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Review

Countermeasures against viral diseases of farmed fish

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ABSTRACT

Farmed fish provide an increasing fraction of the human food supply, and are of major economic importance in many countries. As in the case of terrestrial agriculture, bringing together large numbers of animals of a single species (i.e., monoculture) increases the risk of infectious disease outbreaks, including viral infections. Aquaculture, in which farmed fish are kept at high population densities in close proximity with wild fish reservoirs, is ideal for the emergence of wild-type pathogens that exist benignly in local wild fish and/or the spreading of aquatic pathogens to wild fish that enter into or come into close proximity with net cages and with fish escaping from them. This paper provides a general review for the non-specialist of viral diseases of farmed fish and how they could be prevented or treated. It has five principal objectives: (1) to provide an update on the most important and emerging viral diseases of salmonid aquaculture; (2) to review general aspects of innate antiviral defense against virus infections in fish, including recent advances in antiviral signaling; (3) to discuss current principles and practices of vaccinating fish; (4) to review antiviral drugs that have activity against viruses of farmed fish, and current barriers to employing them in aquaculture; and (5) to discuss the growing use of "functional feeds" in salmonid aquaculture to mitigate viral diseases. In conclusion, despite the challenging aquatic environment, it is expected that well thought-out combinations of vaccination and immunostimulants and/or antiviral drugs could provide solid protection against viral diseases of farmed fish.

1. Introduction

The intensive rearing of fish, shellfish and algae, the fastest growing food-producing industry in the world (Hall et al., 2011), is highly vulnerable to infectious diseases, similarly to captive livestock agriculture (for example the poultry and swine industries). However, in contrast to terrestrial farmed animals, the strains used in aquaculture usually have been very recently derived from wild strains (DFO Science, 2011; Duarte et al., 2007; Gunnarsson, 2007; Rosenlund and Skretting, 2006; Svasand et al., 2004) and may not have had enough time to adapt to high-density confinement in the aquaculture environment (Rodroguez-Ramilo et al., 2011). This chronic stress (Snieszko, 1974; Wedemeyer, 1996; Weyts et al., 1999; Yada and Nakanishi, 2002) provides opportunities for the emergence of diseases caused by pathogens that may be harmless under natural conditions.

The techniques and systems used in farming fish are as diverse as the species currently being raised, and also vary by geographic region, but normally encompass three stages: incubation/hatchery seed production, early rearing and on-growing (briefly reviewed by Roberts and Shepherd, 1974). For example, aquaculture can be carried out in still water ponds, raceway systems, fresh-water gravity tanks and recirculation systems (in large tanks) on land or in net cages in a lake, river or offshore. In the latter cases, farmed fish are reared at high population densities in floating, open-net cages near shore (for marine aquaculture), or in a lake or river (for inland aquaculture), in the same water column as the wild fish reservoir, which are often at a relatively low population density (Hill, 2005). The cages are either square/hexagonal steel platform construction linked together or circular polyethylene rings 10–50 m deep with netting to hold in farmed fish (Fig. 1). Such a cozy aquatic environmental interaction is inevitably ideal for selecting and propagating more virulent variants of wild-type pathogens that exist benignly in local wild fish. This so-called "local effect" is an inherent and highly costly risk of aquaculture around the world (Hill, 2005), although for several viral diseases of economic importance to aquaculture, there is no clear evidence of transmission of virus between wild and farmed fish (Johansen et al., 2011).

The most common causative agents of infectious diseases in aquaculture are bacteria (54.9%), followed by viruses (22.6%), parasites (19.4%) and fungi (3.1%) (McLoughlin, 2006). Viral diseases have been

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Fig. 1. Floating open-net cage aquaculture in New Brunswick, Canada. A fish farm near shore, with several hexagonal cages 10–50 m deep with netting to hold in farmed fish. Each cage is covered with a net to keep fish in and birds out. (Source: Dr. Tillmann Benfrey). See also McAlaster (2011).

more difficult to control, due to the high susceptibility of aquatic animals at an early age, the lack of therapeutics, insufficient knowledge of the pathogenesis of viral infections and limited knowledge of natural resistance mechanisms in aquatic animals. Viruses are therefore the principal pathogens that are negatively impacting aquaculture.

Several strategies that are used to successfully rear terrestrial farm animals in intensive production systems are also employed to prevent and control viral diseases in aquaculture, including selective breeding for increased resistance (Gjedrem, 2010) and vaccination (Rimstad, 2011). Despite the traditional use of antibiotics, there is no field experience of applying antiviral drug treatment to farmed fish. Resistance to infectious salmon anaemia (ISA) has been included in breeding programs for Atlantic salmon in Norway since 1993 (Moen et al., 2007), and since 1997 for resistance to infectious pancreatic necrosis (IPN) (Kjøglum et al., 2008). Atlantic salmon and rainbow trout can be selected for resistance to IPN (Storset et al., 2007; Okamoto et al., 1993). Vaccines are now in widespread use against viral diseases in cattle, sheep, etc., but it is not clear what role antiviral drugs might play against an infection such as foot-and-mouth disease (Goris et al., 2008). In farmed fish, prophylactic antiviral compounds would be best used in broodstock and valuable genetic stocks which are not used for human consumption.

2. Recent viral disease outbreaks in farmed fish

2.1. Virus spread

The spread of diseases is the most feared threat to aquaculture. It is a matter of global concern especially with increased trade and movement of live aquatic animals and their products across national borders. The Food and Agricultural Organization (FAO) calculated the value of the world exports of fish and fishery products in 2008 to be US$102.0 billion, doubling the US$51.5 billion corresponding value in 1998 (FAO, 2010), which illustrates the large volume of international trade. Virus spread can occur through translocation of successfully farmed live aquatic animals to new destinations (e.g., transfer of live non-indigenous fish for farming), particularly animals with low prevalence of clinical disease, subclinical infections or asymptomatic carrier hosts (Gaughan, 2002; Rodgers et al., 2011) or by the shipment of infected/contaminated fish eggs (McAllister and Reyes, 1984; Baudin-Laurencin, 1987; Bovo et al., 1987; Yoshimizu, 1996; Nishizawa et al., 2006; Vike et al., 2009; Mutoloki and Evensen, 2011).

Increased risk for new diseases occurs with the expanding range of emerging farmed fish species such as Atlantic halibut "*Hippoglossus hippoglossus*", Arctic char "*Salvelinus alpinus*", Sablefish "*Anoplopoma fimbria*", and Atlantic cod "*Gadus morhua*", and with new production approaches such as integrated multitrophic aquaculture (McAlaster, 2011). Migratory wild fish (Tucker et al., 1999) can also spread viral pathogens over long distances (Dopazo and Bandín, 2006), similarly to migratory wild birds (Webster et al., 1992). Scavenging wild birds can also act as disease vectors (Peeler et al., 2004). Moreover, improved diagnostic and surveillance efforts, which have accompanied the burgeoning aquaculture industry, have also been accompanied by the discovery of new and emerging viral diseases that are naturally enzootic in wild fish populations (Batts et al., 2011, 2012; Haugland et al., 2011; Lorincz et al., 2011; Murray and Peeler, 2005; Palacios et al., 2010; Walker and Winton, 2010).

Below, outbreaks of 4 of 7 viral diseases on the Organization Internationale des Epizooties (OIE) list of reportable finfish viral diseases (Table 1; OIE, 2012a) are reviewed: viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) outbreaks originating from the wild fish reservoirs; spring viraemia of carp (SVC) linked to koi carp imports in USA; and infectious salmon anaemia (ISA) outbreaks linked to importation of fertilized salmon eggs in Chile, illustrating how viral infections spread among farmed fish, and how they are recognized and controlled.

2.2. Viral haemorrhagic septicaemia (VHS)

Viral haemorrhagic septicaemia (VHS) is an economically important viral disease of farmed rainbow trout "*Oncorhynchus mykiss*" (Smail, 1999) and turbot "*Scopthalmus maximus*" (Schlotfeldt et al., 1991; Ross et al., 1994) primarily in Europe; Japanese flounder "*Paralichthys olivaceus*" in Japan (Isshiki et al., 2001) and olive flounder "*Paralichthys olivaceus*" in Korea. VHS is caused by VHS virus (VHSV), also known as Egtved virus, a member of the genus *Novirhabdovirus*, family *Rhabdoviridae*. Members of the genus have five major structural proteins (designated L [150–225 kDa], G [63–80 kDa], N [38–47 kDa], P [22–26 kDa], and M [17–22 kDa] and a small unique non-virion protein (NV) [12–14 kDa], hence the name *Novirhabdovirus* (Tordo et al., 2005). Genotyping based on VHSV G- and N-genes reveals four groups correlated with geographic location of the isolates (Einer-Jensen et al., 2004; Snow et al., 2004; Elsayed et al., 2006; Inton et al., 2008) (Fig. 2).

VHSV infection is characterized by high mortality rates (up to 100% in fry) with affected fish having abnormal swimming behaviour, distended abdomen (due to ascites), widespread hemorrhaging externally and in internal organs accompanied by necrotic changes in various visceral organs. The severity varies with the species of fish (being most severe in rainbow trout) and VHSV strain. VHSV infection in farmed rainbow trout is considered to be of marine origin (Dixon, 1999; Skall et al., 2005), possibly introduced through the use of unpasteurized marine fish as feed in the past 50 years (Einer-Jensen et al., 2004).

Outbreaks of VHS emerged for the first time in marine aquaculture in sea-farmed rainbow trout in France and Denmark (Castric and De Kinkelin, 1980; Horlyck et al., 1984) and in sea-farmed turbot "*Scopthalmus maximus*" in Germany and Scotland (Schlotfeldt et al., 1991; Ross et al., 1994), and are believed to have been caused by virus from a local wild fish reservoir (Hill, 2005). Turbot is farmed in land-based tanks with seawater (Gunnarsson, 2007). Mortality rates of up 90% have been observed in experimentally infected turbot (Castric and De Kinkelin, 1984). The outbreak in Germany, which began suddenly, was associated with significant mortality (Schlotfeldt et al., 1991). Virus was most likely introduced to the turbot production facility by contaminated seawater. Virus was isolated from fish tissue samples in fathead minnow

Table 1
OIE list of reportable finfish viral diseases.

OIE[a] listed disease	Virus name[b]	Geographical distribution	References
Epizootic haematopoietic necrosis (EHN)	EHN virus, genus *Ranavirus*, family *Iridoviridae*	Australia	Langdon et al. (1986), OIE (2011)
Infectious haematopoietic necrosis (IHN)	IHN virus, genus *Novirhabdovirus*, family Rhabdoviridae	USA, Canada, Asia, Europe	Bootland and Leong (1999), OIE (2011)
Infectious salmon anaemia (ISA)	ISA virus, genus *Isavirus*, family *Orthomyxoviridae*	Norway, Canada, UK, USA, Faroes Islands, Ireland, Chile	Kibenge et al. (2004), OIE (2011)
Koi herpesvirus disease	KHV (Cyprinid herpesvirus 3), genus *Cyprinivirus*, family *Alloherpesviridae*	World wide	Haenen et al. (2004), OIE (2011)
Red sea bream iridoviral disease	RSB iridovirus, family *Iridoviridae*	Asia	Matsuoka et al. (1996), OIE (2011)
Spring viraemia of carp (SVC)	SVC virus, genus *Vesiculovirus*, family *Rhabdoviridae*	China, Europe, former Soviet Union states, Brazil, USA, Canada	Ahne et al. (2002), OIE (2011)
Viral haemorrhagic septicaemia (VHS)	VHS virus (Egtved virus), genus *Novirhabdovirus*, family *Rhabdoviridae*	Europe, USA, Canada, Japan, Korea	Skall et al. (2005), OIE (2011)

[a] The OIE is recognized by the World Trade Organization as the reference and standard-setting body in all issues involving animal health. The OIE develops and establishes health standards for safe trade of animals and animal products. Without this oversight, countries can set laws that result in unfair trade in animals and animal products. OIE Member countries report OIE Listed diseases (OIE, 2012a) to OIE, which disseminates the information to other countries. This transparency allows neighboring countries to take actions to minimize disease entering into their country.
[b] Virus name includes current species, genus and family classification.

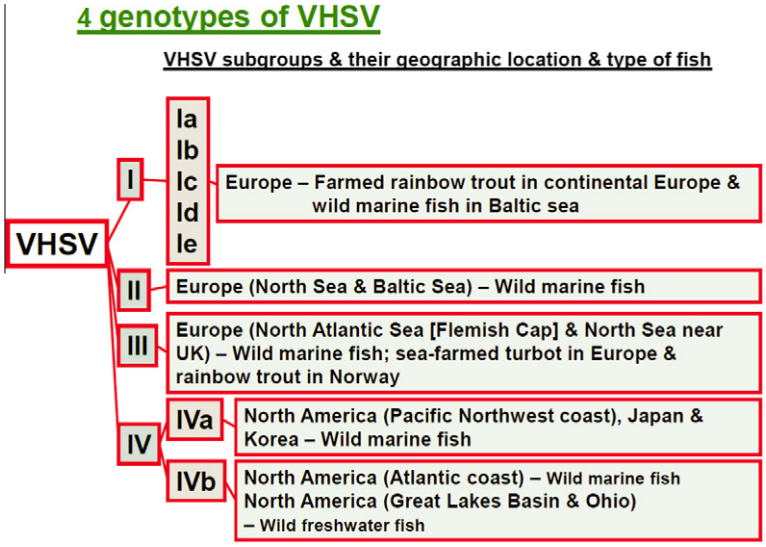

Fig. 2. Phylogeography of viral hemorrhagic septicaemia virus (VHSV), with types of fish from which the VHSV genotypes and subgroups are most commonly isolated (Elsayed et al., 2006; Winton et al., 2008; Johansen et al., 2011). Genotypes I to III are found in Europe. The original genotype IV (now subgroup IVa) occurs in on the Pacific Northwest coast of North America and in Japan and Korea. The new subgroup IVb is found on the Northeast Atlantic coast of North America and in the Great Lakes Basin.

(FHM) and chinook salmon embryo (CHSE-214) cell lines, which showed viral cytopathic effect (CPE) 24 h postinoculation (p.i.) and complete destruction of the cell monolayers by 76 h p.i. (Schlotfeldt et al., 1991). Electron microscopy revealed rhabdovirus-like particles in the infected CHSE-214 cells, and VHSV was confirmed using rabbit antiserum and monoclonal antibodies to VHSV (Lorenzen et al., 1988). Outbreaks in 2007–2009 in rainbow trout in Norway were caused by group III marine VHSV (Fig. 2) previously not considered to be pathogenic to rainbow trout (Johansen et al., 2011).

In Japan, VHSV originating from local wild Japanese flounder "*Paralichthys olivaceus*" (Watanabe et al., 2002) was responsible for the emergence of the disease in farmed flounder (Isshiki et al., 2001). This outbreak had cumulative mortality of 50–70% in market-size fish in 2 farming areas; the isolated virus caused cumulative mortality of 100% in experimental infections of 14–124 gm fish and 60% in larger (1059 gm) fish. The disease was initially thought to be due to a new type of hirame rhabdovirus (HIRRV); which is widely distributed in fish in Japan (Nishizawa et al., 1991), and causes lesions very similar to those observed in VHSV infections. However, it was differentiated from HIRRV by lack of cross-neutralization with antisera against HIRRV and VHSV, and by western blot analyses and RT-PCR (Isshiki et al., 2001).

VHSV has an extremely wide host range, having been isolated from 82 different freshwater and marine fish species in the Northern Hemisphere waters of Europe, North America, and Asia (Hedrick et al., 2003; Skall et al., 2005; OIE, 2011). Marine VHSV isolates have periodically caused large-scale die-offs of wild fish species, but show no to low pathogenicity for rainbow trout and Atlantic salmon "*Salmo salar*" (Skall et al., 2005).

Recently, very large die-offs of at least 28 species of freshwater fish occurred in the Great Lakes Basin of North America, beginning in the Bay of Quinte, Lake Ontario, Canada, in the spring of 2005 (Lumsden et al., 2007). Within 4 days, more than 200 dead or dying drum "*Apoldinotus grunniens*" were found at several locations along some of the shorelines. The number of dead fish declined by the third week, but modeling results indicated that between 20–30,000 freshwater drum died within a 35–40 day period (Lumsden et al., 2007).

Virus was detected by transmission electron microscopy in affected heart tissue and virus isolation from pooled tissues on FHM cells; VHSV was confirmed by enzyme immunoassay, RT-PCR and sequence analysis. Additional mortality events in several species of fish, including muskellunge "*Esox masquinongy*" in Lake St. Clair (Elsayed et al., 2006) occurred in this large outbreak (Bowser, 2009). It is not known with certainty how the virus arrived in the Great Lakes basin, although it is known to have been present as early as 2003 (Elsayed et al., 2006). The strain is a subtype IVb marine VHSV (Fisher and Garner, 2007), similar to isolates from stickleback "*Gasterosteus aculeatus aculeatus*", mummichog "*Fundulus heteroclitus*" (Olivier, 2002), striped bass "*Morone saxatilis*" and wild brown trout "*Salmo trutta*" on the Atlantic coast of North America (Gagné et al., 2007). It is speculated that VHSV was introduced to the Great Lakes via the natural movement of fish up the Saint Lawrence River from near-shore areas of the Atlantic coast of Canada (Groocock et al., 2007; Walker and Winton, 2010). Subtype IVb is the only VHSV strain outside of Europe that has been associated with significant mortality in freshwater species (Fig. 2). The recently discovered broad host range of VHS and significant differences in its pathogenicity in different host species will continue to cause problems for control programs, which are currently based mainly on protection of the significant European rainbow trout industry (OIE, 2011). Control methods include fish health surveillance programs, quarantine and eradication, followed by fallowing.

2.3. Infectious haematopoietic necrosis (IHN)

Infectious haematopoietic necrosis (IHN) is a very severe viral disease of farmed salmonids (Bootland and Leong, 1999; Wolf, 1988b). The disease is caused by IHN virus (IHNV), the type species of the genus *Novirhabdovirus*, family *Rhabdoviridae*. IHNV is enzootic throughout the Pacific Northwest of North America, with a contiguous range extending from Alaska to California and inland to Idaho (Kurath et al., 2003). The virus causes asymptomatic infections in adult salmonids (Pacific salmon including Chinook salmon "*Oncorhynchus tshawytscha*", Sockeye/Kokanee salmon "*O. nerka*", Chum salmon "*O. keta*", Pink salmon "*O. gorbuscha*", masou "*O. masou*", Coho salmon "*O. kisutch*", and Atlantic salmon), rainbow/steelhead trout "*O. mykiss*" (Bootland and Leong, 1999; Traxler et al., 1998; Wolf, 1988b) and Pacific herring "*Clupea harengus*", but can cause mortalities of up to 100% in young salmonids (fry and juveniles) in both fresh and sea water, depending on virus strain and environmental conditions (OIE, 2011; Traxler et al., 1993, 1998; Wolf, 1988b). Smolts can develop clinical disease within 7 days of being introduced to sea water (Saksida, 2004). In British Columbia, sockeye fry migrating from spawning channels have suffered high mortality due to IHNV (Traxler and Rankin, 1989). Clinically affected fish are lethargic or may have whirling behaviour, and have cranial swelling (cephalic bumps), abdominal distension, exophthalmia, darkened skin and pale gills with hemorrhages at the base of the fins. Internal lesions include severe petechial and ecchymotic hemorrhage in the viscera, bloody ascites, and pale livers (Traxler et al., 1998). Surviving fish develop strong protective immunity (LaPatra et al., 1993); they often have scoliosis (Amend et al., 1969). Normally, all infected lots of fish are destroyed. The natural disease must be differentiated from VHS and IPN.

Three different viral genogroups have been identified, each localized to a specific geographic region and a particular salmonid species: the Northwest Coast genogroup (Oregon to Alaska) in Sockeye salmon, the Idaho genogroup in rainbow trout and the California genogroup in Chinook salmon (Hsu et al., 1986; Nichol et al., 1995; Emmenegger et al., 2000; Troyer et al., 2000; Emmenegger and Kurath, 2002). Kurath et al. (2003) confirmed this grouping using phylogenetic analysis based on a 303-nucleotide variable region ("mid-G") in the glycoprotein gene, and designated the three genogroups as U (upper), M (middle), and L (lower), respectively, to correlate with geographic areas in the Pacific Northwest of North America (Fig. 3). Sequences of the Russian IHNV isolates were reported to be indistinguishable from those of the U genogroup, suggesting virus transmission or exposure to a common viral reservoir in the North Pacific Ocean (Rudakova et al., 2007).

The virus is considered to have spread from North America to Europe (initially to France and Italy and later to Germany) (Baudin-Laurencin, 1987; Bovo et al., 1987) and to Japan (Yoshimizu, 1996; Nishizawa et al., 2006) via shipments of IHNV-contaminated rainbow trout eggs or fry. The European and Japanese IHNV isolates cluster with U and M genogroups (Enzmann et al., 2005; Kim et al., 2007). In Europe, rainbow trout is the most affected fish species (Kuzmin et al., 2009). Nishizawa et al. (2006), using phylogenetic analysis of the full-length glycoprotein gene, identified a fourth genogroup consisting of Japanese rainbow trout isolates from 1980 to 1996 and designated it "JRt" (Fig. 3) It shares a common source with the U genogroup. The JRt genogroup was later introduced to Korea (Kim et al., 2007) and possibly Taiwan (Wang et al., 1996). The virus in Japan has caused losses among wild fish stocks (Traxler et al., 1998). An IHNV database is available containing records for more than 1000 individual field isolates, which is updated annually (Kurath, 2012).

The first reported outbreak of IHN occurred in sockeye salmon fry at fish hatcheries in Oregon and Washington in 1953 (Kurath et al., 2003). In 1973–1974, it was responsible for a 90% loss in a population of 20,000 sockeye salmon fingerings at a hatchery in Alaska; in 1974, 99% of 600,000 sockeye salmon alevins and fingerings of broodstock that spawned in 1973 died (Grischkowsky and Amend, 1976). The natural disease first occurred in farmed Atlantic salmon in British Columbia in 1992, representing the first time the virus affected salmon at sea (Armstrong et al., 1993; Traxler et al., 1993). Since then, it has occurred at epizootic levels on Atlantic salmon farms in 1992–1996 (St-Hilaire, 2000) and 2001–2002 with cumulative mortality rates as high as 77% in smolts (<700 g) and 53% in harvest-sized fish (Saksida, 2004). It was hypothesized that the source of these outbreaks is a stock of wild salmon, most likely sockeye, carrying a virulent strain of IHNV. Because of the cyclical nature of the sockeye life history (Groot, 1996), the virulent strain would only appear every 4–5 years with the return of this particular stock to the rivers to spawn (Saksida, 2004). However, cultured salmonid fish such as Chinook salmon (St-Hilaire et al., 2001) and the non-salmonid fish that occur around sea net-pens such as shiner perch "*Cymatogaster aggregate*" and tubesnout "*Aulorhynchus flavidus*" could also serve as carriers of the virus, as they can have high virus titers without showing any clinical signs (Traxler and Richard, 1996).

In May, 2012, IHNV was diagnosed on at least 3 farm sites on the west coast of Vancouver Island, the first such diagnosis in British Columbia since 2003. The virus was initially detected in Atlantic salmon samples collected during routine health screening of a fish farm belonging to one company. Subsequently, the virus was detected on a second farm of the same company, and as a "low positive" in coho salmon samples on a third farm belonging to another company; all companies then started testing their farms for IHNV (Anonymous 4). The first detection triggered a quarantine of the farm site and depopulation of its entire stock of 560,000 one-kilo-sized salmon (Anonymous 5). The affected farm site will be left to fallow, without restocking, for at least 3–4 months. The virus has also been detected in Atlantic salmon on a farm in Washington State, prompting its depopulation. These control measures are intended to limit the spread of the disease, and are consistent with IHN being an OIE-listed disease (Table 1; OIE, 2012a).

