World Journal of Microbiology & Biotechnology **13**, 375–381

Special topic review: Nodaviruses as pathogens in larval and juvenile marine finfish

B.L. Munday* and T. Nakai

Nodaviruses have emerged as major pathogens of a wide range of larval and juvenile marine finfish in aquaculture worldwide. The causative agents are non-enveloped, icosahedral, RNA viruses with diameters in the range of 25–34 nm. They display considerable serological and molecular homology, although the present evidence suggests that there is more than one agent causing disease in a range of species. The diseases produced by these nodaviruses invariably involve the central nervous system and the retina where they usually produce vacuolation and cell necrosis. Virus particles are numerous within the cytoplasm of affected cells and extracellularly. As a result of the lesions, affected larvae/juveniles exhibit a range of neurological signs usually culminating in high mortality rates (not uncommonly 100%). One virus, that of the European sea bass, has recently been cultured in a fish cell line, but to date techniques such as the fluorescent antibody test, enzyme-linked immunosorbent assay and polymerase chain reaction have relied upon the harvest of purified viral antigen from infected tissues rather than obtaining these reagents from viruses grown in cell cultures. The epidemiology of these diseases is only partly understood. All appear to transmit readily by cohabitation of infected fish with naive larvae or juveniles, but vertical transmission has only been recognized with striped jack nervous necrosis and sea bass nervous necrosis viruses. Consequently, some aspects of disease control are based on first principles, rather than application of a full understanding of epidemiological factors.

Key words: Central nervous system, disease, juvenile fish, larval fish, nodavirus, pathology, retina

Background

Disease in larval and juvenile marine finfish attributable to a nodavirus was first described by Yoshikoshi & Inoue (1990) in Japanese parrotfish (*Opelegnathus fasciatus*) in Japan, where the disease was designated as 'viral nervous necrosis' (VNN). Since that time similar diseases have been reported in a wide range of aquaculture species in both hemispheres and from the sub-tropics to cold-temperate regions of the world (Glazebrook *et al*. 1990; Bloch *et al*. 1991; Breuil *et al*. 1991; Renault *et al*. 1991; Chua *et al*. 1995; Danayadol *et al*. 1995). Some papers on VNN-like disease have described the disease as 'barramundi picorna-like virus infection' in barramundi (*Lates calcarifer*) (Glazebrook & Heasman 1992), 'encephalomyelitis' in turbot (*Scophthalmus maximus*) (Bloch *et al.* 1991), or 'fish encephalitis' in sea bass (*Dicentrarchus labrax*) (Breuil *et al.* 1991).

In a number of species, the diseases caused by nodaviruses have been major constaints on aquaculture development (Yoshikoshi & Inoue 1990; Breuil *et al.* 1991; Mori *et al.* 1992; Munday *et al.* 1992; Grotmol *et al.* 1995) and have significantly impeded commercialization of some fish species. At present, the reported fish host species number 19 in 10 families (Table 1). It is reasonable to expect that as more marine finfish are subjected to intensive aquaculture, additional examples of viral nervous necrosis will be recognized.

The Diseases

Table 1 shows the species known to be affected by viral nervous necrosis due to piscine nodaviruses.

There is a great commonality of clinical signs with 'mass mortality' and a variety of neurological abnormalities, as follows: *Lates calcarifer*, uncoordinated darting, corkscrew swimming, pale colour, anorexia, wasting; *Dicentrarchus labrax*, whirling swim pattern, swimbladder hyperinflation, anorexia; *Epinephelus akaara*, whirling swim pattern; *Pseudocaranx dentex*, abnormal swimming behaviour, swimbladder hyperinflation; *Oplegnathus fasciatus*, spiral swimming, dark colour; *Hippoglossus hippoglossus*, lethargy, belly-up at rest, abnormal swimming, pale colour; *Scophthalmus maximus*,

B.L. Munday is with the Department of Aquaculture, University of Tasmania, PO Box 1214, Launceston, Tasmania 7250, Australia; fax: 61 363 243804. T. Nakai is with the Faculty of Applied Biological Science, Hiroshima University, Higashi-hiroshima 739, Japan. *Corresponding author.

