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Aquaculture and the DNA Revolution

Genetics; Microbiome; Vaccines; Treatments; Technology

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Executive Summary

Our global population is predicted to grow to at least 9 billion people by 2050, requiring an increase in protein production of around 50% from today's levels. An increase in protein derived from the aquatic environment is considered to be critical for ensuring the protein gap can be closed. Wild capture has peaked and will (at best), be maintained at current levels with effective management. Aquaculture will therefore contribute ~100% of future growth in aquatic protein production.

Most commentators agree that future expansion of the industry will be dependent upon innovation throughout the value chain. As examples, sustainable diets with nutritional profiles optimized for the health and productivity of each species (and their nutritional value for the consumer) are essential, as are new biosecurity and engineering solutions to minimize environmental impact and improve animal welfare. However, the present report focuses on reducing losses from infectious diseases and parasitic infestations, an ever-present danger which will persist for as long as aquaculture is practiced in open water systems.

The aim of all farmers is to achieve a maximum crop of the best quality. For a fish farmer, this means avoiding mortalities during all phases of production from egg to juvenile to adult. However, mortalities are the tip of the iceberg of the impact of disease on productivity. Morbidity is at least as damaging, typically manifesting as smaller animals of inferior quality that cost the farmer the same or more to produce, but which are ultimately worth significantly less. Effective control of disease is therefore one of the most important leading indicators of improved productivity.

Industrial aquaculture is a very young industry compared with terrestrial livestock farming. For example, Atlantic salmon production began in earnest in the 1970's. Every major species under cultivation has its own genetic and immunological idiosyncrasies and faces a unique set of disease threats which require deconvolution on a case by case basis. Considering this remarkable diversity and complexity, one could be forgiven for turning inward and focusing on individual species and diseases in isolation. Indeed, it is important that a percentage of effort is focussed in this way. However, if one takes a step back, it becomes apparent that many novel healthcare platform technologies originating in human medicine can be successfully adapted for aquaculture. Such platform technologies can be leveraged to provide solutions that transcend species barriers and create excellent opportunities for shared learning and economies of scale. The majority of these platform technologies are made possible by remarkable advances in molecular biology, described by numerous commentators as the DNA revolution.

The premise of the present report is that disease prevention is more cost effective and economically beneficial than an over-dependence on treatment. Three key pillars of prevention are described:

- **Genetic Advantage**. The DNA revolution has provided a rich toolbox for identifying genes associated with disease resistance, thereby massively accelerating the selection of genetically advantaged animals.

- **Healthy Microbiome**. The DNA revolution has enabled a new level of understanding of the importance of managing the health of the meta organism, which comprises both the host and its

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commensal microbiota. New prophylactic and therapeutic interventions to prevent and correct dysbiosis are constantly being developed and many are a very natural fit for aquaculture.

- **Improved Vaccines**. The DNA revolution has completely changed the face of modern vaccinology. Vaccines have proven to be extremely important in aquaculture, as evidenced by the virtual elimination of the use of antibiotics in salmon aquaculture in many countries following their introduction.

Whilst there is a compelling argument for an increased emphasis on prevention in infectious disease management, new and improved treatments are still required for those occasions when prophylaxis fails. The DNA revolution is providing a rich array of new therapeutic tools including gene silencing technologies, affordable platforms for monoclonal antibody production and precision alternatives to antibiotics such as CRISPR-CAS. Translated and delivered in the appropriate manner, these technologies lend themselves to use in aquaculture and we can expect to see some of the most exciting new products of the DNA revolution in this area.

The report concludes by describing an integrated model for the future of healthcare in intensive aquaculture. The model comprises systematic improvements in genetic fitness and microbiome health, complemented by improved prophylactic and therapeutic treatments. Optimal application of these technologies will be informed and coordinated by a plethora of new environmental, physiological and behavioural monitoring tools resulting from the rapid adoption of precision farming methodologies in aquaculture. We live in unprecedented times with respect to technological opportunities. There is every reason to be optimistic that global aquaculture can achieve its full potential if a relentless focus on innovation is maintained throughout the value chain.