Laboratory diagnosis of IHNV infection can be by virus isolation on fish cell lines including EPC (*Epithelioma papulosum cyprini*), BF-2 (bluegill fry), FHM and CHSE-214, followed by immunological or

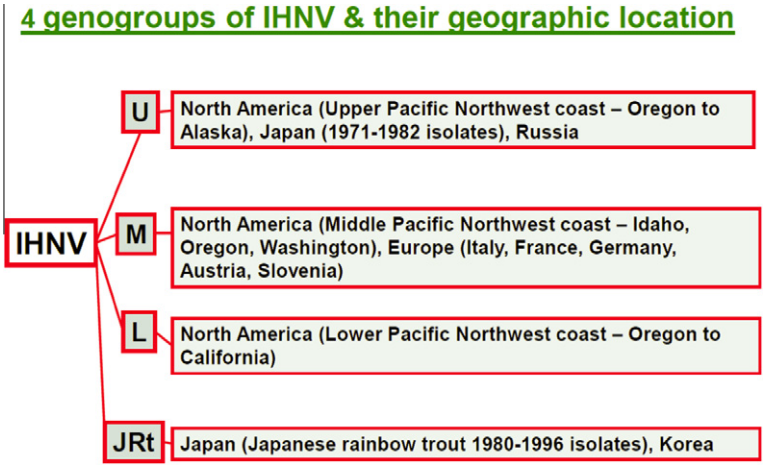

Fig. 3. Differentiation of infectious hematopoietic necrosis virus (IHNV), based on genotypic analysis of the "mid-G" (glycoprotein) gene (303 nts), and its geographic distribution worldwide (Nishizawa et al., 2006; Kim et al., 2007; Rudakova et al., 2007; Grilc Fajfar et al., 2011).

molecular identification of the virus. Viral nucleic acids can also be detected directly in tissue samples by RT-PCR (OIE, 2011). Using polyclonal rabbit antisera, only a single serotype of IHNV has been identified (Engelking et al., 1991).

2.4. Spring viraemia of carp (SVC)

Spring viraemia of carp (SVC) is an economically important viral disease of farmed common carp "*Cyprinus carpio carpio*", but outbreaks can also occur in wild common carp and in several other freshwater species. Common carp have also been selectively bred for the ornamental fish industry, where they are known as koi (Hartman, 2004). The disease is widespread in European carp pond culture, where it causes significant morbidity and mortality (Ahne et al., 2002), but is considered an exotic disease in the USA. SVC is caused by SVC virus (SVCV), also in the family *Rhabdoviridae*, but it is distinct from VHSV and IHNV, and is in the genus *Vesiculovirus*. Moreover, SVCV is unique among fish viruses in that it also replicates in cell cultures of avian and mammalian origins at temperatures of 20–22 °C (Ahne et al., 2002). Outbreaks of disease, particularly in young carp, are associated with mortality up to 70%. Affected fish typically show a distended abdomen (due to ascites), exophthalmia, and petechial hemorrhages of the skin (Fijan et al., 1971). Internally, hemorrhages in the swim bladder, edematous organs and catarrhal enteritis are usually seen (Ahne et al., 2002); Severity varies with the age of the fish and the water temperature. Older fish are more resistant, and warm temperatures (>10 °C but <25 °C) are more lethal, but no mortality occurs above 25 °C. Recovered fish are resistant to reinfection (Ahne et al., 2002).

The first cases of spring viraemia of carp (SVC) in the USA in 2002, as in Switzerland in 2001 (Bernet and Wahli, 2002) and in Denmark in 2002 (OIE, 2011) were linked to koi carp imports without a requirement for health certification of freedom from disease (Hill, 2005). The US outbreak started in April 2002, and involved one of the largest koi farms operating in the states of North Carolina and Virginia (Goodwin, 2002). Virus was isolated on the EPC cell line and confirmed as SVCV by immunohistochemistry. Depopulation, cleaning and disinfection of the affected farm required an international effort, which began in July and ended in October, 2003, when the quarantine was lifted. There were also mass mortalities of wild common carp at Cedar Lake, Wisconsin in 2002 (Dikkeboom et al., 2004) and in farmed koi in the states of Washington and Missouri in 2004 (Shivappa et al., 2008). SVCV was also isolated from apparently healthy wild common carp in the Calumet Sag Channel in Illinois in 2003.

The US SVCV isolates of 2002 (Dikkeboom et al., 2004), 2003 and 2004 (Warg et al., 2007) belonged to SVCV genogroup 1a, which consists of strains from Asia (Stone et al., 2003). The US Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) controls SVCV outbreaks like other foreign animal diseases, through quarantine, depopulation and disinfection of premises, followed by enhanced surveillance. In 2006, SVCV was also isolated for the first time from apparently healthy wild common carp from Lake Ontario, Canada, that were destined for export to France (Garver et al., 2007). The Canadian SVCV isolate grouped with genogroup 1a, together with isolates from Asia and the USA, and was most similar to the 2003 Illinois isolate (Garver et al., 2007).

2.5. Infectious salmon anaemia (ISA)

Infectious salmon anaemia (ISA) is a serious viral disease of marine-farmed Atlantic salmon caused by ISA virus (ISAV), which belongs to the genus *Isavirus*, family *Orthomyxoviridae*. ISA is arguably the most important viral disease of marine-farmed Atlantic salmon, and continues to cause severe economic losses in an increasing number of countries (reviewed in Kibenge et al., 2004). Mortality in a fish cage rises slowly and can vary from 0% to 90% (OIE, 2011). The virus may be present in a cage up to 6 months before significant mortality is noted. The three main gross lesions are exophthalmos (bulging, blood-shot eyes), petechial hemorrhages on the abdomen, and congestion and enlargement of the liver. Histopathologic lesions include intestinal mucosal ulceration and hemorrhage, liver hemorrhage and hepatocyte necrosis, and erythrophagocytosis (Godoy et al., 2008). Diagnostic confirmation requires virus isolation on permissive fish cell lines and virus identification, for example by RT-PCR (OIE, 2011).

The virus occurs in two basic genotypes, North American and European (Kibenge et al., 2001). The European genotype shows more genetic variation than the North American genotype (Kibenge et al., 2007; Nylund et al., 2007) (Fig. 4). The non-pathogenic variant ISAVs called "highly polymorphic region" (HPR0) viruses detected to date are all of European genotype. HPR0 viruses deserve special consideration because their evolutionary status is not clearly understood. The term HPR0 is used to indicate that they have a "full-length" highly polymorphic region (HPR) of their surface envelope glycoprotein haemagglutinin-esterase or HE (Fig. 5). All ISAV isolated to date from clinical disease have amino acid

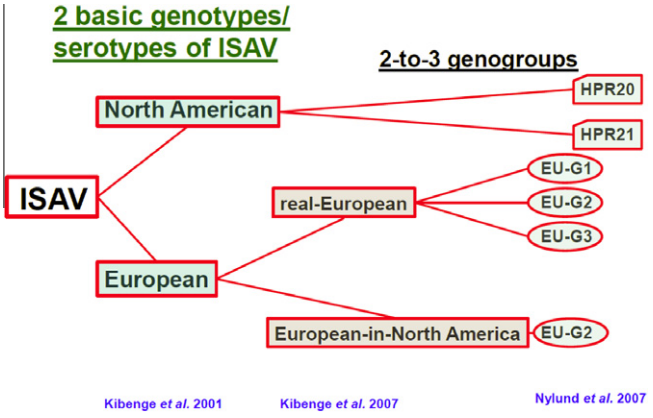

Fig. 4. Infectious salmon anaemia virus (ISAV) strain identification. ISAV occurs in two basic genotypes, North American and European; the European genotype shows more genetic variation (Kibenge et al., 2001; Nylund et al., 2007).

deletions in HPR relative to HPR0, suggesting that HPR is important to ISAV virulence (Kibenge et al., 2007). HPR0 viruses have not yet been cultured; their presence in fish can only be demonstrated using purely nucleic acid-based assays thereby causing diagnostic confusion, which results potentially in the expensive culling of apparently healthy fish. It is evident that ISAV is now enzootic in fish populations in Norway, New Brunswick-Canada, Scotland, the Faroe Islands, Maine-USA, and Chile (Godoy et al., 2008; Kibenge et al., 2009; Debes et al., 2011). Virulent strains of the virus have been replaced by HPR0 viruses (Fig. 4), but the dynamics of this evolution are not known. Only Norway and Canada reported ISA outbreaks in 2012 (OIE, 2012b). The 2012 outbreak in Canada occurred in Nova Scotia and involved ISAV of the North American genotype. There have been reports of ISAV in farmed and wild fish on the west coast of Canada, however, the precise status is unknown (Casselman, 2011). Because there have been no virus isolations to date, the Department of Fisheries and Oceans concluded that there have been no confirmed findings of ISAV in British Columbia (DFO, 2011).

In the Southern hemisphere, ISAV was first detected in Chile in 1999 in marine-farmed Coho salmon, and it was shown to be of the North American genotype (Kibenge et al., 2001). During the winter of 2007, unexplained mortalities following recovery from an outbreak of Piscicricketsiosis were registered in Atlantic salmon of

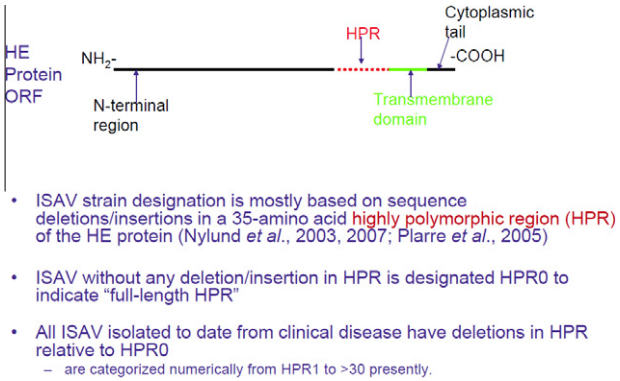

Fig. 5. Infectious salmon anaemia virus (ISAV) strain identification, based on amino acid deletions in the highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) protein (Nylund et al., 2007). The line diagram of the HE protein open reading frame (ORF) shows the location of the HPR (Rimstad et al., 2001; Kibenge et al., 2001; Plarre et al., 2005). ISAV without any amino acid deletion in HPR is designated HPR0 (Cunningham et al., 2002).

3.9 kg average weight at a grow-out site located in central Chiloé in Region X of Chile. The disease was confirmed as ISA, for the first time in its classical presentation and affecting farmed Atlantic salmon in Chile (Godoy et al., 2008). Chile had experienced almost exponential growth of the Atlantic salmon industry from 1987 to 2004, but by 2004, several production indicators started to show a gradual decline and increasing mortality designated as being due to "non-identified" causes. However, the general reaction in the industry was to stock more fish to compensate for losses. Phylogenetic analysis of the ISAV isolates from this outbreak indicates that the virus was introduced from Norway in about 1996 (Kibenge et al., 2009), probably through fertilized salmon eggs (Vike et al., 2009; Cottet et al., 2010). The ISAV responsible for the outbreak (mostly HPR7b) was most similar to isolates from Norway, but had acquired a mutation consisting of a 33 base-pair insert in genome segment 5, which encodes the surface envelope fusion protein (Kibenge et al., 2009).

The last clinical ISA outbreak in Chile was recorded in September 2010; since then, most of all ISAV diagnoses have involved the non-virulent HPR0 virus (Fig. 6). Economic losses due to the outbreak in 2007 were estimated to amount to a 9% reduction in the US$2.24 billion Chilean aquaculture industry (i.e., $20 million) and an approximately 3.0% reduction in the workforce. In 2009, the outbreak accounted for a 60% drop in Atlantic salmon production, from a peak of about 400,000 tons prior to 2007. Projected losses for 2007–2011 will be approximately $1 billion (i.e., 50% of the economic value of the Chilean industry). Full recovery is not expected before 2013.The most important disease control measures introduced in 2009 by the industry and Chilean Government, and responsible for the recovery are (1) all-in, all-out farming, fallowing, and zone management; (2) restriction of fish movements; (3) coordination of sea lice control; (4) ISAV vaccination; (5) use of good quality smolts; (6) reduction of farm stocking numbers; and (6) better surveillance and diagnostic capacities.

2.5.1. ISA virus (ISAV) HPR0

ISAV HPR0 can be found in apparently healthy wild and farmed fish. Three theories have been put forward to explain its relationship to virulent strains of ISAV (reviewed in Kibenge et al., 2009). The first and original theory, also referred to as the "deletion hypothesis," is that HPR0 in wild fish is the ancestor of novel strains that adapt to the new host, possibly through HE gene deletions during intensive aquacultural practices (Mjaaland et al., 2005). This hypothesis has resulted in the culling of apparently healthy fish, considering them at high risk for development of ISA. However, there has been no authentic report linking an initial presence of HPR0 virus to a subsequent clinical outbreak (MacBeath et al., 2009; Murray et al., 2010). Indeed, the risk of emergence of pathogenic ISAV variants from a reservoir of HPR0 is considered to be low (Debes et al., 2011). Moreover, HPR0 viruses are detected late during ISA outbreaks, and persist long after the disease has been contained or eradicated (MacBeath et al., 2009; Kibenge et al., 2009; Debes et al., 2011) (Fig. 6).

The second theory, also referred to as the "insertion hypothesis," is that virulent ISAV undergoes mutations involving insertions into the HPR, resulting in HPR0 and attenuation; the virus adapts and stops killing its host (Kibenge et al., 2007). Support for this hypothesis is found in a recent report indicating that sequence variation in the HPR is most likely due to homologous recombination (copy-choice recombination), presumably because of strand switching by the viral RNA polymerase (Castro-Nallar et al., 2011) during negative-RNA strand synthesis from the nucleic acid template of one virus to another (which has been shown to occur in picorna-, corona-, alpha-, rota- and orbiviruses and influenza viruses). However, no outbreaks caused by a HPR type of ISAV have been observed among wild Atlantic salmon. On the other hand,

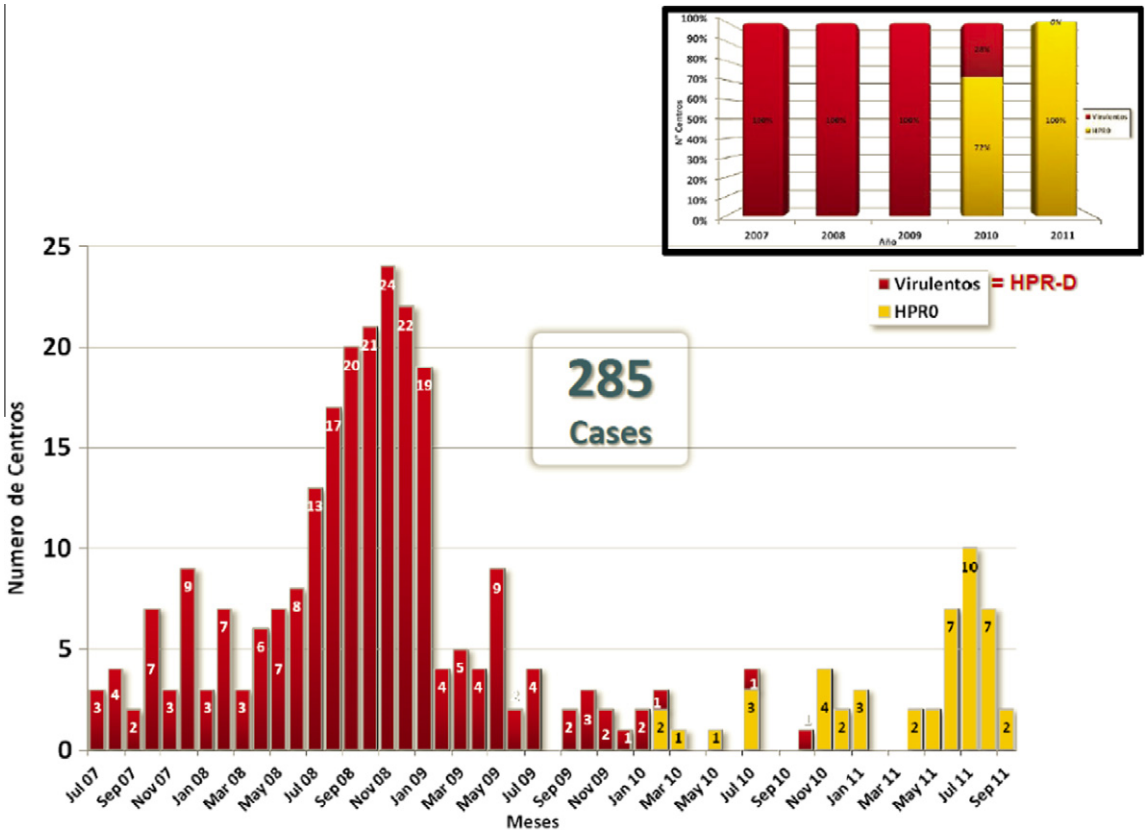

Fig. 6. Prevalence of infectious salmon anaemia virus (ISAV) (virulent or HPR-D and non-virulent or HPR0) positive cases in Chile, July, 2007 to September, 2011. The first case was detected in June, 2007; the peak was in November 2008. Since 2010, ISAV HPR0 has been found in different production stages of Atlantic salmon (fry, pre-smolt, growout in marine farms, & brood stock) without associated clinical signs of ISA. Inset: Proportion of cases with virulent or HPR-D and non-virulent or HPR0 ISAV; 2007–2009: 100% virulent or HPR-D; 2010: 28% virulent or HPR-D and 72% non-virulent or HPR0; 2011: 100% non-virulent or HPR0 ISAV. (Data from National Fisheries Service (Sernapesca), Chile).

HPR0 viruses in the Faroe Islands have been termed low-pathogenicity/low-virulent ISAV (Debes et al., 2011), a term that is consistent with influenza A viruses in wild birds.

The third (and most plausible) theory, also referred to as the "quasispecies" or "mutational cloud" hypothesis, is that HPR0 is the "original" consensus sequence of all HPR-deleted sequences during virus replication in a cell (Kibenge et al., 2009). As predicted by the quasispecies concept (Holland et al., 1992), new HPRs then appear at the periphery of the quasispecies distribution, where they are produced by the erroneous copying of mutants already present; the dominant (well-adapted and advantageous) mutants are easily detected as the cause of disease. This theory would also accommodate the "insertion hypothesis," since it suggests that various HPRs, including HPR0, are present at any one time. In the case of Chile, HPR7b was the principal mutant associated with disease outbreaks (Kibenge et al., 2009).

3. Other emerging viral diseases of farmed fish

3.1. Diseases of economic importance

Most major bacterial diseases of salmonids have been successfully controlled through antibiotics and vaccination (Bowden et al., 2003), whereas vaccines against viral diseases have not performed well to date. Viruses are currently the principal pathogens that are negatively impacting finfish aquaculture. Because of the global nature of aquaculture, the OIE, which provides international standards and guidelines for safe trade in animals and their products (Brückner et al., 2007; Mohan, 2009), essentially defines the economic importance of farmed fish viral diseases by placing them on the OIE list (Table 1; OIE, 2012a). Any diseases listed are notifiable (or reportable) to the OIE because of the risk of spread through commercial trade in fish and their products.

OIE Member Countries report listed diseases to the OIE, which disseminates the information to other countries. This transparency allows neighboring countries to take actions to minimize the disease entering into their country (Hill, 2005). Non-OIE-listed finfish viral diseases are either too new or have been de-listed in recent years because they are enzootic in all regions with susceptible fish hosts. Consequently, the major viral diseases fit the definition of emerging aquatic animal viral diseases (Murray and Peeler, 2005; Walker and Winton 2010; Rimstad, 2011). In addition to the OIE-reportable diseases VHS, IHN, SVC, and ISA, which are reviewed in Section 2 above, some non-OIE listed viral diseases are discussed below.

3.2. Infectious pancreatic necrosis (IPN)

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease of salmonids, which occurs in all major salmon farming countries. It is caused by IPN virus (IPNV), a member of the genus *Aquabirnavirus*, family *Birnaviridae*. Serological classification showed that the IPNV strains are divided into two serogroups (A and B). Within serogroup A, nine distinct serotypes were identified (Hill and Way, 1995), whose classification on the basis of deduced amino acid similarities of VP2 demonstrated that the strains clustered into 6 genogroups (Blake et al., 2001).

Different IPNV strains vary in virulence (Santi et al., 2004). Susceptibility to IPN generally decreases with age (Wolf, 1988a), except for Atlantic salmon smolts which are often affected also after transfer from freshwater to seawater (Jarp et al., 1995; Smail et al., 1992; Roberts and Pearson, 2005). Thus at the freshwater stage affected fish will have abnormal swimming behaviour, exophthalmos, distended abdomen, darkened pigmentation, focal necrotic lesions in the exocrine pancreatic tissue and severe liver necrosis. Similar clinical signs with reduced liver involvement but with petechial hemorrhages of pancreas are seen in larger fish in seawater. The disease has to be differentiated from pancreas disease (PD) caused by salmon alphavirus, which also affects skeletal and heart muscle. Confirmatory diagnosis of IPN can easily be done by virus isolation and RT-PCR. IPN was removed from the OIE list of notifiable aquatic animal diseases in 2005 (Table 1), but it continues to cause significant losses to the aquaculture industry in some European countries (Brun, 2003) and in Chile, and most farmed salmon are vaccinated against it. In the European Union, IPN is still regulated under national control programs within each Member State (in the UK, rainbow trout farms have been exempt since 1995).

IPNV is a very tough virus, able to withstand desiccation, and it can survive in both fresh and sea water for considerable periods. It is resistant to high temperature (for example, while ISAV is inactivated at 55 °C for 5 min, IPNV requires heating to 80 °C for 2 h to achieve significant inactivation) and to low pH, enabling it to survive in ensiled waste (McAllister and Bebak, 1997) and in the gut of piscivorous (fish-eating) birds (McAllister and Owens, 1992). It is about 100 times more resistant to ultraviolet radiation than some other viruses (e.g., IHNV) making it very difficult and costly to sterilize hatchery water supplies by this method. In Sweden, IPNV is controlled by culling, and the virus is officially absent from that country in spite of very high levels in neighboring Norway (Ariel and Olesen, 2002). In 1999–2000 in Scotland, marine IPNV prevalence declined following fallowing and widespread culling to control the 1998–99 ISA outbreak (Stagg, 2001; Murray et al., 2002). However, because IPNV has a wide variety of host species (Hill, 1982) and persistent carriers among recovered hosts (Smail and Munro, 1985), it is difficult to eradicate, once established.

Because fish develop a lifelong persistent infection, farmed fish may be the most important reservoir of IPNV in the aquatic environment (Johansen et al., 2011). However, the worldwide distribution of IPNV is not only a consequence of aquaculture and commercial movements of cultured fish, particularly when wild fish populations are considered (Dopazo and Bandín, 2006). For example, the genetic variation of IPNV isolates in Chile cannot be explained entirely by trade in fish eggs (McAllister and Reyes, 1984; Mutoloki and Evensen, 2011). Analysis of more recent Chilean IPNV isolates has identified a cluster of genogroup 1 isolates (Fig. 7) that are most similar to those made in 1999 by Romero-Brey et al. (2009) from 12 different species of wild fish from the Flemish Cap fishery located in international waters near Newfoundland, on the northeastern coast of Canada (Callejas et al., 2012).