Table 1. Fish species affected by viral nervous necrosis (VNN).*

Order Perciformes	
Family Centropomatidae	
barramundi *Lates calcarifer*	Glazebrook *et al*. 1990
Japanese sea bass *Lateolabrax japonicus*	Jung *et al*. 1996
Family Percichthydae	
sea bass *Dicentrarchus labrax*	Breuil *et al*. 1991
Family Serranidae	
redspotted grouper *Epinephelus akaara*	Mori *et al*. 1991
kelp grouper *E. moara*	Nakai *et al*. 1994
sevenband grouper *E. septemfasciatus*	Fukuda *et al*. 1996
brownspotted grouper *E. malabaricus*	Danayadol *et al*. 1995
greasy grouper *E. tauvina*	Chua *et al*. 1995
Family Carangidae	
striped jack *Pseudocaranx dentex*	Mori *et al*. 1992
purplish amberjack *Seriola dumerili*	Muroga 1995
Family Sparidae	
sea bream *Sparus aurata*	Comps & Raymond 1996
Family Sciaenidae	
shi drum *Umbrina cirrosa*	Comps *et al*. 1996
Family Oplegnathidae	
Japanese parrotfish *Oplegnathus fasciatus*	Yoshikoshi & Inoue 1990
rock porgy *O. punctatus*	Muroga 1995
Order Pleuronectiformes	
Family Pleuronectidae	
barfin flounder *Verasper moseri*	Muroga 1995
halibut *Hippoglossus hippoglossus*	Grotmol *et al*. 1995
Family Bothidae	
Japanese flounder *Paralichthys olivaceus*	Nguyen *et al*. 1994
turbot *Scophthalmus maximus*	Bloch *et al*. 1991
Order Tetraodontiformes	
Family Triodontidae	
tiger puffer *Takifugu rubripes*	Nakai *et al*. 1994

* Modified from Muroga (1995).

spiral and/or looping swim pattern, belly-up at rest, dark colour.

Apart from colour changes and wasting there are no consistent marcroscopic findings in affected fish. However as shown in Table 2, there are considerable variations in the age at which disease is first noted and the period over which mortality occurs. In general, the earlier signs of disease first occur, the greater is the rate of mortality. Although disease occurrence at the juvenile stages in some species is very rare, mass mortalities often occur at juvenile to young stages in the other fish species but usually do not reach 100%, indicating the age-dependence of susceptibility. However, a recent interesting finding was that a nodavirus was detected in young or adult sevenband grouper *Epinephelus septemfasciatus* affected by a disease characterized by upside-down swimming and swimbladder inflation (Fukuda *et al*. 1996).

At the light microscope level, histopathological findings, characterized by vacuolation and necrosis of the central nervous system, are remarkably consistent between the various species. In general, the anterior brain is more severely affected than the posterior brain and spinal cord. Lesions have been described in the spinal ganglia in Japanese parrotfish (Yoshikoshi & Inoue 1990). Larval fish are more severely affected than juveniles. The most characteristic lesion is the presence of vacuoles in the grey matter of the brain; these appear to be intracytoplasmic, but their exact position cannot always be determined. Other lesions noted include pyknosis, shrinkage and basophilia of affected cells (Yoshikoshi & Inoue 1990), focal pyknosis and karyorrhexis of neural cells, granularity of the neuropil and accumulation of eosinophilic material in macrophages and blood vessel walls (Munday *et al*. 1992) and presence of mononuclear cell (macrophage) infiltrates (Grotmol *et al*. 1995). Basophilic, intracytoplasmic inclusions, approximately 1 μm in diameter in Japanese parrotfish (Yoshikoshi & Inoue 1990) and barramundi (Glazebrook *et al*. 1990), 2–5 μm in European sea bass (Breuil *et al*. 1991) and of unspecified size in brownspotted grouper (Boonyaratpalin *et al*. 1996) have been reported. Retinal lesions have also been described in all species where the eye has been examined. In the instances of Japanese parrotfish (Yoshikoshi & Inoue 1990) and turbot (Bloch

Table 2. Important clinical features of viral nervous necrosis of larval and juvenile fish.

