

**VIRAL NERVOUS NECROSIS (VNN) DISEASE CONTROL USING DIFFERENT
CONCENTRATION OF FUCOIDAN IN THE REARING WATER OF ASIAN
SEABASS JUVENILE, *LATES CALCARIFER***

HO SIE KHEE

**THIS DISSERTATION SUBMITTED IN PARTIAL
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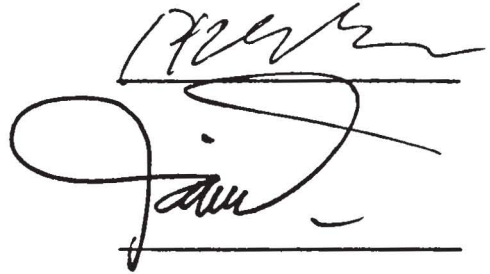


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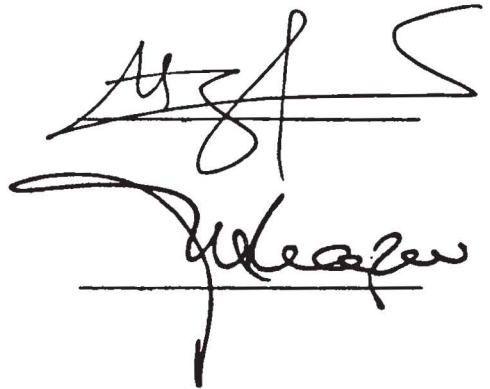
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Abstract

VNN can be categorized as one of the serious problem in larval production and cause mass mortality in grouper and Asian seabass, *Lates calcarifer*. VNN occurs during the larval and early juvenile period as early at 5 dAH up to 45 dAH and can causes mass mortalities up to 100%. In UMS fish hatchery, VNN had occurred in every batch of Asian seabass production. Mass mortalities occurred up to 100%. This study was carried out to determine the possibility of using Fucoidan to control VNN disease occurrence in *L. calcarifer* juvenile with optimum Fucoidan concentration. Early stage of *L. calcarifer* juvenile which affected by VNN from UMS hatchery's rearing tank was real in different 7L tank with different concentration of Fucoidan given daily. In the present study, the survival rate of *L. calcarifer* juvenile was 0% in control while 20 ml/ton, 40 ml/ton, 80 ml/ton and 120 ml/ton had the survival rate of 12.78 %, 25.00 %, 45.55 %, and 9.44 % respectively. This study proved that Fucoidan can control clinical signs associated VNN disease in *L. calcarifer* juvenile with the optimum concentration of 80 ml/tonne. *L. calcarifer* juvenile shows healthy appearance such as swim normally after Fucoidan treatment.

Abstrak

VNN boleh dikategori sebagai salah satu masalah serius dalam proses pengkulturan bembih dan memberi impak yang besar kepada ikan kerapu dan siakap, *Lates calcarifer*. VNN berlaku pada peringkat larva and awal juvenil dari 5 hari sehingga 45 hari selepas menetas dan menyebabkan kadar kematian yang tinggi mencecah 100%. Di hatcheri ikan UMS, penyakit ini telah berlaku di dalam setiap produksi ikan siakap. Kadar kematian mencecah 100%. Penyelidikan ini telah dijalankan untuk mengkajikan kemungkinan penggunaan Fucoidan untuk mengawal juvenil *L. calcarifer* yang telah dijangkiti penyakit VNN dengan kepekatan yang optimum. Juvenil *L. calcarifer* yang telah dijangkiti penyakit VNN dari Ums hatcheri dikumpul dan dikultur di 7L tangki dengan menambah Fucoidan yang berbeza kepekatan setiap hari. Dalam kajian ini, kadar kemandirian juvenil *L. Calcarifer* adalah 0% dalam 0 ml/ton, 12.78% dalam 20 ml/ton, 25.00% dalam 40 ml/ton, 45.55 % dalam 80 ml/ton dan 9.44 % dalam 120 ml/ton. Keputusan penyelidikan menunjukkan bahawa penggunaan Fucoidan untuk mengawal penyakit VNN pada juvenil *L. calcarifer* adalah berkesan dan 80 ml/ton adalah kepekatan yang optimum. Ikan menunjukkan perlakuan yang normal dan sihat selepas perubatan Fucoidan seperti berenang dalam arah yang normal.