3.3. Pancreas disease (PD) and sleeping disease (SD)

Pancreas disease (PD) and sleeping disease (SD) are serious illnesses of farmed salmonids in Europe (Raynard and Houghton 1993; Graham et al., 2011). Affected fish show inappetence, lethargy, increased faecal casts in cages, runting in juveniles, and increased mortality (1–48%) (McLoughlin and Graham, 2007). The disease was first recorded in 1976 in Scotland (Munro et al., 1984). It has re-emerged in the last 8–9 years as a significant threat to sustainable salmon production in Norway (Fig. 8), with site mortalities of up to 60% and 10% of survivors failing to grow after infection (runts) (Anonymous 1). It is caused by salmonid alphavirus (SAV), a new member of the genus *Alphavirus* in the family *Togaviridae* (Weaver et al., 2005). It is referred to as pancreas disease (PD) in marine-farmed Atlantic salmon and rainbow trout, but the same pathogenesis is referred to as sleeping disease (SD) in freshwater-farmed rainbow trout. Affected fish show sleeping behaviour (lying on their side), exophthalmos, and swollen abdomens (McLoughlin and Graham, 2007). Virus isolates from these conditions in Europe in 1991–2007 can be differentiated into 6 different subtypes by genotypic analysis of partial sequences from nsP3 and E2, the two most variable regions of the genome (Fringuelli et al., 2008) (Fig. 9). PD has been described only once outside western Europe, in Canada, but no virus was detected (Kent and Elston, 1987). However, this was years before culture techniques for SAV were developed, and at the time PD was considered to be a nutritional disease (Bell et al., 1991; McVicar, 1987).

Pancreas disease is one of the most economically damaging diseases in Norwegian aquaculture. Interestingly, its emergence in Norway coincided with a significant reduction in ISA outbreaks (Fig. 8). Vertical transmission, while reported (Karlsen et al., 2005), has not been confirmed by molecular epidemiologic studies (Fringuelli et al., 2008). The virus is enzootic, and the disease tends to recur in each successive generation of fish introduced onto infected sites (McLoughlin and Graham, 2007), suggesting a substantial reservoir of infection. Although alphaviruses are transmitted by mosquitoes, the vector for SAV has yet to be incriminated (Forrester et al., 2012). It has been suggested that sea lice may be a vector in Ireland (Roger and Mitchell, 2007).

Sub-clinical SAV infection in marine-farmed Atlantic salmon has been reported in Scotland (Graham et al., 2006), suggesting that other factors related to the environment, host and/or pathogen may have the potential to influence the severity of a PD outbreak in a given population. Moreover, in clinical disease, the lesions can be confused with vitamin E-selenium deficiency (McLoughlin et al., 1992), IPN (Roberts and Pearson, 2005), cardiomyopathy syndrome (CMS) (Ferguson et al., 1990; Hodneland et al., 2005), and heart and skeletal muscle inflammation (HSMI).

3.4. Cardiomyopathy syndrome (CMS)

Cardiomyopathy syndrome (CMS) of farmed Atlantic salmon was first described as a disease entity in Norway in 1985. CMS has been recorded along the entire Norwegian coast, with clinical manifestations throughout the year, peaking in the late fall and late winter/early spring. CMS, also referred to as "heart rupture disease", has also been recorded in Scotland (Rodger and Turnbull, 2000) and the Faroe Islands (Poppe and Sande, 1994), and suspected cases have been reported in Canada (Brocklebank and Raverty, 2002). Affected fish may die suddenly, without clinical signs of disease, or signs may be nonspecific, such as abnormal swimming, lethargy and anorexia (Haugland et al., 2011). The most characteristic necropsy findings are a ruptured atrium (thin wall), enlarged atrium and a pericardial sac filled with blood or blood clots (Ferguson et al., 1990). Economic losses are high, because CMS affects adult farmed Atlantic salmon 14–18 months after sea transfer and close to harvest (Ferguson et al., 1990). The mortality rate is about 6%. Cardiac lesions similar to those of CMS have also been reported in wild Atlantic salmon (Poppe and Seierstad, 2003).

The cause of CMS was recently reported to be a newly discovered dsRNA virus in the family *Totiviridae*, genus *Giardiavirus* (Haugland et al., 2011), the first time such a virus has been found to infect a vertebrate host. It was identified through genome sequencing by random priming methods, using virus isolated on the GF-1 cell line derived from the orange-spotted grouper"*Epinephelus coincides*" (Haugland et al., 2011). Because it causes a necrotizing myocarditis in salmon, the name piscine myocarditis virus (PMCV) was proposed (Haugland et al., 2011). A related virus was previously

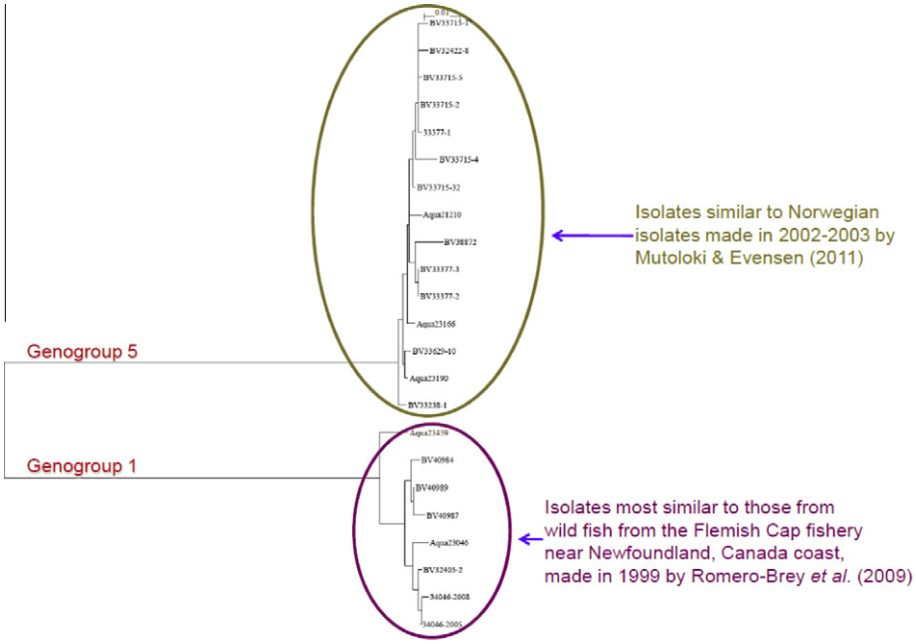

Fig. 7. Phylogenetic tree showing the relationship between recent infectious pancreatic necrosis virus (IPNV) isolates in Chile. The genogroup 5 IPNV isolates were linked to transmission through trade in salmon eyed eggs from Europe in 1999 (Mutoloki and Evensen, 2011). Because there have been no imports of fish or eggs from the eastern Canadian coast, the presence of the different IPNV isolates in Chile cannot be explained entirely by trade. (Kibenge, F.S.B., Wang, Y., Godoy, M.G., and Kibenge, M.J.T., unpublished data).

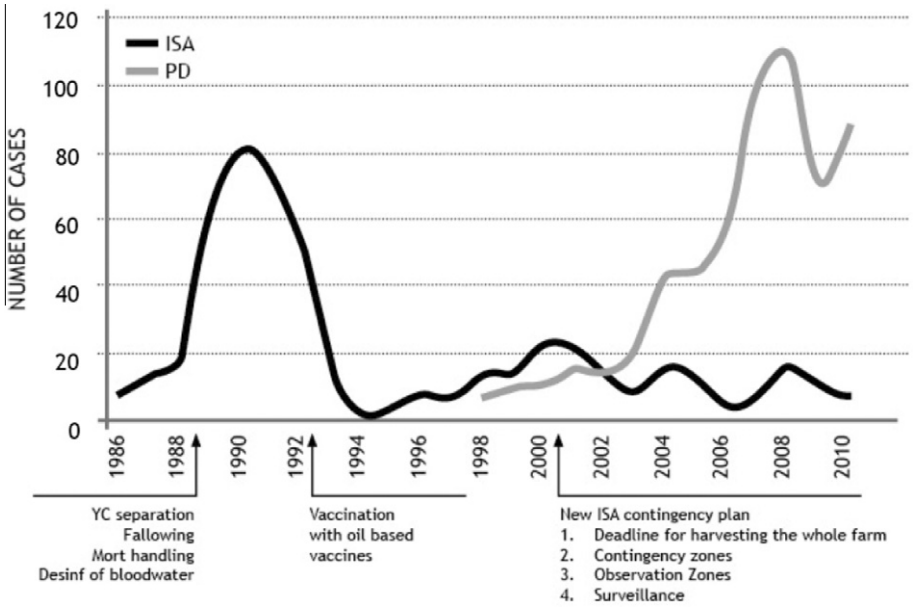

Fig. 8. Incidence of infectious salmon anaemia (ISA) and pancreas disease (PD), 1986–2011 (Norwegian Veterinary Institute reports, http://www.vetinst.no/nor/Temasider/Fiskesykdommer). The emergence of PD in Norway coincided with a significant reduction in ISA outbreaks.

identified as the cause of infectious myonecrosis (IMN) of white Pacific shrimp (Poulos et al., 2006). The disease has been reproduced experimentally, using tissue homogenates from CMS-diseased fish (Ferguson et al., 1990; Fritsvold et al., 2009) and cell culture-grown virus 6 weeks postinoculation (Haugland et al., 2011).

3.5. Heart and skeletal muscle inflammation (HSMI)

Heart and skeletal muscle inflammation (HSMI) is a disease of farmed Atlantic salmon, first recognized in 1999 in Norway (Kongtorp et al., 2004) and subsequently in Scotland (Ferguson et al., 2005). HSMI appears 5–9 months after fish are transferred from fresh water to marine pens. Affected fish show reduced appetite, aberrant swimming and up to 20% mortality, but there are usually no external signs of illness. Internal lesions consist of myocarditis and necrosis of red skeletal muscle.

HSMI has recently been associated with infection by a novel reovirus, termed piscine reovirus (PRV), which was identified through high-throughput pyrosequencing of clinical samples from fish experimentally injected with tissue homogenates from HSMI-

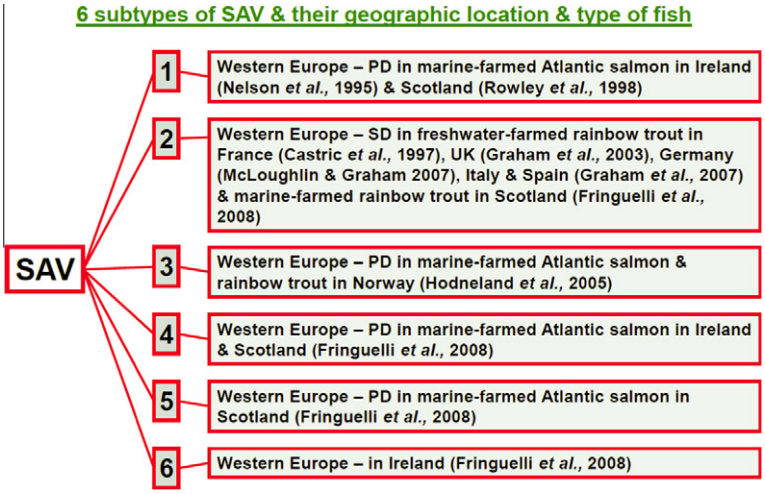

Fig. 9. Differentiation of salmonid alphavirus (SAV), based on genotypic analysis of partial sequences from nsP3 and E2, their geographic distribution within Western Europe and pathogenesis, with the species of fish from which they have been isolated (Fringuelli et al., 2008; Castric et al., 1997; Graham et al., 2007, 2003; Nelson et al., 1995; Rowley et al., 1998).

diseased fish (Palacios et al., 2010). Although it has not yet been reproduced in naïve fish by inoculation with an isolate of PRV, viral RNA was unequivocally detected in HSMI lesions, using *in situ* hybridization. Nonetheless, formal implication of PRV in HSMI requires further investigation. Løvoll et al. (2011), using high-throughput sequencing of heart samples from natural outbreaks and from fish experimentally challenged with material from fish diagnosed with CMS, concluded that PRV behaves as an opportunist, whereas the totivirus might be more directly linked with the development of CMS (Haugland et al., 2011).

Reoviruses are nonenveloped, icosahedral viruses with 10–12 segments of dsRNA that are found in a variety of hosts, including humans (Chappell et al., 2005). PRV is widespread in Norwegian aquaculture, and is also found in wild fish populations. Specifically, it has been detected by RT-qPCR at a low prevalence in certain marine species (Atlantic herring, Capelin "*Mallotus villosus*", Atlantic horse mackerel "*Trachurus trachurus*", and great silver smelt "*Argentina silus*") along the coast of Norway (Wiik-Nielsen et al., 2012). Because none of the positive samples were from fish caught close to fish farms that had registered outbreaks of HSMI, the authors speculated that there is probably a more complex relationship, involving several carriers and virus reservoirs (Wiik-Nielsen et al., 2012). PRV has also been detected in Atlantic salmon in Chile (National Fisheries Service of Chile, Sernapesca), and most recently, in farmed Atlantic salmon and in wild Pacific salmon in British Columbia, Canada.

3.6. Cutthroat trout virus (CTV)

Cutthroat trout virus (CTV) is a newly described salmonid virus in the family *Hepeviridae*, which is widespread in cutthroat trout "*Oncorhynchus clarkia*" in the western USA (Batts et al., 2011). CTV is not associated with disease, and readily forms persistently infected cultures of the CHSE-214 cell line (Batts et al., 2011). It was originally considered to be a picornavirus. CTV has about 40% nucleotide (13–26% amino acid sequence) identity in pairwise alignments of the various regions of the genome with human hepatitis E virus, which is sufficiently low to warrant the creation of a novel genus, *Cutrovirus* (Batts et al., 2011). CTV is 93% identical to HKS virus, which is named after the original clinical presentation of ISA in New Brunswick, Canada, "hemorrhagic kidney syndrome," and was initially identified as a "toga-like" virus (Kibenge et al., 2000; Batts et al., 2011). The virus was discovered as a dual infection with ISAV; on its own, it was shown experimentally to be non-pathogenic to Atlantic salmon (Kibenge et al., 2000).

4. Ontogeny of the salmonid immune system

Bony fish (*Osteichthyes*) such as salmonids possess most components of the immune system we associate with higher vertebrates. While a complete review of ontogenic development is beyond the scope of this paper, the authors refer those interested to a more complete review of teleost immune ontogeny, especially with respect to zebrafish, by Zapata et al. (2006).

The two primary lymphoid organs in teleosts are the thymus and head kidney, or pronephros (Fig. 10). The head kidney is considered to have an analogous function to the bone marrow in higher vertebrates, and it also functions as a secondary lymphoid organ, along with the spleen. Developing B-cell populations in the head kidney have been described as remarkably similar to mouse bone marrow, showing three distinct B-cell stages, from early through late-developing B cells and IgM-secreting cells (Zwollo et al., 2010). In salmonids there is no distinct element separating the head- from the mid-kidney, the latter being glomeruli-rich, with some scattered T and B cells. Furthermore, the presence of a secondary site of lymphopoesis (K4) in the posterior kidney, recently identified by Zwollo et al. (2010), suggests that not only the anterior/head portion of the kidney is important in B-cell generation and development in salmonids. Aside from the lack of true bone marrow, teleosts also lack lymph nodes, germinal centres or a mucosa-associated lymphoid tissue (MALT), as in mammals (summarized in Table 2) (Tort et al., 2003). Recently, however, interbranchial lymphoid tissue (ILT) has been identified in salmonids, and suggested to be "dissimilar to all previously described lymphoid tissue" (Koppang et al., 2010). Morphological differences between the ILT and the thymus suggest different roles in salmonid immunity. Being purely intraepithelial in structure, the ILT differs from mammalian MALTs, and its location suggests that represents an evolutionary forerunner (Koppang et al., 2010). The ILT therefore represents a newly discovered secondary lymphoid organ in teleosts.

Finally, gut-associated lymphoid tissue (GALT) in teleosts is well developed, containing intraepithelial lymphocytes (considered to be T cells) and B lymphocytes within the gut lamina propria (Zapata et al., 2006). Until recently, teleost B cells were thought to produce only two isotypes, IgD and IgM, and IgM was thought to be

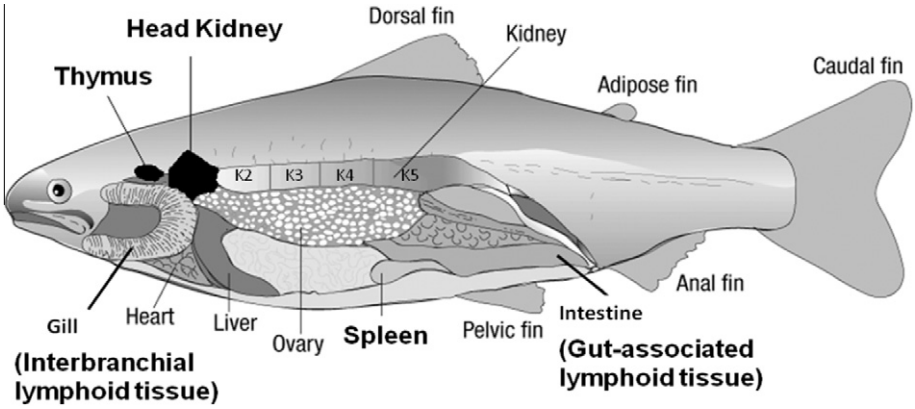

Fig. 10. Teleost anatomy in salmonids, highlighting lymphoid tissues. Kidney regions are approximations based on Zwollo et al. (2005). The figure was adapted from the original Aquatic Animal Health, Product Integrity Animal and Plant Health, AGDAFF.

Table 2
Comparative immune system of teleosts and mammals.

Comparative developmental vertebrate immunology	Teleosts[a]	Mammals
Physical barriers and interfaces	Skin mucus; scales; gills	Skin; respiratory epithelium of the lungs
Immune effector cell types	Neutrophil/heterophil; Eosinophil; Monocyte/Macrophage; Dendritic cell (only identified in trout gill structures so far); NK cell; T lymphocytes (helper, cytotoxic); B lymphocytes (plasma cells)	Neutrophil; Eosinophil; Basophil; Mast cell Monocyte/ Macrophage; Dendritic cell; NK cell; T lymphocytes (helper, cytotoxic); B lymphocytes (plasma cells)
Major antigen presentation cells	Monocyte/macrophage	Dendritic cell, macrophage/monocyte
Lymphoid tissues	Head kidney; Thymus; Spleen; Gut-associated lymphoid tissue (not well organized); Interbranchial lymphoid tissue	Bone marrow; Thymus; Spleen; Lymph nodes; Gut-associated lymphoid tissue; Germinal centres
Antibody		
Ig Diversity	IgM; IgD; IgT	IgM; IgD; IgA; IgE; IgG
Response to challenge/ rechallenge	Slow and weak memory response (temperature dependent)	Fast and strong memory
Affinity maturation (AM)	Low affinity, and low AM	High affinity and high AM

[a] In particular, salmonids (information restricted to development in environments within physiological optima: 10–15 °C).

the only isotype responsive to pathogens in both systemic and mucosal compartments. A third isotype, IgT, appears to be specialized in gut mucosal immunity, suggesting that differentiation of immunoglobulin isotypes occurred prior to tetrapod evolution (Zhang et al., 2011). In contrast to mammals, B cells in fish can apparently mature systemically and migrate to tissues, where they produce antibodies locally. Furthermore, mucosal antibody production has been shown to originate from tissue-specific B cells (Swan et al., 2008). However, it is not known whether IgT-secreting cells are localized in mucosal sites or are transported there systemically.

As in all vertebrates, blood cells of fish are formed from the ventral mesoderm, and hematopoetic tissues give rise to adult lymphoid cells and to all other definitive hematopoetic cell lineages (Zapata et al., 2006). In salmonids, there is a systematic combination of the two hematopoetic derivations observed in other teleosts: (1) exemplified by angel fish, in which initial hematopoesis occurs in the yolk sac, and (2), occurring later in salmonids and exemplified by zebrafish, in which early hematopoesis occurs in the intraembryonic intermediate cell mass (Zapata et al., 2006). In teleosts, the first leucocytes to populate these tissues are generally macrophages, in some cases within the yolk sac before hatch (*Danio rerio*), to the head kidney and spleen (*Cyprinus carpio*), and generally the thymus post-hatch (Mulero et al., 2007). Granulocytes such as heterophils/neutrophils and eosinophils have been observed shortly afterwards, but basophils are absent. Teleosts exhibit both B- and T-cell lineages. While T-helper and cytotoxic T cells are not well described, markers for these cells are beginning to become available for some species, and suggest the presence of similar T-cell subsets (Th-0, -1, -2, -17, T-regs, etc.) in salmonids and other teleosts. Functional polarization of alternative and classical macrophage activation also occurs in teleosts. These multiple activation states are important for the generation of effective initial microbial killing and for downstream memory responses (Fast et al., 2009; Joerink et al., 2006a,b).

Recombination activating gene (RAG) expression in whole rainbow trout has been identified 10 days post-fertilization (dpf), before the appearance of the thymus or kidney, and within the pronephros at hatching; Ig expression occurs from 14 dpf to 4–8 d pre-hatch (Zapata et al., 2006). In comparison, this occurs much faster in zebrafish (3 dpf for RAG; $\leqslant$3 weeks pf for Ig). However, it should be noted that these differing developmental rates are confounded by speciation, life history and poikilothermic physiology. While cold water (14 °C) species, such as rainbow trout, and warm water ($\geqslant$22 °C) species such as zebrafish, each have their own optimal temperature ranges, it is important to contextualize development at different temperatures, since rates would be expected to

decrease with increasing temperature and increase at lower temperatures within the optimal ranges for each species.

The presence of lymphocytes in lymphoid organs also follows this timeline, whereby the thymus is populated from about a week before to shortly after hatch, followed by the kidney at 4–5 days (including Ig + cells) and the spleen 1–2 weeks post-hatch. Although lymphocytes may populate these tissues, there is still a delay before the onset of functional humoral responses and immunocompetence. Ig + cells are not observed in the spleen of rainbow trout until a month after hatch, and humoral responses are not measured before at least 8 weeks (Manning and Grace, 1982; Zapata et al., 2006). However, salmonids such as rainbow trout do show earlier cell-mediated immunity, exhibiting allograft rejection by 2 weeks. Furthermore, antibody production to T-independent antigens occurs more quickly than to T-dependent antigens in fry (Manning and Grace, 1982; Zapata et al., 2006). Such late maturation of antibody and memory responses is further illustrated by the ineffective nature of immersion vaccines in salmonids under 1 g (Johnson et al., 1982). Whereas RAG and Ig expression appear to occur later in salmonids than in common carp and zebrafish, the population of lymphoid organs such as the kidney and spleen and the onset of functional humoral responses appear to occur within the same timeframe in these groups (Zapata et al., 2006).

5. Immunity of farmed fish to viral diseases

5.1. Innate antiviral responses

Virus entry requires initial binding to cellular receptors and penetration of the plasma membrane, with transfer of the genome and accessory proteins into the cytosol through membrane fusion in the case of enveloped viruses and pore formation or membrane lysis in the case of non-enveloped viruses (Flint et al., 2004). Genome transcription and replication produce viral nucleic acids such as dsRNA, single stranded (ss)RNA, and dsDNA in a left-handed Z-conformation, generated through negative supercoiling by RNA polymerases (Rich and Zhang, 2003). Thus ssRNA, dsRNA, and viral glycoproteins constitute the basic "Viral Associated Molecular Patterns" (VAMP) by which pathogen recognition receptors (PRR) detect an invading virus. The production and presentation of VAMPs may occur at multiple stages of the viral life cycle, with cellular compartmentalization determining VAMP-PRR interactions (Thompson and Locarnini, 2007). Several PRRs, including Toll-like receptors (TLRs) and RIG-I-like helicases (RLH) play significant roles in the initial stages of signaling pathways (Kawai and Akira, 2006; Randall and Goodbourn, 2008). Downstream signaling leads to the activation or synthesis of antiviral proteins such as Mx, ISG-15, ISG-56, PKR and OAS (see Workenhe et al., 2010). The stimulation of type I IFN-stimulated genes through this signaling pathway culminates in the production of inflammatory cytokines, chemokines and increased surface expression of co-stimulatory molecules. Teleost fish have been shown to respond to a number of TLR and RLH agonists, resulting in type I IFN-related protein expression similar to that seen in mammals (Purcell et al., 2006; Workenhe et al., 2010; Robertsen, 2008).

