Benchmark®
Genetics

Genetics and ova production of Atlantic salmon for **land-based farming**

Why land-based farming?

Up until recent years, the Atlantic salmon spent most of its lifespan in cages located in the oceans.

This type of production system is still the industry norm; however, during the last decade, the successful introduction of Recirculating Aquaculture Systems (RAS) technology has opened up new opportunities for how and where future production of salmon will be. The main drivers for the change apart of RAS-technology are the currently high salmon prices driven by demand, opportunities to produce close to the consumers and the depressed growth of volumes coming from traditional farming. The fact that producing close to the market is also having a positive effect on the carbon footprint, is another argument for investing in land-based RAS-systems around the world. Sustainability, Sea-lice, disease, limitations of growth by regulators, high production costs are fuelling the farmers and investors to look at projects on land.

Land-based production with RAS-technology has evolved into two different directions:

- Production of large smolt, from 200g up to 1kg on land, reducing the time for grow-out in the sea from 18 to less than 12 months;
- Full-cycle production on land, including the grow-out phase.

This brochure focusses on ova production and genetics adapted for full-cycle land-based production systems.

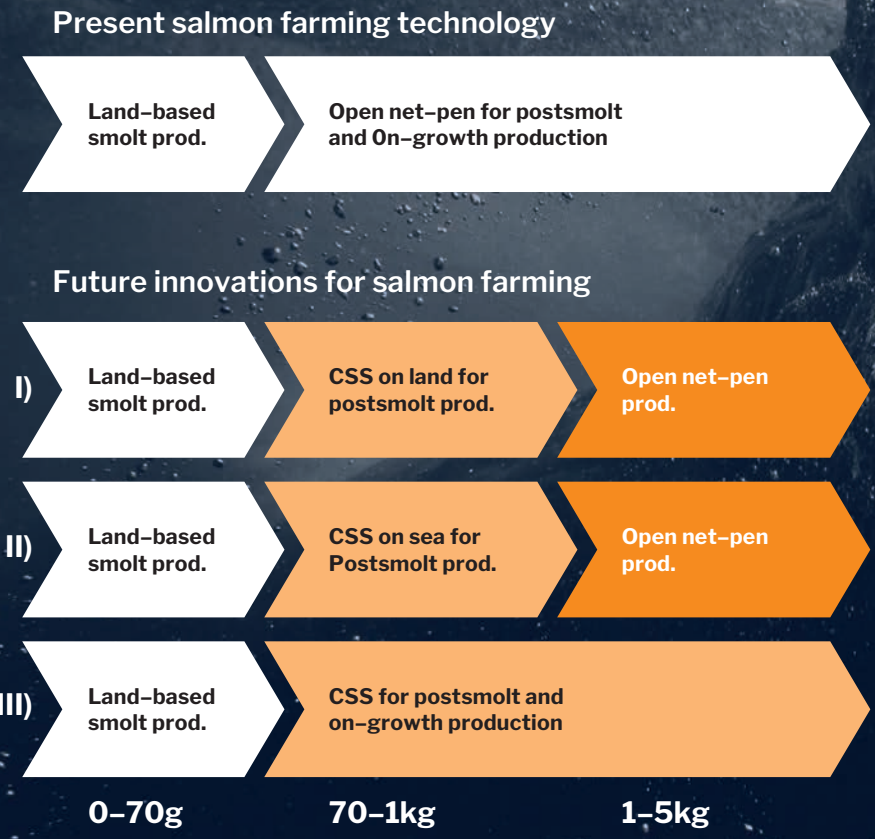


Fig. 1: Traditional systems v.s land-based systems (source: Nofima and CtrlAQUA)

