Epizoological Tools for Acute Hepatopancreatic Necrosis Disease (AHPND) in Thai Shrimp Farming

THESIS SUBMITTED TO THE UNIVERSITY OF STIRLING FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

NATTAKAN SALEETID

JULY 2017

UNIVERSITY of STIRLING

50 YEARS

INSTITUTE OF AQUACULTURE

Declaration

I hereby declare that:

(1) This thesis is composed by me,

(2) The work described is my own work, except for the live shrimp movement network data from Thailand, which I analyse, but which is obtained from the Thailand Department of Fisheries,

(3) The bibliography contains all the literature that I have used in writing the thesis, and

(4) The work contained in thesis has not been submitted for any other degree.

Signature: _____

Signature of supervisor: _____

Date: _____

Abstract

Acute hepatopancreatic necrosis disease (AHPND) is an emerging bacterial infection in shrimp that has been widespread across the major world shrimp producing countries since 2009. AHPND epizootics have resulted in a huge loss of global shrimp production, similar to that caused by white spot disease in the 1990's. The epizootiological understanding of the spread of AHPND is still in its early stages, however, and most of the currently published research findings are based on experimental studies that may struggle to capture the potential for disease transmission at the country scale. The main aim of this research, therefore, is to develop epizootiological tools to study AHPND transmission between shrimp farming sites. Some tools used in this research have already been applied to shrimp epizoology, but others are used here for the first time to evaluate the spread of shrimp diseases.

According to an epizootiological survey of AHPND in Thailand (Chapter 3), the first case of AHPND in the country was in eastern shrimp farms in January 2012. The disease was then transmitted to the south in December 2012. The results obtained from interviews, undertaken with 143 sample farms were stratified by three farm-scales (large, medium and small) and two locations (east and south). Both the southern location and large-scale farming were associated with a delay in AHPND onset compared with the eastern location and small- and medium-scale farming.

The 24 risk factors (mostly related to farming management practices) for AHPND were investigated in a cross-sectional study (Chapter 3). This allowed the development of an AHPND decision tree for defining cases (diseased farms) and controls (non-diseased farms) because at the time of the study AHPND was a disease of unknown etiology. Results of univariate and unconditional logistic regression models indicated that two farming management practices related to the onset of AHPND. First, the absence of pond harrowing before shrimp stocking increased the risk of AHPND occurrence with an odds ratio (OR) of 3.9 (95 % CI 1.3–12.6; P-value = 0.01), whereas earthen ponds decreased the risk of AHPND with an OR of 0.25 (95 % CI 0.06–0.8; P-value = 0.02). These findings imply that good farming management practices, such as pond-bottom

harrowing, which are a common practice of shrimp farming in earthen ponds, may contribute to overcoming AHPND infection at farm level.

For the purposes of disease surveillance and control, the structure of the live shrimp movement network within Thailand (LSMN) was modelled, which demonstrated the high potential for site-to-site disease spread (Chapter 4). Real network data was recorded over a 13-month period from March 2013 to March 2014 by the Thailand Department of Fisheries. After data validation, c. 74 400 repeated connections between 13 801 shrimp farming sites were retained. 77 % of the total connections were inter-province movements; the remaining connections were intra-province movements (23 %).

The results demonstrated that the LSMN had properties that both aided and hindered disease spread (Chapter 4). For hindering transmission, the correlation between in and out degrees was weakly positive, i.e. it suggests that sites with a high risk of catching disease posed a low risk for transmitting the disease (assuming solely network spread), and the LSMN showed in-out disassortative mixing, i.e. a low preference for connections joining sites with high in degree linked to connections with high out degree. However, there were low values for mean shortest path length and clustering. The latter characteristics tend to be associated with the potential for disease epidemics. Moreover, the LSMN displayed the power-law $P(k) \sim k^{-\gamma}$ in both in and out degree distributions with the exponents (γ) 2.87 and 2.17, respectively. The presence of power-law distributions indicates that most sites in the LSMN have a small number of connections, while a few sites have large numbers of connections. These findings not only contribute to a better understanding of disease spread between sites, therefore, but also reveal the importance of targeted disease surveillance and control, due to the detection of scale-free properties in the LSMN.

Chapter 5, therefore, examined the effectiveness of targeted disease surveillance and control in respect to reducing the potential size of epizootics in the LSMN. The study untilised network approaches to identify high-risk connections, whose removal from the network could reduce epizootics. Five disease-control algorithms were developed for the comparison: four of these algorithms were based on centrality measures to

represent targeted approaches, with a non-targeted approach as a control. With the targeted approaches, technically admissible centrality measures were considered: the betweenness (the number of shortest paths that go through connections in a network), connection weight (the frequency of repeated connections between a site pair), eigenvector (considering the degree centralities of all neighbouring sites connected to a specified site), and subnet-crossing (prioritising connections that links two different subnetworks). The results showed that the estimated epizootic sizes were smaller when an optimal targeted approach was applied, compared with the random targeting of high-risk connections. This optimal targeted approach can be used to prioritise targets in the context of establishing disease surveillance and control programmes.

With complex modes of disease transmission (i.e. long-distance transmission like via live shrimp movement, and local transmission), an $SEIRS$ compartmental, individual-based epizootic model was constructed for AHPND (Chapter 6). The $SEIRS$ modelling uncovered the seasonality of AHPND epizootics in Thailand, which were found likely to occur between April and August (during the hot and rainy seasons of Thailand). Based on two movement types, intra-province movements were a small proportion of connections, and they alone could cause a small AHPND epizootic. The main pathway for AHPND spread is therefore long-distance transmission and regulators need to increase the efficacy of testing for diseases in farmed shrimp before movements and improve the conduct of routine monitoring for diseases. The implementation of these biosecurity practices was modelled by changing the values of the long-distance transmission rate (B_{long}). The model demonstrated that high levels of biosecurity on live shrimp movements ($B_{long} < 1$) led to a decrease in the potential size of epizootics in Thai shrimp farming. Moreover, the potential size of epizootics was also decreased when AHPND spread was modelled with a decreased value for the local transmission rate B_{local}. Hence, not only did the model predict AHPND epizootic dynamics stochastically, but it also assessed biosecurity enhancement, allowing the design of effective prevention programmes.

In brief, this thesis develops tools for the systematic epizootiological study of AHPND transmission in Thai shrimp farming and demonstrates that: (1) at farm level, current

Thai shrimp farming should enhance biosecurity systems even in larger businesses, (2) at country level, targeted disease control strategies are required to establish disease surveillance and control measures. Although the epizootiological tools used here mainly evaluate the spread of AHPND in shrimp farming sites, they could be adapted to other infectious diseases or other farming sectors, such as the current spread of tilapia lake virus in Nile tilapia farms.

Acknowledgments

This thesis would not have been possible without the support of many people. First and foremost, I would like to thank my supervisors Darren Green and Francis Murray for providing me with the opportunity to complete my PhD thesis at the University of Stirling.

I especially want to thank my supervisor, Darren Green, who provided me with direction, technical support and became more of a mentor and friend than a supervisor. He read my numerous revisions and helped make some sense of the confusion. I am very grateful for his patience, motivation and immense knowledge in epidemiology.

In addition, thanks are due to my sponsors, the Agricultural Research Development Agency (Public Organization) and the Thailand Department of Fisheries for their financial support throughout my study. My sincere thanks also go to Dr. Jiraporn Kasornchandra and Dr. Putth Songsangjinda for believing in me and supporting me in everything.

Finally, I wish to thank to my parents for their love and support throughout my life, and to my best friends, Paranee, Thidarat and Sooksri, who always help me and believe that I can do it.

Table of contents

List of figures

List of tables

Chapter 1 - General introduction

1.1 Overview of the thesis

Farmed penaeid shrimp are the highest value species in world aquaculture production at approximately USD 22 000 million in 2014 (FAO, 2016b). Shrimp is the most important internationally traded fishery commodity in both the United States of America (USA) and the European Union (EU) markets (FAO, 2016a). Further, shrimp farming drives economic growth for many countries, provides a source of income and better livelihoods for producers, and develops many related businesses in the whole shrimp industry.

Thailand is one of the top shrimp-producing countries (FAO, 2016b), with an annual production of around 500 000–600 000 tonnes based on 2014 figures (Undercurrent News, 2014), and with 85 % of this total sold outside the country (Alam, 2015). The shrimp supply chain in Thailand comprises of hatcheries, ongrowing farmers, traders and brokers, shrimp auction markets and processing plants (Alam, 2015). Farmed shrimp represents one of the major agricultural products driving the growth in annual Thai gross domestic product (GDP) from 2.9 % in 2015 to 3.2 % in 2016 (National Economic and Social Development Board, 2017). Moreover, about 30 % of Thai labourers are in the agriculture sector (The World Bank, 2015). The shrimp farming sector is therefore a key element in allowing exporting countries like Thailand to improve their social and economic circumstances.

Nevertheless, the growth and sustainability of shrimp farming is affected by disease outbreaks, mainly caused by microparasites such as viruses, bacteria, fungi and protozoans. Thitamadee *et al.* (2016) describe recent diseases threatening Asian shrimp farming, the biggest source of shrimp worldwide. They indicate that a new emerging disease, acute hepatopancreatic necrosis disease (AHPND) is of most concern, together with the reoccurrence of viral diseases such as white spot syndrome virus and yellow head virus (Thitamadee *et al.,* 2016).

Importantly, the widespread presence of diseases leads to the use of chemicals and antibiotics in farming (Chen *et al.,* 2015; Holmström *et al.,* 2003; Rico *et al.,* 2012; Uchida

et al., 2016). The residues of these chemicals and antibiotics not only have a potentially adverse effect on human health such as causing the development of antibiotic resistance (Rocha *et al.,* 2016) and some are actually toxic (Somjetlerdcharoen, 2002), but they also lead to international trade disputes (FDA, 2016) and dramatic pollution of the environment (Le and Munekage, 2004). These negative impacts of chemicals and antibiotics on human health, trade and the environment infer that disease prevention and controls (e.g. biosecurity, good farm management and disease surveillance measures) are the best management interventions for shrimp farming (Brugere *et al.,* 2017; Chinabut and Puttinaowarat, 2005).

In terms of disease prevention and controls, in 1998 the Thai authorities launched the National Disease Surveillance and Monitoring Programme for Shrimp Farming (NACA, 2017). Furthermore, the movements of shrimp between source sites and destination sites, the most common pathway for site-to-site disease transmission, are controlled by the aquatic animal trade regulation of Thailand, B.E.2553 (2010). Authorised users record real-life live shrimp movements in a computer system; data that is referred to in this research as the Live Shrimp Movement Network or LSMN. Although the results of network modelling provide a good description of disease spread and are utilised in most control programmes (Green *et al.,* 2012; Keeling and Eames, 2005; Werkman *et al.,* 2011), the data from the LSMN has never been applied in network modelling to examine disease spread.

In order to protect the Thai shrimp farming from AHPND and other diseases, epizootiological studies are needed. Four epizootiological questions are therefore analysed in this research using a variety of tools. The investigated epizootiological questions consist of:

(1) What are the risk factors for the spread of AHPND at farm level? (Chapter 3);

(2) How does the network structure of live shrimp movements influence site-to-site disease transmission? (Chapter 4);

(3) How can we identify those live shrimp movements at high risk of disease transmission from site to site? (Chapter 5); and

(4) What is the overall AHPND prevalence when the disease is widespread through both long-distance and local transmission? (Chapter 6)

These research outcomes can be used to improve existing disease prevention and control measures and regulations, i.e. in respect to biosecurity, certification schemes for shrimp farming and disease surveillance and control programmes. The background of shrimp farming is described in more detail in the next section in order to justify the importance of this research.

1.2 The importance and current status of the shrimp farming sector

Given that the world population is expected to rise to 12.3 billion in 2100 (Gerland *et al.,* 2014), the aquaculture sector has a high potential to produce large amounts of human food compared with the fishery sector. As can be seen in the statistical report of the Food and Agriculture Organization of the United Nations (FAO, 2016b), there is a clear contrast between aquaculture and capture trends over the 29 years from 1985 to 2014: aquaculture production has increased gradually from 10 to 70 million tonnes, while production from fish capture has remained stable at c. 80–90 million tonnes.

Aquaculture offers various food commodities: fish, crustaceans and molluscs. Among the product varieties, penaeid shrimp (tiger shrimp *Penaeus monodon* and whiteleg shrimp *Litopenaeus vannamei*) are two of the dominant species for international trade. They play an important role in food consumption, and drive economic growth and enhance people's livelihood in many agricultural countries (FAO, 2016b). The countries shown in Figures 1.1 and 1.2, respectively, were the major shrimp exporters to the United States of America (USA) and the European Union (EU) in 2014 (FAO, 2015).

Moreover, farmed shrimp is a high-value product. In January 2017, shrimp prices (per kg) were two times more expensive than *Pangasius* sp., a commercial freshwater fish (FAO, 2017). The high levels of income that it is possible to make from shrimp means that shrimp farming has become widespread (Filose, 1995). The net income, for example, of small-scale intensive farming in India was around 2 000 USD per hectare and 9 000 USD per hectare for medium-scale intensive farming (Bhattacharya and Ninan, 2011).

These show the importance of shrimp farming for global food supply and socio-economic status. When disease is widespread, therefore, the huge economic loss is the obvious outcome throughout the supply chain of farmed shrimp.

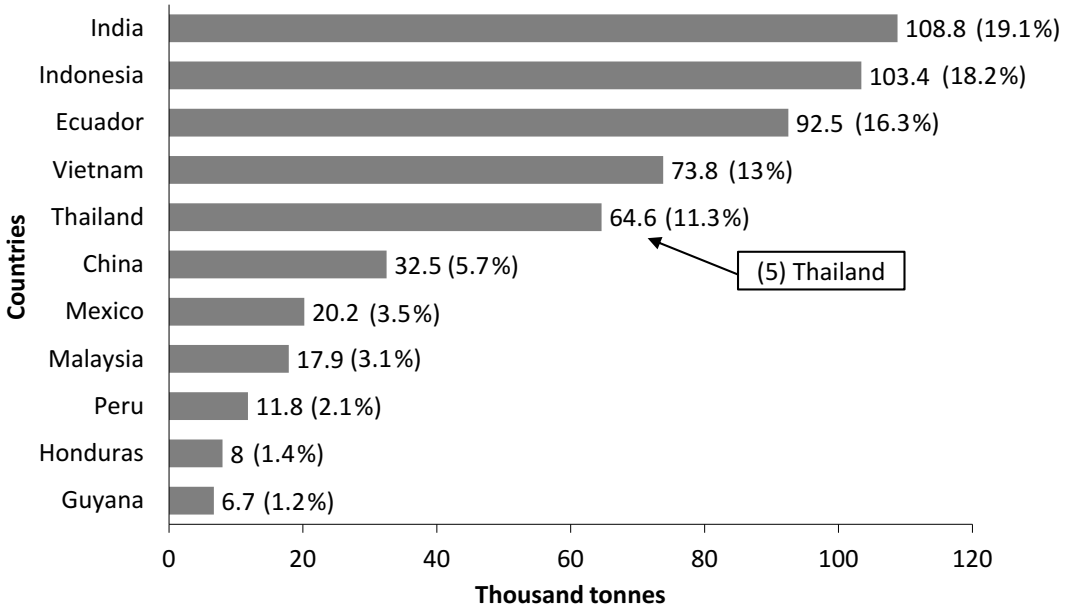


Figure 1.1 Volume and percentage of shrimp products (raw shrimp and value-added shrimp) imports to the USA market by the major producing countries in 2014 (unit: thousand tonnes). The total shrimp imported into USA was around 570 thousand tonnes. Thailand was the fifth-largest supplier (FAO, 2015).

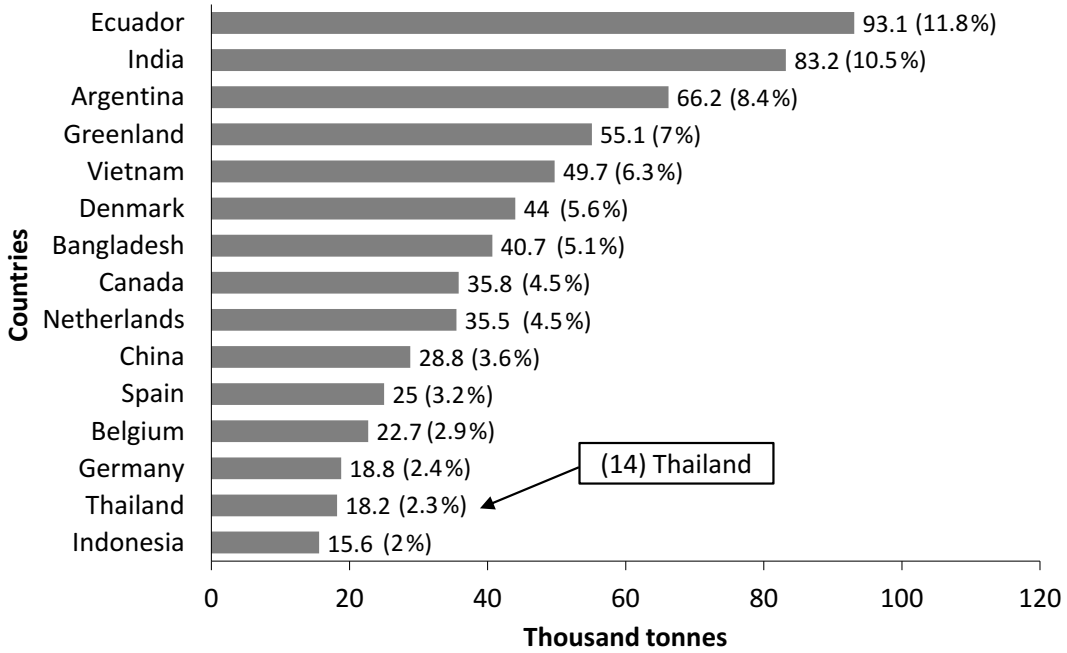


Figure 1.2 Volume and percentage of shrimp products (raw shrimp and value-added shrimp) imports to EU markets by the major producing countries in 2014 (unit: thousand tonnes). The total shrimp imported into the EU was around 790 thousand tonnes. Thailand was the 14th-largest supplier (FAO, 2015).

1.3 The supply chain of farmed shrimp, related regulations for controlling production and social communities of shrimp producers in Thailand

This section outlines the supply chain of Thai shrimp production. It also describes Thai regulations (i.e. movement controls, site certification and farming registration), and the social communities of shrimp producers in Thailand that have been incorporated into our research.

The supply chain for farmed shrimp in Thailand is simple (Figure 1.3). For hatchery production of shrimp seed, the wild broodstock of *Penaeus monodon* is either captured from the sea or cultured in a breeding programme, whereas using broodstock of *Litopenaeus vannamei* from a breeding programme is a common practice (Lebel *et al*., 2010). Alam (2015) and Uddin (2008) demonstrate that the shrimp industry in Thailand entails three steps for passing the product between a hatchery site and domestic and global markets. In this research, however (Chapters 4, 5 and 6), the network modelling of disease epizootics has been focused on the transmission between seed-producing sites (hatcheries and nurseries) and ongrowing sites, a process that denotes a large number of live shrimp movements for farming. The remaining steps (i.e. movements of chilled or frozen shrimp from ongrowing sites to traders, brokers, processing plants or auction markets) should not pose a risk of spreading to shrimp farming sites.

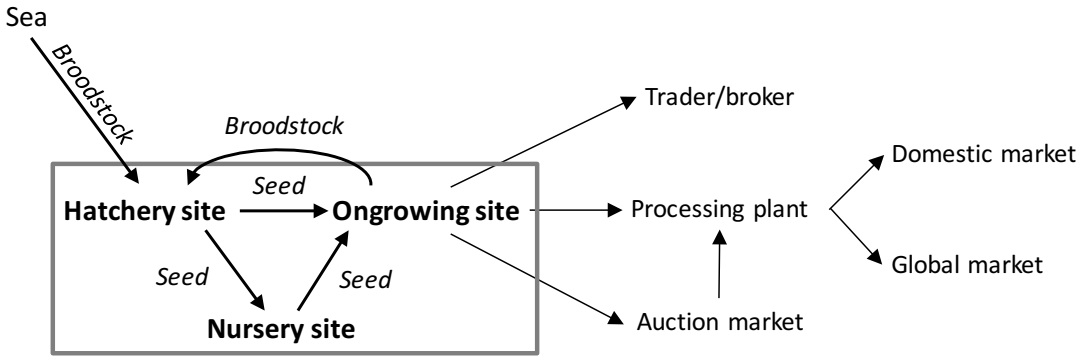


Figure 1.3 The shrimp production chain in Thailand (modified from Alam, 2015 page 103). The live shrimp movement data used in the research demonstrate the movements of live shrimp (shrimp seed) between hatchery, nursery and ongrowing sites, as shown in the box.

Uddin (2008) indicates that the supply chain for farmed shrimp starts from hatchery sites, which produce shrimp seed for ongrowing sites. Instead of direct selling to the ongrowing sites, hatchery sites pass some of their production to nursery sites at nauplius stage or initial postlarval stage. Then, nursery sites rear the seed from the nauplius until the postlarval (PL) stage (mostly PL 10; reared for 20 days) before selling the production to the ongrowing sites (FAO, 2014). Commonly, the production period for whiteleg shrimp (*L. vannamei*) is 105–120 days at pond level with a density at 400 000–500 000 shrimp per hectare, obtaining a harvest size of 21–25 g for processing plants (Wyban, 2007). Some producers practice a higher density stocking of 900 000–1 200 000 shrimp per hectare for a targeted size of 12–18 g in case of partial harvest, and 24 g for final harvest. Currently, shrimp farming can generate a production capacity of two or three cycles per year (Limsuwan, 2009). Shrimp farming with a production capacity of three cycles per year tends to have a high risk of diseases because a fallow period to treat and disinfect pathogens is shorter than that of farming one or two cycles per year (Cock *et al.,* 2009; Muniesa *et al.,* 2015).

The movements of live shrimp in each of the steps mentioned above are closely recorded in the live shrimp movement record (Kongkeo and Davy, 2010; Yamprayoon and Sukhumparnich, 2010). This record follows the aquatic animal trade regulation of Thailand, B.E.2553 (2010). All producers must inform the proper authorities (i.e. the Thailand Department of Fisheries staff and their representatives) about the movements of shrimp. Instead of a paper-based system to collect the shrimp movement data, a computer-based system has been used by Thai authorities since March 2013 and such electronic records subsequently are printed on a paper for checking (Songsanjinda, 2013). Moreover, all shrimp farming sites must be registered legally and their farming management practices should be inspected under the governmental certification schemes (Kongkeo and Davy, 2010). Farming standards and certification are developed to enhance food safety on aquaculture and sustainability including environment and livelihood reasons (Corsin *et al.,* 2007; Piumsombun *et al.,* 2005; Pongthanapanich and Roth, 2006; Yamprayoon and Sukhumparnich, 2010). The criteria for farming standards include good health management of farmed shrimp, disease prevention and control,

and the application of movement documents for traceability in the shrimp production chain (National Bureau of Agricultural Commodity and Food Standards, 2014).

As a regulatory requirement for the control of shrimp production, the live shrimp movement record in Thailand provides new and useful data for epizootiological studies. This official record of live shrimp movements can help to indicate potential routes of infectious disease transmission from site to site, and since 2013 these data are more readily obtained and analysed. The research presented in Chapters 4, 5 and 6 is the first study to use this data source for shrimp epizoology, however. Moreover, prior to this thesis, there is no evidence that the live shrimp movement data has been utilised as a part of disease prevention and control in the Thai farming certification scheme (i.e. for farm monitoring programme).

In addition, social communities of shrimp producers, i.e. shrimp farmer clubs, have an influence on disease prevention and controls. Shrimp farmer clubs support better farming practices (Kassam *et al.,* 2011), contributing to a decrease of disease outbreaks (Kongkeo and Davy, 2010). Importantly, the annual conferences arranged by these clubs generate an exchange of the ideas between producers and help to improve knowledge about farm practices and shrimp health management. When the causal agent of AHPND remained unknown, the Thai shrimp farmer clubs participated in setting up suitable broodstock feeding practices in hatcheries. Shrimp fry from broodstock treated with non-live feeds became a key agreement between shrimp sellers and buyers (Suratthani Shrimp Farmers Club, 2014). Consequently, the role of polychaete worms, bivalve molluscs and other live feeds in disease transmission to farmed shrimp could be decreased. Hence, social farming communities have participated in the effectiveness of disease control strategies in Thailand.

1.4 The characteristics of shrimp farming in Thailand

Figure 1.4 shows the approximately 20 000 shrimp farming sites in Thailand that together generate up to 600 000 tonnes of shrimp production annually (2011). Production decreased substantially in 2013, however, mainly due to disease problems (FAO, 2013a). The figure also illustrates a new era in the Thai shrimp industry in 2003,

when whiteleg shrimp were introduced to the Thailand in 2003. The introduction of new shrimp species, together with the development of new technologies and innovations, led to a large increase in total production with a smaller number of shrimp farming sites. These sites adopt more intensive systems, which often develop poor water quality and stressful condition in farmed shrimp (Kautsky *et al.,* 2000).

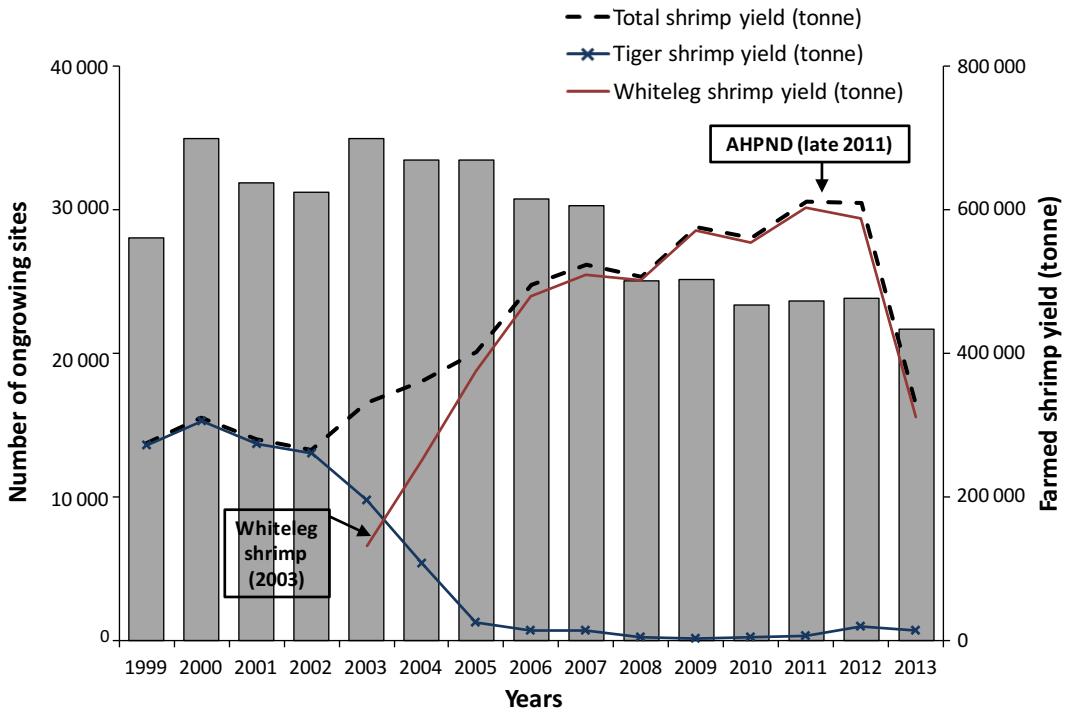


Figure 1.4 Overview of shrimp farming in Thailand for two major shrimp species: tiger shrimp and whiteleg shrimp, 1999–2013. The left axis of the graph presents the number of ongrowing sites. The right axis shows the yield (in tonnes) of farmed shrimp production (modified from Thailand DoF, 2016).

Regarding the geographic location of shrimp farming sites in Thailand (Figure 1.5), Thailand has an approximately 2 600 km-long shoreline along the Gulf of Thailand and the Andaman Sea (Tookwinas *et al.,* 2005), with a large number of shrimp farming sites are intensively established along these coastal areas. The remainder, called inland farming sites, are situated away from the shoreline, drawing water from rivers, canals or lakes. Not only are these water bodies the source of water for farming, but they are also used for discharge of nutrients and, concomitantly, pathogens during water-pond discharge (Barraza-Guardado *et al.,* 2013; Marchand *et al.,* 2014). This means that a group of neighbouring sites that share natural resources have a shared risk of disease transfer through hydrological connectivity.

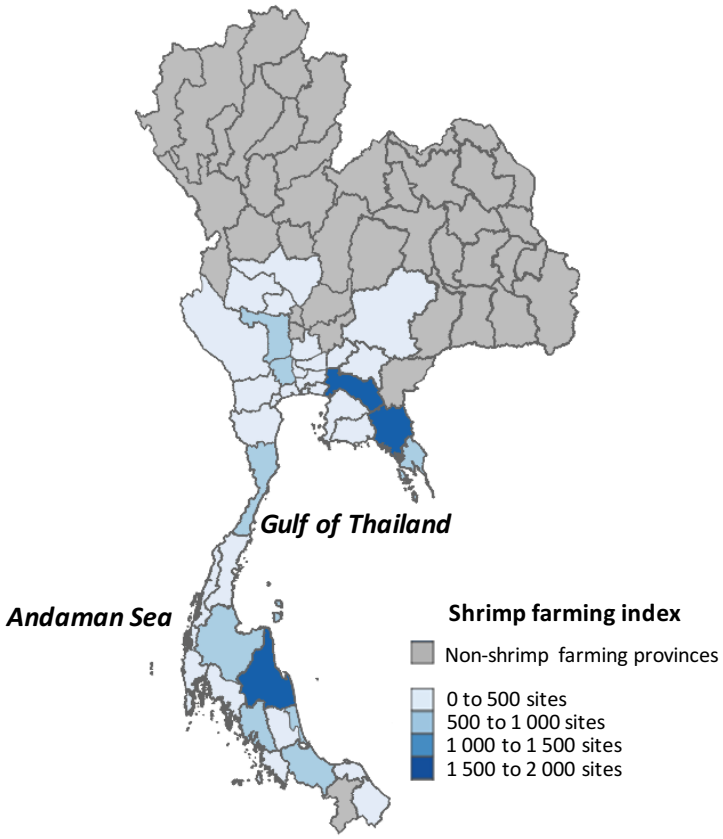


Figure 1.5 Distribution of Thai shrimp farming sites by province. Data summarised from the live shrimp movement data between March 2013 and March 2014 and figure illustrated using the *tmap* package, in the *R* Programme Environment (R foundation for statistical computing, 2015). The data were also used to construct network models in Chapters 4, 5 and 6.

The different geographic locations of shrimp farming sites mean that various water salinity levels are used for rearing shrimp. A high-salinity system, with a salinity range between 10–30 ppt, is applied to shrimp farming located near the coast, whereas the inland farming operates under a low-salinity system of 3–4 ppt (Flaherty *et al.,* 2000; Tookwinas *et al.,* 2005). Importantly, low salinity farming causes a decreased innate immune ability in shrimp and also reduces their resistance to bacterial diseases (Wang and Chen, 2006; Wang and Chen, 2005). The salinity parameter is only one of a number of environmental factors that affect susceptibility to infection in shrimp farming sites. The occurrence of diseases, however, is dependent on the nature of the disease (microparasitic or macroparasitic) and its transmission, which can be explained in terms of the epidemiological triad (Section 1.5.1).

1.5 Infectious diseases in farmed shrimp and their transmission

Microparasites have been a major cause of severe disease outbreaks in Asian shrimp farming over the past 30 years (Flegel, 2012). Sixty percent of losses are due to viral diseases, 20 % due to bacterial diseases (particularly vibriosis as described in Disease box 1), and the remaining losses due to other parasites (Flegel, 2012). Vaccination and immunostimulants are a challenge for inhibiting disease spread in shrimp farming (Johnson *et al.,* 2008; Namikoshi *et al.,* 2004). An example of successful vaccines is provided on vaccination trials with *P. monodon* to induce resistance to white spot syndrome virus (Vaseeharan *et al.,* 2006). Nevertheless, there is evidence that their use rarely succeeds in general farming for two reasons: the lack of an adaptive immune system in shrimp, and the presence of multiple pathogens within sites (Campos *et al.,* 2014; Cock *et al.,* 2009; Gräslund *et al.,* 2003; Gräslund and Bengtsson, 2001; Supungul *et al.,* 2015). Epizootiological studies to describe the occurrence of diseases and their transmission are therefore currently required in the shrimp farming industry.

To increase understanding of the dynamics of disease epizootics in shrimp farming, the nature of major shrimp diseases, i.e. vibriosis, white spot disease, yellow head disease, infectious hypodermal and haematopoietic necrosis, acute hepatopancreatic necrosis disease and taura syndrome, together with their routes of transmission, are briefly outlined in this section (Disease boxes 1–6 and Appendix A). Importantly, in Chapter 3 “Evaluating risk factors for transmission of acute hepatopancreatic necrosis disease (AHPND) in the Thai shrimp farming sector”, the visible clinical signs of these major shrimp diseases have been used in the differentiation of the AHPND-affected sites from sites affected by other diseases, and thus it is important to explain those clinical signs here.

Disease box 1: Vibriosis

Vibrio species that commonly cause vibriosis in farmed shrimp are, for example, *V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus* and *V. penaeicida* (Saulnier *et al.,* 2000). Clinical signs of infected shrimp vary with the type of *Vibrio* spp. Infected shrimp can have pale and opaque muscles and black stripes on the lateral cephalothorax present in infected shrimp (Longyant *et al.,* 2008). A bright-red syndrome is caused by *V. harveyi*, where the infected shrimp shows red discoloration spots on the abdomen (Soto-Rodriguez *et al.,* 2010). Shrimp infected with *V. cholerae* show an exterior visual appearance of leg yellowing (Cao *et al.,* 2015). Vibriosis leads to low survival rates in hatcheries and ongrowing sites. In many cases, outbreaks of vibriosis have caused mass mortality among small shrimp in hatchery sites, such as in Taiwan in 1994 (Liu *et al.,* 1996), and China in 1995 (Vandenberghe *et al.,* 1998). Vibriosis also largely occurred in farmed shrimp in Sri Lanka in 2010 (Heenatigala and Fernando, 2016).

Disease box 2: White spot disease

White spot disease (WSD) is an infection of shrimp by white spot syndrome virus or WSSV (OIE, 2013b). WSD was first reported in Taiwan in 1992 (Chou *et al.,* 1995). WSD spread has among many of the shrimp producing countries in Asia (e.g. China, Japan, Korea, Thailand, India and Bangladesh) within one or two years of its first detection (Escobedo-Bonilla *et al.,* 2008), and in North America in 1995 (Lightner, 1999). Most shrimp infected with WSSV exhibit white spots on their exoskeleton and lesions on the cephalotholax (Cheng *et al.,* 2013; Rodríguez *et al.,* 2003), however poor water quality such as high alkalinity or bacterial disease may also cause these white spots (OIE, 2013b). WSD infection results in high mortality in farmed shrimp, up to 100 % within one or two weeks (Rajendran *et al.,* 1999; Wu *et al.,* 2005).

Disease box 3: Yellow head disease

Yellow head disease (YHD) is an infection of shrimp by yellow head virus or YHV (OIE, 2013b). The first YHD epizootic was identified in Asia in 1990 (Walker and Winton, 2010). The economic losses from YHD outbreaks were reported at an estimated USD 3 million in Thailand (2008), for example (Senapin *et al.,* 2010). Gross signs of diseased shrimp include yellowish colouration and swollen cephalothorax (Lio-Po *et al.,* 2001). Cumulative mortality of 60–70 % has been reported among *P. monodon* and *L. vannamei* cultured in earthen ponds (Senapin *et al.,* 2010).

1.5.1 The occurrence of disease

An understanding of disease occurrence is important to shrimp epizoology. All diseases are multifactorial, as manifested by the epidemiological triad model (Figure 1.6). The diseases exist when there is interaction between pathogen, host and the environment.

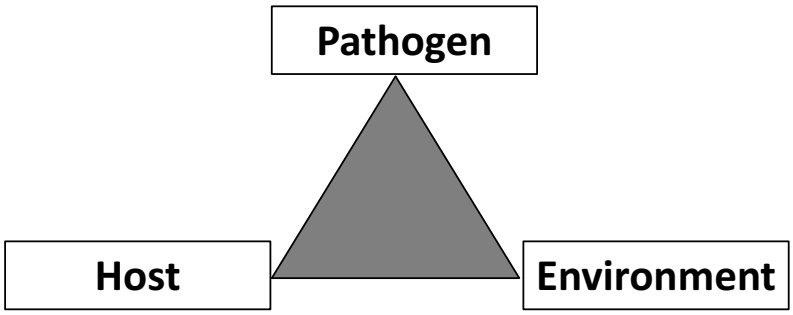


Figure 1.6 The epidemiological triad (from Rockett, 1999 page 10).

Pathogens, in terms of microparasites such as viruses and bacteria, cause numerous infections in shrimp farming, despite the fact that many control measures to prevent microparasitic infections have been developed and implemented. Microparasites are distinguished from macroparasites by their small size, the short time required to complete their generation, and their high ability for direct reproduction within hosts (Anderson and May, 1981). Thus, the incidence and prevalence of disease due to microparasites often tends to be high, particularly in farming conditions like shrimp rearing. One of the important measures to prevent microparasitic infections in shrimp

farming is the use of shrimp seed produced under specific pathogen free (SPF) conditions (Lightner *et al.,* 2009; Moss *et al.,* 2012). This measure, however, may not be sufficient due to the complexity in the life cycles of pathogens. For example, if WSSV is latent, WSD-infected shrimp may not be detected with a commercialised diagnostic test (He and Kwang, 2008; Khadijah *et al.,* 2003). In addition, in SPF conditions, shrimp are only free from specifically targeted pathogens, and thus remain at risk from non-targeted or unknown pathogen (Barman *et al.,* 2012). Hence, stocking SPF shrimp is just one measure for preventing and controlling disease occurrence in shrimp farming. The pathogen may not be detected in all cases of SPF shrimp, and such shrimp farming sites can be infected via other pathways.

Shrimp are hosts of diseases in this thesis. Shrimp are susceptible to a wide variety of pathogens, and especially to viruses (Lightner, 2011; Thitamadee *et al.,* 2016). Shrimp susceptibility to a particular disease is affected by the species concerned (Bell and Lightner, 1984; Lightner *et al.,* 1998; Overstreet *et al.,* 1997), tolerance to infection (Hameed *et al.,* 2000; Witteveldt *et al.,* 2006), and life history stage (Aguirre-Guzmán *et al.,* 2001; Sudha *et al.,* 1998). Many severe viral outbreaks in shrimp are due to persistent infections at a low level (Walker and Winton, 2010). Shrimp can also be infected by multiple pathogens such as by hepatopancreatic parvovirus and monodon baculovirus (Flegel *et al.,* 2004). This evidence demonstrates key conditions of disease occurrence, although, in reality, a variety of environmental factors influence the health of farmed shrimp.

The major environmental factors affecting shrimp farming have been evaluated for their association with diseases. Environmental factors that affect shrimp health include climate (Piamsomboon *et al.,* 2016), seasonality (Boonyawiwat, 2009) and storms (Zhang *et al.,* 2016). The experiments in Rahman *et al*. (2006) and Rahman *et al*. (2007) showed that a high water temperature of 33 °C was related directly to a reduction in shrimp mortality from WSD (Disease box 2), compared with a temperature of 27 °C. The lowest (0.5 %) and highest salinity levels (5.4 %) were associated with high WSD-related mortality in shrimp (Ramos-Carreño *et al.,* 2014). Environmental factors may also trigger disease reoccurrence, such as in the case of infection with IHHN (Disease box 4) and AHPND (Disease box 5). A shrimp pond infected with IHHN has a probability of repeated

IHHN onset when the temperature was below 24 °C (Montgomery-Brock *et al.,* 2007). In addition, a high pH level, at 8.5–8.8, was identified as associated with repeated AHPND onset in affected shrimp ponds in Malaysia (Akazawa and Eguchi, 2013). This evidence demonstrates that the environment affects the susceptibility to disease of farmed shrimp.

Disease box 4: Infectious hypodermal and haematopoietic necrosis

Infectious hypodermal and haematopoietic necrosis (IHHN) is infection of shrimp by infectious hypodermal and haematopoietic necrosis virus (OIE, 2013b). The first occurrence of IHHN in farmed shrimp was observed in Hawaii in the 1980's due to the movement of IHHN-infected postlarvae from Central or South America (Lightner *et al.,* 1983a). The cuticle of the shrimp is found to be whitish in diseased shrimp and, generally, there was high mortality (> 80 %) after shrimp moulting (Lightner *et al.,* 1983b). IHHN-infected shrimp often exhibit runt-deformity syndrome, resulting in a reduced growth rate, and a high presence of rostrum, antennae or cuticle deformity (Chayaburakul *et al.,* 2005; Kalagayan *et al.,* 1991).

Disease box 5: Acute hepatopancreatic necrosis disease

Acute hepatopancreatic necrosis disease (AHPND) occurs when shrimp are infected by specific strains of *Vibrio parahaemolyticus* bacteria (Tran *et al.,* 2013). Further, the bacteria produce a toxin that damages the hepatopancreas of shrimp (Lai *et al.,* 2015). AHPND has been invading major shrimp producing areas: China (2009), Vietnam (2010), Malaysia (2011), Thailand (2011/2012), Mexico (2013), and, most recently, the Philippines (2015). It causes high mortalities, up to 100 % within 35 days post stocking (Eduardo and Mohan, 2012; Kasornchandra, 2014). Gross signs can be seen in ongrowing ponds, i.e. empty gut and stomach, and pale and atrophied hepatopancreas of affected shrimp (NACA, 2014).

1.5.2 Risk factors for vibriosis outbreaks in farmed shrimp

The link between evidence for vibriosis in varied conditions and the actual field conditions and farming practices that leads to AHPND are reviewed here (Table 1.1). The geographic location of the farm has been suggested as an important factor in increasing the productivity of shrimp farming but also in the susceptibility to infection of shrimp farming sites (Zhu and Dong, 2013). Some examples of the relationship between the location of farming sites and the vibriosis are given in Table 1.1 (part 1). Gopal *et al*. (2005) found that the numbers of *Vibrio* in shrimp farming of in India were higher along the west coast than the south coast, at around 10^2 cfu per ml water. The establishment of farms near human communities also increased the risk of vibriosis due to large amounts of heavy organic material from the human community flowing into natural sources (Mohney *et al.,* 1994; Reilly and Twiddy, 1992). Farms located close to estuaries commonly faced widely fluctuating salinity levels, with high fluctuations of salinity from 35 % to 5 and 15 % being associated with an increased risk of *V. alginolyticus* infection in farmed whiteleg shrimp (Wang and Chen, 2005). Farms established in, or close to, agriculture areas risked contamination from methyl parathion (pesticides) that led to increased susceptibility to *V. parahaemolyticus* infection in shrimp (Roque *et al.,* 2005).

Climate appears to influence outbreaks of vibriosis among shrimp farms. The wet season has been related to the growth of *V. cholerae* (Reilly and Twiddy, 1992), while cooler temperatures, at 20 °C, have been related to an increased risk of *V. penaeicida* among cultured blue shrimp *P. stylirostris* (Goarant *et al.,* 2000); in turn, warmer water (changed from 27 to 32 °C) has been related to an increased risk of *V. alginolyticus* for cultured *L. vannamei* (Cheng *et al.,* 2005).

The most important factors that appear to influence vibriosis outbreaks, however, are inappropriate farming management practices. Shrimp fed untreated *Artemia* risked *V. parahaemolyticus* and *V. harveyi* infections (Quiroz-Guzmán *et al.,* 2013). High phytoplankton dynamics incurred an abundance of *Vibrio* spp. within shrimp ponds (Lemonnier *et al.,* 2016). Tho *et al.* (2012) demonstrated that sediment provided a better microenvironment for *Vibrio* than water and, particularly in the rainy season,

pond sediment provided the best habitat for *V. nigripulchritudo* (Goarant *et al.,* 2006; Walling *et al.,* 2010) and *V. cholerae* (Lekshmy *et al.,* 2014). As an attempt to decrease the large amount of pond sediment, therefore, generally earthen ponds have been lined with plastic sheets.

A lined pond is considered to be better practice in terms of reducing the volume of sediment. This is a technique that is generally applicable to intensive shrimp farming. Reilly and Twiddy (1992), however, have demonstrated that intensive farming systems increased the risk of *V. cholerae* infection in farmed shrimp. Rearing high numbers of shrimp within the lined ponds led to a high nutrient concentration in the water pond, which is important as a risk factor for vibriosis (Funge-Smith and Briggs, 1998). That high abundance of *V. cholera* and *V. parahaemolyticus* was caused by the heavy organic matter within shrimp ponds was proposed by Ganesh *et al.* (2010). High pH levels ($>$ 7), high salinity ($>$ 0.5 %) and high ammonia are also related to *Vibrio* abundance in shrimp ponds (Heenatigala and Fernando, 2016; Lekshmy *et al.,* 2014; Liu and Chen, 2004; Lokkhumlue and Prakitchaiwattana, 2013). A high risk of *Vibrio* diseases was detected whenever shrimp ponds lacked diversity of *Vibrio* communities (Sung *et al.,* 1999). Compared with lined ponds, earthen ponds could be fully prepared by drying, harrowing and filling with probiotic *Bacillus*, to reduce the risk of *Vibrio* (Moriarty, 1998; Nimrat *et al.,* 2008).

Vibriosis is a waterborne disease and extraction of culture water from the sea has been identified as a risk factor in terms of increasing the presence of *V. harveyi* and *V. splendidus* (Lavilla-Pitogo *et al.,* 1990), demonstrating the importance of water treatment at the beginning of stocking. Although farms often use recirculated water systems (meaning no extraction of culture water from the sea), *Vibrio* could still grow rapidly, principally due to the poor quality of the water reused in the farms (Colt, 2006). This is possibly related to the poor planning of the recirculating systems, which aids the transmission of diseases from infected ponds to others (Funge-Smith and Briggs, 1998; Mugnier *et al.,* 2013).

Vibriosis in shrimp farming often co-occurs with other pathogens. When farmed shrimp are infected by WSD, Kannapiran *et al.* (2009) as well as Selvin and Lipton (2003) found

that the risk of *V. harveyi* and *V. alginolyticus* infection is increased. Recently, there has been high prevalence of white feces disease (WFD; a protozoan infection) and AHPND incidence at the same shrimp farming sites (Limsuwan, 2010; Sriurairatana *et al.,* 2014). Somboon *et al.* (2012) indicated that the haemolymph and intestine of WFD-infected shrimp has high numbers of *V. vulnificus*.

Anthropogenic factors are shown to be associated with vibriosis in farmed shrimp. Examples of these anthropogenic factors include the lack of pathogen-free broodstock screening, and the use of equipment or facilities without disinfection (Chrisolite *et al.,* 2008).

The risk factors for vibriosis outbreaks in farmed shrimp descibed above will be used in designing our work (Chapter 3).

Table 1.1 Reviews of risk factors for vibriosis in shrimp farming

Risk factor	Host species	Pathogen species
(1) Farming location		
Different geographic location (between the west and the east coastal area of India)	*Penaeus* spp.	*Vibrio* spp. (Gopal, 2005)
Farming location near human communities	*Penaeus* spp.	*V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* (Mohney *et al.,* 1994), and *V. cholerae* (Reilly and Twiddy, 1992)
Farming located close to estuaries	*L. vannamei*	*V. alginolyticus* (Wang and Chen, 2005)
Farming located in or close to agricultural areas	*L. vannamei*	*V. parahaemolyticus* (Roque *et al.,* 2005)
(2) Climate		
Cool temperature (at 20 °C)	*P. stylirostris*	*V. penaeicida* (Goarant *et al.,* 2000)
Warm temperature (at 32 and 34 °C)	*L. vannamei*	*V. alginolyticus* (Cheng *et al.,* 2005)
Wet season	*Penaeus* spp.	*V. cholerae* (Reilly and Twiddy, 1992)
(3) Farming management practices		
Feeding shrimp with untreated *Artemia*	*Penaeus* spp.	*V. parahaemolyticus* and *V. harveyi* (Quiroz-Guzmán *et al.,* 2013)
High phytoplankton dynamics	*P. stylirostris*	*Vibrio* spp. (Lemonnier *et al.,* 2016)
Intensive shrimp aquaculture	*Penaeus* spp.	*V. cholerae* (Reilly and Twiddy, 1992)
Stressed shrimp and large amounts of the pathogen in ponds	*P. stylirostris*	*V. nigripulchritudo* (Mugnier *et al.,* 2013)
Large amounts of pond sediment	*Penaeus* spp.	*V. nigripulchritudo* (Goarant *et al.,* 2006; Walling *et al.,* 2010), *V. cholerae* (Lekshmy *et al.,* 2014), and *Vibrio* spp. (Tho *et al.,* 2012)
Heavy organic matter	*Penaeus* spp.	*V. cholerae* and *V. parahaemolyticus* (Ganesh *et al.,* 2010)
High pH (>7) and high salinity and ammonia levels	*P. monodon*	*V. alginolyticus*, *V. parahaemolyticus*, *V. damsela*, and *V. anguillarum* (Heenatigala and Fernando, 2016)

Table 1. 1 (cont.)

Risk factor	Host species	Pathogen species
Higher salinity level	*L. vannamei*	*V. parahaemolyticus* (Lokkhumlue and Prakitchaiwattana, 2013)
High concentration of ammonia in water	*L. vannamei*	*V. alginolyticus* (Liu and Chen, 2004)
No applications of either pond drying in sunlight or pond harrowing	*Penaeus* spp.	*Vibrio* spp. (Nimrat *et al.,* 2008)
Decreases in the diversity of the *Vibrio* community	*P. monodon*	*Vibrio* spp. (Sung *et al.,* 1999)
Using water sourced from the sea	*P. monodon*	*V. harveyi* and *V. splendidus* (Lavilla-Pitogo *et al.,* 1990)
(4) Viral and protozoan disease outbreaks		
Outbreaks of WSD	*P. monodon*	*V. harveyi* (Kannapiran *et al.,* 2009), and *V. alginolyticus* (Selvin and Lipton, 2003)
Outbreaks of WFD	*L. vannamei*	*V. vulnificus* (Somboon *et al.,* 2012)
(5) Anthropogenic activities		
Transmission of pathogens via broodstock, maturation and spawning facilities in the shrimp hatchery	*P. monodon*	*V. harveyi* (Chrisolite *et al.,* 2008)

1.5.3 Site-to-site transmission of shrimp diseases

In shrimp farming, the following modes appear to be the important routes in site-to-site disease transmission: long-distance via movements of live shrimp (either broodstock or shrimp seed), local spread via close proximity of sites, and sharing of water courses (Figure 1.7). However, not all transmission events fit this pattern and other routes of transmission exist.

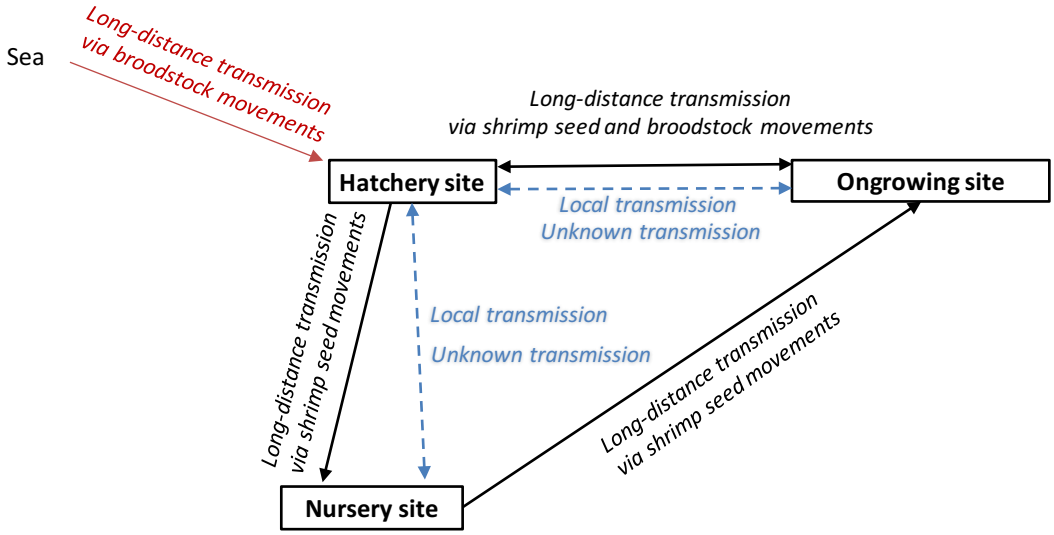


Figure 1.7 Major routes in site-to-site transmission of shrimp diseases

The most common route for transmitting diseases from site to site is long-distance transmission. With long-distance transmission, a susceptible site can be infected via the importation of infected shrimp. The pandemics of WSD, YHD, IHHN and TS (Disease box 2–4 and 6, respectively) in farmed shrimp in the USA were obvious examples of national epizootics resulting from the movements of live infected shrimp seed and broodstock (Lightner, 2003; Lightner *et al.,* 1997). It includes the potential transmission of AHPND, a new emerging outbreak in shrimp farming (OIE, 2013a).

Note that this research (Chapters 4, 5 and 6) focuses on the domestic epizootics in shrimp farming, where all site-to-site movements of live shrimp can serve as long-distance transmission routes at the country scale. Although movements of frozen shrimp products can lead to some infections, e.g. WSSV and YHV (Nunan *et al.,* 1998), these are not included in this research.

Disease box 6: Taura syndrome

Taura syndrome (TS) is the infection of shrimp by taura syndrome virus (OIE, 2013b). It was first detected in farmed shrimp in Ecuador in 1992 (Chaivisuthangkura *et al.,* 2016). TS was observed in Taiwan in 1998 due to introducing TS-infected shrimp from epizootic countries (Tu *et al.,* 1999). Diseased shrimp have a pale reddish colouration, with a red tail fan and pleopods, and are soft-shelled (Bonami *et al.,* 1997; Lightner *et al.,* 1995; Song *et al.,* 2003). Acute mortality of shrimp is possible within three days (ChunI and YenLing, 2000).

Another pathway for site-to-site disease transmission is via local spread, which often occurs due to anthropogenic activities. In Thailand, a few large-scale farms apply closed recirculating systems in shrimp rearing. The remaining farms, however, may conduct water discharge or water exchange (Boyd *et al.,* 2017; Flaherty *et al.,* 2000; Yaemkasem *et al.,* 2017). Although water exchange results in a decrease in the ammonia concentration in shrimp ponds (Hopkins *et al.,* 1993), where the exchange occurs directly between shrimp ponds and natural water courses (e.g. canals, lakes, rivers and the sea), without proper water treatment, this can contribute to widespread disease through hydrological connectivity (Anh *et al.,* 2010; Pruder, 2004; Tendencia *et al.,* 2011). Additionally, the absence of installation of crab and bird fencing in shrimp farming can aid local spread of disease due to physical proximity of sites, as described in Balakrishnan *et al.* (2011) and Kumaran (2009).

The movement of fomites (inanimate objects such as farming facilities, vehicles and staff's clothes) aids local and long-distance transmission by introducing diseases to susceptible sites (Rodgers *et al.,* 2011). Corsin *et al.* (2005) found that there was no strong association between potential fomites (sharing farming facilities and staff) and disease spread in shrimp farming, despite fomites often being subject to disease mitigation measures, such as the sanitation of incoming vehicles and disinfection of facilities in farming (Bondad-Reantaso, 2016; Dvorak, 2009; Mohan *et al.,* 2004; Yanong and Erlacher-Reid, 2012).

For modelling purposes, the final mode for disease transmission between sites is unknown transmission with a usual exposure to risk factor but in which a major transmission pathway is not identified.

The nature of shrimp diseases and their transmission, as presented in this section, are important in forming an epizootiological study for AHPND and other diseases. Crucially, a better understanding of the occurrence and transmission of infectious diseases in farmed shrimp can be achieved with modelling approaches. Among these models, this study is interested in network models and compartmental epidemic models, which are discussed in Chapter 2 “Epidemiological and epizootiological tools for design of disease prevention and control strategies”.

1.6 Research outline

The purpose of this research is to develop epizootiological tools to evaluate the spread of AHPND in the Thai shrimp farming industry. It is important to know the economic and sociological importance of shrimp farming, the characteristics of Thai shrimp farming, the occurrence of diseases and their transmission, and the disease prevention and control measures that have been developed. The thesis, therefore, includes a review of the literature on the epidemiological and epizootiological tools for various sectors, allowing the development of effective tools to prevent and control the spread of disease spread individual site and country levels.

