science (AIMS) and Queensland Government (20).
- The longitude and latitude of each surveillance site were provided via the portal for matching with salinity (parts-per-thousand, ‰) and temperature (degrees Celsius) data at different sea depths. Data was only available at surveillance sites at a depth of 0.5m
- Leading average measurements were calculated by averaging daily salinity and temperature values over a 60, 30 and 14 days prior to trawling date

Institute of Marine and Artic Studies (IMAS) – Marine habitat

- The Seamap Australia dataset from IMAS was spatially joined with longitude and latitude of sites provided by Dr Emma Flukes (2)
- The Seamap Australia contains validated habitat data as of 2017
- It derives both biotic and substratum classifications to create a layer
- Some trawling sites felt outside the classified habitat area; therefore, the nearest habitat classification was used

QFish commercial catch data

- The QFish portal is maintained by the Queensland Government Department of Agriculture and Fisheries (21)
- Data on fishing catch and effort is collected through commercial logbook programs
- Catch information from all forms of commercial fishing for species captured in the surveillance program was extracted for logbook zones W37 and W38 which aligns most closely with the surveillance area (Figure 2)

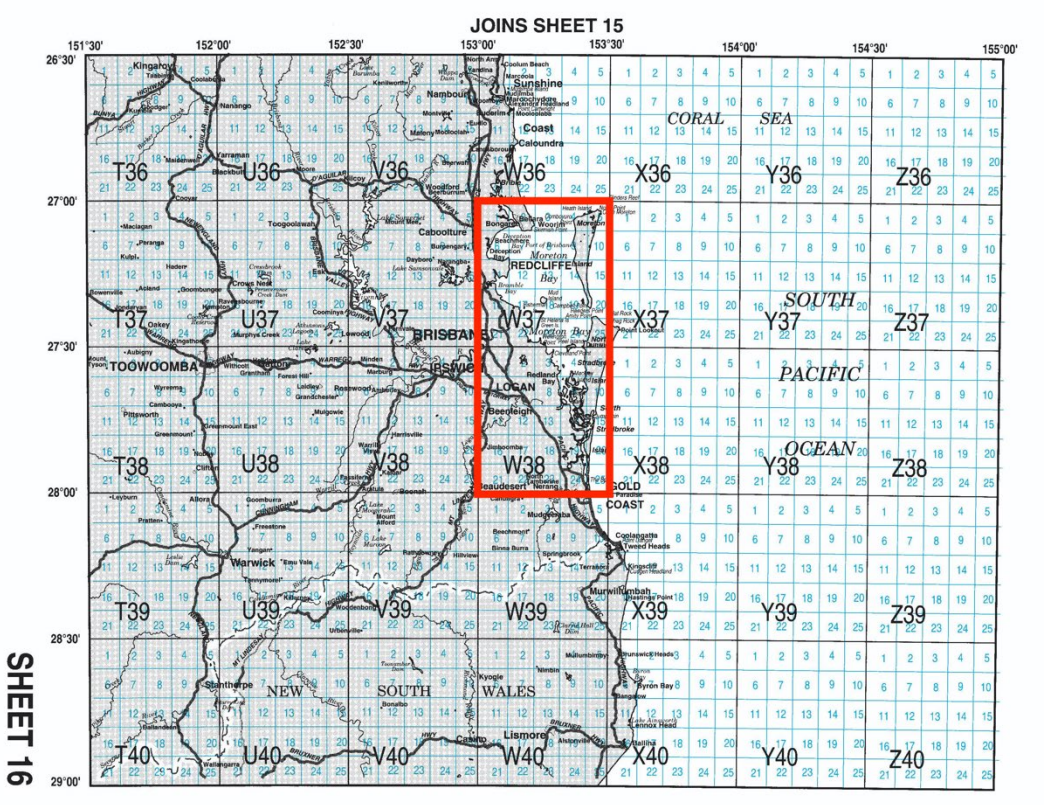

Figure 3 Logbook map displaying grids for commercial fishing. Data used from grids outline in red (22).

- Species included were:
  - All tiger prawns
  - Giant/black tiger prawns *(Penaeus monodon)*
  - Blue swimmer crabs *(Portunus armatus)*
  - Banana prawns *(Fenneropenaeus merguienis)*
  - Greasyback prawns *(Metapenaeus bennettae)*
  - Mud crabs *(Scylla serrata)*

White spot disease Movement Regulated Area map

- The map was sourced from the Queensland Government department of Agriculture and Fisheries as shape files (1)
- This map was used to evaluate the geographical location of current WSSV in relation to the restriction zone

All datasets were integrated in a secure PostgreSQL database and made ready for statistical analysis using RStudio version 1.4.1717 and R version 4.1.0.

Data analysis

The primary outcome of interest throughout the analysis is a WSSV positive sample. A sample is a bag of up to 186 animals of the same species. A sample is deemed positive if one or more individual animals was PCR positive for WSSV. Furthermore, a site was deemed positive if one or more samples were positive for WSSV.

Multivariable logistic regression was used to analyse the relationship between the outcome of sampling and variables including sea surface temperature, salinity, rainfall and captured decapod species. Habitat and seasonality could not be included in the models as they are perfect predictors of the outcome (e.g., it did not need modelling to demonstrate that all positive samples were collected in March). Generalised

estimating equations (GEEs) were used alongside logistic regression to account for the longitudinal nature of the data. This is required as repeated samples are made at each site and there may be dependence between collected observations. Correlation may cause underestimation of variances of association estimates with between-period covariates (e.g., seasons) and overestimation of variance of within-period covariate effects.

Results

Decapod Biology

In theory, WSSV can infect all decapod species. Many prawn species have similar lifecycle and migration patterns. In general, prawns will remain in coastal nurseries (e.g. mangrove areas) for approximately 6 months before moving to oceanic waters (23). They settle in mud, sand, or silt substrates. This outwards migration, or 'recruitment' occurs in summer and autumn; commercial fishing yields the highest catch during these periods (24). Greasyback prawns and brown tiger prawns live mostly within estuarine habitats and have limited migration movements (25,26). The banana prawn and giant tiger prawns can be found in deeper waters (down to 160m in depth) (27,28). *Acetes sp.,* more commonly known as Jelly prawns, are found throughout estuarine and inshore waters (29). Studies have shown that greasyback prawns are very abundant in the Moreton Bay area (28). Greasybacks are distributed from eastern Victoria to Cooktown in northern Queensland and banana prawns are found in all inshore areas on the Queensland coast (30). Both giant tiger prawns and brown tiger prawns are distributed from New South Wales central coast, around the north coast of Australia to Shark Bay in Western Australia (26,31).

Crab species are found in intertidal coastal waters, mainly near mangroves or enclosed habitats. Adult crabs have distinct seasonal movements. Female crabs move into offshore marine waters for oceanic spawning. Larvae disperse and can travel large distances; this helps genetic recruitment from distant populations (32). Only small numbers of individuals will migrate per generation which maintains homogeneity of population genetics. Mud crabs are mostly found in estuarine and sheltered environments, with females migrating offshore for spawning (33). Mangrove swimming crabs are typically found in shallow areas with soft substrates and caught frequently by commercial trawlers (34). Like the mangrove swimming crab, the blue swimming crab is found in sandy and muddy substrata down to 50m in depth (35).

Biosecurity Queensland surveillance data

A total of 1,138 samples were collected over 64 different sites from Moreton Bay, the Logan River and Brisbane River (Figure 1). This resulted in a total animal capture count of 27,614. Four sites were not sampled after the first collection period due to capture yield. The surveillance period occurred between 23 February 2017 and 10 March 2020. Samples were taken over two distinct periods of the year, in February and March (summer months) and August to November (spring months). Approximately 50% more samples were collected in summer compared to spring, with a median of 176 samples in the summer and 116 in spring (Table 1). As most samples were collected during summer, over three quarters of animals were captured during this season.

Table 1 Seasonal summary of sampling of White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20

Season	Number of samples per season	Mean number of samples per season	Median number of samples per season	Range sample count per season	Total animals tested per season	Mean number of animals tested per sample	Median number of animals tested per sample
Summer	783	196	176	167 – 263	24,675	27.7	12
Spring	355	118	116	102 – 137	5,939	16.7	5
Total	1,138						

Fifty-one samples were classified as positive, four as suspect and 1,083 as negative (Table 2). The median number of animals per positive sample was on average six times greater per negative sample.

Table 2 Overall summary of sampling and PCR results of White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20

PCR Results	Site Count	Number of samples	Total number of captured animals	Mean animals per sample	Median animals per sample	Range animal count per sample
Positive		51	2,804	55	54	2 – 125
Suspect		4	90	22.5	17.5	10 – 45
Negative		1,083	24,720	22.8	8	1 – 378
Total	64	1,138	27,614	24.3	9	1 – 378

Positive samples occurred exclusively in March every year except 2019 (Figure 3). No positive samples were collected in 2019 (Figure 4). In total, approximately, 68% (738/1,083) of negative samples had less than 20 animals per sample and approximately 78% (40/51) of positive samples had more than 20 animals per sample (Table 3).

Table 3 **Overall summary of sampling and PCR results of White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20**

PCR Results	Total number of samples by captured animal count								
Summer	**< 10**	**10-20**	**20-30**	**30-40**	**40-60**	**60-80**	**80-100**	**>100**	**Total**
Positive	5	6	2	4	11	12	3	8	51
Negative	334	124	69	46	61	32	23	39	728
Spring									
Negative	241	39	17	14	20	7	5	12	355

Five prawn species including greasyback prawns, banana prawns, giant tiger prawns, brown tiger prawns, and glass shrimp and three crab species including the mangrove swimming crab, mud crab and blue swimmer crab were captured (Table 4). Three species, the giant tiger prawn, glass shrimp and mud crab had no positive samples. These species were less likely captured, as well as having a low number of animals captured per sample. All other species captured had >100 samples and >1,000 individual animals caught. Greasyback prawns had the most samples (n=313) and largest number of animals caught (12,675). Species captured with >100 samples were caught at every surveillance month (Figure 5).

