2.2.14 Viral Encephalopathy and Retinopathy

Stewart C. Johnson[1] and Diana Jaramillo[2]

[1.] Fisheries and Oceans Canada,
Pacific Biological Station
Nanaimo, British Columbia V9R 5K6
Canada

[2] Department of Health Management
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, Prince Edward Island C1A4P3
Canada
Stewart.Johnson@dfo-mpo.gc.ca; djaramillo@upei.ca

A. Name of Disease and Etiological Agent

Viral Encephalopathy and Retinopathy (VER) also known as Viral Nervous Necrosis (VNN) is a serious disease of marine and to a lesser extent freshwater fish. This disease is caused by species of virus belonging to the genus *Betanodavirus* within the family Nodaviridae. The International Committee on the Taxonomy of Virus currently recognizes 4 species of *Betanodavirus*: Striped Jack nervous necrosis virus (SJNNV) which is the type species, Barfin Flounder nervous necrosis virus (BFNNV), Red-spotted Grouper nervous necrosis virus (RGNNV) and Tiger Puffer nervous necrosis virus (TPNNV) http://www.ictvonline.org/virusTaxonomy.asp . For the purposes of this chapter we will follow this taxonomy and will refer to the species of *Betanodavirus* collectively as Nervous Necrosis Virus (NNV).

Betanodaviruses have non enveloped virions that are about 25-30 nm in diameter with icosahedral symmetry (Luo et al. 2014, Chen et al. 2015). The genome is made up of 2 molecules of positive sense single stranded RNA (ssRNA) (Mori et al. 1992, Liu et al. 2012). RNA1 encodes directly an RNA-dependent RNA polymerase and a subgenomic section of RNA1, which is referred to as RNA3 encodes the non-structural proteins B1 and B2 (Sommerset and Nerland 2004). The B2 protein which is only detected during periods of active viral replication, binds and protects double stranded RNA and through this process stops the host siRNA silencing response against the virus (Fenner et al. 2006, Fenner et al. 2006, Fenner et al. 2007, Mezeth et al. 2009). RNA2 encodes the capsid protein. The current classification of *Betanodavirus* as proposed by Nishizawa et al., (1997) is based on sequence analysis of a highly variable region of the RNA2.

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Betanodavirus species are found in wild and cultured fish across a broad geographical range having been isolated from and/or associated with outbreaks of VER in almost every continent (Table 1). Although the majority of these records are for cultured fish in the marine environment the presence of NNV in freshwater has been reported in Singapore, Taiwan, India, Australia, Italy, Greece and Spain. Although NNV or VER has not been reported to be present in South America, NNV genomic material has been detected by molecular means in healthy fish imported from the Amazon region (Gomez et al. 2006). The Fish Pathogen Database (http://www.fishpathogens.eu/website) is a recently developed source for molecular, geographical and epidemiological information for NNV (Mikkelsen et al. 2015).

Table 1: Geographic locations where NNV has been detected and/or diagnosed associated with mortalities in fish. Regions where NNV has been reported in both marine and freshwater are underlined.
[A] Includes a report for Réunion Island in the Indian Ocean.

Continent	Region	Reference
Asia	China	(Lin et al. 2001, Wang et al. 2006, Liu et al. 2012,
	Japan	(Yoshikoshi and Inoue 1990, Nguyen et al. 1994,
	Indonesia	(Pakingking Jr et al. 2009)
	Singapore	(Tan et al. 2001, Hegde et al. 2003, Hegde et al.
	South Korea	(Sohn and Park 1998, Oh et al. 2002, Cha et al. 2007,
	Taiwan	(Chi et al. 1997, Chi et al. 2003, Kuo et al. 2012)
	Philippines	(Maeno et al. 2004, de la Pena et al. 2011)
	India	(Azad et al. 2005, Parameswaran et al. 2008, Binesh
	Thailand	(Starkey et al. 2004, Nopadon et al. 2009,
	Malaysia	(Ransangan and Manin 2010, Ransangan et al. 2011)
	Iran	(Nazari et al. 2014)
	Israel	(Ucko et al. 2004)
Oceania	Australia	(Glazebrook et al. 1990, Munday and Owens 1998,
	French Polynesia	(Renault et al. 1991, Thiéry et al. 2004, David et al.
Europe	France[A]	(Bigarré et al. 2009, Mikkelsen et al. 2015)
	Portugal	(Cutrín et al. 2007, Mikkelsen et al. 2015)
	Italy	(Bovo et al. 1999, Starkey et al. 2004, Toffolo et al.
	Greece	(Le Breton et al. 1997, Athanassopoulou et al. 2003,
	Cyprus	(Mikkelsen et al. 2015)
	Spain	(Starkey et al. 2004, Thiéry et al. 2004, Hodneland et
	Malta	(Skliris et al. 2001, Starkey et al. 2004)
	Norway	(Grotmol et al. 1997, Dannevig et al. 2000, Johansen
	United Kingdom	(Starkey et al. 2000, Starkey et al. 2001, Starkey et
North America and The Caribbean	Canada	(Barker et al. 2002, Johnson et al. 2002, Gagné et al.
	U.S.A	(Curtis et al. 2001)
	Martinique	(Breuil et al. 1991)
Africa	Tunisia	(Chérif et al. 2009, Haddad-Boubaker et al. 2013,

2. Host Species

NNV is recognized for its lack of host specificity (Renault et al. 1991, Thiéry et al. 2004). The majority of the susceptible hosts are marine fish, but susceptible species have also been reported from brackish and freshwater habitats. Since the first reports of VER, the number of susceptible species has increased over the years so that to date, NNV has been reported from more than 60 species of fish from 35 families and 11 orders of cultured fish (Appendix 1). NNV detection is a common occurrence in wild caught fish with large numbers of species identified as carriers in marine surveys conducted in the Adriatic Sea (Ciulli et al. 2007), Canada (Barker et al. 2002), China (Liu et al. 2015, Ma et al. 2015), Italy (Giacopello et al. 2013), Japan (Sakamoto et al. 2008), Korea (Baeck et al. 2007, Kim et al. 2007, Gomez et al. 2008), Norway (Nylund et al. 2008), Spain (Moreno et al. 2014) and the Philippines (de la Pena et al. 2011).

NNV isolates are considered not to be host specific as isolates have been found to be pathogenic across wide ranges of unrelated fish species despite these species having large phylogenetic distances between them and dissimilar environmental niches (Skliris and Richards 1999, Sommer et al. 2004, Bigarré et al. 2009). There are a number of species which are considered resistant to infection and disease. These include the: Japanese Amberjack (*Seriola quinqueradiata)*, Yellowtail Amberjack (*Seriola lalandi),* Red Seabream (*Pagrus major*), Rabbitfish (*Siganus guttatus*), Goldfinned Barb (*Puntius sachsii*), Bronze Coridora (*Corydoras aeneus*), Sailfin Catfish (*Liposarcus multiradiatusand*) and Neon Tetra (*Paracheirodon innesi*) (Arimoto et al. 1993, Furusawa et al. 2007, Maeno et al. 2007). However, NNV resistance should be taken with prudence as naturally occurring outbreaks of VER have been reported in species that were initially considered to be resistant to NNV infection by experimental challenge (Furusawa et al. 2007, Binesh 2013).

C. Epizootiology

1. NNV Genotypic and phenotypic variants

Although a large number of *Betanodavirus* species have been proposed, there are presently only 4 recognized species based on phylogenetic analysis of the variable T4 region. In addition, genetic analysis of the RNA1 and RNA2 has identified the occurrence of reassortant variants that contain nucleic acids from SJNNV and RGNNV and which were likely produced in cells co-infected with these viruses (Olveira et al. 2009, Souto et al. 2015). At this time there is relatively little known about host specificity and pathogenicity of different NNV species and reassortant mutants (Breuil et al. 2001, Toffolo et al. 2007, Olveira et al. 2009). Recently, Vendramin et al., (2014) compared the pathogenicity of 10 strains of NNV in European Sea-bass (*Dicentrarchus labrax*) at temperatures of 20, 23 and 25^{o} C. These strains were isolated from a variety of hosts and host environments and included RGNNV and SJNNV and reassortant variants of these species. Despite significant differences in virulence, as measured by morbidity and sublethal affects such as reduced growth and condition, among strains no patterns were evidenced that could suggest a relationship between genotype and virulence.

2. Susceptible host stages

Outbreaks of disease are most commonly reported in larval and juvenile stages; however outbreaks also occur in market-sized fish of some species such as European Sea-bass, and grouper *Epinephelus sp.* (Fukuda et al. 1996, Le Breton et al. 1997).

3. NNV Transmission

As NNV infects a wide number of species spawned and reared under a wide variety of conditions and health management practices, it is difficult to generalize about the relative importance of routes of transmission of this virus.

Vertical Transmission: Outbreaks of clinical disease in early larval stages and the presence of NNV genetic material within mature gonads have led to the general view that NNV can be transmitted vertically. However, the evidence for vertical transmission is limited and sometimes contradictory. Evidence supporting the occurrence of vertical transmission includes: 1) reports of successful use of ozonation of eggs to prevent the disease (Mori et al. 1998, Grotmol and Totland 2000, Watanabe et al. 2013), however egg disinfection is not always effective (Buchan et al. 2006, Kuo et al. 2012); 2) an association between antibody levels in broodstock and disease occurrence at individual broodstock-larval level (Mushiake et al. 1994), although the association does not always hold true (Mushiake 1993); and 3) the identification of NNV genetic material in eggs and larvae from IP challenge broodstock (Breuil et al. 2002), in this case the possibility of contamination of gonads cannot be ruled out . In addition, Kai et al. (2010) demonstrated that vaccination of Orange-spotted Groupers (*Epinephelus coioides*) resulted in undetectable levels of NNV in eggs from vaccinated fish when compared to those from unvaccinated fish and proposed vaccination as a potential way to reduce the risk of vertical transmission of NNV broodfish subjected to stresses associated with repeated spawning. However this study was limited by a small sample size (n=3) and the conclusions were not supported by statistical analysis. Thus, further epidemiological research is needed to establish the importance of vertical transmission in NNV.

Horizontal Transmission: Horizontal transmission of NNV is widely accepted. Epidemiological studies on VER outbreaks have pointed at the spread of the virus via water through the introduction of an infected or contaminated population (Le Breton et al. 1997) or via water from an environmental source (Curtis et al. 2001, Hick et al. 2011). Horizontal transmission of NNV and the subsequent development of disease have also been demonstrated by experimental infections using immersion and cohabitation challenges in a large number of species. The portal of viral entry and dynamics of NNV shedding are important questions that require further study.

Contamination of Feedstock: Feeding of raw fish or fish meal that hasn't been adequately processed is another route by which NNV is thought to be transmitted (Kim et al. 2007, de la Pena et al. 2011). However, it is not known whether NNV transmission occurs through ingestion or water borne exposure to NNV released from infected food, or both. Juvenile European Sea-bass have been shown to be susceptible to NNV infection via the oral route under laboratory conditions (Peducasse et al. 1999).

