Comments on Docket No. FDA-2011-N-0899
Draft Environmental Assessment for AquAdvantage Salmon and
Preliminary Finding of No Significant Impact, Dated 4 May 2012,
Prepared by the Center for Veterinary Medicine, U.S. Food and Drug Administration
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We respectfully submit the following comments on the May 4, 2012 Draft Environmental Assessment of AquAdvantage Salmon (AAS) and the companion May 4, 2012 Preliminary Finding of No Significant Impact (FONSI). We focus on the scientific quality and scientific reliability of the environmental risk assessment and proposed risk management measures. We are well qualified to provide these comments because we have over two decades of experience in developing and improving science-driven, environmental risk assessment and management methodologies for transgenic fish and in conducting risk assessment experiments on transgenic fish, all of which is documented by our record of peer-reviewed, scientific publications.

We also focus on the scientific adequacy of this Environmental Assessment because c**urrent law and regulations do not require the FDA to seek public comment of a draft environmental assessment (EA) or draft environmental impact statement (EIS) before approving any future commercial applications involving AAS, or any other line of transgenic fish or other animal**. We found major scientific inadequacies in this EA that set an unacceptably low bar for the scientific basis of future EA or EIS documents. Independent scientists with highly relevant expertise and the broader public may not be given a chance to comment on future EA or EIS of applications. The scientific quality and reliability of this EA will thus set the precedent for future applications that may involve larger-scale or less confined commercial production, or involve fish expressing more ecologically disruptive engineered traits.

Our three major recommendations regarding the 2012 draft EA are as follows:

1. We urge that the FDA conduct a failure mode analysis of the multiple confinement measures and include the analysis and results in a revised EA or in a full EIS. The company has proposed these confinement measures to reduce escape of AAS into aquatic ecosystems. We urge this scientific improvement because the preliminary FONSI hinges on the adequacy and assurance of these multiple confinement measures. The failure mode analysis should be as quantitative as possible and, at a minimum, present a fault-tree analysis (Burgman 2005). It should consider how climate change scenarios might alter the frequency and severity of storm events on Prince Edward Island and the highlands of Panama. Failure mode analysis is the state-of-the-art for reducing environmental risks of many technologies (e.g., Bowles and Peláez 1995, Burgman 2005, Hauptmanns 2010, Pillay and Wang 2003). A failure mode analysis of the multiple confinement measures for AAS is needed to substantiate their reliability. And if the failure mode analysis reveals significant weaknesses, this can inform the design of targeted improvements in confinement measures or operational procedures (Pillay and Wang 2003).

We provide more detailed comments relevant to this recommendation in sections III, IV.A and V below.

2. **We strongly recommend that the FDA either fully improve the scientific quality and reliability of the environmental consequences assessment in the draft EA or delete the consequence assessment and base its regulatory decision solely on the adequacy and assurance of the multiple confinement measures.** The draft environmental consequence assessment (in its section 7, and aspects of section 3.2.2, 3.2.3, and 5.2) is full of scientific inadequacies, omissions, and scientific uncertainties, especially linguistic uncertainties (see updated sections IV.B and VI below). The environmental consequences assessment inappropriately focuses on outdated risk assessment ideas (particularly Kapuscinski and Hallerman 1990, 1991) where it should use state-of-the-art environmental risk assessment methods for genetically modified fish, brought together in a book (Kapuscinski et al. 2007) whose scientific peer-review was led by guest editors Dr. Eric Hallerman and Dr. Peter Schei. A scientifically reliable environmental consequences assessment should use state-of-the-art methodologies to: scope the consequences assessment and select quantitative and semi-quantitative methods wherever possible (Hayes et al. 2007); assess escape probabilities and fitness changes in transgenic fish (Kapuscinski et al. 2007a); assess ecological effects (Devlin et al. (2007); and identify and treat all sources of scientific uncertainty (Hayes et al. 2007a, Hayes 2011). In its current form, the draft environmental consequence assessment sets a scientifically unacceptable, low standard for this commercial application and for all future commercial applications involving transgenic fish.

We provide more detailed comments relevant to this recommendation in sections IV.B and VI below.

3. **A fully revised environmental consequence assessment should focus on possible ecological consequences of AAS escapes in the upper reaches of the un-disclosed river in Panama. This is necessary to fill a major scientific gap in the environmental consequence assessment.** The draft EA presents temperatures in the river's upper reaches that would allow escaped AAS to survive and live in the river until they die by natural causes. The draft EA also states that introduced rainbow trout live in the upper reaches of this river, a species whose temperature and other ecological requirements overlap with those of Atlantic salmon. The upper reaches of this river is the ecosystem most likely to experience an ecological effect from escaping AAS, even if escapees don't reproduce in the river and particularly if there are periodic escape events. A revised consequence assessment should use state-of-the art methods from Devlin et al. (2007) and utilize actual data about the fish community and stream ecology of the river's upper reaches to assess potential interactions between AAS and various fish species and other organisms; and follow the steps for assessing ecological effects presented in Box 6.1. in Devlin et al (2007).

