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# Status and future perspectives of vaccines for industrialised fin-fish farming

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ABSTRACT

Fin fish farming is developing from extensive to intensive high industrial scale production. Production of fish in high-density growth conditions requires effective vaccines in order to control persistent and emerging diseases. Vaccines can also have significant positive impact on the reduced usage of antibiotics. This was demonstrated when vaccines were introduced in Norway for Atlantic salmon (*Salmo salar*) in the late eighties and early nineties, resulting in a rapid decline of antibiotics consumption. The present review will focus on current vaccine applications for farmed industrialized fish species such as Atlantic salmon, coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), ayu (*Plecoglossus altivelis*), cod (*Gadus morhua*), sea bass (*Dicentrarchus labrax*), gilt-head sea bream (*Sparus aurata*), yellowtail (*Seriola quinqueradiata*), great amberjack (*Seriola dumerili*), barramundi (*Lates calcarifer*), japanese flounder (*Paralichythys olivaceus*), turbot (*Scophthalmus maximus*), red sea bream (*Pagrus major*), rock bream (*Oplegnathus fasciatus*), seven band grouper (*Epinephelus septemfasciatus*), striped catfish (*Pangasianodon hypophthalmus*), channel catfish (*Ictalurus punctatus*) and tilapia *(Oreochromis niloticus).* This paper will review the current use of licensed vaccines in fin fish farming and describe vaccine administration regimes including immersion, oral and injection vaccination. Future trends for inactivated-, live attenuated - and DNA - vaccines will also be discussed.



## 1. Introduction

About 600 aquatic species are farmed worldwide [21]. This includes both extensive low industrial scale production and more intensive high industrial scale production. The industrialised fish species are usually farmed at high-density under optimized growth conditions (light and temperature regimes) using intensive feeding with artificial feed. For these industrialised fish species the technological sophistication have increased in order to solve the many challenges that occur in an intensive production system [60]. Although nearly 2/3 of the fin fish production consist of extensive production of carps, the industrialisation of fin fish farming is expanding for both high and low value species (Fig. 1). Effective vaccines have probably been one of the most important factors for the growth and success of intensive salmonid farming systems. The introduction of water-in-oil (w/o) emulsion vaccines in Norway has demonstrated a significant impact on the reduction of antibiotics from 50000 kg in 1987 to 1000–2000 kg in 1997, while at the same time the production increased from 50 000 tonnes to 350 000 tonnes [81]. The development of a sustainable industrialised aquaculture industry depends on the development and implementation of vaccines and vaccination regimes that makes the disease situation predictable and manageable under intensive production. This article will focus on current vaccine applications for farmed industrialized fish species and the future perspectives of vaccine technologies. It will focus on the licensed vaccines used in finfish farming, although the non-licensed ones can have a significant local impact on particular segments, as autogenous or other non licensed applications. However, as this use is normally not published and referred, it will not be covered in this review.

## 2. Vaccines for industrial scale fin-fish farming

Vaccines are available for more than 17 species of fish and protect against more than 22 different bacterial diseases and 6 viral diseases. Vaccines are available in more than 40 countries (Fig. 2). Although a large number of countries have some vaccines licensed, vaccination is mostly used in industrialized species in countries

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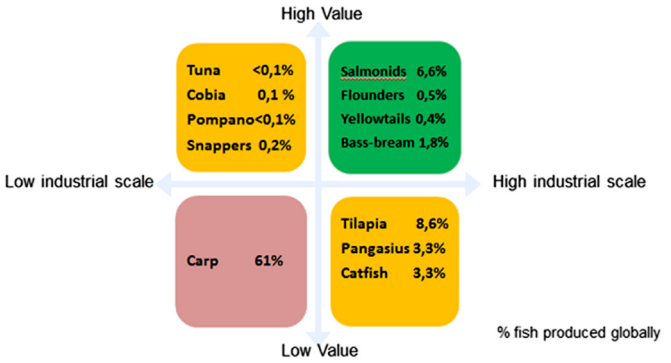


**Fig. 1.** Overview of industrialised farmed fish species categorised into low and high industrialised scale and high and low value of the product. Volume percentage of total fish produced is shown, based on FAO Fish stat+ 2011.

illustrated in Fig. 2. The large industrial scale vaccination was initially developed for vaccination of salmonids, but have now been implemented for several species.

In major fish producing countries like China (the worlds largest producer of farmed fish covering 61% of the total Aquaculture production [21]), vaccination is not common practise, however, the intensive research on fish vaccines in China, shows promise for the development of new fish vaccines in China [99]. Current vaccine application is described for the most important species/groups below. Fish vaccines are produced by a wide range of companies, of which major producers are listed in Table 1.

## 3. Vaccine administration

Administration of vaccines is either performed orally through feed, by immersion in diluted vaccine suspensions or by injection via the intraperitoneal (w/o based vaccines) or intramuscular (DNA vaccines) route. In general, the level and duration of efficacy are highest with the injection method, however, so is also the handling and stress of fish. Fry which are too small to be injected are usually vaccinated by immersion or by the oral route.

### 3.1. Injectable vaccines

Injection vaccination has been important for disease protection of salmon and trout during the production cycle to harvest. In late 1980s the salmonid vaccine market primarily used water-based vaccines against pathogens such as *Listonella anguillarum* (*L. anguillarum*), *Vibrio* (*Aliivibrio*) *salmonicida* (*V. salmonicida*) and *Yersinia ruckeri* (*Y. ruckeri*). However, the introduction of *Aeromonas salmonicida* (*A. salmonicida*) into the Norwegian salmon farming industry resulted in high mortality for which water-based furunculosis vaccines were not sufficiently protective. The introduction of oil-based vaccines in the early 1990s resulted in significant reduction of furunculosis outbreaks and subsequently also a reduction of the use of water-based injection and immersion vaccines as described by Sommerset et al. 2005. The high efficacy and long duration of protection that the water-in-oil emulsion (w/o) adjuvants induce have been essential for the growth of salmonid aquaculture. There are many types of oil-containing emulsions such as oil-in-water (o/w), water-in-oil (w/o), water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) emulsions, for which w/o is primarily used for fish vaccines due the formulations ability to induce long term protection [1]. The introduction of these effective w/o vaccines makes it possible to harvest fish at a larger size, resulting in improved economy for the farmers. W/o based vaccines have been related to injection-site reactions causing reduced growth and downgrading of Atlantic salmon (*Salmo salar*) at harvest [57]. However, this problem has in general been reduced. Studies also show that the inflammatory reactions vary within the