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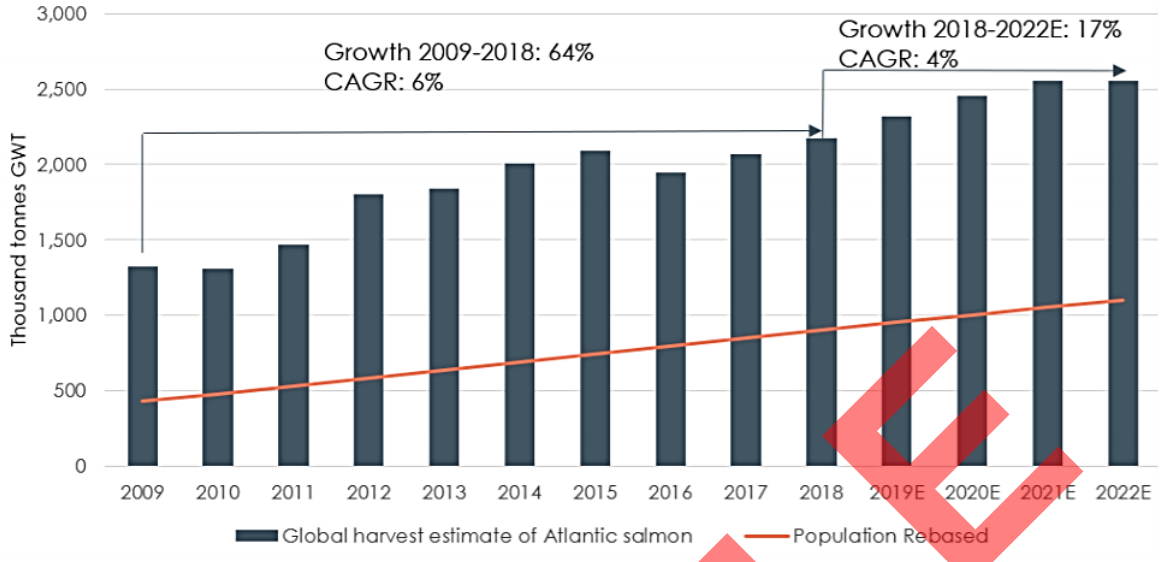
Figure 3: Global salmon production; past performance and future trends



Source: Salmon Farming Industry Handbook 2019, MOWI

https://corpsite.azureedge.net/corpsite/wp-content/uploads/2019/06/Salmon-Industry-Handbook-2019.pdf

The industry is predicted to continue to grow with CAGR of around 4% for the foreseeable future. A number of factors underpin this impressive trajectory, a few of which are listed below:

- **Continued consumer demand;** driven by the social perception of salmon as a "affordable luxury" by the middle classes and its outstanding nutritional qualities, being high in marine Omega 3 fatty acids which are an essential component of a healthy diet.

- **Profitability;** Salmon farmers are envied by other livestock producers. Average earnings before interest, tax, depreciation and amortization (EBITDA) has been estimated at 23.8% for salmon (http://hugin.info/209/R/2239765/882920.pdf) compared with 8.2% for beef (https://jbss.infoinvest.com.br/enu/4980/RAS%202018%20-%20Ingls%20VF.pdf) and 9.3% for pork (http://media-whgroup.todayir.com/20190423164502000334699 51_en.pdf)

- **Food conversion efficiency;** Atlantic salmon have the lowest (most efficient) food conversion rates of any of the major livestock species and also have the lowest greenhouse gas footprint. (Discussed in https://mowi.com/wp-content/uploads/2019/04/2018-salmon-industry-handbook-1.pdf)

The industry is uniquely consolidated, with the top 10 companies controlling at least 75% of production in all markets with the exception of Norway where the figure is only just short of that at 69%.

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Table 4:**Commercially significant diseases of farmed shrimp (Penaeus monodon)**				
Nuclear Polyhedrosis BaculovirosesAlso known as Monodon baculovirus disease (MBV)	Baculovirus	Virus	Lethargy, anorexia, dark coloured shrimp; reduced feeding & growth rates; often increased surface & gill fouling with various epibiotic & epicommensal organisms; severely affected larvae & postlarvae may exhibit a white midgut line through the abdomen; acute MBV causes loss of hepatopancreatic tubule & midgut epithelia &, consequently, dysfunction of these organs, often followed by secondary bacterial infections; linked with high mortalities (>90%) in late postlarvae & juvenile shrimp in many culture facilities; usually juvenile & adult *P. monodon* are more resistant to MBV than larval shrimp; MBV may predispose infected shrimp to infections by other pathogens;	Reduce stocking density, use of chemicals & environmentally induced stress; prevent contamination of fertilized eggs from spawner faeces by washing in formalin or iodophore treated seawater; if infected, culture facility must be disinfected & stock should be removed & sterilized