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LISTS OF SYMBOL AND ABBREVIATION

ANOVA	analysis of variance
BMRI	Borneo Marine Research Institute
cm	Centimeter
Da	Daltons
d AH	day after hatching
°C	degrees celsius
DO	dissolve oxygen
DHA	docosahexaenoic acid
HIV	Human immunodeficiency virus
kDa	Kilodaltons
Kg	Kilogram
L	Liter
MT	matrix ton
m	Meter
mg	Milligram
ml	Milliliter
nm	Nanometer
NNV	Nervous necrosis virus
ppt	Part per thousand
%	Percentage
PCR	polymerase chain reaction
RSV	respiratory syncytial virus
RNA	Ribonucleic acid
RM	Ringgit Malaysia
<i>spp</i>	Species
Km ²	square kilometer
TL	total length
UMS	Universiti Malaysia Sabah
VNN	Viral nervous necrosis
WSSV	white spot syndrome virus

CHAPTER 1

INTRODUCTION

1.1 Asian seabass, *Lates calcarifer*

Asian seabass, *Lates calcarifer* (Bloch, 1790) is belonging to the order of Perciformes, family of Centropomidae, subfamily of Latinae, genus of *Lates*, and species of *calcarifer* (Makaira, 1999). Asian seabass is a catadromous species which like to migrate to other place. Common names of Asian seabass in Australia and Papua New Guinea is “Seabass”, “Giant Sea Perch”, “Barramundi”, in Philippines and Indonesia is “Kakap”, “Akame” in Japanese and “Siakap” in Malaysia (Makaira, 1999).

L. calcarifer are mostly cultured in Australia, Malaysia, Indonesia, India, Thailand and the United States. It is an important species from three principal perspectives. Firstly it is for recreational fishing, for which there exist significant stock enhancement prospect; secondly is as a candidate for commercial aquaculture; and lastly is as a candidate for domestic stocking (Makaira, 1999).

L. calcarifer known as a popular species in aquaculture is due to its rapid growth. It can reach 1.5-3.0kg in one year in ponds under optimum conditions. Nowadays, *L. calcarifer* is popular and sought-after game fish of very considerable economic importance which generates millions of dollars per year in revenue for the sport fishing and tourist industries (Makaira, 1999).

The first artificial spawning of the species in Asia was achieved in 1973 in Thailand (Barnabe, 1995), where annual hatchery production now exceeds 100 million larvae. Most of the cultured fish are now grown from hatchery-reared juveniles. During its early development stages, the industries in Australia and Asia were constrained mainly by a lack of juveniles and the cost and suitability of artificial feeds (Dhert *et al.*, 1992; Pillay, 1993). The adoption of extensive, low-cost larval rearing methods overcame problems associated with seed stock supply and, as the industry has expanded, more efficient, lower-cost diet formulations have been developed and economic, manufactured diets have become available (Cann, 1996). Nowadays, *L. calcarifer* industry reached 4000 tonnes per year in Australian, estimated to exceed 30,000 tonnes in broader Southeast Asian and up to 800 tons in US (Makaira, 1999).

