



**Biotica  
Research  
Today**  
Vol 5:1  
2023

45  
47

## Selective Breeding for Disease Resistance in Aquaculture: Current Methods and Future Directions

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 Open Access

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### Keywords

Disease resistance, Genome editing, Genome selection, Selective breeding

### Article History

Received on: 04<sup>th</sup> January 2023

Revised on: 15<sup>th</sup> January 2023

Accepted on: 16<sup>th</sup> January 2023

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### How to cite this article?

Rakkannan and Priyadarshi, 2023. Selective Breeding for Disease Resistance in Aquaculture: Current Methods and Future Directions. *Biotica Research Today* 5(1):45-47.

### Abstract

Infectious disease is a major constraint for all aquaculture-produced species. The vast majority of farmed fish and shellfish production is based on stocks that have undergone little or no selective breeding. Because disease resistance is universally heritable, there is enormous potential for selecting for improved resistance to key diseases. This article discusses current methods followed for selective breeding in commercially important aquatic animals for disease-resistant species, including species successfully bred and some bottlenecks. Future directions are highlighted, including implementing cost-effective genomic selection for disease resistance trait, to improve genomic prediction in distantly related populations and the potential of genome editing tools for mapping causative variation underlying disease resistance traits.

### Introduction

The aquaculture sector is crucial for increasing fish production around the world and Global production of aquatic animals was estimated at 178 million tonnes in 2020 (FAO, 2022). Aquaculture farming produces nearly one-third of the fish consumed today. New scientific tools are being developed and used to improve aquaculture species production, and knowledge pertaining to biotechnology is playing a very important role and has a wide range of useful applications, particularly in the areas of increasing growth and yield of commercial aquaculture species, improving genetic traits with desired characteristics, strengthening the immune system, fish health and disease management, and boosting nutritional value. Selective breeding programmes have great potential to help address this challenge *via* cumulative improvements in key production traits, such as resistance to disease. Currently, less than 10% of aquaculture production derives from selectively bred stocks, lagging significantly behind the terrestrial animal and plant farming industries.

Genetic gains for aquatic species are generally higher than those for terrestrial farm animals, and that is encouraging. Infectious diseases are a major constraint on aquaculture production, causing high mortality and impaired growth. Disease prevention through management and biosecurity is particularly difficult in marine aquaculture species exposed to the open ocean. As a result, selective breeding to produce stock with improved resistance to key pathogens, utilizing naturally occurring genetic variation (heritability) for resistance in farmed aquaculture populations, is a major and increasingly important component of disease control. Almost all well-powered studies on the genetic basis of disease resistance in aquaculture species have found significant heritability for these traits. As a result, effective selective

breeding programmes, when combined with other prevention and control strategies, can provide cumulative and permanent improvements in host resistance. This article will highlight current methods used to combat disease resistance through selective breeding and will discuss future possibilities made possible by technological advances in genomics and genome-editing technologies.

## What is Disease Resistance?

The ability of the host to prevent infection or its effects by limiting pathogen reproduction is frequently referred to as disease resistance, while the converse is referred to as susceptibility (Bishop and Woolliams, 2014). However, several terms related to characteristics linked to broad-sense disease resistance have been defined, such as “infectivity,” which is the propensity of spreading infection upon contact with a susceptible person, and “tolerance,” which can refer to the ability of the host to reduce the impact of pathogens on performance. Disease resistance will be interpreted broadly for the purposes of this discussion to include all illness features where genetic improvement will result in a decrease in disease incidence or severity. For more than 20 years, aquaculture breeders have targeted the characteristic of disease resistance, and since 1993, the first salmon breeding programmes have centered on disease resistance. However, selective breeding for disease resistance is difficult, in part because accurate and informative disease resistance measures or correlates must be captured. From a practical breeding standpoint, highly pathogenic viral and bacterial infections affecting aquaculture species are frequently the simplest to combat when resistance is defined as the survival (and/or mortality) of individuals during an epidemic or a planned challenge. For a number of significant infectious diseases, it has been demonstrated that this disease binary feature has a moderate to high heritability. Disease-challenge testing can be used to test relatives of the selected candidates in a breeding scheme to prevent compromising biosecurity among broodstock, especially for advanced finfish breeding programmes like salmonids and tilapia. Pathogen or parasite load measured by cell culture or qPCR or biomarkers of the host immune responses are alternative measures of disease resistance (Houston, 2017).

## Current Methods of Breeding for Disease Resistance

The majority of advanced selective breeding schemes for aquaculture species use family selection as the state of the art. Family selection entails maintaining a breeding nucleus with candidate parental brood stock from a large number of genetically diverse families. To obtain family-level data on disease resistance, full siblings of these animals can be placed in field conditions or sent for experimental disease challenge testing. Tagging or genetic markers are used to