	Earliest occurrence of disease	Usual onset of disease	Latest occurrence	Usual mortality rate	Highest mortality rate
L. calcarifer	9 dph*	15–18 dph	≥ 24 dph	50–100%/month	100% in <1 month
D. labrax	10 dph	25–40 dph	Bodyweight approx 5 g	10%/month	
E. akaara	14 dph (7–8 mm tl)	9–10 mm tl	<40 mm tl	80%	Up to 100%
E. malabaricus		20–50 mm tl		50–80%	
P. dentex	1 dph	1–4 dph	<20 dph (8 mm tl)	100%	
O. fasciatus	6–25 mm tl		<40 mm tl		Up to 100%
H. hippoglossus		60–70 dph	juvenile		Up to 100%
P. olivaceus	35 dph (17–18 mm tl)	25 mm tl		100%	
S. maximus	<21 dph		Bodyweight 50–100 mg		Up to 100%

* dph, days post hatch; tl, total length.

et al. 1991) there is no information on this point. Vacuolation involves the cellular components of the retina especially the bipolar and ganglionic nuclear layers (Munday *et al*. 1992), although small vacuoles can be found in the rod and cone layer (Grotmol *et al*. 1995). However, Comps *et al*. (1996) reported nodavirus infection in shi drum (*Umbrina cirrosa*) with clinical signs of 'fish encephalitis virus disease' but unaccompanied by lesions of the central nervous system. However, it is not clear that the authors actually examined the eyes of these fish. This may be significant as Comps and Raymond (1996) have described presumptive nodavirus infection in sea bream (*Sparus aurata*) with light microscopic lesions confined to the retina.

Ultramicroscopic observations have confirmed the light microscopic findings and also revealed the presence of numerous viral particles both intra- and extracellularly. Intracellular particles are present in the cytoplasm, but not the nucleus. Intracellular viruses occur as single particles, in aggregates and as crystalline arrays (Yoshikoshi & Inoue 1990; Breuil *et al*. 1991; Munday *et al*. 1992).

The Agents

Frerichs *et al*. (1996) suggest that there is a single virus, piscine neuropathy virus. However, we believe there is insufficient evidence to support this unitary concept at this time.

The viral particles visualized in the various nervous necrosis syndromes are all non-enveloped and icosahedral in shape with a diameter in the range of 25–34 nm. They exhibit a bright red fluorescence with acridine orange indicating that they consist of single-stranded nucleic acid. Serologically there is considerable antigenic similarity between the viruses from striped jack, barramundi, European sea bass, Japanese parrotfish and redspotted grouper when a fluorescent antibody technique (FAT) incorporating a rabbit antiserum against striped jack nervous necrosis virus (SJNNV) (Mori *et al*. 1992) is used (Office International des Epizooties 1995; Munday *et al*. 1994). However, an enzyme-linked immunosorbent assay (ELISA) using the same antiserum is only specific for SJNNV (Office International des Epizooties 1995). This indicates a close, but not identical antigenic relationship between the agents. Support for this suggestion was provided by studies using monoclonal antibodies against SJNNV which showed that there was evidence for differences between the neutralizing epitopes on the coat protein of SJNNV and other viral nervous necrosis agents (Nishizawa *et al*. 1995a). Additionally, Comps *et al*. (1996), using *in situ* hybridization, demonstrated relatedness between the nodaviruses causing disease (fish encephalitis) in sea bass, barramundi, turbot and shi drum.

Preliminary molecular approaches to the study of SJNNV and the viruses from barramundi and European sea bass revealed that these contained two single-

stranded, positive-sense RNA molecules with molecular weights of 1.01×10^6 Da (RNA1) and 0.49×10^6 Da (RNA2) (Mori *et al*. 1992; Comps *et al*. 1994). RNA1 encodes a non-structural protein of 100 kDa and RNA2 encodes a major coat protein of 42 kDa. As as result of these studies, these viruses have been identified as new members of the family *Nodaviridae*, which had previously been composed entirely of insect viruses (Murphy *et al*. 1995). Subsequently, it has been shown that there is a close relationship between the coat protein genes of a number of piscine nodaviruses (Nishizawa *et al*. 1995b). The SJNNV coat protein gene is 1410 bases in length and contains a single open reading frame of 1023 bases coding for a protein of 340 amino acids. The sequence similarities between the coat protein gene of SJNNV and four known insect nodaviruses (Nodamura virus, black beetle virus, flock house virus, Boolarra virus) (Dasgupta *et al*. 1984; Dasgupta & Sgro 1989; Kaesberg *et al*. 1990) are quite low both at the nucleotide (28.6%) and amino acid (10.6%) levels. In contrast, a highly conserved sequence is found between SJNNV and another four nervous necrosis agents; 75.8% or higher at the nucleotide level and 80.9% or higher at the amino acid level. Based on these results, it was suggested that these piscine nodaviruses should be classified as a new group of the nodaviruses.