We provide additional comments relevant to this recommendation in sections V.C and VI below.

Below, we present comments we submitted on Sept 16, 2010 (for the draft Environmental Assessment released on Sept 3, 2010), followed by updates to address changes– or lack of changes –in the 2012 draft Environmental Assessment.

I. If this application is approved, farming of transgenic AquAdvantage salmon will proliferate in the foreseeable future and other species are likely to follow. Farmed Atlantic salmon is a global commodity, with approximately 1.5 million metric tonnes farmed in 2008 (FAO 2010, Kontali 2009) and typical salmon farms raise 500,000 to 1 million fish in poorly confined growout areas, each as large as four football fields. The applicant will likely want to sell AAS eggs to many growers to be profitable in this global industry. Thus, this is a historic application whose approval could lead to transgenic salmon becoming the first genetically engineered animal farmed on a large scale for human food.

Update: The 2012 draft EA does not try to hide the fact that the intention is to sell AAS eggs for grow-out at other locations than those approved in the present application. However, it emphasizes that this EA is only applicable to the Prince Edward Island site in Canada and the site in Panama. Nevertheless, the precedent-setting nature of this draft EA still holds. Thus, our concerns about major weaknesses in the scientific methodologies and scientific reliability of the draft EA are still relevant.

II. We urge the FDA to extend the public comment period on the scientific issues in this historic application before making a final decision. The time between the release of nearly 260 pages of technical documents on Sept 3 (the day before a national holiday weekend) and the September 16 deadline for written comments was much too short for adequate examination by the community of scientific experts on the genetics and ecology of transgenic fish and on methodologies for environmental risk assessment of transgenic fish. The limited review period did not give us enough time to prepare a more complete set of comments on all relevant scientific issues in the documents. And scientists are only one group among diverse stakeholders in this decision. A rushed process does not build public confidence in a decision that requires weighing complex matters in environmental and food safety risk assessment. A rushed process with extremely limited opportunity for comment only when the agency is finally "nearing a decision" (FDA 2010) contradicts research findings on how to gain public trust in risk decision-making. Best practices involve structured deliberation with relevant stakeholders at a few key points in the risk assessment, especially at the early steps that frame the entire process--problem formulation and identification and prioritization of hazards to address in the rest of the risk assessment (NRC 1996 and 2009, Hayes et al. 2007, Nelson et al. 2007, Nelson et al. 2009, Renn 2008). Even the National Research Council report on biological confinement of genetically engineered organisms advised public participation earlier in the process (NRC 2004:189-190).

Update: The 2012 draft EA was released on the Internet on Friday Dec 21 (with official notice published in the Dec 26 Federal Register) just prior to and during a major holiday week with a comment deadline two months later (February 25, 2013). This still gives the impression of trying to reduce public attention to this precedent-setting document. After pressure from members of Congress to extend the comment period, the FDA announced, on February 13, 2013, an extension of the comment deadline to April 26, 2013.

III. Multiple confinement of these transgenic salmon is crucial to prevent environmental harm especially because of scientific uncertainty regarding their environmental risks (Kapuscinski et al. 2007, Devlin et al 2006). If the physical or geographical confinement measures fail and there are regular escapes of sterile transgenic fish into environments where they can thrive, they could still alter the environment "in permanent ways, especially if transgenic fish overexploit key resources" such as wild fish prey (Devlin et al. 2007:152). Yet virtually no research has been done on how transgenic fish, including AAS, might affect other fish species in environments where they might end up. Biological confinement, alone, is not sufficient to prevent environmental harm. This is why assurance of multiple confinement is crucial. **Thus, we commend the applicant for proposing multiple confinement of the AAS strain at two relatively small facilities**, a hatchery on Prince Edward Island (PEI), Canada and a grow-out facility at an undisclosed location near a river in the highlands of Panama.

Update: The geographical confinement provided by waters near the shore of PEI, Canada and near the mouth of the river in Panama is laid out with more detail than in the 2010 draft EA. The application still hinges on the assumption that escapes will not happen, and even if it would, that water temperatures are too warm for AAS in the lower reaches of the river in Panama to prevent the survival of any escapees for any significant period of time. However, the 2012 draft EA does not present scientific data on these physiological limits in AAS, and such data were also missing from the 2010 EA. Instead, the draft EA conjectures that "there is no *a priori* reason" for a possible change in the upper temperature tolerance of AAS. Unfortunately, this ignores evidence that growth-enhanced transgenic coho salmon showed altered (faster) growth rates at higher temperatures compared to non-transgenic coho salmon (Lõhmus et al. 2010), which provides a scientific reason to test whether growth-enhanced AAS exhibit changes in their tolerance of higher water temperatures. See section V.C below for more detail on this matter.

