April 7, 2014

MEMORANDUM

TO: Tom Weseloh, Joint Committee on Fisheries and Aquaculture

FROM: Brie Lindsey

SUBJECT: Genetically Modified Salmon and Potential to Affect Environment in the Case of an Escape

Growth-Enhanced Genetically Modified Salmon and Effects on the Environment: An Overview

Summary

The U.S. Food and Drug Administration is reviewing an application for genetically modified salmon intended for consumption, called AquAdvantage Salmon (AAS). AAS is an Atlantic salmon, *Salmo salar*, that has been genetically modified to allow it to grow faster than standard Atlantic salmon, achieving market size in half the time of conventionally farmed salmon. Production and grow-out of AAS is proposed to occur in facilities with several containment measures; while the risk of escape is greatly reduced by these safeguards, it is not absent. Though escape is unlikely, there is concern that escaped AAS could impact natural environments and wild salmon through gene flow, competition for food and habitat, or disease. Laboratory experiments and observations have shown a range of consequences of growth-enhancement transgenes in salmon, from increased growth rates, aggression, and risky behaviors, to hunger and
increased metabolism, lower reproductive success, and decreased disease resistance, all of which may affect the spread of transgenes and the impact on wild populations. These traits arise from interactions between genotype and environment, and may vary by salmon species. Because experiments and observations on transgenic salmon must be carried out in contained conditions that have been shown to decrease salmon hardiness, it is difficult to assess how prominent these traits would be in an environment to which transgenic salmon would escape. Therefore, while there is laboratory-based evidence that transgenic salmon escapees can have negative effects on wild salmon populations, the question about the extent of these effects in the natural environment remains open.

**AquAdvantage Salmon: A Transgenic Food Proposal**

The U.S. Food and Drug Administration (FDA) is currently reviewing an application for the first genetically modified animal, AquaBounty Technologies’ AquAdvantage Salmon, for consumption. AquAdvantage Salmon (AAS) is an Atlantic salmon, *Salmo salar*, that has been genetically engineered to be faster-growing through the insertion of a growth hormone-regulating gene from a Pacific chinook salmon and a promoter gene from an ocean pout (often referred to as the “antifreeze” gene). Together, these genes allow the AAS to reach market size more quickly, by growing year-round (instead of only during warm seasons) and about twice as fast as traditionally bred and farmed salmon. Ultimately, the AAS is the same size at maturity as a standard Atlantic salmon, but reaches this size within 16–18 months, rather than three years.

According to the “Environmental Assessment for AquAdvantage Salmon”\(^1\) submitted to the U.S. FDA as part of the evaluation process, AAS is intended for the land-based culture of the salmon for commercial sale and human consumption. FDA approval for AAS would be for a specific set of conditions, including the location and containment conditions: eyed-eggs (eggs developed to the point that eyes are visible) would be produced in Canada on Prince Edward Island, shipped to Panama, grown out in land-based facilities in Panama, and shipped to the United States for retail sale. Multiple,

\(^1\) “Environmental Assessment for AquAdvantage Salmon,” submitted to the Center for Veterinary Medicine, U.S. Food and Drug Administration, for public display, August 25, 2010.

\(^2\) “AquAdvantage Salmon Draft Environmental Assessment,” prepared for the Center for Veterinary Medicine, U.S. Food and Drug Administration, Department of Health and Human Services, May 4, 2012.
simultaneous, and redundant containment measures would be in place to minimize risk of escape and environmental harm; various forms of physical, geographic, and biological containment would be employed.

For the eyed-eggs production site, physical containment includes a main building with barred windows on the ground floor, dead-bolted steel doors, and cipher-locked interior access to the aquaculture facility; a chain-link fence around the building and surrounding facilities; a motion-activated security camera system to monitor the exterior of the building; and remote status notification, in case of a change in operational conditions. The tanks in the aquaculture area have other physical containment mechanisms, consisting of various mesh netting and screens, as well as chemically lethal environments in spawning drain areas. (A thorough description can be found in the "Environmental Assessment for AquAdvantage Salmon." Biological containment of the salmon would also occur at the egg-production site, as AquaBounty would produce all female eggs. These eggs would then be pressure-treated to induce triploidy (three chromosomes per cell, instead of the normal diploid cell, containing two), which leads to sterility. At the production facility, at least 95 percent triploidy is required to accept a batch of eggs. Geographical confinement also will be practiced at the production site: AquaBounty plans to spawn fish only in November and December, when AquaBounty argues environmental conditions in the vicinity of the facility—very cold, saline water—wouldn’t be conducive to the survival of early life stages.