http://www.fao.org/fishery/culturedspecies/Penaeus_vannamei/en

Table 5:**Commercially significant diseases of farmed shrimp (Litopenaeus vannamei)**				
Disease	**Agent**	**Type**	**Syndrome**	**Measures**
White spot (WSD); also known as WSBV or WSSV	Part of the white spot syndrome baculovirus complex (recently renamed in a new family as a nimavirus)	Virus	Acutely infected shrimp show reduced food consumption; lethargy; high mortality of 100% within 3–10 days of onset of clinical signs; loose cuticles with white spots of 0.5–2.0 mm diameter, most apparent inside the carapace; moribund shrimp often have pink to reddish-brown colouration due to expansion of cuticular chromatophores & few if any white spots	Use SPF broodstock; wash & disinfect eggs/nauplii with iodine, formalin; screen broodstock, nauplii, PL & pond stages; avoid rapid changes in water quality; maintain water temperature >30 °C; avoid stress; avoid use of fresh feeds such as trash fish; minimize water exchange to prevent entry of virus carriers; treat infected ponds & hatcheries with 30 ppm chlorine to kill infected shrimp & carriers; disinfect associated equipment
Taura Syndrome (TS); also known as Taura syndrome Virus (TSV) or red tail disease	Single-stranded RNA virus (Picornaviridae)	Virus	Occurs during single moult in juvenile shrimp beginning 5–20 days after stocking, or has a chronic course over several months; weakness, soft shell, empty gut & diffuse expansion of red chromatophores in appendages; mortality varies 5–95%; survivors may have black lesions, & remain carriers for life	Use SPF & SPR broodstock; wash & disinfect eggs & nauplii; clean & disinfect contaminated vehicles & equipment; scare away birds (vectors); destroy all stock & thoroughly disinfect infected facilities

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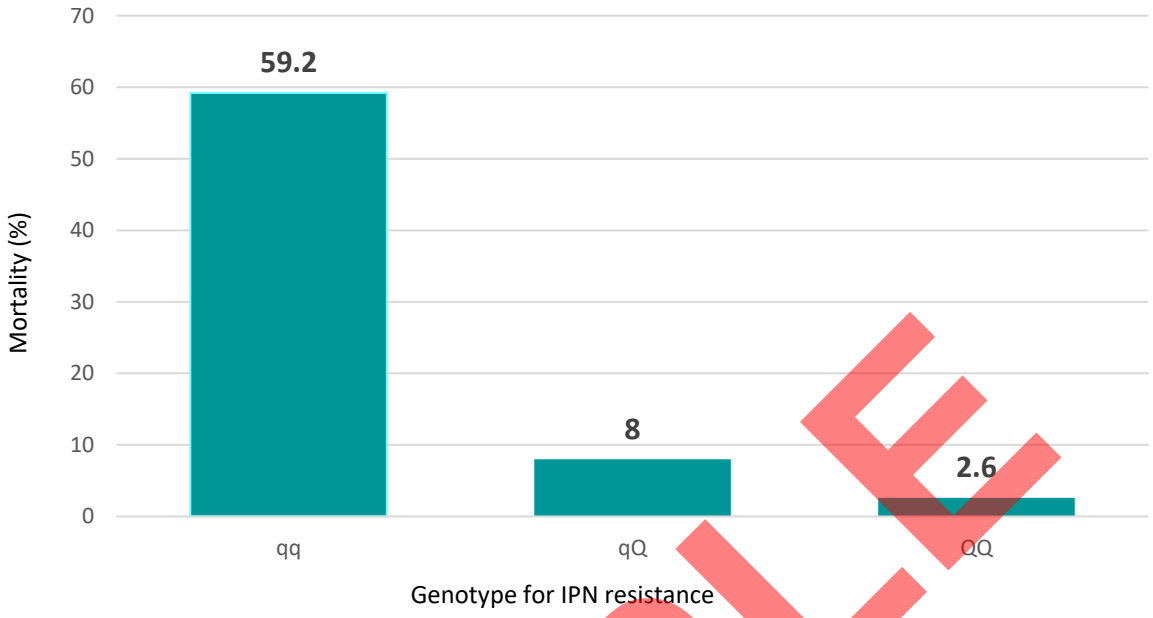
Figure 10: Accumulated mortality in IPN challenge test of salmon fry with different genotypes for IPN resistance



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Similarly, IPNV resistant rainbow trout (*Onchorynchus mykiss*) have been developed and, more recently, two major QTLs have been discovered which show resistance to another major disease of trout caused by the bacterium *Flavobacterium psycrophilum*. In the case of this second disease, trout which are homozygous for both QTLs have survival rates in excess of 80% whereas fully sensitive fish approximate only 30% survival. Strains of trout which have been bred to be resistant to both of these diseases are now entering the market. Vaccines, although available for both, have not been sufficiently successful in controlling the problem. In the near future it is hoped that the combination of these resistant strains, together with vaccines and improved husbandry, will result in their much more effective control. (https://aquagen.no/en/products/trout-eggs/product-documentation-for-rainbow-trout/flavobacteriosis-resistance-for-rainbow-trout/).

The existence of a major genetic component to IPNV resistance is clear from this work but the functional basis of genetic resistance to IPNV in salmon is of importance since the biological mechanisms underlying the genetic resistance and susceptibility might lead to some generalisations applicable to other viral infections.