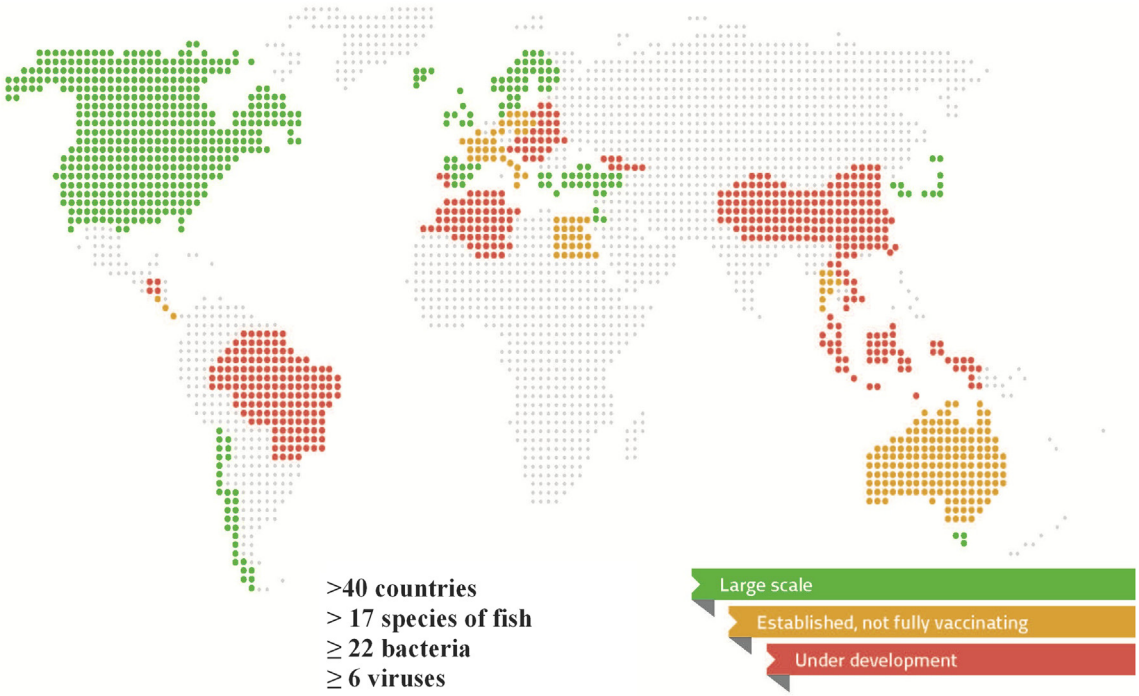


**Fig. 2.** A categorisation of the countries according to the use and implementation of fish vaccination. Green shows countries where vaccination is commonly used. Yellow are countries where vaccination is used, but not fully implemented. Red are countries where fish vaccination is under development. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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**Table 1**
Major producers of licensed fish vaccines listed in alphabetical order.

| Company | Origin |
|---|---|
| Agrovet | Chile |
| Centrovet | Chile |
| DaeSung Micro. Bio. Lab | Korea |
| Dainippon | Japan |
| Goryo B&P | Korea |
| Green Cross | Korea |
| Hipra | Spain |
| JungAn Vaccine | Korea |
| Komi Pharm | Korea |
| Korea BNP | Korea |
| Kyoritsu | Japan |
| MSD | USA |
| Nisseiken | Japan |
| Novartis | Switzerland |
| PHARMAQ | Norway |
| Pfizer | USA |
| Tecnovax | Argentine |
| Recalcine | Chile |
| Veterquimica | Chile |

salmonid species [64]. In other species side effects are usually less severe such as in sea bass (*Dicentrarchus labrax*) [2], cod (*Gadus morhua*) [55], turbot (*Scophthalmus maximus*) [7,9] and yellowtail (*Seriola quinqueradiata*) [32]. The vaccination procedure has usually been performed by vaccination teams manually performing vaccination of anesthetized fish. A skilled vaccinator is capable of vaccinating up to 3500 fish per hour [70]. However, the high labour costs for manual vaccination have in the last decade resulted in development of automated vaccination machines that are capable of handling up to 20 000 fish per hour [56].

DNA vaccines are administered by intra muscular injection such as DNA vaccine against infectious hemorrhagic necrosis, IHN.

### 3.2. Immersion vaccines

Commercial immersion vaccines are primarily in the form of formalin inactivated bacterial suspension or as live bacterial vaccines. The vaccination procedures for inactivated antigens are either by short dip in concentrated antigen suspension or a longer duration of exposure in a more diluted bath. Many inactivated immersion vaccines are used at 1/10 dilution of a concentrated vaccine suspension. The exposure duration is usually between 30 and 60 s. Early studies [87] using radio labelled *A. salmonicida* showed that 1/10 dilution of vaccine suspension gave no significant difference in antigen uptake in rainbow trout between 5 s and 10 min exposure time. Furthermore, a 1/100 dilution for two hours exposure duration resulted in significantly higher uptake compared to 100 s and 10 min exposure time. Inactivated immersion vaccines are also administered by bath directly in the holding tank for 30 min using 1/500 dilution. This is usually performed by reducing the water level in the tank and increasing oxygenation. The reduced handling of fish for bath vaccination compared to dip makes it both less labour intensive and less stressful for the fish. Several techniques have been described for improving immersion vaccine uptake such as hyperosmotic dip [38,90], ultrasound mediated uptake [26–28] and multiple puncture instrument [67]. Although, these methods have showed increased uptake of vaccine components during vaccination, none have yet gained any widely commercial use in the fish vaccination industry.

