Transgenic Salmon Aquaculture: Concerns and Possible Solutions

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Transgenic Salmon Aquaculture: Concerns and Possible Solutions

An Interactive Qualifying Project Report

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WORCESTER POLYTECHNIC INSTITUTE

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Degree of Bachelor of Science

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The overall goal of this project was to identify and evaluate the remaining technical, procedural, and regulatory issues necessary to ensure the safe introduction of commercial transgenic fish aquaculture in the United States, especially for AquaBounty Technologies (ABT) AquAdvantage salmon (AAS). In order to achieve this goal, interviews were conducted with experts in the field and various stakeholders. The group compiled information from these interviews and background research to draw conclusions and make recommendations. We conclude that several key disagreements between ABT and consumer groups have likely resulted from miscommunication issues within the publically available data, and provide recommendations for resolving the disputes.
ACKNOWLEDGEMENTS

We would first like to thank the experts and stakeholders in the area of transgenic salmon and their FDA approval that were willing to speak with us on this project. Specifically, we would like to thank Henry Clifford, the vice-president of marketing and sales at AquaBounty Technologies Inc. (ABT) for providing insight into the views and aims of the company. We would also like to thank Bob King, Colin O’Neil, Daniel Sousa, Siobhan DeLancey, and Eric Nelson for their help understanding and identifying the remaining regulatory and procedural aspects surrounding this topic. Additionally, we would like to thank Tim Schwab, Richard Howard, Fredrick Sundström, Robert H. Devlin, Ian Fleming, Garth Fletcher, Eric Hallerman, Marion Nestle, Joel Bader, and others for their expertise and invaluable information provided on technical and environmental issues surrounding the FDA approval of ABT’s AquAdvantage salmon.

Last, but certainly not least, we would like to thank Professors Dave Adams and Dominic Golding for their combined efforts in aiding us in finding prospective interviewees and rigorously editing our drafts. We would like to specifically thank Professor Dave Adams for supplying us with a wealth of technical information, and Professor Dominic Golding for his guidance and information about legislation and regulation.
EXECUTIVE SUMMARY

The worldwide demand for salmon has increased steadily in recent decades due to its high protein and nutritional content. Aquaculture, the process of raising fish and other aquatic plants or animals under controlled conditions, has come to play an important role in addressing the increasing demand for high-protein food. About two-thirds of the fish consumed in the United States is farmed, 94% of which is imported from around the globe, putting increasing pressure on both the fishing and aquaculture industries.

Transgenic animals contain a foreign gene inserted into the genome for the purpose of imparting new properties to the animal. The aquaculture industry has sought to use transgenics to produce fish better than their wild-type (WT) counterparts. Some transgenic lines have been created that grow faster, while others are resistant to disease or produce anti-freeze protein in their blood to allow their survival in colder temperatures. Although no transgenic animal has yet received approval for human consumption, the Maynard (MA) Biotechnology company AquaBounty Technologies Inc. (ABT or AB) is the closest to achieving FDA approval for a genetically modified (GM) animal for human consumption. AquAdvantage salmon (AAS) is a GM Atlantic salmon with a DNA construct containing Chinook growth hormone (GH) under the control of an oceanic pout (OP) antifreeze protein promoter sequence. ABT has provided data showing that AAS grows up to three times faster than normal Atlantic salmon, but the mechanism for how AAS grow faster is not completely known at this time. The levels of GH in AAS muscle and blood are approximately equal to non-GM Atlantic salmon, but ABT speculates that the pout promoter allows the efficient production of GH in a broad range of tissues in close proximity to GH-receptors which accelerates growth. Faster growing fish would be useful in transgenic aquaculture because they would aid in more quickly producing meat for consumption.
ABT plans to use high pressures to make their fish eggs triploid (containing one extra set of chromosomes) which sterilizes the eggs. The eggs will be produced on Prince Edward Island (Canada), reared inside inland tanks in Panama, and the frozen meat shipped to the U.S. for consumption.

However, some organizations, businesses, and fishermen have voiced strong opposition to GM food, including salmon. The opposition includes the Consumers Union, Food and Water Watch, and the Center for Food Safety. Their collective opinion that AAS should not be approved by the FDA stems from their belief that the ABT studies provided to the FDA underreport or fail to detect health problems and abnormalities in the AAS fish, that the environmental risk assessment performed by the FDA was inadequate, that the FDA’s New Animal Drug Application (NADA) is unsuitable for reviewing transgenic animals for consumption, and that AAS is “still-experimental technology.” (Hansen, 2013) As of April 26, 2013 the public comment period on the Environmental Assessment for AAS closed, and the FDA is in its final deliberations.

The FDA Review of AAS

The United States currently has no legislation specifically regulating transgenic animals. Under the Coordinated Framework for the Regulation of Biotechnology, the FDA regulates GM animals by the Federal Food, Drug, and Cosmetic Act (FFDCA) as a new animal drug application (NADA), also termed an investigational new animal drug application (INAD). Under the FFDCA guidelines, Section 512, if an article is intended to alter the structure or function of the animal it is considered a new animal drug, so all rDNA constructs that change the characteristics of an animal qualify as a new “drug.”
In April 1993, A/F Protein (later renamed AquaBounty Technologies) approached the FDA for guidance on its application, which eventually resulted in a formal filing of an Investigational New Animal Drug (INAD) application on September 14, 1995. The FDA review process included several steps, some of which were unprecedented. As with all new drug applications, the sponsor (ABT) was required to conduct and pay for studies to obtain regulatory compliance. The first step required ABT to define the product for the FDA. The next step included a molecular characterization of the construct, in which ABT’s recombinant DNA (rDNA) construct was assessed. Following this, a step for the Molecular Characterization of the GE Animal Lineage occurred, where studies were submitted to prove that the animal does not change over many generations. The next step was the phenotypic characterization, where the FDA determined whether the rDNA construct was safe for the GM animal. Next, a Durability Assessment was performed where ABT’s plans for ensuring that the produced animals are equivalent to the animals evaluated by the FDA were reviewed. Since AAS is intended for human consumption and is considered a new drug, an extra step was implemented to assess safety for consumption which compared AAS salmon against non-GM salmon. In accordance with the requirements of the National Environmental Policy Act (NEPA), the FDA requested that ABT perform an environmental assessment to evaluate the potential impact AAS might have on the environment. Although the Environmental Protection Agency (EPA) is sometimes involved in this step, in this case it was not because AAS will not be raised in the U.S. Uniquely, for the case of GM animals, the FDA required the formation of a committee of scientific experts and veterinarians (the Veterinary Medicine Advisory Committee, VMAC) to review ABT’s submitted data, and required the VMAC to hold a public meeting after its review to present its findings and receive public comments. Although not required to do so, two weeks in advance of
the September 2010 public meeting, ABT made an unprecedented effort for transparency by making its 172-page VMAC briefing package and 84-page environmental assessment publically available. The VMAC meeting began with a public orientation session, followed by a discussion of the strengths and weaknesses of the data by VMAC members. The next steps included a public commentary period, a charge to the VMAC to expand the data provided for review, further VMAC committee deliberations, and the VMAC Chair’s report submitted to the FDA. The Chairman’s Report (agreed to by all members of the VMAC) concluded that the rDNA construct was safe for the fish, that no harm would come from ingesting the fish, that AAS grew faster, and the environmental risks were appropriately mitigated by ABT’s proposed sterility and containment system. The FDA’s Finding of No Significant Impact (FONSI) was submitted on May 4, 2012 for public comment. The public comment period for the FDA’s Environmental Assessment and FONSI closed on April 26, 2013. The FDA is currently reviewing the public comments as part of its final deliberations prior to making its final decision.

Project Goals and Methodology

The goals of our project were to develop a comprehensive assessment of the origins, technology, and current status of transgenic fish aquaculture; characterize what key stakeholders believe are the remaining hurdles to the introduction of commercial transgenic fish aquaculture and how the hurdles should be addressed; assess the scientific procedures used to help make transgenic aquaculture environmentally safe; and recommend alternative solutions to remaining problems that introducing transgenic fish for human consumption may cause in the United States.
Findings / Conclusions / Recommendations

With respect to technical issues, one problem encountered in our project was the use of different promoters and GH genes in the GH-fish experiments published to date, which made it difficult to directly compare the published studies, especially when trying to make predictions about the effects of GH on AAS fish. A key argument identified in our interviews was ABT’s claim of no elevated GH in plasma or muscle in AAS, while consumer groups argued the VMAC data showed GH elevation in the plasma. Our investigation showed that both sides are partially correct. The VMAC average value for GH in AAS plasma (39.9 ng/ml) was higher in AAS than the comparator groups (28.2 in non-GM siblings and 20.5 in wild-type controls), but the small differences in the means are not statistically significant. So, although AAS appears in the VMAC data to have a slightly higher means (validating the consumer group’s argument) the slightly higher number is not statistically significant (validating ABT claims). Backing up ABT’s argument that AAS fish show no GH elevation is the VMAC data showing that AAS pituitaries appear normal (unlike with some MT-GH-fish that strongly over-express GH and show reduced pituitary sizes), and the AAS show no more morphological abnormalities than is observed in other sterile triploid fish (see food safety section). Interestingly, the GH levels in AAS muscle reported by the VMAC were actually below the limits of detection of the assay (and were also not shown in the table), so this initially made it difficult to explain AAS’ rapid growth. In fact, the mechanism of rapid growth remains unknown to ABT, and they speculate that the pout promoter restricts the GH expression to a wide range of tissues in close proximity to GH receptors, allowing rapid growth without truly elevating the GH levels above those seen in WT fish. We recommend more thoroughly investigating the mechanism of AAS rapid growth to help explain this rapid growth, which should help AAS fish gain public acceptance.
With respect to the FDA’s environmental assessment, our interviews with consumer groups indicated they strongly believe the FDA’s environmental review was not rigorous, that the FDA does not have the appropriate expertise to perform a rigorous review, and that no qualified outside agencies were consulted in the FDA review. Our research identified two agencies with strong expertise in environmental science, the National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (FWS). Public documents identified in our research indicate those two agencies were indeed consulted by the FDA in their review; the NMFS and FWS were provided with the environmental assessment and FONSI, and concurred with the FDA’s finding of “no effect”. Our interview with a member of the FWS indicated he thought the FWS review was mostly informal, so to resolve this issue we recommend that the FWS and NMFS more thoroughly document for the public the extent of their review and any dissenting opinions, if any. All of our interviews with consumer groups agreed that FWS and NMSF had considerable expertise in this area and would be appropriate agencies for rigorously performing environmental assessments.

With respect to bills submitted to congress, our findings indicate that no current U.S. federal law requires the labeling of GM food. Although several bills have been introduced into the senate and house requiring the labeling of GM food, the bills remain under consideration in committees and have not been brought to a vote. We recommend that these bills be passed.

With respect to the issue of whether AAS meat is safe to eat, our interviews with consumer groups indicated that their most serious concern was an apparent elevation of the levels of a hormone, insulin-like growth factor-1 (IGF-1), which is known to cause cancer in humans. The consumer groups argue the VMAC data showed that only 6 AAS fish were evaluated for hormone levels (and the mean IGF-1 levels were elevated), while ABT claimed
that 30 AAS fish were evaluated (and the mean was not elevated). As was the case with GH levels, our investigation indicates that both sides are partially correct. The VMAC data shows IGF-1 levels for only 6 AAS fish (validating the consumer group’s point), but the report text indicates that the FDA-approved protocol requires any values below the lower limits of detection to be excluded (24 fish were beneath the lower limits of detection, so indeed 30 AAS fish were evaluated, validating ABT’s claim). Although the AAS average IGF-1 levels appeared to be slightly elevated (consumer claims), the apparent increase was not statistically significant (ABT’s claim). When the 24 non-included samples were included, the AAS means was actually lower than for non-GM salmon. For the sake of clarity, in the future we recommend including all numerical values in the calculations. Our research also determined that the amount of IGF-1 produced in the human digestive tract daily vastly exceeded the amount that would be consumed daily by eating AAS, so consumer worries about IGF-1 likely are not valid.

With respect to food allergenicity, our findings identified several studies performed to determine whether AAS GH showed similarities to other known allergens, and the findings were negative. In addition, there appears to be no medical consensus about what levels of allergen would constitute a problem, so we recommend that those studies be performed to provide medical guidelines for future GM applications. Although triploid fish in general show a higher incidence of morphological problems, we found no evidence that these would cause problems with meat consumption.

With respect to environmental issues, ABT has proposed using what most of our interviewees agreed are the most sophisticated barriers in the industry to prevent escape, and we see little room for improvement there. However, our findings indicate that no facility can guarantee a lack of escape, and there is evidence for the escape of salmon from inland tanks, so
we deem it important to at least consider the effects of a potential escape. With respect to fish sterility, most stakeholders interviewed agreed that the use of triploid sterile fish in aquaculture is widespread and is the current industry standard. ABT’s own assessment of percent sterility varied from 95-100% based on data from relatively small batches of eggs, so based on this data some fertile AAS fish might be present. However, no data exists for the expected percent sterility for scaled-up batches the size proposed in Panama, so we agree with the FDA’s requirement that ABT monitor the percent sterility of their large batches of eggs, and report the data to the FDA. We also conclude that ABT’s proposed thermal barrier adds an extra security layer in case of an escape. The cold 15°C water in the highland streams surrounding the Panama site is ideal for salmon culture and survival, but salmon do not feed above 23°C, so any escaped AAS according to the VMAC could only survive for a few days in downstream warmer waters. The presence of several dams downstream would hinder the rapid migration of AAS to colder deep ocean waters, increasing their chance of starvation, but we recommend that ABT monitor the surrounding stream waters for AAS escapes, and report that data to the FDA and Panamanian authorities for further monitoring. Although brown trout are capable of cross-breeding (hybridizing) with AAS, ABT’s data indicates that no brown trout are present in Panama highlands and would not survive the upstream migration through warm waters. Even if escaped AAS were to hybridize with brown trout, we identified one published study indicating the AAS-trout hybrid is sterile, and if so, hybridization would not be a long-term problem. However, another study indicated the AAS-trout hybrids might be fertile, so we recommend this topic of whether AAS-hybrids are sterile be further researched.

