Diagnostic Cases of Marine Fish Culture at Floating Cages and Breeding Centre in Tuaran, Sabah

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Abstract: A fish health diagnosis activities were carried out at the hatchery in Tanjung Badak Breeding Centre, Tuaran, Sabah and in the floating cages in Pulau Bhai, Sandakan, Sabah in 2006 and 2007. A total of 116 fish samples comprise of tiger grouper (*Epinephelus fuscogutatus*), red snapper (*Lutjanus erythropterus*), sea bass (*Lates calcarifer*), Papuan black snapper (*Lutjanus goldiei*) and Napoleon wrasse (*Cheilinus undulatus*) were sampled. The fish samples were tested for viruses, parasites and bacteria. A total of 228 samples from 'pooled' organs of kidney, spleen, brain, liver and eye were tested for Iridovirus (RSIVD) and Nodavirus (VNN) using IQ2000™ Qiagen PCR kit. The results indicated that 17.2% of tiger grouper from Tanjung Badak dan Sandakan were positive with RSIVD whereas 7.78% of sea bass and 2.6% of red snapper were positive with VNN. Parasites such as *monogeneans (Diplectanid pseudorhabdosynochus)*, capsalid (*Benedenia* sp.), protozoa *Brooklynella* sp. and leeches were found in tiger grouper and monogeneans (*Haliostrema* sp.) in red snapper. Monogeneans were the most common parasite found in fish at high prevalence between 36.4-100% and mean intensity of 8.8-27, followed by *Trichodina* sp. at prevalence of 4.5-100%. *Capsalid* (*Benedenia* sp.) at prevalence of 20% caused significant mortalities and health problems to fish culture at floating cages. *V. vulnificus* and *V. alginolyticus* were isolated from skin abrasions, skin ulcer, liver and kidney. Bacteria such as *Staphylococcus* sp., and *Micrococcus* sp. were also found on the skin and internal organs of the fish with chronic skin ulcers.

Keywords: Fish, health, monitoring, cage culture, Sabah

Abstrak: Aktiviti diagnosis kesihatan ikan telah dijalankan di hatceri di Pusat Pembenihan Tanjung Badak, Tuaran, Sabah dan di sangkar terapung Pulau Bhai, Sandakan, Sabah pada tahun 2006 dan 2007. Sejumlah 116 ekor ikan daripada spesis ikan kerapu harimau (*Epinephelus fuscogutatus*), ikan merah (*Lutjanus erythropterus*), siakap (*Lates calcarifer*), kanai (*Lutjanus goldiei*) dan maming (*Cheilinus undulatus*) telah disampel. Ikan tersebut telah diperiksa kehadiran parasit, bakteria dan virus. Sebanyak 228 sampel dari organ-organ terkumpul (ginjal, limpa, otak, hati dan mata) telah diperiksa untuk Iridovirus (RSIVD) dan Nodavirus (VNN) menggunakan kit IQ2000™ Qiagen PCR. Keputusan menunjukkan sejumlah 17.2% kerapu harimau dari Tanjung Badak dan Sandakan, positif dengan RSIVD manakala 7.8% ikan siakap dan 2.6% ikan merah positif dengan VNN. Parasit seperti *monogeneans (Diplectanid pseudorhabdosynochus)*, capsalid (*Benedenia* sp.), protozoa *Brooklynella* sp. dan lintah pada kerapu harimau dan monogeneans (*Haliostrema* sp.) pada ikan merah. Monogenean adalah parasit yang paling kerap ditemui dengan prevalen yang tinggi iaitu di antara 36.4-100% dan intensiti purata di antara 8.8-27, diikuti dengan *Trichodina* sp. dengan prevalen di antara 4.5-100%. Infestasi capsalid (*Benedenia* sp.) dengan prevalen 20.0% telah menyebabkan kematian dan masalah kesihatan kepada ikan marin di sangkar terapung. Kecederaan kulit disebabkan oleh parasit dan pengendalian ikan mendedahkan ikan kepada jangkitan sekunder bakteria seperti *V. vulnificus* and *V. alginolyticus*, *Staphylococcus* sp. and *Micrococcus* sp..