Production cycle land-based RAS-systems

The impact of Benchmark Genetics

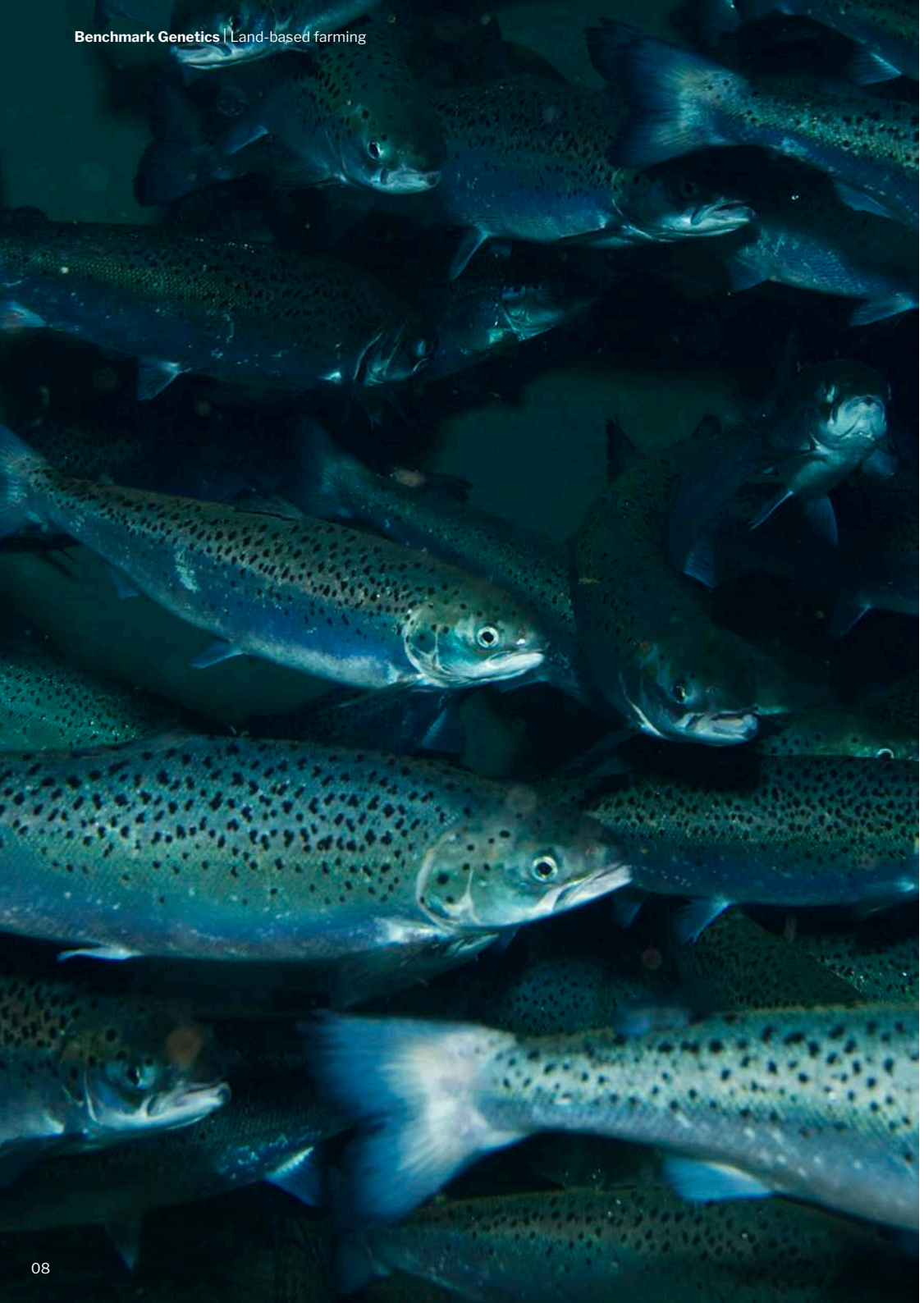
Broodstock	Hatchery	Nursery	Smolt	Post-smolt	Grow-out
Sustainable breeding strategies	Egg quality	Low mortality	Fast growth Robustness	Fast growth Robustness	Fast growth Robustness
Genetic improvement	Biosecurity	Technical support	QTL-IPN	QTL-IPN	QTL-IPN GS-Quality
Biosecurity	Low mortality				

Biology v.s technology

From a genetics perspective, large smolt production is quite similar to the traditional systems, although there are some significant biological concerns associated with early maturation for producers using high temperature (above 12°C) to promote faster growth.

Full-cycle production at large scale on land is, however, still in relatively early development with many new projects in the planning phase or under construction. Many of these projects are even based on full cycles using freshwater throughout the entire lifespan, including the grow-out phase. A key factor is to ensure that the genetic potential is keeping up with the developments of new technology and does not become a showstopper for success.

Benchmark Genetics has been following these developments closely for some time, engaging in R&D projects with academic partners such as The Conservation Fund Freshwater Institute (TCFFI) and The Marine Institute of Norway to understand the biological aspects of land-based farming from a genetics perspective. This brochure gives an introduction to this research and the products we have developed to meet the challenges and needs of the producers on land.



Production strategies for broodstock

Conventionally, broodstock has been produced in the sea and only transferred to land the very last period before spawning for temperature and light manipulation to induce maturation.

Such a production strategy is associated with risks for the producer as the broodstock spends one year more in the sea than the commercial salmon. Risks of losses caused by diseases, the introduction of pathogens, attachment of sea lice, and risks of escape are all associated with production in the sea. Benchmark is continuously seeking ways of producing more sustainably, taking care of both the value of genetics as well as fish welfare. For this reason, a large part of production of broodstock has been moved on land for a more extended period and even the entire life cycle. The so-called Semi-closed strategy means that the broodstock are transferred on land after just one year in the sea, reducing the biological risks significantly.

Such an approach also allows maturation and spawning time to be controlled by using advanced light and temperature regimes developed by the company, resulting in continuous production of ova, all year round. A third option is a Closed strategy, keeping the broodfish on land for the entire cycle — from egg to spawning. A significant part of Benchmark's global production follows this scheme. Just as for the Semi-closed strategy, the Closed approach allows introducing light and temperature regimes, giving complete control of the maturation of the fish. In addition to being able to deliver eggs all year round, this strategy also secures the highest levels of biosecurity, setting a whole new standard for the salmon industry.

