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Review

Viral encephalopathy and retinopathy in aquaculture: a review

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Abstract

Viral encephalopathy and retinopathy (VER), otherwise known as viral nervous necrosis (VNN), is a major devastating threat for aquatic animals. Betanodaviruses have been isolated in at least 70 aquatic animal species in marine and in freshwater environments throughout the world, with the notable exception of South America. In this review, the main features of betanodavirus, including its diversity, its distribution and its transmission modes in fish, are firstly presented. Then, the existing diagnosis and detection methods, as well as the different control procedures of this disease, are reviewed. Finally, the potential of selective breeding, including both conventional and genomic selection, as an opportunity to obtain resistant commercial populations, is examined.

Keywords: betanodavirus, disease resistance, genetics, nervous necrosis virus, selective breeding.

Introduction

Although there is presently no strong evidence highlighting a possible raise of fish disease outbreaks due to climate change, increasing temperatures are

expected to induce the spread of pathogens towards higher latitudes and to provoke negative impacts on fish physiology (Cochrane *et al.* 2009). Among others, the viral encephalopathy and retinopathy (VER), otherwise known as viral nervous necrosis (VNN), is considered one of the most serious viral threats for almost all marine aquaculture fish species and requires a special focus due to the fact that outbreaks mostly happen in warm conditions. This disease, detected in at least 70 cultured or wild marine and fresh water species, already caused serious economic losses in the aquaculture industry in the past decades, and we can anticipate larger impacts of this disease because of global warming.

No simple and effective procedures are available to treat this disease in fish. It is, therefore, important to develop tools and set up new approaches to limit the occurrence and impacts of VNN episodes in aquaculture farms.

To stress that need, we present here an extensive review about VNN disease in aquaculture, including the features of the virus, the available procedures to control this disease and the potential of selective breeding and genomic selection (GS) for resistance to viral diseases, as a prospective way to prevent VNN disease in fish.

Nervous necrosis virus

The causative agent of VNN, the nervous necrosis virus (NNV), was classified as a member of the

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Nodaviridae family (Mori *et al.* 1992), which contains two genera: alphanodavirus and betanodavirus (Van Regenmortel *et al.* 2000). The species of the first genus were originally isolated from insects (Fig. 1), but appear to infect both vertebrates and invertebrates, and to cause the death of insect and mammalian hosts (Adachi *et al.* 2008). Betanodaviruses usually affect the nervous system of marine fish, leading to behavioural abnormalities and extreme high mortalities (Munday, Kwang & Moody 2002). In mammals, the pathogenicity of betanodaviruses is poorly reported, but mice have been demonstrated as non-susceptible, and human cells as not permeable to that genus (Adachi *et al.* 2008). Recently, a new emerging disease, the white tail disease (WTD), which affects the giant freshwater prawn and the whiteleg shrimp *Penaeus vannamei*, has been demonstrated to be caused by the *Macrobrachium rosenbergii* nodavirus (MrNV). Sequence analysis of this virus suggests the existence of a new genus, gammanodavirus, infecting crustaceans (Qian *et al.* 2003; Senapin *et al.* 2012, Fig. 1).

General morphology

Betanodavirus virions were first described as non-enveloped, spherical in shape and have icosahedral symmetry, with a diameter around 25 nm and a capsid formed by 180 copies of a single protein of 42 Kda (Mori *et al.* 1992). A similar virus of 20–34 nm in diameter was detected in infected Asian sea bass *Lates calcarifer* larvae, striped jack *Pseudocaranx dentex*, turbot *Scophthalmus maximus*, European sea bass *Dicentrarchus labrax* (Glazebrook, Heasman & Beer 1990; Yoshikoshi & Inoue 1990; Bloch, Gravningen & Larsen 1991; Munday *et al.* 1992), and many various fish species throughout the world were subsequently

recorded to be infected by betanodaviruses (Munday *et al.* 2002; Shetty *et al.* 2012).

Molecular structure

Betanodavirus contains a bisegmented genome composed of two single-stranded, positive-sense RNA molecules (Mori *et al.* 1992). The sequence of RNA1 is about 3.1 kb and includes an open reading frame (ORF) encoding a RNA-dependent RNA polymerase (RdRp) of 110 kDa catalysing the replication of the virus, also named protein A (Nagai & Nishizawa 1999). The sequence of RNA2 (1.4 kb) encodes the capsid protein (37 kDa), which may have a function in the induction of cell death (Guo *et al.* 2003). In addition, during the virus replication, a subgenomic RNA (RNA3) is synthesized from the 3'-terminus of RNA1 (Ball & Johnson 1999). This RNA3 encodes two other non-structural proteins, B1 (111 amino acids) and B2 (75 amino acids). Protein B1 displays antinecrotic property enhancing the viability of viral host cell (Somerset & Nerland 2004). Protein B2 is an inhibitor of host RNA silencing in either alphanodavirus or betanodavirus, but could also promote mitochondrial fragmentation and cell death induced by hydrogen peroxide production (Su *et al.* 2014).

Classification

Betanodavirus was described for the first time from infected larval striped jack. The name striped jack nervous necrosis virus (SJNNV) was consequently adopted (Mori *et al.* 1992). Subsequently other agents of VNN were isolated from diseased fish species (Munday *et al.* 2002). The first comparative studies between viral strains isolated from different marine fish species were carried out in the middle of the 1990s, where Nishizawa *et al.* reported the sequence of SJNNV and four different fish nodaviruses as well as four different insect nodaviruses (Nishizawa *et al.* 1995). From a phylogenetic analysis of the RNA2 T4 variable region, betanodaviruses were classified into four different species designed as the SJNNV type, the barfin flounder nervous necrosis virus (BFNNV) type, the red-spotted grouper nervous necrosis virus (RGNNV) type and the tiger puffer nervous necrosis virus (TPNNV) type (Nishizawa *et al.* 1997). These species partially correlate with

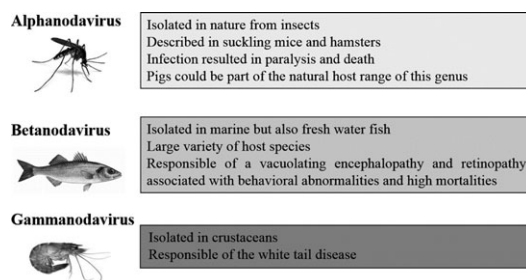


Figure 1 Three genera of Nodaviridae.

three different serotypes determined from virus neutralization using polyclonal antibodies (serotype A for SJNNV species, B for TPNNV species and C for BFNNV and RGNNV species) (Mori *et al.* 2003). Each species corresponds to different host fish and different *in vitro* optimal growth temperatures (Table 1). RGNNV is the most popular species because a variety of fish species, distributed in warm water, are affected (optimal growth temperature of 25–30 °C) (Asian sea bass, European sea bass, groupers), whereas BFNNV is restricted to cold-water (15–20 °C) marine fish species (Atlantic halibut *Hippoglossus hippoglossus*, Atlantic cod *Gadus morhua*, flounders) and TPNNV infects a single species (Tiger puffer *Takifugu rubripes*) at an intermediate temperature (20 °C). The SJNNV type was initially known to affect a few species cultured in Japan at 20–25 °C (Nishizawa *et al.* 1995; Iwamoto *et al.* 2000; Munday *et al.* 2002; Toffan *et al.* 2016). However, it was also recently described in some fish species cultured in southern Europe such as Senegalese sole *Solea senegalensis* in Spain, gilthead sea bream *Sparus aurata* and European sea bass in the Iberian Peninsula (Thiéry *et al.* 2004; Cutrín *et al.* 2007). This capacity to infect such warm-water fish species is probably associated with reassortant RGNNV and SJNNV strains (Iwamoto *et al.* 2004; Toffolo *et al.* 2007; Panzarin *et al.* 2012; Toffan *et al.* 2016; see also Phylogenetic relationships paragraph). Phylogenetic analysis of betanodaviruses was also performed based on the T2 region, which covers a larger RNA2 sequence than T4 (Chi, Shieh & Lin 2003; Johansen *et al.* 2004). This taxonomy has been used to genetically characterized new isolates in various fish species as well as in different areas (Aspehaug, Devold & Nylund 1999; Starkey *et al.* 2000; Dalla Valle *et al.* 2001; Skliris *et al.* 2001; Tan *et al.* 2001; Johnson, Sperker & Leggiadro 2002; Chi *et al.* 2003; Gagné *et al.* 2004; Johansen *et al.* 2004; Sommerset & Nerland 2004; Thiéry *et al.* 2004; Ransangan & Manin 2012; Vendramin *et al.* 2013). Because NNV is detected in many new species as well as new regions, description of new isolates and sequences are regularly published and could lead to evolution in the classification (Table 1). For example, an additional genotype including a turbot betanodavirus strain (TNNV) was described in 2004. This species is currently awaiting classification (Johansen *et al.* 2004).

An alternative classification has been proposed (Thiéry *et al.* 2004). However, this numerical nomenclature (cluster I, II, III and IV), independent from the host species origin, is not extensively used because viruses from different clusters could infect a same host species, for example European sea bass (Thiéry, Raymond & Castric 1999a; Thiéry, Arnauld & Delsert 1999b), and the classification was not consistent with geographical areas (Dalla Valle *et al.* 2001; Thiéry *et al.* 2004; Cutrín *et al.* 2007).