For diseases occurring in the fry stage such as rainbow trout fry syndrome and infectious pancreas necrosis (IPN), it is important to vaccinate as early as possible. However, efficacy is dependent on the development of immunocompetence in the vaccinated fish. A study by Amend and Johnson [4] showed that coho salmon (*Oncorhyncus kisutch*) below 1 g had a delayed and poor response to vaccination, from 1 to 2 g improved onset was achieved but protection declined after 3–4 months. Long term duration of protection was only achieved on sizes above 2 g.

Live vaccines used for catfish production in USA against *Edwardsiella ictaluri* (*E. ictaluri*) and *Flavobacterium columnaris* (*F. columnaris*), consists of frozen vials where each vial of vaccine is sufficient to vaccinate 3.4 kg of catfish in approximately 20 L of water. The vaccine has shown to be efficacious in channel catfish (*Ictalurus punctatus*) fry as early as 7–30 days post hatching [46,78].

### 3.3. Oral vaccines

Oral vaccination with antigens included in the feed is in principle the ideal method of vaccine delivery. Such products are usually sold as suspensions for either directly coating the feed with antigen or mixing antigen into the feed during production. The efficacy of oral vaccines is dependent on antigen content in the feed [30] resistance against gastric degradation and antigen adsorption/uptake in the gut. MSD have a patented encapsulation technology which is used in their oral vaccines, while Centrovet Laboratories uses a patented (developed by Advanced BioNutrition Corp.) MicroMatrix™ Targeted Delivery Systems (TDS) for the prevention of piscirickettsiosis also called salmon rickettsial septicemia (SRS), and infectious salmon anaemia (ISA). Three companies have licensed oral vaccine to prevent disease caused by *Lactococcus garvieae* (*L. garvieae*) in 100–400 g *Seriola* sp. in Japan. Oral vaccines can be used for primary vaccination or as a booster vaccine to improve protection against long-lasting endemic diseases.

### 3.4. Vaccine regimes

For many species, a vaccination regime has been developed in order to keep fish protected all through the production cycle. Vaccination can be performed with up to three administration time points, usually starting with dip-vaccination, followed by booster vaccination (orally or dip/bath) and, finally, an injection vaccination. After injection of w/o based vaccines, subsequent vaccinations are not usually performed. However, oral booster vaccination against piscirickettsiosis after injection vaccination is performed in Chile. Booster vaccination is often performed by bath or by oral delivery in order to reduce the work involved in administering vaccine and, consequently, also the stress on the fish. Immersion (dip or bath) vaccination regimes have been described for sea bass against vibriosis and pasteurellosis [30] or by oral delivery as described in the Vaccination Guide [40].

## 4. Licensed vaccines for industrialised fin fish production

### 4.1. Salmonids

The high value industrialized salmonid species in global aquaculture are Atlantic salmon, coho salmon, rainbow trout (*Oncorhyncus mykiss*) and ayu (*Plecoglossus altivelis*). The type of disease challenges in these species differs, both in severity and occurrence in separate regions. Consequently this reflects the use of different types of vaccines listed in Table 2, as well as the vaccination strategies applied.

#### 4.1.1. Atlantic salmon and coho salmon

Atlantic salmon is by far the most significant salmonid species in global aquaculture today, both in terms of value as well as scale of production, which occurs primarily in Norway, Chile, Canada, Scotland, Ireland, the Faroe Islands, Iceland and Australia. The total

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**Table 2**
Summary of antigens in licensed vaccines, commercially available for anadromous and freshwater salmonids.

| Species | Region | *A. salmonicida* | *L. anguillarum* | *V. salmonicida* | *V. ordalii* | *M. viscosa* | *Y. ruckeri* | *P. salmonis* | *R. salmoninarum* | *F. columnaris* | *L. garvieae* | IPNV | ISAV | PDV | IHNV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atlantic salmon | Norway and Faroe islands | Inj | Inj | Inj | | Inj | Imm | | | | | Inj | Inj | Inj[*] | |
| | Chile | Inj | | | Inj | | Imm | Inj, Ora | | | | Inj, Ora | Inj, Ora | | |
| | UK/Ireland | Inj | | | | | | | | | | Inj | | Inj | |
| | N.America | Inj, Imm | Inj, Imm | Inj | | | Imm | | Inj | | | | Inj | | Inj[a] |
| Rainbow trout | Norway | Inj | Inj | Inj | | Inj | | | | | | | | | |
| | Europe (exclusive Norway) | Inj | Imm, Inj, Ora | | | | Imm, Inj, Ora | | | | Inj | Inj | | | |
| | Chile | Inj | | | Inj | | Imm | Inj, Ora | | | | Inj, Ora | | | |
| | N. America | Inj, Imm | Inj | | | | Imm | | Inj | Imm | | | | | |
| | Japan | | Imm | | | | | | | | | | | | |
| Coho salmon | Chile | Inj | | | Inj | | Imm | Inj, Ora | | | | Inj, Ora | Inj, Ora | | |
| | Japan | | Imm | | | | | | | | | | | | |
| Ayu | Japan | | Imm | | | | | | | | | | | | |

Imm = Immersion vaccine, Ora = Oral vaccine, Inj = Injectable vaccine.
[*] No injectable PD vaccine in Faroe Islands.
[a] DNA vaccine.

global production of Atlantic salmon in 2011 was 1 619 200 tonnes [47].

Several licensed fish vaccines for Atlantic salmon and coho salmon are commercially available. Licensed vaccines against the following diseases are known; furunculosis (*A. salmonicida*), vibriosis (*L. anguillarum* and *Vibrio ordalii (V. ordalii*)), cold-water vibriosis (*V. salmonicida*), winter-ulcer (*Moritella viscosa (M. viscosa*)), yersiniosis- Enteric Red Mouth (ERM (*Y. ruckeri*)), piscirickettsiosis (*Piscirickettsia salmonis (P. salmonis*)), bacterial kidney disease (BKD) (*Renibacterium salmoninarum (R. salmoninarum),* infectious pancreas necrosis (IPN virus), pancreas disease (Salmonid Alpha virus), infectious salmon anaemia (ISA virus) and infectious hemorrhagic necrosis (IHN virus). Most of the vaccines are w/o based vaccines administered by intra-peritoneal injection. However, different vaccination regimes are applied dependent on the regional prevalence of specific diseases.

