VIBRIOSIS IN FLOATING MARINE CAGE-CULTURED FOOD FISHES

NURLIYANA MOHAMAD

FS 2019 67

VIBRIOSIS IN FLOATING MARINE CAGE-CULTURED FOOD FISHES

By

NURLIYANA MOHAMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2019

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

VIBRIOSIS IN FLOATING MARINE CAGE-CULTURED FOOD FISHES

By

NURLIYANA MOHAMAD

July 2019

Supervisor : Assoc. Prof. Mohammad Noor Amal Azmai, PhD
Faculty : Science

Vibriosis is a bacterial disease that is caused by several species from the genus of *Vibrio*. It leads to economic losses among the cage-cultured marine fishes throughout the world, including Malaysia. The lack of epidemiological data on vibriosis in this country has hindered the development of efficient curative and preventive measures to combat the disease. This study aimed to determine the prevalence of *Vibrio* spp., to identify the risk factors, transmission, clinical signs and histopathological changes in infected fish, and to understand the antibiotic resistance profiles and distribution of virulence genes of *Vibrio* spp. isolated from cage-cultured marine food fishes.

A cross-sectional and longitudinal study was conducted from December 2016 to August 2017, at a commercial floating cage farm located at Pulau Ketam, Klang, Selangor, Malaysia. Monthly samplings of Asian seabass, red snapper and hybrid grouper (giant grouper × tiger grouper) were carried out to isolate and identify *Vibrio* spp. At the same time, various biotic factors and environmental parameters of the sampling area were also determined. While sampling the fish, clinical signs and histopathological changes of fish naturally infected with vibriosis were noted. Pathogenicity study was carried out to further confirm the causal agent of disease in cultured fish. In addition, the possible transmission routes of *Vibrio* spp. in cultured fish were analyzed. Lastly, the distributions of virulence genes among *Vibrio* spp. were determined using PCR identification, while the antibiotic resistance patterns of the isolates were determined using disc diffusion method.

The highest occurrence of *Vibrio* spp. was found in the hybrid grouper (59%), followed by Asian seabass (31%) and red snapper (28%). Nine species of *Vibrio* were recovered from cultured fish in this study, where 88.1% of the isolated *Vibrio* belonged to the *Harveyi* clade, followed by the *Vulnificus* (6.6%) and the *Cholera* (0.6%) clades. Fish

mortality and size showed strong associations with the presence of some *Vibrio* spp. The water parameters were inconsistently correlated with *Vibrio* spp. in each fish species; the water SO_4, salinity and rainfall exhibited strong correlation with certain *Vibrio* spp. in the cultured fishes. In addition, fluctuation of physico-chemical properties of water may also impose stress to the cultured fish, thus increase their susceptibility to *Vibrio* infection.

Hybrid groupers naturally infected by *Vibrio harveyi* and *V. alginolyticus* displayed lethargy, excessive mucus production, fins rot, enlargement of spleens and generalize congestion of the brains and internal organs. Experimentally infected Asian seabass demonstrated similar clinical signs and histopathological changes as naturally infected hybrid groupers. Asian seabass infected with single *V. alginolyticus* and co-infected with both *Vibrio* spp. resulted in 100% mortality. However, concurrently infected fish demonstrated severe clinical signs and histopathological changes compared to single infections, indicating bigger impact brought by concurrent infections to cultured fish.

In addition, *pyrH* gene sequences of 160 isolates of *V. alginolyticus*, *V. campbellii*, *V. parahaemolyticus* and *V. harveyi* recovered from cultured fish, wild fish, trash fish, fish fry, water and sediment were used to investigate the degree of relatedness and possible transmission existing between the isolated *Vibrio* spp. The population tree revealed the possible transmission from the newly introduced fish fry and wild fish into the cultured fish, while water also might possibly serve as a natural transmission medium of certain *Vibrio* spp. in this fish farm.

Typical virulence genes produced by pathogenic *V. harveyi* were widely distributed among the 63 *Vibrio* spp. isolated from fish in various geographical regions of Peninsular Malaysia. Multiple antibiotic resistance (MAR) was exhibited in all *Vibrio* strains, particularly against ampicillin, penicillin, polypeptides, cephems, and streptomycin, where 75% of the isolates have MAR index of higher than 0.20. Host species and geographical origin showed no correlation with the presence of virulence genes and the antibiotic resistance pattern of the *Vibrio* spp.

In conclusion, this study demonstrated a yearlong presence of *Vibrio* spp. in cultured fishes, where fluctuating water physico-chemical properties influenced the presence of certain *Vibrio* spp. Exposure of cultured fish to different *Vibrio* spp. may also resulted in synergistic interactions between the pathogens, thus increasing the severity of the infection. In addition, wild and fry fish could transmit the *Vibrio* spp. to the cultured fish in cage farm. Lastly, the majority of *Vibrio* spp. isolated exhibited multiple antibiotic resistances, which represents a real concern and warrants on-going surveillance. The data presented in this thesis offer an updated information on vibriosis in cultured marine fishes in Malaysia, which could help in future effective control measures and contribute to the insights on methods to prevent the disease in cultured fish.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENYAKIT VIBRIOSIS PADA IKAN MARIN MAKANAN DALAM KULTUR SANGKAR TERAPUNG

Oleh

NURLIYANA MOHAMAD

Julai 2019

Penyelia : Prof. Madya Mohammad Noor Amal Azmai, PhD
Fakulti : Sains

Vibriosis adalah merupakan penyakit yang disebabkan oleh beberapa spesies bakteria dari genus *Vibrio*. Penyakit ini telah mendatangkan kerugian ekonomi di seluruh dunia, termasuk Malaysia. Kekurangan data epidemiologi mengenai vibriosis di negara ini telah menghalang perkembangan langkah-langkah kuratif dan pencegahan yang berkesan untuk memerangi vibriosis. Kajian ini dijalankan bertujuan untuk menentukan prevalens, faktor risiko, penyebaran, tanda klinikal dan perubahan histopatologi dalam ikan yang dijangkiti vibriosis, serta untuk memahami profil rintangan antibiotik dan pengagihan gen virulen dalam spesies *Vibrio* yang dipencil dari ikan sangkar.

Satu kajian rentas dan longitudinal telah dijalankan dari Disember 2016 sehingga Ogos 2017, di sangkar terapung berskala komersial yang terletak di Pulau Ketam, Klang, Selangor, Malaysia. Persampelan bulanan terhadap ikan siakap, ikan merah dan kerapu hibrid (kerapu kertang × kerapu harimau) telah dijalankan untuk memencilkan dan mengenal pasti spesies *Vibrio*. Pada masa yang sama, pelbagai faktor biotik dan parameter alam sekitar di kawasan itu juga ditentukan. Tanda-tanda klinikal dan perubahan histopatologi ikan yang dijangkiti vibriosis secara semulajadi telah direkodkan. Ujian patogenisiti dilaksanakan bagi mengesahkan agen penyebab penyakit dalam ikan sangkar. Selain itu, kemungkinan penyebaran spesies *Vibrio* dalam ikan sangkar juga dianalisis. Akhir sekali, pengagihan gen virulen di kalangan spesies *Vibrio* ditentukan dengan menggunakan teknik PCR, manakala profil rintangan antibiotik ditentukan dengan menggunakan kaedah resapan cakera.

Prevalens tertinggi spesies *Vibrio* direkodkan dalam kerapu hibrid (59%), di ikuti oleh siakap (31%) dan ikan merah (28%). Sembilan spesies *Vibrio* telah dipencil dari ikan sangkar dalam kajian ini, di mana 88.1% daripada *Vibrio* yang dipencilkan terdiri daripada kumpulan *Harveyi*, diikuti oleh *Vulnificus* (5.5%) dan *Cholera* (0.6%). Kadar

kematian dan saiz ikan menunjukkan hubungan yang kuat dengan kehadiran beberapa spesies *Vibrio*. Parameter air tidak mempunyai hubungan yang konsisten dengan spesies *Vibrio* dalam setiap spesies ikan. Bagaimanapun, sulfat, kemasinan dan kehadiran hujan menunjukkan hubungan yang kuat dengan kehadiran sesetengah spesies *Vibrio* dalam ikan sangkar. Di samping itu, ketidakseimbangan ciri fiziko-kimia air juga boleh menyebabkan tekanan kepada ikan sangkar, lantas meningkatkan kecendurangan mereka terhadap jangkitan oleh *Vibrio*.

Kerapu hibrid yang dijangkiti oleh *Vibrio harveyi* dan *V. alginolyticus* secara semulajadi telah menunjukkan kelesuan, pengeluaran lendir yang berlebihan, pereputan sirip, pembengkakan limpa dan kesebakan pada otak dan organ dalaman. Siakap yang dijangkiti secara eksperimen menunjukkan tanda-tanda klinikal dan perubahan histopatologi yang sama seperti kerapu hibrid yang dijangkiti secara semulajadi. Siakap yang dijangkiti oleh *V. alginolyticus* dan juga kedua-dua *Vibrio* telah mengakibatkan kematian sebanyak 100%. Walau bagaimanapun, ikan yang dijangkiti oleh dua spesies *Vibrio* serentak menunjukkan tanda-tanda klinikal dan perubahan histopatologi yang lebih teruk berbanding dengan jangkitan tunggal, menunjukkan kesan yang lebih besar yang dibawa oleh jangkitan serentak kepada ikan sangkar.

Selain itu, urutan gen *pyrH* daripada 160 *V. alginolyticus*, *V. campbellii*, *V. parahaemolyticus* dan *V. harveyi* yang dipencil dari ikan sangkar, ikan liar, ikan baja, anak ikan, sampel air dan tanah digunakan untuk menyiasat hubungan filogenetik dan kemungkinan penyebaran di antara *Vibrio* yang terpencil. Analisi filogenetik mendedahkan kemungkinan bahawa anak ikan yang baru dilepaskan dalam sangkar dan ikan liar boleh menyebarkan *Vibrio* kepada ikan sangkar, sementara air berkemungkinan memainkan peranan sebagai medium penghantaran semulajadi *Vibrio* di dalam sangkar ikan.

Gen tipikal yang dihasilkan oleh patogen *V. harveyi* teredar secara meluas di kalangan 63 *Vibrio* yang dipencil dari ikan sangkar dari pelbagai kawasan di Semenanjung Malaysia. Rintangan antibiotik yang tinggi telah dipamerkan oleh semua *Vibrio*, terutamanya terhadap ampisilin, penisilin, polipeptida, cefem, dan streptomisin. Indeks rintangan pelbagai antibiotik adalah di antara 0.06 dan 0.56, dan 75% daripada isolat mempunyai indeks rintangan pelbagai antibiotik yang lebih tinggi daripada 0.20. Spesies perumah dan asal geografi tidak menunjukkan perhubungan di antara kehadiran gen virulen dan corak rintangan antibiotik dalam *Vibrio*.

Kesimpulannya, kajian ini membuktikan kehadiran *Vibrio* sepanjang tahun di dalam ikan sangkar di mana sifat fiziko-kimia kimia air yang tidak stabil mempengaruhi kehadiran spesies *Vibrio*. Pendedahan ikan sangkar kepada pelbagai spesies *Vibrio* juga boleh menyebabkan interaksi sinergistik antara patogen, dan seterusnya meningkatkan impak jangkitan. Di samping itu, ikan liar dan anak ikan boleh menjadi pembawa *Vibrio* kepada ikan laut yang diternak di dalam sangkar. Terakhir, majoriti *Vibrio* menunjukan pelbagai rintangan antibiotik yang membawa kepada kebimbangan dan memerlukan pengawasan yang berterusan. Data yang dikemukakan dalam tesis ini

menyumbang kepada maklumat terkini mengenai vibriosis di kalangan ikan sangkar di Malaysia, yang dapat membantu dalam langkah-langkah kawalan yang lebih berkesan dan menyumbang kepada pandangan mengenai kaedah pencegahan penyakit ini dalam ikan sangkar pada masa akan datang.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful.

Alhamdulillah, all praise to Allah S.W.T. for His blessings and guidance, so that I am able to accomplish this thesis as a partial fulfilment of the requirements for my Doctor of Philosophy degree in Universiti Putra Malaysia.

Foremost, I would like to express my sincere gratitude and appreciation to my main supervisor Assoc. Prof. Dr. Mohammad Noor Amal Azmai for the continuous support of my study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I would also like to thank him for his friendship, empathy and great sense of humor. I could not have imagined having a better advisor and mentor for my PhD study. I also would like to thank to my co-supervisors, Assoc. Prof. Dr. Ina Salwany Md Yasin, Prof. Dr. Mohd Zamri Saad and Assoc. Prof. Dr. Muskhazli Mustafa for their encouragement, insightful comments and suggestions.

I want to express my thanks to my friends for all the fun we had in the past years and for helping me survive all the stress. Most importantly, I want to thank Nur Sharmila Sharip and Nor Royhaila Mohamad, for the stimulating discussions, for the sleepless nights we were working together before deadlines and for the endless moral support. My sincere thanks also go to fellow labmates from Aquatic Laboratory, Department of Biology, Faculty of Science, UPM, for countless assistances and supports.

Last but not least, I would like to thank my beloved parents, Mohamad Senik and Romlah Omar for believing in me and supporting me throughout my life. Also not forgetting my fiancé, Mizanurfakhri Ghazali for his love and care. I would not be who I am today without these people in my life.

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mohammad Noor Amal Azmai, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Ina Salwany Md. Yasin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohd Zamri Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Muskhazli Mustafa, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

_____
ROBIAH BINTI YUNUS, PhD
Professor and Dean School of
Graduate Studies Universiti Putra
Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature:_____ Date: _____

Name and Matric No.: Nurliyana Mohamad (GS48741)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in Rule 41 Rules 2003 (Revision 2012-2013) were adhered to.

Signature : _____
Name of Chairman
of Supervisory Committee : Assoc. Prof. Dr. Mohammad Noor Amal Azmai

Signature : _____
Name of Member
of Supervisory Committee : Assoc. Prof. Dr. Ina Salwany Md. Yasin

Signature : _____
Name of Member of
of Supervisory Committee : Prof. Dr. Mohd Zamri Saad

Signature : _____
Name of Member of
of Supervisory Committee : Assoc. Prof. Dr. Muskhazli Mustafa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AHPND	Acute Hepatopancreatic Necrosis Disease
AFS	Asian Fisheries Society
ARGs	Antibiotic Resistant Genes
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
EO	Essential oil
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
FAO	Food and Agriculture Organization
IPNV	Infectious Pancreatic Necrosis Virus
MABV-F	Marine Birnavirus
MAR	Multiple Antibiotic Resistant
MEGA	Molecular Evolutionary Genetics Analysis
MLSA	Multilocus Sequence Analysis
MS 222	Tricane Methanesulfonate
NCBI	National Center for Biotechnology Information
RAPD	Random Amplification of Polymorphic DNA
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose
TSA	Tryptone Soya Agar
TSB	Tryptone Soy Broth
VNN	Viral Nervous Necrosis

LIST OF FISH SPECIES

Accepted name	Scientific name
African sharptooth catfish	*Clarias gariepinus* (Burchell, 1822)
Arabian surgeon fish	*Acanthurus sohal* (Forsskål, 1775)
Asian seabass	*Lates calcarifer* (Bloch, 1790)
Atlantic cod	*Gadus morhua* Linnaeus, 1758
Atlantic halibut	*Hippoglossus hippoglossus* (Linnaeus, 1758)
Atlantic salmon	*Salmo salar* Linnaeus, 1758
Ayu	*Plecoglossus altivelis* (Temminck & Schlegel, 1846)
Bighead carp	*Hypophthalmichthys nobilis* (Richardson, 1845)
Black seabream	*Acanthopagrus schlegelii* (Bleeker, 1854)
Black rockfish	*Sebastes schlegeli* (Valenciennes, 1828)
Blackspot seabream	*Pagellus bogaraveo* (Brünnich, 1768)
Bluefin tuna	*Thunnus* sp.
Brown spotted grouper	*Epinephelus tauvina* (Forsskål, 1775)
Brown shark	*Carcharhinus plumbeus* (Nardo, 1827)
Camouflage grouper	*Epinephelus polyphekadion* (Bleeker, 1849)
Chinook salmon	*Oncorhynchus tshawytscha* (Walbaum, 1792)
Cobia	*Rachycentron canadum* (Linnaeus, 1766)
Coho salmon	*Oncorhynchus kisutch* (Walbaum, 1792)
Common snook	*Centropomus undecimalis* (Bloch, 1792)
Common sole	*Solea solea* (Linnaeus, 1758)
Crimson snapper	*Lutjanus erythropterus* Bloch, 1790
European eels	*Anguilla anguilla* (Linnaeus, 1758)
European seabass	*Dicentrarchus labrax* (Linnaeus, 1758)
Flounder	*Paralichthys* sp.
Giant grouper	*Epinephelus lanceolatus* (Bloch, 1790)
Gilthead seabream	*Sparus aurata* Linnaeus, 1758
Golden pompano	*Trachinotus ovatus* (Linnaeus, 1758)
Golden trevally	*Gnathanodon speciosus* (Forsskål, 1775)
Grass carp	*Ctenopharyngodon idella* (Valenciennes, 1844)
Green grouper	*Epinephelus coioides* (Hamilton, 1822)
Groupers	*Epinephelus* sp.
Half-smooth tongue sole	*Cynoglossus semilaevis* Günther, 1873
Horse mackerel	*Carangoides malabaricus* (Bloch & Schneider, 1801)