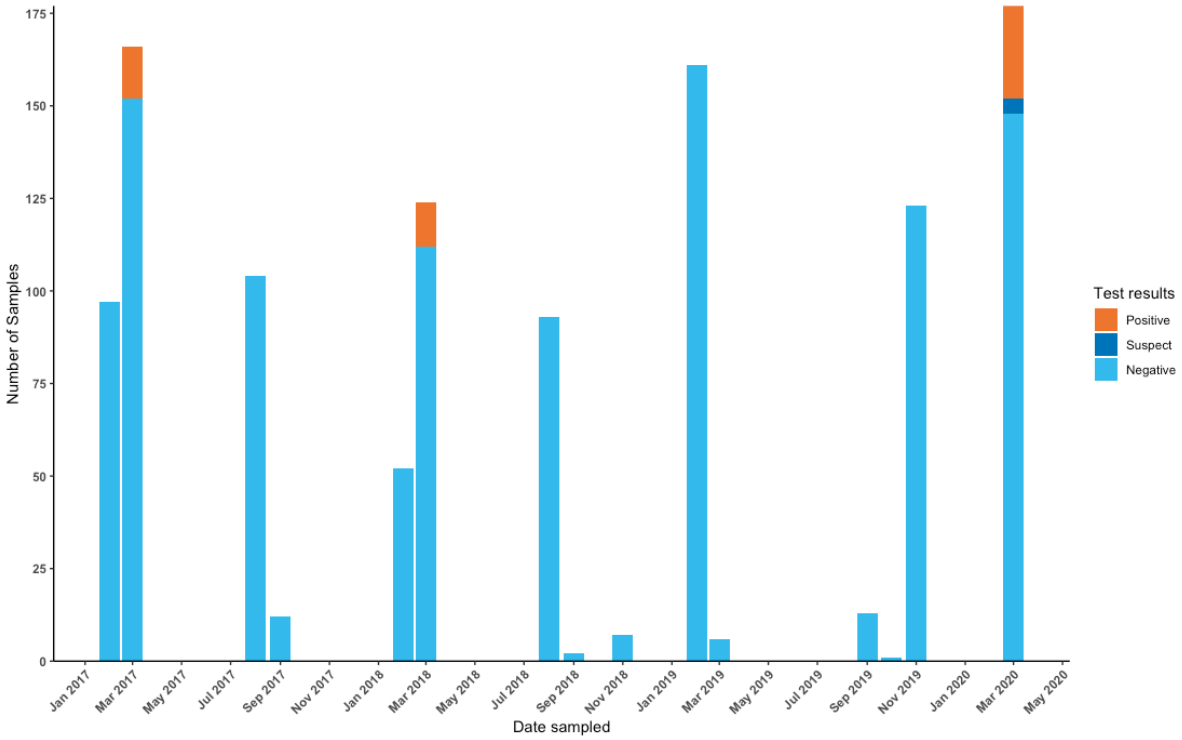

Figure 4 **Temporal distribution of sampling and PCR results of White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20**

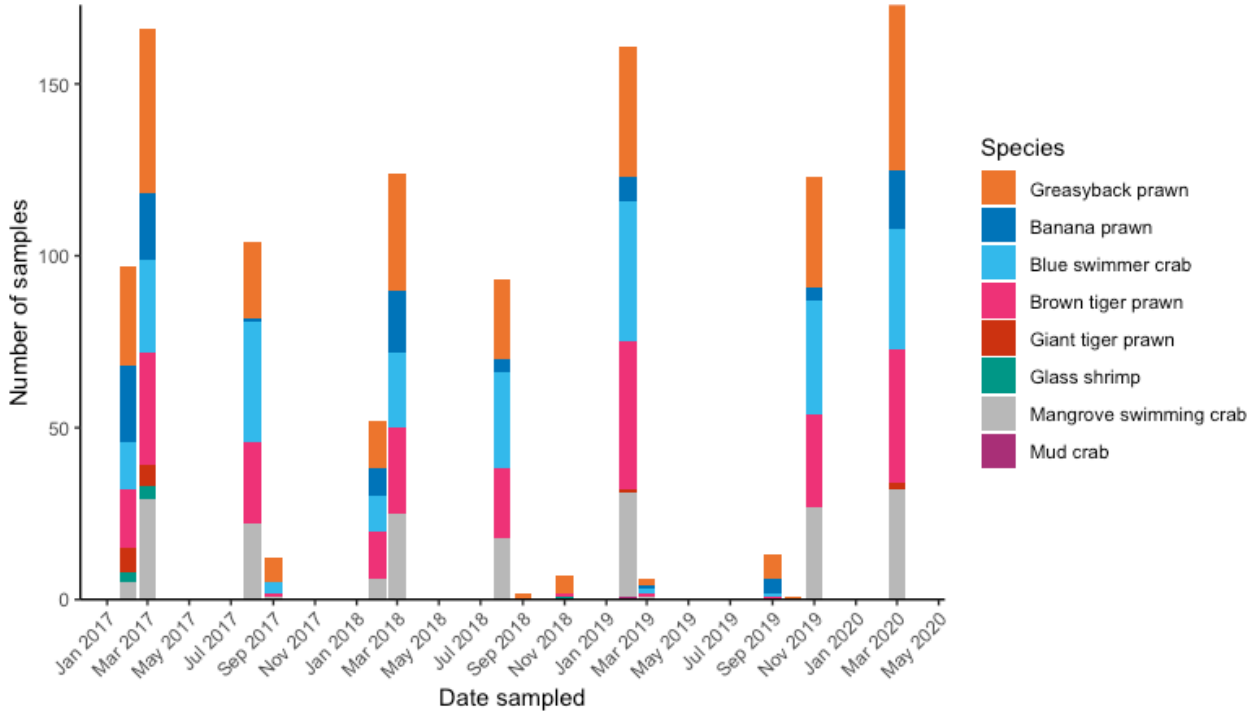

Figure 5 Temporal distribution of sampling and host species captured occurrences during White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20

WSSV was not detected in three species – giant tiger prawn, glass shrimp and the mud crab (Table 4). Greasyback prawn, mangrove swimming crab and brown tiger prawn samples were approximately 2.6 to 3.7 times more likely to have a positive result when compared to blue swimmer crab samples. The 95% confidence interval of the odds ratio for banana prawns span 1.0, providing weak evidence (not significant) of the distinct detection of WSSV relative to blue swimmer crab samples (Table 4).

Table 4 Taxonomic summary and Odds ratios of sampling for White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity during 2017-20

Species	Total sample count	Total individual animals captured	Prevalence of positive trawls	Odds ratio* (95% CI)*
Blue swimmer crab *(Portunus armatus)*	250	3,433	2.0%	Reference species
Greasyback prawn *(Metapenaeus bennettae)*	313	12,675	5.1%	2.65 (1.02 – 8.19)
Mangrove swimming crab *(Thalamita crenata)*	197	3,677	7.1%	3.77 (1.41 – 11.83)
Brown tiger prawn *(Penaeus esculentus)*	248	6,460	5.6%	2.96 (1.11 – 9.87)
Giant/black tiger prawn (*Penaeus monodon)*	16	32	0%	NA
Banana prawn *(Fenneropenaeus merguienis)*	105	1,360	1.9%	0.95 (0.13 – 4.49)
Glass shrimp *(Acetes sp.)*	8	66	0%	NA
Mud crab *(Scylla serrata)*	1	1	0%	NA

**An odds ratio compares the association between an exposure and an outcome. A value greater than 1 indicates a positive association with the outcome, a value less than 1 indicates a negative association with the outcome. For example, compared to the blue swimmer crab, the greasyback prawn is 2.65 times more likely to be positive for WSSV. The banana prawn has an odds ratio less than one, which means the banana prawn is less likely to have positive samples compared to the blue swimmer crab.*

**A 95% confidence interval (95% CI) is used with odds ratios to estimate its precision. If the interval spans 1, there is less confidence that there is a true association between the exposure and the outcome. If a confidence interval is very wide, the odds ratio is less precise, and must be interpreted with caution. For the greasyback prawn, the 95% CI is 1.02 – 8.19, meaning there is evidence that there is a positive association between a positive sample and greasyback prawns. In contrast, the banana prawn 95% CI however crosses 1, therefore the evidence for an association between banana prawns and a positive sample is lacking.*

Samples caught in southern and northern Moreton Bay (TM501-TM549) had an even distribution of captured species (of those species that had over 100 samples in the surveillance period). Species distribution in the Logan River (LR/AL) and Brisbane River (BR) samples was less balanced with most sites having a large proportion of greasyback prawns and an uneven distribution of other species (Figure 6).

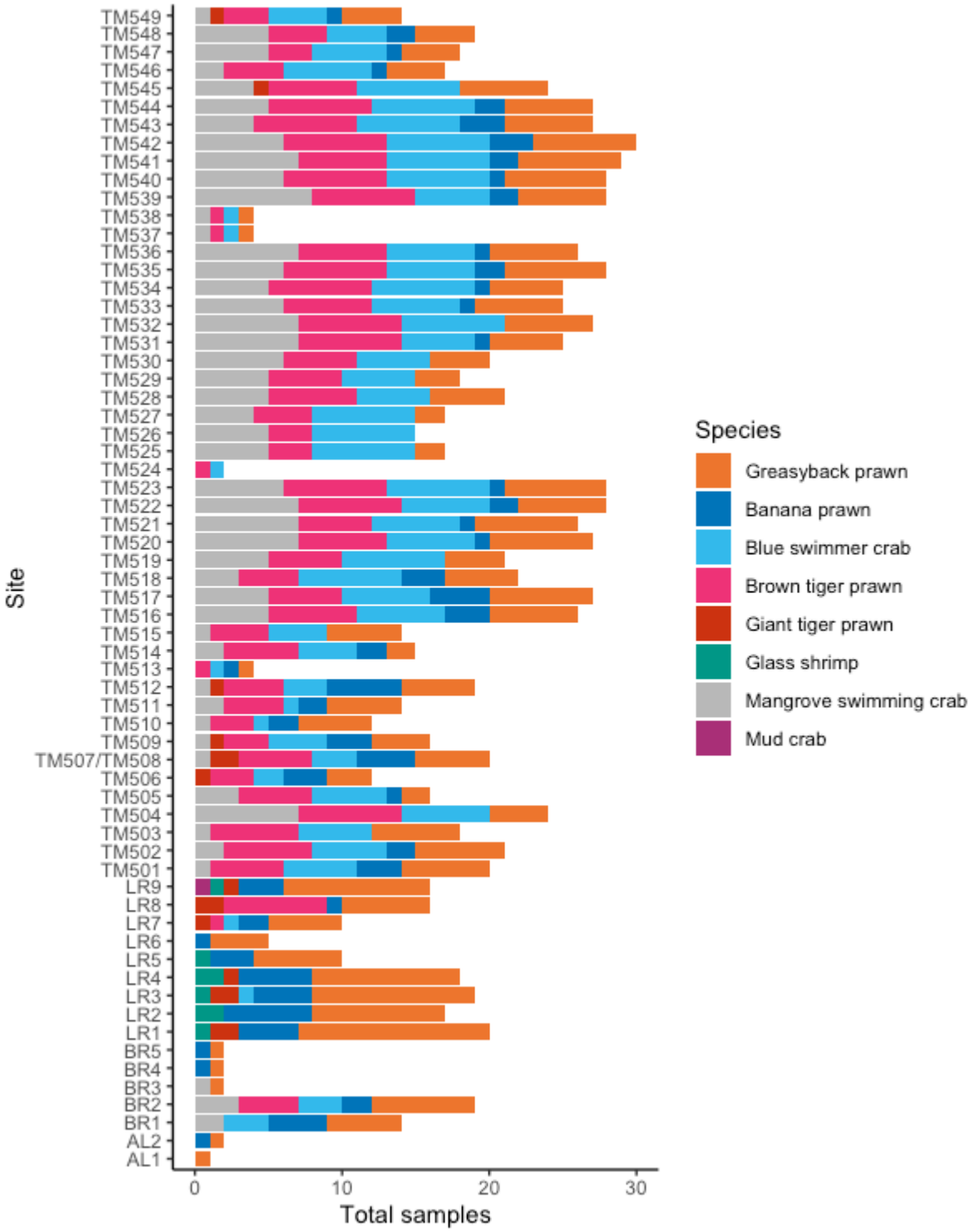

Figure 6 Spatial distribution of sampling and host species captured occurrences by site during White Spot Syndrome Virus surveillance conducted across the Moreton Bay by Biosecurity Queensland during 2017-20

Thirteen of the 64 surveyed sites (20.3%) were positive. All positive sites were located in northern Moreton Bay (Figure 7). The number of positive sites increased from 2017 to 2020, with five close located sites testing positive in 2017, eight sites in 2018 and eleven sites in 2020 (Figure 7).