IV. However, we have two major concerns:

A. How will the FDA assure and verify that multiple confinement is continually achieved at the two facilities and in many future facilities as farming of these fish proliferates? How will the FDA assure that all sales of processed AAS in the USA come from fish grown under successful multiple confinement? How will the FDA assure and audit the company's implementation of an "integrated confinement system" (Table 10 in the Environmental Assessment)? This is a good idea on paper but the actual achievement of multiple confinement depends on many human actions, and the rigor of audit and regulatory oversight. An even greater challenge is how to assure multiple confinement at many, larger facilities in different environments and nations as commercial production of these fish proliferates. Does the FDA have the staff, financial resources and sufficient overseas jurisdiction for adequate surveillance of diverse domestic and foreign hatcheries and grow-out facilities?

Update: The 2012 draft EA emphasizes that the approval will be only for the sites specified and that use of the AA salmon at any other site will require a separate approval for that site. Because the FDA is not required to seek public comment prior to approving potentially larger-scale, future commercial applications, our concerns and questions are still germane.

B. The scope of the Environmental Assessment is too narrow and its methods inadequate for the issues at hand. We urge the FDA to now require a complete environmental risk assessment, as a fully transparent Environmental Impact Statement (EIS). The current Environmental Assessment only assesses the likelihood of transgenic salmon escaping from multiple confinement at the two facilities but the proposed multiple confinement does not absolve the need for a complete environmental risk assessment, given the likely proliferation of sales of AAS eggs for growout beyond one facility in Panama. The Environmental Assessment does not provide the full information needed to predict environmental effects of AAS, some of which we describe below. It focuses on an outdated list of issues (from Kapuscinski and Hallerman 1991) and ignores the major advances in methodologies for assessing environmental risks of transgenic fish (Kapuscinski et al. 2007). These advanced methods systematically integrate information about the environment and the transgenic fish's genotype and phenotype to identify and prioritize hazards upon which to focus the environmental risk assessment (Devlin et al. 2007, Kapuscinski et al. 2007a, Hayes et al. 2007).

All parties will benefit from a full assessment of potential environmental harm and benefit presented in a thorough and transparent EIS. All future regulated growers will benefit from more complete information to guide their investments in grow-out facilities that are more likely to be approved. The public interest will be better served by a more complete and transparent process.

Update: For the use of AA salmon within the two specified sites, physical confinement measures (primarily mechanical barriers to escape) appear to be able to reduce the risk of escape to very low levels. The Panama grow-out site is small-scale, and does not represent the kind or size of possible future facilities for commercial, profit-making grow-out of AAS. We still recommend conducting a quantitative failure mode analysis in order to: scientifically substantiate the adequacy of this application's multiple confinement measures; and set a strong precedent for how future applications should scientifically substantiate the adequacy of risk management measures. The US Food Drug and Cosmetic Act, the law under which the FDA claims regulatory authority over genetically modified animals, requires the FDA to keep confidential all information about a genetically modified animal application (even the mere existence of an application) unless the applicant wishes to share some information with public. It is worrisome that independent scientists with highly relevant expertise, as well as the general public, may not get a chance to comment on an EA or EIS before approval of future applications that will involve larger and less-confined commercial-scale growout. AquaBounty Technologies has probably already identified several initial purchasers of AAS eggs for commercial grow-out. We still recommend the FDA to conduct a scientifically reliable EIS that considers the PEI and Panama facilities and grow-out facilities of the most likely 2-4 initial buyers of AAS eggs.

We urge that the FDA not make a final decision on this application until our major concerns are fully addressed. Below, we further describe these concerns and suggestions to address them.

V. Strengthen the assessment and assurance of multiple confinement measures

The FDA should require a quantitative failure mode analysis for each form of biological, mechanical, chemical, and geographical confinement and for the overall combination of

confinement methods (Burgman 2005). Although the limited scope of the Environmental Assessment is justified by arguing that the AAS will be raised only in the two mentioned facilities, the proposed wording for the product label (Environmental Assessment page 48) and limitations for use (Briefing Packet, page 8) leave open the door for possible production in other FDA-approved facilities. Therefore, we urge doing failure mode analysis for the range of facilities that may obtain AAS eggs in the foreseeable future, as part of a full EIS.

Update: The 2012 draft EA clarifies that this application is only for use of AAS at the PEI, Canada hatchery and Panama grow out facility. However, it still does not present a quantitative failure mode analysis for these two facilities. Please also see our major recommendation, at the start of these 2013 comments, about adding a failure mode analysis.

A. Biological confinement

Production of 100% all-female populations of salmon is well established, which should make this part of a quantitative failure mode analysis relatively easy. It is not as easy to achieve 100% sterility through pressure-shock induction of triploidy. Failure analysis of triploid induction in AAS should quantify the variability in percent triploids across treated batches of eggs and the frequency of "exceptional diploids". Devlin et al. (2010) obtained 97% to 99.8% successful triploidy when they treated batches of 10,000 to 19,000 transgenic coho salmon eggs. They also detected 1.1% exceptional diploids overall among all pressure-treated groups (54,787 fish). Exceptional "diploid" individuals can contain the transgene but their fertility and ability to transmit the transgene to offspring is not yet known. Do exceptional diploids occur among treated AAS? If yes, it is necessary to determine their fertility or devise a proven way to eliminate them from eggs destined for growout.