Carrier States: Betanodaviruses have been identified to be present at low concentrations in fish showing no obvious signs of VER under cultured and wild conditions (Johansen et al. 2002, Munday et al. 2002, Johansen et al. 2004, Rise et al. 2010, Hodneland et al. 2011, Giacopello et al. 2013, Olveira et al. 2013, Ma et al. 2015). In some species such as the Gilthead Seabream (*Sparus aurata*) which is an asymptomatic carrier of NNV and resistant to the development VER, this virus can replicate and persist for up to 3 months in the central nervous system without any gross or histological signs of neurological damage (Castric et al. 2001, Lopez-Munoz et al. 2012). Thus, the presence of NNV carrier states within broodstock populations is thought as a significant challenge with respect to VER management in many species (Breuil et al. 2000).

4. Environmental and Husbandry Factors:

A number of environmental and husbandry-related factors have been associated with clinical disease in cultured fish populations and increased risk of spreading NNV to offspring. Binesh (2014) examined the effects of elevated temperature and crowding on the development of acute disease in Zebrafish (*Brachydanio rerio*) that were asymptomatic carriers of NNV. Elevation of temperature and crowding were significantly related to cumulative mortality due to VER. In a more recent study, juvenile Senegalese Sole (*Solea senegalensis*) were infected by bath challenge and co-habitation with a reassortant Betanodavirus strain and reared at 22, 18 or 16^{o} C (Souto et al. 2015). These authors demonstrated that NNV could be transmitted at all of these temperatures and that virulence decreased with decreasing temperatures with 100%, 75-80% and 8% mortality reported at 22, 18 and 16^{o} C, respectively. Fish held at 16^{o} C remained infected but showed little signs of disease, however, increasing water temperature from 16 to 22^{o} C resulted in a rapid increase in NNV load and the development of clinical disease. Elevated temperature was also shown to play a role in the development of disease in European Eels (*Anguilla anguilla*) (Chi et al. 2003). There is also evidence that stress associated with the induction of spawning and repeated spawning is related to higher risk of VER outbreaks in larval fish (Mushiake et al. 1994). Reduced feeding resulting in low growth was associated with higher levels of NNV in asymptomatic Atlantic Halibut (*Hippoglossus hippoglossus*) (Johansen et al. 2004).

D. Disease Signs

1. Behavioral Changes Associated with the Disease

Marine and Freshwater fish infected with NNV have been reported to show: erratic swimming behavior, hypersensitivity to stimuli, lethargy, and loss of appetite during disease outbreaks (Arimoto et al. 1994, Le Breton et al. 1997, Munday and Nakai 1997, Oh et al. 2002, Maeno et al. 2004, Bovo et al. 2011, Binesh et al. 2013). NNV subclinical carriers may display no behavioral changes associated with infection even in the presence of moderate to severe pathological changes in the eyes and central nervous system (Gjessing et al. 2009).

2. External Gross Signs

In some disease outbreaks darkening of body coloration has been reported. With exception of spinal deformities, no other external signs have been associated with NNV infection (Oh et al. 2002).

3. Internal Gross Signs

Internal lesions are rarely described for NNV infection. However, inflation of the swim bladder, which is not a specific sign of VER, and hyperemia around the cranium have been described with infection (Husgaro et al. 2001, Oh et al. 2002).

4. Histopathological Changes Associated with the Disease

In cases of clinical disease histopathological changes are generally confined to tissues of the nervous system and eye. Vacuolization and necrosis of the brain, spinal cord and/or retina are the most common histopathological findings reported in clinically infected fish from naturally occurring outbreaks (Figure 1) (Grotmol et al. 1997, Johansen et al. 2003, Bigarré et al. 2009, Nopadon et al. 2009). The same pathological changes are generally reported in fish that have

been experimentally infected with NNV by various routes. However, in the case of experimentally challenged larval Atlantic Halibut, lesions were reported to become more severe and widespread being found in the intestine, liver, olfactory epithelium, yolk-sac epithelium, gills and pectoral fins, as well as in the retina and central nervous system at later stages of infection (Grotmol et al. 1999).

Fish that are subclinical carriers of NNV, including survivors of disease outbreaks, may show different histopathological lesions including cell degeneration and reactive changes such as hypercellularity, and proliferation or hypertrophy of glial cells (Johansen et al. 2002, Johansen et al. 2004, Gjessing et al. 2009).
NNV has been found in association with lesions and neuronal tissue in the retina, brain and spinal cord in both diseased and apparently healthy cells by immunohistochemisty (Hellberg et al. 2010).

5. NNV Tissue Tropism

In fish undergoing clinical disease or in those that are asymptomatic carriers NNV genomic material and antigens are generally reported within tissues of the central nervous system and eyes. However, it is important to remember that these are the tissues which show histopathological signs of VER and are therefore the tissues that are most commonly examined. With the use of molecular diagnostics testing it has become possible to identify NNV genetic material in a variety of other tissues in clinically diseased and carrier fish. Intraperitoneal and intramuscular injection challenges have been used to examine tissue tropism in Atlantic Cod (*Gadus morhua*), Atlantic Halibut, European Seabass and Pompano (*Trachinotus ovatus*) over various timeframes post infection (Grove et al. 2003, Korsnes et al. 2009, Lopez-Jimena et al. 2011, Su et al. 2015). Pompano samples were obtained from 4 to 96 hours post-infection (hpi) from an infection that resulted in the onset of clinical disease at 72 hpi (Su et al. 2015). Betanodavirus genomic material was found in all of the tissues tested, with brain tissue having the highest load and liver and intestine only testing positive at the last time point (96 hpi). Atlantic Cod were examined at 25, 130 and 180 days post-infection (dpi) in an experimental challenge that resulted in no morbidity. At 25 dpi NNV was found primarily in the heart, spleen, liver and kidney with only 3/5 and 1/5 individuals testing positive in the eye and brain, respectively (Korsnes et al. 2009). At the later time points NNV levels remained relatively constant in the spleen, liver, and kidney but increased in brain and eye. Intraperitoneal challenge (IP) of juvenile Atlantic Halibut resulted in increasing numbers of fish testing positive for NNV in the brain/eye and liver over time (5 -41 dpi) with the first detection at 13 dpi (Grove et al. 2003). Kidney and intestine tested positive at all-time points, whereas heart tissue was variable and gills never tested positive. To our knowledge there is only 1 report of NNV tissue tropism in naturally infected fish. Chi et al. (2001) using in situ hybridization for the coat protein gene reported the detection of NNV in the brain and retina, gill, skeletal muscle, liver, pyloric gland, intestine and blood cells in the heart of moribund grouper larvae.

E. Disease Diagnostic Procedures

Detailed diagnostic procedures for the detection of NNV are given in Chapter 2.3.11 of the OIE Manual of Diagnostic Tests for Aquatic Animals 2015 http://www.oie.int/international-standard-setting/aquatic-manual/access-online/ . Please note that VER is no longer listed by the OIE and not all tests recommended in the manual have been validated. Readers are encouraged to consult the OIE Manual (2015) for details on the diagnostic procedures described below.

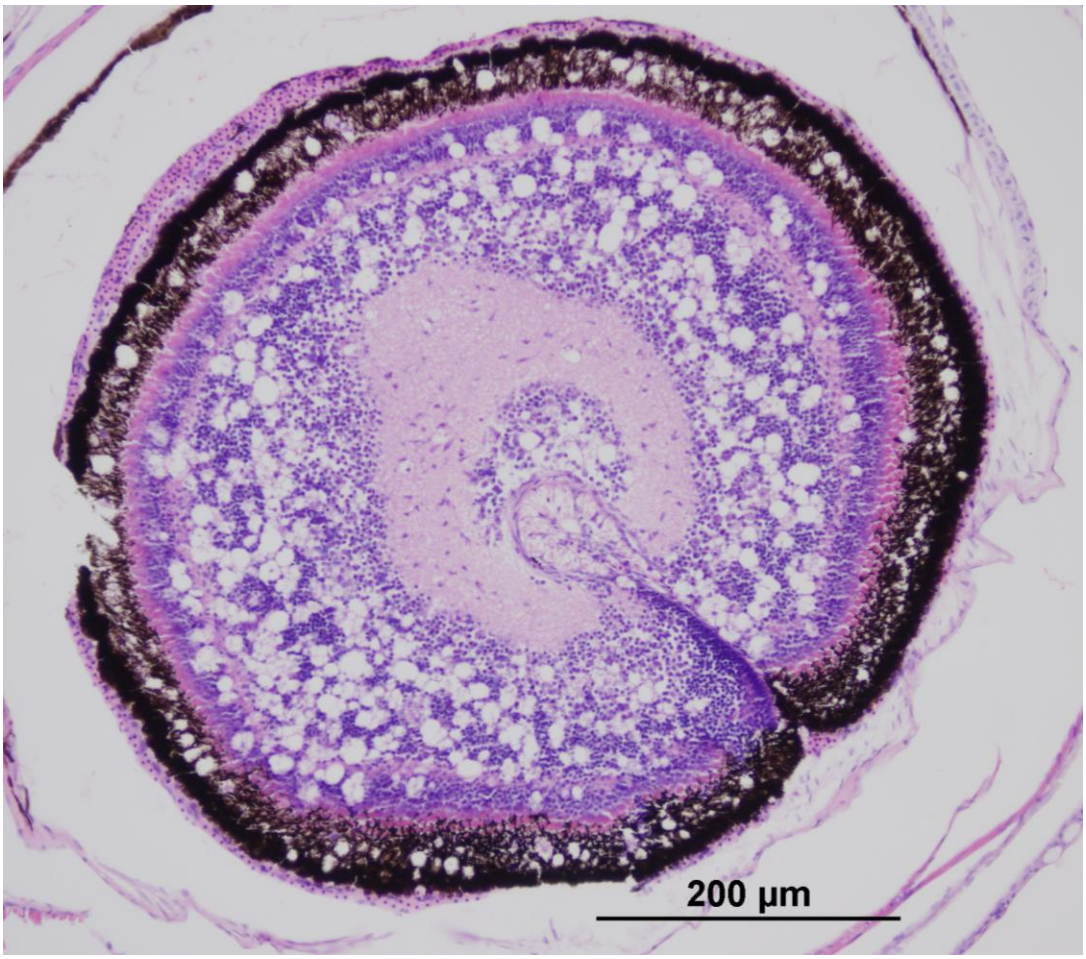

Figure 1: Histopathological changes in retina of severely infected Australian Bass *Macquaria novemaculeata*. All layers of the retina display generalized vacuolation (empty round spaces) and signs of necrosis including pyknosis and karyorrhexis.

1. Presumptive Diagnosis

There are no external or internal gross signs that are specific for this disease; however, behavioral and histopathological changes can be used to support a presumptive diagnosis of VER. Lack of behavioral changes and/or histopathological signs of VER should not be taken as an indication that the fish are free of NNV. Diagnostic testing using cell culture, indirect florescent antibody tests (IFAT), molecular assays or serological tests is required to confirm the presence of NNV.