Hybrid striped bass	*Morone saxatilis* × *M. chrysops*
Iberian toothcarp	*Aphanius iberus* (Valenciennes, 1846)
Indian threadfin	*Alectis indica* (Rüppell, 1830)
Jack crevalle	*Caranx hippos* (Linnaeus, 1766)
Japanese horse mackerel	*Trachurus japonicus* (Temminck & Schlegel, 1844)
Japanese parrotfish	*Oplegnathus fasciatus* (Temminck & Schlegel, 1844)
John's snapper	*Lutjanus johnii* (Bloch, 1792)
Lemon sharks	*Negaprion brevirostris* (Poey, 1868)
Malabar grouper	*Epinephelus malabaricus* (Bloch & Schneider, 1801)
Malabar snapper	*Lutjanus inermis* (Peters, 1869)
Mangrove snapper	*Lutjanus griseus* (Linnaeus, 1758)
Mangrove red snapper	*Lutjanus argentimaculatus* (Forsskål, 1775)
Marine sunfish	*Mola mola* (Linnaeus, 1758)
Meagre	*Argyrosomus regius* (Asso, 1801)
Milkfish	*Chanos chanos* (Forsskål, 1775)
Humpback grouper	*Cromileptes altivelis* (Valenciennes, 1828)
Mullet	*Mugilidae* sp.
Nile tilapia	*Oreochromis niloticus* (Linnaeus, 1758)
Olive flounder	*Paralichthys olivaceus* (Temminck & Schlegel, 1846)
Orange-spotted grouper	*Epinephelus coioides* (Hamilton, 1822)
Pacific salmon	*Oncorhynchus* sp.
Pompano	*Trachinotus blochii* (Lacepède, 1801)
Rainbow trout	*Oncorhynchus mykiss* (Walbaum, 1792)
Redbanded seabream	*Pagrus auriga* Valenciennes, 1843
Red drum	*Sciaenops ocellatus* (Linnaeus, 1766)
Red seabream	*Pagrus major* (Temminck & Schlegel, 1843)
Red snapper	*Lutjanus* sp.
Sagor catfish	*Hexanematichthys sagor* (Hamilton, 1822)
Saithe	*Pollachius virens* (Linnaeus, 1758)
Sea mullet	*Mugil cephalus* Linnaeus, 1758
Sebae clownfish	*Amphiprion sebae* Bleeker, 1853
Senegalese sole	*Solea senegalensis* Kaup, 1858
Silver carp	*Hypophthalmichthys molitrix* (Valenciennes, 1844)
Silver seabream	*Pagrus auratus* (Forster, 1801)
Silver pomfret	*Pampus argenteus* (Euphrasen, 1788)
Silvery black porgy	*Sparidentex hasta* (Valenciennes, 1830)
Southern catfish	*Silurus soldatovi meridionalis* Chen, 1977

Spotted grouper	*Epinephelus analogus* Gill, 1863
Spotted sicklefish	*Drepane punctata* (Linnaeus, 1758)
Striped bass	*Morone saxatilis* (Walbaum, 1792)
Striped jack	*Pseudocaranx dentex* (Bloch & Schneider, 1801)
Summer flounder	*Paralichthys dentatus* (Linnaeus, 1766)
Tiger grouper/Brown-marbled grouper	*Epinephelus fuscoguttatus* (Forsskål, 1775)
Tilapia	*Oreochromis aureus* (Steindachner, 1864)
Turbot	*Scophthalmus maximus* (Linnaeus, 1758)
White perch	*Morone americana* (Gmelin, 1789)
White spotted spinefoot	*Siganus canaliculatus* (Park, 1797)
Yellow catfish	*Tachysurus fulvidraco* (Richardson, 1846)
Yellow croaker	*Larimichthys crocea* (Richardson, 1846)
Yellow grouper	*Epinephelus awoara* (Temminck & Schlegel, 1842)
Yellowtail	*Seriola quinqueradiata* Temminck & Schlegel, 1845
Yellowstreaked snapper	*Lutjanus lemniscatus* (Valenciennes, 1828)
Zhengchuan catfish	*Silurus soldatovi meridionalis* × *S. asotus*

CHAPTER 1

INTRODUCTION

1.1 General introduction

Vibrio takes its name from the Latin word Vibrare, meaning 'to wave' (Nishiguchi and Jones, 2004). *Vibrio*, which belongs to the family Vibrionaceae, is motile Gram-negative bacteria widely distributed in marine and estuarine environment. *Vibrio* can be found in various vertebrate and invertebrate marine animals, aquatic plants and sediments or freely living in the water column (Chase *et al*., 2015). This bacterium is highly adaptable, robust and able to persist in aquatic environment even during harsh conditions (Vezzulli *et al.,* 2015). Some species of *Vibrio* are important for natural systems such as the nutrient cycle (Thompson *et al*., 2004). However, *Vibrio* received the attention of many as the causative agent of a disease in aquatic animals.

Vibriosis is a disease that develops following infection by several species of pathogenic *Vibrio* (Haenen *et al*., 2014). Vibriosis has been recognised since 1718. However, the first confirmed *Vibrio* infection in fish was reported in 1893, where it causes massive mortality among migrating wild eels (Canestrini, 1893). The diseases that develop following infection by *Vibrio* had started to receive serious attention when it became a threat to the economically important farmed fish such as salmonids in America and Europe (Sinderman *et al*., 1990; Kashulin *et al*., 2017). Since then, vibriosis is recognised as one of the notable diseases with huge economic impact on aquaculture worldwide (Abdel-Aziz *et al*., 2013; Marudhupandi *et al*., 2017). Previously, this disease has severely affected the shrimp aquaculture industry causing a significant economic loss of more than USD 1 billion (Zorriehzahra and Banaederakhshan, 2015). In recent years, losses due to vibriosis among cage-cultured fish have been reported in Asian country including Japan, India, Thailand, Vietnam and China (Mohi *et al*., 2010; Khouadja *et al*., 2013; Haenen *et al*., 2014; Dong *et al*., 2017; Rameshkumar *et al*., 2017; Zhu *et al*., 2017).

1.2 Problem statements

Epidemiology of vibriosis in Malaysian aquaculture sector remains poorly understood. To date, no comprehensive study has been conducted on the epidemiology of vibriosis in cage-cultured marine fish in Malaysia. Predominant species of *Vibrio* among cage-cultured marine food fish are not clearly known, especially when large number of *Vibrio* species can be present in marine environment (You *et al*., 2016). Given that many *Vibrio* species shared high similarity in biochemical characteristic, biochemical identification-based methods used in previous studies may lead to misidentifying

and/or underestimating the prevalence of certain species of *Vibrio* in cultured fish (Croci *et al*., 2007).

Besides, the influence of various biotic and abiotic factors on the occurrence and abundance of *Vibrio* spp. in cage-cultured marine food fish are not completely understood. The distribution, composition and abundance of *Vibrio* spp. in aquatic environment are known to be dependant on the geographical location and the environmental conditions (Takemura *et al*., 2014). However, the correlation between the environmental factors and the occurrence of individual *Vibrio* spp. in cage-cultured marine fish were not extensively discussed.

The presence of *Vibrio* spp. in cultured fish does not always cause a disease problem to the fish. Depending on the various factors such as environmental stressors and host health status, *Vibrio* manifestation in fish may resulted in either acute or chronic infections, producing various clinical signs, gross lesions and pathological changes (Bellos *et al.,* 2015; Liu *et al.,* 2016). Clinical signs and gross lesions particularly are very important as they serve as an indicator for *Vibrio* infection, ensuring rapid recognition of the disease (Noga *et al*., 2010). However, clinical signs demonstrated by fish suffered from vibriosis are often non-distinguishable from other bacterial and viral infection. In addition, the presence of multiple infections by *Vibrio* spp. in cultured fishes are often negligible. Since cultured fishes are always exposed to various pathogens, it is important to understand the consequences of concurrent infection in cultured fish.

Horizontal transmission was reported as the most probable route in vibriosis (Leong and Colorni, 2002; Takemura *et al*., 2014). However, the role of water, sediment, fry fish, wild fish and feed in transmission of vibriosis had not been studied. Assessing the possible routes of infection is important for the biosecurity of the farm, to prevent the introduction of potentially pathogenic *Vibrio* into grow-out cages and control the spread of the infection when it occurs.

Lastly, the virulence factors associated with the pathogenicity of *Vibrio* spp. in Malaysian cage-cultured marine fish were yet clarified. With the emerging concern on the multiple antibiotic resistant (MAR) bacterial populations, which threatens both aquaculture sectors and public health, it is essential to monitor the MAR among *Vibrio* species as well as preventing their widespread. On the other hand, expression of the virulence genes by the *Vibrio* spp. is important to assist the bacteria to enter and colonize the host, avoiding host defences and causing damage to the host (Ruwandeepika *et al*., 2010). Thus, information on the distribution and expression of virulence genes in *Vibrio* spp. may contribute invaluable insights to a more environmentally friendly approaches to treat *Vibrio* infection in cage-cultured fish. Better understanding of the epidemiology of vibriosis will facilitate further development of efficient curative and preventive measures to combat this disease.

1.3 Hypotheses

1. Objective 1:
   H_0: The presence of *Vibrio* spp. in cultured fishes will not be influenced by different host species, host size and host health condition.
   $H\alpha$: The presence of *Vibrio* spp. in cultured fishes will be influenced by different host species, host size and host health condition.

2. Objective 2:
   H_0: Water physico-chemical parameters have no influence on the presence of *Vibrio* spp. in cultured fishes.
   $H\alpha$: Water physico-chemical parameters will influence on the presence of *Vibrio* spp. in cultured fishes.

3. Objective 3:
   H_0: Cultured fishes affected with vibriosis will not demonstrate certain clinical signs, gross lesions and pathological changes.
   $H\alpha$: Cultured fishes affected with vibriosis will demonstrate certain clinical signs, gross lesions and pathological changes.

4. Objective 4:
   H_0: No possible transmission of *Vibrio* spp. from other sources such as newly introduced fry, wild fish, feed and from surrounding environments including water and sediments.
   $H\alpha$: *Vibrio* spp. may be transmitted or spread from other sources such as newly introduced fry, wild fish, feed and from surrounding environments including water and sediments.

5. Objective 5:
   H_0: *Vibrio* spp. in cultured fishes does not possess virulence genes associated with fish and human disease. In addition, antibiotic resistant *Vibrio* strains are not detected in cultured fishes.
   $H\alpha$: *Vibrio* spp. in cultured fishes posses virulence genes associated with fish and human disease. In addition, antibiotic resistant *Vibrio* strains will be detected in cultured fishes.

1.4 Objectives

1. To determine the prevalence of *Vibrio* spp. in floating cage-cultured Asian seabass, red snapper, hybrid grouper (giant grouper × tiger grouper) and its culturing environment.
2. To identify the risk factors that influences the presence of *Vibrio* spp. in the floating marine cage-cultured food fishes.
3. To describe the associated clinical signs and histopathological changes of the fish naturally and experimentally infected with vibriosis.
4. To determine the possible transmission routes of *Vibrio* spp. in the floating marine cage-cultured food fishes.
5. To analyse the distribution of virulence genes and the antibiotic resistance patterns of *Vibrio* spp. isolated from floating marine cage-cultured food fishes.

The objectives in this thesis could be integrated as described below:

1. Objective 1: This objective determined the prevalence of *Vibrio* spp. in Asian seabass, red snapper and hybrid grouper that were cultured in a farm between December 2016 and August 2017. The correlation between the presence of *Vibrio* spp. in cultured fishes with biotic factors such as the host species, clinical signs, gross lesions, mortality and size were also investigated. Data of Objectives 1 and 2 had been published in Journal of Aquatic Animal Health, 31: 154-167, 2019. DOI: 10.1002/aah.10062.

2. Objective 2: For this objective, the risk factors that influence the presence of *Vibrio* spp. in cultured Asian seabass, red snapper and hybrid grouper in Objective 1 were identifed. Environmental parameters including temperature, pH, salinity, turbidity, dissolved oxygen, micronutrients and rainfall were recorded throughout the sampling period and analysed by using statistical analysis. Data of Objectives 1 and 2 had been published in Journal of Aquatic Animal Health, 31: 154-167, 2019. DOI: 10.1002/aah.10062.

3. Objective 3: This objective described the clinical signs, gross lesions and histopathological changes of fish naturally and experimentally infected with *Vibrio* spp. An outbreak of vibriosis had occurred during the data collection for Objectives 1 and 2. The pathogens were isolated and identified, while the pathogenicity test was carried out in the laboratory to confirm the etiological agent of the outbreak and to reproduce the observed clinical signs, gross lesions and histopathological changes in model fish. Data of Objective 3 had been published in Journal of Aquatic Animal Health, 31(1): 88-96, 2019. DOI: 10.1002/aah.10055.

4. Objective 4: This study determined the possible transmission routes of *Vibrio* spp. in floating cage-cultured marine food fishes. Representative

Vibrio spp. that were isolated from various biological and environmental sources including cultured fish, fry and wild fish, water and sediment in Objectives 1 and 2 were used in this study. Their genetic sequences were compared by using phylogenetic analyses in order to determine the relatedness between the *Vibrio* strains of the same species. Data of Objective 4 had been published in Letters in Applied Microbiology, 68(66): 485-496, 2019. DOI: 10.1111/lam.13146.

5. Objective 5: For this objective, the distribution of virulence genes and the antibiotic resistance patterns of *Vibrio* spp. isolated from Objectives 1 and 2, together with *Vibrio* isolates that were colleted from previous studies were analysed. Firstly, the presence of selected virulence associated genes in the representative *Vibrio* spp. were investigated. Then, the extent of antibiotic resistance of *Vibrio* spp. isolated from marine fishes cultured in various farms in Peninsular Malaysia were discussed. Data of Objectives 5 had been published in BMC Veterinary Research, 15:176, 2019. DOI: 10.1186/s12917-019-1907-8.

REFERENCES

Abdel-Aziz, M., Eissa, A. E., Hanna, M., and Okada, M. A. (2013). Identifying some pathogenic *Vibrio/Photobacterium* species during mass mortalities of cultured gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) from some Egyptian coastal provinces. *International Journal of Veterinary Science and Medicine,* 1(2): 87-95.

Abdel-Aziz, M., Ibrahem, M. D., Ibrahim, M. A., Abu-elala, N. M., and Abdel-moneam, D. A. (2017). Monitoring of different *Vibrio* species affecting marine fishes in Lake Qarun and Gulf of Suez: Phenotypic and molecular characterization. *The Egyptian Journal of Aquatic Research*, 43(2): 141-146.

Abdullah, A., Ramli, R., Ridzuan, M. S. M., Murni, M., Hashim, S., Sudirwan, F., Abdullah, S. Z., Mansor, N. N., Amira, S., Zamri-Saad, M., and Amal, M. N. A. (2017). The presence of Vibrionaceae, *Betanodavirus* and *Iridovirus* in marine cage cultured fish: Role of fish size, water physicochemical parameters and relationships among the pathogens. *Aquaculture Reports,* 7: 57-65.

Abu-Elala, N. M., Abd-Elsalam, R. M., and Marzouk, M. S. (2015). Molecular and Immunohistochemical diagnosis of *Photobacterium damselae* subspecies *piscicida* during naturally occurring disease in Egypt. *Journal of the World Aquaculture Society,* 46(6): 583-595.

