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A Case Study on the Mortality of Cobia (*Rachycentron canadum*) Cultured in Traditional Cages

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Abstrak: Kematian secara besar-besaran ikan haruan tasik, *Rachycentron canadum* dalam tempoh 2–3 hari telah dilaporkan oleh 3 penternak di Bukit Tambun, Pulau Pinang pada bulan Februari dan Mac 2007. Hanya ikan haruan tasik dengan berat badan 3–4 kg yang terlibat. Kebanyakan ikan haruan tasik yang dijangkiti menunjukkan corak berenang di permukaan air dan cubaan melompat keluar dari air. Kesemua sampel menunjukkan jangkitan ringan dan sederhana positif penyakit *viral nervous necrosis* (VNN). Jangkitan lintah laut (*Zeylanicobdella arugamensis)*, monogenean badan (*Benedenia* sp.) dan copepod (*Caligus* sp.) juga dikesan. Walau bagaimanapun tiada patogen bakteria dikesan. Parameter untuk kualiti air berada dalam julat sesuai untuk ternakan ikan haruan tasik. Punca sebenar penyebab kematian tinggi ikan haruan tasik semasa siasatan adalah tidak jelas. Namun, kami percaya kematian ikan haruan tasik ini boleh berpunca dari jangkitan VNN yang boleh mencetuskan tekanan dan ditambah pula dengan ruang yang terhad untuk ikan haruan tasik yang membesar. Hal ini seterusnya bertindak sebagai faktor pendorong yang menekankan dan melemahkan ikan haruan tasik dan menyebabkan kematian yang tinggi. Sangkar tradisional dengan saiz 2 (panjang) x 2 (lebar) x 1 m (kedalaman) hanya sesuai digunakan untuk menternak ikan haruan tasik yang bersaiz 1 kg ke bawah. Selain itu, ikan haruan tasik peringkat kecil perlu dilakukan pemantauan untuk jangkitan VNN sebelum dimasukkan ke dalam sangkar ternakan.

Kata kunci: Ikan Haruan Tasik, *Viral Nervous Necrosis* (VNN), *Rachycentron canadum*, Ektoparasit

Abstract: The mass mortality of cobia (*Rachycentron canadum*) within 2–3 days was reported by 3 private farms in Bukit Tambun, Pulau Pinang, in February and March 2007. Only cobia with body weights of 3–4 kg were affected. Most diseased cobia swam on the surface and displayed flashing behaviour. All samples were positive for viral nervous necrosis (VNN) with low to medium levels of infection. Infestations by leeches (*Zeylanicobdella arugamensis*), body monogeneans (*Benedenia* sp.) and copepods (*Caligus* sp.) were also found, but no pathogenic bacteria were isolated. All water quality parameters monitored were within optimal ranges for culturing cobia. The main causes of high mortality in cobia remain unclear during the study. However, we believe that the mass mortality of cobia could be probably due to VNN infection and that the rate of mortality will increase further when cobia are subjected to aquaculture-related stresses (e.g., limited space). Traditional cages with a size of 2 (length) x 2 (width) x 1 m (depth) should only be used for rearing cobia below 1 kg in weight given the species' natural behaviours. In addition, cobia fingerlings should be screened for VNN prior to stocking them in cages.

Keywords: Cobia, Viral Nervous Necrosis (VNN), *Rachycentron canadum*, Ectoparasite

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INTRODUCTION

Cobia (*Rachycentron canadum*), known locally as *haruan tasik*, is one of the most popular species for aquaculture in Malaysia because of its rapid growth rate, low feed conversion ratio and high market price for its firm texture. In 2004, the state of Pulau Pinang imported 1000 fingerling cobia with a retail value of RM1064.00, and this number increased to 10000 fingerling with a retail value of RM152000.00 by 2006. The number of imported fingerling from Taiwan increased to 55470 in 2007 (Department of Fisheries Malaysia 2007).

