

**OCCURRENCE OF IRIDOVIRUSES AND  
BACTERIAL PATHOGENS IN BIVALVES,  
SCAVENGING FISH AND TRASH FISH IN  
MARINE AQUACULTURE FACILITIES IN  
SABAH, MALAYSIA**



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**2015**

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SABAH, MALAYSIA**



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**THIS SUBMITTED IN FULFILLMENT FOR  
THE DEGREE OF MASTER OF SCIENCE**

**BORNEO MARINE RESEARCH INSTITUTE  
UNIVERSITI MALAYSIA SABAH**

**2015**

## DECLARATION

I hereby declare that material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

26 August 2015

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**CERTIFICATION**

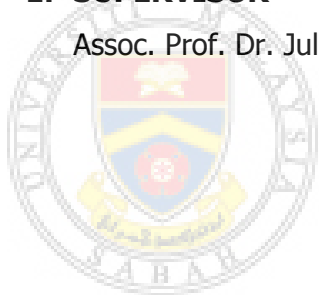
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TITLE : **OCCURRENCE OF IRIDOVIRUS AND BACTERIAL  
PATHOGENS IN BIVALVES, SCAVENGING FISH AND TRASH  
FISH IN MARINE AQUACULTURE FACILITIS IN SABAH,  
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DEGREE : **MASTER OF SCIENCE (MARINE BIOTECHNOLOGY)**  
VIVA DATE : **05 AUGUST 2015**

**DECLARED BY**

**1. SUPERVISOR**

Assoc. Prof. Dr. Julian Ransangan

Signature



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## ABSTRACT

Study was carried out to determine the occurrence of iridovirus and bacterial pathogen in bivalves, scavenging fish and trash fish collected from marine aquaculture facilities in Sabah. Bivalves and scavenging fish were collected from Kota Marudu, Kuala Penyu and Tuaran over a period of year from May 2012 to April 2013. Meanwhile, trash fish specimens were collected from fisheries landing stations Kudat, Semporna, Lahad Datu, Kuala Penyu and Kota Kinabalu. From the Polymerase Chain Reaction (PCR) analysis, specimens of bivalves and scavenging fish (n=2068) were detected positive for iridovirus (266), *Photobacterium damselae* (53), *Vibrio harveyi* (135), *Vibrio alginolyticus* (44) and *Vibrio parahaemolyticus* (147). Meanwhile, 35 of trash fish (n=221) specimens were also detected positive for iridovirus using PCR. The DNA of positive iridovirus samples was then characterized by DNA sequencing. From the DNA sequencing analysis, it was shown that the iridovirus found on bivalves, scavenging fish and trash fish are from the genus Megalocytivirus (*Iridoviridae*). Further strain divergence analysis suggested that the megalocytivirus from bivalves and scavenging fish are from the same strain that is the Infectious Spleen Kidney and Necrosis Virus (ISKNV) with the average percentage of nucleotide similarity of 98.77%. Similarly, the megalocytivirus from trash fish also showed the highest average percentage nucleotide similarity to ISKNV (99.40%), which suggested that the megalocytivirus in Sabah are from the strain of ISKNV. Finding of this study revealed that some of the bivalves and scavenging fish which are abundance in the aquaculture facilities particularly floating net cage, are carrier of iridovirus, *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Photobacterium damselae*. These animals can become the hosts of viral and bacterial pathogens and possibly transmit them to cultured fish. Finding of this study also showed that the trash fish can become the carrier of iridovirus and may transmit it to cultured fish during feeding. Therefore, the study suggests that the aquaculture facilities should often be cleaned from biofouling organisms such as bivalves to prevent disease outbreak. Furthermore, the use of trash fish should be minimized for the similar reason.

## **ABSTRAK**

### **KEJADIAN IRIDOVIRUS DAN BAKTERIA DI DALAM BIVALVIA, IKAN KECIL DAN MAKANAN IKAN DI DALAM FASILITI AKUAKULTUR MARIN**