AFS. Annual Fisheries Statistic (2016). Annual Fisheries Statistic. Department of Fisheries, Ministry of Agriculture and Agro-Based Industry, Malaysia.

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): 1395-1404.

Akayli, T., Timur, G., Aydemir, B., Kiziler, A. R., Coskun, O., Albayrak, G., and Arican, E. (2008). Characterization of *Vibrio alginolyticus* isolates from diseased culture gilthead sea bream, *Sparus aurata*. *Israeli Journal of Aquaculture-Bamidgeh*, 60(2): 89-94.

Akayli, T., Timur, G., Albayrak, G., and Aydemir, B. (2010). Identification and genotyping of *Vibrio ordalii*: a comparison of different methods. *Israeli Journal of Aquaculture-Bamidgeh,* 62(1): 9-18.

Al-Othrubi, S. M., Kqueen, C. Y., Mirhosseini, H., Hadi, Y. A., and Radu, S. (2014). Antibiotic resistance of *Vibrio parahaemolyticus* isolated from cockles and shrimp sea food marketed in Selangor, Malaysia. *Clinical Microbiology,* 3(3): 148-154.

Al-Sunaiher, A. E., Ibrahim, A. S. S., and Al-Salamah, A. A. (2010). Association of *Vibrio* species with disease incidence in some cultured fishes in the Kingdom of Saudi Arabia. *World Applied Sciences Journal,* 8(5): 653-660.

Albert, V., and Ransangan, J. (2013). Effect of water temperature on susceptibility of culture marine fish species to vibriosis. *International Journal of Research in Pure and Applied Microbiology,* 3(3): 48-52.

Alexopoulos, A., Plessas, S., Voidarou, C., Noussias, H., and Stavropoulou, E. (2011). Microbial ecology of fish species on growing in Greek sea farms and their watery environment. *Anaerobe*, 17(6): 264-266.

Alves Jr, N., Neto, O. S. M., Silva, B. S., De Moura, R. L., Francini-Filho, R. B., Barreira e Castro, C., Paranhos, R., Bitner-Mathe, B. C., Kruger, R. H., Vicente, A. C. P., Thompson, C. C., and Thompson, F. L. (2010). Diversity and pathogenic potential of vibrios isolated from Abrolhos Bank corals. *Environmental Microbiology Reports,* 2(1): 90-95.

Amal, M. N. A., Zarif, S. T., Suhaiba, S. M., Aidil, M. R. M., Shaqinah, N. N., Zamri-Saad, M., and Ismail, A. (2018). The effects of fish gender on susceptibility to acute *Streptococcus agalactiae* infection in Javanese medaka *Oryzias javanicus*. *Microbial Pathogenesis*, 114: 251-254.

Amalina, N. Z., and Ina-Salwany, M. Y. (2016). Recent advancements in molecular detection of *Vibrio* species in aquatic animals: A review. *Bioscience Biotechnology Research Communications,* 9(3): 349-356.

Amaro, C., Biosca, E. G., Fouz, B., Alcaide, E., and Esteve, C. (1995). Evidence that water transmits *Vibrio vulnificus* biotype 2 infections to eels. *Applied and Environmental Microbiolology*, 61(3), 1133-1137

Amel, B. K. N., Amine, B., and Amina, B. (2008). Survival of *Vibrio fluvialis* in seawater under starvation conditions. *Microbiological Research,* 163(3): 323-328.

Amin, A. K. M. R., Tanaka, M., Al- saari, N., Feng, G., Mino, S., Ogura, Y., Hayashi, T., Meirelles, P. M., Thompson, F. L., Gomez-Gil, B., Sawabe, T., and Sawabe, T. (2017). *Thaumasiovibrio occultus* gen. nov. sp. nov. and *Thaumasiovibrio subtropicus* sp. nov. within the family Vibrionaceae, isolated from coral reef seawater off Ishigaki Island, Japan. *Systematic and Applied Microbiology,* 40(5): 290-296.

Angelidis, P., Karagiannis, D., and Crump, E. M. (2006). Efficacy of a *Listonella anguillarum* (syn. *Vibrio anguillarum*) vaccine for juvenile sea bass *Dicentrarchus labrax*. *Diseases of Aquatic Organisms,* 71(1): 19-24.

Arias, C. R., Pujalte, M. J., Garay, E., and Aznar, R. (1998). Genetic relatedness among environmental, clinical, and diseased-eel *Vibrio vulnificus* isolates from different geographic regions by ribotyping and randomly amplified polymorphic DNA PCR. *Applied and Environmental Microbiology,* 64(9): 3403-3410.

Arif, M., Suprapto, H., and Sulmartiwi, L. (2016). Bacteria associated with mass mortality of hybrid grouper *Epinephelus* sp. in East Java Province Indonesia. *International Journal of Fisheries and Aquatic Studies*, 4(6): 439-441.

Aslizah, M. A. (2018). Development of *Vibrio harveyi* ptotease deletion mutant as live attenuated vaccine against vibriosis in *Epinephelus fuscoguttatus* (Forsskal, 1775). PhD. Thesis, UPM, Malaysia.

Assis, G. B., Tavares, G. C., Pereira, F. L., Figueiredo, H. C., and Leal, C. A. (2016). Natural coinfection by *Streptococcus agalactiae* and *Francisella noatunensis* subsp. *orientalis* in farmed Nile tilapia (*Oreochromis niloticus* L.). *Journal of Fish Diseases,* 40(1): 51-63.

Austin, B., Pride, A. C., and Rhodie, G. A. (2003). Association of a bacteriophage with virulence in *Vibrio harveyi*. *Journal of Fish Diseases,* 26(1): 55-58.

Austin, B., and Austin, D. A. (2012). *Bacterial fish pathogens*. Heidelberg, Germany: Springer.

Austin, B. and Austin, D. A. (2016) *Bacterial fish pathogens: Disease of farmed and wild fish (6th edition)*. Switzerland. Springer International Publishing.

Austin, B., and Zhang, X. H. (2006). *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. *Letters in Applied Microbiology*, 43(2): 119-124.

Avendaño-Herrera, R., Toranzo, A. E., and Magariños, B. (2006). Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. *Diseases of Aquatic Organisms*, 71(3): 255-266.

Azad, I. S., Thirunavukkarasu, A. R., Kailasam, M., and Rajan, J. J. S. (2004). Virulence and histopathology of *Vibrio anguillarum* like (VAL) bacterium isolated from hatchery produced juveniles of *Lates calcarifer* (Bloch). *Asian Fisheries Science Journal,* 17: 101-110.

Bagge, J., and Bagge, O. (1956). *Vibrio anguillarum* som arsag til ulcussygdom has torsk (*Gadus callarias* L.) (*Vibrio anguillarum* as cause of the ulcer disease in cod). *Nordisk Veterinaer Medicin,* 8: 481-492.

Bai, F., Pang, L., Qi, Z., Chen, J., Austin, B., and Zhang, X. H. (2008). Distribution of five *vibrio* virulence-related genes among *Vibrio harveyi* isolates. *The Journal of General and Applied Microbiology*, 54(1): 71-78.

Baker-Austin, C., Trinanes, J., Gonzalez-Escalona, N., and Martinez-Urtaza, J. (2017). Non-cholera *Vibrios*: the microbial barometer of climate change. *Trends in Microbiology,* 25(1): 76-84.

Baker-Austin, C., and Oliver, J. D. (2018). *Vibrio vulnificus*: new insights into a deadly opportunistic pathogen. *Environmental Microbiology*, 20(2): 423-430.

Balebona, M. C., Zorrilla, I., Moriñigo, M. A., and Borrego, J. J. (1998). Survey of bacterial pathologies affecting farmed gilt-head sea bream (*Sparus aurata* L.) in southwestern Spain from 1990 to 1996. *Aquaculture,* 166(1-2): 19-35.

Banerjee, G., and Ray, A. K. (2017). Bacterial symbiosis in the fish gut and its role in health and metabolism. *Symbiosis*, 72(1): 1-11.

Bauer, A. W., Kirby, W. M. M., Shenis, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology,* 45(4): 493-496.

Bej, A. K., Patterson, D. P., Brasher, C. W., Vickery, M. C. L., Jones, D. D., and Kaysner, C. A. (1999). Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tl*, *tdh* and *trh*. *Journal of Microbiological Methods,* 36(3): 215-225.

Ben-Haim, Y., Thompson, F. L., Thompson, C. C., Cnockaert, M. C., Hoste, B., Swings, J., and Rosenberg, E. (2003). *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *International Journal of Systemic and Evolutionary Microbiology,* 53(Pt 1): 309-315.

Benediktsdóttir, E., Verdonck, L., Spröer, C., Helgason, S., and Swings, J. (2000). Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographical locations: a proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *International Journal of Systematic and Evolutionary Microbiology,* 50(2): 479-488.

Bellos, G., Angelidis, P., and Miliou, H. (2015). Effect of temperature and seasonality principal epizootiological risk factor on vibriosis and photobacteriosis outbreaks for European sea bass in Greece (1998-2013). *Journal of Aquaculture Research and Development*, 6(5).

Bergman, A. M. (1909). Die roten Beulenkrankheit des Aales. *Ber Bayerischen Biol Versuchs-Station,* 2: 10-54. (in German).

Bierring, E., Madhun, A., Isachsen, C. H., Omdal, L. M., Einen, A. C. B., Garseth, Å. H., Bjorn, P. A., Nilesen, R., and Karlsbakk, E. (2012). *Annual report on health monitoring of wild anadromous salmonids in Norway. Annual Report 2012, No 6-2013.* Bergen: Institute of Marine Research.

Bilung, L. (2014). Incidence of *Vibrio parahaemolyticus* at Estuarine Region of Sg. Sarawak (Sarawak) Influenced by Physicochemical Parameters. *Bioremediation Science and Technology Research,* 2(1): 9-13.

Birkbeck, T. H., and Treasurer, J. W. (2014). *Vibrio splendidus*, *Vibrio ichthyoenteri* and *Vibrio pacinii* isolated from the digestive tract microflora of larval ballan wrasse, *Labrus bergylta Ascanius*, and goldsinny wrasse, *Ctenolabrus rupestris* (L.). *Journal of Fish Diseases,* 37(1): 69-74.

Bisharat, N., Agmon, V., Finkelstein, R., Raz, R., Ben-Dror, G., Lerner, L., Soboh, S., Colodner, R., Cameron, D. N., Wykstra, D. L., Swerdlow, D. L., and Farmer, J. J (1999). Clinical, epidemiological, and microbiological features of *Vibrio vulnificus* biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. *The Lancet*, 354(9188): 1421-1424.

Bjelland, A. M., Fauske, A. K., Nguyen, A., Orlien, I. E., Østgaard, I. M., and Sørum, H. (2013). Expression of *Vibrio salmonicida* virulence genes and immune response parameters in experimentally challenged Atlantic salmon (*Salmo salar* L.). *Frontiers in Microbiology,* 4: 401.

Boran, H., Terzi, E., Altinok, I., Capkin, E., and Bascinar, N. (2013). Bacterial diseases of cultured Mediterranean horse mackerel (*Trachurus mediterraneus*) in sea cages. *Aquaculture*, 396(399): 8-13.

Bordas, M. A., Balebona, M. C., Rodriguez-Maroto, J. M., Borrego, J. J., and Morinigo, M. A. (1998). Chemotaxis of pathogenic *Vibrio* strains towards mucus surfaces of gilt-head sea bream (*Sparus aurata* L.). *Applied and Environmental Microbiology,* 64(4): 1573-1575.

Bowden, T. J., Bricknell, I. R., and Preziosi, B. M. (2018). Comparative pathogenicity of *Vibrio* spp*., Photobacterium damselae* spp. *damselae* and five isolates of *Aeromonas salmonicida* ssp. *achromogenes* in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). *Journal of Fish Diseases*, 41(1): 79-86.

Brunt, J., Newaj-Fyzul, A., and Austin, B. (2007). The development of probiotics for the control of multiple bacterial diseases of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases,* 30(10): 573-579.

Bruun, A. F., and Heiberg, B. (1932). The "red disease" of the eel in Danish waters. *Meddelelser fra Kommissionen for Danmarks Fiskeri-og Havundersøgelser. Serie: Fiskeri,* 9: 1-17. (in German).

Bunlipatanon, P., and U-taynapun, K. (2017). Growth performance and disease resistance against *Vibrio vulnificus* infection of novel hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*). *Aquaculture Research*, 48(8): 1711-1723.

Bunpa, S., Sermwittayawong, N., and Vuddhakul, V. (2016). Extracellular enzymes produced by *Vibrio alginolyticus* isolated from environments and diseased aquatic animals. *Procedia Chemistry,* 18: 12-17.

Burke, J., and Rodgers, L. (1981). Identification of pathogenic bacteria associated with the occurrence of red spot in sea mullet, *Mugil cephalus* L., in south-eastern Queensland. Bacterial Haemorrhagic Septicemia in Silver Mullet (*Mugil curema*). *Journal of Fish Diseases*, 4(2): 153-159.

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., and Buschmann, A. H. (2013). Antimicrobial use in aquaculture re-examined: its

relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology*, 15(7): 1917-1942.

Callol, A., Pajuelo, D., Ebbesson, L., Teles, M., MacKenzie, S., and Amaro, C. (2015). Early steps in the European eel (*Anguilla anguilla*)-*Vibrio vulnificus* interaction in the gills: Role of the RtxA1 3 toxin. *Fish and Shellfish Immunology,* 43(2): 502-509.

Camkerten, I., Avsever, M. L., and Aksoy, A. (2017). First isolation of *Vibrio furnissii* (emerging *Vibrio*) from mussels (Mediterranean mussel and bearded mussel) in Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 64(3): 205-209.

Canestrini, G. (1893). La mallattia dominante dellc angiiille. *Atti dell'Istituto veneto di scienze lettere,* 7: 809-814.

Cano-Gomez, A., Bourne, D. G., Hall, M. R., Owens, L., and Høj, L. (2009). Molecular identification, typing and tracking of *Vibrio harveyi* in aquaculture systems: current methods and future prospects. *Aquaculture,* 287(1): 1-10.

Cardinaud, M., Barbou, A., Capitaine, C., Bidault, A., Dujon, A. M., Moraga, D., and Paillard, C. (2014). *Vibrio harveyi* adheres to and penetrates tissues of the European abalone *Haliotis tuberculata* within the first hours of contact. *Applied and Environmental Microbiology,* 80(20): 6328-6333.

Ceccarelli, D., and Colwell, R. R. (2014). *Vibrio* ecology, pathogenesis, and evolution. *Frontiers in Microbiology,* 5: 256.

Chan S. M. (1997). Identification of *Vibrio* spp. isolated from cultured seabass *Lates calcarifer* Bloch and their antibiogram relationship with plasmid profiles. M.Sc. Thesis, UPM, Malaysia.

Chart, H. (2012). *Vibrio*, mobiluncus, gardnerella and spirillum: *Cholera*; vaginosis; rat bite fever. *Medical Microbiology,* 8: 314-323.

Chase, E., Young, S., and Harwood, V. J. (2015). Sediment and vegetation as reservoirs of *Vibrio vulnificus* in the Tampa Bay Estuary and Gulf of Mexico. *Applied and Environmental Microbiology,* 81(7): 2489-2494.

Chatterjee, S., and Haldar, S. (2012). *Vibrio* related diseases in aquaculture and development of rapid and accurate identification methods. *Journal of Marine Science Research and Development,* S1: 2.

Chen, Q., Yan, Q., Wang, K., Zhuang, Z., and Wang, X. (2008). Portal of entry for pathogenic *Vibrio alginolyticus* into large yellow croaker *Pseudosciaena crocea*, and characteristics of bacterial adhesion to mucus. *Diseases of Aquatic Organisms,* 80(3): 181-188.

Cheng, S., Zhang, W. W., Zhang, M., and Sun, L. (2010). Evaluation of the vaccine potential of a cytotoxic protease and a protective immunogen from a pathogenic *Vibrio harveyi* strain. *Vaccine,* 28(4): 1041-1047.

Chimetto, L. A., Brocchi, M., Gondo, M., Thompson, C. C., Gomez-Gil, B., and Thompson, F. L. (2009). Genomic diversity of *Vibrios* associated with the Brazilian coral *Mussismilia hispida* and its sympatric zoanthids (*Palythoa caribaerorum*, *Palythoa variabilis*, and *Zoanthus solanderi*). *Journal of Applied Microbiology,* 106(6): 1818-1826.