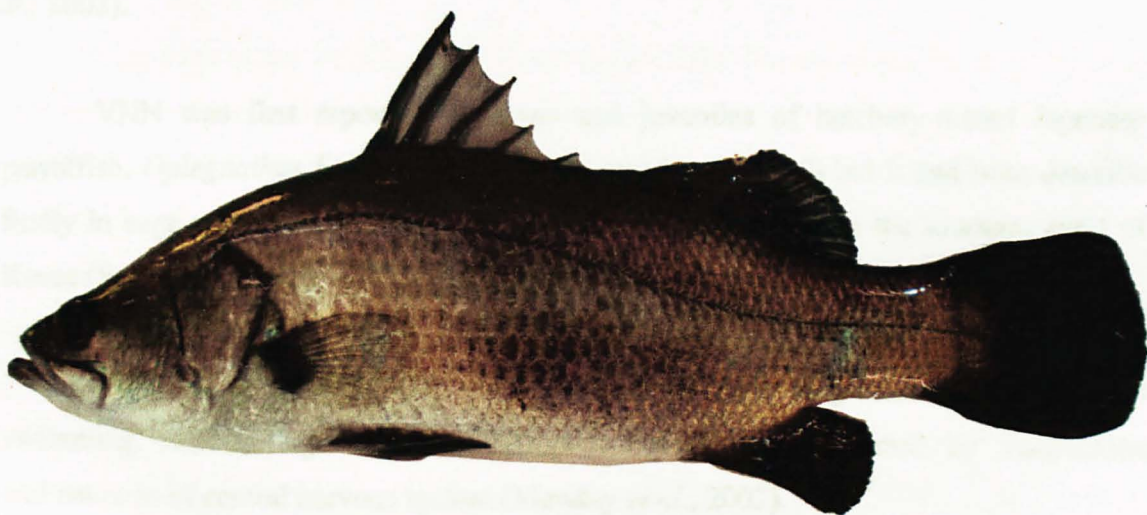


Photo 1.1 Asian seabass, *L. calcarifer*

However, *L. calcarifer* is reported to be easily susceptible to various diseases such as virus, bacterial, parasite, and also environmental diseases. In South Asian country such as Thailand, Indonesia, Philippine and as well as Malaysia *L. calcarifer* is a closely related and mostly reported infected by VNN, lymphocystis, irridovirus, and vibriosis. VNN is the major worldwide disease affecting many species including *L. calcarifer*.

1.2 Viral Nervous Necrosis

Viral nervous necrosis (VNN) also known as viral encephalopathy and retinopathy (VER), viral vacuolating encephalopathy and retinopathy, paralytic syndrome, spinning grouper disease, fish encephalitis, piscine neuropathy and whirling disease (Mori *et al.*, 1992; Muroga, 2001).

VNN is a devastating disease (Nguyen *et al.*, 1996) which can cause extremely high mortality to the cultured fish. Betanodavirus infections that caused viral nervous necrosis (VNN) have emerged as major constraints on the culture and sea ranching of marine fish in almost all parts of the world. More than 30 species of marine fish have been devastatingly affected during the seedling period and the culture process (Munday *et al.*, 2002).

VNN was first reported in larvae and juveniles of hatchery-reared Japanese parrotfish, *Oplegnathus fasciatus* (Yoshikoshi and Inoue, 1990) but it had been describe firstly in cage cultured groupers (*Epinephelus septemfasciatus*) on the southern coast of Korea (Sohn *et al.*, 1998).

Affected fishes with VNN showed abnormal swimming behaviours with spiral swimming and darting and were histopathologically characterized by vacuolation and necrosis of central nervous system (Munday *et al.*, 2002).

L. calcarifer with VNN appearance is clearly shown at fish hatchery of Borneo Marine Research Institute (BMRI) of Universiti Malaysia Sabah (UMS). Previous study conducted confirmed the VNN occurred and it can be observed on *L. calcarifer* age ranged 5 to 45 days after hatched, early juvenile stage. Mortality can reached up to 100% when reach critical level. Two or more, or combination of clinical signs can observed from the affected *L. calcarifer* such as lethargy, abnormal swimming behaviour, pin head, and dark coloration.

1.3 Fish Disease Control

Aquaculture is becoming an increasingly important source of fish available for human consumption nowadays and expected that any long term rise in seafood production will depend on the future progress of aquaculture (FAO, 2000). As the number of aquaculture facilities grows, so does the need to develop safe and effective drug for treating fish diseases.

Fish health management is a term used in aquaculture to describe management practices which are designed to prevent fish disease. In fish health management, there are three main principals which are pathogenesis, diagnosis and control. Pathogenesis of a disease is the mechanism by which an etiological factor causes the disease.

Diagnosis is the identification of the nature of the disease, either by the process of elimination or other analytical methods while control is use some chemical or vaccination or other method to control the disease to continues affect the fish. Disease control is appropriate when nonobligate pathogen are involve or when effective chemotherapeutic and management practice are available. The purpose of control is to reduce pathogen level in an environment or host so that the disease will not occur (Plumb, 1999).

An understanding of such relationships is important for the prevention, control, and treatment of disease in aquaculture (Villena, 2003). Recent researched proved Fucoidan extracted from *Cladosiphon okamuranus* was reported able to control white spot syndrome disease (WSSV) in *Penaeus japonicus* (Takahashi *et al.*, 1998). Results of previous study shown that the 12-15g shrimps were fed with crude Fucoidan of 200mg/kg everyday give highest survival rate than others which is 93%. Therefore, Fucoidan might be an alternative control measure in controlling viral diseases such as VNN.

1.4 Objectives of Study

The main objective of this study is to solve the problem of the juvenile rearing and thus improve the productivity of this species in hatchery. To achieve this main objective, two specific objectives which are outlined as followed:

- I. To determine the possibility of using Fucoidan to control VNN disease occurrence in *L. calcarifer* juvenile
- II. To determine the optimum Fucoidan concentration for VNN disease control

CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture

Aquaculture is cultivation of aquatic life included finfish, shrimp, seaweed, algae, plankton, molluscs, crustaceans and other aquatic plants. Unlike fishermen, aquaculture cultivates or rears the aquatic population under controlled conditions. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators; and the organisms must also be harvested by an individual or corporate body which has owned them throughout their rearing period (FAO, 2001).