These all-female, triploid eyed-eggs would then be transported to the grow-out facility in Panama. There, they would be cultured in freshwater tanks (six indoors for fry and four outdoors for older salmon grow-out). Physical containment at this facility includes rigid cages of netting surrounding outdoor tanks, a series of filters and screens for drains, a remote site location, gated bridge entry onto the site, an exterior fence with barbed wire, on-site manager residence, and guard dogs. If an AAS does escape into the river flowing downhill from the facility—where a population of rainbow trout (a salmonid) is known to be successful—the fish is expected to be limited in its movements and survival by the substantial increase in both water temperature and clarity downstream, as well as an established water diversion project that acts as a barrier to fish movements within the watershed. Finally, AquaBounty argues that AAS, grown in freshwater for the entire period of culture, would lose the ability to adjust to saline environments, and therefore not be able to survive in the estuary or ocean if able to migrate all the way downstream.
Though many safeguards will be in place to greatly minimize risk of escape, the risk cannot be reduced to zero. Fish can be lost from facilities by damages caused to nets by predators or birds, or even poachers. Catastrophic events, such as floods or earthquakes, can damage facilities and make them vulnerable to fish losses. In egg-producing facilities, human error in handling can lead to escapes, such as eggs carried in water droplets on clothing.\(^3\) And an escapee from the grow-out facility in Panama may survive the geographic particularities (e.g., salinity) expected to confine it; a study in New Brunswick, Canada, provides clear evidence that wild landlocked Atlantic salmon are able to migrate from their streams to the ocean and survive in coastal waters after remaining in freshwater for three to seven years.\(^4\) Furthermore, the biological confinement method of induced triploidy is not always 100 percent effective, meaning some females produced in the AquaBounty facility may be able to reproduce.\(^1\)

Taken together, the likelihood of escape, survival, and reproduction is extremely remote, but is not absent. This very small risk also does not consider the potential for illegal or malicious release outside the scope of what would be the FDA-approved conditions of use. For these reasons, it is still important to consider potential impacts to the surrounding environment and wild populations should an escape or accidental or malicious release occur.

The introduction of transgenic salmon to natural environments may impact wild populations in at least three general ways: through genes, competition, or disease. The degree of harm done via the first, gene flow, will depend on the net fitness of the transgenic fish and its ability to mate with wild fish. The second, competition, will depend on interactions between transgenic and wild fish and different aspects of their environment. The third, disease transmission, will depend on a variety of factors, including the presence of disease, the vulnerability of transgenic and wild fish, and the likelihood of contact between the two.

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Gene Flow

Escaped commercially produced fish that can reproduce with wild fish can introduce their genes—different from the wild stock as a result of selective breeding for traits that make the fish desirable for farming—to the population. Whether these genes and their associated traits flow into the natural population depends on the farmed fish’s net fitness, or its likelihood to survive and reproduce. Net fitness arises from the following six traits:

- Juvenile viability (chances of surviving to sexual maturity)
- Adult viability (chances of surviving to procreate)
- Fecundity (number of eggs produced by a female)
- Fertility (percent of eggs successfully fertilized by male sperm)
- Mating success (success at securing mates)
- Age at sexual maturity

Research on the fitness of farmed fish and their offspring with wild fish has suggested that “domestication selection,” or natural selection for fish well-adapted to the hatchery environment, makes them less fit for ocean survival, resulting in problems for the natural population. Observation of a Pacific salmon hatchery program showed that farmed Pacific salmon did not reproduce as well as wild salmon when they were in natural habitats, and suggested that interbreeding would produce less successful Pacific salmon.\(^5\) Even more distressing, another study looked at Atlantic salmon and found that, in addition to reducing wild smolt production through competition for habitat with the larger farmed parr, breeding between the wild and farmed Atlantic salmon produced less viable hybrids.\(^6\) The authors of this study suggested that the offspring production might be so low that it would lead to the eventual extinction of the wild population.

