# Public consultation on Environmental impact assessment of genetically modified sterile VIRGIN® Atlantic salmon for use in research trials in aquaculture sea-cages

We are happy to contribute to the public consultation on GM sterile virgin salmon and we are positive to field trials to gather necessary data on important parameters for fish welfare and risk for negative impacts according to the Gene Technology Act. This is the first application for field trials of a GM fish, let alone a genome edited fish, and thus has a great public interest. It is therefore important that the data collected are comprehensive and relevant with a high transparency and low threshold for discussing implications among stakeholders, scientists, regulators/legislators, and the wider public.

## Impact on/risk to health and environment

This application is put forward by the Institute of Marine Research for trial release of genetically modified Atlantic salmon and has been thoroughly assessed by the Norwegian Scientific Committee (VKM), which identified several shortcomings, which we agree with. Most notably, VKM found that the data from the applicant does not fully support the assumption that all genotypes of the experimental fish with double allelic mutations, leads to sterility. VKM evaluated the risk of spreading the sterility gene to the wild population, as high, due to the high consequences for the wild salmon population. This is a field trial release that concerns 303 F1 VIRGIN fish and it is technically possible to map the genetic status of each fish, the mosaic nature of the edit raises serious question on how this can be managed in an upscaled trial or as a commercial production system. Single allelic spread due to escapees, could be disastrous and the applicant's argument for a stable integration and maintenance of the deletion would imply persistent changes, which makes this a serious threat to wild salmon.

In addition to the risk of genetic exchange to wild salmon population, the behavioural patterns of the VIRGIN fish are uncertain at this point. A recent published study in zebrafish shows that *dnd1* knock outs becomes male and performs normal courtship behaviour with wild type female fish (Chu et al., 2023). We are not aware of any such studies in salmon, but if similar behaviour is observed with VIRGIN fish, it can affect wild salmon population by causing wasteful spawning by wild female salmon.

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## Fish Welfare

The collection of fish welfare date could be broader than the applicant suggests. This is the first trial release of GM salmon in Europe so welfare parameters should be chosen as broad as possible as the results from the trial are of major public interest. We feel that a comprehensive list of welfare indicators, for instance as suggested by Noble et al. (2019), would significantly improve transparency in the development of genome edited fish and ultimately build public trust and acceptance of GM fish in aquaculture.

We agree with VKM in the assessment that many unresolved questions about fish welfare and gene expression can be answered in land-based trials. We observe that for some important parameters that are already reported on, the sample size is too small to conclude, and this can be improved by further studies before field release. For instance, increased vertebra deformities in female VIRGIN fish during specific life stages was reported by Kleppe et al., 2022. Even though vertebra strength and composition were claimed to be similar between VIRGIN fish and wild type fish, the reported sample size was too small to conclude (6 or 5 in each group). Applicants have reported that in VIRGIN fish there is a minuscule change in gene expression in the pituitary. Interestingly, some of the altered genes (e.g. Chac1, https://www.ncbi.nlm.nih.gov/gene/79094#gene-expression) are linked to oxidative damage and poor organ health (tumour development) in humans (Li et al., 2021). Indeed, recent literature has linked *dnd1* with several somatic cancers (Zhang et al., 2021). To what extent this is also the case for fish and salmon, is unknown.

Since VIRGIN fish do not have to invest energy for maturation and gonad development, questions remain on the infection probability and chance of VIRGIN fish becoming a carrier of disease (without symptom) in relation to current farmed fish. If VIRGIN fish become reservoir of infectious disease, it will have severe interactions compared to regular farmed fish and the cage environment and upon escape.

The expression of *dnd1* gene controls germ cell development in a variety of species, including fish, but the literature is not clear on if the expression of this gene in salmon is confined only to the germ cells. *dnd1* appears to be expressed in lower levels in a variety of tissue types in several organisms (Kashem et al., 2018) and there is also an indication of expression in heart and brain of adult zebrafish (Wong et al., 2013). A sequencing effort reported in zebrafish also found that *dnd1* is expressed in blastodisc, brain and heart in addition to germ cells *(https://www.ncbi.nlm.nih.gov/gene/373074)*. The concern is that the VIRGIN fish with *dnd1* knockouts, have changes induced in other tissues where *dnd1* is expressed. These alterations could have an impact on long term fish welfare.

## Ethical considerations, sustainability, and benefit to society.

We disagree with the applicant claims that ethical considerations, sustainability, and benefit to society are not relevant considering the application is concerning an experimental release. The Norwegian Gene Technology Act relates to the production and use of genetically modified organisms (GMOs) and the purpose is to ensure that the production and use of GMOs take place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development and without adverse effects on health and the environment. This also relates to field trials. As evident both by the applications suggested marked interest, suggested potential benefits in commercial production and the description of the RRI activities/studies which will be implemented (open meetings, interviews, and online surveys), the field release must be assessed with this end goal, also discussing potential negative effects of release. In line with this, we feel that the list of stakeholders warrants expansion. Considering the potential of escape and the following non-/recombinational and genetic interference (Bradbury et al. 2020) of VIRIGN salmon in wild salmon stocks, other relevant parties could include sea fishers, local population, and salmon farming companies (Blix and Myhr 2023) in addition to salmon egg producers and the retail customers. The outcomes would be a more detailed descriptions of the impact on each stakeholder with regards to ethical norms such as trust, autonomy, respecting integrity, justice, etc (see Antonsen et al. 2021, Forsberg et al. 2019, Kjeldaas et al. 2023) and could greatly improve public trust and acceptance. The applicant states that this assessment is difficult to perform before the public consultation is finished and refers to the NOU-report “Genteknologi i en bærekraftig fremtid”. It is worth mentioning that this report is not unanimous in its conclusions, nor is it yet published in its final format as it has not been on a public consultation and thus not yet processed by the Department of Climate and Environment.

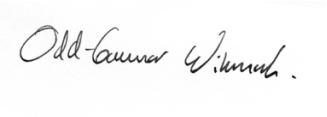
We think it would be beneficial to demonstrate the marked interest in VIRGIN salmon before committing to a field trial. The salmon farming industry’s willingness to pay for and apply the suggested solution of genome edited sterile salmon is unclear and most likely tied to public acceptance.

The assessment of sustainability is mainly leaning on and referring to the impact assessment. Even though some of the questions in the sustainability assessment is not relevant, which is expected, those that are – especially global effects and ecological boundaries, needs to be elaborated. For instance, it is unclear what a low value of the ecosystem represents? If this represents low biodiversity, it is not in line with the Kunming-Montreal Global Biodiversity Framework targeting conservation of 30% of the marine and coastal areas by 2030 (CBD 2022).

## Conclusions

In conclusion, we support VKM in the conclusions of its risk assessment and recommends that the field trials are not conducted with the current experimental design. To minimize risk of escaped fish, data should be collected with land-based trials where possible. Due to the high public interest, we believe that it is important to expand the data for fish welfare and perform a thorough assessment of the ethical and sustainability considerations and to open up a broad discussion on benefits to society with a transparent discussion, with broad stakeholder and public involvement.

Regards,



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