Production strategies Benchmark Genetics

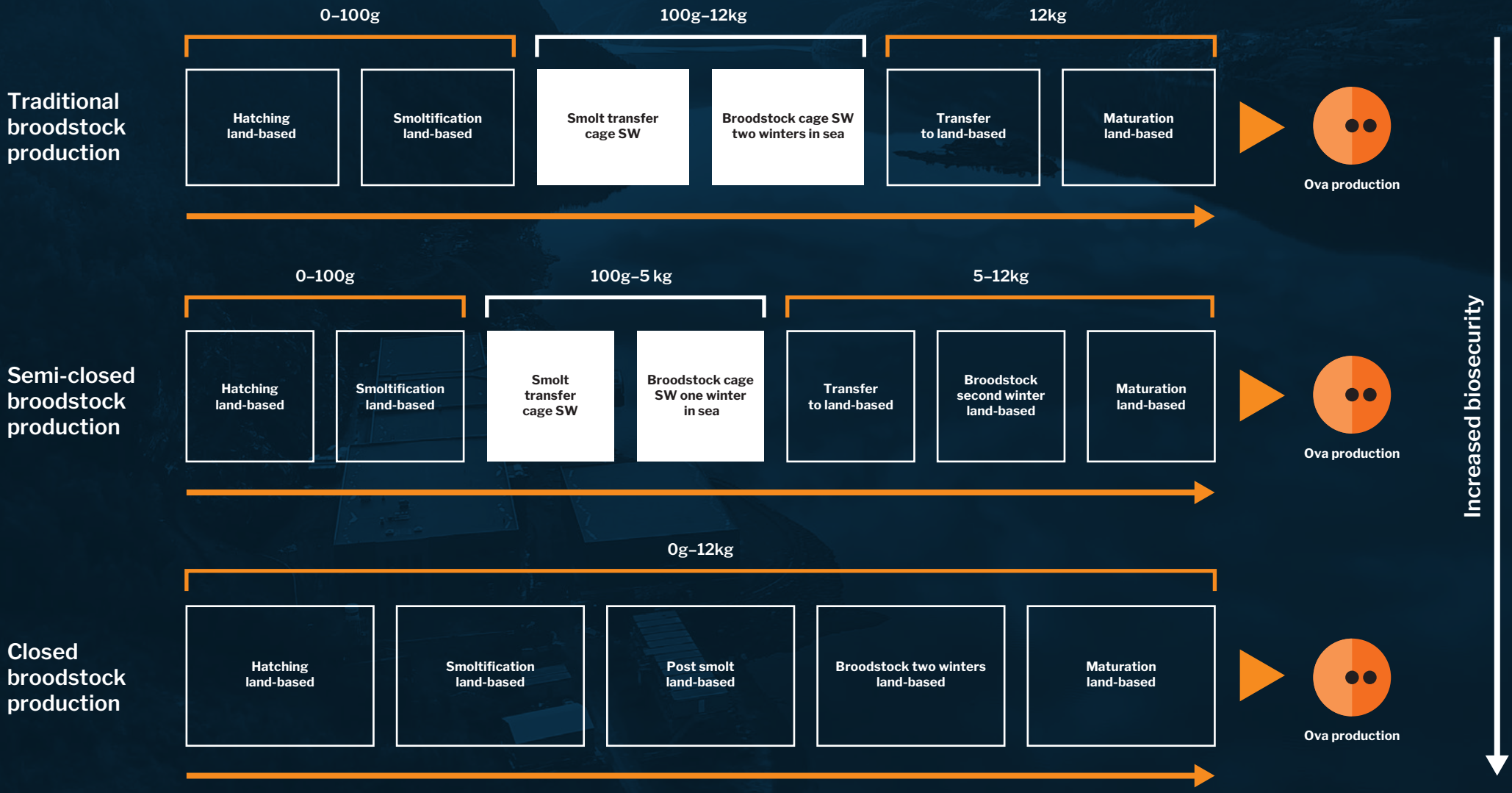


Fig. 2: Production strategies

Sharing the experience across the world

It is valid to say that Benchmark Genetics has the most extensive experience in the industry of land-based broodstock farming, starting in Iceland back in 1991.

At our two broodstock facilities, Vogarvik and Kalmanstjörn, clean and pathogen-free fresh and seawater are pumped from deep-drilled boreholes and naturally filtered through porous lava stone, providing the best starting point for farming broodstock. Experiments with light and temperature regimes in Benchmark Genetics has resulted in a set of standard operation procedures (SOP) for Closed broodstock production that is unique to the industry and is currently undergoing an international patenting.

Sharing the SOP's developed in Iceland across the entities in the division has put Benchmark Genetics at the forefront of broodstock production worldwide. The newly opened facility in Salten is combining the Semi-closed and Closed production strategies to optimize the capacity of the ova production. The same will be the case for our new operations in Ensenada and Curacalco in Chile, both to operate on Closed land-based production cycles.