Phylogenetic relationships

Among the different species of betanodaviruses, amino acid sequences of RdRp protein and capsid protein share 87–99% and 77–100% of identity, respectively [82–98% for the complete RNA1 nucleic sequence and 76–99% for the RNA2 segment (Okinaka & Nakai 2008)]. The topology of phylogenetic trees based on RNA1 and RNA2 distinguishes several clades, suggesting a high diversity despite relatively strong purifying selection on most codons (Panzarin *et al.* 2012). This important variability can be explained by a significant substitution rate but also by a reassorting process specific to segmented viruses (Panzarin *et al.* 2012).

Distribution and transmission

Distribution

Viral encephalopathy and retinopathy is one of the most widespread viral diseases of marine fish species cultured worldwide. A large number of species have been reported to be affected, especially larval and juvenile stages in which high mortalities were recorded (Munday *et al.* 2002; Shetty *et al.* 2012). Based on clinical signs, VNN disease has been documented since 1985 in Japanese parrotfish *Oplegnathus fasciatus* larvae and juveniles in Japan, while the pathogen was first observed in the brain of reared Japanese parrotfish (Yoshikoshi & Inoue 1990). Three years later, it was recorded in European sea bass produced in Martinique (West Indies, France) and French Mediterranean (Breuil *et al.* 1991). Since then, similar clinical signs with encephalitis associated with picorna-like viral particles were observed in the Asian sea bass *Lates calcarifer* cultured in Australia (Glazebrook *et al.* 1990; Munday *et al.*

Table 1 Species of the genus Betanodavirus

Species	GenBank accession no.	Optimal temperature for replication	Serotype	Main hosts effected	Key Ref.
Species in the genus Betanodavirus					
Barfin flounder nervous necrosis virus – BFNNV-BF93Hok	RNA1 (EU826137 = NC_011063) RNA2 (EU826138 = NC011064)	15–20 °C	C	Atlantic cod (<i>Gadus morhua</i>) Barfin flounder (<i>Verasper moseri</i>) Atlantic halibut (<i>Hippoglossus hippoglossus</i>)	Munday et al. (2002), Iwamoto et al. (2000), Mori et al. (2003), Vendramin et al. (2013)
Red-spotted grouper nervous necrosis virus – RGNNV-SGWak97	RNA1 (AY324869 = NC_008040) RNA2 (AY324870 = NC_008041)	25–30 °C	C	Sevenband grouper (<i>Epinephelus septemfasciatus</i>) Red-spotted grouper (<i>Epinephelus akaara</i>) Keip grouper (<i>Epinephelus moara</i>) Orange-spotted grouper (<i>Epinephelus coioides</i>)	Munday et al. (2002), Iwamoto et al. (2000), Mori et al. (2003), Vendramin et al. (2013, 2014)
Striped jack nervous necrosis virus – SJNNV-SJ93Nag	RNA1 (AB056571 = NC_003448) RNA2 (AB056572 = NC_003449)	20–25 °C	A	Dragon grouper (<i>Epinephelus lanceolatus</i>) Greasy grouper (<i>Epinephelus tauvina</i>) Humpback grouper (<i>Cromileptes altivelis</i>) Barramundi (<i>Lates calcarifer</i>) Japanese sea bass (<i>Lateolabrax japonicus</i>) European sea bass (<i>Dicentrarchus labrax</i>) Japanese striped jack (<i>Pseudocaranx dentex</i>)	Nishizawa et al. (1997), Iwamoto et al. (2000), Mori et al. (2003), Thiéry et al. (2004), Vendramin et al. (2013, 2014)
Tiger puffer nervous necrosis virus – TPNNV-TPKag93	RNA1 (EU236148 = NC_013640) RNA2 (EU236149 = NC_013641)	20 °C	B	Gilthead sea bream (<i>Sparus aurata</i>) Senegalese sole (<i>Solea senegalensis</i>) Tiger puffer (<i>Takifugu rubripes</i>)	Iwamoto et al. (2000), Mori et al. (2003), Vendramin et al. (2013)
Other genotypes which have not been approved as species					
Atlantic cod nervous necrosis virus – ACNNV	RNA1 (EF433472) RNA2 (EF433468)	15–20 °C	C	Atlantic cod (<i>Gadus morhua</i>)	Nylund et al. (2008), Johnson et al. (2002)
Atlantic halibut nodavirus – AHNV	RNA1 (AJ401165) RNA2 (AJ245641)	15–20 °C	C	Atlantic halibut (<i>Hippoglossus hippoglossus</i>)	Grottmoll & Toitland (2000), Johnson et al. (2002), Sommeret & Nerland (2004)
Dicentrarchus labrax encephalitis virus – DIEV	RNA2 (U39876)	25–30 °C	C	Sea bass (<i>Dicentrarchus labrax</i>)	Dalla Valle et al. (2001), Johnson et al. (2002)
Dragon grouper nervous necrosis virus – DGNNV	RNA1 (AY721616) RNA2 (AY721615)	25–30 °C	C	Dragon grouper (<i>Epinephelus laceolatus</i>)	Panzarin et al. (2012), Johnson et al. (2002)
Greasy grouper nervous necrosis virus – GGNNV	RNA1 (AF319555) RNA2 (AF318942)	25–30 °C	C	Greasy grouper (<i>Epinephelus tauvina</i>)	Tan et al. (2001), Johnson et al. (2002), Sommeret & Nerland (2004)
Japanese flounder nervous necrosis virus – JFNNV	RNA1 (FJ748760) RNA2 (D38527)	25–30 °C	C	Japanese flounder (<i>Paralichthys olivaceus</i>)	Panzarin et al. (2012), Johnson et al. (2002)

Table 1 Continued

Species	GenBank accession no.	Optimal temperature for replication	Serotype	Main hosts effected	Key Ref.
Lates calcarifer encephalitis virus – LcEF	RNA2 (AF175516)	25–30 °C	C	Barramundi (<i>Lates calcarifer</i>)	Skiris <i>et al.</i> (2001)
Malabaricus grouper nervous necrosis virus – MGNNV	RNA2 (AF245003)	25–30 °C	C	Malabaricus grouper (<i>Epinephelus malabaricus</i>)	Johnson <i>et al.</i> (2002)
Seabass nervous necrosis virus – SBNNV	RNA2 (Y08700)	20–25 °C	A	Sea bass (<i>Dicentrarchus labrax</i>)	Thiéry <i>et al.</i> (2004)
Solea senegalensis nervous necrosis virus – SSNNV	RNA1 (FJ803911) RNA2 (AJ698113)	20–25 °C	A	Senegalese sole (<i>Solea senegalensis</i>)	Panzarin <i>et al.</i> (2012), Thiéry <i>et al.</i> (2004)
Turbot nodavirus – TNV	RNA2 (AJ608266)	Undefined	Undefined	Turbot (<i>Scophthalmus maximus</i>)	Johansen <i>et al.</i> (2004)
Macrobrachium rosenbergii nodavirus – MrNV	RNA1 (AY231436) RNA2 (AY231437)	25–30 °C	Undefined	Giant freshwater prawn (<i>Macrobrachium rosenbergii</i>)	Senapin <i>et al.</i> (2012), Bonami & Widada (2011), Senapin <i>et al.</i> (2012), Tang <i>et al.</i> (2007)
Penaeus vannamei nodavirus – PvNV	RNA1 (FJ751226) RNA2 (FJ751225)	25–30 °C	Undefined	Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	

2002), as well as in turbot *Scophthalmus maximus* (Bloch *et al.* 1991), red-spotted grouper *Epinephelus akaara* (Nishizawa *et al.* 1995), striped jack *Pseudocaranx dentex* (Mori *et al.* 1992), Japanese flounder *Paralichthys olivaceus* (Nishizawa *et al.* 1995), tiger puffer *Takifugu rubripes*, kelp grouper *Epinephelus moara* (Munday *et al.* 2002) and barfin flounder *Verasper moseri* in Japan (Nishizawa *et al.* 1995), and recently in golden grey mullet *Liza aurata* and leaping mullet *Liza saliens* in the Caspian Sea (Zorriehzahra *et al.* 2016).

Infections caused by NNV have been detected all around the world, with the notable exception of South America (Crane & Hyatt 2011; Shetty *et al.* 2012). It was the cause of mass mortality in Atlantic halibut in Norway and Scotland (Grotmol *et al.* 1997; Starkey *et al.* 2000) and in juvenile greasy grouper *Epinephelus tauvina* in Singapore (Hegde *et al.* 2002) and in groupers in Taiwan (Chi *et al.* 1997). Betanodaviruses have been the cause of high economical losses in aquaculture industry throughout the Mediterranean area. Mass mortalities have been repeatedly recorded since 1991 on larvae and juvenile stages in European sea bass in France (Breuil *et al.* 1991) as well as on grow-out size sea bass in Greece, Italia and Tunisia (Le Breton *et al.* 1997; Bovo *et al.* 1999; Thiéry *et al.* 2004; Haddad-Boubaker *et al.* 2013). Grey mullet *Mugil cephalus*, red drum *Sciaenops ocellatus* and barramundi cultured in Israel were also reported to be affected by NNV (Ucko, Colorni & Diamant 2004). Farmed Senegalese sole *Solea senegalensis* were reported as infected by RGNNV and SJNNV in Spain (Thiéry *et al.* 2004; Hodneland *et al.* 2011). More recently, RGNNV, SJNNV genotypes and reassortant RGNNV/SJNNV and SJNNV/RGNNV viruses have been reported to infect several fish species (European sea bass, sea bream, Senegalese sole) in Mediterranean Sea (Toffolo *et al.* 2007; Oliveira *et al.* 2009; Panzarin *et al.* 2012; Haddad-Boubaker *et al.* 2013; Toffan *et al.* 2016). A strain belonging to the RGNNV species caused mass mortality in white sea bass *Atractoscion nobilis* reared in South California in 1999 (Curtis *et al.* 2001). NNV was also found in Atlantic cod and haddock *Melanogrammus aeglefinus* juvenile stages on the Atlantic coast of North America (Johnson *et al.* 2002). Furthermore, betanodaviruses do not only affect reared fish species, but have also been found in a variety of wild fish species, as reported in Table 2.