The rapid expansion and intensification of aquaculture have resulted in an increased incidence of diseases outbreaks. Generally, the occurrence of diseases is one of the major factors limiting the growth and production of cage-cultured fish. Cobia, like other species reared through aquaculture, are vulnerable to infectious diseases that can be parasitic, bacterial or viral. Parasitic diseases, which have caused economic losses in captive fish, are particularly common due to ectoparasitic infestations. The most frequently reported ectoparasitic infestations in cultured cobia include crustacean parasites, skin flukes, myxosporeans and protozoans. McLean *et al*. (2008) reported 10 species of crustacean parasites that infest cultured cobia, such as *Caligus lalandei* and *Caligus epidemicus* (Ho *et al*. 2004; Chang & Wang 2000). Skin monogeneans (*Neobenedenia girellae*) have been recorded in juvenile stages of cultured cobia (Ogawa *et al*. 2006; Lopez *et al*. 2002). Cobia with heavy ectoparasitic infestations experience severe damage on the skin surface, which can lead to death or secondary infections (McLean *et al*. 2008). Chiau *et al*. (2004) also observed that *Streptococcus spp.* and skin monogenean infestations in cobia have led to blindness. Myxosporidian parasites have also been linked to mass mortality in cultured cobia. Species such as *Myxidium*, *Ceratomyxa, Myxobolus* and *Kudoa* have the potential to infect cultured cobia (Blaylock *et al.* 2004). Infestations by *Sphaerospora*-like myxosporideans were reported to have caused 90% mortality in juvenile stages of cultured cobia in Taiwan (Chen *et al*. 2001). Juvenile cobia have also been observed with infestations of cryptocaryoniasis, *Brooklynella hostiles, Ichthyobodo* spp*.,* and *Amyloodinium ocellatum* [Food and Agriculture Organization (FAO) 2007; Bunkley-Williams & Williams 2006; Kaiser & Holt 2005].

Studies have recently reported increasing numbers of bacterial disease outbreaks throughout the culture cycle in cobia. Vibriosis, mycobacteriosis, furunculosis and streptococcosis are the most common bacterial diseases reported (Liao *et al.* 2004). Juvenile moribund cobia infected by *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* have been reported to have a 45% mortality rate (Liu *et al.* 2004; Rajan *et al.* 2001). *Photobacterium* spp. have caused 80% mortality and have been identified as a major emerging threat for cobia (Liu *et al.* 2003; Rajan *et al.* 2003; Lopez *et al.* 2002). Infections by various bacteria, such as *Aeromonas hydrophila*, *Citrobacter* spp. and *Mycobacterium marinum,* were also reported in juvenile cobia (Lowry & Smith 2006).

Viral diseases also often cause high losses and reductions in production of aquacultured fish species (Munday *et al.* 2002). Chi *et al.* (2003) reported that the nervous necrosis virus (VNN) is usually associated with mass mortalities in larval marine fish. Fish infected with VNN often display spiral swimming behaviour, and this disease can be transmitted vertically and horizontally. In addition to VNN, lymphocystis has also been reported in juvenile cobia in Taiwan (Liao *et al.* 2004). In Malaysia, the only reports of VNN infections causing mass mortalities are for greasy grouper (*Epinephelus tauvina*; Bondad-Reantaso *et al.* 2001) and humpback grouper fry (*Cromileptes altivelis;* Chuah & Kua 2003). However, no mass mortalities due to VNN infections have been reported in farmed cobia.

A total of 3 cases of mass mortality within 2–3 days were reported to the National Fish Health Research Centre (NaFisH), Pulau Pinang. Here, we describe these cases to highlight that VNN and ectoparasite infestations in farmed cobia may have potentially caused these mass mortality events.

MATERIALS AND METHODS

Animal Samples

Cobia fingerlings (approximately 6 inches in length) were obtained by fish importer, from Taiwan on 19 May 2006 and were distributed to two culture sites at Sg. Udang, Kedah and Bt. Tambun, Pulau Pinang. The fish were cultured in cages for 8–9 months without displaying symptoms or dying until 27 Feb 2007 for Farm A and until 2 March 2007 for Farms B and C. Four fish (weight ranged from 3 to 4 kg) were sampled from the 2 culture sites (Table 1) and analysed at NaFisH. Gross observation of all samples revealed no clinical signs.

Table 1: Information related to the sampling sites and cobia populations examined in this study.

Location	Date of sampling / number of sample	Source of fingerling	Mortality start to be observed (date)	Cumulative mortality	Temperature of cultured water (^{o}C)
Sg. Udang	No samples	Taiwan	16 Feb 2007	>90%	30.40–30.80
Farm A (Bt. Tambun)	28 Feb 2007 / n=3	Taiwan	27 Feb 2007	>80%	30.40–30.80
Farm B (Bt. Tambun)	No samples	Taiwan	3 March 2007	>90%	30.40–30.80
Farm C (Bt. Tambun)	3 March 2007 / n=1	Taiwan	2 March 2007	>80%	30.40–30.80

Parasitological Examination

The fish were examined for ectoparasites and endoparasites based on the methods of Kabata (1985).