With respect to fish behavior as it relates to potential escapes, we identified two main issues: potential alterations in the pituitary gland in GH-salmon in general (which could
negatively affect fish physiology), and the potential spread of the transgene into WT fish (Trojan gene effect, which would potentially negatively affect the WT salmon population). The former issue was shown in published studies to occur in GM salmon using a different metallothionein (MT) promoter that elevates GH in the serum, so the consumer worries about this issue may be valid for MT-GH-salmon. But ABT’s data indicates there is no alteration to the pituitary gland in AAS, likely due to the lack of elevated serum GH, so this likely is not a major problem. But in keeping with best practice, we recommend that ABT analyze gene expression patterns more thoroughly in the AAS pituitary glands to obtain a more thorough assessment of potential changes. The Trojan gene effect was shown in published reports to theoretically be capable of leading to extinction of WT fish populations if the GM fish showed a strong mating advantage, so it is easy to see where the public concerns come from on this topic. However, subsequent experiments performed by several leading fish experts showed that AAS actually showed a reduced mating success with non-GM Atlantic salmon, so a Trojan effect is likely not possible in this case. Published studies showing that AAS can hybridize with brown trout or Atlantic salmon makes it easy for consumer groups to focus on just that data. But we deem it equally important to consider whether such hybrids are actually more fit than WT populations.

With respect to sustainability, ABT indicates that one of the main reasons they wish to farm GH-salmon is that it will reduce demands on the WT salmon populations. Our findings indicate that some scientists believe that farming the animal at the top of the food chain may not be sustainable, and may actually further diminish the supply of natural food for this top predator. Sustainability environmental studies are relatively few for Atlantic salmon, so we recommend that such studies be continued.
With respect to economics, some of the stakeholders interviewed in this project pointed out that even if ABT works at maximal capacity in the Panama facility, the profit earned may not be sufficient to bring the company out of debt, unless ABT’s expands into other countries or into the U.S. itself. Given this, it is possible that the FDA in the near future may receive an application from ABT for approving AAS culture in the U.S. If so, the environmental impact studies will need to be expanded to include a variety of U.S. streams and species, and based on our interviews with consumer groups would likely receive strong opposition.
## AUTHORSHIP

<table>
<thead>
<tr>
<th>Section</th>
<th>Primary Author</th>
<th>Secondary Author</th>
<th>Primary Editor</th>
<th>Secondary Editor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 Introduction</td>
<td>MC, MG, CM, JP, CR</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>2.0 Literature Review</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>2.2</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
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<tr>
<td>2.3</td>
<td>MG</td>
<td>----</td>
<td>MG</td>
<td>----</td>
</tr>
<tr>
<td>2.4</td>
<td>JP</td>
<td>----</td>
<td>JP</td>
<td>----</td>
</tr>
<tr>
<td>2.6.1</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>2.6.2</td>
<td>JP</td>
<td>----</td>
<td>JP</td>
<td>----</td>
</tr>
<tr>
<td>2.6.3</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>2.6.4</td>
<td>CR, CM</td>
<td>----</td>
<td>CR, CM</td>
<td>----</td>
</tr>
<tr>
<td>2.6.9 - 2.6.11</td>
<td>JP</td>
<td>----</td>
<td>JP</td>
<td>----</td>
</tr>
<tr>
<td>2.6.12</td>
<td>MC</td>
<td>----</td>
<td>MC</td>
<td>----</td>
</tr>
<tr>
<td>3.0 Methods</td>
<td>MC, MG, CM, JP, CR</td>
<td>----</td>
<td>CR, CM</td>
<td>----</td>
</tr>
<tr>
<td>4.0 Results/Findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>MC, JP</td>
<td>----</td>
<td>JP</td>
<td>----</td>
</tr>
<tr>
<td>4.2.1 – 4.2.1A</td>
<td>CR</td>
<td>----</td>
<td>CR</td>
<td>----</td>
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<tr>
<td>4.2.1B</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>4.2.2</td>
<td>MC, CR</td>
<td>----</td>
<td>CR</td>
<td>----</td>
</tr>
<tr>
<td>4.2.3</td>
<td>MG</td>
<td>----</td>
<td>MG</td>
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<tr>
<td>4.2.4</td>
<td>CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>4.2.5</td>
<td>CR</td>
<td>----</td>
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<td>4.3.1</td>
<td>MC, CM</td>
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<td>CM</td>
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<td>4.3.2</td>
<td>MC</td>
<td>----</td>
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<td>4.3.3</td>
<td>MC, CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>4.3.4</td>
<td>MC, MG</td>
<td>----</td>
<td>MG</td>
<td>----</td>
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<td>4.4</td>
<td>MC</td>
<td>----</td>
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<td>----</td>
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<tr>
<td>4.5</td>
<td>MG, CM</td>
<td>----</td>
<td>MG, CM</td>
<td>----</td>
</tr>
<tr>
<td>5.0 Conclusions/Recommendations</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>5.1</td>
<td>JP</td>
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<td>JP</td>
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<tr>
<td>5.2</td>
<td>CR</td>
<td>----</td>
<td>CR</td>
<td>----</td>
</tr>
<tr>
<td>5.3</td>
<td>MG</td>
<td>----</td>
<td>MG</td>
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<tr>
<td>5.4</td>
<td>MG</td>
<td>----</td>
<td>MG</td>
<td>----</td>
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<tr>
<td>5.5</td>
<td>MC, CM</td>
<td>----</td>
<td>CM</td>
<td>----</td>
</tr>
<tr>
<td>5.6</td>
<td>MG, CM</td>
<td>----</td>
<td>MG, CM</td>
<td>----</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iii</td>
</tr>
<tr>
<td>Executive Summary</td>
<td>iv</td>
</tr>
<tr>
<td>Authorship</td>
<td>xiv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>xv</td>
</tr>
<tr>
<td>Figures</td>
<td>xvi</td>
</tr>
<tr>
<td>Tables</td>
<td>xvi</td>
</tr>
<tr>
<td><strong>1.0 Introduction</strong></td>
<td>01</td>
</tr>
<tr>
<td><strong>2.0 Literature Review</strong></td>
<td>05</td>
</tr>
<tr>
<td>2.1 Current Condition of the World’s Fisheries</td>
<td>05</td>
</tr>
<tr>
<td>2.2 Background on Aquaculture</td>
<td>06</td>
</tr>
<tr>
<td>2.3 Salmon Aquaculture</td>
<td>10</td>
</tr>
<tr>
<td>2.4 Transgenic Technology Introduction</td>
<td>12</td>
</tr>
<tr>
<td>2.5 Transgenic Fish</td>
<td>14</td>
</tr>
<tr>
<td>2.6 AquaBounty’s AquAdvantage Salmon</td>
<td>17</td>
</tr>
<tr>
<td><strong>3.0 Methods</strong></td>
<td>40</td>
</tr>
<tr>
<td><strong>4.0 Results/Findings</strong></td>
<td>44</td>
</tr>
<tr>
<td>4.1 Technical Issues</td>
<td>44</td>
</tr>
<tr>
<td>4.2 Regulatory Issues</td>
<td>55</td>
</tr>
<tr>
<td>4.3 Environmental Issues</td>
<td>66</td>
</tr>
<tr>
<td>4.4 Ecological Issues</td>
<td>73</td>
</tr>
<tr>
<td>4.5 Economic/ Sustainability Issues</td>
<td>77</td>
</tr>
<tr>
<td><strong>5.0 Conclusions/Recommendations</strong></td>
<td>80</td>
</tr>
<tr>
<td>References</td>
<td>97</td>
</tr>
<tr>
<td><strong>Appendix</strong></td>
<td>106</td>
</tr>
<tr>
<td>Appendix A: Salmon Growth Cycle</td>
<td>106</td>
</tr>
<tr>
<td>Appendix B: Initial List of Interview Questions</td>
<td>108</td>
</tr>
<tr>
<td>Appendix C: Interview Preamble</td>
<td>111</td>
</tr>
<tr>
<td>Appendix D: Sample of Amended Interview Questions</td>
<td>112</td>
</tr>
</tbody>
</table>
FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure-1</td>
<td>Global Expansion of Fishing Operations</td>
<td>05</td>
</tr>
<tr>
<td>Figure-2</td>
<td>Annual Fish Consumption</td>
<td>07</td>
</tr>
<tr>
<td>Figure-3</td>
<td>Stagnating Wild Catch: Growing Aquaculture</td>
<td>08</td>
</tr>
<tr>
<td>Figure-4</td>
<td>Production Cycle of <em>Salmo salar</em></td>
<td>11</td>
</tr>
<tr>
<td>Figure-5</td>
<td>Regulatory Review Process for GE Animals</td>
<td>20</td>
</tr>
<tr>
<td>Figure-6</td>
<td>Regulatory Environmental Assessment Steps Taken for AAS</td>
<td>23</td>
</tr>
<tr>
<td>Figure-7</td>
<td>Schematic Summary of Containment Measures at the Grow-out Facility</td>
<td>34</td>
</tr>
</tbody>
</table>

TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table-1</td>
<td>Summary of GH and Cold Tolerant Transgenic Salmon</td>
<td>16</td>
</tr>
<tr>
<td>Table-2</td>
<td>Key Components of Physical Containment Measures</td>
<td>33</td>
</tr>
<tr>
<td>Table-3</td>
<td>Summary of Organizations and Stakeholders Interviewed</td>
<td>42</td>
</tr>
<tr>
<td>Table-4</td>
<td>Current Legislation Regarding Transgenic Fish</td>
<td>65</td>
</tr>
<tr>
<td>Table-5</td>
<td>Global Salmon Escape Events (mostly Atlantic salmon)</td>
<td>67</td>
</tr>
</tbody>
</table>
1.0 INTRODUCTION

Overfishing, a consequence of the world’s continuous growth, has placed a tremendous stress on the global ecosystem causing a domino effect of changes within the fishing industry (SOFIA, 2012). According to the United Nations Food and Agricultural Organization (FAO), eighty-five percent of the world’s marine stocks are either fully exploited or overfished, driving accelerated growth in the farmed seafood industry (SOFIA, 2012). Aquaculture, the process of raising fish and other aquatic plants or animals in controlled conditions, plays an important role in addressing the increasing demand for high-protein food. A large variety of species have been cultured including carp, tilapia, catfish, salmon, lobsters, oysters and scallops (Nash, 2011). The potential benefits of aquaculture include the ability to raise fish in a more controlled environment away from natural predators, the ability to raise increased biomass in less space, providing more fish for consumption, producing meat faster, saving diminishing fish populations, and reducing cost to the consumer. Globally, in 2008, aquaculture accounted for 45.7 percent of the world’s fish food production for human consumption (Nash, 2011). Not all cultured fish go directly to the supermarket; aquaculture products can be used to replenish fish stocks in lakes or rivers that are popular sport fishing locations, used in landscaping water fountains, or sold as bait. But aquaculture has several potential disadvantages, such as the increased spread of fish diseases, increased costs associated with pens, feed, and sanitation, and negative potential effects on the environment.

The use of transgenic fish represents a new development in aquaculture intended to enhance productivity. But at the same time, this new approach raises a variety of new scientific, legal, ethical, and public relations issues. Transgenics is the use of genetic engineering to introduce a foreign gene into the DNA genome of a host animal or plant for the purpose of
giving the host heritable new and useful properties. Transgenic technology has already been used to create genetically modified (GM) animals in many species, including fish, pigs, rabbits, mice, sheep and cattle. For example, transgenic animals have been created that produce drugs in their milk for treating patients with blood clotting disorders, provide disease models for testing new therapies for Parkinson’s disease, cancer, Alzheimer’s disease, and AIDS, or produce pig organs that may be immunologically compatible with human patients awaiting transplants. Transgenic fish have been created to enhance growth, increase cold tolerance, increase respiratory performance, and improve nutritional value.

With respect to transgenic food sources, Calgene’s Flavr Savr tomato was approved for human consumption in 1994 (U.S. Department of Agriculture, 1994), but no transgenic animal has yet received approval for human consumption. Maynard (MA) Biotechnology company AquaBounty Technologies Inc. (ABT or AB) is the closest to achieving FDA approval for a GE animal for human consumption. Their AquAdvantage Salmon (AAS) has been under review since September 14, 1995, and there is currently no date set to receive FDA approval. ABT indicates the AAS grows up to three times faster than normal Atlantic salmon. Faster growing fish would be useful in transgenic aquaculture because they would aid in more quickly producing meat for consumption, which is needed since the global population in October 2011 reached the seven billion mark with estimates of it reaching nine billion within the next few decades (Holtzman, 2013).

The potentially imminent commercialization of the first genetically engineered animal for human consumption is the focus of this IQP project. Although the need to manufacture more food for the growing population is undeniable, the desire to pursue the marketing of genetically modified salmon in an attempt to help solve this problem is not shared by all parties. The greatest
advocate of GM salmon is AquaBounty Technologies Inc., who claim AAS is an environmentally sustainable alternative to current farmed salmon and poses no threat to wild salmon populations. Additionally, ABT’s data indicates that AAS have the potential to grow to market size in half the time of conventional salmon, but in all other respects are identical to the wild type (AquaBounty Technologies Inc., 2013). However, many organizations, businesses, and fishermen have voiced strong opposition to GE salmon, with the key players being the Consumers Union, Food and Water Watch, and the Center for Food Safety. Their collective opinion, that AAS should not be approved by the FDA, stems from their belief that the ABT studies provided to the FDA underreport or fail to detect health problems and abnormalities in the AAS fish, that the environmental risk assessment performed by the FDA was inadequate, that the FDA’s New Animal Drug Application (NADA) is unsuitable for reviewing transgenic animals for consumption, and that AAS is “still-experimental technology.” (Hansen, 2013) As of April 26, 2013 the public comment period on the Environmental Assessment for AquAdvantage salmon closed, and the FDA is in final deliberations on the approval of the GE salmon.