Recent research has identified a number of pathways that feed into this canonical response. In particular, studies over the past few years have unveiled previously unappreciated roles for mitochondria in innate immune responses, and it is becoming increasingly apparent that mitochondria participate in RLH signaling, antibacterial immunity and sterile inflammation. Recognition of the role of mitochondria in innate antiviral signaling began with the identification of an adaptor protein, mitochondrial antiviral signaling protein (MAVS), in RLH signaling. One of the most fascinating properties of MAVS is its association with the outer mitochondrial membrane (OMM), a location that is required for proper antiviral signaling (Seth et al., 2005). Follow-up research has yielded additional insights into signaling by MAVS downstream of RLHs, demonstrating that mitochondria are centrally positioned in innate antiviral responses. On another note, oxidative phosphorylation in mitochondria generates the majority of reactive oxygen species (ROS) in any given cell. Although ROS can damage cellular proteins, lipids and nucleic acids via oxidation, they also serve as crucial second messengers in various redox-sensitive signaling pathways. Interestingly, ROS have been implicated as both positive and negative modulators of RLH signaling (Jin et al., 2010; Soucy-Faulkner et al., 2010; Tal et al., 2009). Although the MAVS gene has been cloned and functionally characterized, the details of mitochondrial involvement in innate antiviral immunity of fish is not clearly established; further research should address these gaps.

5.2. Immune enhancement through feed additives

Fish feeds with additives that enhance growth and overall health beyond basic nutrition, often referred to as "functional feeds," are becoming more common as preventative and intervention strategies against aquatic pathogens. These include broad-based groups such as pro- and pre-biotics, immunostimulants and nucleotides (Burrells et al., 2001; Carrington and Secombes, 2006; Tacchi et al. 2011). In particular, the absence of chemotherapeutic intervention options, and the opportunities for synergism with acquired immune stimulation (i.e., vaccination), have driven research and application of feed additives for protection of fish against viral infection. There are examples of pre- (manno/fructo-oligosaccharides; MOS/FOS) and probiotics (lactobacillus/lactococcus and bifidobacterium) associated with enhanced innate responses such as inflammatory cytokine signaling, mucus production, increased phagocytic capacity, respiratory burst and complement activity (Tacchi et al., 2011). However, their application with respect to viral diseases is sparse. Harikrishnan et al. (2010) describe the use of Lactobacil® and Sporolac® (Inter Care Ltd.) in the olive flounder and its positive effects on complement, phagocytosis and oxidative burst. In this case, flounder were previously infected (30 days) with an iridovirus, lymphocystis diseases virus, and the enhanced innate responses were credited with a 45% reduction in mortality (Harikrishnan et al., 2010). At the time of this writing, the only probiotic feed additive available for use in salmonids in the EU is Bactocell®. However, it was initially released for its ability to reduce vertebral compression syndrome in salmonids, and there has been no published work on its use in the prevention of treatment of viral diseases.

5.2.1. Classical immunostimulants

In comparison to pro- and pre-biotics, which are often crude extracts used to enhance gut health, digestion and overall nutrition, immunostimulants are often purified products that aim to enhance innate immunity through pathogen-associated molecular patterns (PAMPs). The most common immunostimulant used in fish has been β-glucan, either through injection or in particular in feed, as in the 1–3, 1–6 β-glucan, Macrogard®. via i.p. injection, β-glucans have induced higher survival (40% at 10 mg/kg β-glucan mycelia of *Poria coccos* 85), more rapid and greater duration of Mx expression in grass carp "*Ctenopharyngodon idella*" experimentally exposed to grass carp hemorrhage virus (GCHV) (Kim et al., 2009), as well as protection against IHNV in salmonids (LaPatra et al., 1998). While Macrogard® has been used for years as a feed inclusion to combat bacterial disease and improve gut health, through enhancement of macrophage activity (Burrells et al., 2001), except for IHNV, there is only anecdotal evidence of its use against ISAV and other important viruses of salmonids.

Another group of immunostimulants are unmethylated cytosine-guanine oligodinucleotides (CpGs). CpGs have been shown to produce antiviral responses in salmonids (Jorgensen et al., 2003); type A and B CpGs show synergistic induction of type I and II interferons (Strandskog et al., 2008). CpGs have only recently been incorporated into feeds and administered to determine their effects on ectoparasitic infection in salmon (Covello et al., 2011a). In these studies, CpGs fed at 20 mg/kg were shown to transiently induce inflammatory signals such as IL-1β, IL-8 and TLR-9 signaling in head kidney and spleen tissues. This feed additive also showed protective responses against ectoparasitic infection (>40% reductions in infection load), both from initial exposure and enhanced responses after re-infection (>20% reductions in infection load above re-exposed controls), providing further evidence of the enhancement of innate and adaptive immune pathways (Covello et al., 2011a,b). The signaling pathways in these and i.p. studies with CpG, recruitment through GALT and interaction with other hematopoetic tissues (head kidney and spleen) are still not understood. Although evidence of anti-viral responses to injection has been observed, as previously mentioned, protection against viral exposure through CpG in feed has not been investigated, but will provide an interesting area of future research, especially in conjunction with oral vaccination and CpGs' proven efficacy as a vaccine adjuvant (Hartmann et al., 2000; Weeratna et al., 2000).

5.2.2. Commercial combination feeds

Feed additives have also been combined in various formulations by the world's leading salmon feed companies, EWOS and Skretting, to exploit synergisms. In 2008, Skretting released React PD2®, including glucans, probiotics and anti-oxidants to combat pancreas disease (PD); it has been suggested to reduce necrosis, inflammation and damage to pancreas and skeletal muscle, respectively, and mortality due to PD and HSMI. When combined with a 'proactive diet' and vaccination, React PD2® has also been shown to reduce mortality by 85% (McGurk et al., 2011). EWOS Opal PD® is another marketed feed additive targeting these pathologies, which also contains a mixture of immunostimulants and other components. Despite their use in commercial production and application, most feed additives are still largely untested in the public realm for their effects on antiviral responses and immunity to viral infection.

5.3. Vaccination of fish

Vaccination is a very effective way of protecting animals against infectious disease. Where properly applied in aquaculture, it has significantly reduced the need for antibiotic use as a compensation method for the immunosuppression associated with the intensification of farming fish (Fig. 11). However, fish respond differently from avians and mammals to vaccination. Major differences between fish and other vertebrates are that their metabolism and immune response are temperature-dependent (Rijkers et al., 1980; Harrahy et al., 2001). Moreover, fish produce antibodies with lower affinity for antigens (Pilstrøm, 2005). Affinity maturation does not occur to the same degree as in mammals (sub-populations of high-affinity lymphocytes emerge >15 weeks post-immunization, and affinity increases only 2–3 *x*); the nature of long-term immunological memory is still poorly understood (Kaatari et al., 2002; Ye et al., 2011). One extreme example is the Atlantic cod, which does not produce specific antibodies to natural infection or vaccination, contains no major histocompatability complex (MHC) II or CD 4 genes. While having greatly expanded MHC I and Toll-like-receptors (TLR) genes, it can be specifically protected against disease by vaccination (Pilstrøm, 2005; Barr et al., 2011). These unique features of the piscine immune system have meant that, in contrast to poultry and swine, vaccinated fish are not normally monitored for antibody responses in standard serological tests (Kibenge et al., 2002). However, specific antibody responses in Atlantic salmon were strongly correlated with vaccine protection against a virulent *Aeromonas salmonicida* infection (Bricknell et al., 1999; O'Dowd et al., 1999), and one would expect this to be true also for highly efficacious vaccines against fish viral diseases.

It is generally accepted that successful fish farming depends on the use of vaccination (Evensen, 2009; Plant and LaPatra, 2011; Sommerset et al., 2005; Thorarinsson and Powell, 2006), particularly when pathogen eradication is unlikely to be successful. With the expansive development of aquaculture in the 1990s, much effort was dedicated to vaccine development and the use of vaccines in fish farming became popular (Ellis, 1997; Gudding et al., 1999; Lillehaug, 1991; Newman, 1993; Håstein et al., 2005). However, in contrast to bacterial vaccines (Håstein et al., 2005), vaccines against viral diseases have not performed well to date, and antiviral drugs have not yet found their way into aquaculture. As a consequence, opportunities for synergism with vaccination have driven research and use of feed additives as immunostimulants to enhance innate immunity for protection of fish against viral infection. Today, farmed Atlantic salmon are routinely vaccinated against a number of bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral (IPN, ISA, PD, red sea bream iridoviral disease) diseases before sea transfer (Fig. 12) (McLoughlin and Graham, 2007; OIE, 2011). Thus, there are relatively few effective and officially licensed virus vaccines (Biering et al., 2005), and fish vaccinology is still a young and maturing science (Evensen, 2009).

5.3.1. Vaccine types

In the marine-farmed Atlantic salmon production cycle, fish are vaccinated when they are 30–40 g in weight, at the fingerlings stage,while in freshwater in hatcheries before smoltification and before being moved to the high-risk environment in sea cage grow-out sites (Sommerset et al., 2005) (Fig. 12). Of the five categories of vaccines (attenuated/modified live, inactivated, subunit, recombinant, and nucleic acid/naked DNA), the two main types currently in use are inactivated (killed) virus and sub-unit vaccines. These are either monovalent or polyvalent/multivalent (combination), and typically contain mineral-based adjuvants (e.g., aluminium hydroxide), oil-based adjuvants (e.g., mineral oil or vegetable oil) (Kuroda et al., 2004) or lipo-adjuvants such as liposomes to enhance immunogenicity (Audibert and Lise, 1993; Evensen et al., 2005). In Europe, the salmonid industry relies on a single injection of a multivalent (6–7 antigen) vaccine. Commercially available subunit fish vaccines include those against IPNV and ISAV.

Despite the numerous advantages of DNA vaccines (Heppell and Davis, 2000) and a number of technological improvements, including getting plasmids into cells, increasing protein production once they are inside, and modifications of the vaccine proteins that increase their recognition and response by the immune system (Mikalsen et al., 2004), to date there is only one commercially licensed DNA vaccine (against IHNV) for fish (Lorenzen and LaPatra, 2005; Kurath et al., 2007; Kurath, 2008).

A modified live vaccine consisting of attenuated koi herpesvirus (KHV), further mutated by UV irradiation (Perelberg et al., 2005), is currently licensed for use in Israel, and has been widely used in carp farms across the country (OIE, 2011). However, because of ecosafety concerns for the aquatic environment, requiring that an attenuated virus be proven non-pathogenic to wild fish, this is an isolated example, as there are no attenuated/modified live vaccines (Benmansour and De Kinkelin, 1997) widely licensed for use in fish (Sommerset et al., 2005). In addition, concern has been expressed that use of live modified vaccines without suitable markers may cause diagnostic confusion during cell culture-based certification examinations (Wolf, 1988b).

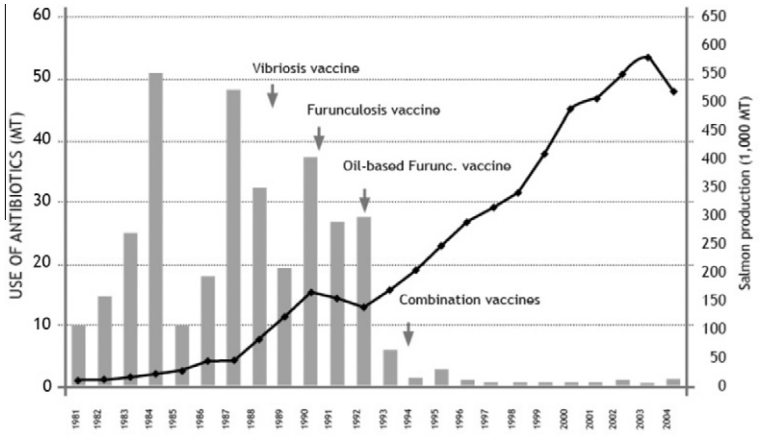

Fig. 11. Antibiotic use in fish (grey bars) in relation to Norwegian salmon production (black line joining square dots), 1981–2004. Note the dramatic drop in antibiotic use following the introduction of oil-based vaccines in 1992. These vaccines offered high protection against vibriosis caused by *Listonella (Vibrio) anguillarum* and furunculosis caused by *Aeromonas salmonicida* (Håstein et al., 2005).

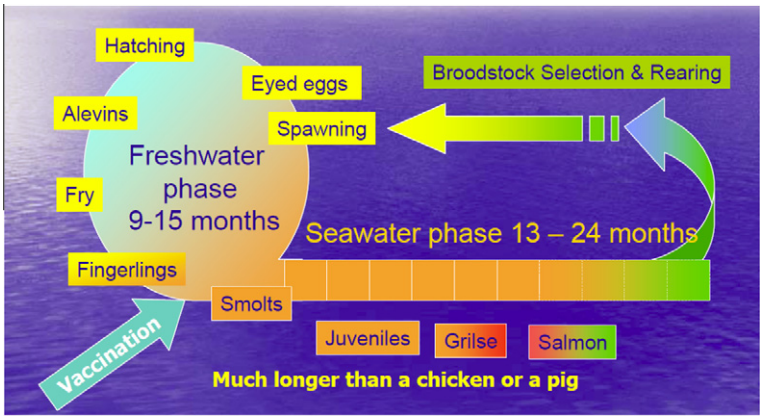

Fig. 12. Farmed Atlantic salmon production cycle (McLoughlin 2006). Because Atlantic salmon are anadromous (Anonymous 3), they have 2 phases. In the freshwater phase, adult salmon spawn and the eggs hatch into alevins and develop into fry, then fingerlings, or "parr", in freshwater rivers (or in the hatchery, in case of farmed fish). In the seawater phase, after the fingerlings undergo a parr-smolt transformation, the smolts migrate to the sea, or are moved to grow-out cages in the sea in case of farmed fish. Farmed fish are routinely vaccinated before sea transfer. Wild adult salmon stay in the ocean for 1–4 years before returning to their native rivers to spawn. Those returning after 1 year are called "grilse" (Anonymous 3; McCormick et al., 1998).

Table 3
Comparison of fish vaccination methods.

	Injection vaccination	Immersion vaccination	Oral vaccination
Target	Systemic	Skin and gills	Digestive system
Ease of administration	+(labour intensive)	++	+++ (mass vaccination of fish any size)
Fish weight	>15 g	1–5 g	1–5 g
Stress	Severe (requires anaesthetization & handling); Moderate for automated vaccination	None for baths; Moderate for spray; Severe for dip	None or negligible
Cost	Cost-effective for high value species[a]	Cost-effective for fish <10 g	Moderate
Efficacy/Potency	+++ (with adjuvants)	++	Weak (Inferior)
Side-effects	Severe with oil-adjuvant	None or negligible	None or negligible
Duration of immunity	6–12 months	Shorter	Shortest

[a] High value species include Atlantic salmon and Rainbow trout.

5.3.2. Vaccine delivery methods

The method of vaccine delivery is a major factor to consider in terms of efficacy, cost and side-effects. Three principal methods of administration are available: injection, immersion and oral vaccination; each has its advantages and disadvantages (Table 3). Of these, injection and immersion are the main methods (Bravo and Midtlyng, 2007); until recently, oral vaccination has been inferior in terms of efficacy (Ellis, 1997; Vandenberg, 2004; Plant and LaPatra, 2011).

5.3.2.1. Injection. Injection vaccination is either intraperitoneal (i.p.), with or without adjuvants and usually without boosters, or intramuscular (i.m.) for DNA vaccines (Corbeil et al., 2000). Injection is not the most economical method of vaccine delivery on a

large scale. Because fish have to be removed from the water and anesthesized, injections are labor-intensive and stressful. Moreover, injection requires fish to be over a certain size (>15–20 g), making vaccination of fry difficult (Plant and LaPatra, 2011). While it is clear that maternal transfer of some components of innate and adaptive immunity occurs in teleosts, resulting in enhanced protection of offspring, the mechanistic or pathway contributions are uncertain (Lillehaug et al., 1996; Tanaka et al., 1999) and functional targeting or boosting in larvae has therefore been limited (Mulero et al., 2007).

In large-scale vaccination of Atlantic salmon fingerlings, fish are transported in pipes from the rearing tank to an anesthetic bath, and are then injected manually by a professional team using air-powered syringes (Ellis, 2002) or by automated vaccination machines (Plumb and Hanson, 2010; Sharpe, 2007; Sommerset et al., 2005). This is commonly associated with serious side-effects, primarily inflammatory reactions (adhesions between intraperitoneal organs and melanin deposits on internal organs and on the abdominal wall) at the injection site (Midtlyng et al., 1996; Mutoloki et al., 2004), which may lead to a reduced growth rate (up to 10% reduction over 18 months) and death (up to 25% mortality). Reduced meat quality, carcass down-grading and spinal deformities (Berg et al., 2006; Midtlyng and Lillehaug, 1998; Sørum and Damsgård, 2004; Vandenberg, 2004); granulomatous uveitis (Koppang et al., 2004), and systemic autoimmunity are also seen (Koppang et al., 2008). This raises both economical and ethical concerns regarding current fish vaccination methods (Engelstad, 2005). Interestingly, vaccination of Atlantic cod results in rapid encapsulation of vaccine, less pronounced adhesions between internal organs and the abdominal wall and no pigmentation (Maira et al., 2008).

5.3.2.2. Immersion. The second principal method of vaccine delivery to fish is immersion in a suspension of vaccine (Antipa et al., 1980; Sommerset et al., 2005). Immersion can be by bath, dip, or spray. Spray vaccination is used for larger fish (for 2–5 s), where injection is not possible. Dips are usually for a very short time (5–60 s) in a concentrated vaccine, whereas baths are of longer duration (>60 min) in diluted vaccine (Egidius and Anderson, 1979; Ellis, 1988a; Horne and Ellis, 1988; Yanong, 2008). Immersion vaccination can also be by flush (Anderson et al., 1979). Such methods are cheap and easy (Plant and LaPatra, 2011), and are effective for several bacterial vaccines. However, they require large volumes of vaccine (Nakanishi and Ototake, 1997), and there is no good control for vaccine dose. Moreover, the degree and duration of immune protection induced by immersion vaccination is variable (Tatner, 1987; Tatner and Horne, 1983).

When fish are injected with vaccines, the protective antigens can be localized in the spleen and kidney (Tatner et al., 1984), which are the main organs of phagocytic filtration and the main sites for protective immunity. However, after immersion in the vaccine, the antigens are almost exclusively located on the outer surfaces, skin and gills. In fact, whereas injection induces a systemic immune response in spleen and kidney, it is believed that immersion induces mainly an integumentary immune response in the mucous membranes of skin, gills and gut (Ellis, 1988a). This explains the findings that for all studied vaccines, injection provides far better immune protection than immersion (Gudding et al., 1999; Midtlyng et al., 1996; Tatner et al., 1984).

A better strategy is to use immersion vaccination of 5 g fish, to protect them until they are big enough for injection. A newer version of immersion vaccination uses ultrasound (Plant and LaPatra, 2011), a high-frequency sound wave of approximately 20 kHz that enhances cell permeability. The method was found to be as effective as i.p. injection at providing protection against challenge (Zhou et al., 2002a,b). Another novel and equally effective method uses a multiple-puncture instrument to produce small lesions in the sides of fish while they are immersed in the vaccine (Nakanishi et al., 2002).

5.3.2.3. Oral vaccination. Oral vaccination, in which vaccine is administered in feed, is the most sought after method of delivery, since it is suitable for mass administration to fish of all sizes (Ellis, 1988a; Vandenberg, 2004). The exposure time is 1 week or longer (Horne and Ellis, 1988). The vaccine is mixed or bioencapsulated into the feed before feeding. When live food (e.g., rotifers or brine shrimp) is used, it is first added to a concentrated vaccine solution and allowed to take up the vaccine, then fed to fry or small fingerlings (Yanong, 2008). Oral vaccination is ideally suited for the salmon industry, particularly during the seawater growth stage in which injection vaccination is not possible while vaccine-induced immune responses from the freshwater stage decay (Tobar et al., 2011).

Despite the initial promise of oral vaccination (i.e., being completely non-stressful, as it does not interfere with routine husbandry practices), it was found to confer only a very limited level of immune protection (Newman, 1993). This mainly reflects the destruction of antigen in the stomach and upper intestinal tract before it reaches immune tissues in the lower intestinal tract (Ellis, 1988a; Sommerset et al., 2005; Georgopoulou et al., 1985; Rombout et al., 1985; Ellis, 1998; Vandenberg, 2004), plus the requirement for all fish to be feeding. In some cases, oral administration of antigens even resulted in suppression of immune responses (Lillehaug, 1989). Effective oral immunization (Vandenberg, 2004) therefore requires a delivery system that

- protects antigens from degradation in the acid environment of the stomach;
- ensures that antigens remain in the intestinal tract long enough to be taken up by the GALT; and
- controls the antigen dose, to ensure that every fish ingests the vaccine (Quentel and Vigneulle, 1997; Rombout et al., 2011).

To reduce leaching into the water and/or to provide some protection against digestion in the fish gut, a coating agent is often used (Ellis, 1995, 1998; Joosten et al., 1997; Vandenberg, 2004; Sommerset et al., 2005).

Plant-based vaccines have recently come into prominence, because they have the potential to be administered as oral vaccines with minimal expense in downstream processing and application (Companjen et al., 2005, 2006), while offering better protection against digestive degradation due to their fibrous tissues (Richter and Kipp, 1999). Other oral vaccine delivery systems for fish, including the microalgae *Chlamydomonas reinhardtii* expressing foreign antigens (i.e., transgenic microalgae) (Siripornadulsil et al., 2007) were recently reviewed by Plant and LaPatra (2011). The acid resistant cell wall of *Chlamydomonas* allows antigens to reach the immune tissues in the lower intestinal tract (Siripornadulsil et al., 2007). Vandenberg et al. (2003) have developed an Oralject™ method which delivers the vaccine with antiproteases and membrane permeability enhancers, allowing antigen to escape digestion in the foregut, resulting in enhanced uptake and immunogenicity (Shoemaker et al., 2006). Most recently, an oral delivery vehicle consisting of a bioadhesive cationic polysaccharide formulation (MicroMatrix™) has been developed (Harel, 2009) that is capable of inducing a lasting and specific immune response against viral pathogens such as ISAV (Murias, 2012).

5.3.3. Type and longevity of immune response

Although fish can and do regulate their body temperature, the immune response is affected by body temperature, which normally equals their surroundings. Water temperature during immunization determines how quickly immunity develops. Thus, fish immu-

nity is based on "degree-days", determined by multiplying the water temperature each day with the number of days post-vaccination (CVMP, 2011). A disadvantage of this method of assessing immune protection is that it does not predict immune memory, which can only be predicted by demonstration of specific immune responses (Ellis, 1988a). Another factor that complicates assessment of the potency of fish vaccines is that experimental waterborne challenge, which because it resembles natural exposure is more appropriate than injection, very rarely achieves high levels of morbidity with most fish pathogens (Biering et al., 2005). Thus, in most cases, it is compelling to assess vaccine effectiveness by using injection challenge to obtain the required minimum morbidity or mortality of 60% in control fish and <24% in the vaccinated fish (Ellis, 1988a; Amend, 1981). However, there is quite a lot of resistance to using injection challenges to assess vaccine performance (Nordmo, 1997). Clearly, a better understanding of the immune system of fish (Lieschke and Trede, 2009) will contribute to improved evaluation and application of vaccination in finfish aquaculture. For example, it has been shown that, when pathogen encounter occurs, fish injected with an optimal vaccine dose mount an extremely quick IL-22 response in the gill in contrast to non-immunised fish, and this may be key to mucosal immune protection from disease (Corripio-Miyar et al., 2009).