*Kajian telah dijalankan untuk memastikan kejadian iridovirus dan bakteria di dalam bivalvia, ikan kecil dan makanan ikan yang dikumpul daripada fasiliti akuakultur. Bivalvia dan ikan kecil diambil daripada Kota Marudu, Kuala Penyu dan Tuaran, pengambilan sampel telah dijalankan selama setahun daripada Mei 2012 sehingga April 2013. Manakala sampel makanan ikan diambil daripada Kudat, Semporna, Lahad Datu, Kuala Penyu dan Kota Kinabalu. Daripada analisis Polymerase Chain Reaction (PCR), sampel bivalvia dan ikan kecil (n=2068) telah didapati positif iridovirus (266), Photobacterium damsela (53), Vibrio harveyi (135), Vibrio alginolyticus (44) dan Vibrio parahaemolyticus (147). Di samping itu, 35 daripada sampel makanan ikan (n=221) juga telah didapati positif iridovirus dengan menggunakan PCR. Kemudian, DNA sampel yang positif iridovirus telah dianalisis menggunakan kaedah DNA sequencing untuk pencirian iridovirus yang dijumpai didalam sampel. Keputusan analisis DNA sequencing menunjukkan bahawa iridovirus yang dijumpai di dalam bivalvia, ikan kecil dan makanan ikan adalah daripada genus megalocytivirus (Iridoviridae). Analisis untuk penentuan strain menunjukkan yang megalocytivirus yang dijumpai didalam bivalvia dan ikan kecil adalah daripada strain Infectious Spleen Kidney and Necrosis Virus (ISKNV) dengan purata peratus kesamaan nukleotida sebanyak 98.77%. megalocytivirus yang dijumpai didalam makanan ikan juga adalah daripada ISKNV dengan purata peratus kesamaan nukleotida sebanyak 99.40 yang mana menunjukkan bahawa megalocytivirus di Sabah adalah daripada strain ISKNV. Berdasarkan keputusan kajian; bivalvia dan ikan kecil yang berada di fasiliti akuakultur boleh menjadi pembawa kepada iridovirus, Vibrio harveyi, Vibrio parahaemolyticus, Vibrio alginolyticus dan Photobacterium damsela. Bivalvia dan ikan kecil boleh menjadi perumah kepada virus dan bakteria patogen dan boleh melepaskan patogen tersebut kedalam ikan kultur. Kajian ini juga menunjukkan bahawa makanan ikan juga boleh menjadi pembawa kepada iridovirus. Penggunaan makanan ikan didalam akuakultur boleh menjadi salah satu cara untuk menjangkitkan iridovirus kepada ikan yang dikultur. Oleh itu, kajian ini mencadangkan untuk selalu membersihkan kawasan fasiliti akuakultur untuk mengurangkan kehadiran organisma biofouling seperti bivalvia. Oleh itu, penggunaan makanan ikan harus dikurangkan yang digunakan juga harus dikaji terlebih dahulu untuk memastikan makanan ikan tersebut tidak dijangkiti patogen supaya penyakit dalam akuakultur dapat dikurangkan.*

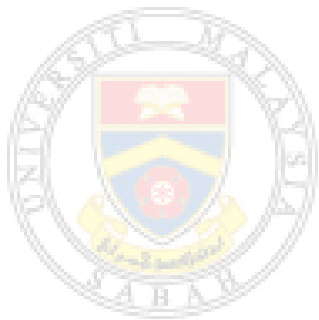
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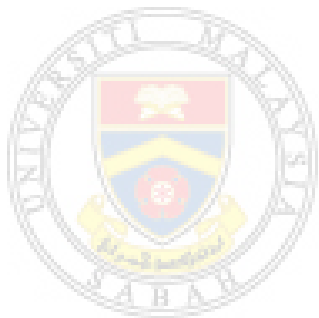
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## LIST OF ABBREVIATION

<b>AFCD</b>	Aquaculture Fisheries Division
<b>ATPase</b>	Adenosine Triphosphate
<b>ATV</b>	Ambystoma tigrinum virus
<b>BIV</b>	<i>Bohle iridovirus</i>
<b>BLAST</b>	Basic Local Alignment Search Tool
<b>BLASTN</b>	Basic Local Alignment Search Tool Nucleotide
<b>CDS</b>	Coding Sequences
<b>CTAB</b>	Cetyltrimethylammonium Bromide
<b>DNA</b>	Deoxyribonucleotide Acid
<b>DNTPs</b>	Deoxynucleotide Triphosphates
<b>DTAB</b>	Dodecyltrimethylammonium Bromide
<b>dsDNA</b>	Double stranded Deoxyribonucleotide Acid
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>EDTA</b>	Ethylenediaminetetraacetic Acid
<b>EtBr</b>	Ethidium Bromide
<b>FAO</b>	Food and Agriculture Organization
<b>FLD</b>	Fish Lymphocystis Disease
<b>FV-3</b>	Frog Virus 3
<b>GIVD</b>	Grouper Iridovirus Disease
<b>GSIVD</b>	Grouper Spawner Iridovirus Disease
<b>IBC</b>	Inclusion Body-bearing Cell
<b>ICTV</b>	International Committee of Taxonomy of Virus
<b>IIV</b>	<i>Invertebrate iridescent virus</i>
<b>IIV-1</b>	<i>Invertebrate iridescent virus 1</i>
<b>IIV-3</b>	<i>invertebrate iridescent virus 3</i>
<b>IIV-6</b>	<i>Invertebrate iridescent virus 6</i>
<b>IPTG</b>	Isopropyl $\beta$ -D-1-thiogalactopyranoside
<b>ISKNV</b>	Infectious Spleen Kidney Necrosis Virus
<b>LAMP</b>	Loop-mediated isothermal amplification
<b>LB</b>	Luria-Bertani
<b>LCDV</b>	<i>Lymphocytivirus disease virus</i>

