



UNIVERSITI PUTRA MALAYSIA

***PREVALENCE, RISK FACTORS AND TRANSMISSION OF VIRAL
NERVOUS NECROSIS IN A HATCHERY PRODUCING HYBRID
GROUPEL [♂ *Epinephelus lanceolatus* (Bloch, 1790) × ♀ *Epinephelus
fuscoguttatus* (Forsskål, 1775)] FRY***

ATIRAH KHAIRIAH AHMAD

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By

ATIRAH KHAIRIAH AHMAD

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfillment of the
Requirement for the Degree of Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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August 2018

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Institute : Institute of Bioscience

Fisheries and aquaculture are essential source of food, nutrition and also earnings for hundreds of millions of people around the world. One of the most cultured marine fish all around the world was grouper. Grouper is one of the high value and demanded cultured marine fish especially in the Southeast Asia countries. However, the supply of groupers from aquaculture is often limited due to diseases, mainly from *Betanodavirus*. The diseases caused by these viruses are commonly known as Viral Nervous Necrosis (VNN). VNN has been recognized to give tremendous mass mortality of fish all around the world and this virus is primarily affecting the larval and juvenile stages of fish. Therefore, this study investigates the prevalence, risk factors and transmission of viral nervous necrosis in a hatchery producing hybrid grouper [♂ *Epinephelus lanceolatus* (Bloch, 1790) × ♀ *Epinephelus fuscoguttatus* (Forsskål, 1775)] fry.

The experiment was conducted in a marine fish hatchery located in Terengganu, Malaysia. Artificial breeding of hybrid groupers fry was conducted at monthly interval for 12 months from March 2016 to April 2017. A total of 61 broodstocks with 28 male giant groupers (*E. lanceolatus*), and 33 female tiger groupers (*E. fuscoguttatus*) were used and sampled in this experiment. In general, the number of broodstocks sampling for each month were between one to three broodstocks for giant grouper (GG), while for tiger grouper (TG), it was between two to four broodstocks. Three artificial breeding attempts with different VNN status of broodstocks, which were : 1) VNN-positive GG × VNN-positive TG (GG+ × TG+); 2) VNN-positive GG × VNN-negative TG (GG+ × TG-); and 3) VNN-negative GG × VNN-negative TG (GG- × TG-) were conducted. Then, after fertilization, the fry was observed for VNN status at 5, 10, 20, 30, 40, 60, 90 and 120 days' post hatching, while the water quality during the study period were recorded. The detected VNN were proceed for VNN transmission study and molecular characterization analysis. Then, the sequences obtained in this study were compared with those of the other typical VNN isolates available in the

GenBank/EMBL nucleotide database. The phylogenetic tree was constructed by neighbor-joining analysis with the MEGA7 program.

The prevalence of VNN in broodstocks throughout 12 months of sampling periods showed low detection of VNN. Viral Nervous Necrosis only detected in April 2016 with a percentage of 50% for GG and 33.33% for TG broodstocks. There were significant correlations between the presence of VNN in GG, TG and hybrid grouper (HG) fry in selected water quality parameters. Iron and ammonia consistently showed significant positive and strong relationship with the presence of VNN in GG, TG and produced HG fry. Viral Nervous Necrosis transmission study and molecular characterization analysis suggested possible horizontal and vertical transmission of VNN between the groupers in this hatchery. The VNN might be transmitted between broodstock to broodstock, fry to fry and broodstocks to fry or *vice versa*, and circulated inside the hatchery culture system. In addition, detected VNN strains in this study were closely related between each other and distinctively grouped from other VNN strains, including from East Malaysia.

Therefore, this study provides significant information for control and prevention of VNN to farmers in this country that producing HG fry at hatchery level.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**PREVALEN, FAKTOR RISIKO DAN TRANSMISI VIRAL NERVOUS
NECROSIS DI DALAM PUSAT PENETASAN ANAK IKAN KERAPU HIBRID**
[♂ *Epinephelus lanceolatus* (BLOCH, 1790) × ♀ *Epinephelus fuscoguttatus*
(FORSSKÅL, 1775)]

Oleh

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Perikanan dan akuakultur adalah sumber penting bagi makanan, nutrisi dan juga pendapatan untuk beratus-ratus juta orang di seluruh dunia. Salah satu ikan laut yang paling banyak diternak di seluruh dunia adalah kerapu. Kerapu merupakan ikan laut yang mempunyai nilai pasaran dan permintaan yang tinggi terutamanya di Asia Tenggara. Walau bagaimanapun, bekalan kerapu dari akuakultur sering terhad disebabkan oleh penyakit, terutamanya dari *Betanodavirus*. Penyakit yang disebabkan oleh virus ini biasanya dikenali sebagai Viral Nervous Necrosis (VNN). VNN telah dikenalpasti sebagai virus yang menyebabkan kematian besar-besaran kepada ikan di seluruh dunia dan virus ini terutamanya memberi kesan kepada larva dan juvenil ikan. Kajian ini menyiasat prevalen, faktor risiko dan transmisi viral nervous necrosis di dalam pusat penetasan anak ikan kerapu hibrid [♂ *Epinephelus lanceolatus* (Bloch, 1790) × ♀ *Epinephelus fuscoguttatus* (Forsskål, 1775)].