The most comprehensive w/o based injectable vaccine for Atlantic salmon is composed of seven different antigens protecting against furunculosis, vibriosis, cold-water vibriosis, winter-ulcer, IPN and ISA. However, in Norwegian salmon farming more than 95% of Atlantic salmon smolts, equivalent to approximately 300 million fish on an annual basis per August 2012, were vaccinated with six-component w/o based vaccines containing antigens protecting against furunculosis, vibriosis, cold-water vibriosis, winter ulcer and IPN [73]. The vaccine is administered at the freshwater site as a single injection. Two to three weeks or at least 230° days prior to this, approximately 30% of Atlantic smolts were vaccinated with a monovalent w/o based vaccine against PD, a condition which causes major losses in the Norwegian industry [5].

In the Faroe Islands, vaccination against ISA in Atlantic salmon was included as part of the national contingency plan against this disease [17]. Today more than 90% of salmon smolts in the Faroe Islands are vaccinated with a seven-component vaccine.

In the Atlantic salmon industries in Scotland and Ireland combined w/o based vaccines against furunculosis and IPN are most commonly in use. In addition, a large proportion of pre-smolts are vaccinated with a single, w/o based monovalent PD- vaccine, prior to vaccination with the divalent vaccine.

In Canada, Atlantic salmon is produced both on the east Atlantic coast and the west Pacific coasts. The different disease challenges, the large distances between the coasts and also within the east coasts warrant different vaccine strategies. East coast producers typically vaccinate against furunculosis, vibriosis and cold-water vibriosis with w/o based vaccines. In addition a multivalent w/o based vaccine also including ISA-virus is commonly is use. On the west coast it is routinely vaccinated against the same bacterial diseases as on the east coast. In addition, a separate intra muscular injection with a monovalent saline based DNA vaccine against IHN, is commonly used. This is so far the only licensed DNA vaccine for aquaculture. The USA has a small Atlantic salmon industry with presence on both coasts (Maine and Washington States). Vaccine use reflects use on the Canadian side on respective coasts, however there is no licensed IHN-vaccine available in the Washington State. In the fresh water fry stage Atlantic salmon are commonly vaccinated against ERM and furunculosis. In USA and Canada there is also an attenuated *Arthrobacter*-based commercial vaccine against BKD available.

In Chile, vaccines designed for Atlantic salmon have been developed over the past 11 year period. The first w/o based vaccine in use was a monovalent vaccine against IPN followed by divalent w/o based vaccines against furunculosis and IPN. Further, trivalent vaccines including antigen protecting against *V. ordalii* were licensed a few years later followed by a four-component vaccine also protecting against piscirickettsiosis in 2009. No ISA-vaccines were licensed before 2007 when ISA hit the industry and led to a

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serious downturn in Atlantic salmon industry production. Different vaccines were licensed in Chile and today all Atlantic salmon are vaccinated with w/o based vaccines. Currently, the most common vaccination regime for Atlantic salmon includes injection with five-component vaccines against furunculosis, vibriosis, piscirickettsiosis, IPN and ISA. Some farmers have also included oral vaccination of Atlantic salmon at 1–1.5 kg against piscirickettsiosis as part of their vaccination regime. At the early freshwater stage mortality from IPN is frequently seen [16] and as part of their vaccination program, some farmers apply IPN-immersion vaccines at 2–3 g size and/or IPN-monovalent vaccines administered by injection to 8–10 g fish.

The current vaccination regime for coho salmon in Chile is vaccination against IPN and piscirickettsiosis with w/o based divalent vaccines.

In Australia (Tasmania), the production of Atlantic salmon in 2011 were 36 000 tonnes [47]. There are no commercial, registered vaccines available to the industry except four different autogenous vaccines for fish. Amoebic gill disease (AGD) is the most serious health problem in Atlantic salmon cultured in Tasmania [10].

In Japan, the production of salmonids (ayu, coho and trout) were 30 000 tonnes in 2010 [24]. The fish are vaccinated by immersion against *L. anguillarum* serotype J-O-1 and J-O-3 [65]. The serotype J-O-1 is European serotype O2, and J-O-3 is equivalent to serotype O1 [94].

#### 4.1.2. Rainbow trout

The production of rainbow trout can be differentiated into large trout production in seawater/brackish water and freshwater pan sized/inland production. The primary producing areas are in Europe, North America, Chile, Japan and Australia. Chile is currently the largest producer [21].

The diseases affecting rainbow trout in seawater production of large trout versus freshwater rainbow trout production differ in the context of vaccines available and vaccination regimes most frequently used. Licensed vaccines against the following diseases are known; furunculosis (*A. salmonicida*), vibriosis (*L. anguillarum* and *V. ordalii*), winter-ulcer (*M. viscosa*), yersiniosis-ERM (*Y. ruckeri*), lactococcosis (*L. garvieae)*, flavobacteriosis (*F. columnare*), piscirickettsiosis (*P. salmonis)* and IPN. Most of the vaccines for large rainbow trout are w/o based injectable vaccines compared to mainly water-based vaccines used for freshwater rainbow trout and at early fry stage.

*4.1.2.1. Large rainbow trout production in seawater.* The majority of the global large trout production takes place in Norway, Finland, Sweden, Denmark and Chile. The total global harvest of farmed large trout in 2011 was 316 700 tonnes [47].

In Norway rainbow trout are routinely vaccinated with w/o based vaccines against furunculosis and vibriosis. However, winter-ulcer in trout is common and multivalent vaccines including a *M. viscosa* component are often preferred.

In Finland, Sweden and Denmark vaccination against furunculosis and vibriosis with w/o based vaccines is a standard vaccination regime for large rainbow trout. In Denmark, most rainbow trout fry are primarily immersion vaccinated against yersiniosis at 3–5 g. Due to an increased prevalence of yersiniosis in brackish farm sites in the Baltic Sea over the past years [84], simultaneous vaccination by injection of a water-based ERM-vaccine and a w/o based furunculosis/vibriosis vaccine is more commonly seen.