2. Confirmatory Diagnosis

Virus isolation in cell culture is the only *in vitro* technique that allows the confirmation of the infectivity of viruses and therefore it is considered by many to be the "gold standard" for NNV diagnosis. Because of its high permissivity to all known NNV species: BFNNV, RGNNV, TPNNV and SJNNV, the SNN-1 cell line (ECACC 96082808) is widely used for NNV isolation (Frerichs et al. 1996, Iwamoto et al. 1999). It consists of a mixed cell population which is persistently infected with a C-type endogenous retrovirus SnRV (Frerichs et al. 1991). Concern that the presence of the mixed cell population could impair the performance of the SNN-1 for NNV diagnosis led to the development of the clone cell line E-11 (ECACC 01110916) (Iwamoto

et al. 2000, Bandín et al. 2014). According to the OIE both SSN-1 and E-11 cell lines are suitable for culture of all betanodaviruses (OIE 2015).
More details on these cell lines and the conditions for their maintenance can be found at the European collection of Authenticated cell lines website:
http://www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=96082808&collection=ecacc_gc and
http://www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=01110916&collection=ecacc_gc .
Other NNV susceptible cell lines include SAF-1 cell line(Bandin et al. 2006)(ECACC 00122301), free from SnRV :
http://www.phe-culturecollections.org.uk/products/celllines/generalcell/detail.jsp?refId=00122301&collection=ecacc_gc
and GF-1 (grouper fin: ATCC PTA-859)(Chi et al. 1999, Lai et al. 2003, Qin et al. 2006). More information about this cell line can be found at the USPTO patent database website:
http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=6,436,702.PN.&OS=PN/6,436,702&RS=PN/6,436,702 .

The OIE manual provides a detailed description of cell culture methods and optimal temperatures for viral growth: 25-30^{o}C for RGNNV, 20-25^{o}C for SJNNV, 20^{o}C for TPNNV and 15-20^{o}C for BFNNV (OIE 2015). An example of extensive and methodic optimization of RGNNV culture and numeration in SSN-1 can be found in the publication by Hick et al., (2011).

Cell cultures (inoculated and positive controls) are usually monitored for cytopathic effects (CPE) for a period of 10 days (OIE 2015) (Figure 2). In cases where CPE is not detected in the first round of culture (10 days) the OIE recommends sub-cultivation on fresh cells or passages. To confirm that NNV is the causative agent of the CPE, an additional identification step is needed using alternative testing by IFAT, conventional PCR or Real Time PCR (SYBR green or probe-based) can be used. These techniques can also be used to identify NNV proteins or nucleic acids directly from tissues and histological sections (described below).

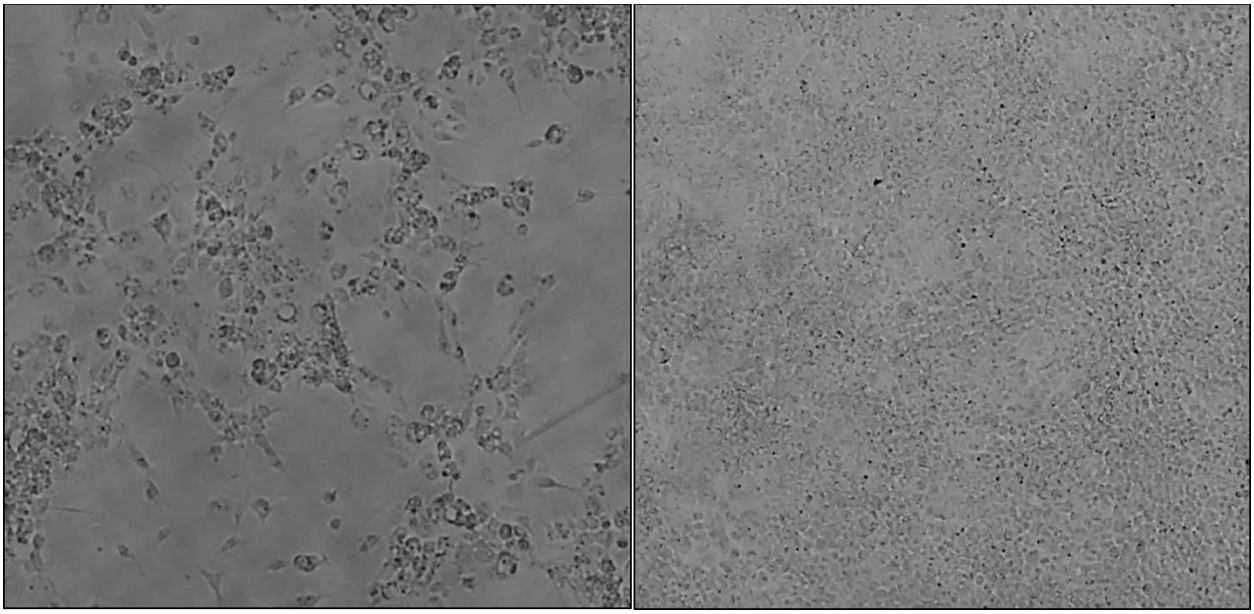

Figure 2: A) Typical CPE of NNV on SSN-1 cell culture showing round intra-cellular vacuoles, shrinkage (cells look round and dark) and detachment (clear spaces) B) non-infected SSN-1 cells

Indirect florescent antibody tests:

Indirect florescent antibody tests (IFAT) can be used to identify the presence of NNV antigens in nervous tissue imprints, histological sections and cell culture (OIE 2015). Commercially produced antibodies are available for a number of betanodavirus; however, prior optimization validation is recommended for the recognition of each NNV species or strain/s of interest. Procedures for conducting IFAT are described in detail in the OIE manual (2015). These tests are not fully validated therefore results should be interpreted with caution.

Nucleic Acid Based Detection Methods:

Molecular detection of NNV RNA serves as a rapid and sensitive screening method that facilitates higher laboratory throughput. At present most molecular diagnostic testing for NNV uses Real Time Quantitative PCR (RT-qPCR) as this method has been demonstrated to have better analytical performance than the conventional or nested PCR methods that were initially developed (Hick and Whittington 2010) and even higher analytical sensitivity than cell culture (Dalla Valle et al. 2000, Grove et al. 2006). Testing procedures using conventional or nested PCR are described in detail in the OIE Manual and will not be described here (OIE 2015).

There are two types of chemistries used to detect PCR products using real-time PCR instruments these are: SYBR®-Green Based Detection and TaqMan®-Based Detection. Regardless of the chemistry which is used the most common diagnostic protocols for NNV use a two-step real time PCR method that involves a separate first strand cDNA synthesis (reverse transcription reaction (RT)) and then the adding of an aliquot of the resulting cDNA to the PCR reaction. The two-step method allows for the optimization of both the RT and real-time PCR steps which can make this method more sensitive and a better choice when developing diagnostic tests for new isolates/strains. An important feature of the two-step method is that it generates a cDNA pool that is suitable for storage should additional testing be required.

There are numerous published RT-q PCR tests for different species and strains of NNV. The majority of these tests use primers and probes which, due to a large amount of genetic diversity within species and strains of NNV, do not amplify all species and in some cases not all strains within species. Dalla Valle et al. (2000) developed a SYBR-green assay using primers designed against RNA1 and RNA2 capable of detecting all 4 species of NNV. Hicks and Whittington (2010) developed and validated a TaqMan RT-qPCR assay which is reported to identify all 4 species of NNV, but with different sensitivities in tissue and cell culture. The most thoroughly validated test is a TaqMan RT-qPCR designed to identify all 4 species of NNV, as well as the genetically distinct isolates from Atlantic cod and Atlantic halibut, in a variety of sample matrices (Panzarin et al. 2010). Taken together these 3 diagnostic procedures provide a good starting point for those wishing to apply molecular methods for the detection of NNV. Step by step details of the test method of Panzarin et al. (Panzarin et al. 2010) are provided in the OIE manual (2015).

More recently Baud et al. (Baud et al. 2015) reported the development of a one-step TaqMan RT-qPCR assay targeting the RNA2 region of the genome. This assay was tested against a variety of isolates belonging to all 4 species of NNV in tissue and cell culture samples. The biggest difference of this assay is the use of a one-step PCR method. This method is faster, simpler, reduces the risk of contamination and can potentially be more sensitive, though potential drawbacks include lack of individual control of reaction conditions for the RT and PCR, NNV primers and probe are present during the RT reaction risking formation of primer-dimers, and the entire cDNA pool is used in the reaction and therefore not available for additional testing. The one-step PCR method can be applied to any of the above mentioned procedures.

Isothermal methods for detection of Betanodaviruses include loop-mediated isothermal amplification (LAMP) (Sung and Lu 2009, Xu et al. 2010, Wang et al. 2011, Suebsing et al. 2012, Mekata et al. 2015, Su et al. 2015, Hwang et al. 2016), nucleic acid sequence based amplification (NASBA) (Starkey et al. 2004) and cross-priming isothermal amplification (CPA) (Su et al. 2015). It is widely reported that these isothermal amplification methods for the detection of NNV are highly sensitive, up to 100 times more sensitive than RT-PCR or nested RT-PCR (Starkey et al. 2004, Xu et al. 2010, Wang et al. 2011, Suebsing et al. 2012, Mekata et al. 2015, Su et al. 2015, Hwang et al. 2016) and equivalent to RT-qPCR methods (Su et al. 2015). However, the specificity of these methods is unknown as formal validation has not been conducted to date.

All of the molecular tests described above do not distinguish viable from non-viable NNV. Detected segments could consist of RNA from replication intermediates, or fragments from viruses that are no longer viable or infectious. For these reasons positive molecular results especially those that are not confirmed by pathological/clinical signs or other test methods, such as cell culture, should be interpreted with caution.

F. Procedures for Detecting Subclinical Infections

As mentioned previously, Betanodaviruses are often found in low abundance in fish showing no obvious clinical or histopathological signs of VER (Johansen et al. 2002, Munday et al. 2002, Johansen et al. 2004, Rise et al. 2010, Hodneland et al. 2011, Giacopello et al. 2013, Olveira et al. 2013, Ma et al. 2015). Some species of fish can be asymptomatic carriers of NNV, supporting the replication and persistence of the virus, and serve as reservoirs of infection (Castric et al. 2001, Lopez-Munoz et al. 2012). Due to the tissue tropism of NNV, tissues from the CNS and the eyes from juvenile and adult fish, and whole bodies of larval fish, are the tissues of choice for diagnostic testing. Identification of NNV carriers is especially problematic in populations, such as broodstock, where lethal sampling is not possible. In such situations other tissues (eggs, ovarian and seminal fluids, gonadal biopsies, fin tissue etc.) can be used. However it is important to remember that these tissues often test negative in carrier fish, meaning that only positive results from these tissues should be view as conclusive.

Cell culture and molecular methods can both be used for the detection of subclinical infections keeping in mind limitations and relative sensitivity of these methods as previously discussed. More important for detection of subclinical infections is the collection of appropriate numbers and types of samples for analysis. Large sample sizes from suspect populations, multiple tissue samples from each fish, and replicate testing of individuals will increase the chance of identifying subclinical infections.

G. Procedures for Determining Prior Exposure to the Etiological Agent

Although serological methods cannot be used to assess NNV status of fish, they are valuable in determining whether fish have been previously exposed to NNV or vaccinated against NNV.

ELISA protocols for the detection of anti-NNV antibodies have been described (Breuil et al. 2000, Watanabe et al. 2000, Jaramillo et al. 2016, Jaramillo et al. 2016). Recent validation efforts of an antibody based ELISA for NNV shows that serological methods can be relatively accurate at detecting previous exposure to NNV, with a 81.8% sensitivity and 86.7% specificity performance in subclinical populations (Jaramillo et al. 2016). There are a variety of commercially available NNV and anti-fish Ig antibodies on the market, many of which cross react

between species, suggesting that these ELISA protocols may be adaptable for a wide range of species.