Moen *et al.* discovered SNPs associated with the putative QTL genotype in the cadherin-1 gene (CDH1) gene which encodes a protein that co-locates with the IPN virus in liver cells and can bind to the IPN virus *in vitro*. This suggested a possible role for CDH1 in the entry of the virus to host cells and may form part of the underlying mechanism of the QTL. They postulated that failure to enter the host cells was the primary mechanism controlling resistance. Robledo *et al* (2016) however pointed out that Moen had described data only from fish 34 days post infection by which time the virus may have been cleared from resistant fish.

Robledo *et al* (op cit) challenged both resistant and susceptible strains and examined the gene expression profiles of each following challenge at several time points. Significant viral titres were observed in both resistant and susceptible fish at all timepoints, although generally at higher levels in susceptible fish. However,

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Chapter 2: Microbiome

Understanding the microbiome

The DNA revolution and the meteoric rise in interest in the microbiome seen in the last decade are inevitably and inextricably linked. It is not the case that we were unaware of the microbiome prior to the DNA revolution, but most early studies focused on individual microorganisms under defined conditions *in vitro* or *ex vivo* models which lacked the context of the ecosystem from which they were plucked. We lacked the molecular tools and the bioinformatics capabilities required to describe, quantify and manipulate a complex microbial community comprising an estimated 100 trillion cells. The last decade has seen an exponential increase in activity. In 2012, the NIH Human Microbiome Project reported the results of 5 years of intensive research https://www.hmpdacc.org/hmp/. Harnessing the power of transformational advances in DNA sequencing technology, researchers purified all human and microbial DNA from more than 5,000 samples obtained from 242 male and female volunteers. Using newly emerging bioinformatics techniques, researchers sorted through 3.5 terabases (3.5 x 10^{12} base pairs) of genome sequence data to identify specific genetic signals found only in bacteria. One aspect of the study revealed that our microbiome outnumbers our own cells by at least 10:1 in absolute numbers and contributes up to 3% of our body mass. The human genome comprises ~22,000 genes but this is comprehensively trumped by our microbiome which comprises >1000 species and contributes >8 million protein encoding genes.

As a result of such research, it is now understood that multicellular organisms exist as meta-organisms in which the host and its commensal microbiome are truly interdependent.