In Canada, the rainbow trout in salt or brackish water are produced on the east coast. The fish are vaccinated against furunculosis and/or vibriosis prior to transfer to the marine environment. Both w/o based multivalent and monovalent water based vibrio vaccines are in use. In the fry stage the trout is commonly vaccinated against ERM with water- based vaccines.

In Chile the use of vaccines for rainbow trout began in mid nineties' with immersion vaccination against *Y. ruckeri* and *Flavobacterium psychrophilum* (*F. psychrophilum*). Vaccination against yersiniosis is still a common practice in 2012 whilst vaccines against flavobacteriosis are no longer available. By the late nineties' vaccines with a water-based *P. salmonis* component against piscirickettsiosis was licensed, shortly followed by an introduction of a w/o based vaccine. The current vaccination regime for large rainbow trout is vaccination to protect against IPN and piscirickettsiosis with w/o based divalent vaccines. Some farmers use a trivalent w/o based vaccine against vibriosis, IPN and piscirickettsiosis. In addition, some farmers also vaccinate with an oral vaccine protecting against piscirickettsiosis at 1–1.5 kg. At the early freshwater stage of rainbow trout, as for Atlantic salmon, mortality due to IPN is seen, and as part of their vaccination program, some farmers apply IPN-immersion vaccines to rainbow trout at 2–3 g size and/or IPN-monovalent vaccines administered by injection to 8–10 g fish.

*4.1.2.2. Freshwater rainbow trout production.* Fresh water rainbow trout is the most commonly farmed salmonid throughout the mainland Europe where each country shares a common disease profile due to the nature of the industry which imports/exports live fish regularly and the general farming practises which are employed.

The two major bacterial disease challenges to freshwater rainbow trout production in Europe are yersiniosis (also called enteric redmouth disease) and flavobacteriosis (caused by *F. psychrophilum*) known as rainbow trout fry syndrome (RTFS). Although flavobacteriosis is a significant disease threat to the industry in most rainbow trout producing countries there are no fully licensed immersion- or injectable vaccines available. Furunculosis in freshwater rainbow trout production is frequently described, and recently vibriosis caused by *L. anguillarum*, found in Turkish freshwater rainbow trout was reported to rapidly spread amongst farms [86]. Lactococcosis caused by *L. garvieae* is seen in several rainbow trout producing countries especially in southern Europe [95], and documented as a major bacterial disease in Iran, a significant producer of trout estimated to be the largest in the Middle East and equal to Turkey.

Water-based injection- and immersion vaccines against yersiniosis and vibriosis in rainbow trout are licensed in several European countries as well as oral vaccines against the same diseases [34]. As a general regime, most rainbow trout fry are vaccinated against yersiniosis by one immersion at 3–5 g size only, during the production cycle. However, a booster vaccination should always be considered. Vaccination using a w/o based injectable vaccine against lactococcosis is part of a specific vaccine regime in some European countries.

Rainbow trout is produced in North America, both in the USA and Canada. The main area of food fish production in the USA is Idaho, with around 70% of the volume, typically producing 500–600 g portion sized trout [96]. Ontario is Canada's largest food trout production region, targeting mostly 1 kg rainbow trout [22].

Major bacterial endemic diseases in North American rainbow trout production include ERM and flavobacteriosis, furunculosis and BKD. Water-based immersion vaccines are commercially available against the three diseases first listed, and fish are typically immersed once or twice from 2 to 3 g and booster vaccinated at around 5 g. A live attenuated *Arthrobacter*-based injection vaccine is available against BKD, injected intraperitoneally to fish of 10 g or more. An overview over licensed, commercial fish vaccines in Canada and USA are publically available online [8].

The major viral disease of concern is IHN, which is endemic in the western part of North America. The DNA vaccine APEX-IHN is licensed for salmonids, however it is not commonly used in trout.

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### 4.2. Marine species

#### 4.2.1. Cod

Most of the farmed cod is produced in Norway. The production in 2011 was 15 000 tonnes [22]. Atypical furunculosis and vibriosis are the most typical bacterial diseases in cod today. The prevalence of francisellosis caused by *Francisella noatunensis* (*F. noatunensis*) in Norwegian cod farming is significantly reduced since 2008 and no vaccines are currently available. Water-based vibrio vaccines for immersion are applied according to a standard regime recommending vaccination at 1 g fish size followed by booster vaccination at 5 g. A w/o based injection vaccine against vibriosis and atypical furunculosis is available in Norway for vaccination of cod at minimum 30 g size Table 3.

#### 4.2.2. Sea bass and sea bream

The Mediterranean countries are the leading producers of farmed European sea bass (*Dicentrarchus labrax*) and gilt-head sea bream (*Sparus aurata*). The production was 129 000 tonnes of sea bass and 161 000 tonnes of sea bream in 2011 [48]. The bacterial diseases most often found are vibriosis (*L. anguillarum*) and pasteurellosis (*Photobacterium damselae* (*P. damselae*)). The prevalence of vibrio infections in sea bass is higher compared to sea bream. Since the mid nineties a recommended injection vaccination regime applied for sea bass has been successful. A combined water-based immersion vaccine against vibrio and pasteurellosis is recommended at 1 g and re-vaccination at 5 g followed by injection of a combined w/o based vaccine at minimum 15 g size. Monovalent water-based vibrio and pasteurella vaccines for immersion are available in selected markets (Table 3).

#### 4.2.3. Yellowtail and great amberjack

Seriola species as yellowtail (*Seriola quinqueradiata*) and great amberjack (*Seriola dumerili*) are mainly produced in Japan. The production was 139 000 tonnes in 2010 [20]. Immersion vaccine against *L. anguillarum* J-O-3, is available for fish at 1–3 g. Vaccination started with the use of oral vaccine against *L. garvieae* in 1997. The vaccine is recommended used from 100 to 400 g. Later through a serial of mono and divalent vaccines, trivalent water-based injection vaccines containing iridovirus, *L. anguillarum* (J-O-3) and *L. garvieae* or *L. garvieae*, *L. anguillarum* and *Streptococcus dysgalactiae* (*S. dysgalactiae*) are now used from 10 g in weight. Also two w/o based vaccines, one divalent and one trivalent, recommended for fish from 30 g up. The trivalent w/o based vaccine protects against *P. damsela, L. garvieae* and *L. anguillarum* (J-O-3) [66]. Vaccination of yellowtail against pasturellosis, was not reported to cause any significant side effects [31] (Table 3).