Table 2 Fish species influenced by viral encephalopathy and retinopathy (VER)/viral nervous necrosis (VNN)

Host species		Latin name		Species	Key ref.
Oder	Family	Common name	Latin name	Species	Key ref.
Marine species					
Farmed species					
Decapoda	Penaeidae	Whiteleg shrimp	<i>Litopenaeus vannamei</i>	PVNV	Tang et al. (2007)
Scorpaeniformes	Sebastidae	Black rockfish	<i>Sebastes inermis</i>	RGNNV	Gomez et al. (2004)
		Oblong rockfish	<i>Sebastes oblongus</i>		
		Spotbelly rockfish	<i>Sebastes pachycephalus</i>		
Pempheriformes	Lateolabracidae	Chinese seabass	<i>Lateolabrax sp.</i>	SJNNV	Cutrin et al. (2007)
Perciformes	Sparidae	Red seabream	<i>Pagrus major</i>	SJNNV	Yoshikoshi & Inoue (1990), Nishizawa et al. (1997)
	Oplegnathidae	Gillhead sea bream	<i>Sparus aurata</i>		
		Japanese parrotfish	<i>Oplegnathus fasciatus</i>		
		(Barred knifejaw)			
	Centropomidae	Japanese sea bass	<i>Lateolabrax japonicus</i>	RGNNV	Mori et al. (2003)
	Sciaenidae	White sea bass	<i>Atractoscion nobilis</i>	RGNNV	Curtis et al. (2001)
	Percichthyidae	European sea bass	<i>Dicentrarchus labrax</i>	RGNNV/SJNNV	Breuil et al. (1991), Thiéry et al. (2004)
	Scombridae	Pacific bluefin tuna	<i>Thunnus orientalis</i>	RGNNV	Sugaya et al. (2009)
	Rachycentridae	Cobia	<i>Rachycentron canadum</i>	RGNNV	Chi et al. (2003)
	Carangidae	Yellow-wax pompano	<i>Trachinotus falcatus</i>		
		Striped jack	<i>Pseudocaranx dentex</i>	SJNNV/TPNNV	Mori et al. (1992), Nishizawa et al. (1997)
		Golden pompano	<i>Trachinotus blochii</i>	RGNNV	Ransangan et al. (2011)
	Serranidae	Humpback grouper	<i>Cromileptes altivelis</i>	RGNNV	Yuasa, Koesharyani & Mahardika (2007)
		Dragon grouper	<i>Epinephelus lanceolatus</i>	RGNNV	Lin et al. (2001)
		Red-spotted grouper	<i>Epinephelus akaara</i>	RGNNV	Nishizawa et al. (1997)
		Black-spotted grouper	<i>Epinephelus fuscogutatus</i>	RGNNV	Chi et al. (1997)
		Sevenband grouper	<i>Epinephelus septemfasciatus</i>	SJNNV	Fukuda et al. (1996)
		Greasy grouper	<i>Epinephelus tauvina</i>	GGNNV	Hegde et al. (2002), Tan et al. (2001)
		Orange-spotted grouper	<i>Epinephelus coioides</i>	RGNNV	Chi et al. (1999)
		Brown-spotted grouper	<i>Epinephelus malabaricus</i>	RGNNV	Nishizawa et al. (1997)
		Yellow grouper	<i>Epinephelus awoara</i>	RGNNV	Lai et al. (2001)
		Kelp grouper	<i>Epinephelus moara</i>	Undefined	Munday et al. (2002), Nishizawa et al. (1997)
		Tiger puffer	<i>Takifugu rubripes</i>	TPNNV	Thiéry et al. (2004)
	Tetraodontidae	Senegalese sole	<i>Solea senegalensis</i>	SJNNV	Nishizawa et al. (1995)
	Soleidae	Barfin flounder	<i>Verasper moseri</i>	BFNNV	Grotmol et al. (1997)
	Pleuronectidae	Atlantic halibut	<i>Hippoglossus hippoglossus</i>	BFNNV	Nishizawa et al. (1995)
		Japanese flounder	<i>Paralichthys olivaceus</i>	SJNNV	Johansen et al. (2004)
	Paralichthyidae	Turbot	<i>Scophthalmus maximus</i>	TNV	Bloch et al. (1991)
	Scophthalmidae	Barramundi/Asian sea bass	<i>Lates calcarifer</i>	RGNNV	Mori et al. (2003)
Perciformes	Centropomidae	Pacific cod	<i>Gadus macrocephalus</i>	BFNNV	Johnson et al. (2002)
Gadiformes	Gadidae	Atlantic cod	<i>Gadus morhua</i>	BFNNV	Gagné et al. (2004)
		Haddock	<i>Melanogrammus aeglefinus</i>	BFNNV	

Table 2 Continued

Host species	Family	Common name	Latin name	Species	Key ref.
Wild species					
Oder					
	Epigonidae	Cardinal fish	<i>Epigonus telescopus</i>	Undefined	Giopello et al. (2013)
	Serranidae	Wild dusky grouper	<i>Epinephelus marginatus</i>	RGNNV	Vendramin et al. (2013)
	Sparidae	Wild golden grouper	<i>Epinephelus costae</i>	RGNNV	Ciulli et al. (2007)
	Mugilidae	Bogue	<i>Boops boops</i> (L.)	RGNNV	Zorriehzahra et al. (2016)
		Flathead grey mullet	<i>Mugil cephalus</i> (L.)	RGNNV	Ciulli et al. (2007)
		Golden grey mullet	<i>Liza aurata</i>	RGNNV	
		Leaping mullet	<i>Liza saliens</i>	RGNNV	
	Gobiidae	Red mullet	<i>Mullus barbatus barbatus</i> (L.)	RGNNV	
	Carangidae	Black goby	<i>Gobius niger</i> (L.)	RGNNV	Gomez et al. (2004)
		Horse mackerel	<i>Trachurus trachurus</i>	RGNNV	
		Japanese scad	<i>Decapterus maruadsi</i> (Temminck & Schlegel)	RGNNV	
	Lepisosteidae	Garpike (Longnose Gar)	<i>Lepisosteus osseus</i>	RGNNV	Ciulli et al. (2007)
	Pleuronectidae	Wild winter flounder	<i>Pleuronectes americanus</i>	BFNNV	Gagné et al. (2004)
	Notacanthidae	Shortfin spiny eel	<i>Notacanthus bonaparte</i>	Undefined	Giopello et al. (2013)
	Beryciformes	Mediterranean slimehead	<i>Hoplostethus mediterraneus mediterraneus</i>		
	Gadiformes	Glasshead grenadier	<i>Hymenocephalus italicus</i> (Giglioli)	RGNNV	Ciulli et al. (2007)
	Gadidae	Whiting	<i>Merlangi merlangus</i> (L.)		
	Merlucciidae	European hake	<i>Merluccius merluccius</i> (L.)		
	Clupeidae	European pilchard	<i>Sardina pilchardus</i> (Walbaum)		
	Triglidae	Gurnard	<i>Chelidonichthys lucerna</i> (L.)		
	Sebastidae	Marbled rockfish	<i>Sebastes marmoratus</i> (Cuvier)	RGNNV	Gomez et al. (2004)
	Monacanthidae	Threadtail filefish	<i>Stephanolepis cirrifer</i> (Temminck & Schlegel)		
		Black scrapper	<i>Thamnaconus modestus</i> (Gunther)		
	Portunidae	Charybdis crab	<i>Charybdis bimaculata</i>	RGNNV	Gomez et al. (2008)
	Pandalidae	Southern humpback shrimp	<i>Pandalus hypsinotus</i>		
	Mytilidae	Mediterranean mussel	<i>Mytilus galloprovincialis</i>		
Freshwater species					
Farmed species					
	Acipenseriformes	Sturgeon	<i>Acipenser gueldenstaedi</i>	SJNNV	Athanassopoulou et al. (2004)
	Anguilliformes	European eels	<i>Anguilla anguilla</i>	RGNNV	Chi et al. (2003)
	Siluriformes	Chinese catfish	<i>Parasilurus asotus</i>	Undefined	Shetty et al. (2012)
		Australian catfish	<i>Tandanus tandanus</i>	Undefined	
		Sleepy cod	<i>Oxyeleotris lineolatus</i>	RGNNV	Bovo et al. (2011)
		Largemouth black bass	<i>Micropterus salmoides</i> (Lacepede)		
		Pike-perch	<i>Sander lucioperca</i>		
		Tilapia	<i>Oreochromis niloticus</i>	RGNNV	Bigarré et al. (2009)
		Giant freshwater prawn	<i>Macrobrachium rosenbergii</i>	MNV	Bonami & Widada (2011)