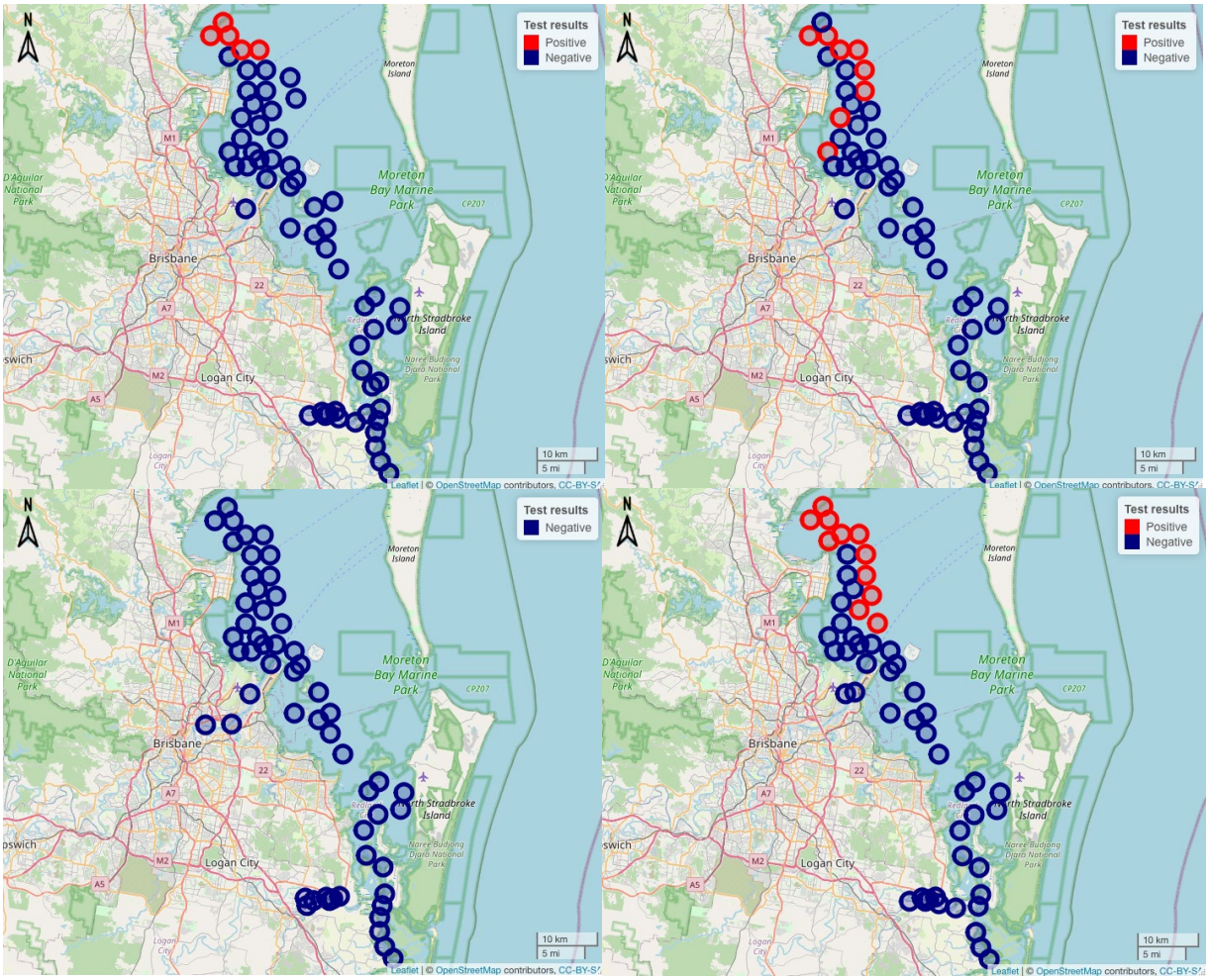

Figure 7 **Yearly distribution of sites with a positive White Spot Syndrome Virus detection during WSSV surveillance conducted across the Moreton Bay by Biosecurity Queensland (top left 2017, top right 2018, bottom left 2019, bottom right 2020).**

Environmental data including salinity, temperature, and rainfall

Southeast Queensland has a sub-tropical climate, with increased rainfall, temperatures and humidity in summer months and dry, mild winters (36). This is reflected in monthly rainfall data from the Brisbane Aero weather station. This weather station is located approximately in the middle of the surveillance area, near the Brisbane Airport. In 2019, cumulative rainfall in summer months (214.8mm) was approximately half of 2018 (396.8mm) (Table 5). Australia wide, 2019 was the driest year on record since 1902, with the national cumulative annual rainfall 40% below the 1961-90 annual average (37).

Table 5 **Cumulative rainfall (mm) for summer surveyed months (February & March) and spring surveyed months (August – November) from the Brisbane Aero weather station, Queensland, 2017-20.**

Year	February-March	August-November
2017	325.2	235.8
2018	396.8	206.0
2019	214.8	123.4
2020	583.0	-

Sea surface temperature across the surveyed sites fluctuated with the seasons, with summer months approximately five degrees warmer than spring (Figure 8). Significant decreases in salinity were seen in March 2017 and March 2020. Precipitation has been shown to decrease sea surface salinity, and overtime, salinity dilution can reach deeper ocean depths (38). Increased rainfall occurred in the days prior to a decrease in average sea surface salinity (Figure 7).

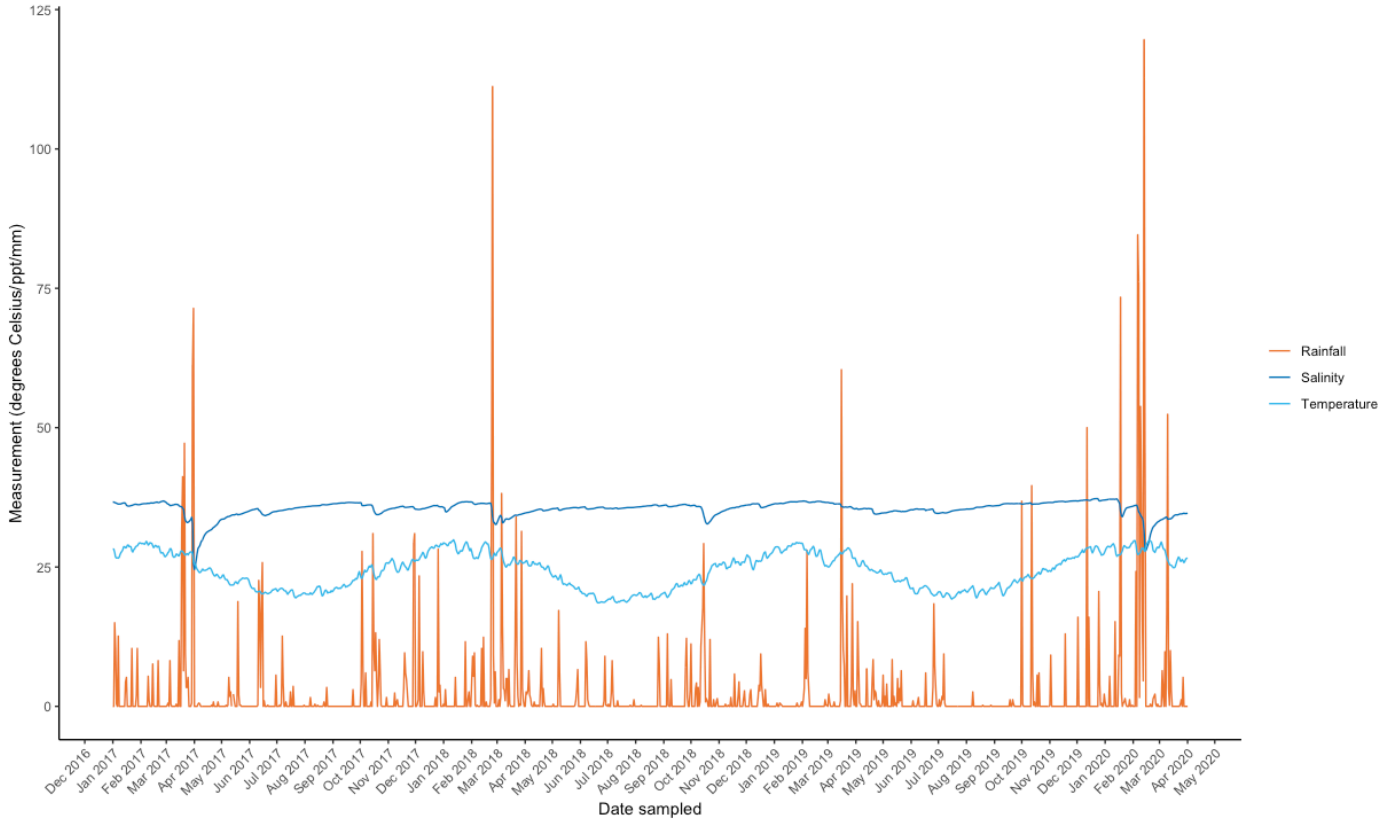

Figure 8 **Sea surface salinity (ppt) and temperature (degree Celsius) averaged across all White Spot Syndrome Virus surveillance sites with total rainfall (millimetres) recorded at the Brisbane Aero weather station, across the Moreton Bay sites surveyed by Biosecurity Queensland during 2017-20**

Habitat

Most samples were collected from mixed soft substrata, with all positive samples collected from this habitat (Figure 9). Substratum is defined based on composition and size of seabed materials (39). A mixed soft substrata is composed of a mixture of coarse and fine sediment, neither component exceeding 80% (40). The distinction between hard and soft substratum is important as hard substrata can support reef ecosystems while soft cannot (40).