Figure 11: The concept of the meta-organism in its environment

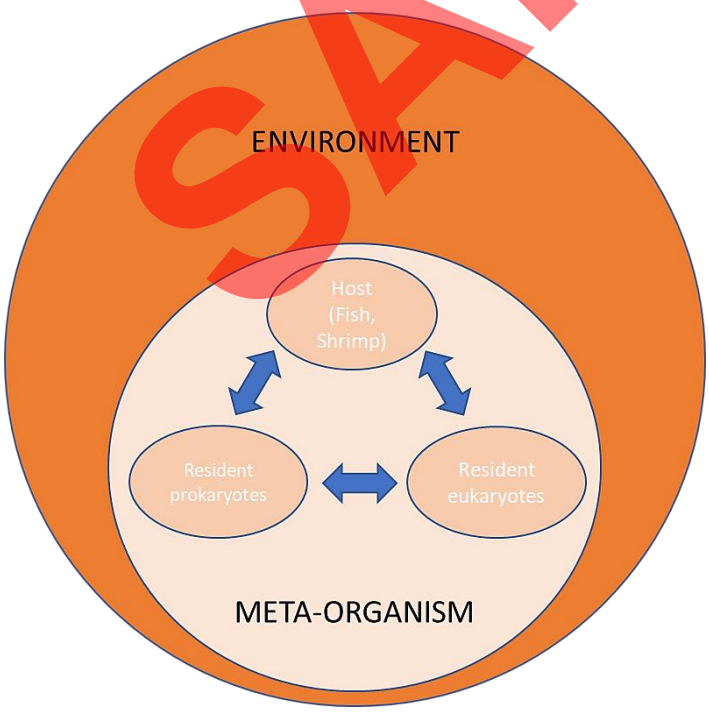

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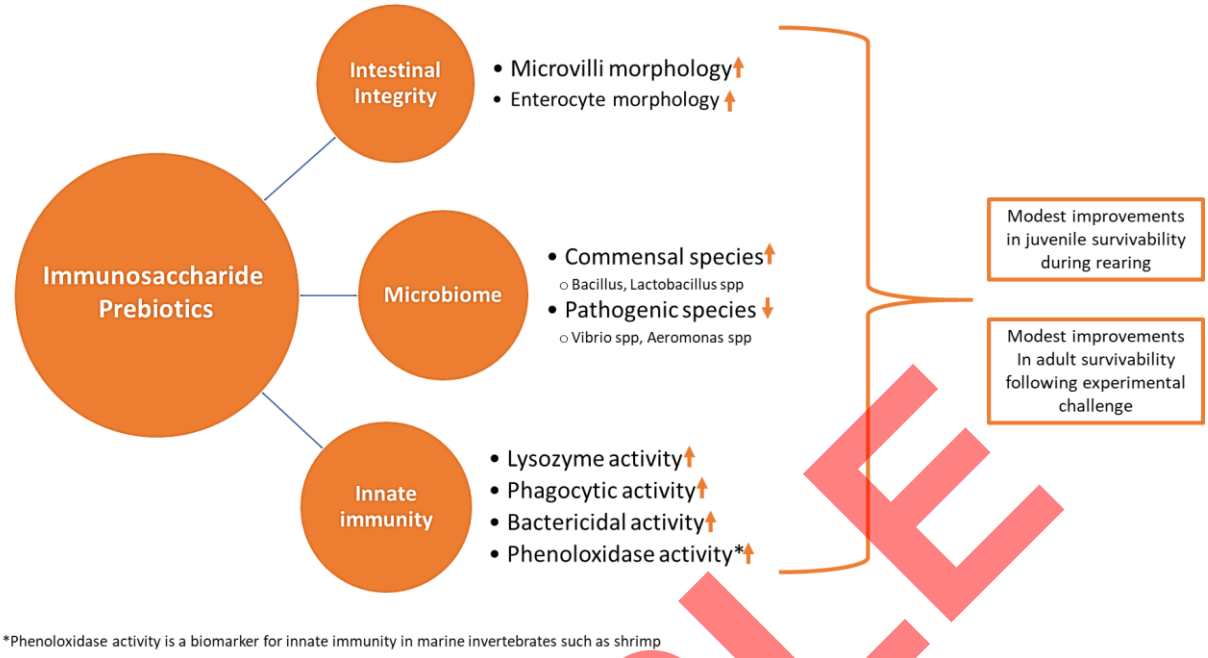
Figure 14: Host benefits from Immunosaccharide Prebiotics



The number of recent publications on prebiotics (last two years) is relatively low compared to those considering the microbiome as a whole and indeed compared to those relating to probiotics. What the literature reveals is a continued interest in three main areas;

- Expanding the existing data set to include more aquaculture species.
- Deeper analysis of the modes of action using improved molecular microbiology and immunology tool sets.
- The concept of Synbiotics; i.e. co-administered prebiotic and probiotic combinations.

This new data is in the main incremental and complimentary to the body of work which already exists. One area that offers potential for further improvements in efficacy are the synbiotic approaches where an optimized probiotic strain is co-administered with a complimentary prebiotic. However, this body of literature is very new. Larger, well controlled studies executed under field conditions will be necessary to build a broad church of commercial confidence.

Translation of research into commercial products

The following is an indicative list of some of the better described prebiotic products which are currently commercially available for intensively reared aquaculture species.

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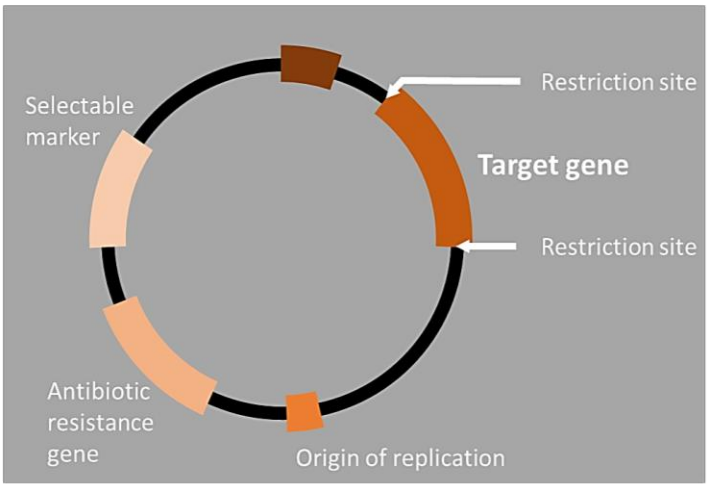
Figure 16: The Anatomy of a simple plasmid DNA vaccine



Key Components

- **Promotor** drives expression of the inserted gene. The most commonly used promotor is that derived from human Cytomegalovirus (CMV)
- **Restriction sites** facilitate cloning of the inserted gene
- **Origin of replication** enables the plasmid to be propagated in the production strain, usually *E. coli.*
- **Antibiotic resistance genes** were historically included to enable positive selection pressure to be maintained during production but would now be replaced by auxotrophic markers
- **Selectable markers** have historically included genes encoding enzymes like Beta Galactosidase to enable colour based selection of recombinant plasmids

The concept has its origins in the observations of Jon Wolff and Philip Felgner as described in (Wolff *et al*., 1990). These researchers were surprised to see expression of protein in mice following injection of naked DNA, which was included as a control group in their studies on cationic lipid mediated plasmid transfection. In the immediate aftermath of their discovery, and indeed for a number of years after, DNA vaccines were heralded as a cornerstone technology in the future of vaccination. The reasons for the excitement were clear:

- Recombinant proteins were expensive and complex to produce whereas plasmid DNA was relatively simple to propagate.
- The transfection process resembled viral infection and could reasonably be expected to stimulate the authentic signals that are so essential for effective training of the immune system
- The DNA was non-replicating, there was no vector or live virus involvement, so expectations around safety were very high.