Introduction

Global aquaculture has been growing steadily over the last two decades, especially within Southeast Asian Region. Asia with its annual yield of about 10% of the world aquaculture production, four countries (Indonesia, Thailand, Vietnam and Philippines) ranked among the top ten aquaculture producers in the world (Nagasawa and Cruz-Lacierda, 2004). Following the steps of our neighbouring countries, Malaysia too has taken serious measures and initiatives to increase aquaculture production. Aquaculture sector in Sabah has proven to be an important supplier of animal protein and has contributed significantly to the State's economy. By 2010, Sabah was expected to produce 30,700 metric tonnes of marine fish worth RM 1.23 billion (Anon., 2008). The Fisheries Department in Sabah has analyzed the potential for marine cage farming area and has identified the Sandakan Bay area with high potential where there is already some cage farming development. Pulau Bhai, situated in Sandakan Bay is safely located without any natural disaster, strong current or waves.

Disease outbreaks are one of the major constraints to aquaculture production. Production losses as a result of fish mortality have often been associated with diseases apart from poor water quality. Infectious diseases caused by viruses, bacteria and parasites have resulted in reduction of aquaculture production. Red Seabream Iridovirus Disease (RSIVD) has caused ecological and economic impacts on marine fish farming in recent years. RSIVD has been isolated from many grouper species (*Epinephelus coioides, E. fuscoguttatus*) in Japan, Taiwan, Thailand, Malaysia and Indonesia (Gilda and Leobert, 2004). Viral Nervous Necrosis disease (VNN) has been reported in marine fishes such as groupers, sea bass, and red snapper (Yukio *et al.*, 2007). This disease caused high mortalities in the affected fish with typical clinical signs of cock-screw swimming followed with acute death. The causative agent of VNN was identified as a member of the family Nodaviridae.

The objective of this study is to diagnose fish health problems encountered at floating cages Sandakan and hatchery units in Tuaran, Kota Kinabalu, Sabah.

Materials and Methods

A total of 116 marine fishes including tiger grouper (*Epinephelus fuscogutatus*) from hatchery (n=36 pcs) and the floating cages (n=37 pcs), sea bass (*Lates calcarifer*) (n=9 pcs), red snapper (*Lutjanus erythropterus*) (n=18 pcs), Papuan black snapper (*Lutjanus goldiei*) (n=10 pcs) and Blue Green Napoleon wrasse (*Cheilinus undulatus*) (n=6 pcs) were sampled and tested for virus, bacteria and parasites. Monitoring activities were carried out from January 2006 until June 2007. Monthly sampling was carried out with sampling number range between 5 to15 pcs of fish per visit and selection was made from the cages or tanks with health problems. The healthy and sick fish were selected in equal number from the same cages at each sampling based on the observation of physical appearance and behavioral changes. The total samples tested for virus were (n=266), bacteria (n=75) and parasites (n=75). The samples were stored in ice (4°C) during transportation and analysed upon reaching the lab at the National Fish Health Research Centre (NaFish), Batu Maung, Penang.

Viral detection

RSIVD was detected using nested polymerase chain reaction (PCR) whereas VNN was tested with reverse transcriptase-polymerase chain reaction (RT-PCR) using kit IQ2000™ (Qiagen GmbH, German). Sample preparations for DNA or RNA extraction procedures were carried out separately according to the DNA and RNA extraction kit manual (Farming IntelliGene Tech. Co. Taiwan). Thermal cycling program for nested PCR of RSIVD were as follows: initialization: 95°C for 5 min, denaturation; 94°C 20 sec; annealing; 62°C 20 sec; extension/elongation; 72°C 30 sec; 20°C 30 sec at the end of final cycle. The amplification reaction was set for 45 cycles. RT-PCR amplification profile for VNN were as follows: reverse transcription; 42°C 30 min; initialization; 94°C 2 min; denaturation; 94°C 20 sec; annealing; 62°C 20 sec; extension; 72°C 30 sec, and 20°C 30 sec at the end of the final cycle. The amplification reaction was set for 45 cycles. After nested reaction was completed, the samples were prepared for electrophoresis and gel staining with Ethidium Bromide for final data assay.