Table 2 Continued

Host species	Order	Family	Common name	Latin name	Species	Key ref.
Ornamental/ model fish species	Cyprinodontiformes	Poeciliidae	Guppy	<i>Poecilia reticulata</i>	RGNNV	Hegde <i>et al.</i> (2003)
		Cyprinidae	Zebrafish	<i>Danio rerio</i>	RGNNV	Lu <i>et al.</i> (2008)
	Perciformes	Blenniidae	Goldfish	<i>Carassius auratus</i>	RGNNV	Binesh (2013)
			Freshwater blenny	<i>Salaria fluviatili</i>	RGNNV	Vendramin <i>et al.</i> (2012)
			Medaka	<i>Oryzias latipes</i>	RGNNV	Furusawa, Okinaka & Nakai (2006)

Regarding environment, although NNV is mostly known for infecting aquatic animals in marine and brackish water, the reports of freshwater species infected by NNV have been increasing (Table 2). NNV infection was observed in freshwater eel and catfish aquaculture systems in Taiwan (Chi *et al.* 2003) as well as in other freshwater species including sturgeon *Acipenser gueldenstaedtii* (Athanasopoulou, Billinis & Prapas 2004), tilapia *Oreochromis niloticus* (Bigarré *et al.* 2009), largemouth bass *Micropterus salmoides*, pike-perch *Sander lucioperca*, striped bass × white bass, *Morone saxatilis* × *Morone chrysops* (Bovo *et al.* 2011), guppy *Poecilia reticulata* (Hegde *et al.* 2003), Australian catfish *Tandanus tandanus* and sleepy cod *Oxyeleotris lineolatus* (Munday *et al.* 2002). Zebrafish *Danio rerio* and goldfish *Carassius auratus* were also found to be infected (Binesh 2013). Furthermore, the freshwater blenny *Salaria fluviatili*, which is an endangered species endemic to watersheds of the Mediterranean Basin, was also reported as affected by NNV (Vendramin *et al.* 2012). To date, the susceptibility of Mandarin fish *Siniperca chuatsi* to RGNNV, an important economical species in freshwater aquaculture in China, has been demonstrated (Tu *et al.* 2016). At present, at least 70 host species belonging to 32 families of 16 orders have been described as carriers of betanodavirus (Table 2) and this disease is widely reported all over the world, with the exception of South America.

Transmission

NNV is characterized by both vertical and horizontal transmissions (Munday *et al.* 2002; see also Fig. 2). Vertical transmission was early described in a number of different fish species where betanodaviruses were detected in broodstock gonads or in early larval stages with typical symptomatic signs. It can occur from broodstock to larvae through germplasm, including the eggs or genital fluids as reported in striped jack, in barfin flounder or in European sea bass (Mushiaki *et al.* 1994; Nishizawa, Muroga & Arimoto 1996; Mori, Mushiaki & Arimoto 1998; Dalla Valle *et al.* 2000; Watanabe, Nishizawa & Yoshimizu 2000; Breuil *et al.* 2002).

Horizontal transmission is a very difficult route to control because betanodavirus can easily spread during an outbreak via water but also rearing

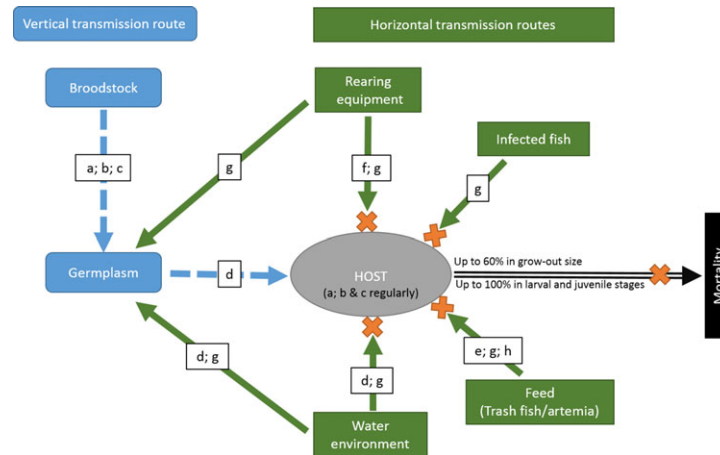


Figure 2 The different transmission routes of betanodaviruses and possible prevention modes. Blue discontinuous arrows represent vertical transmission routes; green arrows represent horizontal transmission routes; orange crosses display possible actions of genetics (by improving for fish natural barriers to infections or resistance/tolerance – see section ‘Selective breeding to nervous necrosis virus (NNV) resistance: prospective procedure’); host represents either larvae/juvenile/grow-out size or broodstock; the possible prevention modes are as follows: a: vaccination; b: serological diagnostic (ELISA) to screen and eliminate seropositive individuals; c: direct diagnostic (RT-qPCR) to screen and eliminate positive individuals or germplasm; d: ozone/UV/bleach water treatments; e: strict control of feed input to avoid NNV infected trash fish; f: unique equipment kit for each tank/pond/cage and adapted decontamination of equipment after use; g: biosecurity measures during all production cycles; h: ozone treatment of artemia before feeding.

equipment (Mori *et al.* 1998; Watanabe *et al.* 1998). Horizontal transmission has been experimentally demonstrated by several routes: contact between healthy fish and diseased larvae (Arimoto *et al.* 1993), bathing fish in water containing betanodavirus-infected tissue homogenates (Arimoto *et al.* 1993; Tanaka, Aoki & Nakai 1998; Grotmoll, Berghl & Totland 1999), contamination using strains isolated from symptomatic fish (Koch postulate) (Thiéry *et al.* 1997; Peducasse *et al.* 1999) or contact of healthy fish with asymptomatic carriers (Skliris & Richards 1999; Breuil *et al.* 2002).

Once in the aquatic environment, betanodavirus can persist without host for a long time and can be spread widely by tide, aquatic transport means or migration of the wild hosts (Gomez *et al.* 2004, 2008; Giacobello *et al.* 2013). As NNV was reported in sand worms belonging to the family *Nereidae* (Liu *et al.* 2006a) but also in crabs and mussels (Gomez *et al.* 2008), several studies are carried out to clarify the existence of non-fish carriers or vectors of NNV such as raw fish (trash fish), brine shrimp *Artemia salina* and mollusks used as feed for marine culture (Gomez *et al.* 2010; Costa & Thompson 2016). Commercial trade of aquatic animals should also be regarded

as an important potential source of virus diffusion (Gomez *et al.* 2006).

Diagnosis/detection

First diagnostic approaches

In the early 1990s, the structure of NNV was already clearly known, but virus isolation using cell lines was not successful. Therefore, the method of VNN diagnostic relied on the observation of characteristic clinical signs. VNN is characterized by typical behavioural abnormalities (erratic swimming patterns such as spiralling or whirling, lying down at the tank bottom, rapid swimming, darker coloration) associated with an impairment of the nervous system (Fig. 3) (Yoshikoshi & Inoue 1990; Breuil *et al.* 1991; Chi *et al.* 1997). Gross pathology examination frequently reveals a hyperinflation of the swim bladder and haemorrhages on the brain tissue. The most common microscopical findings consist of vacuolation and necrosis of nervous cells of the spinal cord, brain and/or retina, particularly in larvae and juveniles stages. The infection is rarely accompanied by inflammatory processes. In the presence of these typical signs, diagnosis must be confirmed