Information on how long NNV antibodies persist following exposure (or vaccination) in different host species under different culture conditions and whether carriers of NNV retain their antibody titers would further improve the interpretation of serological tests results.

H. Procedures for Transport and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

Cell Culture: Although NNV can remain viable in saline water at 15°C for over to 6 months (Frerichs et al. 2000), changes in the viability of NNV in tissue following the death of the host and/or long term frozen storage have not been determined. For this reason tissues should be kept on ice or refrigerated and processed as quickly as possible into cell culture, preferably within 48 hours. It is possible to isolate viable NNV from tissues that have been stored at -80°C, however it is expected that virus titer will decrease with time.

Molecular Testing: To ensure NNV RNA integrity, tissues for molecular testing should be either frozen immediately after collection (-80°C) or placed into a suitable RNA stabilizing reagent, such as RNA*late*®*r* (Ambion®) or AVL buffer (Qiagen®). It is important to follow the manufacturers' instructions with respect to tissue size, tissue/reagent ratios and storage conditions to ensure that the viral RNA is adequately protected.

Tissues that have been stored frozen for brief periods of time can still be used for diagnostic testing. However it should be expected that some degradation of viral RNA will occur which may have an effect on test performance.

The use of endogenous controls "reference gene assay", to determine the quality of the original tissue with respect to RNA degradation is considered a desirable practice (Sepulveda et al. 2013). This procedure provides an indication of the level of decomposition of the fish sample by comparing it to a well preserved sample. However, the procedure makes the assumption that host RNA degrades at a similar rate to pathogen RNA which may not be the case for all viruses.

It is also possible to conduct molecular tests on fixed and/or paraffin embedded samples and commercially available kits for total RNA extraction are available. Only positive results should be considered conclusive and it is important to remember that contamination of tissues during histological processing may occur. Negative results from such tissues should also be interpreted cautiously as these processes can have significant effects on RNA integrity.

Appendix 1: NNV- susceptible species.

Family	Species	Common name	Habitat	Reference
Acipenseridae	*Acipenser sp.*	Sturgeon	Freshwater/ Anadromous	(Athanassopoulou et al. 2004)
	Acipenser gueldestaedtii.	Russian Sturgeon	Freshwater/ Anadromous	(Xylouri et al. 2007)
Ephippidae	*Platax orbicularis*	Orbicular Batfish	Marine	(David et al. 2010)
Scaridae	*Calatomus japonicas*	Japanese Parrotfish	Marine	(Yoshikoshi and Inoue 1990, Muroga 2001)
Scombridae	*Thunnus oleintalis*	Pacific Bluefin Tuna	Marine	(Nishioka et al. 2010)
Serranidae	*Epinephelus akaara*	Red-spotted Grouper /Hong Kong Grouper	Marine	(Chi et al. 1997, Lin et al. 2001)
	Epinephelus awoara	Yellow Grouper	Marine	(Lai et al. 2001)
	Epinephelus costae	Golden Grouper	Marine	(Vendramin et al. 2013)
	Epinephelus fuscoguttatus	Brown-marbled Grouper, Flowery Cod	Marine	(Chi et al. 1997)
	Epinephelus coioides	Orange-spotted Grouper	Marine	(Lin et al. 2001, Ma et al. 2012, Wu et al. 2012)
	Epinephelus lanceolatus	Giant Grouper, Dragon Grouper	Marine	(Liu et al. 2005)
	Epinephelus marginatus	Dusky Grouper	Marine	(Vendramin et al. 2013)
	Plectropomus maculatus	Spotted coral Grouper	Marine	(Nopadon et al. 2009)
	Cromileptes altivelis	Barramundi Cod	Marine	(Moody et al. 2009)
	Epinephelus aeneus	White Grouper,	Marine	(Ucko et al. 2004)
	Epinephelus malabaricus	Malabar Grouper	Marine	(Boonyaratpalin et al. 1996, Starkey et al. 2004)
	Epinephelus stemphasciatus	Seven- band Grouper	Marine	(Fukuda et al. 1996, Sohn and Park 1998, Tanaka et al. 1998, Tanaka et al. 2004)
	Epinephelus moara	Kelp Grouper	Marine	(Nishizawa et al. 1997)
	Cromileptes altivelis	Humpback Grouper	Marine	(Chi et al. 2003)
	Epinephelus tauvina	Greasy Grouper	Marine	(Tan et al. 2001)
Moronidae	*Dicentrarchus labrax*	European Sea-bass	Marine/ euryhaline	(Breuil et al. 1991, LeBreton et al. 1997, Thiery et al. 1999, Starkey et al. 2004, Chérif et al. 2009, Hodneland et al. 2011, Haddad-Boubaker et al. 2013, Vendramin et al. 2013)
	Morone saxatilis X Morone chrysops	Hybrid Striped x White Bass	Freshwater	(Bovo et al. 2011)

Latidae	*Lates calcarifer*	Barramundi (Asian Seabass)	Diadromous	(Munday et al. 1992, Chi et al. 2003, Maeno et al. 2004, Azad et al. 2005, Parameswaran et al. 2008, Moody et al. 2009, Hick et al. 2011)
Lateolabracidae	*Lateolabrax japonicus*	Japanese Sea-bass	Diadromous	(Nishizawa et al. 1997)
Latridae	*Latris lineata*	Striped Trumpeter	Marine	(Moody et al. 2009)
Sparidae	*Sparus aurata*	Seabream	Marine	(Castric et al. 2001, Thiéry et al. 2004, Bitchava et al. 2007, Chérif et al. 2009, Haddad-Boubaker et al. 2013)
	Pagrus major	Red Seabream	Marine	(Nishizawa et al. 1997)
	Diplodus sargus	White Bream	Marine	(Toffolo et al. 2007)
Carangidae	*Pseudocaranx dentex*	White Trevally, Striped Jack	Marine	(Mori et al. 1992, Nakajima et al. 1998, Muroga 2001, Starkey et al. 2004)
	Trachinotus blochii	Snubnose Pompano, Golden Pompano	Marine	(Ransangan et al. 2011)
	Trachinotus ovatus	Pompano	Marine	(Wang et al. 2006)
	Trachinotus falcatus	Yellow-wax Pompano , Permitfish	Marine	(Chi et al. 2003)
	Seriola dumerili	Purplish Amberjack	Marine	(Nishizawa et al. 1997)
Oplegnathidae	*Oplegnathus fasciatus*	Rock Bream, Knifejaw	Marine	(Cha et al. 2007)
	Oplegnathus punctatus	Spotted Knifejaw	Marine	(Starkey et al. 2004)
	Lutjanus erythropterus	Firespot Snapper	Marine	(Chi et al. 2003)
Lutjanidae	*Sciaenops ocellatus*	Red Drum	Marine	(Oh et al. 2002, Ucko et al. 2004, Oh et al. 2005)
Sciaenidae	*Atractoscion nobilis*	White Sea-bass,	Marine	(Curtis et al. 2001)
	Umbrina cirrosa	Shi Drum	Marine	(Katharios and Tsigenopoulos 2010)
Percichthyidae	*Argyrosomus regius*	Meagre	Marine	(Thiéry et al. 2004)
	Macquaria novemaculeata	Australian Bass	Diadromous	(Moody et al. 2009, Jaramillo et al. 2016)
Cichlidae	*Oreochromis niloticus*	Tilapia	Freshwater-euryhaline	(Bigarré et al. 2009, Keawcharoen et al. 2015)
Acanthuridae	*Acanthurus triostegus*	Convict Surgeonfish,	Marine	(Thiéry et al. 2004)
Apogonidae	*Apogon exostigma/ Pristiapogon exostigma*	Narrow-stripe Cardinal	Marine	(Thiéry et al. 2004)
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	Freshwater	(Bovo et al. 2011)
Rachycentridae	*Rachycentron canadum*	Cobia	Marine	(Chi et al. 2003)
Elotridae	*Oxyeleotris lineolata*	Sleepy Cod	Freshwater	(Moody et al. 2009)
Pomacentridae	*Amphiprion sebae*	Sebae Clownfish	Marine	(Binesh et al. 2013)
Mugilidae	*Liza aurata*	Golden-grey Mullet	Catadromous	(Nazari et al. 2014)
	Mugil cephalus	Grey Mullet	Diadromous	(Cha et al. 2007, Toffolo et al. 2007)
Paralichthyidae	*Paralichthys olivaceus*	Japanese Flounder	Marine	(Nguyen et al. 1994, Nishizawa et al. 1997, Suzuki 2006)

Soleidae	*Solea senegalensis*	Senegalese Sole	Marine	(Thiéry et al. 2004)
Scophtalmidae	*Scophthalmus maximus*	Turbot	Marine	(Bloch 1991, Olveira et al. 2013)
Pleuronectidae	*Hippoglossus hippoglossus*	Atlantic Halibut	Marine	(Totland et al. 1999, Dannevig et al. 2000, Johansen et al. 2004, Nylund et al. 2008)
	Pleuronectes americanus	Winter Flounder	Marine	(Barker et al. 2002)
	Verasper moseri	Barfin Flounder	Marine	(Nishizawa et al. 1997, Suzuki 2006)
Sebastidae	*Sebastes oblongus*	Oblong Rockfish	Marine	(Oh et al. 2005)
Siluridae	*Silurus asotus*	Anur Catfish, Japanese Catfish, Chinese Catfish	Freshwater	(Chi et al. 2003)
Monacanthidae	*Stephanolepis cirrhifer*	Thread-sail Filefish	Marine	(Pirarat et al. 2009)
Tetraodontidae	*Takifugu rubripes*	Tiger puffer	Marine	(Nishizawa et al. 1997)
Gadidae	*Gadus morhua*	Atlantic Cod	Marine	(Johnson et al. 2002, Gagné et al. 2004, Starkey et al. 2004, Patel et al. 2007, Nylund et al. 2008)
	Gadus macrocephalus	Pacific Cod	Marine	(Nishizawa et al. 1997)
	Melanogrammus aeglefinus	Haddock	Marine	(Gagné et al. 2004, Murray et al. 2010)
Cyprinidae	*Danio rerio*	Zebrafish	Freshwater	(Binesh 2013)
	Carassius auratus	Goldfish	Freshwater	(Binesh 2013)
Anguillidae	*Anguilla anguilla*	European Eel	Diadromous	(Chi et al. 2003)
Poeciliidae	*Poicelia reticulate*	Guppy	Freshwater	(Hegde et al. 2003)

References

Arimoto, M., K. Maruyama and I. Furusawa.1994. Epizootiology of viral nervous necrosis (VNN) in striped jack. Fish Pathology 29: 19-24.

Arimoto, M., K. Mori, T. Nakai, K. Muroga and I. Furusawa. 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.

Athanassopoulou, F., C. Billinis and T. Prapas. 2004. Important disease conditions of newly cultured species in intensive freshwater farms in Greece: first incidence of nodavirus infection in Acipenser sp. Diseases of Aquatic Organisms 60: 247-252.

Athanassopoulou, F., C. Billinis, V. Psychas and K. Karipoglou. 2003. Viral encephalopathy and retinopathy of Dicentrarchus labrax (L.) farmed in fresh water in Greece. Journal of Fish Diseases 26: 361-365.