So far, the early potential has not been fully realized in human health. Simple intramuscular injection of naked DNA has proven very inefficient, requiring prohibitively high doses, formulation with complex transfection agents and electroporation (painful and so far impractical for mass vaccination). A second wave of interest in DNA prime/protein boost regimes has since followed but there have been no game changing clinical success stories here either. Companies like Inovio https://www.inovio.com/ continue to push forward, not only in terms of improving the vaccines themselves, but also in terms of delivery devices, including the CELLECTRA® electroporation device which shows promise as an enabling technology for mass vaccination campaigns. However, Interest in naked DNA vaccines has somewhat waned in human health and we now live in an era where RNA is considered to be the "new DNA" in the minds of many human vaccine designers.

However, that's is not the end of the naked DNA story by any means. Remarkably, DNA vaccine development is a field where aquaculture leads rather than follows. DNA vaccination has delivered some outstanding clinical results in fish, resulting in two breakthrough commercial products so far, namely APEX IHN® for IHNV and Clynav™ for Salmon Pancreas Disease Virus (SPDV) also known as Salmonid Alphavirus (SAV), both marketed by

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per salmon significantly exceeds that invested in an individual broiler chicken or indeed tilapia or catfish. Herein lies the vaccine developer's dilemma; how best to provide safe and efficacious products for current and emerging diseases at the right price for a given market? No single technology can solve all the challenges.

The issue of emerging diseases presents a fascinating conundrum. Most Atlantic salmon are on-grown in open sea pens. This means that there is little means to control the water to which the fish are exposed, unlike in a broiler house, for example, where biosecurity and air handling can be used to minimize exposure to the external environment. If wild fish chose to congregate around the pens or migrate through waters in which those pens are located, those interactions may lead to unpredictable exposure to infectious agents. Furthermore, larger environmental changes generally attributable to global warming may have a profound impact on the potential for emerging diseases in aquaculture. The case of migration patterns of mackerel is a good example where in recent years, Atlantic populations have moved north west into Icelandic and Faroese territorial waters https://thefishsite.com/articles/northeast-atlantic-mackerel-stocks. This brings the potential for farmed salmon to be exposed to different water bodies and different wild fish populations with unpredictable consequences for emergence of new diseases.

The following table summarizes the main diseases impacting farmed Atlantic salmon and the corresponding technologies used to address them:

Table 15: **Vaccine technologies currently used in Atlantic salmon aquaculture**

Pathogen type	Pathogen Classification	Clinical Condition	Traditional vs. Molecular
Bacteria	Aeromonas salmonicida	Furunculosis	Traditional
	Vibrio spp. (particularly Salmonicida, anguillarum and ordalii)	Vibriosis	Traditional
	Renibacterium salmoninarum	Bacterial Kidney Disease	Traditional
	Moritella viscosa	Winter Ulcer	Traditional
	Tenacibaculum spp.	Tenacibaculosis	Not yet available (presumed traditional)
	Piscirikketsia salmonis	Salmonid Rikketsial Septicaemia (SRS)	Traditional and molecular
Viruses	Aquabirnavirus	Infectious Pancreatic Necrosis (IPN)	Traditional and molecular
	Novirhabdovirus	Infectious Haematopoetic necrosis (IHN)	Molecular
	Aqua Orthomyxovirus	Infectious salmon anaemia (ISA)	Traditional and molecular
	Salmonid Alphavirus (SAV)	Pancreas Disease (PD)	Traditional and molecular
	Aqua Totivirus	Cardiomyopathy Syndrome (CMS)	Not yet available (presumed molecular)
	Piscine Reovirus (PRV)	Heart & skeletal muscle inflammation HSMI)	Not yet available (presumed molecular)

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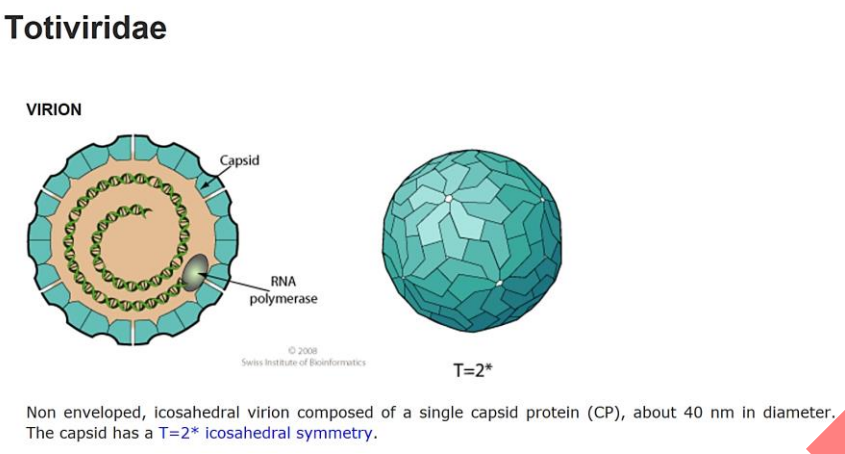
Figure 22: General virion structure described for Totiviridae