Bacteriological Examination

Several internal organs, such as the kidneys, spleen, eyes and brain, were inoculated directly onto blood agar (BA) and brain heart infusion agar (BHI) to measure total heterotrophic flora and onto thiosulfate citrate bile saccharose agar (TCBS) to identify *Vibrio* spp. The agar plates were incubated at 30°C for 24 hr, after which the grown colonies were re-streaked before obtaining pure cultures. Biochemical profiles were determined using commercial Analytical Profile Index (API) kits (Biomerieux, Marcy l'Etoile, France).

Virological Examination

RNA was extracted from brain, spleen and kidney tissue samples and examined using PCR techniques with an IQ2000 VNN detection and prevention system according to the included instructions (Farming Intelligent Tech. Corp., Taipei, Taiwan). Briefly, samples were homogenised with 0.5 ml RNA extraction solution and then left to stand at room temperature for 5 min. Chloroform was then added to each sample, and the tubes were mixed and centrifuged at 12000 x g for 15 min. The upper aqueous phase was transferred to a new tube, supplemented with 0.5 ml isopropanol, centrifuged and washed with 75% ethanol. After the ethanol had been removed, the remaining pellet was dried and dissolved with 0.2 ml of diethylpyrocarbonate (DEPC) water.

Two types of premixed reagents, including primers, were prepared in the following manner: 15 µl of nested PCR premixed reagent was added to 8 µl of RT-PCR premixed reagents. The mixture was then amplified under the following conditions: the RT-PCR reaction was initiated at 42°C for 30 min and run at 94°C for 2 min. The PCR reaction was then run at 94°C for 20 s, 62°C for 20 s and 72°C for 30 s (repeated for 15 cycles), followed by 72°C for 30 s and 20°C for 30 s to complete the cycle. After the completion of this cycle, 15 µl of nested reaction reagent was added to the tube, and the PCR conditions were set to 94°C for 20 s, 62°C for 20 s and 72°C for 30 s (repeated for 30 cycles), followed by 72°C for 30 s and 20°C for 30 s to complete the cycle using a master cycler (Biometra, Gottingen, Germany). The PCR products were then electrophoresed on a 2% gel and stained with ethidium bromide for 30 min before viewing with the Gene Genius gel documentation system (Syngene, Frederick, Maryland, USA). The results were based on the formation of bands at 289 bp (light infection), 289–479 bp (medium infection), or 479–1160 bp (severe infection). Bands formed at 665 bp served as the internal control.

RESULTS AND DISCUSSION

On-site investigations on 28 Feb 2007 and on 2 and 3 March 2007 showed that infected fish swam in circles on the surface and displayed flashing behaviours (Fig. 1). Clinical signs, such as swimming in a spiral, were accompanied by mortality rates ranging from 80% to 100% within 2–3 days of the first symptoms. During the site investigation, it was observed that only cobia showed clinical signs of infection, while other species, such as grouper (*Epinephelus* spp.) and Asian sea bass (*Lates calcarifer*), remained asymptomatic. All cobia samples were positive for low to medium levels of infection by VNN, reflected by bands at 289–479 bp after agarose gel electrophoresis. Infestations by leeches (*Zeylanicobdella arugamensis*), body monogeneans (*Benedenia* spp.) and copepods (*Caligus* spp.) were also found, but no pathogenic bacteria were isolated. Water quality parameters monitored at the time of the investigation were within optimal ranges for culturing fish, with the exception of ammonia levels, which were higher than 0.01 ppm (Table 2).

The spiral swimming patterns by infected cobia were consistent with swimming irregularities generally displayed by fish infected with VNN or *Mycobacterium marinum*. No bacteria were isolated during the study; however, infected cobia were VNN positive. On the other hand, water quality was found to be within the optimal range for culturing cobia with the exception of ammonia level. We believe that cobia displaying abnormal swimming behaviours were infected by VNN. In addition to VNN, cobia were also infected with ectoparasites, such as leeches, body monogeneans and copepods. However, the mean intensity for each ectoparasitic infestation was less than two parasites per fish. Because multiple cultured species are cultured in the same area, co-infestations by multiple species of ectoparasites are often common in farm cages. The main cause of the VNN disease outbreak during the investigation period is still unknown. However, in the present study, high ammonia levels (>0.01 ppm) could further stress and weaken cobia, increasing the likelihood that they would succumb to pathogens such as VNN and ectoparasites. In a related study of older sea bass and grouper infected with VNN, fish showed clinical signs only in high water temperatures (Tanaka *et al.* 1998). In the current study, cobia were reared in traditional cages measuring 2 (length) x 2 (width) x 1 m (depth) at initial stocking levels of 1000 fish/cage and 100–150 g/fish. Loads were reduced to 50–70 fish/cage when fish reached 3–4 kg after 6 months of culture. These conditions may have caused additional stress for the cobia, which require larger spaces due to their pelagic nature. Moreover, cobia can gain 1 kg of body weight per month (Lunger *et al.* 2007). Based on our observations, we recommend that cobia should be transferred to larger, deeper cages or to open sea cages when they reach more than 1 kg of body weight.