Azad, I. S., M. S. Shekhar, A. R. Thirunavukkarasu, M. Poornima, M. Kailasam, J. J. S. Rajan, S. A. Ali, M. Abraham and P. Ravichandran. 2005. Nodavirus infection causes mortalities in hatchery produced larvae of Lates calcarifer: first report from India. Diseases of Aquatic Organisms 63: 113-118.

Baeck, G. W., D. K. Gornez, K. S. Oh, J. H. Kim, C. H. Choresca, Jr. and S. C. Park 2007. Detection of piscine nodaviruses from apparently healthy wild marine fish in Korea. Bulletin of the European Association of Fish Pathologists 27: 116-122.

Bandin, I., J. G. Olveira, J. J. Borrego, C. P. Dopazo and J. L. Barja. 2006. Susceptibility of the fish cell line SAF-1 to betanodavirus. Journal of Fish Diseases 29: 633-636.

Bandín, I., S. Souto, J. M. Cutrín, C. López-Vázquez, J. G. Olveira, C. Esteve, E. Alcaide and C. P. Dopazo. 2014. Presence of viruses in wild eels Anguilla anguilla L, from the Albufera Lake (Spain). Journal of Fish Diseases 37: 597-607.

Barker, D. E., A.-M. MacKinnon, L. Boston, M. D. B. Burt, D. K. Cone, D. J. Speare, S. Griffiths, M. Cook, R. Ritchie and G. Olivier. 2002. First report of piscine nodavirus infecting wild winter flounder Pleuronectes americanus in Passamaquoddy Bay, New Brunswick, Canada. Diseases of Aquatic Organisms 49: 99-105.

Baud, M., J. Cabon, A. Salomoni, A. Toffan, V. Panzarin and L. Bigarré (2015). First generic one step real-time Taqman RT-PCR targeting the RNA1 of betanodaviruses. Journal of Virological Methods 211: 1-7.

Bigarré, L., J. Cabon, M. Baud, M. Heimann, A. Body, F. Lieffrig and J. Castric. 2009. Outbreak of betanodavirus infection in tilapia, Oreochromis niloticus (L.), in fresh water. Journal of Fish Diseases 32: 667-673.

Binesh, C. 2013. Mortality due to viral nervous necrosis in zebrafish Danio rerio and goldfish Carassius auratus. Diseases of Aquatic Organisms 104: 257-260.

Binesh, C. P. 2013. Mortality due to viral nervous necrosis in zebrafish Danio rerio and goldfish Carassius auratus. Diseases of Aquatic Organisms 104: 257-260.

Binesh, C. P. 2014. Elevation of temperature and crowding trigger acute viral nervous necrosis in zebra fish, Brachydanio rerio (Hamilton-Buchanan), subclinically infected with betanodavirus. Journal of Fish Diseases 37: 279-282.

Binesh, C. P., K. Renuka, N. Malaichami and C. Greeshma. 2013. First report of viral nervous necrosis-induced mass mortality in hatchery-reared larvae of clownfish, Amphiprion sebae Bleeker. Journal of Fish Diseases 36: 1017-1020.

Bitchava, K., E. Xylouri, E. Fragkiadaki, F. Athanassopoulou, M. Papanastassopoulou and O. Sabatakou. 2007. First incidence of clinical signs of nodavirus infection in sea bream, Sparus auratus L. Israeli Journal of Aquaculture-Bamidgeh 59: 3-9.

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

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

Bovo, G., A. Gustinelli, F. Quaglio, F. Gobbo, V. Panzarin, A. Fusaro, F. Mutinelli, M. Caffara and M. L. Fioravanti. 2011. Viral encephalopathy and retinopathy outbreak in freshwater fish farmed in Italy. Diseases of Aquatic Organisms 96: 45-54.

Bovo, G., T. Nishizawa, C. Maltese, F. Borghesan, F. Mutinelli, F. Montesi and S. De Mas. 1999. Viral encephalopathy and retinopathy of farmed marine fish species in Italy. Virus Research 63: 143-146.

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

Breuil, G., O. Mouchel, C. Fauvel and J. Pepin. 2001. Sea bass Dicentrarchus labrax nervous necrosis virus isolates with distinct pathogenicity to sea bass larvae. Diseases of aquatic organisms 45: 25-31.

Breuil, G., J. F. Pepin, J. Castric, C. Fauvel and R. Thiery. 2000. Detection of serum antibodies against nodavirus in wild and farmed adult sea bass: Application to the screening of broodstock in sea bass hatcheries. Bulletin of the European Association of Fish Pathologists 20: 95-100.

Breuil, G., J. F. P. Pépin, S. Boscher and R. Thiéry. 2002. Experimental vertical transmission of nodavirus from broodfish to eggs and larvae of the sea bass, Dicentrarchus labrax (L.). Journal of Fish Diseases 25: 697-702.

Buchan, K. A. H., D. J. Martin-Robichaud, T. J. Benfey, A. M. MacKinnon and L. Boston. 2006. The efficacy of ozonated seawater for surface disinfection of haddock (Melanogrammus aeglefinus) eggs against piscine nodavirus. Aquacultural Engineering 35: 102-107.

Castric, J., R. Thiery, J. Jeffroy, P. de Kinkelin and J. C. Raymond. 2001. Sea bream Sparus aurata, an asymptomatic contagious fish host for nodavirus. Diseases of Aquatic Organisms 47: 33-38.

Cha, S. J., J. W. Do, N. S. Lee, E. J. An, Y. C. Kim, J. W. Kim and J. W. Park 2007. Phylogenetic analysis of betanodaviruses isolated from cultured fish in Korea. Diseases of Aquatic Organisms 77: 181-189.

Chen, N.-C., M. Yoshimura, H.-H. Guan, T.-Y. Wang, Y. Misumi, C.-C. Lin, P. Chuankhayan, A. Nakagawa, S. I. Chan, T. Tsukihara, T.-Y. Chen and C.-J. Chen. 2015. Crystal Structures of a Piscine Betanodavirus: Mechanisms of Capsid Assembly and Viral Infection. PLoS Pathogens 11(10).

Chérif, N., R. Thiéry, J. Castric, S. Biacchesi, M. Brémont, F. Thabti, L. Limem and S. Hammami. 2009. Viral encephalopathy and retinopathy of Dicentrarchus labrax and Sparus aurata farmed in Tunisia. Veterinary research communications 33: 345-353.

Chi, S., J. Shieh and S. Lin. 2003. Genetic and antigenic analysis of betanodaviruses isolated from aquatic organisms in Taiwan. Diseases of Aquatic Organisms 55: 221-228.

Chi, S. C., W. W. Hu and B. J. Lo. 1999. Establishment and characterization of a continuous cell line (GF-1) derived from grouper, Epinephelus coioides (Hamilton): a cell line susceptible to grouper nervous necrosis virus (GNNV). Journal of Fish Diseases 22: 173-182.

Chi, S. C., B. J. Lo and S. C. Lin. 2001. Characterization of grouper nervous necrosis virus (GNNV). Journal of Fish Diseases 24: 3-13.

Chi, S. C., C. F. Lo, G. H. Kou, P. S. Chang, S. E. Peng and S. N. Chen. 1997. Mass mortalities associated with viral nervous necrosis (VNN) disease in two species of hatchery-reared grouper, Epinephelus fuscogutatus and Epinephelus akaara (Temminck & Schlegel). Journal of Fish Diseases 20: 185-193.

Chi, S. C., J. R. Shieh and S. J. Lin. 2003. Genetic and antigenic analysis of betanodaviruses isolated from aquatic organisms in Taiwan. Diseases of Aquatic Organisms 55: 221-228.

Ciulli, S., E. Galletti, M. Grodzki, A. Alessi, M. Battilani and S. Prosperi. 2007. Isolation and genetic characterization of Betanodavirus from wild marine fish from the Adriatic sea. Veterinary Research Communications 31: 221-224.

Curtis, P. A., M. Drawbridge, T. Iwamoto, T. Nakai, R. P. Hedrick and A. P. Gendron. 2001. Nodavirus infection of juvenile white seabass, Atractoscion nobilis, cultured in southern California: first record of viral nervous necrosis (VNN) in North America. Journal of Fish Diseases 24: 263-271.

Cutrín, J. M., C. P. Dopazo, R. Thiéry, P. Leao, J. G. Olveira, J. L. Barja and I. Bandín. 2007. Emergence of pathogenic betanodaviruses belonging to the SJNNV genogroup in farmed fish species from the Iberian Peninsula. Journal of Fish Diseases 30: 225-232.

Dalla Valle, L., L. Zanella, P. Patarnello, L. Paolucci, P. Belvedere and L. Colombo. 2000. Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. Journal of Fish Diseases 23: 321-327.

Dannevig, B., R. Nilsen, I. Modahl, M. Jankowska, T. Taksdal and C. M. Press. 2000. Isolation in cell culture of nodavirus from farmed Atlantic halibut Hippoglossus hippoglossus in Norway. Diseases of Aquatic Organisms 43: 183-189.

David, R., C. Treguier, C. Montagnani, C. Belliard, P. Levy, G. Nedelec, V. Joufoques, G. Remoissenet, Y. Gueguen and N. Cochennec-Laureau. 2010). Molecular detection of betanodavirus from the farmed fish, Platax orbicularis (Forsskal) (Ephippidae), in French Polynesia. Journal of Fish Diseases 33: 451-454.

de la Pena, L. D., V. S. Suarnaba, G. C. Capulos and M. N. M. Santos. 2011. Prevalence of viral nervous necrosis (VNN) virus in wild-caught and trash fish in the Philippines. Bulletin of the European Association of Fish Pathologists 31: 129-138.

Fenner, B. J., W. Goh and J. Kwang. 2006. Sequestration and protection of double-stranded RNA by the betanodavirus B2 protein. Journal of Virology 80: 6822-6833.

Fenner, B. J., W. Goh and J. Kwang. 2007. Dissection of double-stranded RNA binding protein B2 from betanodavirus. Journal of Virology 81: 5449-5459.

Fenner, B. J., R. Thiagarajan, H. K. Chua and J. Kwang. 2006. Betanodavirus B2 is an RNA interference antagonist that facilitates intracellular viral RNA accumulation. Journal of Virology 80: 85-94.

Frerichs, G., D. Morgan, D. Hart, C. Skerrow, R. Roberts and D. Onions. 1991. Spontaneously productive C-type retrovirus infection of fish cell lines. The Journal of general virology 72: 2537-2539.

Frerichs, G., H. Rodger and Z. Peric. 1996. Cell culture isolation of piscine neuropathy nodavirus from juvenile sea bass, Dicentrarchus labrax. Journal of general virology 77: 2067-2071.

Frerichs, G. N., A. Tweedie, W. G. Starkey and R. H. Richards 2000. Temperature, pH and electrolyte sensitivity, and heat, UV and disinfectant inactivation of sea bass (Dicentrarchus labrax) neuropathy nodavirus. Aquaculture 185: 13-24.

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

Furusawa, R., Y. Okinaka, K. Uematsu and T. Nakai. 2007. Screening of freshwater fish species for their susceptibility to a betanodavirus. Diseases of Aquatic Organisms 77: 119-125.

Gagné, N., S. C. Johnson, M. Cook-Versloot, A. M. MacKinnon and G. Olivier. 2004. Molecular detection and characterization of nodavirus in several marine fish species from the northeastern Atlantic. Diseases of Aquatic Organisms 62: 181-189.