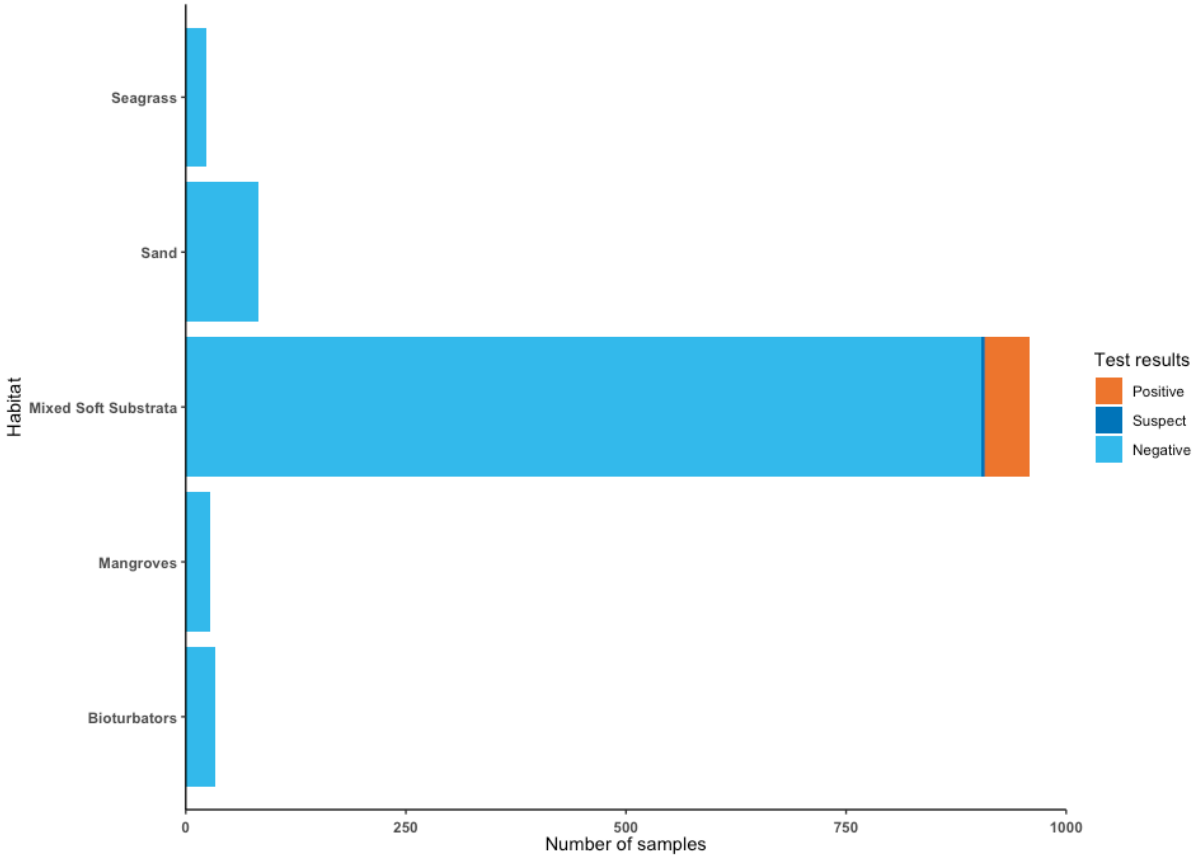

Figure 9 Number of samples per habitat, by PCR test result between 2017-20

Suitable decapod habitats, such as soft substrata and mangroves, extend beyond the boundary of the current Movement Regulated Area (Figure 10). Susceptible species, such as those in the surveillance program, have a wide distribution along the eastern coast of Australia. This surveillance program was designed to recover Australia's freedom from disease status, therefore, all sampling occurred within the current Movement Regulated Area and all positive samples were at the northern most surveyed sites (Figure 10). Other surveillance activities also occurred within the Movement Regulated Area and were conducted to establish the distribution of WSSV within Moreton Bay (3).

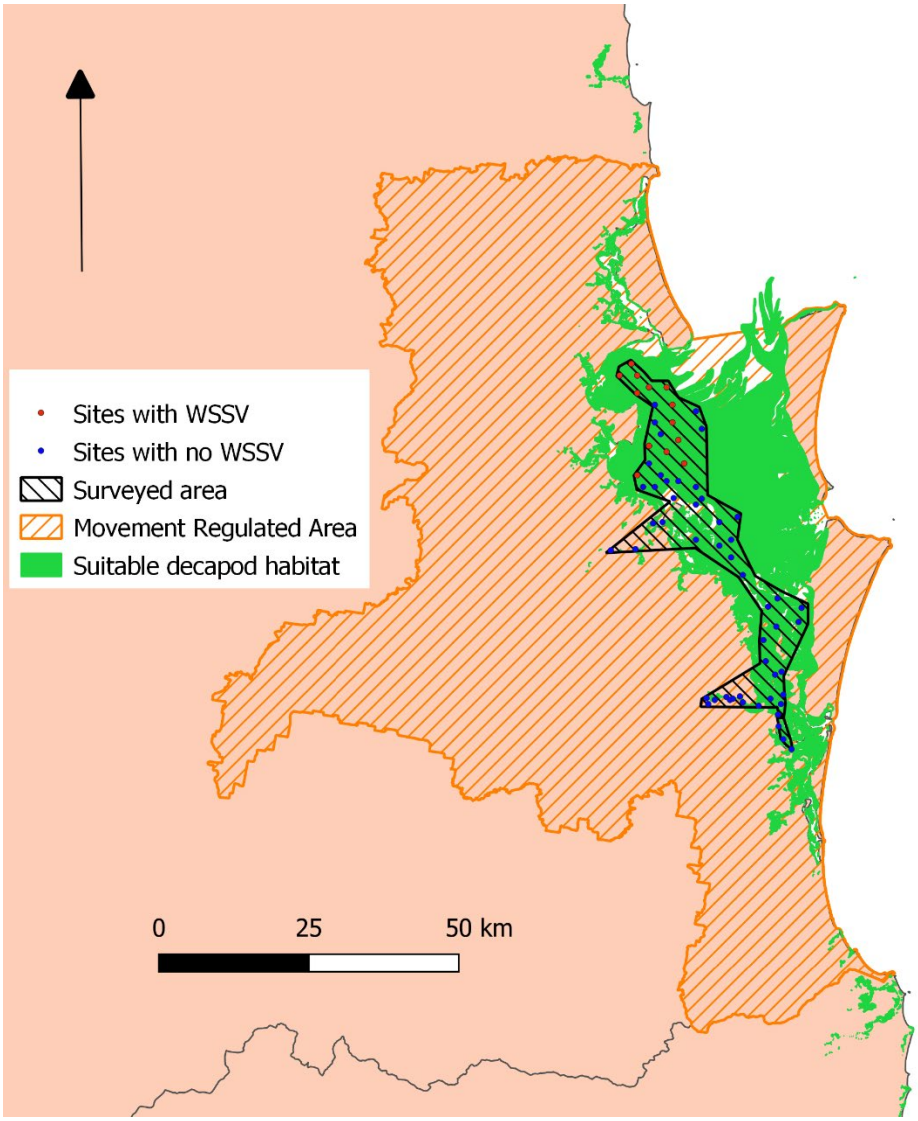

Figure 10 Broader distribution of habitat types similar to where decapods were sampled in Moreton Bay (green), current Movement Regulated Area (dashed orange) and surveillance sites with at least one positive sample (red dot) or without detection (blue dot) for White Spot Syndrome Virus in Moreton Bay region Queensland, Australia (1,2)

Zoning and surveillance

AQUAVETPLAN for WSD outlines the zoning strategies recommended following detection of an outbreak of WSSV (41). The area with known WSSV is the infected area, a restricted area should be a zone surrounding this. The control area allows for a buffer between the infected and free area. Together, the infected, restricted and control areas contain all declared WSSV prevalent areas (41). Continued sampling in the restricted and control areas will allow for early warning of disease spread (Figure 11). Movement restrictions in the restricted and control area will reduce the likelihood of spread to the free area. Whilst these formalised zones may not be perfectly fit the current situation, the principles of a buffer area surrounding the infected area is relevant to protect prawn producers outside the infected area.

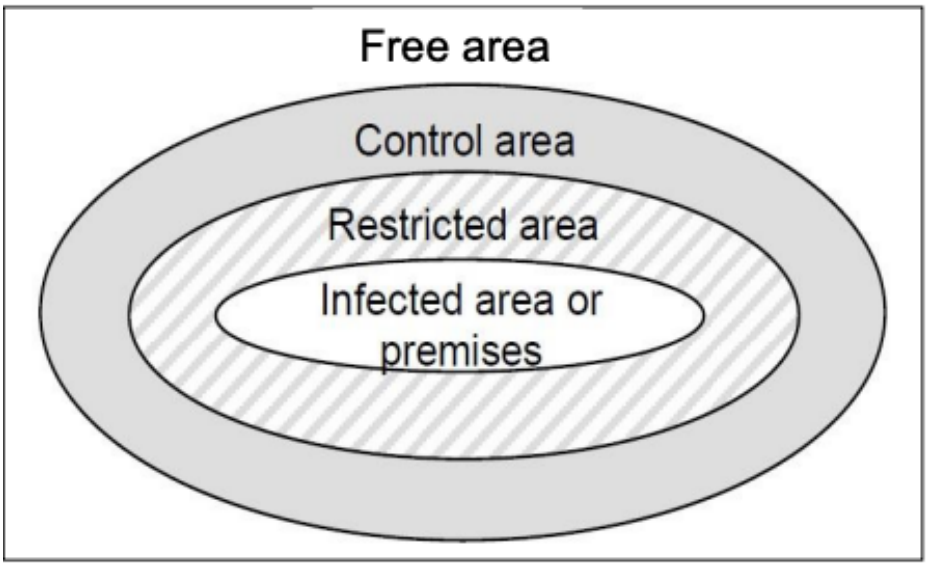

Figure 11 Zoning recommendations for outbreaks of White Spot Virus Syndrome (adapted from AQUAVETPLAN Disease Strategy (41))

The shape and size of any future surveillance or buffer zones surrounding the infected WSSV area should be guided by an understanding of the currently known infected area, where surveillance has occurred and suitable decapod habitat that may indicate where transmission between populations may be possible. Figure 10 outlines the distribution of habitat where prawns were successfully sampled but close to the restricted area, surveillance sites, and infected sites. From the data provided, it is clear that infected sites were detected at the edge of the surveillance zone, indicating it is possible that infection may have extended beyond the surveyed area, but that this may not yet be known.

Alternatively, it is possible that by good planning or chance, the surveillance reached the exact edge of the infected area, and that infection does not extend beyond the surveyed area, or that infection has faded out. However, taken together with the suitable habitat where prawns were successfully sampled during the surveillance (represented by a green in Figure 10 and Figure 13) it is possible that WSSV has extended beyond the sampled area, and this is not yet known. This possible extension of the infected area may be a short distance, for example in suitable habitat within or just outside the Movement Regulated Area. Alternatively, it is possible that spread could be more extensive for example to a large area of suitable habitat found north of the sampled area near Fraser Island.

Commercial fishing

Commercially caught decapod species, by all fishing methods, captured in the surveillance program include giant tiger prawns, brown tiger prawns, banana prawns, greasyback prawns, mud crabs and blue swimmer crabs. Except blue swimmer crabs, commercial fishing data shows higher yields decapods in summer months and a decline through winter (Figure 12). The year 2019 saw an overall decline in catch, with banana prawns and greasyback prawns seeing a significant decline in catch. All tiger prawns saw a slight increase in catch in 2019 (Figure 12).