Bacterial detection

Swab was taken from the internal organs separately (spleen, kidney, brain, skin ulcer and wound) with aseptic procedures and placed in a tube containing transport medium. Blood agar or brain heart infusion agar BHI (Oxoid, England) was used for bacterial isolation. Bacterial isolates was identified using API systems; API 20E, API Staph 20 (Bio-Merieux, France).

Parasite identification

Samples of mucus were scraped from the skin and fins, the tips of several primary lamellae were cut with a pair of scissors and transferred to a slide. The mucus/gill lamellae were then spread on a slide using a scalpel blade, air dried, fixed with methanol, stained with Giemsa, mounted with neutral mountant DPX and examined under light microscope (10-40x magnification). For capsalid test, few fishes (3-5 pcs depending on size) were placed in a plastic bag containing fresh water and kept for 15 min. After 15 min, the capsalid will dislodged from the fish skin into the water and they were then collected for identification. The

term prevalence (%) and mean intensity are used in accordance with the definitions established in Margolis *et al.* (1982). The prevalence (%) was calculated as the percentage of the total number of fish infested out of the total number of fish examined. The mean intensity was calculated as the average number of parasites in the total number of the infected fish.

Results

Viral detection

At Tanjung Badak hatchery unit, 6.03% of first batch tiger grouper fingerlings were diagnosed with RSIVD between January-March, 2006 (Fig. 1). The occurrence of RSIVD increased to 12.93% until April-June 2007. The increase of RSIVD was due to recurrence of RSIVD from the first batch as well as the new cases diagnosed from two batches of fry received at floating cages, Pulau Bhai in April and June 2007. Based on clinical observation, 65% of RSIVD fish were showing signs of losing weight, skin abrasion or ulcers on the body and mouth (Fig. 3) whereas 25% of healthy fish live as a carrier and become a potential source of infection to others. RSIVD was also detected with 10% occurrence of dropsy (hyperinflated swim bladder) in *Napoleon wrasse*. An acute episode of high mortalities with cumulative of 80% was reported at the end of June 2006 in sea bass fry one month after admission into the cages. VNN with occurrence of 7.76% and 2.59% in sea bass and red snapper respectively were diagnosed in July 2006. Since the sampling was carried out after the mortalities have subsided, the cause of mortalities cannot be ascertained. However, the infected fish and the remaining fish in the group did not show any clinical signs of VNN disease throughout the year.

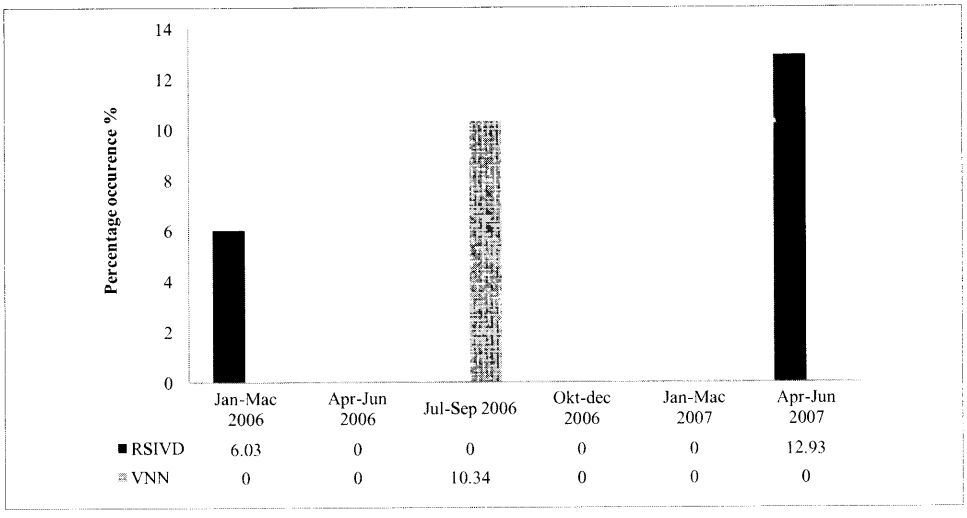

Figure 1: Percentage occurrence of RSIVD and VNN in marine fish culture at Breeding Centre, Tanjung Badak and floating cages, Sandakan, Sabah from January 2006 to June 2007