Thus, the focus of our project is AquaBounty’s AAS fish, with the overall goal of identifying and evaluating the remaining technical, procedural, and regulatory issues necessary for helping ensure the safe introduction of commercial transgenic fish aquaculture in the United States, especially AAS. To accomplish this goal, we began by developing a comprehensive assessment of the origins, technology, and current status of transgenic aquaculture. We characterized what key stakeholders believe are the remaining hurdles for introducing commercial transgenic fish aquaculture and how the remaining the hurdles or misunderstandings should be addressed. We also evaluated the benefits and risks associated with transgenic fish consumption, and assessed the scientific procedures used to help make transgenic aquaculture
environmentally safe. Our findings indicate that the format for ABT’s data presented to the VMAC has resulted in miscommunication on both sides of the argument, and we make recommendations for avoiding such miscommunications in the future.
2.0 LITERATURE REVIEW

AquaBounty Technologies Inc. (ABT’s) AquAdvantage salmon (AAS) may soon be the first genetically modified animal approved by the FDA for human consumption. Due to the industrialization of fishing, there has been a collapse in several wild-type fish populations. As a way to cope with the depletion of our natural fish sources, aquaculture has increased worldwide in an attempt to feed the growing population’s increasing demand for fish, but it also comes with environmental and food safety concerns.

ABT is seeking FDA approval for their AAS, whose genetic material has been manipulated to grow to maturity within half the time of conventional salmon. ABT has been working on getting FDA approval of AAS since September 14, 1995, 18 years, having to conduct a series of environmental and consumer health risk assessments, and then waiting for the FDA review process to conclude. Stakeholders have a wide range of opinions on this topic regarding regulatory, environmental, food safety, and sustainability concerns.

2.1 Current Condition of the World’s Fisheries

In the 1950s and 1960s, the industrialization of fishing led to a major boost in global fish catches, causing widespread over-fishing and the collapse of many fish stocks (Allsopp, 2009).

From the 1950s to 2005 (Figure-1), the annual global catch increased from 19 million tons to 80 million tons, due to the continued southward expansion of fishing operations and the exploitation of new areas (Palk, 2011). As of 2008, total world production was 142 million tons of fish, of which 100 million tons were marine fish. Nearly 80 million tons of marine fish were captured, while the remaining 20 million was produced from aquaculture. Out of the 142 million tons produced both from fresh water and marine sources, approximately 115 million tons was used for human food, with the rest used for fishmeal, fish oil, and other products. The accession of fully exploited or over-exploited fish correlates with a slow decline of marine captures over the past several years. (SOFIA, 2012)

In particular, there has been a notable decline in the world’s predatory fish. Scientists from the Future of Marine Animal Populations (FMAP) project of the Census of Marine Life program claim that up to 90 percent of all large predatory fish such as cod, sharks, salmon, and tuna have been depleted (Worm et al., 2005). The mean trophic level has declined to only eleven percent relative to 1950, which is indicative of the amount of pressure that human exploitation is imposing on the ocean (Christensen, 2003).

2.2 Background on Aquaculture

Although not without problems of its own, aquaculture is being increasingly viewed as a potential solution to the world’s depleting fisheries. The term aquaculture refers to the cultivation of both marine and freshwater species, and can range from land-based to open-ocean production (Maine Department of Marine Resources Staff, 2006). Aquaculture is an ancient practice. Australian aboriginals are thought to have cultured eels as early as 6000 BC, and in 2500 BC the Chinese are thought to have raised carp in freshwater lakes (Bardach et al., 1972; McCoy, 1987;
Nash, 2011). Species commonly produced by aquaculture today include carp, salmon, tilapia, catfish, shrimp, crayfish, crabs, lobsters, oysters, and scallops. However, modern aquaculture is a recent phenomenon, with the domestication of fish being described as having taken place ‘overnight’ (Howarth, 2006). A greater understanding of fish reproduction and genetics, as well as technological strides in the equipment used to hold and rear fish have led to expedited productivity.

Aquaculture currently supplies approximately 55 million tons of fish out of the total 118 million tons consumed annually by humans, and a further increase in aquaculture production could potentially reduce pressures on the wild fish populations. (SOFIA, 2012) Worldwide, fish meat consumption is increasing while wild type fish populations are declining (Nash, 2011). The wild type fish have declined due to various environmental issues such as pollution and the damming of rivers, but over-fishing is widely recognized as the root of the problem (Allsopp, 2009). Data from the United Nation’s Food and Agricultural Organization (FAO) shown in Figure-2 illustrates the steady increase in fish consumption in the last 50 years, increasing from 9.9 kg per capita in the 1960s to 17 kg per capita in 2007.

The consumption of fish products is expected to increase strongly in the coming decade, reaching 20.6 kg in 2022, up from 19 kg on average in 2010-12 (SEAFOOD.COM NEWS, 2013). Despite the U.S. and other countries attempts to help stocks recover by putting fishing quotas in place, the world’s fish stocks are gradually becoming over-exploited or depleted, increasing the need for aquaculture. In 2009, the United Nations Food and Agriculture Organization (FAO) determined that aquaculture has grown in the tons of fish produced by 8% per year for the past 30 years, and predicted that the world’s increased demand for fish consumption will only be met by aquaculture (Naylor et al., 2009; FDA VMAC, 2010). Figure-3 shows the increasing role that aquaculture will likely play in meeting the demand for fish in the future (FAO, 2012).

![Figure 3: Stagnating Wild Catch: Growing Aquaculture. Retrieved from http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%202018.juli_h%C3%B8y%20tl.pdf](http://www.marineharvest.com/PageFiles/1296/2012%20Salmon%20Handbook%202018.juli_h%C3%B8y%20tl.pdf)

As wild fish catches have decreased slowly over time, aquaculture has grown to meet the demand for fish. In 2004, aquaculture provided 32% of the world’s fish production (Naylor et al.,
and by 2011 aquaculture provided 42% of the fishery output for human consumption (Marine Harvest, 2012). The FAO estimates that by 2030, aquaculture will have increased from 45 million tons to 85 million tons of fish per year (Marine Harvest, 2012).

2.2.1 Aquaculture Systems

Aquaculture can be conducted in either freshwater or salt water, using inland tanks or open water net pens. Freshwater aquaculture can occur in either naturally or artificially created ponds that are most usually on agricultural land areas. Marine aquaculture usually takes place in cages or net pens directly placed in coastal waters, but sometimes can involve ponds built along the coast that are filled with seawater. Land-based aquaculture can take the form of one of two systems: re-circulating or raceway. In a re-circulating system, the fish remain in tanks where the water is treated and re-circulated. In a raceway system, water from local sources is directed through the fish farming operation. At the same time, there are various degrees of cultivation used by fish farmers. At one end of the spectrum all required food, pest and disease control drugs are provided to the fish by the fish farmers, and at the other end of the spectrum the fish are left to fend for themselves, feeding off the available food. (Allsopp, 2009)

2.2.2 Environmental Hazards of Aquaculture

Due to dead fish, fish feces, and uneaten food pellets, expansion of aquaculture has been associated with eutrophication and stimulation of unwanted algal blooms. When the waste decomposes, organic and inorganic nutrients are released either directly or indirectly into the ocean, causing algal growth, and in some cases oceanic dead zones due to the depletion of oxygen in the water (Allsopp, 2009). In terms of increased spread of disease and parasites among
wild type populations, evidence shows that this is due in large part to the result of infested farmed fish escaping from their holding pens and transferring their diseases and parasites directly to the wild type (Allsopp, 2009). A major environmental concern of aquaculture is the potential for escape and interbreeding with wild type fish (Allsopp, 2009). Many studies have indicated that the inter-breeding of farmed and wild salmon can result in reduced lifetime success, lowered fitness, and lower production over at least two generations (Thorstad et al., 2008).

2.3 Salmon Aquaculture

Due to its high content of protein and beneficial omega-3 fatty acids, the consumption of Salmon meat has increased considerably. Salmon consumption in the U.S. increased nine-fold between 1987 and 1999 (Knapp et al., 2007; FDA VMAC, 2010). In 1989, salmon comprised about 5% of the fish consumed in the U.S., and by 2004 that amount had almost tripled to 13% (Knap et al., 2007). The National Marine Fisheries Service (NMFS) estimated that about two-thirds of the salmon consumed in the United States is farmed, and 94% is imported from Canada, Chile, Norway, and Scotland (FDA VMAC, 2010). These findings place strong demands on the aquaculture industry to increase production.

Salmon belong to the family Salmonidae, consisting of the genus Salmo (containing Salmo salar, the Atlantic salmon) and the genus Oncorhynchus (containing the Pacific salmon). Worldwide, there are three main populations of Atlantic salmon: North American, European, and Baltic (FDA VMAC, 2010). All three populations are native to the North Atlantic ocean. The Atlantic salmon differ from the Pacific in that they do not die after spawning, so the adults can return to the ocean. The longer Atlantic salmon live in the ocean before spawning, the larger they can grow. A more complete explanation of the salmon life and growth cycle is shown in
Appendix A. For salmon aquaculture, key factors in monetary return are how fast the fish can grow and how efficiently they can metabolize the feed.

The production cycle for Atlantic salmon in aquaculture is relatively simple as shown in a diagram prepared by the FAO in Figure 4. The eggs are retrieved from pregnant females in a process called stripping (diagram left) and cultured in cold fresh water less than 10°C (to mimic the winter) in trays until they hatch (diagram lower left). The hatched fry or alevins are cultured in freshwater tanks or stream cages through the smolt and parr stages (lower center), and after 8-16 months are transferred to seawater cages (lower right). The broodstock are grown for about 2 years (upper right), and are then harvested for food or breeding.

Figure 4: Production Cycle of Salmo salar. Retrieved from http://www.fao.org/fishery/culturedspecies/Salmo_salar/en
This production cycle mimics the time spent between fresh and salt water that a wild salmon would experience, and also allows for the potential acceleration of growth. Fish grown without light and temperature manipulation produce ‘S1’ smolts in the next spring, but if the growth environment is altered, light and temperature regimes can induce smoltification earlier. The most intensive of these systems are sometimes used to maintain fish at as high a density as 50 kg/m$^3$. Once they have completed the freshwater portion of the growth cycle, the salmon are confirmed to have adapted for salt water and are transferred to saltwater net pens or land-based seawater systems that when placed together form ‘seasites.’ These seasites normally only contain a single generation of fish at a time and can grow this generation for up to two years, with at least 6 weeks of fallow between generations. These land-based tanks, while more separate from any wild populations, can be very susceptible to damage or escape caused by natural disasters such as hurricanes or large storms, and can be more costly because of the electricity required to pump the saltwater from the shore to the tanks (FAO, 2013). Production costs in salmon aquaculture can vary greatly, but are usually dependent on farm size, the health status of the stock, location, and the availability of feed and fish.

2.4 Transgenic Technology Introduction

Transgenics is the use of genetic engineering tools to insert foreign genes into the DNA genomes of host plants and animals for the purpose of providing the host with useful heritable properties. Transgenic organisms represent a subset of genetically modified (GM) organisms with the focus on the host acquiring a gene (transgene) that species does not normally possess in nature. The creation of transgenic organisms has allowed modern agriculture and scientists to
develop new ways to overcome old problems. Before transgenics, farmers would select the traits they wanted in their crops from a current year’s adult plants, and then use their seeds for the following year’s fields. This “natural selection” approach, performed for thousands of years, developed plants that were heartier and yielded more food. However, in the modern age, specific genes encoding useful traits can be identified, cloned, and inserted into host genomes, without the natural reproductive limits of species barriers. New transgenic crops can be resistant to disease, better capable of withstanding drought, or able to grow in poor soil. Transgenic animals have been created to provide disease models for testing new therapies, create bioreactors for producing life-saving human drugs, create xenotransplanters for providing organs for transplant into patients, and create scientific models for testing the function of newly discovered genes.

Creating a transgenic organism uses recombinant DNA (rDNA) techniques. The technology was initially developed in the late 1960’s and early 1970’s to identify, isolate, clone (amplify) and insert specific genes into DNA. Inserting cloned DNA into vectors (plasmids or viruses) can be used to create genomic libraries of DNA, replicate (amplify) the inserted DNA, or transfer the foreign DNA into a second organism. Techniques for transferring the foreign DNA into a cell include DNA microinjection, embryonic stem cell-mediated transfer, and retrovirus-mediated transfer (Federation of American Scientists, 2013). Transgenic technology was first used to create a transgenic organism in 1973 (Cohen et al., 1973) to create E. coli transformed with a plasmid expressing a foreign gene. The first transgenic animal was a mouse containing foreign SV-40 viral sequences inserted into the genome, but the SV-40 sequences were not expressed (there was no observable transgenic phenotype) (Jaenisch and Mintz, 1974). The first animal to express its transgene was a mouse containing a rat growth hormone gene
under the control of a metallothionein promoter, which produced a larger than normal mouse (supermouse) (Palmiter et al., 1982).

Two techniques are usually used for creating transgenic animals: pronuclear injection (Costantini and Lacy, 1981) and embryonic stem cell (ESC) manipulation (Bradley et al., 1984; Bronson and Smithies, 1994). In the first process, following the cloning of the desired transgene into a plasmid, the transgene is injected into the male pronucleus of a newly fertilized egg prepared by in vitro fertilization (IVF). The zygote is grown for about 5 days in vitro, and is then implanted into the uterus of a foster mother to create the transgenic offspring. In the second technique, the transgene is cloned into a virus which is used to infect embryonic stem cells. The ESCs are grown, screened to select for cells containing the transgene, and then injected into a 5-day old blastocyst. The blastocyst is then implanted into the uterus as described above.