A number of workers have attempted to culture these nodaviruses on a wide range of cell lines, but generally without success (Breuil *et al*. 1991; Munday *et al*. 1992; Nguyen *et al*. 1994; Grotmol *et al*. 1995). However, successful culture of the piscine neuropathy nodavirus from sea bass has now been achieved in a fish cell line (SSN-1) derived from striped snakehead (*Channa striatus*) (Frerichs *et al*. 1996). The SSN-1 cell line is derived from whole fry tissue and the identity of the cells in the line is not known (Frerichs *et al*. 1991; G.N. Frerichs personal communication, 1996).

Arimoto *et al*. (1996) examined a number of potential virucidal agents using SJNNV as the target virus in order to assess its resistance to chemical and physical agents: formalin at 1600 μg/ml was ineffective and cresol was only effective at 10000 μg/ml; sodium hypochlorite, calcium hypochlorite, benzalkonium chloride and iodine were suitable disinfectants at 50 μg/ml; ethanol and methanol were effective at 60% and 50% respectively; the virus was inactivated by a temperature of 60 °C for 10 min and by a pH of 12; effective levels of u.v. light irradiation and ozone were 1.0×10^5 μW/s.cm^2 and 0.1 μg/ml total residual oxidant for a minimum of 2.5 min, respectively.

Experimental Infections

The most comprehensive report of experimental reproduction of viral nervous necrosis is that of Arimoto *et al*. (1993) using SJNNV. In larvae of striped jack 1 and 9 days old, mortalities occurred over the period 2–6 days post-exposure (dpe); exposure was by immersion in a homogenate of diseased tissue or purified virus, or by cohabitation with the infected larvae. Mortality of 100% was induced by levels of virus as low as 0.1 ng/ml. Juvenile striped jack (81 days old) and 1-day-old red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*) and goldstriped amberjack (*S. lalandi*) did not show signs of infection or disease when exposed to 100 ng/ml of purified virus. A study on the progression of SJNNV in larval stiped jack revealed that the initial multiplication site of the virus is the spinal cord, from which the virus spreads to the brain and finally to the retina (Nguyen *et al*. 1996). Virus multiplication was also observed in epidermal cells with hyperplasia, however, the role of skin as a portal of entry for the virus remains unclear. Mori *et al*. (1991) exposed juvenile redspotted grouper (*Epinephelus akaara*) to a homogenate of diseased larval redspotted grouper by immersion or intraperitoneal injection; the mortality rate in these relatively advanced fish was 10–30% and clinical signs did not occur until 10–14 dpe with mortality occurring 3 days later. Boonyaratpalin *et al*. (1996) reported a 4–10 day incubation period in fingerling brown spotted grouper (*E. malabaricus*) with a mortality of 40–60% and Galzebrook *et al*. (1990) reported an incubation period of approximately 4 days for barramundi exposed by cohabitation or exposure to water from infected tanks (M.P. Heasman personal communication, 1990). These experiments all indicate that horizontal spread of the viruses is a potent means of dispersing and amplifying these agents.

Diagnosis

Presumptive diagnosis is possible at the light-microscope level in those instances where typical pathology occurs i.e. usually in the epizootic situation. However, it is not unusual to find vacuolation of the central nervous system and/or retina of fish which does not have the typical appearance or distribution of viral nervous necrosis but which, nonetheless, have to be conclusively proven not to be due to a nodavirus. This can be achieved by the use of electron microscopy and/or serological or molecular biological techniques (Office International des Epizooties 1995).

Negative staining of brain or eye homogenates for electron microscopy is a relatively rapid means of confirming viral nervous necrosis. Non-enveloped, round to icosahedral particles about 25–30 nm in diameter are present and it may be possible to detect capsomeres. Ideally, the tissues should be fresh or frozen, but it is possible to see particles in formalin-fixed material (Oliver 1990). Positive staining for electron microscopy requires appropriate fixation of tissues in 1–2% glutaraldehyde

although, again, formalin-fixation may still permit adequate, but not optimal, visualization of viral particles.

A fluorescent antibody test using the anti-SJNNV rabbit serum has proved to be a rapid and group-specific test for at least five nervous necrosis viruses when applied to frozen or paraffin-embedded sections (Office International des Epizooties 1995). Logically, it should also be suitable for impression smears of affected tissues. While suitable for detecting virus in clinically affected animals, this technique is not sensitive enough to detect carrier or inapparent infection status.