A global team of experts in aquaculture genetics

1

Iceland

Line of business:
Atlantic salmon ova

Sites:

- 2 land-based broodstock sites, Vogarvik and Kalmanstjörn
- Freshwater family production
- Incubation center Vogarvik

Capacity: 200m ova/year

2 3 4

Norway

Line of business:
Atlantic salmon ova and technical genetics improvement services

Sites:

- Land-based broodstock and incubation site Benchmark Genetics Salten
- Freshwater family production Lønningdal
- 50% J.V Salmar Genetics
- Partnerships Bolaks, Salten Stamfisk, Lerøy and Salmar

Capacity: 300m ova/year incl. partners

Offices:

- Sunndalsøra and Ås

R&D and geneticists team:
18 employees

5

Chile

Line of business:
Atlantic salmon ova and technical genetics improvement services

Sites:

- Land-based broodstock and incubation site Ensenada
- Freshwater family production Curacalco

Capacity: 50m ova/year

Biosecurity

At land-based RAS facilities, it is extremely important to be in control of any biological material entering the site, both water and fish. In particular, it is crucial to avoid pathogens to establish reservoirs in the systems.

Getting rid of the pathogen will require extensive and costly operations of cleaning and disinfecting, and at worst case fish may have to be culled.

Benchmark Genetics' production sites have been designed to hold the highest standards of biosecurity, including closed hygienic compartments, extremely high levels of UV water treatment, bore-hole water (Iceland and Chile) and avoiding any contact with wild fish.

Besides, a comprehensive screening program is performed to eliminate broodfish carrying unwanted pathogens. We are proud to say that all of Benchmark Genetics' sites today are officially recognized as free of ISA (HPR deleted), IPN, PD/SAV, CMS/PCMV/Totivirus, IHN, VHS and BKD.

In 2015 Benchmark Genetics biosecurity system was taken to another level with compartmentalization according to the guidelines of the OIE (World Organization for Animal Health).

Our implemented compartment is officially recognized by the Icelandic Food and veterinarian authorities (MAST) and the Chilean Fisheries and Aquaculture Authorities, Sernapesca. As a result of this recognition, Benchmark Genetics is currently the only company permitted to export Atlantic salmon ova to Chile.

Although our broodstock in Iceland is free of the listed pathogens, some customers require additional screening for security reasons. We are offering individual screening for the following pathogens:

- ISAV
- SAV/PD
- IPNV
- PMCV/CMS
- PRV/HSMI
- VHSV
- IHNV
- OMV
- BKD
- Yersiniosis

Genetic traits for closed land-based production cycles

Advantages in producing on land are highly controlled production systems, improved growth, no sea-lice issues and high levels of biosecurity.

On the other hand, typical biological challenges associated with land-based farming are early maturation of males, the unreleased potential for growth and several problems related to operations (high densities, handling of large fish) and water quality parameters. Gill-health issues and specific diseases such as Yersiniosis are also commonly experienced in RAS systems.

Fewer traits — higher genetic gain

Farming salmon in open cages in the sea is far more biological challenging than farming on land. The genetics product offering has up to recently been tailored to improve resistance for many diseases occurring in the sea. As most of these diseases are not prevalent in closed land-based systems, the number of traits required for the genetics products are fewer. This means that we can tailor the genetic pool by putting higher pressure on the traits in focus and thereby obtaining significantly higher genetic gains per generation, to the benefit of land-based farming operations.

Type of trait	Demand from farming in sea-cages	Demand from closed land-based systems
Production efficiency traits	Freshwater growth	Freshwater growth
	Seawater growth	(Seawater growth)*
	Late maturation	(Late maturation)**
Disease resistance traits	IPN	IPN
	PD	
	ISA	
	CMS	
	SRS	
	Sea-lice	
	AGD	
Physical quality traits	Body shape	Body shape
	Slaughter Yield	Slaughter Yield
	Fat	Fat
	Pigmentation	Pigmentation

* Relevant for land-based system using seawater in the grow-out process
** Can be solved by All-Female/All-Female-Triploid products

Table 1: Selection pressure on traits — comparison between cage and land-based farming

1. Growth

Fast growth is the single most important trait for producers using land-based systems. Benchmark Genetics has since the very start had a substantial focus on improving the growth rate, both in freshwater and in the sea.

Since 1991, we have been using family selection and adding on Genomic Selection (GS) for seawater growth in 2017.

To gain more insights to growth in full-cycle land-based farming, Benchmark Genetics have since 2012 been running trials, both in-house and with The Conservation Fund Freshwater Institute (TCFFI). Results from these trails and how they are being implemented in the commercial production of ova are shown in the sections below.