Christensen, H. (2003). Characterization of *Vibrio* strains isolated from turbot, *Scophthalmus maximus* culture by phenotypic analysis, ribotyping and 16S rRNA gene sequence comparison. *Journal of Applied Microbiology,* 95(4): 693-703.

CLSI. Methods for dilution antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline-2nd ed., CLSI document M45-A2. (2010). Clinical and Laboratory Standards Institute, Wayne, Pennsylvania 19087, USA.

CLSI. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement, CLSI document M100-S23. (2013). Clinical and Laboratory Standards Institute, Wayne, Pennsylvania 19087, USA.

Collin, B., Ann-Sofi, R. H., Stina-Mina, E. B., Aidate, M., and Bodil, H. (2013). Characteristics of potentially pathogenic *Vibrios* from subtropical Mozambique compared with isolates from tropical India and boreal Sweden. *FEMS Microbiology Ecology*, 83(2): 255-264.

Colquhoun, D. J., and Sørum, H. (2001). Temperature dependent siderophore production in *Vibrio salmonicida*. *Microbial Pathogenesis,* 31(5): 213-219.

Colquhoun, D. J., Aase, I. L., Wallace, C., Baklien, A., and Gravningen, K. (2004). First description of *Vibrio ordalii* from Chile. *Bulletin-European Association of Fish Pathologists,* 24(4): 185-188.

Colt, J. (2006). Water quality requirements for reuse systems. *Aquaculture Engineering,* 34(3): 143-156.

Colwell, R. R., and Grimes, D. J. (1984). *Vibrio* diseases of marine fish populations. *Helgoländer Meeresuntersuchungen,* 37(1-4): 265-287.

Croci, L., Suffredini, E., Cozzi, L., Toti, L., Ottaviani, D., Pruzzo, C., Serratore, P., Goffredo, E., Loffredo, G., and Mioni, R. (2007). Comparison of different biochemical and molecular methods for the identification of *Vibrio parahaemolyticus*. *Journal of Applied Microbiology,* 102(1): 229-237.

Crothers-Stomps, C., Høj, L., Bourne, D. G., Hall, M. R., and Owens, L. (2010). Isolation of lytic bacteriophage against *Vibrio harveyi*. *Journal of Applied Microbiology,* 108(5): 1744-1750.

Croxatto, A., Chalker, V. J., Lauritz, J., Jass, J., Hardman, A., Williams, P., Camara, M., and Milton, D. L. (2002). *VanT*, a homologue of *Vibrio harveyi LuxR*, regulates serine, metalloprotease, pigment, and biofilm production in *Vibrio anguillarum*. *Journal of Bacteriology*, 184(6): 1617-1629.

Croxatto, A., Lauritz, J., Chen, C., and Milton, D. L. (2007). *Vibrio anguillarum* colonization of rainbow trout integument requires a DNA locus involved in exopolysaccharide transport and biosynthesis. *Environmental Microbiology,* 9(2): 370-383.

Crumlish, M., Thanh, P. C., Koesling, J., Tung, V. T., and Gravningen, K. (2010). Experimental challenge studies in Vietnamese catfish, *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. *Journal of Fish Diseases*, 33(9): 717-722.

Culver, D. A., and Geddes, M. C. (1993). Limnology of rearing ponds for Australian fish larvae: relationships among water quality, phytoplankton, zooplankton, and the growth of larval fish. *Marine and Freshwater Research,* 44(4): 537-551.

Das, P. C. (2018). Grow-out Fish Farming in Freshwater: Principles & Practices. *Mass Breeding and Culture Technique of Catfishes*, 97.

Deepanjali, A., Kumar, H. S., and Karunasagar, I. (2005). Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. *Applied and Environmental Microbiology,* 71(7): 3575-3580.

Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., and Bossier, P. (2008). Quorum sensing and quorum quenching in *Vibrio harveyi*: lessons learned from *in vivo* work. *ISME Journal*, 2: 19-26.

Department of Environment (DOE) (2011). Malaysia Marine Water Quality Criteria and Standard. https://www.doe.gov.my/portalv1/wp-content/uploads/ 2019/04/ BOOKLET-BI. pdf. Assessed on 16 August 2019.

Department of Environment (DOE) (2012). National Water Quality Standards for Malaysia.https://www.doe.gov.my/portalv1/wp-content/uploads/2019/05/ Standard-Kualiti-Air-Kebangsaan.pdf. Assessed on 16 August 2019.

Department of Fisheries Malaysia (DOF) (2017) Annual fisheries statistics. Ministry of Agriculture and Agro-Based Industry, Putrajaya.

DePaola, A., Nordstrom, J. L., Bowers, J. C., Wells, J. G., and Cook, D. W. (2003). Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in

Alabama oysters. *Applied and Environmental Microbiology,* 69(3): 1521-1526.

De Schryver, P., Defoirdt, T., and Sorgeloos, P. (2014). Early mortality syndrome outbreaks: a microbial management issue in shrimp farming? *PLoS Pathogens,* 10(4): 1003919.

De Silva, S. S., and Phillips, M. J. (2007). A review of cage aquaculture: Asia (excluding China). *FAO Fisheries Technical Paper*, 498: 21.

Dierckens, K., Rekecki, A., Laureau, S., Sorgeloos, P., Boon, N., Van den Broeck, W., and Bossier, P. (2009). Development of a bacterial challenge test for gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. *Environmental Microbiology,* 11(2): 526-533.

Diggles, B. K., Moss, G. A., Carson, J., and Anderson, C. D. (2000). Luminous vibriosis in rock lobster *Jasus verreauxi (Decapoda: Palinuridae*) phyllosoma larvae associated with infection by *Vibrio harveyi*. *Diseases of Aquatic Organisms,* 43(2): 127-137.

Di Pinto, A., Ciccarese, G., Tantillo, G., Catalano, D., and Forte, V. T. (2005). A collagenase-targeted multiplex PCR assay for identification of *Vibrio alginolyticus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. *Journal of Food Protection,* 68(1): 150-153.

Dong, H. T., Nguyen, V. V., Phiwsaiya, K., Gangnonngiw, W., Withyachumnarnkul, B., Rodkhum, C., and Senapin, S. (2015). Concurrent infections of *Flavobacterium columnare* and *Edwardsiella ictaluri* in striped catfish, *Pangasianodon hypophthalmus* in Thailand. *Aquaculture*, 448: 142-150.

Dong, H. T., Taengphu, S., Sangsuriya, P., Charoensapsri, W., Phiwsaiya, K., Sornwatana, T., Khunrae, P., Rattanarojpong, T., and Senapin, S. (2017). Recovery of *Vibrio harveyi* from scale drop and muscle necrosis disease in farmed barramundi, *Lates calcarifer* in Vietnam. *Aquaculture*, 473: 89-96.

Elmahdi, S., DaSilva, L. V., and Parveen, S. (2016). Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. *Food Microbiology*, 57: 128-134.

Elston, R. A., Hasegawa, H., Humphrey, K. L., Polyak, I. K., and Häse, C. C. (2008). Re-emergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: severity, environmental drivers, geographic extent and management. *Diseases of Aquatic Organisms,* 82(2): 119-134.

FAO. *Responsible use of antibiotics in aquaculture*. (2005). FAO Fisheries Technical Paper No. 469, Rome.

FAO. *Characteristics, structure and resources of the sector*. (2013). *FAO Country Notes*, 1380: 1-16.

FAO. *Food and Agriculture Organization of the United Nations (UN FAO).* (2016). The State of World Fisheries, FAO, Rome.

FAO. *FishStatJ, a Tool for Fishery Statistics Analysis*. (2017). FAO Fisheries and Aquaculture Department, FIPS - Statistics and information, Rome.

FAO. *Food and Agriculture Organization of the United Nations (UN FAO).* (2018). The State of World Fisheries, FAO, Rome.

Fernández, J., and Avendaño-Herrera, R. (2009). Analysis of 16S-23S rRNA gene internal transcribed spacer of *Vibrio anguillarum* and *Vibrio ordalii* strains isolated from fish. *FEMS Microbiology Letters,* 299(2): 184-192.

Fernández-Delgado, M., Sanz, V., Giner, S., Suárez, P., Contreras, M., Michelangeli, F., and García-Amado. (2016). Prevalence and distribution of *Vibrio* spp. in wild aquatic birds of the Southern Caribbean Sea, Venezuela, 2011–12. *Journal of Wildlife Diseases,* 52(3): 621-626.

Fernández-Delgado, M., Suárez, P., Giner, S., Sanz, V., Peña, J., Sánchez, D., and García-Amado, M. A. (2017). Occurrence and virulence properties of *Vibrio* and *Salinivibrio* isolates from tropical lagoons of the southern Caribbean Sea. *Antonie van Leeuwenhoek*, 110(6): 833-841.

Firdaus-Nawi, M., Zamri-Saad, M., Nik-Haiha, N. Y., Zuki, M. A. B., and Effendy, A. W. M. (2013). Histological assessments of intestinal immuno-morphology of tiger grouper juvenile, *Epinephelus fuscoguttatus*. *SpringerPlus*, 2(1): 611.

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

Frans, I., Michiels, C. W., Bossier, P., Willems, K. A., Lievens, B., and Rediers, H. (2011). *Vibrio anguillarum* as a fish pathogen: virulence factors, diagnosis and prevention. *Journal of Fish Diseases*, 34(9): 643-661.

Froelich, B., Bowen, J., Gonzalez, R., Snedeker, A., and Noble, R. (2013). Mechanistic and statistical models of total *Vibrio* abundance in the Neuse River Estuary. *Water Research,* 47(15): 5783-5793.

Froelich, B. A., Weiss, M. J., and Noble, R. T. (2014). The evaluation of four recent culture-based methods for the isolation and enumeration of *Vibrio vulnificus* bacteria from oyster meat. *Journal of Microbiological Methods,* 97: 1-5.

Froehlich, H. E., Smith, A., Gentry, R. R., and Halpern, B. S. (2017). Offshore aquaculture: I know it when I see it. *Frontiers in Marine Science*, 4: 154.

Fouz, B., Roig, F. J., and Amaro, C. (2007). Phenotypic and genotypic characterization of a new fish-virulent *Vibrio vulnificus* serovar that lacks potential to infect humans. *Microbiology*, 153(6): 1926-1934.

Fukui, Y., and Sawabe, T. (2007). Improved one-step colony PCR detection of *Vibrio harveyi*. *Microbes and Environments,* 22(1): 1-10.

GAA (2013). Cause of EMS shrimp disease identified. GAA News Releases. Available: http://www.gaalliance.org/newsroom. Accessed 29 March 2014.

Gabriel, U. U., and Akinrotimi, O. A. (2011). Management of stress in fish for sustainable aquaculture development. *Researcher*, 3(4): 28-38.

Gardner, I., Saksida, S., Dixon, B., McKenzie, P., and Johnson, S. (2014). Pathogen exchange between wild and farmed finfish: evidence to assess pathogen source and factors associated with clinical disease occurrence. *Bulletin of the Aquaculture Association of Canada,* 111(3): 1-35.

Gauthier, D. T. (2015). Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections. *The Veterinary Journal,* 203(1): 27-35.

Geng, Y., Liu, D., Han, S., Zhou, Y., Wang, K. Y., Huang, X. L., Chen, D. F., Peng, X., and Lai, W. M. (2014). Outbreaks of vibriosis associated with *Vibrio mimicus* in freshwater catfish in China. *Aquaculture*, 433, 82-84.

George, M. R., John, K. R., Iyappan, T., and Jeyaseelan, M. J. P. (2005). Genetic heterogeneity among *Vibrio alginolyticus* isolated from shrimp farms by PCR fingerprinting. *Letters in Applied Microbiology,* 40(5): 369-372.

Georgiadis, M. P., Gardner, I. A., and Hedrick, R. P. (2001). The role of epidemiology in the prevention, diagnosis, and control of infectious diseases of fish. *Preventive Veterinary Medicine*, 48(4): 287-302.

Givens, C. E., Bowers, J. C., DePaola, A., Hollibaugh, J. T., and Jones, J. L. (2014). Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus*–potential roles for fish, oyster, sediment and water. *Letters in Applied Microbiology,* 58(6): 503-510.

Goh, S. G., Bayen, S., Burger, D., Kelly, B. C., Han, P., Babovic, V., and Gin, K. Y. H. (2017). Occurrence and distribution of bacteria indicators, chemical tracers and pathogenic *Vibrios* in Singapore coastal waters. *Marine Pollution Bulletin,* 114(1): 627-634.

Gomez-Gil, B., Thompson, C. C., Matsumura, Y., Sawabe, T., Iida, T., Christen, R., Thompson, F., and Sawabe, T. (2014). Family Vibrionaceae (Chapter 225). In: E. Rosenberg, E. F. DeLong, F. L. Thompson, S. Lory, E. Stackebrandt (Eds). *The Prokaryotes*, New York, United States. Springer.

Gómez-León, J., Villamil, L., Lemos, M. L., Novoa, B., and Figueras, A. (2005). Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from aquacultured carpet shell clam (*Ruditapes decussatus*) larvae associated with mass mortalities. *Applied and Environmental Microbiology,* 71(1): 98-104.

Gudding, R., and Van Muiswinkel, W. B. (2013). A history of fish vaccination: science-based disease prevention in aquaculture. *Fish and Shellfish Immunology,* 35(6): 1683-1688.

Guzmán, G. A., Martínez, J. G. S. R., Castãneda, P., Monzón, A. P., Rodríguez, T. T., and Cruz-Hernandez, N. I. D. L. (2010). Pathogenicity and infection route of *Vibrio parahaemolyticus* in American white shrimp, *Litopenaeus vannamei. Journal of the World Aquaculture Society,* 41(3): 464-470.

Granada, L., Sousa, N., Lopes, S., and Lemos, M. F. (2016). Is integrated multitrophic aquaculture the solution to the sectors' major challenges? – a review. *Reviews in Aquaculture*, 8(3): 283-300.

Grimes, D. J., Colwell, R. R., Stemmler, J., Hada, H., Maneval, D., Hetrick, F. M., May, E. B., Jones, R. T., and Stoskopf, M. (1984a). *Vibrio* species as agents of elasmobranch disease. *Helgoländer Meeresuntersuchungen,* 37(1-4): 309-315.

Grimes, D. J., Stemmler, J., Hada, H., May, E. B., Maneval, D., Hetrick, F. M., Jones, R. T., Stoskopf, M., and Colwell, R. R. (1984b). *Vibrio* species associated with mortality of sharks held in captivity. *Microbial Ecology,* 10(3): 271-282.

Haenen, O. L. M., Fouz Rodríguez, B., Amaro González, C., Isern, M. M., Mikkelsen, H., Zrnčić, S., Travers, M. A., Renault, T., Hellstrom, A., and Dalsgaard, I. (2014). Vibriosis in aquaculture. 16th EAFP Conference, Tampere, Finland, 4thSeptember 2013. *Bulletin of the European Association of Fish Pathologists*, 34(4): 138-147.

Haldar, S., Maharajan, A., Chatterjee, S., Hunter, S. A., Chowdhury, N., Hinenoya, A., Asakura, M., and Yamasaki, S. (2010). Identification of *Vibrio harveyi* as a causative bacterium for a tail rot disease of sea bream *Sparus aurata* from research hatchery in Malta. *Microbiological Research,* 165(8): 639-648.

Haldar, S., Chatterjee, S., Sugimoto, N., Das, S., Chowdhury, N., Hinenoya, A., Asakura, M., and Yamasaki, S. (2011). Identification of *Vibrio campbellii* isolated from diseased farm-shrimps from south India and establishment of its pathogenic potential in an *Artemia* model. *Microbiology,* 157(Pt 1): 179-188.

Halwart, M., Soto, D., and Arthur, J. R. (2007). *Cage aquaculture: regional reviews and global overview*. *FAO Fisheries Technical Paper.*

Hamdan, R. H., Peng, T. L., Ong, B. L., Suhana, M. Y. S., Hamid, N. H., Afifah, M. N. F., and Raina, M. S. (2017). Antibiotics resistance of *Vibrio* spp. isolated from diseased seabass and tilapia in cage culture. *Proceedings of International Seminar on Livestock Production and Veterinary Technology*. Bali, Indonesia.

Han, Y., Mo, Z., Xiao, P., Hao, B., Li, J., and Yang, G. (2011). Characterization of *EmpA* protease in *Vibrio anguillarum* M3. *Journal of Ocean University of China*, 10(4): 379-384.