This first chapter has been written to give a general introduction to epizootiological tools for AHPND and other known diseases in the Thai shrimp farming sector, and to demonstrate the research outline here. The remainder of the research aims to:

- Investigate the risk factors for acute hepatopancreatic necrosis disease (AHPND);
- Demonstrate the structure of the live shrimp movement network (LSMN), which poses potential for site-to-site transmission of AHPND and other known infectious diseases;

- Identify connections in the LSMN posing a high risk for disease transmission, leading towards the development of disease surveillance and control algorithms for Thai shrimp farming; and,

- Model the dynamics of AHPND epizootics in shrimp farming sites, where the model considers both long-distance and local transmission.

The research has been divided into seven chapters; an overview of the chapters and their linkage is given in Figure 1.8.

The following chapter (Chapter 2) discusses the relevant literature. It explores: (1) study designs in epidemiology and epizoology, (2) graph or network theory, and (3) epidemic models and network models.

In the third chapter, as a recent outbreak in shrimp farming, the risk factors for AHPND were investigated by an epizootiological survey at farm level. A cross-sectional study is described. This study linked with the data from the Sustaining Ethical Aquaculture Trade (SEAT), EU FP7 research project. Importantly, this survey data, i.e. disease mitigation measures, farming management practices and the cumulative AHPND incidence, has also been used in interpreting results of Chapter 4 and in modelling the spread of AHPND of Chapter 6.

In the fourth chapter, graph or network theory is applied for the first time to the Thai shrimp farming sector. The chapter demonstrates the industry susceptibility to infection via long-distance transmission (i.e. live shrimp movement) based on the real network of live shrimp movements in Thailand (LSMN). It contains a quantitative analysis of the LSMN including properties such as degrees, average path length, clustering coefficients and assortativity. The results of this chapter inform the fifth chapter.

Disease-control algorithms are developed to identify high-risk connections in the Thai shrimp farming network (Chapter 5). These algorithms include various network centrality measurements (e.g. betweenness, eigenvector and degree), and their capacities in reducing the potential epizootic size in the LSMN are measured by the reachability of sites (in network terminology, nodes) and connected components. The

best algorithm can be used as a control strategy. Moreover, disease outbreaks do not only lead to financial losses for producers and interruption of business, but they also influence annual government expenditure (FAO, 2013b). The disease-control algorithm developed in this chapter will therefore be concerned with operating costs as well as effectiveness.

In the sixth chapter, the dynamics of AHPND epizootics are explained using a network-based epidemic model. The results indicate the seasonality of AHPND spread, and the effect of long-distance and local transmission on the AHPND epizootic dynamics in Thailand; they also suggest disease prevention and control measures to explore.

All the results of the research are discussed in the final chapter, including the contribution of the research to the Thai shrimp farming sector, and potential future work.

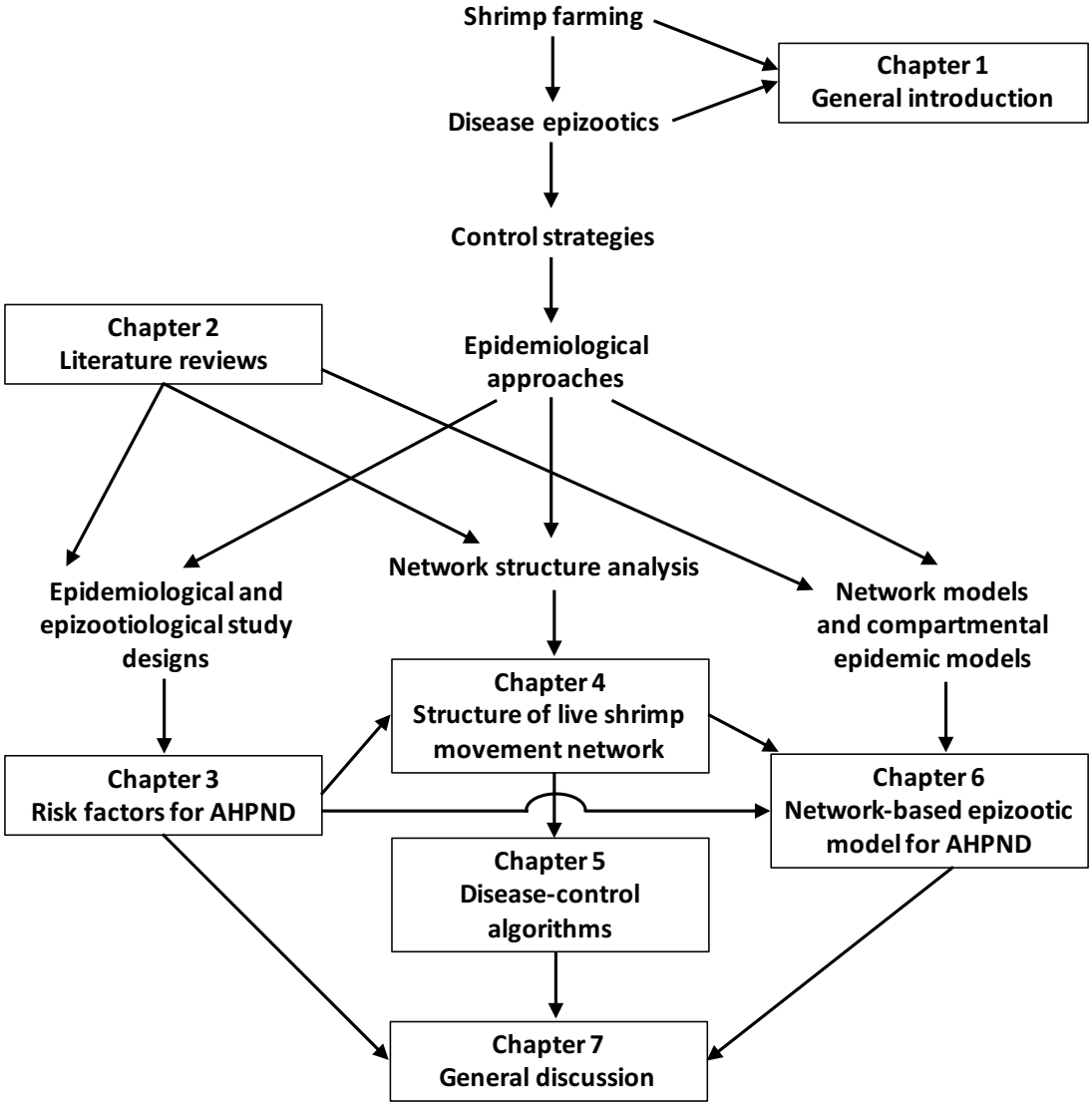


Figure 1.8 Outline of the "Epizootiological tools for AHPND in Thai shrimp farming" research.

1.7 References

Aguirre-Guzmán, G., Vázquez-Juárez, R. and Ascencio, F. (2001) Differences in the susceptibility of American white shrimp larval substages (*Litopenaeus vannamei*) to four *Vibrio* species. *Journal of Invertebrate Pathology,* 78 (4), pp. 215–219.

Akazawa, N. and Eguchi, M. (2013) Environmental trigger for EMS/AHPNS identified in Agrobest shrimp ponds. Global Aquaculture Advocate July/August 2013, pp. 16–17.

Alam, S.N. (2015) Safety in the shrimp supply chain. In: P. Vishweshwaraiah *et al.*, ed. *Regulating safety of traditional and ethnic foods.* Oxford: Academic Press, pp. 99–124.

Anderson, R.M. and May, R.M. (1981) The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 291 (1054), pp. 451–524.

Anh, P.T., Kroeze, C., Bush, S.R. and Mol, A.P.J. (2010) Water pollution by intensive brackish shrimp farming in south-east Vietnam: causes and options for control. *Agricultural Water Management,* 97 (6), pp. 872–882.

Balakrishnan, G., Peyail, S., Ramachandran, K., Theivasigamani, A., Savji, K.A., Chokkaiah, M. and Nataraj, P. (2011) Growth of cultured white leg shrimp *Litopenaeus vannamei* (Boone 1931) in different stocking density. Advances in Applied Science Research, 2 (3), pp. 107–113.

Barman, D., Kumar, V., Roy, S. and Mandal, S.C. (2012) Specific pathogen free shrimps: Their scope in aquaculture. *World Aquaculture,* 43 (1), pp. 67.

Barraza-Guardado, R.H., Arreola-Lizarraga, J.A., Lopez-Torres, M.A., Casillas-Hernandez, R., Miranda-Baeza, A., Magallon-Barrajas, F. and Ibarra-Gamez, C. (2013) Effluents of shrimp farms and its influence on the coastal ecosystems of Bahia de Kino, Mexico. *The Scientific World Journal,* 2013, pp. 1–8.

Bell, T.A. and Lightner, D.V. (1984) IHHN virus: infectivity and pathogenicity studies in *Penaeus stylirostris* and *Penaeus vannamei*. *Aquaculture,* 38 (3), pp. 185–194.

Bhattacharya, P. and Ninan, K.N. (2011) Social cost-benefit analysis of intensive versus traditional shrimp farming: a case study from India. *Natural Resources Forum,* 35 (4), pp. 321–333.

Bonami, J.R., Hasson, K.W., Mari, J., Poulos, B.T. and Lightner, D.V. (1997) Taura syndrome of marine penaeid shrimp: characterisation of the viral agent. *The Journal of General Virology,* 78 (Pt 2), pp. 313–319.

Bondad-Reantaso, M.G. (2016) Acute hepatopancreatic necrosis disease (AHPND) of penaeid shrimps: Global perspective. In: R. V. Pakingking Jr. and E. G. T. de Jesus-Ayson, ed. *Proceedings of the ASEAN Regional Technical Consultation on EMS/AHPND and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia*, 22nd to 24th February 2016. Aquaculture Department, Southeast Asian Fisheries Development Center, pp. 16–23.

Boonyawiwat, V. (2009) *Traditional and molecular epidemiology to determine risk factors for outbreaks of shrimp white spot disease in Thailand.* PhD, Kasetsart University.

Boyd, C.E., McNevin, A.A., Racine, P., Tinh, H.Q., Minh, H.N., Viriyatum, R., Paungkaew, D. and Engle, C. (2017) Resource use assessment of shrimp, *Litopenaeus vannamei* and *Penaeus monodon*, production in Thailand and Vietnam. *Journal of the World Aquaculture Society*, 48 (2), pp. 201–226.

Brugere, C., Onuigbo, D.M. and Morgan, K.L. (2017) People matter in animal disease surveillance: challenges and opportunities for the aquaculture sector. *Aquaculture*, 467, pp. 158–169.

Campos, L.N.S., Herrera, F.D., Araujo, A.D.R. and Sánchez, R.A.G. (2014) *Litopenaeus vannamei* immunestimulated with *Macrocystis pyrifera* extract: improving the immune response against *Vibrio campbellii*. *Journal of Coastal Life Medicine,* 2 (8), pp. 617–624.

Cao, H., An, J., Zheng, W. and He, S. (2015) *Vibrio cholerae* pathogen from the freshwater-cultured whiteleg shrimp *Penaeus vannamei* and control with *Bdellovibrio bacteriovorus*. *Journal of Invertebrate Pathology,* 130, pp. 13–20.

Chaivisuthangkura, P., Vaniksampanna, A., Pasookhush, P., Longyant, S. and Sithigorngul, P. (2016) Taura syndrome virus. In: D. Liu, ed. *Molecular detection of animal viral pathogens.* New York: CRC Press, pp. 17–25.

Chayaburakul, K., Lightner, D.V., Sriurairattana, S., Nelson, K.T. and Withyachumnarnkul, B. (2005) Different responses to infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Penaeus monodon* and *P. vannamei*. *Diseases of Aquatic Organisms,* 67 (3), pp. 191–200.

Chen, H., Liu, S., Xu, X., Liu, S., Zhou, G., Sun, K., Zhao, J. and Ying, G. (2015) Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Marine Pollution Bulletin,* 90 (1–2), pp. 181–187.

Cheng, L., Lin, W., Wang, P., Tsai, M., Hsu, J. and Chen, S. (2013) White spot syndrome virus epizootic in cultured Pacific white shrimp *Litopenaeus vannamei* (Boone) in Taiwan. *Journal of Fish Diseases,* 36 (12), pp. 977–985.

Cheng, W., Wang, L. and Chen, J. (2005) Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to *Vibrio alginolyticus*. *Aquaculture*, 250 (3–4), pp. 592–601.

Chinabut, S. and Puttinaowarat, S. (2005) The choice of disease control strategies to secure international market access for aquaculture products. *Developments in Biologicals*, 121, pp. 255–261.

Chou, H., Huang, C., Wang, C., Chiang, H. and Lo, C. (1995) Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Diseases of Aquatic Organisms,* 23 (3), pp. 165–173.

ChunI, Y. and YenLing, S. (2000) Outbreaks of taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Fish Pathology,* 35 (1), pp. 21–24.

Cock, J., Gitterle, T., Salazar, M. and Rye, M. (2009) Breeding for disease resistance of penaeid shrimps. *Aquaculture,* 286 (1–2), pp. 1–11.

Colt, J. (2006) Water quality requirements for reuse systems. *Aquacultural Engineering*, 34 (3), pp. 143–156.

Corsin, F., Funge-Smith, S. and Clausen, J. (2007) *A qualitative assessment of standards and certification schemes applicable to aquaculture in the Asia–Pacific region.* RAP Publication 2007/25. Bangkok: FAO. Available: http://www.fao.org/3/a-ai388e.pdf [Accessed: 18 March 2017].

Corsin, F., Turnbull, J.F., Mohan, C.V., Hao, N.V. and Morgan, K.L. (2005) Pond-level risk factors for white spot disease outbreaks. *Diseases in Asian Aquaculture V,* pp. 75–92.

Dvorak, G. (2009) *Biosecurity for aquaculture facilities in the north central region.* NCRAC Fact Sheet. Paper 8. Iowa State: North Central Regional Aquaculture Center. Available: http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1007&context=ncrac_factsheets [Accessed: 13 March 2015].

Eduardo, M.L. and Mohan, C.V. (2012) *Early mortality syndrome (EMS)/Acute hepatopancreatic necrosis syndrome (AHPNS): an emerging threat in the Asian shrimp industry.* Bangkok: NACA. Available: http://library.enaca.org/Health/DiseaseLibrary/disease-advisory-ems-ahpns.pdf [Accessed: 15 August 2014].

Escobedo-Bonilla, C.M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M. and Nauwynck, H. (2008) A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *Journal of Fish Diseases,* 31 (1), pp. 1–18.

FAO (2013a) *GLOBEFISH - Analysis and information on world fish trade (Shrimp - September 2013).* Available: http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/338065/ [Accessed: 12 November 2015].

FAO (2013b) *Report of the FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304).* FAO Fisheries and Aquaculture Report No. 1053. Rome: FAO. Available: http://www.fao.org/docrep/018/i3422e/i3422e00.htm [Accessed: 11 October 2013].

FAO (2014) *Cultured aquatic species information programme Penaeus vannamei (Boone, 1931).* Rome: FAO. Available: http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en [Accessed: 29 April 2017].

FAO (2015) *A quarterly update on world seafood markets.* Issue 2/2015. Rome: FAO. Available: http://www.fao.org/3/a-bc009e.pdf [Accessed: 29 April 2017].

FAO (2016a) *Food outlook, biannual report on global food markets.* ISSN 0251-1959. Rome: FAO. Available: http://www.fao.org/3/a-I5703E.pdf [Accessed: 29 April 2017].

FAO (2016b) *The state of world fisheries and aquaculture 2016.* ISBN 978-92-5-109185-2. Rome: FAO. Available: http://www.fao.org/3/a-i5555e.pdf [Accessed: 8 July 2016].

FAO (2017) *European price report.* Issue 1/2017. Rome: FAO. Available: http://www.fao.org/3/a-br288e.pdf [Accessed: 29 April 2017].

FDA (2016) *FDA issues import alert on imported shrimp and prawns from Peninsular Malaysia.* Available: http://www.fda.gov/food/newsevents/constituentupdates/ucm496475.htm [Accessed: 22 June 2016].

Filose, J. (1995) Factors affecting the processing and marketing of farm raised shrimp. In: C.L. Browdy and J.S. Hopkins, ed. *Swimming through Troubled Water: Proceedings of the Special Session on Shrimp Farming.* California, 1st to 4th Feburary 1995. Baton Rouge, Louisiana: World Aquaculture Society, pp. 227–234.

Flaherty, M., Szuster, B. and Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio,* 29 (3), pp. 174–179.

Flegel, T.W. (2012) Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology,* 110 (2), pp. 166–173.

Flegel, T.W., Nielsen, L., Thamavit, V., Kongtim, S. and Pasharawipas, T. (2004) Presence of multiple viruses in non-diseased, cultivated shrimp at harvest. *Aquaculture,* 240 (1–4), pp. 55–68.

Funge-Smith, S.J. and Briggs, M.R.P. (1998) Nutrient budgets in intensive shrimp ponds: implications for sustainability. *Aquaculture*, 164 (1–4), pp. 117–133.

Ganesh, E.A., Das, S., Chandrasekar, K., Arun, G. and Balamurugan, S. (2010) Monitoring of total heterotrophic bacteria and *Vibrio* spp. in an aquaculture pond. *Curr.Res.J.Biol.Sci*, 2 (1), pp. 48–52.

Gerland, P., Raftery, A.E., Sevcikova, H., Li, N., Gu, D., Spoorenberg, T., Alkema, L., Fosdick, B.K., Chunn, J., Lalic, N., Bay, G., Buettner, T., Heilig, G.K. and Wilmoth, J. (2014) World population stabilization unlikely this century. *Science (New York, N.Y.),* 346 (6206), pp. 234–237.

Goarant, C., Ansquer, D., Herlin, J., Domalain, D., Imbert, F. and De Decker, S. (2006) "Summer Syndrome" in *Litopenaeus stylirostris* in New Caledonia: Pathology and epidemiology of the etiological agent, *Vibrio nigripulchritudo*. *Aquaculture*, 253 (1–4), pp. 105–113.

Goarant, C., Herlin, J., Brizard, R., Marteau, A., Martin, C. and Martin, B. (2000) Toxic factors of *Vibrio* strains pathogenic to shrimp. *Diseases of Aquatic Organisms*, 40 (2), pp. 101–107.

Gopal, S. (2005) The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. *International Journal of Food Microbiology*, 102 (2), pp. 151–159.

Gräslund, S. and Bengtsson, B. (2001) Chemicals and biological products used in south-east Asian shrimp farming, and their potential impact on the environment — a review. *Science of the Total Environment,* 280 (1–3), pp. 93–131.

Gräslund, S., Holmström, K. and Wahlström, A. (2003) A field survey of chemicals and biological products used in shrimp farming. *Marine Pollution Bulletin,* 46 (1), pp. 81–90.

Green, D.M., Werkman, M. and Munro, L.A. (2012) The potential for targeted surveillance of live fish movements in Scotland. *Journal of Fish Diseases,* 35 (1), pp. 29–37.

Hameed, A.S., Charles, M.X. and Anilkumar, M. (2000) Tolerance of *Macrobrachium rosenbergii* to white spot syndrome virus. *Aquaculture,* 183 (3), pp. 207–213.

He, F. and Kwang, J. (2008) Identification and characterization of a new E3 ubiquitin ligase in white spot syndrome virus involved in virus latency. *Virology Journal,* 5 (1), pp. 151.

Heenatigala, P. and Fernando, M. (2016) Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures. *Sri Lanka Journal of Aquatic Sciences,* 21 (1), pp. 1–17.

Holmström, K., Gräslund, S., Wahlström, A., Poungshompoo, S., Bengtsson, B. and Kautsky, N. (2003) Antibiotic use in shrimp farming and implications for environmental impacts and human health. *International Journal of Food Science & Technology,* 38 (3), pp. 255–266.

Hopkins, J.S., Hamilton, R.D., Sandier, P.A., Browdy, C.L. and Stokes, A.D. (1993) Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. *Journal of the World Aquaculture Society,* 24 (3), pp. 304–320.

Johnson, K.N., van Hulten, M.C.W. and Barnes, A.C. (2008) "Vaccination" of shrimp against viral pathogens: Phenomenology and underlying mechanisms. *Vaccine,* 26 (38), pp. 4885–4892.

Kalagayan, H., Godin, D., Kanna, R., Hagino, G., Sweeney, J., Wyban, J. and Brock, J. (1991) IHHN virus as an etiological factor in runt-deformity syndrome (RDS) of juvenile *Penaeus vannamei* cultured in Hawaii. *Journal of the World Aquaculture Society,* 22 (4), pp. 235–243.

Kannapiran, E., Ravindran, J., Chandrasekar, R. and Kalaiarasi, A. (2009) Studies on luminous, *Vibrio harveyi* associated with shrimp culture system rearing *Penaeus monodon*. *Journal of Environmental Biology*, 30 (5), pp. 791−795.

Kasornchandra, J. (2014) *The major diseases of shrimp and marine fish and the epidemic prevention.* Bangkok: Thailand Department of Fisheries (DoF).

Kassam, L., Subasinghe, R. and Philips, M. (2011) *Aquaculture farmer organizations and cluster management.* FAO Fisheries Technical Paper No. 563. Rome: FAO. Available: http://www.fao.org/docrep/014/i2275e/i2275e.pdf [Accessed: 29 April 2017].

Kautsky, N., Rönnbäck, P., Tedengren, M. and Troell, M. (2000) Ecosystem perspectives on management of disease in shrimp pond farming. *Aquaculture,* 191 (1–3), pp. 145−161.

Keeling, M.J. and Eames, K.T. (2005) Networks and epidemic models. *Journal of the Royal Society Interface,* 2 (4), pp. 295–307.

Khadijah, S., Neo, S.Y., Hossain, M.S., Miller, L.D., Mathavan, S. and Kwang, J. (2003) Identification of white spot syndrome virus latency-related genes in specific-pathogen-free shrimps by use of a microarray. *Journal of Virology,* 77 (18), pp. 10162–10167.

Kongkeo, H. and Davy, F.B. (2010) Backyard hatcheries and small scale shrimp and prawn farming in Thailand. In: S.S. De Silva and F.B. Davy, eds. *Success stories in Asian aquaculture.* Dordrecht: Springer, pp. 67–83.

Kumaran, M. (2009) Group approach to shrimp farming: the key to sustainability. Aquac Asia, 14 (3), pp. 18–21.

Lai, H., Ng, T.H., Ando, M., Lee, C., Chen, I., Chuang, J., Mavichak, R., Chang, S., Yeh, M., Chiang, Y., Takeyama, H., Hamaguchi, H., Lo, C., Aoki, T. and Wang, H. (2015) Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. *Fish & Shellfish Immunology,* 47 (2), pp. 1006–1014.

Lavilla-Pitogo, C.R., Baticados, M.C.L., Cruz-Lacierda, E.R. and de la Pena, L.D. (1990) Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture*, 91 (1), pp. 1–13.

Le, T.X. and Munekage, Y. (2004) Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam. *Marine Pollution Bulletin,* 49 (11–12), pp. 922–929.

Lebel, L., Mungkung, R., Gheewala, S.H. and Lebel, P. (2010) Innovation cycles, niches and sustainability in the shrimp aquaculture industry in Thailand. *Environmental Science & Policy*, 13 (4), pp. 291–302.

Lekshmy, S., Nansimole, A., Mini, M., Athira, N. and Radhakrishnan, T. (2014) Occurrence of *Vibrio cholerae* in shrimp culture environments of Kerala, India. *Indian Journal of Scientific Research,* 5 (2), pp. 151.

Lemonnier, H., Lantoine, F., Courties, C., Guillebault, D., Nézan, E., Chomérat, N., Escoubeyrou, K., Galinié, C., Blockmans, B. and Laugier, T. (2016) Dynamics of phytoplankton communities in eutrophying tropical shrimp ponds affected by vibriosis. *Marine Pollution Bulletin,* 110 (1), pp. 449–459.

Lightner, D.V. (1999) The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV. *Journal of Applied Aquaculture,* 9 (2), pp. 27–52.

Lightner, D.V. (2003) The penaeid shrimp viral pandemics due to IHHNV, WSSV, TSV and YHV: history in the Americas and current status. *UJNR Aquaculture Panel Symposium.* California, USA, 17th to 18th and 20th November 2003, pp. 17–20.

Lightner, D.V. (2011) Virus diseases of farmed shrimp in the western hemisphere (the Americas): a review. *Journal of Invertebrate Pathology,* 106 (1), pp. 110–130.

Lightner, D.V., Hasson, K., White, B. and Redman, R. (1998) Experimental infection of western hemisphere penaeid shrimp with Asian white spot syndrome virus and Asian yellow head virus. *Journal of Aquatic Animal Health,* 10 (3), pp. 271–281.

Lightner, D.V., Redman, R.M. and Bell, T.A. (1983b) Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. *Journal of Invertebrate Pathology,* 42 (1), pp. 62–70.

Lightner, D.V., Redman, R.M., Arce, S. and Moss, S.M. (2009) Specific pathogen-free shrimp stocks in shrimp farming facilities as a novel method for disease control in crustaceans. *Shellfish Safety and Quality,* pp. 384–424.

Lightner, D.V., Redman, R.M., Bell, T.A. and Brock, J.A. (1983a) Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. *Journal of the World Mariculture Society,* 14 (1–4), pp. 212–225.

Lightner, D.V., Redman, R.M., Hasson, K.W. and Pantoja, C.R. (1995) Taura syndrome in *Penaeus vannamei* (Crustacea: Decapoda): gross signs, histopathology and ultrastructure. *Diseases of Aquatic Organisms,* 21 (1), pp. 53–59.

Lightner, D.V., Redman, R.M., Poulos, B.T., Nunan, L.M., Mari, J.L. and Hasson, K.W. (1997) Risk of spread of penaeid shrimp viruses in the Americas by the international movement of live and frozen shrimp. *Revue Scientifique Et Technique (International Office of Epizootics),* 16 (1), pp. 146–160.

Limsuwan, C. (2009) *Experiences cultivating white shrimp in Thailand, summary of presentations in Peru, Colombia, and Guatemala, in February 2009.* Nicovita-Alicorp Technical Service. Available: http://www.nicovita.com.pe/en/extranet/Boletines/Abr-Jun_09.pdf [Accessed: 3 March 2015].

Limsuwan, C. (2010) *White feces disease in Thailand.* Nicovita-Alicorp Technical Service. Available: http://www.nicovita.com.pe/cdn/Content/CMS/Archivos/Documentos/DOC_271_2.pdf [Accessed: 27 April 2017].

Lio-Po, G.D., Lavilla, C.R. and Cruz-Lacierda, E.R. (2001) *Health Management in aquaculture.* Iloilo, Philippines: SEAFDEC.

Liu, C. and Chen, J. (2004) Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, 16 (3), pp. 321–334.

Liu, P., Lee, K., Yii, K., Kou, G. and Chen, S. (1996) News & Notes: isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicus*. *Current Microbiology*, 33 (2), pp. 129–132.

Lokkhumlue, M. and Prakitchaiwattana, C. (2013) Influences of cultivation conditions on microbial profiles of Pacific white shrimp (*Litopenaeus vannamei*) harvested from eastern and central Thailand. *Chiang Mai University Journal of Natural Science*, pp. 1–9.

Longyant, S., Rukpratanporn, S., Chaivisuthangkura, P., Suksawad, P., Srisuk, C., Sithigorngul, W., Piyatiratitivorakul, S. and Sithigorngul, P. (2008) Identification of *Vibrio* spp. in vibriosis *Penaeus vannamei* using developed monoclonal antibodies. *Journal of Invertebrate Pathology*, 98 (1), pp. 63–68.

Marchand, C., Molnar, N., Deborde, J., Patrona, L. and Meziane, T. (2014) Seasonal pattern of the biogeochemical properties of mangrove sediments receiving shrimp farm effluents (New Caledonia). *Journal of Aquaculture Research & Development*, 5 (5), pp. 1–13.

Mohan, C., Corsin, F. and Padiyar, P. (2004) Farm-level biosecurity and white spot disease (WSD) of shrimp. *Aquaculture Health International,* 3, pp. 16–20.

Mohney, L.L., Lightner, D.V. and Bell, T.A. (1994) An epizootic of vibriosis in ecuadorian pond-reared *Penaeus vannamei* Boone (Crustacea: Decapoda). *Journal of the World Aquaculture Society*, 25 (1), pp. 116–125.

Montgomery-Brock, D., Tacon, A.G.J., Poulos, B. and Lightner, D.V. (2007) Reduced replication of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Litopenaeus vannamei* held in warm water. *Aquaculture*, 265 (1–4), pp. 41–48.

Moriarty, D.J. (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164 (1), pp. 351–358.

Moss, S.M., Moss, D.R., Arce, S.M., Lightner, D.V. and Lotz, J.M. (2012) The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. *Journal of Invertebrate Pathology*, 110 (2), pp. 247–250.

Mugnier, C., Justou, C., Lemonnier, H., Patrois, J., Ansquer, D., Goarant, C. and Lecoz, J. (2013) Biological, physiological, immunological and nutritional assessment of farm-reared *Litopenaeus stylirostris* shrimp affected or unaffected by vibriosis. *Aquaculture*, 388–391, pp. 105–114.

Muniesa, A., Perez-Enriquez, R., Cabanillas-Ramos, J., Magallón-Barajas, F.J., Chávez-Sánchez, C., Esparza-Leal, H. and Blas, I. (2015) Identifying risk factors associated with white spot disease outbreaks of shrimps in the Gulf of California (Mexico) through expert opinion and surveys. *Reviews in Aquaculture*, pp. 1–9.

NACA (2014) *Diseases of crustaceans: acute hepatopancreatic necrosis syndrome (AHPNS).* Available: http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf [Accessed: 1 May 2015].

NACA (2017) *Quarterly aquatic animal disease report (Asia and Pacific Region) 1998-2016.* Thailand: NACA. Available: http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report [Accessed: 29 April 2017].

Namikoshi, A., Wu, J.L., Yamashita, T., Nishizawa, T., Nishioka, T., Arimoto, M. and Muroga, K. (2004) Vaccination trials with *Penaeus japonicus* to induce resistance to white spot syndrome virus. *Aquaculture*, 229 (1–4), pp. 25–35.

National Bureau of Agricultural Commodity and Food Standards (2014) *Good aquaculture practices for marine shrimp farm.* Available: http://www.acfs.go.th/standard/download/GAP-FOR-MARINE-SHRIMP-FARM.pdf [Accessed: 10 March 2017].

National Economic and Social Development Board (2017) *Gross domestic product, chain volume measures: Q4/2016.* Bangkok: Office of the National Economic and Social Development Board. Available: http://www.nesdb.go.th/nesdb_en/ewt_news.php?nid=4340&filename=index [Accessed: 20 March 2017].

Nimrat, S., Suksawat, S., Maleeweach, P. and Vuthiphandchai, V. (2008) Effect of different shrimp pond bottom soil treatments on the change of physical characteristics and pathogenic bacteria in pond bottom soil. *Aquaculture*, 285 (1), pp. 123–129.

Nunan, L.M., Poulos, B.T. and Lightner, D.V. (1998) The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture*, 160 (1), pp. 19–30.

OIE (2013a) *Acute hepatopancreatic necrosis disease, aetiology epidemiology diagnosis prevention and control references.* Available: http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/AHPND_DEC_2013.pdf [Accessed: 12 August 2014].

OIE (2013b) *Aquatic animal health code. Section 9: diseases of crustaceans.* World Organisation for Animal Health (OIE). Available: http://www.oie.int/fileadmin/Home/eng/Health_standards/aahc/2010/en_titre_1.9.htm [Accessed: 12 August 2014].

Overstreet, R.M., Lightner, D.V., Hasson, K.W., McIlwain, S. and Lotz, J.M. (1997) Susceptibility to Taura syndrome virus of some penaeid shrimp species native to the Gulf of Mexico and the southeastern United States. *Journal of Invertebrate Pathology*, 69 (2), pp. 165–176.

Piamsomboon, P., Inchaisri, C. and Wongtavatchai, J. (2016) Climate factors influence the occurrence of white spot disease in cultured penaeid shrimp in Chanthaburi province, Thailand. *Aquaculture Environment Interactions*, 8, pp. 331–337.

Piumsombun, S., Rab, M.A., Dey, M.M. and Srichantuk, N. (2005) The farming practices and economics of aquaculture in Thailand. *Aquaculture Economics & Management*, 9 (1–2), pp. 265–287.

Pongthanapanich, T. and Roth, E. (2006) Voluntary management in Thai shrimp farming. *Aquaculture Economics & Management*, 10 (3), pp. 265–287.

Pruder, G.D. (2004) Biosecurity: application in aquaculture. *Aquacultural Engineering*, 32 (1), pp. 3–10.

Quiroz-Guzmán, E., Balcázar, J.L., Vázquez-Juárez, R., Cruz-Villacorta, A.A. and Martínez-Díaz, S.F. (2013) Proliferation, colonization, and detrimental effects of *Vibrio parahaemolyticus* and *Vibrio harveyi* during brine shrimp hatching. *Aquaculture*, 406, pp. 85–90.

R foundation for statistical computing (2015) *R: a language and environment for statistical computing.* Available: https://www.R-project.org/ [Accessed: 5 December 2015].

Rahman, M.M., Corteel, M., Dantas-Lima, J.J., Wille, M., Alday-Sanz, V., Pensaert, M.B., Sorgeloos, P. and Nauwynck, H.J. (2007) Impact of daily fluctuations of optimum (27 °C) and high water temperature (33 °C) on *Penaeus vannamei* juveniles infected with white spot syndrome virus (WSSV). *Aquaculture,* 269 (1–4), pp. 107–113.

Rahman, M.M., Escobedo-Bonilla, C.M., Corteel, M., Dantas-Lima, J.J., Wille, M., Sanz, V.A., Pensaert, M.B., Sorgeloos, P. and Nauwynck, H.J. (2006) Effect of high water temperature (33 °C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei*. *Aquaculture,* 261 (3), pp. 842–849.

Rajendran, K.V., Vijayan, K.K., Santiago, T.C. and Krol, R.M. (1999) Experimental host range and histopathology of white spot syndrome virus (WSSV) infection in shrimp, prawns, crabs and lobsters from India. *Journal of Fish Diseases*, 22 (3), pp. 183–191.

Ramos-Carreño, S., Valencia-Yáñez, R., Correa-Sandoval, F., Ruíz-García, N., Díaz-Herrera, F. and Giffard-Mena, I. (2014) White spot syndrome virus (WSSV) infection in shrimp (*Litopenaeus vannamei*) exposed to low and high salinity. *Archives of Virology,* 159 (9), pp. 2213–2222.

Reilly, P.J.A. and Twiddy, D.R. (1992) *Salmonella* and *Vibrio cholerae* in brackishwater cultured tropical prawns. *International Journal of Food Microbiology*, 16 (4), pp. 293–301.

Rico, A., Satapornvanit, K., Haque, M.M., Min, J., Nguyen, P.T., Telfer, T.C. and Van Den Brink, P. J. (2012) Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. *Reviews in Aquaculture*, 4 (2), pp. 75–93.

Rocha, R.d.S., Sousa, O.V.d. and Vieira, Regine Helena Silva dos Fernandes. (2016) Multidrug-resistant *Vibrio* associated with an estuary affected by shrimp farming in Northeastern Brazil. Marine Pollution Bulletin, 105 (1), pp. 337-340.

Rockett, I.R. (1999) Population and health: an introduction to epidemiology. *Population Bulletin*, 54 (4), pp. 1.

Rodgers, C., Mohan, C. and Peeler, E. (2011) The spread of pathogens through trade in aquatic animals and their products. *Rev Sci Tech Off Int Epiz*, 30 (1), pp. 241–256.

Rodríguez, J., Bayot, B., Amano, Y., Panchana, F., De Blas, I., Alday, V. and Calderón, J. (2003) White spot syndrome virus infection in cultured *Penaeus vannamei* (Boone) in Ecuador with emphasis on histopathology and ultrastructure. *Journal of Fish Diseases,* 26 (8), pp. 439–450.

Roque, A., Abad, S., Betancourt-Lozano, M., de la Parra, L.M.G., Baird, D., Guerra-Flores, A.L. and Gomez-Gil, B. (2005) Evaluation of the susceptibility of the cultured shrimp *Litopenaeus vannamei* to vibriosis when orally exposed to the insecticide methyl parathion. *Chemosphere*, 60 (1), pp. 126–134.

Saulnier, D., Haffner, P., Goarant, C., Levy, P. and Ansquer, D. (2000) Experimental infection models for shrimp vibriosis studies: a review. *Aquaculture*, 191 (1), pp. 133–144.

Selvin, J. and Lipton, A. (2003) *Vibrio alginolyticus* associated with white spot disease of *Penaeus monodon*. *Diseases of Aquatic Organisms,* 57 (1), pp. 147–150.

Senapin, S., Thaowbut, Y., Gangnonngiw, W., Chuchird, N., Sriurairatana, S. and Flegel, T.W. (2010) Impact of yellow head virus outbreaks in the whiteleg shrimp, *Penaeus vannamei* (Boone), in Thailand. *Journal of Fish Diseases*, 33 (5), pp. 421–430.

Somboon, M., Purivirojkul, W., Limsuwan, C. and Chuchird, N. (2012) Effect of *Vibrio* spp. in white feces infected shrimp in Chantaburi, Thailand. *Kasetsart University Fisheries Research Bulletin*, 36 (1), pp. 7–15.

Somjetlerdcharoen, A. (2002) Chloramphenicol concerns in shrimp culture. *Aquaculture Asia*, 7(1), pp. 51–55.

Song, Y., Yu, C., Lien, T., Huang, C. and Lin, M. (2003) Haemolymph parameters of Pacific white shrimp (*Litopenaeus vannamei*) infected with taura syndrome virus. *Fish & Shellfish Immunology*, 14 (4), pp. 317–331.

Songsanjinda, P. (2013) *The application of aquatic animal movement document database in Thailand.*

Soto-Rodriguez, S.A., Gomez-Gil, B. and Lozano, R. (2010) 'Bright-red'syndrome in Pacific white shrimp *Litopenaeus vannamei* is caused by *Vibrio harveyi*. *Diseases of Aquatic Organisms*, 92 (1), pp. 11–19.

Sriurairatana, S., Boonyawiwat, V., Gangnonngiw, W., Laosutthipong, C., Hiranchan, J. and Flegel, T.W. (2014) White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. *PloS One*, 9 (6), e99170. Available: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0099170&type=printable [Accessed: 1 March 2015].

Sudha, P.M., Mohan, C.V., Shankar, K.M. and Hegde, A. (1998) Relationship between white spot syndrome virus infection and clinical manifestation in Indian cultured penaeid shrimp. *Aquaculture*, 167 (1–2), pp. 95–101.

Sung, H., Li, H., Tsai, F., Ting, Y. and Chao, W. (1999) Changes in the composition of *Vibrio* communities in pond water during tiger shrimp (*Penaeus monodon*) cultivation and in the hepatopancreas of healthy and diseased shrimp. *Journal of Experimental Marine Biology and Ecology*, 236 (2), pp. 261–271.

Supungul, P., Jaree, P., Somboonwiwat, K., Junprung, W., Proespraiwong, P., Mavichak, R. and Tassanakajon, A. (2015) A potential application of shrimp antilipopolysaccharide factor in disease control in aquaculture. *Aquaculture Research*, 43 (3), pp. 1–13.

Suratthani Shrimp Farmers Club (2014) *Suratthani Shrimp Farmers Club.* Available: http://suratthanishrimp.com.

Tendencia, E.A., Bosma, R.H. and Verreth, J.A.J. (2011) White spot syndrome virus (WSSV) risk factors associated with shrimp farming practices in polyculture and monoculture farms in the Philippines. *Aquaculture*, 311 (1–4), pp. 87–93.

Thailand DoF (2016) *Number of farms, area under culture and yield by species and provinces, 1999-2013.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.fisheries.go.th/it-stat/yearbook/Index.htm [Accessed: 27 June 2016].

The World Bank (2015) *Employment in agriculture (% of total employment).* Available: http://data.worldbank.org/indicator/SL.AGR.EMPL.ZS?locations=TH [Accessed: 26 April 2017].

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W. and Itsathitphaisarn, O. (2016) Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 452, pp. 69–87.

Tho, N., Merckx, R. and Ut, V.N. (2012) Biological characteristics of the improved extensive shrimp system in the Mekong delta of Vietnam. *Aquaculture Research*, 43 (4), pp. 526–537.

Tookwinas, S., Chiyakum, K. and Somsueb, S. (2005) *Aquaculture of white shrimp Penaeus vannamei in Thailand.* Iloilo, Philippines: SEAFDEC. Available: https://repository.seafdec.org.ph/bitstream/handle/10862/855/RTC-P.vannamei_p74-80.pdf?sequence=1&isAllowed=y [Accessed: 1 April 2014].

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. and Lightner, D.V. (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Diseases of Aquatic Organisms*, 105 (1), pp. 45–55.

Tu, C., Huang, H., Chuang, S., Hsu, J., Kuo, S., Li, N., Hsu, T., Li, M. and Lin, S. (1999) taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Diseases of Aquatic Organisms*, 38 (2), pp. 159–161.

Uchida, K., Konishi, Y., Harada, K., Okihashi, M., Yamaguchi, T., Do, M.H.N., Thi Bui, L., Duc Nugyen, T., Do Nguyen, P. and Thi Khong, D. (2016) Monitoring of antibiotic residues in aquatic products in urban and rural areas of Vietnam. *Journal of Agricultural and Food Chemistry*, 64 (31), pp. 6133-6138.

Uddin, M.T. (2008) *Value chains and standards in shrimp export.* Available: http://ir.nul.nagoya-u.ac.jp/jspui/bitstream/2237/10939/1/169.pdf.

Undercurrent News (2014) *Thai shrimp export volumes down 32% through August.* UK: Undercurrent News. Available: https://www.undercurrentnews.com/2014/10/27/thai-shrimp-export-volumes-down-32-through-august [Accessed: 18 June 2016].

Vandenberghe, J., Li, Y., Verdonck, L., Li, J., Sorgeloos, P., Xu, H. and Swings, J. (1998) Vibrios associated with *Penaeus chinensis* (Crustacea: Decapoda) larvae in Chinese shrimp hatcheries. *Aquaculture*, 169 (1), pp. 121–132.

Vaseeharan, B., Prem Anand, T., Murugan, T. and Chen, J. (2006) Shrimp vaccination trials with the VP292 protein of white spot syndrome virus. Letters in Applied Microbiology, 43 (2), pp. 137–142.

Walker, P.J. and Winton, J.R. (2010) Emerging viral diseases of fish and shrimp. *Veterinary Research*, 41 (6), pp. 51.

Walling, E., Vourey, E., Ansquer, D., Beliaeff, B. and Goarant, C. (2010) *Vibrio nigripulchritudo* monitoring and strain dynamics in shrimp pond sediments. *Journal of Applied Microbiology*, 108 (6), pp. 2003−2011.

Wang, F. and Chen, J. (2006) Effect of salinity on the immune response of tiger shrimp *Penaeus monodon* and its susceptibility to *Photobacterium damselae* subsp. *damselae*. *Fish & Shellfish Immunology*, 20 (5), pp. 671–681.

Wang, L. and Chen, J. (2005) The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. *Fish & Shellfish Immunology*, 18 (4), pp. 269–278.

Werkman, M., Green, D.M., Murray, A.G. and Turnbull, J.F. (2011) The effectiveness of fallowing strategies in disease control in salmon aquaculture assessed with an SIS model. *Preventive Veterinary Medicine*, 98 (1), pp. 64–73.

Witteveldt, J., Vlak, J.M. and van Hulten, M.C.W. (2006) Increased tolerance of *Litopenaeus vannamei* to white spot syndrome virus (WSSV) infection after oral application of the viral envelope protein VP28. *Diseases of Aquatic Organisms*, 70 (1/2), pp. 167–170.

Wu, W., Wang, L. and Zhang, X. (2005) Identification of white spot syndrome virus (WSSV) envelope proteins involved in shrimp infection. *Virology*, 332 (2), pp. 578–583.

Wyban, J. (2007) Domestication of pacific white shrimp revolutionizes aquaculture. *Global Aquaculture Advocate*, 10 (4), pp. 42–44.

Yaemkasem, S., Boonyawiwat, V., Kasornchandra, J. and Poolkhet, C. (2017) Risk factors associated with white spot syndrome virus outbreaks in marine shrimp farms in Rayong Province, Thailand. Diseases of Aquatic Organisms, 124 (3), pp. 193–199.

Yamprayoon, J. and Sukhumparnich, K. (2010) Thai aquaculture: achieving quality and safety through management and sustainability. *Journal of the World Aquaculture Society*, 41 (2), pp. 274–280.

Yanong, R.P. and Erlacher-Reid, C. (2012) Biosecurity in Aquaculture, Part 1: An Overview. *USDA Southern Regional Aquaculture Center*, 4707, pp. 1–16.

Zhang, J., Li, Z., Wen, G., Wang, Y., Luo, L., Zhang, H. and Dong, H. (2016) Relationship between white spot syndrome virus (WSSV) loads and characterizations of water quality in *Litopenaeus vannamei* culture ponds during the tropical storm. *Iranian Journal of Veterinary Research*, 17 (3), pp. 210.

Zhu, C. and Dong, S. (2013) Aquaculture site selection and carrying capacity management in the People's Republic of China. In: L.G. Ross, T.C. Telfer, L. Falconer, D. Soto and J. Aguilar-Manjarrez, ed. *Site Selection and Carrying Capacities for Inland and Coastal Aquaculture.* Stirling, UK, 6th to 8th December 2010. Rome: FAO, pp. 219–230. Available: http://www.fao.org/3/contents/7358f8b4-00c1-55bd-83fd-01f93bc68ac1/i3322e.pdf#page=231 [Accessed: 15 January 2015].

Chapter 2 - Epidemiological and epizootiological tools for design of disease prevention and control strategies

N. Saleetid; D.M. Green; F.J. Murray

Preface

This chapter describes the theoretical literature and empirical evidence that relates to the development of disease prevention and control strategies. The chapter covers epidemiological and epizootiological study designs: experimental, observational and theoretical studies. These approaches will allow the (1) investigation of the risk factors for acute hepatopancreatic necrosis disease (AHPND) in Chapter 3 (most of these have been previously been shown to be useful historical in shrimp diseases); (2) study of the structure of the live shrimp movement network in Chapter 4; (3) design of the algorithms for targeted disease surveillance and control in Chapter 5; and (4) running of network-based models of AHPND epizootic dynamics in Chapter 6. The final part describes the application to AHPND.

Chapter 2 - Epidemiological and epizootiological tools for design of disease prevention and control strategies

Advances in technology and innovation have led to an increase in shrimp farming. In turn, dramatic decreases in global shrimp production due to widespread infectious diseases have been widely reported (FAO, 2013; FAO, 2016). There have thus been many attempts to design disease prevention and control measures, both for the early detection of diseases, and for maintaining the sustainability of the shrimp farming sector (Bondad-Reantaso *et al.,* 2005). This chapter is intended to: (1) review the epidemiological and epizootiological tools that have been the most useful historically to examine the distribution (pattern and frequency) and risk factors for disease transmission among a population, and (2) identify gaps in current epizootiological research into disease outbreaks in shrimp farming. Epidemiological studies cover experimental, observational and theoretical approaches, however, this thesis focuses on the two latter types of epidemiological studies because they are more useful for determining disease transmission in the whole shrimp farming sites.

2.1 Experimental studies

Evaluating risk factors for disease is one of the major tasks in epidemiology (WHO, 2016) that can be conducted with experiments. Experimental studies are used where the effect of exposure to a risk factor is evaluated by assigning that exposure alongside controls to a study population such as a clinical trial of new drug for white spot disease (WSD) in shrimp (Ocampo *et al.*, 2014). Much of the experimental study of diseases has been done in the context of testing a particular hypothesis in the laboratory. For example, the occurrence of WSD in farmed shrimp was found to be associated with chemical components, such as an exposure of 20 mg L^{-1} total ammonia nitrogen (Liang *et al.,* 2016), a low water temperature of 27 °C (Rahman *et al.,* 2006), and a rapid change in salinity from 22 to 14 ppt (Liu *et al.,* 2006). The epizoology of vibriosis in shrimp was studied experimentally to assess their association with pesticide-contaminated shrimp (Labrie *et al.,* 2003), and the presence of WSD before vibriosis (Phuoc *et al.,* 2008). From this literature, it is observed that such experimental studies keep other environmental

factors constant, while in fact the complexity of the natural environment effects the occurrence of diseases.

2.2 Observational studies

The second type of epidemiological studies is observational studies (where the effect of exposure to a risk factor is observed by experimenters without prior assignment of any exposure to a study population) (Jepsen *et al.,* 2004). Observational studies often take place in a natural environment to test multiple hypothesises, for example, where multiple risk factors are thought to be associated with the occurrence of disease. An example of this can be seen in Corsin *et al.* (2001). Using a cohort study design, about 100 potential risk factors have been investigated for their association with WSD occurring in farmed shrimp (Corsin *et al.*, 2001). This is one of the three main types of observational studies as described following.

The three main types of observational studies are cross-sectional, cohort and case–control studies. With different temporal designs, cross-sectional studies are mainly used to determine prevalence, meaning the number of cases (diseased individuals) in a population at a particular point in time. Cohort studies may be prospective or retrospective. Whereas, case–control studies are generally retrospective (Mann, 2003; Song and Chung, 2010). Among these, if a population sampled is already defined as disease cases and controls, case–control studies show comparative advantages, particularly through being able to limit the effect of confounding factors (risk factors for a disease in non-exposed individuals, which are associated with the exposure of interest in the studied population) (Mann, 2003; Salas *et al.,* 1999). To deal with any confounding factor, cases and controls are matched using specific criteria such as the age and gender of participants (McCreadie and Scottish Comorbidity Study Group, 2002; Yusuf *et al.,* 2004), or the geographic location of farming sites (Boonyawiwat *et al.,* 2016).

Investigations with case–control studies also minimise the incompleteness of data collection that is often caused by death or inability to contact cases during follow-up periods. This problem happens in prospective cohort studies with a large sample size (Holme *et al.,* 2016; Swerdlow *et al.,* 2015). In addition, since finding cases is more

expensive in cross-sectional studies, some bias may arise due to the recruitment effort needed to complete those studies, as demonstrated in Van Schayck *et al*. (2002). Regarding these limitations, case–control studies are an effective and feasible strategy to find risk factors for diseases, compared with other study designs (Diomidus, 2002; Schlesselman, 1982; Schulz and Grimes, 2002).

To illustrate this further, the following three examples draw upon the three main types of observational studies: cross-sectional, cohort and case–control studies. The first two examples studied WSD; another studied AHPND. Looking at other diseases and the risk factors for them, however, will provide ideas and hypotheses for this work, as well as for other emerging or different diseases like AHPND.

A cross-sectional study was conducted by Tendencia *et al.* (2011) to investigate the risk factors for WSD infection in shrimp farms in the Philippines during a 14-month survey of the disease. Cases (WSD-infected farms) were identified by the principal clinical sign of WSD, i.e. white spots appeared on the body of farmed shrimp, or by a polymerase chain reaction (PCR) test for confirmation of WSD. The result strongly suggested that feeding fresh molluscs to shrimp was a major risk factor of WSD infection. It was expected that, as filter feeders, molluscs could act as WSSV carriers by obtaining the pathogens in soil and water with shrimp predation of the molluscs leading to the transfer of these pathogens. This research has a weakly temporal association between the presence of disease and the risk factor because both data were measured simultaneously. Thus, it may difficult to determine whether the presence of disease followed exposure to the risk factor in time or exposure to the potential risk factor resulted from the presence of disease (Song and Chung, 2010). However, there are many other published works that support the finding that the use of fresh molluscs and fish in shrimp rearing is a potential risk factor for WSD and other viral diseases (Hameed *et al.,* 2002; Vijayan *et al.,* 2005)

As a forward-looking approach, a prospective cohort study investigates individual groups moving forwards from exposure to a risk factor to the later presence (or absence) of a study disease (Grimes and Schulz, 2002). In shrimp farming, a prospective cohort study was conducted by Corsin *et al.* (2001) to investigate the risk factors for

WSD. The authors designed the cohort study with the 24 shrimp ponds of a Vietnamese farm, and a six-month follow-up of the study disease. Cases (infected ponds) were defined by a PCR test. The main result indicated that earthen shrimp ponds established close to the sea were associated with an increased risk of WSD presence. This research could utilise a cohort study because of the short production cycle of farmed shrimp (around six months) and the use of a small number of epizootiological units (24 shrimp ponds of a farm).

In contrast to prospective cohort studies and cross-sectional studies, a case–control study is a retrospective or backward-looking approach (Hoffmann and Lim, 2007; Pearce, 2012). Study individuals are already divided into two groups: cases (denoted diseased individuals) and controls (referred non-diseased individuals), and then exposure to candidate risk factors in the past in each group is examined (Mann, 2003). Recently, a case–control study was designed to identify the risk factors for AHPND (Boonyawiwat *et al.,* 2016). Cases were obtained from reporting of disease occurrences by farmers to local staff of the Thailand Department of Fisheries. During the study period, the pathogenic agent of this disease remained unknown (the PCR test was unavailable), but the cases were confirmed by histopathology based on the major AHPND signs given in NACA (2014). This research suggested five factors which related to increased risk of AHPND occurrence at shrimp pond level, i.e. the use of chlorine for water treatment, the availability of a water reservoir, the culture of predator fish in water preparation ponds, the stocking of multiple shrimp species, and increased density of shrimp stocking. A case–control study design was most efficient for this research for two reasons. First, being a new disease at the time, AHPND cases remained rare or hard to confirm. Second, the presence of AHPND among shrimp farming sites was suspected to be related to multiple risk factors, especially in respect to farm management practices.

This section has described the main types of observational study designs for epidemiology that are used to evaluate the risk factors for shrimp diseases. To gain more knowledge of disease spread and to design effective disease prevention and control measures, many recent epidemiological studies have also used theoretical approach

with mathematical models to enhance the understanding of disease epidemic dynamics. These will be discussed next.

2.3 Theoretical studies

In theoretical study design of epidemiology, the dynamics of disease spread can be determined by means of a wide variety of mathematical models such as compartmental epidemic models, mass-action models, network models and individual-based models. This section, therefore, gives details of the mathematical modelling approaches. These provide more understanding of the challenges in studying the dynamics of infectious diseases transmitted among shrimp farming sites in Thailand, since these epizootic dynamics remain poorly understood. Some of the discussed epidemic modelling approaches have never been applied to shrimp epizoology.

2.3.1 Compartmental epidemic models for microparasites *versus* macroparasites

Most disease outbreaks in shrimp farming are caused by microparasites (Flegel *et al.,* 2008). Severe infections from macroparasites occur less frequently due to chemical treatment and good management practices in farming. Nevertheless, macroparasites can serve as a vector in the transmission of microparasites, leading to large epidemics (Lo *et al.,* 1996; Vijayan *et al.,* 2005; Zhang *et al.,* 2008). To model their spreads, the nature of the life cycle and how it is appropriately modelled are important (Anderson and May, 1991).

The life cycles of microparasites contrast with those of macroparasites. A microparasite can complete its life cycle (multiply) within an individual host, while a macroparasite utilises one or more hosts, or is partly free living. A microparasite (viruses, bacteria, fungi and protozoans) is a very small organism but a macroparasite (e.g. arthropods and worms) is larger and can be counted. Nevertheless, it should be noted that although some microparasites are “micro” in scale, their nature tends to be “macro” in modelling. An example is free-living ciliate protozoans. Recently, they have been shown to cause ectoparasitic diseases of farmed shrimp in Iran, i.e. mostly by species of the genus *Zoothamnium* (Afsharnasab, 2015), and of farmed Nile tilapia in Saudi Arabia, i.e. *Trichodina maritinkae*, *T. centrostrigeata* and *T. frenata* (Abdel-Baki *et al.,* 2017).

To model the dynamics of microparasite transmission, the Kermack-McKendrick mathematical model is widely used (Diekmann *et al.,* 1995; Kermack and McKendrick, 1927). Table 2.1 shows three well-known mathematical model types and their application to microparasite infections (often in human diseases), in which an individual can be in one of three compartments: S (susceptible), I (infectious) or R (recovery, removed, death or quarantine). Thus, they are also called compartmental models.