1.1 Environmental factors and growth

The environmental factors have a significant impact on growth performance, and adjustments in water quality, temperature, light, nutrition and standard operation procedures (SOP) can give immense results, as we recently experienced in our broodstock facility, Kalmanstjörn in Iceland. Back in 2016, we made changes related to equipment, management training, SOP's and nutrition, resulting in a doubling of the growth rate, shortening the production time significantly and improving the fish welfare. Our broodstock can achieve smolt to 4kg in less than 12 months in our flow-through system.

As growth is strongly correlated with temperature, we are using Thermal Growth Coefficient (TGC) when measuring growth. It should also be noted that research and experience from commercial production have shown that farming at temperatures above 12°C induces early maturation, particularly in males. Losses due to maturation can reduce the total production efficiency even if the TGC is higher for the non-mature population. However, there are solutions for overcoming the maturation challenge that is explained in section 2. It is generally accepted that the optimum temperature for seawater growth in Atlantic Salmon is 11.9°C, and this is considered to be optimum for growth in RAS-systems until further analysis can be completed.

A trial at TCFFI was designed using light regimes inducing artificial winter, resulting in higher growth than the parallel groups undergoing 12:12 light:dark regimes and ambient photoperiods. By gaining more experience and knowledge of the impacts of environmental factors such as temperature and photoperiods, there is potential to improve the TGC significantly in the future. With the current production systems, protocols and genetics, producers in land-based systems will typically use between 24 and 26 months from the reception of ova to reaching harvest weight of about 4.5kg. A test group of All-female diploids and triploids at TCFFI was reported to have reached an average weight of 6.5kg in 27 months (source: TCFFI, 2019). Improved technology, production protocols and further adaptation of the genetics is likely to reduce the time from egg to harvest to 20 months.

1.2 Selection for growth based on systems

In Benchmark Genetics, the correlation between growth in different farming systems; RAS, Land-based flow-through and sea cages, by parallel comparison trials using a large number of families has been measured. Growth in RAS-systems is clearly below the growth in the other two technologies, indicating that there is potential for improving growth in RAS production by using breeding and genetics strategies. We also found that there is a relatively high genetic correlation, 0.7, between growth in land-based flow-through systems in Iceland and the RAS systems used by our customers, meaning that we have an excellent base for selection for growth in our Icelandic facilities. Trials also show that the families that perform best in RAS are also the same that grow fastest in flow-through systems.

Genetic Correlations of growth

Station	Vogarvik	RAS	Kalmanstjörn
Vogarvik			
RAS	0.67 ± 0.06		
Kalmanstjörn	0.85 ± 0.03	0.72 ± 0.07	
Test Harvest (Cage)	0.51 ± 0.07	0,30 ± 0.10	0.56 ± 0.07

Table 2: Correlations of growth between different production systems

- Good correlation between our two land-based flow-through systems (Vogarvik and Kalmanstjörn)
- Good correlation between land-based RAS-systems and land-based flow-through systems
- Significantly lower correlation between land-based flow-through systems and open cage systems
- Low correlation between land-based RAS and open cages

* Generic correlation indicate if the traits are dependent on each other.
+1 = positive correlation, -1 = negative correlation, 0 = independent traits

2. Early maturation

Early sexual maturation of males, also known as "precocious maturation", is a major concern when producing on land. When the fish goes into sexual maturation, it stops growing and converts muscle and fat into testes and ovaries.

The red pigmentation is extracted from the fillet to the gonads and the skin, resulting in salmon with pale fillet and brown skin which is not commercially acceptable. There are many theories behind why some fish are triggered to mature at an early stage. However, research and experience have shown that the maturation ratio tends to increase with water temperatures above 12°C. Sudden changes in temperature also seems to trigger the sexual maturation and should be avoided at all times. TCFFI estimates that an average of 15% of fish in RAS systems mature ahead of schedule. However, a rate of 20–30% is common, and some companies have even experienced levels as high as 50%, which is devastating for the profitability of their operations (source: TCFFI/Undercurrent News).

Benchmark Genetics has been selecting for late sexual maturation in the breeding programmes since the early 1970's; starting with phenotypic selection, moving into family selection in 1991 and further introducing Genomic Selection (GS) in 2017. In addition to GS, populations are monitored for the so-called "salmon puberty gene", published in Nature in 2015 (https://www.nature.com/articles/nature16062).

At Benchmark Genetics in Iceland, we have also been introducing specific treatments of broodstock and eggs, resulting in a significant reduction in sexual maturation; All-Female and triploid. These products are now gaining increased popularity with land-based customers, as they are experiencing improved production output and profitability by greatly reducing the rates of early maturation.

2.1 All-Female Ova

The All-Female method is a complex breeding strategy where female broodstock are sex-reversed in a process called masculinization. These males produce sperm cells that all carry X-chromosomes giving offspring populations that only consist of females, resulting in the "All-Female" product. As females have much lower incidence of early maturation, this significantly reduces the early maturity challenge that many land-based farms are experiencing with mixed-sex populations. The All-Female product has become very popular with full-cycle land-based customers, who experience a higher number of individuals fully grown to the ideal harvest weight and a more homogeneous weight distribution.