An ELISA using the anti-SJNNV serum has been developed and is sensitive enough to detect this virus (at 5 ng/well) in affected striped jack larvae and the ovaries of carrier striped jack broodstock (Arimoto *et al*. 1992). Unfortunately, as previously stated, this test is not applicable to other nervous necrosis viruses. Mushiake *et al*. (1992) developed an indirect ELISA procedure for serum antibody detection which revealed that striped jack broodstocks had antibodies to SJNNV at high frequencies.

Based on the sequence data of SJNNV coat protein gene (RNA2), a reverse transcription–polymerase chain reaction (RT–PCR) technique has been developed to detect SJNNV (Nishizawa *et al*. 1994). This technique is able to amplify approximately 100 fg of SJNNV nucleic acid and has been successfully used to detect the virus in striped jack at early stages of infection when SJNNV antigens were not detectable by ELISA. The RT–PCR technique also confirmed that juvenile striped jack surviving epizootics of VNN carried the virus for at least 3 months. Thus, PCR is particularly suitable for the diagnosis of asymptomatic carrier fish. Furthermore, since the conserved target region among fish nodaviruses was successfully amplified from diseased fish other than striped jack, PCR using primers designed for SJNNV has a broad application for the diagnosis of VNN in a number of species (Nishizawa *et al*. 1995b).

Comps *et al*. (1996) described an *in situ* hybridization assay using a probe (N12) which hybridizes to RNA2. Digoxigenin-11-dUTP and radio-labelled versions of the probe could detect 10 and 5 pg respectively of sea bass nodavirus genomic RNA. This probe also hybridized with nodaviruses from barramundi, turbot, and shi drum, but not with rotifer birnavirus or Taura syndrome (picorna-like) virus.

Epidemiology

Except for striped jack nervous necrosis, the epidemiology of these diseases is poorly understood. In striped jack, the virus has been detected in the ovaries of striped jack spawners at high frequency (65%) and fertilized eggs, although it is unknown whether the virus is present on the surface of, or inside the eggs (Arimoto *et al*. 1992). This indicates that the spawners are important virus reservoirs and virus propagating in the ovaries is shed with eggs into the environmental water. Also, the risk of spread increases with hormonal induction of spawning and multiple spawnings (Mushiake *et al*. 1994). Horizontal spread occurs after hatching frequently leading to total mortalities in the first few days. More recently, viral RNA has been detected in the ovaries of sea bass (Comps *et al*. 1996).

The initial source of infection for other species is not known. Munday *et al*. (1992) suggested that water pumped from nearby estuaries could contain virus excreted by carrier juveniles. However, until adequate examinations of spawners using sensitive techniques such as PCR are undertaken, the role of vertical transmission in species other than striped jack and sea bass will remain an enigma.

Even though there are no published data on the resistance of nervous necrosis nodaviruses to environmental conditions, it is reasonable to expect that they will be relatively robust and, therefore, likely to be transferred on clothing, equipment, etc. Also, the possibility of transport over significant distances in aerosols needs to be considered, although limited trials did not demonstrate transmission of the barramundi nodavirus by this route (M.P. Heasman, personal communication, 1990).

Control

Exclusion of infected animals would be the most certain means of control. At present, the elimination or segregation of virus-carrying spawners, as detected by PCR, is the best choice to prevent disease in striped jack, even though a negative PCR does not mean complete absence of virus in the fish (Mushiake *et al*. 1994). This is presumably because the stress of multiple spawnings activates residual, extraovarian virus. Consequently, it is recommended that all positive spawners are rejected and provisionally 'clean' fish are not induced to spawn more than 10 times in a season (Mushiake *et al*. 1994). As an alternative control procedure, a basic investigation on vaccination has just started in striped jack using a recombinant coat protein of SJNNV (K. Mori, M. Arimoto, T. Nakai & K. Muroga, unpublished work). As injection with the recombinant protein induced virus neutralizing antibodies in adult striped jack, use of vaccines seems to be a potential approach for the control of viral nervous necrosis.

In Australia, attempts have been made to prevent translocation of infected juvenile barramundi by subjecting a sample of a shipment to light-microscopic examination. Although this technique detected some infected batches, it is not sufficiently sensitive to ensure absolute exclusion of inapparent, carrier fish.