Immunocompetence in fish is based on developmental stage, rather than size, and is often a reflection of degree-days (Horne and Ellis, 1988). Salmonids therefore do not develop a very effective level of immunity when vaccinated below 0.5–1 g (Tatner and Horne, 1983) and do not develop prolonged immune memory until about 4 g (Johnson et al., 1982; Ellis, 1988b).

While fish lack some of the specialized cellular and tissue components of the mammalian GALT, there is considerable evidence for the ability of enterocytes, especially in the hind gut segment (Georgopoulou et al., 1985), to take up antigens and translocate them to macrophages and lymphocytes in the lamina propria and, under certain circumstances, to systemic lymphoid organs, i.e., the kidney and spleen (Ellis, 1998; Rombout and Van den Berg, 1989; Eldridge et al., 1990). In carp, studies suggest that oral delivery of antigens stimulates antibody production in the gut, gill and skin, but not in the kidney and blood, while parenteral injection stimulates the systemic compartment, but not the mucosal compartment (Hoel et al., 1997; Ellis, 1998). In salmonids, in contrast, oral immunization appears to stimulate only low responses in the gill, gut and skin, while injected antigen stimulates both systematic and mucosal responses, and stimulates the latter more effectively than oral immunization (Ellis, 1998). However, this finding may reflect measurement of IgM, rather than IgT *per se*. If only IgM was measured, it would show a better response to injection in all areas, whereas we may expect to see enhanced IgT during oral immunization, showing it may still be an option. Thus, while there is evidence of a common mucosal immune system in fish, there appears to be some species variation in the extent of its compartmentalization, and in the nature of antigen uptake by enterocytes (Ellis, 1998). For example, in carp, only soluble antigens are effectively taken up, through pinocytosis, while salmonid enterocytes can take up whole bacterial cells as well as soluble antigens (Ellis, 1998). However, for orally delivered antigens, the site in the gut where uptake occurs influences immunogenicity.

Longevity of immune protection is a major concern for vaccination of farmed fish. Only rarely is the duration of protection induced by a single vaccination longer than one year (Horne and Ellis, 1988; Newman 1993; Mitchell, 1995), while rearing time to harvest is much longer, commonly two years (Fig. 12). Consequently, many of the available fish vaccines are of limited potency. Booster vaccinations of large fish, usually by injection, are required to increase the duration of protection. This involves considerable labor, handling, and stress to the fish, which may result in mortality. No delivery systems are currently used in fish to prolong bioavailability of antigens. In addition, a number of potential adjuvants that might be used as antigen depots to increase the degree of immune protection cannot be used in aquaculture because they cause severe reactions or are toxic (Adams et al., 1988; Gudding et al., 1999; Midtlyng and Lillehaug, 1998; Evensen et al., 2005).

5.3.4. Vaccination against specific diseases

5.3.4.1. Vaccination against ISAV. Control of ISA by vaccination has been used in North America since 1999 and in the Faroe Islands since 2004, with variable results (OIE, 2011). Vaccination for ISA was allowed in most parts of Norway in 2010 (Johansen et al., 2011). The unprecedented spread of ISA in the Atlantic salmon industry in Chile in 2007–2011 has created a high demand for ISA vaccines, which has resulted in improved vaccine products being developed and marketed in Chile (Murias, 2012). However, their efficacy in the presence of wide-spread HPR0 infections is not known. Most vaccines consist of inactivated whole ISAV emulsified with adjuvant, administered by i.p. injection (Kibenge et al., 2003). It has recently been shown that the level of protection correlates with the amount of ISAV antigen in the vaccine; fish immunized with large amounts of antigen produce ISAV-specific and neutralizing antibodies and have a relative percent survival (RPS) as high as 86% (Lauscher et al., 2011). Virus is eliminated more rapidly from vaccinated than unvaccinated fish (Christie et al., 2003).

5.3.4.2. Vaccination against IPNV. Vaccine development has been problematic mainly for economic reasons, because the cost of producing inactivated viral vaccines from virus grown in fish cell lines is very high (Dorson, 1988). Recently, the use of improved culture methods and recombinant vaccine production has led to more affordable vaccines. Commercially available IPNV vaccines include inactivated whole virus and recombinant VP2 protein vaccines, produced in *Escherichia coli* or in yeast (Christie, 1997). They are incorporated into existing commercial vaccines against a variety of bacterial diseases and delivered by injection some months before smolts are transferred to sea water (Fig. 12). However, protection is not complete, and IPN continues to cause significant losses to the aquaculture industry in some European countries (Brun, 2003) and in Chile (Callejas et al., 2012) where different strains of virus co-circulate in some regions. The efficacy of IPNV vaccines in protecting against mortality in post-smolts is still uncertain because of the lack of reliable challenge models (Biering et al., 2005). Moreover, salmon broodstock are regularly tested for IPNV by culture methods, which is expensive and results in the destruction of eggs from infected parents. Large savings could be made if vaccines could prevent broodstock from becoming IPNV carriers.

Because vaccination may markedly reduce the number of carrier broodstock, but is unlikely to eliminate them completely, the need to test broodstock may not be eliminated, but the wastage of eggs from carrier parents would be greatly reduced. One problem with the efficacy of IPNV vaccines may be related to the prevalence of the virus in smolt farms. For example, 30–40% of hatcheries in Norway experience an outbreak of IPN each year, and its prevalence increases during the production cycle (Brun, 2003). This means that, in many cases, fish are already infected with IPN when they are vaccinated; it is not known if this has a negative impact on vaccine efficacy (Biering et al., 2005).

5.3.4.3. Vaccination against VHSV. Several attempts have been made to develop a safe and efficacious vaccine against VHSV using inactivated virus, live virus, and sub-unit protein (VHSV G protein), but with disappointing results (reviewed by De Kinkelin, 1988, and by Biering et al., 2005). A DNA vaccine expressing the VHSV G glycoprotein is efficacious in rainbow trout (Lorenzen et al., 1998;

Lorenzen and LaPatra, 2005). Most recent research has focused on a reverse-genetics approach (Biacchesi, 2011).

5.3.4.4. Vaccination against IHN. As in the case of VHS, several types of vaccines are effective experimentally against IHN (reviewed by Winton, 1997), but they have shown poor efficacy in field testing (Traxler et al., 1998). A DNA vaccine encoding the G glycoprotein was efficacious in rainbow trout, Atlantic salmon, chinook salmon and sockeye salmon (reviewed by Kurath, 2005), and is currently licensed for use in British Columbia (Kurath, 2008). An extensive field vaccination programme of 1.6 million marine-farmed Atlantic salmon undertaken in British Columbia in 2004/2005 in support of licensure demonstrated an RPS of 64% at 17 months post-vaccination in laboratory experiments (Salonius et al., 2007), but due to the absence of natural viral challenge at the testing sites, field efficacy was never confirmed. The recent IHNV outbreak in marine-farmed Atlantic salmon in British Columbia (Anonymous 4) involved unvaccinated fish. Vaccination against IHN is not allowed in areas of the European Union that have been declared disease-free.

5.3.5. Major hurdles in vaccine development

Major hurdles in the development of efficacious vaccines for aquaculture, are the costs, which include the vaccine dose, requirements for specialists (for example, labour-intensive, time-consuming handling of fish in injection vaccination), the costs of equipment and anaesthesia, plus the adverse effects of injection vaccination resulting in stress, injection site necrosis, adhesions of tissues, reduced growth rates and even death. Because fish generally require a larger antigen dose than terrestrial animals, inactivated viral vaccines have proven easy to develop, but they are difficult to make cost-effective (Sommerset et al., 2005; Biering et al., 2005). Although injection multivalent vaccines containing a combination of bacterins and inactivated virus or viral proteins are cost-effective, they require thorough evaluation to avoid antigenic competition, interference between different antigens and non-specific immunosuppression (Busch, 1997). More research is also needed to find safer alternatives to the oil-based adjuvants used in injection vaccines. Thus, vaccines that provide robust immunity against viral diseases are needed, and alternative methods for rapid and efficient delivery need to be developed and employed. Fortunately, the high level of knowledge of the molecular virology of important fish viruses now allows innovative approaches for vaccine development, including novel approaches to attenuation that may lead to a new generation of live, attenuated vaccines (Biacchesi, 2011; Lauring et al., 2010).

Oral administration of vaccines is by far the most sought-after method of delivery in the aquaculture industry (Vandenberg, 2004). The major advantage of in-feed (oral) vaccines is that they enable the farmer to immunize fish *en masse* from the moment they become immunocompetent, with a minimum of handling, significantly decreasing labour costs and improving fish production. Oral vaccines also permit multiple vaccinations, inducing intensive, long-term immune protection, and provide the only method suitable for mass immunization of fish of all sizes. Moreover, oral booster vaccinations can be performed quickly, for example during anticipated disease outbreaks. Because they require no change in husbandry practices, unlike the current i.p. injection method, stress to the fish is reduced, improving growth and shortening the production cycle. Oral vaccines would also avoid side-effects associated with injection. However, a main disadvantage of oral vaccines, which has never been considered, is the fact that there is a hierarchy in feeding, in which dominant fish would eat the majority of vaccine-containing food, while those lower down the pecking order get a lower than therapeutic dose. To date, little work has been carried out to assess the value of combining different vaccination methods (Ellis, 1988b), for example priming with injection in the hatchery stage, followed by oral vaccination as a boosting method in sea cages.

There is an on-going need for new formulations to increase the efficacy of fish viral vaccines. The inclusion of immunoregulatory molecules in a vaccine may have value in driving the immune response in a particular direction (Secombes, 2011), as has been demonstrated for terrestrial farm animals and humans (Charerntantanakul, 2009; Kayamuro et al., 2009). The use of polymeric micro- and nanoparticles in delivery systems represents a novel approach (Ellis, 1998), "immunobioengineering", in the context of aquaculture (Nielsen et al., 2011). This combined knowledge of materials sciences and immunology (Hubbell et al., 2009) has already improved the oral delivery of fish vaccines (Harel, 2009). The use of low-toxic and biodegradable materials such as polylactide-co-glycolic acids (PLGAs) to make micro- and nano-sized vaccine antigen carriers can improve antigen availability and/or presentation over time (Ellis, 1998; Lecaroz et al., 2006; Dobrovolskaia and McNeil, 2007; Elamanchili et al., 2007; Schlosser et al., 2008; Schliehe et al., 2011), increasing the duration of immunity of fish vaccines. PLGAs may also improve biosafety for salmon DNA vaccines (Faurez et al., 2010). Nielsen et al. (2011) have reviewed the research gaps and regulatory issues that remain to be addressed to facilitate the development and application of nanoparticle-based salmon vaccines.

6. Therapeutics for farmed fish viral diseases

It is generally accepted that the strict parasitic relationship between virus and host, which makes most antiviral agents toxic, is the principal obstacle to effective drug treatment of viral diseases. The best strategy for developing antiviral drugs is therefore to target processes unique to virus replication, i.e., those not essential for host-cell metabolism (Table 4). However, this means that clinically useful antiviral drugs characteristically have a narrow spectrum of activity, often against a single virus in a limited number of animal species, as any broader spectrum of action is normally associated with toxicity. In humans, antiviral drugs are largely restricted to the treatment of hepatitis, influenza and herpes- and retroviral infections. Much progress in antiviral drug therapy has been driven by the development of antiretroviral drugs (De Clercq, 2010) and increased knowledge of the biochemistry of viral replication (Murphy et al., 1999).

Although there are several limitations in veterinary medicine for the use of antiviral drugs, there are also many potential applications. The principal restriction is that no specific antiviral compounds are currently licensed solely for veterinary use, because of the high cost of developing and registering a new drug, especially for livestock (cattle, pigs, sheep, goats and poultry), in which extensive safety, toxicity and residue data are required. Chemotherapy in aquaculture has utility for some parasitic, fungal and bacterial diseases, but in aquatic animals farmed for human consumption, the risk of residues remaining in the tissues and being detected by food safety authorities is a major concern (Hill, 2005).

Because virus replication and pathology are usually advanced by the time clinical signs appear, antiviral compounds against infections in fish and other animals may be of value only:

- to treat apparently healthy animals that have been in contact with sick animals;
- as prophylaxis, in anticipation of stress (transport, market, mixing, racing, shows, competitions, etc.) which might reactivate latent herpesvirus infections or depress the immune system, and therefore increase susceptibility to viral infection;
- as an adjunct to emergency vaccination against an OIE-listed viral disease such as foot-and-mouth disease (FMD), to bridge the "immunity-gap" between vaccination and the development of an immune response (Goris et al., 2008);

Table 4
Targets for antiviral drugs.[a]

Virus replication step	Probable mechanism of action	Examples of drugs
Attachment of virion to cell receptor	Receptor analogs, Capsid-binding drugs	Pleconaril (ViroPharma VP-63843)
Penetration by Fusion	Viral fusion inhibitors	T-20 (Fuzeon)
Uncoating	Inhibit the uncoating of influenza A viruses by blocking the ion-channel activity of the viral M2 protein	Amantadine (Symmetrel), Rimantadine (Flumadine)
Early transcription from viral genome	Transcriptase inhibitors	Ribavirin, Isoprinosine, oligonucleotides
Reverse transcription	Nucleoside inhibitors	Azidothymidine (AZT, Zidovudine)
	Non-nucleoside inhibitors	Nevirapine
Translation of viral RNA into protein	Inhibition of translation of early viral mRNA	Interferon-α (IFN-α, Intron), IFN-β and IFN-γ
	Inhibition of translation of late mRNA of poxviruses by disintegrating the polyribosomes	Marburan
Posttranslational cleavage of proteins	Protease inhibitors	Indinavir, Ritonavir, Invirase, Nelfinavir
Replication of viral DNA genome	Nucleoside inhibitors	Acyclovir (Acycloguanosine)
	DNA polymerase inhibitor	Foscarnet (Foscovir)
Replication of viral RNA genome	Replicase inhibitors	
Assembly & Maturation	Protease inhibitors	Glycosylation inhibitors, Ritonavir
Release	Neuraminidase inhibitors	Zanamivir (Relenza), Oseltamivir (Tamiflu)

[a] Reviewed by Field and Laughlin (1999) and Murphy et al. (1999).

- to treat breeding stock, including artificial insemination and embryo-transfer animals such as cattle, horse, pigs, and sheep, against viruses that cause reproductive viral diseases; and
- to treat chronic or recurrent viral diseases in pets.

Although aquaculture drug approvals by the US Food and Drug Administration occurred as early as 1964, only eight active ingredients, sold as 18 different drug products, are currently approved in the USA (Bowker and Gaikowski, 2012). Antiviral drugs have been studied to identify *in vitro* activity against viruses of fish, but have not yet found commercial use in aquaculture.

The antiviral activity of acyclovir (acycloguanosine) on the fish herpesviruses *Oncorhynchus masou* virus (OMV) and channel catfish virus (CCV) was studied *in vitro*, using rainbow trout gonad (RTG-2) cells. (Kimura et al., 1983a). Viral replication was completely suppressed by 2.5 μg/ml of acyclovir, whereas the cells were considerably inhibited at 10 times that dose (Kimura et al., 1983a). However, acyclovir was ineffective when evaluated against OMV in chum salmon fry *in vivo*, probably because an effective drug level could not be maintained in the fish (Kimura et al., 1983b). A number of other compounds, including 1-β-d-Arabinofuranosyl-E-5-bromovinyluracil (BVaraU), 1-β-d-arabinofuranosyl-E-5-iodovinyluracil (IVaraU), 1-β-d-arbinofuranosyl-E-5-chlorovinyluracil (CVaraU) and 1-β-d-arabinofuranosyl-5-vinyluracil (VaraU), were also highly effective against OMV *in vitro*, using Yamame (*Oncorhynchus masou*) kidney cells (YNK), but *in vivo* treatment had no effect on the life span of infected fish (Suzuki et al., 1988).

Hasobe and Saneyoshi (1985) identified 11 compounds that inhibited replication of IHNV *in vitro*, but further testing of 5 of them (6-thioinosine, 5-hydroxyuridine, 9-[*S*]-(2,3-dihdroxypropyl)adenine, virazole, and chloroquin) *in vivo*, using steelhead trout fry did not show significant reductions in mortality (14–34% survival in treated groups, compared to 8% survival of untreated fish) (reviewed by Winton, 1991). Hasobe et al. (1986) further showed enhanced activity of nucleoside antibiotics against IHNV when combined with guanine-7-*N*-oxide. Ribavirin, amantadine, methisazone and bis-benzimidazole were also shown to be effective against IHNV in rainbow trout cell cultures (Hudson et al., 1988). Ribavirin was also effective against IPNV (Hudson et al., 1988).

Several other compounds have also shown *in vitro* inhibitory activity against viral diseases of fish. A natural product, 46NW-04A, extracted from *Pseudomonas fluorescens* was effective against enveloped viruses of fish, including IHNV and the salmon herpesvirus OMV (Kimura et al., 1990). The synthetic purine derivative Isoprinosine (Methisoprinol®, Imunovir®, Inosiplex®) with both antiviral (inhibits virus replication by suppressing viral RNA synthesis) and immunomodulatory properties (Anonymous 2) was shown to inhibit the replication of SVCV *in vitro*, but has not been tested under carp culture conditions (Siwicki et al., 2003).

In humans, recombinant IFN-α is widely used as an antiviral drug (Field and Laughlin, 1999). IFN-α has also been shown to protect pigs against FMDV (Chinsangaram et al., 2003). There are several reports of the antiviral effects of recombinant IFN against fish viruses *in vitro* (Robertsen et al., 2003; Long et al., 2004; Kitao et al., 2009; Wang et al., 2006; Ooi et al., 2008; Zhang et al., 2007). For example, Wang et al. (2006) reported that purified recombinant IFN expressed in *E. coli* had antiviral activity against SVCV and IHNV; the activity was higher on the EPC cell line than on a grass carp ovary cell line. Recombinant IFN has also been shown to be effective *in vivo* against IHNV in rainbow trout (Ooi et al., 2008), infectious spleen and kidney necrosis virus (ISKNV) infection in zebrafish (Li et al., 2010), and against red-spotted grouper nervous necrosis virus (RGNNV) infection in sevenband grouper "*Epinephelus septemfasciatus*" (Ohta et al., 2011).

The IFN-induced Mx protein is one of the best studied components of the antiviral state induced by type I IFN in many vertebrate species (Haller and Kochs, 2002). The Atlantic salmon Mx1 (ASMx1) protein has been shown to inhibit the replication of IPNV (Larsen et al., 2004) and ISAV *in vitro* (Kibenge et al., 2005). In the latter study, CHSE-214 cells constitutively expressing ASMx1 showed increased resistance to ISAV infection, manifested as delayed development and a significant reduction in the severity of CPE and a 10-fold reduction in virus yield. However, by real-time RT-PCR, no significant difference was observed in the mean threshold cycle (Ct) values of ISAV RNA levels, suggesting that ASMx1 activity occurs at a post-transcription step of virus replication, possibly in the cytoplasm (Kibenge et al., 2005).

Most recently, it has been demonstrated that the broad-spectrum antiviral drug ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (Virazole, Rebetol) is effective in inhibiting ISAV replication both *in vitro* and *in vivo* (Rivas-Aravena et al., 2011). Ribavirin is a nucleoside analog which, following intracellular phosphorylation, is a competitive inhibitor of the enzyme inosine-5′-monophosphate dehydrogenase (IMPDH), which catalyzes the conversion of IMP to XMP, an essential step in the *de novo* biosynthesis of guanine nucleotides (Markland et al., 2000). Inhibition of IMPDH reduces the level of intracellular GTP and dGTP pools (De Clercq, 1993), inhibiting viral RNA synthesis (Wray et al., 1985).

When ribavirin is used to treat human viral infections, a side-effect is anaemia, which results from the accumulation of the triphosphate form of the drug in erythrocytes (Markland et al., 2000), but this does not appear to be an issue when the drug is used to treat ISAV in fish cell lines and in Atlantic salmon (Rivas-Aravena et al., 2011). Moreover, the IMPDH-inhibitory activity of ribavirin is a minor intracellular component (Smee and Huggins, 1999), and it is possible that other proposed mechanisms of action (Gilbert and Knight, 1986; De Clercq, 1993) are more important in inhibiting ISAV, including inhibition of proinflammatory mediators induced by viral infection (Ning et al., 1998; Tam et al., 1999); the induction of lethal mutagenesis after ribavirin is incorporated into viral RNA (Crotty et al., 2001); the inhibition of guanyl transferase, reducing mRNA capping; or direct inhibition of the viral RNA polymerase (Field and Laughlin, 1999).

Antiviral strategies based on gene silencing (knockdown) by RNA interference (RNAi) are also being investigated as potential therapeutic tools against viral infections (Tan and Yin, 2004; Haasnoot et al., 2007). RNAi is an evolutionarily conserved process in plant and animal cells, in which double-stranded, short interfering RNAs (siRNAs) trigger a sequence-specific posttranscriptional gene silencing process, by specifically targeting a homologous sequence for cleavage by cellular ribonucleases (Hannon, 2002). RNAi targets viral mRNA in a sequence-specific, dose-dependent manner; it has been used to inhibit the replication of a number of viruses *in vitro* and/or *in vivo*. siRNA approaches against various fish viruses are being developed, including against iridovirus-tiger frog virus (Xie et al., 2005), VHSV (Schyth et al., 2006; Ruiz et al., 2009), red sea bream iridovirus (Dang et al., 2008), and rock bream iridovirus (Zenke et al., 2010).

7. Concluding remarks

Aquaculture is a millennia-old industry which is practiced at many levels in the modern world, from sustainable aquaculture to industrialized salmon farming, where it is significantly limited by viral diseases. Although several strategies used to control viral diseases in terrestrial farm animals have been applied to aquaculture, the aquatic environment poses a number of unique challenges, including the lack of physical barriers to wild fish populations, which act as an unavoidable pathogen reservoir for farmed fish. Because pathogen eradication cannot be accomplished in such an environment, vaccination is the only sustainable method for controlling viral diseases. However, there are relatively few efficacious vaccines, and fish vaccinology is still a young and maturing science, with many vaccines still under experimentation. Recent advances in immunology, pathogenesis and the biochemistry of viral replication and the knowledge gained from sequencing fish genomes will potentially affect vaccination strategies. Unlike bacterial diseases, for which there are both efficacious vaccines and antibiotics, antiviral drugs have not yet found commercial use in aquaculture. To enhance immunity to viral diseases, there is a growing use of "functional feeds" in salmonid aquaculture. A rational combination of vaccines, antiviral drugs and immunostimulant feeds would provide formidable protection against viral diseases of farmed fish.