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While there is ample evidence that gene flow from conventional farmed fish to wild populations may have negative effects, the outcome is less clear with transgenic farmed fish. Genetic engineering expands the range of potential traits that can be expressed in a fish strain. Some of these traits may decrease net fitness of a fish, making it less likely to survive and pass its genes onto future generations. Other introduced genes may improve net fitness and increase the impact the genetically modified fish could have on wild fish populations. Because these genes weren’t previously present in the fish population, it is difficult to predict how these genetic changes might alter fish behaviors or disturb ecosystem processes. Environmental consequences of this gene flow range from benign to harmful, depending on a variety of factors, including the traits expressed by the genes.3

Three scenarios could arise from the addition of transgenes into a wild population:

Purge/Elimination of Transgenes

One possible scenario involves a transgene for a trait that puts a fish at a disadvantage for living in the wild habitat and also makes the fish less successful at reproducing. Poor reproductive success would result in less frequent introduction of the transgenes, and offspring carrying the genes will be selected against by the environment in which they don’t fare well. In this case, the transgene will eventually be eliminated from the wild population. Though the final population is composed of the wild genotype, there may still be important disturbances of the gene pool while the transgene is being bred out over multiple generations.

Spread/Invasion of Transgenes

If the transgenes make the transgenic fish equally or more fit than the wild fish, the genes of the transgenic fish are likely to spread through the population, as the offspring carrying the transgene would not be selected against by their environment. Recent studies suggest that age at maturity may be the most important fitness factor in this scenario, followed by juvenile viability.7

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Trojan Gene Hypothesis

Finally, if a transgenic fish has a mating advantage but also has traits for reduced survival in a particular habitat, the tendency would be toward rapid decline or extinction of the wild population. In this scenario, the transgene would be driven into the natural population by more successful transgenic mates and rapidly spread the introduced genes. The population size would quickly shrink as the resulting offspring fare poorly in the environment to which they are ill-suited.

A simplified model examining the effects of two salmon fitness traits, mating success and juvenile viability, estimated the likelihood that a transgene would become part of the wild genotype. Of the possible combinations of mating advantage and juvenile viability (from high-mating advantage and high viability to low-mating advantage and low viability), two-thirds of the modeled populations included the transgene. With a very strong mating advantage and low probability arising from the transgene, the wild population would be driven extinct, according to the modeling exercise.

Several experiments have attempted to determine whether transgenic salmon are at a mating advantage or disadvantage relative to wild salmon. Because experiments with transgenic salmon cannot be performed in completely natural settings, their results must be interpreted with the understanding that confinement has been shown to reduce reproductive performance in salmonids. One study compared how transgenic coho salmon and captively reared non-transgenic coho salmon competed against wild coho salmon for mating with spawning females. The authors of the study found that wild fish performed more courtship behaviors, were more reproductively aggressive, released more, faster-swimming sperm in their ejaculate, and sired far more offspring than transgenic fish. Though the effects of genotype-environment (i.e., captivity) interactions could not be controlled for, the authors noted that captively reared non-transgenic salmon also sired more offspring than transgenic fish.

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Another experiment compared the breeding performance of transgenic and wild Atlantic salmon for two different types of males: mature anadromous males (males that mature sexually upon their return from the ocean) and “precocious parr” (males that mature early, before ever leaving the river).\textsuperscript{11} While precocious males make up only a small portion of natural populations, they often contribute a large portion (11 percent to 65 percent) of the fertilized eggs because larger males don’t see them as competitors and the parr are able to sneak fertilizations. Results from the experiment showed that wild Atlantic salmon were more successful at reproduction than transgenic salmon for both types of male. The authors pointed out, however, that the transgenic salmon did show interest and had some success in mating, and would therefore still have the potential to contribute genes to future generations of a wild population.

There is recent evidence that the transgenes can be passed not only to wild populations of the same species, but also to natural populations of closely related fish.\textsuperscript{12} In crosses between growth-hormone transgenic Atlantic salmon mothers and wild brown trout fathers, hybrid offspring were as viable as salmon offspring and brown trout offspring. No studies have yet been carried out to see the reproductive success of these transgenic, hybrid offspring, but the authors suggested that there may be novel ecological impacts, especially arising from competition between wild populations and the transgenic hybrids. In this case, the transgenic hybrids grew significantly faster than either transgenic or wild parents (and faster than non-transgenic hybrids) under hatchery (well-fed) conditions. In more natural conditions, fish in populations comprised of only transgenic or only wild salmon had comparable growth rates, but when both were present in the same setting, transgenic hybrids actually reduced wild salmon growth rates.