Table 1: Percentage of VNN and RSIVD detected in fish samples from Tg. Badak and Sandakan from January 2006 to June 2007

Site	Fish	No. of fish	VNN	RSIVD	Total Percentage (%)
Tg. Badak Sandakan	Grouper sp.	36	0	12	10.34
	Grouper sp.	37	0	8	6.89
	Wrasse sp.	6	0	2	1.72
	Sea bass	9	9	0	7.76
	Red snapper	18	3	0	2.59
	Black snapper	10	0	0	0
Total		116	12	22	29.3

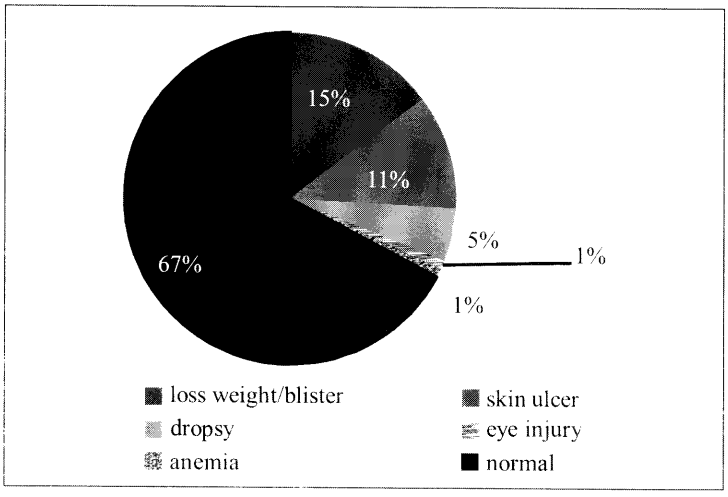

Figure 2: Percentage of pathological signs or changes observed in the fish examined

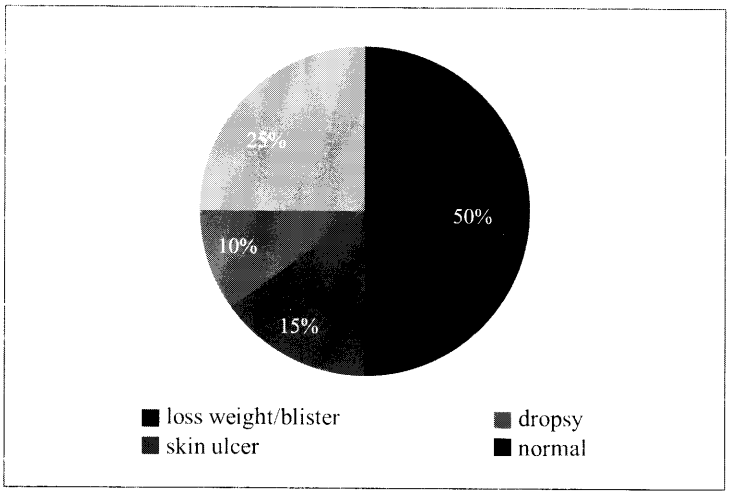

Figure 3: Percentage of pathological signs or changes observed in fish dianosed with RSIVD

Bacterial detection

V. vulnificus and *V. alginolyticus* were isolated from skin abrasions and skin ulcer. These bacterial strains were also isolated from liver and kidney. Other bacteria such as *Staphylococcus* sp., and *Micrococcus* sp. were found on the skin and internal organs of the fish with chronic skin ulcers.

Parasites detection

A capsalid (*Benedenia* sp.) infestation was encountered in April-June 2006 with some mortality in heavily infected fish (Fig. 4). M*onogeneans (Pseudorhabdosynochus*) in tiger grouper or *Haliostrema* sp. in red snapper were common findings in gills at prevalence between 36.4-100% with intensity of 8.8-27. In December 2006, 80.0% prevalence of *Brooklynella sp.* infestation was reported with some mortality and observations of severe skin ulcers in grow-out tiger grouper in cages. Other common parasites found on the skin smear were *Trichodina* sp. (prevalence 4.5-100%) and leech (via observations and physical examinations).