Table 2.1 Three well-known compartmental models and their application to microparasite infections

Model	Host	Microparasite disease
SIR	Human	Measles (Bjørnstad *et al.,* 2002; Sattenspiel and Dietz, 1995; Shulgin *et al.,* 1998), influenza (Brauer, 2008), dengue fever (Feng and Velasco-Hernández, 1997), chickenpox, measles, mumps and influenza (Allen, 2008; Coburn *et al.,* 2009; Feng and Velasco-Hernández, 1997), and Zika virus (Bewick *et al.,* 2016)
	Livestock	Foot-and-mouth disease (Hagenaars *et al.,* 2011; Heath *et al.,* 2008; Keeling, 2005b)
	Salmon	Furunculosis (Ogut *et al.,* 2005)
	Shrimp	White spot disease (Hernandez-Llamas *et al.,* 2013; Lotz and Soto, 2002), and taura syndrome (Lotz *et al.,* 2003)
SIS	Human	Sexually transmitted diseases, i.e. hepatitis B (Kribs-Zaleta and Martcheva, 2002) and Gonorrhoea (Gray *et al*., 2011)
SI	Human	Sexually transmitted diseases HIV/AIDS (Bozkurt and Peker, 2014; Lloyd-Smith *et al.,* 2004)

SIR models are appropriate for diseases with full immunity or permanent removal by death while SIS models (susceptible-infectious-susceptible) have been used for diseases with little or no immunity (Allen, 1994; Blyuss and Kyrychko, 2005; Feng and Velasco-Hernández, 1997; Keeling and Eames, 2005). Hence, in the SIS models an individual can become susceptible after that individual recovers from an initial infection. Finally, SI models apply to diseases that remain infectious for life and are never "removed" (Kim *et al.,* 2013; Tassier, 2013). Because these compartmental models apply to individuals, the interpretation of them at an individual scale differs between smaller and larger scales.

A unique compartmental model may not be sufficient to model all the various mechanisms for disease transmission. In many cases of studying epidemiological patterns (e.g. spread by multiple transmission routes), researchers have added extra compartments into the standard mathematical model to gain more understanding of the realistic disease transmission (Johnson *et al.,* 2016; Ng *et al.,* 2003; Tien and Earn, 2010). Two examples using $SIRS$ models where an individual achieves temporary immunity, and $SEIR$ models (with compartment term "E" denoted "exposed") where during an incubation period an individual has the disease but is not yet infectious, such as for influenza (Degli Atti *et al.,* 2008), tuberculosis (Marais *et al.,* 2004) and measles (Robinson *et al.,* 2014). These additional compartments extend the entire life cycles of pathogens into the modelling of disease outbreaks.

In another type of modelling shrimp diseases, macroparasite infections primarily cause a decrease in growth rate and strength, and an increase in the occurrence of deformity in fish (Williams and Bunkley-Williams, 2000). Macroparasites that are commonly found in farmed fish and shrimp include helminths, e.g. monogeneans, cestodes and nematodes (Domínguez-Machín *et al.,* 2011; Soler-Jiménez *et al.,* 2016), and copepods, e.g. sea lice (Igboeli *et al.,* 2014). Due to the complex life cycles of these organisms, May and Anderson (1979) propose that compartmental models are unlikely to be helpful for studying their transmission. They state that the dynamics of macroparasite infections are much more dependent on the number of parasites in a host. Thus, modelling based on the number of hosts, parasites, and free-living infective stages becomes more efficient than standard compartmental models (May and Anderson, 1979).

For the outbreak of AHPND (a microparasite infection) in Thai shrimp farming, the $SEIRS$ compartmental model is a useful tool to model the epizootic dynamics at site level. Four compartments in the $SEIRS$ model: susceptible (S), exposed (E), infected (I) and removed (R) cover the actual patterns of AHPND occurrence in shrimp farming sites. Other models may work well, however. For example, macroparasite models can work at site level if the within-site prevalence varies a lot. Alternatively, simpler models, such as $SIRS$ models, can be used. Additionally, compartmental models of the spread of infectious disease that utilise mass-action or network approaches might be applicable. These are discussed in the following sub-section.

2.3.2 Mass-action *versus* network models

Mass-action and network models have important roles in modelling and assessing disease transmission both between persons or animal farming sites, and within a pond. The distinguishing contrast between mass-action and network models is outlined here.

2.3.2.1 Mass-action models

Mass-action models assume disease transmission in a homogenous population of humans, animals or farming sites (Hethcote and Van Ark, 1987; Nold, 1980). For example, the following SI epidemic model divides S and I into two compartments that are differentially susceptible and infectious. The rate of individual in class i being infected at time t is:

$$\frac{dS_i}{dt} = -\alpha_i S_i \sum_j \beta_j I_j$$

where $S_i + I_i = N_i$ at all times, the parameters α and β are a constant contact rate and transmission rate per unit time, respectively.

In addition, the direction of disease transmission is neglected in most mass-action models. This does not include the mass-action model that is naturally directed. The direction of transmission effects epidemic dynamics because an individual can act as either a source or a sink of infection, or as both a source and sink of infection (Chaves and Hernandez, 2004; Wesolowski *et al.,* 2012). For example, in the Thai shrimp farming, the potential pathway of transmission through live shrimp movements is from hatchery sites (sources of infection) to nursery sites or to ongrowing sites (sinks for infection), although there are also a small number of upstream movements (from ongrowing sites to hatchery sites) to reproduce new generations of shrimp, such as for genetic improvement programmes (see Figure 2.1: this structure has been applied to Chapters 4, 5 and 6). Thus, neglect of these real epidemiological patterns may lead to over- or under-estimation of disease epidemics in mass-action models (Meyers, 2007).

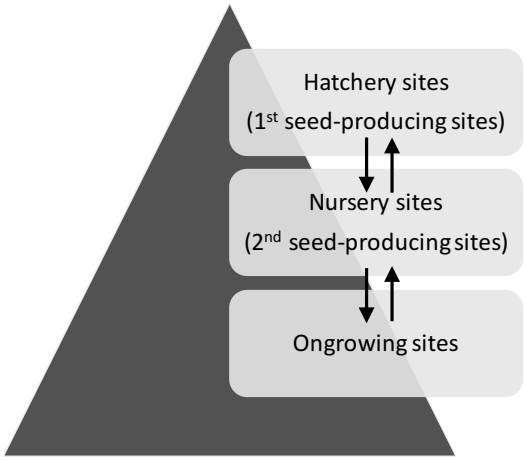


Figure 2.1 Potential pathway for disease transmission via live shrimp movements in Thailand. The main pathway is downstream towards the ongrowing sites. There are a small number of upstream movements (from ongrowing sites to seed-producing sites) for reproducing new generations of shrimp.

2.3.2.2 Network models

Owing to model-based approaches, several recent epidemic models on diseases have taken the real-life disease spread, i.e. the heterogeneity of infection in a population, and the direction of transmission into consideration. These are typically known as network models (Funk *et al.,* 2010; Perisic and Bauch, 2009). The major difference between network models and mass-action models is that the network models can capture the reality that the population tends to be heterogeneous with non-random mixing (Hethcote and Van Ark, 1987; Nold, 1980). Keeling (2005a) stated that in mass-action models each infected individual can transmit to all other individuals, while the disease spread in network models requires connections between individuals that represent a transmission route of diseases to be specified. Hence, the chance of transmission from infected individuals in network models is dependent on contact networks that are generally degree-heterogeneous (Keeling, 2005a; Volz *et al.,* 2011).

In support of this view, Woolhouse *et al.* (1997) explained the heterogeneity of infection with the 80/20 rule. The rule suggests that often 20 % of individuals contribute at least 80 % of the infection in the whole population. Therefore, whereas the network models are estimated using the 80/20 rule, the mass-model assumption disregards the heterogeneity of infection in a population, i.e. in the number of connections between

individuals (Finkenstadt *et al.,* 2002; Germann *et al.,* 2006; Kane *et al.,* 1999), and the time of host-parasite interactions (Miller *et al.,* 2012).

Basically, the modelling of a network is based on graph theory, in which a network refers to a group of sites (network terminology, nodes) connected by direct or indirect connections. Networks with direct connections potentially allow the modelling of disease spread to address the sources and sinks for disease transmission because the direction of connections is already known (Gates and Woolhouse, 2015). In many cases, network models are reconstructed through simulated networks, while real networks are less often modelled due to the unavailability of reliable network data, or lack of a data recording system (Keeling and Eames, 2005). A simple simulated network is that of a node connecting with two neighbours in a ring. Additionally, Witten and Poulter (2007) presented network types that have often been used in epidemic modelling: random, lattice, Watt–Strogatz small world and Barabasí–Albert scale-free networks. Demonstrations of these simulated networks are shown in Figure 2.2.

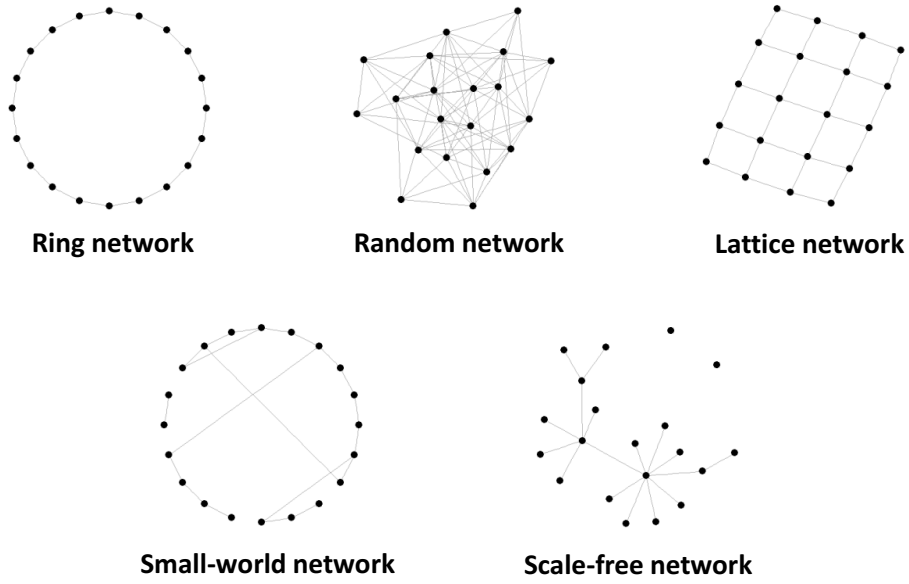


Figure 2.2 Epidemic network models often are often characterised by these five simulated networks. All networks contain 20 nodes. The probability of each connection is 0.4 for drawing the random network, and 0.1 for drawing the small-world network.

The theoretical distinguishing properties in simulated networks are useful to determine disease spread in many real networks including farmed animal movement networks

(Christley *et al.,* 2005a; Kiss *et al.,* 2006). Poisson degree distribution, a major characteristic of random networks, is less often found in reality (Newman *et al.,* 2002), but many real networks are found to have other properties, such as those of small-world networks and scale-free networks. A small-world network is characterised by high clustering, and short path lengths (distances between sites) (Watts and Strogatz, 1998). As measured by a clustering coefficient, clustering is defined as the tendency for triangles of connections to exist in networks (Newman, 2008). Small-world networks with short mean path lengths also link to the small-world experiment of Milgram *et al.* (1992) who studied the 'six degrees of separation' theory. They propose that individuals can get a piece of information (or a disease) via a connection of no more than six intermediates (Milgram *et al.,* 1992; Watts, 1999). Infection within small-world networks is commonly fast, mainly due to their short path lengths (the number of paths traversed between a site pair) (Kiss *et al.,* 2006; Newman, 2008). For scale-free networks, degree distributions lie on a power-law form $P(k) \sim k^{-\gamma}$ (Barabasi, 2009; Pastor-Satorras and Vespignani, 2001). Most sites in a scale-free network have a small number of connections, but a few sites have a large numbers of connections. The heterogeneity in these site degrees becomes more interesting in terms of disease prevention and control, particularly in designing a control strategy at the most highly connected sites (Barthélemy *et al.,* 2005).

In terms of the application of theoretical studies in epidemiology, most of them can represent disease spread in whole populations and lead to the development of efficient disease surveillance and control measures. In order to achieve the aim of this thesis, therefore, network models for targeted disease surveillance and control will be addressed in the next sub-section.

2.3.2.3 Network models for targeted disease surveillance and control

Targeted disease surveillance and control using a risk-based approach is one of the potential strategies to make the farming sector more sustainable (Peeler and Taylor, 2011). For farmed shrimp, according to NACA (2017), the Asian aquaculture industry has established two important types of surveillance to protect the health of farmed aquatic animals: active and passive surveillance. Active surveillance is undertaken with the goal

of investigating targeted pathogens, while passive surveillance utilises laboratory samples submitted for disease testing purposes (Burgess and Morley, 2015). There are several limitations with these surveillance programmes, however, such as a lack of suitable resources for surveillance, and the effective diagnosis of diseases (Bondad-Reantaso *et al.,* 2005). Cost-effective interventions for disease controls can be improved if targeted surveillance and control is developed and implemented.

For the spread of disease on social or animal movement networks, the idea of targeted surveillance and control is that high-risk connections serve strongly as a potential transmission route for infectious diseases; and thus their removal from the network leads to a decrease in transmission (Duan *et al.,* 2005; Green *et al.,* 2012; Lou and Ruggeri, 2010). Bajardi *et al.* (2012) argue that targeting disease surveillance and control approaches based on centrality measures may fail to minimise the epidemic in the network due to temporal fluctuations. Measures of centrality, however, play an important role in targeting disease surveillance and control for farmed animal diseases, as shown in Table 2.2.

Table 2.2 also demonstrates that an optimal centrality measure for one network may not perform well for others, because such networks have particular structures. For example, the algorithm based on betweenness centrality related well to the specific properties of the network of livestock movements in France, which displayed a scale-free network and a large connected component (a giant strongly connected component or GSCC) over the network (Rautureau *et al.,* 2011). Accordingly, these centrality measures, which already apply to farmed animal networks, are used to form potential candidate disease-control algorithms in the LSMN (Chapter 5).

Table 2.2 Centrality measures studied in five networks of farmed animal movements resulting in optimal strategies for targeted disease surveillance and control

Centrality measure	Live fish in Scotland[a]	Livestock in the UK[b]	Livestock in Italy[c]	Livestock in Argentina[d]	Livestock in France[e]
Degree	x	x	x*	x*	x
Betweenness	x	x*	x	x	x*
Community-bridging	x				
Greedy	x*				
Eigenvector	x		x		
Closeness					x

x Examined for this network

* The optimal centrality measure

[a] Live fish movements in Scotland (Green *et al.,* 2012).

[b] Livestock movements in the UK (Ortiz-Pelaez *et al.,* 2006).

[c] Livestock movements in Italy (Natale *et al.,* 2009).

[d] Livestock movements in Argentina (Aznar *et al.,* 2011).

[e] Livestock movements in France (Rautureau *et al.,* 2011).

Degree = the number of connections of each node

Betweenness = the number of shortest paths that go through nodes, or alternatively, connections

Community-bridging = the connections that link between two sub-networks

Greedy = the ability of connections in terms of the greatest reduction to either mean and maximum reach

Eigenvector = the degree centralities of all neighbouring nodes connected to a specified node

Closeness = the number of shortest paths from a specified node to all neighbouring nodes

We finish this section by remarking that in Thailand no work has been done to study shrimp epizoology with network modelling. To fill this gap, the network structure of live shrimp movements of Thailand is analysed, and the potential of the network in respect to disease spread evaluated in Chapter 4. In addition, disease-control algorithms for the live shrimp movement network of Thailand are examined in Chapter 5 in terms of their efficacy in reducing the potential epizootic size.

While the modelling of the dynamics of disease epidemics becomes more and more complex, another epidemiological tool, individual-based modelling, might be an appropriate tool. This type of model is discussed in the next sub-section.

2.3.3 Individual-based simulation models

Individual-based modelling is a robust tool for epizoology. The key distinguishing feature of individual-based modelling is the ability to track individuals in a population. In individual-based models, the transmission of diseases can be tracked at microscale interactions (e.g. disease spread between infected sites and susceptible sites, where "individual" refers to the site) in order to interpret the epidemic dynamics of a whole population in a specific timing and location (Al-Mamun *et al.,* 2016; Kisjes *et al.,* 2014). Individual-based models are increasingly important for examining many severe disease outbreaks, and supporting decision-makers in the development of control strategies. They have been mainly applied in recent epidemiological studies of human diseases such as tuberculosis (Cardona and Prats, 2016), and animal diseases such as vibriosis (Paillard *et al.,* 2014)

An important reason for choosing individual-based models is that the modelling of infectious disease transmission has become very complex (Gu *et al.,* 2003). The complexities in modelling epidemic dynamics are illustrated in the following examples. Demographics (the age or gender of persons) affect the spread of influenza type A (Arima *et al.,* 2013; Donaldson *et al.,* 2009; Quandelacy *et al.,* 2014). Pathogens such as influenza, malaria or dengue contain multiple strains causing diverse epidemiological patterns in populations (Lourenço and Recker, 2013). Individuals may carry out various behaviours such as avoiding contact with infected persons (Van Segbroeck *et al.,* 2010), and choosing longer distances of animal movements for aquaculture (Keeling *et al.,* 2001).

In addition, many biological processes have a stochastic nature. For example, a site is exposed to a disease, but is not infected. Black and McKane (2012) indicate that there has been a recent increase in the number of individual-based models and stochastic models for predicting infectious disease dynamics. To incorporate stochasticity in individual-based models, Rattana *et al.* (2013) used the Gillespie algorithm to determine next events according to event times distributed exponentially. Instead of using this exponential distribution, Vergu *et al.* (2010) used a gamma distribution (i.e. a positively

skewed distribution) to evaluate the changes from infectious to recovered states. This served explicitly to simulate the biology of individuals.

An important concept that is established in many individual-based models is that of metapopulation concept. This concept emphasises that individuals often characterise two or more structured populations (Levin, 1974). Keeling *et al.* (2010) indicate that individual-based models that do not maintain this individuals' structure overestimate the spatial spread and potential size of the epidemic. An example of individual-based metapopulation models is diseases transmission in fish farming sites modelled on two structured populations: the site and the fish (Green, 2010). This structure also appears in farmed shrimp production, in which site stucture is simplified, giving rise to a single level model, as in the Chapter 6 of this thesis.

In summary, the dynamics of disease epidemics are affected by the heterogeneity of individuals and the stochastic nature of the transmission process. These dynamics are difficult to determine by using equation-based models, but it can be achieved with individual-based models.

2.4 Application to acute hepatopancreatic necrosis disease (AHPND)

This chapter presents tools that are potentially useful for examining AHPND spread in Thailand. AHPND, a new disease with high mortalities of farmed shrimp, has occurred in eastern Thailand since 2011/2012 from whence it was transmitted to other shrimp farming areas. These epizootics resulted in the lowest production of farmed shrimp in the ten years from 2003 to 2013 (Thailand DoF, 2016).

In order to investigate the risk factors for AHPND that still had an unknown etiology at the time of commencement of this study, farmers' knowledge of shrimp diseases is directly relevant in order to develop a case definition for AHPND. Thai farmers have long experience in farming shrimp, combined with the experience of losses due to diseases (Tookwinas *et al.,* 2005). When infections occur, farmers are able to submit farmed shrimp for diagnosis of diseases in many aquatic animal health services both private and governmental laboratories; the laboratory results give the farmers better

knowledge about shrimp diseases. Thai farmers are therefore able to differentiate AHPND cases from other shrimp diseases.

For Thai shrimp farming, data on live shrimp movements from source sites to destination sites are available (SEAFDEC/MFRDMD, 2016). This kind of data is important for studying recent epizoology. The potential transmission of AHPND, and other infectious diseases of farmed shrimp, can therefore be modelled as a network that contains all sites and their connections. The analysis of the network structure allows disease epizootics to be evaluated, and assists in the development of control strategies at a country level, such as targeted disease surveillance and control.

In the real pattern of AHPND spread, latent periods are evident in site-to-site AHPND transmission. During these periods, farmed shrimp are infected by AHPND, but no clinical signs of that disease appear at site level (Tran *et al.,* 2013). Nonetheless, these exposed sites remain infectious. After a site is infected with the disease, different fallowing periods are presented given different farming management practices for removing the disease. Then, the sites start a new crop and become at risk of re-infection (this evidence was observed in the epizootiological survey reported in Chapter 3). This indicates that the SI model for AHPND epidemic dynamics requires extra compartments, i.e. exposed (E) and removed (R) states.

Another epidemiological tool that can be applied for studying AHPND epizootic dynamics in Thai shrimp farming is the compartmental, individual-based epidemic model. This model is helpful in testing control strategies by changing parameters such as lower rates of long-distance transmission, denoting better disease control arrangements in the country (Riley *et al.*, 2003).

To conclude, the epidemiological and epizootiological tools that we present in this chapter are useful for shrimp disease epizoology in Thailand. Not only are these epizootiological tools suitable for AHPND, but they are also useful for other infectious diseases of farmed shrimp such as WSD, YHD and other vibriosis.

2.5 References

Abdel-Baki, A.S., Al Ghamdi, A. and Al-Quraishy, S. (2017) First record of three African trichodinids (Ciliophora: Peritrichida) in cultured Nile tilapia (*Oreochromis niloticus*) in Saudi Arabia with re-evaluation of their host specificity. *Parasitology Research*, 116 (4), pp. 1–7.

Afsharnasab, M. (2015) Prevalence and intensity of protozoan ectoparasite of the white leg shrimp (*Penaeus indicus*) in Helleh site, South of Iran. *Iranian Journal of Aquatic Animal Health*, 2 (1), pp. 17–23.

Allen, L.J. (1994) Some discrete-time SI, SIR, and SIS epidemic models. *Mathematical Biosciences*, 124 (1), pp. 83–105.

Allen, L.J. (2008) An introduction to stochastic epidemic models. In: F. Brauer *et al.*, ed. *Mathematical Epidemiology.* Berlin: Springer, pp. 81–130.

Al-Mamun, M.A., Smith, R.L., Schukken, Y.H. and Gröhn, Y.T. (2016) Modeling of Mycobacterium avium subsp. paratuberculosis dynamics in a dairy herd: An individual based approach. *Journal of Theoretical Biology*, 408, pp. 105–117.

Anderson, R.M. and May, R.M. (1991) *Infectious diseases of humans.* Oxford: Oxford university press.

Arima, Y., Zu, R., Murhekar, M., Vong, S. and Shimada, T. (2013) Human infections with avian influenza A (H7N9) virus in China: preliminary assessments of the age and sex distribution. *Western Pacific Surveillance and Response*, 4 (2), pp. 1–3.

Aznar, M.N., Stevenson, M.A., Zarich, L. and León, E.A. (2011) Analysis of cattle movements in Argentina, 2005. *Preventive Veterinary Medicine*, 98 (2–3), pp. 119–127.

Bajardi, P., Barrat, A., Savini, L. and Colizza, V. (2012) Optimising surveillance for livestock disease spreading through animal movements. *Journal of the Royal Society, Interface,* 9 (76), pp. 2814–2825.

Barabasi, A.L. (2009) Scale-free networks: a decade and beyond. *Science (New York, N.Y.)*, 325 (5939), pp. 412–413.

Barthélemy, M., Barrat, A., Pastor-Satorras, R. and Vespignani, A. (2005) Dynamical patterns of epidemic outbreaks in complex heterogeneous networks. *Journal of Theoretical Biology*, 235 (2), pp. 275–288.

Bewick, S., Fagan, W.F., Calabrese, J.M. and Agusto, F. (2016) Zika virus: endemic versus epidemic dynamics and implications for disease spread in the Americas. *bioRxiv*, pp. 041897.

Bjørnstad, O.N., Finkenstädt, B.F. and Grenfell, B.T. (2002) Dynamics of measles epidemics: estimating scaling of transmission rates using a time series SIR model. *Ecological Monographs*, 72 (2), pp. 169–184.

Black, A.J. and McKane, A.J. (2012) Stochastic formulation of ecological models and their applications. *Trends in Ecology & Evolution*, 27 (6), pp. 337–345.

Blyuss, K.B. and Kyrychko, Y.N. (2005) On a basic model of a two-disease epidemic. *Applied Mathematics and Computation*, 160 (1), pp. 177–187.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff, M. (2005) Disease and health management in Asian aquaculture. *Veterinary Parasitology,* 132 (3–4), pp. 249–272.

Boonyawiwat, V., Patanasatienkul, T., Kasornchandra, J., Poolkhet, C., Yaemkasem, S., Hammell, L. and Davidson, J. (2016) Impact of farm management on expression of early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) on penaeid shrimp farms in Thailand. *Journal of Fish Diseases*, 40 (5), pp. 649-659.

Bozkurt, F. and Peker, F. (2014) Mathematical modelling of HIV epidemic and stability analysis. *Advances in Difference Equations*, 2014 (1), pp. 1.

Brauer, F. (2008) Epidemic models with heterogeneous mixing and treatment. *Bulletin of Mathematical Biology*, 70 (7), pp. 1869–1885.

Burgess, B.A. and Morley, P.S. (2015) Veterinary hospital surveillance systems. *Veterinary Clinics of North America: Small Animal Practice*, 45 (2), pp. 235–242.

Cardona, P. and Prats, C. (2016) The small breathing amplitude at the upper lobes favors the attraction of polymorphonuclear neutrophils to Mycobacterium tuberculosis lesions and helps to understand the evolution toward active disease in an individual-based model. *Frontiers in Microbiology*, 7, pp. 354.

Chaves, L.F. and Hernandez, M. (2004) Mathematical modelling of American cutaneous leishmaniasis: incidental hosts and threshold conditions for infection persistence. *Acta Tropica*, 92 (3), pp. 245–252.

Christley, R., Robinson, S., Lysons, R. and French, N. (2005) Network analysis of cattle movement in Great Britain. *Proc.Soc.Vet.Epidemiol.Prev.Med*, pp. 234–243.

Coburn, B.J., Wagner, B.G. and Blower, S. (2009) Modeling influenza epidemics and pandemics: insights into the future of swine flu (H1N1). *BMC Medicine*, 7 (1), pp. 30.

Corsin, F., Turnbull, J., Hao, N., Mohan, C., Phi, T., Phuoc, L., Tinh, N. and Morgan, K. (2001) Risk factors associated with white spot syndrome virus infection in a Vietnamese rice-shrimp farming system. *Diseases of Aquatic Organisms*, 47 (1), pp. 1–12.

Degli Atti, M.L.C., Merler, S., Rizzo, C., Ajelli, M., Massari, M., Manfredi, P., Furlanello, C., Tomba, G.S. and Iannelli, M. (2008) Mitigation measures for pandemic influenza in Italy: an individual based model considering different scenarios. *PloS One*, 3 (3).

Diekmann, O., Heesterbeek, J. and Metz, J. (1995) The legacy of Kermack and McKendrick. *Epidemic Models: Their Structure and Relation to Data (D.Mollison, Ed.)*, pp. 95–115.

Diomidus, M. (2002) Epidemiological study designs. *Studies in Health Technology and Informatics*, 65, pp. 126–135.

Domínguez-Machín, M.E., Hernández-Vergara, M.P., Jiménez-García, I., Simá-Álvarez, R. and Rodríguez-Canul, R. (2011) Survey of protozoan, helminth and viral infections in shrimp *Litopenaeus setiferus* and prawn *Macrobrachium acanthurus* native to the Jamapa River region, Mexico. *Diseases of Aquatic Organisms*, 96 (2), pp. 97–103.

Donaldson, L.J., Rutter, P.D., Ellis, B.M., Greaves, F.E., Mytton, O.T., Pebody, R.G. and Yardley, I.E. (2009) Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. *BMJ (Clinical Research Ed.)*, 339, pp. b5213.

Duan, W., Chen, Z., Liu, Z. and Jin, W. (2005) Efficient target strategies for contagion in scale-free networks. *Physical Review E*, 72 (2), pp. 026133.

FAO (2013) *Report of the FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304).* FAO Fisheries and Aquaculture Report No. 1053. Rome: FAO. Available: http://www.fao.org/docrep/018/i3422e/i3422e00.htm [Accessed: 11 October 2013].

FAO (2016) *The state of world fisheries and aquaculture 2016.* ISBN 978-92-5-109185-2. Rome: FAO. Available: http://www.fao.org/3/a-i5555e.pdf [Accessed: 8 July 2016].

Feng, Z. and Velasco-Hernández, J.X. (1997) Competitive exclusion in a vector-host model for the dengue fever. *Journal of Mathematical Biology*, 35 (5), pp. 523–544.

Finkenstadt, B.F., Bjornstad, O.N. and Grenfell, B.T. (2002) A stochastic model for extinction and recurrence of epidemics: estimation and inference for measles outbreaks. *Biostatistics (Oxford, England)*, 3 (4), pp. 493–510.

Flegel, T.W., Lightner, D.V., Lo, C.F. and Owens, L. (2008) Shrimp disease control: past, present and future. In: M.G. Bondad-Reantaso, C.V. Mohan, M. Crumlish and R.P. Subasinghe, ed. *Diseases in Asian Aquaculture.* Sri Lanka, 25th to 28th October 2005. Manila, Philippines: Asian Fisheries Society, pp. 355–378.

Funk, S., Salathe, M. and Jansen, V.A. (2010) Modelling the influence of human behaviour on the spread of infectious diseases: a review. *Journal of the Royal Society, Interface*, 7 (50), pp. 1247–1256.

Gates, M.C. and Woolhouse, M.E. (2015) Controlling infectious disease through the targeted manipulation of contact network structure. *Epidemics*, 12, pp. 11–19.

Germann, T.C., Kadau, K., Longini, I.M., Jr and Macken, C.A. (2006) Mitigation strategies for pandemic influenza in the United States. *Proceedings of the National Academy of Sciences of the United States of America*, 103 (15), pp. 5935–5940.

Gray, A., Greenhalgh, D., Hu, L., Mao, X., & Pan, J. (2011) A stochastic differential equation SIS epidemic model. *SIAM Journal on Applied Mathematics*, 71(3), pp. 876–902.

Green, D.M. (2010) A strategic model for epidemic control in aquaculture. *Preventive Veterinary Medicine*, 94 (1), pp. 119–127.

Green, D.M., Werkman, M. and Munro, L.A. (2012) The potential for targeted surveillance of live fish movements in Scotland. *Journal of Fish Diseases*, 35 (1), pp. 29–37.

Grimes, D.A. and Schulz, K.F. (2002) Cohort studies: marching towards outcomes. *The Lancet,* 359 (9303), pp. 341–345.

Gu, W., Killeen, G.F., Mbogo, C.M., Regens, J.L., Githure, J.I. and Beier, J.C. (2003) An individual-based model of *Plasmodium falciparum* malaria transmission on the coast of Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 97 (1), pp. 43–50.

Hagenaars, T.J., Dekker, A., de Jong, M.C. and Eble, P.L. (2011) Estimation of foot and mouth disease transmission parameters, using outbreak data and transmission experiments. *Revue Scientifique Et Technique (International Office of Epizootics)*, 30 (2), pp. 467–477.

Hameed, A.S., Murthi, B.L.M., Rasheed, M., Sathish, S., Yoganandhan, K., Murugan, V. and Jayaraman, K. (2002) An investigation of Artemia as a possible vector for white spot syndrome virus (WSSV) transmission to *Penaeus indicus*. *Aquaculture*, 204 (1), pp. 1–10.

Heath, M.F., Vernon, M.C. and Webb, C.R. (2008) Construction of networks with intrinsic temporal structure from UK cattle movement data. *BMC Veterinary Research,* 4, pp. 11.

Hernandez-Llamas, A., Magallon-Barajas, F.J., Perez-Enriquez, R., Cabanillas-Ramos, J., Esparza-Leal, H.M. and Portillo-Clark, G. (2013) Pond shutdown as a strategy for preventing outbreaks of white spot disease in shrimp farms in Mexico. *Reviews in Aquaculture*, 5, pp. 1–8.

Hethcote, H.W. and Van Ark, J.W. (1987) Epidemiological models for heterogeneous populations: proportionate mixing, parameter estimation, and immunization programs. *Mathematical Biosciences*, 84 (1), pp. 85–118.

Hoffmann, R.G. and Lim, H.J. (2007) Observational study design. In: W.T. Ambrosius, ed. *Topics in Biostatistics* New Jersey: Humana Press, pp. 19–31.

Holme, I., Retterstøl, K., Norum, K. and Hjermann, I. (2016) Lifelong benefits on myocardial infarction mortality: 40-year follow-up of the randomized Oslo diet and antismoking study. *Journal of Internal Medicine*, 280, pp. 221–227.

Igboeli, O.O., Burka, J.F. and Fast, M.D. (2014) Lepeophtheirus salmonis: a persisting challenge for salmon aquaculture. *Anim Front*, 4 (1), pp. 22–32.

Jepsen, P., Johnsen, S.P., Gillman, M.W. and Sorensen, H.T. (2004) Interpretation of observational studies. *Heart (British Cardiac Society)*, 90 (8), pp. 956–960.

Johnson, T.L., Landguth, E.L. and Stone, E.F. (2016) Modeling relapsing disease dynamics in a host-vector community. *PLoS Negl Trop Dis,* 10 (2), e0004428. Available: http://journals.plos.org/plosntds/article/file?id=10.1371/journal.pntd.0004428&type=printable [Accessed: 1 February 2017].

Kane, A., Lloyd, J., Zaffran, M., Simonsen, L. and Kane, M. (1999) Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: model-based regional estimates. *Bulletin of the World Health Organization*, 77 (10), pp. 801–807.

Keeling, M. (2005a) The implications of network structure for epidemic dynamics. *Theoretical Population Biology*, 67 (1), pp. 1–8.

Keeling, M.J. and Eames, K.T. (2005) Networks and epidemic models. *Journal of the Royal Society Interface*, 2 (4), pp. 295–307.

Keeling, M.J. (2005b) Models of foot-and-mouth disease. *Proceedings of the Biological Sciences / the Royal Society*, 272 (1569), pp. 1195–1202.

Keeling, M.J., Danon, L., Vernon, M.C. and House, T.A. (2010) Individual identity and movement networks for disease metapopulations. *Proceedings of the National Academy of Sciences of the United States of America*, 107 (19), pp. 8866–8870.

Keeling, M.J., Woolhouse, M.E., Shaw, D.J., Matthews, L., Chase-Topping, M., Haydon, D.T., Cornell, S.J., Kappey, J., Wilesmith, J. and Grenfell, B.T. (2001) Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogeneous landscape. *Science (New York, N.Y.)*, 294 (5543), pp. 813–817.

Kermack, W.O. and McKendrick, A.G. (1927) A contribution to the mathematical theory of epidemics. *Proc. R. Soc. Lond. A,* 115 (772), pp. 700–721.

Kim, K.I., Lin, Z. and Zhang, Q. (2013) An SIR epidemic model with free boundary. *Nonlinear Analysis: Real World Applications*, 14 (5), pp. 1992–2001.

Kisjes, K.H., Duintjer Tebbens, R.J., Wallace, G.S., Pallansch, M.A., Cochi, S.L., Wassilak, S.G. and Thompson, K.M. (2014) Individual-based modeling of potential poliovirus transmission in connected religious communities in North America with low uptake of vaccination. *The Journal of Infectious Diseases*, 210 Suppl 1, pp. S424–S433.

Kiss, I.Z., Green, D.M. and Kao, R.R. (2006) The network of sheep movements within Great Britain: Network properties and their implications for infectious disease spread. *Journal of the Royal Society, Interface/the Royal Society*, 3 (10), pp. 669–677.

Kribs-Zaleta, C.M. and Martcheva, M. (2002) Vaccination strategies and backward bifurcation in an age-since-infection structured model. *Mathematical Biosciences*, 177–178, pp. 317–332.

Labrie, L., Roque, A., Gomez-Gil, B. and Turnbull, J.F. (2003) Effect of methyl parathion on the susceptibility of shrimp *Litopenaeus vannamei* to experimental vibriosis. *Diseases of Aquatic Organisms*, 57 (3), pp. 265–270.

Levin, S.A. (1974) Dispersion and Population Interactions. *The American Naturalist*, 108 (960), pp. 207–228.

Liang, Z., Liu, R., Zhao, D., Wang, L., Sun, M., Wang, M. and Song, L. (2016) Ammonia exposure induces oxidative stress, endoplasmic reticulum stress and apoptosis in hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*). *Fish & Shellfish Immunology*, 54, pp. 523–528.

Liu, B., Yu, Z., Song, X., Guan, Y., Jian, X. and He, J. (2006) The effect of acute salinity change on white spot syndrome (WSS) outbreaks in *Fenneropenaeus chinensis*. *Aquaculture*, 253 (1–4), pp. 163–170.

Lloyd-Smith, J.O., Getz, W.M. and Westerhoff, H.V. (2004) Frequency-dependent incidence in models of sexually transmitted diseases: portrayal of pair-based transmission and effects of illness on contact behaviour. *Biological Sciences*, 271 (1539), pp. 625–634.

Lo, C., Ho, C., Peng, S., Chen, C., Hsu, H., Chiu, Y., Chang, C., Liu, K., Su, M. and Wang, C. (1996) White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Diseases of Aquatic Organisms*, 27 (3), pp. 215–225.

Lotz, J.M. and Soto, M.A. (2002) Model of white spot syndrome virus (WSSV) epidemics in *Litopenaeus vannamei*. *Diseases of Aquatic Organisms*, 50 (3), pp. 199–209.

Lotz, J.M., Flowers, A.M. and Breland, V. (2003) A model of taura syndrome virus (TSV) epidemics in *Litopenaeus vannamei*. *Journal of Invertebrate Pathology*, 83 (2), pp. 168–176.

Lou, J. and Ruggeri, T. (2010) The dynamics of spreading and immune strategies of sexually transmitted diseases on scale-free network. *Journal of Mathematical Analysis and Applications*, 365 (1), pp. 210–219.

Lourenço, J. and Recker, M. (2013) Natural, persistent oscillations in a spatial multi-strain disease system with application to dengue. *PLoS Comput Biol*, 9 (10), e1003308. Available: http://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1003308&type=printable [Accessed: 20 August 2015].

Mann, C.J. (2003) Observational research methods. Research design II: cohort, cross sectional, and case–control studies. *Emergency Medicine Journal: EMJ*, 20 (1), pp. 54–60.

Marais, B., Gie, R., Schaaf, H., Hesseling, A., Obihara, C., Starke, J., Enarson, D., Donald, P. and Beyers, N. (2004) The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era [State of the Art]. *The International Journal of Tuberculosis and Lung Disease*, 8 (4), pp. 392–402.

May, R.M. and Anderson, R.M. (1979) Population biology of infectious diseases: Part II. *Nature,* 280 (5722), pp. 455–461.

McCreadie, R.G. and Scottish Comorbidity Study Group. (2002) Use of drugs, alcohol and tobacco by people with schizophrenia: case–control study. *The British Journal of Psychiatry: The Journal of Mental Science*, 181, pp. 321–325.

Meyers, L. (2007) Contact network epidemiology: Bond percolation applied to infectious disease prediction and control. *Bulletin of the American Mathematical Society*, 44 (1), pp. 63–86.

Milgram, S., Sabini, J.E. and Silver, M.E. (1992) *The individual in a social world: essays and experiments.* New York: Mcgraw-Hill Book Company.

Miller, J.C., Slim, A.C. and Volz, E.M. (2012) Edge-based compartmental modelling for infectious disease spread. *Journal of the Royal Society, Interface / the Royal Society*, 9 (70), pp. 890–906.

NACA (2014) *Diseases of crustaceans: acute hepatopancreatic necrosis syndrome (AHPNS).* Available: http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf [Accessed: 1 May 2015].

NACA (2017) *Quarterly aquatic animal disease report (Asia and Pacific Region) 1998–2016.* Thailand: NACA. Available: http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report [Accessed: 29 April 2017].

Natale, F., Giovannini, A., Savini, L., Palma, D., Possenti, L., Fiore, G. and Calistri, P. (2009) Network analysis of Italian cattle trade patterns and evaluation of risks for potential disease spread. *Preventive Veterinary Medicine*, 92 (4), pp. 341–350.

Newman, M.E. (2008) The mathematics of networks. *The New Palgrave Encyclopedia of Economics*, 2 (2008), pp. 1–12.

Newman, M.E., Watts, D.J. and Strogatz, S.H. (2002) Random graph models of social networks. *Proceedings of the National Academy of Sciences of the United States of America*, 99 Suppl 1, pp. 2566–2572.

Ng, T.W., Turinici, G. and Danchin, A. (2003) A double epidemic model for the SARS propagation. *BMC Infectious Diseases*, 3 (1), pp. 1.

Nold, A. (1980) Heterogeneity in disease-transmission modeling. *Mathematical Biosciences*, 52 (3), pp. 227–240.

Ocampo, L., Chavez, B., Tapia, G., Ibarra, C. and Sumano, H., 2014. Efficacy of a pharmaceutical preparation based on glycyrrhizic acid in a challenge study of white spot syndrome in white shrimp (*Litopenaeus vannamei*). Aquaculture, 428, pp.280–283.

Ogut, H., LaPatra, S. and Reno, P. (2005) Effects of host density on furunculosis epidemics determined by the simple SIR model. *Preventive Veterinary Medicine*, 71 (1), pp. 83–90.

Ortiz-Pelaez, A., Pfeiffer, D., Soares-Magalhaes, R. and Guitian, F. (2006) Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (FMD) epidemic in the UK. *Preventive Veterinary Medicine*, 76 (1), pp. 40–55.

Paillard, C., Jean, F., Ford, S.E., Powell, E.N., Klinck, J.M., Hofmann, E.E. and Flye-Sainte-Marie, J. (2014) A theoretical individual-based model of brown ring disease in Manila clams, *Venerupis philippinarum*. *Journal of Sea Research*, 91, pp. 15–34.

Pastor-Satorras, R. and Vespignani, A. (2001) Epidemic spreading in scale-free networks. *Physical Review Letters*, 86 (14), pp. 3200.

Pearce, N. (2012) Classification of epidemiological study designs. *International Journal of Epidemiology*, 41 (2), pp. 393–397.

Peeler, E.J. and Taylor, N.G. (2011) The application of epidemiology in aquatic animal health-opportunities and challenges. *Veterinary Research*, 42 (1), pp. 94.

Perisic, A. and Bauch, C.T. (2009) Social contact networks and disease eradicability under voluntary vaccination. *PLoS Comput Biol*, 5 (2), e1000280. Available: http://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1000280&type=printable [Accessed: 3 May 2016].

Phuoc, L., Corteel, M., Nauwynck, H., Pensaert, M., Alday-Sanz, V., Van Den Broeck, W., Sorgeloos, P. and Bossier, P. (2008) Increased susceptibility of white spot syndrome virus-infected *Litopenaeus vannamei* to *Vibrio campbellii*. *Environmental Microbiology*, 10 (10), pp. 2718–2727.

Quandelacy, T.M., Viboud, C., Charu, V., Lipsitch, M. and Goldstein, E. (2014) Age- and sex-related risk factors for influenza-associated mortality in the United States between 1997-2007. *American Journal of Epidemiology*, 179 (2), pp. 156–167.

Rahman, M.M., Escobedo-Bonilla, C.M., Corteel, M., Dantas-Lima, J.J., Wille, M., Sanz, V.A., Pensaert, M.B., Sorgeloos, P. and Nauwynck, H.J. (2006) Effect of high water temperature (33 °C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei*. *Aquaculture*, 261 (3), pp. 842–849.

Rattana, P., Blyuss, K.B., Eames, K.T. and Kiss, I.Z. (2013) A class of pairwise models for epidemic dynamics on weighted networks. *Bulletin of Mathematical Biology*, 75 (3), pp. 466–490.

Rautureau, S., Dufour, B. and Durand, B. (2011) Vulnerability of animal trade networks to the spread of infectious diseases: a methodological approach applied to evaluation and emergency control strategies in cattle, France, 2005. *Transboundary and Emerging Diseases*, 58 (2), pp. 110–120.

Robinson, M., Conan, A., Duong, V., Ly, S., Ngan, C., Buchy, P., Tarantola, A. and Rodo, X. (2014) A model for a chikungunya outbreak in a rural Cambodian setting: implications for disease control in uninfected areas. *PLoS Negl Trop Dis*, 8 (9), e3120. Available: http://journals.plos.org/plosntds/article/file?id=10.1371/journal.pntd.0003120&type=printable [Accessed: 4 November 2016].

Salas, M., Hotman, A. and Stricker, B.H. (1999) Confounding by indication: an example of variation in the use of epidemiologic terminology. *American Journal of Epidemiology*, 149 (11), pp. 981–983.

Sattenspiel, L. and Dietz, K. (1995) A structured epidemic model incorporating geographic mobility among regions. *Mathematical Biosciences*, 128 (1), pp. 71–91.

Schlesselman, J.J. (1982) *Case–control studies: design, conduct, analysis.* New York: Oxford University Press.

Schulz, K.F. and Grimes, D.A. (2002) Case–control studies: research in reverse. *The Lancet*, 359 (9304), pp. 431–434.

SEAFDEC/MFRDMD (2016) *Traceability of fish and fishery products: capture fishery products.* Available: http://www.seafdec.org/documents/sc16_wp10.pdf [Accessed: 3 March 2016].

Shulgin, B., Stone, L. and Agur, Z. (1998) Pulse vaccination strategy in the SIR epidemic model. *Bulletin of Mathematical Biology*, 60 (6), pp. 1123–1148.

Soler-Jiménez, L.C., Paredes-Trujillo, A.I. and Vidal-Martínez, V.M. (2016) Helminth parasites of finfish commercial aquaculture in Latin America. *Journal of Helminthology*, 91, pp. 1–27.

Song, J.W. and Chung, K.C. (2010) Observational studies: cohort and case–control studies. *Plastic and Reconstructive Surgery*, 126 (6), pp. 2234–2242.

Swerdlow, A.J., Cooke, R., Albertsson-Wikland, K., Borgstrom, B., Butler, G., Cianfarani, S., Clayton, P., Coste, J., Deodati, A., Ecosse, E., Gausche, R., Giacomozzi, C., Kiess, W., Hokken-Koelega, A.C., Kuehni, C.E., Landier, F., Maes, M., Mullis, P.E., Pfaffle, R., Savendahl, L., Sommer, G., Thomas, M., Tollerfield, S., Zandwijken, G.R. and Carel, J.C. (2015) Description of the SAGhE cohort: a large European study of mortality and cancer incidence risks after childhood treatment with recombinant growth hormone. *Hormone Research in Paediatrics*, 84 (3), pp. 172–183.

Tassier, T. (2013) *The economics of epidemiology.* New York: Springer.

Tendencia, E.A., Bosma, R.H. and Verreth, J.A.J. (2011) White spot syndrome virus (WSSV) risk factors associated with shrimp farming practices in polyculture and monoculture farms in the Philippines. *Aquaculture*, 311 (1–4), pp. 87–93.

Thailand DoF (2016) *Number of farms, area under culture and yield by species and provinces, 1999-2013.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.fisheries.go.th/it-stat/yearbook/Index.htm [Accessed: 27 June 2016].

Tien, J.H. and Earn, D.J. (2010) Multiple transmission pathways and disease dynamics in a waterborne pathogen model. *Bulletin of Mathematical Biology*, 72 (6), pp. 1506–1533.

Tookwinas, S., Chiyakum, K. and Somsueb, S. (2005) *Aquaculture of white shrimp Penaeus vannamei in Thailand.* Iloilo, Philippines: SEAFDEC. Available: https://repository.seafdec.org.ph/bitstream/handle/10862/855/RTC-P.vannamei_p74-80.pdf?sequence=1&isAllowed=y [Accessed: 1 April 2014].

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. and Lightner, D.V. (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Diseases of Aquatic Organisms*, 105 (1), pp. 45–55.

Van Schayck, C.P., Loozen, J.M., Wagena, E., Akkermans, R.P. and Wesseling, G.J. (2002) Detecting patients at a high risk of developing chronic obstructive pulmonary disease in general practice: cross sectional case finding study. *BMJ (Clinical Research Ed.)*, 324 (7350), pp. 1370.

Van Segbroeck, S., Santos, F.C. and Pacheco, J.M. (2010) Adaptive contact networks change effective disease infectiousness and dynamics. *PLoS Comput Biol*, 6 (8), e1000895. Available: http://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1000895&type=printable [Accessed: 12 May 2016].

Vergu, E., Busson, H. and Ezanno, P. (2010) Impact of the infection period distribution on the epidemic spread in a metapopulation model. *PloS One*, 5 (2), pp. e9371.

Vijayan, K., Raj, V.S., Balasubramanian, C., Alavandi, S., Sekhar, V.T. and Santiago, T. (2005) Polychaete worms—a vector for white spot syndrome virus (WSSV). *Diseases of Aquatic Organisms,* 63 (2–3), pp. 107–111.

Volz, E.M., Miller, J.C., Galvani, A. and Meyers, L.A. (2011) Effects of heterogeneous and clustered contact patterns on infectious disease dynamics. *PLoS Comput Biol*, 7 (6), e1002042. Available: http://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1002042&type=printable [Accessed: 12 May 2015].

Watts, D.J. (1999) Networks, dynamics, and the small-world phenomenon 1. *American Journal of Sociology*, 105 (2), pp. 493–527.

Watts, D.J. and Strogatz, S.H. (1998) Collective dynamics of 'small-world'networks. *Nature*, 393 (6684), pp. 440–442.

Wesolowski, A., Eagle, N., Tatem, A.J., Smith, D.L., Noor, A.M., Snow, R.W. and Buckee, C.O. (2012) Quantifying the impact of human mobility on malaria. *Science (New York, N.Y.)*, 338 (6104), pp. 267–270.

WHO (2016) *The terminology of epidemiology.* Available: http://www.who.int/topics/epidemiology/en/ [Accessed: 14 July 2016].

Williams, E. and Bunkley-Williams, L. (2000) Multicellular parasite (macroparasite) problems in aquaculture. *Encyclopedia of Aquaculture.* New York: Wiley, pp. 562–579.

Witten, G. and Poulter, G. (2007) Simulations of infectious diseases on networks. *Computers in Biology and Medicine*, 37 (2), pp. 195–205.

Woodward, M. (2013) *Epidemiology: study design and data analysis.* Third ed. New York: CRC press.

Woolhouse, M.E., Dye, C., Etard, J.F., Smith, T., Charlwood, J.D., Garnett, G.P., Hagan, P., Hii, J.L., Ndhlovu, P.D., Quinnell, R.J., Watts, C.H., Chandiwana, S.K. and Anderson, R.M. (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America*, 94 (1), pp. 338–342.

Yusuf, S., Hawken, S., Ôunpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J. and Lisheng, L. (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case–control study. *The Lancet*, 364 (9438), pp. 937–952.

Zhang, J., Dong, S., Dong, Y., Tian, X. and Hou, C. (2008) Bioassay evidence for the transmission of WSSV by the harpacticoid copepod *Nitocra* sp. *Journal of Invertebrate Pathology*, 97 (1), pp. 33–39.

Chapter 3 - Evaluating risk factors for transmission of acute hepatopancreatic necrosis disease (AHPND) in the Thai shrimp farming sector

N. Saleetid; D.M. Green; F.J. Murray

Preface

Building on the author's master's degree study in 2012/2013, the investigation of risk factors for site-to-site transmission of AHPND is intensively assessed in this chapter. An extra phase of data collection (Phase 4: Cross-checking of the risk factors) was implemented and more advanced statistical approaches were applied, insofar as these are technically admissible. In the extra phase, the data were collected using face-to-face interviews with nine key informants. More advanced statistical analyses (i.e. receiver operating characteristic curve and cross-validation analysis) were applied than those used in the original master's thesis. The survey data, i.e. farming management practices and the cumulative AHPND incidence, has also been used in interpreting results of Chapter 4 and in modelling the spread of AHPND of Chapter 6.

At the time of data collection for this observational epizootiological study, the cause of the initial outbreak of AHPND was unknown. Thus, the designation of cases and controls was based on the AHPND decision tree, which was developed as an epizootiological tool in the cross-sectional study. Note that this chapter is designed for publication. This study utilised the data from the Sustaining Ethical Aquaculture Trade (SEAT), EU FP7 research project.

Chapter 3 - Evaluating risk factors for transmission of acute hepatopancreatic necrosis disease (AHPND) in the Thai shrimp farming sector

3.1 Abstract

In this study, the risk factors for site-to-site transmission of AHPND were evaluated in Thailand using a cross-sectional approach. An unbiased sample frame of 206 shrimp farms (previously participated in the Sustaining Ethical Aquaculture Trade (SEAT), EU FP7 research project) were engaged in four consecutive structured surveys in 2013–2014. The outcome led to the development of a decision tree for AHPND case determination and risk estimation using univariate and unconditional logistic regression analysis.

Interviews were successfully performed with 143 of the above 206 shrimp farms (70 %). 35 % of the 143 met the AHPND case definition, with a higher proportion in the east. Southern farms showed a delay in AHPND onset, and large-scale farms that usually invested in more biosecurity resources than others also showed a delayed onset of AHPND. The cumulative incidence of AHPND in southern and large-scale farms increased sharply after the first occurrence AHPND, however. Two risk factors for AHPND transmission were found: earthen ponds were less risky with an odds ratio (OR) of 0.25 (95 % CI 0.06–0.8; P-value = 0.02) compared with shrimp rearing in fully plastic-lined ponds; and the absence of pond harrowing was higher risky with an OR of 3.9 (95 % CI 1.3–12.6; P-value = 0.01) compared with the presence of pond harrowing. These findings imply that the Thai shrimp farming industry should enhance biosecurity systems, and that a simple good farming management practice, such as harrowing pond bottom which are a common practice of shrimp farming in earthern ponds, may protect farms against AHPND.

3.2 Introduction

Acute hepatopancreatic necrosis disease (AHPND) is the most economically damaging epizootic pandemic to affect the farmed penaeid shrimp sector since the outbreak of white spot syndrome in the 1990's (Flegel, 1997). In 2013 the total annual losses were

estimated as USD 5 billion (Rosenberry, 2013). AHPND, also described as early mortality syndrome (EMS) prior to the recent detection of a bacterial pathological agent (OIE, 2013; Thitamadee *et al.,* 2016) was first detected in China in 2009. It was subsequently reported in Vietnam in 2010, Malaysia and Thailand in 2011 (Eduardo and Mohan, 2012), Mexico in 2013 (Nunan *et al.,* 2014) and the Philippines in 2014 (Leobert *et al.,* 2015), whereas mass mortalities attributed to *Vibrio* species in Indian shrimp farms were non-AHPND (Kumar *et al.,* 2014).

AHPND (most Thai farmers called this disease "EMS" or "EMS/AHPND") was reported in Thailand at first time in the east central provinces in late 2011 (FAO, 2013) and in southern producer provinces in late 2012. In each instance, the incidence rose sharply in the months following the first detection, resulting in widespread precautionary fallowing of ponds as the primary farmer response (Flegel, 2012). Although the disease affects a wide range of important commercial penaeid shrimp species, including *Litopenaeus vannamei*, *Penaeus monodon* and *P. chinensis* farmed over a range of production intensities (OIE, 2013), the highest risk of loss appears to be associated with more intensive farming practices (FAO, 2013), the norm for *L. vannamei* production.

The former “EMS” title describes the occurrence of mass mortalities (> 70 %) during the first 35 days of culture in newly prepared ponds (FAO, 2013b; Flegel and Lo, 2014; Lightner *et al.,* 2012; Thitamadee *et al.,* 2016). AHPND, meanwhile, describes the characteristic degenerative pathology in the vital digestive and glandular midgut-organ. Toxin-producing strains of *Vibrio parahaemolyticus* have been confirmed as a primary causative agent Tran *et al.* (2013). These pathogens also had a high chance of antibiotic resistance (Han *et al.,* 2015a; Han *et al.,* 2015b; Lai *et al.,* 2015). Several *Vibrio* species are zoonotic (Austin, 2010). Fortunately, the three strains of *V. parahaemolyticus* implicated in AHPND infection in Thailand do not appear to present a public health-risk (Chonsin *et al.,* 2016; Kondo *et al.,* 2014).

The clinical signs of AHPND do not differ significantly in some cases from those of shrimp infected with different types of vibriosis. For example, as a hepatopancreatic infection, *V. harveyi* causes necrosis of the hepatopancreatic cells, and a thicker abnormal basal

lamina than in normal shrimp (Jiravanichpaisal *et al.,* 1994; Tran *et al.,* 2013). Clear case definition is therefore very important to evaluate the risk factors for AHPND occurrence.

A range of studies has positively correlated loss-severity with various environmental risk factors. The general abundance of *Vibrio* spp. and other pathogens in shrimp has been linked to high phytoplankton levels (Peterson *et al.,* 2010) and pH level (> 8.2, Costa *et al.,* 2010), and Akazawa and Eguchi (2013) found such a correlation between elevated pH and AHPND in Malaysia. In China, outbreaks of AHPND have been associated with elevated salinity levels and older ponds (Panakorn, 2012). Co-location of semi-intensive and intensive farming systems in southern Vietnam pointed to an increased probability of AHPND mortalities in intensive systems (FAO, 2013). There has been a general lack of systematic epizootiological studies underpinning such observations, however.