Update: Diploid AAS do occur and the 2012 draft EA specifies a program to detect such females. It will use a statistical sampling program to assure that no more than 5% of the all-female individuals are diploid (pages 40-41) and if a batch fails to meet this criterion, the batch will be destroyed. This approach does not allow 100% certainty of preventing diploid females from entering the grow-out facility; and is tolerable for the Panama site only because there are no Atlantic salmon males in the colder, upper reaches of the accessible river. In the future, if this approach is proposed for a grow-out facility in a region where there are Atlantic salmon, it would be imperative to conduct a quantitative risk assessment of possible interbreeding between diploid AAS females and wild or feral Atlantic salmon males and of the genetic and ecological consequences of such interbreeding.

B. Physical and chemical confinement

Physical and chemical confinement measures are especially prone to equipment failures, power failures, operational wear, and human error (Mair et al. 2007). Failure mode analysis of these confinement measures is critical. We commend the applicant's proposed "integrated confinement system" plan that aims to reduce these sources of failure. But this does not remove the need for a quantitative failure assessment.

Update: The 2012 draft EA provides some new details and has deleted some details compared to the 2010 draft EA. These details do not change the importance of our recommendation to present a failure mode analysis that is as quantitative as possible.

C. Geographical confinement
Failure analysis of geographical confinement should include data on how AAS respond to changes in temperature and season. The Environmental Assessment suggests water temperatures in lower reaches of the Panamanian River and Pacific Ocean will be lethal to AAS but has the thermal tolerance of AAS been measured? In coho salmon, the optimal growth temperature, and presumably other physiological traits relevant for thermal tolerance, at the freshwater stage changed with growth-hormone transgenesis. Growth-enhanced transgenic coho salmon grew faster at 18ºC than at 12 ºC (and the upper thermal limit for their fast growth is unknown because they were not tested above 18 ºC) whereas wild-type coho salmon did not grow significantly faster at 18ºC than at 12 ºC (Lõhmus et al. 2010). How has growth-hormone transgenesis affected thermal tolerance and optimal growth temperature in AAS? Water temperatures given for the high-elevation portions of the Panamanian river (near the grow-out facility) range from 15º to 19ºC (Environmental Assessment, Table 3). Are there data on whether the transgenic Atlantic salmon continue to grow fast, are able to survive or perish at these temperatures, in fresh as well as saltwater?

Overall, the Environmental Assessment does not give sufficient data on seasonal variation and habitat complexity in the receiving environments around the Canadian hatchery and Panamanian grow-out facility to identify if AAS escapes could thrive in certain locations and seasons and to estimate the likelihood of this hazard. In Panama, this needs to be examined for the watershed, with full consideration of seasonal variation and habitat complexity. Oceanographic conditions are also very complex in the Gulf of Panama and Pacific coast of Panama. During the dry season, for instance, upwelling of colder and food-rich waters could be hospitable to adult salmon if they make it downriver. Finally, a full EIS should consider seasonal and spatial variation and complexity in environments surrounding the range of possible grow-out facilities.

Update: The 2012 draft EA still does not present data on temperature tolerance by AAS. The EA still lacks the information needed to assess if AAS could survive in some upper parts of the river in Panama, where they could interact ecologically with other organisms in the river. We do appreciate the following improvements in the 2012 version: provision of additional information on why near-shore, ocean conditions around the PEI hatchery site and near the mouth of the river in Panama are non-conducive for AA salmon survival.

VI. Require a Scientifically Rigorous Environmental Impact Statement before making a decision on the AAS application

A. The Environmental Assessment does not give the full information needed to predict environmental effects of AAS. It focuses on completing only the "exposure" step of risk assessment, and concludes there is "extremely small" likelihood of exposure due to multiple confinement at the two facilities, thus no consequence and no need to assess consequences. As scientists, we cannot agree with this approach because it assumes 100% achievement of multiple confinement without having presented the failure mode analysis that is standard practice in technology risk assessment. Even if actual exposure is very close to zero, it is still necessary to assess ecological consequences, from low to high severity consequences, and then estimate the overall risk. We also disagree with this approach because of the likely proliferation of farming

AAS in numerous grow-out facilities where multiple confinement will be harder to implement and assure (Mair et al. 2007).

Update: The 2012 draft EA does present an environmental consequence assessment (section 7, starting on page 65) but it is full of scientific inadequacies and omissions. The consequence assessment assumes that escape is unlikely but the EA lacks a quantitative failure mode analysis of the multiple confinement measures to substantiate this assumption. The consequence assessment assumes that escapees will not survive based on two assertions not backed up by science.