#### 4.2.4. Asian sea bass/barramundi

Asian sea bass or barramundi (*Lates calcarifer*) is produced in many Asian countries and in Australia. The production volume is 66 000 tonnes in 2010, of which 1/3 is from Malaysia [21]. *Streptococcus iniae* (*S. iniae*) causes mortality particularly in bigger fish. A vaccine against *S. iniae*, is licensed in Singapore and Indonesia (Neil Wendover, pers com), the vaccine is inactivated water-based and can be administered by immersion or injection [59]. Vaccine for irridoviral disease is also available in Singapore. No side effects have been reported in connection with vaccination of Asian sea bass. Other diseases reported are tenacibaculosis (caused by *Tenacibaculum maritimum* (*T. maritimum*)), and viral neural necrosis (VNN) [98] (Table 3).

#### 4.2.5. Flatfish (olive flounder and turbot)

Olive flounder (*Paralichythys olivaceus*) is produced in Japan, China and Korea. In Japan, injection vaccines against *S. iniae* are available and recommended for fish at 30–300 g [66]. In Korea, an immersion vaccine is available against *Edwardsiella tarda* (*E. tarda*) [72]. However, injection vaccination is more used. Through successive development of monovalent injection vaccines, a trivalent water-based vaccine against *S. iniae*, *Streptococcus parauberis* (*S. parauberis*) and *E. tarda* is developed and approved by the Korean animal, plant & fisheries quarantine & inspection agency. The development is now moving towards five valent vaccines including *L. anguillarum* and *T. maritimum* [42]. In China, there is a vaccine available for *Vibrio* sp and *E. tarda* (Dr. Jie Huang, pers com).

Production of farmed turbot is geographically wide spread in Europe with the largest production volumes in Spain. According to the Business Association of Marine Aquaculture Producers (Apromar) the production reached 7755 tonnes. A range of different bacterial pathogens challenge the European turbot industry, e. g. *E. tarda, L. anguillarum, T. maritimum, S. parauberis* and atypical *A. salmonicida*. Prevalence of the different bacteria and disease challenges varies geographically. Vaccines against the bacteria listed above are developed, but to a variable degree available. Vaccines are also offered as autogenous products by vaccine manufacturers. There are commercial *L. anguillarum* vaccines available for immersion and injection administration [68,93], containing a mixture of serotypes O1 and/or O2a. However, a licensed w/o based vaccine (GAVA-3), marketed by Hipra Laboratories (Spain), is to our knowledge the only vaccine covering all three serotypes O1, O2a and O2b of *L. anguillarum* [95] (Table 3).

#### 4.2.6. Other marine fish

Red sea bream (*Pagrus major*) is produced mainly in Japan and the production was 68 000 tonnes in 2010 [21]. Iridovirus causes disease in many marine species [65]. The inactivated red sea bream

**Table 3**
Summary of antigens in licensed vaccines, commercially available for marine and brackish water fish.

| Species | Region | Atypical *A. salmonicida* | *E. tarda* | *P. damsella* | *L. anguillarum* | *S. iniae* | *S. parauberis* | *S. dysgalactiae* | *L. garvieae* | Iridovirus | VNNV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cod | Norway | Inj | | | Inj, Imm | | | | | | |
| Gilt-head sea bream | Greece, Turkey | | | Inj, Imm | Inj, Imm | | | | | | |
| European sea bass | Greece Turkey | | | Inj, Imm | Inj, Imm | | | | | | |
| Asian sea bass | Indonesia, Malaysia | | | | | Inj | | | | | |
| | Singapore | | | | | | | | | Inj | |
| Red sea bream | Japan | | | | | | | | | Inj | |
| Rock bream | Korea | | | | | | | | | Inj | |
| Yellowtail | Japan | | | Inj | Inj | | | Inj | Inj | Inj | |
| Great amberjack | Japan | | | Inj | Inj | | | Inj | Inj, Oral | Inj | |
| Striped Jack | Japan | | | | | | | | | Inj | |
| Grouper | Japan | | | | | | | | | Inj | Inj |
| Turbot | Spain | | | | Inj, Imm | | | | | | |
| Olive flounder | Korea, Japan | | Inj, Imm | | | Inj | Inj | | | | |
| Flatfish | China | | Inj, Imm | | Inj | | | | | | |

Imm = Immersion vaccine, Ora = Oral vaccine, Inj = Injectable vaccine.

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iridovirus (RSIV) vaccine were first licensed for red sea bream in Japan and is administered by injection for 5–20 g fish [66]. The use of this vaccine has later been extended to seriola species that are injected at 10–100 g, striped jack at 10–70 g, and grouper at 5–50 g.

21 000 tonnes of rock bream (*Oplegnathus fasciatus*) was produced in Korea [21]. A recombinant vaccine is licensed for rock bream in Korea [72].

Recently a vaccine was licensed to protect seven band grouper (*Epinephelus septemfasciatus*) against VNN in Japan [69].

### 4.3. Fresh water species

#### 4.3.1. Grass carp

The production of grass carp (*Ctenopharyngodon idellus)* is more than 4.3 mill tonnes globally, where of China produces 4.2 mill tonnes (FAO fish stat+ 2012).

A live attenuated vaccine has been launched for grass carp in China for spring viraemia of carp (Prof Huang Jie, pers. Com). It is also described by Ref. [99] that two inactivated vaccines against grass carp haemorrhage and *Aeromonas hydrophila* (*A. hydrophila*) have veterinary certificate of China, however with no real industrial applications when described in 2008 (Table 4).

#### 4.3.2. Catfish (striped catfish (pangasius) and channel catfish)

The production of catfish is more than 3 mill tonnes globally [21]. The main countries are Vietnam (38%), China (26%), Indonesia (12%) and USA (7%). The major species are striped catfish (*Pangasianodon hypophthalmus*) and channel catfish.