<b>LCDV-1</b>	<i>Lymphocytivirus disease virus 1</i>
<b>LCDV-2</b>	<i>Lymphocytivirus disease virus 2</i>
<b>LCDV-C</b>	<i>Lymphocytivirus disease virus in China</i>
<b>LYCIV</b>	Large Yellow Croaker Iridovirus
<b>MCP</b>	Major Capsid Protein
<b>MgCl<sub>2</sub></b>	Magnesium Chloride
<b>NaCl</b>	Sodium Chloride
<b>NCBI</b>	National Center for Biotechnology Information
<b>OIE</b>	World Organization for Animal Health
<b>ORFs</b>	Open Reading Frames
<b>OSGIV</b>	Orange-Spotted Grouper Iridovirus
<b>PCR</b>	Polymerase Chain Reaction
<b>RBIV</b>	Rock Bream Iridovirus
<b>RFLP</b>	Restriction Fragment Length Polymorphism
<b>RSIV</b>	Red Sea bream Iridovirus
<b>RSIVD</b>	Red Sea Bream Iridoviral disease
<b>RT-PCR</b>	Reverse Transcriptase Polymerase Chain Reaction
<b>SBIV</b>	Seabass Iridovirus
<b>SGD</b>	Sleepy Grouper Disease
<b>SGIV</b>	Singapore Grouper Iridovirus
<b>SGIVD</b>	Singapore Grouper Iridovirus Disease
<b>SOC</b>	Super Optimum Growth
<b>TAE</b>	Tris-acetate-EDTA buffer
<b>TE</b>	Tris-EDTA buffer
<b>TFV</b>	Tiger Frog Virus
<b>TGIV</b>	Taiwan Grouper Iridovirus
<b>TGIVD</b>	Taiwan Grouper Iridovirus Disease
<b>TRBIV</b>	Turbot Reddish Body Iridovirus
<b>UV</b>	Ultraviolet
<b>UPGMA</b>	Unweighted Pair Group Method with Arithmetic Mean
<b>X-GAL</b>	5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside

## LIST OF SYMBOLS

<b>%</b>	Percentage
<b>°C</b>	Degree Celsius
<b>µl</b>	Microliter
<b>µM</b>	Micromolar
<b>2X</b>	2 times concentrated
<b>5X</b>	5 times concentrated
<b>10X</b>	10 times concentrated
<b>1X</b>	working concentration
<b>bp</b>	basepair
<b><i>et al.</i></b>	And others
<b>g</b>	Gram
<b>M</b>	Molar
<b>mg/ml</b>	milligram per milliliter
<b>mg/L</b>	milligram per liter
<b>ml</b>	milliliter
<b>mM</b>	Milimolar
<b>ng/ µl</b>	nanogram per microliter
<b>nM</b>	Nanomolar
<b>nm</b>	Nanometer
<b>ppt</b>	Part per Thousand
<b>rpm</b>	Revolution per minute
<b>sp.</b>	Species
<b>U</b>	Unit
<b>V</b>	Voltage

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## CHAPTER 1

### GENERAL INTRODUCTION

#### 1.1 Aquaculture in Malaysia

Aquaculture in Malaysia was first introduced in 1920's. The aim of aquaculture introduction was to improve country's economy in term of exports revenue and alternative seafood supply to satisfy the increasing demand for seafood (FAO, 2008). According to Othman (2008), Malaysia has high potential for aquaculture development because of its favorable climate as well as vast natural resources. Malaysia is also known as one of the countries that has high fish consumption. Furthermore, fishes are one of the cheapest sources of animal protein.

Aquaculture is defined as the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Aquaculture can be divided into various stages namely; hatchery operation, nursery operation and grow-out operation. And depending on the species being farmed, aquaculture can be carried out in freshwater, brackish water or marine water. Therefore, aquaculture can be used to produce fish, mollusks, crustaceans and aquatic plants for both human consumption and ornamental purposes.

Aquaculture production in Malaysia is increasing every year. Table 1.1 shows the estimated aquaculture production in Malaysia from 2008 to 2012. While Tables 1.2 and 1.3 shows the estimated production by states from 2008 to 2012 of marine/brackish water aquaculture and freshwater aquaculture, respectively. Obviously, Sabah has been the biggest contributor in term of aquaculture production of marine/brackish water in Malaysia.