Giacopello, C., M. Foti, T. Bottari, V. Fisichella and G. Barbera 2013. Detection of viral encephalopathy and retinopathy virus (VERV) in wild marine fish species of the South Tyrrhenian Sea (Central Mediterranean). Journal of Fish Diseases 36: 819-821.

Gjessing, M. C., A. Kvellestad, K. Ottesen and K. Falk 2009. Nodavirus provokes subclinical encephalitis and retinochoroiditis in adult farmed Atlantic cod, Gadus morhua L. Journal of Fish Diseases 32: 421-431.

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

Gomez, D. K., G. W. Baeck, J. H. Kim, C. H. Choresca, Jr. and S. C. Park. 2008. Molecular detection of betanodavirus in wild marine fish populations in Korea. Journal of Veterinary Diagnostic Investigation 20: 38-44.

Gomez, D. K., D. J. Lim, G. W. Baeck, H. J. Youn, N. S. Shin, H. Y. Youn, C. Y. Hwang, J. H. Park and S. C. Park. 2006. Detection of betanodaviruses in apparently healthy aquarium fishes and invertebrates. Journal of Veterinary Science 7: 369-374.

Gomez, D. K., S. Matsuoka, K.-i. Mori, Y. Okinaka, S. C. Park and T. Nakai. 2009. Genetic analysis and pathogenicity of betanodavirus isolated from wild redspotted grouper Epinephelus akaara with clinical signs. Archives of Virology 154: 343-346.

Grotmol, S., Bergh Ø and T. GK. 1999. Transmission of viral encephalopathy and retinopathy (VER) to yolk-sac larvae of the Atlantic halibut Hippoglossus hippoglossus:occurrence of nodavirus in various organs and a possible route of infection. Diseases of Aquatic Organisms 36: 95-106.

Grotmol, S. and G. K. Totland. 2000. Surface disinfection of Atlantic halibut Hippoglossus hippoglossus eggs with ozonated sea-water inactivates nodavirus and increases survival of the larvae. Diseases of Aquatic Organisms 39: 89-96.

Grotmol, S., G. K. Totland, K. Thorud and B. K. Hjeltnes. 1997. Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: A probable cause of mass mortality of cultured larval and juvenile Atlantic halibut Hippoglossus hippoglossus. Diseases of Aquatic Organisms 29: 85-97.

Grove, S., R. Faller, K. B. Soleim and B. H. Dannevig. 2006. Absolute quantitation of RNA by a competitive real-time RT-PCR method using piscine nodavirus as a model. Journal of Virological Methods 132: 104-112.

Grove, S., R. Johansen, B. H. Dannevig, L. J. Reitan and T. Ranheim. 2003. Experimental infection of Atlantic halibut Hippoglossus hippoglossus with nodavirus: tissue distribution and immune response. Diseases of Aquatic Organisms 53: 211-221.

Haddad-Boubaker, S., L. Bigarre, N. Bouzgarou, A. Megdich, M. Baud, J. Cabon and N. Ben Chehida. 2013. Molecular epidemiology of betanodaviruses isolated from sea bass and sea bream cultured along the Tunisian coasts. Virus Genes 46: 412-422.

Haddad-Boubaker, S., W. Boughdir, S. Sghaier, J. Ben Souissi, A. Megdich, R. Dhaouadi, A. Amara, V. Panzarin and E. Fakhfakh. 2014. Outbreak of Viral Nervous Necrosis in Endangered Fish species Epinephelus costae and E. marginatus in Northern Tunisian Coasts. Fish Pathology 49: 53-56.

Hegde, A., H. Teh, T. Lam and Y. Sin. 2003. Nodavirus infection in freshwater ornamental fish, guppy, Poicelia reticulata comparative characterization and pathogenicity studies. Archives of virology 148: 575-586.

Hegde, A., H. C. Teh, T. J. Lam and Y. M. Sin. 2003. Nodavirus infection in freshwater ornamental fish, guppy, Poicelia reticulata - comparative characterization and pathogenicity studies. Archives of Virology 148: 575-586.

Hellberg, H., A. Kvellestad, B. Dannevig, G. Borno, I. Modahl, R. N. Haldorsen, F. Vik-Mo, K. Ottesen, E. M. Saetre and H. Sindre. 2010. Outbreaks of viral nervous necrosis in juvenile and adult farmed Atlantic cod, Gadus morhua L., in Norway. Journal of Fish Diseases 33: 75-81.

Hick, P., G. Schipp, J. Bosmans, J. Humphrey and R. Whittington. 2011. Recurrent outbreaks of viral nervous necrosis in intensively cultured barramundi (Lates calcarifer) due to horizontal transmission of betanodavirus and recommendations for disease control. Aquaculture 319: 41-52.

Hick, P., A. Tweedie and R. J. Whittington. 2011. Optimization of Betanodavirus culture and enumeration in striped snakehead fish cells. Journal of Veterinary Diagnostic Investigation 23: 465-475.

Hick, P. and R. J. Whittington. 2010. Optimisation and validation of a real-time reverse transcriptase-polymerase chain reaction assay for detection of betanodavirus. Journal of Virological Methods 163: 368-377.

Hodneland, K., R. García, J. A. Balbuena, C. Zarza and B. Fouz. 2011. Real-time RT-PCR detection of betanodavirus in naturally and experimentally infected fish from Spain. Journal of Fish Diseases 34: 189-202.

Husgaro, S., S. Grotmol, B. K. Hjeltnes, O. M. Rodseth and E. Biering. 2001. Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot Scophthalmus maximus and Atlantic halibut Hippoglossus hippoglossus, and evaluation of a vaccine against SJNNV. Diseases of Aquatic Organisms 45: 33-44.

Hwang, J., S.-S. Suh, M. Park, M.-J. Oh, J.-O. Kim, S. Lee and T.-K. Lee. 2016. Detection of coat protein gene of nervous necrosis virus using loop mediated isothermal amplification. Asian Pacific Journal of Tropical Medicine 9: 230-235.

Iwamoto, T., K.-i. Mori, M. Arimoto and T. Nakai. 1999. High permissivity of the fish cell line SSN-1 for piscine nodaviruses. Diseases of Aquatic Organisms 39: 37-47.

Iwamoto, T., T. Nakai, K. Mori, M. Arimoto and I. Furusawa. 2000. Cloning of the fish cell line SSN-1 for piscine nodaviruses. Diseases of Aquatic Organisms 43: 81-89.

Jaramillo, D., S. Duerr, P. Hick and R. Whittington. 2016. Bayesian estimation of diagnostic sensitivity and specificity of a nervous necrosis virus antibody ELISA. Preventive Veterinary Medicine 123: 138-142.

Jaramillo, D., P. Hick, K. Deece, A. Tweedie, P. Kirkland, E. Arzey and R. J. Whittington. 2016. Comparison of ELISA formats for detection of antibodies specific for nervous necrosis virus (Betanodavirus) in the serum of immunized barramundi Lates calcarifer and Australian bass Macquaria novemaculeata. Aquaculture 451: 33-38.

Johansen, R., M. Amundsen, B. H. Dannevig and A. I. Sommer. 2003. Acute and persistent experimental nodavirus infection in spotted wolffish Anarhichas minor. Diseases of Aquatic Organisms 57: 35-41.

Johansen, R., S. Grove, A. K. Svendsen, I. Modahl and B. Dannevig. 2004. A sequential study of pathological findings in Atlantic halibut, Hippoglossus hippoglossus (L.), throughout one year after an acute outbreak of viral encephalopathy and retinopathy. Journal of Fish Diseases 27: 327-341.

Johansen, R., T. Ranheim, M. K. Hansen, T. Taksdal and G. K. Totland. 2002. Pathological changes in juvenile Atlantic halibut Hippoglossus hippoglossus persistently infected with nodavirus. Diseases of Aquatic Organisms 50: 161-169.

Johnson, S. C., S. A. Sperker, C. T. Leggiadro, D. B. Groman, S. G. Griffiths, R. J. Ritchie, M. D. Cook and R. R. Cusack. 2002. Identification and characterization of a piscine neuropathy and nodavirus from juvenile Atlantic cod from the Atlantic coast of North America. Journal of Aquatic Animal Health 14: 124-133.

Kai, Y. H., H. M. Su, K. T. Tai and S. C. Chi. 2010. Vaccination of grouper broodfish (Epinephelus tukula) reduces the risk of vertical transmission by nervous necrosis virus. Vaccine 28: 996-1001.

Katharios, P. and C. S. Tsigenopoulos. 2010. First report of nodavirus outbreak in cultured juvenile shi drum, Umbrina cirrosa L., in Greece. Aquaculture Research 42: 147-152.

Keawcharoen, J., S. Techangamsuwan, A. Ponpornpisit, E. D. Lombardini, T. Patchimasiri and N. Pirarat. 2015. Genetic characterization of a betanodavirus isolated from a clinical disease outbreak in farm-raised tilapia Oreochromis niloticus (L.) in Thailand. Journal of Fish Diseases 38: 49-54.

Kim, J. H., D. K. Gomez, C. H. Choresca, Jr. and S. C. Park. 2007. Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. Aquaculture 272: 105-110.

Korsnes, K., E. Karlsbakk, M. Devold, A. H. Nerland and A. Nylund. 2009. Tissue tropism of nervous necrosis virus (NNV) in Atlantic cod, Gadus morhua L., after intraperitoneal challenge with a virus isolate from diseased Atlantic halibut, Hippoglossus hippoglossus (L.). Journal of Fish Diseases 32: 655-665.

Kuo, H.-C., T.-Y. Wang, H.-H. Hsu, P.-P. Chen, S.-H. Lee, Y.-M. Chen, T.-J. Tsai, C.-K. Wang, H.-T. Ku and G.-B. Lee. 2012. Nervous necrosis virus replicates following the embryo development and dual infection with iridovirus at juvenile stage in grouper. PloS one 7(4).

Kuo, H.-C., T.-Y. Wang, H.-H. Hsu, S.-H. Lee, Y.-M. Chen, T.-J. Tsai, M.-C. Ou, H.-T. Ku, G.-B. Lee and T.-Y. Chen. 2012. An Automated Microfluidic Chip System for Detection of Piscine Nodavirus and Characterization of Its Potential Carrier in Grouper Farms. Plos One 7(8).

Lai, Y. S., J. A. C. John, C. H. Lin, I. C. Guo, S. C. Chen, K. Fang and C. Y. Chang. 2003. Establishment of cell lines from a tropical grouper, Epinephelus awoara (Temminck & Schlegel), and their susceptibility to grouper irido- and nodaviruses. Journal of Fish Diseases 26: 31-42.

Lai, Y. S., S. Murali, H. C. Chiu, H. Y. Ju, Y. S. Lin, S. C. Chen, I. C. Guo, K. Fang and C. Y. Chang. 2001. Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus-susceptible cell line from yellow grouper, Epinephelus awoara (Temminck & Schlegel), brain tissue. Journal of Fish Diseases 24: 299-309.

Le Breton, A., L. Grisez, J. Sweetman and F. Ollevier. 1997. Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass,Dicentrarchus labrax (L.). Journal of Fish Diseases 20: 145-151.

Lin, L., J. G. He, K. Mori, T. Nishioka, J. L. Wu, S. P. Weng, K. Mushiake, M. Arimoto and T. Nakai. 2001. Mass mortalities associated with viral nervous necrosis in hatchery-reared groupers in the People's Republic of China. Fish Pathology 36: 186-188.