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References

Adams, A. et al., 1988. The potency of adjuvanted injected vaccines in rainbow trout (*Salmo gairdneri* Richardson) and bath vaccines in Atlantic salmon (*Salmo salar* L.) against furunculosis. Aquaculture 69, 15–26.
Ahne, W. et al., 2002. Spring viraemia of carp (SVC). Dis. Aquat. Org. 52, 261–272.
Amend, D.F., 1981. Potency testing of fish vaccines. Dev. Biol. Standard. 49, 447–454.
Amend, D.F., Yasutake, W.T., Mead, R.W., 1969. A hematopoietic virus disease of rainbow trout and sockeye salmon. Trans. Am. Fish. Soc. 98, 796–804.
Anderson, D.P., Robertson, B.S., Dixon, O.W., 1979. Induction of antibody-producing cells in rainbow trout, Salmo gairdneri Richardson – by flush exposure. J. Fish Biol. 15, 317–322.
Anonymous 1: Intervet Schering-Plough Animal Health, 20671 Compact PD Technical Manual. (Published 2009)
Anonymous 2: Isoprinosine, <http://www.anti-aging-drugs.com/biblio/isoprinosine_biblio.php> (Published 2011; Consulted February 16, 2012).
Anonymous 3: Life cycle of Atlantic salmon, <http://www.marine.ie/home/services/operational/stock/Life+Cycle+of+the+Atlantic+Salmon.htm> (Consulted February 27, 2012).
Anonymous 4: IHN outbreak may be spreading. http://fis.com/fis/worldnews/worldnews.asp?l=e&country=0&special=aquaculture&monthyear=&day=&id=52379&ndb=1&df=0 (Published 2012; Consulted May 22, 2012).
Anonymous 5: IHN virus found in farmed fish. <http://www.fishfarmingxpert.com/index.php?page_id=76&article_id=94883> (Published 2012; Consulted May 17, 2012).
Antipa, R. et al., 1980. Vibrio anguillarum vaccination of sockeye salmon, *Onchorhynchus nerka* (Walbaum) by direct and hyperosmotic immersion. J. Fish Dis. 3, 161–165.
Ariel, E., Olesen, N.J., 2002. Finfish in aquaculture and their diseases – a retrospective view on the European community. Bull. Eur. Assoc. Fish Pathol. 22, 72–84.
Armstrong, R., Robinson, J.R., Rymes, C., Needham, T., 1993. Infectious hematopoietic necrosis virus in Atlantic salmon in British Columbia. Can. Vet. J. 34, 312–313.
Audibert, F.M., Lise, L.D., 1993. Adjuvants: current status, clinical perspectives and future prospects. Immunol. Today 14, 281–284.
Barr, B. et al., 2011. The genome sequence of Atlantic cod reveals unique immune system. Nature 477, 207–210.
Batts, W.N. et al., 2012. Genetic analysis of a novel nidovirus from fathead minnows. J. Gen. Virol. 93, 1247–1252.
Batts, W.N. et al., 2011. A novel member of the family *Hepeviridae* from cutthroat trout (*Oncorhynchus clarkii*). Virus Res. 158, 116–123.
Baudin-Laurencin, F., 1987. IHN in France. Bull. Eur. Ass. Fish Pathol. 7, 104.
Bell, J.G. et al., 1991. High dietary linoleic acid affects the fatty acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*): association with stress susceptibility and cardiac lesion. J. Nutr. 121, 1163–1172.
Benmansour, A., De Kinkelin, P., 1997. Live fish vaccines: history and perspectives: In: Gudding, R., Lillehaug, A., Midtlyng, P.J., Brown, F. (Eds), Fish Vaccinology, Developments in Biological Standardization, vol. 90. Karger, Basel, Switzerland, pp. 279–289.
Berg, A.E. et al., 2006. Time of vaccination influences development of adherences, growth and spinal deformities in Atlantic salmon (*Salmo salar* L.). Dis. Aquat. Org. 69, 239–248.
Bernet, D., Wahli, T., 2002. First report of SVC infection in koi carp in Switzerland. Bull. Eur. Assoc. Fish Pathol. 22, 229–231.
Biacchesi, S., 2011. The reverse genetics applied to fish RNA viruses. Vet. Res. 42, 12.
Biering, E., Villoing, S., Sommerset, I., Christie, K.E., 2005. Update on viral vaccines of fish. In: Midtlyng, P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 97–113.
Blake, S. et al., 2001. Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. Dis. Aquat. Org. 45, 89–102.
Bootland, L.M., Leong, J.C., 1999. Infectious haematopoietic necrosis virus. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Diseases and Disorders, Viral, vol. 3. Bacterial and Fungal Infections, CAB International, Oxon, UK, pp. 57–121.
Bovo, G., Giorgetti, G., Jorgensen, P.E.V., Olesen, N.J., 1987. Infectious haematopoietic necrosis: first detection in Italy. Bull. Eur. Ass. Fish Pathol. 7, 124.
Bowden, T., Bricknell, I., Ellis, A.E., 2003. Fish vaccination. An overview. Bodø, Norway: IntraFish; http://www.intrafish.com (Published 2003; Consulted March 27, 2003).
Bowker, J.D., Gaikowski, M.P., 2012. New aquaculture drugs under FDA review: products include antibiotics, oxidizing agents, sedative. Global Aquaculture Advocate January/February, 36–39.
Bowser, P.R. 2009. Fish Diseases: Viral Hemorrhagic Septicaemia (VHS). Northeastern Regional Aquaculture Center (NRAC), Publication No. 201–2009.
Bravo, S., Midtlyng, P.J., 2007. The use of fish vaccines in the Chilean salmon industry 1999–2003. Aquaculture 270, 36–42.
Bricknell, I.R., King, J.A., Bowden, T.J., 1999. Duration of protective antibodies, and the correlation with protection in Atlantic salmon (*Salmo salar* L.), following vaccination with an *Aeromonas salmonicida* vaccine containing iron-regulated outer membrane proteins and secretory polysaccharide. Fish Shellfish Immunol. 9, 139–151.
Brocklebank, J., Raverty, S., 2002. Sudden mortality caused by cardiac deformities following seining of preharvest farmed Atlantic salmon (*Salmo salar*) and by

cardiomyopathy of postintraperitoneally vaccinated Atlantic salmon parr in British Columbia. Can. Vet. J. 43, 129–130.
Brückner, G. et al., 2007. The OIE network of reference laboratories. J. Wildlife Dis. 43, S60–S63.
Brun, E. 2003. Epidemiology. In: Skjelstad, B. (Ed.), IPN in Salmonids: A review. ISBN 82-91743-16-9.
Burrells, C., Williams, P.D., Forno, P.F., 2001. Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to disease in salmonids. Aquaculture 199, 159–169.
Busch, R.A., 1997. Polyvalent vaccines in fish: the interactive effects of multiple antigens. Dev. Biol. Stand. 90, 245–256.
Callejas, et al. 2012. Use of reverse transcription-real time polymerase chain reaction (RRT-PCR) assays with Universal Probe Library (UPL) probes for the detection and genotyping of infectious pancreatic necrosis virus strains isolated in Chile. J. Virol. Meth. 183, 80–85.
Carrington, A.C., Secombes, C.J., 2006. A review of CpGs and their relevance to aquaculture. Vet. Immunol. Immunopathol. 112, 87–101.
Casselman, A., 2011. More salmon shenanigans in British Columbia. Nature News Blog Dec 02 2011. <http://blogs.nature.com/news/2011/12/more_salmon_shenanigans_in_bri.html>. (Published and Consulted December 2, 2011).
Castric, J., De Kinkelin, P., 1980. Occurrence of viral haemorrhagic septicaemia in rainbow trout *Salmo gairdneri* Richardson reared in sea water. J. Fish Dis. 3, 21–27.
Castric, J., De Kinkelin, P., 1984. Experimental study of the susceptibility of two marine fish species, sea bass (Dicentrarchus labrax) and turbot (Scophthalmus maximus), to viral haemorrhagic septicaemia. Aquaculture 41, 203–212.
Castric, J. et al., 1997. Isolation of the virus responsible for sleeping disease in experimentally infected rainbow trout (*Oncorhynchus mykiss*). Bull. Eur. Ass. Fish Pathol. 17, 27–30.
Castro-Nallar, E. et al., 2011. Molecular phylodynamics and protein modeling of infectious salmon anaemia virus (ISAV). BMC Evolutionary Biology 11, 349.
Chappell, J.D. et al., 2005. Genus Orthoreovirus. In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, pp. 447–454.
Charerntantanakul, W., 2009. Adjuvants for porcine reproductive and respiratory syndrome virus vaccines. Vet. Immunol. Immunopathol. 129, 1–13.
Chinsangaram, J. et al., 2003. Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. J. Virol. 77, 1621–1625.
Christie, K.E., 1997. Immunization with viral antigens: infectious pancreatic necrosis. Dev. Biol. Stand. 90, 191–199.
Christie, K.E., et al. 2003. Vaccination against infectious salmon anaemia: onset and duration of protection and carrier status post challenge. Abstract. 11th International Conference of the EAFP, Malta, September 2003.
Companjen, A.R. et al., 2006. Improved uptake of plant-derived LTB-linked proteins in carp gut and induction of specific humoral immune responses upon infeed delivery. Fish Shellfish Immunol. 21, 251–260.
Companjen, A.R., et al. 2005. Development of a cost-effective oral vaccination method against viral disease in fish. In: Midtlyng P.J. (Ed), Progress in Fish Vaccinology, Developments in Biological Standardization. Karger, Basel, Switzerland, vol. 121, pp. 143–150.
Corbeil, S., Kurath, G., LaPatra, S.E., 2000. Fish DNA vaccine against infectious hematopoietic necrosis virus: efficacy of various routes of immunization. Fish Shellfish Immunol. 10, 711–723.
Corripio-Miyar, Y. et al., 2009. Identification of interleukin-22 in gadoids and examination of its expression level in vaccinated fish. Mol. Immunol. 46, 2098–2106.
Cottet, L. et al., 2010. Bioinformatic analysis of the genome of infectious salmon anaemia virus associated with outbreaks with high mortality in Chile. J. Virol. 84, 11916–11928.
Covello, J., et al., 2011a. Orally administered immunostimulants affect inflammatory gene expression and sea lice (Lepeophtheirus salmonis) burdens on Atlantic salmon (*Salmo salar*). In: 15th International Conference on Diseases of Fish and Shellfish, Radisson Blu Hotel, Split, Croatia, September 12–16, 2011.
Covello, J., et al., 2011b. The effects of orally administered immunostimulants on Atlantic salmon (*Salmo salar*) immune gene expression and sea lice (Lepeophtheirus salmonis) infection. In: 52nd Annual Western Fish Disease Workshop, Vancouver Island Convention Centre, Nanaimo, British Columbia, June 14–16, 2011.
Crotty, S., Cameron, C.E., Andino, R., 2001. RNA virus error catastrophe: direct molecular test by using ribavirin. Proc. Natl. Acad. Sci. USA 98, 6895–6900.
Cunningham, C.O. et al., 2002. A novel variant of the infectious salmon anaemia virus (ISAV) haemagglutinin gene suggests mechanisms for virus diversity. Bull. Eur. Ass. Fish Pathol. 22, 366–374.
CVMP: Committee for Medicinal Products for Veterinary Use; Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines. November 14, 2011. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/11/WC500118226.pdf (downloaded January 1 2012).
Dang, L.T. et al., 2008. Inhibition of red sea bream iridovirus (RSIV) replication by small interfering RNA (siRNA) in a cell culture system. Antiviral Res. 77, 142–149.
Debes, H.C. et al., 2011. A low-pathogenic variant of infectious salmon anaemia virus (ISAV-HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (*Salmo salar* L.) in the Faroe Islands. J. Gen. Virol. 92, 909–918.
De Clercq, E., 2010. Antiretroviral drugs. Curr. Opin. Pharmacol. 10, 507–515.
De Clercq, E., 1993. Antiviral agents: characteristic activity spectrum depending on the molecular target with which they interact. Academic Press, Inc., New York, NY, pp. 1–55.
De Kinkelin, P., 1988. Vaccination against viral haemorrhagic septicaemia. In: Ellis, A.E. (Ed.), Fish Vaccination. Academic Press Limited, London, pp. 172–192.
DFO Science, 2011. Halibut aquaculture. <http://www.dfo-mpo.gc.ca/science/Publications/article/2007/01-10-2007-eng.htm> (Published 2011; Consulted February 11, 2012).
DFO, 2011. Infectious salmon anaemia (ISA) virus – Accepted testing methods. November 2011. <http://www.dfo-mpo.gc.ca/media/back-fiche/2011/20111108-eng.htm> (Published and Consulted November 08, 2011).
Dikkeboom, A.L. et al., 2004. First report of spring viraemia of carp virus (SVCV) in wild common carp in North America. J. Aquat. Anim. Health 16, 169–178.
Dixon, P.F., 1999. VHSV came from the marine environment: clues from the literature, or just red herrings? Bull. Eur. Ass. Fish Pathol. 19, 60–65.
Dopazo, C.P., Bandín, I., 2006. Global epidemiology: Worldwide distribution of IPNV is not only a consequence of aquaculture and commercial movements of cultured fish. DIP Newsletter 30, VESO, Norway.
Dobrovolskaia, M.A., McNeil, S.E., 2007. Immunological properties of engineered nanomaterials. Nat. Nanotechnol. 2, 469–478.
Dorson, M., 1988. Vaccination against infectious pancreatic necrosis. In Ellis, A.E. (Ed,), Fish Vaccination. London, Academic Press Limited, pp. 162–171.
Duarte, C.M., Marbá, N., Holmer, M., 2007. Rapid domestication of marine species. Science 316, 382–383.
Egidius, E.C., Anderson, K., 1979. Bath immunization – a practical and non-stressing method of vaccinating sea farmed rainbow trout *Salmo gairdneri* Richardson against vibriosis. J. Fish Dis. 2, 405–410.
Einer-Jensen, K. et al., 2004. Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. J. Gen. Virol. 85, 1167–1179.
Elamanchili, P. et al., 2007. "Pathogen-Mimicking" nanoparticles for vaccine delivery to dendritic cells. J. Immunother. 30, 378–395.
Eldridge, J.H. et al., 1990. Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. J. Controlled Release 11, 205–214.
Ellis, A.E., 2002. Health and disease in Atlantic salmon farming. In: Stead, S.M., Laird, L.M. (Eds.), Handbook of salmon farming. Springer Praxis, Chichester, pp. 373–401.
Ellis, A.E., 1998. Meeting the requirements for delayed release of oral vaccines for fish. J. Appl. Ichthyol. 14, 149–152.
Ellis, A., 1997. Vaccines for farmed fish. In: Pastoret, P.P., Blancou, J., Vannier, P., Verschueren, C. (Eds.), Veterinary Vaccinology. Elsevier, Amsterdam, pp. 411–417.
Ellis, A.E., 1995. Recent development in oral vaccine delivery systems. Fish Pathol. 30, 293–300.
Ellis, A.E., 1988a. General principles of fish vaccination. In: Ellis, A.E. (Ed.), Fish vaccination. Academic Press Limited, London, pp. 1–19.
Ellis, A.E., 1988b. Optimizing factors for fish vaccination. In: Ellis, A.E. (Ed.), Fish vaccination. Academic Press Limited, London, pp. 32–46.
Elsayed, E. et al., 2006. Isolation of viral hemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St. Clair, Michigan, USA reveals a new sub-lineage of the North American genotype. J. Fish Dis. 29, 611–619.
Emmenegger, E.J., Kurath, G., 2002. Genetic characterization of infectious hematopoietic necrosis virus of coastal salmonid stocks in Washington State. J. Aquat. Anim. Health 14, 25–34.
Emmenegger, E.J. et al., 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Dis. Aquat. Org. 40, 163–176.
Engelking, H.M., Harry, J.B., Leong, J.C., 1991. Comparison of representative strains of infectious hematopoietic necrosis virus by serological neutralization and cross-protection assays. Appl. Environ. Microbiol. 57, 1372–1378.
Engelstad, M., 2005. Vaccination and consumer perception of seafood quality. In: Midtlyng, P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 245–254.
Enzmann, P.J. et al., 2005. Infectious hematopoietic necrosis virus: monophyletic origin of European IHNV isolates from North-American genogroup M. Dis. Aquat. Org. 66, 187–195.
Evensen, Ø., 2009. Development in fish vaccinology with focus on delivery methodologies, adjuvants and formulations. Options Méditerranéennes, A/No. 86, The use of veterinary drugs and vaccines in Mediterranean aquaculture.
Evensen, Ø., Brudeseth, B., Mutoloki, S., 2005. The vaccine formulation and its role in inflammatory processes in fish – effects and adverse effects. In: Midtlyng, P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 117–125.
FAO, 2010. The State if World Fisheries and Aquaculture 2010. Food and Agriculture Organization of the United Nations, Rome.
Fast, M.D. et al., 2009. Mutation within the *Aeromonas salmonicida* type III secretion system affects host macrophage activation and downstream immune responses. Fish Shellfish Immunol. 27, 721–728.
Faurez, F. et al., 2010. Biosafety of DNA vaccines: new generation of DNA vectors and current knowledge on the fate of plasmids after injection. Vaccine 28, 3888–3895.
Ferguson, H.W., Poppe, T., Speare, D.J., 1990. Cardiomyopathy in farmed Norwegian salmon. Dis. Aquat. Org. 8, 225–231.
Ferguson, H.W. et al., 2005. An outbreak of disease resembling heart and skeletal muscle inflammation in Scottish farmed salmon, *Salmo salar* L., with observations on myocardial regeneration. J. Fish Dis. 28, 119–123.

Field, A.K., Laughlin, C.A., 1999. Antivirals. In: Granoff, A., Webster, R.G. (Eds), Encyclopedia of Virology, second ed., vol. 1. Academic Press Ltd. pp 54–68.
Fijan, N. et al., 1971. Isolation of the viral causative agent from the acute form of infectious dropsy of carp. Veterinarski Archiv 41, 125–138.
Fisher, M.C., Garner, T.W.J., 2007. The relationship between the emergence of Batrachochytrium dendrobatidis, the international trade in amphibians and introduced amphibian species. Fungal Biol. Rev. 21, 2–9.
Flint, S.J. et al., 2004. Principles of Virology. ASM Press, Washington, DC.
Forrester, N.L. et al., 2012. Genome-scale phylogeny of the alphavirus genus suggests a marine origin. J. Virol. 86, 2729–2738.
Fringuelli, E. et al., 2008. Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. J. Fish Dis. 31, 811–823.
Fritsvold, C. et al., 2009. Experimental transmission of cardiomyopathy syndrome (CMS) in Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 87, 225–234.
Gagné, N. et al., 2007. Isolation of viral haemorrhagic septicaemia virus from mummichog, stickleback, striped bass and brown trout in eastern Canada. J. Fish Dis. 30, 213–223.
Garver, K.A. et al., 2007. First detection and confirmation of spring viraemia of carp virus in common carp, *Cyprinus carpio* L., from Hamilton Harbour, Lake Ontario. Can. J. Fish Dis. 30, 665–671.
Gaughan, D.J., 2002. Disease-translocation across geographic boundaries must be recognized as a risk even in the absence of disease identification: the case with Australian *Sardinops*. Rev. Fish Biol. Fish. 11, 113–123.
Georgopoulou, U., Sire, M.F., Vernier, J.M., 1985. Macromolecular absorption of proteins by epithelial cells of the posterior intestinal segment and their intracellular digestion in the rainbow trout. Ultrastructural and biochemical study. Biol. Cell 53, 269–282.
Gjedrem, T., 2010. The first family-based breeding program in aquaculture. Rev. Aquacult. 2, 2–15.
Gilbert, B.E., Knight, V., 1986. Biochemistry and clinical applications of ribavirin. Antimicrob. Agents Chemother. 30, 201–205.
Godoy, M.G. et al., 2008. First detection, isolation and molecular characterization of infectious salmon anaemia virus associated with clinical disease in farmed Atlantic salmon (*Salmo salar*) in Chile. BMC Vet. Res. 4, 28.
Goodwin, A.E., 2002. First report of spring viraemia of carp virus (SVCV) in North America. J. Aquat. Anim. Health 14, 161–164.
Goris, N., Vandenbussche, F., De Clercq, K., 2008. Potential of antiviral therapy and prophylaxis for controlling RNA viral infections of livestock. Antiviral Res. 78, 170–178.
Graham, D.A. et al., 2007. First laboratory confirmation of salmonid alphavirus infection in Italy and Spain. J. Fish Dis. 30, 569–572.
Graham, D.A. et al., 2006. Sub-clinical infection of farmed Atlantic salmon (*Salmo salar* L.) with salmonid alphavirus – a prospective longitudinal study. Dis. Aquat. Org. 72, 193–199.
Graham, D.A. et al., 2003. First isolation of sleeping disease virus from rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom. J. Fish Dis. 26, 691–694.
Graham, D.A. et al., 2011. A comparative study of marine salmonid alphavirus subtypes 1–6 using an experimental cohabitation challenge model. J. Fish Dis. 34, 273–286.
Fajfar, Grilc. et al., 2011. Genotyping of Slovenian infectious hematopoietic necrosis virus isolates based on the "mid-G" region sequences of the glycoprotein gene. Bull. Eur. Ass. Fish Pathol. 31, 47–57.
Grischkowsky, R.S., Amend, D.F., 1976. Infectious hematopoietic necrosis virus: prevalence in certain Alaskan sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Board Can. 33, 186–188.
Groocock, G.H. et al., 2007. Detection of viral haemorrhagic septicaemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. Dis. Aquat. Org. 76, 187–192.
Groot, C., 1996. Salmonid life histories. In: Principles of Salmonid Culture. In: Pennell, W., Barton, B.A. (Eds.), Developments in Aquaculture and Fisheries Science, vol. 29, Elsevier, Amsterdam, pp. 97–230.
Gudding, R., Lillehaug, A., Evensen, O., 1999. Recent developments in fish vaccinology. Vet. Immunol. Immunopathol. 72, 203–212.
Gunnarsson, V.I., 2007. http://www.fisheries.is/aquaculture/species/cod/; http://www.fisheries.is/aquaculture/species/arctic-charr/; http://www.fisheries.is/aquaculture/species/atlantic-salmon/; http://www.fisheries.is/aquaculture/species/atlantic-halibut/; http://www.fisheries.is/aquaculture/species/turbot/; http://www.fisheries.is/aquaculture/species/other-species/ (Published 2007; Consulted February 3, 2012).
Haasnoot, J., Westerhout, E.M., Berkhout, B., 2007. RNA interference against viruses: strike and counterstrike. Nat. Biotechnol. 25, 1435–1443.
Haenen, O.L.M. et al., 2004. The emergence of koi herpesvirus and its significance to European aquaculture. Bull. Eur. Ass. Fish Pathol. 24, 293–307.
Hall, S.J., et al., 2011. Blue Frontiers: Managing the environmental cost of aquaculture. The Worldfish Center, Penang, Malaysia.
Haller, O., Kochs, G., 2002. Interferon induced Mx proteins: Dynamin like GTPases with antiviral activity. Traffic 3, 710–717.
Hannon, G.J., 2002. RNA interference. Nature 418, 244–251.
Harel, M., 2009. Inventor; Advanced Bionutrition., assignee. Encapsulated vaccines for the oral vaccination and boostering of fish and other animals. United States Patent US 20090238845.
Harikrishnan, R., Balasundaram, C., Heo, M.-S., 2010. Effect of probiotics enriched diet on *Paralichthys olivaceus* infected with lymphocystis disease virus. Fish Shellfish Immunol. 29, 868–874.
Harrahy, L.N.M., Schreck, C.B., Maue, A.G., 2001. Antibody-producing cells correlated to body weight in juvenile Chinook saslmon (*Oncorhynchus tshawytscha*) acclimated to optimal and elevated temperatures. Fish Shellfish Immunol. 11, 653–659.
Hartmann, G. et al., 2000. Delineation of a CpG phosphorothioate oligodeoxynucleotide for activating primate immune responses in vitro and in vivo. J. Immunol. 164, 1617–1624.
Hartman, K.H. 2004. Koi Herpesvirus (KHV) Disease. University of Florida IFAS Extension VM-149. http://edis.ifas.ufl.edu/pdffiles/VM/VM11300.pdf (Published 2004, Revised 2008; Consulted February 16, 2012).
Hasobe, M., Saneyoshi, M., 1985. On the approach to the viral chemotherapy against infectious hematopoietic necrosis virus (IHNV) *in vitro* and *in vivo* on salmonid fishes. Fish Pathol. 20, 343–351.
Hasobe, M., Saneyoshi, M., Isono, K., 1986. The synergism of nucleoside antibiotics combined with guanine-7-*N*-oxide against a rhabdovirus, infectious hematopoietic necrosis virus (IHNV). J. Antibiotics 39, 1291–1297.
Håstein, T., Gudding, R., Evensen, Ø. 2005. Bacterial vaccines for fish – An update of the current situation worldwide. In: Midtlyng P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 55–74.
Haugland, Ø. et al., 2011. Cardiomyopathy syndrome of Atlantic salmon (*Salmo salar* L.) is caused by a double-stranded RNA virus of the *Totiviridae* family. J. Virol. 85, 5275–5286.
Hedrick, R.P. et al., 2003. Host and geographic range extensions of the North American strain of viral hemorrhagic septicaemia virus. Dis. Aquat. Org. 55, 211–220.
Heppell, J., Davis, H.L., 2000. Application of DNA vaccine technology to aquaculture. Adv. Drug Deliv. Rev. 43, 29–43.
Hill, B.J., 1982. Infectious pancreatic necrosis virus and its virulence. In: Roberts, R.J. (Ed.), Microbial Diseases of Fish. Academic Press, London, pp. 91–114.
Hill, B.J., 2005. The need for effective disease control in international aquaculture. In: Midtlyng, P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 3–12.
Hill, B.J., Way, K., 1995. Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. Ann. Rev. Fish Dis. 5, 55–77.
Hodneland, K. et al., 2005. New subtype of salmonid alphavirus (SAV), *Togaviridae*, from Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* in Norway. Dis. Aquat. Org. 66, 113–120.
Hoel, K., Salonius, K., Lillehaug, A., 1997. *Vibrio* antigens of polyvalent vaccines enhance the humoral response of *Aeromonas salmonicida* antigens in Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol. 7, 71–80.
Holland, J.J., De La Torre, J.C., Steinhauer, D.A., 1992. RNA virus populations as quasispecies. Curr. Top. Microbiol. Immunol. 176, 1–20.
Horlyck, V. et al., 1984. Occurrence of VHS in Danish maricultured rainbow trout. Bull. Eur. Assoc. Fish Pathol. 4, 11–13.
Horne, M.T., Ellis, A.E., 1988. Strategies of fish vaccination. In: Ellis, A.E. (Ed.), Fish Vaccination. Academic Press Limited, London, pp. 55–66.
Hubbell, J.A., Thomas, S.N., Swartz, M.A., 2009. Materials engineering for immunomodulation. Nature 462, 449–460.
Hudson, J.B., Graham, E.A., Simpson, M.F., 1988. The efficacy of amantadine and other antiviral compounds against two salmonid viruses *in vitro*. Antiviral Research 9, 379–385.
Hsu, Y., Engelking, H.M., Leong, J.C., 1986. Occurrence of different types of infectious hematopoietic necrosis virus in fish. Appl. Environ. Microbiol. 52, 1353–1361.
Isshiki, T. et al., 2001. An outbreak of VHSV (viral haemorrhagic septicaemia virus) infection in farmed Japanese flounder Paralichthys olivaceus in Japan. Dis. Aquat. Org. 47, 87–99.
Jarp, J. et al., 1995. Risk factors for furunculosis, infectious pancreatic necrosis and mortality in post-smolt of Atlantic salmon, *Salmo salar* L.. J. Fish Dis. 18, 67–78.
Jin, L., Lenz, L., Cambier, J., 2010. Cellular reactive oxygen species inhibit MPYS induction of IFNβ. PLoS ONE 5, e15142.
Joerink, M. et al., 2006a. Head kidney-derived macrophages of common carp (*Cyprinus carpio* L.) show plasticity and functional polarization upon differential stimulation. J. Immunol. 177, 61–69.
Joerink, M., Savelkoul, H.H.J., Wiegertjes, G.F., 2006b. Evolutionary conservation of alternative activation of macrophages: Structural and functional characterization of arginase 1 and 2 in carp (*Cyprinus carpio* L.). Mol. Immunol. 43, 1116–1128.
Johnson, K.A., Flynn, J.K., Amend, D.F., 1982. Onset of immunity in salmonid fry vaccinated by direct immersion in *Vibrio anguillarum* and *Yersinia ruck*eri bacterins. J. Fish Dis. 5, 197–206.
Johansen, L.-H. et al., 2011. Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. Aquaculture 315, 167–186.
Joosten, P.H.M. et al., 1997. Oral vaccination of fish against *Vibrio anguillarum* using alginate microparticles. Fish Shellfish Immunol. 7, 471–485.
Jorgensen, J.B., Johansen, L.-J., Steiro, K., Johansen, A., 2003. CpG DNA induces protective antiviral immune responses in Atlantic salmon (*Salmo salar* L.). J. Virol. 77, 11471–11479.
Kaatari, S.L., Zhang, H.L., Khor, I.W., Kaatari, I.M., Shapiro, D.S., 2002. Affinity maturation in trout: clonal dominace of high affinity antibodies late in the immune response. Dev. Comp. Immunol. 26, 191–200.
Karlsen, M. et al., 2005. Genetic stability within the Norwegian subtype of salmonid alphavirus (family *Togaviridae*). Arch. Virol. 151, 861–874.
Kawai, T., Akira, S., 2006. Innate immune recognition of viral infection. Nat. Immunol. 7, 131–137.