Within hatcheries, strict hygiene has been shown to assist with controlling viral nervous necrosis. Anderson *et al*. (1993) reported that a regime of no recycling of water, chemical sterilization of influent seawater and decontaminating half of the tanks during each hatching cycle was successful in a barramundi hatchery. Nakai *et al*. (1995) recommended disinfection of eggs with iodine at 50 μg/ml for 10 min and utensils with chlorine at 50 μg/ml for 10 min, rearing of each batch of larvae/juveniles in separate tanks supplied with sterilized (u.v. at 1.0×10^5 μW/s.cm^2 or ozone at 0.1 μg/ml for 2.5 min) seawater and rigorous separation of larval and juvenile striped jack from broodfish.

Alternatively, the stocking density can be reduced to ≤10 larvae/l in 'green ponds' and thereby reduce transmission and disease to a negligible level (Anderson *et al*. 1993). This technique has been successful with barramundi, although the low levels of infection which sometimes occur produce regulatory problems when fish are shipped across regional borders.

The Future

It is apparent that nervous necrosis nodaviruses and their respective diseases are widespread in the Indo-Pacific region (Yoshikoshi & Inoue 1990; Mori *et al*. 1991, 1992; Muroga *et al*. 1994; Munday 1994). Such viruses have also been reported from the Mediterranean, France and Scandinavia (Breuil *et al*. 1991; Renault *et al*. 1991; Bloch *et al*. 1991; Grotmol *et al*. 1995). As the barramundi nodavirus has been introduced into China with infected larvae (Y. Zhang, personal communication, 1994), it is possible that other translocations have occurred and not been reported. If marine aquaculture continues to be conducted under existing philosophies in the future, nodavirus infections as well as other infectious diseases will further increase on both a geographic and a host species basis. Further detailed virological and molecular biological investigations among piscine nodaviruses will be required to understand these diseases. In particular, an urgent requirement is the comparison of infectivity among the viruses from different sources; something which should be facilitated by the recent success in culturing sea bass nodavirus (Frerichs *et al*. 1996). This system will be particularly advantageous for quantitative analysis of infectivity and the determination of epitopes with monoclonal antibodies, in addition to propagation of the piscine nodaviruses that have not yet been purified. In addition, the cell line will be a more useful tool than current methods, such as PCR, for epidemiological purposes including the identification of initial sources of infection for fish species other than striped jack, and the detection of infective virions in the environment. However, as the establishment of infection generally depends on a balance between the amount of invading agent and the defence mechanisms of the host, the most important prophylactic method is to reduce various stress factors on the fish; to ignore this basic precept is to court disaster.

References

Anderson, I., Barlow, C., Fielder, S., Hallam, D., Heasman, M. & Rimmer, M. 1993 Occurrence of the picorna-like virus infecting barramundi. *Austasia Aquaculture* **7**, 42–44.

Arimoto, M., Mushiake, K., Mizuta, Y., Nakai, T., Muroga K. & Furusawa, I. 1992 Detection of striped jack nervous necrosis virus (SJNNV) by enzyme-linked immunosorbent assay (ELISA). *Fish Pathology* **27**, 191–195.

Arimoto, M., Mori, K., Nakai, T., Muroga, K. & Furusawa, I. 1993 Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack, *Pseudocaranx dentex* (Bloch & Schneider). *Journal of Fish Diseases* **16**, 461–469.

Arimoto, M., Sato, J., Maruyama, K., Mimura, G. & Furusuwa, I. 1996 Effect of chemical and physical treatments on the inactivation of striped jack nervous necrosis virus (SJNNV). *Acquaculture* **143**, 15–22.

Bloch, B., Gravningen, K. & Larsen, J.L. 1991 Encephalomyelitis among turbot associated with a picornavirus-like agent. *Diseases of Aquatic Organisms* **10**, 65–70.

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J. & Hoffmann, R.W. 1996 Picorna-like virus associated with mortality and a spongionus encephalopathy in grouper *Epinephelus malabaricus*. *Diseases of Aquatic Organisms* **26**, 75–80.

Breuil, G., Bonami, J.R., Pepin, J.F. & Pichot, Y. 1991 Viral infection (picorna-like virus) associated with mass mortalities in hatchery-reared sea-bass (*Dicentrarchus labrax*) larvae and juveniles. *Aquaculture* **97**, 109–116.