Taking a cross-sectional approach, the Thai shrimp farming sites were sampled over a study period to seek paired groups, with or without AHPND clinical signs. Both groups were identified and compared on the basis of potential causal attributes. The study aimed to assess (1) the association of environmental and farm-management risk factors with the geographical prevalence and incidence of putative AHPND cases in Thailand in order to (2) draw inferences regarding its transmission and to suggest possible mitigation strategies.

3.3 Methods

3.3.1 Development of a case definition and decision tree for AHPND

Since the causal agent of AHPND was not known at this time, case definitions in this study were based on clinical signs described in the AHPND disease advisory of NACA (2014), through key informant and farmer perceptions (Section 3.3.4), and a review of other secondary literature on Thai and global AHPND outbreaks. Based on this information, an 'AHPND case decision-tree' was constructed around four specific and measurable indicators: (1) date of onset of the first clinical signs of AHPND, (2) the age of the affected shrimp, (3) mortality rates, and (4) three characteristic and easily observed clinical signs of AHPND infection: i.e. location of dead shrimp, diseased shrimp behaviour and the presence of a pale/whitish hepatopancreas. To assist differentiation

in this final step, picture cards showing gross AHPND whitish and atrophied hepatopancreas pathology (FAO, 2013; NACA, 2014) were shown alongside those showing clinical signs of other high-prevalence shrimp diseases (white spot disease, yellow head disease, taura syndrome, vibriosis and infectious hypodermal haematopoietic necrosis virus: Murray *et al.*, 2013). The picture cards are shown in Appendix A at the end of this thesis.

3.3.2 Candidate risk factors for AHPND occurrence at farm level

Candidate farm-level risk (i.e. independent) factors for AHPND occurrence were reviewed from secondary data (Akazawa and Eguchi, 2013; FAO, 2013; Panakorn, 2012) and key informant opinion (Thai Department of Fisheries or DoF staff and farmers: Section 3.3.4). In addition, potential risk factors were also mined from an earlier 'Integrated Farmer Survey' (Section 3.3.3). A diverse range of 24 factors (Table 3.1) was short-listed covering farm scale and location, farming experience, management practices, farm infrastructure and water management characteristics.

Table 3.1 Candidate risk factors for AHPND occurrence at farm level

Main factor	Candidate risk factors
Farm scale and location	Farm scale and region
Farming experience	Age of farm
Management practices	Age of pond affected, pond drying duration, stocking density, pond harrowing before stocking, effluent treatment, sediment removal and sediment fate
Farm infrastructure	Ongrowing pond type, maximum water depth, clean water storage, alternative clean water storage and growing pond, and sediment pond
Water management characteristics	Water source, discharge method, water storage method, recirculate and reuse water, maximum water replacement, water exchange frequency, maximum salinity, average pH and average alkalinity

3.3.3 Sample design

An 'Integrated Farmer Survey' (IFS: Murray *et al.,* 2013), separately enumerated between December 2010 and July 2011 (i.e. immediately prior to the onset of AHPND), provided a sample frame of 206 farms for this research. The primary selection phase of 206 sample farms was based on district-wise production data collected from provincial DoF offices between 2009 and 2014 (Thailand DoF, 2016), followed by randomised selection of farm clusters and individual farms within clusters. The results of the current study are therefore expected to be generalisable to wider Thai shrimp production conditions.

In more detail, this frame was based on a multi-phase, random sampling approach stratified on farm-scale and location, i.e. being conducted across the principal Thai farming areas in east central (Chachoengsao and Chanthaburi provinces) and south Thailand (mainly Suratthani province and some areas in Songkhla province). Farm selection was stratified on three scale levels—large, medium and small—based on indicators of business ownership, labour pattern, farm management and the number of ongrowing ponds, as shown in Table 3.2 (Murray *et al.,* 2013).

Table 3.2 Criteria used for classifying Thai shrimp farms into three scales: small, medium and large (from Murray *et al.*, 2013)

Criteria	Farm scale		
	Small	Medium	Large
Ownership of business	Household or extended family	Household or external owner	Corporate (i.e. joint stock company)
Full-time labour (non-family)	$<$ 3 Full-time labour	Yes	Yes
Management	Household or extended family	Household or external salaried manager	Salaried manager
Number of ongrowing ponds	$<$ 3	$>$ 2	$>$ 2

As a key variable to determine AHPND spread, the location of farms corresponded to different onset-times for AHPND, i.e. first in the east followed by the south, and also co-varied with scale to some degree, as farms tended to be larger on average in the south. Informal organisation of farmers through shrimp farmer clubs also tended to be more advanced in the south (Kassam *et al.,* 2011) and, therefore, hypothetically, also their ability to coordinate collective action in response to disease outbreaks.

3.3.4 Survey design

Primary data collection was based on four iterative survey phases conducted between March 2013 and December 2014, as described below. All interviews were conducted with permission of the farmers in the sample-frame. The interviews took place at a convenient time for the farmers, and were conducted in the Thai language by the main author who is a Thai native speaker.

Phase 1 Brief telephone survey. An attempt was made to contact by telephone all 206 farmers in the sample-frame. With a short 15–20 minute structured survey, respondents were asked about disease problems and possible associated risk factors on their farms since the first reports of AHPND in Thailand at the late of 2011. Questions covered the timing (month) of the first AHPND-type losses, clinical signs, numbers of ponds affected, mortality rates, age of diseased shrimp, management practices, and measures taken to mitigate disease transmission or recurrence, as shown in Appendix B at the end of this thesis.

Phase 2 In-depth face-to-face interview. This more in-depth structured survey incorporated the pre-prepared decision-tree and disease cards described above. A total of 143 farmers who has responded at Phase-1 farmers provided the sample-frame for this phase. A random-selection method was used to select 14–15 farms for interview in each of the three farm-scale and three provinces (i.e. two eastern and one southern). An interview was arranged in their farm. It took approximately 30–45 minutes per interview. Note that the number of farms in each scale category was less balanced due to limits on total numbers in each area.

Phase 3 Follow-up gap-filling and data validation. This phase was conducted with Phase-2 farmers by phone between June and September 2013. It included collection of additional data on stocking density characteristics, age of affected ponds, pond drainage and sludge removal practices, and water quality conditions (pH, salinity and alkalinity)

Phase 4 Cross-checking of the risk factors. The final phase was to explore farmers' perspectives on the risk factors in respect to AHPND obtained from the cross-sectional analysis, and any change in local shrimp farming practices. Several open-questions were used to ask nine skilled persons from within the sample frame in December 2014. An interview was arranged in their farm and took approximately 50 minutes to 1 hour. This interview allowed the farmers' opinions and beliefs in all respects.

3.4 Data Analysis

3.4.1 Descriptive analysis of the occurrence of AHPND and other diseases

The prevalence of putative AHPND cases, and other diseases, was plotted against the location and farm-scale stratification variables. The cumulative monthly incidence of new AHPND cases was also plotted against the same variables, calculated as the number of new cases per month time divided by the number of the total responding population at risk in each stratification category. Based on the timing of the first reported cases within the sample frame, we used two location-specific exposure times for case definitions. First, cases in the eastern provinces ranged from January 2012 to May 2013. Second, cases in the southern provinces ranged from December 2012 to May 2013.

3.4.2 Statistical analysis of the risk factors for AHPND

The aim of the statistical analysis was to evaluate the risk factors for AHPND occurrence at farm level. All statistical analyses were conducted within the R Programme Environment (R foundation for statistical computing, 2015). The risk of AHPND was estimated as an odds ratio (OR); this measure is often used to investigate risks associated with rare diseases (Schmidt and Kohlmann, 2008):

$$R_{exposed} = \frac{\text{Cases with risk factor}}{\text{Non-cases with it}}$$

$$R_{control} = \frac{\text{Cases without risk factor}}{\text{Non-cases without it}}$$

$$OR = \frac{R_{exposed}}{R_{control}}$$

where $OR > 1$: risk factor for increased risk of AHPND

$OR < 1$: risk factor for decreased risk of AHPND

$OR = 1$: risk factor is independent of AHPND presence.

Using two-step methods, the 24 candidate variables (Section 3.3.2) were screened using univariate tests followed by multivariate tests. For the univariate tests, the significance of the OR was assessed through 95 % confidence intervals (CI), Fisher's Exact Test or binary logistic regression. Multivariate tests were done using unconditional logistic regression models to evaluate the potential risk factors that were obtained from the univariate tests above. All variables with a P-value < 0.1 in univariate models were kept to test using backward stepwise (variables were removed from the model) regression procedure. Given several competing models, the AIC (Akaike's Information Criterion) was used as a comparison tool, in which the better-fitting model showed a lower value of AIC (Akaike, 1974).

The predictive performance of the models was evaluated in two ways:

(1) Cross-validation technique: Using a random-selection method, farm samples in a group of cases and controls were divided into two approximately equal-size subsets: D_1 and D_2. Models were constructed on D_1 as a training set, and the predictive ability of the models was tested using D_2 as a testing set (Mori *et al.,* 1999). Then, we computed the proportion of observations, which were correctly predicted. Cross validation was performed both ways round (swapping the sets), and an average of the accuracies was calculated and reported.

(2) Receiver operating characteristic (ROC) curve: The ROC approach was used to judge the performance of models in terms of predicting AHPND occurrence with two possible outcomes: cases (diseased farms) and controls (non-disease farms). We used a range of different cut-off points obtained from estimated probabilities (log odds) to define predicted cases and non cases. The y coordinates on the ROC curve for a model were derived as true positives (sensitivity), and the x coordinates were derived as false positives (1-specificity) at each cut-off point. Based on the area under the ROC curve (AUC), the model was classified to be either an informative model ($AUC > 0.5$; the predictive result of model is better than a random model) or an uninformative model ($AUC = 0.5$; the predictive result of model is not different from a random model) (Alonzo and Pepe, 2002; Kumar and Indrayan, 2011).

To aid understanding, the overall methodology is shown in Figure 3.1.

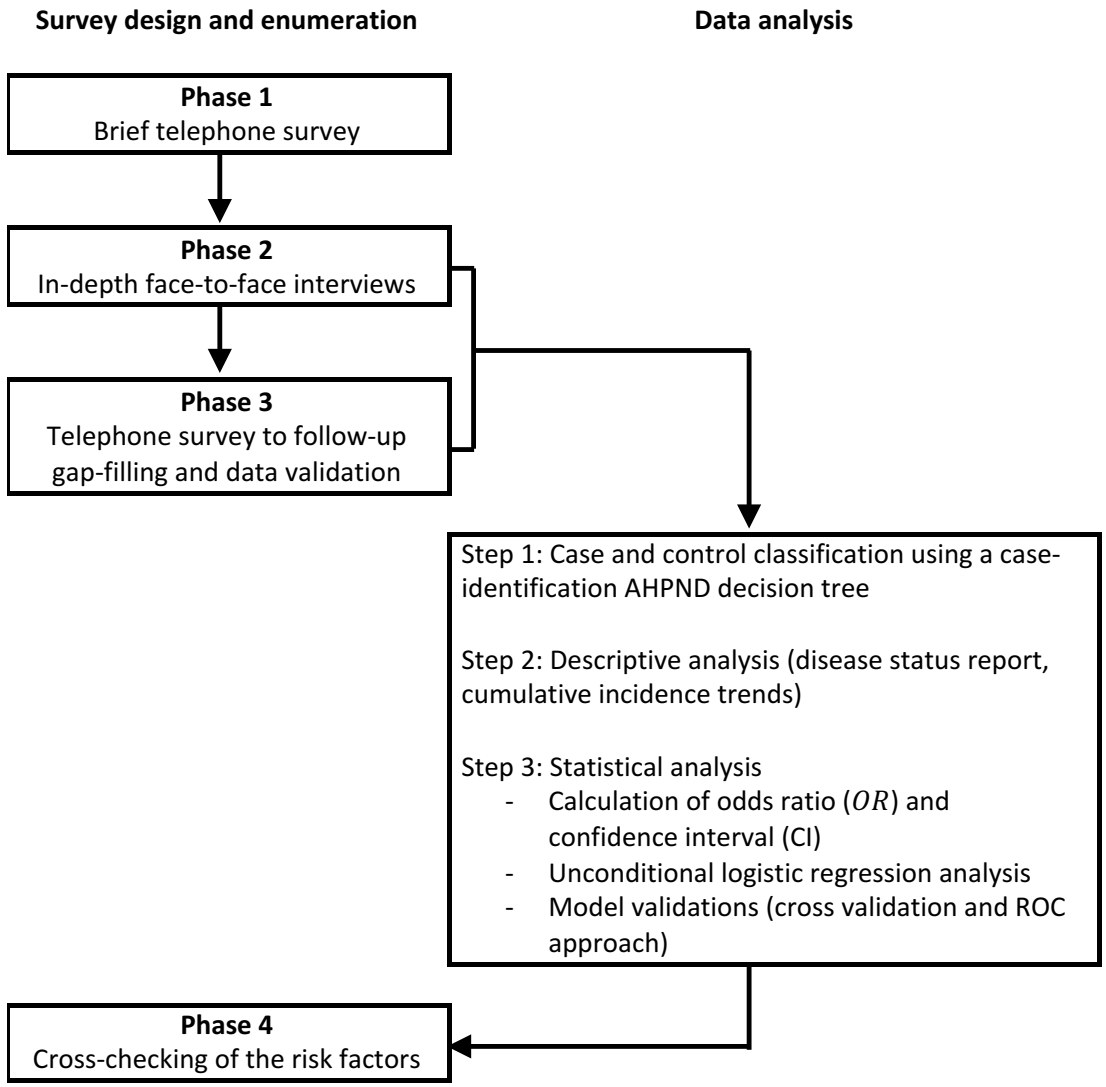


Figure 3.1 A flow chart of the methodology used in evaluating risk factors for transmission of acute hepatopancreatic necrosis disease (AHPND) in Thai shrimp farming. The survey design contained four major phases. Data analysis evaluated the risk factors for AHPND in three steps.

3.5 Results

3.5.1 Identification of AHPND cases and controls

3.5.1.1 Case-identification AHPND decision tree

A case-identification AHPND decision tree was developed for this study because, at the time, AHPND was a disease of unknown etiology. Four indicators of putative AHPND infection were incorporated into this decision tree, resulting in farms being assigned to one of three mutually exclusive infection categories: higher or lower probability of

AHPND infection, or no AHPND (Figure 3.2). Justifications for the indicators, constituting four tiers in the decision-tree were as follows:

Tier 1 Date of onset of the first AHPND clinical signs. The onset timing of putative cases had to occur after the first reported cases of AHPND officially reported by the Thailand Department of Fisheries (DoF) in different regions, i.e. the fourth quarter of 2011 (October to December) in eastern provinces (FAO, 2013), and the fourth quarter of 2012 in southern provinces, according to our survey.

Tier 2 Age of affected shrimp. Based on the reports of Flegel and Lo (2014) and FAO (2013), any infection that occurred beyond 35 days after first stocking of post-larvae was excluded from being a putative AHPND case.

Tier 3 Mortality rates of infected farm. Akazawa and Eguchi (2013) reported average mortality rates of 70–80 % in 'AHPND affected ponds in a large commercial shrimp farm in Malaysia (Akazawa and Eguchi, 2013) whilst mortalities approaching 100 % have been observed in other countries (Eduardo and Mohan, 2012). Consequently, cases with 70–100 % mortality rate were classed as having *higher* probability of APHND infection whilst farms with rates between 10–70 % were classified as having *lower* AHPND probability.

Tier 4 Multiple visible clinical signs of the AHPND pathology. Based on the NACA advisory (2014), cases classified as lower or higher probability AHPND cases in Tier 3 were subjected to further confirmation according to the following additional behavioural gross pathological signs:

(1) Mortality location: Mortalities concentrated around the pond edge and pond bottom.

(2) Shrimp behaviour (spiral or swirling swimming to edge of pond and turning belly-up), and

(3) Pale/whitish/atrophy of the hepatopancreas (HP; also HP hard to crush by hand), and discolouration of abdominal muscle (opaque; pinkish or white).

Having defined higher and lower probability cases from Tier 3, the higher probability cases needed to have only (3) described above to be confirmed, whilst two, one of these was (3), or more clinical signs were required to confirm lower probability cases. In both instances, failure to recognise any of these clinical signs resulted in demotion of the case to a non-AHPND loss cause. The cases may have co-infection of other shrimp diseases such as white spot disease and yellow head disease.

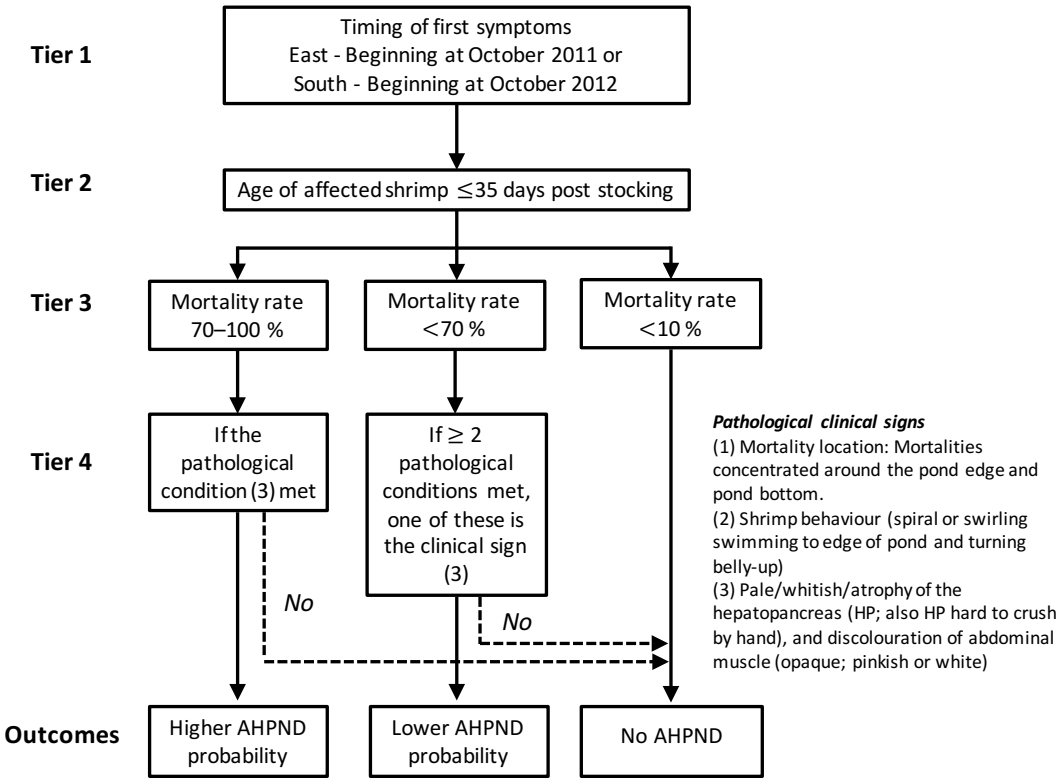


Figure 3.2 The AHPND decision tree for determination of higher AHPND probability, lower AHPND probability, and no AHPND. The decision was based upon four tiers including the significant behavioural gross pathological clinical signs of AHPND.

3.5.1.2 Completed interviews

Interviews were completed for 143 of the 206 farms in the original sample (70 %). Reasons for non-response were as follows: 48 farms (76 %) did not respond to calls or

their numbers were no longer valid; 15 farms (24 %) had ceased to engage in shrimp farming (Table 3.3).

Table 3.3 The outcome from the telephone survey (Phase 1) followed by face-to-face interviews (Phase 2)

Region	East						South						Total
Province	Chanthaburi			Chachoengsao			Suratthani			Songkhla			
farm scale	S	M	L	S	M	L	S	M	L	S	M	L	
Completed interview	22	7	3	43	9	0	27	25	4	0	0	3	143
Non-response for calling	4	2	0	7	2	0	6	10	0	0	0	4	35
Invalid contact number	3	0	1	3	1	0	4	1	0	0	0	0	13
Not engaged in shrimp farming	3	2	0	3	1	0	5	0	0	0	0	1	15
Total	**32**	**11**	**4**	**56**	**13**	**0**	**42**	**36**	**4**	**0**	**0**	**8**	**206**

S = small farm; M = medium farm; L = large farm

3.5.1.3 Case and control samples

Based on the decision-tree outcomes, the 143 responding farms were each assigned to one of three groups: (1) 51 were classed as having a high probability of AHPND, (2) 55 farms reported no disease loss or clinical signs consistent with other diseases, whilst (3) the remaining 37 farms had an indeterminate status, i.e. low-probability of AHPND. To increase statistical power, we omitted these 37 farms with the indeterminate status of group 3 from further analysis. The first two groups were assigned as case and control groups, respectively (Table 3.4).

Table 3.4 Cross-tabulation of outcomes for case and control samples

Farm Scale	Geographic Location				Total	
	East region		South region			
	Case	Control	Case	Control	Case	Control
Small	26	24	5	14	31	38
Medium	8	3	9	12	17	15
Large	2	0	1	2	3	2
Total	**36**	**27**	**15**	**28**	**51**	**55**

3.5.2 Descriptive epizoology of AHPND

3.5.2.1 Report of disease status

The disease status of our sample farms is shown in Figure 3.3. AHPND presented a major disease problem for shrimp farms in the sample frame in both regions of Thailand, with a higher AHPND occurrence of 43 % (36 of 84) of sample farms in the east compared to 25 % (15 of 59) of sample farms in the south. Consistent with the earlier onset of the disease in the east, Chanthaburi province had the highest AHPND prevalence, accounting for 44 % (14 of 32) of sample farms in Chanthaburi, followed by 42 % (22 of 52) of sample farms in Chachoengsao, and 37 % (15 of 56) of sample farms in Suratthani. The figure also shows that farms infected with white spot disease also had a high probability of AHPND infection, as found in sample farms in Chanthaburi and Suratthani. Farms without AHPND clinical signs were infected with white spot disease, yellow head disease, taura syndrome and white feces syndrome. The results, therefore, provided an overview of the disease problems facing the Thai shrimp farming sector during this epizootiological survey.

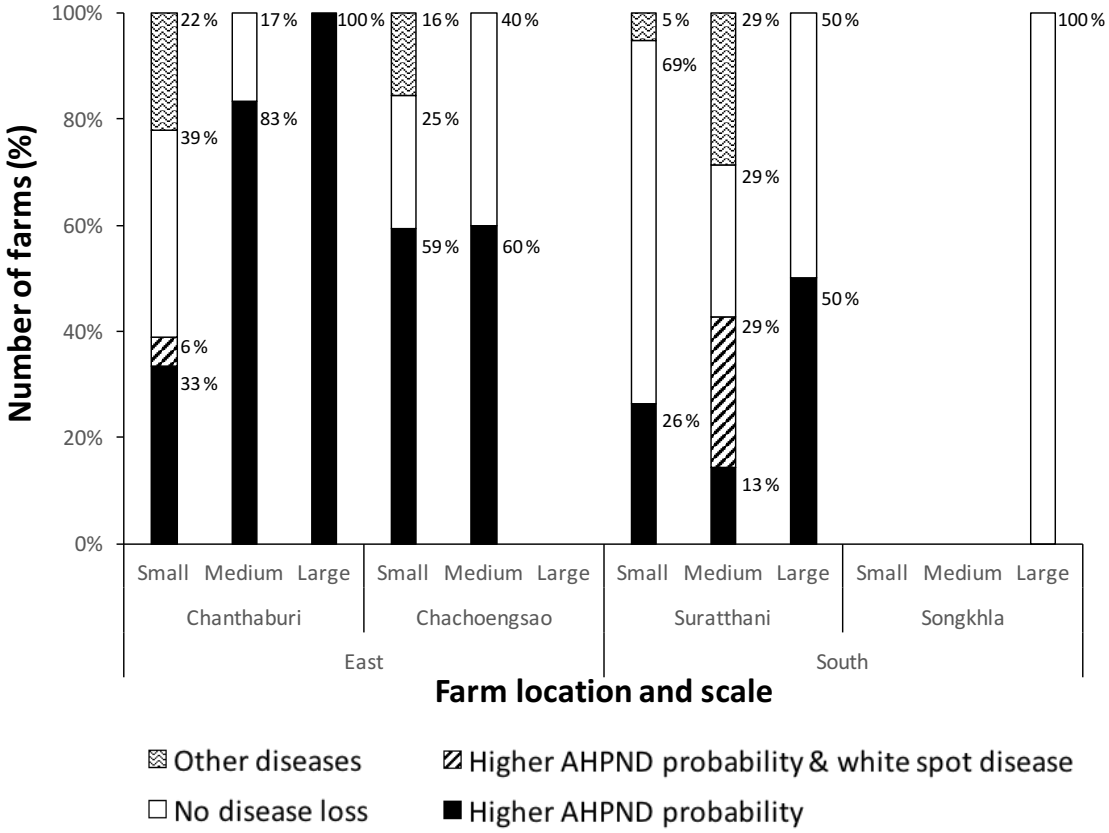


Figure 3.3 Report of disease status stratified according to geographic location and farm-scale between January 2012 and May 2013. AHPND was the main disease problem for Thai shrimp farming. Other diseases included white spot disease, yellow head disease, taura syndrome and white feces syndrome.

3.5.2.2 Cumulative incidence trends graphed

In the eastern districts, the cumulative AHPND incidence was 43 cases per 100 farms in 17 months or two cases per 100 farm-months, and in the south was 25 cases per 100 farms in six months or four cases per 100 farm-months (Figure 3.4). It was seen clearly that both the southern location and the large-scale variables showed a delay of AHPND onset compared with remaining factors (the eastern location, and the small- and medium-scale variables), but their cumulative incidence increased sharply after the first incidence of AHPND.

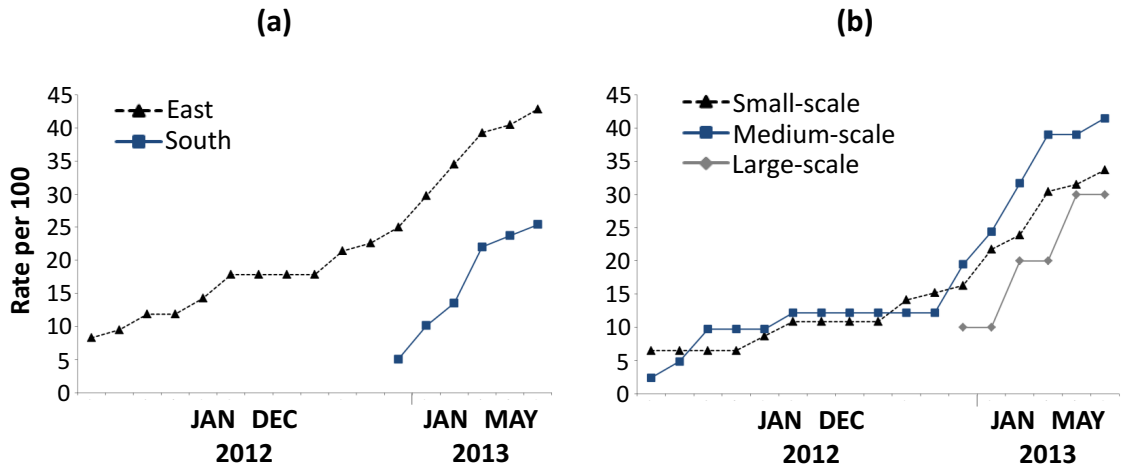


Figure 3.4 The cumulative incidence of AHPND between January 2012 and May 2013, accounting to two regions (a) and three farm-scales (b).

3.5.3 Risk factors for AHPND transmission at farm level

The results of the univariate analysis are summarised in Table 3.5. Two of the 24 variables were associated with AHPND at farm-level at the 0.05 significance level. The first variable was the earthen pond–a significant risk factor for AHPND with an OR of 0.25 (CI 0.06–0.8; P-value = 0.02); the second was the absence of pond harrowing–a significant risk factor for AHPND with an OR of 3.9 (CI 1.3–12.6; P-value = 0.01). These two variables were modelled in further analysis, whereas the remaining 22 variables which were not predictive of AHPND according to these univariate tests (P-value $>$ 0.1) were excluded.

Table 3.5 The statistically significant risk factors for AHPND with odds ratios (ORs) and 95 % confidence intervals

Variable	Case No.	Control No.	P-value	OR (CI)
Ongrowing pond type[1]				
Earthen pond[2]	32	46	0.02	0.25 (0.06–0.8)
Lined pond	14	5		
Pond management[1]				
No pond harrowing[2]	33	24	0.01	3.9 (1.3–12.6)
Pond harrowing	7	20		

Note–(1) Fisher's exact test and (2) Reference level

Further analysis to evaluate risk factors for AHPND was performed using unconditional logistic regression models. Variables with a P-value < 0.1 were kept to be run in backward stepwise unconditional logistic regression models. The models only included cases in the data where all data fields of the two variables (ongrowing pond type and pond management) were completed.

The results of the unconditional logistic regression models that fitted the data best are shown in Table 3.6. The first unconditional logistic regression model has the lower AIC value. The first nested model contained ongrowing pond type and pond management (AIC = 102.19); the second model contained pond management (AIC = 107.29).

Table 3.6 Unconditional logistic regression analysis of risk factors for AHPND

Model	Variable	Case No.	Control No.	Exp(coefficient)[(1)]	SE (coef)
Model 1	Ongrowing pond type	39	41	0.19	0.67
AIC=102.19	Pond management			4.35	0.47
Model 2	Pond management			3.95	0.52
AIC=107.29					

Note–(1) Maximum likelihood estimation odds ratios

For model validation, the cross-validation analysis is presented in Table 3.7. The mean percentage correct of the two models obtained from the unconditional logistic regression is around 65 % according to our data sets (referred to as "D_1" and "D_2").

Table 3.7 Cross-validation results on the AHPND models obtained from unconditional logistic regression

Model	Testing on D_1	Testing on D_2	Mean
Model 1: Ongrowing pond type and pond management	68 %	63 %	66 %
Model 2: Pond management	65 %	63 %	64 %

Using the ROC approach, both models were plotted for their ability to predict AHPND presence at farm level. The proportions in a binary outcome (disease or non-disease farm) were modelled by using a prediction of the log odds and using various cut-off points to define the predicted outcomes. The true positives (sensitivity) of the model were presented on the vertical axis, and the false positives (1-specificity) were shown on the horizontal axis.

The ROC results are presented in Figure 3.5. The area under the ROC curve (AUC) of both models was *circa* 68 %, i.e. a higher value than an uninformative model (AUC of 0.5).

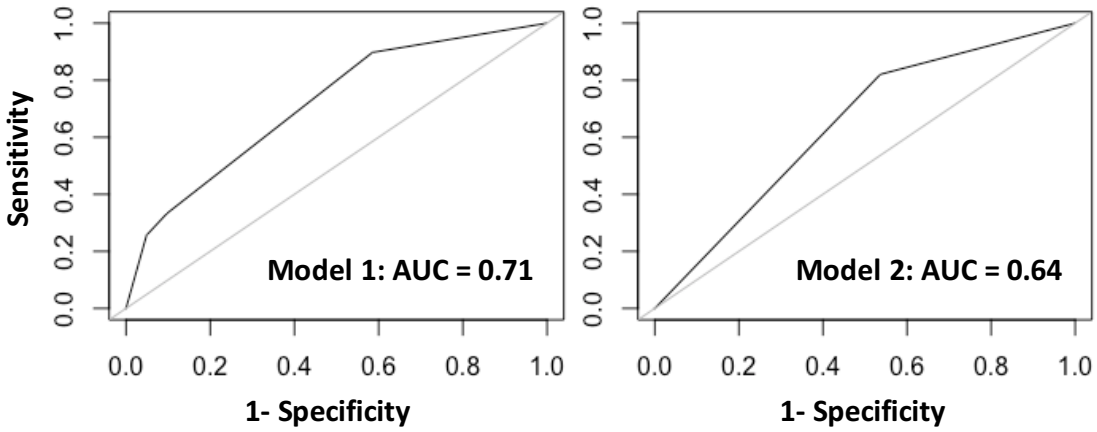


Figure 3.5 ROC curves for AHPND models. Model 1 with two variables: ongrowing pond type and pond management. Model 2 with one variable: pond management. Both models obtain the AUC > 0.5 (better results than random). The diagonal line indicates the prediction from a random model.

These risk models of AHPND and their validation implied that, in the complete subset, there were significant interactions between pond harrowing practice, ongrowing pond type and the incidence of putative AHPND cases occurring at farm-level. With these two risk factors, it can be suggested that shrimp farmers should apply pond harrowing as one of the most important farming practices. This suggestion follows the computation of the OR which interprets that the sample farms where the absence of pond harrowing before stocking were infected with AHPND 3.9 times more often than the sample farms that applied pond harrowing.

3.6 Discussion

The initial AHPND distribution in Thailand during January 2012 and May 2013 was examined in the sample frame of this observational epizoology study. The first incidence occurred in the eastern provinces in January 2012, and delayed incidence in the south in December 2012, at a higher cumulative incidence. A need for improvements in biosecurity in Thai shrimp farming is implied through this research, given that the incidence of AHPND occurred in all farm scales, even the large commercial farms which generally invest more biosecurity resources in shrimp farming than others.

In this research, the identified risk factors for AHPND transmission emphasise the importance of environmental farming managements. One of these risk factors is

earthen ponds to raise shrimp. Earthen ponds with a large area of pond soil play an important role in shrimp farming production. For example, they provide a higher capacity to accumulate and absorb nutrients (nitrogen and phosphorus) and organic matter (i.e. uneaten feed, faeces and moribund shrimp), compared with lined ponds (Burford *et al.,* 2003; Funge-Smith, 1996). Moriarty (1997) proposes that earthen ponds provide a larger habitat for microorganisms, which is a major mechanism to enhance the food web within ponds, while lined ponds have a limited capacity in this regard. The accumulated sediment, however, may exceed the capacity of ponds to decompose the nutrients, causing poor water quality, and toxicity to farmed shrimp, i.e. from nitrite and ammonia (Avnimelech and Ritvo, 2003; Boyd *et al.,* 2002; Hargreaves, 1998). Predominantly, shrimp farm sediment is one of the important habitats for vibrios (Lekshmy *et al.,* 2014; Tho *et al.,* 2012; Walling *et al.,* 2010), including the pathogenic agent of AHPND (Kongkumnerd, 2014).

In contrast, lined ponds increase the risk of AHPND infection. Thitamadee *et al.* (2016) note that the utilisation of pond lining is an effective tool for disease prevention, but fully lined ponds may contain gaps in the plastic sheets from installing aerators or feeding trays, and due to the short lifetime of plastics of around 2–5 years. These leaks allow anaerobic organisms to grow underneath the plastic sheets and increment the risk of AHPND. The disadvantage of pond lining has been stated by Boyd (2014), i.e. phytoplankton blooms, low-alkalinity water, and high amounts of sludge (organic matter). Hence, lined ponds are also likely to be related to environmental problems in shrimp farming.

With earthen ponds, the farmers can fully manage the anaerobic conditions of AHPND pathogens, and other comparative organisms through proper pond management techniques, such as liming, pond drying, and pond bottom harrowing. According to our research, not performing harrowing before stocking was a factor that increased the risk of AHPND transmission. This finding is explained by the advantages of harrowing in enhancing the shrimp pond environment. When pond soil is exposed to the air, soil respiration increases (Boyd and Pippopinyo, 1994; Egna and Boyd, 1997; Xinglong and Boyd, 2006). Predominantly, it contributes to a lower amount of toxic gases, such as hydrogen sulphide and nitrite, in pond soil because these toxic gases are oxidised to

non-toxic forms (Adhikari *et al.,* 2012; Boyd *et al.,* 2002). Therefore, both a habitat of facultative anaerobic bacteria such as *V. parahaemolyticus* (Youngren-Grimes *et al.,* 1988) and toxic gases should be decreased in shrimp ponds that are harrowed. In addition, pond harrowing with pond drying and probiotics loading is able to enhance decomposition in sediment, and to eliminate the numbers of *Vibrio* living in sediment efficiently (Boyd, 2003; Moriarty, 1998; Moriarty, 1999; Nimrat *et al.,* 2008). De Schryver *et al.* (2014) also suggest that good microbial management within shrimp ponds can be an excellent strategy for AHPND prevention rather than using disinfecting.

The use of a case-identification AHPND decision tree to determine case and control was discussed here. The case-identification AHPND decision tree is a flexible and intelligent epizootiological tool: it supports epizoology in terms of being a quicker and less expensive analysis. Furthermore, the case-identification AHPND decision tree can be adapted when there is recurrence of AHPND, or the gross signs of disease pathology can be changed when there is incidence of other new diseases. Identifying cases with an AHPND decision tree may be less accurate than by using laboratory histopathology and PCR testing as diagnostic tools. The main reason is that this case-identification AHPND decision tree was developed for a disease of unknown etiology, thus we have to have an identified pathogen in order to have a definitive case definition.

This research has several limitations. The possibility for self-selection bias may arise in the survey design because the sample farms may have lower operating costs, lower quality facilities, or misunderstanding of appropriate biosecurity practices, meaning that the sample farms are more likely to get infections because they are inherently riskier. We found, however, that self-selection according to biosecurity practices had only a small bias on risk estimations. As seen by the cumulative incidence of AHPND (Figure 3.4), large-scale farms tended to have a slower incidence of AHPND than medium- and small-scale farms. This showed that stratification could minimise the self-selection bias in the research. Moreover, with prior business in Thai shrimp farming and experience with disease problems, the Phase 1 population should provide good representatives in Phase 2 in terms of providing information about the association between AHPND occurrence and risk factors.

All the identified risk factors convey the need for changes in farm management practices at shrimp farming sites. Other risk factors for AHPND may be included in further research and cross-sectional studies, however. Effective strategies to develop disease prevention and control at the country level are also needed. This can be achieved through analysing the structure of the live shrimp movement network to gain more understanding of how disease epizootic dynamics play out across the whole shrimp farming sector in Thailand.

3.7 References

Adhikari, S., Lal, R. and Sahu, B.C. (2012) Carbon sequestration in the bottom sediments of aquaculture ponds of Orissa, India. *Ecological Engineering*, 47, pp. 198–202.

Akaike, H. (1974) A new look at the statistical model identification. *Automatic Control, IEEE Transactions On*, 19 (6), pp. 716–723.

Akazawa, N. and Eguchi, M. (2013) Environmental trigger for EMS/AHPNS identified in Agrobest shrimp ponds. Global Aquaculture Advocate July/August 2013, pp. 16–17.

Alonzo, T.A. and Pepe, M.S. (2002) Distribution-free ROC analysis using binary regression techniques. *Biostatistics (Oxford, England)*, 3 (3), pp. 421–432.

Al-Tawfiq, J.A., Hinedi, K., Ghandour, J., Khairalla, H., Musleh, S., Ujayli, A. and Memish, Z.A. (2014) Middle East respiratory syndrome coronavirus: a case–control study of hospitalized patients. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 59 (2), pp. 160–165.

Austin, B. (2010) Vibrios as causal agents of zoonoses. *Veterinary Microbiology*, 140 (3–4), pp. 310–317.

Avnimelech, Y. and Ritvo, G. (2003) Shrimp and fish pond soils: processes and management. *Aquaculture*, 220 (1–4), pp. 549–567.

Boyd, C.E. and Pippopinyo, S. (1994) Factors affecting respiration in dry pond bottom soils. *Aquaculture,* 120 (3–4), pp. 283–293.

Boyd, C.E. (2003) Bottom soil and water quality management in shrimp ponds. *Journal of Applied Aquaculture*, 13 (1–2), pp. 11–33.

Boyd, C.E. (2014) Relationships between pond bottom soil management and water quality in aquaculture ponds. Adelaide, South Australia, 7–11 June 2014. Adelaide: World Aquaculture Society, pp. 1–29. Available: https://www.was.org/documents/MeetingPresentations/WA2014/WA2014_0263.pdf [Accessed: 11 April 2017].

Boyd, C.E., Wood, C. and Thunjai, T. (2002) *Aquaculture pond bottom soil quality management.* Oregon: Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C. (2003) Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, 219 (1), pp. 393–411.

Chonsin, K., Matsuda, S., Theethakaew, C., Kodama, T., Junjhon, J., Suzuki, Y., Suthienkul, O. and Iida, T. (2016) Genetic diversity of *Vibrio parahaemolyticus* strains isolated from farmed Pacific white shrimp and ambient pond water affected by acute hepatopancreatic necrosis disease outbreak in Thailand. *FEMS Microbiology Letters*, 363 (2), pp. 1–8.

Chrisolite, B., Thiyagarajan, S., Alavandi, S.V., Abhilash, E.C., Kalaimani, N., Vijayan, K.K. and Santiago, T.C. (2008) Distribution of luminescent *Vibrio harveyi* and their bacteriophages in a commercial shrimp hatchery in South India. *Aquaculture*, 275 (1–4), pp. 13–19.

Costa, R.A., Silva, G.C., Peixoto, J.R., Vieira, G.H. and Vieira, R.H. (2010) Quantification and distribution of *Vibrio* species in water from an estuary in Ceará-Brazil impacted by shrimp farming. *Brazilian Journal of Oceanography*, 58 (3), pp. 183–188.

De Schryver, P., Defoirdt, T. and Sorgeloos, P. (2014) Early mortality syndrome outbreaks: a microbial management issue in shrimp farming? *PLoS Pathogens,* 10 (4), pp. e1003919.

Eduardo, M.L. and Mohan, C.V. (2012) *Early mortality syndrome (EMS)/Acute hepatopancreatic necrosis syndrome (AHPNS): an emerging threat in the Asian shrimp industry.* Bangkok: NACA. Available: http://library.enaca.org/Health/DiseaseLibrary/disease-advisory-ems-ahpns.pdf [Accessed: 15 August 2014].

Egna, H.S. and Boyd, C.E. (1997) *Dynamics of pond aquaculture.* CRC press.

FAO (2013) *Report of the FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304).* FAO Fisheries and Aquaculture Report No. 1053. Rome: FAO. Available: http://www.fao.org/docrep/018/i3422e/i3422e00.htm [Accessed: 11 October 2013].

Flegel, T.W. (1997) Major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. *World Journal of Microbiology and Biotechnology*, 13 (4), pp. 433–442.

Flegel, T.W. and Lo, C. (2014) *Announcement regarding free release of primers for specific detection of bacterial isolates that cause acute hepatopancreatic necrosis disease (AHPND).* Bangkok: NACA. Available: http://www.enaca.org/publications/health/disease-cards/ahpnd-detection-method-announcement.pdf [Accessed: 5 December 2014].

Flegel, T.W. (2012) Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology*, 110 (2), pp. 166–173.

Funge-Smith, S.J. (1996) Water and sediment quality in different intensive shrimp culture systems in southern Thailand. *Coastal Aquaculture and Environment: Strategies for Sustainability.ODA Research Project*, 6011, pp. 1–27.

Han, J.E., Tang, K.F., Tran, L.H. and Lightner, D.V. (2015a) *Photorhabdus* insect-related (Pir) toxin-like genes in a plasmid of *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp. *Dis.Aquat.Org*, 113, pp. 33–40.

Han, J.E., Mohney, L.L., Tang, K.F.J., Pantoja, C.R. and Lightner, D.V. (2015b) Plasmid mediated tetracycline resistance of *Vibrio parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps. *Aquaculture Reports*, 2, pp. 17–21.

Hargreaves, J.A. (1998) Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture*, 166 (3–4), pp. 181–212.

Jiravanichpaisal, P., Miyazaki, T. and Limsuwan, C. (1994) Histopathology, biochemistry, and pathogenicity of *Vibrio harveyi* infecting black tiger prawn *Penaeus monodon*. *Journal of Aquatic Animal Health*, 6 (1), pp. 27–35.

Kassam, L., Subasinghe, R. and Philips, M. (2011) *Aquaculture farmer organizations and cluster management.* FAO Fisheries Technical Paper No. 563. Rome: FAO. Available: http://www.fao.org/docrep/014/i2275e/i2275e.pdf [Accessed: 29 April 2017].

Kautsky, N., Rönnbäck, P., Tedengren, M. and Troell, M. (2000) Ecosystem perspectives on management of disease in shrimp pond farming. *Aquaculture,* 191 (1–3), pp. 145–161.

Kondo, H., Tinwongger, S., Proespraiwong, P., Mavichak, R., Unajak, S., Nozaki, R. and Hirono, I. (2014) Draft genome sequences of six strains of *Vibrio parahaemolyticus* isolated from early mortality syndrome/acute hepatopancreatic necrosis disease shrimp in Thailand. *Genome Announcements,* 2 (2), e00221–14. Available: http://genomea.asm.org/content/2/2/e00221-14.full [Accessed: 2 May 2014].

Kongkumnerd, J. (2014) *The current status of EMS outbreak in Thai marine shrimp farming.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.shrimpaqua.com/download/EMS/situation-EMS.pdf [Accessed: 10 December 2014].

Kumar, R. and Indrayan, A. (2011) Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatrics*, 48 (4), pp. 277–287.

Kumar, B.K., Deekshit, V.K., Raj, J.R.M., Rai, P., Shivanagowda, B.M., Karunasagar, I. and Karunasagar, I. (2014) Diversity of *Vibrio parahaemolyticus* associated with disease outbreak among cultured *Litopenaeus vannamei* (Pacific white shrimp) in India. *Aquaculture*, 433, pp. 247–251.

Lai, H., Ng, T.H., Ando, M., Lee, C., Chen, I., Chuang, J., Mavichak, R., Chang, S., Yeh, M., Chiang, Y., Takeyama, H., Hamaguchi, H., Lo, C., Aoki, T. and Wang, H. (2015)

Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. *Fish & Shellfish Immunology*, 47 (2), pp. 1006–1014.

Le, T.X. and Munekage, Y. (2004) Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam. *Marine Pollution Bulletin*, 49 (11–12), pp. 922–929.

Lekshmy, S., Nansimole, A., Mini, M., Athira, N. and Radhakrishnan, T. (2014) Occurrence of *Vibrio cholerae* in shrimp culture environments of Kerala, India. *Indian Journal of Scientific Research,* 5 (2), pp. 151.

Leobert, D., Cabillon, N.A.R., Catedral, D.D., Amar, E.C., Usero, R.C., Monotilla, W.D., Calpe, A.T., Fernandez, D.D. and Saloma, C.P. (2015) Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines. *Dis Aquat Org*, 116, pp. 251–254.

Lightner, D.V., Redman, R.M., Pantoja, C.R., Noble, B.I. and Tran, L. (2012) Early mortality syndrome affects shrimp in Asia. *Global Aquaculture Advocate Magazine*, 40 Available: https://pdf.gaalliance.org/pdf/GAA-Lightner-Jan12.pdf [Accessed: 31 March 2013].

Mori, Y., Takahashi, H. and Oka, R. (1999) Image-to-word transformation based on dividing and vector quantizing images with words. *First International Workshop on Multimedia Intelligent Storage and Retrieval Management.* Citeseer, pp. 1–9.

Moriarty, D.J. (1997) The role of microorganisms in aquaculture ponds. *Aquaculture*, 151 (1), pp. 333–349.

Moriarty, D.J. (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164 (1), pp. 351–358.

Moriarty, D.J. (1999) Disease control in shrimp aquaculture with probiotic bacteria. In: C.R. Bell, M. Brylinsky and P. Johnson-Green, ed. *International Symposium on Microbial Ecology.* Halifax, Canada, 9th to 14th August 1998. Kentville N.S.: Atlantic Canada Society for Microbial Ecology, pp. 237–243. Available: http://socrates.acadiau.ca/isme/symposium08/moriarty.pdf [Accessed: 25 March 2016].

Murray, F.J., Haque, M.M., Zhang, W., Thanh, L.P., Nietes Satapornvanit, A. and Little, D.C. (2013) *Defining boundaries towards understanding sustainable ethical aquaculture trade between Asia and Europe.* SEAT Project Report Ref: D2.82.8. Stirling, UK. www.SEATglobal.eu: University of Stirling.

NACA (2014) *Diseases of crustaceans: acute hepatopancreatic necrosis syndrome (AHPNS).* Available: http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf [Accessed: 1 May 2015].

Nimrat, S., Suksawat, S., Maleeweach, P. and Vuthiphandchai, V. (2008) Effect of different shrimp pond bottom soil treatments on the change of physical characteristics and pathogenic bacteria in pond bottom soil. *Aquaculture*, 285 (1), pp. 123–129.

Nunan, L., Lightner, D.V., Pantoja, C. and Gomez-Jimenez, S. (2014) Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. *Diseases of Aquatic Organisms*, 111 (1), pp. 81–86.

OIE (2013) *Acute hepatopancreatic necrosis disease, aetiology epidemiology diagnosis prevention and control references.* Available: http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/AHPND_DEC_2013.pdf [Accessed: 12 August 2014].

Panakorn, S. (2012) Opinion article: more on early mortality syndrome in shrimp. *Aquaculture Asia Pacific*, 8 (1), pp. 8–10.

Pearce, N. (2016) Analysis of matched case–control studies. *BMJ (Clinical Research Ed.)*, 352, i969. Available: http://dx.doi.org/10.1136/bmj.i969 [Accessed: 19 June 2017].

Peterson, O., Asplund, M., Karunasagar, I. and Godhe, A. (2010) Phytoplankton community composition and diversity effects on the growth of marine *Vibrio* bacteria. In: P. Pagou and G. Hallegraeff, ed. *International Conference on Harmful Algae.* Crete, Greece, 1st to 5th November 2010. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO 2013, pp. 147-151.

R foundation for statistical computing (2015) *R: a language and environment for statistical computing.* Available: https://www.R-project.org/ [Accessed: 5 December 2015].

Rosenberry, B. (2013) *Early mortality syndrome: managing the perfect killer, a webinar organized by the Global Aquaculture Alliance and sponsored by SeafoodSource.com. Ho Chi Minh City, Vietnam. December 10–13, 2013.* Shrimp News International. Available: http://www.shrimpnews.com/FreeReportsFolder/NewsReportsFolder/VietnamGAAwebinarEMS.html [Accessed: 15 April 2014].

Schmidt, C.O. and Kohlmann, T. (2008) When to use the odds ratio or the relative risk? *International Journal of Public Health*, 53 (3), pp. 165–167.

Schwartz, J. (2004) Is the association of airborne particles with daily deaths confounded by gaseous air pollutants? An approach to control by matching. *Environmental Health Perspective*, 112 (5), pp. 557–561.

Thailand DoF (2016) *Number of farms, area under culture and yield by species and provinces, 1999-2013.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.fisheries.go.th/it-stat/yearbook/Index.htm [Accessed: 27 June 2016].

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W. and Itsathitphaisarn, O. (2016) Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 452, pp. 69–87.

Tho, N., Merckx, R. and Ut, V.N. (2012) Biological characteristics of the improved extensive shrimp system in the Mekong delta of Vietnam. *Aquaculture Research*, 43 (4), pp. 526–537.

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. and Lightner, D.V. (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Diseases of Aquatic Organisms*, 105 (1), pp. 45–55.

Van Den Eeden, S.K., Tanner, C.M., Bernstein, A.L., Fross, R.D., Leimpeter, A., Bloch, D.A. and Nelson, L.M. (2003) Incidence of parkinson's disease: variation by age, gender, and race/ethnicity. *American Journal of Epidemiology*, 157 (11), pp. 1015–1022.

Xinglong, J. and Boyd, C.E. (2006) Relationship between organic carbon concentration and potential pond bottom soil respiration. *Aquacultural Engineering,* 35 (2), pp. 147–151.

Xu, S., Shetterly, S., Cook, A.J., Raebel, M.A., Goonesekera, S., Shoaibi, A., Roy, J. and Fireman, B. (2016) Evaluation of propensity scores, disease risk scores, and regression in confounder adjustment for the safety of emerging treatment with group sequential monitoring. *Pharmacoepidemiology and Drug Safety*, 25 (4), pp. 453–461.

Youngren-Grimes, B.L., Grimes, D.J. and Colwell, R.R. (1988) Growth of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* under strict anaerobic conditions. *Systematic and Applied Microbiology*, 11 (1), pp. 13–15.

Chapter 4 - Analysis of the network structure of the live shrimp movements relevant to AHPND epizootic

N. Saleetid; D.M. Green; F.J. Murray

Preface

The fourth chapter analyses the structure of the live shrimp movement network of Thailand (LSMN) using graph theory and network approaches. The analysis is aimed at finding the real structure of the LSMN and to thereby suggest potential disease transmission mechanisms, which is an important step towards the prevention and control of a disease epizootic. The epizootiological survey conducted in Chapter 3 provides information on disease mitigation measures as an aid in interpreting results in this chapter. The chapter is designed for publication, and thus, the spread of acute hepatopancreatic necrosis disease (AHPND) is described again in the introduction section. The first part describes the general characteristics of the LSMN. Second, the network is visualised according to the provincial borders of Thailand. Then it is quantified using statistical and mathematical measures. The final part discusses the network structure relevant to the spread of AHPND. This chapter contributes to the development of a control strategy in the fifth chapter and epizootic models of AHPND in the sixth chapter.

Impact statement

The LSMN are well recorded, but have been used in a limited extent to assess the spread of disease. To increase understanding of the spread of disease from site to site, the LSMN was modelled and its structure examined in terms of relating these factors to potential disease epizootics between sites. This is the first project to use network modelling to characterise potential disease transmission in the shrimp farming sector and provides authoritative information to the Thailand Department of Fisheries for targeted disease surveillance and control with limited operating resources.

Chapter 4 - Analysis of the network structure of the live shrimp movements relevant to AHPND epizootic

4.1 Abstract

This research models and analyses the live shrimp movement network of Thailand (LSMN), which has a potential effect on site-to-site disease transmission. The movement data were collected over a 13-month period from March 2013 to March 2014. Importantly, the spread of acute hepatopancreatic necrosis disease (AHPND), and other known diseases, occurred during the period covered. Results show that large-scale connectivity in the LSMN typically relies on inter-province movements with an average distance of around 200 km. The LSMN was examined by network modelling and found to have a mixture of characteristics both hindering and aiding disease spread. For hindering transmission, the correlation between in and out degrees was weakly positive, i.e. sites with a high risk of catching disease posed a low risk for transmitting the disease (assuming solely network spread), and the LSMN showed disassortative mixing in $r(in, out)$, i.e. a low preference for connections join sites with high in degree link to connections with high out degree. However, there were low values for mean shortest path length and clustering coefficient. These latter characteristics tend to be associated with the potential for disease epizootics. In addition to the small-world property (i.e. short mean path length) presented in the LSMN, the network exhibited power-law distributions of in and out degrees with exponents of 2.87 and 2.17, respectively, indicating the scale-free phenomenon. This result showing the heterogeneity in site degrees demonstrates that a targeted strategy can potentially perform well to minimise the scale of a disease epizootic.

4.2 Introduction

Shrimp farming has been involved in the socio-economic growth of Thailand since the 1980s (Szuster, 2006). Nevertheless, the invasion of infectious diseases presents as the major barrier to success in the sector. The historical diseases of Asian shrimp farming over the past 30 years had been reviewed in Flegel (2012). Microparasites, i.e. viruses and bacteria, are a major cause of Asian farmed shrimp deaths, representing a relatively

huge economic loss (Flegel, 2012). This is a reason why the World Organisation for Animal Health (OIE) requires all member countries to develop disease surveillance and control measures for aquatic animals.

In terms of pathways of shrimp disease transmission, long-distance transmission (i.e. live shrimp movements) poses a high potential for site-to-site disease spread. To take a recent example, acute hepatopancreatic necrosis disease (AHPND, also known as EMS) has hit Thai shrimp farming since late 2011, and the disease has been transmitted across Thai regions through the movements of infectious live shrimp (FAO, 2013b; OIE, 2013a). This epizootic disease damaged Thai shrimp production by an estimated 500 000 tonnes of shrimp during a 3-year period from 2011 to 2014 (Songsanjinda, 2015). Although there have been many worldwide efforts to stop the spread of AHPND, such as movement restrictions, biofloc technology, genetics improvements, and enhanced breeding techniques (Hong *et al.,* 2016; Pakingking Jr *et al.,* 2016), no such control strategies for AHPND emerge from network modelling.

Network modelling is playing an increasingly important role in epizoology. Its application relies on graph theory. A graph, or network, includes a set of sites (in network terminology: nodes) and their connections. Most often, weights of connections, such as the frequencies of connections between the same pairs of sites are ignored, by analysing non-weighted networks. There are two reasons for this: (1) weighted networks are more complex to analyse and (2) there has been a lack of off-the-shelf tools that can be used to analyse them, whereas many tools have been designed for non-weighted networks (Newman, 2004; Robinaugh *et al.,* 2016; Wei *et al.,* 2013; Yang *et al.,* 2012). However, weighted networks generate an improved representation and a more realistic structure than non-weighted ones (Barrat *et al.,* 2004; Wiedermann *et al.,* 2013). Consequently, there are a growing number of tools used for weighted networks. This means that this study can evaluate two forms of live shrimp movement network: non-weighted and weighted networks.