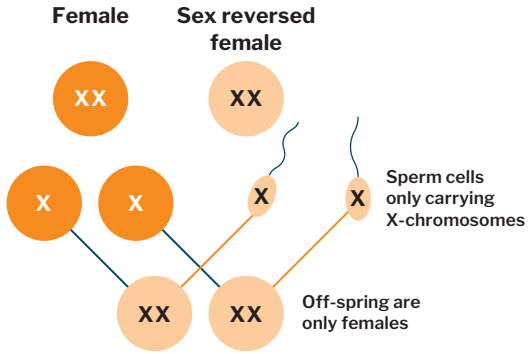


Fig. 3: All-Female process

2.2 All-Female and Triploid Ova

Some producers using combinations of high temperature and full-cycle freshwater are still experiencing 12–15% losses on All-female due to maturation. It is possible to eliminate the problem by letting the All-Female eggs go through a process called Triploidization. Triploid salmon carry three sets of chromosomes instead of two, are sterile and never mature. Triploids occur naturally at low levels in all plants and animals and are commonly used in crop production (e.g. tomatoes) and aquaculture (e.g. oysters).

In salmon, triploidy can be induced by exposing eggs to pressure shortly after fertilsation. Although the number of chromosomes is increased through this reproductive technique, no genetic modification is involved, and triploid crops and animals are not GMO. Triploid salmon are slightly more sensitive to temperature during incubation, and low oxygen levels during growth. Nutritional requirements are also slightly different.

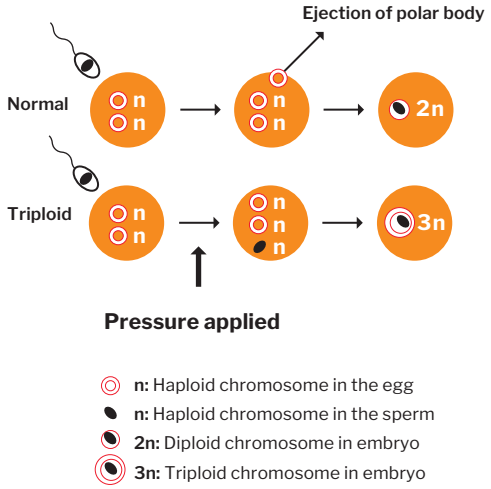


Fig. 4: All-Female Triploid process

3. Disease resistance

Modern genetic technologies are being applied to develop salmon that are more resistant to various diseases that are common in farming environments.

Using Quantitative Trait Loci (QTL) and Genomic Selection (GS) allows the use of genomic information to select the best performing breeders to improve resilience for specific pathogens and parasites in the offspring.

3.1 Infectious Pancreatic Necrosis

Farming on land means reduced risks of disease outbreaks. However, the disease Infectious Pancreatic Necrosis (IPN) is a severe and highly contagious viral disease of salmonids that usually affects the fish during early life stages and in the first phase in the grow-out stage.

The condition, caused by the infectious pancreatic necrosis virus (IPNV), may lead to significant levels of mortality. The virus is mainly spread through horizontal transmission (i.e. via infected water and from one individual to the next), although a vertical path of spread has also been suggested (i.e. from parents to the offspring). The outbreaks are usually sudden and can often cause a high number of mortalities and significant economic losses. Following infection, any animals that survive the outbreak can develop a lifelong illness.

Benchmark Genetics offers IPN robust fish using QTL selection, which started on the year-class 2007 and was initially commercialized in 2010. Resistance to IPN is highly heritable, and can, to a large degree, be explained by genetic variation in a single gene. Across salmon populations in Benchmark Genetics, approximately 80–90% of the animals that carry the resistant form of the gene resist exposure to IPNV when tested in controlled trials. The introduction of the IPN QTL has resulted in a significant reduction in numbers of IPN cases in Norway and other salmon producing countries.

3.2 Other disease and parasite challenges

In Benchmark Genetics, we are continuously following the developments of emerging diseases and evaluating the need to include more traits into our breeding programmes. As examples, Yersiniosis, Pox and skin blisters are health issues we are closely monitoring for product developments associated with RAS-systems.

Diseases such as Vibriosis and Furunculosis have been detected in land-based farming systems, and vaccination should be considered if the farming operation is located in an environment where these pathogens are prevalent in the water sources.