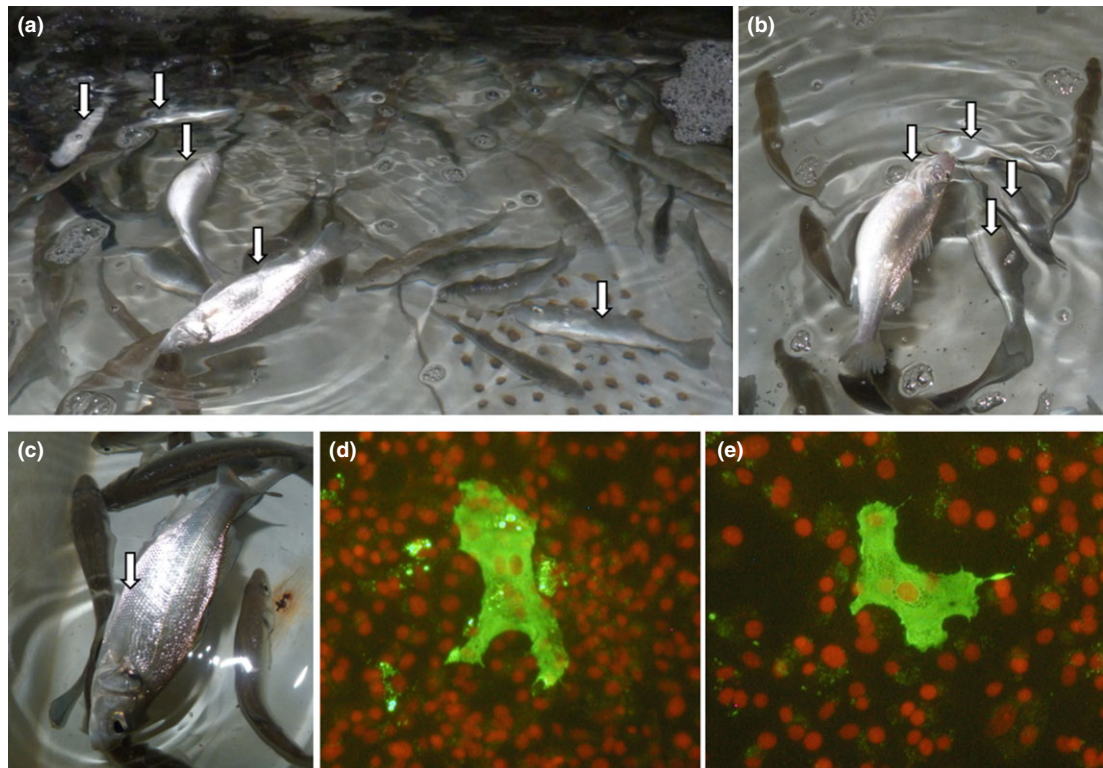


Figure 3 (a–c) Typical clinical signs observed during experimental nervous necrosis virus (NNV) infection in European sea bass (arrows show impacted fish). (d, e) Positive immunofluorescence antibody test signal (in green) obtained for betanodaviruses on SSN1 cell line. Source: Anses, Ploufragan-Plouzané Laboratory, Viral diseases of fish Unit.

by a laboratory test. Electronic microscopy allowed observation of virus particles free or membrane bound by endoplasmic reticulum in cells collected from infected organs (brain, retina) and revealed icosahedral, non-enveloped viruses with a commonly reported diameter of 20–34 nm (Glazebrook *et al.* 1990; Yoshikoshi & Inoue 1990; Bloch *et al.* 1991; Breuil *et al.* 1991; Mori *et al.* 1992; Grotmol *et al.* 1997). Over two decades, the reference method to detect betanodavirus was isolation in permissive cell culture (striped snakehead cells SSN-1 or E11) followed by immunological (indirect fluorescent antibody test – IFAT, immunohistochemistry, enzyme-linked immunosorbent assay – ELISA; Nuñez-Ortiz *et al.* 2015) or molecular identification (RT-PCR, nested RT-PCR, real-time RT-PCR). However, cell culture is time-consuming and requires a great experience, and some NNV strains are not always easy to detect because of a poor cultivability and/or the absence of induction of clear cytopathic effects. This is why molecular methods, particularly real-time RT-PCR, have been increasingly used (Munday *et al.* 2002; Shetty *et al.* 2012).

Direct molecular methods

Numerous RT-PCR protocols have been described for the detection of VNN (Table 3). The first RT-PCR published designed a set of primers (F2/R3) directed against 430 bp from the T4 variable region of the RNA2 segment of a SJNNV strain isolated from striped jack (Nishizawa, Nakail & Muroga 1994). Later on, the same region was amplified from other isolates, such as red-spotted grouper (Nishizawa *et al.* 1995). This test, recommended by the World Organization for Animal Health (OIE) until 2006, was extensively used for routine diagnostic and genotyping of betanodavirus and led to the current classification (Nishizawa *et al.* 1995, 1997). However, the sensitivity of this method is limited not only by a low viral load but also by the genetic diversity of the T4 region that leads to mismatches between the F2/R3 primers and their targets (Nishizawa *et al.* 1996; Thiéry *et al.* 1997; Dalla Valle *et al.* 2001). In some cases, it has been illustrated that betanodavirus in brain could be detected by

Table 3 Primers/probes sets used for betanodavirus detection by RT-PCR

Primer/Probe	Target	GenBank accession number ^a	Sequence 5' – 3'	Position	Key Ref.
VNNV1	RNA2	AB056572	ACACTGGAGTTTGAATTCA	343–362	Dalla Valle <i>et al.</i> (2000)
VNNV2			GTCTTTGTTGAAGTTGTCCCA	953–934	
VNNV3			ATTGTGCCCGCAAACAC	366–383	
VNNV4			GACACGTTGACCACATCAGT	620–601	
AH95-F1	RNA2	AJ245641	AGTGTGTGTGCTGGAGTG	577–596	Grotmoll & Totlandl (2000)
AH95-R1			CGCCCTGTGTGAATGTTTTG	917–898	
F2	RNA2	AB056572	CGTGTCAATCATGTGTGCT	592–611	Nishizawa <i>et al.</i> (1994)
R3			CGAGTCAACACGGGTGAAGA	1017–998	
F'2	RNA2	Y08700	GTTCCCTGTACAACGATTCC	677–693	Thiéry <i>et al.</i> (1999a,b)
R'3			GGATTTGACGGGGCTGTCTA	970–951	
Q-CP-1	RNA2	D38636	CAACTGACAACGATCACACCTTC	234–256	Dalla Valle <i>et al.</i> (2005)
Q-CP-2			CAATCGAACACTCCAGCGACA	463–443	
P1	RNA2	AJ245641	GGTATGTGCGAGAATCGCCC	141–159	Grove <i>et al.</i> (2006)
P2			TAACCACCGCCCGTGT	351–335	
Probe			TTATCCCAGCTGGCACCGGC ^b	183–202	Hick & Whittington (2010)
qR2TF	RNA2	<i>LcNNV09_07</i> ^c	CTTCCTGCCTGATCCAACTG	378–397	
qR2TR			GTTCTGCTTTCCACCATTTG	470–451	
R2probe2			CAACGACTGCACCACGAGTTG ^b	448–428	
RNA2 FOR RNA2	RNA2	DQ864760	CAACTGACARCGAHCACAC	392–410	Panzarin <i>et al.</i> (2010)
REV probe			CCCACCAYTTGGCVAC	460–445	
			TYCARGRACTCGTGGTGCVG ^b	422–442	
Nod1f	RNA2	EF617335; AY744705;	TTCCAGCGATACGCTGTTGA	322–341 ^d	Hodneland <i>et al.</i> (2011)
Nod1r		AF175511; AB056572;	CACCGCCCGTGTGTTGC	376–391 ^d	
		AJ608266; D38637;	AAATTCAGCCAATGTGC ^b	356–372 ^d	
		D38635			
Nod2f	RNA2	EF617335; AY744705;	CTGGGACACGCTGCTAGAATC	301–321 ^d	Hodneland <i>et al.</i> (2011)
Nod2r		AF 175511; AB056572;	TGGTCTGTGTCAGTTGGATCA	414–434 ^d	
		AJ608266; D38637;	AAATTCAGCCAATGTGC ^b	356–372 ^d	
		D38635			
RG-RNA2-F2:	RNA2	D38636	CGTCCGCTGTCCATTGACTA	624–643	Lopez-Jimena <i>et al.</i> (2011)
RG- RNA2-R2:			CTGCAGGTGTGCCAGCATT	723–705	
oPVP111	RNA2	AF245003; AF245004;	TCCTGCCTGAYCCAATGAC	381–400 ^e	Bigarré <i>et al.</i> (2010)
oPVP88		AF281657; AF499774;	TGGTCATCMACGATACGCAC	1058–1039 ^e	
		AJ245641; AJ608266;			
		D30814; U39876;			
		EF433468; AY549548;			
		EU236149			
Q-RdRP-1	RNA1	D38636	GTGTCCGGAGAGGTTAAGGATG	589–610	Dalla Valle <i>et al.</i> (2005)
Q-RdRP-2			CTTGAATTGATCAACGGTGAACA	861–839	
RG-RNA1-F:	RNA1	AY369136	GGCTCAGATCTGGTAATGTTCAA	2144– 2167	Lopez-Jimena <i>et al.</i> (2011)
RG-RNA1-R:			CAAAGCCAAGGGAAGAAGCA	2206–2187	
oPVP154	RNA1	AJ401165; EF617335;	TCCAAGCCGGTCTAGTCAA	2717–2736 ^f	Baud <i>et al.</i> (2015)
oPVP155		EU826137; AB025018;	CACGAACGTKCGCATCTCGT	2884–2865 ^f	
Taqman-Probe		AB056571; AF319555;	CGATCGATCAGCACCTSGTC ^a		
		GQ402010; GQ402012;			
		AY690597			

^aSequences from which the primers or probes have been designed; ^bLabel position on probes; ^cThe design of primers and probe was achieved on an isolate obtained from a infected barramundi sampled but not reported in GenBank (Hick & Whittington 2010); ^dThe position of the primers and probe is based on SJNNV genome (AB056572); ^eThe position of the primers and probe is based on BFNNV genome (AY549548); ^fThe position of the primers and probe is based on BFNNV genome (AJ401165).

immunohistochemistry, whereas the same samples were negative by RT-PCR (Thiéry *et al.* 1997). In addition, low or false-positive and also false-negative results were reported in different fish species like striped jack, barfin flounder, European sea bass, shi drum *Umbrina cirrosa* and gilthead sea bream (Nishizawa *et al.* 1996; Mori *et al.* 1998; Thiéry *et al.* 1999a,b; Dalla Valle *et al.* 2000;

Watanabe *et al.* 2000). To improve the performance of this test and take into account genetic diversity reported in newly available sequences, further generations of tests were developed. Primers specific to more conserved region of the RNA2 or allowing to discriminate Mediterranean and Atlantic viral strains were published as well as nested-PCR approaches allowing to improve the

sensitivity by at least 100 times (Thiéry *et al.* 1999a,b; Dalla Valle *et al.* 2000). More recently, Bigarré and colleagues designed a new set of primers in a highly conserved region (680 bp) named T6 in RNA2, which perfectly matches with a wide range of published sequences and detects at least three of the five described species namely RGNNV, SJNNV and BFNNV (Bigarré *et al.* 2010).