Network model approaches can be helpful conveniently to plot and mathematically describe the connections within contact networks, and to predict disease dynamics and persistence from their derived structure (Kurvers *et al.,* 2014; Meyers *et al.,* 2003). Many

studies of disease outbreaks have used both simulated and real network models to evaluate and compare the effectiveness of control strategies (Cai *et al.,* 2014; Guinat *et al.,* 2016; Hartvigsen *et al.,* 2007; Ma *et al.,* 2013; Werkman *et al.,* 2011). Huang *et al.* (2016), however, suggested that real networks reconstructed from real-life connections facilitated more reliable prediction of disease epidemics than using simulated networks. Hence, the real network model is becoming increasingly important in various sectors such as aquaculture (Green *et al.,* 2012; Munro and Gregory, 2009; Taylor *et al.,* 2010), livestock production (Aznar *et al.,* 2011; Büttner *et al.,* 2013; Kiss *et al.,* 2006), and plant trade (Moslonka-Lefebvre *et al.,* 2011).

The structure of the live shrimp movement network of Thailand (LSMN) has been examined in this research, to explain its susceptibility to disease transmission. The computer-based recording of real live shrimp movements between sites served as a data source here, following from the aquatic animal trade regulation of Thailand, B.E.2553 (2010). The recording of movements is operated by authorised users, providing unique records in that the sources and destinations of daily shrimp batch movements can illustrate the spread of diseases from site to site. Furthermore, this research indicates that movement records have a key role in shrimp epizoology, contributing to the enhancement of disease surveillance and control strategies in the Thai shrimp farming sector.

4.3 Methods

4.3.1 Data sources

The live shrimp movement network (LSMN) data—an electronic and Thai government database—was provided by the Thailand Department of Fisheries. It contains records collected from daily batch movements consisting of: (1) the farm registration number (the first two digits denote provincial site location as shown in Appendix C at the end of this thesis; in the third and fourth positions, *01* denote ongrowing site and *02* denote seed-producing site), (2) the source site and destination site of live shrimp, (3) the date of movement, and (4) the seed quantity. Importantly, the data included the 13-month period of AHPND spread from March 2013 to March 2014 during which AHPND spread

around the country (NACA, 2017), and covered up to three production cycles of farmed shrimp (Flaherty *et al.,* 2000). Unfortunately, unreported movements, mostly generated from non-commercial farming with low productivity and for breeding improvement purposes were not available. This represented a limitation of the study, although the expected numbers affected are very small.

Microsoft Access was used to combine *circa* 99 000 available records, combining multiple records of a batch moved within a day as one connection. We omitted records having no recorded data in any of the fields. Furthermore, 167 records were chosen randomly from the entire dataset for data validation in terms of the physical location of source and destination because failures in this regard could affect the reliability of the network modelling. This sample size of the 167 records was obtained from the online EpiTools epidemiological calculators (http://epitools.ausvet.com.au). Then we checked the physical location of source and destination in such chosen records against the Thai shrimp farm registration database obtained from Coastal Aquaculture Research and Development Division (2013), resulting in four errors at village level (more detail of the Thai local governmental units was given by Nagai *et al.,* 2008). Thus, the 95 % confidence interval for the population proportion of such errors was calculated as 0.9 % to 6 %, which was computed by using the R Programme Environment with the *Hmisc* (*binconf*) package (Harrell, 2015; R foundation for statistical computing, 2015). These errors would decrease reliability in our results if villages were used to identify site locations, but are not important in this chapter.

4.3.2 Identification of live shrimp movement types by provincial scale

Using the provincial borders of Thailand, the live shrimp movements could be divided into two types. The use of provincial borders corresponds with the provincial administration of Thailand Department of Fisheries (www4.fisheries.go.th). Firstly, intra-province movement is a connection between two sites within the same province; secondly, inter-province movement is a connection between two different provinces. Furthermore, the distance between two sites—i and j—was computed as straight-line distance (d) basing on the geographic coordinates of the Thai sub-districts available from Google Earth (2015). The obtained results represented the expected distances of infection between

sites as proposed in Bogel *et al.* (1976). The formula for the straight-line distance (4.1) shown in Dubé *et al.* (2008) is:

$$d_{ij} = \frac{t}{360} \times (x^2 + y^2)^{\frac{1}{2}} \tag{4.1}$$

$$x = (longitude_{site\,j} - longitude_{site\,i}) \times cos(latitude_{site\,i})$$
$$y = latitude_{site\,j} - latitude_{site\,i}$$

where *t* is the circumference of the earth (= 40 075 km).

4.3.3 Provincial visualisation for the live shrimp movement network (LSMN)

According to the two movement types (intra- and inter-province movements), the LSMN provincial movements for 37 provinces were visualised by using Pajek Software (Mrvar and Batagelj, 1996). The frequency of connections for the intra-province and inter-province movements were presented with different line colours and scaled line widths on a log scale due to the wide variation in sizes. In an effort to illustrate the movement network based on national administrative boundaries, the position of 37 provinces was approximated to the geographic locations on a map of Thailand (Kaesler, N.A.), using Inkscape software (www.inkscape.org).

4.3.4 LSMN adjacency matrix for network representation and analysis at site level

The quantitative analysis of the LSMN structure was performed in the R Programme Environment (R foundation for statistical computing, 2015). The LSMN was represented by an adjacency matrix. The *igraph* software package (Csardi and Nepusz, 2006) was used since it is more flexible for analysing a large complex network with a sparse adjacency matrix (a square matrix used to represent a network whose elements are mostly zeros). The package provides many network analysis tools, e.g. matrix eigenvalues, and network rewiring algorithms used in this research (Csardi and Nepusz, 2006). In addition to the *igraph* package, we also used the *tnet* package from Opsahl (2009) to calculate weighted shortest paths and weighted clustering.

In the case of the LSMN, the weights of these connections were taken into account. A weight w_{ij} implied the frequency of connections that varied in such site pairs (i, j). Thus, the LSMN was represented by the adjacency matrix h, which was the element-wise multiplication of matrix a by matrix w as explained above. a_{ij} took the value 0 if there was no connection from site i to site j and a_{ij} took the value 1 otherwise, representing a pathway of disease transmission from i to j. About 300 self-loop connections (a_{ii} = 1) were removed from the analysis because these self-loops did not contribute to further spread of diseases (Britton *et al.,* 2011; Draief *et al.,* 2008).

A directed network can be used to quantify the local network structure of each site by calculating in and out degrees, and undirected degrees, as in (4.2)–(4.4), respectively. In degrees indicate the number of connections move into each site and out degrees indicate the number of connections move from each site. Undirected degrees denote the number of connections in either direction of each site. These non-weighted degree calculations (no repeated connections) are shown in Barrat *et al.* (2004) and Green *et al.* (2009).

$$k_i^{in} = \sum_j a_{ji} \tag{4.2}$$

$$k_i^{out} = \sum_j a_{ij} \tag{4.3}$$

$$k_i^{und} = k_i^{in} + k_i^{out} - \sum_j (a_{ij})(a_{ji}) \tag{4.4}$$

Additionally, weighted measures were carried out for comparison proposes, as in (4.5)–(4.7).

$$k_i^{in} = \sum_j a_{ji} w_{ji} \tag{4.5}$$

$$k_i^{out} = \sum_j a_{ij} w_{ij} \tag{4.6}$$

$$k_i^{und} = k_i^{in} + k_i^{out} \tag{4.7}$$

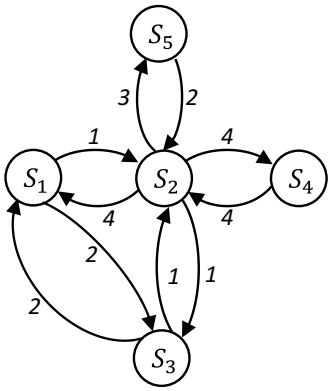
The total number of connections (M) was equal to the total number of either in degrees or out degrees (4.8).

$$M = \sum_j k_i^{in} = \sum_j k_i^{out} \quad (4.8)$$

The degree distributions of the LSMN (the weighted network) were statistically analysed for a power-law characteristic and exponents were estimated, using the Kolmogorov-Smirmov test with the *igraph* software package (Csardi and Nepusz, 2006). Its implementation uses the method of Clauset *et al.* (2009) and Newman (2005); the null hypothesis is that the LSMN is generated from a power-law distribution. Other basic statistics of the degrees, i.e. summary, mean, maximum and minimum, and coefficient of variation, were also presented in the results.

The shortest path L_{ij} denotes a connection $i \rightarrow j$ in a network. The L_{ij} for non-weighted directed networks was calculated by using the Dijkstra's algorithm (Csardi and Nepusz, 2006; Dijkstra, 1959). Weighted directed networks require more computations, however; for these, the L_{ij} was computed as in Opsahl (2009): first the L_{ij} was calculated by using the Dijkstra's algorithm, then it was divided by the mean connection weight of the network (the total number of repeated connections divided by the total number of connections), giving an adjacency matrix of the weighted shortest paths for the LSMN. To explain Opsahl's algorithm, a small network is shown in Figure 4.1. Noted that, in some cases, the weighted shortest paths do not have a clear meaning in real life such as giving the decimal values of shortest path lengths.

(a) **(b)**



1. Calculate the mean edge weight

$$Mean\ weight = \frac{Total\ number\ of\ repeated\ connections}{Total\ number\ of\ connections}$$

$$= 24/10 = 2.4$$

2. Evaluate the shortest paths (An example for a site 'S_1')

$L_{S1\to S2}$ = 2.4/1 = 2.4

$L_{S1\to S3}$ = 2.4/2 = 1.2

$L_{S1\to S4}$ = 2.4/1 + 2.4/4 = 3.0

$L_{S1\to S5}$ = 2.4/1 + 2.4/3 = 3.2

3. Give the matrix of shortest paths

		To				
		S1	*S2*	*S3*	*S4*	*S5*
From	*S1*	NA	2.4	1.2	3.0	3.2
	S2	0.6	NA	1.8	0.6	0.8
	S3	1.2	2.4	NA	3.0	3.2
	S4	1.2	0.6	2.4	NA	1.4
	S5	1.8	1.2	3.0	1.8	NA

Figure 4.1 A small weighted directed network (a) and its matrix of the shortest paths L_{ij} (b) computed by the algorithm of Opsahl (2009). The average shortest path length $\langle L \rangle$ of this network is 1.84 (= 36.8/20).

The average shortest path length $\langle L \rangle$ was computed following Mao and Zhang (2013) (4.9):

$$\langle L \rangle = \frac{1}{\sum_{i \neq j} [L_{ij} \neq \infty]} \sum_{i \neq j}^{N} L_{ij} \tag{4.9}$$

where $[X]$ is the Iverson bracket denoting 1 where condition X is true and 0 otherwise.

Clustering refers to the presence of grouped sites that the disease can transmit through (Shirley and Rushton, 2005). The characteristic of clustering was demonstrated by the clustering coefficient (C) derived from the concept of '*any friend of yours is a friend of mine*'. This was similar to the study of Green *et al.* (2012). C was calculated as a ratio of the number of triangles to the number of triples. Ahnert and Fink (2008) defined a triangle as a set of three sites where with $\{S_1 \to S_2 \to S_3, S_1 \to S_3\}$, meaning that both direct and indirect routes for $S_1 \to S_3$ exist, and where such a triangle corresponded to a triple

$S_1 \rightarrow S_2$ and $S_1 \rightarrow S_3$. Thus, the C is the proportion of triples where a direct route of transmission also exists.

We computed the assortativity coefficient (r) to represent the assortative mixing by degree in the LSMN. Assortative mixing by degree is common in contact networks of persons and animals. In addition to being a network property that aids disease transmission, assortative mixing inhibits the effectiveness of targeted vaccination strategies due to the persistence of giant components (the largest number of sites in a network that are interconnected by directed connections) in the network (Newman, 2003). The extent of assortative mixing shows the tendency of sites in a network to connect to other sites with similar degrees, i.e. high-degree sites tend to be connected to other high-degree sites (Newman, 2003). In the context of epizoology, this can indicate that disease is more easily spread in the network, leading to a higher basic reproduction number (R_0) compared with a network that has a negative value of assortativity coefficient (disassortativity). Foster *et al.* (2010) state that the assortativity of directed networks can be represented by four measures: $r(out, in)$, $r(in, out)$, $r(out, out)$, and $r(in, in)$. Among these four measures, however, the most interesting for epidemiological study is the $r(in, out)$—i.e. directed connections joining sites with a high in degree link to directed connections joining sites with high out degree—because this measure describes the patterns of network connections, which lead to epidemics. Its equation is shown in Green *et al.* (2009), and is written below by (4.10):

$$r(in, out) = \frac{M\sum_{i\rightarrow j} k_i^{in} k_j^{out} - \left(\sum_{i\rightarrow j} k_i^{in}\right)\left(\sum_{i\rightarrow j} k_j^{out}\right)}{\sqrt{\left[M\sum_{i\rightarrow j}(k_i^{in})^2 - \left(\sum_{i\rightarrow j} k_i^{in}\right)^2\right]\left[M\sum_{i\rightarrow j}(k_j^{out})^2 - \left(\sum_{i\rightarrow j} k_j^{out}\right)^2\right]}} \quad (4.10)$$

where k_i^{in} and k_j^{out} denote the in degree of source and out degree of destination for connection $i \rightarrow j$ respectively, and M is the total number of connections in the network.

Heesterbeek and Dietz (1996) define the basic reproduction ratio (R_0) as the expected number of secondary cases generated by a typical case during its transmission period in a particular group of susceptible individuals. In order to estimate the epidemic threshold

based on network topology, one estimate for R_0 from a degree-based estimation is given in Green *et al.* (2012) (4.11):

$$R_0 \sim \frac{\langle k^{in} k^{out} \rangle}{\sqrt{\langle k^{in} \rangle \langle k^{out} \rangle}} \tag{4.11}$$

where $\langle X \rangle$ represents an average of the X value.

An estimated $R_0 > 1$ indicates that an outbreak can spread though the network upon introduction (Heesterbeek and Dietz, 1996; Jones, 2007). In addition, the largest eigenvalue λ of the LSMN's adjacency matrix h was calculated, since this closely relates to the epidemic threshold in the network (Becker and Hall, 1996; Chakrabarti *et al.,* 2008; Prakash *et al.,* 2010). With few closed cycles in the network, however, this measure could easily be zero, or highly non-representative of the network. Thus, a simple adjustment was made, following Green *et al.* (2009), i.e. adding a constant number (K = 0.5) to all connections, as in (4.12).

$$h_{ij}^{adjust} = \frac{K w_{ij}}{N} + h_{ij} \tag{4.12}$$

Then, the largest eigenvalue λ was computed with the *eigen_centrality* function in the *igraph* package (Csardi and Nepusz, 2006). This result was obtained by solving the equation $hV = V\lambda$, where V is called an eigenvector of h corresponding to an eigenvalue (Restrepo *et al.,* 2007).

4.3.5 Rewiring the network

To evaluate the small-world property of the network, a structural comparison was made for the LSMN, for either C or $\langle L \rangle$, by comparing it with randomly rewired networks, while preserving the number of sites and degree distribution from the original one (Evans, 2007; Kiss and Green, 2008; Maslov and Sneppen, 2002; Noldus and Van Mieghem, 2013). One thousand rewired networks were developed, where in such rewired networks the probability of rewiring was set at one, resulting in all the two-pair connections in the LSMN being swapped. For example, two connections $S_1 \rightarrow S_2$ and $S_3 \rightarrow S_4$ are replaced by the simulated connections $S_1 \rightarrow S_4$ and $S_3 \rightarrow S_2$ (see Figure

4.2). The Mann-Whitney U test was used to compare for $\langle L \rangle$ in the original network with the distribution of rewired values. The result addressed the question of whether the original network is consistent with the rewired ones.

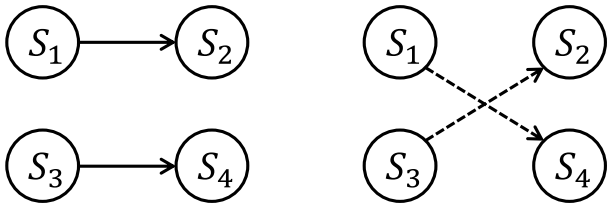


Figure 4.2 An example of a rewiring process which generates a new network by swapping the endpoints of two-pair connections in a network.

Connections can be rewired locally or non-locally, resulting in changing the susceptibility of the network to infection. Thus, the effect of the rewiring process on the potential epizootic size was also evaluated here. The effect of the rewiring process on the potential epizootic size in the 1 000 rewired LSMNs was assessed by the measures explained below.

Site reach. This network measure was proposed in Green *et al.* (2012). The number of sites reachable from any other (R_i) in an adjacency matrix of shortest paths (L_{ij}) was counted as in (4.13):

$$R_i = \sum_{j \neq i} [L_{ij} \neq \infty] \tag{4.13}$$

where $[X]$ is the Iverson bracket again.

The maximum reach in each rewired network was used to represent an estimate of the worst-case epidemic size, and the mean reach implied a typical epidemic size (Green *et al.,* 2012). Both maximum and mean reach are defined in (4.14) and (4.15), respectively:

$$\text{Max reach} = \max_i(R_i) \tag{4.14}$$

$$\text{Mean reach} = \frac{\sum R_i}{N} \tag{4.15}$$

where N is the total number of sites in the network.

Giant strongly connected components (GSCC) for directed networks. A GSCC refers to the largest number of sites in a network that are interconnected by directed connections. This has often been used to assess the potential epidemic size of networks (Kao *et al.,* 2007; Kiss *et al.,* 2006; Rautureau *et al.,* 2011). Danon *et al.* (2011) explained that all sites in a GSCC were able to catch the disease if the infection was seeded by any of those sites. In other words, all sites in the GSCC were equally at risk from transmission (Green *et al.,* 2009; Yatabe *et al.,* 2015). In addition to strongly connected components, all connections were also considered as bidirectional in order to measure the giant weakly connected component or GWCC (the largest number of sites in a network that are interconnected by undirected connections) (Pastor-Satorras *et al.,* 2015).

The GSCC was sought by two consecutive depth-first searches based on the method of Tarjan (1972). The GWCC, meanwhile, was searched by a simple breadth-first algorithm (Korf, 1985). Both implementations were run on the R Programme Environment package *igraph* (Csardi and Nepusz, 2006; R foundation for statistical computing, 2015). To provide a better understanding of the possible outcomes, examples of the implementation of the algorithms are given in Figure 4.3 (for SCC) and Figure 4.4 (for WCC).

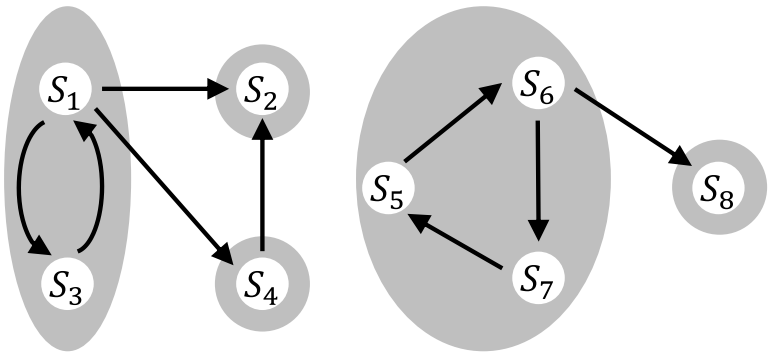


Figure 4.3 Strongly connected component of a directed network with eight sites. This small network has five strongly connected components (SCCs), which are shown by grey shading. The size of the giant strongly connected component (GSCC) is equal to three and contains sites S_5, S_6 and S_7.

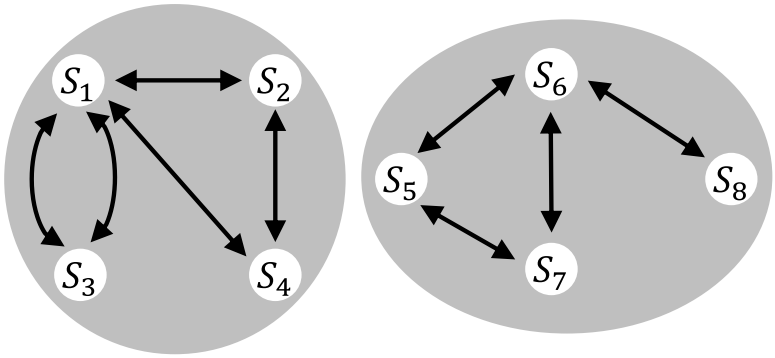


Figure 4.4 Weakly connected component of a bidirectional network with eight sites. This small network has two weakly connected components (WCCs), which are shown by grey shading. The size of the giant weakly connected component (WSCC) is equal to four, with a tie between the two sets of sites $\{S_1, S_2, S_3, S_4\}$ and $\{S_5, S_6, S_7, S_8\}$.

4.4 Results

The network modelling reported in this section considers the live shrimp movement data over a 13-month study period from March 2013 to March 2014.

4.4.1 General characteristics of the live shrimp movement network of Thailand (LSMN)

4.4.1.1 The number of sites

Figure 4.5 displays the general characteristics of the LSMN. *Circa* 13 800 shrimp farming sites were located in 37 provinces of five regions, i.e. south (5 665 sites; 41 % of the total), east (4 874 sites; 35 %), central (1 949 sites; 14 %), west (1 312 sites; 9 %), and one site in the northeast. The highest number of seed-producing sites denoted both hatcheries and nurseries was in the eastern region (379 sites; 47 % of the total 804 seed producing sites). Whereas 11 provinces have no seed-producing sites. The range and mean of the ratio of seed producing sites to ongrowing sites was 0–1.22 and 0.09, respectively.

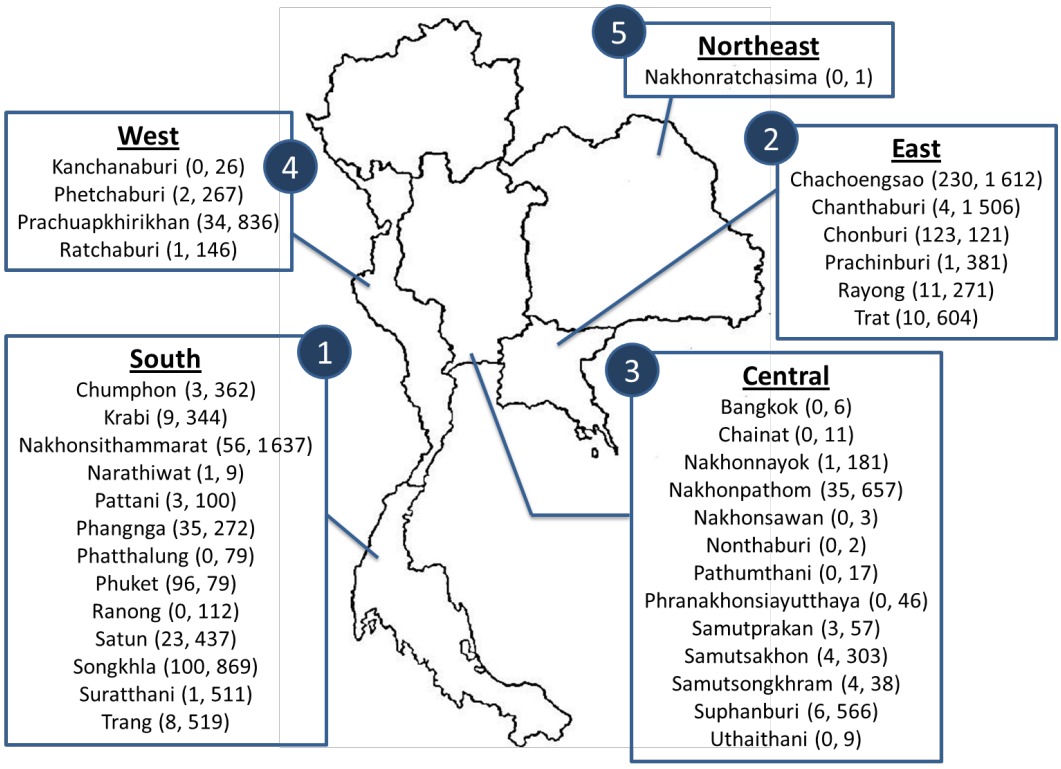


Figure 4.5 Circa 13 800 shrimp farming sites located in five regions and 37 provinces of Thailand. Values in brackets show the number of seed-producing sites and ongrowing sites, respectively. Among regions, the highest number of sites is in south (1) and the lowest number is in northeast (5). These data were collected from the live shrimp movements in Thailand from March 2013 to March 2014.

4.4.1.2 The characteristics of live shrimp movements

The diagrammatic representation of LSMN, which demonstrates the Thai shrimp farming industry structure, is shown in Figure 4.6. A few hatcheries obtain broodstock *L. vannamei* from ongrowing sites with broodstock improvement programmes. Instead of direct selling the shrimp seed at PL 10 to the ongrowing sites, the hatcheries pass some of their production to the nurseries at nauplius stage. Then, many nurseries rear the seed from the nauplius until PL 10 before selling the production to the ongrowing sites. The figure also indicates that there are a few number of shrimp seed (PL 12–14) movements between the on-growing sites as these sites are conducted by relatives of family.

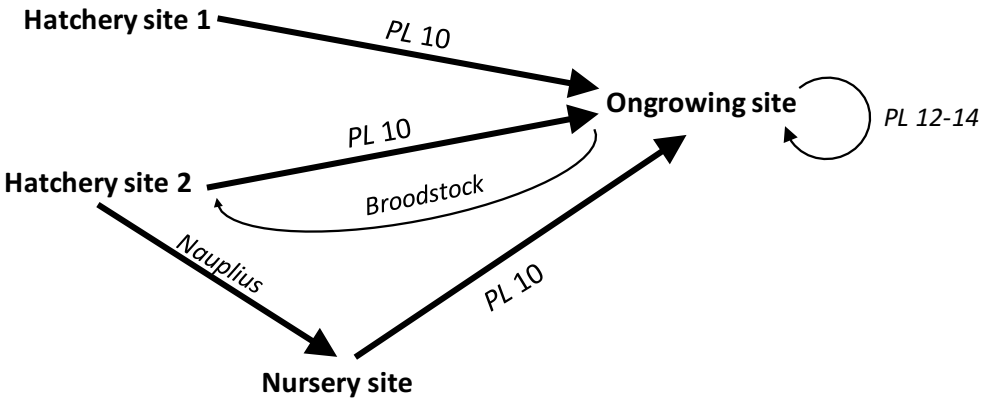


Figure 4.6 Diagrammatic representation of LSMN demonstrating the Thai shrimp farming industry structure.

The characteristics of live shrimp movements are summarised in Figures 4.7 and 4.8. Overall, *circa* 13 800 sites were involved in 33 720 site-to-site movements. As shown in Figure 4.7, these movements contained 74 462 repeated connections that included 57 281 connections entailing inter-province movements (77 % of the total repeated connections), and the monthly maximum was reached at about 6 000 connections in March 2014. The remaining connections were intra-province movements (23 %), with the maximum for these being reached at about 2 000 connections in September 2013.

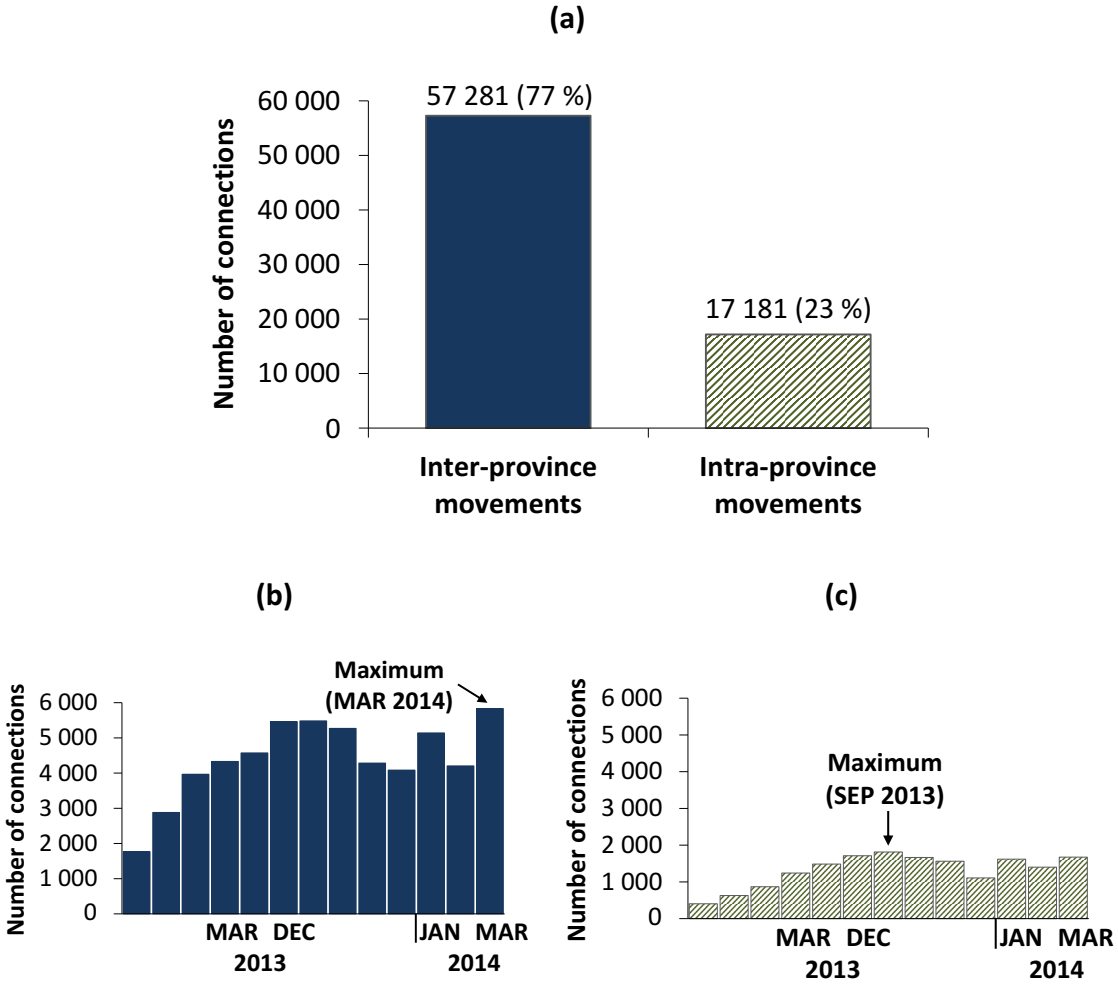


Figure 4.7 Distribution of the number of repeated connections over the 13-month study period (March 2013–March 2014) of live shrimp movements in Thailand. (a) The total number of connections are stratified by two movement types: inter- and intra-province movements. (b) The monthly distribution by inter-province movements. (c) The monthly distribution by intra-province movements.

Figure 4.8 gives the number of shrimp moved. Most shrimp were moved between sites via the inter-province movements (83 % of the 161 133 $\times 10^9$ shrimp). The maximum was reached at about 13 000 billion shrimp in March 2014. The remaining shrimp were moved by the intra-province movements, counting around 17 %, with the maximum was reached at about 3 000 billion shrimp in January 2014.

The results suggest that the movements, whether counted by the number of connections or the number of shrimp moved, can contribute to the spread of diseases across provinces. Importantly, the provincial controls of inter- and intra-province

movements (i.e. movement restriction) have the potential for preventing and controlling disease spread in Thai shrimp farming.

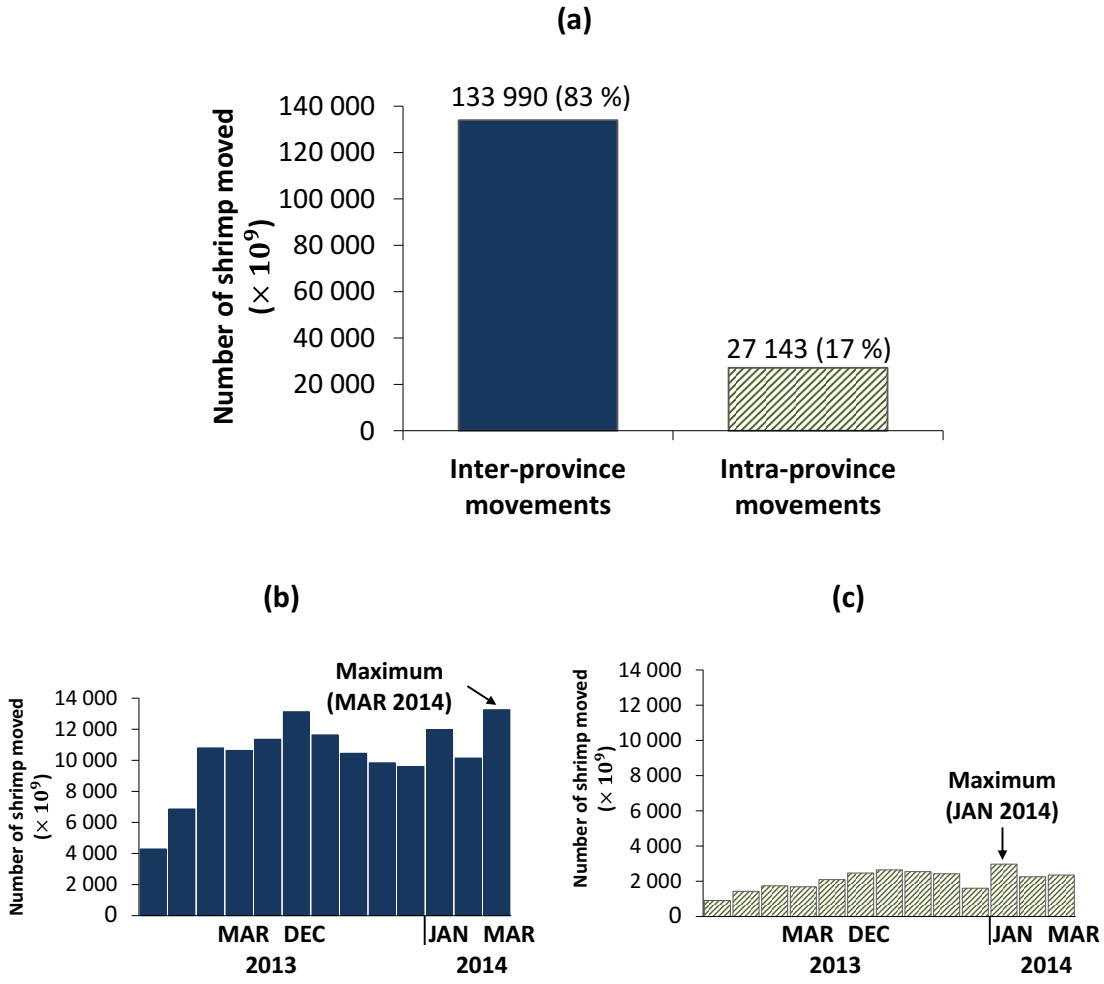


Figure 4.8 Distribution of the number of shrimp moved over the 13-month study period (March 2013–March 2014) of live shrimp movements in Thailand. (a) The total number of shrimp moved were stratified by two movement types: inter- and intra-province movements. (b) The monthly distribution by inter-province movements. (c) The monthly distribution by intra-province movements.

As measured geographically on the Thai map, the mean straight-line distances of the intra- and inter-province movements were 24 and 192 km, respectively. The 75th percentile of straight-line distance (km) for the inter-province movements was approximately 200 km, and around 30 km for the intra-province movements. These characteristics of live shrimp movements can be used to describe disease epizootics due to long-distance transmission.

4.4.2 Visualising the LSMN based on national provincial centres

The LSMN visualisation based on national provincial centres is presented in Figure 4.9. The figure also displays a subset of an example of provincial dataset, which is the movements of live shrimp from all three hatcheries in Samutprakan (SPK) to ongrowing sites, as shown in the box. The lines and arrows in the figure indicate the connections of live shrimp between source sites and destination sites with frequencies of connections corresponding to the colour and width (on a log scale) of the line. For disease surveillance and control in the Thai shrimp farming, the provincial control of movements has potential in terms of practicality because Thailand has local DoF offices in all 77 provinces, which play important roles in disease prevention and control implementation. This can be supported by a better understanding of two main types of the movements (inter- and intra-province movements).

In Figure 4.9, The inter-province movements with the highest number of repeated connections ($>$ 1 500 connections) are displayed in the orange and thicker lines, corresponding to Chonburi (CBI) → Chanthaburi (CTI), Chonburi (CBI) → Chachoengsao (CCO), Chachoengsao (CCO) → Chanthaburi (CTI), Trat (TRT) → Chanthaburi (CTI), and Chumphon (CPN) → Suratthani (SNI). Using the same key, the intra-province movements (i.e. a loop from a province to itself) with the highest number of connections were found in Chachoengsao (CCO), Nakhonsithammarat (NRT), Phuket (PKT), Songkhla (SKA), and Prachuapkhirikhan (PKN).

The visualised and quantitative outcomes of the LSMN bolsters understanding of disease transmission via long-distance transmission in the Thai shrimp farming sector, and provide information for the planning and designing of basic disease surveillance and control measures. For example, in the context of network models, provinces with a high number of connections out of the provincial boundaries may have an important role in transmitting diseases to other provinces. Hence, regulators can use the results of this work to allocate a larger amount of surveillance resources to those provinces, such as to Chonburi (CBI) and Trat (TRT).

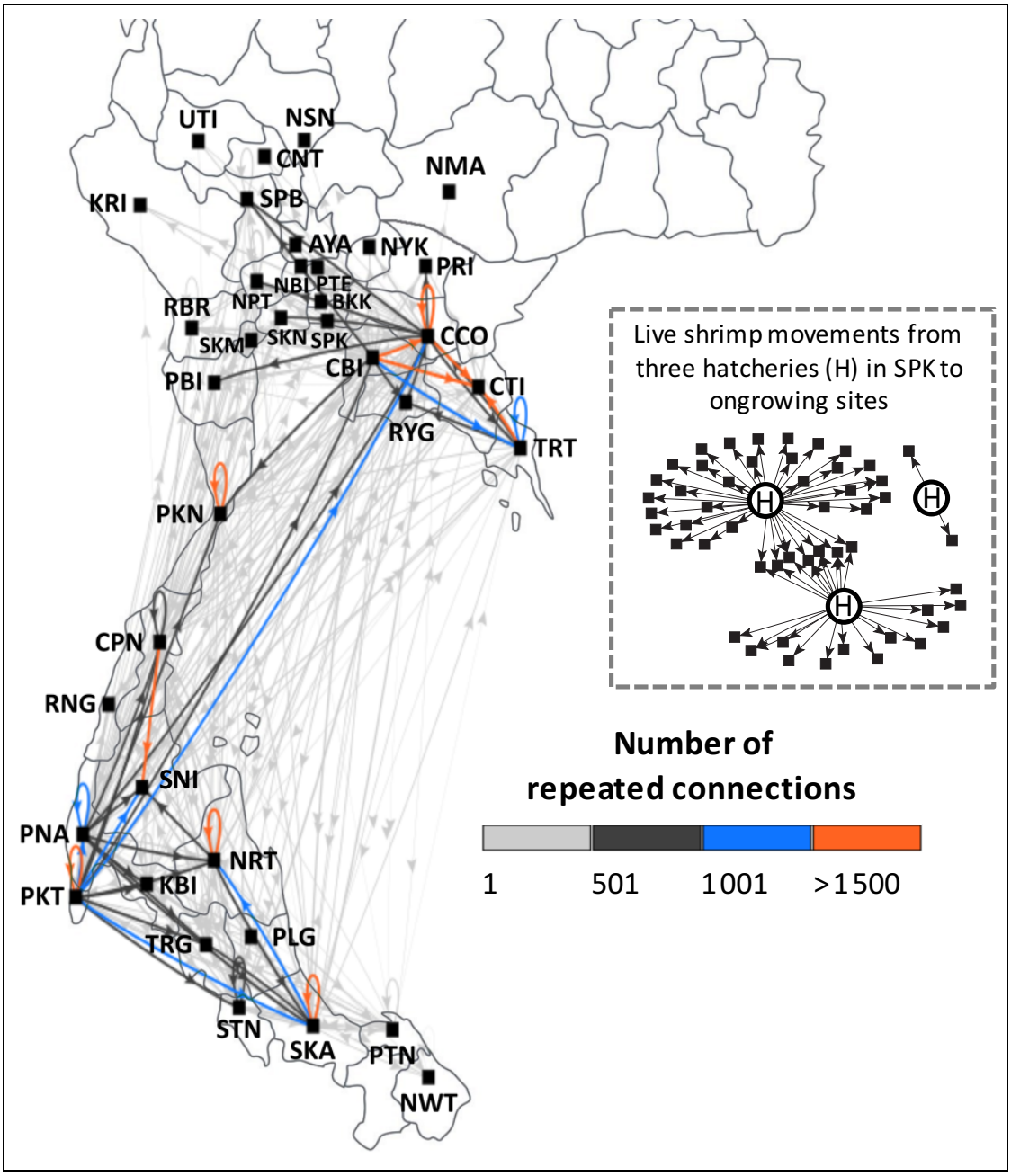


Figure 4.9 The provincial structure of the live shrimp movement network of Thailand (LSMN) over a 13-month period (March 2013–March 2014). The shrimp farming sites are located in 37 provinces of Thailand. The line width is plotted on a log scale according to the actual number of repeated connections. Four classifications of connections are shown by the different line colours: (1) 1–500 connections with grey, (2) 501–1 000 connections with dark grey, (3) 1 001–1 500 connections in blue, and (4) > 1 500 connections in orange. The figure also displays a subset of an example of provincial dataset, which is the movements of live shrimp from all three hatcheries in Samutprakan (SPK) to ongrowing sites, as shown in the box. The abbreviation list of national provincial centres is shown in Appendix C.

4.4.3 Descriptive analysis of the live shrimp movement network (LSMN) at site level

The site degrees of the LSMN were measured, and the results are summarised in Table 4.1. It appears that there are differences between using the non-weighted degrees (not including repeated connections) and the weighted degrees (including repeated connections).

Computing the LSMN as non-weighted, the mean (and coefficient of variation) for the in degree k^{in} was 2.4 (0.9) and 2.4 (9.2) for the out degree k^{out}. The undirected degree k^{und} was 4.9 (4.6). In degree k^{in} had a narrow range of degrees between 0 and 30. In contrast, k^{out} had a larger range between 0 and 932. Using the Pearson product-moment correlation, the correlation between the site degrees k^{in} and k^{out} was weakly positive with a value of 0.03 (N = 13 801 and P-value $<$ 0.01). The weak positive value of degree correlation indicated that sites with a high risk of catching disease posed a low risk for transmitting the disease (assuming solely network spread). This also reflected the fragmented nature of the network for hindering disease transmission (Kiss *et al.,* 2006).

Examining the site degrees from the weighted network, the degree properties were about two times larger than for the non-weighted one. The mean (and coefficient of variation) for k^{in} was 5.4 (1.7) and for k^{out} was 5.4 (13.4). The value for k^{und} was 10.8 (6.9). In degree k^{in} had a narrow range of degrees between 0 and 232. In contrast, k^{out} had a larger range between 0 and 5 839. The correlation between the site degrees k^{in} and k^{out} was weakly positive with a value of 0.24 (N = 13 801 and P-value $<$ 0.01).

The fact that a much weaker correlation between the site degrees k^{in} and k^{out} was observed with the non-weighted LSMN than with the weighted one suggests that studying the non-weighted network would miss this correlation, and it would lead to underestimation of epizootic spread.

Table 4.1 Degree properties of the live shrimp movement network of Thailand (LSMN). With two types of site degree calculations (non-weighted and weighted). The properties measured are total number of sites, total degrees, mean degrees, variation coefficient of degrees, and degree correlation.

Property	Non-weighted degree	Weighted degree
Total number of sites (N)	13 801	
Total degrees		
– in degree	33 720	74 462
– out degree	33 720	74 462
– $undirected$ degree	67 414	148 924
Mean degree		
– in degree	2.4	5.4
– out degree	2.4	5.4
– $undirected$ degree	4.9	10.8
Coefficient of variation		
– in degree	0.9	1.7
– out degree	9.2	13.4
– $undirected$ degree	4.6	6.9
Degree correlation	0.03	0.24

Considering weighted degree distribution, it was found that the LSMN demonstrated a power-law $P(k) \sim k^{-\gamma}$ for both in and out degree distributions with the exponents (γ) 2.87 and 2.17, respectively (Figure 4.10). The exponents of site degree distributions in the LSMN were in the range of two to three, similar to many scale-free networks, as proposed in Goh *et al.* (2002). Additionally, the Kolmogorov-Smirmov test accepted the power-law as a plausible model with large P-values > 0.05 (P-values of k^{in} = 0.7 and k^{out} = 0.54). These results indicate that the LSMN displays a scale-free topology.

A scale-free property is of major interest for epizoology (Boguná *et al.,* 2003; Shirley and Rushton, 2005). In respect to the LSMN, the scale-free property indicated that most sites had very low degrees of connections and few sites had high degrees of connections. The transmission capacity of this small group could be compared with the 80/20 rule as

proposed in Woolhouse *et al.* (1997). These authors suggested that, given the heterogeneity in site degrees, infection in 20 % of the total sites is sufficient to lead to the infection of the remaining sites.

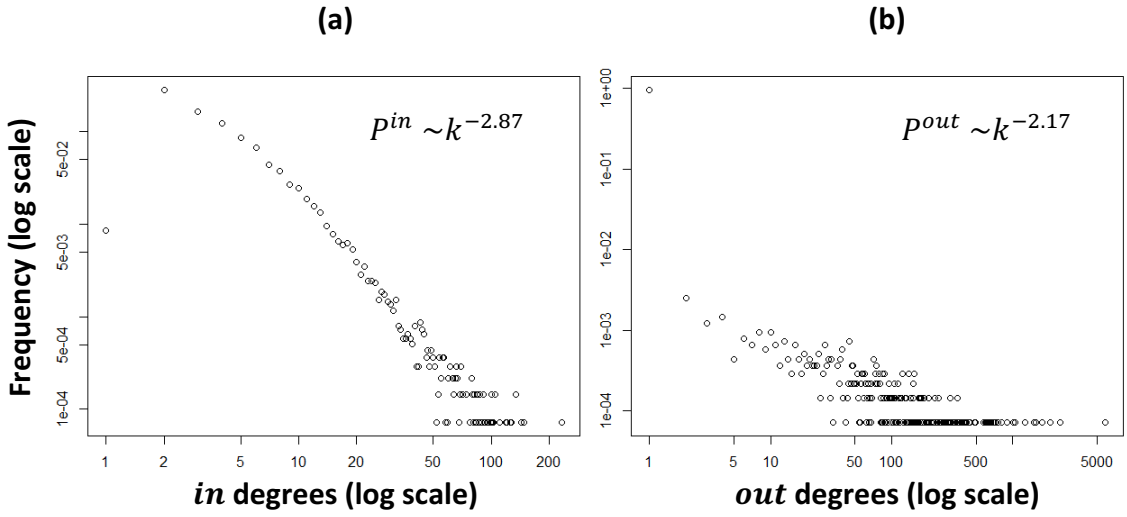


Figure 4.10 The weighted degree distributions for the LSMN plotted on a log–log scale. The sites with higher *in* degrees have a greater chance of being infected (a), and the sites with higher *out* degrees have a greater risk for transmitting disease (b).

Based on the weighted LSMN, the R_0 estimated by the degree-based calculation, was high ($\sim$ 34.5), compared to the largest eigenvalue = 16.2. The high value of R_0 can indicate that the connectivity of the LSMN obeys a bipartite structure: the network consists of two types of sites, i.e. seed-producing sites and ongrowing sites. To aid understanding of the bipartite structure, Table 4.2 shows the description of the total number of connections between two site types in the weighted LSMN. The bipartite structure is also evident in the non-weighted LSMN (Table 4.3). From these tables, both the non-weighted and weighted LSMN demonstrate a high number of connections between different site types ($>$ 80 % of the total connections). Nevertheless, the remaining connections join sites within the same type of site. Since a small number of connections disobeying such this bipartite structure, this may give rise to some difficulty in interpreting R_0 and in describing what R_0 means to seed-producing or ongrowing sites.

Table 4.2 Description of the number of connections between seed-producing sites and ongrowing sites based on the weighted degree of the LSMN. Their proportions are given in brackets.

Weighted degree		Destination	
		Seed-producing site	Ongrowing site
Source	Seed-producing site	10 775 (14.5 %)	63 596 (85.4 %)
	Ongrowing site	7 (0 %)	84 (0.1 %)

Table 4.3 Description of the number of connections between seed-producing sites and ongrowing sites based on the non-weighted degree of the LSMN. Their proportions are given in brackets.

Non-weighted degree		Destination	
		Seed-producing site	Ongrowing site
Source	Seed-producing site	2 047 (6.1 %)	31 589 (93.7 %)
	Ongrowing site	7 (0 %)	77 (0.2 %)

As measured by the weighted degree, the LSMN showed in-out disassortative mixing with a value of -0.09. This implies a low preference for connections in the LSMN that join sites with a high in degree link to those with a high out degree. This results in less extensive transmission of the disease to other sites. This finding also confirms that the LSMN is a hierarchical network (e.g. a few sites are sources for many connections in the LSMN), similar to what has been observed in relation to pig production in the United States (Lee *et al.,* 2017). According to Barthélemy *et al.* (2004), these few sites can become superspreaders for transmitting diseases and induce fast epidemics. In addition, using the same assortativity measure, the result was similar in the rewired networks. This result demonstrates that the dynamics of connections do not affect assortative mixing pattern in the LSMN.

A key property of small-world networks (i.e. small mean path lengths) is shown in both the non-weighted and weighted LSMN. Focusing on the non-weighted network, the value of the mean path length $\langle L \rangle$ was small (3.47), with 0.5 % of potential total paths

$N(N-1)$. For the weighted LSMN, $\langle L \rangle$ was equal to 2.99, with 0.14 % of potential total paths. The smaller value of $\langle L \rangle$ in the weighted network provides a good explanation for the speed of disease transmission in the network. For example, if the connections between two sites (i, j) are more frequent, as measured by the weighted network, a disease might be transmitted quicker through this network structure than the non-weighted one (Opsahl, 2009), and this is reflected in the low value of L_{ij}.

To further investigate the emergence of the small-world property, the distribution of weighted path lengths is plotted in Figure 4.11. This clearly demonstrates that the network includes many short paths and few long paths. In addition, the results of $\langle L \rangle$ were compared with the small-world experiment of Milgram *et al.* (1992) who studied the 'six degrees of separation' theory. This suggests that sites can get a disease via a connection of no more than six intermediates, showing a small-world network. In contrast with the small-world networks studied in Watts and Strogatz (1998), the LSMN characterised a small $\langle L \rangle$ but with low clustering as measured by a non-weighted clustering coefficient C = 0.0051 (the weighted C = 0.1). This low value of C implies that there are few strong ties (triangles) in the LSMN.

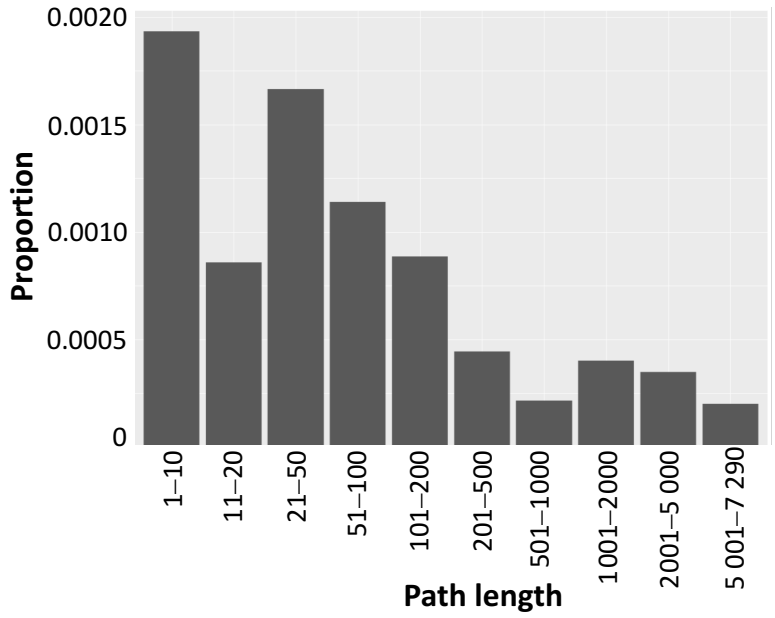


Figure 4.11 The distribution of weighted path lengths in the live shrimp movement network of Thailand (LSMN) is shown as a fraction of total connections.

To provide more evidence in respect to this small-world property, the important properties of the LSMN compared with rewired networks are summarised in Table 4.4. Using the non-parametric Mann–Whitney U test comparing the mean path length $\langle L \rangle$ for the original network and rewired ones, we concluded that the $\langle L \rangle$ of the original network and the rewired ones did not differ at a 0.05 significance level (P-value = 0.84). For 1 000 randomly rewired networks with rewiring probabilities = 1 (conserving the original LSMN's weighted degree distribution and the number of sites N), the average clustering coefficient C was 0.06 (SD = 0.008), and the average of $\langle L \rangle$ was 3.84 (SD = 2.12), with 0.5 % of potential paths existing. These indicate that the clustering coefficient (C), and the average shortest path length $\langle L \rangle$ within the LSMN are close to values computed from the corresponding randomly rewired networks. Thus, even in the event of the connections being changed, the LSMN still has a property of a small-world network and a small value of clustering coefficient.

Both properties of the LSMN were also compared with two networks of animal movements. The first was the network structure of Scottish live fish movements (561 sites and 1 340 connections) that had C = 0.07 and $\langle L \rangle$ = 5.92 (Green *et al.,* 2012). The second was the static network of pig movements in Germany (97 980 sites and 315 333 connections) that had $\langle L \rangle$ = 5.5 (Lentz *et al.,* 2016). A small characteristic path length and a low clustering coefficient denote that site-to-site transmission of disease in the LSMN is much easier and quicker than in these two alternative examples.

Table 4.4 also shows that the estimated maximum potential epizootic size in the LSMN was smaller than in the 1 000 rewired networks. This result was assessed by two network measures: (1) site reach, including repeated connections, and (2) the giant strongly connected component (GSCC) and the giant weakly connected component (GWCC).

With the worst-case epizootics, the average maximum reach for all fully rewired LSMNs was higher than in the original LSMN, an increase from 7 290 to 11 000 (SD = 449.7). As measured by mean reach, the estimated typical epizootic size also increased from 19.5 to 72.5 (SD = 20.2). Similarly, the mean size of GSCC was greater for the rewired LSMNs compared with the original one, an increase from 5 to 27.4 (SD = 17.8). In contrast, there

was no difference in the size of GWCC between the original network and the rewired LSMNs.

Table 4.4 Estimated maximum and mean reach, size of giant strongly connected components (GSCCs), and size of giant weakly connected component (GWCCs) for both the LSMN and the rewired LSMNs

Network measure	LSMN	Fully rewired LSMNs
		Average (SD)
Average path length $\langle L \rangle$	2.99	3.84 (2.12)
Clustering coefficient C	0.1	0.06 (0.008)
Total reach	268 000	1 005 000 (284 000)
Maximum reach	7 290	11 000 (450)
Mean reach	19.5	72.5 (20.2)
Size of GSCC	5	27.4 (17.8)
Size of GWCC	13 000	13 000 (3.5)

4.5 Discussion

Many infectious diseases transmit among populations via network spread, such as dengue disease in humans (Reiner *et al.,* 2014; Stoddard *et al.,* 2013), foot-and-mouth disease in domestic and wild animals (Sobrino and Domingo, 2017), and pancreas disease in farmed fish (Stene *et al.,* 2014). Thus, using graph theory and a network approach, the structure of the live shrimp movement network of Thailand (LSMN) over the 13-month study period was investigated in respect to the potential spread of shrimp diseases, and the important epizootiological properties both aiding and hindering the spread of diseases were characterised. Because the movements of live shrimp play a crucial role in infectious disease transmission from site to site, particularly in respect to the recent outbreak of AHPND (OIE, 2013) and other known shrimp diseases (Lightner, 1983), these results are a step towards designing an effective disease surveillance and control programme for Thai shrimp farming.