Hardy-Smith, P. (2006). Biosecurity at the Farm Level—How to Create a State of Mind. *Aquaculture Biosecurity: Prevention, Control, and Eradication of Aquatic Animal Disease,* 10: 149-154.

Harikrishnan, R., Balasundaram, C., and Heo, M. S. (2010). Molecular studies, disease status and prophylactic measures in grouper aquaculture: Economic importance, diseases and immunology. *Aquaculture*, 309(1-4): 1-14.

Hasegawa, H., Lind, E. J., Boin, M. A., and Häse, C. C. (2008). The extracellular metalloprotease of *Vibrio tubiashii* is a major virulence factor for pacific oyster (*Crassostrea gigas*) larvae. *Applied and Environmental Microbiology,* 74(13): 4101-4110.

Hashem, M., and El-Barbary, M. (2013). *Vibrio harveyi* infection in Arabian sturgeon fish (*Acanthurus sohal*) of Red Sea at Hurghada, Egypt. *The Egyptian Journal of Aquatic Research*, 39: 199-203.

Hatje, E., Neuman, C., Stevenson, H., Bowman, J. P., and Katouli, M. (2014). Population dynamics of *Vibrio* and *Pseudomonas* species isolated from farmed Tasmanian Atlantic salmon (*Salmo salar* L.): a seasonal study. *Microbial Ecology,* 68(4): 679-687.

Hazeri, M., Hassan, M. D., Abba, Y., Omar, A. R., Allaudin, Z. N., Soltani, M., Hamdan, R. H., Faten, A. M. N., Raina, M. S., and Vishkaei, M. S. (2016). Histopathological evaluation and molecular detection of natural Iridovirus infection in cultured grouper fish in Malaysia. *Comparative Clinical Pathology*, 25(5): 965-971.

Hellberg, H., Mikalsen, J., Colquhoun, D., Hansen, H., Bornø, G., and Nilsen, A. (2009). *The health situation in farmed marine fish 2008*. Norwegian National Veterinary Institute, Oslo.

Hellberg, H., Nilsen, H. K., Bornø, G., Skjelstad, H. R., Colquhoun, D., and Jensen, B. B. (2010). *The health situation in farmed marine fish 2009*. Norwegian National Veterinary Institute, Oslo.

Henke, J. M., and Bassler, B. L. (2004). Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *Journal of Bacteriology*, 186(20): 6902-6914.

Hernández-Robles, M. F., Álvarez-Contreras, A. K., Juárez-García, P., Natividad-Bonifacio, I., Curiel-Quesada, E., Vázquez-Salinas, C., and Quiñones-Ramírez, E. (2016). Virulence factors and antimicrobial resistance in environmental strains of *Vibrio alginolyticus*. *International Microbiology*, 19(4): 191-198.

Heuer, O. E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., and Angulo, F. J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases,* 49(8): 1248-1253.

Hickey, M. E., and Lee, J. L. (2017). A comprehensive review of *Vibrio* (*Listonella*) *anguillarum*: ecology, pathology and prevention. *Reviews in Aquaculture,* 10(3): 1-26.

Higuera, G., Bastías, R., Tsertsvadze, G., Romero, J., and Espejo, R. T. (2013). Recently discovered *Vibrio anguillarum* phages can protect against experimentally induced vibriosis in Atlantic salmon, *Salmo salar*. *Aquaculture,* 392: 128-133.

Hiyoshi, H., Kodama, T., Iida, T., and Honda, T. (2010). Contribution of *Vibrio parahaemolyticus* virulence factors to cytotoxicity, enterotoxicity, and lethality in mice. *Infection and Immunity*, 78(4): 1772-1780.

Hispano, C., Nebra, Y., and Blanch, A. R. (1997). Isolation of *Vibrio harveyi* from an ocular lesion in the short sunfish (*Mola mola*). *Bulletin-European Association of Fish Pathologists,* 17: 104-107.

Hjerde, E., Lorentzen, M. S., Holden, M. T., Seeger, K., Paulsen, S., Bason, N., and Sanders, S. (2008). The genome sequence of the fish pathogen *Aliivibrio salmonicida* strain LFI1238 shows extensive evidence of gene decay. *BMC Genomics,* 9(1): 616.

Hsiao, H. I., Jan, M. S., and Chi, H. J. (2016). Impacts of climatic variability on *Vibrio parahaemolyticus* outbreaks in Taiwan. *International Journal of Environmental Research and Public Health*, 13(2): 188.

Hsu, P. Y., Lee, K. K., Hu, C. C, and Liu, P. C. (2014). Purification and characterization of a phospholipase by *Photobacterium damselae* subsp. p*iscicida* from cobia *Rachycentron canadum*. *Journal of Basic Microbiology,* 54(9): 969-975.

Hu, Y. H., and Sun, L. (2011). A bivalent *Vibrio harveyi* DNA vaccine induces strong protection in Japanese flounder (*Paralichthys olivaceus*). *Vaccine,* 29(26): 4328-4333.

Hussin, M. A., Nik Daud, N. S., and Nik Razali, N. L. (1996). Natural spawning and larval rearing of tiger grouper, *Epinephalus fuscoguttatus* (Forskal). *A preliminary result. Paper presented at the 5th Fisheries Research Institute Conference*. 8 – 10 July 1997. Fisheries Research Institute Penang Malaysia.

Igbinosa, E. O. (2016). Detection and antimicrobial resistance of *Vibrio* isolates in aquaculture environments: implications for public health. *Microbial Drug Resistance*, 22(3): 238-245.

Immanuel, G., Vincybai, V. C., Sivaram, V., Palavesam, A., and Marian, M. P. (2004). Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. *Aquaculture,* 236(1-4): 53-65.

Immanuel, G., Uma, R. P., Iyapparaj, P., Citarasu, T., Punitha Peter, S. M., Michael Babu, M., and Palavesam, A. (2009). Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. *Journal of Fish Biology,* 74(7): 1462-1475.

Ina-Salwany, M. Y., Al-saari, N., Mohamad, A., Fathin-Amirah, M., Mohd, A., Amal, M. N. A., Kasai, H., Mino, S., Sawabe, T., and Zamri-Saad, M. (2018). Vibriosis in fish: a review on disease development and prevention. *Journal of Aquatic Animal Health,* 31(1): 3-22.

Ishimaru, K., and Muroga, K. (1997). Taxonomical re-evaluation of two pathogenic *Vibrio* species isolated from milkfish and swimming crab. *Fish Pathology,* 32(1): 59-64.

Islam, G. M. N., Tai, S. Y., and Kusairi, M. N. (2016). A stochastic frontier analysis of technical efficiency of fish cage culture in Peninsular Malaysia. *SpringerPlus*, 5(1): 1127.

Izumiya, H., Furukawa, M., Ogata, K., Isobe, J., Watanabe, S., Sasaki, M., Ichinose, K., Arakawa, E., Morita, M., Kurane, I., and Ohnishi, M. (2017). A double-quadratic model for predicting *Vibrio* species in water environments of Japan. *Archives of Microbiology*, 199(9): 1293-1302.

Jin, S., Wang, G., Zhao, Q., Zheng, T., and Chen, Y. (2004). Epidemiology of vibriosis in large yellow croaker *Pseudosciaena crocea* (Richardson) in marine cage culture. *Fisheries Science*, 24(1): 17-19.

Johansen, L. H., Eggset, G., and Sommer, A. I. (2009). Experimental IPN virus infection of Atlantic salmon parr; recurrence of IPN and effects on secondary bacterial infections in post-smolts. *Aquaculture,* 290(1): 9-14.

Johansen, L. H., Jensen, I., Mikkelsen, H., Bjørn, P. A., Jansen, P. A., and Bergh, Ø. (2011). Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. *Aquaculture,* 315(3): 167-186.

Johnson, C. N., Flowers, A. R., Noriea, N. F., Zimmerman, A. M., Bowers, J. C., DePaola, A., and Grimes, D. J. (2010). Relationships between environmental factors and pathogenic Vibrios in the Northern Gulf of Mexico. *Applied and Environmental Microbiology*, 76(21): 7076-7084.

Johnson, C. N., Bowers, J. C., Griffitt, K. J., Molina, V., Clostio, R. W., Pei, S., Laws, E., Paranjpye, R. N., Strom, M. S., Chen, A., Hasan, N. A., Huq, A., Norlea III, A. N. F., Grimes, J., and Colwell, R. R. (2012). Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Applied and Environmental Microbiology,* 78(20): 7249-7257.

Johnson, C. N. (2015). Influence of Environmental Factors on *Vibrio* spp. in Coastal Ecosystems. *Microbiology spectrum*, 3(3).

Joshi, J., Srisala, J., Truong, V. H., Chen, I. T., Nuangsaeng, B., Suthienkul, O., Lo, C. F., Flegel, T. W., Sritunyalucksana, K., and Thitamadee, S. (2014). Variation in *Vibrio parahaemolyticus* isolates from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND). *Aquaculture,* 428-429: 297-302.

Jun, J. W., Shin, T. H., Kim, J. H., Shin, S. P., Han, J. E., Heo, G. J., Zoys, M. D., Shin, G. W., Chai, J. Y., and Park, S. C. (2014). Bacteriophage therapy of a *Vibrio parahaemolyticus* infection caused by a multiple-antibiotic resistant O3:K6 pandemic clinical strain. *The Journal of Infectious Diseases,* 210(1): 72-78.

Jung, S. J., Kim, S. R., Joung, I. Y., Kitamura, S. I., Ceong, H.T., and Oh, M. J. (2008). Distribution of marine birnavirus in cultured olive flounder *Paralichthys olivaceus* in Korea. *The Journal of Microbiology,* 46(3): 265-273.

Kahla-Nakbi, A. B., Chaieb, K., and Bakhrouf, A. (2009) Investigation of several virulence properties among *Vibrio alginolyticus* strains isolated from diseased cultured fish in Tunisia. *Diseases of Aquatic Organanism,* 86(1): 21-28.

Kang, C. H., Shin, Y., Jang, S., Yu, H., Kim, S., An, S., and So, J. S. (2017). Characterization of *Vibrio parahaemolyticus* isolated from oysters in Korea: Resistance to various antibiotics and prevalence of virulence genes. *Marine Pollution Bulletin*, 118(1-2): 261-266.

Kar, D. (2015). *Epizootic ulcerative fish disease syndrome*. Academic Press, Oxford UK.

Karlsen, C., Vanberg, C., Mikkelsen, H., and Sorum, H. (2014). Co-infection of Atlantic salmon (*Salmo salar*), by *Moritella viscosa* and *Aliivibrio wodanis*, development of disease and host colonization. *Veterinary Microbiology,* 171(1-2): 112-121.

Karthik, R., Hussain, A. J., and Muthezhilan, R. (2014). Effectiveness of *Lactobacillus* sp (AMET1506) as probiotic against vibriosis in *Penaeus monodon* and *Litopenaeus vannamei* shrimp aquaculture. *Biosciences Biotechnology Research Asia,* 11: 297-305.

Kashulin, A., Seredkina, N., and Sørum, H. (2017). Cold-water vibriosis. The current status of knowledge. *Journal of Fish Diseases*, 40(1): 119-126.

Kawatsu, K., Sakata, J., Yonekita, T., and Kumeda, Y. (2015). Evaluation of an immunochromatographic assay for direct identification of thermostable direct hemolysin-producing *Vibrio parahaemolyticus* colonies on selective agar plates. *Journal of Microbiological Methods,* 119: 4-6.

Kent, M. L., Dungan, C. F., Elston, R. A., and Holt, R. A. (1988). *Cytophaga* sp. (Cytophagales) infection in seawater pen-reared Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms,* 4(3): 173-180.

Khouadja, S., Lamari, F., and Bakhrouf, A. (2013). Characterization of *Vibrio parahaemolyticus* isolated from farmed sea bass (*Dicentrarchus labrax*) during disease outbreaks. *International Aquatic Research,* 5(1): 13.

Kim, M. N., and Bang, H. J. (2008). Detection of marine pathogenic bacterial *Vibrio* species by multiplex polymerase chain reaction (PCR). *Journal of Environmental Biology,* 29(4): 543-546.

Kim, J. Y., and Lee, J. L. (2017). Correlation of total bacterial and *Vibrio* spp. populations between fish and water in the aquaculture system. *Frontiers in Marine Science,* 4: 147.

Kiriyakit, A., Gallardo, W. G., and Bart, A. N. (2011). Successful hybridization of groupers (*Epinephelus coioides* x *Epinephelus lanceolatus*) using cryopreserved sperm. *Aquaculture*, 320(1-2): 106-112.

Klein, S. L., West, C. K. G., Mejia, D. M., and Lovell, C. R. (2014). Genes similar to the *Vibrio parahaemolyticus* virulence-related genes *tdh*, *tlh*, and *vscC2* occur in other Vibrionaceae species isolated from a pristine estuary. *Applied and Environmental Microbiology,* 80(2): 595-602.

Kopprio, G. A., Streitenberger, M. E., Okuno, K., Baldini, M., Biancalana, F., Fricke, A., Martínez, A., Neogi, S. B., Koch, B. P., Yamasaki, S., and Lara, R. J. (2017). Biogeochemical and hydrological drivers of the dynamics of *Vibrio* species in two Patagonian estuaries. *Science of the Total Environmental*, 576: 646-656.

Korun, J., Ince, A. G., and Karaca, M. (2013). Antibiotic resistance and plasmid profile of *Vibrio alginolyticus* strains isolated from cultured European seabass (*Dicentrarchus labrax*, L.). *Bulletin of the Veterinary Institute in Pulawy,* 57(2): 173-177.

Korzen, L., Abelson, A., and Israel, A. (2016). Growth, protein and carbohydrate contents in *Ulva rigida* and *Gracilaria bursa-pastoris* integrated with an offshore fish farm. *Journal of Applied Phycology*, 28(3): 1835-1845.

Kotob, M. H., Menanteau-Ledouble, S., Kumar, G., Abdelzaher, M., and El-Matbouli, M. (2016). The impact of co-infections on fish: a review. *Veterinary Research,* 47(1): 98.

Kraxberger-Beatty, T., McGarey, D. J., Grier, H. J., Lim, D. V. (1990). *Vibrio harveyi*, an opportunistic pathogen of common snook, *Centropomus undecimalis* (Bloch), held in captivity. *Journal of Fish Diseases,* 13(6): 557-560.

Kriem, M. R., Banni, B., El Bouchtaoui, H., Hamama, A., El Marrakchi, A., Chaouqy, N., Robert-Pillot, A., and Quilici, M. L. (2015). Prevalence of *Vibrio* spp. in raw shrimps (*Parapenaeus longirostris*) and performance of a chromogenic medium for the isolation of *Vibrio* strains. *Letters in Applied Microbiology*, 61(3), 224-230.

Krumperman, P. H. (1985). Multiple antibiotic indexing of *E. coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*, 46(1):165-170.

Krupesha-Sharma, S. R., Rathore, G., Verma, D. K., Sadhu, N., and Philipose, K. K. (2013). *Vibrio alginolyticus* infection in Asian seabass (*Lates calcarifer*, Bloch) reared in open sea floating cages in India. *Aquaculture Research*, 44(1): 86-92.

Kua, B. C., Iar, A., Siti-Zahrah, A., Irene, J., Norazila, J., Nik Haiha, N. Y., Fadzillah, Y., Mohammed, M., Siti Rokhaiya, B., Omar, M., and Teoh, T. P. (2016). Current status of acute hepatopancreatic necrosis disease (AHPND) of farmed shrimp in Malaysia. *In Proceedings of the ASEAN Regional Technical Consultation on EMS/AHPND and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia*, 22-24 February 2016, Makati City, Philippines. Aquaculture Department, Southeast Asian Fisheries Development Center.

Kumar, S. R., Parameswaran, V., Ahmed, V. I., Musthaq, S. S., and Hameed, A. S. (2007). Protective efficiency of DNA vaccination in Asian seabass (*Lates calcarifer*) against *Vibrio anguillarum*. *Fish and Shellfish Immunology,* 23(2): 316-326.

Kumar, P., Thirunavukkarasu, A. R., Subburaj, R., and Thiagarajan, G. (2015). Concept of stress and its mitigation in aquaculture. In: S. Perumal, A.R. Thirunavukkarasu, P. Pachiappan (Eds.), *Advances in Marine and Brackish Water Aquaculture*. New Delhi: Springer.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA 7: Molecular evolutionary genetics analysis version 7.0 for bigger dataset. *Molecular Biology and Evolution*, 33(7): 1870-1874.