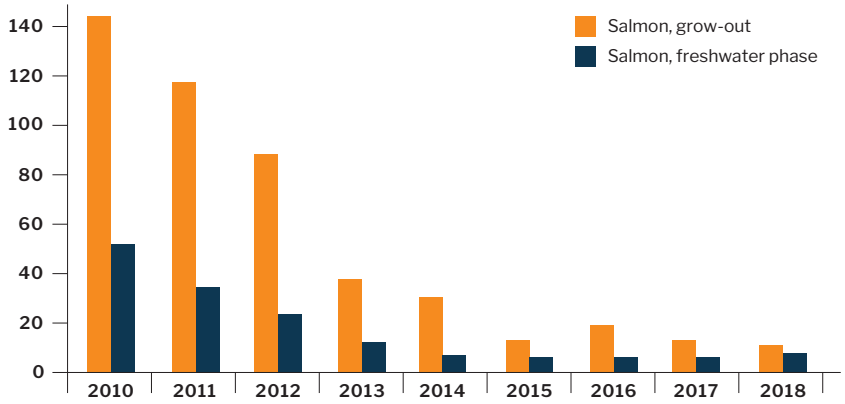


Fig. 5: Reduction in the number of IPN outbreaks in salmon production in Norway since 2010 (source: The Veterinarian Institute of Norway)

4. Quality

With fewer disease resistance traits needed, land-based farming allows an increased focus on improvements of the performance on harvest and quality traits such as pigmentation. This trait is characteristic of the end product and therefore have large effects on profitability of the production.

Physical quality traits, having been included in the breeding programme using phenotypic selection since the very start, are since 2017 also based on the latest genetic technology, Genomic Selection (GS). Pigmentation is controlled by a large number of genes, each contributing with a small effect. This is where the strength of the GS method lies, as it allows the use of information from thousands of genetic markers, covering the entire length of the salmon's genome. By genotyping these markers, the accuracy of the breeding values calculated for each animal is increased allowing the best animals for breeding to be precisely identified.

Using GS for quality traits allows the best broodstock for these traits to be used in the nucleus and production of commercial eggs for RAS-systems.

Selection of the best broodstock for RAS-production has been improved in two ways: increased selection pressure on the important traits for RAS-production by reducing the pressure on disease resistance and introduction of GS to increase the accuracy with which desirable broodstock are identified.

Product range for land-based farming

SalmoRAS® 4+ SalmoRAS® 4+ IPN

Product	Selection pressure — traits	Standard treatment	Optional additional treatment
SalmoRAS4+	Fast growth	All-Female	Triploid – 3N
SalmoRAS4+IPN	Fast growth and QTL-IPN	All-Female	Triploid – 3N

The tailor-made product range for full-cycle production in land-based farms offers the best combinations of characteristics to suit growth and fish welfare in recirculation systems (RAS). Combining different traits and treatments, we have aimed to make it easy to choose the product specifically suited for each customer.

The standard product is treated as All-Female, however Triploid can be chosen in addition to fully eliminate problems with early maturation.

Cryopreservation — choosing the best males at any time

Conventionally, males and females with the best characteristics had to sexually mature at the same time to produce the desired products. Advances in cryopreservation of Atlantic salmon milt now allows the sperm from desirable males to be stored for use when required.

In three cryolabs around the world (Norway, Iceland and Chile) we can freeze and store milt from the best-performing males to be used at any time of the year, according to order planning. Milt from the same male can be used for several orders to the same customer, resulting in high consistency of the delivered eggs.

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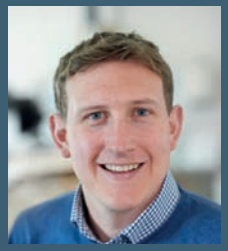

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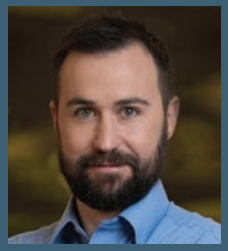

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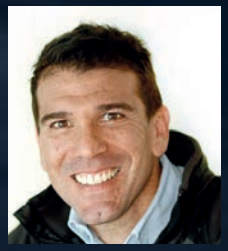

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Benchmark at a glance

Our mission is to enable food producers to improve their sustainability and profitability.

By providing solutions in genetics, advanced nutrition and health which improve yield, quality, resilience and animal welfare.

Genetics

Improved genetics provide a crucial starting point for production efficiencies and health resilience.