Network visualisation provides a good source for studying disease spread within real complex networks. Visualising H1N1 influenza pandemic worldwide (Brockmann and Helbing, 2013), for example, gives a better understanding of influenza spread via persons who travelled across c. 4 000 airports in 2009. The LSMN as visualised using the 37 provincial borders, gives a good illustration of the connections in the Thai shrimp farming. Visualising the LSMN as a direct movement network, it can be clearly seen which provinces have high connections in and out. The nodes denoting hatcheries have high risk of being source of infection; the nodes denoting nurseries have high risk of being source and sink of infection; the nodes denoting ongrowing sites have high risk of being sink of infection. Additionally, the provincial visualisation with two movement types (intra- and inter-province movements) emphasises that the regulators should increase efforts in respect to movement controls even during normal 'peacetime' situation.

The results also demonstrate these properties of the LSMN that either hinder or aid the spread of disease. For hindering the transmission, the LSMN had weak correlation between site degrees. For aiding transmission, the LSMN displayed a small characteristic path length and low clustering. It should be noted that clustering is a very local clustering measurement, as measured by the clustering coefficient. Not all clustering coefficients are necessary all that local, while there is lots of clustering at large scales due to local trading being common in the LSMN (e.g. within province). Thus, when the clustering was removed by rewiring, it was not surprising that the estimated potential epizooic size also increased.

For Thai shrimp farming, repeated connections between sites are common because there are a few shrimp seed producers in Thailand. The pattern of connections that often repeat may relate to the rapid transmission of AHPND and other infectious diseases in the network. As measured by mean path length $\langle L \rangle$, the weighted LSMN (including repeated connections) displayed a shorter value of $\langle L \rangle$ than the non-weighted one (not including repeated connections). In this case, Shirley and Rushton (2005) describe that a network with the shortest value of $\langle L \rangle$ is more likely to have the fastest rate of infection during periods of epizootic. Fast decision making is therefore required

to prevent disease epizootics in the Thai shrimp farming, in particular when the number of repeated connections increases.

The structure of the LSMN is scale-free (exhibiting power-law in-and out-degree distributions with the exponents γ = 2.87 and 2.17, respectively). These results corresponded to the studies of Pastor-Satorras and Vespignani (2002). These authors demonstrated that the variances of power-law distributions $0 < \gamma \leq 3$ became infinite, resulting in zero epidemic thresholds and disease spread and persistence in scale-free networks at any transmission rate, while exponents above three indicated finite variances. Chatterjee and Durrett (2009) also found a non-zero epidemic threshold in random networks with power-law distributions $\gamma > 3$. A disease-control strategy focused on reducing the transmission probability would probably apply to the cases where $\gamma > 3$ (Newman, 2002). To prevent disease spread in such power-law networks, therefore, control strategies focused on keeping non-zero epidemic threshold, such as by targeting highly connected sites, are more effective (Dezső and Barabási, 2002). Nevertheless, these are theoretical considerations. Any actual dataset has a finite mean and variance.

The important properties of LSMN that influence on the spread of diseases are detected by modelling the 1 000 rewired networks in order to examine small-world phenomenon and estimate epizootic sizes. In these rewired networks, the mean path length $\langle L \rangle$, reachability, and giant strongly connected components (GSCC) tended to be larger than in the original network, while the clustering coefficient (C) became smaller. In addition, the assortative mixing remained constant throughout all the rewired networks. Among these properties, the increases in either the maximum reach or giant connected components after network rewiring can be explained through the behaviour of Thai shrimp farmers that was observed during the face-to-face interviews in the Chapter 3 “Evaluating risk factors for AHPND transmission in the Thai shrimp farming sector”. The ongrowing farmers are likely to make a new contact when they feel dissatisfied with the seed quality. Consequently, this behaviour contributes to an increase in the estimated epizootic size in the Thai shrimp farming.

The LSMN that we study here does not contain both unreported movements and about 300 self-loop connections. This is an acknowledged limitation of the study. Unreported movements are typically generated from non-commercial farming with low productivity and for breeding improvement purposes, whereas self-loops are caused by sites that act as both seed-producing and ongrowing sites, which hold same farm registration number. The structure of the LSMN may change if the network analysis includes data set in respect to unreported movements and self-loops.

The movements between seed producing sites represented the movements of shrimp seed with nauplius or initial PL stages from hatcheries to nurseries. The data covered a few movements from ongrowing sites to seed producing sites (hatcheries) for breeding improvement purposes. The movements between ongrowing sites appeared to be sharing shrimp seed between relatives of family such as father's and son's farms, for example.

In summary, our network analysis describes the scope of potential disease transmission among the Thai shrimp farming sites via live shrimp movements. The LSMN is characterised by important epizootiological properties that both aid and hinder disease transmission. Because scale-free properties are found in the LSMN, we emphasise that optimal targeted disease surveillance and control can reduce the spread of epizootics in the Thai shrimp farming. Moreover, not only can a targeted strategy offer more effective prevention of disease epizootics, but it also provides a more efficient use of limited resources for disease surveillance and control. These considerations lead us to further research in the next chapter.

4.6 References

Ahnert, S.E. and Fink, T. (2008) Clustering signatures classify directed networks. *Physical Review E*, 78 (3), pp. 036112.

Aznar, M.N., Stevenson, M.A., Zarich, L. and León, E.A. (2011) Analysis of cattle movements in Argentina, 2005. *Preventive Veterinary Medicine*, 98 (2–3), pp. 119–127.

Barrat, A., Barthelemy, M., Pastor-Satorras, R. and Vespignani, A. (2004) The architecture of complex weighted networks. *Proceedings of the National Academy of Sciences of the United States of America*, 101 (11), pp. 3747–3752.

Barthélemy, M., Barrat, A., Pastor-Satorras, R. and Vespignani, A. (2004) Velocity and hierarchical spread of epidemic outbreaks in scale-free networks. *Physical Review Letters*, 92 (17), pp. 1–4.

Becker, N.G. and Hall, R. (1996) Immunization levels for preventing epidemics in a community of households made up of individuals of various types. *Mathematical Biosciences*, 132 (2), pp. 205–216.

Bogel, K., Moegle, H., Knorpp, F., Arata, A., Dietz, K. and Diethelm, P. (1976) Characteristics of the spread of a wildlife rabies epidemic in Europe. *Bulletin of the World Health Organization*, 54 (4), pp. 433–447.

Boguná, M., Pastor-Satorras, R. and Vespignani, A. (2003) Absence of epidemic threshold in scale-free networks with degree correlations. *Physical Review Letters*, 90 (2), pp. 028701.

Britton, T., Deijfen, M. and Liljeros, F. (2011) A weighted configuration model and inhomogeneous epidemics. *Journal of Statistical Physics*, 145 (5), pp. 1368–1384.

Brockmann, D. and Helbing, D. (2013) The hidden geometry of complex, network-driven contagion phenomena. *Science (New York, N.Y.)*, 342 (6164), pp. 1337–1342.

Büttner, K., Krieter, J., Traulsen, A. and Traulsen, I. (2013) Static network analysis of a pork supply chain in Northern Germany—Characterisation of the potential spread of infectious diseases via animal movements. *Preventive Veterinary Medicine*, 110 (3–4), pp. 418–428.

Cai, C., Wu, Z. and Guan, J. (2014) Effect of vaccination strategies on the dynamic behavior of epidemic spreading and vaccine coverage. *Chaos, Solitons & Fractals*, 62, pp. 36–43.

Chakrabarti, D., Wang, Y., Wang, C., Leskovec, J. and Faloutsos, C. (2008) Epidemic thresholds in real networks. *ACM Transactions on Information and System Security (TISSEC)*, 10 (4), pp. 1.

Chatterjee, S. and Durrett, R. (2009) Contact processes on random graphs with power law degree distributions have critical value 0. *The Annals of Probability*, 37 (6), pp. 2332–2356.

Clauset, A., Shalizi, C.R. and Newman, M.E. (2009) Power-law distributions in empirical data. *SIAM Review*, 51 (4), pp. 661–703.

Coastal Aquaculture Research and Development Division (2013) Thai shrimp farm registration database.

Csardi, G. and Nepusz, T. (2006) The igraph software package for complex network research. *InterJournal*, Complex Systems (http://igraph.org), pp. 1695.

Danon, L., Ford, A.P., House, T., Jewell, C.P., Keeling, M.J., Roberts, G.O., Ross, J.V. and Vernon, M.C. (2011) Networks and the epidemiology of infectious disease. *Interdisciplinary Perspectives on Infectious Diseases*, 2011, pp. 1–28.

Dezső, Z. and Barabási, A. (2002) Halting viruses in scale-free networks. *Physical Review E*, 65 (5), pp. 055103.

Dijkstra, E.W. (1959) A note on two problems in connexion with graphs. *Numerische Mathematik*, 1 (1), pp. 269–271.

Draief, M., Ganesh, A. and Massoulié, L. (2008) Thresholds for virus spread on networks. *The Annals of Applied Probability*, 18 (2), pp. 359–378.

Dubé, C., Ribble, C., Kelton, D. and McNab, B. (2008) Comparing network analysis measures to determine potential epidemic size of highly contagious exotic diseases in fragmented monthly networks of dairy cattle movements in Ontario, Canada. *Transboundary and Emerging Diseases*, 55 (9–10), pp. 382–392.

Evans, T. (2007) Exact solutions for network rewiring models. *The European Physical Journal B*, 56 (1), pp. 65–69.

FAO (2013) *Report of the FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304).* FAO Fisheries and Aquaculture Report No. 1053. Rome: FAO. Available: http://www.fao.org/docrep/018/i3422e/i3422e00.htm [Accessed: 11 October 2013].

Flaherty, M., Szuster, B. and Miller, P. (2000) Low salinity inland shrimp farming in Thailand. *Ambio*, 29 (3), pp. 174–179.

Flegel, T.W. (2012) Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology*, 110 (2), pp. 166–173.

Foster, J.G., Foster, D.V., Grassberger, P. and Paczuski, M. (2010) Edge direction and the structure of networks. *Proceedings of the National Academy of Sciences of the United States of America*, 107 (24), pp. 10815–10820.

Goh, K.I., Oh, E., Jeong, H., Kahng, B. and Kim, D. (2002) Classification of scale-free networks. *Proceedings of the National Academy of Sciences of the United States of America*, 99 (20), pp. 12583–12588.

Google Earth (2015) *Geographic coordinates of the sub-districts across Thailand.* Google Inc. Available: http://www.google.com/earth/index.html [Accessed: 3 March 2015].

Green, D.M., Werkman, M. and Munro, L.A. (2012) The potential for targeted surveillance of live fish movements in Scotland. *Journal of Fish Diseases*, 35 (1), pp. 29–37.

Green, D.M., Gregory, A. and Munro, L.A. (2009) Small- and large-scale network structure of live fish movements in Scotland. *Preventive Veterinary Medicine*, 91 (2–4), pp. 261–269.

Guinat, C., Relun, A., Wall, B., Morris, A., Dixon, L. and Pfeiffer, D.U. (2016) Exploring pig trade patterns to inform the design of risk-based disease surveillance and control strategies. *Scientific Reports*, 6, pp. 28429.

Harrell, F.E. (2015) *Hmisc: Harrell miscellaneous. R package version 3.16-0.* Available: http://CRAN.R-project.org/package=Hmisc [Accessed: 6 December 2015].

Hartvigsen, G., Dresch, J.M., Zielinski, A.L., Macula, A.J. and Leary, C.C. (2007) Network structure, and vaccination strategy and effort interact to affect the dynamics of influenza epidemics. *Journal of Theoretical Biology*, 246 (2), pp. 205–213.

Heesterbeek, J.A.P. and Dietz, K. (1996) The concept of R_0 in epidemic theory. *Statistica Neerlandica*, 50 (1), pp. 89–110.

Hong, X., Lu, L. and Xu, D. (2016) Progress in research on acute hepatopancreatic necrosis disease (AHPND). *Aquacult Int*, 24, pp. 577–593.

Huang, C., Liu, X., Sun, S., Li, S.C., Deng, M., He, G., Zhang, H., Wang, C., Zhou, Y. and Zhao, Y. (2016) Insights into the transmission of respiratory infectious diseases through empirical human contact networks. *Scientific Reports,* 6, pp. 31484.

Jones, J.H. (2007) *Notes on R_0.* Califonia: Department of Anthropological Sciences. Available: https://people.stanford.edu/jhj1/sites/default/files/file/jones-r0-notes.pdf [Accessed: 12 May 2015].

Kaesler, D. (N.A.) *Map of Thailand (License: royalty free).* Available: http://www.dreamstime.com [Accessed: 19 August 2014].

Kao, R.R., Green, D.M., Johnson, J. and Kiss, I.Z. (2007) Disease dynamics over very different time-scales: foot-and-mouth disease and scrapie on the network of livestock movements in the UK. *Journal of the Royal Society, Interface / the Royal Society*, 4 (16), pp. 907–916.

Kiss, I.Z. and Green, D.M. (2008) Comment on "Properties of highly clustered networks". *Physical Review E*, 78 (4), pp. 048101.

Kiss, I.Z., Green, D.M. and Kao, R.R. (2006) The network of sheep movements within Great Britain: Network properties and their implications for infectious disease spread. *Journal of the Royal Society, Interface / the Royal Society*, 3 (10), pp. 669–677.

Korf, R.E. (1985) Depth-first iterative-deepening: An optimal admissible tree search. *Artificial Intelligence,* 27 (1), pp. 97–109.

Kurvers, R.H.J.M., Krause, J., Croft, D.P., Wilson, A.D.M. and Wolf, M. (2014) The evolutionary and ecological consequences of animal social networks: emerging issues. *Trends in Ecology & Evolution*, 29 (6), pp. 326–335.

Lee, K., Polson, D., Lowe, E., Main, R., Holtkamp, D. and Martínez-López, B. (2017) Unraveling the contact patterns and network structure of pig shipments in the United States and its association with porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks. *Preventive Veterinary Medicine*, 138, pp. 113–123.

Lentz, H.H., Koher, A., Hövel, P., Gethmann, J., Sauter-Louis, C., Selhorst, T. and Conraths, F.J. (2016) Disease spread through animal movements: a static and temporal network analysis of pig trade in Germany. *PloS One,* 11 (5), e0155196. Available: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0155196&type=printable [Accessed: 12 January 2017].

Lightner, D.V., Redman, R.M., Bell, T.A. and Brock, J.A. (1983a) Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. *Journal of the World Mariculture Society,* 14 (1–4), pp. 212–225.

Ma, J., van den Driessche, P. and Willeboordse, F.H. (2013) The importance of contact network topology for the success of vaccination strategies. *Journal of Theoretical Biology*, 325, pp. 12–21.

Mao, G. and Zhang, N. (2013) Analysis of average shortest-path length of scale-free network. *Journal of Applied Mathematics*, 2013, pp. 1–5.

Maslov, S. and Sneppen, K. (2002) Specificity and stability in topology of protein networks. *Science (New York, N.Y.)*, 296 (5569), pp. 910–913.

Meyers, L.A., Newman, M., Martin, M. and Schrag, S. (2003) Applying network theory to epidemics: control measures for *Mycoplasma pneumoniae* outbreaks. *Emerging Infectious Diseases*, 9 (2), pp. 204–210.

Milgram, S., Sabini, J.E. and Silver, M.E. (1992) *The individual in a social world: essays and experiments.* New York: Mcgraw-Hill Book Company.

Moslonka-Lefebvre, M., Finley, A., Dorigatti, I., Dehnen-Schmutz, K., Harwood, T., Jeger, M.J., Xu, X., Holdenrieder, O. and Pautasso, M. (2011) Networks in plant epidemiology: from genes to landscapes, countries, and continents. *Phytopathology*, 101 (4), pp. 392–403.

Mrvar, A. and Batagelj, V. (1996) *Pajek: Analysis and Visulisation of Large Networks.* Available: http://pajek.imfm.si [Accessed: 20 August 2014].

Munro, L. and Gregory, A. (2009) Application of network analysis to farmed salmonid movement data from Scotland. *Journal of Fish Diseases*, 32 (7), pp. 641–644.

NACA (2017) *Quarterly aquatic animal disease report (Asia and Pacific Region) 1998-2016.* Thailand: NACA. Available: http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report [Accessed: 29 April 2017].

Nagai, F., Mektrairat, N. and Funatsu, T. (2008) *Local government in Thailand: analysis of the local administrative organization survey.* Chiba, Japan: Institute of developing economies.

Newman, M.E. (2002) Spread of epidemic disease on networks. *Physical Review E*, 66 (1), pp. 016128.

Newman, M.E. (2003) Mixing patterns in networks. *Physical Review E*, 67 (2), pp. 026126.

Newman, M.E. (2004) Analysis of weighted networks. *Physical Review E*, 70 (5), pp. 056131.

Newman, M.E. (2005) Power laws, Pareto distributions and Zipf's law. *Contemporary Physics*, 46 (5), pp. 323–351.

Noldus, R. and Van Mieghem, P. (2013) Effect of degree-preserving, assortative rewiring on OSPF router configuration. *Teletraffic Congress (ITC), 2013 25th International.* IEEE, pp. 1–4.

OIE (2013) *Acute hepatopancreatic necrosis disease, aetiology epidemiology diagnosis prevention and control references.* Available: http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/AHPND_DEC_2013.pdf [Accessed: 12 August 2014].

Opsahl, T. (2009) *Structure and evolution of weighted networks.* University of London (Queen Mary College), London, UK. Available: https://toreopsahl.com/tnet [Accessed: 1 December 2015].

Pakingking Jr, R.V., de Jesus-Ayson, Evelyn Grace T and Acosta, B.O. (2016) Addressing acute hepatopancreatic necrosis disease (AHPND) and other transboundary diseases for improved aquatic animal health in Southeast Asia. *Proceedings of the ASEAN Regional Technical Consultation on EMS/AHPND and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia.* Makati City, Philippines, 22nd to 24th February 2016. Iloilo, Philippines: SEAFDEC Available: https://repository.seafdec.org.ph/handle/10862/3096 [Accessed: 20 February 2017].

Pastor-Satorras, R., Castellano, C., Van Mieghem, P. and Vespignani, A. (2015) Epidemic processes in complex networks. *Reviews of Modern Physics*, 87 (3), pp. 925.

Pastor-Satorras, R. and Vespignani, A. (2002) Epidemic dynamics in finite size scale-free networks. *Physical Review E*, 65 (3), pp. 035108.

Prakash, B.A., Chakrabarti, D., Faloutsos, M., Valler, N. and Faloutsos, C. (2010) Got the flu (or mumps)? check the eigenvalue! *arXiv Preprint arXiv:1004.0060*, pp. 1–26.

R foundation for statistical computing (2015) *R: a language and environment for statistical computing.* Available: https://www.R-project.org/ [Accessed: 5 December 2015].

Rautureau, S., Dufour, B. and Durand, B. (2011) Vulnerability of animal trade networks to the spread of infectious diseases: a methodological approach applied to evaluation and emergency control strategies in cattle, France, 2005. *Transboundary and Emerging Diseases*, 58 (2), pp. 110–120.

Reiner, R.C., Stoddard, S.T. and Scott, T.W. (2014) Socially structured human movement shapes dengue transmission despite the diffusive effect of mosquito dispersal. *Epidemics*, 6, pp. 30–36.

Restrepo, J.G., Ott, E. and Hunt, B.R. (2007) Approximating the largest eigenvalue of network adjacency matrices. *Physical Review E*, 76 (5), pp. 056119.

Robinaugh, D.J., Millner, A.J. and McNally, R.J. (2016) Identifying highly influential nodes in the complicated grief network. *Journal of Abnormal Psychology*, 125 (6), pp. 747.

Shirley, M.D.F. and Rushton, S.P. (2005) The impacts of network topology on disease spread. *Ecological Complexity*, 2 (3), pp. 287–299.

Sobrino, F. and Domingo, E. (2017) *Foot-and-mouth disease virus: current research and emerging trends.* Norfolk, UK: Caister Academic Press.

Songsanjinda, P. (2015) Crisis of Thai shrimp production. Bangkok, 16th to 20th August 2015. Thailand Research Expo 2015: National Research Council of Thailand, pp. 1–35.

Stene, A., Viljugrein, H., Yndestad, H., Tavornpanich, S. and Skjerve, E. (2014) Transmission dynamics of pancreas disease (PD) in a Norwegian fjord: aspects of water transport, contact networks and infection pressure among salmon farms. *Journal of Fish Diseases*, 37 (2), pp. 123–134.

Stoddard, S.T., Forshey, B.M., Morrison, A.C., Paz-Soldan, V.A., Vazquez-Prokopec, G.M., Astete, H., Reiner, R.C., Jr, Vilcarromero, S., Elder, J.P., Halsey, E.S., Kochel, T.J., Kitron, U. and Scott, T.W. (2013) House-to-house human movement drives dengue virus transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 110 (3), pp. 994–999.

Szuster, B. (2006) Coastal shrimp farming in Thailand: searching for sustainability. In: T.H. Chu, ed. *Environment and livelihoods in tropical coastal zones: managing agriculture-fishery-aquaculture conflicts.* Norfolk, UK: CAB International, pp. 86–97.

Tarjan, R. (1972) Depth-first search and linear graph algorithms. *SIAM Journal on Computing*, 1 (2), pp. 146–160.

Taylor, N., Way, K., Jeffery, K. and Peeler, E. (2010) The role of live fish movements in spreading koi herpesvirus throughout England and Wales. *Journal of Fish Diseases*, 33 (12), pp. 1005.

Watts, D.J. and Strogatz, S.H. (1998) Collective dynamics of 'small-world'networks. *Nature*, 393 (6684), pp. 440–442.

Wei, D., Deng, X., Zhang, X., Deng, Y. and Mahadevan, S. (2013) Identifying influential nodes in weighted networks based on evidence theory. *Physica A: Statistical Mechanics and its Applications*, 392 (10), pp. 2564–2575.

Werkman, M., Green, D.M., Murray, A.G. and Turnbull, J.F. (2011) The effectiveness of fallowing strategies in disease control in salmon aquaculture assessed with an SIS model. *Preventive Veterinary Medicine*, 98 (1), pp. 64–73.

Wiedermann, M., Donges, J.F., Heitzig, J. and Kurths, J. (2013) Node-weighted interacting network measures improve the representation of real-world complex systems. *EPL (Europhysics Letters)*, 102 (2), pp. 28007.

Woolhouse, M.E., Dye, C., Etard, J.F., Smith, T., Charlwood, J.D., Garnett, G.P., Hagan, P., Hii, J.L., Ndhlovu, P.D., Quinnell, R.J., Watts, C.H., Chandiwana, S.K. and Anderson, R.M. (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America*, 94 (1), pp. 338–342.

Yang, Z., Fu, D., Tang, Y., Zhang, Y., Hao, Y., Gui, C., Ji, X. and Yue, X. (2012) Link prediction based on weighted networks. *AsiaSim 2012.* Berlin: Springer, pp. 119–126.

Yatabe, T., More, S., Geoghegan, F., McManus, C., Hill, A.E. and Martínez-López, B. (2015) Characterization of the live salmonid movement network in Ireland: implications for disease prevention and control. *Preventive Veterinary Medicine*, 122 (1), pp. 195–204.

Chapter 5 - Target priority for targeted disease surveillance and control in the live shrimp movement network of Thailand

N. Saleetid; D.M. Green; F.J. Murray

Preface

Chapter 5 uses the data introduced in Chapter 4. The aim of this chapter is to develop a targeted disease-control algorithm for the Thai shrimp farming sector based on the analysis of the structure of the live shrimp movement network (LSMN) undertaken in Chapter 4. This chapter is designed for publication; thus, in its introduction part information on the spread of AHPND in shrimp farming sites is given again. One of the keys to working successfully with the algorithm is writing R scripts. These R scripts were developed by programming work done by the author, but with advice from D.M. Green, and are different from those in previous studies.

Impact statement

Disease surveillance and control can lead to reduced population losses from infectious diseases by detecting and treating epizootics at an early stage. In the case of Thai shrimp farming, unless targeted disease surveillance and control is established, diseases could spread and persist among sites, due to the structure of the LSMN. Thus, this study adopted network approaches to identify high-risk connections whose removal from the network could reduce epizootics. The targeted disease-control algorithms obtained from this study provide the Thailand Department of Fisheries with a good strategy for planning the annual disease surveillance and control programme for farmed shrimp and other aquatic species.

Chapter 5 - Target priority for targeted disease surveillance in the live shrimp movement network of Thailand

5.1 Abstract

Targeted disease surveillance and control by using network approaches has been shown to contribute to reducing the spread of diseases in various farm animal sectors such as cattle, pig and fish, but has never been previously assessed in shrimp, even though the live shrimp movement network makes sites very vulnerable to disease epizootics. To implement targeted disease surveillance and control, therefore, five disease-control algorithms were evaluated and their efficacy compared in terms of reducing a potential epizootic in the large-scale network of live shrimp movements of Thailand (LSMN). The results of these algorithms show that an effective strategy to control disease spread in the Thai shrimp farming can be achieved by removing a small number of targeted high-risk connections. Specially, two disease-control algorithms based on betweenness centrality (the number of shortest paths between possible pairs of sites that run along it) and subnet-crossing (connections crossing subnets within large network) are proposed for use to prioritise targets for disease surveillance and control measures in Thai shrimp farming.

5.2 Introduction

Infectious diseases have continually hit shrimp farming sectors. Over the last 30 years, it is microparasites that have mainly caused severe infections in shrimp (Flegel *et al.,* 2008; Thitamadee *et al.,* 2016). Particularly, outbreaks of viral white spot disease in the 1990's did not only resulted in socio-economic losses, but also contributed to the enhancement of many mitigation measures such as biosecurity (Lightner, 2005) and diagnostic techniques (Flegel *et al.,* 2008). Consequently, a huge financial investment has been made in the development of disease surveillance programmes at national and international scales (Bondad-Reantaso *et al.,* 2005). Nonetheless, no approach has been proposed to target high-risk connections in live shrimp movement networks.

Recently, farmed shrimp have become vulnerable to a bacterial disease named acute hepatopancreatic necrosis (AHPND, also known as EMS). Its outbreak caused a strong decrease in shrimp exports from two major shrimp-producing countries (i.e. China and Thailand) in a 5-year period from 2009 to 2013 (Portley, 2016). Most concernedly, AHPND caused the Thai shrimp production sector to decline from around 500 000 tonnes in 2011 to 200 000 tonnes in 2014 (a 56 % decrease; Songsanjinda, 2015). In addition to an annual operating cost of about USD 2 Million for shrimp disease surveillance and control (Planning Division, 2016), in 2014/2015 the Thai government used a new USD 2.7 million investment for the mitigation of AHPND (Kongkumnerd, 2014). Due to the high cost of disease surveillance and control, regulators should design disease surveillance and control programmes strategically, i.e. identifying high-risk shrimp farming sites or connections, and this can be done with network approaches.

Many epidemiological studies have performed centrality measures for the identification of high-risk individuals whose removal from the network minimises potential disease spread. The identification of high-risk individuals in a network relates to the 80/20 rule proposed by Woolhouse *et al*. (1997). The authors developed this rule to explain the effects of heterogeneities in site (in network terminology, nodes) degrees on disease transmission, in that generally 20 % of infectious sites corresponded to 80 % of the transmission. Thus, disease surveillance and control targeted at the "core" 20 % group can be the most effective strategy (Woolhouse *et al.,* 2005).

Due to the range of network structures, however, any given measure for identifying influential sites or connections may not suitable for all networks (Newman and Park, 2003). Degree centrality is proposed by many epidemiologists as a means to demonstrate the most important individuals in networks (Bohm *et al.,* 2009; Christley *et al.,* 2005b; Zhang *et al.,* 2010). Algorithms based on betweenness centrality have been shown to be the most efficient way to reduce potential epidemic size in terms of component structure in the case of the network of cattle movements in France (Rautureau *et al.,* 2011), and the network of pig movements in Germany (Lentz *et al.,* 2016). The algorithm based on betweenness centrality also reduces potential epidemic size in terms of site reachability in the live fish movement network in Scotland (Green *et al.,* 2009). For comparison, other measures of centrality include closeness centrality

(Fournie *et al.,* 2013) and eigenvector centrality (Herrera *et al.,* 2016). Hence, it is important to address three key questions in this research: (1) does an algorithm work for a given system? (2) how does this algorithm relate to the properties of the network? and (3) which is appropriate for Thai shrimp farming?

This research, therefore, has aimed to find the optimal disease-control algorithm for the Thai shrimp farming system. Disease-control algorithms with and without targeted approaches are evaluated and compared using the movement records of live shrimp movements in Thailand (LSMN) over the 13-month period from March 2013 to March 2014. The outcome of this research can form part of the process of implementing mitigation measures for management areas in real time during an epizootic period, and for developing disease surveillance and control programmes in conventional non-epizootic periods, not only for the Thai shrimp farming sector but also other shrimp producing countries.

5.3 Materials and methods

5.3.1 Data source for the live shrimp movement network (LSMN)

As with Chapter 4, the LSMN with its c. 74 400 repeated connections in the period from March 2013 and March 2014 was the data source for this research. These official data were recorded by authorisers of the Thailand Department of Fisheries, following the aquatic animal trade regulation of Thailand, B.E.2553 (2010). The recorded data indicated the farm registration number, the source and destination of the live shrimp movement, the date of the movement, and the seed quantity.

The LSMN was represented by a connection-weighted adjacency matrix h, an element-wise multiplication of matrix a by matrix w. The term "site" refers to "node" in conventional network terminology. All connections between sites were shown in a matrix a_{ij}. An element a_{ij} took the value 0 if there was no connection from site i to j and 1 otherwise, which in this case represented a potential pathway for site-to-site disease transmission. The weight w_{ij} denoted the frequency of connections of such a_{ij}. About 300 self-loop connections (a_{ii} = 1) were removed from the analysis because

these self-loops had no effect on site-to-site disease transmission (Britton *et al.,* 2011; Draief *et al.,* 2008).

5.3.2 Disease-control algorithms for targeted disease surveillance and control

Disease-control algorithms were developed to target high-risk connections between two sites (i and j) in the LSMN, whose removal from the network reduced the potential transmission of disease. The algorithms used in this research were developed from those described by Green *et al.* (2012), which contain four targeted approaches based on betweenness, connection weight, eigenvector centrality, and subnet-crossing to identify targets for removal and—as a control—a non-targeted approach. An effective algorithm should have high performance in reducing the susceptibility of a network to a disease epizootic with relatively few removals.

Figure 5.1 demonstrates the disease-control algorithm. The algorithm begins with the whole network. Then, the connections a from i to j in the network are listed in descending order according to one of the following criteria. If connections have equal values, these connections are selected at random for each replicate.

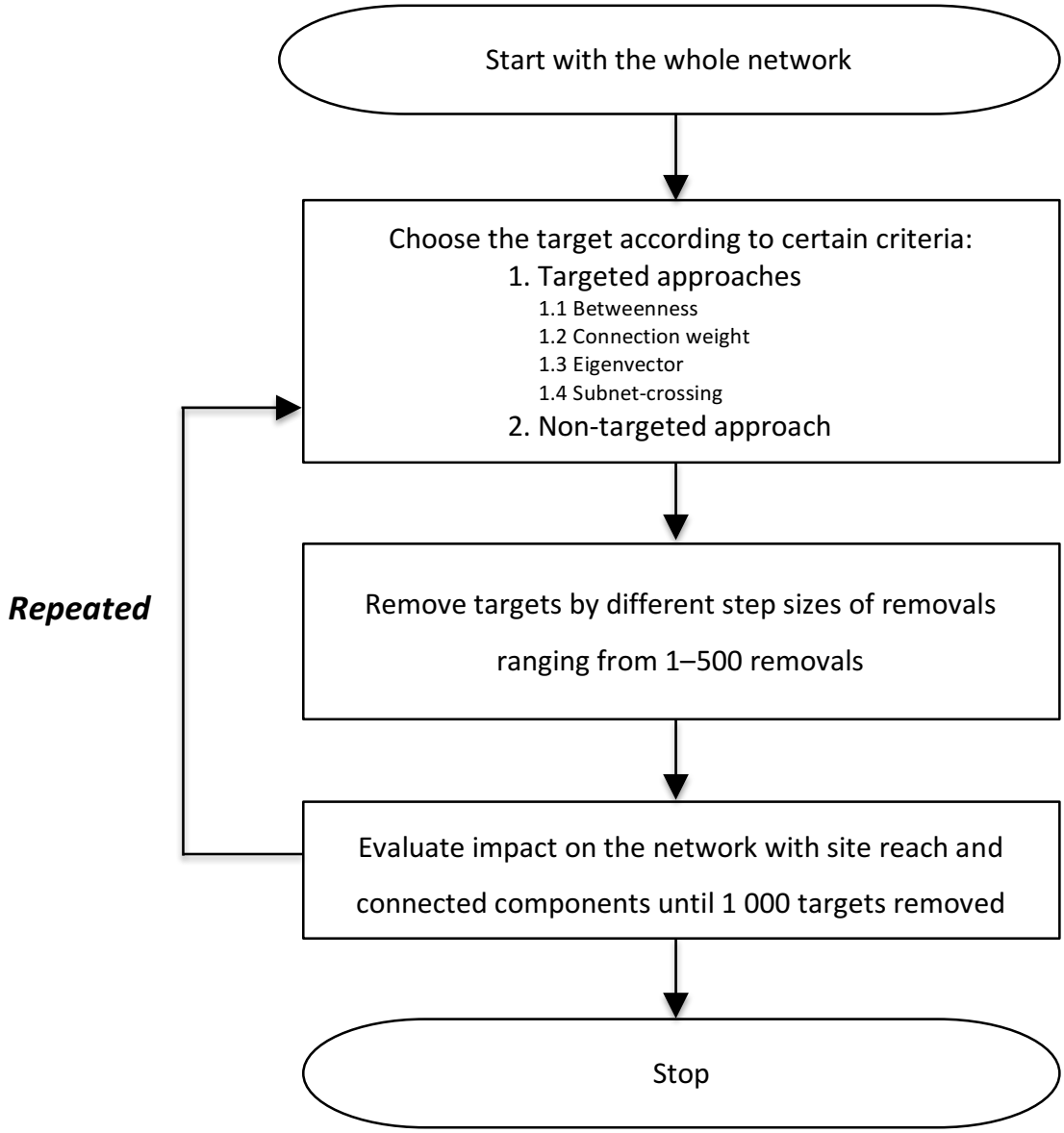


Figure 5.1 Schematic explaining disease-control algorithms with and without targeted approaches for targeted disease surveillance and control for the live shrimp movement network of Thailand (LSMN). The process is stopped when 1 000 targeted connections are removed.

Betweenness. The betweenness centrality value was calculated for connections a_{ij} in the network. The betweenness of a connection a_{ij} was defined by Girvan and Newman (2002) as the number of shortest paths between possible pairs of sites that run along it (not compute repeated connections). The authors proposed the measure to answer which connections in a network were most "between" other pairs of sites. High-risk connections were detected using a betweenness centrality criterion contained in the

edge-betweenness function in the *igraph* package (Brandes, 2001; Csardi and Nepusz, 2006).

Connection weight. With simple degree centrality, a site with a high degree centrality score (high number of connections a_{ij}) is considered more important for disease transmission in a network than other sites with a lower degree centrality (Christley *et al.,* 2005b; Tanaka *et al.,* 2014). Since this research focused on connections (i, j) with a high weight w_{ij}, the connection a_{ij} with highest weight was considered as a target for removal from the network.

Eigenvector centrality. Eigenvector centrality has been found to be worth studying a network where some high degree sites are connected to many low degree sites (Bonacich, 2007). The eigenvector centrality value of a site could be expressed by the matrix form $hV = V\lambda$, where λ = an eigenvalue of the network (a constant), V = an eigenvector corresponding with the eigenvalue λ, and h = a large, sparse, non-symmetric matrix (Lehoucq and Scott, 1996). For the LSMN, the high-risk sites were detected using an eigenvector criterion within the *evcent* function in the *igraph* package (Csardi and Nepusz, 2006). In general, the site with the highest eigenvector centrality value contains lots of connections. In this case, targeted connections a_{ij} belonging to this site were chosen at random.

Subnet-crossing. A subnet (subnetwork) corresponds to a group of sites that are more densely interconnected than between groups. The concept behind the subnet-crossing algorithms-based approach is to remove connections a_{ij} crossing subnets within large networks. Here, the *fastgreedy.community* subnet detection function in the *igraph* package was used (Clauset *et al.,* 2004; Csardi and Nepusz, 2006). Then, connections that link two different subnets were indicated with the command *crossing* (Clauset *et al.,* 2004; Csardi and Nepusz, 2006). Often, subnet-crossing connections were high in number, thus a random method was used to select a target for removal.

Non-targeted approach (as control). In this research, a non-targeted approach was used to compare with the targeted approaches. A connection a_{ij} in the LSMN was targeted simply by using a randomised selection.

In order to demonstrate the effect of the removal of connections on the network structure, after a connection removal the site reach and network components were calculated.

Site reach. The number of sites reachable from others R_i represent potential targets for disease spread (Green *et al.,* 2012). Site reach was calculated from an adjacency matrix of shortest paths (L_{ij}), here computed by Dijkstra's algorithm (Csardi and Nepusz, 2006; Dijkstra, 1959). If there is a path from a focal site i to another j, the matrix L_{ij} gives a positive value. On the other hand, if there is no path from a focal site to other the L_{ij} is defined as infinity. For the LSMN, the adjacency matrix of shortest paths (L_{ij}) was calculated through the *shortest.paths* function in the *igraph* package (Csardi and Nepusz, 2006). Then, the number of sites that reached R_i is equal to the number of positive values of that focal site i as defined in (5.1):

$$R_i = \sum_{j \neq i} [L_{ij} \neq \infty] \tag{5.1}$$

where $[X]$ is the Iverson bracket denoting 1 where condition X is true and 0 otherwise.

The maximum value of R_i across all sites served as an estimate of the worst-case epidemic size, and the mean number of R_i serves as an estimate of typical epidemic size, defined in (5.2) and (5.3), respectively. These measures were used to quantify the susceptibility of the network to an epidemic (Green *et al.,* 2012):

$$\text{Max reach} = \max_i(R_i) \tag{5.2}$$

$$\text{Mean reach} = \frac{\sum R_i}{N} \tag{5.3}$$

where N is the total number of sites in the network.

Connected component. We computed the maximum size of the strongly connected component (SCC), called the giant strongly connected component (GSCC), and a mean size of SCC for the whole network. The GSCC determined the largest number of sites connected by direct connections within the network. The weakly connected component

sizes (WCC) were calculated in the same way as either the maximum or mean size, in which the network was considered as undirected (Pastor-Satorras *et al.,* 2015).

The SCC was implemented by two consecutive depth-first searches, and the WCC was searched via a simple breadth-first algorithm. Both kinds of network components were computed with the *clusters* function (mode "strong" used for SCC, and mode "weak" used for WCC) in the *igraph* package (Csardi and Nepusz, 2006).

In the calculation of the mean connected component size, the contraharmonic mean was used, instead of the arithmetic mean. The contraharmonic mean $L_2(\mathbb{C})$ computes as the arithmetic mean of the squares of the values divided by the arithmetic mean of the values, defined in (5.4, Pahikkala, 2010):

$$L_2(\mathbb{C}) = \frac{\sum_i \mathbb{C}_i^2}{\sum_i \mathbb{C}_i} \tag{5.4}$$

where $\mathbb{C}_i$ is the number of sites in component i.

This form of average behaves better in terms of not being so affected by small isolated groups of sites (Moskovitz, 1933). An example of the arithmetic mean of 3, 30 and 50 is (3+30+50)/3 = 27.7, whereas the contraharmonic mean $L_2(\mathbb{C})$ of those values is (9+900+2500)/(3+30+50) = 41.

5.3.3 Using the disease-control algorithms

Each algorithm was repeated over 1 000 connection removals using the R Programme Environment (R foundation for statistical computing, 2015). All algorithms were set to recalculate the network properties at the start of each iteration. In addition, due to the stochastic nature of all targeted approach algorithms, such algorithms were repeated 1 000 times and average results were presented in the results section.

Some of these algorithms are complex and require recalculation of network properties at each step. For example, a step size of one would naively be expected to work best, but its computation was costly, i.e. needed much computer memory or slowly computed. Thus, as an attempt to minimise its computational complexity, in each

approach, the highest-ranked connections were removed from the network, with the number of connections removed at each step varied from one to 500. For example, 1 000 removals were performed for the step size of one connection removal at a removal, and 100 removals were performed for the step size of 10 connection removals at a removal.

5.3.4 Characterising the targeted connections

All targets were characterised in terms of the connection length (km) as the straight-line distance between two sites (Dubé *et al.,* 2008). Basing on the geographic coordinates of the Thai sub-districts available from Google Earth (2015), the straight-line distance (d) was calculated according to the (4.1) in Chapter 4.

5.4 Results

5.4.1 The number of sites reached in the network

The results of using the five disease-control algorithms with step size one are shown in Figure 5.2. Each algorithm demonstrates different abilities to reduce both the maximum reach and mean reach of sites in the LSMN. Once the total of 1 000 connections a_{ij} was removed, the betweenness-based algorithm performed well for both network measures. The maximum and mean reach were reduced to 50 % after 400 targets from the network. The other algorithms (connection weight-, eigenvector-, subnet crossing-, and random-based) performed relatively poorly. The betweenness-based algorithm still performed well when a step size of more than one removal was applied (results not shown).

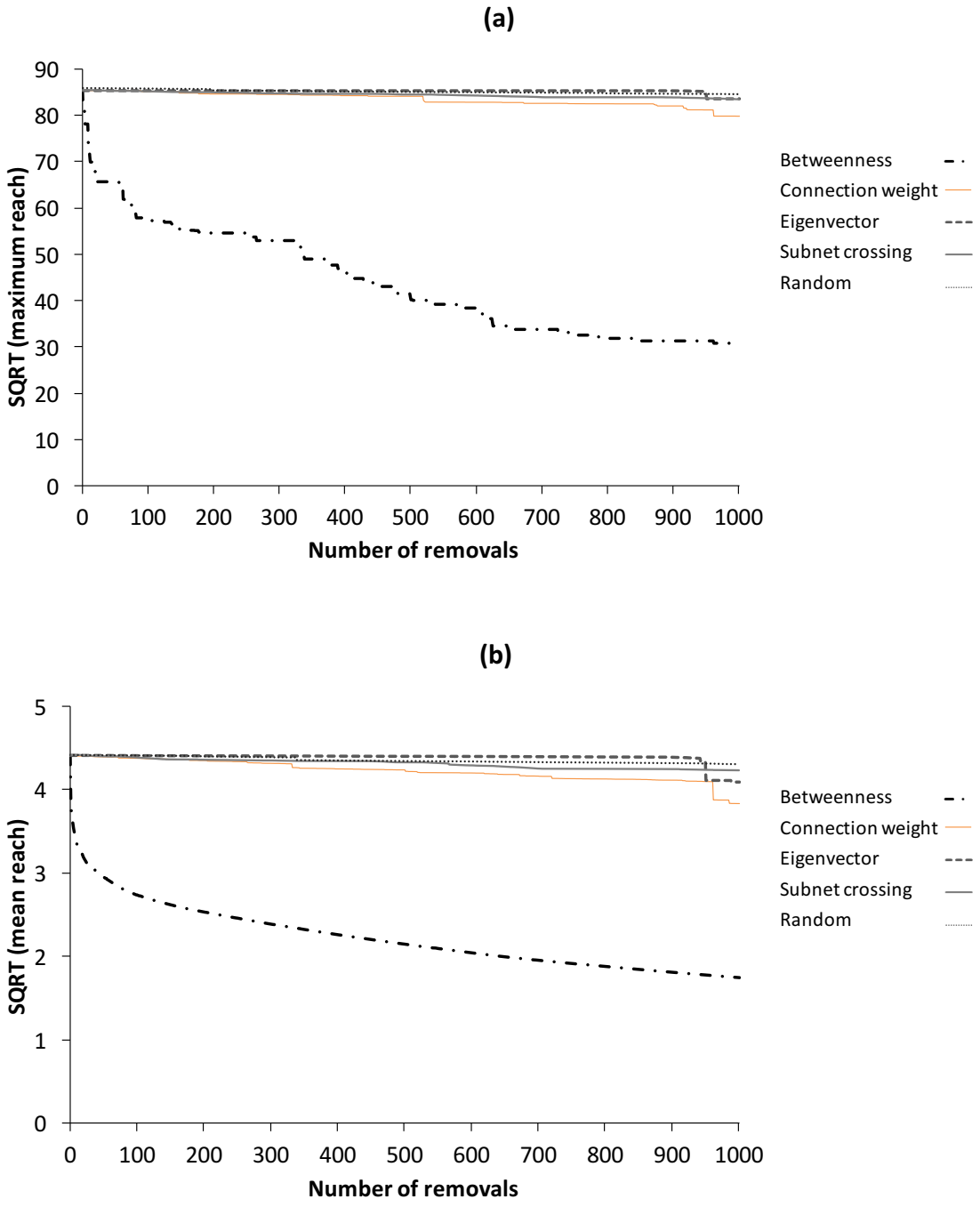


Figure 5.2 Evaluating the disease-control algorithms against the network reachability. The betweenness algorithm performs well for both measures: (a) maximum reach and (b) mean reach. The graphs (y-axis) are plotted on a square-root (SQRT) scale to aid reading.

With regards to using different step sizes, the network snapshot at 250-removed connections with the betweenness algorithm and the random algorithm are presented and compared in Figure 5.3. The figure shows little or no difference in both maximum reach and mean reach whether a step size of one is used or a larger step size.

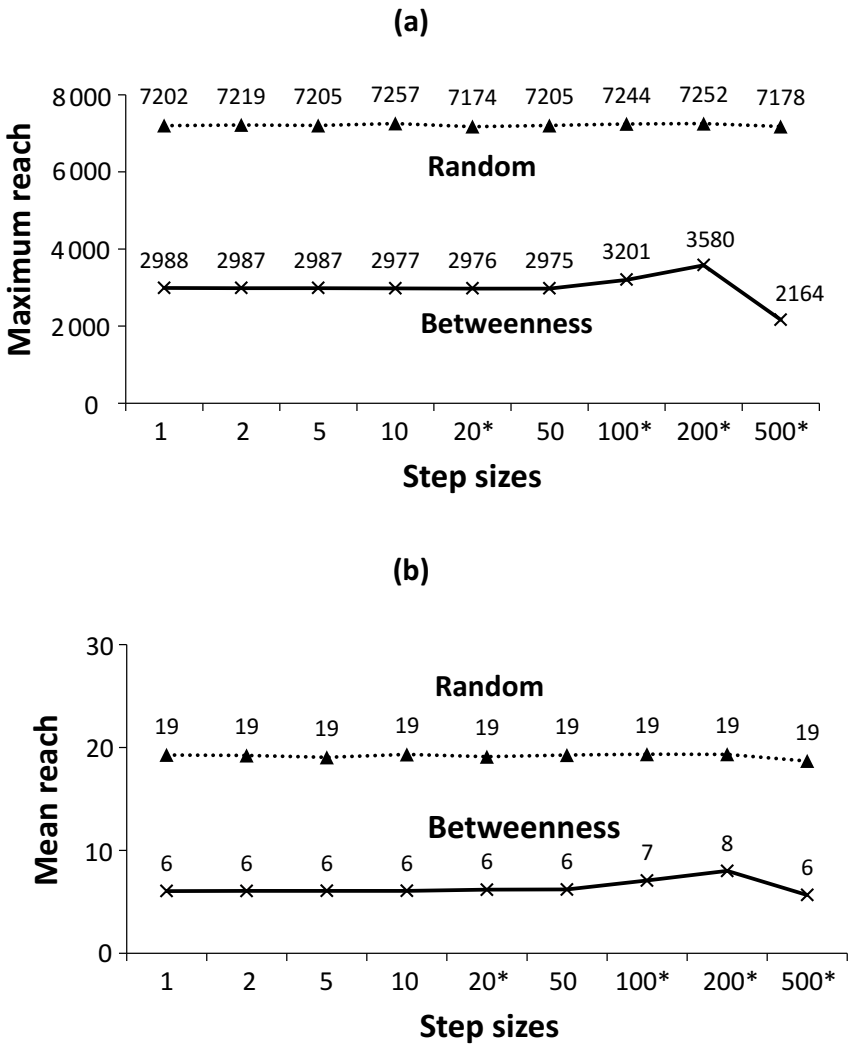


Figure 5.3 Results of different step sizes of the betweenness algorithm compared to the random algorithm at 250 removals. The estimated epizootic sizes were measured by (a) maximum reach measure and (b) mean reach. Note that the results of 20-, 100-, 200-, and 500-step sizes with (*) are 240, 200, 200, and 500 removals, as these do not divide neatly into 250.

5.4.2 Reducing connected components in the network

In terms of reducing connected components, the results of using the five disease-control algorithms with a step size of one are shown in Figure 5.4. The effects of the algorithms on the strongly connected component (SCC) are not shown here, however, since this network measure gave very small values for the GSCC ranging from one to five (with a mean SCC of around one), whereas the LSMN had a giant weakly connected component (GWCC) composed of almost all sites (13 786 of 13 801 sites; mean WCC = 13 771). Thus, we focused on the WCC in both the GWCC and mean WCC.

With the total of 1 000 connections removed, the subnet-crossing algorithm performed well to reduce the GWCC and mean WCC in the LSMN. The GWCC slightly decreased from 13 786 to 13 509, while the mean WCC decreased from 13 771 to 13 490. The other algorithms (betweenness-, connection weight-, eigenvector-, and random-based) performed relatively less well in terms of reducing the GWCC and the mean WCC. The results of other step sizes also demonstrated the good performance of the subset-crossing algorithms (results not shown).

Interestingly, the betweenness algorithm had a very small impact in respect to reducing both the GWCC and mean WCC in the LSMN. This contrasts with the earlier result in the Section 5.4.1. This is presumably a result of the GWCC being generated by a large number of sites, and lots of connections in the GWCC showing similar betweenness centrality scores.

Figure 5.5 shows the network snapshots at 250 connections removed. It can be seen that, overall, with the subnet-crossing and random algorithms there is only a small difference in both GWCC and mean WCC whether using the 1-removal step size or bigger step sizes.

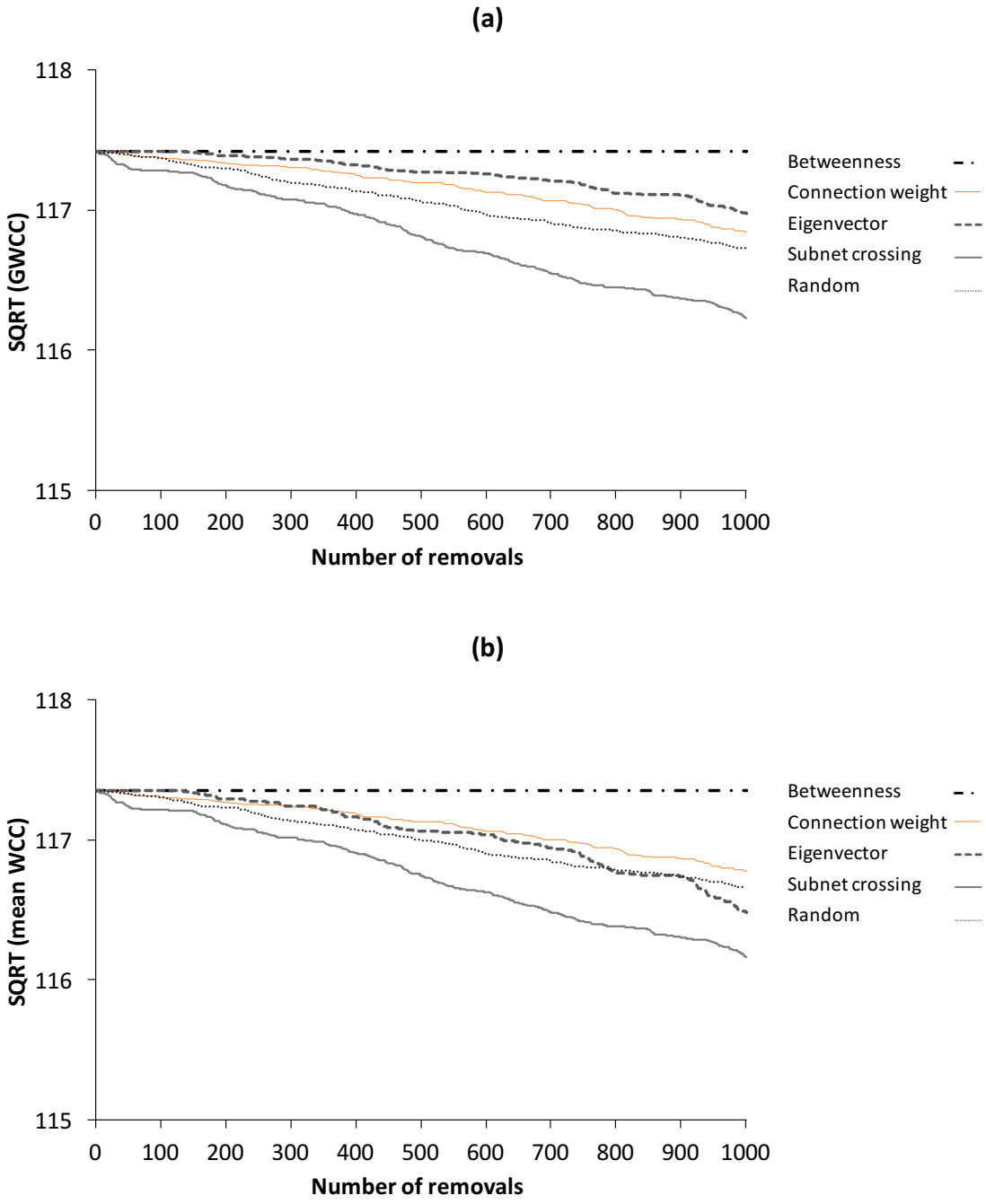


Figure 5.4 Evaluating the disease-control algorithms against the weakly connected components (WCC). The subnet-crossing algorithm performed well for both measures: (a) GWCC and (b) mean WCC. The graphs (y-axis) are plotted on a square-root (SQRT) scale to aid reading. Note that the y-axis does not start at zero.

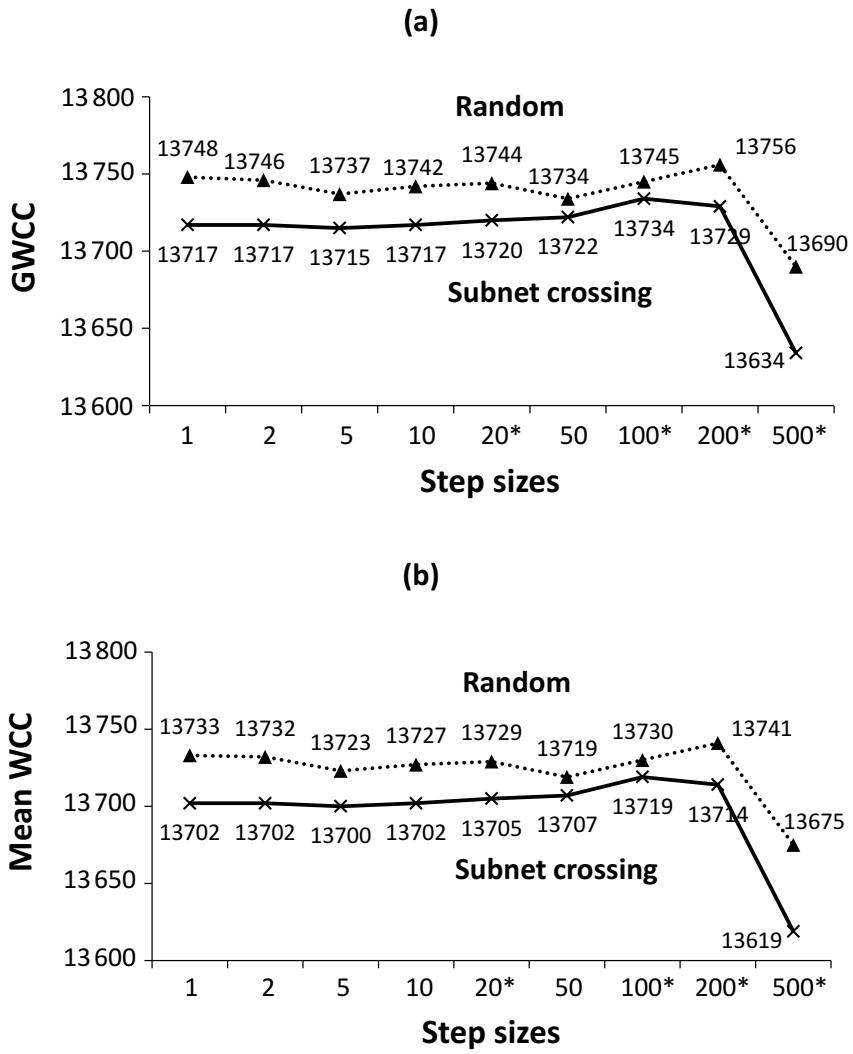


Figure 5.5 Results of different step sizes when comparing the subnet-crossing algorithm and random algorithm at 250 removals. The estimated epizootic sizes were measured by (a) GWCC and (b) mean WCC. Note that the y-axis does not start at zero, and the results of 20-, 100-, 200-, and 500-step sizes with (*) are 240, 200, 200, and 500 removals, as these do not divide neatly into 250.