**Competition**

Beyond the potential for gene flow into wild populations, there is some concern that escaped transgenic salmon may interfere with wild populations through competition for food or habitat. The threat of transgenic Atlantic salmon establishing populations in the areas into which they escape appears to be small, since there is a long history of failed intentional introduction along the U.S. West Coast. A review of scientific


literature performed to evaluate risks of farmed Atlantic salmon to Puget Sound, Washington, asserts there has not been “one successful self-reproducing population anywhere.”¹³ In a history of fishes introduced to California, the authors wrote that the species “has achieved no lasting success,” with introductions between the late 1800s and throughout the 1900s not yielding the hoped-for angler opportunities.¹⁴ But these authors also stated, “It is known that *Salmo salar* [Atlantic salmon] will survive when transplanted to the Pacific coast.” And they noted a successful population of Atlantic salmon in Hosmer Lake, Oregon, but this population is supported by a hatchery and the young fish require “special care, space, and constant observation.” Despite the historical difficulties in establishing Atlantic salmon populations on the West Coast, there is evidence of surviving and reproducing Atlantic salmon populations in British Columbia. There, scientists reported successful natural spawning of aquaculture-escaped Atlantic salmon and suggested these had escaped to underutilized areas that allowed the species to establish.¹⁵

This evidence suggests the possibility for escaped farmed Atlantic salmon to establish a population in the wild, under a narrow set of favorable conditions. The issue is less clear for transgenic Atlantic salmon—whose behavior or abilities in the wild may be substantially different from conventional farmed salmon—because no experiments or observations can be done in nature. Again, scenarios must be envisioned based on the results of laboratory observations and experiments with transgenic salmon.

One study of Atlantic salmon feeding behavior in the presence of a predator found that transgenic salmon were far more willing to take risks than non-transgenic salmon.¹⁶ Not being as strongly deterred by the predator, the transgenic salmon moved twice as quickly and were able to eat about five times as much food as the non-transgenic salmon. This behavior would confer a competitive feeding advantage to the transgenic salmon over wild salmon, but would also make them vulnerable to predation. Moving more quickly might allow the transgenic salmon to escape predators more easily, but

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might also increase the likelihood they would encounter more predators overall. At the same time, transgenic salmon lose their parr marks—dark bars on their bodies that provide camouflage against a stream bed background—and therefore appear more silvery than wild salmon of the same developmental stage, which might make them even more vulnerable to predators than wild fish. It is impossible to determine which effect of the feeding behavior observed would have the larger impact in the wild: the ability to feed more, faster, or the vulnerability to predation.

Another study attempted to determine the role that food availability would play on populations of coho salmon when made up of transgenics and non-transgenics at a critical stage early in life. The results of the experiments showed that when transgenic coho salmon were a part of the population in an environment with scarce food, the population always crashed or went extinct. In contrast, a population made up entirely of wild salmon had good survival in a food-scarce environment. The explanation for these crashes and extinctions was that in each instance, transgenic individuals would emerge and outcompete all other fish (both transgenic and non-transgenic) for food. These dominant individuals were far more aggressive than the others and sometimes were cannibalistic. The authors pointed out that cannibalism is a common result of hunger and therefore high growth rate in salmonids; because salmon with enhanced growth transgenes tend to be hungrier, they inferred that healthy salmon with the growth hormone transgene would be likely to exhibit the behavior.

But the effects of transgenic salmon in natural environments may depend largely on when they escape from a high-food environment; several studies suggest that salmon with a growth enhancement transgene must be reared in high-food environments in order to express the growth trait for which they have been engineered. The effects of different rearing environments on feeding behaviors have been shown to persist over time, even when the salmon have been subsequently released into different food environments. Experiments performed under natural-like settings to determine transgenic coho salmon effects on populations of other species found that the transgenic coho raised under hatchery conditions (plenty of food) suppressed the growth and

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reduced survival of wild coho salmon, chinook salmon, and steelhead trout once in a
more natural setting (less food). But this negative effect was not observed when
transgenic coho salmon were raised under natural settings—in fact, all fish did equally
well.\textsuperscript{19} The authors concluded that the main \textit{competitive} threat from escaped transgenic
salmon on wild salmon populations may come during the first generation of escapees.
And based on these results, future generations of transgenic salmon would not be
expected to fare worse than wild salmon, even if food is scarce.