Source: https://viralzone.expasy.org/646?outline=all_by_species

Clinical diagnosis of CMS is increasing in significance year on year. 76 cases were recorded in 2007 and this had increased to 107 cases in 2014, with cases recorded in all of the Norwegian salmon farming areas. CMS is of particular economic concern because losses are typically seen in the second year at sea when fish are approaching harvest weight. Disease outbreaks may correlate with significant acute mortalities, but more chronic infections are also apparent, reviewed by (Garseth *et al.*, 2018). As described for HSMI, CMS significantly impacts cardiac function. This means that stressful husbandry interventions, particularly sea lice treatments, are associated with increased risk of mortality in fish manifesting significant clinical signs of CMS.

Vaccine development was initiated as soon as the causative agent of the disease was confirmed, with Pharmaq (Zoetis) at the forefront of efforts along with a consortium of Norwegian academics https://thefishsite.com/articles/cms-virus-identified-in-atlantic-salmon. However, as for HSMI, efforts have been constrained by the challenges of finding a permissive cell line that is suitable for commercial vaccine development. It is therefore highly likely that any effective vaccine for CMS will be a rationally designed product built using molecular biology tools and utilizing one of the platforms described earlier in this section of the report. There are very few published studies regarding progress toward a vaccine, but it is reasonable to assume Pharmaq will be at the forefront of those efforts as they also hold significant intellectual property rights relating to this virus, including VLP based approaches to vaccination: https://worldwide.espacenet.com/publicationDetails/biblio?CC=GB&NR=2452363&KC=&FT=E&locale=en_EP# and http://www.freepatentsonline.com/y2018/0215793.html respectively. Given the extent of the data provided in these patents, it is reasonable to assume Pharmaq may be able to design a commercially viable vaccine.

Sea Lice

No discussion of future challenges in fish vaccination would be complete without mentioning ectoparasites. By far the most significant of these are sea lice, parasitic copepods from the genera Lepeophtheirus and Caligus which are a scourge of the industry in both hemispheres. Sea lice are traditionally controlled with pharmaceuticals rather than vaccines and the subject of more detailed discussion in the upcoming Treatments chapter. From an immunological perspective, sea lice are formidable foes:

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appear on the market, e.g. (BiomX http://www.biomx.com/, Eligo https://eligo.bio/ and Locus https://www.locus-bio.com/).

In aquaculture too, as in other sectors, a low level of interest has consistently been shown but no significant uptake of the technology has taken place. The current status was reviewed by Richards in 2014 and again by Pal (2015). Richards listed a number of trial applications where phages were used in aquaculture (Table 17, below):

Table 17: **Studies involving phage therapy in fish and shellfish**

Pathogen	Name of illness	Fish/shellfish evaluated	Treatment effective	Reference
Aeromonas hydrophila Aeromonas salmonicida	Hemorrhagic septicaemia; tail and fin rot; red-fin disease Furnunculosis	Loach (Misgurnus anguillicaudatus) Brooktrout (Salvelinus fontinalis) Rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar)	Yes Yes Yes No	Wu *et al*. (1981) Jun *et al*. (2013) Imbeault *et al*. (2006) Verner-Jeffreys *et al*. (2007)
Edwardsiella tarda	Edwardsiellosis	Loach (Misgurnus anguillicaudatus)	Yes	Wu *et al*. (1981)
Flavobacterium columnare	Columnaris disease	Catfish (Clarias batrachus)	Yes Yes	Wu and Chao (1982) Prasad *et al*. (2011)
Flavobacterium psychrophilum	Rainbow trout fry and syndrome and in salmonids bacterial	Rainbow trout (O. mykiss) Rainbow trout and Atlantic salmon (Salmo salar)	Yes Yes	Madsen *et al*. (2013) Castillo *et al*. (2012)
Lactococcus spp.	Lactococcosis	Yellowtail (Seriola quinqueradiata)	Yes	Nakai *et al*. (1999)
Pseudomonas aeruginosa	Ulcerative lesions on skin	Freshwater catfish (Clarias gariepinus)	Yes	Khairnar *et al*. (2013)
Psudomonasplecoglossicida hemorrhagicascites	Bacterial disease	Ayu (Plecoglossusaltivelis)	Yes Yes	Park *et al.* (2000) Park and Nakai
Streptococcusiniae	Streptococcosis	Japanese flounder (Para licht hys olivia ceus)	Yes (2007)	Matsuoka *et al.*
Vibrioharveyi	Luminousvibriosis	Shrimp (Penaeus monodon)	Yes Yes	Vinod *et al*. (2006) Karunasa gar *et el*