Firstly, contemporary understanding in evolutionary biology is ignored in the assumption (page 19) that animals show maximal fitness in an environment where they evolved and thus AAS must be less fit in a new environment. Instead, it is now understood that animals are adapted adequately (not necessarily maximally) to their native environment and can show equal or higher fitness in a new environment, as shown by successful establishment of numerous alien aquatic species (e.g. Casal 2006).

Secondly, the 2012 EA assumes that AAS are so highly domesticated and dependent on artificial feeds that they would quickly die outside of captivity (pages 20, 76, and 78) but provides no data on AAS to support this idea. These statements ignore published studies showing that growth-enhanced transgenic coho salmon do as well or better than wild-type salmon under most food limited conditions (Sundström and Devlin 2010), as discussed further in our comments below in section VI.D.d. Instead, the statements on page 20 and 76 cite Kapuscinski et al (2007a) but misconstrue the cited passage (Kapuscinski et al 2007a:125), which recommends obtaining data to assess whether domesticated transgenic fish display traits that disrupt their ability to mate with wild relatives. And the statement on page 78 claims that escaped farmed salmon will starve before learning to seek natural prey but cites a source (Muir 2004) that presents no scientific data and no citation of other studies with scientific data to substantiate this claim.

B. Where the Environmental Assessment and Briefing Packet do present some quantitative data related to environmental risk, they omit information required to scientifically verify the stated conclusions. Frequently missing information includes: sample sizes (or the given sample sizes seem too small to reliably assess the scientific value of the experimental outcome), standard errors, statistical power, or description of statistical tests used to reach the stated conclusion. Although we focused on sections dealing with environmental risks, we noticed similar omissions in the Briefing Packet's presentation of data for other scientific issues. Such incomplete analysis and presentation of data does not meet commonly accepted scientific standards.

Update: There is still no information available on how much of the presented data have been obtained, experimental design, sample size, statistical tests and results, etc.

C. The Environmental Assessment does not adequately consider the growing body of research on genetic and ecological risks of transgenic fish. This research shows there will be high scientific uncertainty in predicting the overall fitness and ecological effects of AAS if they enter nature because it is extremely challenging to extrapolate from experiments using semi-

natural conditions (reviewed in Devlin et al 2007, Devlin et al. 2006, Kapuscinski et al. 2007). This is due to key biological complexities including gene-environment interactions, background genetic effects, pleiotropic effects, tradeoffs between traits expressed across different life stages, persistent effects of the environment experienced early in life, evolution of fertile transgenic fish after escape, ecological variability, and poorly understood ecological processes (Devlin et al. 2004b, 2007, Kapuscinski et al. 2007, Neregard et al. 2008, Pennington et al. 2010, Pennington and Kapuscinski 2011, Sundström et al 2007b, 2009).

Overall, this research indicates it could be very misleading to base an environmental risk assessment on data for only a few traits that do not span the whole life-cycle and measured under a limited range of environmental conditions. We are therefore concerned about overly simplistic statements of "poor fitness" of AAS without the kinds of scientific evidence required to support such a claim (e.g. Environmental Assessment, Table 11 on p. 71; Briefing Packet, p. 43 possible implication that higher critical oxygen level of AAS leads to overall poor fitness). Also, the Environmental Assessment gave an unacceptably cursory mention of uncertainty (two paragraphs on page 73) with no application of scientific methods of uncertainty analysis (Hayes et al.2007a, Hayes 2011).[1]

Update: The 2012 draft EA mentions the problem of genotype-environment interactions but largely ignores the need to explicitly address it through scientifically reliable methods of environmental risk assessment and uncertainty analysis. Section 5.2.2.3 points to this problem, but reports no data on the phenotypic plasticity of AA salmon, which is surprising as Atlantic salmon probably are more plastic in many traits than are coho salmon, for which phenotypic plasticity has been found in published studies on a growth-enhanced transgenic line. The 2012 draft deals more with different life stages than the 2010 draft, but the reasoning is mainly based on knowledge from wild-type and farmed Atlantic salmon rather than AA salmon and this weakens the scientific reliability of the assessment. Finally, although two weak paragraphs on uncertainty analysis were deleted, the 2012 draft EA completely lacks a scientific uncertainty analysis to identify and treat each source of uncertainty (Hayes 2007a, Hayes 2011).

D. The environmental analysis of AAS presented to the public largely avoids facing the complexity and uncertainty inherent to environmental risk assessment of transgenic fish. We strongly urge the FDA to require a science-driven environmental risk assessment that treats the complexity and uncertainty directly and honestly, using the most current methodologies (Kapuscinski et al. 2007, Burgman 2005). Such an environmental risk assessment in an EIS should follow standard ecological risk assessment steps:

1. Conduct a problem formulation and options assessment that integrates scientific analysis and stakeholder deliberation (Nelson et al. 2007) and define conceptual models of the human and environmental system at issue (Landis 2003);

2. Identify all possible hazards and prioritize which hazards to fully assess (Gong et al. 2007, Kapuscinski et al 2007a, Devlin et al. 2007);

[1] On these 2010 comments we have updated the Pennington et al (2010) date and cited two relevant publications from 2011.