Chua, F.H.C., Loo, J.J. & Wee, J.Y. 1995 Mass mortality in juvenile greasy grouper, *Epinephelus tauvina*, associated with vacuolating encephalopathy and retinopathy. In *Diseases in Asian Aquaculture II*, ed Schariff, M., Arthur, J.R. & Subasinghe, R.P. pp. 235–241. Manila: Fish Health Section, Asian Fisheries Society.

Comps, M. & Raymond, J.C. 1996 Virus-like particles in the retina of the sea-bream, *Sparus aurata*. *Bulletin of the European Association of Fish Pathologists* **16**, 151–153.

Comps, M., Pepin, J.F. & Bonami, J.R. 1994 Purification and characterization of two fish encephalitis viruses (FEV) infecting *Lates calcarifer* and *Dicentrarchus labrax*. *Aquaculture* **123**, 1–10.

Comps, M., Trindade, M. & Delsert, C. 1996 Investigation of fish encephalitis viruses (FEV) expression in marine fishes using DIG-labelled probes. *Aquaculture* **143**, 113–121.

Danayadol, Y., Direkbusarakom, S & Supamattaya, K. 1995 Viral nervous necrosis in brownspotted grouper, *Epinephelus malabaricus*, cultured in Thailand. In *Diseases in Asian Aquaculture II* ed Schariff, M., Arthur, J.R. & Subasinghe, R.P. pp. 227–233. Manila: Fish Health Section, Asian Fisheries Society.

Dasgupta, R., Ghosh, A., Dasmahapatra, B., Guarino, L.A. & Kaesberg, P. 1984 Primary and secondary structure of black beetle virus RNA2, the genomic messenger for BBV coat protein precursor. *Nucleic Acids Research* **12**, 7215–7223.

Dasgupta, R. & Sgro, J.-Y. 1989 Nucleotide sequences of three Nodavirus TRNA2's: the messengers for their coat protein precursors. *Nucleic Acids Research* **17**, 7525–7526.

Frerichs, G.N., Morgan, D., Hart, D., Skerrow, C., Roberts, R.J. & Onions, D.E. 1991 Spontaneously productive C-type retrovi-

rus infection of fish cell lines. *Journal of General Virology* **72**, 2537–2539.

Frerichs, G.N., Rodger, H.D. & Peric, Z. 1996 Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, *Dicentrarchus labrax*. *Journal of General Virology* **77**, 2067–2071.

Fukuda, Y., Nguyen, H.D., Furuhashi, M. & Nakai, T. 1996 Mass mortality of cultured sevenband grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. *Fish Pathology* **31**, 165–170.

Glazebrook, J.S. & Heasman, M.P. 1992 Diagnosis and control of picorna-like virus infection in larval barramundi, *Lates calcarifer* Bloch. In *Diseases in Asian Aquaculture I*, ed Schariff, I.M., Subasinghe, R.P. & Arthur, J.R. pp. 267–272. Manila: Fish Health Section, Asian Fisheries Society.

Glazebrook, J.S., Heasman, M.P. & de Beer, S.W. 1990 Picorna-like viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* (Bloch). *Journal of Fish Diseases* **13**, 245–249.

Grotmol, S., Totland, G.K., Kvellestad, A., Fjell, K. & Olsen, A.B. 1995 Mass mortality of larval and juvenile hatchery-reared halibut (*Hippoglossus hippoglossus L.*) associated with the presence of virus-like particles in the central nervous system and retina. *Bulletin of the European Association of Fish Pathologists* **15**, 176–180.

Jung, S.-J., Miyazaki, T., Miyata, M. & Oishi, T. 1996 Histopathological studies on viral nervous necrosis in a new host Japanese sea bass *Lateolabrax japonicus*. *Bulletin of the Faculty of Bioresources, Mie University* **16**, 9–16.

Kaesberg, P., Dasgupta, R., Sgro, J.-Y., Wery, J.-P., Selling, B.H., Hosur, M.V. & Johnson, J.E. 1990 Structural homology among four nodaviruses as deduced by sequencing and X-ray crystallography. *Journal of Molecular Biology* **214**, 423–435.

Mori, K., Nakai, T., Nagahara, M., Muroga, K., Mekuchi, T. & Kanno, T. 1991. A viral disease in hatchery-reared larvae and juveniles of redspotted grouper. *Fish Pathology* **26**, 209–210.

Mori, K., Nakai, T., Muroga, K., Arimoto, M., Mushiake, K. & Furusawa, I. 1992 Properties of a new virus belonging to Nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* **187**, 368–371.