Aquaculture products are grown either in earthen ponds, freshwater lakes and bays, or in the open sea. Medium involved in aquaculture is water. The types of water used vary and depends on the cultivation species, it generally divide into freshwater, brackish water and seawater.

The rearing species are fed and cared to ensure its optimum health and product quality. Once the rearing species reached an appropriate size, the crops were harvested, processed and shipped to market, generally arriving within hours of leaving the water to ensure the product quality.



Recently seafood demand increased rapidly. When the role of aquaculture as a food producing sector is considered in combination with the importance of fish in the diets of many of the worlds' poorest nations, it seems that aquaculture is assured a central role in efforts to meet the challenge to reduce poverty and hunger by half by 2015. The contribution of aquaculture to food security has been put forward by numerous authors and organisations where the direct provision of food and the indirect benefits of employment and income are cited amongst the benefits arising from aquatic animal production (Natasja *et al.*, 2008).

Hence, good planning, management and efforts are necessary to improve aquaculture industry in term of making mass production of commercial aquatic live in high quality with the aid of modern technology and bio-technology.

2.2 Aquaculture in Malaysia

The aquaculture industry in Malaysia will be able to generate revenue of up to RM20 billion. There is much potential in aquaculture, a sub-sector of agriculture, as Malaysia is surrounded by water. The National Aquaculture Development Plan was launched in late 2007 as part of the government's move to improve the fishing industry. The Fisheries Department of Malaysia is targeting 507,558MT of aquaculture products by 2010. The products would come from 49 aquaculture industrial zones, covering 28,000 hectares, in the country (NNN-Bernama, 2010).

Malaysia started the aquaculture field in the year of 1920 until today and there are over 19 species finfish, crustaceans and shellfish cultivated. There are many important commercial species are suitable for aquaculture development in Malaysia due to the geomorphological features. Most of these species possess all the desirable biological attributes for culture, including rapid growth, hardiness, large size and short reproductive cycle. The main freshwater species cultured in Malaysia nowadays are Tilapia, Catfish

and Carp, whereas brackish water species are cockles, prawns, mussels and Asian seabass (FAO, 2008).

Malaysia is a country which mostly bounded by sea with 547,000 km², enormous amount of freshwater resources from the river, lakes and streams all over the country and climatological conditions favouring aquaculture. There are about 6,410 km² of mangrove areas along the coasts, which provide breeding and nursery grounds for fish and crustaceans, besides ensuring the supply of shelter and food to other marine organisms and protecting the coastline from erosion (Yusoff *et al.*, 1997).

2.3 Taxonomy of *L. calcarifer*

L. calcarifer belongs to the family Centropomidae. Members from this family inhabit waters from coastal marine, estuaries to freshwater including mangrove estuaries and rocky to coral reefs. Some of its species are popular and sought-after with high economic importance (Makaira, 1999). On the other word, *L. calcarifer* is a euryhaline species. The taxonomic classification and description of *L. calcarifer* is given below (Lai, 2008).

Taxonomy classification:

- Kingdom : Animalia
- Phylum : Chordata
- Subphylum : Vertebrata
- Class : Actinopterygii

Subclass	: Teleostomi
Order	: Perciformes
Family	: Centropomidae
Genus	: <i>Lates</i>
Species	: <i>Lates calcarifer</i> (Bloch, 1970)

2.4 Morphology of *L. calcarifer*

Body of *L. calcarifer* is elongate, compressed, with a deep caudal peduncle. Its head is pointed with concave dorsal profile becoming convex in front of the dorsal fin. Its mouth is large in size if compared with other species, slightly oblique, upper jaw reaching to behind eye and the teeth are villiform. The scales on the body of *L. calcarifer* are large (Lai, 2008).