Liu, H., Y. Teng, X. Zheng, Y. Wu, X. Xie, J. He, Y. Ye and Z. Wu. 2012. Complete sequence of a viral nervous necrosis virus (NNV) isolated from red-spotted grouper (Epinephelus akaara) in China. Archives of virology 157: 777-782.

Liu, W., C.-H. Hsu, Y.-R. Hong, S.-C. Wu, C.-H. Wang, Y.-M. Wu, C.-B. Chao and C.-S. Lin. 2005. Early endocytosis pathways in SSN-1 cells infected by dragon grouper nervous necrosis virus. Journal of general virology 86: 2553-2561.

Liu, X. D., J. N. Huang, S. P. Weng, X. Q. Hu, W. J. Chen, Z. D. Qin, X. X. Dong, X. L. Liu, Y. Zhou, M. Asim, W. M. Wang, J. G. He and L. Lin. 2015. Infections of nervous necrosis virus in wild and cage-reared marine fish from South China Sea with unexpected wide host ranges. Journal of Fish Diseases 38(6): 533-540.

Lopez-Jimena, B., M. del Carmen Alonso, K. D. Thompson, A. Adams, C. Infante, D. Castro, J. Jose Borrego and E. Garcia-Rosado. 2011. Tissue distribution of Red Spotted Grouper Nervous Necrosis Virus (RGNNV) genome in experimentally infected juvenile European seabass (Dicentrarchus labrax). Veterinary Microbiology 154: 86-95.

Lopez-Munoz, A., M. P. Sepulcre, D. Garcia-Moreno, I. Fuentes, J. Bejar, M. Manchado, M. C. Alvarez, J. Meseguer and V. Mulero. 2012. Viral nervous necrosis virus persistently replicates in the central nervous system of asymptomatic gilthead seabream and promotes a transient inflammatory response followed by the infiltration of IgM(+) B lymphocytes. Developmental and Comparative Immunology 37: 429-437.

Luo, Y.-C., C.-H. Wang, Y.-M. Wu, W. Liu and C.-S. Lin. 2014. Crystallization and X-ray diffraction of virus-like particles from a piscine betanodavirus. Acta Crystallographica Section F-Structural Biology Communications 70: 1080-1086.

Ma, H., W. Wen, Y. Su, J. Feng, L. Xu, C. Peng and Z. Guo. 2015. Epidemiological characterization of VNNV in hatchery-reared and wild marine fish on Hainan Island, China, and experimental infection of golden pompano (Trachinotus ovatus) juveniles. Archives of Virology 160: 2979-2989.

Ma, H., J. Xie, S. Weng, T. Zhou and J. He. 2012. Co-infection of megalocytivirus and viral nervous necrosis virus in a very severe mass mortality of juvenile orange-spotted groupers (Epinephelus coioides). Aquaculture 358: 170-175.

Maeno, Y., L. D. De La Pena and E. R. Cruz-Lacierda. 2004. Mass mortalities associated with viral nervous necrosis in hatchery-reared sea bass Lates calcarifer in the Philippines. Jarq-Japan Agricultural Research Quarterly 38: 69-73.

Maeno, Y., L. D. De la Pena and E. R. Cruz-Lacierda. 2007. Susceptibility of Fish Species Cultured in Mangrove Brackish Area to Piscine Nodavirus. Japan Agricultural Research Quarterly 41: 95-99.

Mao, M.-G., S.-H. Wen, A. Peralvarez-Marin, H. Li, J.-L. Jiang, Z.-Q. Jiang, X. Li, H. Sun and H.-Q. Lu. 2015. Evidence for and characterization of nervous necrosis virus infection in Pacific cod (Gadus macrocephalus). Archives of Virology 160: 2237-2248.

Mekata, T., J. Satoh, M. Inada, S. Dinesh, P. Harsha, T. Itami and R. Sudhakaran. 2015. Development of simple, rapid and sensitive detection assay for grouper nervous necrosis virus using real-time loop-mediated isothermal amplification. Journal of Fish Diseases 38: 873-879.

Mezeth, K. B., S. Patel, H. Henriksen, A. M. Szilvay and A. H. Nerland. 2009. B2 protein from betanodavirus is expressed in recently infected but not in chronically infected fish. Diseases of Aquatic Organisms 83: 97-103.

Mikkelsen, S. S., V. Panzarin, S. P. Jonstrup, L. Bigarre, M. Baud, T. Gray, P. M. Agapow and N. J. Olesen. 2015. Fishpathogens.eu/noda: a free and handy online platform for Betanodavirus targeted research and data sharing. Journal of Fish Diseases 38: 755-760.

Moody, N., P. Horwood, A. Reynolds, T. Mahony, I. Anderson and H. Oakey. 2009. Phylogenetic analysis of betanodavirus isolates from Australian finfish. Diseases of Aquatic Organisms 87: 151-160.

Moreno, P., J. G. Olveira, A. Labella, J. Manuel Cutrin, J. C. Baro, J. Jose Borrego and C. P. Dopazo. 2014. Surveillance of Viruses in Wild Fish Populations in Areas around the Gulf of Cadiz (South Atlantic Iberian Peninsula). Applied and Environmental Microbiology 80: 6560-6571.

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

Mori, K., K. Mushiake and M. Arimoto. 1998. Control measures for viral nervous necrosis in striped jack. Fish Pathology 33: 443-444.

Munday, B. L., J. Kwang and N. Moody. 2002. Betanodavirus infections of teleost fish: a review. Journal of Fish Diseases 25: 127-142.

Munday, B. L., J. S. Langdon, A. Hyatt and J. D. Humphrey. 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. and T. Nakai. 1997. Nodaviruses as pathogens in larval and juvenile marine finfish. World Journal of Microbiology & Biotechnology 13: 375-381.

Munday, B. L. and L. Owens. 1998. Viral diseases of fish and shellfish in Australian mariculture. Fish Pathology 33: 193-200.

Muroga, K. 2001. Viral and bacterial diseases of marine fish and shellfish in Japanese hatcheries. Aquaculture 202: 23-44.

Murray, W. J., J. Black, K. MacNeish, M. Snow and E. S. Munro. 2010. First isolation of a piscine nodavirus from wild caught Haddock, Melanogrammus aeglefinus G. (Gadidae). Bulletin of the European Association of Fish Pathologists 30: 15-24.

Mushiake, K., Nakai, T., Muroga, K., Sekiya, S. and I. Furusawa, 1993. Viral nervous necrosis of striped jack: effects of plasma antibody level of spawners and spawning conditions on the occurrence of the disease in their offsprings. Fish Pathology 41: 327– 332.

Mushiake, K., T. Nishizawa, T. Nakai, I. Furusawa and K. Muroga. 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.

Mushiake, K., T. Nishizawa, T. Nakai, I. Furusawa and K. Muroga. 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.

Nakajima, K., K. Inouye and M. Sorimachi. 1998. Viral diseases in cultured marine fish in Japan. Fish Pathology 33: 181-188.

Nazari, A., M. D. Hassan, G. Bovo, M. J. Zorriehzahra, T. I. Azmi and S. S. Arshad. 2014. Pathogenicity of viral nervous necrosis virus for Guppy fish, Poecilia reticulata. Iranian Journal of Fisheries Sciences 13: 168-177.

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

Nguyen, H. D., T. Mekuchi, K. Imura, T. Nakai, T. Nishizawa and K. Muroga. 1994. Occurrence of viral nervous necrosis (VNN) in hatchery-reared juvenile Japanese flounder Paralichthys olivaceus. Bulletin of the Japanese Society of Scientific Fisheries 60: 551-554.

Nishioka, T., K.-i. Mori, T. Sugaya, N. Tezuka, T. Takebe, H. Imaizumi, K. Kumon, S. Masuma and T. Nakai. 2010. Involvement of Viral Nervous Necrosis in Larval Mortality of Hatchery-reared Pacific Bluefin Tuna Thunnus olientalis. Fish Pathology 45: 69-72.

Nishizawa, T., M. Furuhashi, T. Nagai, T. Nakai and K. Muroga. 1997. Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. Applied and Environmental Microbiology 63: 1633-1636.

Nopadon, P., P. Aranya, T. Tipaporn, N. Toshihiro, K. Takayuki, M. Masashi and E. Makoto. 2009. Nodavirus associated with pathological changes in adult spotted coralgroupers (Plectropomus maculatus) in Thailand with viral nervous necrosis. Research in Veterinary Science 87: 97-101.

Nylund, A., E. Karlsbakk, S. Nylund, T. Isaksen, M. Karlsen, K. Korsnes, S. Handeland, R. Martinsen, T. M. Pedersen and K. Ottem. 2008. New clade of betanodaviruses detected in wild and farmed cod (Gadus morhua) in Norway. Archives of virology 153: 541-547.

Nylund, A., E. Karlsbakk, S. Nylund, T. E. Isaksen, M. Karlsen, K. Korsnes, S. Handeland, R. Martinsen, T. M. Pedersen and K. F. Ottem. 2008. New clade of betanodaviruses detected in wild and farmed cod (Gadus morhua) in Norway. Archives of Virology 153: 541-547.

Oh, M. J., S. J. Jung, S. R. Kim, K. V. Rajendran, Y. J. Kim, T. J. Choi, H. R. Kim and J. D. Kim. 2002. A fish nodavirus associated with mass mortality in hatchery-reared red drum, Sciaenops ocellatus. Aquaculture 211: 1-7.

Oh, M. J., S. J. Jung and S. I. Kitamura. 2005. Comparison of the coat protein gene of nervous necrosis virus (NNV) detected from marine fishes in Korea. Journal of the World Aquaculture Society 36: 223-227.

OIE. 2010. Manual of diagnostic tests for aquatic animals. from http://www.oie.int/international-standard-setting/aquatic-manual/access-online/.

O.I.E. World Organization of Animal Health. 2015 . Manual of Diagnostic Tests for Aquatic Animals , from http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online/

Olveira, J. G., S. Souto, C. P. Dopazo and I. Bandin. 2013. Isolation of betanodavirus from farmed turbot Psetta maxima showing no signs of viral encephalopathy and retinopathy. Aquaculture 406: 125-130.

Olveira, J. G., S. Souto, C. P. Dopazo, R. Thiery, J. L. Barja and I. Bandin. 2009. Comparative analysis of both genomic segments of betanodaviruses isolated from epizootic outbreaks in farmed fish species provides evidence for genetic reassortment. Journal of General Virology 90: 2940-2951.

Pakingking Jr, R., R. Seron, L. Dela Peña, K. Mori, H. Yamashita and T. Nakai. 2009. Immune responses of Asian sea bass, Lates calcarifer Bloch, against an inactivated betanodavirus vaccine. Journal of Fish Diseases 32: 457-463.

Panzarin, V., P. Patarnello, A. Mori, E. Rampazzo, E. Cappellozza, G. Bovo and G. Cattoli. 2010. Development and validation of a real-time TaqMan PCR assay for the detection of betanodavirus in clinical specimens. Archives of Virology 155: 1193-1203.

Parameswaran, V., S. R. Kumar, V. P. I. Ahmed and A. S. S. Hameed 2008. A fish nodavirus associated with mass mortality in hatchery-reared Asian Sea bass, Lates calcarifer. Aquaculture 275: 366-369.