2.5 Transgenic Fish

Compared to making transgenic mammals, it is usually easier to make a transgenic fish because fertilization and growth occur externally. So, the IVF procedures were easier to devise and the manipulated eggs do not need to be implanted (Federation of American Scientists, 2013). The field of transgenics may help provide a solution for increasing salmon production by creating fish that can grow at faster rates than their wild type counterparts. Table-1 summarizes the transgenic fish created to date that show increased growth or cold tolerance compared to wild-type fish. Some of these studies used metallothionein (MT) as a promoter that over-expressed GH in the serum and muscle, while others used an ocean pout (op) promoter to express either GH or an antifreeze protein in Atlantic salmon. In the table next to the type of construct used is the desired effect of the mutation. Many of the early transgenic studies used the
strong metallothionein promoter (MT GH-fish) to elevate GH levels in the plasma, so these MT GH-fish are difficult to directly compare to AAS. A large variety of other studies (discussed in the next section and elsewhere in the report) (e.g., Du et al., 1992; Devlin et al., 1995; Cook et al., 2000; Deitch et al., 2006; Yaskowiak et al., 2006; Sundstrom et al., 2007; Hobbs and Fletcher, 2008; Butler and Fletcher, 2009; Moreau et al., 2011; Moreau and Fleming, 2012; Oke et al., 2013) were performed on op-GH-salmon (similar to or identical to AAS), and these fish are among the best characterized of all the transgenic fish.

Other types of transgenic fish have also been created. The spread of disease is a main concern of aquaculture; as of 2012 the largest losses of cultured fish occurred from disease, with a rate of 45.4% (FDA, 2012). So, transgenic research is also creating transgenic fish that are resistant to disease. Another goal in transgenic aquaculture is growing fish that have a higher rate of feed conversion, so the fish gain the same nutritional benefits as wild type fish, but with a lower feed mass (Wodarg, 2004).
Table 1: Summary of Growth Hormone or Cold Tolerant Transgenic Salmon Created to Date

<table>
<thead>
<tr>
<th>Genetic Modification</th>
<th>Potential Benefit</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH &amp; AFP</td>
<td>Enhanced growth and cold tolerance</td>
<td></td>
<td>Melamed et al., 2002.</td>
</tr>
<tr>
<td>AFP</td>
<td>Enhanced cold tolerance</td>
<td>Precursor AFP has only 70% activity of mature AFP.</td>
<td>Hew et al., 1999; Hew &amp; Fletcher, 2001.</td>
</tr>
<tr>
<td>GH</td>
<td>Enhanced growth</td>
<td>These GM fish may have different respiratory and swimming performance than WT. Oxygen demand of GM fish is 1.6 times higher than WT.</td>
<td>Stevens et al., 1998</td>
</tr>
<tr>
<td>GH &amp; AFP</td>
<td>Enhanced growth and cold tolerance</td>
<td></td>
<td>Hwe et al., 1995</td>
</tr>
<tr>
<td>AFP</td>
<td>Enhanced cold tolerance</td>
<td>Established stable transgenic lines</td>
<td>Fletcher et al., 1992</td>
</tr>
<tr>
<td>GH</td>
<td>Enhanced growth</td>
<td>Only 9 out of 450 fingerlings were GM by PCR analysis</td>
<td>Du et al., 1992</td>
</tr>
<tr>
<td>GH &amp; AFP</td>
<td>Enhanced growth and cold tolerance</td>
<td>GM fish grow on average four times faster than wild-type (WT)</td>
<td>Fletcher et al., 1992</td>
</tr>
<tr>
<td>GH</td>
<td>Enhanced growth</td>
<td>At 1 year of age, the GM fish were 2 to 6 fold larger than WT</td>
<td>Du et al., 1992</td>
</tr>
<tr>
<td>AFP</td>
<td>Enhanced growth and cold tolerance</td>
<td>Only 24 of 137 progeny carried the AFP gene</td>
<td>Shears et al., 1991</td>
</tr>
<tr>
<td>AFP</td>
<td>Increased cold tolerance</td>
<td>Stable integration but a low level of expression</td>
<td>Shears et al., 1988</td>
</tr>
</tbody>
</table>

GH = Growth hormone.  AFP = Anti-freeze protein. WT = wild type.
2.6  AquaBounty’s AquAdvantage Salmon

2.6.1  Background

One of the modern-day leaders in the creation of transgenic fish is AquaBounty Technologies, Inc. (ABT), a Maynard (MA) biotechnology company. ABT is currently waiting for FDA approval for their AquaAdvantage salmon (AAS) to be sold in the U.S. for consumption. According to ABT’s website, AAS grow to market size faster:

“Our mission is to play a significant part in “The Blue Revolution” – bringing together biological sciences and molecular technology to enable an aquaculture industry capable of large-scale, efficient, and environmentally sustainable production of high quality seafood. Increased growth rates, enhanced resistance to disease, better food-conversion rates, manageable breeding cycles, and more efficient use of aquatic production systems are all important components of a sustainable aquaculture industry of the future.”

(www.aquabounty.com)

ABT’s planned approach for their product is to produce eyed-eggs at a specific FDA-approved facility on Prince Edward Island (Canada), ship the eyed-eggs to a specific FDA-approved site in Panama for grow-out, and then process and ship table-ready fish from Panama back to the United States (FDA EA section-1.5, 2010). AAS fish are triploid (mostly sterile), eyed-eggs from a proprietary line of Atlantic salmon, genetically engineered to increase their growth rate and reduce the overall time-to-market (FDA EA section-1.5, 2010).

2.6.2  AAS Genetic Description

The AAS genetic modification includes a copy of a Chinook salmon GH transgene (opAFP-GHc2) integrated at the α-locus to create an EO-1α transgenic line (FDA EA section-2.1, 2010). The founder animal of the AAS transgenic line was created approximately in 1989 (Du et al., 1992; Fox, 2010) by injecting an Atlantic salmon egg with plasmid opAFP-GHc2
containing the GH gene from a Chinook salmon under the control of an ocean pout antifreeze protein (AFP) gene promoter. The pout promoter and Chinook GH genes were chosen to potentially enhance consumer acceptance; the fish genes should be more widely accepted than using human GH genes or viral promoters (Du et al., 1992). The pout promoter contrasts with the Atlantic salmon natural GH promoter, the latter only switches on seasonally in response to increasing day lengths and warmer temperatures (Bjornsson, 1997). The published GH work with the pout promoter (Du et al., 1992; Hobbs and Fletcher, 2008) showed that the GH was expressed at a low level in a wide range of tissues, without increasing GH in the serum or muscle, so the authors speculated the expression occurred efficiently near GH-receptors which created the accelerated growth (topic discussed more fully in the hormone section).

The Chinook and Atlantic salmon GH genes and proteins are very similar; 198 of the 210 GH amino acids are identical. The original DNA construct opAFP-GHc2 upon integration into the genome of the founder animal (and its subsequent breeding), underwent a rearrangement (termed EO-1α) in which a portion of the pout promoter integrated into the GH gene. Amazingly this gene rearrangement did not appear to affect expression or activity of the GH (Yaskowiak et al., 2006; Butler and Fletcher, 2009), and this rearranged EO-1α construct continues today in the AAS salmon line. A potentially important result with respect to the pout promoter is that its expression depends on the environment of the fish. For example, transgenic op-GH fish grown in well fed cultured conditions grew almost three times longer than WT controls, while transgenic fish grown in a simulated natural environment grew only 20% longer than WT controls (Sundström et al., 2007). Thus, the size of the AAS fish might be smaller if they escape their artificial enclosures.
2.6.3 Overview of Health and Environmental Impact of AAS

Though AAS salmon offer a potential solution to economic and sustainability problems with aquaculture, several consumer groups have concerns about the potential health issues and environmental impacts of potential escape. Specifically, there are concerns about possible malformations in AAS, increased levels of IGF-1 a cancer-causing hormone, increased allergenicity, cross-breeding of escaped fish and their effects on the wild-type populations.

2.6.4 The FDA Review of AAS Fish

If approved by the FDA, AAS would become the first genetically engineered animal approved for human consumption. The United States currently has no legislation specifically regulating transgenic animals. Under the Coordinated Framework for the Regulation of Biotechnology, the FDA regulates GM animals by the Federal Food, Drug, and Cosmetic Act (FFDCA) as a new animal drug application (NADA) (VanEenennaam et al., 2013, page-1). Under the FFDCA guidelines, Section 512, if an article is intended to alter the structure or function of the animal it is considered a new animal drug, so all rDNA constructs that change the characteristics of an animal qualify as a new “drug” (FDA Guidance for Industry, 2009). As with all new drug applications, the sponsor (ABT) was required to conduct and pay for studies to obtain regulatory compliance.

In April of 1993, A/F Protein (later renamed AquaBounty Technologies) initially approached the FDA for guidance on its application. Their guidance eventually resulted in ABT formally filing an Investigational New Animal Drug (INAD) application on September 14, 1995, and it has been under consideration for the past 18 years. The FDA review process included several steps, some of which were unprecedented.
The FDA approval process of AAS began with a several-step procedure called GFI 187, which was intended as a method of finding potential risks to the health of the GE animal, to subsequent generations of the transgenic line, or to any consumers (FDA VMAC, section-5). The GFI 187 procedure is illustrated in **Figure 5** below.


First, the submitted data was reviewed for information about the product’s definition (first tier at the bottom in the diagram). In the second step, termed the Molecular Characterization of the Construct (second tier in the diagram), the FDA reviewed how the rDNA construct was made. The rDNA construct was checked to determine whether it contains any foreign DNA segments or organisms that could pose potential health risks to the GE animal itself or any possible animal or human consumers. The DNA construct was evaluated for any potential segments of DNA that could integrate with similar segments or viruses to create new health risks, either by creating a mutated virus strain or by creating a new protein that could have a negative effect on either the animal or the consumer.
Third, the FDA reviewed ABT’s data to determine the effect of rDNA incorporation on the animal and its activity over multiple generations. This is termed Molecular Characterization of the GE Animal Lineage (third tier in the diagram). Included in this portion of the evaluation was an analysis of construct genome location over time, and whether the animals continue to express the intended trait introduced by the construct.

Fourth, the FDA determined whether the rDNA construct is safe for the GE animal line by performing a Phenotypic Characterization (fourth tier in the diagram). This was done by reviewing studies submitted by the applicant about the physiology of the GE animals in comparison to the wild-type. The FDA also inquired about the general health of the animals, including disease-resistance and developmental progress relative to wild-type specimens. A safety evaluation was performed to establish any abnormalities in the GE specimen that would not occur in the wild-type, as well as evaluating the general functions of the animal’s organ systems and the chemical composition of the animal’s edible tissues.

The fifth step included a “Durability Assessment” that reviewed plans the sponsor (ABT) will follow to ensure that produced animals (if approved) will be equivalent to the animals evaluated in the pre-approval review. This process helps ensure that the construct remains stable through multiple generations, and that the animals of subsequent generations are healthy and exhibit and equivalent phenotype.

Since the GE animal is intended for consumption, a sixth step was implemented which assessed the salmon’s “safety for consumption” to determine if the meat differs from those of the wild-type in a way that is unsafe, as well as what differences exist nutritionally from non-GE counterparts (sixth tier in the diagram). Experts evaluated the nutritional content for comparison to the equivalent ranges for vitamins, proteins, fats, and minerals from conventional animals.
Any differences must be examined by the FDA to ensure there is no potential harm to the animal or consumers. For AAS, the tests submitted to the FDA included allergenicity risks, because finfish (like salmon) and shellfish represent some of the major groups of food allergens. This test was designed to compare the potential allergenicity of the AAS to wild-type fish.

The final portion of the GFI 187 review was an “environmental assessment” associated with the specific proposed conditions under which the sponsor wishes to raise the GE animals. The FDA must meet National Environmental Policy Act (NEPA) requirements, and determine whether the proposed agency action (i.e., approval of the AAS under the conditions specified in the application) would likely have an effect on the human environment of the US. In addition to analyzing ABT’s submitted data, the FDA also conducted its own environmental assessment, including site visits to the specific locations at which the AAS were to be raised and consultations with other federal agencies. The site visit to the Panama location was attended by experts in aquaculture from FDA, as well as by an expert in aquaculture from the National Marine Fisheries Services.

Following these inspections, on August 25, 2010, the FDA prepared a draft environmental assessment (FDA EA, 2010) for the conditions specific to ABT’s application, and submitted that draft, and its preliminary findings to the public for a 120 day comment period. Since no adverse impacts were found, on May 4, 2012, the FDA published a Finding of No Significant Impact (FONSI, 2012). If the FDA had determined AAS would significantly impact the environment, a further assessment would have been required. Currently, AquaBounty is not seeking approval to set up any transgenic aquafarms in the U.S., so they will not need to seek permits from the U.S. Fish and Wildlife Service to proceed in Canada and Panama.
Despite the fact that the regulatory review of AAS by the FDA has spanned almost two decades now, the review process has accelerated in the last three years. In addition to the steps mentioned above, in 2010 the FDA presented their findings to the public in a series of steps outlined in Figure-6 (FDA VMAC, 2010).