Currently, two live attenuated vaccines have been developed and are commercially available for channel catfish in the U.S. for *E. ictaluri* and *F. columnare* [6] (Table 4). Bebak and Wagner reported that 9.7% of the fingerling production used one or both vaccines in 2009, while among the total industry 12.3% vaccinated against *Edwardsiella ictaluri*, and 17.4% vaccinated against *F. columnaris*. They also concluded that for striped catfish, there are no commercial vaccines available. However, an inactivated injection vaccine against *E. ictaluri* obtained a commercial license for Vietnam in 2013 (www.pharmaq.com), but large scale vaccination using the injection vaccine against *E. ictaluri* took place through an observation license during the last year [88]. There are no reports of side effects in connection with vaccination of catfish.

In addition to the diseases mentioned above, *A. hydrophila* was recently reported to cause severe mortality in catfish [74].

#### 4.3.3. Tilapia

Tilapia is one of the main species produced in aquaculture world-wide. The total production of tilapia was 3.5 million tonnes in 2010 [21] and the main producing countries are China, Egypt, Indonesia, the Philippines, Thailand and Brazil. The most common specie is *Oreochromis niloticus* with 2.5 million tonnes produced in 2010.

The main disease problem is streptococcosis, predominantly caused by infection of *Streptococcus agalactiae* (*S. agalactiae*), prevalent in temperate and tropical regions. This bacterial species, which constitutes at least 10 serotypes [80], is a well known pathogen towards humans and animals. In tilapia, three different serotypes are known to cause infections, serotype Ia [18], serotype Ib [97] and serotype III [85].

In tilapia production there is not a widespread use of vaccines. The relative low value of each individual fish at harvest makes it difficult for small fish farms to invest in effective vaccines. However, the trend in tilapia production is an increasingly intensive production and the emergence of larger farm companies with focus on reducing the use of antibiotics. Consequently, on-going development and testing of improved vaccines is an important aim for farmers and pharmaceutical companies.

A commercial vaccine for the control of *S. agalactiae* is available in Indonesia, Brazil and several Central American countries [15], in addition an inactivated w/o based vaccine has recently obtained approval from the authorities in Singapore against iridovirus (Genus Megalocytivirus) [23].

## 5. Future trends and technologies in fish vaccinology

To further support sustainable fin fish production in aquaculture there are many fish health related issues to be addressed which are important for fish welfare, profitability and the environment. These issues are the driving forces for the development of new vaccines and efficient vaccination programs against emerging and persistent diseases in aquaculture.

Developing fish vaccines is costly and time consuming, and it would probably not be profitable or realistic to develop vaccines against all pathogens identified. In order to increase availability of vaccines, contract production of different autogenous vaccines is one future scenario. However, the overall importance to be emphasized is vaccine product quality together with best practice vaccination and fish health management.

Most of the vaccines available are based on classical fermentation, cultivation or recombinant technologies. Future vaccines including inactivated vaccines, live attenuated vaccines and DNA vaccines will be reviewed in this section.

### 5.1. Adjuvants for inactivated antigens

For inactivated vaccines, adequate and long-lasting protection is only achieved when adjuvants are included to increase the potency of the formulation. In fish vaccinology, injectable w/o based adjuvants have remained the gold standard since the commercial introduction in the early 1980s. Although detailed knowledge

**Table 4**
Summary of antigens in licensed vaccines, commercially available for fresh water fish.

| Species | Region | *E. ictaluri* | *F. columnare* | *S. agalactiae* | Iridovirus | Spring viraemia of carp |
|---|---|---|---|---|---|---|
| Channel catfish | USA | Imm[a] | Imm[a] | | | |
| Striped catfish | Vietnam | Inj[b] | | | | |
| Tilapia | Brazil | | | Inj | | |
| Tilapia | Costa Rica | | | Inj | | |
| Tilapia | Indonesia | | | Inj | | |
| Tilapia | Singapore | | | | Inj | |
| Grass carp | China | | | | | Imm[a] |

Imm = Immersion vaccine, Ora = Oral vaccine, Inj = Injectable vaccine.
[a] Attenuated.
[b] Inactivated injection.

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about the mode of action for w/o-adjuvants remains to be fully elucidated, numerous studies have documented their ability to depot antigens at the site of injection [62,64], consequently upholding the pivotal contact between vaccine antigens and immune cells [19] to support sustained and robust pro-inflammatory [25,63] and Humoral responses [58].

In general, the injection vaccines presently on the market induce excellent protection against bacterial diseases such as vibriosis, furunculosis and winter ulcers [54]. However, there are some challenges associated with using w/o emulsions as adjuvants. First, the use of these adjuvants may cause adverse effects such as melanization, organ adhesions and autoimmunity [49,57]. A cardinal challenge has been to balance vaccine efficacy with the reactogenic nature manifested at the injection site. Vaccine improvements by formulation optimization and dose volume reduction have nevertheless drastically reduced the severity of side effects. The second main challenge that still remains is to develop vaccines that are equally potent in inducing immunity against intracellular pathogens as what has been documented for the bacterial diseases. For this purpose, w/o adjuvants are sub-optimal as they generally promote strong humoural immune responses [13]. Furthermore, modern fish vaccines for high value species such as salmonids are preferably multivalent containing antigens from several pathogens. The w/o adjuvant is an excellent platform for multivalent vaccines as numerous inactivated antigens may easily be incorporated, it does however demand for high titer production of pathogens. The latter may prove to be a key challenge for development of efficacious antiviral vaccines, since high antigen doses are advantageous for induction of protective immunity against viral diseases [61,89].