Patel, S., K. Korsnes, O. Bergh, F. Vik-Mo, J. Pedersen and A. H. Nerland. 2007. Nodavirus in farmed Atlantic cod Gadus morhua in Norway. Diseases of Aquatic Organisms 77: 169-173.

Peducasse, S., J. Castric, R. Thiery, J. Jeffroy, A. Le Ven and F. B. Laurencin. 1999. Comparative study of viral encephalopathy and retinopathy in juvenile sea bass Dicentrarchus labrax infected in different ways. Diseases of Aquatic Organisms 36: 11-20.

Pirarat, N., T. Katagiri, M. Maita, T. Nakai and M. Endo. 2009. Viral encephalopathy and retinopathy in hatchery-reared juvenile thread-sail filefish (Stephanolepis cirrhifer). Aquaculture 288: 349-352.

Qin, Q. W., T. H. Wu, T. L. Jia, A. Hegde and R. Q. Zhang. 2006. Development and characterization of a new tropical marine fish cell line from grouper, Epinephelus coioides susceptible to iridovirus and nodavirus. Journal of Virological Methods 131: 58-64.

Ransangan, J. and B. O. Manin. 2010. Mass mortality of hatchery-produced larvae of Asian seabass, Lates calcarifer (Bloch), associated with viral nervous necrosis in Sabah, Malaysia. Veterinary Microbiology 145: 153-157.

Ransangan, J., B. O. Manin, A. Abdullah, Z. Roli and E. F. Sharudin. 2011. Betanodavirus infection in golden pompano, Trachinotus blochii, fingerlings cultured in deep-sea cage culture facility in Langkawi, Malaysia. Aquaculture 315: 327-334.

Renault, T., P. Haffner, L. F. Baudin, G. Breuil and J.-R. Bonami. Mass mortality of 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.

Rise, M. L., J. R. Hall, M. Rise, T. S. Hori, M. J. Browne, A. K. Gamperl, S. Hubert, J. Kimball, S. Bowman and S. C. Johnson. 2010. Impact of asymptomatic nodavirus carrier state and intraperitoneal viral mimic injection on brain transcript expression in Atlantic cod (Gadus morhua). Physiological Genomics 42: 266-280.

Sakamoto, T., Y. Okinaka, K.-I. Mori, T. Sugaya, T. Nishioka, M. Oka, H. Yamashita and T. Nakai. 2008. Phylogenetic Analysis of Betanodavirus RNA2 Identified from Wild Marine Fish in Oceanic Regions. Fish Pathology 43: 19-27.

Sepulveda, D., H. Bohle, A. Labra, H. Grothusen and S. H. Marshall. 2013. Design and evaluation of a unique RT-qPCR assay for diagnostic quality control assessment that is applicable to pathogen detection in three species of salmonid fish. BMC Veterinary Research 9.

Skliris, G. P., J. V. Krondiris, D. C. Sideris, A. P. Shinn, W. G. Starkey and R. H. Richards. 2001. Phylogenetic and antigenic characterization of new fish nodavirus isolates from Europe and Asia. Virus Research 75: 59-67.

Skliris, G. P. and R. H. Richards. 1999. Nodavirus isolated from experimentally infected tilapia, Oreochromis mossambicus (Peters). Journal of Fish Diseases 22: 315-318.

Sohn, S. G. and M. A. Park. 1998. Viral diseases of cultured marine fish and shrimp in Korea. Fish Pathology 33: 189-192.

Sommer, A.-I., M. A. Strand, E. Rasmussen and S. Mennen. 2004. Susceptibility of spotted wolffish Anarhichas minor to experimental infection with nodavirus and infectious pancreatic necrosis virus. Diseases of Aquatic Organisms 59: 101-108.

Sommerset, I. and A. H. Nerland. 2004. Complete sequence of RNA1 and subgenomic RNA3 of Atlantic halibut nodavirus (AHNV). Diseases of Aquatic Organisms 58: 117-125.

Souto, S., E. Merour, S. Biacchesi, M. Bremont, J. G. Olveira and I. Bandin. 2015. In vitro and in vivo characterization of molecular determinants of virulence in reassortant betanodavirus. Journal of General Virology 96: 1287-1296.

Souto, S., J. G. Olveira and I. Bandin. 2015. Influence of temperature on Betanodavirus infection in Senegalese sole (Solea senegalensis). Veterinary Microbiology 179: 162-167.

Starkey, W. G., J. H. Ireland, K. F. Muir, A. P. Shinn, R. H. Richards and H. W. Ferguson. 2000. Isolation of nodavirus from Scottish farmed halibut, Hippoglossus hippoglossus (L). Journal of Fish Diseases 23: 419-422.

Starkey, W. G., J. H. Ireland, K. F. Muir, A. P. Shinn, R. H. Richards and H. W. Ferguson. 2001. Nodavirus infection of Atlantic cod, Dover sole, and halibut in the United Kingdom. 10th International Conference of the European Association of Fish Pathologists. Dublin, Ireland.

Starkey, W. G., R. M. Millar, M. E. Jenkins, J. H. Ireland, K. F. Muir and R. H. Richards. 2004. Detection of piscine nodaviruses by real-time nucleic acid sequence based amplification (NASBA). Diseases of Aquatic Organisms 59: 93-100.

Su, Y., H. Xu, H. Ma, J. Feng, W. Wen and Z. Guo. 2015. Dynamic distribution and tissue tropism of nervous necrosis virus in juvenile pompano (Trachinotus ovatus) during early stages of infection. Aquaculture 440: 25-31.

Su, Z. D., C. Y. Shi, J. Huang, G. M. Shen, J. Li, S. Q. Wang and C. Fan. 2015. Establishment and application of cross-priming isothermal amplification coupled with lateral flow dipstick (CPA-LFD) for rapid and specific detection of red-spotted grouper nervous necrosis virus. Virology Journal 12.

Suebsing, R., M.-J. Oh and J.-H. Kim. 2012. Development of a Reverse Transcription Loop-Mediated Isothermal Amplification Assay for Detecting Nervous Necrosis Virus in Olive Flounder Paralichthys olivaceus. Journal of Microbiology and Biotechnology 22: 1021-1028.

Sung, C.-H. and J.-K. Lu. 2009. Reverse transcription loop-mediated isothermal amplification for rapid and sensitive detection of nervous necrosis virus in groupers. Journal of Virological Methods 159: 206-210.

Suzuki, K. 2006. Detection of DNAs homologous to betanodavirus genome RNAs in barfin flounder Verasper moseri and Japanese flounder Paralichthys olivaceus. Diseases of Aquatic Organisms 72: 225-239.

Tan, C., B. Huang, S. F. Chang, G. H. Ngoh, B. Munday, S. C. Chen and J. Kwang. 2001. Determination of the complete nucleotide sequences of RNA1 and RNA2 from greasy grouper (Epinephelus tauvina) nervous necrosis virus, Singapore strain. Journal of general virology 82: 647-653.

Tanaka, S., H. Aoki and T. Naka. 1998. Pathogenicity of the nodavirus detected from diseased sevenband grouper. Fish Pathology 33: 31-36.

Tanaka, S., M. Takagi and T. Miyazaki. 2004. Histopathological studies on viral nervous necrosis of sevenband grouper, Epinephelus septemfasciatus Thunberg, at the grow-out stage. Journal of Fish Diseases 27: 385-399.

Thiery, R., C. Arnauld and C. Delsert. 1999. Two isolates of sea bass, Dicentrarchus labrax L., nervous necrosis virus with distinct genomes. Journal of Fish Diseases 22: 201-207.

Thiéry, R., J. Cozien, C. de Boisséson, S. Kerbart-Boscher and L. Névarez. 2004. Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. Journal of general virology 85: 3079-3087.

Toffolo, V., E. Negrisolo, C. Maltese, G. Bovo, P. Belvedere, L. Colombo and L. D. Valle. 2007. Phylogeny of betanodaviruses and molecular evolution of their RNA polymerase and coat proteins. Molecular Phylogenetics and Evolution 43: 298-308.

Totland, G. K., S. Grotmol, Y. Morita, T. Nishioka and T. Nakai. 1999. Pathogenicity of nodavirus strains from striped jack Pseudocaranx dentex and Atlantic halibut Hippoglossus hippoglossus, studied by waterborne challenge of yolk-sac larvae of both teleost species. Diseases of Aquatic Organisms 38: 169-175.

Ucko, M., A. Colorni and A. Diamant. 2004. Nodavirus infections in Israeli mariculture. Journal of Fish Diseases 27: 459-469.

Vendramin, N., F. Padros, T. Pretto, E. Cappellozza, V. Panzarin, G. Bovo, A. Toffan and C. Terregino. 2012. Viral encephalopathy and retinopathy outbreak in restocking facilities of the endangered freshwater species, Salaria fluviatilis (Asso). Journal of Fish Diseases 35: 867-871.

Vendramin, N., P. Patarnello, A. Toffan, V. Panzarin, E. Cappellozza, P. Tedesco, A. Terlizzi, C. Terregino and G. Cattoli. 2013. Viral Encephalopathy and Retinopathy in groupers (Epinephelus spp.) in southern Italy: a threat for wild endangered species? BMC Veterinary Research 9.

Vendramin, N., A. Toffan, M. Mancin, E. Cappellozza, V. Panzarin, G. Bovo, G. Cattoli, I. Capua and C. Terregino. 2014. Comparative pathogenicity study of ten different betanodavirus strains in experimentally infected European sea bass, Dicentrarchus labrax (L.). Journal of Fish Diseases 37: 371-383.

Wang, C.-H., K.-Y. Lien, T.-Y. Wang, T.-Y. Chen and G.-B. Lee. 2011. An integrated microfluidic loop-mediated-isothermal-amplification system for rapid sample pre-treatment and detection of viruses. Biosensors & Bioelectronics 26: 2045-2052.

Wang, J., Z. Guo, J. Huang and J. Feng. 2006. Investigation on mass mortality of young fish Trachinotus ovatus [J]. South China Fisheries Science 3: 009.

Watanabe, K.-i., K. Ide, T. Iwasaki, J. Satoh, K.-i. Mori and T. Mekata. 2013. Disinfection of Fertilized Grouper Eggs with Electrolyzed Seawater for Prevention of Viral Nervous Necrosis. Fish Pathology 48: 5-8.

Watanabe, K.-i., T. Nishizawa and M. Yoshimizu. 2000. Selection of brood stock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. Diseases of Aquatic Organisms 41: 219-223.

Wu, M. S., C. W. Chen, C. H. Lin, C. S. Tzeng and C. Y. Chang. 2012. Differential expression profiling of orange-spotted grouper larvae, Epinephelus coioides (Hamilton), that survived a betanodavirus outbreak. Journal of Fish Diseases 35: 215-225.

Xu, H.-D., J. Feng, Z.-X. Guo, Y.-J. Ou and J.-Y. Wang. 2010. Detection of red-spotted grouper nervous necrosis virus by loop-mediated isothermal amplification. Journal of Virological Methods 163: 123-128.

Xylouri, E., Y. P. Kotzamanis, F. Athanassopoulou, L. Dong, I. S. Pappas, A. Argyrokastritis and E. Fragkiadaki. 2007. Isolation, characterization, and sequencing of nodavirus in sturgeon (Acipenser gueldestaedi L.) reared in freshwater facilities. Israeli Journal of Aquaculture-Bamidgeh 59: 36-41.

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