Since 2005, numerous real-time RT-PCR assays were developed to regularly adapt the primer sets and probes to newly published sequences (Dalla Valle *et al.* 2005; Fenner *et al.* 2006a; Hick & Whittington 2010; Panzarin *et al.* 2010; Hodneland *et al.* 2011; Baud *et al.* 2015). These real-time RT-PCR assays, targeting RNA1 or RNA2, are now currently used for the diagnosis of betanodavirus because they are less time-consuming than classical approaches and significantly decrease cross-contamination occurring during post-amplification procedures (Hick & Whittington 2010; Hodneland *et al.* 2011). Recently, a one-step generic TaqMan[®] method targeting sequences found in a vast majority of known viral genotypes was validated and efficiently used to detect NNV in different geographical regions and host species (Panzarin *et al.* 2010; Baud *et al.* 2015), and an optimized loop-mediated isothermal amplification has been developed to detect NNV in *Epinephelus septemfasciatus* (Hwang *et al.* 2016). This last method showed improved sensitivity compared with PCR.

Detection of different NNV species coexisting in the same host is still complex and may require a combination of approaches (Lopez-Jimena *et al.* 2010). An ubiquitous assay detecting all species would be desirable, but because of the high genetic diversity of betanodavirus, selection of specific and wide spectrum primers allowing the detection of all possible variants still remains a big challenge (Hodneland *et al.* 2011).

Indirect serological methods

Serological investigations have been developed for several viral fish diseases, but only few of them are used for routine surveillance, despite the fact that diseases survivors often become latent carriers with significant antibody response. The major reasons for this are poor knowledge on the kinetics of the antibody response in fish at various water temperatures and lack of validation data. Nevertheless,

several ELISA or serum neutralization tests described and improved over time proved their efficiency to detect antibodies specific to VNN (Watanabe *et al.* 1998; Huang *et al.* 2001; Fenner *et al.* 2006b; Scapigliati *et al.* 2010; Choi *et al.* 2014; Jaramillo *et al.* 2016b). For ELISA tests, the determination of the cut-off point is critical to make the distinction between virus free status and viral infection. These indirect methods are routinely used by several fish farms to regularly screen breeders. They have the advantage to be no-lethal and safe for fish and allow a regular screening of the VNN serological status of a population at an individual level (Breuil & Romestand 1999; Watanabe *et al.* 2000; Breuil *et al.* 2002; Nuñez-Ortiz *et al.* 2015; Jaramillo *et al.* 2016a).

Control procedures

There are no simple and effective procedures to treat the viral disease in fish once established. Therefore, efforts were concentrated on the means and tools to prevent entry, diffusion and persistence of the virus, mostly strict hygiene, vaccination and eradication of infected populations (Gomez-Casado, Estepa & Coll 2011; Shetty *et al.* 2012).

In hatcheries, an important route of virus entry is infected asymptomatic breeders (Mushiaki *et al.* 1994; Watanabe *et al.* 1998). Although ozonation can seemingly prevent NNV transmission from infected broodstock, it is not fully efficient because betanodavirus is not only present on the surface of the eggs but also inside the eggs, and can also penetrate the egg via spermatozoa (Kuo *et al.* 2012). A positive point is that vertical transmission can be controlled effectively in hatcheries by combining detection via serological tests (ELISA) to detect anti-VNN specific antibodies (in the blood serum of broodstock) or/and sensitive RT-PCR assays to recognize viral RNA (in the eggs or genital fluids), combined with the elimination of positive individuals (Mushiaki *et al.* 1994; Breuil & Romestand 1999; Watanabe *et al.* 2000; Breuil *et al.* 2002; Hodneland *et al.* 2011). Ozonation and ultraviolet light are also used to clean fertilized eggs and control water quality during rearing larval and juvenile stages. Even if treatment of larvae requires complicated procedures, these treatments appear effective to prevent horizontal transmission (Arimoto *et al.* 1996; Watanabe *et al.* 1998; Grotmoll &

Totlandl 2000). Although betanodaviruses can be prevented effectively in hatchery by the management of betanodavirus-free broodstock and disinfecting the hatchery water, the fish can be infected by betanodaviruses from the environment when they are cultured at grow-out stages.

Vaccination has been considered as an effective procedure for controlling VNN disease. A number of vaccines made with inactivated NNV, virus-like particles (VLPs), recombinant C protein and synthetic peptides from the C protein have been tested (Gomez-Casado *et al.* 2011). Recombinant betanodavirus coat proteins expressed in *Escherichia coli* was firstly proposed in different fish species like sevenband grouper *Epinephelus septemfasciatus* and humpback grouper *Cromileptes altivelis* (Tanaka *et al.* 2001; Yuasa *et al.* 2002), turbot and Atlantic halibut (Húsgarð *et al.* 2001; Sommerset *et al.* 2005). More recent constructions combined to artemia or *Vibrio anguillarum* induced significant levels of protection in larvae of orange-spotted grouper *Epinephelus coioides* (Lin *et al.* 2007; Chen *et al.* 2011), and enhanced virus-neutralizing antibody response was observed after immunization at grow-out stages with recombinant C protein (Sommerset *et al.* 2005). Virus-like particles have also been developed to create a more effective procedure to control VNN disease (Liu *et al.* 2006b; Thiéry *et al.* 2006). To date, the efficiency of the pFNCPE42-DNA vaccine, which has been developed using the capsid protein gene of an Indian isolate of fish nodavirus, has been illustrated in Asian sea bass with a high relative percentage survival of 77.33% (Vimal *et al.* 2016). All these types of vaccines are usually applied by injection method. Consequently, they are only really effective on grow-out size fish or to prevent vertical transmission in breeding, while the VNN disease often occurs in early larval and juvenile stages at which the size of fish is too small to allow vaccination by injection (Sommerset *et al.* 2005; Kai & Chi 2008; Brudeseth *et al.* 2013). A water-delivery strategy (immersion) could represent a more interesting way of control (Kai & Chi 2008) but still needs to be improved.

The viral diversity of betanodavirus with at least four different species described is another challenge to overcome for which DNA vaccines have numerous advantages compared with traditional antigen vaccines (Gomez-Casado *et al.* 2011). However, no licence has been delivered to date for potential applications in commercial fish farms

in some areas such as Europe (Gomez-Casado *et al.* 2011; Brudeseth *et al.* 2013). The vaccine application is usually expensive in fish and the protection generated often lasts for in short time because of the low immune reactivity in early stages of life (Sommerset *et al.* 2005). For these disadvantages, although a variety of vaccinations for NNV have been experienced (Table 4), only one inactivated RGNNV vaccine against NNV of sevenband grouper was commercialized in Japan (Brudeseth *et al.* 2013). Nevertheless, work in progress to better understand the immune mechanisms involved during NNV infection (Carballo *et al.* 2016; Costa & Thompson 2016; Wu *et al.* 2016) will likely result in the near future in the improvement in the prophylactic strategies, like the use of preventive administration of interferons at the larval stage (Kuo *et al.* 2016) or of ribavirin as antiviral agent (Huang *et al.* 2016).

Selective breeding to NNV resistance: prospective procedure

While selective breeding programmes have been mostly targeting productivity traits like growth and carcass quality (Gjedrem & Thodesen 2005), disease resistance remains a major goal for breeding programmes, as mortality caused by diseases is a major threat to aquaculture. Selecting fish with increased resistance to specific diseases seem to be feasible for most diseases (reviewed by Gjedrem 2015). Moreover, it provides cumulative and permanent improvement in resistance over generations at the population level, thus providing unique benefits when compared to other methods. Due to its cost however, the selective breeding strategy towards resistant cultured fish is particularly interesting when other prevention methods are inefficient. The use of resistant populations would not only reduce outbreaks, but also lower the cost of fish production (Ødegård *et al.* 2011; Yáñez, Houston & Newman 2014a).