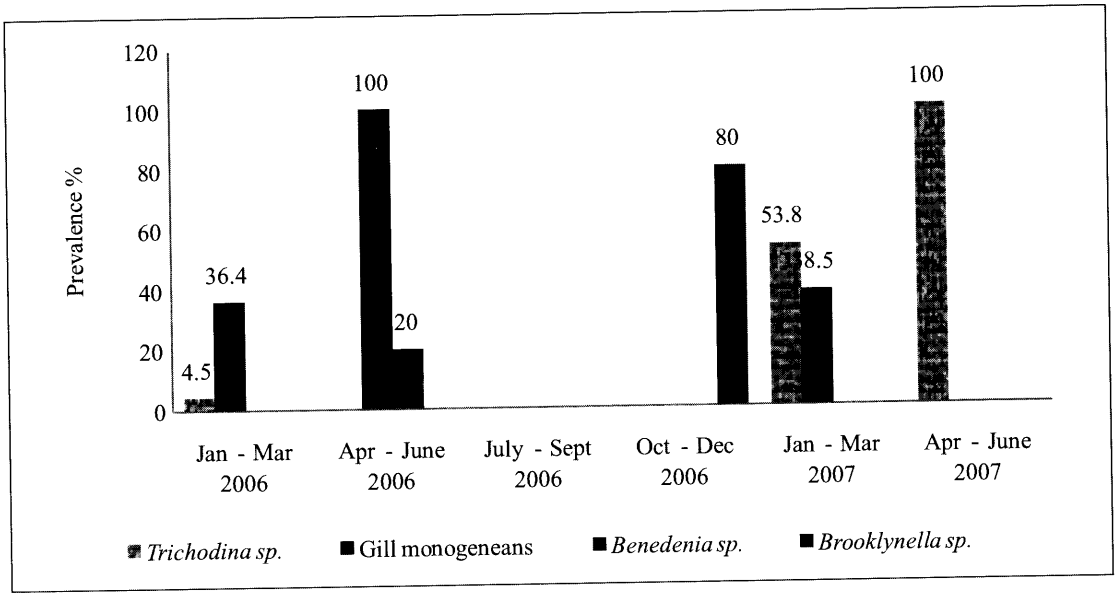

Figure 4: Prevalence (%) of parasites found from wet smear of gills and skin preparations

Photo1: Monogenean (*Diplectanid pseudorhabdosynochus*) on gills of tiger grouper (40 x magnifications)

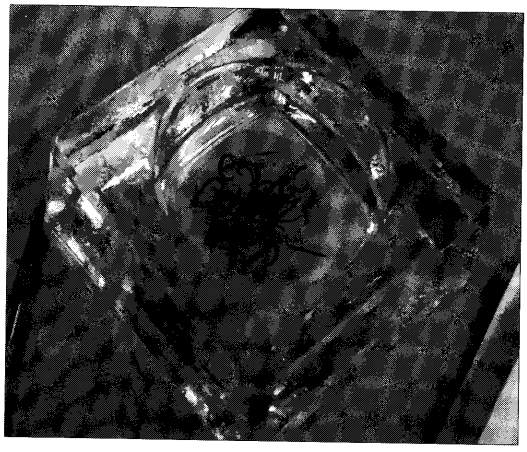

Photo 2: Marine leech from the skin of fish

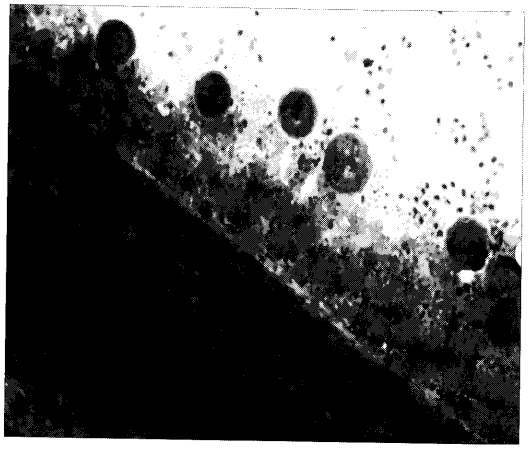

Photo 3: *Trichodina* sp. on gill smear of tiger grouper fry (40 x magnification)