Kayamuro, H. et al., 2009. The use of a mutant TNF-alpha as a vaccine adjuvant for the induction of mucosal immune responses. Biomaterials 30, 5869–5876.
Kent, M.L., Elston, R.A., 1987. Pancreas disease in pen-reared Atlantic salmon in North America. Bull. Eur. Ass. Fish Pathol. 7, 29–31.
Kibenge, F.S.B. et al., 2009. Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. Virol. J. 6, 88.
Kibenge, F.S.B. et al., 2007. Mapping of putative virulence motifs on infectious salmon anaemia virus surface glycoprotein genes. J. Gen. Virol. 88, 3100–3111.
Kibenge, F.S.B. et al., 2004. Infectious salmon anaemia virus: causative agent, pathogenesis and immunity. Anim. Health Res. Rev. 5, 65–78.
Kibenge, F.S.B., et al. 2003. The development of infectious salmon anemia virus vaccines in Canada. In: Miller, O., Cipriano, R.C. (Tech. Coords.), International response to infectious salmon anemia: prevention, control, and eradication: proceedings of a symposium; 3–4 September 2002; New Orleans, LA. Tech. Bull. 1902. Washington, DC: US Department of Agriculture, Animal and Plant Health Inspection Service; US Department of the Interior, US Geological Survey; US Department of Commerce, National Marine Fisheries Service, pp. 39–49.
Kibenge, F.S.B. et al., 2001. Antigenic variation among isolates of infectious salmon anaemia virus (ISAV) correlates with genetic variation of the viral haemagglutinin gene. J. Gen. Virol. 82, 2869–2879.
Kibenge, F.S.B. et al., 2000. A dual infection of infectious salmon anaemia (ISA) virus and a togavirus-like virus in ISA of Atlantic salmon *Salmo salar* in New Brunswick. Can. Dis. Aquat. Org. 42, 11–15.
Kibenge, M.J.T., Munir, K., Kibenge, F.S.B., 2005. Constitutive expression of Atlantic salmon Mx1 protein in CHSE-214 cells confers resistance to infectious salmon anaemia virus. Virol. J. 2, 75.
Kibenge, M.T. et al., 2002. Serological evidence of infectious salmon anaemia virus (ISAV) infection in farmed fish, using an indirect enzyme-linked immunosorbent assay (ELISA). Dis. Aquat. Org. 51, 1–11.
Kim, W.-S. et al., 2007. Genotyping of Korean isolates of infectious hematppoietic necrosis virus (IHNV) based on the glycoprotein gene. Arch. Virol. 152, 2119–2124.
Kim, Y.-S., Ke, F., Zhang, Q-Y., 2009. Effect of beta glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. Fish Shellfish Immunol. 27, 336–340.
Kimura, T. et al., 1990. An antiviral agent (46NW-04A) produced by *Pseudomonas* sp. and its activity against fish viruses. J. Aquat. Anim. Health 2, 12–20.
Kimura, T., Suzuki, S., Yoshimizu, M., 1983a. In vitro antiviral effect of 9-(2-hydroxyethoxymethyl) guanine on the fish herpesvirus, *Oncorhynchus masou* virus (OMV). Antiviral Res. 3, 93–101.
Kimura, T., Suzuki, S., Yoshimizu, M., 1983b. In vivo antiviral effect of 9-(2-hydroxymethyl) guanine on experimental infection of chum salmon (Oncorhynchus keta) fry with Oncorhynchus masou virus (OMV). Antiviral Res. 3, 103–108.
Kitao, Y. et al., 2009. Characterization and expression analysis of type I interferon in common carp *Cyprinus carpio* L.. Mol. Immunol. 46, 2548–2556.
Kjøglum, S. et al., 2008. Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. Aquacult. Res. 39, 498–505.
Kongtorp, R.T. et al., 2004. Heart and skeletal muscle inflammation in Atlantic salmon Salmo salar *L*: a new infectious disease. J. Fish Dis. 27, 351–358.
Koppang, E.O., Fischer, U., Moore, L., Tranulis, M.A., Dijkstra, J.M., Kollner, B., Aune, L., Jirillo, E., Hordvik, I., 2010. Salmonid T cells assemble in the thymus, spleen, and in novel interbranchial lymphoid tissue. J. Anat. 217, 728–739.
Koppang, E.O. et al., 2008. Vaccination-induced systemic autoimmunity in farmed Atlantic salmon. J. Immunol. 181, 4807–4814.
Koppang, E.O. et al., 2004. Granulomatous uveitis associated with vaccination in the Atlantic salmon. Vet. Pathol. 41, 122–130.
Kurath, G. 2012. An online database for IHN virus in Pacific salmonid fish: MEAP-IHNV. http://pubs.usgs.gov/fs/2012/3027/pdf/fs20123027.pdf (Published 2012; Consulted May 29, 2012).
Kurath, G., 2008. Biotechnology and DNA vaccines for aquatic animals. Rev. Sci. Tech. Off. Int. Epiz. 27, 175–196.
Kurath, G., 2005. Overview of recent DNA vaccine development for fish. In: Midtlyng P.J. (Ed), Progress in Fish Vaccinology, Developments in Biological Standardization. vol 121. Karger, Basel, Switzerland, pp. 201–213.
Kurath, G., Purcell, M.K., Garver, K.A., 2007. Fish rhabdovirus models for understanding host response to DNA vaccines. CAB Reviews 2, 1–12.
Kurath, G. et al., 2003. Phylogeography of infectious haematopoietic necrosis virus in North America. J. Gen. Virol. 84, 803–814.
Kuroda, Y. et al., 2004. Distinctive patterns of autoimmune response induced by differenttypes of mineral oil. Toxicol. Sci. 78, 222–228.
Kuzmin, I.V. et al., 2009. The rhabdoviruses: biodiversity, phylogenetics, and evolution. Infection, Genetics and Evolution 9, 541–553.
Langdon, J.S. et al., 1986. First virus isolation from Australian fish: an iridovirus-like pathogen from redfin perch. *Perca fluviatilis* L.. J. Fish Dis. 9, 263–268.
LaPatra, S.E., Lauda, K.A., Jones, G.R., Shewmaker, W.S., Bayne, C.J., 1998. Resistance to IHN virus infection in rainbow trout is increased by glucan while subsequent production of serum neutralizing activity is decreased. Fish Shellfish Immunol. 8, 435–446.
LaPatra, S.E. et al., 1993. Characterization of the humoral response of rainbow trout to infectious hematopoietic necrosis virus. J. Aquat. Anim. Health 5, 165–171.
Larsen, R., Røkenes, T.P., Robertsen, B., 2004. Inhibition of infectious pancreatic necrosis virus replication by Atlantic salmon Mx1 protein. J. Virol. 78, 7938–7944.
Lauring, A.S., Jones, J.O., Andino, R., 2010. Rationalizing the development of live attenuated virus vaccines. Nat. Biotechnol. 28, 573–579.
Lauscher, A. et al., 2011. Immune responses in Atlantic salmon (*Salmo salar*) following protective vaccination against infectious salmon anaemia (ISA) and subsequent ISA virus infection. Vaccine 29, 6392–6401.
Lecaroz, C. et al., 2006. Biodegradable micro- and nanoparticles as long-term delivery vehicles for gentamicin. J. Microencapsul. 23, 782–792.
Li, Z. et al., 2010. Administration of recombinant IFN1 protects zebrafish (Danio rerio) from ISKNV infection. Fish Shellfish Immunol. 29, 399–406.
Lieschke, G.J., Trede, N.S., 2009. Fish immunology. Current Biology 19, R678–R682.
Lillehaug, A., 1991. Vaccination of Atlantic salmon (*Salmo salar* L.) against cold-water vibriosis – duration of protection and effect on growth rate. Aquaculture 92, 99–107.
Lillehaug, A., 1989. Oral immunization of rainbow trout, *Salmo gairdneri* Richardson, against vibriosis with vaccines protected against digestive degradation. J. Fish Dis. 12, 579–584.
Lillehaug, A., Svatdal, S., Endal, T., 1996. Passive transfer of specific maternal antibody does not protect Atlantic salmon (*Salmo salar* L.) fry against yersiniosis. Fish Shellfish Immunol. 6, 521–535.
Long, S. et al., 2004. Identification of a cDNA encoding channel catfish interferon. Dev. Comp. Immunol. 28, 97–111.
Lorenzen, N., LaPatra, S.E., 2005. DNA vaccines for aquacultured fish. Rev. Sci. Technol. Off. Int. Epiz. 24, 201–213.
Lorenzen, N. et al., 1998. Protective immunity to VHS in rainbow trout (Onchorhynchus mykiss, Walbaum) following DNA vaccination. Fish Shellfish Immunol. 8, 261–270.
Lorenzen, N., Olesen, N.J., Vestergard-Jørgensen, P.E., 1988. Production and characterization of monoclonal antibodies to four Egtved virus structural proteins. Dis. Aquat. Org. 4, 35–42.
Lorincz, M. et al., 2011. First detection and analysis of a fish circovirus. J. Gen. Virol. 92, 1817–1821.
Løvoll, M. et al., 2011. A novel totivirus and piscine reovirus (PRV) in Atlantic salmon (*Salmo salar*) with cardiomyopathy syndrome (CMS). Virol. J. 7, 309.
Lumsden, J.S. et al., 2007. Mortality event in freshwater drum *Aplodinotus grunniens* from Lake Ontario, Canada, associated with viral haemorrhagic septicaemia virus, Type IV. Dis. Aquat. Org. 76, 99–111.
MacBeath, A.J.A., Bain, N., Snow, M., 2009. Surveillance for infectious salmon anaemia virus HPR0 in marine Atlantic salmon farms across Scotland. Dis. Aquat. Org. 87, 161–169.
Maira, C., et al., 2008. Side effects of injectable vaccines in cod – Adapting the side-effect scale to cod. Fish Farming Xpert 3/2008, pp 57–61. http://www.pharmaq.no/sfiles/31/8/file/side_effects_of_injectable_vaccines_in_cod_kyst_s57-61.pdf (downloaded January 1, 2012).
Manning, M.J., Grace, M.F., 1982. Developmental aspects of immunity and tolerance in fish. In: Roberts, R.J. (Ed.), Microbial diseases of fish. Academic Press. pp 31–46.
Markland, W. et al., 2000. Broad-spectrum antiviral activity of the IMP dehydrogenase inhibitor VX-497: a comparison with Ribavirin and demonstration of antiviral additivity with alpha interferon. Antomicrob. Agents Chemother. 44, 859–866.
Matsuoka, S., Inouye, K., Nakajima, K., 1996. Cultured fish species affected by red sea bream iridoviral disease from 1991 to 1995. Fish Pathol. 31, 233–234.
McAlaster, T. 2011. Engineered ecosystem for salmon. http://www.theglobeandmail.com/life/food-and-wine/engineered-ecosystem-for-salmon/article1891038/?from=1891039 (Published and Consulted February 1, 2011).
McAllister, P.E., Bebak, J., 1997. Infectious pancreatic necrosis virus in the environment: relationship to effluent from aquaculture facilities. J. Fish Dis. 20, 201–207.
McAllister, P.E., Owens, W.J., 1992. Recovery of infectious pancreatic necrosis virus from the faeces of wild piscivorous birds. Aquaculture 106, 227–232.
McAllister, P.E., Reyes, X., 1984. Infectious pancreatic necrosis virus: isolation from rainbow trout, *Salmo gairdneri* Richardson, imported into Chile. J. Fish Dis. 7, 319–322.
McCormick, S.D. et al., 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 55, 77–92.
McGurk, C., et al. 2011. Blood biochemistry for assessing the impact of functional nutrition on pathogenesis of salmon pancreas disease (PD). In: 15th International Conference on Diseases of Fish and Shellfish, Radisson Blu Hotel, Split, Croatia, September 12–16, 2011.
McLoughlin, M., 2006. Fish vaccination – A brief overview. <http://www.imb.ie/images/uploaded/documents/Fish%20Vaccine%20Overview.pdf> (Published January 25, 2006; Consulted February 27, 2012).
McLoughlin, M.F., Graham, D.A., 2007. Alphavirus infections in salmonids – a review. J. Fish Dis. 30, 511–531.
McLoughlin, M.F., Kennedy, S., Kennedy, D.G., 1992. Vitamin E responsive myopathy in rainbow trout *Oncorhynchus mykiss* fry. Vet. Rec. 130, 224–226.
McVicar, A.H., 1987. Pancreas disease of farmed Atlantic salmon, *Salmo salar*. Scotland: Epidemiology and Early Pathology 67, 71–78.
Midtlyng, P., Reitan, L.J., Speilberg, L., 1996. Experimental studies on the efficacy and side effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish Shellfish Immunol. 6, 335–350.