Disease resistance heritability in fish

Improving a trait by artificial selection basically requires sufficient genetic variation for this trait in the population. Genetic variation in disease resistance has been observed for many diseases, and most likely variation can be observed for all diseases (Bishop & Woolliams 2014). While heritability for resistance to viral diseases has been

Table 4 The different types of nervous necrosis virus (NNV) vaccine tested in fish

Type of vaccinations	Species	Method	Results/RPS	Key Ref.
<i>Inactivated vaccines</i>				
BEI-inactivated HGNNV vaccine	Orange-spotted grouper	Immersion	RPS = 79%	Kai & Chi (2008)
Formalin-inactivated vaccines	<i>Epinephelus coioides</i> (early larval stage-40 dph with average body weight (BW) of 0.2 g and TBL of 2.4 cm)		(BEI-inactivated NNV vaccines) 39% (Formalin-inactivated NNV vaccines)	
Formalin-inactivated vaccine (RGNNV)	Sevenband grouper <i>Epinephelus septemfasciatus</i> (juvenile-25.4 g)	Injection	60% in fish groups immunized with $10^{7.5}$ TCID ₅₀ per fish or higher doses.	Yamashita <i>et al.</i> (2009)
BEI-inactivated HGNNV vaccine	Adult Orange-spotted grouper <i>Epinephelus coioides</i> (mean body weight of 1.35 kg)	Injection	High efficiency	Kai <i>et al.</i> (2010)
Formalin-inactivated vaccine (RGNNV type)	Brown-marbled grouper <i>Epinephelus fuscoguttatus</i> (5 g)	Injection	86–100%	Pakingking <i>et al.</i> (2010)
<i>Recombinant vaccines</i>				
Recombinant capsid protein vaccine (Artemia-encapsulated recombinant <i>Escherichia coli</i> expressing the NNV capsid protein gene)	Orange-spotted grouper <i>Epinephelus coioides</i> (Larvae-35 dph)	Oral	64.5%.	Lin <i>et al.</i> (2007)
Recombinant capsid protein (<i>Vibrio anguillarum</i> -based oral vaccine)	Orange-spotted grouper <i>Epinephelus coioides</i> (fry)	Oral	78.3%	Chen <i>et al.</i> (2011)
Recombinant capsid protein (rT2 vaccine)	Turbot <i>Scophthalmus maximus</i> [weighing from 1 to 3 g (mean 1.8 g)]	Injection	82%	Húsgarð <i>et al.</i> (2001)
Recombinant capsid protein vaccine (recAHNV-C) & vaccine plasmid (called pAHNV-C)	Turbot <i>Scophthalmus maximus</i> (Juvenile-mean weight 2.2 g)	Injection	50% in fish groups immunized with recAHNV-C (10 mg) + pAHNV-C (5 mg) 57% in fish groups immunized with recAHNV-C (10 mg)	Sommerset <i>et al.</i> (2005)
Recombinant protein vaccine- <i>E. coli</i> BL21 (DE3)	Sevenband grouper <i>Epinephelus septemfasciatus</i> (28 g)	Injection	88% in fish groups immunized with $10^{3.4}$ TCID ₅₀ per fish	Tanaka <i>et al.</i> (2001)
<i>Virus-like particles (VLPs) vaccines</i>				
VLPs of GNNV	Dragon grouper <i>Epinephelus lanceolatus</i> (20 g) Malabar grouper <i>Epinephelus malabaricus</i> (20 g)	Injection	Significant efficiency	Liu <i>et al.</i> (2006b)
VLPs MGNNV VLPs (trial 1) SB2 VLPs (trial 2)	European sea bass <i>Dicentrarchus labrax</i> 66 g 22 g	Injection	71.7–89.4% 27.4–88.9%	Thiéry <i>et al.</i> (2006)
<i>DNA vaccines</i>				
pFNCPE42-DNA vaccine	Asian sea bass <i>Lates calcarifer</i> (juvenile stage)	Injection	77.33%	Vimal <i>et al.</i> (2016)

estimated in many species, it remains that most studies have been conducted in salmonids.

The heritability of resistance to viral diseases has been shown to be moderate to high in fish (Table 5). In the first place, resistance to VHS virus (VHSV) was found highly heritable

($h^2 = 0.57–0.63$) in rainbow trout (*Oncorhynchus mykiss*) when assessed by mortality (Dorson *et al.* 1995; Henryon *et al.* 2005), while it was little heritable ($h^2 = 0.11 \pm 0.10$ and 0.13) when resistance was assessed as the time until death following challenge (Henryon *et al.* 2002, 2005).

Table 5 Recent heritability estimates of resistance to viral diseases in farmed fish species

Pathogen	Species (host)	Heritability: h^2 (\pm SE)		Notes	Key ref.
		Binary traits	Time until death		
Viral Nervous Necrosis Viruses	Atlantic cod (<i>Gadus morhua</i>)	$h^2 = 0.75$ (± 0.11)		Threshold model (on the underlying scale)	Ødegård <i>et al.</i> (2010b)
		$h^2 = 0.68$ (± 0.14)		Threshold model (on the underlying scale)	Bangera <i>et al.</i> (2011)
VHS virus	Rainbow trout (<i>Oncorhynchus mykiss</i>)	$h^2 = 0.91$		CURE model	Bangera <i>et al.</i> (2013)
		$h^2 = 0.63$ (± 0.26)		Linear model (angular transformation)	Dorson <i>et al.</i> (1995)
Infectious salmon anaemia virus	Atlantic salmon (<i>Salmo salar</i>)		$h^2 = 0.13$	On the logarithmic-time scale	Henryon <i>et al.</i> (2002)
		$h^2 = 0.57$	$h^2 = 0.11$ (± 0.10)	Survival, liability scale	Henryon <i>et al.</i> (2005)
			$h^2 = 0.13$ (± 0.03) (O.S.)	Linear model (Observable scale)/On the underlying liability scale	Gjøen <i>et al.</i> (1997)
		$h^2 = 0.24$ (± 0.03)		Threshold model using cross-sectional data	Olesen <i>et al.</i> (2007)
		$h^2 = 0.318$ (± 0.022)		Threshold model (on the underlying scale)	Ødegård <i>et al.</i> (2007a)
		$h^2 = 0.319$ (± 0.022)		Threshold model (on the underlying scale)	Ødegård <i>et al.</i> (2007b)
Infectious pancreatic necrosis virus	Atlantic salmon (<i>Salmo salar</i>)	$h^2 = 0.37$		On the underlying liability scale	Kjøglum <i>et al.</i> (2008)
		$h^2 = 0.40$ (± 0.04)		On the underlying liability scale	Gjerde <i>et al.</i> (2009)
		$h^2 = 0.43$	$h^2 = 0.16$	Transformed to the liability scale/Observed	Guy <i>et al.</i> (2006)
		$h^2 = 0.31$		Linear model (Observable scale)	Wetten <i>et al.</i> (2007)
Salmon pancreas disease virus	Atlantic salmon (<i>Salmo salar</i>)	$h^2 = 0.55$		On the underlying liability scale	Kjøglum <i>et al.</i> (2008)
		$h^2 = 0.38$ (± 0.017)		On the underlying liability scale	Guy <i>et al.</i> (2009)
			$h^2 = 0.21$ (± 0.005)	Transformed to the liability scale/Linear model (Observable scale)	Norris <i>et al.</i> (2008)
Koi herpesvirus	Common carp (<i>Cyprinus carpio</i>)	$h^2 = 0.79$ (± 0.14)		On the underlying liability scale	Ødegård <i>et al.</i> (2010a)

Moderate-to-high heritabilities have been estimated for infectious salmon anaemia virus (ISAV), ranging from 0.13 to 0.26 on the observable scale and from 0.19 to 0.40 on the liability scale (Gjøen *et al.* 1997; Ødegård *et al.* 2007a; Olesen, Hung & Ødegård 2007; Kjøglum *et al.* 2008; Gjerde *et al.* 2009), while the heritability of infectious pancreatic necrosis virus (IPNV) resistance was also found to be moderate to high, ranging between 0.16 and 0.55 (Guy *et al.* 2006, 2009; Wetten *et al.* 2007; Kjøglum *et al.* 2008). Other viral diseases in fish also show moderate-to-high heritability, such as resistance to salmon pancreas disease virus (SPDV) in Atlantic salmon (*Salmo salar*) with a liability scale estimate of

0.21 ± 0.05 (Norris, Foyle & Ratcliff 2008), and koi herpesvirus (KHV) resistance ($h^2 = 0.79 \pm 0.14$) in common carp (*Cyprinus carpio*) (Ødegård *et al.* 2010a).

To date, a high heritability for NNV has been demonstrated, but only in Atlantic cod (Ødegård, Sommer & Præbel 2010b; Bangera *et al.* 2011, 2013). Ødegård *et al.* (2010b) compared the NNV resistance of three different groups of Atlantic cod including Norwegian coastal cod (CC), Northeast Atlantic cod (NEAC) and their F1 crossbreds. They showed that the highest survival was observed in CC (56%), followed by crosses (31%), whereas the survival rate of NEAC was only 10%. The estimated heritability for NNV

resistance was high on the observed scale (0.43 ± 0.07) and very high on the underlying scale (0.75 ± 0.11) (Ødegård *et al.* 2010b). Besides that, a high heritability for NNV resistance was also recorded (0.68 ± 0.14) by Bangera *et al.* (2011) who later on reported an extremely high heritability (0.91 using a cure model) for NNV resistance in the same species (Bangera *et al.* 2013). In addition, the genetic correlation between resistance to NNV and to a bacterial disease (vibriosis) was shown not to significantly differ from zero (Bangera *et al.* 2011). This lack of correlation is similar to other studies in salmonids which estimated the genetic correlation between resistance against ISAV and furunculosis (Gjøen *et al.* 1997; Ødegård *et al.* 2007b; Kjøglum *et al.* 2008) or VHSV and enteric red-mouth disease as well as rainbow trout fry syndrome (Henryon *et al.* 2005).

The heritability of resistance to viral disease is moderate to high in almost existing studies, indicating viral disease resistance can be improved significantly based on selective breeding in farmed fish – and the prospects for NNV resistance are especially good, due to the high to very high heritability estimate (only in Atlantic cod for the moment).

