INDONESIAN HATCHERY REARED SEABASS LARVAE (*Lates calcarifer*), ASSOCIATED WITH VIRAL NERVOUS NECROSIS (VNN)

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ABSTRACT

Mass mortality amongs seabass larvae, *Lates calcarifer*, reared in hatcheries in East Java and Bali due to viral nervous necrosis were investigated. Outbreaks of the disease occurred from August to November 1997. Each time the disease occurred, cumulative mortality of the larvae reached 100%. Infected fish were characterized by abnormal behaviour such as swimming upside down or sinking to the bottom. Bacteria or parasites associated with the disease were not detected in infected fish. Histopathologically, necrosis and vacuolation in the brain and retina were observed. Abundant spherical viral particles, 30 nm in diameter, were found in the cytoplasm of affected nerve cells. These findings revealed that the mortalities among seabass larvae were due to viral nervous necrosis (VNN) caused by nodavirus.

KEYWORDS: seabass, *Lates calcarifer*, viral nervous necrosis, nodavirus.

INTRODUCTION

Seed production techniques for various marine fish and shellfish have been developed in Indonesia. Seabass, *Lates calcarifer* is one of the species marked as new and potential commodity for aquaculture. However, mass mortality in the larval stages due to unidentified diseases have led to frequent decreases in production in recent years.

In 1997, mass mortalities of seabass larvae, *Lates calcarifer* occurred at hatcheries in Situbondo, East Java, and in Gerokgak, Bali in August and then in Banyuwangi in November. The mortality rate reached 100% in all cases. Clinical signs of the infected fish were characterized by abnormal behavior such as swimming upside down or sinking to the bottom.

Mass mortalities in seabass larvae due to a viral infection called viral nervous necrosis (VNN) (Glazebrook *et al.*, 1990; Renaulte *et al.*, 1991; Munday *et al.*, 1992) have been reported in Australia and the Mediterranean. VNN has also been reported in a wild variety of cultured marine fish species, and has caused serious damage to the industry in Japan (Yoshikoshi & Inoue, 1990; Mori *et al.*, 1991).

In this study, a histopathological study of the mass mortalities at three seabass hatcheries in Indonesia was conducted to elucidate the cause of the mortalities.

MATERIALS AND METHODS

Fish Examined

One to two-month-old larvae of seabass reared in East Java and Bali showing abnormal behavior such as swimming upside down or sinking to the bottom, were examined for this study. The origin of the eggs distributed to the these hatcheries identical. Water temperatures were 27-29° C when outbreaks of the disease occurred.

Parasitological and Bacteriological Examinations

The gills and body surface were examined for parasites by using light microscope. Bacteria from the liver and brain were isolated using marine agar and TCBS agar, then incubated at 27° C and 35° C respectively for 48 hours.

Histological Examination

About 20 of moribund seabass larvae from each hatchery were used for histological examination and 5 were used for observation transmission electron microscopy. The whole body of moribund larvae was fixed in 10% phosphate buffered formalin. The sample was then embedded in paraffin wax and the section was stained with haematoxylin-eosin (H&E). For transmission electron microscopy, the sample was re-fixed in a 2.5% glutaraldehyde- 2% paraformaldehyde mixture (pH 7.4), then post-fixed with 1% osmium

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tetroxide, and embedded in Quetol 812. A thin section was stained with 1% uranyl acetate and 1% lead citrate, then examined by electron microscope.

RESULTS AND DISCUSSION

Parasitological and Bacteriological Examinations

Neither parasites nor bacteria were detected from the fish samples.

Histopathological Examination

All of the examined fish showed same histopathological appearance. Vacuolation and degeneration of nerve cells were observed in the brain (Figure 1) and retina (Figure 2), but no histopathological changes were observed in other organs. Numerous round-shaped, unenveloped virus particles, about 30 nm in diameter, were observed in the cytoplasm of the degenerated nerve cells (Figure 3). In both the brain and retina, the virions had the same size and morphology. Normal appearance of the brain and retina of seabass was shown in Figure 4.