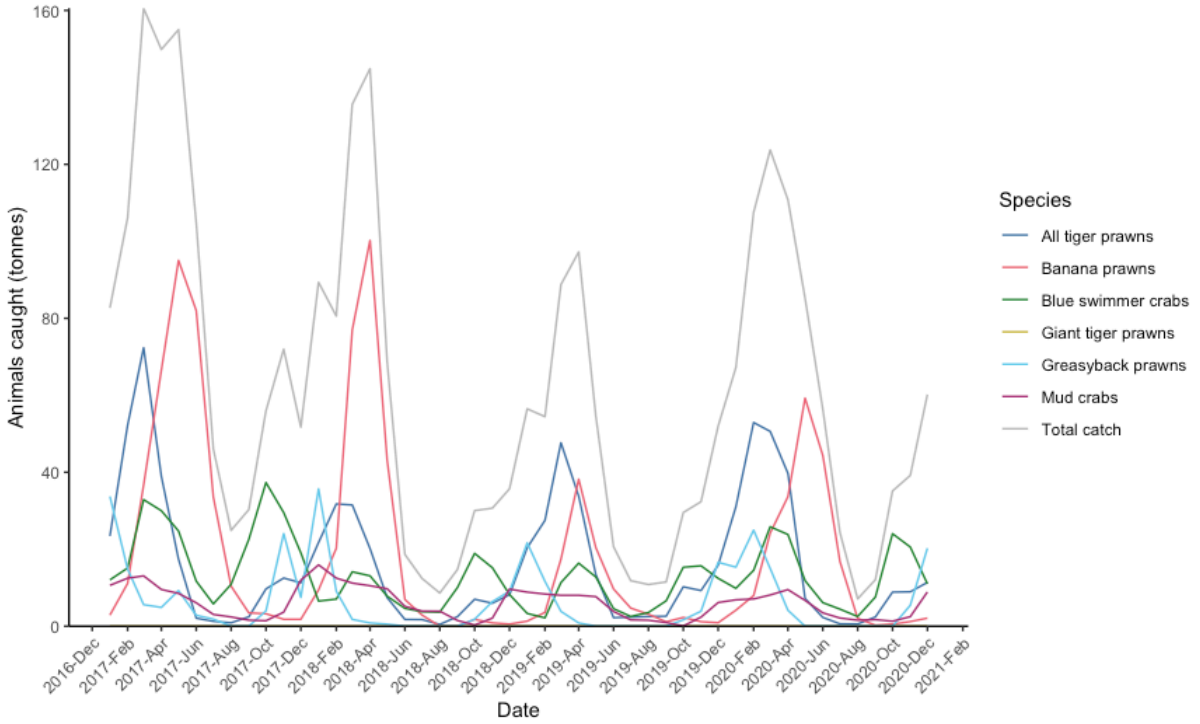

Figure 12 Total tonnes of commercially caught decapods by all fishing methods, by species, for the Moreton Bay area, between 2017 – 2020.

Modelling

Generalised linear models (GLMs) with a logit link function and binomial distribution were used for logistic regression. Three models, using different calculations of average rainfall, sea salinity and temperature were compared. Average rainfall, sea salinity and temperature were calculated for periods 14-, 30- and 60-days prior to the sampling date. Some sites did not have sea salinity or temperature data and were excluded during data cleaning. These results can be found in Appendix 3.

Considering the longitudinal nature of the data and the possible correlation between observations at each site, GEEs, an extension of GLMS, were also used for modelling. GEEs are a nonparametric way to handle possible correlation between observations at each site. GEEs will use the most appropriate within-subject covariance structure and uses moment assumptions to iteratively choose the best β. The covariance structure chosen for modelling is an autoregressive covariance structure. This structure implies that species with observations at the same site, each sampling period, have similar covariance and variance.

$$logit(p_{ij}) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature14 + \beta_3 meansalinity14 + \beta_4 meanrainfall14 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab$$ - Model 1

$$logit(p_{ij}) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature30 + \beta_3 meansalinity30 + \beta_4 meanrainfall30 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab$$ - Model 2

$$logit(p_{ij}) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature60 + \beta_3 meansalinity60 + \beta_4 meanrainfall60 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab$$ - Model 3

Table 6 Odds ratios and 95% confidence intervals for GEE models for the outcome of PCR test result for White Spot Syndrome Virus

Model	Variable	Odds ratio	95% Confidence intervals
Model 1	Average sea surface temperature 14d prior (°C)	6.24*	2.42 – 16.13
	Average sea surface salinity 14d prior (ppt)	0.98	0.85 – 1.14
	Average daily rainfall 14d prior (mm)	1.12*	1.07 – 1.18
	Greasyback prawn (cf. blue swimmer crab)	2.56*	1.01 – 6.53
	Mangrove swimming crab (cf. blue swimmer crab)	4.09*	1.48 – 11.29
	Brown tiger prawn (cf. blue swimmer crab)	2.36	0.77 – 7.22
	Giant tiger prawn (cf. blue swimmer crab)	0.00	0.00 – 0.00
	Banana prawn (cf. blue swimmer crab)	1.39	0.24 – 8.18
	Total count of decapods captured per sample	1.02*	1.01 – 1.02
	Year	1.00	1.00 -1.00
Model 2	Average sea surface temperature 30d prior (°C)	2.87*	1.36 – 6.02
	Average sea surface salinity 30d prior (ppt)	1.80*	1.14 – 2.84
	Average daily rainfall 30d prior (mm)	1.23*	1.08 – 1.41
	Greasyback prawn (cf. blue swimmer crab)	2.79*	1.10 – 6.97
	Mangrove swimming crab (cf. blue swimmer crab)	4.28*	1.57 – 11.68
	Brown tiger prawn (cf. blue swimmer crab)	2.23	0.75 – 6.68
	Giant tiger prawn (cf. blue swimmer crab)	0.00	0.00 – 0.00
	Banana prawn (cf. blue swimmer crab)	1.23	0.19 – 8.14
	Total count of decapods captured per sample	1.01*	1.01 – 1.02
	Year	1.00	1.00 – 1.00
Model 3	Average sea surface temperature 60d prior (°C)	9.75*	1.94 – 48.92
	Average sea surface salinity 60d prior (ppt)	7.99*	1.68 – 38.10
	Average daily rainfall 60d prior (mm)	1.45*	1.13 – 1.85
	Greasyback prawn (cf. blue swimmer crab)	2.32*	1.16 – 4.63
	Mangrove swimming crab (cf. blue swimmer crab)	3.50*	1.50 – 8.18
	Brown tiger prawn (cf. blue swimmer crab)	1.83	0.88 – 3.78
	Giant tiger prawn (cf. blue swimmer crab)	0.00	0.00 – INF
	Banana prawn (cf. blue swimmer crab)	1.41	0.35 – 5.17
	Total count of decapods captured per sample	1.02*	1.01 – 1.02
	Average sea surface temperature 14d prior (°C)	1.00	1.00 – 1.00

We could not include an indicator variable for season (orthagonality due to all outbreaks being detected in summer), including sea surface temperature and average daily rainfall variables allowed us to control the confounding effects of season in the model. The descriptive analysis showed that sea surface temperature and rainfall were, on average, higher during the summer months than spring months in this dataset. Therefore, these variables are able to act as a proxy for season in our models. The odds of a

positive sample increased in response to increasing sea surface temperature and rainfall, therefore, with the positive samples all detected in late summer, and the modelled association with increasing temperature and rainfall it can be inferred that sampling in summer months (or at least warm wet periods), increases the odds of a positive sample. In Model 1, there was no association between a positive sample and sea surface salinity, however in both Model 2 and 3 there was evidence for an association between increasing sea surface salinity and a positive sample. Due to the wide confidence intervals for sea surface salinity in Model 3, the odds must be interpreted with caution due to information loss in this model. As the results for sea surface salinity, results were inconsistent across models, this variable will not be included as a risk factor for positive samples of WSSV.

Secondly, the odds of a positive sample increased by approximately 1-2% in all models for each additional animal per sample. Lastly, the odds of a positive sample were approximately 2.5 times higher for a sample of greasyback prawns and 4 times higher for a sample of mangrove swimming crabs compared to a sample of blue swimmer crabs.

Modelling using interaction terms between rainfall, sea surface salinity and sea surface temperature was examined. These models added unnecessary complexity to the analysis and did not change the results of the analysis significantly. Therefore, these models were not included from this report.

Discussion

In many countries around the world, WSSV is enzootic in wild decapod populations (6). Once an introduction occurs into the wild it is rare for an established infection to fade out and disappear (42). Through the results of this analysis and expert opinion, it could be assumed it is possible that WSSV should be considered enzootic (or established) in Moreton Bay. Positive samples were found in a wide range of decapods through multiple surveillance activities and over multiple years (3).

Risk factors – what factors increase the chance of a WSSV positive sample?

Host species is an important factor to account for WSSV surveillance. A wide range of decapods and zooplankton were found to be positive for WSSV (3). Odds of WSSV was highest in greasyback prawns, mangrove swimming crabs and brown tiger prawns. Modelling showed strong evidence that, when compared to a sample of blue swimmer crabs, greasyback prawn and mangrove swimming crab samples had higher odds of a positive sample. There was little evidence to show a difference in odds of a positive sample between blue swimmer crabs and brown tiger prawns. Therefore, prioritising sampling of mangrove swimming crabs and greasyback prawns, while limiting testing of blue swimmer crabs, may increase the likelihood of positive samples in areas where WSSV is present, both because prevalence was high and that these are abundant species in the infected area.

Due to the sampling procedure, unknown number of true positive animals per sample due to possible contamination and the reporting of surveillance results, interpretation of results at an individual animal level was impossible. Our study analysed samples that were stored collectively from each field sampling site with potential cross contamination between them. This precludes calculation of sample size for demonstration of freedom from disease (or detection of disease) at each site. However, it could be shown empirically that an increase in the number of animals per sample increased the odds of a positive detection. This makes sense as intuitively; larger sample sizes would increase the probability of collecting at least one infected animal at each site if infection was present. For example, although positive detections were made in samples with as little as 2 animals, the majority (78%) of positive samples had >20 individual animals. Therefore, in this scenario, a minimum of 20 to up to 186 animals per sample is recommended based on empirical data, recognising uncertainties as there is no gold standard in this analysis and we could not formally calculate sample size for disease detection surveys. However, we would recommend that individual decapods are stored separately to reduce cross contamination, noting that this would still not prevent possible contamination that could occur during the sorting process, so that prevalence can be estimated accurately, and future sample size calculations and greater understanding of prevalence at sites can be achieved. The OIE recommends targeting the cuticular epithelium and subcuticular connective tissues for sampling (18).

If individual animals are stored separately and prevalence estimated, in the future, a sample can be calculated to demonstrate freedom at each site according to the methods of Cameron and Baldock (1998) (43). Table 7 demonstrates freedom sample sizes for a site under various scenarios of assumed prevalence at each site. As understanding of what prevalence may be expected is gained, refinement of sample sizes can occur. This will ensure sensitive surveillance is conducted, and if prevalence is higher than expected, smaller samples can be taken which may save money.

Table 7 Sample sizes per site required to detect WSSV infection in Decapods

The calculations assume that the test specificity and sensitivity of the PCR is 99% and that the prevalence of WSSV varies between 1 and 10%. It is assumed that 95% confidence and power is required and that a smaller population is present (hypergeometric distribution used for calculations).