Finally, a growth-enhancement transgene may be expressed differently by various
salmon species. For instance, some early studies on transgenic coho salmon suggested
that the advantage given transgenic salmon fry by their higher foraging motivation and
quicker motions might be balanced out by an increased metabolic demand that would
equate to lower survival in the end. But a recent study showed that at a critical early
life stage, transgenic Atlantic salmon did not, in fact, have lower survival than
non-transgenic Atlantic salmon.\textsuperscript{20} The implications of this finding are that a greater
proportion of transgenic individuals might survive a critical early life stage than
previously thought based on observations of older transgenic salmon. The authors
suggested this result might be a consequence of delayed onset of transgene expression
in the Atlantic salmon, particularly in comparison with coho salmon, meaning the
metabolic costs of fast growth may not be as expensive for young Atlantic salmon as
other species of transgenic salmon. It is therefore important to consider species-specific
characteristics of transgenic fish when evaluating risks.

\textbf{Disease}

Transgenic salmon, like any farmed fish, may place additional stress on wild
populations as vectors of disease. Disease is among the greatest problems facing
aquaculture; the high densities of fish kept in farms and closed containers, alterations
from natural diets, and nutrient deficiencies are common consequences of conventional
aquaculture and can all contribute to increased disease exposure or impaired immune

\textsuperscript{19} L. Fredrik Sundstrom et al., “Growth-Enhanced Coho Salmon Invading Other Salmon Species
\textsuperscript{20} Darek T.R. Moreau et al., “Growth Hormone Transgenesis Does Not Influence Territorial Dominance
or Growth and Survival of First-Feeding Atlantic Salmon \textit{Salmo salar} in Food-Limited Stream
function in farmed fish.\textsuperscript{21} An article in \textit{Science} estimated an outbreak of sea lice in a salmon farm located along a wild salmon migration route could lead to a 99 percent collapse of pink salmon within only four salmon generations.\textsuperscript{22} The effects of a parasitic outbreak have been estimated to be far-reaching, too; one set of field and modeling results suggested that a single salmon farm increased infection risk by as much as four or five orders of magnitude over natural rates of infection near the farm, and elevated the risk of infection as far as 75 kilometers away.\textsuperscript{23}

A salmon with a transgene for disease resistance would clearly be expected to succumb to fewer of these outbreaks, but what about salmon with transgenes for enhanced growth, like AAS? Genetics are expected to play some role in immune system function, and if more energy is going to more rapid growth, a fish might be expected to be more vulnerable to pathogens. Studying disease resistance in transgenic coho salmon, a group of scientists found that growth-enhanced transgenic coho smolts (but not fry) were more susceptible to infection by a bacterial pathogen than non-transgenic coho.\textsuperscript{24} While this was true at first exposure to the bacteria, the transgenic coho were less susceptible to infection after subsequent exposures, suggesting that their innate immune system was impaired, but the acquired immune response worked well. In order to get a larger picture of transgenic immune system function, a different group of researchers exposed the same strain of transgenic salmon to a different bacterial pathogen and also compared various aspects of its immune system response to that of non-transgenic coho salmon.\textsuperscript{21} They found that transgenic salmon were more susceptible than wild coho to infection by this bacteria as well. Not only that, but examination of a suite of immunological factors showed that the transgenic salmon had impaired immune function overall. In addition to the genetic differences in immune function shown for transgenic salmon, the sterility technique AAS will undergo during production—induced triploidy—has been shown to significantly impact disease resistance in salmon. In the earlier study on disease resistance discussed above,

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transgenic coho fry—as a group of both triploid (sterile) and diploid (normal) individuals—were overall no less resistant to disease than non-transgenic fry. But when infection resistance of just the triploid transgenic coho fry was compared with that of non-transgenic fry, it was clear that the triploid transgenic fry were significantly more susceptible to disease.24

Conclusion

Evidence of potential impacts to wild salmon exists, though it is difficult to gauge how applicable it is to the natural environment. While escape from the proposed AquaBounty facilities is unlikely, it is not impossible. Once in a natural environment, however, it is unclear with our present understanding how the many factors arising from an altered genome might combine to the advantage or detriment of AAS and wild salmon. The higher growth rates of transgenic salmon may be an advantage because larger fish tend to survive better in the wild, mate more successfully, and outcompete smaller fish around them. At the same time, consequences of the altered growth rate and contained rearing, such as higher metabolism, riskier behaviors, decreased camouflage in young fish, poor reproductive performance, and less-developed secondary sexual characteristics (red coloring or a kype)9 in mature fish may be costly in natural arenas. Ultimately, because the evidence is based on transgenic salmon that are necessarily kept in strict confinement, and confined rearing has acknowledged negative effects on the hardiness of fish, it is difficult to say how significant these effects would be in real rivers and streams.

If I can be of further assistance, please do not hesitate to contact me at (916) 651-1500.

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