The main causes of high mortality in cobia remain unclear. The ectoparasites found during the investigation are normally associated with low mortality rates, with the exception of instances of massive infestations (Mark 2004). Limited space for growth may have stressed and weakened the cobia, allowing the low to medium levels of infection to lead to high mortality rates. We believe that PCR screenings for spawners and fingerlings brought into Malaysia should be conducted to prevent the introduction of VNN, which is vertically transmitted. A larger net cage or reduced stocking for adult cobia should also be used because traditional cages at 2 (length) x 2 (width) x 1 m (depth) are only suitable for rearing cobia below 1 kg weight due to the natural behaviours of these fish.

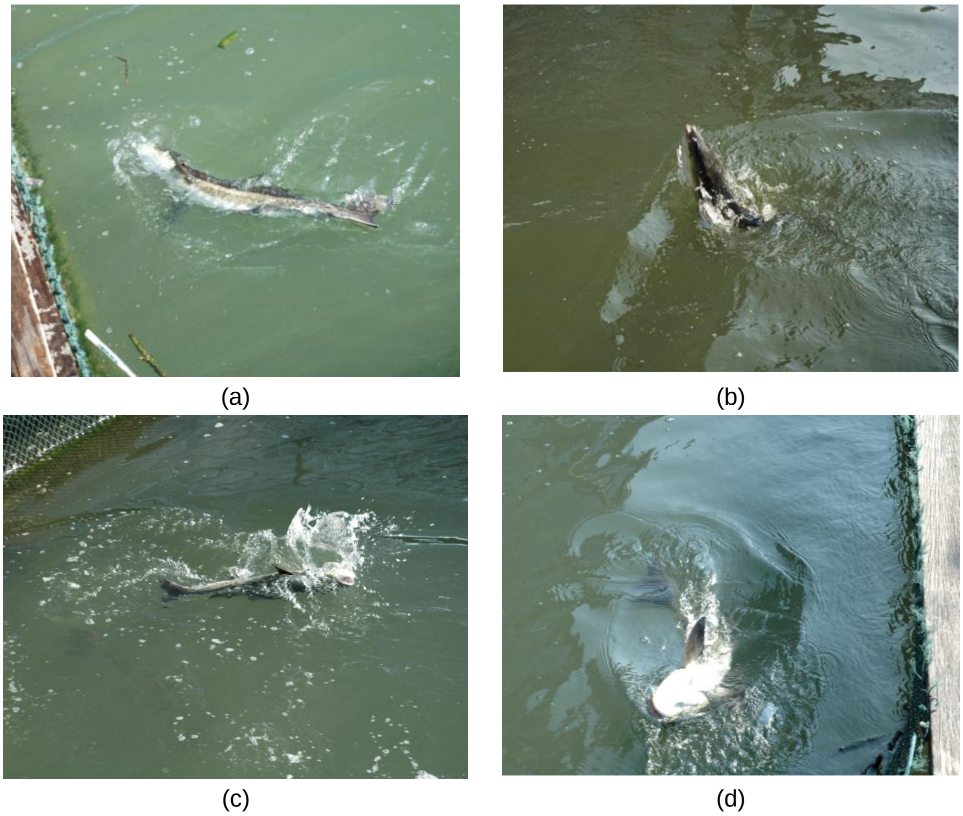

Figure 1: Examples of infected fish swimming in circles on the surface and displaying flashing behaviours.

Table 2: Water quality parameters measured during the investigation at Bt. Tambun.

Location	Water sampling site	Temperature (^{o}C)	Salinity (ppt)	DO (ppm)	pH	Ammonia * (ppm)
Farm A	Surface	30.80	30.90	6.68	7.90	0.44
	Middle	30.57	30.90	6.51	7.90	0.24
	Bottom	30.45	30.88	6.30	7.90	0.45
Farm B	Surface	30.70	30.80	6.77	7.90	0.13
	Middle	30.68	30.80	6.70	7.90	0.11
	Bottom	30.45	30.88	6.60	7.90	0.37
Farm C	Surface	30.70	30.80	7.20	7.90	0.07
	Middle	30.60	30.80	7.20	7.90	0.53
	Bottom	30.50	30.80	6.70	7.90	0.15

Notes: *Optimal ammonia conditions for cultured fish are <0.01 ppm; DO: Dissolved oxygen

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