TOTAL BACTERIAL AND *VIBRIO* COUNT OF WATER IN A TIGER PRAWN COMMERCIAL HATCHERY

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I here by declare that this thesis contains my original research work. Source of findings reviewed here in have been duly acknowledged.

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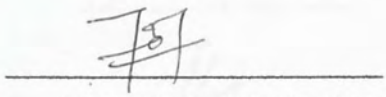

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ABSTRACT

A study on the total bacterial count was conducted at Ko-Nelayan, Kg. Laya-laya. Sampling of the water was done twice a month for 14 weeks from June until December 2004. Sampling was conducted in the morning and four water parameters were taken, which were temperature, dissolved oxygen, pH and salinity. The sampling sites were divided into three different station. Besides that, total vibrio was counted at the three different sampling sites. Result showed that total bacteria and *Vibrio* had different patterns but remain high (more than 1X 10^5 cfu/ml) and it not significant by water quality (temperature, pH, salinity and dissolved oxygen).

ABSTRAK

Kajian terhadap jumlah koloni bakteria telah dilakukan di Ko-Nelayan, Kg. Laya-laya. Penyampelan ke atas air adalah dua kali dalam sebulan bermula dari Jun hingga Disember 2004 di mana terdapat 14 minggu untuk penyampelan dijalankan. Penyampelan dilakukan pada waktu pagi dan empat parameter air telah di ukur iaitu suhu, kandungan oksigen terlarut, pH dan tahap kemasinan dimana terdapat tiga tempat penyampelan yang berbeza. Selain daripada itu, jumlah koloni *"Vibrio"* juga dikira bagi ketiga-tiga tempat penyampelan tersebut. Keputusan menunjukkan jumlah koloni bakteria dan *Vibrio* bagi ketiga-tiga tempat adalah berbeza tetapi masih lagi dalam jumlah yang tinggi (lebih dari 1×10^5 cfu/ml) dan ia tidak signifikan terhadap kualiti air (suhu, pH, tahap kemasinan dan kandungan oksigen terlarut).

CONTENT

LIST OF FIGURES

LIST OF SYMBOLS

NH_3	Ammonia
$CaCO_3$	Calcium carbonate
NaCl	Natrium chloride
ppt	Part per thousand
ppm	Part per million
°C	Celsius degree
ml	Milliliter
g	Gram
Cfu/ml	Colony forming unit per milliliter
Mg/l	Milligram per liter

CHAPTER 1

INTRODUCTION

The demand of fish and shell fish is increasing but production remains still or in some countries begin to decrease. Around 95 million tonnes of fish are harvested from wild stocks (FAO, 2002). However, most of the world's major marine fisheries have collapsed or declined so much so that aquaculture is recognized an important source of good quality animal proteins that is high in unsaturated fatty acids. Aquaculture has been identified as an important sector for providing proteins to the increasing number of human population. There is based on the fact that aquaculture utilizes even unfertile land for cultivation of aquatic animals. Aquaculture can also be operated in an integrated manner in that it can be incorporated with other animal production such as poultry or what is termed integrated aquaculture.

Data from the Food and Agriculture Organization (FAO), collected at global scale, suggests that 47% of fish stock are already exploited to their maximum sustainable limits, while 18% are reported as over-exploited and 10% are depleted (FAO 2002). Exploitation of fish via aquaculture on one hand is good for providing source of protein but on the other hand it causes quite a number of problems particularly on water quality of management of catchments and coastal environments.

In an open area, pollution is difficult to control because waste from factories for example, and human activities are directly linked to marine systems. Once in the waters, pollutants can affect aquatic animals including fishes.

Poor management of water quality leads to environmental degradation. Environmental conditions vary considerably at different times of the year, and the bacterial and fungal, load of sea water also varies (Biswas, 1992). Situations like these have been widely reported to increase the occurrences of disease outbreaks and mass mortality of aquaculture animals such as fish and shellfish. The poorly managed water quality creates stressful environment to the aquatic animals, which eventually weakened them and make them prone or susceptible to diseases brought about bacterial infections.

In aquaculture, bacteria play an important role to maintain water condition. These microorganisms involve in nutrient cycles as well as disease outbreaks. Nutrients such as nitrogen, sulfur, calcium and magnesium are major nutrients for their growth. Although these microorganisms play an important role in maintaining the balance of eco-chemical of water body, some of them can cause disease, especially when the fish have been weakened by stressful environmental conditions.