Kumara, K. R. P. S., and Hettiarachchi, M. (2016). Regular monitoring and controlling *Vibrio*-a critical biosecurity measure for Sri Lankan shrimp (*Penaeus monodon*) hatcheries. *Fish Pathology,* 51: 46-53.

Kusuda, R., and Salati, F. (1993). Major bacterial diseases affecting mariculture in Japan. *Annual Review of Fish Diseases,* 3: 69-85.

Kutateladze, M., and Adamia, R. (2010). Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends in Biotechnology,* 28(12): 591-595.

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., Powell, E. N., Rondeau, D., and Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual Review of Marine Science,* 7: 471-496.

Laganà, P., Caruso, G., Minutoli, E., Zaccone, R., and Delia, S. (2011). Susceptibility to antibiotics of *Vibrio* spp. and *Photobacterium damsela* ssp. *piscicida* strains isolated from Italian aquaculture farms. *New Microbiologica,* 34(1): 53-63.

Larsen, A. M., Rikard, F. S., Walton, W. C., and Arias, C. R. (2015). Temperature effect on high salinity depuration of *Vibrio vulnificus* and *V. parahaemolyticus* from the eastern oyster (*Crassostrea virginica*). *International Journal of Food Microbiology,* 192: 66-71.

Leano, E. M., and Mohan, C. V. (2012). Early mortality syndrome threatens Asia's shrimp farms. *Global Aquaculture Advocate,* 7-8: 38-39.

Lee, J. H., Ahn, S. H., Kim, S. H., Choi, Y.H., Park, K. J., and Kong, I. S. (2002). Characterization of *Vibrio mimicus* phospholipase A (*PhlA*) and cytotoxicity on fish cell. *Biochemical and Biophysical Research Communications,* 298(2): 269-276.

Lee, H., Choi, S., Shin, H., Lee, J., and Choi, S. (2014). *Vibrio vulnificus* bacteriophage SSP002 as a possible biocontrol agent. *Applied and Environmental Microbiology,* 80(2): 515-524.

Leong, T. S., and Colorni, A. (2002). Infectious diseases of warmwater fish in marine and brackish water. In: P.T.K. Woo, D.W. Bruno and L.H.S. Lim (Eds.), *Diseases and disorders of finfish in cage culture* (pp. 193–230). CABI publishing.

Letchumanan, V., Pusparajah, P., Tan, L. T. H., Yin, W. F., Lee, L. H., and Chan, K. G. (2015). Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. *Frontiers in Microbiology,* 6: 1417.

Letchumanan, V., Chan, K. G., Pusparajah, P., Saokaew, S., Duangjai, A., Goh, B. H., Ab Mutalib, N. S., and Lee, L. H. (2016). Insights into bacteriophage application in controlling *Vibrio* species. *Frontiers in Microbiology*, 7: 1114.

Letunic, I., and Bork, P. (2007) Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics,* 23(1): 127-128.

Leung, T. L. F., and Bates, A. E. (2013). More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. *Journal of Applied Ecology*, 50(1): 215-222.

Li, M. F., Wang, C. L., and Sun, L. (2011). A pathogenic *Vibrio harveyi* lineage causes recurrent disease outbreaks in cultured Japanese flounder (*Paralichthys olivaceus*) and induces apoptosis in host cells. *Aquaculture,* 319(1): 30-36.

Li, L., Mou, X., and Nelson, D. R. (2013). Characterization of *Plp*, a phosphatidylcholine-specific phospholipase and hemolysin of *Vibrio anguillarum*. *BMC Microbiology,* 13(1): 271.

Li, Y., Li, Y., Qureshi, S., Kappas, M., and Hubacek, K. (2015). On the relationship between landscape ecological patterns and water quality across gradient zones of rapid urbanization in coastal China. *Ecological Modelling*, 318: 100-108.

Liang, H. Y., Xia, L. Q., Wu, Z. H., Jian, J. C., and Lu, Y. S. (2010a). Expression, purification and antibody preparation of flagellin *FlaA* from *Vibrio alginolyticus* strain HY9901. *Letters in Applied Microbiology,* 50(2): 181-186.

Liang, H. Y., Wu, Z. H., Jian, J. C., and Huang, Y. C. (2010b). Protection of red snapper (*Lutjanus sanguineus*) against *Vibrio alginolyticus* with a DNA vaccine containing flagellin *flaA* gene. *Letters in Applied Microbiology,* 52(2): 156-161.

Liang, B., Jiang, F., Zhang, S., Yue, X., Wang, H., and Liu, B. (2017). Genetic variation in *Vibrio* resistance in the clam *Meretrix petechialis* under the challenge of *Vibrio parahaemolyticus*. *Aquaculture,* 468(Pt 1): 458-463.

Liu, P. C., Lin, J. Y., and Chuang, W. H. (2004). Isolation and characterization of pathogenic *Vibrio harveyi* (*V. carchariae*) from the farmed marine cobia fish *Rachycentron canadum* L. with gastroenteritis syndrome. *World Journal of Microbiology and Biotechnology*, 20(5):495-499.

Liu, L., Ge, M., Zheng, X., Tao, Z., Zhou, S., and Wang, G. (2016). Investigation of *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay, China. *Aquaculture Reports*, 3: 220-224.

Lopez, J. R., PIeiro-Vidal, M., Garcia-Lamas, N., De la Herran, R., Navas, J. I., Hachero-Cruzado, I., and Santos, Y. (2010). First isolation of *Tenacibaculum soleae* from diseased cultured wedge sole, *Dicologoglossa cuneata* (Moreau), and brill, *Scophthalmus rhombus* (L.) *Journal of Fish Diseases*, 33(3): 273-278.

Lorenz, N., Reiger, M., Toro-Nahuelpan, M., Brachmann, A., Poettinger, L., Plener, L., Lassak, J., and Jung, K. (2016). Identification and initial characterization of prophages in *Vibrio campbellii*. *PloS one*, 11(5): e0156010.

Luo, Z. H., Yu, Y. P., Jost, G., Xu, W., and Huang, X. L. (2015). Complete genome sequence of a giant *Vibrio* bacteriophage VH7D. *Marine Genomics,* 24(Pt 3): 293-295.

Macián, M. C., Arias, C. R., Aznar, R., Garay, E., and Pujalte, M. J. (2000a). Identification of *Vibrio* spp. (other than *V. vulnificus*) recovered on CPC agar from marine natural samples. *International Microbiology,* 3(1): 51-53.

Macián, M. C., Garay, E., González-Candelas, F., Pujalte, M. J., and Aznar, R. (2000b). Ribotyping of *Vibrio* populations associated with cultured oysters (*Ostrea edulis*). *Systematic and Applied Microbiology,* 23(3): 409-417.

Magi, G. E., Avendano-Herrera, R., Magarinos, B., Toranzo, A. E., and Romalde, J. L. (2007). First reports of flexibacteriosis in farmed tub gurnard (*Chelidonichthys lucernus* L.) and wild turbot (*Scophthalmus maximus*) in Italy. *Bulletin of the European Association of Fish Pathologists*, 27(5): 177-184.

Mancuso, M., Genovese, M., Guerrera, M. C., Casella, G., Genovese, L., Piccolo, G., and Maricchiolo, G. (2015). First episode of vibriosis in wild specimens of *Pagellus bogaraveo* (Brünnich, 1768) in the Mediterranean Sea. *Cahiers de Biologie Marine,* 56: 355-361.

Manilal, A., Sujith, S., Selvin, J., Shakir, C., Gandhimathi, R., and Kiran, G. S. (2010). Virulence of *vibrios* isolated from diseased black tiger shrimp, *Penaeus monodon*, Fabricius. *Journal of the World Aquaculture Society*, 41(3): 332-343.

Martinez-Urtaza, J., Lozano-Leon, A., Varela-Pet, J., Trinanes, J., Pazos, Y., and Garcia-Martin, O. (2008). Environmental determinants of the occurrence and distribution of *Vibrio parahaemolyticus* in the rias of Galicia, Spain. *Applied and Environmental Microbiology*, 74(1): 265-274.

Marudhupandi, T., Kumar, T. T. A., Prakash, S., Balamurugan, J., and Dhayanithi, N. B. (2017). *Vibrio parahaemolyticus* a causative bacterium for tail rot disease in ornamental fish, *Amphiprion sebae*. *Aquaculture Reports,* 8: 39-44.

Mateus, L., Costa, L., Silva, Y. J., Pereira, C., Cunha, A., and Almeida, A. (2014). Efficiency of phage cocktails in the inactivation of *Vibrio* in aquaculture. *Aquaculture,* 424-425: 167-173.

Matz, C., McDougald, D., Moreno, A. M., Yung, P. Y., Yildiz, F. H., and Kjelleberg, S. (2005). Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proceedings of the National Academy of Sciences*, 102(46): 16819-16824.

Mechri, B., Medhioub, A., Medhioub, M. N., and Aouni, M. (2012). Diversity of Vibrionaceae associated with *Ruditapes decussatus* hatchery in Tunisia. *Annals of Microbiology*, 62(2): 597-606.

Mikkelsen, H., Lund, V., Martinsen, L. C., Gravningen, K., and Schrøder, M. B. (2007). Variability among *Vibrio anguillarum* O2 isolates from Atlantic cod (*Gadus morhua* L.): characterisation and vaccination studies. *Aquaculture,* 266(1): 16-25.

Mohd-Yusof, N. Y., Monroig, O., Mohd-Adnan, A., Wan, K. L., and Tocher, D. R. (2010). Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, *Lates calcarifer*. *Fish Physiology and Biochemistry*, 36(4): 827-843.

Mohi, M. M., Kuratani, M., Miyazaki, T., and Yoshida, T. (2010). Histopathological studies on *Vibrio harveyi* infected tiger puffer, *Takifugu rubripes* (Temminck et Schlegel), cultured in Japan. *Journal of Fish Diseases*, 33(10): 833-840.

Mondal, M., Nag, D., Koley, H., Saha, D. R., and Chatterjee, N. S. (2014). The *Vibrio cholerae* extracellular chitinase ChiA2 is important for survival and pathogenesis in the host intestine. *PLoS One*, 9(9): e103119.

Montes, M., Farto, R., Pérez, M. J., Nieto, T. P., Larsen, J. L., and Christensen, H. (2003). Characterization of *Vibrio* strains isolated from turbot (*Scophthalmus maximus*) culture by phenotypic analysis, ribotyping and 16S rRNA gene sequence comparison. *Journal of Applied Microbiology,* 95(4): 693-703.

Munro, J., Oakey, J., Bromage, E., and Owens, L. (2003). Experimental bacteriophage-mediated virulence in strains of *Vibrio harveyi*. *Diseases of Aquatic Organisms*, 54(3):187-194.

Musa, N., Wei, L. S., and Wee, W. (2008). Phenotypic and genotypic characteristics of *Vibrio harveyi* isolated from black tiger shrimp (*Penaeus monodon*). *World Applied Sciences Journal*, 3(6): 885-902.

Nagai, T., Iida, Y., Iwamoto, E., and Nakai, T. (2008). A new vibriosis of cultured ayu *Plecoglossus altivelis*. *Fish Pathology,* 43(1): 49-54.

Najiah, M., Lee, K. L., Hassan, M. D., and Shariff, M., and Mohd-Azmi, M. L. (2003). Preliminary study on genetic distance of *Vibrio parahaemolyticus* isolates from diseased fish and shrimp brackishwater ponds by random amplified polymorphic DNA (RAPD) in Malaysia. *Asian Fisheries Science,* 16: 299-305.

Nakamura, Y., Itoh, T., Matsuda, H., and Gojobori, T. (2004). Biased biological functions of horizontally transferred genes in prokaryotic genomes. *Nature Genetics*, 36(7): 760-766.

Nakhamchik, A., Wilde, C., and Rowe-Magnus, D. A. (2008). Cyclic-di-GMP regulates extracellular polysaccharide production, biofilm formation, and rugose colony development by *Vibrio vulnificus*. *Applied and Environmental Microbiology*, 74(13): 4199-4209.

Natrah, F. M. I., Defoirdt, T., Sorgeloos, P., and Bossier, P. (2011). Disruption of bacterial cell-to-cell communication by marine organisms and its relevance to aquaculture. *Marine Biotechnology*, 13(2): 109-126.

Nguyen, H. N. K, Van, T. T. H., and Coloe, P. J. (2016). Antibiotic resistance associated with aquaculture in Vietnam. *Microbiology Australia*, 37(3): 108-111.

Nishiguchi, M. K., and Jones, B. W. (2004). Microbial biodiversity within the Vibrionaceae. In *Origins* (pp. 533-548). Springer, Dordrecht.

Noga, E. J. (2010). *Fish Disease: Diagnosis and Treatment (2nd edition)*. Mosby-Year Book, Inc., St. Louis, Missouri, US.

Noorlela, M. Y. (2014) Fish Hatchery the B-Area [PowerPoint slides]. Retrieved from https://www.slideshare.net/LelaMy/fish-hatchery-the-barea.

Noorlis, A., Ghazali, F. M., Cheah, Y. K., Tuan Zainazor, T. C., Ponniah, J., Tunung, R., Tang, J. Y. H., Nishibuchi, M., Nakaguchi, Y., and Son, R. (2011). Prevalence and quantification of *Vibrio* species and *Vibrio parahaemolyticus* in freshwater fish at hypermarket level. *International Food Research Journal*, 18(2): 689-695.

Oakey, H. J., and Owens, L. (2000). A new bacteriophage, VHML, isolated from a toxin-producing strain of *Vibrio harveyi* in tropical Australia. *Journal of Applied Microbiology,* 89(4): 701-709.

Odeyemi, O. A., and Ahmad, A. (2017). Population dynamics, antibiotics resistance and biofilm formation of *Aeromonas* and *Vibrio* species isolated from aquatic sources in Northern Malaysia. *Microbial Pathogenesis*, 103: 178-185.

Oh, M. J., Kim, W. S., Kitamura, S. I., Lee, H. K., Son, B. W., Jung, T. S., and Jung, S. J. (2006). Change of pathogenicity in olive flounder *Paralichthys olivaceus* by co-infection of *Vibrio harveyi*, *Edwardsiella tarda* and marine birnavirus. *Aquaculture,* 257(1-4): 156-160.

Ohnishi, K., Nakahira, K., Unzai, S., Mayanagi, K., Hashimoto, H., Shiraki, K., and Yanagihara, I. (2011). Relationship between heat-induced fibrillogenicity and hemolytic activity of thermostable direct hemolysin and a related hemolysin of *Vibrio parahaemolyticus*. *FEMS Microbiology Letters*, 318(1): 10-17.

Orruño, M., Parada, C., Ogayar, E., Kaberdin, V. R., and Arana, I. (2019). Effects of abiotic and biotic factors on *Vibrio harveyi* ATCC 14126T survival dynamics in seawater microcosms. *Aquatic Microbial Ecology*, 83(2): 109-118.

Ottaviani, D., Bacchiocchi, I., Masini, L., Leoni, F., Carraturo, A., Giammarioli, M., and Sbaraglia, G. (2001). Antimicrobial susceptibility of potentially pathogenic halophilic *vibrios* isolated from seafood. *International Journal of Antimicrobial Agents*, 18(2): 135-140.

Ottaviani, D., Leoni, F., Talevi, G., Masini, L., Santarelli, S., Rocchegiani, E., Susini, F., Motagna, C., Monno, R., D'Annibale, L., Manso, E., Oliva, M., and Pazzani, C. (2013). Extensive investigation of antimicrobial resistance in *Vibrio parahaemolyticus* from shellfish and clinical sources, Italy. *International Journal of Antimicrobial Agents,* 42(2): 191-193.

O'Toole, R., Von Hofsten, J., Rosqvist, R., Olsson, P. E., and Wolf-Watz, H. (2004). Visualisation of zebrafish infection by GFP-labelled *Vibrio anguillarum*. *Microbial Pathogenesis,* 37(1): 41-46.

Parvathi, A., George, J., and Kumar, S. (2009). Prevalence and heterogeneity of hemolysin gene *vhh* among hatchery isolates of *Vibrio harveyi* in India. *Current Microbiology,* 59(1): 42-47.

Pascual, J., Macián, M. C., Arahal, D. R., Garay, E., and Pujalte, M. J. (2010) Multilocus sequence analysis of the central clade of the genus *Vibrio* by using the 16S rRNA, *recA*, *pyrH*, *rpoD*, *gyrB*, *rctB* and *toxR* genes. *International Journal of Systematic and Evolutionary Microbiology,* 60(1): 154-165.