Recently, a viral infection, viral nervous necrosis (VNN) was reported in larvae and juveniles of Japanese parrotfish (Yoshikoshi & Inoue, 1990), in stripped jack (Mori *et al.*, 1992), larvae and juveniles of redspotted grouper (Mori *et al.*, 1991), kelp grouper and tiger puffer (Nakai *et al.*, 1994), adult sevenband grouper (Fukuda *et al.*, 1996), barramundi in Australia (Glazebrook *et al.*, 1990;

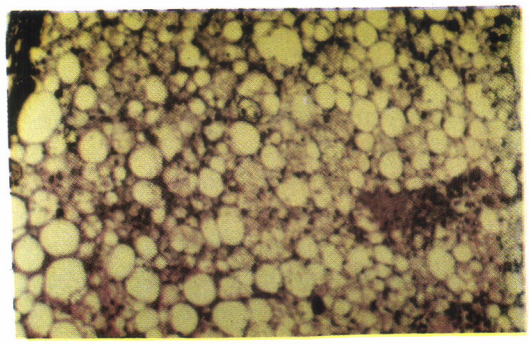


Figure 1. Light microscopy showing necrosis and vacuolation in the brain of diseased seabass larvae (H&E, x 100).

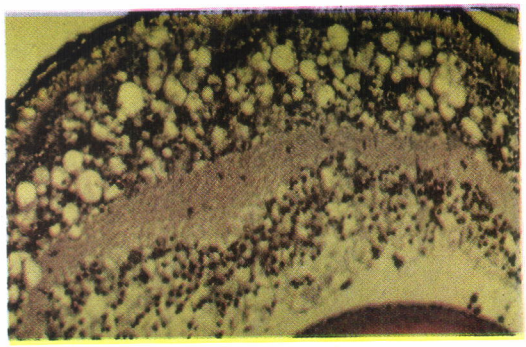


Figure 2. Light microscopy showing necrosis and vacuolation in the retina of a diseased seabass larvae (H&E, x 100).

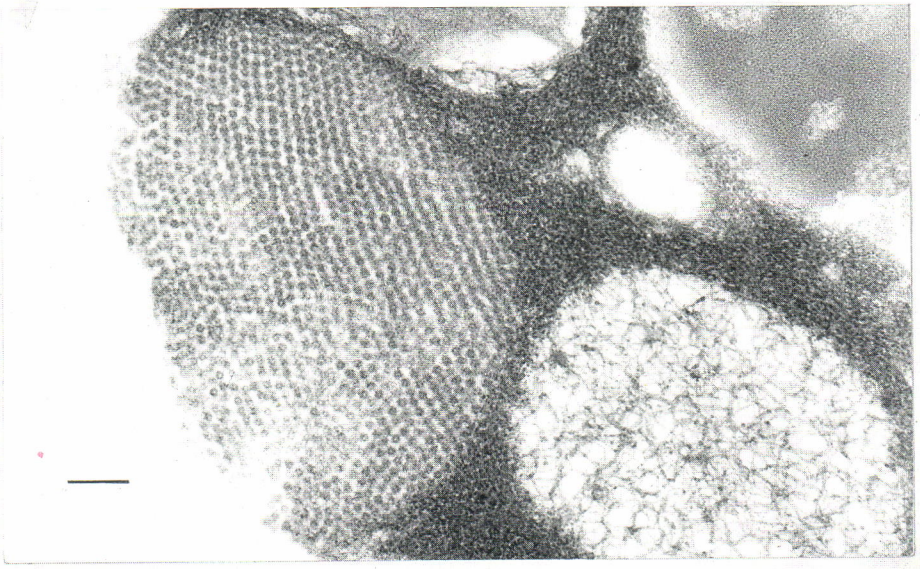
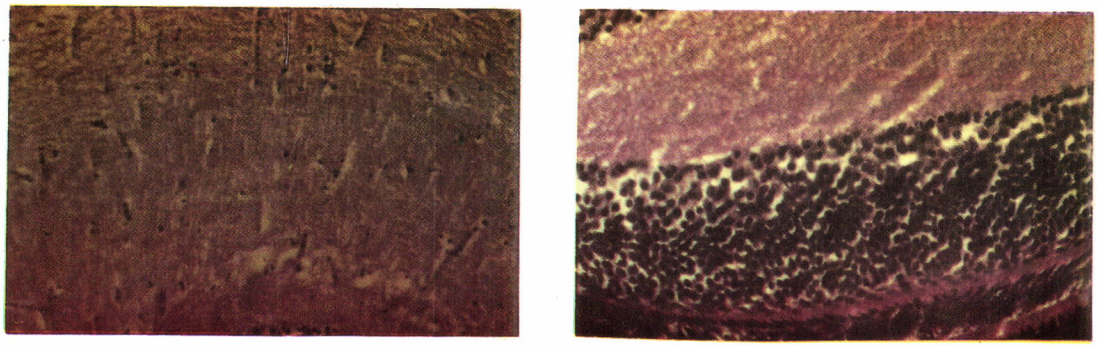