**Table 1.1: Estimated production from aquaculture, Malaysia, 2008 to 2012**

	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>
<b>Number of aquaculture culturist</b>	30,634	23,986	26,291	28,599	29,482
<b>Aquaculture production ('000 Tonnes)</b>	354	473	581	526	634
<b>Freshwater</b>					
<b>Quantity ('000 Tonnes)</b>	96	153	155	122	164
<b>Value (RM million)</b>	471.80	704.30	760.34	684.15	992.39
<b>Brackishwater/Marin</b>					
<b>Quantity ('000 Tonnes)</b>	259	320	426	404	471
<b>Value (RM million)</b>	1,268.25	1,617.66	2,038.46	1,821.22	1,765.71

Source: Fisheries Department, Malaysia, 2012



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**Table 1.2: Production of brackishwater/marine aquaculture by State, 2008 to 2012**

State	2008		2009		2010		2011		2012	
	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)
<b>Perlis</b>	268	5,799	363	4,851	354	4,330	118	1,462	89	1,241
<b>Kedah</b>	1,468	17,150	4,379	45,133	4,896	43,633	3,281	30,787	2,659	33,467
<b>Pulau Pinang</b>	22,676	174,344	21,497	230,054	31,859	347,837	34,168	433,414	34,498	444,726
<b>Perak</b>	48,884	213,756	46,766	252,917	48,191	270,132	36,279	241,633	31,025	165,348
<b>Selangor</b>	26,629	182,730	43,517	211,424	53,179	222,649	37,792	207,189	20,900	173,759
<b>Negeri Sembilan</b>	447.49	5,381	1,164	15,035	1,405	17,165	1,418	17,394	1,016	12,440
<b>Melaka</b>	183.74	2,612	212	2,391	216	2,063	141	1,993	124	2,987
<b>Johor</b>	27,028	303,266	30,347	289,096	32,278	302,880	18,194	228,919	16,290	217,902
<b>Pahang</b>	5,917	123,243	10,670	224,354	12,260	260,632	4,216	80,632	3,807	67,678
<b>Terengganu</b>	1,953	24,582	2,072	26,778	2,076	26,118	2,165	29,606	4,150	59,453
<b>Kelantan</b>	229	3,278	1,079	14,880	598	7,676	410	5,372	609	8,496
<b>Sarawak</b>	4,143	66,741	5,459	66,305	8,604	129,902	9,592	145,394	9,434	142,529
<b>Sabah</b>	118,754	146,365	152,153	234,445	229,734	403,384	256,514	397,424	364,013	435,594
<b>Total</b>	258,581	1,268,248	319,676	1,617,663	425,650	2,038,402	404,287	1,821,220	470,620	1,765,714

Source: Fisheries Department, Malaysia, 2012

**Table 1.3: Production of freshwater aquaculture by State, 2008 to 2012**

State	2008		2009		2010		2011		2012	
	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)
<b>Perlis</b>	254	1,072	531	1,765	151	511	67	246	153	548
<b>Kedah</b>	869	8,003	6,221	20,453	3,801	20,133	3,154	10,396	3,973	13,419
<b>Pulau Pinang</b>	6,849	6,654	4,817	12,854	6,264	17,661	5,337	17,278	4,994	17,000
<b>Perak</b>	13,889	121,578	51,701	224,381	70,319	329,205	53,618	288,129	92,264	558,629
<b>Selangor</b>	8,629	65,568	15,251	70,150	14,867	65,385	14,365	63,522	14,857	69,946
<b>Negeri Sembilan</b>	431	29,853	12,082	50,787	8,882	41,777	7,187	41,308	6,983	38,627
<b>Melaka</b>	96	21,922	28,605	90,119	13,810	45,588	7,248	23,622	7,966	27,637
<b>Johor</b>	10,816	55,382	12,783	42,772	14,196	47,558	10,628	38,934	10,511	42,131
<b>Pahang</b>	5,842	52,737	7,413	74,814	9,361	78,048	9,341	102,965	8,779	104,080
<b>Terengganu</b>	133	22,568	3,644	20,932	3,561	22,920	3,101	22,006	4,219	30,979
<b>Kelantan</b>	80	3,124	2,162	9,295	2,365	11,061	1,695	8,449	2,033	10,326
<b>Sarawak</b>	3,380	26,238	2,560	27,837	2,929	22,060	2,396	18,374	3,054	31,003
<b>Sabah</b>	5,852	57,099	4,859	58,144	4,871	58,429	4,081	48,921	3,971	47,614
<b>Total</b>	57,118	471,798	152,631	704,303	155,399	760,335	122,219	684,149	163,757	992,386

Source: Fisheries Department, Malaysia, 2012