Passalacqua, P. L., Zavatta, E., Bignami, G., Serraino, A., and Serratore, P. (2016). Occurrence of *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* in the clam *Ruditapes philippinarum* (Adams & Reeve, 1850) from Emilia Romagna and Sardinia, Italy. *Italian Journal of Food Safety,* 5(1): 5709.

Paydar, M., The, C. S. J., and Thong, K. L. (2013). Prevalence and characterisation of potentially virulent *Vibrio parahaemolyticus* in seafood in Malaysia using conventional methods, PCR and REP-PCR. *Food Control,* 32(1): 13-18.

Pedersen, K., Skall, H. F., Lassen-Nielsen, A. M., Nielsen, T. F., Henriksen, N. H., and Olesen, N. J. (2008). Surveillance of health status on eight marine rainbow trout, *Oncorhynchus mykiss* (Walbaum), farms in Denmark in 2006. *Journal of Fish Diseases,* 31(9): 659-667.

Preheim, S. P., Boucher, Y., Wildschutte, H., David, L. A., Veneziano, D., Alm, E. J., and Polz, M. F. (2011). Metapopulation structure of Vibrionaceae among coastal marine invertebrates. *Environmental Microbiology*, 13(1): 265-275.

Pujalte, M. J., Sitjà-Bobadilla, A., Macián, M. C., Belloch, C., Alvarez-Pellitero, P., Pérez-Sánchez, J., Uruburu, F., and Garay, E. (2003). Virulence and molecular typing of *Vibrio harveyi* strains isolated from cultured dentex, gilthead sea bream and European sea bass. *Systemic and Applied Microbiology,* 26(2): 284-292.

Qi, W. (2002). *Social and economic impacts of aquatic animal health problems in aquaculture in China*. FAO Fisheries Technical Paper.

Qin, Y. X., Wang, J., Su, Y. Q., Wang, D. X., and Chen, X. Z. (2006). Studies on the pathogenic bacterium of ulcer disease in *Epinephelus awoara*. *Acta Oceanologica Sinica,* 25: 154-159.

Raguenes, G., Christen, R., Guezennec, J., Pignet, P., and Barbier, G. (1997). *Vibrio diabolicus* sp. nov., a New Polysaccharide-Secreting Organism Isolated from a Deep-sea Hydrothermal Vent Polychaete Annelid, *Alvinella pompejana*. *International Journal of Systematic Bacteriology*, 47(4): 989-995.

Rahman, M. S., Martino, M. E., Cardazzo, B., Facco, P., Bordin, P., Mioni, R., Novelli, E., and Fasolato, L. (2014) *Vibrio* trends in the ecology of Venice Lagoon. *Applied and Environmental Microbiology,* 80(8): 2372-2380.

Rahman, M. S., Carraro, R., Cardazzo, B., Carraro, L., Boscolo Meneguolo, D., Martino, M. E., Andreani, N. A., Bordin, P., Mioni, R., Novelli, E., Balzan, S., and Fasolato, L. (2017). Molecular typing of *Vibrio parahaemolyticus* strains isolated from mollusks in the North Adriatic Sea. *Foodborne Pathogens and Disease,* 14(8): 454-464.

Raida, M. K., and Buchmann, K. (2008). Development of adaptive immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. *Fish and Shellfish Immunology,* 25(5): 533-541.

Raja, R. A., and Jithendran, K. P. (2015). Aquaculture Disease Diagnosis and Health Management. In *Advances in Marine and Brackishwater Aquaculture*. Springer. India.

Rajan, P. R., Lopez, C., Lin, J. H., and Yang, H. (2001). *Vibrio alginolyticus* infection in cobia (*Rachycentron canadum*) cultured in Taiwan. *Bulletin of the European Association of Fish Pathologists,* 21(6): 228-234.

Ramamurthy, T., Chowdhury, G., Pazhani, G. P., and Shinoda, S. (2014). *Vibrio fluvialis*: an emerging human pathogen. *Frontiers in Microbiology,* 5: 91.

Rameshkumar, P., Kalidas, C., Tamilmani, G., Sakthivel, M., Nazar, A. A., Maharshi, V. A., Rao, S. K. S., and Gopakumar, G. (2014). Microbiological and histopathological investigations of *Vibrio alginolyticus* infection in cobia *Rachycentron canadum* (Linnaeus, 1766) cultured in sea cage. *Indian Journal of Fisheries,* 61(1): 124-127.

Rameshkumar, P., Nazar, A. K. A., Pradeep, M. A., Kalidas, C., Jayakumar, R., Tamilmani, G., Sakthivel, M., Samal, A. K., Sirajudeen, S., Venkatesan, V., and Nazeera, B. M. (2017). Isolation and characterization of pathogenic *Vibrio alginolyticus* from sea cage cultured cobia *Rachycentron canadum* (Linnaeus 1766) in India. *Letters in Applied Microbiology*, 65(5): 423-430.

Randrianarivelo, R., Danthu, P., Benoit, C., Ruez, P., Raherimandimby, M., and Sarter, S. (2010). Novel alternative to antibiotics in shrimp hatchery: effects of the essential oil of *Cinnamosma fragrans* on survival and bacterial concentration of *Penaeus monodon* larvae. *Journal of Applied Microbiology,* 109(2): 642-650.

Ransangan, J., and Mustafa, S. (2009). Identification of *Vibrio harveyi* isolated from diseased Asian seabass (*Lates calcarifer*) by use of 16S ribosomal DNA sequencing. *Journal of Aquatic Animal Health*, 21(3): 150-155.

Ransangan, J., Lal, T. M., and Al-Harbi, A. H. (2012). Characterization and experimental infection of *Vibrio harveyi* isolated from diseased Asian seabass (*Lates calcarifer*). *Malaysian Journal of Microbiology*, 8(2): 104-115.

Ransangan, J., Manin, B. O., Lal, T. M. M., Lu, K. C., Sade, A., and Abdullah, A. (2013a). Betanodavirus infection in marine fish aquaculture in Malaysia. *Research Journal of Animal, Veterinary and Fishery Sciences*, 1(7), 10-15.

Ransangan, J., Imm, L. K. L., Lal, T. M., and Sade, A. (2013b). Phenotypic characterization and antibiotic susceptibility of *Vibrio* spp. isolated from aquaculture waters on the west coast of Sabah, Malaysia. *International Journal of Research in Pure and Applied Microbiology*, 3(3): 58-66.

Rao, B. M., and Lalitha, K. V. (2015). Bacteriophage for aquaculture: are they beneficial or inimical. *Aquaculture,* 437: 146-154.

Raszl, S. M., Froelich, B. A., Vieira, C. R., Blackwood, D. A., and Noble, R. T. (2016). *Vibrio parahaemolyticus* and *Vibrio vulnificus* in South America: Water, Seafood, and Human Infections. *Journal of Applied Microbiology,* 121(5): 1201-1222.

Razak, A. A., Ransangan, J., & Sade, A. (2014). First report of *Megalocytivirus* (Iridoviridae) in grouper culture in Sabah, Malaysia. *International Journal of Current Microbiology and Applied Sciences*, 3(3): 896-909.

Rico, R. M., Tapia-Paniagua, S., Martínez-Manzanares, E., Balebona, M. C., and Moriñigo, M. A. (2008). Characterization of *Vibrio harveyi* strains recovered from diseased farmed Senegalese sole (*Solea senegalensis*). *Journal of Applied Microbiology,* 105(3): 752-760.

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): 75-93.

Rimmer, M. A., Phillips, M. J., and Sim, S. Y. (2005). Aquaculture of groupers in Asia and the Pacific. *Economics and marketing of the live reef fish trade in Asia Pacific, proceeding of workshop, ACIAR Working Paper no. 60.* Noumea, New Caledonia.

Rong, R., Lin, H., Wang, J., Khan, M. N., and Li, M. (2014). Reductions of *Vibrio parahaemolyticus* in oysters after bacteriophage application during depuration. *Aquaculture,* 418-419: 171-176.

Rønneseth, A., Castillo, D., D'Alvise, P., Tønnesen, Ø., Haugland, G., Grotkjær, T., Engell-Sørensen, K., Nørremark, L., Bergh, Ø., Wergeland, H. I., and Gram, L. (2017). Comparative assessment of *Vibrio* virulence in marine fish larvae. *Journal of Fish Diseases*, 40(10): 1373-1385.

Ruiz, P., Poblete, M., Yáñez, A. J., Irgang, R., Toranzo, A. E., and Avendaño-Herrera, R. (2015). Cell-surface properties of *Vibrio ordalii* strains isolated from Atlantic salmon *Salmo salar* in Chilean farms. *Diseases of Aquatic Organisms,* 113(1): 9-23.

Ruiz, P., Balado, M., Toranzo, A. E., Poblete-Morales, M., Lemos, M. L., Avendaño-Herrera, R. (2016). Iron assimilation and siderophore production by *Vibrio*

ordalii strains isolated from diseased Atlantic salmon *Salmo salar* in Chile. *Diseases of Aquatic Organisms*, 118(3): 217-226.

Ruwandeepika, H. A. D., Defoirdt, T., Bhowmick, P. P., Shekar, M., Bossier, P., and Karunasagar, I. (2010). Presence of typical and atypical virulence genes in *vibrio* isolates belonging to the *Harveyi* clade. *Journal of Applied Microbiology*, 109(3): 888-899.

Saad, T. T., and Atallah, S. T. (2014). Studies on bacterial infection in marine fish. *Journal of the Arabian Aquaculture Society,* 9(1): 1-20.

Saeed, M. O. (1995). Association of *Vibrio harveyi* with mortalities in cultured marine fish in Kuwait. *Aquaculture,* 136(1-2): 21-29.

Saalah, S., Shapawi, R., Othman, N. A., and Bono, A. (2010). Effect of formula variation in the properties of fish feed pellet. *Journal of Applied Sciences*, 10(21): 2537-2543.

Sahilah, A. M., Laila, R. A. S., Sallehuddin, H. M., Osman, H., Aminah, A., and Azuhairi, A. A. (2014). Antibiotic resistance and molecular typing among cockle (*Anadara granosa*) strains of *Vibrio parahaemolyticus* by polymerase chain reaction (PCR)-based analysis. *World Journal of Microbiology and Biotechnology*, 30(2): 649-659.

Sainz-Hernández, J. C., and Maeda-Martínez, A. N. (2005). Sources of *Vibrio* bacteria in mollusc hatcheries and control methods: a case study. *Aquaculture Research,* 36(16): 1611-1618.

Salama, A. J., Satheesh, S., Balqadi, A. A., and Kitto, M. R. (2016). Identifying suitable fin fish cage farming sites in the eastern Red Sea Coast, Saudi Arabia. *Thalassas: An International Journal of Marine Sciences*, 32(1): 1-9.

Samuelsen, O. B., and Bergh, Ø. (2004). Efficacy of orally administered florfenicol and oxolinic acid for the treatment of vibriosis in cod (*Gadus morhua*). *Aquaculture,* 235(1): 27-35.

Samuelsen, O. B., Nerland, A. H., Jørgensen, T., Schrøder, M. B., Svåsand, T., and Bergh, Ø. (2006). Viral and bacterial diseases of Atlantic cod *Gadus morhua*, their prophylaxis and treatment: a review. *Diseases of Aquatic Organisms,* 71(3): 239-254.

Sandlund, N., Rødseth, O. M., Knappskog, D. H., Fiksdal, I. U., and Bergh, Ø. (2010). Comparative susceptibility of turbot, halibut, and cod yolk-sac larvae to challenge with *Vibrio* spp. *Diseases of Aquatic Organisms,* 89(1): 29-37.

Santhyia, A. V., Mulloorpeedikayil, R. G., Kollanoor, R. J., and Jeyaseelan, P. M. (2015). Molecular variations in *Vibrio alginolyticus* and *V. harveyi* in shrimp-farming systems upon stress. *Brazilian Journal of Microbiology,* 46(4): 1001-1008.

Saravanan, V., Sanath, K. H., Karunasagar, I., and Karunasagar, I. (2007). Putative virulence genes of *Vibrio cholerae* from seafoods and the coastal environment of Southwest India. *International Journal of Food Microbiology*, 119(3): 329-333.

Sarjito, S., Radjasa, O. K., Sabdono, A., Prayitno, S. B., and Hutabarat, S. (2009). Phylogenetic diversity of the causative agents of vibriosis associated with groupers fish from Karimunjawa Islands, Indonesia. *Current Research in Bacteriology*, 2(1): 14-21.

Sarter, S., Randrianarivelo, R., Ruez, P., Raherimandimby, M., and Danthu, P. (2011). Antimicrobial effects of essential oils of *Cinnamosma fragrans* on the bacterial communities in the rearing water of *Penaeus monodon* larvae. *Vector-Borne and Zoonotic Diseases,* 11(4): 433-437.

Sawabe, T., Kita-Tsukamoto, K., and Thompson, F. L. (2007). Inferring the evolutionary history of *Vibrios* by means of multilocus sequence analysis. *Journal of Bacteriology,* 189(21): 7932-7936.

Sawabe, T., Ogura, Y., Matsumura, Y., Feng, G., Amin, A. R., Mino, S., Nakagawa, S., Swabe, T., Kumar, R., Fukui, Y., Satomi, M., Matsushima, R., Thompson, F. L., Gomez-Gil, B., Christen, R., Maruyama, F., Kurokawa, K., and Hayashi, T. (2013). Updating the *Vibrio* clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of *Vibrio tritonius* sp. nov. *Frontiers in Microbiology,* 4: 414.

Sharifuzzaman, S. M., and Austin, B. (2010). Kocuria SM1 controls vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology,* 108(6): 162-170.

Shen, G. M., Shi, C. Y., Fan, C., Jia, D., Wang, S. Q., Xie, G. S., Li, G. Y., Mo, Z. L., and Huang, J. (2017). Isolation, identification and pathogenicity of *Vibrio harveyi*, the causal agent of skin ulcer disease in juvenile hybrid groupers *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂. *Journal of Fish Disease,* 40(10): 1351-1362.

Shinoda, S. (2005). Pathogenic factors of vibrios with special emphasis on *Vibrio vulnificus*. *Journal-Pharmaceutical Society of Japan*, 125(7): 531-547.

Sindermann, C. J. (1984). Disease in marine aquaculture. *Helgoländer Meeresuntersuchungen,* 37(1): 505.

Sindermann, C. J. (1990). *Principal diseases of marine fish and shellfish: Diseases of Marine Fish* (Vol. 1). Academic Press.

Silva-Rubio, A., Acevedo, C., Magarinos, B., Jaureguiberry, B., Toranzo, A. E., and Avendaño-Herrera, R. (2008). Antigenic and molecular characterization of *Vibrio ordalii* strains isolated from Atlantic salmon *Salmo salar* in Chile. *Diseases of Aquatic Organisms,* 79(1): 27-35.

Sivakumar, N., Selvakumar, G., Varalakshmi, P., and Ashokkumar, B. (2014). *Lactobacillus* sp. a potent probiotic for disease free shrimp aquaculture. *International Journal of Recent Scientific Research,* 5: 1031-1045.

Småge, S. B., Frisch, K., Brevik, Ø. J., Watanabe, K., and Nylund, A. (2016). First isolation, identification and characterisation of *Tenacibaculum maritimum* in Norway, isolated from diseased farmed sea lice cleaner fish *Cyclopterus lumpus* L. *Aquaculture,* 464: 178-184.

Sommerset, I., Krossoy, B., Biering, E., and Frost, P. (2005). Vaccines for fish in aquaculture. *Expert Review of Vaccines,* 4(1): 89-101.

Soffientino, B., Gwaltney, T., Nelson, D. R., Specker, J. L., Mauel, M., and Gomez-Chiarri, M. (1999). Infectious necrotizing enteritis and mortality caused by *Vibrio carchariae* in summer flounder *Paralichthys dentatus* during intensive culture. *Diseases of Aquatic Organisms,* 38(3): 201-210.

Somga, J. R., Somga, S. S., and Reantaso, M. B. (2002). *Impacts of disease on small-scale grouper culture in the Philippines*. FAO Fisheries Technical Paper.