5.4.3 The characteristics of targeted connections

The targeted connections from the disease-control algorithms above are denoted as the high-risk connections for disease spread in the LSMN. In order to characterise these high-risk connections, all targets from the betweenness and subnet-crossing algorithms were characterised in terms of connection lengths (km). We found that, when using the betweenness-based algorithm, the geographic distances of the targeted connections were (on average) longer, compared to the mean connection length in the whole connections (around 200 km). When using the subnet-crossing algorithm, however, the

geographic distances of the targeted connections were (on average) shorter. With the betweenness algorithm, the mean connection length was 271 km (SD = 281), compared to 170 km (SD = 180) with the subnet crossing-based

Moreover, the types of source-destination pairs for the 1 000 removals, are quite different between using the two algorithms. Tables 5.1 shows that, based on the betweenness algorithm, most targeted connections join other sites within the same type of site, i.e. seed-producing sites. Table 5.2 demonstrates that a high number of connections between different site types, i.e. from seed-producing sites to ongrowing sites, are the priority of the subnet-crossing algorithm.

Table 5.1 Source and destination site types of the top 1 000 removals from the betweenness-based algorithm shown by probabilities (in percentages) in the total number of removals, and in the whole connections (values in brackets).

Betweenness algorithm		Destination	
		Seed-producing site	Ongrowing site
Source	Seed-producing site	99.3 % (2.9 %)	0 %
	Ongrowing site	7 % (0 %)	0 %

Table 5.2 Source and destination site types of the top 1 000 removals from the subnet-crossing based algorithm shown by probabilities (in percentages) in the total number of removals, and in the whole connections (values in brackets).

Subnet-crossing algorithm		Destination	
		Seed-producing site	Ongrowing site
Source	Seed-producing site	7.5 % (0.2 %)	92.1 % (2.7 %)
	Ongrowing site	0.1 % (0 %)	0.3 % (0 %)

5.5 Discussion

Several approaches based on real network models of animal movements have been widely proposed for examining the potential for disease transmission and for designing control strategies. A recent example is the network of livestock movements in New

Zealand, which shows that removing a small proportion of sites decreases the estimated epizootic size (Marquetoux *et al.,* 2016), whereas the network model approach has now been implemented in shrimp farming sector in this research (Chapter 4 "Analysis of the network structure of the live shrimp movements relevant to AHPND epizootic").

As the main result of Chapter 4, the live shrimp movement network of Thailand (LSMN) displays small-world and scale-free properties. Its structure demonstrates that random connection removal tends to become highly inefficient and costly as a control strategy (Dezső and Barabási, 2002; Eubank *et al.,* 2004; May and Lloyd, 2001; Pastor-Satorras and Vespignani, 2002b). Hence, five disease-control algorithms were evaluated in our research and their effectiveness compared on the real structure of the LSMN. In order to limit the amount of surveillance resources, each algorithm is specified to allow a maximum of 1 000 removals, accounting for 3 % of all site-to-site connections a_{ij} in the LSMN. The optimal algorithm will provide a smaller scale of estimated potential epizootic indicated by two network measures, i.e. site reach (maximum and mean reach) and connected components (SCC and WCC). Essentially, maximum reach is almost like a compromise between GSCC and GWCC, and in some ways more epizootiologically useful.

In terms of site reach, the betweenness-based algorithm had high performance in reducing the susceptibility of our studied network to a disease epizootic with few removals (this is network structive dependent; since if the structure of the network changes, other centrality measures may become more effective). The removal of the 3 % of connections targeted by the betweenness criterion strongly corresponds to a decrease in site reach of at least 80 % in the LSMN. Girvan and Newman (2002) defined the betweenness centrality of a connection as the number of shortest paths between pairs of sites that passes through it. Connection removals with high betweenness centrality scores mean that the preferential pathways of many pairs of sites disappear from the network. Consequently, the site reach in the LSMN becomes smaller.

Considering the LSMN data, most of those high-betweenness connections were the connections between seed-producing sites, which played an important role in distributing shrimp seed (with their pathogens) to many ongrowing sites in the network.

It implies that further connections can occur shortly after the first connection, corresponding to the life cycle of shrimp (Quispe *et al.,* 2016). For example, nursery sites rear the seed from nauplius until postlarval stage with a short period of 20 days before selling the production to the ongrowing sites (FAO, 2014). Given this short period of shrimp seed production, the operation of the disease surveillance and control measures along these high-betweenness connections (e.g. tests for shrimp diseases) needs to be fast to achieve an early detection of disease outbreaks.

Although the concepts of reachability and WCC are not different, in our network study the two sets of results are different. In terms of WCC, targeting based on the subnet-crossing algorithm was suitable for reducing the GWCCs and the mean size of WCCs in the LSMN. It can be presumed that the component-based measures are being tested against the measures themselves. Thus, it is likely to be rather efficient in that case. As seen, however, given that with 1 000 targeted connections removed there is only a small decrease of GWCCs and mean WCCs, the component-based measure may require a higher number of targeted connections if it is to reduce a high estimated epizootic size. The need for the removal of more targeted connections would result in a higher cost of intervention in terms of disease surveillance and controls, and thus the subnet-crossing algorithm may not be a suitable strategy.

Although the GSCC was chosen as an indicator of maximum potential epizootic size in the network analysis of Marquetoux *et al.* (2016), it is not a good measure in the LSMN. Marquetoux *et al.* analysed the livestock movements from site to site in New Zealand, with a large number of sites contained in its GSCC (accounting for 79 % of the total number of sites; 129 of 164). In contrast, the GSCC in the LSMN only included 0.04 % of all sites. The study of epidemics in directed networks, however, often uses the GSCC in order to determine an estimated epidemic size within the networks, such as with human social networks (Eubank *et al.,* 2004), and animal contact networks (Kiss *et al.,* 2006; Rautureau *et al.,* 2011). One major advantage of the GSCC measure was proposed by Volkova *et al.* (2010) and Kao *et al.* (2007). They described that the GSCC approached the lower bound of the final epidemic size in the absence of control strategies, as with the late development of pathogen diagnosis in the case of AHPND. Thus, if the structure of LSMN possesses a large strongly connected component, the GSCC may become

compelling in designing targeted disease surveillance and controls for Thai shrimp farming.

Although, the use of a larger step size had little or no effect on the reduction of estimated epidemic sizes, whether measured by site reach and WCC, it gave an important advantage, allowing fast computations for a large-complex network like the LSMN. A few fast algorithms for computing network centrality have already been developed (Bader *et al.,* 2007; Brandes, 2001; Madduri *et al.,* 2009; Shi and Zhang, 2011). It appears, however, that most algorithms in the literature use one-step removals. The study of Green *et al.* (2012), for example, conducted one-step removal to evaluate the effects of targeted removal for the directed network of fish farms. By removing more than one connection, a closely related example is presented in Natale *et al*. (2009). They specified the number of targets before simulating the epidemic network models such as the 1 % of sites with the highest centrality scores. As well as allowing faster computations, the use of bigger step sizes (more than one removal) may help to design disease-control algorithms.

In summary, in an attempt to reduce the spread of diseases in the LSMN by removing a limited number of connections between sites, the targeted strategies performed relatively well compared to a non-targeted approach. This research was done on the single network layer of live shrimp movement. Different results might arise, if local contact or water-borne contact were included. This complex mode of disease transmission leads us to use epizootic network models into AHPND, as addressed in the following chapter.

5.6 References

Bader, D.A., Kintali, S., Madduri, K. and Mihail, M. (2007) Approximating betweenness centrality. In: A. Bonato and F.R.K. Chung, eds. *International Workshop on Algorithms and Models for the Web-Graph.* Germany: Springer, pp. 124–137.

Bohm, M., Hutchings, M.R. and White, P.C. (2009) Contact networks in a wildlife-livestock host community: identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. *PloS One*, 4 (4), e5016. Available: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0005016 [Accessed: 29 April 2009].

Bonacich, P. (2007) Some unique properties of eigenvector centrality. *Social Networks,* 29 (4), pp. 555–564.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff, M. (2005) Disease and health management in Asian aquaculture. *Veterinary Parasitology,* 132 (3–4), pp. 249–272.

Brandes, U. (2001) A faster algorithm for betweenness centrality*. *Journal of Mathematical Sociology*, 25 (2), pp. 163–177.

Britton, T., Deijfen, M. and Liljeros, F. (2011) A weighted configuration model and inhomogeneous epidemics. *Journal of Statistical Physics*, 145 (5), pp. 1368–1384.

Christley, R.M., Pinchbeck, G.L., Bowers, R.G., Clancy, D., French, N.P., Bennett, R. and Turner, J. (2005) Infection in social networks: using network analysis to identify high-risk individuals. *American Journal of Epidemiology*, 162 (10), pp. 1024–1031.

Clauset, A., Newman, M.E. and Moore, C. (2004) Finding community structure in very large networks. *Physical Review E*, 70 (6).

Csardi, G. and Nepusz, T. (2006) The igraph software package for complex network research. *InterJournal*, Complex Systems (http://igraph.org), pp. 1695.

Dezső, Z. and Barabási, A. (2002) Halting viruses in scale-free networks. *Physical Review E,* 65 (5), pp. 055103.

Dijkstra, E.W. (1959) A note on two problems in connexion with graphs. *Numerische Mathematik*, 1 (1), pp. 269–271.

Draief, M., Ganesh, A. and Massoulié, L. (2008) Thresholds for virus spread on networks. *The Annals of Applied Probability*, 18 (2), pp. 359–378.

Dubé, C., Ribble, C., Kelton, D. and McNab, B. (2008) Comparing network analysis measures to determine potential epidemic size of highly contagious exotic diseases in fragmented monthly networks of dairy cattle movements in Ontario, Canada. *Transboundary and Emerging Diseases*, 55 (9–10), pp. 382–392.

Eubank, S., Guclu, H., Kumar, V.A., Marathe, M.V., Srinivasan, A., Toroczkai, Z. and Wang, N. (2004) Modelling disease outbreaks in realistic urban social networks. *Nature*, 429 (6988), pp. 180–184.

FAO (2014) *Cultured aquatic species information programme Penaeus vannamei (Boone, 1931).* Rome: FAO. Available: http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en [Accessed: 29 April 2017].

Flegel, T.W., Lightner, D.V., Lo, C.F. and Owens, L. (2008) Shrimp disease control: past, present and future. In: M.G. Bondad-Reantaso, C.V. Mohan, M. Crumlish and R.P. Subasinghe, ed. *Diseases in Asian Aquaculture.* Sri Lanka, 25th to 28th October 2005. Manila, Philippines: Asian Fisheries Society, pp. 355–378.

Fournie, G., Guitian, J., Desvaux, S., Cuong, V.C., Dung do, H., Pfeiffer, D.U., Mangtani, P. and Ghani, A.C. (2013) Interventions for avian influenza A (H5N1) risk management in live bird market networks. *Proceedings of the National Academy of Sciences of the United States of America*, 110 (22), pp. 9177–9182.

Girvan, M. and Newman, M.E. (2002) Community structure in social and biological networks. *Proceedings of the National Academy of Sciences of the United States of America*, 99 (12), pp. 7821–7826.

Google Earth (2015) *Geographic coordinates of the sub-districts across Thailand.* Google Inc. Available: http://www.google.com/earth/index.html [Accessed: 3 March 2015].

Green, D.M., Werkman, M. and Munro, L.A. (2012) The potential for targeted surveillance of live fish movements in Scotland. *Journal of Fish Diseases*, 35 (1), pp. 29–37.

Green, D.M., Gregory, A. and Munro, L.A. (2009) Small- and large-scale network structure of live fish movements in Scotland. *Preventive Veterinary Medicine*, 91 (2–4), pp. 261–269.

Herrera, J.L., Srinivasan, R., Brownstein, J.S., Galvani, A.P. and Meyers, L.A. (2016) Disease surveillance on complex social networks. *PLoS Comput Biol*, 12 (7), e1004928. Available: http://journals.plos.org/ploscompbiol/article/file?id=10.1371/journal.pcbi.1004928&type=printable [Accessed: 30 January 2017].

Kao, R.R., Green, D.M., Johnson, J. and Kiss, I.Z. (2007) Disease dynamics over very different time-scales: foot-and-mouth disease and scrapie on the network of livestock movements in the UK. *Journal of the Royal Society, Interface / the Royal Society*, 4 (16), pp. 907–916.

Kiss, I.Z., Green, D.M. and Kao, R.R. (2006) The network of sheep movements within Great Britain: Network properties and their implications for infectious disease spread. *Journal of the Royal Society, Interface / the Royal Society*, 3 (10), pp. 669–677.

Kongkumnerd, J. (2014) *The current status of EMS outbreak in Thai marine shrimp farming.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.shrimpaqua.com/download/EMS/situation-EMS.pdf [Accessed: 10 December 2014].

Lehoucq, R. and Scott, J. (1996) An evaluation of software for computing eigenvalues of sparse nonsymmetric matrices. *Preprint MCS-P547*, 1195, pp. 5.

Lentz, H.H., Koher, A., Hövel, P., Gethmann, J., Sauter-Louis, C., Selhorst, T. and Conraths, F.J. (2016) Disease spread through animal movements: a static and temporal network analysis of pig trade in Germany. *PloS One*, 11 (5), e0155196. Available: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0155196&type=printable [Accessed: 12 January 2017].

Lightner, D.V. (2005) Biosecurity in shrimp farming: pathogen exclusion through use of SPF stock and routine surveillance. *Journal of the World Aquaculture Society*, 36 (3), pp. 229–248.

Madduri, K., Ediger, D., Jiang, K., Bader, D.A. and Chavarria-Miranda, D. (2009) A faster parallel algorithm and efficient multithreaded implementations for evaluating betweenness centrality on massive datasets. *Parallel & Distributed Processing*, *2009. IPDPS 2009. IEEE International Symposium On.* IEEE, pp. 1–8.

Marquetoux, N., Stevenson, M.A., Wilson, P., Ridler, A. and Heuer, C. (2016) Using social network analysis to inform disease control interventions. *Preventive Veterinary Medicine*, 126, pp. 94–104.

May, R.M. and Lloyd, A.L. (2001) Infection dynamics on scale-free networks. *Physical Review E*, 64 (6), pp. 066112.

Moskovitz, D. (1933) An alignment chart for various means. *The American Mathematical Monthly*, 40 (10), pp. 592–596.

Natale, F., Giovannini, A., Savini, L., Palma, D., Possenti, L., Fiore, G. and Calistri, P. (2009) Network analysis of Italian cattle trade patterns and evaluation of risks for potential disease spread. *Preventive Veterinary Medicine*, 92 (4), pp. 341–350.

Newman, M.E. and Park, J. (2003) Why social networks are different from other types of networks. *Physical Review E*, 68 (3), pp. 036122.

Pahikkala, J. (2010) On contraharmonic mean and Pythagorean triples. *Elemente Der Mathematik*, 65 (2), pp. 62–67.

Pastor-Satorras, R., Castellano, C., Van Mieghem, P. and Vespignani, A. (2015) Epidemic processes in complex networks. *Reviews of Modern Physics*, 87 (3), pp. 925.

Pastor-Satorras, R. and Vespignani, A. (2002) Immunization of complex networks. *Physical Review E*, 65 (3), pp. 036104.

Planning Division (2016) *Annual plans 2016.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.fisheries.go.th/planning/index.php?option=com_content&view=article&id=68:2010-11-30-09-28-07&catid=9:2009-09-12-10-16-07&Itemid=5 [Accessed: 23 September 2016].

Portley, N. Portley, N. (2016) *SFP report on the shrimp sector: Asian farmed shrimp trade and sustainability.* Sustainable Fisheries Partnership Foundation. Available: www.sustainablefish.org [Accessed: 21 September 2016].

Quispe, R.L., Justino, E.B., Vieira, F.N., Jaramillo, M.L., Rosa, R.D. and Perazzolo, L.M. (2016) Transcriptional profiling of immune-related genes in Pacific white shrimp (*Litopenaeus vannamei*) during ontogenesis. *Fish & Shellfish Immunology*, 58, pp. 103–107.

R foundation for statistical computing (2015) *R: a language and environment for statistical computing.* Available: https://www.R-project.org/ [Accessed: 5 December 2015].

Rautureau, S., Dufour, B. and Durand, B. (2011) Vulnerability of animal trade networks to the spread of infectious diseases: a methodological approach applied to evaluation and emergency control strategies in cattle, France, 2005. *Transboundary and Emerging Diseases*, 58 (2), pp. 110–120.

Shi, Z. and Zhang, B. (2011) Fast network centrality analysis using GPUs. *BMC Bioinformatics,* 12 (1), pp. 1.

Songsanjinda, P. (2015) Crisis of Thai shrimp production. Bangkok, 16th to 20 th August 2015. Thailand Research Expo 2015: National Research Council of Thailand, pp. 1–35.

Tanaka, G., Urabe, C. and Aihara, K. (2014) Random and targeted interventions for epidemic control in metapopulation models. *Scientific Reports*, 4, pp. 1–8.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W. and Itsathitphaisarn, O. (2016) Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 452, pp. 69–87.

Volkova, V.V., Howey, R., Savill, N.J. and Woolhouse, M.E. (2010) Sheep movement networks and the transmission of infectious diseases. *PLoS One*, 5 (6), e11185. Available: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0011185&type=printable [Accessed: 23 April 2015].

Woolhouse, M.E., Dye, C., Etard, J.F., Smith, T., Charlwood, J.D., Garnett, G.P., Hagan, P., Hii, J.L., Ndhlovu, P.D., Quinnell, R.J., Watts, C.H., Chandiwana, S.K. and Anderson, R.M. (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America*, 94 (1), pp. 338–342.

Woolhouse, M.E., Shaw, D.J., Matthews, L., Liu, W.C., Mellor, D.J. and Thomas, M.R. (2005) Epidemiological implications of the contact network structure for cattle farms and the 20-80 rule. *Biology Letters,* 1 (3), pp. 350–352.

Zhang, H., Zhang, J., Zhou, C., Small, M. and Wang, B. (2010) Hub nodes inhibit the outbreak of epidemic under voluntary vaccination. *New Journal of Physics,* 12 (2), pp. 023015.

Chapter 6 - Epizootic disease modelling in farmed shrimp using compartmental epizootic network-based simulations

N. Saleetid; D.M. Green; F.J. Murray

Preface

The dynamics of an acute hepatopancreatic necrosis disease (AHPND) epizootic are modelled in order to acquire a better understanding of its epizoology and to examine the effect of two potential pathways for AHPND epizootics (long-distance and local spread) within the Thai shrimp farming industry. The live shrimp movement data (LSMN) used in Chapters 4 and 5 also serves as a major data source in this chapter, as well as the survey data collected in Chapter 3 provides information on incubation and fallow periods for AHPND, and a chance of infection via local spread. The *R* codes for the model simulation were written by the author, with input from D.M. Green. The chapter is designed in the format of publications to help us prepare a potential journal paper, and thus, nature of AHPND is described again in this introduction section.

Impact statement

Although testing for infectious diseases before movements of farmed shrimp is a well-established principle in Thailand, in some cases diseases may not be detected. This makes it more difficult to design disease controls, and provides a motivation for modelling AHPND epizootic dynamics, especially since outbreaks of AHPND have caused massive shrimp production losses in Thailand. The purpose of this model is to evaluate the effect of long-distance and local spread relative to AHPND epizootics in Thailand and to suggest mitigation measures. Poor biosecurity practices (e.g. water discharge and low efficiency of disease screening of live shrimp movements) are therefore assumed in the model. The results of the model could emphasise the importance of biosecurity in stopping the spread of disease across shrimp farming sites.

Chapter 6 - Epizootic disease modelling in farmed shrimp using compartmental epizootic network-based simulations

6.1 Abstract

Since the first outbreak of acute hepatopancreatic necrosis disease (AHPND) in Thailand in 2011/2012, there has been little in the way of epizootiological study of its epizootic dynamics. Here, therefore, an $SEIRS$ compartmental, individual-based epizootic model is used to explore potential AHPND spread within the real live shrimp movement network of Thailand (LSMN) in a 13-month period. In addition, the model examines the effect of two potential pathways for the spread of AHPND: long-distance transmission via live shrimp movements and local transmission (e.g. shared water bodies for farming and sites located in close proximity). The results reveal that AHPND epizootics in Thailand are more likely to occur during hot and rainy seasons (between April and August). The inter-province movements are potentially a major source of AHPND epizootics. With lower rates of long-distance transmission in the model ($\beta_{long} < 1$), the number of infected sites was smaller. In the model, local transmission alone (β_{long} was 0 and β_{local} was 0.002) was responsible for very small epizootic sizes. The models therefore suggest that the AHPND epizootic dynamics in Thai shrimp farming can be minimised by enhancing biosecurity measures in respect to live shrimp movements, and interventions to reduce local spread should be considered as part of current disease surveillance and control measures.

6.2 Introduction

Acute hepatopancreatic necrosis disease (AHPND) is a new epizootic bacterial disease in farmed shrimp, first identified by Lightner *et al.* (2012). It causes severe mortality of shrimp of up to 100 % in infected sites. Diseased shrimp have obvious visible signs of AHPND, i.e. empty gut and stomach, and pale or atrophied hepatopancreas. Most sites infected by AHPND have been found to have increased shrimp mortality within 10–35 days post stocking of postlarvae (FAO, 2013; NACA, 2014).

The scale of bacterial epizootics in shrimp farming is not less than of that viral epizootic. AHPND has caused huge global economic losses due to the fact that its epizootics occur throughout the major producing countries, i.e. China, Vietnam, Thailand, Malaysia, Mexico and the Philippines (Dabu *et al.,* 2015; Eduardo and Mohan, 2012; Nunan *et al.,* 2014). Since AHPND first appeared in Southern China in 2009, it still persists in the shrimp farming sector (NACA, 2017; Zorriehzahra and Banaederakhshan, 2015). Importantly, the emergence of AHPND is encouraging shrimp stakeholders throughout the world to undergo a dramatic transition in farming practices (Eduardo and Mohan, 2012; FAO, 2013). The era of the AHPND epizootic is therefore well recognised by many shrimp stakeholders as being either a crisis for shrimp aquaculture or the beginning of much-needed improvements in shrimp farming

Movements of infected shrimp are a major route for AHPND transmission (OIE, 2013a; Tran *et al.,* 2013b). Alternatively, the specific strains of *Vibrio parahaemolyticus*, the causative agent of AHPND, can spread via the live feeds commonly used in seed-producing sites such as polychaete worms and bivalves (Ushijima, 2014). The case–control study of Boonyawiwat *et al.* (2016) also found that the source of shrimp seed related to the occurrence of AHPND at site level. Shrimp farmers have also observed that mass mortalities of shrimp have occurred in AHPND-infected sites have occurred even in newly prepared ponds (FAO, 2013). According to this evidence, many farmers suspect that seed-producing sites generate long-distance transmission of AHPND, as described above in Chapter 3.

Disease outbreaks via physical proximity of sites and their hydrological connectivity are classified as local transmission with non-contact network spread. This follows from common activities such as sharing of water supplies (Galappaththi and Berkes, 2015; Smith, 1999) and discharging water from cultured ponds (Muniesa et al., 2015; Patil et al., 2002; Vandergeest et al., 1999). Importantly, pond water and soil serve as a good medium for the causative agent of AHPND to grow (Chonsin et al., 2016; Kongkumnerd, 2014; Tran et al., 2013a). Hence, there is likely to be more than one pathway influencing the dynamics of AHPND epizootic dynamics, making it more difficult for regulators to design disease controls.

In many cases, efficient disease controls can be achieved by using epidemic and network modelling approaches. Epidemic network models attempt to capture the epidemic dynamics in a population in which real connections exist between individuals and allow potential disease transmission (Keeling and Eames, 2005). In human diseases, the SIR (susceptible-infectious-recovered) epidemic model has been recently modelled for the spread of Ebola in Liberia (Rizzo *et al.,* 2016). Epidemic models for the spread of foot-and-mouth disease in the real networks of livestock movements (Durand and Mahul, 2000; Rvachev and Longini, 1985) have also provided many new insights and epizootiological tools that can be applied to other farmed animal diseases, such as carp (Taylor *et al.,* 2011) and salmon (Werkman *et al.,* 2011). Importantly, these models have resulted in the development of effective disease prevention and control methods. An AHPND model therefore represents a very powerful tool to assist in the development of suitable measures to prevent large AHPND outbreaks.

An improved understanding of the dynamics of the AHPND epizootic among sites is required in order to support disease prevention and control. In addition, although we know much about the infection at site level, this does not mean that we know the epizootic at country scale. Thus, we have developed an $SEIRS$ compartmental, individual-based epizootic model in this research to: (1) examine AHPND epizootic dynamics in Thai shrimp farming sites, and (2) analyse the effect of long-distance and local transmission, including the effect of established biosecurity measures on live shrimp movements. This is the first epizootic modelling of bacterial disease based on the real network of live shrimp movements.

6.3 Materials and method

6.3.1 The live shrimp movement network (LSMN)

A traceability system for farmed shrimp developed by the Thailand Department of Fisheries provided access to the records of daily live shrimp movements among 13 801 sites N_{total} (seed-producing sites = 804, and ongrowing sites = 12 997) in the 396-day period from 1 March 2013 to 31 March 2014. Over this period there had been onsets of AHPND and other shrimp diseases such as white spot disease, yellowhead disease, and

taura syndrome (NACA, 2017). The records contained a source site of shrimp, a destination site of shrimp, a movement date, and a quantity of shrimp seed, useful in determining the country-wide dynamics of infectious diseases. Multiple records representing a batch moved within a day were combined as one record. In total, our model was based on *circa* 74 000 records.

In this research, an adjacency matrix h_{ijt} of size $N_{total}\times N_{total}\times$ 396 days was used to represent the LSMN mathematically such that the timing was an actual connection date from site i to site j on day t. The h_{ijt} was the element-wise multiplication of a matrix a_{ijt} by matrix w_{ijt} on day t. Element a_{ijt} took the value 1 if there was a connection from a site i to a site j implying a possible path for disease transmission from site i to j, and a_{ijt} took the value 0 otherwise. About 300 self-loop connections (a_{iit} = 1) were removed from the analysis because these self-loops did not contribute to further spread of diseases (Britton *et al.,* 2011; Draief *et al.,* 2008).

Unlike in Chapters 4 and 5, where the weight w_{ijt} denoted the frequency of connections, each element addressed in this part was the number of shrimp seed in the connections between the same pairs of sites (in units of a billion shrimp) under an assumption that a connection with a larger number of infected shrimp would offer more risk of pathogen transmission than connections with fewer infected shrimp.

6.3.2 Local contacts between shrimp farming sites

Either physical proximity of sites or their hydrological connectivity was assumed to pose a risk of local transmission for AHPND. The sub-district area was used to determine the location of sites (more detail of the Thai local governmental units was given by Nagai *et al.,* 2008). An undirected block matrix b_{ij} of size $N_{total}\times N_{total}$ determined the local connections between sites in the same sub-district (not including self-loops). The model assumed that the local non-network spread within sub-districts was of a mixing random type: each site could infect all members, excepting for sub-districts with only one site. A site located in a sub-district with a higher number of infected and exposed sites would presumably have more chance of becoming infected than others.

The frequency of the number of site members for the 748 sub-districts relevant to the LSMN is illustrated in Figure 6.1. 75 % of the sub-districts had equal to or less than 21 site members (i.e. the 75th percentile for 748 sub-districts = 21).

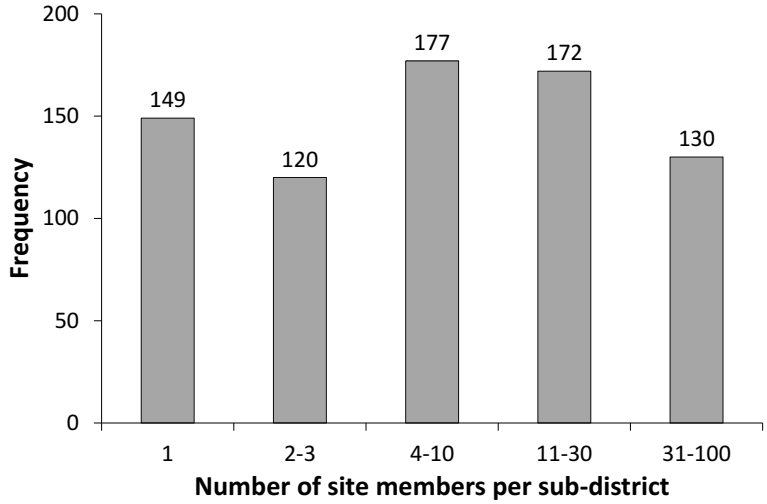


Figure 6.1 Frequency of number of site members per sub-district. Most sub-districts had ≤ 21 site members (the 75th percentile for 748 sub-districts = 21).

6.3.3 An $SEIRS$ compartmental, individual-based epizootic model for acute hepatopancreatic necrosis disease (AHPND)

An $SEIRS$ compartmental, individual-based epizootic model was constructed to explore AHPND transmission among the Thai shrimp farming sites. Two constructs were involved in the model: (1) all shrimp farming sites were taken from the LSMN, i.e. seed-producing sites and ongrowing sites, and (2) the directed network of live shrimp movements (Section 6.3.1) and the undirected network of local spread (Section 6.3.2) with the former encompassing two scenarios: scenario A (both intra- and inter-province movements) and scenario B (intra-province movements alone).

All sites were classified into one of four states: susceptible (S), exposed (E), infected (I) and removed (R); their states varied over time. The 'S' state was not infected, but was stocked and could be infected. The 'E' state was infected but without either visible signs of AHPND or unusual shrimp mortality. Sites at 'E' state, however, could pose a risk to other sites. The next state, 'I', was infected and had increased shrimp mortality with clinical signs of AHPND. Similar to the exposed sites, the 'I' could pose a risk to others.

The final state 'R' was fallow, meaning that the site was not stocked and in use for a shrimp production. Accordingly, the 'R' site could not be infected.

To simulate an epizootic, the model building consisted of the following three steps:

6.3.3.1 Initialisation of model simulation

Epizootics were started in each month to explore the seasonal effects. During each simulation, the initial infected sites (seeds) with a finite fraction of seeds at 0.02 were selected at random from the 804 seed-producing sites in the LSMN (= 16 seeds). All other sites were assumed to be susceptible. Hence, a vector E_{it} represented the state of site i at initialisation $t = t_0$: 0 for susceptible, infected and removed, and 1 for exposed.

Model parameters were estimated based on the details of the spread of AHPND and Thai shrimp farming activities reported in the literature, and the surveyed data that was collected in the Chapter 3. The probabilities of infection via a live shrimp movement (f) and local spread (g) are described in section 6.3.3.2.

Incubation, infectious, and fallow periods for each site were different. To characterise two of these periods (incubation and fallow) three distributions were fitted to the data collected during the epizootiological survey in the Chapter 3 (Weibull, lognormal, and gamma) (Figure 6.2). These candidate distributions have recently been employed in the research of Tojinbara *et al.* (2016). The lognormal distribution was found to be the best fit to the observed incubation periods of a microparasitic disease, followed by the gamma distribution, whereas the Weibull did not fit well (Bénet *et al.,* 2013). Nevertheless, the Weibull distribution is usually found to be the best fit to the emergence of bacterial diseases (Chen, 2007; Henryon *et al.,* 2005; Lai *et al.,* 2016).

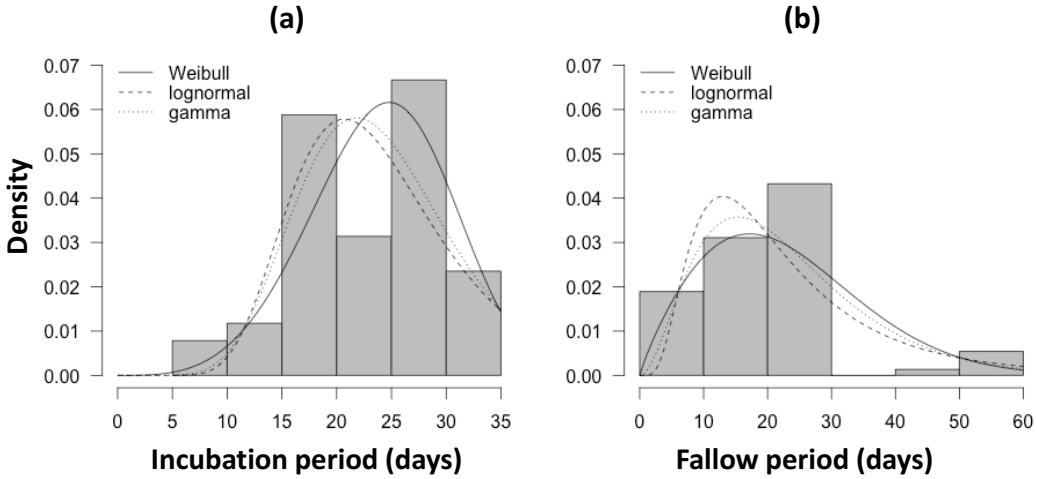


Figure 6.2 Density plots of fitted distributions of the data for incubation periods (a) and fallow periods (b) in days.

The best fitting distribution provided a lower Akaike's Information Criterion (AIC) value as in Leclerc *et al.* (2014) and Bénet *et al.* (2013). Table 6.1 shows the results of fitting of the three distributions to the data in this study by maximum likelihood. Considering the AIC values, the Weibull distribution was the best fit to the observed AHPND incubation periods (AIC = 339), and the gamma distribution was the best fit to the observed fallow periods (AIC = 576). In the modelling, therefore, the sites were randomly given different periods, distributed according to these appropriate distributions. These steps were computed using the R Programme Environment with the *fitdistrplus* package (Delignette-Muller and Dutang, 2015; R foundation for statistical computing, 2015).

Table 6.1 Akaike's Information Criterion (AIC) values of fitted distributions. The Weibull distribution gives the smallest AIC value for incubation period data while the gamma distribution gives the smallest AIC value for fallow period data.

Observed data	Weibull	Lognormal	Gamma
Incubation period	339	351	346
Fallow period	578	582	576

When the sites were infectious, over half of the farmers (65 %, 61 of 94) in the epizootiological survey of the Chapter 3 indicated their intention to stop stocking suddenly after infection (here we called this farmer behaviour "type I"). This behavioural

intention implied a short-term infection at the sites. In contrast, the remaining farmers tried to treat the infected stock by stopping feeding, loading with probiotics, and water exchange within the pond, resulting in either moderate (28 %, 26 of 94: type II) or long-term infection (7 %, 7 of 94: type III). Thus, the model proceeded using the following two steps: (1) the model assigned one of these three types of behaviour to each site (both seed-producing and ongrowing sites) while keeping all proportions constant (65 % of N_{total} for type I, 28 % of N_{total} for type II, and 7 % of N_{total} for type III), and (2) the infection period was generated randomly for each site under three assumptions: a 1–7 day short-term infection for a group of farmers behaviour type I, an 8–30 day moderate-term infection for a group of farmers behaviour type II, and a 31–120 day long-term infection for a group of farmers behaviour type III.

The model fixed the expected periods for incubation, infectious and fallow for each site before running a simulation. This meant that if a site was infected twice it behaved the same each time.

6.3.3.2 Simulation approach

The epizootics were modelled using a 1-day time step. Assuming that a connection with a larger number of shrimp would carry more risk of pathogen transmission than connections with fewer shrimp, we used the number of shrimp moved, c (6.1) as a weight for the infectiousness of shrimp, ω (6.2):

$$c_{jt} = \sum_{i} (E_{it} + I_{it}) h_{ijt} \tag{6.1}$$

$$\omega_{jt} = c_{jt}^{\mu} \tag{6.2}$$

where μ = 1 denoting the infectiousness of shrimp act proportional to their number.

At each 1-day time step, a vector f represented a probability of destination sites (j) becoming exposed via the connections of live shrimp from exposed E and infected I source sites (i), as in (6.3):

$$f_{jt} = 1 - (1 - \beta_{long})^{\omega_{jt}} \quad (6.3)$$

where β_{long} = 1 implies very high risk of infection via the connections of infected shrimp since, in the present circumstance, any $\omega > 0$ causes infection.

The f included the stochastic nature of the epizootic process (sites successfully or unsuccessful treated for AHPND). A vector Q of size N_{total} relied on a $Bernoulli$ distribution: 0 for non-exposed and 1 for exposed with a probability = f, as in (6.4).

$$Q_{jt} \sim Bernoulli(f_{jt}) \quad (6.4)$$

Additionally, an effect of local transmission was dependent on the number of infections (both exposed E and infected I sites) within close proximity, z (6.5). The close proximity of sites was based on the modelled sub-district areas. The maths here worked in parallel to the above.

$$z_{jt} = \sum_{i} (E_{it} + I_{it}) b_{ij} \quad (6.5)$$

The z was used as a weight for the infectiousness of site (η), as in (6.6):

$$\eta_{jt} = z_{jt}^{\epsilon} \quad (6.6)$$

where ϵ = 1 denotes the infectiousness of sites, acting proportional to the number of their neighbours.

A vector g represented the probability of sites becoming exposed via local spread, as in (6.7):

$$g_{jt} = 1 - (1 - \beta_{local})^{\eta_{jt}} \quad (6.7)$$

where β_{local} = 0.002 per day as the chance of infection due to local non-network spread. This was estimated from the number of AHPND cases (2012–2013) in Thailand (via local

and long-distance spread), bases on the results from the Chapter 3. Additionally, β_{local} = 0.1 per day were modelled to evaluate an increase of local spread effect.

Although a susceptible site was at risk of acquiring disease caused by infection via local spread, the site might not be infected as a result of good farming management and control. To take account of this in the model, g was included to capture the stochastic nature of the epizootic processes. A new vector Y of size N_{total} was generated by the same procedure as used for the vector Q, as in (6.8), but 1 denoting exposed relied on a probability = g.

$$Y_{jt} \sim Bernoulli(g_{jt}) \tag{6.8}$$

The new state vector after exposure to infection was:

$$E_{i,t+1} = E_{it} + (1 - E_{it})(1 - (1 - Q_{jt})(1 - Y_{jt})) \tag{6.9}$$

where $E_i + S_i + I_i + R_i = 1$

Then, the model ran over the time period over which infection occurred. As described in the initialisation section, each site had a given length of time spent in its state following each period for incubation, infection, and fallow. Hence, when a site was exposed to infection, it was in the 'E' state for the incubation period. After the incubation period ended, the site entered the 'I' state ($E \rightarrow I;\ I_i = 1, E_i = 0$). The further changes of 'I' and 'R' were dependent on the infectious period (for $I \rightarrow R;\ R_i = 1, I_i = 0$) and the fallow period (for $R \rightarrow S;\ S_i = 1, R_i = 0$), respectively.

6.3.3.3 Final results

At the final stage of the model simulation, the number of infected sites 'I' was counted and plotted each day over the 13-month network data period. Overall, the modelling process can be summed up as shown in Figure 6.3.

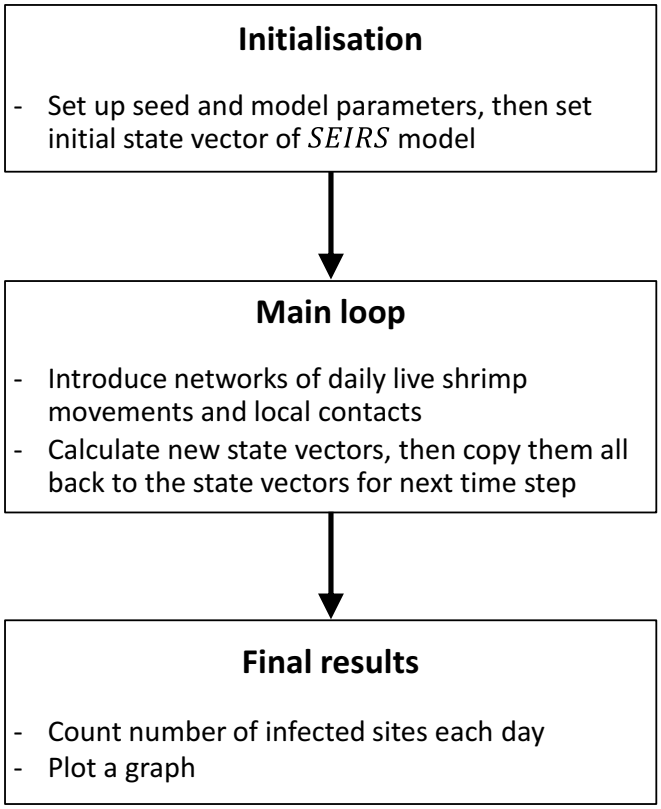


Figure 6.3 Design and implementation of an algorithm for an *SEIRS* compartmental, individual-based epizootic model for shrimp disease in Thailand

The epizootic simulation model was carried out in the R Programme Environment (R foundation for statistical computing, 2015). The model was run 80 times for each epizootic started. In total, 1 040 results were obtained (13 months × 80 simulations) per parameter set studied; these were used to compute the mean number of infected sites, representing an estimated epizootic size for AHPND.

Geographic distributions of AHPND prevalence at provincial level of Thailand were shown in the result. The number of infected sites (I) during a given period was obtained by the modelling of β_{long} = 1 and β_{local} = 0.002. The prevalence of AHPND at each province was calculated as the proportions (%) of sites infected with AHPND (6.10).

$$\text{Proportions} = \frac{\text{Number of infected sites in a province}}{\text{Total number of infected sites}} \times 100 \qquad (6.10)$$

The AHPND-infected site proportions were plotted in map form to represent the geographic distributions of AHPND prevalence in Thailand. A map of Thailand as a

shapefile was downloaded from http://www.diva-gis.org/gdata. This map was modified using the *rgdal* package in the R Programme Environment (Bivand *et al.,* 2015; R foundation for statistical computing, 2015), as well as the *tmap* package (Tennekes, 2017).

To evaluate the accuracy of the disease prevalence prediction generated using the $SEIRS$ model, the model estimates were compared against the real pattern of AHPND epizootics in Thailand at provincial level reported in July 2013 by FAO (2013). The real AHPND epizootics in Thai regions and provinces are shown in Table 6.2.

Table 6.2 Real pattern of AHPND epizootics within shrimp farming sites of Thailand reported in July 2013 (FAO, 2013)

Region	Disease presence	Province
West	No AHPND case	
Northeastern	No AHPND case	
Central	AHPND case	Nakhonpathom
East	AHPND case	Chachoengsao, Chanthaburi, Rayong, and Trat
South	AHPND case	Chumphon, Krabi, Nakhonsithammarat, Phuket, Songkhla, and Suratthani

Moreover, the receiver operating characteristic (ROC) curve was used to evaluate the model performance in predicting a binary outcome (Park *et al.,* 2004). Here we presented three tests on the ROC analysis:

(1) Disease prevalence of the $SEIRS$ model against disease prevalence in the field as reported by FAO (2013),

(2) Disease prevalence of a simpler null model with the number of sites against disease prevalence in the field as reported by FAO (2013), and

(3) Disease prevalence of a simpler null model with the number of connections (inter-province movements 'connections in' and intra-province movements) against disease prevalence in field as reported by FAO (2013).

We used different cut-off values of a "predictor" to make the predictor binary in the ROC analysis. The predictor varied with the predictive disease prevalence obtained from each test model. With the area under the ROC curve (AUC), the model was classified to be either an informative model (AUC $>$ 0.5; the predictive result of model is better than random) or an uninformative model (AUC = 0.5; the predictive result of model is not different from random) (Alonzo and Pepe, 2002; Kumar and Indrayan, 2011). The ROC analysis was done in the R Programme Environment (R foundation for statistical computing, 2015).

6.4 Results

6.4.1 Seasonality of AHPND epizootic dynamics

The mean number of sites infected by AHPND due to long-distance and local spread (default parameters β_{long} was 1 and β_{local} was 0.002) is shown in Figure 6.4. These results are obtained from the simulations of the $SEIRS$ models, and they based on the real network of live shrimp movements of Thailand (LSMN) over the 13-month period from March 2013 to March 2014.

The figure illustrates that inter-province movements are the major source of large epizootics according to our models. We can see that there is a difference in the number of infected sites between two scenarios: A (both intra- and inter-province movements), and B (intra-province movements alone).

With scenario A, where the larger epizootics are generated from both intra- and inter-province movements, the results of the $SEIRS$ models reveal that AHPND epizootics in Thailand are more likely to occur during the hot and rainy seasons (between April and August), and are less likely to happen throughout the rest of the year (mainly cool season). With scenario B, meanwhile, where the epizootics are generated from the intra-province movements alone, the $SEIRS$ models gave a much lower number of infected sites and a shorter period for the risk of AHPND (between April and June), compared with the scenario A.

The model results for the two scenarios suggest that control strategies of restrictions on inter-province movements of live shrimp are obviously required to minimise the spread of AHPND.

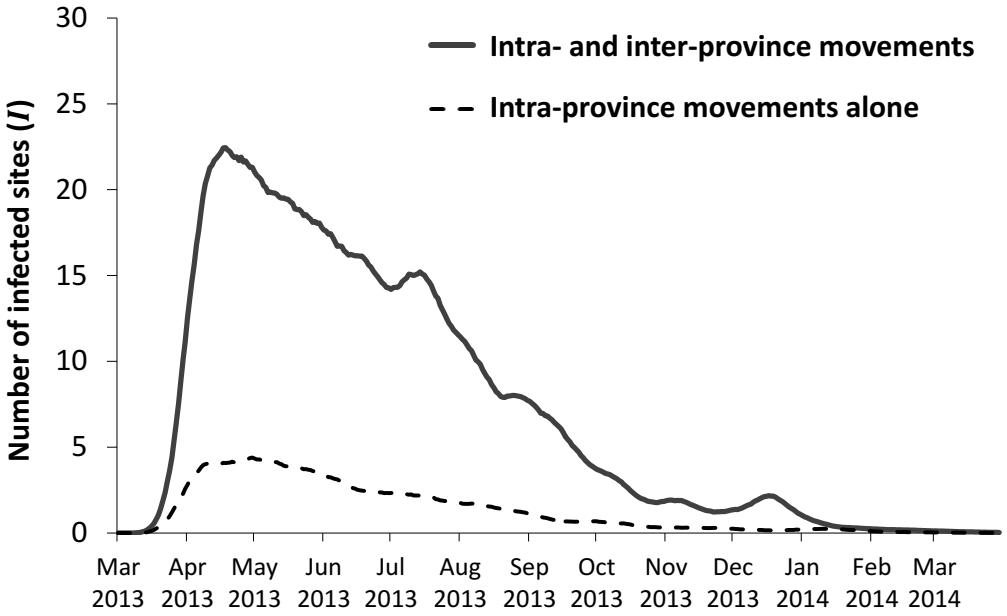


Figure 6.4 Mean number of infected sites per seed for one-month epizootics. Infection occurs via long-distance and local spread (default parameters β_{long} was 1 and β_{local} was 0.002). The averaged values of 1 040 epizootics are shown, where 16 seeds were selected at random in each month (seed fraction was 0.02). The results were generated from two data sets: both intra- and inter-province movements (scenario A; solid line), and intra-province movements alone (scenario B; dash line).

6.4.2 Effect of long-distance and local transmission on AHPND epizootic dynamics

The model also provided the chance to simulate trial biosecurity measures, i.e. testing for diseases in farmed shrimp before movements, and disease surveillance programmes. The models with scenario A used different trial values of β_{long} = 0, 0.5 and 1. Parameter β_{local} was 0.002, and the seed fraction was 0.02.

The results are shown in Figure 6.5. If the movements relied on biosecurity measures (β_{long} was lower), the number of infected sites decreased markedly and the remaining number of infected sizes was very small. Importantly, in the models, local spread alone could support the AHPND epizootics in the Thai shrimp farming (β_{long} was 0 and β_{local} was 0.002). This indicated that even if the Thai shrimp farming sector fully applied

biosecurity on live shrimp movements, a small number of seeds (16 seeds) could cause an AHPND epizootic via local spread.

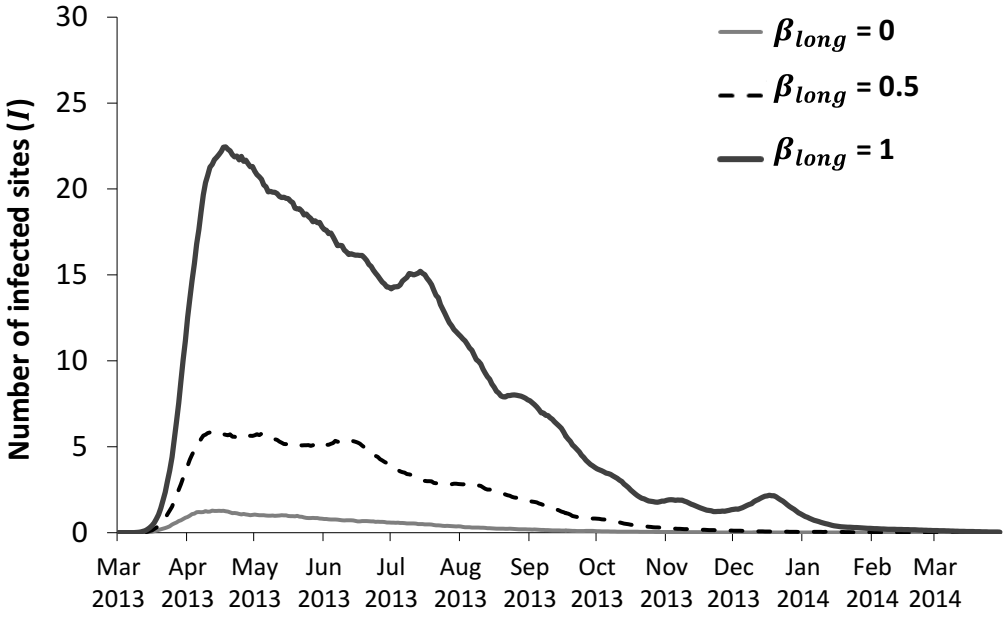


Figure 6.5 Expected outcomes of the application of biosecurity measures on live shrimp movements in Thailand. With β_{long} varied from 0 to 1, the lower β_{long} gave smaller epizootics. The model set β_{local} at 0.002, and the seed fraction at 0.02.

To evaluate the effect of increased local spread, the epizootics were compared with two values of β_{local} (0.002 and 0.01 per day) in scenario A. The default parameter β_{long} was set to be one. Figure 6.6 shows that the greater local spread results in a higher number of infected sites. Interventions to reduce local spread should therefore be considered as part of current disease surveillance and control measures.

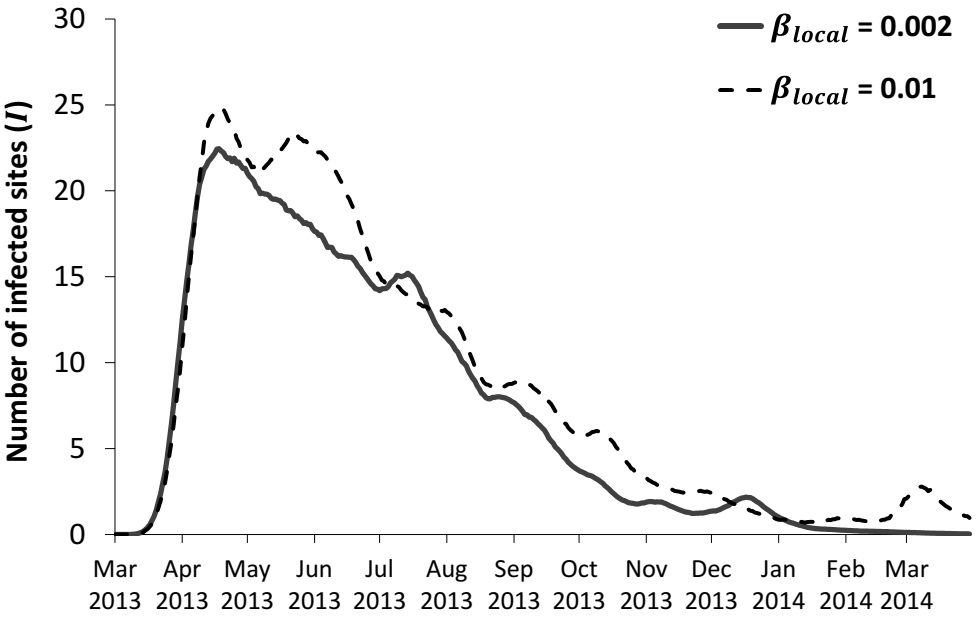


Figure 6.6 Effects of larger local spread in Thai shrimp farming sectors. The higher local spread (β_{local} was 0.01) caused a higher number of infected sites, compared with setting β_{local} at 0.002. The model set β_{long} at 1, and the seed fraction at 0.02.

6.4.3 Geographic distributions of AHPND prevalence at provincial level in Thailand

According to the model, the geographical distributions of AHPND prevalence on day 153 (July 2013, 31) is presented in Figure 6.7a. The model shows that the southern and eastern provinces of Thailand were at greater risk of AHPND than other areas. The results were similar to the real pattern of AHPND epizootics in Thailand reported in July 2013 as shown in Figure 6.7b (FAO, 2013 see Table 6.1). In respect to the western provinces, however, the model clearly overestimated the number of AHPND, as in reality none occurred in the west during the studied period.

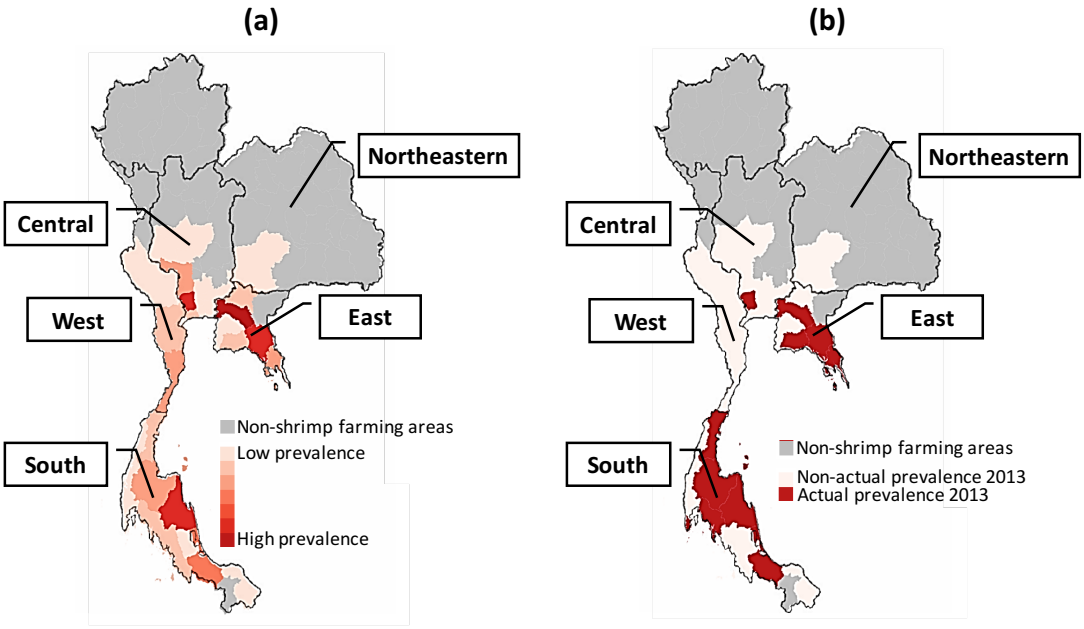


Figure 6.7 Geographic distributions of AHPND-infected provinces in Thailand. (a) The prevalence of AHPND between March 1st 2013 and July 31st 2013, are modelled using the $SEIRS$ models. The epizootic models started with 16 seeds in each month. Model parameters of β_{long} and β_{local} were 1 and 0.002, respectively. (b) The real AHPND presence in each province of Thailand as reported in July, 2013 (FAO, 2013).

6.4.4 Predictive performance of the $SEIRS$ models

To further test the model validity, the ROC approach was used to show the AUC values for three test models: the $SEIRS$ model, the simpler null model with the number of sites, and the simpler null model with the number of connections.