![Figure 6: Regulatory Environmental Assessment Steps Taken for AAS.](image)

Uniquely, for the case of AAS involving a GM animal for consumption, the FDA required the formation of a committee of scientific experts and veterinarians (the Veterinary Medicine Advisory Committee, VMAC) to review ABT’s submitted data, and required the VMAC to hold a public meeting after its review to present its findings and receive public comments. The VMAC meeting began with a public orientation session, followed by a discussion of the strengths and weaknesses of the data by VMAC members. Several industry leaders and scientific experts presented their knowledge on the subject for the purpose of introducing the topic to the committee. Although not required to do so, two weeks in advance of the September 19-20, 2010 public meeting (diagram left side), ABT made an unprecedented effort for transparency by making its 172-page VMAC briefing package (FDA VMAC, 2010) and 84-page environmental assessment (FDA EA, 2010) publically available.
Following the public meeting was a period of public commentary. During this time, several legislators wrote to the FDA in October 2010 expressing their concerns, organized in two letters, one from several senators and one from several congressmen. The letter submitted to the senate was signed by Senators Mark Begich and Lisa Murkowski of Alaska, Kirsten Gillibrand and Sheldon Whitehouse of New York, Barbara Mikulski of Maryland, Maria Cantwell and Patty Murray of Washington, Jon Tester of Montana, Barbara Boxer and Dianne Feinstein of California, Bernard Sanders of Vermont and Jeff Merkley of Oregon. The letter submitted to the house was signed by Congressmen: Reps. Earl Blumenauer of Oregon, George Miller, Sam Farr, John Garamendi, Lynn Woolsey, Barbara Lee, Jackie Speier, and Lois Capps of California, Raúl Grijalva of Arizona, Maurice Hinchey, Jerrold Nadler, and Louise Slaughter of N.Y., Betty McCollum and Keith Ellison of Minnesota, Dennis Moore of Kansas, Jim Moran of Virginia, Peter Welch of Vermont, David Wu and Kurt Schrader of Oregon, Madeleine Bordallo of Guam, Jim McDermott and Norm Dicks of Washington, Lloyd Doggett of Texas, and Donna Christensen of the Virgin Islands. Due to these concerns, and over 800 public comments received, the FDA extended the comment period. The original comment period was scheduled to end in February of 2013, but was extended to April 2013 to satisfy due process and allow for more public commentary. During this time a charge was given to the VMAC to answer the questions: 1) Do the data and information demonstrate that the rDNA construct is safe to AquAdvantage salmon? 2) Do the data and information demonstrate that there is a reasonable certainty of no harm from consumption of foods derived from AquAdvantage salmon? 3) Do the data indicate that AquAdvantage Salmon grow faster than their conventional counterparts? 4) Are any potential environmental impacts from AquAdvantage Salmon production adequately mitigated by AquaBounty Technologies’ proposed conditions of use?
Following the charge, the VMAC had time to deliberate the questions relative to the data given, and provide their committee response as an updated draft environmental assessment (published May 4, 2012) and the VMAC Chair submitted a Chairman’s report to the FDA (FDA Chairman’s Report, 2010) (agreed to by all members of the VMAC) which concluded the rDNA construct was safe for the fish, that no harm would come from ingesting the fish, that AAS grew faster, and the environmental risks were appropriately mitigated by ABT’s proposed sterility and containment system (FDA Chairman’s Report, 2010). Since their draft environmental assessment indicated no environmental impact, also on May 4, the FDA published their preliminary Finding of No Significant Impact statement (FONSI, 2012), and made it available for public comment.

On September 15, 2012, a letter signed by 56 scientific experts and industry leaders was sent to President Obama, complaining of the long deliberation of ABT’s application by the FDA, and requesting that the President instruct the FDA to expedite the process. The signees included several scientists interviewed for our project, such as Eric Hallerman, Head, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute; and William Muir, Professor, Department of Animal Sciences, Purdue University. The signees also included distinguished names such as John Connelly, President of the National Fisheries Institute; Allison VanEenennaam, University of California Davis; John Bonner, CEO, Council for Agricultural Science and Technology; Jim Sartin, President, American Society of Animal Science; Terry Etherton, Distinguished Professor of Animal Nutrition, Penn State University; Steve Strauss, University Distinguished Professor, Oregon State University; Christine Bruhn, Director, Center for Consumer Research, Department of Food Science, University of California, Davis; Gilbert
Ross, Executive Director, American Council on Science and Health; and Bruce Ames, Senior Scientist, Children’s Hospital Oakland Research Center, University of California, Berkeley.

The public comment period for the FDA’s Environmental Assessment and FONSI closed on April 26, 2013. The FDA is currently reviewing the public comments as part of its final deliberations prior to making its final decision.

With its application in the final stages of approval, AAS has been the focus of fierce debate by various environmental groups, scientists, and consumers who are raising concerns in terms of the science of the technology and the extent of the environmental assessment. Their key concerns are food safety (is AAS entirely safe for humans to eat), and the potential for AAS escape (will the AAS be able to escape from their tanks and impact the environment or other fish).

2.6.5 Potential AAS Developmental Alterations

If an increased percentage of developmental alterations are found in AAS fish relative to other farmed salmon or to other triploid fish, this could affect the survival and mating behavior of escaped AAS fish. Two studies have shown that over-expressing GH in salmon can alter their behavior, swimming ability, and body structure (Witten et al., 2009; Hu and Zhu 2010), but those studies were done on fish in which GH was elevated in the serum, so are not directly comparable to AAS. The alterations in body structure were predicted to result from the rapid growth of the TG fish, as they were similar to structural alterations seen in rare WT individuals showing rapid growth. The structural alterations were predicted to slow the swimming of TG fish relative to WT.
AAS salmon showed body malformations at a higher incidence than WT diploid fish, but the malformations were comparable to those observed in other triploid fish (FDA VMAC, 2010, page-25). So, some of the observed AAS malformations may result from the triploid process itself, not from the GH transgene. “ABT reported a higher prevalence of gill lesions among triploid fish than diploid fish. The lesions included structural abnormalities of the gill filaments along with increased segmental lamellar epithelial hyperplasia” (FDA VMAC, 2010 pg. 39).

Triploids also show a reduced immune function, resulting from either the elevated pressure used to create the triploids or from the extra set of chromosomes (Ching et al., 2010). But, none of these morphological changes appears to have been created in AAS from the presence of the GH transgene, and the VMAC concluded that AAS “show no demonstrable differences from the comparator fish population when reared under growth conditions in ABT’s facility” (FDA VMAC, page-31).

2.6.6 Triploid Fish

ABT’s submitted plan to the FDA includes the use of sterile triploid fish. WT fish are normally diploid (containing two copies of each chromosome), but AAS fish eggs will be pressure-treated to make them triploid (containing three copies of each chromosome). Triploid fish do not produce gametes (eggs and sperm), so are usually sterile. Triploidy has been used worldwide for the past decade to sterilize trout, carp, and salmon for the sport fishing industry because triploid fish grow larger as fewer resources are spent on reproduction (Piferrer et al., 2009).

Triploidy can be artificially induced by treating fish eggs with elevated pressure, elevated temperature, or with chemicals. ABT’s triploid AAS eggs will be produced at Prince Edward
Island in Canada using high pressure (FDA VMAC, 2010), and the eggs will be shipped to Panama for raising the fry. The process must be carefully adjusted for each species to avoid negative effects on the fish. ABT’s data indicates the pressure process they developed will create about 98-100% triploids, so the remaining 1-2% should be diploid (and presumably fertile) (FDA VMAC, 2010), and because of this we deem it important to at least consider the potential effects on the environment and other fish of fertile AAS escape. Because no data currently exists for large-scale pressure treatment of eggs, the FDA has required that ABT test each batch of eggs produced at the Prince Edward Island site, and discard any batch exceeding 5% diploid eggs (FDA VMAC, 2010). In most species, triploid and diploid fish are indistinguishable until after maturity, when diploids begin to use energy for reproduction while triploids use their energy to grow.

Triploid females usually show a complete sterility, and ABT is proposing using only triploid females, but for the opposite sex some triploid males remain fertile, so in its submission to the FDA AquaBounty indicated its plan was to raise only females. The males used to provide sperm for the AAS eggs will be converted from AAS females by treatment with the male hormone 17-methyltestosterone, a common procedure in modern aquaculture, and their sperm will be used to fertilize WT salmon eggs to create more females.

2.6.7 Potential AAS Hormonal Alterations

ABT was required by the FDA to monitor potential alterations in hormonal levels in their AAS fish; if the levels were found to be altered relative to triploid fish or to normal farmed fish, this could affect AAS behavior which becomes important in the event of an escape and the AAS interact with other fish. Physiologically, all mammals respond to the production of one hormone
by altering the levels of other related hormones. For example, the release of a large quantity of thyroxin from the thyroid gland leads to a decrease in the release of thyroid stimulating hormone (TSH) from the pituitary gland which decreases the release of thyroxin from the thyroid, restoring it to normal levels. Thus, producing GH in AAS might be expected to alter the levels of other related hormones, and in particular to reduce the size of the pituitary gland and its secreted hormones in a negative feedback loop. In fact, Mori and Devlin (1999) showed that MT GH-salmon (producing elevated levels of GH under the control of a strong metallothionein promoter) contain pituitary glands that are smaller on average than controls. However, this study was not performed with AAS salmon.

With respect to GH levels, the original paper describing AAS (Du et al., 1992) speculated the GH would only be locally expressed in close proximity to GH receptors, driving AAS growth without showing elevated GH in plasma or muscle. A later paper (Hobbs and Fletcher, 2008) showed broad but low levels of GH in all the tissues analyzed, supporting the hypothesis of a low level but more regionally focused expression. With respect to the data submitted to the FDA, Table 13 of the FDA VMAC Report (2010, page-66) reports the mean plasma GH levels as 39.9 ng/ml in AAS, 28.2 ng/ml in non-GM siblings, and 20.5 ng/ml in WT controls, but none of the values was significantly different. So, unlike MT-GH-fish that show strong elevation of GH in the serum and a decrease in pituitary size, AAS fish with no observed increase in plasma GH levels likely will not show alterations in other physiologically related hormones, and this agrees with VMAC data showing that AAS did not have statistically different concentrations of estradiol, testosterone, 17-ketotestosterone, thyroxine (T4), or triiodothyronine (T3) hormone levels compared to non-GM fish (FDA VMAC, pages-66 and 68).
With respect to other hormonal levels, consumers have placed a strong interest in the levels of insulin-like growth factor-1 (IGF-1), which is known to cause cancer in humans (Giovannucci et al., 2003). Table 15 in the FDA VMAC report (2010, page-68) shows the IGF-1 values for AAS versus controls. The average IGF-1 value (total tissue) for AAS was 10.26 ng/ml (N=6) with a maximum of 18.43 ng/ml, while non-GM controls were 7.34 ng/ml (N=11), with a maximum of 12.24 ng/ml, but none of the differences was statistically different. However, the data was provided to the VMAC is in a format that we predict will cause serious miscommunication with consumer groups, because any value beneath the lower limit of detection of the assay was eliminated from the Table. For AAS, 24 such values were discarded (30 total AAS fish were analyzed), which if included would have produced an AAS IGF-1 mean lower than non-GM fish. With only 6 AAS fish actually listed in the Table, we predict this will cause a miscommunication with consumer groups.

2.6.8 Potential AAS Allergenicity

WT salmon are known to be an allergen to some individuals, so AAS is expected to cause allergies in some individuals. But the main health concern with eating AAS is whether its GH transgene product causes increased allergenicity relative to WT salmon. Chinook GH has an amino acid sequence different than any other previously characterized allergen (FDA VMAC, 2010), so Chinook GH is not predicted to cause serious allergies. The VMAC concluded that AAS salmon pose no additional allergenic risk relative to control Atlantic salmon (FDA VMAC, 2010, page-106). In another study, diploid salmon expressing GH using a metallothionein promoter showed no increased allergenicity relative to normal diploid salmon (Nakamura et al., 2009). In addition, there appears to be no medical consensus about what level of an allergen
actually constitutes a risk to the public (Goodman et al., 2008; VanEenennaam and Muir, 2011), so without such studies evaluating allergenicity potentials remains difficult.

2.6.9 Physical and Environmental Containment at ABT’s Production Facility

Several physical containment barriers have been implemented at ABT’s egg production facility at Prince Edward Island (Canada) to help prevent the release of live eggs into the environment. The majority of physical containment comes from a series of redundant screening and filtration of water flows, and as a final precaution, chlorine will be used on all drains to kill any eggs that are accidently discarded down the drain (FDA, EA, 2010).

With respect to escaped eggs hatching and interacting with local fish, the FDA’s environmental assessment states that the geographical location of the production facility itself is sufficient for minimizing escape issues because no WT populations remain in Canada due to acid rain, high salinity, and over-fishing. Thus, any eggs accidently discharged into the sewerage (and into the ocean) as they grow would likely find no Atlantic salmon for inter-breeding. And with respect to temperature, the winter water temperatures are too cold for salmon survival, but they could survive in summer (FDA, EA, 2010).

2.6.10 Physical and Environmental Containment at ABT’s Grow-Out Facility

ABT’s procedure submitted to the FDA proposes to raise AAS fish at a specific site in Panama. Because 1-2% of AAS females might be fertile, the grow-out facility must have physical containment barriers to help prevent escape. The physical containment barriers were outlined in the environmental assessment for AAS and are shown in Table-2 and Figure 7. The physical escape barriers include screens wherever water flows out of the system, security in the form of netting and fencing topped with barbed wire surrounding the fry and grow-out tanks, and
downstream hydro-electric plants to prevent passage of any escaped fish downstream. A minimum of 11 sequential physical barriers are in place between the fry tanks and the local river, with seven of these barriers installed following outflow from the grow-out tanks (FDA, EA, 2010).