accurately track families and pedigrees. For several species and diseases, family selection for disease resistance has been extremely successful (Bishop and Woolliams, 2014). However, it has some drawbacks, such as the high cost of routine disease challenge data collection and the inability to capitalize on half of the genetic variation (the within-family component). Marker-assisted selection is one method for expanding on family selection and learning about the comparative disease resistance of candidates from a full-sibling family. The principle of marker-assisted selection is based on detecting quantitative trait loci (QTL) affecting the trait of interest and selecting animals based on whether they carry favourable alleles at the QTL. Aquaculture species are typically closely related to their wild ancestors, and the farm environment’s relatively new selection and disease pressures raise the possibility that major-effect loci segregate within populations. The case of infectious pancreatic necrosis resistance in Atlantic salmon is a successful example of QTL analyses applied to selective breeding, in which a major QTL explains the majority of the genetic variance for resistance. Other cases of QTL-affecting disease resistance include salmonid alphavirus, ISAV, and *Gyrodactylus salaris* in salmon, lymphocystis disease in Japanese flounder, bonamiosis in the European Flat Oyster. However, in animal breeding, marker-assisted selection based on a single QTL has not been consistently successful, partly because most economically important traits have a polygenic genetic architecture. While recent domestication of aquaculture species may result in an oligogenic design for disease resistance traits, it is also important to note that the effect of any given QTL may vary depending on the environment and genetic background of the population. For modern selective breeding schemes in aquaculture, genomic selection (GS) is the cutting-edge technology. Genome-wide markers are used in GS to calculate genomic breeding values without knowing the underlying QTL affecting the trait of interest. While the initial concept of GS was to detect and exploit population-wide linkage disequilibrium between genome-wide markers and QTL, the advantages of genomic selection also include an accurate estimate of the genetic relationship between any two individuals that could be provided by pedigree records alone, particularly within families. To date, the use of GS has resulted in higher prediction accuracy of breeding values than the use of pedigree information alone in all studies of aquaculture species. A platform for generating high-density SNP marker genotypes across animal populations is required for genomic selection, and SNP arrays have been developed for several aquaculture species, including Atlantic salmon, rainbow trout, common carp, and catfish. The cost of high-density genotyping in large numbers of individuals is a significant disadvantage of GS. Furthermore, while GS is effective when the training and test populations are closely related (*e.g.*, within a breeding program’s year group), the ability to predict breeding values in animals more distantly

related to the training population is rather limited (Houston, 2017).

## Future Strategies for Aquaculturists (Houston, 2017)

For new and emerging aquaculture species, the steps taken to enable selective breeding for disease resistance may change with technological advances. Reference genome sequences, SNP genotyping platforms, and other genomic tools, for example, can now be evident from the beginning. Once suitable selection goals have been established, this can inform the composition of the base population from which to begin a breeding scheme and can enable rapid progression to family or even marker-based selection techniques to ensure rapid gain and minimal inbreeding.

1. Improving response to selection in multiple environments will be a major goal for certain aquaculture species with more advanced breeding schemes (*e.g.*, based on a family selection with sib-testing). As a result, when optimizing a breeding programme for the species stock, which is typically disseminated to several countries and diverse farming systems, it is critical to quantify and incorporate GE interaction, and the high fecundity may facilitate trait recording in multiple environments. Resistance to sea lice is an obvious target trait in Atlantic salmon production (*L. salmonis* in Europe and *Caligus* spp. in Chile).

2. In aquaculture species with high fecundity and large full-sibling families, the marker density required for step changes in breeding value prediction improvement over pedigree methods is relatively low (*e.g.*, 5K genome-wide SNP in a typical salmon breeding programme), and even lower when using within-family selection. Advances in genotyping by sequencing, which has great potential for genomic selection in farmed animals, will drive down the cost of generating SNP datasets of this magnitude.

3. Imputation techniques are also expected to play an important role in improving the cost-effectiveness of genomic selection for disease resistance and other important traits. With ever-improving reference genome sequences and genetic maps, the opportunity now exists to genotype selected animals (*e.g.*, parents) at high density and others (*e.g.*, offspring) at very-low density, but impute to high density - a technique that is relatively commonplace in terrestrial livestock breeding and has shown promise in Atlantic salmon breeding.

4. The current implementation of GS is based on a combination of linkage disequilibrium between markers and causative variants and estimation of realized relationships between relatives. To improve genomic prediction in distantly related populations, causative variants and/or markers in linkage

disequilibrium with causative variants must be identified and used. This is likely to require very large reference population sizes, genotyped at high density, or fully sequenced for a typically polygenic trait. Besides that, functional annotation of reference genomes will be critical for prioritizing putative causative variants, which will be facilitated by the recent Functional Annotation of All Salmonid Genomics initiative.

5. Genome editing technology, which allows the deletion, change, or addition of base pairs at highly specific and targeted locations, is likely to be a key tool in the identification of causative variation underlying disease resistance in farmed animals. The major techniques include zinc-finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats, which can be used to induce new mutations in specific target loci. The most obvious initial applications of GE in disease resistance breeding are to increase the frequency of known resistance alleles at major effect loci and potentially fix them. Genome editing in cell line models may be an important intermediate step in targeting and validating putative causative genes for viral and bacterial disease resistance traits.

## Conclusion

Disease resistance is almost universally heritable and is a key goal of existing selective breeding schemes. Several success stories of mass selection, family selection, and marker-assisted selection are evident. Gathering appropriate phenotypes from disease challenges or field experiments is pertinent for making genetic progress. Genomic selection is the state-of-the-art for modern aquaculture breeding schemes and offers substantial improvements in selection accuracy over pedigree-based methods. Predicting disease resistance of distantly related animals to those with trait records is a major future challenge, which is directly related to the identification of causative variants. Genome editing technology is likely to play a key role in identifying causative variation and has the potential for breeding disease-resistant animals in aquaculture.

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