Figure 6.8 shows that all test models have good predictive performance compared with an uninformative model (AUC of 0.5). The AUC values of the $SEIRS$ model, site number model, and connection number model were 0.88, 0.87, and 0.91, respectively. Although the AUC of the $SEIRS$ model is close to that other of the other models, the $SEIRS$ model is a systematic approach and a more flexible tool for predicting sites at risk to AHPND. If the connections between shrimp farming site have the consistency in term of network structure, the $SEIRS$ model does not require the real-time information for modelling.

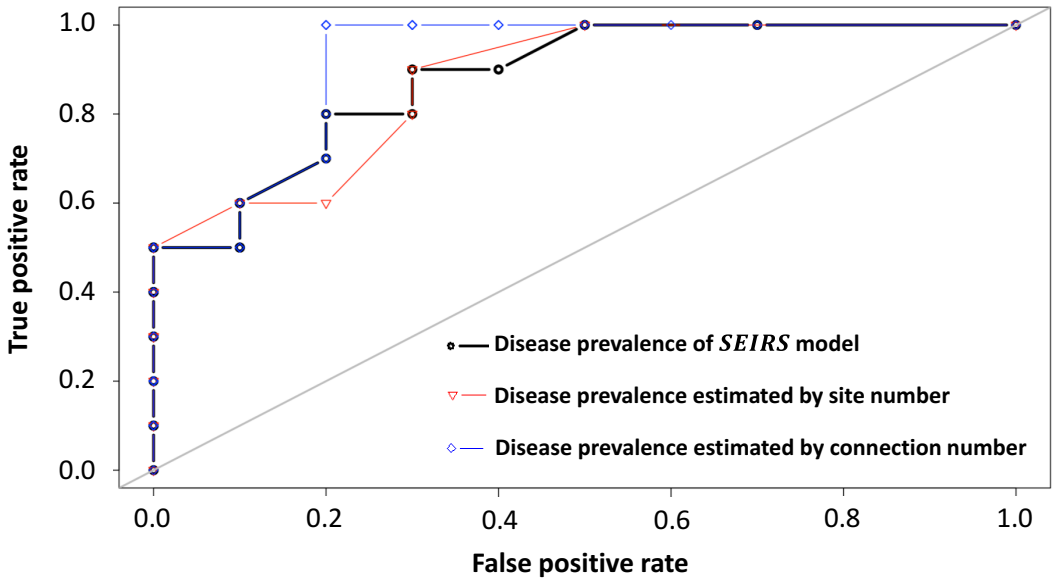


Figure 6.8 ROC curves of three test models identifying the presence of AHPND in Thai shrimp farming sites. The ROC curves are plots of true positives, i.e. where the model gave positives for provinces identified as having AHPND (plotted on the y axis) versus false positives, i.e. where the model gave positives for provinces identified as having no AHPND (plotted on the x axis). The disease presence in the field reported by FAO (2013) was used to compare with the disease presence in the *SEIRS* model, and for disease presence in the two simpler null models: prediction by number of sites, and number of connections.

6.5 Discussion

This study has modelled the epizootiological pattern of a new bacterial disease named acute hepatopancreatic necrosis disease (AHPND, also known as EMS) in Thai shrimp farming, and has examined the effect of long-distance and local transmission on its epizootic dynamics. The epizootic estimation relied on an $SEIRS$ (susceptible-exposed-infected-remove-susceptible) model. In addition, the epizootic model simulation was controlled by the real network of live shrimp movements of Thailand (LSMN) over a 13-month period from March 2013 to March 2014. As a consequence, the model systematically characterised the importance of two studied pathways for AHPND transmission, thereby informing the design of disease surveillance and control for whole shrimp farming sites or at a country-wide scale.

When live shrimp move from a location of high AHPND incidence, they carry the risk of infection. Thus, patterns of live shrimp movements can reflect the seasonality of AHPND

epizootics in Thailand. Our model demonstrated that AHPND was likely to occur between April and August (during the hot and rainy seasons in Thailand). During this period, the number of site connections totally increased by about 50 %, as shown in the Chapter 4 “Analysis of the network structure of the live shrimp movements relevant to AHPND epizootic”, with approximately half of these having originally moved from the south. To implement successful control strategies, seed-producing sites in southern areas should be monitored closely for the AHPND pathogen, such as increasing the sampling frequency prior to the occurrence of regular outbreaks.

In terms of long-distance transmission effects, inter-province movements play a major role in AHPND epizootics, while intra-province movements alone also generate small epizootic sizes. Although an important biosecurity practice, i.e. testing for AHPND in farmed shrimp before moving the shrimp from site to site has been implemented in the Thai shrimp farming sector (Uddin, 2008; Yamprayoon and Sukhumparnich, 2010), one concern is whether current disease control measures are based on the sensitivity of diagnostic techniques and delays in diagnosis (i.e. the time between symptom onset and establishment of diagnosis) among infected sites (Ahmed *et al.,* 2016; Lightner and Redman, 1998; Liu *et al.,* 2016; Saulnier *et al.,* 2000a; Sithigorngul *et al.,* 2007). Additionally, previous work has indicated that vibriosis can be found in healthy shrimp, which makes the infectious shrimp difficult to detect and treat at the initial stage (Aguirre-Guzmán *et al.,* 2004; Goarant *et al.,* 2006; Gomez-Gil *et al.,* 1998; Vincent and Lotz, 2005). From this evidence, it is possible that the infected shrimp are still being accidentally moved between sites.

Moreover, local transmission alone can cause AHPND epizootics according to our modelling, although these epizootics are not large. Nevertheless, in this case if the disease occurs in areas with a high number of sites, or in a site with high connections, many infected sites may be observed across large areas. This local spread can be generated by common activities within Thai shrimp farming such as sharing of water bodies, as well as by the close proximity of shrimp farming sites (Boonyawiwat *et al.,* 2016; Hazarika *et al.,* 2000). Water discharge from infected sites to canals, rivers or the sea, also causes local spread increases, if those sites do not conduct appropriate water disinfection. Additionally, vibrios are often found growing in aquatic environments

(Kongkumnerd, 2014), making shrimp farming sites vulnerable to receiving pathogens via local spread. These pathways driving local spread need to be eliminated.

The disease control strategies suggested by this proposed model are biosecurity on live shrimp movements and monitoring of diseases in the natural environment. The model is useful for testing control strategies by changing model parameters. First, applying a lower chance of infection via the connections of infected shrimp (β_{long}) in the modelling resulted in smaller sizes of potential AHPND epizootics. This illustrated that applying biosecurity measures to shrimp movements could effectively control AHPND epizootic dynamics. Another test was changing the chance of infection due to local non-network spread (β_{local}). The increased likelihood of local spread led to larger epizootics. This emphasised the importance of monitoring diseases in the natural environment of shrimp farms and also the need for water disinfection for shrimp rearing. These are common practices of biosecurity, but they might be neglected in recent Thai shrimp farming.

The removed sites (R) in our $SEIRS$ model might be infected. Nevertheless, if they are drying out, for example, and not connected to water sources or carrying shrimp (called site fallowing), they probably do not pose on onward risk of infection because of a lack of host-pathogen-environment interactions (Rockett, 1999). In real practice, Thai shrimp farmers conduct the fallowing process in varying periods from 3 to 60 days (data obtained from the epizootiological survey in Chapter 3), although the Thailand Department of Fisheries recommends that all sites should be fallowed for between one and four weeks after harvesting shrimp (National Bureau of Agricultural Commodity and Food Standards, 2014; Thailand DoF, 2010). Thus, an appropriate period of fallowing requires further modelling.

A model will never address exactly the real-world complexity. It just means that a simple solution to a complex problem may work. For example, in our models, the occurrence of AHPND was overestimated in some areas. Nevertheless, the model provided more accurate predictions of AHPND occurrence than random guess models. For comparing the performance of the three different test models, the value of the ROC curves obtained from the $SEIRS$ model was close to that of the two simpler null models (as

described in the results section). The $SEIRS$ epizootic model was heavily driven by the connection data (live shrimp movements), thus the obtained result was not unexpected. Also of connections very closely with site count that was not unexpected either since the site counts are known to relate to low or high numbers of connections. Nevertheless, note that data here aggregates at a high level (province), and aggregation at a site level may therefore show different results.

One of the limitations of this research was that the model was not greatly concerned with types of shrimp farming sites, i.e. large, medium and small scales. Different types of site provide various farming management practices (Erler *et al.,* 2007; Lebel *et al.,* 2010). The model did, however, consider the number of shrimp. Large-scale sites that commonly relate to stocking a high number of shrimp should have high vulnerability to infection compared with small and medium scales. Taking account of this would be expected to make the model more accurate.

To summarise, the AHPND epizootic dynamics were modelled in the Thai shrimp farming industry using an $SEIRS$ compartmental, individual-based epizootic model. The results suggested that both long-distance and local transmission influenced the dynamics of AHPND epizootic in Thai shrimp farming. Of the efforts to control these epizootics at the country scale, biosecurity of live shrimp movements and interventions to reduce the local spread (e.g. monitoring of pathogens in natural environments) are required. In future modelling of AHPND epizootic dynamics, control strategies in respect to local spread will be evaluated.

6.6 References

Aguirre-Guzmán, G., Mejia Ruíz, H. and Ascencio, F. (2004) A review of extracellular virulence product of *Vibrio* species important in diseases of cultivated shrimp. *Aquaculture Research*, 35 (15), pp. 1395–1404.

Ahmed, R., Rafiquzaman, S., Hossain, M.T., Lee, J. and Kong, I. (2016) Species-specific detection of *Vibrio alginolyticus* in shellfish and shrimp by real-time PCR using the groEL gene. *Aquaculture International*, 24 (1), pp. 157–170.

Alonzo, T.A. and Pepe, M.S. (2002) Distribution-free ROC analysis using binary regression techniques. *Biostatistics (Oxford, England)*, 3 (3), pp. 421–432.

Bénet, T., Voirin, N., Nicolle, M., Picot, S., Michallet, M. and Vanhems, P. (2013) Estimation of the incubation period of invasive aspergillosis by survival models in acute myeloid leukemia patients. *Medical Mycology*, 51 (2), pp. 214–218.

Bivand, R., Keitt, T. and Rowlingson, B. (2015) rgdal: Bindings for the geospatial data abstraction library.

Boonyawiwat, V., Patanasatienkul, T., Kasornchandra, J., Poolkhet, C., Yaemkasem, S., Hammell, L. and Davidson, J. (2016) Impact of farm management on expression of early mortality syndrome/acute hepatopancreatic necrosis disease (EMS/AHPND) on penaeid shrimp farms in Thailand. *Journal of Fish Diseases*, 40 (5), pp. 649–659.

Britton, T., Deijfen, M. and Liljeros, F. (2011) A weighted configuration model and inhomogeneous epidemics. *Journal of Statistical Physics*, 145 (5), pp. 1368–1384.

Chen, H. (2007) Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. *Food Microbiology*, 24 (3), pp. 197–204.

Chonsin, K., Matsuda, S., Theethakaew, C., Kodama, T., Junjhon, J., Suzuki, Y., Suthienkul, O. and Iida, T. (2016) Genetic diversity of *Vibrio parahaemolyticus* strains isolated from farmed Pacific white shrimp and ambient pond water affected by acute hepatopancreatic necrosis disease outbreak in Thailand. *FEMS Microbiology Letters*, 363 (2), pp. 1–8.

Dabu, I.M., Lim, J.J., Arabit, P.M.T., Orense, Sharlaine Joi Ann B, Tabardillo, J.A., Corre, V.L. and Maningas, M.B.B. (2015) The first record of acute hepatopancreatic necrosis disease in the Philippines. *Aquaculture Research*, pp. 1–8.

Delignette-Muller, M.L. and Dutang, C. (2015) fitdistrplus: An R Package for Fitting Distributions. *Journal of Statistical Software*, 64 (4), pp. 1–34.

Draief, M., Ganesh, A. and Massoulié, L. (2008) Thresholds for virus spread on networks. *The Annals of Applied Probability*, 18 (2), pp. 359–378.

Durand, B. and Mahul, O. (2000) An extended state-transition model for foot-and-mouth disease epidemics in France. *Preventive Veterinary Medicine*, 47 (1–2), pp. 121–139.

Eduardo, M.L. and Mohan, C.V. (2012) *Early mortality syndrome (EMS)/Acute hepatopancreatic necrosis syndrome (AHPNS): an emerging threat in the Asian shrimp industry.* Bangkok: NACA. Available: http://library.enaca.org/Health/DiseaseLibrary/disease-advisory-ems-ahpns.pdf [Accessed: 15 August 2014].

Erler, D., Songsangjinda, P., Keawtawee, T. and Chaiyakam, K. (2007) Nitrogen dynamics in the settlement ponds of a small-scale recirculating shrimp farm (*Penaeus monodon*) in rural Thailand. *Aquaculture International*, 15 (1), pp. 55–66.

FAO (2013) *Report of the FAO/MARD technical workshop on early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304).* FAO Fisheries and Aquaculture Report No. 1053. Rome: FAO. Available: http://www.fao.org/docrep/018/i3422e/i3422e00.htm [Accessed: 11 October 2013].

Galappaththi, E.K. and Berkes, F. (2015) Can co-management emerge spontaneously? Collaborative management in Sri Lankan shrimp aquaculture. *Marine Policy*, 60, pp. 1–8.

Goarant, C., Ansquer, D., Herlin, J., Domalain, D., Imbert, F. and De Decker, S. (2006) "Summer Syndrome" in *Litopenaeus stylirostris* in New Caledonia: Pathology and epidemiology of the etiological agent, *Vibrio nigripulchritudo*. *Aquaculture*, 253 (1–4), pp. 105–113.

Gomez-Gil, B., Tron-Mayén, L., Roque, A., Turnbull, J.F., Inglis, V. and Guerra-Flores, A.L. (1998) Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei*. *Aquaculture*, 163 (1–2), pp. 1–9.

Hazarika, M., Samarakoon, L., Honda, K., Thanwa, J., Pongthanapanich, T., Boonsong, K. and Luang, K. (2000) Monitoring and impact assessment of shrimp farming in the east coast of Thailand using remote sensing and GIS. *International Archives of Photogrammetry and Remote Sensing,* 33 (B7/2; PART 7), pp. 504–510.

Henryon, M., Berg, P., Olesen, N.J., Kjær, T.E., Slierendrecht, W.J., Jokumsen, A. and Lund, I. (2005) Selective breeding provides an approach to increase resistance of rainbow trout (*Onchorhynchus mykiss*) to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. *Aquaculture*, 250 (3–4), pp. 621–636.

Huitric, M., Folke, C. and Kautsky, N. (2002) Development and government policies of the shrimp farming industry in Thailand in relation to mangrove ecosystems. *Ecological Economics*, 40 (3), pp. 441–455.

Keeling, M.J. and Eames, K.T. (2005) Networks and epidemic models. *Journal of the Royal Society Interface*, 2 (4), pp. 295–307.

Kongkumnerd, J. (2014) *The current status of EMS outbreak in Thai marine shrimp farming.* Bangkok: Thailand Department of Fisheries (DoF). Available: http://www.shrimpaqua.com/download/EMS/situation-EMS.pdf [Accessed: 10 December 2014].

Kumar, R. and Indrayan, A. (2011) Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatrics*, 48 (4), pp. 277–287.

Lai, C.C., Ji, D.D., Wu, F.T., Mu, J.J., Yang, J.R., Jiang, D.D., Lin, W.Y., Chen, W.T., Yen, M.Y., Wu, H.S. and Chen, T.H. (2016) Etiology and risk factors of acute gastroenteritis in a Taipei Emergency Department: clinical features for bacterial gastroenteritis. *Journal of Epidemiology/Japan Epidemiological Association*, 26 (4), pp. 216–223.

Lebel, L., Mungkung, R., Gheewala, S.H. and Lebel, P. (2010) Innovation cycles, niches and sustainability in the shrimp aquaculture industry in Thailand. *Environmental Science & Policy*, 13 (4), pp. 291–302.

Leclerc, M., Doré, T., Gilligan, C., Lucas, P., Filipe, J. and Nishiura, H. (2014) Estimating the delay between host infection and disease (incubation period) and assessing its. *PloS One*, 9 (1), e86568. Available: http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0086568&type=printable [Accessed: 1 May 2016].

Lightner, D.V., Redman, R.M., Pantoja, C.R., Noble, B.I. and Tran, L. (2012) Early mortality syndrome affects shrimp in Asia. *Global Aquaculture Advocate Magazine,* 40 Available: https://pdf.gaalliance.org/pdf/GAA-Lightner-Jan12.pdf [Accessed: 31 March 2013].

Lightner, D.V. and Redman, R.M. (1998) Shrimp diseases and current diagnostic methods. *Aquaculture*, 164 (1–4), pp. 201–220.

Liu, X., Guan, Y., Cheng, S., Huang, Y., Yan, Q., Zhang, J., Huang, G., Zheng, J. and Liu, T. (2016) Development of a highly sensitive lateral immunochromatographic assay for rapid detection of *Vibrio parahaemolyticus*. *Journal of Microbiological Methods*, 131, pp. 78–84.

Muniesa, A., Perez-Enriquez, R., Cabanillas-Ramos, J., Magallón-Barajas, F.J., Chávez-Sánchez, C., Esparza-Leal, H. and Blas, I. (2015) Identifying risk factors associated with white spot disease outbreaks of shrimps in the Gulf of California (Mexico) through expert opinion and surveys. *Reviews in Aquaculture*, pp. 1–9.

NACA (2014) *Diseases of crustaceans: acute hepatopancreatic necrosis syndrome (AHPNS).* Available: http://www.enaca.org/publications/health/disease-cards/ahpnd-disease-card-2014.pdf [Accessed: 1 May 2015].

NACA (2017) *Quarterly aquatic animal disease report (Asia and Pacific Region) 1998–2016.* Thailand: NACA. Available: http://www.enaca.org/modules/library/publication.php?tag_id=279&label_type=1&title=quarterly-aquatic-animal-disease-report [Accessed: 29 April 2017].

Nagai, F., Mektrairat, N. and Funatsu, T. (2008) *Local government in Thailand: analysis of the local administrative organization survey.* Chiba, Japan: Institute of Developing Economies.

National Bureau of Agricultural Commodity and Food Standards (2014) *Good aquaculture practices for marine shrimp farm.* Available: http://www.acfs.go.th/standard/download/GAP-FOR-MARINE-SHRIMP-FARM.pdf [Accessed: 10 March 2017].

Nunan, L., Lightner, D.V., Pantoja, C. and Gomez-Jimenez, S. (2014) Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. *Diseases of Aquatic Organisms,* 111 (1), pp. 81–86.

OIE (2013) *Acute hepatopancreatic necrosis disease, aetiology epidemiology diagnosis prevention and control references.* Available: http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/AHPND_DEC_2013.pdf [Accessed: 12 August 2014].

Park, S.H., Goo, J.M. and Jo, C.H. (2004) Receiver operating characteristic (ROC) curve: practical review for radiologists. *Korean Journal of Radiology,* 5 (1), pp. 11–18.

Patil, A.A., Annachhatre, A.P. and Tripathi, N.K. (2002) Comparison of conventional and geo-spatial EIA: A shrimp farming case study. *Environmental Impact Assessment Review,* 22 (4), pp. 361–375.

R foundation for statistical computing (2015) *R: a language and environment for statistical computing.* Available: https://www.R-project.org/ [Accessed: 5 December 2015].

Rizzo, A., Pedalino, B. and Porfiri, M. (2016) A network model for Ebola spreading. *Journal of Theoretical Biology*, 394, pp. 212–222.

Rockett, I.R. (1999) Population and health: an introduction to epidemiology. *Population Bulletin*, 54 (4), pp. 1.

Rvachev, L.A. and Longini, I.M. (1985) A mathematical model for the global spread of influenza. *Mathematical Biosciences*, 75 (1), pp. 3–22.

Saulnier, D., Avarre, J., Le Moullac, G., Ansquer, D., Levy, P. and Vonau, V. (2000) Rapid and sensitive PCR detection of *Vibrio penaeicida*, the putative etiological agent of Syndrome 93 in New Caledonia. *Diseases of Aquatic Organisms*, 49 (2), pp. 109–115.

Sithigorngul, P., Rukpratanporn, S., Pecharaburanin, N., Suksawat, P., Longyant, S., Chaivisuthangkura, P. and Sithigorngul, W. (2007) A simple and rapid immunochromatographic test strip for detection of pathogenic isolates of *Vibrio harveyi*. *Journal of Microbiological Methods*, 71 (3), pp. 256–264.

Smith, P.T. (1999) Application of GIS to the Thai shrimp farm survey. In: P.T. Smith, ed. *Towards Sustainable Shrimp Culture in Thailand and the Region.* Songkhla, Thailand, 28th October to 1st November 1996. Canberra, Australia: Australian Centre for International Agricultural Research, pp. 113-130. Available: http://aciar.gov.au/files/node/2196/pr90_pdf_11112.pdf [Accessed: 17 November 2014].

Taylor, N.G., Norman, R.A., Way, K. and Peeler, E.J. (2011) Modelling the koi herpesvirus (KHV) epidemic highlights the importance of active surveillance within a national control policy. *Journal of Applied Ecology*, 48 (2), pp. 348–355.

Tennekes, M. (2017) *tmap: Thematic maps.*

Thailand DoF (2010) *Thai good aquaculture practices (GAP) certification standards for marine shrimp farms.* Available: http://www.fisheries.go.th/thacert/images/pdf/SF_GAPcriteria.pdf [Accessed: 1 May 2015].

Tojinbara, K., Sugiura, K., Yamada, A., Kakitani, I., Kwan, N.C.L. and Sugiura, K. (2016) Estimating the probability distribution of the incubation period for rabies using data from the 1948–1954 rabies epidemic in Tokyo. *Preventive Veterinary Medicine*, 123, pp. 102–105.

Tran, L., Nunan, L., Redman, R.M., Lightner, D.V. and Fitzsimmons, K. (2013a) *EMS/AHPNS: infectious disease caused by bacteria.* Global Aquaculture Advocate. Available: http://apfa.com.au/wp-content/uploads/2017/03/GAA-Tran-July13.pdf [Accessed: 6 May 2016].

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. and Lightner, D.V. (2013b) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Diseases of Aquatic Organisms*, 105 (1), pp. 45–55.

Uddin, M.T. (2008) Value Chains and Standards in Shrimp Export.

Ushijima, B. (2014) *Pooling Knowledge: Devising next-generation technologies to detect diseases affecting aquaculture.* Japan: Public Relations Office of the Government of Japan. Available: http://dwl.gov-online.go.jp/video/cao/dl/public_html/gov/pdf/hlj/20141101/24-25.pdf [Accessed: 6 May 2016].

Vandergeest, P., Flaherty, M. and Miller, P. (1999) A political ecology of shrimp aquaculture in Thailand. *Rural Sociology*, 64 (4), pp. 573–596.

Vincent, A.G. and Lotz, J.M. (2005) Time course of necrotizing hepatopancreatitis (NHP) in experimentally infected *Litopenaeus vannamei* and quantification of NHP-bacterium using real-time PCR. *Diseases of Aquatic Organisms*, 67 (1–2), pp. 163–169.

Werkman, M., Green, D.M., Murray, A.G. and Turnbull, J.F. (2011) The effectiveness of fallowing strategies in disease control in salmon aquaculture assessed with an SIS model. *Preventive Veterinary Medicine*, 98 (1), pp. 64–73.

Yamprayoon, J. and Sukhumparnich, K. (2010) Thai aquaculture: achieving quality and safety through management and sustainability. *Journal of the World Aquaculture Society*, 41 (2), pp. 274–280.

Zorriehzahra, M. and Banaederakhshan, R. (2015) Early mortality syndrome (EMS) as new emerging threat in shrimp industry. *Adv.Anim.Vet.Sci*, 3 (2s), pp. 64–72.

Chapter 7 - General discussion

7.1 Summary

This final chapter summarises the main conclusions of the research. In the last part of this chapter, we suggest some directions for further work.

The main objective of this research was to apply epizootiological tools to study of AHPND and other infectious disease spread in the Thai shrimp farming industry. Chapter 3 (the first research chapter), we evaluated the risk factors for AHPND occurrence at site level using a cross-sectional study design. The AHPND-infected cases were identified from a decision tree that was developed basing on gross signs of AHPND and non-laboratory confirmation. The results from univariate analysis and conditional logistic regression indicated that no pond harrowing increased the risk of AHPND and the use of earthen ponds for shrimp rearing decreased the risk of the disease occurrence.

Chapter 4 utilised the data of the live shrimp movement network (LSMN) to examine the spread of infectious diseases in shrimp farming sites. The results emphasised that the LSMN had a high number of inter-province movements, which increased the chance of disease epizootics across province and region borders of Thailand. Moreover, the LSMN represented a scale-free network with small-world properties. This suggests that regulators and decision makers need to pose more attention in efforts to design disease surveillance and control strategies.

Consequently, in Chapter 5 disease-control algorithms were developed using four targeted approaches and a non-targeting approach. These aimed to find optimal control strategies to reduce the potential epizootic size in the LSMN. The results from the studied network demonstrate that targeted strategies, i.e. biosecurity measures in respect to targeted live shrimp movements, support the success of disease surveillance and control in Thai shrimp farming, and improve the cost-effectiveness of surveillance resources.

Chapter 6 modelled the spread of AHPND from site to site, based on two transmission routes for the disease (long-distance and local transmission). An $SEIRS$ compartmental, individual-based epizootic model showed that AHPND was likely to occur during the hot and rainy seasons of Thailand (between April and August). During this outbreak period, the movements were mainly sourced from the areas in the south. Although the number of intra-province movements was small (23 % of total connections), they also affected the estimated epizootic size. For disease prevention and control, the models indicated that lower long-distance transmission rates ($\beta_{long} < 1$, and β_{local} was 0.002) strongly reduced the number of infected sites. Local spread alone, however, could still cause epizootics (β_{long} was 0, and β_{local} was 0.002). Given these two major routes for AHPND transmission, two measures are suggested in this research. First is an increase in biosecurity on live shrimp movements (effective testing of diseases in farmed shrimp before movements, and targeted disease surveillance and control of disease spread in the live shrimp movement network). The second is interventions to reduce local transmission such as monitoring of diseases in natural environments.

7.2 General discussion

7.2.1 Disease case confirmation

Chapter 3 developed an AHPND decision tree for the identification of AHPND-infected cases, based on the disease at the point being an unknown etiology. This effective tool was able to narrow the gap period for disease prevention and controls. The tool also could also help the decision of farmers to deal with the infection at site level, providing evidence to support the early stopping of any pathway for transmitting pathogens, such as stopping water discharge to natural sources. The outcome should lead to smaller sized epidemics, as proposed in Ferguson *et al.* (2001). A matter of great concern is that waiting for laboratory results caused the delayed outbreak reporting, and consequently the late implementation of mitigation measures, as happened with a waterborne outbreak of Giardiasis (a gastrointestinal disease) in the Norwegian population in 2004 (Nygård *et al.,* 2006). Hence, until there is a rapid diagnostic method for infectious diseases, the decision tree will remain useful to the study epidemiology of these diseases.

The AHPND decision tree can reflect on current disease surveillance and control programmes in Thailand. Shrimp samples obtained from both active and passive surveillances are confirmed by the PCR testing (a diagnosis tool for AHPND). This test is quite costly (approximately 25 USD per sample) and time-consuming (around two days) (Coastal Aquatic Animal Health Research Institute, 2016b). Thus, the decision tree can be used as a primary screen to detect cases of AHPND. The use of the decision tree technique as a screening tool for diseases has already been shown to be effective in the epidemiological investigation of rheumatoid arthritis in a large database of around two million patients (Zhou *et al.,* 2016). For another purpose, the decision tree gives decision makers a tool to choose optimised control measures for foot-and-mouth disease transmitted between livestock animals (Tomassen *et al.,* 2002).

7.2.2 Shrimp farming data used for epizoology

7.2.2.1 Data recorded on shrimp farming management and practices

During the epizootiological survey in Chapter 3, a key success for the data collection was that many interviewees had written their daily farming practices in their own diary books or electronic files. The recording follows the requirement of good aquaculture practice (GAP) standards for Thai shrimp farming (National Bureau of Agricultural Commodity and Food Standards, 2014), with most shrimp farming sites in Thailand now being certified by the GAP standard (Fisheries Commodity Standard System and Traceability Division, 2017). Thus, the interviewees could provide accurate data to us, leading to robust epizootiological studies. The recording of farming activities is most helpful in epizoology if such recording is collected for purposes of disease surveillance and controls (Birkhead *et al.,* 2015; Wendt *et al.,* 2015).

7.2.2.2 Live shrimp movement network data

It should be emphasised that the live shrimp movement network data provides robust epizootiological information. Modelling these data as a network provided a powerful tool for the understanding of disease spread between sites (Chapter 4). Additionally, its modelling aided the design of disease surveillance and control strategies that might be applied given limitations of surveillance resources (Chapter 5). Nonetheless, the

network may change its structure over the long term because patterns of connections change (Green *et al.,* 2012; Stoddard *et al.,* 2013). Implementations of control strategies such as vaccination (McReynolds *et al.,* 2014), and case isolation and quarantine (Lipsitch *et al.,* 2003; Pandey *et al.,* 2014) also influence the structure of networks. Thus, further research is needed to determine the consistency of this network structure, in which such historical data of the live shrimp movement network provide a benchmark.

The network data that we obtained from the Thailand Department of Fisheries can be considered to be highly accurate for two reasons. First, the data were based on official reports; second, such information were kept in computerised database. Nonetheless, there were some minor failures that should be mentioned. Errors were found in respect to site addresses within the village details. If we relied on the location of sites at the lowest level of the village, therefore, these errors would affect the reliability of our results. Hence, computer data are only as good as that which are entered.

7.2.3 Modelling disease epizootic dynamics

The network modelling in Chapters 4 and 5 considers the potential route of disease transmission (live shrimp movements) but in reality most shrimp diseases can transmit from site to site via other pathways, such as hydrological connectivity and the physical proximity of sites (see Chapter 1). In addition, the non-random mixing and heterogeneity of connections affect disease transmission. Thus, we modelled the live shrimp movements as a weighted network, and took the effect of local non-network spread and the number of shrimp into account while modelling AHPND epizootic dynamics (Chapter 6).

The capacity of farmers to manage their sites after infection also plays an important role in disease epizootics. Wu *et al.* (2001) proposed that the mortality of white spot disease-challenged shrimp that were stocked at high density became critical if moribund shrimp were not immediately removed as to minimise the cannibalism and waterborne transmission. The evidence indicates that models for disease spreads between animal farm sites should consider the farmers' behaviour in disease prevention and control as an example in Chapter 6.

7.3 Future work

7.3.1 Control strategies for local non-network spread

To control disease spread via live shrimp movements, Thailand applied a process of testing farmed shrimp for diseases before their movements (National Bureau of Agricultural Commodity and Food Standards, 2015). Additionally, Thai regulators routinely monitor for diseases in farmed shrimp through active and passive surveillance programmes (Coastal Aquatic Animal Health Research Institute, 2016a). Here, a targeted disease surveillance approach was developed in Chapter 5 using network modelling approaches with the aim of improving these surveillance programmes. While the effect of the local non-network spread on the AHPND epizootic dynamics was demonstrated in Chapter 6, the benefit of the targeted strategies for local non-network spread was not clear in practice.

Commonly, *Vibrio* spp. are found to be free living in aquatic environments (Thompson *et al.,* 2004). Their life cycles often relate to local non-network spread of diseases (i.e. hydrological transmission) for farmed shrimp (Apanakapan *et al.,* 2016; Heenatigala and Fernando, 2016; Naganathan *et al.,* 2014; Pui *et al.,* 2014). Importantly, the abundance of *Vibrio* spp. in natural water sources can be promoted by cohabitation with protozoans (Laskowski-Arce and Orth, 2008). Thus, targeting of network spread may not sufficient. For this case, hydrodynamic models may need to be a part of the design of control strategies. Parallel examples for this include salmon lice, which are abundant on both wild and farmed fish (Torrissen *et al.,* 2013), and for which many hydrodynamic models have already been developed (Gillibrand and Willis, 2007; Murray and Gillibrand, 2006).

7.3.2 Coinfection epizootic models

During AHPND outbreaks, farmed shrimp in Asia face another infectious disease named hepatopancreatic microsporidiosis (HPM) caused by a microsporidian *Enterocytozoon hepatopenaei* (NACA, 2015; Thitamadee *et al.,* 2016). HPM does not lead to mass mortalities, but it is associated with slow growth in affected stocks (Salachan *et al.,* 2017). Nevertheless, recently, Aranguren *et al.* (2017) found that HPM-infected shrimp have a high risk of AHPND infection. The evidence shows that shrimp are infected by

both HPM and AHPND, leading to more complex dynamics of AHPND epizootics than our models in Chapter 6. Additional parameters, such as a probability of HMP infection, therefore need to be added to the models developed in this research.

7.3.3 Geographical information systems (GIS) for shrimp farming sites

With approaches of computerisation and geographical information systems (GIS), the description of a disease epidemic in terms of real spatial results can be undertaken (Cromley, 2003; Tami *et al.,* 2016). This is because GIS can work on areas, while many network models such as ours treat sites essentially as point sources. For human disease models, GIS has been used to examine transmission of tuberculosis (Moonan *et al.,* 2004), chikungunya fever (Rodriguez-Morales *et al.,* 2016), and dengue disease (Palaniyandi *et al.,* 2014), for example. GIS has also recently been used in the modelling of livestock diseases such as bovine tuberculosis (Ribeiro‐Lima *et al.,* 2016). For aquatic animals, GIS is typically applied to identify suitable areas for aquatic animal farming (Giap *et al.,* 2005; Salam *et al.,* 2003; Salam *et al.,* 2005), nevertheless GIS has been less widely used in shrimp and fish epizoology (Bayot *et al.,* 2008).

For Thai shrimp farming, the GIS approach could allow us more accuracy in site location to determine a change in the distance of movements caused by any rewiring. Additionally, the geographic distribution of AHPND, and the spread of other shrimp diseases in Thailand, could be drawn at site level instead of province level as shown in Chapter 6.

7.4 Conclusions

- The use of epidemiological and epizootiological tools demonstrated four main epizootiological findings for AHPND spread in Thai shrimp farming.

- A cross-sectional study revealed a significant association between AHPND transmission at site level and farming management practices, i.e. types of ongrowing ponds and harrowing. No pond harrowing increased the risk of AHPND onset, while earthen ponds decreased the risk of AHPND occurrence in ongrowing sites.

- Moreover, case confirmation with the AHPND decision tree allowed rapid investigations of the risk factors for the AHPND transmission, given that at the time of data collection, AHPND was a disease with an unknown etiology. This case-identification AHPND decision tree can be adapted when there is recurrence of AHPND, or the gross signs of disease pathology can be changed when there is incidence of other new diseases.

- In order to prevent and control infectious disease outbreaks at the country scale, network modelling is applied here for the first time to the shrimp farming sector. This illustrated the properties of the live shrimp movement network in Thailand (LSMN) in aiding and hindering the transmission of diseases. Most importantly, our research into network modelling emphasised that the random disease surveillance and control could not effectively minimise disease spread site to site via long-distance transmission, compared with the optimal targeted disease surveillance and control.

- According to our $SEIRS$ compartmental, individual-based epizootic model, the inter-province movements were the main source of AHPND spread in the Thai shrimp farming. The $SEIRS$ modelling strongly demonstrated that the increase of biosecurity within the live shrimp movement network could lead to smaller potential AHPND epizootics. Additionally, it suggested that there was a need for disease surveillance and control in natural sources or other pathways relative to local non-network spread.

- Our network epizootic model may apply to other diseases and industries. The model can be applied to other diseases by using different parameter values, or adding other pathways for disease transmission. The model can be applied to other industries by including other types of sites (e.g. fish, mussel and crab farming, or processing plants) and their connections if the data is available. Importantly, the model is useful to evaluate current regulations or desired strategies to prevent and control disease outbreaks such as biosecurity and surveillance strategies.

The epidemiological and epizootiological tools used in this research provide unique contributions to the study of shrimp epizoology. The results give more understanding of site-to-site transmission of AHPND and other infectious diseases in farmed shrimp. Thai regulators and decision makers can use them in improvement of disease surveillance and control.

7.5 References

Apanakapan, J., Chuchird, N., Tongsri, P., Taemmesub, T., Taparhaudee, W. and Sritunyalucksana, K. (2016) Effect of spore-forming bacteria on *Vibrio* spp., survival rate and water qualities in the larval rearing of the Pacific white shrimp (*Litopenaeus vannamei*). *Kasetsart University Annual Conference.* Kasetsart University, 2nd to 5th February 2016, pp. 648–655. Available: https://www.cabdirect.org/cabdirect/FullTextPDF/2016/20163376046.pdf [Accessed: 29 April 2017].

Aranguren, L.F., Han, J.E. and Tang, K.F. (2017) Enterocytozoon hepatopenaei (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN) in the Pacific white shrimp *Penaeus vannamei*. *Aquaculture,* 471, pp. 37–42.

Bayot, B., Sonnenholzner, S., Ochoa, X., Guerrerro, J., Vera, T., Calderón, J., de Blas, I., del Pilar Cornejo-Grunauer, M., Stern, S. and Ollevier, F. (2008) An online operational alert system for the early detection of shrimp epidemics at the regional level based on real-time production. *Aquaculture,* 277 (3), pp. 164–173.

Birkhead, G.S., Klompas, M. and Shah, N.R. (2015) Uses of electronic health records for public health surveillance to advance public health. *Annual Review of Public Health,* 36, pp. 345–359.

Coastal Aquatic Animal Health Research Institute (2016a) *Disease surveillance and control projects in aquatic animals.* Available: http://www.aquathai.org/wed/projects/ [Accessed: 23 May 2017].

Coastal Aquatic Animal Health Research Institute (2016b) *Procedures for aquatic animal health services.* Available: http://www.aquathai.org/wed/home/service/ [Accessed: 22 May 2017].

Cromley, E.K. (2003) GIS and disease. *Annual Review of Public Health,* 24 (1), pp. 7–24.

Ferguson, N.M., Donnelly, C.A. and Anderson, R.M. (2001) Transmission intensity and impact of control policies on the foot and mouth epidemic in Great Britain. *Nature,* 413 (6855), pp. 542–548.

Fisheries Commodity Standard System and Traceability Division (2017) *List certified farm and operator for GAP CoC and organic on fisheries and aquaculture standards.* Available: http://www.fisheries.go.th/thacert [Accessed: 29 May 2017].

Giap, D.H., Yi, Y. and Yakupitiyage, A. (2005) GIS for land evaluation for shrimp farming in Haiphong of Vietnam. *Ocean & Coastal Management,* 48 (1), pp. 51–63.

Gillibrand, P.A. and Willis, K.J. (2007) Dispersal of sea louse larvae from salmon farms: modelling the influence of environmental conditions and larval behaviour. *Aquatic Biology*, 1 (1), pp. 63–75.

Green, D.M., Werkman, M. and Munro, L.A. (2012) The potential for targeted surveillance of live fish movements in Scotland. *Journal of Fish Diseases*, 35 (1), pp. 29–37.

Heenatigala, P. and Fernando, M. (2016) Occurrence of bacteria species responsible for vibriosis in shrimp pond culture systems in Sri Lanka and assessment of the suitable control measures. *Sri Lanka Journal of Aquatic Sciences*, 21 (1), pp. 1–17.

Laskowski-Arce, M.A. and Orth, K. (2008) Acanthamoeba castellanii promotes the survival of *Vibrio parahaemolyticus*. *Applied and Environmental Microbiology*, 74 (23), pp. 7183–7188.

Lipsitch, M., Cohen, T., Cooper, B., Robins, J.M., Ma, S., James, L., Gopalakrishna, G., Chew, S.K., Tan, C.C., Samore, M.H., Fisman, D. and Murray, M. (2003) Transmission dynamics and control of severe acute respiratory syndrome. *Science (New York, N.Y.)*, 300 (5627), pp. 1966–1970.

McReynolds, S.W., Sanderson, M.W., Reeves, A. and Hill, A.E. (2014) Modeling the impact of vaccination control strategies on a foot and mouth disease outbreak in the central United States. *Preventive Veterinary Medicine*, 117 (3), pp. 487–504.

Moonan, P.K., Bayona, M., Quitugua, T.N., Oppong, J., Dunbar, D., Jost, K.C., Burgess, G., Singh, K.P. and Weis, S.E. (2004) Using GIS technology to identify areas of tuberculosis transmission and incidence. *International Journal of Health Geographics,* 3 (1), pp. 23.

Murray, A. and Gillibrand, P. (2006) Modelling salmon lice dispersal in Loch Torridon, Scotland. *Marine Pollution Bulletin*, 53 (1), pp. 128–135.

NACA (2015) *Diseases of Crustaceans: hepatopancreatic microsporidiosis caused by Enterocytozoon hepatopenaei (EHP).* Available: http://enaca.org/publications/health/disease-cards/ehp-disease-card-2015.pdf [Accessed: 10 March 2017].

Naganathan, S., Muruganandam, M. and Venkatesan, V. (2014) Ecological and quorum sensing potential of *Vibrio* sp. from prawn farms of Tamil Nadu coastal regions. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3 (3), pp. 1102–1112.

National Bureau of Agricultural Commodity and Food Standards (2014) *Good aquaculture practices for marine shrimp farm.* Available: http://www.acfs.go.th/standard/download/GAP-FOR-MARINE-SHRIMP-FARM.pdf [Accessed: 10 March 2017].

National Bureau of Agricultural Commodity and Food Standards (2015) *Guidance on the application of Thai agricultural standard TAS 7401(G)-2015.* Available: http://www.acfs.go.th/standard/download/GAP_MARINE-SHRIMP-FARM.pdf [Accessed: 1 April 2017].

Nygård, K., Schimmer, B., Søbstad, Ø, Walde, A., Tveit, I., Langeland, N., Hausken, T. and Aavitsland, P. (2006) A large community outbreak of waterborne giardiasis-delayed detection in a non-endemic urban area. *BMC Public Health,* 6 (1), pp. 141.

Palaniyandi, M., Anand, P. and Maniyosai, R. (2014) GIS based community survey and systematic grid sampling for dengue epidemic surveillance, control, and management: a case study of Pondicherry Municipality. *Int.J of Mosquito Research*, 1 (4), pp. 72–78.

Pandey, A., Atkins, K.E., Medlock, J., Wenzel, N., Townsend, J.P., Childs, J.E., Nyenswah, T.G., Ndeffo-Mbah, M.L. and Galvani, A.P. (2014) Strategies for containing Ebola in west Africa. *Science (New York, N.Y.)*, 346 (6212), pp. 991–995.

Pui, C., Bilung, L., Mohd, N., Zainal, N., Vincent, M. and Apun, K. (2014) Risk of acquiring *Vibrio parahaemolyticus* in water and shrimp from an aquaculture farm. *Kuroshio Sci*, 8 (1), pp. 59–62.

Ribeiro-Lima, J., Carstensen, M., Cornicelli, L., Forester, J. and Wells, S. (2016) Patterns of cattle farm visitation by white-tailed deer in relation to risk of disease transmission in a previously infected area with bovine tuberculosis in Minnesota, USA. *Transboundary and Emerging Diseases*, pp. 1–11.

Rodriguez-Morales, A.J., Bedoya-Arias, J.E., Ramírez-Jaramillo, V., Montoya-Arias, C.P., Guerrero-Matituy, E.A. and Cárdenas-Giraldo, E.V. (2016) Using geographic information system (GIS) to mapping and assess changes in transmission patterns of chikungunya fever in municipalities of the Coffee-Triangle region of Colombia during 2014–2015 outbreak: Implications for travel advice. *Travel Medicine and Infectious Disease*, 14 (1), pp. 62–65.

Salachan, P.V., Jaroenlak, P., Thitamadee, S., Itsathitphaisarn, O. and Sritunyalucksana, K. (2017) Laboratory cohabitation challenge model for shrimp hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP). *BMC Veterinary Research*, 13 (1), pp. 9.

Salam, M.A., Ross, L.G. and Beveridge, C.M. (2003) A comparison of development opportunities for crab and shrimp aquaculture in southwestern Bangladesh, using GIS modelling. *Aquaculture*, 220 (1), pp. 477–494.

Salam, M.A., Khatun, N.A. and Ali, M.M. (2005) Carp farming potential in Barhatta Upazilla, Bangladesh: a GIS methodological perspective. *Aquaculture*, 245 (1), pp. 75–87.

Stoddard, S.T., Forshey, B.M., Morrison, A.C., Paz-Soldan, V.A., Vazquez-Prokopec, G.M., Astete, H., Reiner, R.C., Jr, Vilcarromero, S., Elder, J.P., Halsey, E.S., Kochel, T.J., Kitron, U. and Scott, T.W. (2013) House-to-house human movement drives dengue virus transmission. *Proceedings of the National Academy of Sciences of the United States of America*, 110 (3), pp. 994–999.

Tami, A., Grillet, M.E. and Grobusch, M.P. (2016) Applying geographical information systems (GIS) to arboviral disease surveillance and control: A powerful tool. *Travel Medicine and Infectious Disease*, 14 (1), pp. 9–10.

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P.V., Sritunyalucksana, K., Flegel, T.W. and Itsathitphaisarn, O. (2016) Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 452, pp. 69–87.

Thompson, F.L., Iida, T. and Swings, J. (2004) Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews*, 68 (3), pp. 403–431.

Tomassen, F.H.M., de Koeijer, A., Mourits, M.C.M., Dekker, A., Bouma, A. and Huirne, R.B.M. (2002) A decision-tree to optimise control measures during the early stage of a foot-and-mouth disease epidemic. *Preventive Veterinary Medicine*, 54 (4), pp. 301–324.

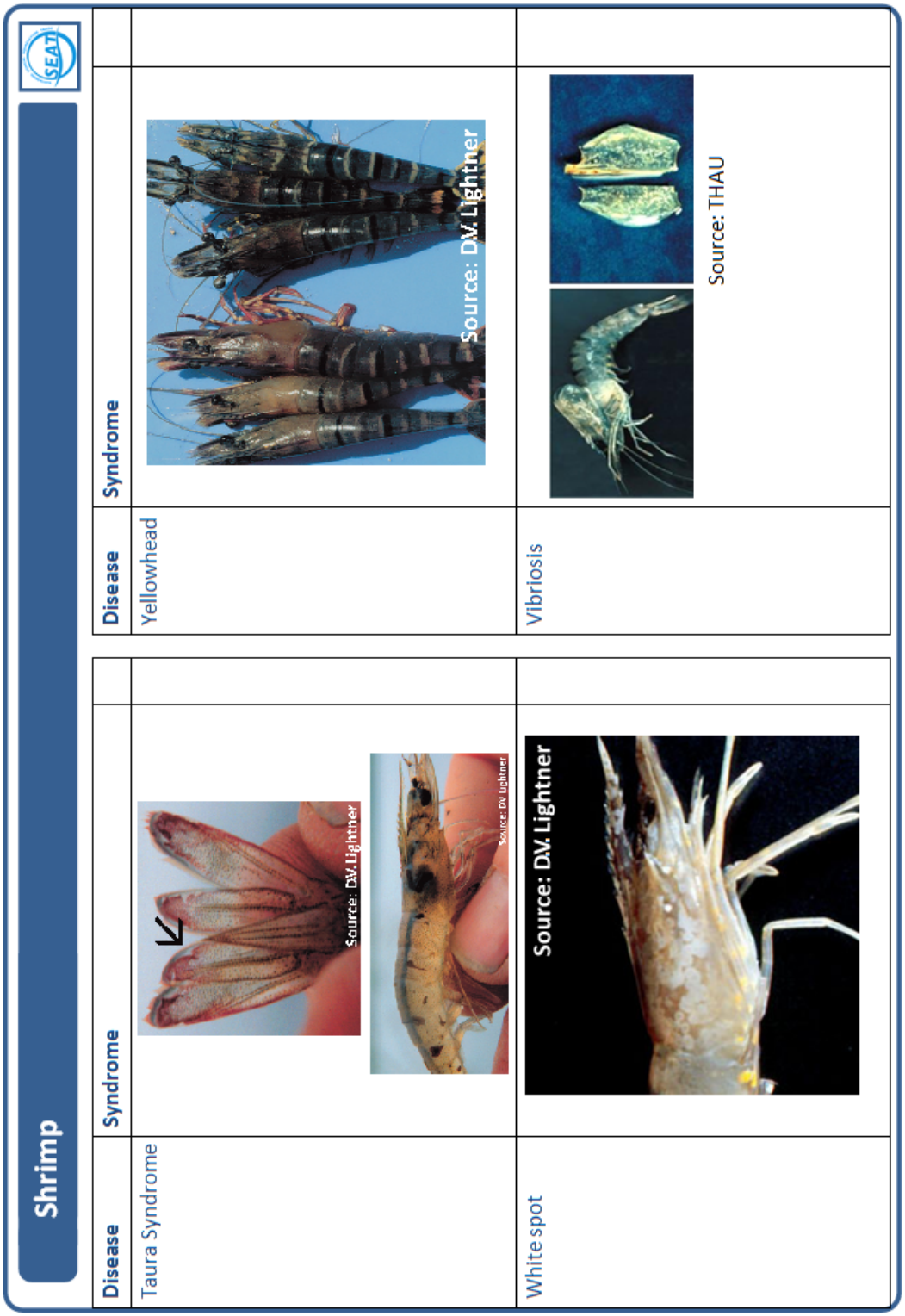
Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F., Horsberg, T.E. and Jackson, D. (2013) Salmon lice–impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases*, 36 (3), pp. 171–194.

Wendt, A., Kreienbrock, L. and Campe, A. (2015) Zoonotic disease surveillance–inventory of systems integrating human and animal disease information. *Zoonoses and Public Health*, 62 (1), pp. 61–74.

Wu, J.L., Namikoshi, A., Nishizawa, T., Mushiak, K., Teruya, K. and Muroga, K. (2001) Effects of shrimp density on transmission of penaeid acute viremia in *Penaeus japonicus* by cannibalism and the waterborne route. *Diseases of Aquatic Organisms*, 47 (2), pp. 129–135.

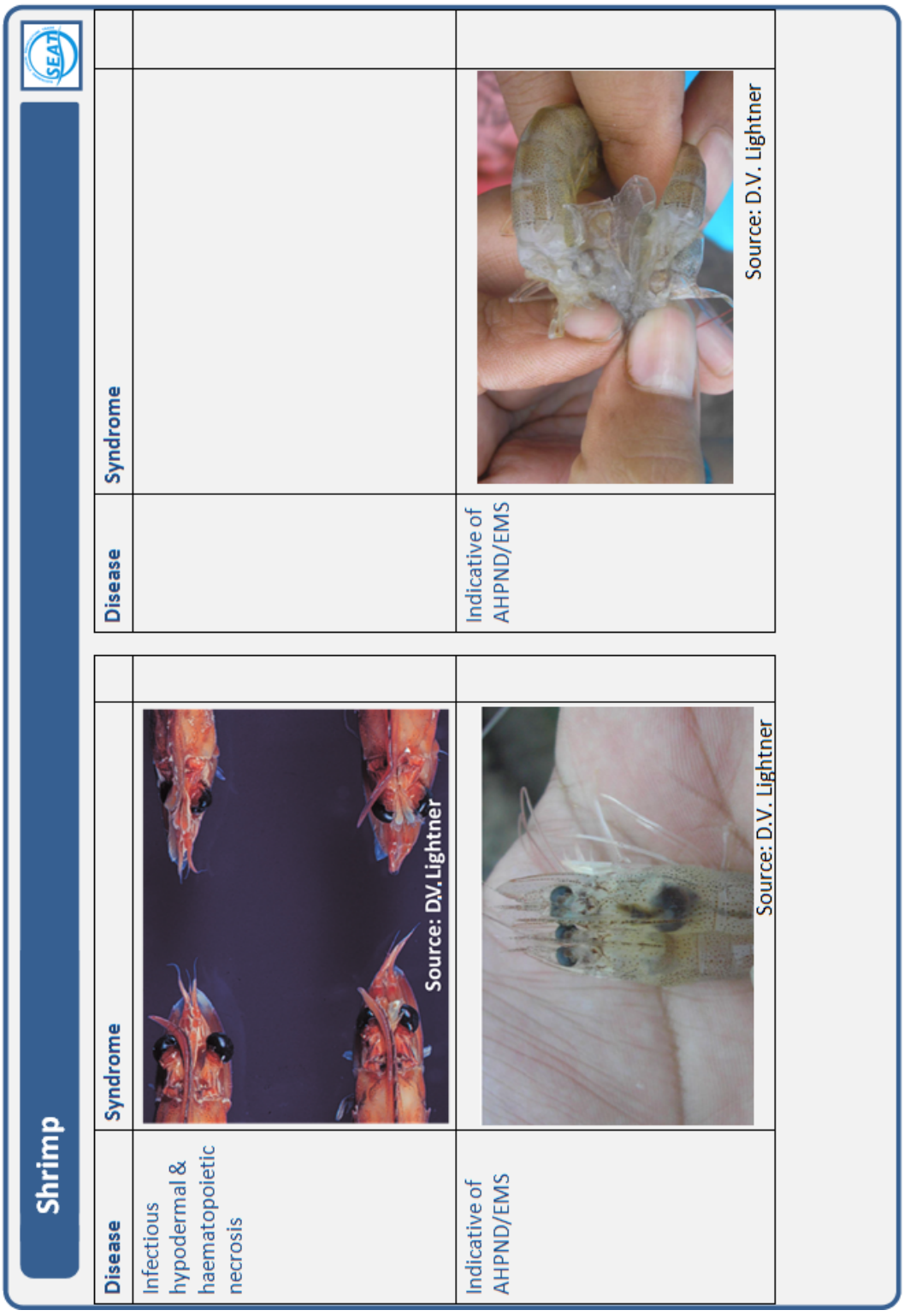
Zhou, S., Fernandez-Gutierrez, F., Kennedy, J., Cooksey, R., Atkinson, M., Denaxas, S., Siebert, S., Dixon, W.G., O'Neill, T.W. and Choy, E. (2016) Defining disease phenotypes in primary care electronic health records by a machine learning approach: a case study in identifying rheumatoid arthritis. *PloS One*, 11 (5), pp. e0154515.

Appendix A: Shrimp disease pictures



Picture cards showing gross signs of AHPND were shown alongside those showing clinical signs of other high-prevalence shrimp diseases while collecting data for AHPND cases and non-cases.

Appendix A: Shrimp disease pictures



Picture cards showing gross signs of AHPND were shown alongside those showing clinical signs of other high-prevalence shrimp diseases while collecting data for AHPND cases and non-cases.

Appendix B: Questions used in brief telephone survey

(***Note that the answers base on shrimp farming from year 2011 to current***)

Questions:

1. Does your farm face any disease problem? If 'yes' please tell me the name of disease?
2. If the farm is infected by AHPND, When do you see the AHPND-type losses for the first time? What are the signs of the shrimp that are clinically ill (Thai speakers call กุ้งป่วย/Kûng-Pèwy/) including the age of AHPND-diseased shrimp?
3. How many ponds affected with AHPND? What are the mortality rates in each affected pond?
4. Please give me the details about general management practices
   - Features of ongrowing ponds
   - Pond drying duration
   - Pond harrowing before stocking
5. What measures do you take to mitigate disease transmission or recurrence?

Appendix C: National provincial centres and abbreviation

Province code	National provincial centre	Abbreviation
11	Samutprakan	SPK
18	Chainat	CNT
73	Nakhonpathom	NPT
72	Suphanburi	SPB
61	Uthaithani	UTI
74	Samutsakhon	SKN
13	Pathumthani	PTE
14	Phranakhonsiayutthaya	AYA
26	Nakhonnayok	NYK
75	Samutsongkhram	SKM
10	Bangkok	BKK
60	Nakhonsawan	NSN
12	Nonthaburi	NBI
23	Trat	TRT
24	Chachoengsao	CCO
21	Rayong	RYG
20	Chonburi	CBI
22	Chanthaburi	CTI
25	Prachinburi	PRI
30	Nakhonratchasima	NMA
77	Prachuapkhirikhan	PKN
70	Ratchaburi	RBR
71	Kanchanaburi	KRI
76	Phetchaburi	PBI
90	Songkhla	SKA
94	Pattani	PTN
80	Nakhonsithammarat	NRT
84	Suratthani	SNI
86	Chumphon	CPN
82	Phangnga	PNA
81	Krabi	KBI
83	Phuket	PKT
96	Narathiwat	NWT
91	Satun	STN
85	Ranong	RNG
92	Trang	TRG
93	Phatthalung	PLG