Sopinka, N. M., Donaldson, M. R., O'Connor, C. M., Suski, C. D., and Cooke, S. J. (2016). Stress indicators in fish (Chapter 11). In: C. B. Schreck, L. Tort, A. P. Farrell, C. J. Brauner. (Eds). *Biology of stress in fish: Fish physiology*, Academic Press.

Sorroza, L., Padilla, D., Acosta, F., Román, L., Grasso, V., Vega, J., and Real, F. (2012). Characterization of the probiotic strain *Vagococcus fluvialis* in the protection of European sea bass (*Dicentrarchus labrax*) against vibriosis by *Vibrio anguillarum*. *Veterinary Microbiology,* 155(2): 369-373.

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): 11-19.

Soto-Rodriguez, S. A., Gomez-Gil, B., Lozano-Olvera, R., Betancourt-Lozano, M., and Morales-Covarrubias, M. S. (2015). Field and experimental evidence of Vibrio parahaemolyticus as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in Northwestern Mexico. *Applied and Environmental Microbiology,* 81(5): 1689-1699.

Spinos, E., Kokkoris, G. D., and Bakopoulos, V. (2017). Prevention of sea bass (*Dicentrarchus labrax*, L. 1758) photobacteriosis and vibriosis. Long term efficacy study of intraperitoneally administered bivalent commercial vaccines. *Aquaculture,* 471: 172-184.

Sterk, A., Schets, F. M., Husman, R., Maria, A., Nijs, T., and Schijven, J. F. (2015). Effect of climate change on the concentration and associated risks of *Vibrio* spp. in Dutch recreational waters. *Risk Analysis,* 35(9): 1717-1729.

Subasinghe, R. (2017). Regional review on status and trends in aquaculture development in Asia-Pacific-2015. *FAO Fisheries and Aquaculture Circular*, (C1135/5): I.

Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., and Al-Habsi, S. (2012). Comparative pathogenomics of bacteria causing infectious diseases in fish. *International Journal of Evolutionary Biology,* 2012: 457264.

Sugita, H., Yamamoto, S., Asakura, C., and Morita, T. (2005). Occurrence of *Listonella anguillarum* in seed production environments of Japanese flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture Research,* 36(9): 920-926.

Sujeewa, A. K., Norrakiah, A. S., and Laina, M. (2009). Prevalence of toxic genes of *Vibrio parahaemolyticus* in shrimps (*Penaeus monodon*) and culture environment. *International Food Research Journal*, 16(1).

Sun, B., Zhang, X. H., Tang, X., Wang, S., Zhong, Y., Chen, J., and Austin, B. (2007). A single residue change in *Vibrio harveyi* hemolysin results in the loss of phospholipase and haemolytic activities and pathogenicity for turbot (*Scophthalmus maximus)*. *Journal of Bacteriology,* 189(6): 2575-2579.

Sun, Y., Yang, H., Ling, Z., Chang, J., and Ye, J. (2009a). Gut microbiota of fast and slow growing grouper *Epinephelus coioides*. *African Journal of Microbiology Research,* 3(11): 637-640.

Sun, K., Zhang, W. W., Hou, J. H., and Sun, L. (2009b). Immunoprotective analysis of VhhP2, a *Vibrio harveyi* vaccine candidate. *Vaccine,* 27(21): 2733-2740.

Sun, Y., Huang, Y., Hu, G., Zhang, X., Ruan, Z., Zhao, X., and Shi, Q. (2016). Comparative transcriptomic study of muscle provides new insights into the growth superiority of a novel grouper hybrid. *PLoS ONE*, 11:e0168802.

Sukui, M., Nakagawa, Y., Harayama, S., and Yamamoto, S., (2001). Phylogenetic analysis and taxonomic study of marine Cyto- phaga-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb.nov.*and Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 51(Pt 5): 1639-1652.

Tacon, A. G. J., and Halwart, M. (2007). Cage aquaculture: a global overview. In: M. Halwart, D. Soto, and J.R. Arthur (Eds.). *Cage aquaculture – Regional reviews and global overview,* (pp. 1-16). FAO Fisheries Technical Paper. No. 498. Rome, FAO.

Takemura, A. F., Chien, D. M., and Polz, M. F. (2014). Associations and dynamics of *Vibrionaceae* in the environment, from the genus to the population level. *Frontiers in Microbiology*, 5: 38.

Tall, A., Hervio-Heath, D., Teillon, A., Boisset-Helbert, C., Delesmont, R., Bodilis, J., and Touron-Bodilis, A. (2013). Diversity of *Vibrio* spp. isolated at ambient environmental temperature in the Eastern English Channel as determined by *pyrH* sequencing. *Journal of Applied Microbiology*, 114(6): 1713-1724.

Tan, Z., Komar, C., and Enright, W. J. (2007). *Health management practices for cage aquaculture in Asia - a key component for sustainability*. Intervet Norbio Singapore Pte Ltd.

Tan, D., Gram, L., and Middelboe, M. (2014). Vibriophages and their interactions with the fish pathogen *Vibrio anguillarum*. *Applied and Environmental Microbiology,* 80(10): 3128-3140.

Tanil, G. B., Radu, S., Nishibuchi, M., and Rahim, R. A. (2005). Characterization of *Vibrio parahaemolyticus* isolated from coastal seawater in peninsular Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health,* 36(4): 940.

Tao, Z., Larsen, A. M., Bullard, S. A., Wright, A. C., and Arias, C. R. (2012). Prevalence and population structure of *Vibrio vulnificus* on fishes from the northern Gulf of Mexico. *Applied and Environmental Microbiology,* 78(21): 7611-7618.

Tendencia, E. A. (2002). *Vibrio harveyi* isolated from cage-cultured seabass *Lates calcarifer* Bloch in the Philippines. *Aquaculture Research,* 33(6): 455-458.

Tendencia, E. A., and Lavilla-Pitogo, C. R. (2004). Chapter 2. Bacterial diseases. In: K. Nagasawa and E.R. Cruz-Lacierda (Eds.). *Diseases of cultured groupers*. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Thompson, F. L., Iida, T., and Swings, J. (2004). Biodiversity of *Vibrios*. *Microbiology and Molecular Biology Reviews* 68(3): 403-431.

Thompson, F. L., Gevers, D., Thompson, C. C., Dawyndt, P., Naser, S., Hoste, B., Munn, C. B., and Swings, J. (2005). Phylogeny and molecular identification of vibrios on the basis of multilocus sequence analysis. *Applied and Environmental Microbiology,* 71(9): 5107-5115.

Toranzo, A. E., Magariños, B., and Romalde, J. L. (2005). A review of the bacterial fish diseases in mariculture systems. *Aquaculture,* 246(1-4): 37-61.

Toranzo, A. E., Magariños, B., and Avendano-Herrera, R. (2017). Vibriosis: *Vibrio anguillarum*, *V. ordalii* and *Aliivibrio salmonicida*. In: P.T.K. Woo and R.C. Cipriono (Eds). *Fish Viruses and Bacteria: Pathobiology and Protection*. Wallingford: CAB International.

Touraki, M., Karamanlidou, G., Karavida, P., and Chrysim, K. (2012). Evaluation of the probiotics *Bacillus subtilis* and *Lactobacillus plantarum* bioencapsulated in *Artemia nauplii* against vibriosis in European sea bass larvae

(*Dicentrarchus labrax*, L.). *World Journal of Microbiology and Biotechnology,* 28(6): 2425-2433.

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): 45-55.

Turner, J. W., Tallman, J. J., Macias, A., Pinnell, L. J., Elledge, N. C., Azadani, D. N., Nilsson, W. B., Paranjpye, R. N., Armbrust E. V., and Strom, M. S. (2018). Comparative genomic analysis of *Vibrio diabolicus* and six taxonomic synonyms: a first look at the distribution and diversity of the expanded species. *Frontiers in Microbiology*, 9: 1893.

Valiente, E., Lee, C. T., Hor, L. I., Fouz, B., and Amaro, C. (2008). Role of the metalloprotease Vvp and the virulence plasmid pR99 of *Vibrio vulnificus* serovar E in surface colonization and fish virulence. *Environmental microbiology*, 10(2), 328-338.

Vandenberghe, J., Thompson, F., Gomez-Gil, B., and Swings, J. (2003). Phenotypic diversity amongst isolates from marine aquaculture systems. *Aquaculture,* 219(1-4): 9-20.

Verma, S. C., and Miyashiro, T. (2013). Quorum sensing in the squid-*Vibrio* symbiosis. *International Journal of Molecular Sciences,* 14(8): 16386-16401.

Vezzulli, L., Pezzati, E., Moreno, M., Fabiano, M., Pane, L., and Pruzzo, C. (2009). Benthic ecology of *Vibrio* spp. and pathogenic *Vibrio* species in a coastal Mediterranean environment (La Spezia Gulf, Italy). *Microbial Ecology,* 58(4): 808-818.

Vezzulli, L., Pezzati, E., Stauder, M., Stagnaro, L., Venier, P., and Pruzzo, C. (2015). Aquatic ecology of the oyster pathogens *Vibrio splendidus* and *Vibrio aestuarianus*. *Environmental Microbiology,* 17(4): 1065-1080.

Warner, E., and Oliver, J. D. (2007). Refined medium for direct isolation of *Vibrio vulnificus* from oyster tissue and seawater. *Applied and Environmental Microbiology,* 73(9): 3098-3100.

Weber, B., Chen, C., and Milton, D. L. (2010). Colonization of fish skin is vital for *Vibrio anguillarum* to cause disease. *Environmental Microbiology Reports,* 2(1): 133-139.

Westrich, J. R., Ebling, A. M., Landing, W. M., Joyner, J. L., Kemp, K. M., Griffin, D. W., and Lipp, E. K. (2016). Saharan dust nutrients promote *Vibrio* bloom formation in marine surface waters. *Proceedings of the National Academy of Sciences,* 113(21): 5964-5969.

Whittington, R. J., Becker, J. A., and Dennis, M. M. (2010). Iridovirus infections in finfish–critical review with emphasis on ranaviruses. *Journal of fish diseases*, 33(2): 95-122.

WHO. *Risk assessment of Vibrio vulnificus in raw oysters: interpretative summary and technical report*. (2005). WHO Library Cataloguing-in-Publication Data.

WHO. *Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance*. (2006). Seoul, Republic of Korea.

Willey, E. O., and Lieberman, B. S. (2011). *Phylogenetics: the theory of phylogenetic systemantics, (2nd Edition)*. Wiley-Backwell, Hoboken.

Williams, T., Barbosa-Solomieu, V., and Chinchar, V. G. (2005). A decade of advances in iridovirus research. *Advances in virus research*, 65: 173-248.

Williams, T. C., Froelich, B., and Oliver, J. D. (2013). A new culture-based method for the improved identification of *Vibrio vulnificus* from environmental samples, reducing the need for molecular confirmation. *Journal of Microbiological Methods,* 93(3): 277-283.

Won, K. M., and S. I. Park. (2008). Pathogenicity of *Vibrio harveyi* to cultured marine fishes in Korea. *Aquaculture*, 285(1-4): 8-13.

Wu, H., Aoki, A., Arimoto, T., Nakano, T., Ohnuki, H., Murata, M., Ren, H., and Endo, H. (2015). Fish stress become visible: A new attempt to use biosensor for real-time monitoring fish stress. *Biosensors and Bioelectronics*, 67: 503-510.

Xu, Y., Wang, C., Zhang, G., Tian, J., Liu, Y., Shen, X., and Feng, J. (2017). ISCR2 is associated with the dissemination of multiple resistance genes among *Vibrio* spp. and *Pseudoalteromonas* spp. isolated from farmed fish. *Archives of Microbiology*, 199(6): 891-896.

Yáñez, J. M., Houston, R. D., and Newman, S. (2014). Genetics and genomics of disease resistance in salmonid species. *Frontiers in Genetics,* 5: 415.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., Ban, M., and Aue-Umneoy, D. (2014). Prevalence and antimicrobial susceptibility of *Vibrio* species related to food safety isolated from shrimp cultured at inland ponds in Thailand. *Food Control,* 38: 30-45.

Yanong, R. P., and Erlacher-Reid, C. (2012). *Biosecurity in aquaculture, Part 1: an overview*. USDA Southern Regional Aquaculture Center.

Yardimci, R. E., and Timur, G. (2015). Isolation and Identification of *Tenacibaculum maritimum*, the Causative Agent of Tenacibaculosis in Farmed Sea Bass (*Dicentrarchus labrax*) on the Aegean Sea Coast of Turkey. *The Israeli Journal of Aquaculture -Bamidgeh*, 1172.

Yeh, R. Y., Shiu, Y. L., Shei, S. C., Cheng, S. C., Huang, S. Y., Lin, J. C., and Liu, C. H. (2009). Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, *Cinnamomum kanehirae*, and the effects on immunity and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology,* 27(1): 26-32.

Yildiz, F. H., and Visick, K. L. (2009). *Vibrio* biofilms: so much the same yet so different. *Trends in Microbiology,* 17(3): 109-118.

Yoon, J. H., Bae, Y. M., and Lee, S. Y. (2016). Evaluation of diluents and a selective agar for enumerating the viable number of *Vibrio* spp. in laboratory broth and on mackerel. *Journal of Food Safety,* 36(3): 332-340.

Yoshikoshi, K., and Inoue, K. (1990). Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathusfasciatus* (Temminck and Schlegel), *Journal of Fish Disease,* 13(1): 69-77.

You, K. G., Bong, C. W., and Lee. C. W. (2016). Antibiotic resistance and plasmid profiling of *Vibrio* spp. in tropical waters of Peninsular Malaysia. *Environmental Monitoring and Assessment*, 188(3): 171.

Yu, Q., Niu, M., Yu, M., Liu, Y., Wang, D., and Shi, X. (2016). Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shellfish in Shanghai. *Food Control,* 60: 263-268.

Yue, X., Liu, B., Xiang, J., and Jia, J. (2010). Identification and characterization of the pathogenic effect of a *Vibrio parahaemolyticus*-related bacterium isolated from clam *Meretrix meretrix* with mass mortality. *Journal of Invertebrate Pathology,* 103(2): 109-115.

Zago, V., Zambon, M., Civettini, M., Zaltum, O., and Manfrin, A. (2017). Virulence-associated factors in *Vibrio cholerae* non-O1/non-O139 and *V. mimicus* strains isolated in ornamental fish species. *Journal of Fish Diseases,* 40(12): 1857-1868.

Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L. A., and Fadda, G. (2001). In vitro susceptibility of *Vibrio* spp. isolated from the environment. *International Journal of Antimicrobial Agents*, 17(5): 407-409.

Zhang, X., Li, Y. W., Mo, Z. Q., Luo, X. C., Sun, H. Y., Liu, P., and Dan, X. M. (2014). Outbreak of a novel disease associated with *Vibrio mimicus* infection in fresh water cultured yellow catfish, *Pelteobagrus fulvidraco*. *Aquaculture*, 432: 119-124.

Zhang, X., Wang, S., Chen, S., Chen, Y., Liu, Y., Shao, C., Wang, Q., Lu, Y., Gong, G., Ding, S., and Sha, Z. (2015). Transcriptome analysis revealed changes of multiple genes involved in immunity in *Cynoglossus semilaevis* during *Vibrio anguillarum* infection. *Fish and Shellfish Immunology,* 43(1): 209-218.

Zhong, Y., Zhang, X. H., Chen, J., Chi, Z., Sun, B., Li, Y., and Austin, B. (2006). Overexpression, purification, characterization and pathogenicity of *Vibrio harveyi* hemolysin VHH. *Infection and Immunity,* 74(10): 6001-6005.

Zhu, Z. M., Dong, C. F., Weng, S. P., and He, J. G. (2017). The high prevalence of pathogenic *Vibrio harveyi* with multiple antibiotic resistance in scale drop and muscle necrosis disease of the hybrid grouper, *Epinephelus fuscoguttatus* (♀) × *E. lanceolatus* (♂), in China. *Journal of Fish Diseases,* 41(4): 589-601.

Zorriehzahra, M. J., and Banaederakhshan, R. (2015). Early mortality syndrome (EMS) as new emerging threat in shrimp industry. *Advanced in Animal and Veterinary Sciences,* 3(2s): 64-72.

Zorrilla, I., Arijo, S., Chabrillon, M., Diaz, P., Martinez-Manzanares, E., Balebona, M. C., and Morinigo, M. A. (2003). *Vibrio* species isolated from diseased farmed sole*, Solea senegalensis* (Kaup), and evaluation of the potential virulence role of their extracellular products. *Journal of Fish Diseases,* 26(2): 103-108.