Assumed prevalence of WSSV in decapods at a site (design prevalence)	Cut point reactors before a sample considered infected (i.e. false positives allowed before a site considered infected)	Sample size required at each site*
1%	NA	Cannot detect disease with a specificity of 99% and prevalence of 1% as not possible to distinguish between false positives and infection.
2%		450 (Large sample size required, difficult to detect WSSV at low prevalence)
5%	3	127
10%	2	56

**Calculated using Ausvet's epitools: https://epitools.ausvet.com.au/freecalctwo*

Lifecycle and migration patterns are important factors to consider when developing surveillance strategies for WSSV. It is known that prawn species, such as banana prawns, greasyback prawns and brown tiger prawns are highly abundant in the mixed soft substrata, especially during summer months (33,44,45). This was the most common habitat in the sampling sites. Giant tiger prawns are usually found in deeper waters and glass shrimp are only found in freshwater river systems, and as these habitats were not targeted during surveillance, lower numbers of these species were caught.

Considering the likely enzootic (established) nature of this disease, season is the primary factor that must be considered when sampling for WSSV. Positive samples were only seen in warmer months, suggesting that expression of disease is more likely during this time. As previously stated, the migration behaviour of prawns and crabs are also affected by seasonality. Prawns especially, are more likely to be found in shallow oceanic waters ('recruitment' areas), during summer months. Therefore, sampling in mixed soft substrata during summer months is likely to yield a higher number of animals and increase chances of detection for a positive sample of WSSV if present in the sampling area.

We could not model season explicitly (all positives were found in summer which mean that season could not be added to the model). However, season is associated with environmental factors such as rainfall and sea temperature and a rise in either of these factors was strongly associated an increased odds of a positive sample. Practically, this means that sampling in summer when rainfall and sea surface temperature are highest, is recommended as this may increase chances of detection of positive samples if WSSV is present in the sampling area.

Rainfall was shown to have a significant impact on the result of a sample. Waiting one to two months after a significant rainfall period may further increase the likelihood of a positive sample. There were no positive samples in 2019, a year with substantially less rainfall than all other years during the surveillance program. Several studies have demonstrated a correlation between freshwater flow from rivers, rainfall and increased commercial catch (46–48). A study involving banana prawns suggested that increased rainfall affects levels of salinity within the estuary. As the salinity decreases it prompts the juveniles to migrate downstream and they are eventually carried into oceanic waters (49). Higher rainfall has also been associated with increased recruitment in fishing areas, due to facilitating the downstream migration of juveniles (50). As well as increasing the number of animals in suitable surveillance sites, rainfall is known to affect sea salinity. It is known that stressors such as sudden

changes in temperature and salinity will increase expression of disease in prawns. Therefore, it is hypothesised that after large rainfall or storm events, more disease (and replicating virus detectable by PCR) may be present in wild populations.

What is the current geographic distribution of WSSV?

Currently, all positive samples that were collected by BQ were located at the northern most edge of the study surveillance area (Figure 10), but in our dataset no samples occurred outside the Movement Regulated Area. WSSV detection was also reported in Beachmere and the Caboolture River, areas outside of the BQ study surveillance area, but still within the Movement Regulated Area using a separate survey (3). Published and unpublished data from samples collected just north of the Movement Regulated Area by BQ have shown no positive results at the time of writing this report. This suggests that WSSV may be contained within the Movement Regulated Area or that sample sizes were too small to detect infection outside the known area due to a possible low prevalence of infection. Regardless, the boundaries of the current geographical distribution of WSSV in the Moreton Bay remains to be defined. Suitable decapod habitat and susceptible host species extend beyond the boundaries of the Movement Regulated Area and it is biological plausible that the infection exist beyond these boundaries.

Extended surveillance coverage to establish a well circumscribed area of distribution of WSSV would be useful to enable delineation of the infected area which would enable other prawn producers to understand their risks for future outbreaks of WSD. For example, if it is understood where the area of WSSV extends to, a buffer area could be implemented, and surveillance conducted to understand transmission dynamics over time. This would allow early warning to producers and fishers outside the currently understood infected area. To understand the geographic distribution of WSSV would require further systematic sampling extending out from the current surveillance area (see *Future Surveillance)*. It is uncertain, at this stage, how far this sampling would need to extend. Essentially, sampling could continue in detail up the coast as long as infection was detected. Large sample sizes in appropriate species, times of year and sometime after heavy rain (at least 14 days up to 60 days post rainfall event) may enhance detection probabilities.

What are possible transmission pathways from Moreton Bay?

There are three hypothesised pathways for spread of WSSV from the Moreton Bay area. Firstly, WSSV may spread via natural and seasonal migration of host species. Most species caught during the surveillance period have sedentary life cycles, with distance limited migration from estuaries and intertidal areas. Some prawn species, such as the eastern king prawn, are known to migrate north as they grow, some more than 100km (51). Due to the mixing of adult populations and genetics, there is suggestion of one stock along the entire eastern coast (52). The risk this species poses to the spread of WSSV is unknown, as it has not been widely tested for WSSV. Although risk of spread from migration of host species is low due to their life cycles, mixing of populations occurs rarely, to allow for genetic mixing of subpopulations (49). This mechanism of spread must be taken into consideration when planning future surveillance.

Secondly, crabs, similar to prawns, are mostly sedentary in nature, only migrating offshore for spawning events. However, many crabs have long larval phases, forming part of zooplankton. A small number of studies have demonstrated that zooplankton can become infected and transmit WSSV (53). Dr. Ben Diggles also found positive zooplankton samples in the Logan River, adjacent to farms experiencing WSD on the property (3,53). Zooplankton drift with oceanic currents. The prolonged larval stage and potential for drift, creates the possibility for wide dispersal of larvae before settling into an estuarine environment (54). Therefore, zooplankton may lead to spread of WSSV outside of the Moreton Bay region.

Lastly, mechanical transmission of WSSV may occur via movement of contaminated equipment or animals out of the Moreton Bay region. Recreational or commercial fishers may move infected material unknowingly out of the area. The commercial fishing industry is large and recent surveys have shown

that up to 400,000 recreational fishers use the Moreton Bay area (21). With high traffic through this region, mechanical transmission must be considered as a potential method of disease spread.

To further understand the mechanisms of possible transmission and spread of WSSV, genotyping is critical. This will allow investigators to explore the relationship among WSSV variants and aid in determining the most likely introduction and transmission routes of the virus. If WSSV is found outside of the current restriction zone, the three following scenarios should be considered:

1. The spread of one strain of WSSV from one wild population to another, i.e., WSSV spread from Moreton Bay to the Logan River or to other areas or vice versa
2. Separate incursions of WSSV from imported, infected stock
3. Separate endemic populations along the eastern coast of Australia.

Future surveillance

As previously stated, defining the current geographic distribution of WSSV disease in (or outside) Moreton Bay is essential for proper zoning, implementation of future surveillance efforts and understanding the risk to the broader prawn farming sector in Australia.

The original purpose of the surveillance program we analysed was to establish Australia's proof of freedom from disease. The surveillance program implemented was ideal to address these concerns and implemented well and disease was detected for several years. However, the dataset available was able to be analysed to address the objectives of this report, whilst acknowledging the data was not designed for risk factor analyses. The analysis of the surveillance data showed the site prevalence of disease is increasing steadily in the Northern Moreton Bay area, with approximately 8% of sites positive in 2017 growing to 17% of sites positive in 2020. Therefore, future surveillance may allow confirmation of whether WSSV should be considered endemic in Australia. Will the disease fade out of the Moreton Bay area over time or is it now established? Further surveillance by expanding the surveillance area is required to delimit the infected area in Moreton Bay. At the time of the previous BQ surveillance program, this was not achievable due to the structure of the program focused on detection of disease. Based on the results of the analysis, it is recommended that when sampling occurs it should follow these principles:

- Sample in the warmer months – primarily March
    - WSSV is likely seasonal but probably endemic with increased detection in summer/warmer months
- If possible, sample after high average rainfall periods (at least 14 days up to 60 days post rainfall event)
    - Increased rainfall will increase the odds of a detection.
- Target greasyback prawns and mangrove swimming crabs
    - These species are more abundant, distributed along the east coast of Australia and had higher prevalence of WSSV than other species in the surveillance program (although bias is possible)
- Store animals individually for sampling, if possible, to reduce cross contamination and allow calculation of prevalence
    - Cross contamination between individual animals that were transported together from each site precluded prevalence calculations and hence accurate recommendations about sample sizes required to detect disease reliably at each site. However, based on the sampling methods used, large sample sizes are required (a minimum of 20 up to 186 animals). If large samples cannot be taken, the sensitivity of surveillance at a site is reduced and some positive sites may be missed. Collect more than 20 individual samples per site.
- Sample from mixed soft substrata
    - Animals are known to inhabit this environment during summer months.

    - Literature has shown susceptible host species frequently inhabit mangrove, sand and soft substrates during summer months and may also be targeted for sampling (28,33,34,55)
- Expand the surveyed area considerably past the last positive site to ensure that there is a chance of detecting the edge of the infected area.

To give industry confidence and allow preparedness for WSSV we recommend continuing surveillance efforts conducted by BQ and adapting them to ensure better delineation of the infected area in and around Moreton Bay. That is, positive samples were detected in the most northern surveillance sites in the WSSV study surveillance area in Moreton Bay, and although no positive samples have been detected outside the area at this stage, it is possible that infection may extend outside this WSSV study surveillance area, perhaps still in the Movement Regulated Area or just outside it (Figure 10). This sampling should continue to occur to identify if and where infection has spread from Moreton Bay.

To do this, we first assume that WSSV in Moreton Bay is a point outbreak that has the potential to expand and infect other areas to the north or south of Moreton Bay. However, we also acknowledge that without further surveillance, including discriminatory molecular epidemiology, this may not be the type of introduction that occurred and that other outbreak scenarios are possible. Regardless, if this is a point outbreak or to provide further evidence about the type of outbreak, we recommend extension of the surveillance program conducted from 2017 - 2020. The method we used to decide upon a surveillance strategy and the type of surveillance we recommend is detailed in the following steps:

1. Identify suitable Decapod habitat based on where prawns were sampled in Moreton Bay

We used a dataset from the Institute of Marine and Arctic Studies (IMAS), a marine habitat map. This was analysed with a geographical information system (within R) to identify suitable habitat where prawn samples were collected in the Moreton Bay area (see the green area within Figure 13). The habitat types where prawns were sampled included predominantly mixed soft substrate but also to a lesser extent seagrass, mangroves and bioturbators habitat.

2. Map these habitats as suitable habitat extending north and south from Moreton Bay

These suitable habitats were then mapped to illustrate where similar prawn populations (e.g. density and species composition) may potentially be found around Moreton Bay, including north and south. This included suitable habitat immediately around Moreton Bay, and large areas of suitable habitat extending north and south from Moreton Bay.

