

# ABSTRACTS

## SESSION 12: ETHICS, FOOD SAFETY AND ENVIRONMENTAL RISK

# GENERATION OF GENE-EDITED BLUEGILL *LEPOMIS MACROCHIRUS* FOR CONTROL AND ERADICATION OF INVASIVE SPECIES BY GENE INDUCED SUPPRESSION FOR ALIEN POPULATION (GISAP)

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## Abstract

As a new method of controlling invasive alien species, we are working on the development of a gene suppression method that releases males with sterilization genes whose functions are deficient in female-specific reproduction-related genes. We have developed whole-genome information necessary to produce males with the sterilization genes, and have developed fundamental technologies for the basic breeding of bluegill and microinjection required for gene editing in bluegill. Next, we aimed to create gene-edited F<sub>0</sub> individuals as the founders of demonstration fish to acquire biological characteristic data of males carrying the sterilization genes. To inhibit the functions of *fshr* and *foxl2*, which are expressed in the female gonads and are important for sexual differentiation and maintenance of ovarian function, gene-edited F<sub>0</sub> individuals were produced using the CRISPR/Cas system. Approximately 500 F<sub>0</sub> individuals were fused for *fshr*-independent editing, and primary selection was performed with HMA using fish-derived DNA; seven of these individuals were judged to be positive for editing. HMA and DNA sequence analysis of six individuals that had grown to adulthood and survived again revealed that a male and a female were positive for editing, while the remaining four were negative. In contrast, in the group in which co-editing of *fshr* and *foxl2* was attempted, five individuals with positivity for *foxl2* alone, and three with positivity for both *fshr* and *foxl2*, were obtained. These F<sub>0</sub> edited individuals are currently being developed as parent fish for the creation of F<sub>1</sub> individuals.

This research was supported by the Environment Research and Technology Development Fund (4-1408 and 4-1703) of the Environmental Restoration and Conservation Agency.

**Keywords:** *invasive alien species, eradication, GISAP, bluegill*

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## THE UNACKNOWLEDGED UNCERTAINTY OF BIOPATENTING; A CASE STUDY OF THE AQUABOUNTY PATENT IN THE EUROPEAN PATENT SYSTEM

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### Abstract

The post normal science (PNS) approach first launched by Funtowicz & Ravetz (1995) addresses current challenges in scientific advice; where science is practiced as the equivalent to Kuhnian normal science (NS) in situations with high uncertainties and high stakes. Numerous biotechnologies, including transgenic animals involve the manipulation of genetic material that embody irreducible uncertainty and complexity with regard to the impact on the organism and its environment (Funtowicz & Strand, 2007). In this paper we apply PNS approach outside its usual domains, but still within the domain of science and technology policy through application of scientific knowledge: in the patent system. In this paper we show from a case study on the patent on a transgenic salmon how patenting practices and procedures related specifically to biotechnology often evade fundamental scientific, ethical and societal uncertainties. The scientific uncertainty and the conflicting value positions in the European public in relation to biopatents are not necessarily considered in the decision-making process in the current patent practices of the European Patent Council. In particular, where science to an increasing degree embrace complexity and uncertainty, patent law seems to continuously evolve towards reducing ambiguity and uncertainty (Calvert and Joly, 2011). Our case of AquaBounty patent in the European patent system provides an example where the assessment of competent ethical expertise was deemed mostly irrelevant, illustrating the consistently narrow interpretation of the scope of ethically relevant considerations from the EPO Boards of Appeal in recent years. Our case study also illustrates a situation where decisions of vital ethical and societal significance are left to a techno-legal institution where alternative expertise or the public has little or no influence.

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*Keywords: ethics, patents, GMO, salmon*

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# FITNESS EFFECTS OF INTROGRESSION OF ESCAPED FARMED SALMON INTO WILD POPULATIONS

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## Abstract

Every year, several thousands of farmed salmon escape from Norwegian aquaculture farms. Despite low survival and spawning success, some escapees enter Norwegian rivers and interbreed with wild salmon. This introgression affects functional traits and reduce fitness of the wild populations. Stochastic simulations was used to quantify fitness consequences of introgression of farmed salmon into wild populations. Fitness was recorded before and during introgression and also after introgression stopped and the population was recovering to its natural state. The software Nemo, which simulates individuals with individual genomes, was used for the simulations. Fitness was modeled as a Gaussian function of one quantitative trait, which was controlled by 80 independent genes with Mendelian inheritance. A simple life cycle, consisting of mating and offspring generation, viability selection, straying and reduction to patch capacity was simulated for 2000 generations to obtain mutation-drift-selection balance. The wild population consisted of five subpopulations with 100-800 parents per generation in each subpopulation, 5 % straying between the subpopulations and slightly different environments between the subpopulations. Strength of natural selection was set to give realistic decline in survival for domesticated fish. Founders of the farmed population was sampled from all of the 5 subpopulations over two generations and domestication mimicked by running natural selection towards an extreme optimum. After 10 generations of domestication in the farmed population, farmed fish was migrated into the wild sub-populations to mimic escapees.

When introgression was kept constant for 85 generations, fitness declined gradually for 10-15 generations before stabilizing at a level of 95 %, 80 % or 67-75 % of the original fitness, with 1, 5, or 10 % introgression, respectively. If introgression was stopped after 10 generations, 70 % of the damage was removed after one generation of natural selection and 90 % after two generations. However, 20 generations was needed before all subpopulations had recovered to their original fitness level. The results show that systematic introgression over time can reduce fitness, but the effect is reversible and the populations will recover naturally when the escapes stop.

*Keywords: stochastic simulation; introgression; salmon; fitness*

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## A NATIONAL TRACEABILITY SYSTEM FOR ESCAPED FARMED SALMON.

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### Abstract

The number of escaped salmon from Norwegian fish farms has decreased significantly since 2006 as a result of targeted preventive work against escapes. The NYTEK regulation (2011) has improved the technical standard of aquaculture structures, and the organisation OURO (2015) coordinates removal of escaped salmon from rivers. OURO is funded by an annual fee per licence for fish farmers without traceable salmon. Only roughly 10% of all Norwegian farmed salmon can currently be traced back to the owner. In 2014, AquaGen launched the TRACK product; using the farmed salmon's own DNA to trace it back to its owner. A unique combination of parent fish is used to make an egg batch, which can only be sold to one hatchery. Fifty thousand genetic markers (SNPs) are analyzed per parent fish. The DNA results are stored in a database, together with the delivery number. A DNA sample from suspected escapees are analyzed for the same SNPs, the results are checked against the database, and potential escapees can be traced back to their parents and the hatchery. Logistic systems are used to narrow down the number of fish farms escapees may come from. Several blind tests have validated the TRACK concept. In one test, DNA-samples from 407 salmon of unknown origin were genotyped. Although the researchers performing the parental assignment did not know that 187 of the fish had TRACK and 220 did not, all TRACK fish were correctly assigned to their parents and no non-TRACK fish were falsely assigned. Results from all validation tests show that >99.9% of potential salmon escapees are traced with TRACK. No physical tagging of the fish is required in the TRACK system. An evaluation of all methods used for tagging and tracing salmon (Tevasvold et al. 2017) recommended that a tracing system be based on a combination of DNA analysis (high density SNP-chip) and rare element analysis of salmon scales. The concept is currently being developed as a national tracing system in Norway, in an initiative from The Norwegian Seafood Federation. The system can potentially also be used to quantify genetic interactions between farmed and wild salmon.

*Keywords: salmon, DNA, traceability, escapes.*

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