There are some bacteria that are reported to cause diseases to aquaculture fish and shellfish. These include the *Aeromonas* spp, which cause hemorrhagic septicemia, furunculosis, fin rot disease and shell erosion in shellfish; *Mycobacterium* that causes columnaris and bacteria gill disease; *Corynebacterium* causes bacterial kidney disease

to fingerling and adult fish, especially salmonids; a number of *Pseudomonas* species also infect fish and shellfish; *Vibrio* spp. can affect to both fish and prawn, and responsible for vibriosis. Unfortunately, these bacteria are frequently associated with physio-chemical parameters of the water such as low dissolved oxygen, acidic condition on the bottom of aquaculture ponds, drastic change in temperature, fluctuation in salinity and pH, and high concentration of ammonia and nitrite. This study is not intending to measure all water quality parameters but rather focusing on a specific aspect of bacterial count available in water used in tiger prawn production. In this respect, total bacterial count and total *Vibrio* count would be enumerated in different waters used in different stages of tiger prawn culture. A long the way, some physical parameters of water such as temperature, salinity, dissolved oxygen and pH would be measured and statistically correlate with the bacterial count. In this study, it has been hypothesized that the source water contains more viable bacteria than either filter or culture water.

CHAPTER 2

LITERATURE REVIEW

2.0 Importance of Aquaculture to the Malaysian Economy

Water pollution is one of the many environmental issues, which has caught attention of many. Uncontrolled wastes from human activities and pollutants released by industries are affecting environment very much to the limit that it is unable to be equilibrated by natural processes. One of the effective ways in which pollutants spread to environment is through diffusion of the pollutants in the waters. Unfortunately, waters are culture medium of aquatic animals.

The demand for proteins from fish is increasingly important but many of the important fishery sources are now beginning to decline so much so that aquaculture is seen as an effective way of filling up the gap between demand and supply for fish proteins. Malaysia is lucky in the sense that it has a long costal line and has much water resources for aquaculture related activities. However, Malaysia has not fully made use of its coastal waters for aquaculture except of some areas such as in Sabah coastal waters or in mangrove areas where tiger prawn is intensively cultured.

Considerable amount of our aquaculture products is exported to China, Hong Kong, Japan, Singapore and to some western countries. This helps improve the Malaysia economy growth in the sense that it is bringing in foreign currencies into the country.

2.1 Types of Aquaculture

Mustafa and Rahman (2000) have defined aquaculture as a farming of aquatic animals and plant useful to humans. They also have classified aquaculture in Malaysia into three categories, which are freshwater aquaculture, brackish water aquaculture and mariculture. The freshwater aquaculture is normally done in water that has the salinity of zero ppt while brackish water aquaculture is conducted in waters having salinity from 1-10 ppt, and mariculture is a type of aquaculture that conducted in sea waters that have salinities ranging from 28 to 35 ppt.

These aquaculture activities have been conducted either in intensive or semi-intensive culture. In an intensive culture, high protein diet, controlled environment and water quality are taken into consideration. In semi-intensive culture, a least complex culture normally conducted using low stocking densities. Modern farming systems should include hatchery, nursery, growth out system and live food culture. Hatchery is where seeds are produced, nursery for rearing early larval stages, growth out for growing fingerlings up to marketable size and live food culture to provide feed for early stage larvae.

2.2 Aquaculture Production in Malaysia

Malaysia is a tropical country in which temperatures are almost consistent and high (27-32^{0}C) throughout the year. This sort of temperature range is suitable for commercially important tropical marine species such as tiger prawn, groupers, sea bass and snappers. It contributed 43,456 metric tonnes of freshwater in 2003 (Berita Harian, 2003) but this is far from the country's target of producing 1.7 million tonnes of freshwater fish products alone.

2.3 Future of Aquaculture in Malaysia

Malaysian population has been on the increase and so has the demand for fish continues year by year. The awareness of having high quality protein in daily diet among Malaysian has further increased the demand for fish. At present, the 1.35 million tonnes through capture and culture fisheries would not be sufficient to fill in the gap of demand for fishery product by the year 2010 so much so that a total of 1.93 million tonnes have to come from aquaculture (Mustafa and Rahman, 2000). This means something has to be done to improve production through aquaculture, and research institutions should be more active in playing their role in search for better techniques in breeding, nursery and grow-out of aquatic animals.

2.4 Problems in Aquaculture

Pathogenic bacteria are affecting aquaculture worldwide. Viral disease already serious problem for prawn farmer in South-east Asia and are likely to affect the Australian industry. Disease develops through the interaction of the fish (the host), the causal agent (the pathogen), and the environment. In the presence of a susceptible host, a pathogen and predisposing environmental conditions (poor water quality), overstocking, frequent handling and inadequate food, disease is very like to occur.

Water quality is difficult to control especially at the net cage systems, some times waste from factory, human activities, plantation waste and water pollution are occurred unexpected. Physical and chemical factors are also fluctuating that stress fish. When the fish under stress, pathogens (viruses, bacterial, parasites, fungal) are easily attacked the fish host and cause disease.

Algae bloom, especially red tides is also threatening aquaculture. Unfortunately, the bloom of these planktons is associated with poor water quality. Some of pathogens are also associated with the planktons.