It is important to realise however, that the IMAS dataset on habitat is not complete in all areas. For example, there is little marine habitat mapping completed between Fraser Island and Moreton Bay. In addition, other areas may have additional habitats that are suitable for decapods, or the decapod species surveyed in Moreton Bay may occupy alternative habitats in other areas away from Moreton Bay. This means that there may be suitable habitat that could sustain large decapod populations, and be suitable for surveillance to identify WSSV, but these could not be identified with our approach. Despite this, the areas we have identified for sampling may allow a point estimate of the presence or absence of WSSV at distances from Moreton Bay. If more detailed delineation is required, for example between a positive and negative area, more detailed sampling between surveillance locations can be conducted following more detailed habitat mapping between surveillance locations. Other data sources such as catch return data or alternative habitat mapping may also be useful but is beyond the scope of this project.

3. Progressive delineating sampling

We recommend continued surveillance beyond the Movement Regulated Area and gradually expanding the surveillance area to gain greater confidence of where WSSV may or may not have

spread. Firstly, the area to the immediate north of Moreton Bay should be examined in more detail. This is displayed as Priority A areas in

Figure 13. Whilst southward sampling close to Moreton Bay could be considered as a Priority A area, we have not listed that area. This is because considerable negative southern sampling occurred within Moreton Bay. In addition, areas outside Moreton Bay are predominantly less inshore and nearshore and more offshore locations with more sand than mixed soft substrata.

Next, large areas of suitable habitat some distance to the north and south of Moreton Bay should be surveyed for WSSV. These are termed Priority B areas. This includes the inshore and near shore areas to the west of Fraser Island near Hervey Bay and areas just to the north of Byron Bay, recognising that NSW surveillance areas are more open beaches and with more sand than mixed substrate, so may potentially be less suitable for prawns than areas in Qld we have identified. It may be necessary to sample estuaries rather than open beaches. After these areas are surveyed, further decision making will be required. For example, if no disease is detected in Priority B areas, more detailed surveillance could be conducted between Priority A and B areas if further discrimination of infected areas is believed warranted. If disease is detected at priority B areas, further surveillance extending north to the commercial prawn farms near Bundaberg or active surveillance of these farms and south to Priority C areas should be conducted. The priority C areas identified are prawn farming operations located in the Bundaberg region and in NSW near Yamba.

After this point, if priority C areas are infected further decision making will be required to determine whether further delineating surveillance north and south is required as the area that was considered infected would now be very large.

See Figure 13 and Table 8 for further information on these priority surveillance areas.

Table 8 Suggested surveillance sites to further delineate the WSSV infected area north and south of Moreton Bay, assuming that Moreton Bay is a point outbreak.

Location	Priority	Latitude ranges where sampling should occur	Comments
North of WSSV study surveillance area but within Movement Regulated Area (or just outside it)	Priority A	-26.796 to -27.111 (S)	East and west of Bribie Island
West of Fraser Island from Pelican Bank south to Tin Can Bay	Priority B	-25.2200 to -25.9600 (S)	Inshore and near shore from Fraser Island
North of Byron Bay	Priority B	-28.6420 (S)	Noting this may not be productive prawn habitat as it is mostly exposed beaches, and not inshore as was the case in Moreton Bay.
Commercial farms near the Bundaberg Area	Priority C	-23.7660 to -24.0900 (S)	Location of multiple prawn farms on the Elliott River, sampling may also occur in the Burnett River
Yamba area	Priority C	-29.3737	Near Yamba.

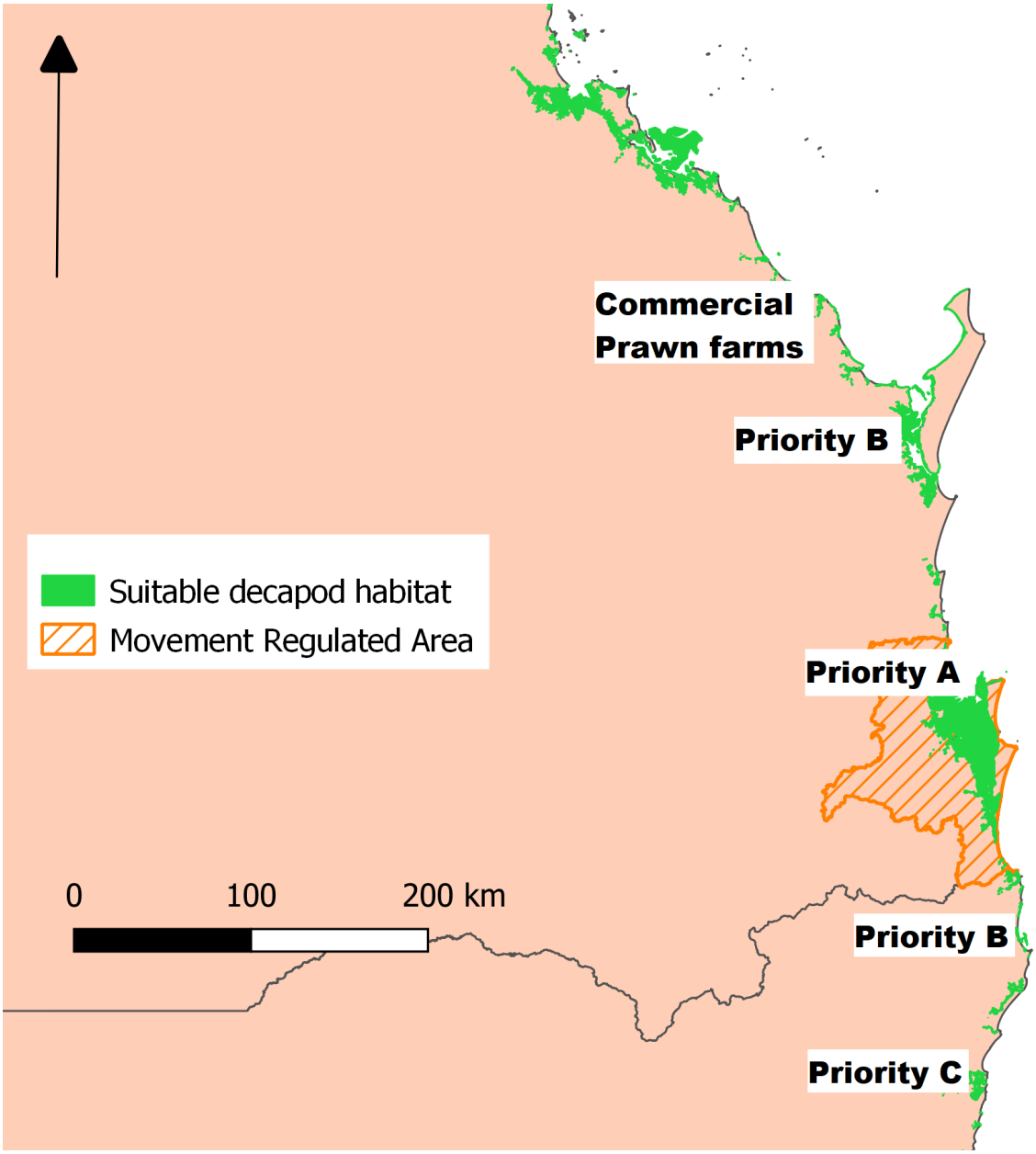

Figure 13 **Broader distribution of habitat types similar to where decapods were sampled in Moreton Bay (green), current Movement Regulated Area (dashed orange) and suggested priority surveillance sites (Priority A – C) for White Spot Syndrome Virus in Queensland and New South Wales, Australia (1,2)**

Other considerations

The decline of habitat due to urbanisation and climate change may impact the distribution of WSSV in the Moreton Bay region. In 2014, urbanisation in the region led to a decline in both habitat and water quality around the bay (56). Decreasing water and habitat quality may impact, not only the distribution of species in this area, but the prevalence of WSSV. It is known that climate change is associated with warmer temperatures and more intense flooding and drought events (57). As the expression of WSD is associated with sudden changes in temperature, salinity and pH, more frequent, severe weather events may lead to an increase in WSSV prevalence. Both urbanisation and climate change may impact the distribution of WSSV in the future.

An understanding of disease spread was desired from this project. Unfortunately, the surveillance data available was not amendable to calculating disease spread, except at the very local area within the northern Moreton Bay. In further detail, we did note small increases of the infected area in the northern Moreton Bay area did occur year on year as additional sampling points became infected. However, APFA's main interest was to determine the likelihood of long distance spread so that threats to prawn farmers in areas remote from the Logan River could be assessed. The surveillance data was not amenable to estimating this possible spread. Firstly, the surveillance data did not allow us to assess whether the outbreak was indeed a point outbreak in just the Moreton Bay area (although this is likely based on our understanding of other surveillance that has been communicated to us verbally). Secondly, no surveillance data outside the Moreton Bay area to the north and south of the study area were made available to Ausvet. If further understanding of the risks of spread of WSSV to the north and south of the Moreton Bay area is required, we would recommend further surveillance outside the Moreton Bay area and simulation modelling. Simulation modelling could comprehensively include information about tides, particle transmission, suitable habitat, water current, population and ecology of decapods and epidemiology.

Conclusion

WSSV should now likely be considered enzootic in Moreton Bay. It appears from limited surveillance data that we have that WSSV distribution is expanding within the area. To allow optimal management and preparation by industry, enhanced surveillance efforts are recommended to establish and monitor the current geographic distribution of WSSV in Moreton Bay and beyond (if relevant), to create appropriate zoning measures (e.g., surveillance outside the infected area to ensure that spread is detected early). This report highlights important factors to consider when developing surveillance to improve efficiency and detectability (e.g., species, sample size, season, and environmental risk factors including season, rainfall, and sea surface temperature). Virus genotyping should play a key role in future outbreak investigation and tracing to determine the most likely introduction and spread pathways for WSSV.

Implications

This project provides industry and government with surveillance strategies to improve understanding of the distribution and spread of WSSV in SE Qld. The project also identified risk factors to support the implementation of risk-based surveillance for more successful and efficient detection of WSSV. Enhanced surveillance strategies can provide early warning to prawn farmers in other areas of Australia that could be crucial to prevent the devastating production and economic losses seen in the 2016 outbreaks.

Recommendations

This project outlines recommendations for effective sampling of animals to detect WSSV. These are as follows:

- Sample in warmer months – particularly March
- Target greasyback prawns and mangrove swimming crabs
- Sample from mixed soft substrata and other suitable habitats (i.e., mangroves, bioturbators), assuming that the ecology of decapods is similar to that observed ion Moreton Bay
- Store animals individually for sampling if possible to reduce cross contamination and allow calculation of prevalence
    - Cross contamination between individual animals that were transported together from each site precluded prevalence calculations and hence accurate recommendations about sample sizes required to detect disease reliably at each site. However, based on the sampling methods used, large sample sizes are required (a minimum of 20 up to 186 animals). If large samples cannot be taken, the sensitivity of surveillance at a site is reduced and some positive sites may be missed
- Sample after high average rainfall periods (at least 14 days up to 60 days after rainfall event), if possible
- Expand the surveyed area considerably past the last positive site to ensure that there is a chance of detecting the edge of the infected area.