Eksperimen ini dijalankan di pusat penetasan ikan laut yang terletak di Terengganu, Malaysia. Pembiakan kumpulan kerapu hibrid dijalankan selama 12 bulan dari Mac 2016 hingga April 2017. Sebanyak 61 induk, dengan 28 kerapu kertang jantan (*E. lanceolatus*), dan 33 kerapu harimau betina (*E. fuscoguttatus*) telah digunakan dan disampel dalam kajian ini. Secara amnya, bilangan induk yang disampel pada setiap bulan adalah antara satu hingga tiga induk untuk kerapu kertang (GG) manakala untuk kerapu harimau (TG), ia adalah antara dua hingga empat induk. Tiga percubaan pembiakan kerapu hibrid telah dijalankan dengan induk yang mempunyai status VNN yang berbeza iaitu, 1) VNN-positif GG × VNN-positif TG (GG + × TG +); 2) GG × positif VNN-negatif TG (GG + × TG-); dan 3) VNN-negatif GG × VNN-negatif TG (GG- × TG-). Kemudian, selepas persenyawaan, anak ikan yang menetas diperhatikan untuk status VNN pada penetapan 5, 10, 20, 30, 40, 60, 90 dan 120 hari selepas menetas, manakala kualiti air semasa tempoh kajian dicatatkan. VNN yang dikesan positif diteruskan untuk kajian transmisi VNN dan analisis pencirian molekul.

Kemudian, isolat VNN yang diperolehi dalam kajian ini dibandingkan dengan jenis isolat VNN yang terdapat dalam pangkalan data nukleotida GenBank / EMBL dan pokok filogenetik dibina dengan program MEGA7.

Kajian transmisi virus VNN dalam induk selama 12 bulan dalam tempoh persampelan menunjukkan prevalen VNN yang rendah. Viral Nervous Necrosis hanya dikesan pada April 2016 dengan peratusan 50% untuk induk GG dan 33.33% untuk induk TG. Terdapat korelasi yang ketara antara kehadiran VNN dalam GG, TG dan anak ikan kerapu hibrid (HG) dengan parameter kualiti air terpilih. Besi dan amonia secara konsisten menunjukkan hubungan yang positif dan kuat dengan kehadiran VNN dalam GG, TG dan anak ikan HG. Kajian transmisi VNN dan analisis pencirian molekul mencadangkan kemungkinan transmisi VNN secara melintang dan menegak antara ikan kerapu di pusat penetasan ini. Viral Nervous Necrosis mungkin dihantar antara induk kepada induk, anak ikan kepada anak ikan dan induk kepada anak ikan atau sebaliknya, dan disebar di dalam pusat penetasan itu sendiri. Di samping itu, isolat VNN yang dikesan dalam kajian ini berkait rapat antara satu sama lain dan dikelaskan secara tersendiri dari VNN yang lain, termasuk dari timur Malaysia.

Oleh itu, kajian ini menyediakan maklumat penting untuk mengawal dan mencegah VNN kepada penternak yang menghasilkan anak ikan HG di peringkat penetasan di negara ini.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

MT	Million tones
VNN	Viral Nervous Necrosis
Kg	Kilogram
m	Meter
mm	Millimeter
cm	Centimeter
TGGG	Tiger grouper x giant grouper
GG	Giant grouper
TG	Tiger grouper
dph	Days post hatch
SJNNV	Striped jack Nervous Necrosis Virus
RGNNV	Red-spotted grouper Nervous Necrosis Virus
TPNNV	Tiger puffer Nervous Necrosis Virus
BFNNV	Barfin flounder Nervous Necrosis Virus
ELISA	Enzyme-linked immunosorbent assay
RT	Reverse transcription
PCR	Polymerase chain reaction
SSN-1	Snakehead cell line
HCG	Human chorionic gonadotropin
°C	Celsius
IU/kg	International Unit per kilogram
mL	Millilitre
rpm	Revolutions per minute
µL	Microlitre

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Fish has always been the main source of food for the people in the world. In 2010, up to 148 million tones (MT) of fisheries product was traded globally (FAO, 2010). From this, 128 MT was utilized as food for people, providing an apparent per capita supply of 20.7 kg (FAO, 2012). Fish as a food to human consumption continues to become of the most traded food around the world with more than half of the value of fish exports derived from developing countries (FOA, 2016). Recent reports highlight, tremendous potential of the oceans and inland aquatic sources to contribute significantly in food security and adequate nutrition for a global population which expected to reach 9.7 billion by 2050 (FOA, 2016).