Midtlyng, P.J., Lillehaug, A., 1998. Growth of Atlantic salmon Salmo salar after intraperitoneal administration of vaccines containing adjuvant. Dis. Aquat. Org. 32, 91–97.
Mikalsen, A.B. et al., 2004. Protection of Atlantic salmon (*Salmo salar*) against infectious pancreatic necrosis after DNA vaccination. Dis. Aquat. Organ. 60, 11–20.
Mitchell, H., 1995. Choosing a furunculosis vaccine: points to consider. Bull. Aquacult. Assoc. Can. 95, 30–37.
Mjaaland, S. et al., 2005. Susceptibility and immune responses following experimental infection of MHC compartible Atlantic salmon (*Salmo salar* L.) with different infectious salmon anaemia virus isolates. Arch. Virol. 150, 2195–2216.
Moen, T. et al., 2007. Mapping of a quantitative trait locus for resistance against infectious salmon anaema in Atlantic salmon (*Salmo salar*): comparing survival analysis with analysis on affected/resistant data. BMC Genetics 8, 53.
Mohan, C.V., 2009. Health: National strategies for aquatic animal health management. Available at: http://thefishsite.com/articles/623/health-national-strategies-for-aquatic-animal-health-management (accessed on July 19, 2011).
Mulero, I., Garcia-ayala, A., Meseguer, J., Mulero, V., 2007. Maternal transfer of immunity and ontogeny of autologous immunocompetence of fish: a mini review. Aquaculture 268, 244–250.
Munro, A.L.S. et al., 1984. An exocrine pancreas disease of farmed Atlantic salmon in Scotland. Helgoländer Meeresunters 37, 571–586.
Murias, A. 2012. Global agreement expands ISA vaccine distribution. FIS – Worldnews. <http://www.fis.com/fis/worldnews/worldnews.asp?l=e&country=0&special=&monthyear=&day=&id=49095&ndb=1&df=0> (Published and Consulted January 13, 2012).
Murphy, F.A. et al., 1999. Vaccination against viral diseases. In: Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., Studdert, M.J. (Eds.), Veterinary Virology, third ed. Academic Press, London, pp. 241–244.
Murray, A.G. et al., 2010. Epidemiological investigation into the re-emergence and control of an outbreak of infectious salmon anaemia in the Shetland Islands, Scotland. Dis. Aquat. Org. 91, 189–200.
Murray, A., Peeler, E.J., 2005. A framework for understanding the potential for emerging diseases in aquaculture. Prev. Vet. Med. 67, 223–235.
Murray, A.G., Smith, R.J., Stagg, R.M., 2002. Shipping and the spread of infectious salmon anemia in Scottish aquaculture. Emerg. Infect. Dis. 8, 1–5.
Mutoloki, S., Evensen, Ø., 2011. Sequence similarities of the capsid gene of Chilean and European isolates of infectious pancreatic necrosis virus point towards a common origin. J. Gen. Virol. 92, 1721–1726.
Mutoloki, S., Alexandersen, S., Evensen, Ø., 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. Fish Shellfish Immunol. 16, 633–644.
Nakanishi, T., Kiryu, I., Ototake, M., 2002. Development of a new vaccine delivery method for fish: percutaneous administration by immersion with application of multiple puncture instrument. Vaccine 20, 3764–3769.
Nakanishi, T., Ototake, M., 1997. Antigen uptake and immune responses after immersion vaccination. Dev. Biol. Stand. 90, 59–68.
Nelson, R.T. et al., 1995. Isolation of a toga-like virus from farmed Atlantic salmon *Salmo salar* with pancreas disease. Dis. Aquat. Org. 22, 25–32.
Newman, S.G., 1993. Bacterial vaccines for fish. Ann. Rev. Fish Dis. 3, 145–185.
Nielsen, K.N., Fredriksen, B.N., Myhr, A.I., 2011. Mapping uncertainties in the upstream: the case of PLGA nanoparticles in salmon vaccines. Nanoethics 5, 57–71.
Nichol, S.T., Rowe, J.E., Winton, J.R., 1995. Molecular epizootiology and evolution of the glycoprotein and non-virion protein genes of infectious hematopoietic necrosis virus, a fish rhabdovirus. Vir. Res. 38, 159–173.
Ning, Q. et al., 1998. Ribavirin inhibits viral-induced macrophage production of TNF, IL-1, the procoagulant fgl2 prothrombinase and preserves Th1 cytokine production but inhibits TH2 cytokine response. J. Immunol. 22, 3487–3493.
Nishizawa, T. et al., 2006. Nucleotide diversity of Japanese isolates of infectious hematopoietic necrosis virus (IHNV) based on the glycoprotein gene. Dis. Aquat. Org. 71, 267–272.
Nishizawa, T. et al., 1991. Characterization of structural proteins of hirame rhabdovirus, HRV. Dis. Aquat. Org. 10, 167–172.
Nordmo, R., 1997. Strengths and weaknesses of different challenge methods. Dev. Biol. Stand. 90, 303–309.
Norwegian Veterinary Institute reports. Available at: <http://www.vetinst.no/nor/Forskning/Publikasjoner/Fiskehelserapporten>.
Nylund, A. et al., 2007. Transmission of infectious salmon anaemia virus (ISAV) in farmed populations of Atlantic salmon (*Salmo salar*). Arch. Virol. 152, 151–179.
O'Dowd, A.M. et al., 1999. The primary and secondary antibody responses to IROMP antigens in Atlantic salmon (*Salmo salar* L.) immunised with A+ and A– Aeromonas salmonicida bacterins. Fish Shellfish Immunol. 9, 125–138.
Ohta, T. et al., 2011. Anti-viral effects of interferon administration on sevenband grouper. Epinephelus septemfasciatus. Fish Shellfish Immunol. 30, 1064–1071.
OIE, 2011. Manual of Diagnostic Tests for Aquatic Animals 2011. <http://www.oie.int/international-standard-setting/aquatic-manual/access-online/> (Published 2011; Consulted February 11, 2012).
OIE, 2012a. OIE Listed Diseases. <http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/> (Published 2012; Consulted February 11, 2012).
OIE, 2012b. Infectious salmon anaemia. <http://web.oie.int/wahis/public.php?WAHIDPHPSESSID=7c523a0d118dfebe366ba309085c0324&page=disease_outbreak_map&disease_type=Aquatic&disease_id=160> (Published 2012; Consulted May 26, 2012).
Okamoto, N. et al., 1993. Resistance of a rainbow trout strain to infectious pancreatic necrosis. Aquaculture 117, 71–76.
Olivier, G., 2002. Disease interactions between wild and cultured fish – perspectives from the American Northeast (Atlantic Provinces). Bull. Eur. Ass. Fish Pathol. 22, 103–109.
Ooi, E.L. et al., 2008. Innate immunomodulation with recombinant interferon-α enhances resistance of rainbow trout (Oncorhynchus mykiss) to infectious hematopoietic necrosis virus. Dev. Comp. Immunol. 32, 1211–1220.
Palacios, G. et al., 2010. Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLoS One 5, e11487.
Peeler, E.J., Gardiner, R., Thrush, M.A., 2004. Qualitative risk assessment of routes of transmission of the exotic fish parasite Gyrodactylus salaries between river catchments in England and Wales. Prev. Vet. Med. 64, 175–189.
Perelberg, A. et al., 2005. Protection of cultured Cyprinus carpio against a lethal viral disease by an attenuated virus vaccine. Vaccine 23, 3396–3403.
Pilstrøm, L., 2005. Adaptive immunity in teleosts: humoral immunity. In: Midtlyng P.J. (Ed.), Progress in Fish Vaccinology, Developments in Biological Standardization, vol. 121. Karger, Basel, Switzerland, pp. 23.
Plant, K.P., LaPatra, S.E., 2011. Advances in fish vaccine delivery. Dev. Comp. Immunol. 35, 1256–1262.
Plarre, H. et al., 2005. Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway. Dis. Aquat. Org. 66, 71–79.
Plumb, J.A., Hanson, L.A., 2010. Health maintenance and principle microbial diseases of cultured fishes, third ed. Wiley-Blackwell, Ames, Iowa.
Poppe, T.T., Sande, R.D., 1994. Cardiomyopathy in farmed Atlantic salmon: a review, introducing an ultrasound technique for clinical examination. Norwegian School of Veterinary Science. Oslo.
Poppe, T.T., Seierstad, S.L., 2003. First description of cardiomyopathy syndrome (CSM)-related lesions in wild Atlantic salmon Salmo salar in Norway. Dis. Aquat. Org. 56, 87–88.
Poulos, B.T. et al., 2006. Purification and characterization of infectious myonecrosis virus of penaeid shrimp. J. Gen. Virol. 87, 987–996.
Purcell, et al., 2006. Conservation of Toll-Like Receptor Signaling Pathways in Teleost Fish. Comp. Biochem. Physiol. Part D. Genomics. Proteomics 1, 77–88.
Quentel, C., Vigneulle, M., 1997. Antigen uptake and immune responses after oral vaccination. Dev. Biol. Stand. 90, 69–78.
Randall, R.E., Goodbourn, S., 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J. Gen. Virol. 89, 1–47.
Raynard, R.S., Houghton, G., 1993. Development towards an experimental protocol for the transmission of pancreas disease of Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 15, 123–128.
Rich, A., Zhang, S., 2003. Timeline Z-DNA: the long road to biological function. Nat. Rev. Genet. 4, 566–572.
Richter, L., Kipp, P.B., 1999. Transgenic plants as edible vaccines. Curr. Top. Microbiol. Immunol. 240, 159–176.
Rijkers, G.T., Frederix-Wolters, E.H., van Muiswinkel, W.B., 1980. The immune system of cyprinid fish. Kinetics and temperature dependence of antibody-producing cells in carp (*Cyprinus carpio*). Immunology 41, 91–97.
Rimstad, E., 2011. Examples of emerging virus diseases in salmonid aquaculture. Aquacult. Res. 42, 86–89.
Rimstad, E. et al., 2001. Characterization of the infectious salmon anaemia virus genomic segment that encodes the putative haemagglutinin. J. Virol. 75, 5352–5356.
Rivas-Aravena, A. et al., 2011. Inhibitory effect of a nucleotide analog on ISAV infection. J. Virol. 85, 8037–8045.
Roberts, R.J., Pearson, M.D., 2005. Infectious pancreatic necrosis in Atlantic salmon, *Salmo salar* L.. J. Fish Dis. 28, 383–390.
Roberts, R.J., Shepherd, C.J., 1974. Handbook of trout and salmon diseases. Fishing News, Surrey, England, pp. 40–42.
Robertsen, B., 2008. Expression of interferon and interferon-induced genes in salmonids in response to virus infection, interferon-inducing compounds and vaccination. Fish Shellfish Immunol. 25, 351–357.
Robertsen, B. et al., 2003. Atlantic salmon interferon genes: cloning, sequence analysis, expression, and biological activity. J. Interferon Cytokine Res. 23, 601–612.
Roger, H., Mitchell, S., 2007. Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. J. Fish Dis. 30, 157–167.
Rodger, H., Turnbull, T., 2000. Cardiomyopathy syndrome in farmed Scottish salmon. Vet. Rec. 146, 500–501.
Rodgers, C.J., Mohan, C.V., Peeler, E.J., 2011. The spread of pathogens through trade in aquatic animals and their products. Rev. Sci. Tech. Off. Int. Epiz. 30, 241–256.
Rodroguez-Ramilo, S.T. et al., 2011. QTL detection for *Aeromonas salmonicida* resistance related traits in turbot (*Scophthalmus maximus*). BMC Genomics 12, 541.
Rombout, J.H. et al., 2011. Teleost intestinal immunology. Fish Shellfish Immunol. 31, 616–626.
Rombout, J.H.W.M., Van den Berg, A.A., 1989. Immunological importance of the second gut segment in carp I. Uptake and process of antigens by epithelial cells and macrophages. J. Fish Biol. 35, 13–22.
Rombout, J.H.W.M. et al., 1985. Uptake and transport of intact macromolecules in the intestinal epithelium of carp (*Cyprinus carpio*) and the possible immunological implications. Cell and Tissue Res. 239, 519–530.
Romero-Brey, I. et al., 2009. Genetic analysis of aquabirnaviruses isolated from wild fish reveals occurrence of natural reassortment of infectious pancreatic necrosis virus. J. Fish Dis. 32, 585–595.

Rosenlund, G., Skretting, M., 2006. Worldwide status and perspectives on gadoid culture. ICES J. Marine Sci. 63, 194–197.
Ross, K. et al., 1994. An outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. Bull. Eur. Assoc. Fish Pathol. 14, 213–214.
Rowley, H.M. et al., 1998. Isolation of salmon pancreas disease virus (SPDV) from Scottish farmed salmon. J. Fish Dis. 21, 469–471.
Rudakova, S.L., Kurath, G., Bochkova, E.V., 2007. Occurrence and genetic typing of infectious hematopoietic necrosis virus in Kamchatka, Russia. Dis. Aquat. Org. 75, 1–11.
Ruiz, S. et al., 2009. New tools to study RNA interference to fish viruses: fish cell lines permanently expressing siRNAs targeting the viral polymerase of viral hemorrhagic septicaemia virus. Antiviral Res. 82, 148–156.
Saksida, S., 2004. Investigation of the 2001–2003 IHN epizootic in farmed Atlantic salmon in British Columbia. <http://www.agf.gov.bc.ca/ahc/fish_health/IHNV_report_2003.pdf>. (Published February 18, 2004; Consulted May 16, 2012).
Salonius, K. et al., 2007. The road to licensure of a DNA vaccine. Curr. Opin. Invest. Drugs 8, 635–641.
Santi, N., Vakharia, V.N., Evensen, Ø., 2004. Identification of putative motifs involved in the virulence of infectious pancreatic necrosis virus. Virology 322, 31–40.
Schliehe, C. et al., 2011. Microencapsulation of inorganic nanocrystals into PLGA microsphere vaccines enables their intracellular localization in dendritic cells by electron and fluorescence microscopy. J. Control Release 151, 278–285.
Schlosser, E. et al., 2008. TLR ligands and antigen need to be coencapsulated into the same biodegradable microsphere for the generation of potent cytotoxic T lymphocyte responses. Vaccine 26, 1626–1637.
Schlotfeldt, H.-J. et al., 1991. Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – a natural outbreak. Bull. Eur. Ass. Fish Pathol. 11, 105–107.
Schyth, B.D., Lorenzen, N., Pedersen, F.S., 2006. Antiviral activity of small interfering RNAs: specificity testing using heterologous virus reveals interferon-related effects overlooked by conventional mismatch controls. Virology 349, 134–141.
Secombes, C.J., 2011. Fish immunity: the potential impact on vaccine development and performance. Aquacult. Res. 42, 90–92.
Seth, R.B. et al., 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3. Cell 122, 669–682.
Sharpe, C.S., 2007. Physiological stress responses to automated and hand vaccine injection procedures in yearling coho salmon. N. Am. J. Aquacult. 69, 180–184.
Shivappa, R., et al. 2008. Spring viraemia of carp in the United States of America: evaluation of current diagnostics, in: Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M., and Subasinghe, R.P. (Eds.), Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 143-156.
Shoemaker, C.A. et al., 2006. Efficacy of a Oralject (TM) *Streptococcus iniae* modified bacterin delivered using technology in Nile tilapia (*Oreochromis niloticus*). Aquaculture 255, 151–156.
Siripornadulsil, S., Dabrowski, K., Sayre, R., 2007. Microalgal vaccines. Adv. Exp. Med. Biol. 616, 122–128.
Siwicki, A.K. et al., 2003. Influence of Methisoprinol on the replication of rhabdoviruses isolated from carp (*Cyprinus carpio*) and catfish (*Ictalurus melas*): in vitro study. Polish J. Vet. Sci. 6, 47–50.
Skall, H.F., Olesen, N.J., Mellergaard, S., 2005. Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. J. Fish Dis. 28, 509–529.
Smail, D.A., 1999. Viral haemorrhagic septicaemia. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish diseases and disorders, Volume 3: Viral, bacterial and fungal infections. CAB International, Oxon, UK, pp. 123–147.
Smail, D.A. et al., 1992. Infectious pancreatic necrosis (IPN) virus Sp serotype in farmed Atlantic salmon, Salmo salar L., post-smolts associated with mortality and clinical disease. J. Fish Dis. 15, 77–83.
Smail, D.A., Munro, A.L.S., 1985. Infectious pancreatic necrosis virus persistence in farmed Atlantic salmon (*Salmo salar*). In: Ellis, A.E. (Ed.), Fish and shellfish pathology. Academic Press, London, pp. 277–288.
Smee, D.F., Huggins, J.W., 1999. Mode of action of ribavirin against cowpox and monkeypox viruses. Antiviriral Res. 41, A52.
Snieszko, S.F., 1974. The effects of environmental stress on outbreaks of infectious diseases of fish. J. Fish Biol. 6, 197–208.
Snow, M. et al., 2004. Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). Dis. Aquat. Org. 61, 11–21.
Sommerset, I. et al., 2005. Vaccines for fish in aquaculture. Expert Rev. Vaccines 4, 89–101.
Sørum, U., Damsgård, B., 2004. Effects of anaesthetization and vaccination on feed intake and growth in Atlantic salmon (*Salmo salar* L.). Aquaculture 232, 333–341.
Soucy-Faulkner, A. et al., 2010. Requirement of NOX2 and reactive oxygen species for efficient RIG-I-mediated antiviral response through regulation of MAVS expression. PLoS Pathog. 6, e1000930.
Stagg, R.M. et al. 2001. Epizootiological investigations into an outbreak of infectious salmon anemia (ISA) in Scotland, FRS Marine Laboratory Report 13/01, p. 60 (Aberdeen, UK).
St-Hilaire, S. 2000. Epidemiology of infectious hematopoietic necrosis disease in net-pen reared Atlantic salmon in British Columbia, Canada. PhD Thesis, University of Guelph, Guelph, Ontario.
St-Hilaire, S. et al., 2001. Infectious hematopoietic necrosis virus antibody profiles in naturally and experimentally infected Atlantic salmon Salmo salar. Dis. Aquat. Org. 46, 7–14.
Stone, D.M. et al., 2003. Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. Dis. Aquat. Org. 53, 203–210.
Storset, A. et al., 2007. Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). Aquaculture 272, 62–68.
Strandskog, G. et al., 2008. Double-stranded RNA- and CpG DNA-induced immune responses in Atlantic salmon: Comparison and synergies. Vaccine 26, 4704–4715.
Suzuki, S., Machida, H., Saneyoshi, M., 1988. Antiviral activity of various 1-β-d-arabinofuranosyl-E-5-halogenvinyluracils and E-5-bromovinyl-2′-deoxyuridine against salmon herpes virus, Oncorhynchus masou virus (OMV). Antiviral Res. 7, 79–86.
Svasand, T. et al., 2004. The status and perspectives for the species. In: Moksness, E., Kjorsvik, E., Olsen, Y. (Eds.), Culture of Cold-water Marine Fish. Blackwell Publishing Ltd., Edinburgh, UK, pp. 433–474.
Swan, C.M., Lindstrom, N.M., Cain, K.D., 2008. Identification of a localized mucosal immune response in rainbow trout, *Oncorhynchus mykiss* (Wlabaum), following immunization with a protein-hapten antigen. J. Fish Dis. 31, 383–393.
Tacchi, L., Bickerdike, R., Douglas, A., Secombes, C.J., Martin, S.A.M., 2011. Transcriptomic responses to functional feeds in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol. 31, 704–715.
Tam, R.C., Pai, B., Bard, J., Lim, C., Averett, D.R., Phan, U.T., Milovanovic, T., 1999. Ribavirin polarizes human T cell responses towards a type 1 cytokine profile. J. Hepatol. 30, 376–382.
Tal, M.C. et al., 2009. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. Proc. Natl Acad. Sci. USA 106, 2770–2775.
Tan, F.L., Yin, J.Q., 2004. RNAi, a new therapeutic strategy against viral infection. Cell Res. 14, 460–466.
Tanaka, T. et al., 1999. Transfer of maternal antibody from mother to egg may have no protective meaning for larvae of Red sea bream *Pagnus major*, a marine teleost. Fish Sci. 65, 240–243.
Tatner, M.F., 1987. The quantitative relationship between vaccine dilution, length of immersion time and antigen uptake, using a radiolabeled *Aeromonas salmonicida* bath in direct immersion experiments with rainbow trout, *Salmo gairdneri*. Aquaculture 62, 173–185.
Tatner, M.F., Horne, M.T., 1983. Susceptibility and immunity to *Vibrio anguillarum* in post-hatching rainbow trout fry, *Salmo gairdneri*. Dev. Comp. Immunol. 7, 465–472.
Tatner, M.F., Johnson, C.M., Horne, M.T., 1984. The tissue localization of *Aeromonas salmonicida* in rainbow trout, *Salmo gairdneri*, following 3 methods of administration. J. Fish. Biol. 25, 95–108.
Thompson, A.J., Locarnini, S.A., 2007. Toll-like receptors, RIG-I like RNA helicases and the antiviral innate immune response. Immunol. Cell Biol. 85, 435–445.
Thorarinsson, R., Powell, D.B., 2006. Effects of disease risk, vaccine efficacy, and market price on the economics of fish vaccination. Aquaculture 256, 42–49.
Tobar, J.A. et al., 2011. Oral vaccination of Atlantic salmon (Salmo alar) against salmonid rickettsial septicaemia. Vaccine 29, 2336–2340.
Tordo, N. et al., 2005. Family *Rhabdoviridae*. In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA, pp. 623–644.
Tort, L., Balasch, J.C., Mackenzie, S., 2003. Fish immune system: a crossroads between innate and adaptive responsesa. Immunologia 22, 277–286.
Traxler, G.S., Richard, J.B., 1996. First detection of infectious hematopoietic necrosis virus in marine fishes. Fish Health Section/American Fisheries Society Newsletter 24, 7.
Traxler, G.S., Rankin, J.B., 1989. An infectious hematopoietic necrosis epizootic in sockeye salmon Oncorhynchus nerka in Weaver Creek spawning channel, Fraser River system, B.C., Canada. Dis. Aquat. Org. 6, 221–226.
Traxler, G.S., Kent, M.L., Poppe, T.T., 1998. Viral diseases. In Diseases of seawater netpen-reared salmonid fishes. Kent, M.L., Poppe, T.T. (Eds.), Fisheries and Oceans Canada, 138pp.
Traxler, G.S., Roome, J.R., Kent, M.K., 1993. Transmission of infectious hematopoietic necrosis virus in seawater. Dis. Aquat. Org. 16, 111–114.
Troyer, R.M., LaPatra, S., Kurath, G., 2000. Genetic analyses reveal unusually high diversity of infectious hematopoietic necrosis virus in rainbow trout aquaculture. J. Gen. Virol. 81, 2823–2832.
Tucker, S. et al., 1999. Detecting pan-Atlantic migration in salmon (*Salmo salar*) using ^{137}Cs. Can. J. Fish. Aquat. Sci. 56, 2235–2239.
Vandenberg, G.W., 2004. Oral vaccines for finfish: academic theory or commercial reality? Anim. Health Res. Rev. 5, 301–304.
Vandenberg, G.W. et al., 2003. A novel system for oral vaccination of salmonids against furunculosis In: Proceedings of the Aquaculture America, Louisville, KY.
Vike, S., Nylund, S., Nylund, A., 2009. ISA virus in Chile: evidence of vertical transmission. Arch. Virol. 154, 1–8.
Walker, P.J., Winton, J.R., 2010. Emerging viral diseases of fish and shrimp. Vet. Res. 41, 51.
Wang, L. et al., 2006. In vitro effects of recombinant zebrafish IFN on spring viraemia of carp virus and infectious hematopoietic necrosis virus. J. Interferon Cytokine Res. 26, 256–259.
Wang, W.-S. et al., 1996. Detection of infectious hematopoietic necrosis virus in rainbow trout Oncorhynchus mykiss from an outbreak in Taiwan by serological and polymerase chain reaction assays. Dis. Aquat. Org. 26, 237–239.
Warg, J.V. et al., 2007. Comparison of multiple genes of spring viraemia of carp viruses isolated in the United States. Virus Genes 35, 87–95.

Watanabe, L. et al., 2002. Isolation of aquabirnavirus and viral haemorrhagic septicaemia virus (VHSV) from wild marine fishes. Fish Pathol. 37, 189–191.
Weaver, S.C. et al., 2005. Togaviridae. In: Fauquet, C.M. et al. (Eds.), Virus taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, England, pp. 999–1008.
Webster, R.G. et al., 1992. Evolution and ecology of influenza A viruses. Microbiological Reviews 56, 152–179.
Wedemeyer, G., 1996. Physiology of Fish in Intensive Culture Systems. Chapman & Hall, London.
Weeratna, R.D., Payette, P.J., Davis, H.L., 2000. The potential of CpG oligodeoxynucleotides as mucosal adjuvants. Crit. Rev. Immunol. 21, 310.
Weyts, F.A.A. et al., 1999. Interactions between the immune system and the hypothalamo-pituitary-interrenal axis in fish. Fish Shellfish Immunol. 9, 1–20.
Wiik-Nielsen, C.R. et al., 2012. First detection of piscine reovirus (PRV) in marine fish species. Dis. Aquat. Org. 97, 255–258.
Winton, J.R., 1997. Immunization with viral antigens: infectious haematopoietic necrosis. Dev. Biol. Stand. 90, 211–220.
Winton, J.R., 1991. Recent advances in detection and control of infectious hematopoietic necrosis virus in aquaculture. Ann. Rev. Fish Dis. 1, 83–93.
Winton, J.R., Kurath, G., Batts, W., 2008. Molecular Epidemiology of Viral Hemorrhagic Septicemia Virus in the Great Lakes Region. US Geological Survey Fact Sheet 2008–3003.
Workenhe, S.T. et al., 2010. The fight between teleost fish immune response and aquatic viruses. Mol. Immunol. 47, 2525–2536.
Wolf, K., 1988a. Infectious pancreatic necrosis. In: Fish viruses and fish viral diseases. Cornell University Press, Ithaca, pp. 115–157.
Wolf, K., 1988b. Infectious hematopoietic necrosis: Fish viruses and fish viral diseases. Cornell University Press, Ithaca, In, pp. 83–114.
Wray, S.K., Gilbert, B.E., Knight, V., 1985. Effect of ribavirin triphosphate on primer generation and elongation during influenza virus transcription in vitro. Antiviriral Res. 5, 39–48.
Xie, J. et al., 2005. Inhibition of reporter gene and iridovirus-tiger frog virus in fish cell by RNA interference. Virology 338, 43–52.
Yada, T., Nakanishi, T., 2002. Interaction between endocrine and immune systems in fish. Int. Rev. Cytol. 220, 35–92.
Yanong, P.E., 2008. Use of vaccines in finfish aquaculture. University of Florida IFAS Extension FA156. <http://edis.ifas.ufl.edu/pdffiles/FA/FA15600.pdf> (Published 2008, Reviewed 2011; Consulted February 12, 2012).
Ye, J., Kaattari, I.M., Kaattari, S.L., 2011. The differential dynamics of antibody subpopulation expression during affinity maturation in a teleost. Fish Shellfish Immunol. 30, 372–377.
Yoshimizu, M., 1996. Disease problems of salmonid fish in Japan caused by international trade. Rev. Sci. Tech. Off. Int. Epiz. 15, 533–549.
Zhang, Y.-B. et al., 2007. The innate immune response to grass carp hemorrhagic virus (GCHV) in cultured *Carassius auratus* blastulae (CAB) cells. Dev. Comp. Immunol. 31, 232–243.
Zapata, A. et al., 2006. Ontogeny of the immune system of fish. Fish Shellfish Immunol. 20, 126–136.
Zenke, K., Nam, Y.K., Kim, K.H., 2010. Development of siRNA expression vector utilizing rock bream β-actin promoter: a potential therapeutic tool against viral infection in fish. Appl. Microbiol. Biotechnol. 85, 679–690.
Zhang, Y.-A., Salinas, I., Sunyer, J.O., 2011. Recent findings on the structure and function of teleost IgT. Fish Shellfish Immunol. 31, 627–634.
Zhou, Y.C. et al., 2002a. Vaccination of the grouper, Epinephalus awoara, against vibriosis using the ultrasonic technique. Aquaculture 203, 229–238.
Zhou, Y.C. et al., 2002b. Ultrasonic immunization of sea bream, *Pagrus major* (Temminck & Schlegel), with a mixed vaccine against *Vibrio alginolyticus* and *V. anguillarum*. J. Fish Dis. 25, 325–331.
Zwollo, P., Cole, S., Bromage, E., Kaattari, S., 2005. B Cell heterogeneity in the teleost kidney: Evidence for a maturation gradient from anterior to posterior kidney. J. Immunol. 174, 6608–6616.
Zwollo, P., Mott, K., Barr, M., 2010. Comparative analyses of B cell populatyions in trout kidney and mouse bone marrow; establishing "B cell signatures". Dev. Comp. Immunol. 34, 1291–1299.