Further development

A key early objective of the project was to determine the rate of spread so that industry could determine their risks (e.g., prawn farmers in north Qld). Due to the nature of the data, we only had a signal that localised spread and expansion of the infected area in Morton Bay was occurring. We could not address the speed and nature of spread from Morton Bay if indeed this is occurring. We have recommended that sampling outside the infected area of Morton Bay occur. Further development of this project could include a detailed surveillance plan to delineate the infected area. In addition, as that data becomes available, this could be used to enable establishment of a disease simulation model, similar to particle tracking modelling produced by the CSIRO for the QLD government in response to the outbreak in 2016.

Extension and Adoption

This project will be adapted to present to APFA members and industry personnel.

We will produce the following:

- A full scientific report
- A condensed version of the report that is easier reading
- A PowerPoint presentation (with video).

These will be presented to APFA (and the PowerPoint presented to an APFA conference along with Ausvet Attendance to answer questions) and the Queensland Seafood Industry Association for dissemination to their members in the Morton Bay area.

A condensed version highlighting the main outcomes and recommendations will be produced and disseminated to industry members. We will circulate the report to the relevant scientific community (through FRDC and APFA).

We will produce a PowerPoint presentation in video form for either presentation at APFA fora or the broader community.

Project coverage

None

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Appendices

Appendix 1: Consultants and Project Staff

Consultant/Project Staff	Company
Dr. Rachel Nye	Ausvet
Dr. Brendan Cowled	Ausvet
Associate Professor Charles Caraguel	The University of Adelaide
Tony Charles	Australian Prawn Farmers Association

Appendix 2: Additional modelling

Generalised linear mixed models (GLMs) with a logit link function and binomial distribution were used for logistic regression. Each surveillance site was randomly selected from within the surveillance area and there is repeated sampling at each site over 4 years. To account for correlation between samples, site was added to the models as a random effect. Furthermore, some sites may naturally have a lower sea surface temperature or number of available individual animals for capture. Three models, using different calculations of average rainfall, sea salinity and temperature were compared. Average rainfall, sea salinity and temperature were calculated for periods 14-, 30- and 60-days prior to the sampling date. Some sites did not have sea salinity or temperature data and were excluded during data cleaning.

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature14 + \beta_3 meansalinity14 + \beta_4 meanrainfall14 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab + random\ effect\ (site)$$ - Model 1

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature30 + \beta_3 meansalinity30 + \beta_4 meanrainfall30 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab + random\ effect\ (site)$$ - Model 2

$$\log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 totaltested + \beta_2 meantemperature60 + \beta_3 meansalinity60 + \beta_4 meanrainfall60 + \beta_5 blueswimmercrab + \beta_6 browntigerprawn + \beta_7 gianttigerprawn + \beta_8 greasybackprawn + \beta_9 mangroveswimmingcrab + random\ effect\ (site)$$ - Model 3

Table 9. Odds ratios and 95% confidence intervals for logistic regression models with site variable as random effect for the outcome of PCR test result for WSSV

Model	Variable	Odds ratio	95% Confidence intervals
Model 1	Average sea surface temperature 14d prior (°C)	3.49*	1.18 – 10.32
	Average sea surface salinity 14d prior (ppt)	0.79*	0.64 – 0.987
	Average daily rainfall 14d prior (mm)	1.05	0.94 – 1.17
	Greasyback prawn (cf. blue swimmer crab)	1.94	0.56 – 6.68
	Mangrove swimming crab (cf. blue swimmer crab)	3.11	0.94 – 10.24
	Brown tiger prawn (cf. blue swimmer crab)	2.36	0.73 – 7.69
	Giant tiger prawn (cf. blue swimmer crab)	0.00	0.00 – Inf
	Banana prawn (cf. blue swimmer crab)	1.20	0.20 – 7.07
	Total count of decapods captured in sample	1.017*	1.01 – 1.03
	Year	1.00	1.00 -1.00
Model 2	Average sea surface temperature 30d prior (°C)	6.18	0.91 – 42.13
	Average sea surface salinity 30d prior (ppt)	19.18*	1.78 – 208.14
	Average daily rainfall 30d prior (mm)	2.71*	1.75 – 4.20
	Greasyback prawn (cf. blue swimmer crab)	2.45	0.60 – 10.01
	Mangrove swimming crab (cf. blue swimmer crab)	3.53	0.91– 13.70
	Brown tiger prawn (cf. blue swimmer crab)	3.32	0.89 – 12.31
	Giant tiger prawn (cf. blue swimmer crab)	0.00	0.00 – INF
	Banana prawn (cf. blue swimmer crab)	1.23	0.18 – 8.63
	Total count of decapods captured in sample	1.02*	1.01– 1.04
	Year	1.00	1.00 – 1.00
Model 3	Average sea surface temperature 60d prior (°C)	28.48	0.49 – 1653.76
	Average sea surface salinity 60d prior (ppt)	19,011.73*	175.76 – 2,056,509.30
	Average daily rainfall 60d prior (mm)	3.92*	2.18 – 7.03
	Greasyback prawn (cf. blue swimmer crab)	2.60	0.55 – 12.31
	Mangrove swimming crab (cf. blue swimmer crab)	3.12	0.68 – 14.30
	Brown tiger prawn (cf. blue swimmer crab)	4.70*	1.08 – 20.43
	Giant tiger prawn (cf. blue swimmer crab)	0.00	NA
	Banana prawn (cf. blue swimmer crab)	1.71	0.20 – 14.71
	Total count of decapods captured in sample	1.03*	1.02 – 1.05
	Year	1.00	1.00 – 1.00

*Odds Ratio shows significance at 5% threshold

Mixed-effect modelling was performed including site as a random effect (Table 9). Across all models, there was evidence that an increase in total count of decapods in a sample, increased the odds of a positive sample by approximately 1 – 3% per animal captured. In Model 1, there was some evidence that, on average, increasing sea surface salinity (OR 0.79, 95% CI 0.67 – 0.987) would decrease the odds of a positive sample. The odds of a positive sample were 3.5 times higher for an average increase in sea surface temperature of 1 degree Celsius 14 days prior to sampling.

As models with random effects take into the same error as a fixed effects model plus an additional component, the variation is increased compared to a standard logistic regression model. The variation is also affected by decreasing sample size in this situation, as some observations were removed due to lack of data. Those variables with wide confidence intervals, such as sea surface salinity, sea surface temperature and the brown tiger prawn in Model 2 and 3, must be interpreted with caution. Models 2 and 3 show strong evidence that the odds of a positive sample increases with an increase in average rainfall prior to sampling and total count of decapods per sample. Due to the wide confidence intervals, there is minimal evidence that there is an association between a positive sample and sea surface salinity.

In Model 3, there was weak evidence that a sample of brown tiger prawns had an increased odds of a positive outcome when compared to a sample of blue swimmer crabs. Although these models may appear inconsistent at times, the association between a positive outcome and total count of decapods and environmental factors such as sea surface temperature and rainfall cannot be dismissed.

Appendix 3: WSSV Sampling Methodology provided by Biosecurity Queensland

Site Selection

Moreton Bay

The survey area in Moreton Bay extended from Deception Bay in the north, to the Gold Coast Broadwater in the south and included the adjacent Brisbane and Logan Rivers. For safety reasons, areas of high vessel traffic (e.g. navigational channels, commercial shipping channels) were avoided during the site selection process. A minimum depth of 5 m (at lowest astronomical tide) was used to exclude areas too shallow for the research vessel to trawl.

Trawl sites were initially selected at random in Moreton Bay, with a small number of additional sites added post the random selection process to ensure coverage across the whole study area. Selected sites were then sampled twice annually until the program ceased.

Logan River

Trawl sites were selected for monitoring during the white spot disease response period in areas of the river adjacent to active prawn farms. A small number of additional sites were then added upstream of the prawn farms for greater coverage. Selected sites were then sampled twice annually until the program ceased.

Brisbane River

Trawl sites were selected towards the mouth of the river, considering suitable trawl ground, avoiding the commercial shipping channels and port facilities. Selected sites were then sampled twice annually until the program ceased.

Timing of trawl surveys

Trawl surveys were conducted during the periods February-March and August-November each year. Abundance of selected crustacean species in Moreton Bay based on local knowledge from the commercial fisheries, surveillance results collected during the WSD response period, and international standards set out by the World Organisation for Animal Health (OIE) for demonstrating disease freedom following an outbreak, were all considered when determining the timing of trawl surveys. Surveys were also subject to weather conditions and logistical considerations including the availability of research vessels.

The survey period in February - March is known to be a period of high abundance for species selected for sampling in the bay and the timing of previous detections of WSSV in wild crustaceans in northern Moreton Bay.

August – November was selected as the second sampling period because crustacean abundance generally begins to increase in Moreton Bay during this period following winter and provided the required temporal separation between sampling events according to international standards set out by the OIE (i.e., $\geq$ 3 months apart).

Species selection

All decapod crustaceans are considered susceptible to white spot syndrome virus and so were identified as suitable species for targeted surveillance during the WSSV national surveillance program. The following decapod crustacean species were targeted for sampling in Moreton Bay and associated river systems, because they were known to be susceptible to WSSV after testing positive for the virus during the WSD response period and were considered abundant enough to be sampled during both surveillance periods:

- Greasyback prawn *Metapenaeus bennettae*
- Banana prawn - *Fenneropenaeus merguiensis*
- Blue swimmer crab - *Portunus armatus*
- Mangrove swimming crab - *Thalamita crenata*
- Brown tiger prawn - *Penaeus esculentus*

Additionally, a small number of other decapod species were collected opportunistically and tested for WSSV during surveillance. These species were not regularly caught within Moreton Bay using the described sampling methodology but had previously returned positive test results for WSSV during the WSD response period. In particular, the Giant tiger prawn - *Penaeus monodon,* the species cultured in prawn aquaculture facilities along the Logan River impacted by the WSD outbreak in 2026-2017.

Trawl gear and deployment

Moreton Bay

In Moreton Bay, beam trawl gear was towed using the 14.5m fisheries research vessel 'Tom Marshall'. The beam is deployed at or towed through the selected site co-ordinates and towed for 0.5 nautical miles once it has settled on the bottom. However, if the seafloor conditions of the coordinates were not practical for trawling, the shot was conducted within two-tenths of a nautical mile (approximately 370 m) of the site. Trawls were conducted with gear of the following specifications:

- 5 m beam
- 8 mm stainless steel wire warp and bridles
- 3.5 fathom (6.4 m) net (3.5 fathom headline), 1½ inch (38 mm) mesh body and 1⅛ inch (29 mm) codend, 8 mm stainless steel ground chain, 6 mm stainless steel tickler chain (approx. 8 m length)
- net attached to the back of the beam, no sweeps
- codend 100 meshes long by 100 meshes round and top opening turtle excluder device (50 mm spacing)

Brisbane and Logan River

In the Brisbane and Logan River systems, while the trawl methods were consistent with that used in Moreton Bay, a smaller research vessel (approx. 5m) was used due to the restricted working areas in some sections of the rivers. Trawl duration was reduced where river sites were near one another (e.g., ≤ 0.5 nautical miles apart) or river features restricted possible trawlable ground. The trawl gear on the smaller vessel was downsized to a 3m beam with proportionately smaller net size.

FRDC FINAL REPORT CHECKLIST

The final report checklist can now be filled in when submitting your final report deliverable in (21).