In Panama, the fish-raising facility is located at a high altitude near a river that drains to the Pacific Ocean. With respect to temperature on AAS escape, ABT’s Panama facility is at a high elevation of approximately 5,000 feet, with water supplied by a nearby spring whose temperature is a relatively constant 15ºC year round. This temperature in the surrounding water is near the cold-optimum for AAS, and likely was one of the reasons the site was chosen for salmon aquaculture. So, any AAS escape would not immediately be hindered by the ideal cold local highland water temperatures. But downstream, the river temperature rapidly increases to about 26-28ºC as it approaches the Pacific Ocean (FDA VMAC, 2010, page 123). This range is at (or near) the 28ºC incipient lethal level for Atlantic salmon (the temperature that would kill the fish after 7 days) (Elliott, 1991). In addition, Atlantic salmon do not feed at temperatures above ~23ºC (FDA EA, 2010, page 26), so the longer the fish remain in the warmer downstream water, the higher the chance of starvation. Moreover, the presence of several dams downstream would physically hinder the rapid migration of AAS from the fatal warm water to any colder ocean water further downstream, further increasing their chance of starvation in the warmer water. Although unlikely, it is not clear from the VMAC report whether it is feasible for a rapidly moving escaped AAS fish to survive long enough in its migration through warm water to make it to the deeper cool ocean waters further downstream. Would such a salmon be able to retain enough energy for such a swim while not feeding? And if so, would it cross-breed with any other fish?
Table-2: Key Components of Physical Containment Measures at the Grow-Out Facility

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Feature or Component</th>
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| **Primary containment**                                                 | Center standpipe cut below tank rim to ensure water level is always below rim  
To prevent escape from the fry tanks via water                        | Netting stretched taut over the top of each tank  
Collar-sleeve screens inserted into top of standpipes to prevent fish from entering standpipe by swimming  
Metal screen made standpipe at base of basket screen impedes fish that entered standpipe (by jumping) from leaving the tank  
Rigid circular plastic screens surrounding the center standpipes  
Perforated gravel floor around each tank allows downward percolation of overflow water but traps any fish in the overflow |
| To prevent escape from the fry tanks by avian predators                | The building is covered and sealed by netting  
Netting stretched taut over the top of each tank  
A single external (so no fish can jump into it) standpipe cut below tank rim to ensure water level is always below rim  
A 1 cm thick rigid PVC slotted drain plate affixed by screws to the only drain in the tank  
Perforated gravel floor around each tank allows downward percolation of overflow water but traps any fish in the overflow |
| To prevent escape from the grow-out tanks via water                    | Each tank is entirely covered by netting stretched over and around the tank on a rigid support structure  
Netting stretched taut over the top of each tank  
Sock filter (500 μm) on the terminal end of the only drain pipe receiving effluent from the fry tanks |
| To prevent escape from fry tanks into drains                          | Sealed metal cage (affixed to ground) through which all effluent from grow-out tanks must pass before entering drain canal  
Concrete structure and containment sump through which all water must pass  
Rigid metal screen affixed to bottom of containment sump through which all water must pass |
| To prevent escaped fish from passing through the drain canal to the sedimentation ponds | Rigid metal screens on the outlet of each pond |
| To prevent escaped fish from passing from one sedimentation pond to another | Four sedimentation ponds in series, each with its own outlet screen |
| To prevent escaped fish from entering the river from the drain canal   | The project is in a very remote location  
The project is built on the opposite side of the river from the road  
A narrow pedestrian bridge crosses the river, with access controlled by a locked metal fence  
Tall barbed wire security fence completely surrounding the perimeter of the fish rearing tanks, with locked entry gates  
Permanent presence of aggressive dogs |

(FDA, VMAC, 2010)
2.6.11 Potential Effects of AAS Escape on the Environment

The impact AAS fish may have on the environment was a required area of investigation for the FDA application. If the fish escape their pens in Panama, will the 1-2% of AAS fish that are not sterile interbreed with WT salmon or brown trout (Sea trout) in the nearby streams? The likelihood of this happening depends on the effectiveness of the process ABT uses to create sterile triploid fish (biological containment), the effectiveness of ABT’s physical barriers in Panama, the presence of fish in Panama that might interbreed with AAS fish, and how that breeding would be altered by the Panama environment (environmental containment). With
respect to fish that AAS might hybridize (cross-breed) with if they escape and are fertile, Oke et al. (2013) showed that wild brown trout (Salmo trutta) can hybridize with AAS in either artificial tanks or in stream experiments, and the offspring are fertile. However, three other studies indicated that salmon/brown-trout hybrids are sterile (Galbreath and Thorgaard, 1994; 1995; 1997). If so, hybridization would not be a long-term problem. Other studies have shown that AAS can hybridize with other WT salmon (Moreau et al., 2011; Moreau and Fleming, 2012), although with a great reduction in mating success relative to WT-WT mating. So, although several published studies indicate that AAS can indeed hybridize with other fish (and predict the consumer groups will focus on this data), with respect to the hybrid’s potential effects on the environment we consider it equally important to consider whether such hybrids are actually more fit than WT populations.

2.6.12 Opposition to AAS

If ABT’s application is approved, it would make AAS the first GM animal approved for human consumption. Several consumer groups monitor GM foods, and they have strong opinions on ABT’s FDA proposal. For example, the organization Food and Water Watch is currently opposed to the approval of transgenic organisms for human consumption. In 2012, Food and Water Watch stated:

“Move over Grinch. The FDA is doing everything in its power to give American consumers a terrible holiday gift this year. Today they took the final step toward approving genetically engineered (GE) salmon, the first GE food animal. Even after countless Americans have expressed their deep concerns about this frankenfish, the FDA has turned a deaf ear moving forward with this reckless approval. That’s why we’re asking Congress to block the approval of GE salmon” (Food and Water Watch, 2012).
In addition, another consumer group, the Center for Food Safety (CFS) based in Washington DC, is a public interest non-profit organization that works with the government to investigate issues related to food safety. Like Food and Water Watch, the CFS is opposed to the approval of GM animals for human consumption. They have stated that the FDA’s review of AAS fish was not thorough, and that the FDA is not qualified to perform comprehensive risk assessments for GM fish (personal communication, Colin O'Neil, Director of Government Affairs at the Center for Food Safety, 8/5/13). The CFS stated that the level of risk assessment on transgenic salmon is inadequate, with many gaps occurring in the information obtained thus far from ABT and the FDA, especially on the effects of eating AAS. The CFS points to the FDA VMAC data that their analysis of hormonal levels in the fish was only obtained from 6 fish. They also mentioned that although differences were seen in the levels of some AAS hormones relative to other triploid fish, ABT claims the observed differences were not statistically significant even though the analysis was done on a low number of animals. CFS is on record as indicating that it is up to the public interest groups, journalists, and members of the public and independent researchers to dig into the topic of AAS and try to figure out what is going on with this product. They have indicated we are at a point where legislators may need to step in and reassess the FDA’s entire approach on the regulatory process of GM food (personal communication, Colin O'Neil, Center for Food Safety, 8/5/13).

These consumer groups do not stand alone on the issue. Over 1.8 million people wrote (Hansen, 2013) to the FDA in response to their 2010 Environmental Assessment (FDA ES, 2010), and their May 4, 2012 Finding of No Significant Impact (FONSI, 2012). One of the public’s major concerns is the potential for cancer caused by the apparent elevation of IGF-1 in the AAS fish relative to WT fish, as previously mentioned in 2.6.7 of the introduction. Many
members of the public and some experts also have environmental and ecological concerns related to fish escape and their possible hybridization with other fish. “Interspecific hybridization is a route for transgenes from genetically modified (GM) animals to potentially invade wild populations, yet the ecological effects and potential risks that may emerge from such hybridization are unknown” (Oke et al., 2013). Some opponents have indicated there is no advantage whatsoever to approving AAS fish, as the growth rates are unverified relative to WT salmon. “AquaBounty Technologies, the makers of GE salmon, have managed to convince everyone that GE salmon can grow two times faster than non-GE salmon, which is supposed to revolutionize aquaculture. Unfortunately, AquaBounty can’t prove any of this. And independent salmon growers and scientists have called these purported growth rates misleading” (Schwab, 2013). They argue that since there is no obvious advantage for producing AAS fish, the biotech industry is seeking to set a precedent for approval of GM animal food, to facilitate future applications. As Tim Schwab states:

“If GE salmon can’t actually grow fast—or offer any other obvious benefit—why exactly is the FDA spending precious resources trying to convince the public that it needs AquaBounty’s GE salmon, which introduces new risks to consumer health and the environment? The answer is that the biotech industry has an enormous interest in seeing GE salmon approved through the FDA, regardless of whether it fails in the marketplace. Moving the first GE food animal successfully through the FDA’s weak approval process will guarantee that this friendly regulatory channel will be in place for biotech animals in the future. In short, GE salmon is the sacrificial lamb.” (Schwab, 2013)

The GMO opponents worry that once ABT gets FDA approval, other biotech companies could more easily gain approval for their GE animals.

In response to the current lack of federal legislation requiring GM food to be labeled, several bills have been submitted to the senate and house requiring GM-food labeling (discussed in more detail in the Results section), but those bills remain under discussion in committees and have not been brought to a vote. In response to a lack of federal requirements, several U.S. states
have introduced labeling laws to inform consumers about GM food in case ABT’s application is approved. For example, in 2013 Connecticut passed the nation’s first GM food labeling law (WholeFoods, 2013), and in June, 2013 Maine was the second state able to pass a GMO labeling law (Caldwell, 2013). Washington State failed to get enough votes to pass their GM food labeling law (WholeFoods, 2013). A few other states are in the process of introducing GM food labeling laws.

Other consumer concerns include those discussed earlier in the report, such as the worry that AAS fish will escape and affect WT fish populations, and that consuming GE salmon with elevated levels of IGF-1 (linked to cancer in humans) will be harmful. On June 4, 2012, Tim Schwab of the Food and Water Watch indicated that ABT’s analysis showed 40% higher rates of hormone IGF-1 linked to cancer (Schwab, 2012). Other concerns brought up by consumer and environmental groups include whether ABT will eventually apply for permission to raise AAS within the U.S. Within Panama, what effects will the fish have on the ecosystem if they escape? If the behavior of AAS fish is altered relative to WT, does that affect courtship behavior if the fish escape, making them more successful at mating than WT fish? One of the greatest fears among consumer groups is that AAS fish will escape from the highlands of Panama to the open ocean and eradicate WT salmon populations either from their increased food needs or by passing their transgene to WT populations. “The potential impact from escaping GE salmon could be severe, with researchers suggesting that a small number of GE fish escapees could cause extinction of wild populations in as little as 40 generations” (Food and Water Watch, 2010).

In addition, some consumers have complained that farming AAS will never be commercially viable. The inland tanks are expensive, scale-up is expensive to pressure-treat for sterility, and in the end it is not clear whether consumers will actually buy the product. “The
FDA’s apparent impotency over the course of a [18]-year (and counting) approval process isn’t surprising. Why should an agency that focuses on drugs and is advised by veterinary medicine experts be expected to know anything about ecology? It may turn out that dumping 100 boatloads of AquAdvantage salmon in the ocean is perfectly safe. But the precedent set by this approval process isn’t” (LeVaux, 2013).

Overall, our review of the available literature summarizes the world’s increased fish consumption to help satisfy a growing hungry population, the attempt of the fish aquaculture industry to help meet this growing demand for fish, the potential role that transgenic fish may play in the aquaculture industry, and the attempt by AquaBounty Technologies to gain FDA approval for their transgenic AquaAdvantage Salmon. Our literature survey also identified several potential problems including the format of the VMAC data which could easily cause confusion, the FDA’s ABT review as a New Animal Drug Application (NADA) which potentially limited the FDA’s requirement to perform a rigorous environmental assessment, the ability of AAS to hybridize with brown trout or other salmon, potential negative effects of the spread of GH transgenes into wild-type populations, a potential lack of interest in the consumer eating genetically modified (GM) fish, and potential problems associated with the lack of federal laws mandating the labeling of GM fish. Extending the information available in the publically available literature, our Methodology was designed to gain additional information on these potential problems from various stakeholders using interviews.
3.0 METHODS

The overall goal of this project was to identify and evaluate the remaining technical, procedural, and regulatory issues necessary to ensure the safe introduction of commercial transgenic fish aquaculture in the United States. In order to achieve this goal, the team identified four primary objectives.

1. Develop a comprehensive assessment of the origins, technology, and current status of transgenic fish aquaculture.
2. Characterize what key stakeholders believe are the remaining hurdles to the introduction of commercial transgenic fish aquaculture and how the hurdles should be addressed.
3. Assess the scientific procedures used to help make transgenic aquaculture environmentally safe.
4. Recommend alternative solutions to remaining problems that introducing transgenic fish for human consumption may cause in the United States.

To accomplish these objectives we began by conducting an extensive review of current research literature, including reputable academic journal articles, relevant books, government reports, legislative documents, scholarly websites, and other pertinent materials.

Building on the information collected during our background research, we conducted an extensive set of semi-structured, in-depth interviews with various stakeholders to determine the range of opinions about the regulation and safety of commercial transgenic fish aquaculture. Initially, we hoped to interview a broad array of stakeholders included federal and state legislators and staff who have or are proposing legislation on the commercial introduction of transgenic fish; representatives from various federal and state agencies (e.g. the FDA and EPA) responsible for regulating all aspects associated with the commercialization of transgenic fish (including the development of transgenic fish, their use in fish farming, and their safety as part of the human food supply); representatives of biotech companies engaged in the commercial
development of transgenic fish; academic experts on the technical, legal, and other issues of concern, and other stakeholders, such as groups representing commercial fishermen, consumers, and environmentalists. Some of the stakeholders were identified by referral from our project advisors, while other stakeholders were identified from the academic and related literature, and by referral from other interviewees (i.e., a snowball sample).

We initially contacted prospective interviewees by email and/or phone. If no response was received after one week, we followed up with a second email or phone call. We soon discovered that it was very difficult to secure interviews with prospective respondents. Some prospective respondents refused to respond to our calls or e-mails at all, while others demurred due to lack of time or avowed lack of knowledge or interest.

Table-3 shows the number of initial contacts we identified according to stakeholder category as well as the number of interviews conducted in-person, by phone, or by e-mail. We would have preferred to conduct our interviews in person whenever possible, but this was not feasible given the distant locations of some respondents, and many respondents preferred to conduct the interview by e-mail. The table also indicates those respondents with whom we had multiple phone and/or e-mail exchanges in order to clarify or expand upon points raised in previous interviews. We developed our preliminary interview questions based on our background research. A list of preliminary questions is shown in Appendix C at the end of the report. We tailored the questions asked according to the expertise of the interviewee. We anticipated that the questions would evolve based on what the interviewee said, so the questions were adjusted based on the direction of the conversation. The questions in the Appendix show the full range of topics addressed in this study.
Table-3: Summary of Organizations and Stakeholders Interviewed

<table>
<thead>
<tr>
<th>Organizations</th>
<th>Initial Contacts</th>
<th>Interviewed</th>
<th>Follow up</th>
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<tbody>
<tr>
<td><strong>Federal Agencies &amp; Legislators</strong></td>
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Notes: E = email; P = phone

With respect to the method of the interview, we first explained the nature of our project, then requested permission to interview, and finally requested permission to quote and gave them the right to review any quotes we used from them prior to submission of our report. We started with an initial set of general questions, but modified them according to stakeholder knowledge/perspective, and also adapted the questions as the project progressed and we learned...
more about the key issues. For phone interviews we asked if the interviewee would consent to be digitally recorded, but in most cases written notes were our primary method of recording the in-person interviews (see Interview Preamble in the Appendix D). A list of amended questions that were asked to various organizations and stakeholders interviewed can be found in Appendix E.