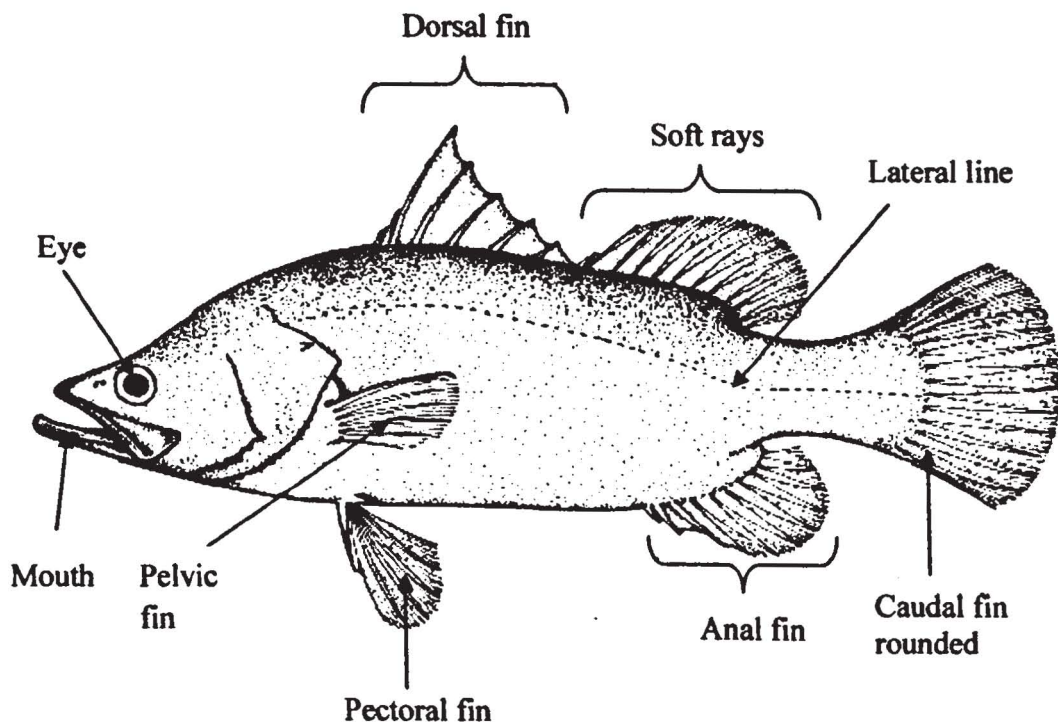


Figure 2.1 Morphological characteristics of *L. calcarifer* (Modified from FAO, 2008)

The dorsal and anal fins of *L. calcarifer* have scaly sheaths. There are seven to nine spines on the dorsal fin and ten to eleven soft rays; a very deep notch almost dividing spiny from soft part of fin. Its pectoral fin is short and rounded, several short, strong serrations above its base. The anal fin is rounded which had three spines and seven to eight short rays. The caudal fin is rounded too. The colour of this fish normally is olive brown above with silver sides and belly, usually juveniles or green or blue above and silver below. No spots or bars are present on the fins or body of *L. calcarifer* (Makaira, 1999).

2.5 Biology of *L. calcarifer*

Asian seabass, *L. calcarifer*, locally known as 'Siakap' and also known as Barramundi was first described by Bloch (1790) is an economically important finfish in Malaysia. However, the local names of *L. calcarifer* vary from place to place.

The *L. calcarifer* is a protandrous hermaphrodite and catadromous species. The change to female usually takes place at about seven years of age and a body length about 80 cm, but is apparently more related to age than to body length. The body length at which sex change occurs varies somewhat across its extensive geographic range, probably due to habitat, food and genetic differences (Lai, 2008).

L. calcarifer are highly fecund which mean a single female in 120 cm (TL) may produce 30–40 million eggs. Hence, even for a large-scale hatchery production only small amount of broodstock are necessary to produce large amount of larvae.

Spawning season of *L. calcarifer* varies within the range of this species. *L. calcarifer* in northern Australia spawn between September and March. In Philippines, *L. calcarifer* spawn from late June to late October, while in Thailand *L. calcarifer* spawning is associated with the monsoon season, with two peaks during the northeast monsoon, August until October and the southwest monsoon, February until June. Spawning occurs near river mouths or around coastal headlands. *L. calcarifer* spawn after the full and new

moons during the spawning season, and the spawning activity is usually associated with incoming tides in which can help to bring the eggs and larvae into the estuary (Rimmer, 2006).

2.6 Distribution of *L. calcarifer*

L. calcarifer can be found in coastal, estuaries and fresh water habitats. It has a very extensive range in tropical and semi-tropical areas of indo-West Pacific. Its distribution ranges from western India, around Sri Lanka to Bay of Bengal, and through the whole of Southeast Asia to eastern Papua New Guinea and northern Australia (Lai, 2008).



Figure 2.2 Distribution of *L. calcarifer* as indicated in red colour block (Australia Musuem Biomaps, 2009)

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