3. Identify and agree upon measurable assessment endpoints--based on identifying possible environmental consequences--for each prioritized hazard (Kapuscinski et al. 2007b);

4. Estimate exposure, i.e., the likelihood of transgenic salmon escaping into and living in natural environments – quantify as much as possible;

5. Estimate likelihood and severity of environmental consequences identified for each prioritized hazard – quantify as much as possible;

6. Identify and appropriately treat uncertainties throughout the exposure and consequence assessment, using contemporary methods (Hayes et al. 2007a, Hayes 2011); and

7. Characterize the overall risk (exposure x consequences), with explicit presentation of uncertainties that affect exposure and consequence assessment.

The most comprehensive peer-reviewed literature on the biology and ecology of any transgenic fish is for a line of transgenic coho salmon bearing a different salmon growth-hormone construct. Conclusions from this body of research point to the key issues that should be investigated for AAS (Table 1 below). These issues must be pursued for AAS, even though the outcomes may differ between AAS and the transgenic coho salmon due to strain-specific differences in altered traits. The environmental analysis in both the Environmental Assessment and Briefing Packet did not adequately incorporate insights from this body of research. For instance, do gene-environment interactions occur in AAS? If yes, how will this be incorporated into the environmental risk assessment, especially as sales of AAS eggs proliferate to many grow-out facilities?

Update: The 2012 draft EA largely ignores these concerns and suggestions. There is some reference to the coho salmon work, but it is not extensively used to understand the concepts of changes induced by inserting growth-hormone transgenes into the genome of Atlantic salmon. The FDA has ignored our recommendation to conduct a full EIS. Instead, the FDA has issued a preliminary "finding of no significant impact" on the USA environment from possible AAS escapees. Unfortunately, the most likely ecosystem to be affected by escapees is the local environment where live fish will be kept, especially the upper reaches of the river in Panama.

The peer-reviewed literature, to date, suggests that, at a minimum, a scientifically reliable environmental risk assessment of AAS should address the following issues:

a. Environmental conditions can lead to very different phenotypes and behavior of hatchery-reared versus stream-reared fish, which affects the relationship between transgenic and non-transgenic fish (genotype by environment interactions) (e.g. Bessey et al. 2004; Devlin et al. 2004b; Sundström et al. 2007b). Whereas this specific research was done on coho salmon, Atlantic salmon show even more plasticity in terms of life-history and behavior. Hence, the age at which AAS might escape from confinement could have dramatically different effects on their phenotype and on how they function in a natural environment. Further, if AAS are reared in

different locations, specific conditions in each location are likely to affect the phenotype in a specific way.

The documents released by FDA lack data on how the environmental conditions affect the phenotypes of AAS at different life stages. These data are crucial for assessing how AAS might behave after escape and, thus, what possible impacts they may have on the ecosystem.

Update: These data are still missing from the 2012 draft EA. They are needed for assessing what effects escapes of AAS would have on fish and other organisms in the stream ecosystem in the Panamanian river, including influences on terrestrial animals that may feed on AAS in the river. Such an assessment should especially consider the river's upper reaches where conditions may be suitable for AAS.

b. Enhanced appetite alters behavior in transgenic salmon (Devlin et al. 1999; Raven et al. 2006). Hence, they are likely to explore novel prey and novel areas (Sundström et al. 2003, 2004b, 2007a). And they also may expose themselves to predation risk (Sundström et al. 2004a).

Hence, using the behavior and habitat selection of wild-type salmon may only partly reveal the extent of impact by transgenic fish as they may venture into areas where wild-type fish do not exist. This should be assessed in conjunction with effects of transgenesis on environmental tolerance (e.g. thermal tolerance pointed out above). It will be very difficult to quantify the tradeoff between possible expansion of prey species versus heightened exposure to predation. What will be the overall effect of this tradeoff on fitness of transgenic salmon in nature? What will be the impact on predators from consuming AAS? How will the trophic role of AAS in the food chain affect the overall ecosystem? These questions can be addressed by conducting ecologically appropriate experiments, applying state-of-the-art methods of uncertainty analysis or a combination of both approaches (Hayes et al. 2007a).

Update: The 2012 draft EA provides some additional information on feeding habits, but these traits of AAS have not been experimentally studied (or, at least, have not been published in peer-reviewed scientific articles) and the statements are based primarily on assumptions.

c. Transgenic salmon may have different responses to temperature and season, compared to wild salmon and to domesticated farmed salmon. For instance, growth-enhanced transgenic coho salmon will likely remain active during winter and thrive at higher temperatures (Devlin et al. 1994 and 2004a; Lõhmus et al. 2008 and 2010). Thus, ecological traits of AAS should be tested under a range of seasonal conditions, including temperature changes.