Munday, B.L. 1994. Occurrence of the picorna-like virus infecting barramundi. *Austasia Aquaculture* **8**, 52.

Munday, B.L., Langdon, J.S., Hyatt, A. & Humphrey, J.D. 1992 Mass mortality associated with a viral-induced vacuolating encephalopathy and retinopathy of larval and juvenile barramundi, *Lates calcarifer* Bloch. *Aquaculture* **103**, 197–211.

Munday, B.L., Nakai, T. & Nguyen, H.D. 1994 Antigenic relationship of the picorna-like virus of the larval barramundi, *Lates calcarifer* Bloch to the nodavirus of larval striped jack, *Pseudocaranx dentex* (Bloch and Schneider). *Australian Veterinary Journal* **71**, 384.

Muroga, K. 1995 Viral and bacterial diseases in larval and juvenile marine fish and shellfish: a review. *Fish Pathology* **30**, 71–85.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds) 1995 Virus taxonomy. Sixth report of the international committee on taxonomy of viruses. *Archives of Virology* **10** (supplement). New York and Vienna: Springer-Verlag.

Mushiake, K., Arimoto, M., Furusawa, T., Furusawa, I., Nakai, T. & Muroga, K. 1992 Detection of antibodies against striped jack nervous necrosis virus (SJNNV) from brood stocks of striped jack. *Nippon Suisan Gakkaishi* **58**, 2351–2356.

Mushiake, K., Nishizawa, T., Nakai, T., Furusawa, I. & Muroga, K. 1994 Control of VNN in striped jack: Selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). *Fish Pathology* **29**, 177–182.

Nakai, T., Nguyen, H.D., Nishizawa, T., Muroga, K., Arimoto, M. & Ootsuki, K. 1994 Occurrence of viral nervous necrosis in kelp grouper and tiger puffer. *Fish Pathology* **29**, 211–212.

Nakai, T., Mori, K., Nishizawa, T. & Muroga, K. 1995 Viral nervous necrosis of larval and juvenile marine fish. *Proceedings of the International Symposium on Biotechnology Applications in Aquaculture*, Asian Fisheries Society Special Publication No. 10, pp. 147–152.

Nguyen, H.D., Mekuchi, T., Imura, K., Nakai, T., Nishizawa, T. & Muroga, K. 1994 Occurrence of viral nervous necrosis (VNN) in hatchery-reared juvenile Japanese flounder *Paralichthys olivaceus*. *Fisheries Science* **60**, 551–554.

Nguyen, H.D., Nakai, T. & Muroga, K. 1996. Progression of striped jack nervous necrosis virus (SJNNV) infection in naturally and experimentally infected striped jack *Pseudocaranx dentex* larvae. *Diseases of Aquatic Organisms* **24**, 99–105.

Nishizawa, T., Mori, K., Nakai, T., Furusawa, I. & Muroga, K. 1994 Polymerase chain reaction (PCR) amplification of RNA of striped jack nervous necrosis virus (SJNNV). *Diseases of Aquatic Organisms* **18**, 103–107.

Nishizawa, T., Kise, M., Nakai, T. & Muroga, K. 1995a Neutralizing monoclonal antibodies to striped jack nervous necrosis virus (SJNNV). *Fish Pathology* **30**, 111–114.

Nishizawa, T., Mori, K., Furuhashi, M., Nakai, T., Furusawa, I. & Muroga, K. 1995b Comparison of the coat protein genes of five fish nodaviruses, the causative agents of nervous necrosis in marine fish. *Journal of General Virology* **76**, 1563–1569.

Office International des Epizooties 1995 Diagnostic Manual for Aquatic Animal Diseases. Paris: O.I.E. pp. 85–90.

Oliver, R. 1990 Cited in acknowledgements by Glazebrook, J.S., Heasman, M.P. and de Beer, S.W. 1990. Picorna-like viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* (Bloch). *Journal of Fish Diseases* **13**: 245–249.

Renault, T., Haffner, P., Baudin Laurencin, F., Breuil, G. & Bonami, J.R. 1991 Mass mortalities in hatchery-reared sea bass (*Lates calcarifer*) larvae associated with the presence in the brain and retina of virus-like particles. *Bulletin of the European Association of Fish Pathologists* **11**, 68–73.

Yoshikoshi, K. & Inoue, K. 1990 Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathus fasciatus* (Temminck & Schlegel). *Journal of Fish Diseases* **13**, 69–77.