Figure 3. Electron micrographs showing numerous virus particle in the cytoplasm of a retinal cell.



(a) (b)

Figure 4. Light microscopy showing normal appearance of the brain (a) and retina (b) of a healthy seabass larvae (H&E, x 100).

Munday *et al.*, 1992; Munday & Owens, 1997), larvae and juveniles of seabass in France (Breuil *et al.*, 1991), and in Tahiti (Renault *et al.*, 1991). The causative virus VNN was tentatively identified as picorna-like virus/picornaviridae (Glazebrook *et al.*, 1990; Mori *et al.*, 1991; Breuil *et al.*, 1991) while more recent publications identified it as nodavirus/ nodaviridae (Mori *et al.*, 1992; Nakai *et al.*, 1994; Fukuda *et al.*, 1996; Nguyen *et al.*, 1996; Munday & Owens, 1997). The VNN disease was characterized by abnormal swimming behaviour such as a whirling or corkscrew motion, heavy vacuolation of the central nervous tissue, and spherical virus particle (25-30 nm in diameter) observed in the cyptoplasm of affected nerve cells (Nakai *et al.*, 1995 *in* Fukuda *et al.*, 1996). Fukuda *et al.* (1996) reported that fish infected with VNN were characterized by upside down swimming behaviour, swimbladder inflation and degeneration of nervous tissue (necrosis and vacuolation).

In the present study, the infected fish were characterized by abnormal swimming or sinking to the bottom, anorexia, slight blackening of the body, and degeneration of brain tissue and retina (necrosis and vacuolation). No parasites nor bacteria associated with the disease were found, but small round-shaped virus particles (30 nm in diameter) were observed in the cytoplasm of affected nerve cells in the central nervous tissues and retina from all samples. These findings are identical as VNN infection cases reported before (Yoshikoshi & Inoue, 1990; Mori *et al.*, 1991; 1992; Nakai *et al.*, 1994; Fukuda *et al.*, 1991; 1996; Glazebrook *et al.*, 1990; Munday *et al.*, 1992; Munday & Owens, 1997; Breuil *et al.*, 1991; Renault *et al.*, 1991). This indicates, that present seabass disease was associated with VNN. This is the first report of VNN of seabass larvae reared in a hatchery in Indonesia.

Fukuda *et al.* (1996) suspected a higher rearing water temperature in summer season (25-28°C) is a possible predisposing factor which enabled the viral invasion into the central nervous system. Mori *et al.* (1991) also reported that disease occurred in larval and juveniles of redspotted grouper were reared at 25-27°C in August to September. The effect of rearing water temperature on the defence mechanisms of seabass larvae should be examined in further experiments.

VNN in Japan was detected from gonads of broodstocks (Mori *et al.*, 1997; Watanabe *et al.*, 1997), indicating possible vertical transmission of the virus from spawners to the offsprings. One effective method to prevent VNN is by using broodstocks free of the virus in the hatchery. But, Glazebrook *et al.* (1990) reported that the virus is highly infectious, being transmitted from diseased to healthy fish within 4 days of contact. Up to now, no chemical agent has been found effective against this disease.

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