Update: The 2012 draft EA did not fill this gap that we had found in the 2010 draft EA.

d. Reduced prey availability will not necessarily be a disadvantage to transgenic salmon. The flexible development (plasticity) of transgenic salmon means that these fish are not dependent upon high amounts of food to survive. Studies on coho salmon also show that under most food limited conditions they do as well or better than wild-type salmon, and the transgenic individuals can survive for at least 5 months without showing much growth (Sundström and

Devlin 2010). In the extreme, the stronger competitive ability of transgenic individuals may eventually result in cannibalism on outcompeted wild-type (Devlin et al. 2004b). Hence, the statement in the Ecological Assessment document (4.2.2) that "these macroinvertebrates, however, are not abundant." does not exclude persistence of AAS. Thus, AAS should be tested under different food availability conditions to determine potential survival and spread throughout possible receiving environments.

Update: This concern was not addressed in the 2012 draft EA. Instead, the EA compares AA salmon to farmed salmon that are said to starve during a period following escape (with no supporting data, not even in the cited source, Muir 2004) and, thus, that AA salmon would also starve and put them at a disadvantage. Have there been scientific studies on how well AA salmon adapt to novel prey after being fed only on artificial pellets? A scientifically reliable approach is to properly design and conduct an experiment to determine whether there is a true difference, not to make unsubstantiated assumptions. Many assumptions in this EA, such as this one about feeding and the assumption that AAS do not show better tolerance of warmer water temperatures, could be validated with relatively simple experiments.

e. Lower fitness of transgenic fish when they first escape does not translate into permanently lower environmental risk. One successfully breeding individual transmitting the transgene is likely to result in a very different phenotype with a different fitness potential relative to its parents (Kapuscinski et al. 2007a). Over the long term, evolutionary processes will exert selection on background genetics to compensate for reductions in fitness caused by the transgene (Ahrens and Devlin 2010).

Update: The 2012 draft EA did not address this comment to any extent, presumably because the growout facility in Panama will rear all-female AAS and the nearby river has no Atlantic salmon, hence no suitable male mates. Any future applications for commercial production of AAS that do not include these biological and geographical confinement measures will need to address this issue.

E. The Environmental Assessment and Briefing Packet compare traits of AAS to traits of farmed salmon but this is not an adequate comparator for understanding environmental effects. It is necessary to assess ecological differences between AAS and wild fish populations that fill a similar ecological niche in the accessible ecosystems. Following this fundamental ecological principle, **appropriate comparator specimens** for the environmental risk assessment of these transgenic Atlantic salmon are:

1. wild Atlantic salmon, including Atlantic salmon populations in possible escape zones and accessible ecosystems,

2. other salmon and trout species in the accessible ecosystems, which may fill a similar ecological niche and, thus, with which the transgenic salmon could compete, and

3. other fish species in the accessible ecosystems filling a similar niche and, thus, with which the transgenic salmon could compete.

The environmental risk assessment should include all three categories of comparators unless AAS will be farmed only near ecosystems that clearly lack a particular category, for example, in an area where there are no wild Atlantic salmon.

Update: The 2012 draft EA still primarily compares AAS with farmed salmon. It should be revised to compare AAS with hatchery-stocked Atlantic salmon and rainbow trout found in ecosystems accessible from the PEI hatchery (page 60 of the EA); and to consider rainbow trout and other fish species that might fill a similar niche in the river adjoining the Panama site (page 63 of the EA).

4. In places where there is farming of Atlantic salmon, the environmental risk assessment should assess if transgenic salmon pose additional ecological risks beyond those already posed by farmed salmon escapees. Risks that the transgenic salmon pose to the salmon farms themselves should also be examined.

Domesticated salmon are currently grown in commercial aquaculture and their environmental effects, as escapees from salmon farms into nature, are currently under significant scientific debate. Published research on this concern is growing. In spite of many similarities between domesticated and growth-enhanced transgenic salmon, the AAS are unlikely to pose the same environmental risks as domesticated salmon. This is because the genetic consequences of transgenic fish interbreeding with wild relatives are very different from those of domesticated salmon interbreeding with wild relatives: any individual inheriting the transgene maintains its phenotypic expression across generations, whereas the effect of integration of domesticated genotypes into wild populations are halved at each generation. To use farmed fish as comparators for the risk-assessment of transgenic fish may therefore not be valid. At a minimum, relevant comparative experimental evidence of phenotypic traits and their consequences should be provided for both farmed and transgenic lines.

Update: The 2012 draft EA assumes that escapes will not happen from the PEI hatchery, and if they do, fish will soon die. Thus, the draft 2012 EA devotes little attention to this issue.