In Malaysia, aquaculture is now promoted as an important growth engine and ultimately becoming the pillar of the country's economy (Yusoff, 2015). Located in a region with an abundant supply of land and water, Malaysia is always working to ensure that this sector is not set aside in their development efforts. In addition, with Malaysian growing populations, approximately the annual fish demand was expected to increase from 1.7 MT in 2011 to 1.93 MT in 2020 (Yusoff, 2015).

Grouper (*Epinephelus* sp.) is one of the high value cultured marine fish especially in the Southeast Asia countries, including Malaysia, Indonesia, Thailand and Philippines (Liao et al., 2001; Yashiro, 2008). In 1997, the volume of live fish traded in Southeast Asia was estimated at 53,000 MT, including approximately 30,000 MT of groupers (Johannes and Riepen, 1995).

However, the supply of groupers from aquaculture is often limited due to diseases, which occur throughout the production cycle (Hyatt and Whittington, 2002; Bondad-Reantaso et al., 2005; Harikrisnan et al., 2010; Asrazitah et al., 2014). In recent years, eggs, fish larvae and even broodstocks were imported. Such practices contribute to the spread of fish pathogens especially viruses (Walker and Mohan, 2009; Manin et al., 2011). *Betanodavirus* has become one of the major problems in fish culture around the world (Patel and Nerland, 2013). *Betanodavirus*, was responsible for serious problem in various cultured fish (Shetty, 2012). These virus are easily transmitted between individuals in intensive aquaculture (Shetty, 2012), and one of the most devastating diseases that affects a large variety of cultured marine fish is caused by viral nervous necrosis (VNN) (Gudding et al., 2014).

Report in Philippines showed that broodstocks and hatchery-reared larvae of orange-spotted grouper (*Epinephelus coioides*) has mortality rate of 100% in 10 days due to VNN (Maeno et al., 2002). During the same year, a mass mortality of hatchery-reared barramundi (*Lates calcarifer*) larvae were also associated with VNN (de la Pena et al.,

2008). In Malaysia, VNN was reported to cause disease in Asian seabass (Ransangan et al., 2010), golden pompano (*Trachinotus blochii*) (Ransangan et al., 2011), and brown-marbled grouper (*E. fuscoguttatus*) (Manin et al., 2011). It was reported that VNN can easily spread within and between hatcheries through contaminated equipments (Munday et al., 1997), contaminated water (Ransangan et al., 2012) and through physical contact with asymptomatic carrier fish (Manin et al., 2011).

Viral Nervous Necrosis infection in marine fish aquaculture in Malaysia is already at a worrying stage (Ransangan et al., 2013). The detection of the virus infection in the widely cultured fish species such as Asian sea bass, brown-marbled grouper and golden pompano indicates that there is high possibility of other fish species could experience some degrees of infection. Such situation can cause great impact to the development and economic aspects of aquaculture in Malaysia (Ransangan et al., 2013).

1.2 Problem statements

Viral Nervous Necrosis is considered to be a severe disease against various marine fish species, characterized by major losses associated with vacuolating lesions of the retina and central nervous system (Sandra et al., 2016). The highest prevalence is usually detected from imported grouper fry and fingerlings, causing 100% mortality in larval stages (Chi et al., 2003). Moreover, preliminary detection methods, control, and prevention strategies of VNN in Malaysia are not fully determined yet, due to the non-systematic reporting system and supervision (Sandra et al., 2016). Therefore, this study is needed to provide significant information for control and prevention of VNN to farmers that producing hybrid grouper fry at hatchery level.

1.3 Significance of the study

Information on the epidemiology of VNN will eventually help the aquaculture hatchery operators to prevent and control the outbreaks of VNN. Furthermore, VNN is reported to be vertically transmitted but it is still unclear on how the virus is transmitted from broodstocks to its fry, due to the inconsistency detection of VNN in the broodstocks itself, especially during breeding time. Hence, information on the epidemiology and disease management of this disease is needed to ensure the broodstocks and fry are protected against the disease.

1.4 Objectives of the study

The aims of this study are:-

- 1) To determine the prevalence of VNN in grouper broodstocks, (tiger grouper *Epinephelus fuscoguttatus* x giant grouper *E. lanceolatus*) at hatchery level.
- 2) To determine the environmental risk factors on the presence of VNN in grouper broodstocks and hybrid grouper fry at hatchery level.
- 3) To investigate the possible transmission routes of VNN at hatchery producing hybrid grouper fry.

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