The progress in adjuvant technology for commercial injectable, inactivated fish vaccines have hitherto mainly been due to refinement rather than replacement of the existing w/o based adjuvants. The challenge of increasing the vaccine potency, predominantly against intracellular pathogens underlines the need to search for alternative adjuvants and delivery systems. During the last two decades a number of different adjuvants have been suggested. These adjuvants could be classified in two broad groups, namely soluble or particular. The former group includes receptor ligands, such as Toll-receptors agonists, which have the advantage of directly binding intra- or extracellularly on innate and adaptive immune cells [33,71]. Thus, unlike for many of the established particle based adjuvants/delivery systems such as aluminium salts (alum), MF59 (o/w) and w/o-formulations, the cellular and molecular mechanisms of action of these soluble adjuvants are well defined and may prove useful for modulating responses to vaccination towards cellular immunity. In Atlantic salmon, synergistic antiviral responses have been reported after vaccination with unmethylated CpG oligodeoxynucleotides (ODN) and poly IC (synthetic double stranded RNA) against salmonid alpha virus (SAV3) [83]. The capacity of other TLR ligands, such as flagellin [39] and imiquimod [44] to induce immune responses have also been reported, but there are still few reports on the efficacy of these soluble adjuvants in protecting fish against disease. In studies on mammalian species, conjugation of TLR ligands to antigens has been shown to increase antigen potency by ensuring temporal and spatial co-delivery of immune modulators (TLR ligands) and antigens, which have been demonstrated to have several benefits over non-conjugated antigens [35].

Particle based adjuvants/delivery systems constitute a large number of biocompatible compounds that are partly or fully biodegradable, including particles made of poly (lactide-co-glycolide) acid (PLGA), chitosan, alginate, liposomes, virosomes, immune stimulating complexes (ISCOMs) and emulsions (w/o and o/w) [13]. Similar to what has been reported for soluble adjuvants, antigen-particle association by absorption and entrapment is known to increase immune responses and vaccine potency/efficacy [36,77]. For non-emulsion particle based delivery systems, size is recognized as a highly important determinant for cellular uptake and antigen processing [43] where sub-micron particles have been demonstrated to support the MHC I/CTL pathway while microparticles, with release of antigens to the extracellular junctions, may support MHCII/Th2 characterized responses [41]. Particulate adjuvants that exists as single entities (e.g PLGA, ISCOMs, liposomes, virosomes etc) work as antigen vehicles and may thereby contribute to protect antigens from degradation, increase the cellular uptake and thus be in support of the depot concept [76]. This quality is specifically important to avoid premature antigen degradation during oral vaccine delivery [50,91,92].

Generally, the novel adjuvants and delivery systems that so far have been examined in fish seem to only induce transient immune responses, resulting in sup-optimal long term protection. We therefore advocate and renew the statement by Evensen et al. [19] who viewed it as unlikely that the w/o adjuvant will be replaced in the immediate future. However, to meet the requirements for improved antiviral responses to vaccination, future fish vaccines are expected to include refinements of the existing w/o-based adjuvant platform rather than fully replacing it.

### 5.2. Future trends – DNA vaccines

The DNA vaccine concept has been a promising candidate in the defence against pathogens, particularly pathogens such as viruses and intracellular bacteria that might require cell mediated antigen presentation for the correct activation of immunity. Indeed, aquaculture is one of the few areas where a DNA vaccine has actually entered the market. The IHNV DNA vaccine licensed for use in Canada has shown good protection against several IHN virus strains [12]. Also DNA vaccines against other Rhabdoviruses show good effect, such as the results from research on a viral hemorrhagic septicemia DNA vaccine [53]. The G-protein of the *Rhabdoviridae* seems to induce an initial unspecific boost of the innate immune system, and hence can serve as an adjuvant and increase the effect of the DNA vaccine [45,79]. This might suggest that with a good adjuvant, DNA vaccines against pathogens outside of the *Rhabdoviridae* family that have not shown as promising results, might perform better [52]. Work on genetic adjuvants (DNA plasmids encoding proteins that are immune-stimulatory), as well as adjuvants that are a part of the vaccine, but not encoded on a plasmid, will be an important step towards better functioning DNA vaccines. Also improved transfection efficiency through new administration methods might serve to optimize the effect of DNA vaccination. Particularly this will be a challenge if multivalent DNA-vaccines are developed.

Another aspect is that the DNA vaccinated fish in some countries will be labelled as a Genetically Modified Organism (GMO). Public perception of GMOs as source of food, as well as restrictive legislation from the authorities on this matter might cause challenges for the aquaculture industry and will have to be addressed.

### 5.3. Future trends – live attenuated vaccines

The use of live vaccines is not a new concept, and the first known vaccine to protect humans against small pox was derived from a live virus used as a vaccine during the 1770s [82].

Despite the advantages of modified live vaccines compared to inactivated vaccine with respect to protection against difficult intracellular pathogens, few vaccines are on the market. Attenuated live vaccines survive and replicate within the host, which results in a strong cellular immune response that confers protection of long

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duration. The live vaccine also has the ability to stimulate the humoral and mucosal immunity [11].

The major disadvantage of using modified live vaccines is related to safety concerns. The possible reversion to virulence means extensive work to document that this will not be a problem in practice is required before such a vaccine can be made commercially available. Several attenuated microorganisms have been shown to be unstable and revert to virulence [3,37,100]. A recent paper demonstrates that attenuated vaccines can recombine to form virulent field viruses in the poultry industry [51]. The important and comprehensive bio-safety assessments include biological safety to aquatic animals and environment as well as purity and efficacy of the vaccine. Presence of the pathogen in the natural environment and the ability to infect people are among the important considerations.

Traditionally, attenuation was achieved by multiple passages of the microorganism on appropriate growth medium which resulted in random mutations that lead to attenuation. Chemical or physical mutagenesis results in random mutation(s) in the pathogen. An environmental bacterium mimicking the antigenic structure of the pathogen is also used (*Arthrobacter davidanieli)* to protect against BKD caused by *Renibacterium salmoninarum* [14,75]. Novel molecular methods enable the development of GMOs targeted to specific genes. GMOs constructed by genetic engineering display an advantage over random mutagenesis, enabling a more targeted safety testing. Another approach is the introduction of foreign genes into a live vaccine strain which expand the protective activity against more diseases. However, attention must be paid to the biological safety assessments as the recombinant hosts acquire new properties. There are regulatory aspects related to the use of live GMO vaccines as they are released into the environment. The regulatory restrictions in many countries are significantly stricter than those for the release of conventional live vaccines. This is in contrast to biological safety considerations where defined genetic modifications allow for much better control and safety assessment than random, mostly unknown mutations in a conventionally attenuated vaccine [29].

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