Source: Richards 2014

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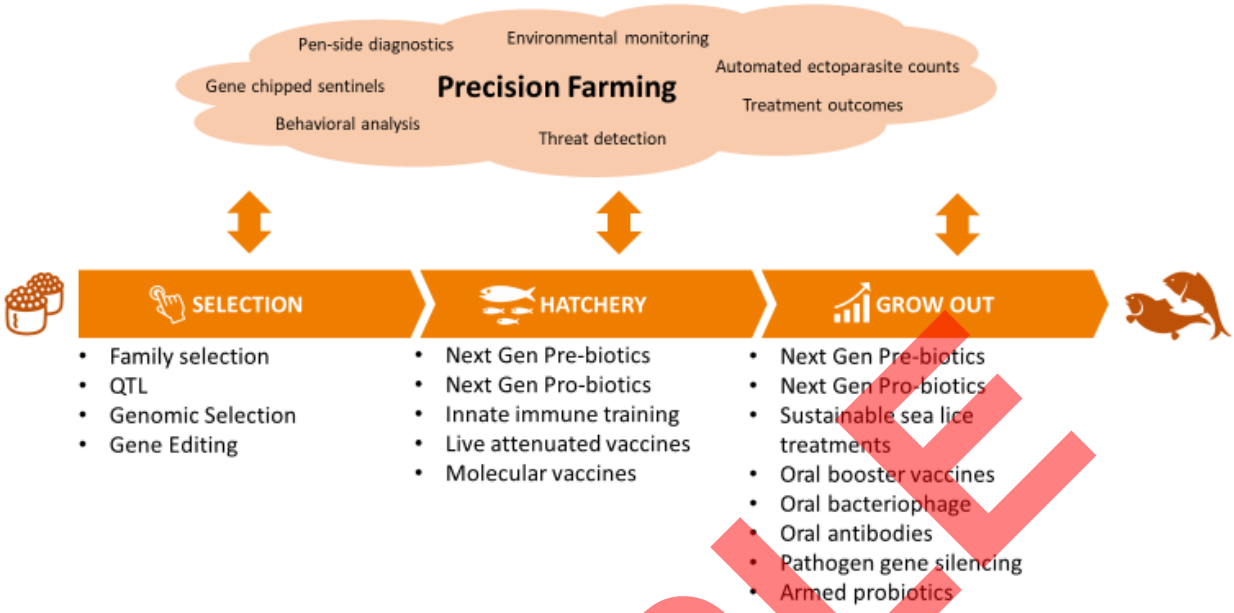
Figure 31: The future of healthcare in aquaculture; disease management in the era of precision farming



This figure describes a "near future" model for aquatic animal health which begins with a genetically advantaged animal being produced for the specific environment in which it will be raised. From the point of fertilization, this generation of animals will be programmed with a genetic advantage over their predecessors. They will be more resistant to infectious diseases, partially in some cases, almost completely in others. They will also be less susceptible to parasite infection, meaning less management intervention, less stress, fewer secondary infections, higher productivity and superior welfare. In the hatchery, the nutritional health of these genetically advantaged juveniles will be the subject of special attention to ensure the microbiome is optimized for gut health and immune system training. In the event bacterial diseases do occur and need to be addressed, a variety of alternatives to antibiotics will be available and these will be characterised by their precision, removing the bad actors without inducing dysbiosis. As the juveniles prepare to leave the hatchery environment and move into the grow-out phase where they will be exposed to the natural environment in raceways, ponds or cages, they will be further prepared with a new and improved generation of vaccines and long acting pharmaceuticals with superior safety profiles, cross reactivity and durational efficacy compared with current products. During grow out phase, the full benefits of precision farming will be seen. The reader is referred to (Føre *et al.*, 2018) for a contemporary discussion of the future of precision aquaculture. Direct sea lice counts will enable treatment regimens to be optimized and the stress of interventions will be reduced. Environmental sensors will give an early warning of any deteriorations in water quality so mitigating actions can be taken. Behavioural monitoring and metabolic sensing will provide early warnings of disease threats which will be swiftly interrogated with advanced molecular diagnostic tools. The next generation of fast acting interventions can then be deployed to stop disease outbreak in its tracks, for example in the form of orally delivered antibodies to neutralize a bacterial infection or gene silencing technologies to shut down viral replication. Prophylactic treatments will also continue in the form of improved oral booster vaccines to ensure the protection initiated by priming of juveniles can be consistently maximized for the entire production life of the animal.

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