2.4.1 Disease Problems in Prawn Farms

The main problem of prawn culture is the outbreak of diseases which are primarily due to poor management of water and lack of adequate technical knowledge on animal health. The major diseases causing mass mortality in prawn farms include the

White Spots Syndrome Virus (WSV), Vibriosis, soft shell syndrome, black gill syndrome, muscle necrosis, fungal and protozoan infections, metamorphosis molt mortality syndrome, bacterial necrosis, larvae mid-cycle disease and white prawn disease. Larvae, post larvae, juveniles and adults of *P.monodon* are affected by the disease due to chemical contaminants like cadmium, copper, oil, ammonia and nitrate in rearing water (Biswas, 1992).

2.4.1.1 Viral Diseases

Prawns, including tiger prawn (*Penaeus monodon*) are prone to viral disease. Many viruses are know to cause disease to prawn, which include Picorna-like virus, that causes hypodermic and haematopoitic necrosis, Baculovirus that causes Penaeid baculovirus (Barnabe, 1994) and White Spot Virus. Monodon Baculovirus (MBV) causes baculovirus disease (Deloach. *et al.,* 1991) in *P.monodon.* This virus affects all stages of *P.monodon.* Hepatopancreatic pravolike virus (HPV), causes cell death and shrinkage of the hepatopancreas, leading abnormal metabolism and eventually death of organism, reaching as high as 50 % within 4-8 weeks of onset of disease (Biswas, 1992). Baculoviral midgut gland necrosis (BMN) also one of the viral disease where can effect the shrimp (Deloach. *et al.,* 1991). Reo-like virus of the hepatopancreas (REO) (Deloach. *et al.,* 1991).

2.4.1.2 Bacterial Disease

Vibrio is a gram negative and fermentative bacterium, short rod, axis curved or straight, 0.5 – 3.0 µm, single or occasionally united into S shape or spirals. The bacterium is motile by a single polar flagellum, it is oxidase-positive, facultative anaerobic and halophilic. Marine vibrios are found in all saline water. They are more frequent static water and soft benthos occurs in combination with high organic load (Inglis *et al.,* 1993).

Some of the several *Vibrio* species that have been reported to cause disease in shrimps include *Vibrio harveyi, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus,* and *Vibrio anguillarium* (Biswas, 1992). These halophilic bacteria may be found extensively in marine and brackish waters and are normal flora in shrimp but cause secondary infections, especially when shrimps are in stressful condition. These bacteria have also been reported to occur in conjunction with excessive level of hydrogen sulfide in the sludge and in an overgrowth of *Cynobacteria* spp.(www.alken-murray.com/Vibrio.htm).

2.4.2 Water Quality Problems

Changes in water quality would directly effect of fish. When the water in the shrimp ponds are not properly monitored, the ammonia (NH_3) concentration increases due to uneaten or feed left-over and partially from the shrimp's faeces, which are rich in proteins when discomposed would produce ammonia. The ammonia level is partially

converted to non-toxic form, nitrate by nitrifying bacteria. Ammonia (NH_3) will be furthered oxidize to ammonium, a toxic form when the pH drops below 8.0. It has been reported that shrimps still can tolerate up to 0.5 ppm of ammonia. Beside ammonia, there are some minerals dissolved in the water and these dissolved minerals are measured in term of hardness. The optimum hardness level in which shrimp can tolerate is up to 140 to 400 ppm (Biswas, 1992). The hardness has the buffering capability to some of the heavy metals that are toxic to fish, especially copper and zink (Biswas, 1992).

The normal range of pH, the measure of hydrogen ions dissolved in the water is from 6.5 to 9.0. At higher temperature, fish are more sensitive to pH changes. At low pH, gill damage would become major problem in aquaculture. Thus, maintaining pH at normal range would be useful for successful aquaculture.

The basic requirement for successful aquaculture is that the water quality must be in excellent condition. Deterioration of water quality would promote the growth of microorganisms that would potentially infective when shrimps are weak due to poor water quality. Excessive growth of bacteria would provide a situation where competition for dissolved oxygen is critical and eventually would cause shrimp to become weak and susceptible to infections.

2.5 Integrated Health Management for Shrimp Aquaculture

Improved environmental condition, healthy stocks and in absence of disease agents would therefore lessen the chance of a disease outbreak. Time and accurate disease diagnostics are very important in aquaculture system. The traditional diagnostic methods are time consuming and require trained manpower and sophisticated equipments to be useful. Therefore, the search for new and rapid diagnostic tests would be of priority in shrimp aquaculture.

2.5.1 Improvement of Water Quality

Environment conditions vary considerably at different times of the year, the bacteria and fungal load of sea water also varies. In pond, the water purifying bacteria decreased ammonia and phosphorus and increased both fish feed organisms and fish production.

Seeding purifying bacteria can help reducing sludge ammonia, phosphorus, aeration needs and treatment cost in waste water treatment in aquaculture and other effluents. Efficient water purifying requires balanced bacteria communities containing strains to degrade organic waste, ammonia and nitrate in sufficient number bacterial augmentation result in increased natural food availability in culture systems. Green mussel (*Perna* spp) has shown promise as biological filters for improving quality of effluents when cultured near to the shrimp farm, terrestrial farms, industries and house hold.

CHAPTER 6

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