VII. Conclusion

Any failure of a multiple confinement system means that, once AquAdvantage salmon escape, the release cannot be undone because these fish are mobile organisms with very low but not zero likelihood of having some fertile escapees. Thus, we conclude it is crucial to conduct a full EIS that assesses the potential genetic and ecological impacts that AquAdvantage salmon could have on wild fish and other aspects of the environment. This is even more crucial because of the scientific uncertainty surrounding how these transgenic salmon will function in different environments, the importance of Atlantic salmon as a major global commodity, and the existing commitment of US society to restore threatened and endangered salmon populations and conserve aquatic biodiversity.

Update: In this regard, the 2012 draft EA has not changed from the 2010 draft.

Table 1. Research findings relevant for an EIS of AAS, drawn from peer-reviewed literature on growth-hormone transgenic coho salmon

Key Insights from Research on Growth-Hormone Transgenic Coho Salmon	**Scientific Literature Reference**
Background genetics can strongly modify the effects of a transgene. Need to understand the evolution of transgenic fish in nature to accurately predict risk in the long term. Modeling shows that effects may occur on non-transgenic fish in populations due to the presence of the transgene.	Ahrens and Devlin 2010, Devlin et al. 2001, Neregard et al. 2008
Transgenic fish can show many changes in behaviors and phenotypes (feeding motivation, migration, dispersal, predation, spawning ability). Fish reared in naturalized stream conditions can show very different phenotypes from those reared in hatchery tanks (gene-environment interactions). Thus, data from the latter may only apply for first generation escapes and the former may require separate risk-assessment.	Bessey et al. 2004, Devlin et al 1999, Devlin et al. 2004b, Sundström et al. 2004b, Sundström et al. 2007a, Sundström et al. 2007b, Sundström et al. 2009, Sundström et al. 2010, Sundström and Devlin 2010
Transgenics showed altered swimming ability, respiration rate, oxygen demand, and antioxidant activity	Farrell et al. 1997, Huang et al. 2004, Lee et al. 2003, Leggatt et al. 2003, Leggatt et al. 2007, Stevens and Devlin 2000b, Sundt-Hansen et al. 2007
Environmental conditions affect the phenotypic difference between wild type and transgenic fish.	Lõhmus et al. 2009, Lõhmus et al. 2010, Sundström et al. 2007b, Sundt-Hansen et al. 2007
Growth-hormone transgenesis can affect fitness arising from predation effects, but effects are stage and environment dependent.	Sundström et al. 2003, Sundström et al. 2004a, Sundström et al. 2005, Tymchuk et al. 2005
Seasonal regulation of feeding is disrupted in transgenics (i.e. they do not slow down in winter as do wild types). Transgenics also show stronger growth response to increasing temperatures and food availability.	Devlin et al 2004b, Lõhmus et al. 2008, Lõhmus et al. 2010
Transgenics have altered use of dietary energy (i.e. carbohydrates), and preferentially use lipid as an energy source, sparing protein. Given all the food they want, behavioral effects of GH cause fish to deposit large amounts of fat, whereas under ration limiting conditions, the fish have lower lipid levels. Gut surface area, feed conversion, and digestive capacity enhanced. Starvation endurance is not greatly affected.	Blier et al. 2002, Higgs et al. 2009, Leggatt et al. 2009, Oakes et al. 2007, Raven et al. 2006, Stevens and Devlin 2000a, Stevens et al. 2005, Sundström and Devlin 2010
Disease resistance is lower in the growth-hormone transgenic coho strain	Jhingan et al. 2003
Growth-hormone transgenesis strongly affects expression of many genes. Growth-hormone transgenesis and domestication affect gene expression in similar, but not identical, ways.	Devlin et al. 2009, Mori et al. 2007, Mori and Devlin 2009, Rise et al. 2006, Roberts et al. 2004
GH and IGF-I elevated, the latter being strongly affected by growth rate (i.e. transgenics kept to a wild-type growth rate have normal IGF-I levels). Thyroid hormone systems strongly affected in growth-hormone transgenics.	Devlin et al. 2000, Eales et al. 2004, Kang and Devlin 2004, Raven et al. 2008
Abnormalities in cellular structure and organism morphology can occur in some strains of transgenic salmon.	Devlin et al. 1995b, Hill et al. 2000, Ostenfeld et al. 1998
Transgene structure is complex, and DNA integrates near integrated horizontally transmitted DNA (i.e. from infectious agents (parasites).	Uh et al. 2006
Detection of transgenic by molecular methods can be reliable	Masri et al. 2002, Rehbein et al. 2002
Triploidy induction does not produce 100% triploids in transgenics. Exceptions are gynogens and aneuploid individuals arising from incomplete retention of paternal chromosomes. These exceptions can contain the transgene, but their ability to transmit it to progeny is not yet known.	Devlin et al. 2010
The traits of every growth-enhanced strain are unique and triploidy impairs growth.	Devlin et al. 1994, Devlin et al. 1995a, Devlin et al. 2004a

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