With respect to the total number of interviews, the group stopped interviewing additional subjects when sufficient information was obtained to represent all sides of the main problems, or when all unclear points had been clarified. We completed our project with 17 interviews; 9 were phone interviews and the rest were email correspondence.

For objectives 3 and 4, the group synthesized the information collected in the literature research, interviews, and follow-up interviews to create recommendations and alternative solutions to the problems that still remain for introducing transgenic fish for human consumption. We went back to some respondents several times to clarify items, especially as we learned from our other interviews. Several interviewees pointed us towards new articles, websites, etc. that we were not aware of previously. We then took all these disparate pieces and integrated them into our Results and Findings section.
4.0 RESULTS & FINDINGS

This section contains a summary of the project interviews with respect to ABT’s battle for FDA approval placed within the context of the main problems identified in our literature review, and the identification of the issues left to be solved for GE fish to be produced and sold for human consumption. Section 4.1 addresses some of the remaining scientific issues with the alteration of these fish as well as some of the solutions. Section 4.2 describes the concerns about existing regulations and the proposed legislation pertaining to AAS and other GMO foods. Section 4.3 weighs some of the potential threats that the escaped AAS fish may pose to the environment and assesses the systems put in place by the FDA to mitigate the environmental impacts. Section 4.4 focuses on the ecological issues that may arise if the fish were inadvertently introduced into the wild. Section 4.5 assesses the sustainability of salmon aquaculture in general, and the economic viability of raising the fish in Panama while selling them elsewhere.

4.1 Technical Issues

In section 4.1, we discuss the scientific problems associated with the AAS, beginning with the genetic and chemical differences between the AAS and other GH-salmon and WT-salmon. The difference in constructs is important because of the difference in their effects on the fish. We then address some of the other side-effects that the produced GH may have on the fish endocrine system. This is potentially important because elevated levels of GH might alter the levels of other hormones which can alter fish behavior. We also examine the possible differences in AAS meat versus other GH-fish in comparison to wild type and normal farmed fish. Lastly, in the technical section we consider potential problems associated with inducing triploidy as a means of inducing sterility.
4.1.1 Differences in DNA Constructs:

One of the initial problems encountered in literature review was the difference between the DNA constructs used to make transgenic GH fish, which made direct comparisons of the various studies difficult. The choice of gene promoter is important because it dictates where and when the GH is produced (expressed) in the fish. Much of the original transgenic fish literature focused on describing MT-GH-fish, while later studies focused extensively on op-GH-fish (AAS). In addition, some studies used Pacific salmon GH, while others used Chinook salmon GH (AAS), which can have different activities in fish.

With respect to GH levels, in some of the MT-GH-fish the GH levels were almost 40 times that of wild-type (WT) salmon, while studies with op-GH fish (AAS) showed that the GH levels never exceeded that of WT salmon. This difference was made very clear by ABT’s Vice President of Marketing and Sales, Henry Clifford when he stated that:

“The FDA examined this topic very closely in regards to our salmon, including obligating us to quantify six different types of growth hormone in our salmon, all of which were statistically not different from the levels of the same growth hormones in conventional Atlantic salmon. The genetic construct in AAS does not result in higher levels of growth hormone; it merely results in more continuous and efficient metabolic use of the salmon’s endogenous growth hormone over a greater number of tissues in the GM salmon” (personal communication with Henry Clifford, Vice President of Marketing and Sales, AquaBounty Technologies, 6/15/13).

Henry Clifford’s indication that the GH levels are not significantly elevated in AAS fish are backed up from the information we obtained in our review of the literature. Table 13 in the FDA’s VMAC report (2010, page-66) showed that the plasma GH levels for AAS averaged to 39.9 ng/ml, compared to 28.2 for non-GM siblings and 20.5 ng/ml for WT controls, and none of the values was significantly different. In addition, Table 15 of the FDA’s VMAC report (2010, page-68) showed that the GH levels of all three types of salmon are below the limits of detection. ABT’s speculative working hypothesis for the accelerated growth observed for AAS, discussed
in the refereed literature (Du et al., 1992) and in the VMAC data (FDA VMAC, 2010, page-66), is that the op promoter drives GH expression at low levels in a broad array of tissues in close proximity to GH receptors, providing more efficient growth. For AquaBounty, the levels of GH are a key point, because if the AAS showed highly elevated levels of GH, it could make public acceptance more difficult, as some of the hormonal alterations and reduction in pituitary gland sizes seen with MT-GH-fish (cited previously in the Literature Review hormonal section) would come into play. Reaffirming this position (although not going into much mechanistic detail), Henry Clifford stated that:

“The AquAdvantage genetic construct is comprised primarily of the growth hormone gene from the Chinook salmon (a cousin of the Atlantic salmon) coupled with a promoter sequence from a antifreeze protein of a marine fish (Ocean Pout). The promoter controls the expression of the growth hormone gene, which in turn accelerates the growth of the AAS salmon” (personal communication with Henry Clifford, 7/23/13).

Armed with only a speculative mechanism of action of GH in their AAS fish, ABT might have improved public acceptance of the fish by performing some more extensive studies on how op-GH drives the accelerated growth without being elevated in the plasma and muscle. And by eliminating GH values in the VMAC tables that were beneath the limits of detection of the assay (were not numerically accurate at such low levels), only the values above the limits of detection were included in the tables, which appeared to create a slightly higher numerical average, which we predict will gain the attention of consumer groups.

Some scientists believe that op-GH-fish and MT-GH-fish might show similar phenotypes. For example, Robert Devlin, an extremely well-respected scientist and one of the “fathers” of fish transgenesis, stated “We have used the AAS construct before this had all been commercialized in the early 1990’s … we inserted the DNA construct to be used in the AAS into Coho salmon and we got very similar effects as to what we see with the [MT] gene constructs
that we made independently here, so I think they are doing very similar things” (personal communication with Robert Devlin, Center for Aquaculture and Environmental Research, Vancouver, Canada, 8/20/13). Clearly, Devlin has directly experimented with different promoters in GH-salmon, so his testimony carries some weight. But regardless of the exact mechanism of the accelerated AAS growth, their pituitaries appear to be normal (unlike with some MT-GH-fish that strongly over-express GH), and the AAS show no more morphological abnormalities than is observed in other sterile triploid fish (see food safety section), so the presence of the transgene appears to only alter growth rates.

Some critics dispute the purported fast growth rates of GE salmon. For example, Tim Schwab, researcher of Food and Water Watch stated “There is no available evidence that GE salmon grow faster at all, they may even grow slower than current commercially existing salmon” (personal communication, Tim Schwab, Food and Water Watch, 6/20/13). “Sufficient research has not been performed on the growth rates in food, so the FDA doesn’t have enough data to really make determinations about this product” (personal communication, Tim Schwab, Food and Water Watch, 6/20/13). However, our research indicates that in spite of Tim Schwab’s information, the accelerated growth of op-GH-AAS has been thoroughly publically documented in the refereed literature since the very first creation of the AAS fish (Du et al., 1992), and in the subsequent experiments (e.g., Cook et al., 2000; Deitch et al., 2006; Oke et al., 2013). The accelerated growth was also documented by ABT for the FDA in the Environmental Assessment (2010, page 20) where they showed the mean body weight of AAS (261.00 ± 60.93 g) was significantly greater (P < 0.0001) than WT diploid salmon (72.63 ± 17.80 g) grown under the same culture conditions.
4.1.2 Effects of GH on Fish Pituitary Glands and Hormones:

In the information provided to the FDA, ABT indicates the op-GH produced in AAS fish does not alter the levels of any other key hormone in the fish (FDA Draft Environmental Assessment, 2012, page 27, Section 5.2.1.1). For MT-GH-fish, our literature review identified a few studies showing that those GM fish show reduced pituitary sizes and altered levels of pituitary hormones (e.g., Mori and Devlin, 1999); likely the reduction in pituitary size results from the elevated levels of GH produced by the strong MT promoter in a negative physiological feedback loop. Using behavioral alterations as an end-stage indicator of potential pituitary alterations, for AAS fish the only behavioral alteration we identified was a decrease in mating fitness relative to WT salmon (see Environmental section, Trojan gene effect), which would likely not harm the environment in the event of an escape.

With respect to other hormones, our interviews with consumer groups indicated that their most serious concern was an apparent elevation of the levels of a hormone, insulin-like growth factor-1 (IGF-1), which is known to cause cancer in humans (see literature review section). For example, Tim Schwab stated, “GE salmon have been shown to have elevated levels of insulin growth factor-1 (IGF-1), a hormone known to be linked to cancer” (personal communication, Tim Schwab, Food and Water Watch, 6/20/13). Likely Tim Schwab is referring to the small numerical increase apparent in the means, not whether the small increase was statistically significant. The consumer groups argue that the VMAC data show that only 6 AAS fish were evaluated for hormone levels (and the mean IGF-1 levels were elevated), while ABT claimed that 30 AAS fish were evaluated (and the IGF-1 mean was not elevated). Our investigation indicates that both sides of the argument are partially correct. The VMAC data in Table 15
(FDA VMAC, 2010, page-68) shows IGF-1 levels for only 6 AAS fish (validating the consumer group’s point), but the report text indicates that the FDA-approved protocol requires any values below the lower limits of detection to be excluded from the table. The IGF-1 levels for 24 AAS fish were beneath the lower limits of detection, so indeed 30 AAS fish were evaluated by ABT, validating ABT’s claim. Although the AAS average IGF-1 levels appeared to be slightly elevated (10.26 ng/ml for AAS versus 7.34 ng/ml for non-GM siblings) (consumer claims), the apparent increase was not statistically significant (see NS not-significant, in the table) (validating ABT’s claim). When the 24 non-included samples were included in the calculation of the means, the AAS IGF-1 mean was actually lower than the non-GM salmon. To prevent confusion in the future, we recommend including all numerical values in the calculations shown in the highly visible data tables (the tables cited most often by consumer groups), and if it is critical to also show the means using only those values above the limits of detection to do so side-by-side with the more inclusive data.

Even if the IGF-1 levels in AAS had been elevated, our research determined that the amount of IGF-1 produced in the human digestive tract daily greatly exceeds (by 100-fold) the amount that would be consumed daily eating AAS meat. According to the Joint Food and Agricultural Organization of the United Nations and World Health Organization Expert Committee on Food Additives (FAO-WHO, 1998), on average humans produce about 10,000,000 nanograms (ng) of IGF-1 per day in the body (Table 1 in the citation), and indirectly consume (through secretions into the digestive tract) about 380,000 ng of IGF-1 per day (page-10 in the citation). Table 19 in the VMAC data (FDA VMAC, 2010, page-73) indicates that someone eating AAS daily would consume about 3.7 micrograms (3,700 ng) of IFG-1 per day, about 100-times less than the amount produced daily by that person’s normal digestive secretions.
Importantly, in an experiment performed on animals, 7 days of oral administration of high doses of IGF-1 in milk did not significantly increase the levels of IGF-1 observed in their blood (FAO-WHO, 1998, page-9), indicating the consumed IGF-1 was fully digested and not absorbed intact (without degradation) into the bloodstream. Given the importance of measuring the levels of IGF-1 (a potential carcinogen) in AAS, the VMAC data extended the IGF-1 analysis to include a Margin of Exposure Assessment (MOE) (FDA VMAC, 2010, page-70), which showed that the levels of IGF-1 expected by ingesting AAS daily lie within the range of daily human exposures and do not constitute a food ingestion hazard. So our results indicate that the extensive consumer worries about IGF-1 likely are not valid.

With respect to other hormones, the VMAC data (FDA VMAC, 2010, Table 15, page-68) shows no significant alterations in estradiol, 11-keto-testosterone, T3, or T4 hormones in AAS fish compared to non-GM siblings or WT salmon. Backing up this point, Henry Clifford indicated that “The FDA required us to assay the levels of 6 different hormones in AAS fish, all of which were found to be statistically equivalent to WT salmon” (personal communication, Henry Clifford, ABT, 7/1/13). The noted fish expert Robert Devlin stated that, “I think there really isn’t a lot of published data on the hormone levels on their fish” (personal communication Robert Devlin, 8/20/13). Dr. Ian Fleming reminded us that perhaps the health of the AAS is not very important per se, so long as they manufacture meat well; “Obviously a big advantage of GH fish, and the reason that growth is selected for in aquaculture facilities, has nothing to do with the health of the fish. It has everything to do with getting the fish up to a size they can be harvested in a quick and cost effective manner” (personal communication, Ian Fleming, Department of Ocean Sciences, University of Newfoundland, 8/13/13).
4.1.3 Effects of Consumed GH Meat:

Our Literature Review identified no studies directly conducted with humans eating GH salmon, but the VMAC data included an extensive analysis of the key hormone levels in the meat (discussed previously), and the AAS meat’s potential allergenicity. The hormone analysis of the meat, as discussed previously, showed GH levels beneath the limits of detection of the assay used. With respect to whether any small amounts of ingested GH (in AAS or WT meat) would affect humans, Dr. Eric Hallerman, a noted fish expert, stated that; “[The growth hormone] gets broken down into short fragments of a few peptides. These fragments are not bioactive. They may even be fully degraded into amino acids which may be taken up [as nutrients], especially the ones that are essential (i.e., that we must get from our diet)” (personal communication, Eric Hallerman, Virginia Tech, 7/14/13). It seems likely that the very tiny amounts of GH produced in these fish (at the same level as WT fish) is not harmful considering it is the same sequence of GH already produced in Chinook salmon which is already being consumed by humans large scale. Eric Hallerman goes on to say that even if the GH is not degraded in the digestive system, “Salmon GH is not bioactive in human consumers. It does not bind our GH receptors. This point is also supported by Henry Clifford who stated that, “Due to interspecies differences in [GH] receptor sites, salmonid GH is not bioactive in humans” (personal communication, Henry Clifford, ABT, 7/1/13). Thus, based on the testimony of fish experts and the FDA data, our findings indicate that the Chinook GH if ingested likely would not harm humans.