Advanced Nutrition

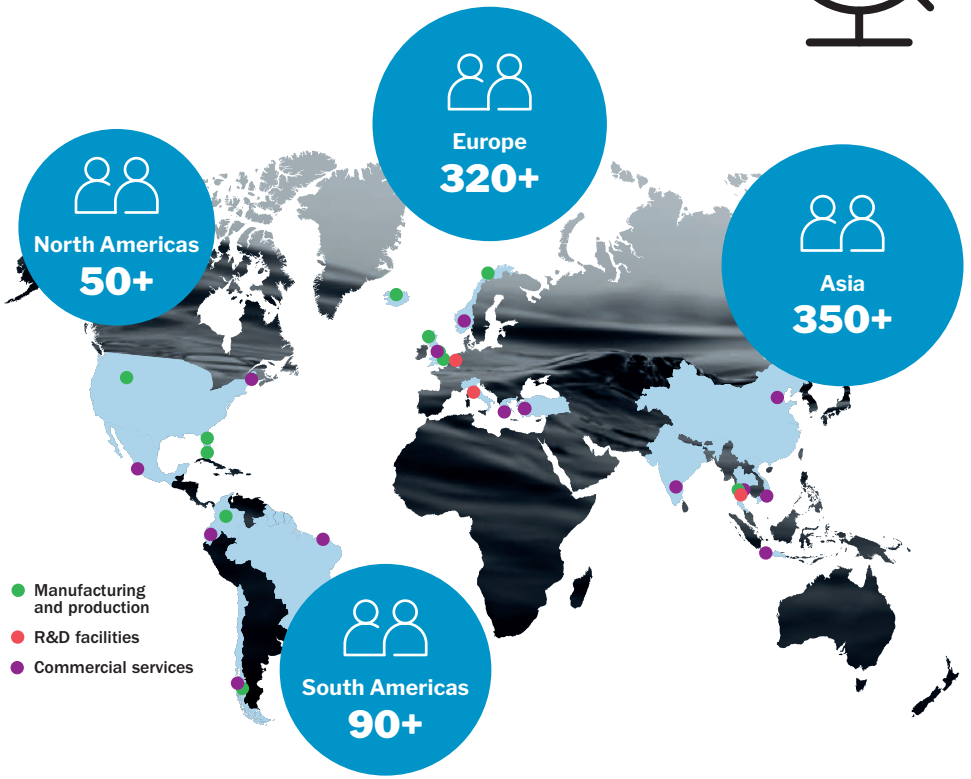
High performance nutritional solutions for shrimp and marine fin fish enhancing fish health and production efficiency.

Animal Health

Solutions for some of the most persistent disease and fish welfare challenges.

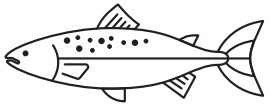
> The aquaculture industry is at an early stage with enormous potential for growth. Benchmark's solutions in genetics, health and advanced nutrition supports the needs for aquaculture producers to develop in a sustainable way.
>
> **Trond Williksen**
> CEO, Benchmark

Global presence



We are present in every major aquaculture market and species.

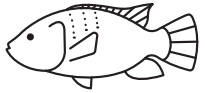
Salmon



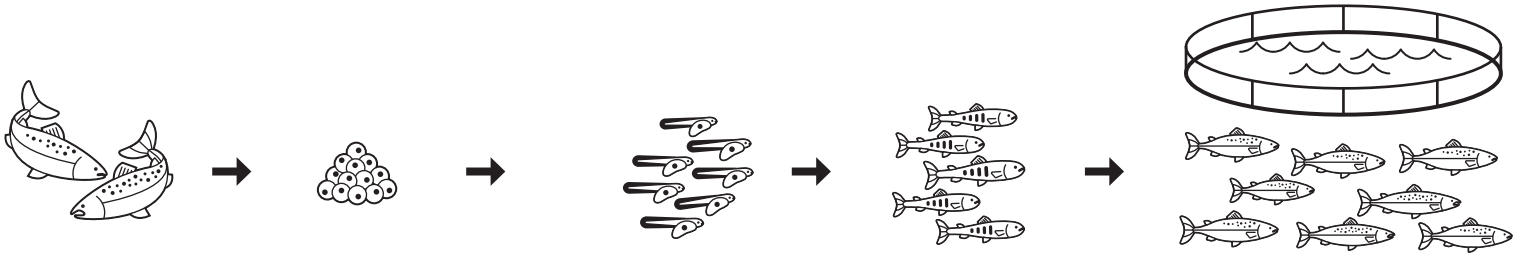
Sea bass/bream

Shrimp

Tilapia



Benchmark's offering



BUSINESS AREA	Broodstock	Hatchery	Nursery	Grow-out	OUTPUTS
Genetics Improved genetics provide a crucial starting point for production efficiencies and health resilience.	Eggs, breeding (parent stock) animals for salmon, shrimp and tilapia Genetic improvement services to a broad range of industry players across 12 species	Hatchery stage fish and shrimp			**Employees** Our growth and continued success is down to the hard work, talent and dedication of every member of our team. Our people strategy ensures that we offer rewarding careers where employees are motivated and inspired to make a difference.
Advanced Nutrition High performance nutritional solutions for shrimp and marine fin fish enhancing fish health and production efficiency.	Broodstock diets*	Probiotics Hatchery diets Enrichment diets			**Customers** Investment in our products and services has a high return relative to the substantial costs resulting from major disease challenges. Our offering drives consistency in supply and supports the long-term growth and sustainability of our customers' business — improving yield, quality and animal health and welfare.
Animal Health Solutions for some of the most persistent disease and fish welfare challenges.	Medicines			Sea lice treatment Purification system	**Shareholders** We are securing the technology at the heart of the 'blue revolution' — driving shareholder value as the industry grows. **Environment** We care for our planet by operating our business responsibly and by developing sustainable solutions that tackle some of the key environmental challenges in our industry. For example, Benchmark's CleanTreat® purification system eliminates the discharge of medicinal bath treatments into the ocean and the development of modern probiotics is reducing the need for antibiotics.

*For marine species and shrimp

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