Genetic selection to viral disease resistance in fish

Following promising heritability estimates, experimental selective breeding for disease resistance has been undertaken and shown to be an effective solution to prevent the outbreak of viral diseases in farmed fish. In the end of the 1980s, selective breeding for resistance to VHSV in rainbow trout was successfully tried in France, resulting in an improved resistance in the second generation, with 0–10% mortality, compared with 70–90% in the control group (Dorson *et al.* 1995). In Denmark, relatively VHSV-resistant broodstock were selected from a challenge test and used to produce first- and second-generation gynogenetic offspring (Bishop & Woolliams 2014). Salmon commercial breeding programmes have included resistance to furunculosis, ISAV and IPNV since 1993 in Norway (Gjøen *et al.* 1997; Moen *et al.* 2009; Yáñez *et al.* 2014a,b). The effective of selective breeding for IPNV resistance in Atlantic salmon was illustrated by Storset *et al.* (2007), where the fish belonging to low- and high-resistant families were

challenged in both fresh water and sea water and obtaining significant differences in mortalities, which ranged from 29 to 32% in high resistance families to 66–79% in low resistance families in both fresh water and sea water.

Quantitative trait loci mapping for resistance to viral diseases. Identifying portions of the genome called quantitative trait loci (QTLs) linked to the disease resistance phenotype is expected to speed up the selection process using marker-assisted selection (MAS; Massault *et al.* 2008; Bishop & Woolliams 2014).

Most of the QTLs identified for resistance to viral diseases in cultured fish have been identified in Salmonids, the most successful example being the IPNV resistance QTL. Three highly significant QTLs were first identified using microsatellite and AFLP markers in a backcross of rainbow trout strains showing high and low resistance to IPNV, each explaining 13–15% of the phenotypic variance of the total phenotypic variance (Ozaki *et al.* 2001, 2007). For IPNV resistance in Atlantic salmon, even more significant QTLs have been identified (Houston *et al.* 2008, 2010; Moen *et al.* 2009; Gheyas *et al.* 2010), leading to a breakthrough with respect to the implementation of QTL in salmon breeding. A first QTL, producing a 75% difference in IPNV mortality between the alternative homozygotes, was mapped to linkage group 21 (LG21) (Houston *et al.* 2008). The same QTL was independently reported in 2009 in Norwegian population, where it explained 29% of the phenotypic variance (Moen *et al.* 2009). Gheyas *et al.* (2010) confirmed the resistance effect of the QTL from LG21 at the fry stage in fresh water, with a QTL heritability of 0.45 ± 0.07 on the liability scale and 0.25 ± 0.05 on the observed scale. In one family, 100% of the offspring homozygous for the susceptible QTL alleles died, whereas 100% of the offspring homozygous for the resistant QTL alleles survived (Gheyas *et al.* 2010).

QTLs for resistance to other viral diseases in Salmonids include QTLs for IHNV resistance (Palti, Parsons & Thorgaard 1999; Palti *et al.* 2001; Miller *et al.* 2004; Rodriguez *et al.* 2004; Barroso *et al.* 2008), ISAV resistance (Moen *et al.* 2004, 2007), VHSV resistance (Verrier *et al.* 2013) and salmonid alphavirus (SAV) resistance (Gonen *et al.* 2015). Like for IPNV, the IHNV QTLs explained a high part of the phenotypic

variance (up to 32.5% according to Barroso *et al.* 2008), while it was more limited for the ISAV QTL (6% of the phenotypic variance, Moen *et al.* 2007). In both cases, a significant association with MHC alleles was later demonstrated (Palti *et al.* 2001; Miller *et al.* 2004 for IHNV; Kjøglum *et al.* 2006 for ISAV).

About NNV resistance, five genomewide significant QTLs, explaining 68% of the phenotypic variance for resistance, detected based on 161 microsatellite markers in Atlantic cod (Baranski *et al.* 2010), a very high amount, which can be paralleled to the very high heritability of NNV resistance reported earlier. A later analysis with a 12K SNP array confirmed both the high proportion of variance explained by genomic markers, and the location of three of these QTLs (Yu *et al.* 2014). The latest QTLs related to NNV resistance identified based on 146 microsatellite markers in Asian sea bass. In that study, Liu *et al.* (2016) detected multiple QTLs for NNV resistance and survival time. However, a few proportion of the phenotypic variation were explained by those QTLs (2.2–4.1% for resistance and 2.2–3.3% for survival time).

Taken altogether, this information about the QTLs for resistance to viral diseases in fish is very promising for increasing the rate of resistance through selective breeding, especially as in many cases QTLs seem to be of large effect, which gives good prospects to improve genetic resistance in a relatively short term, by direct MAS or by introgression of QTLs from different populations (Bishop & Woolliams 2014). This possibility may especially develop as SNP markers become more and more available and affordable, due to their abundance and to fast technological developments, making both detection and selection of QTLs more economically realistic.

MAS and GS for viral disease in fish. Breeding-resistant fish based on survivors of challenge trials, although sometimes done, is generally undesirable due to the risk of vertical transmission of the pathogen. The usual way to overcome this limitation in conventional breeding is to perform sib selection. In sib selection, breeding candidates are kept in a pathogen-free environment and selected using family-wise estimated breeding values obtained from the survival of fish from the same families challenged with the disease. Another possible way to select resistant fish without exposing

them to the pathogen is the identification of relevant QTL and the application of molecular markers for MAS, or the direct use of genotype data to perform GS. With both methods, fish are selected based only on their genotype, either at specific QTL-linked markers in the case of MAS, or at many markers, which may not all be linked to the resistance in the case of GS. This allows to avoid any contact between the breeding candidate and the pathogen. In terms of efficiency, the advantage of MAS compared with conventional selection is expected to be largest when the trait under selection has a low heritability – which is not generally the case for viral disease resistance in fish – or when the trait is not measured on the breeding candidates – which conversely is typically the case for disease resistance (Gjedrem 2015). With simulated traits and populations, the accuracy of selection was improved significantly using MAS, compared with non-MAS in selective breeding in aquaculture (Sonesson 2007). Practical application of MAS in aquaculture breeding has been implemented for IPNV resistance Atlantic salmon in both Norwegian (Moen *et al.* 2009) and Scottish populations (Houston *et al.* 2010). Still, the limitation of MAS is that it requires prior knowledge of alleles that are associated with the traits of interest, which moreover have to be validated in the specific populations or even families under selection. Furthermore, MAS exploits only a limited part of the genetic differences between individuals, as it does not exploit the polygenic background variation, which may account for a large part of the genetic variance (Meuwissen, Hayes & Goddard 2016).

An alternative approach for more polygenic traits is GS. In this approach, genetic markers are used to cover the whole genome so that all QTLs, even non-statistically significant, are in linkage disequilibrium (LD) with at least one marker and selection is based on genetic values predicted from all the markers (Meuwissen, Hayes & Goddard 2001; Goddard & Hayes 2007; Meuwissen *et al.* 2016). The availability of high-density SNP arrays in livestock and now increasingly in aquaculture species is making both GS and genomewide association studies (GWAS) feasible. GWAS approaches allow studies of the genetic architecture of quantitative traits, while GS will improve the accuracy of selection in breeding programmes (Houston *et al.* 2014). In terms of present realization of these approaches, GWAS showed highly

significant association of several SNPs with resistance to IPNV, as well as population-level LD in salmon commercial populations (Houston *et al.* 2012). The implementation of such approaches is dependent on the development of SNP genotyping arrays, which for the time being have mostly been developed in salmonids, like a 130 K array for farmed and wild Atlantic salmon in Scotland (Houston *et al.* 2014), 160 K SNP markers were validated based on 200 K SNPs applied to different wild and farmed populations of Atlantic salmon (Europe population, North America population and Chile population) (Yáñez *et al.* 2014b), and a 57 K SNP chip which is now available for rainbow trout (Palti *et al.* 2014). A 12 K SNP array has been also developed in Atlantic cod, containing markers distributed across all 23 chromosomes (Yu *et al.* 2014). It was already used in a GWAS analysis for NNV resistance which revealed 29 genomewide significant SNPs for binary survival and 36 genome-wide significant SNPs for number of days fish survived, as well as high genomic heritabilities of 0.49 and 0.81 for the same traits, respectively (Bangera, Baranski & Lien 2014). Identification of SNPs is being performed in other species for which NNV resistance is a key issue, such as European sea bass (Tine *et al.* 2014; Palaiokostas *et al.* 2015) or Asian sea bass (Wang *et al.* 2015), which is promising for the development of GWAS or GS for NNV resistance in those species.

Conclusion

Viral encephalopathy and retinopathy is widespread all over the world except in South America. While many of the main marine species in aquaculture are affected by this disease, no simple and effective procedures are available to treat it. Even though VNN can be prevented in hatcheries based on efficient diagnostic methods to monitor the breeders and biosecurity measures during hatchery rearing, this disease still occurs on grow-out sites. Vaccination may be an efficient way to prevent disease occurrence, but because of the specific drawbacks of present vaccination methods and the difficulty to efficiently protect early larval stages, this tool is not fully effective in the case of VNN. Selective breeding has been demonstrated as an effective solution to select resistant aquaculture populations for several diseases, and new genomics-based methods allow to foresee even higher

efficiency of selective breeding for disease resistance in the near future. However, to reach the expectations of a practical GS, more genetic resources and more advanced studies are required for the vast majority of aquaculture species affected by NNV.

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