Discussion

Sick fish were showing signs of abnormal behavior such as isolation from the rest of the group, appeared lethargic, becoming inactive and not eating. High percentage of RSIVD (10.34%) was diagnosed at Hatchery Tanjung Badak, probably due to the infected fry received from Indonesia and Taiwan. A RSIVD 'carrier' fish that survived the disease may have been brought to cages in Sandakan and become a source of virus spreading within the group apart from the addition of two new batches of tiger grouper fry received in April and June 2007. An injury during handling and fish movement has increased the chances of spreading the infections via contaminated water or direct contact between them. A dropsy or distended abdomen with an abnormal swimming was predominately seen in *Napoleon wrasse*. The fish appeared floating with upside down body on the water surface and failed to control its movement. Following two weeks observations of similar case in isolation, no changes were seen after antibiotic treatment and manual removal of blocked air in the swim bladder.

Similarly with an acute outbreak of VNN, the clinical signs of erratic swimming and darkening of the skin color might have been missed. The presence of VNN 'carrier' fish in the affected batch after the mortalities episode indicate the possibility of an outbreak associated with the transportation stress and fish injury.

Some mortality in fry and fingerling was also found to be associated with high infestations of *Benedenia* sp. or leeches however the intensity of the infestations cannot be determined since the fish had been treated with fresh water bath or formalin before sampling. Heavily infected fish showed excessive mucous secretion with haemorrhagic spot and skin ulcers. The parasite has been reported worldwide from subtropical and tropical areas with several species of grouper acting as hosts (Leong and Wong, 1988). Heavy infection may cause not only haemorrhagic and abrasive lesions, but also mortalities in cultured marine fish (Leong, 2001).

Vibriosis caused by *V alginolyticus* and *V. vunificus* were common in tiger grouper fry and grow-out red snapper in February, March, April and June 2006. Secondary bacterial infections with *Staphyloccocus* sp and *Micrococcus* sp. were isolated mainly from the wound and ulcerated skin. Frequent rain fall and low salinity (9-25 ppt) were reported in February 2006, October and December of 2007 (data not shown) which coincide with many incidences of parasitic infestations such as *Brooklynella* sp. and monogeneans.

No effective chemical agents have been reported able to control viral diseases. Early screening of fish with PCR will provide very useful information to the aquaculturist on their decision to bring in or prevent the infected fish from entering into their culture system. Biosecurity in aquaculture practice provides the best control from infectious agents however biosecurity practice in fish farms is relatively new with little knowledge and awareness among workers. In an open system with intensive culture, the potential of disease spreading via direct contact with the pathogen, infected fish and through water becomes greater. Hence, the goal should be modified to eliminate or control the source of infection within the facility. The potential sources of entry for an infectious agent into an aquaculture facility via human contact, animals or equipments can be reduced with regular disinfection or washing of hand, foot or equipments before and after each usage. Asymptomatic carrier in existing stock or new stock should be evaluated and continuously tested. Eradication of the infected fish will be the best solution to eliminate the source of infections however considering the value of the fish cultured, culling was considered as the last resort. Proper record keeping, waste management and general husbandry including strict hygienic practices in cages are of paramount importance for the success of biosecurity program.

Conclusion

Infected fry with RSIVD or VNN could be the main cause of fish mortalities at Tanjung Badak Hatchery and Pulau Bhai floating cages. Disease spreading between the two places as well as cross contamination between local seed or imported fry occurred through admission of the infected fish into a new culture system. Hence, biosecurity program should be included in the daily operation. Rapid screenings of fry and broodstocks for infectious organisms such as parasites, VNN and RSIVD need to be implemented without failed for selection of a healthy fry.

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