With respect to non-hormonal chemical alterations of AAS meat compared to WT meat, the FDA found that, “Based on all previous criteria including statistical analyses, we conclude that levels of all analytes in ABT salmon are similar to levels in appropriate comparator salmon
(e.g. either the sponsor provided controls, regular farmed salmon, data from the literature reports, or some combination of the three)” (VMAC Briefing packet page 96, 2010). Our literature search and interviews identified no sources that disagreed with this assessment of no chemical alterations. Thus, with almost no difference in composition of meat nutrients reported, we agree with most scientists that the AAS meat likely is safe for human consumption.

4.1.4 Potential Problems with Fish Triploidy:

Our Literature Review identified triploidy as the best current practice and main standard of the aquaculture industry for sterilizing fish in a variety of species, so ABT’s use of triploidy to create sterile fish appears to be state-of-the-art. But the elevated pressures used to induce triploidy are not 100% effective (leaving 2-5% of the fish fertile), and can sometimes harm the fish. What types of morphological changes have been observed for AAS and other triploid fish, and would the ingestion of AAS meat harm humans? If approved by the FDA, ABT will need to scale-up their egg production capacity, but currently no data exists on the percent sterility in large pressure-treated batches.

With respect to morphological changes, the triploidy method of sterilization can cause jaw erosions, fin lesions, heart abnormalities, focal inflammation, fin abnormalities, and other physical deformities as reported by the FDA who stated, “Macroscopic observations of gill, fin, and heart abnormalities are most likely due to the induction of triploidy, rather than as a result of fish containing the AquAdvantage construct. In addition to this, [non-GH] triploids grow slower and have impaired immune systems” (VMAC FDA Briefing, 2010, page 41). Thus, although triploidy is a standard method in aquaculture for inducing sterility in a variety of species, triploid fish in general tend to be less healthy than diploid fish.
Triploidy is also not 100% effective at inducing sterility. According to Dr. Robert Devlin, “We’ve only been able to get 99.8% at the highest in terms of percent sterility, and while that seems pretty high if this technology were ever to be used in an open net pen situation where fish obviously escape quite regularly, even a 0.2% or 1% rate of failure of that kind of technology would still release quite a few unsterile animals into nature” (personal communication, Robert Devlin, 8-20-13). Dr. Devlin goes on to indicate that even the smallest chance of fertile fish escaping still poses a risk to the environment. Dr. Ian Fleming also spoke on the technique of triploidy stating that “It will certainly reduce the viability of any escapees, but of course it’s not 100%, so there is always associated risks. But ABT’s use of triploid fish is certainly an improvement over using diploid fish” (personal communication, Dr. Ian Fleming, 8-13-13). Both Dr. Fleming and Dr. Devlin are experts in fish transgenesis and have investigated transgenic GH-fish for many years. In the field of Biology, it is difficult to say that anything occurs with 100% frequency, especially an artificial process; there will usually be a small but measurable rate of failure. ABT’s Vice President of Marketing agrees, saying “some batches are 100% sterile, but some are as low as 99% sterile. On average, we typically can produce batches with 99.9% sterility” (personal communication, Henry Clifford, ABT, 7/1/13). Dr. Eric Hallerman stated that, “Sterilization with triploidy commonly ranges from 95-100%, but is not always 100%. Hence, verifying triploidy with each batch is essential” (personal communication, Eric Hallerman, Virginia Tech, 7/14/13). These statements indicate that although triploidy is very effective at inducing sterility, the success rate is not high enough to ensure that all escaped AAS fish will be sterile, so it becomes important to discuss the potential effect of an escaped fertile AAS fish into the Panama environment and ways of assaying the percent triploidy in each AAS batch produced.
To match the demand for fish eggs to be sent to Panama, ABT’s batches of triploid eggs prepared in Canada will be larger than the batches that were previously used to provide the FDA data. Karyotyping (analyzing chromosome numbers by microscopy) is the most popular way to verify triploidy, but it is time consuming, so karyotyping the nuclei of every AAS egg produced in Canada would not be feasible. However, a faster assay might involve measuring nuclear size, because triploid nuclei contain 150% of the DNA and are usually larger. However, this method can be somewhat unreliable. The most reliable method for measuring triploidy would be cytofluorometric analysis (the staining of the nuclear chromatin with a fluorescent dye whose signal can be quantitated), but this is a very expensive method and would make fish aquaculture less cost effective (Felip et al., 2001). With respect to the percent sterility for larger batches of pressure-treated eggs, no facilities as large as ABT’s have yet been constructed, so there is no data on whether the same range of 95-100% sterility will remain in effect after scale-up. Dr. Robert Devlin indicated, “We’ve induced triploidy large-scale in some studies, inducing up to 60,000 eggs at once. This was done with little compromise to the success rate; however this is still not at the same scale that AquaBounty would be doing if they produce AAS eggs at a commercial level” (personal communication, Robert Devlin, 8/20/13). Although 60,000 eggs may seem like a large number, ABT is proposing to sterilize batches in the hundreds of thousands. So our findings indicate that currently no data exists demonstrating the percent sterility that can be expected at ABT’s proposed level of scale-up.

Another problem with triploidy is the possible re-diploidization of the fish. In this process, previous triploid genomes revert to the diploid state by extruding one extra set of chromosomes during mitosis, and are fertile after they are grown. In theory, this would mean that previously sterile AAS fish could regain the ability to breed. Although re-diploidization has been observed
in some oyster species, Dr. Eric Hallerman notes that re-diploidization has *not* been observed in fish” (personal communication, Eric Hallerman, 7/15/13). He continues that, “AquaBounty agreed with the FDA to assess and report the triploidy status of all of their produced stocks. If a batch is shown to be triploid (sterile), it should remain triploid throughout the lives of the fish.” Overall, with respect to triploid sterilization, our findings indicate that fish experts agree it is currently the most reliable method of inducing sterility, even if it is not always 100% effective, that re-diploidization is not likely to happen in fish, and that no data exists on the percent effectiveness expected by ABT upon scale-up. So, we conclude that it is important to monitor the percent sterility of all batched produced, and to consider the potential consequences of a fertile AAS escape.

### 4.2 Regulatory Issues

Regulatory issues were major concerns of many people that we interviewed for this project. Their concerns discussed in this section included whether the FDA review process was thorough, whether GM-fish should be labeled as such for the consumer, whether the approval process was sufficient to ensure food safety, and whether an FDA-approval would open the flood-gates for future approvals of other GE animals. We end this section with a discussion of some of the legislation that has been proposed to address some of these issues.

#### 4.2.1 Regulatory Process:

The FDA’s review of ABT’s AAS has been a very long drawn out process that has been criticized by several different stakeholders. Their review of ABT’s application as a New Animal Drug has drawn criticism, as has their environmental assessment.
4.2.1.A FDA Review as a New Drug Application:

The U.S. currently has no legislation specifically regulating transgenic animals. Currently, the FDA regulates GM animals under the Federal Food, Drug, and Cosmetic Act (FFDCA) as a new animal drug application (NADA) (VanEenennaam et al., 2013, page-1). The FDA did not “choose” (as some consumer groups believe) to review the application as an NADA, it was required to do so under the FFDCA guidelines, Section 512 which states that if an article is intended to alter the structure or function of the animal it is considered a new animal drug, so all rDNA constructs that change the characteristics of an animal qualify as a new “drug” (FDA Guidance for Industry, 2009). As with all new drug applications, the sponsor (ABT) was required to conduct and pay for studies to obtain regulatory compliance. Clarifying their review of ABT’s application as a NADA, our interview with a representative of the FDA indicated that “GE animals are evaluated under the regulatory framework of the New Animal Drug provisions of the Federal Food, Drug, and Cosmetic Act, as described in Guidance 187. This is because FDA considers the piece of DNA that is inserted into the genome of the animal to affect its structure or function to meet the definition of a new animal drug. It’s the recombinant construct in the animal that’s regulated under the new animal provisions of the Act; the animal itself is not considered a drug” (personal communication, Siobhan DeLancey, FDA, 6/5/13).

But by reviewing the application as a NADA, some legislators interviewed for our project believe that “the FDA’s regulatory process is intended for drugs given to animals. Genetically engineered fish intended for human consumption should be subject to a different review process which is more appropriate for this use” (personal communication, Daniel Sousa, NOAA Sea Grant Fellow for Congressman Mike Thompson, D-CA, 8/13/13). The Center for Food Safety agrees that “there are no laws governing how federal agencies should regulate GE animals. They
are using outdated statutes” (personal communication, Colin O'Neil, Center for Food Safety, 8/5/13). These stakeholders believe that new legislation should be introduced for specifically reviewing GE animals, and that doing so would enhance the credibility of the review process.

4.2.1.B FDA Environmental Assessment:

As a part of the FDA’s review as a New Animal Drug Application, an environmental assessment was required to determine the impact the GE fish may have on the environment. However, some of the consumer groups argue the FDA’s environmental assessment was not transparent enough and not extensive enough for this novel GM food application, and these are topics of heated debate.

With respect to transparency, although several of our consumer group interviews pointed to a general lack of transparency in the FDA’s review process, our research indicates that the review had at least two unprecedented steps towards transparency initiated by the FDA in recognition of the fact that this would be the first GM animal for consumption. The first was the FDA’s unprecedented requirement for the formation of a committee of scientific experts and veterinarians (the Veterinary Medicine Advisory Committee, VMAC) to review ABT’s submitted data, and required the VMAC to hold a public meeting after its review to present its findings and receive public comments. And second, although not required to do so, two weeks in advance of the September 19-20, 2010 public meeting, ABT made an unprecedented effort for transparency by making its 172-page VMAC briefing package (FDA VMAC, 2010) and 84-page environmental assessment (FDA EA, 2010) publically available for comment.

With respect to whether the FDA environmental assessment was rigorous enough, some consumer groups and politicians have argued that the FDA does not have the expertise to
rigorously assess environmental impact of transgenic animals. Several respondents pointed to the Fish and Wildlife Services (FWS) or the National Oceanic and Atmospheric Administration (NOAA) as appropriate agencies with the relevant expertise to conduct and environmental assessment. However, our research identified publically available documents showing those exact two agencies were *indeed formally* consulted by the FDA in their review. The NMFS and FWS were provided with the environmental assessment, VMAC briefing material, and FONSI, and concurred with the FDA’s finding of no effect. Appendix-D.1 of the 2012 FDA Environmental Assessment (pages-135 and 136) shows copies of two signed letters. The first letter is dated December 16, 2010, and is signed by Richard Sayers, Chief of the Division of Conservation, U.S. Fish and Wildlife Service. Dr. Sayers states that “Given the nature of the [Panama] facilities described, any of these outcomes [hybridization, competition, consumption] appears to be extremely unlikely, and your “no effect” determination seems well supported for this approval”. Dr. Sayers also provides his comments on potential future ABT applications, saying “We understand that the use of any other facilities to breed or raise AquaAdvantage salmon for sale in the U.S. would require additional environmental review and consideration of the potential need for consultation under the Endangered Species Act”. The second letter is dated July 6, 2011, and is signed by James Lecky, the Director of the Office of Protected Resources, U.S. National Marine Fisheries Service. In the letter, Dr. Lecky lists the exact dates (8 different days over 4 months in 2011) that the NMFS members met with FDA officials to formally discuss the AquaAdvantage salmon. Moreover, with respect to consulting with other agencies for its environmental review, page-100 of the 2012 FDA Environmental Assessment states that the FDA in 2002 hosted a meeting to exchange information and discuss environmental risk assessment issues for the AquAdvantage Salmon. The 45 meeting participants included
representatives from several FDA offices (the FDA’s Center for Veterinary Medicine, Center for Food Safety and Applied Nutrition, Office of the Commissioner, and Office of International Programs), and several agencies outside the FDA, including the National Marine Fisheries Service, U.S. Fish and Wildlife Service, Canadian Food Inspection Agency, Environment Canada, Canada’s Department of Fisheries and Oceans, Australia’s Department of Primary Industries, Academics from Harvard University, Southampton University, and the University of New Brunswick.

Our interview with one member of the FWS indicated he thought the FWS review was mostly informal conversations over casual lunches, but the signed letters submitted to the FDA point to more formal multiple day-long meetings. All of our interviews with consumer groups agreed that the FWS and NMSF had considerable expertise in this area and would be appropriate agencies for rigorously performing environmental assessments. Henry Clifford of ABT agrees that the “NOAA was indeed consulted by the FDA during their review” (Henry Clifford, 7/1/13). Daniel Sousa, NOAA Sea Grant Fellow for Congressman Mike Thompson, D-CA, noted that the FDA does not have to follow the advice of informal reviews. But this [lack of formal consultation] is why many share the belief that “a more thorough, unbiased data collection and review is needed to better inform the review process” (personal communication, 8/13/13). With respect to which division of the NOAA might be best suited for performing a rigorous review, Bob King, Assistant for Senator Mark Begich, D- AK, indicated it could appropriately be conducted by the National Marine and Fisheries Services (NMFS) within the NOAA (personal communication, 7/22/13).
Joel Bader of the U.S. Fish and Wildlife Service (FWS) mentioned in his interview that he was informally and formally consulted for his opinion on science-related AAS issues when he stated:

“A lot of what we do when we see each other is informal consultations, like we would meet and talk to friends at lunch or something. But they can also ask us in a formal way when they ask us to respond to public drafts [as with the AAS review], and then informally they may ask us ‘what do you think about the science?’ Or ‘a paper has come out, and how do you react to that?’ What we also do for instance, as when ABT’s application was submitted and the FDA told us they were leaning towards a finding of no significant impact. So, they asked us for our opinion both formally and informally. We officially, on the record, said that ‘we stand on the sideline unless there is some Endangered Species Act (ESA) issue we have not already evaluated’ and you can make a decision without us because we have no authority over what a human consumes. Our regulator authority involves typically live animal imports, in this case Salmonid fish. If this product comes in as a dead eviscerated (gutted) carcass for human consumption and we have no ESA concerns, we are not likely to have an issue with this original application. If [the U.S. Fish and Wildlife Service] felt that a dead animal was going to affect Atlantic salmon that is endangered in the United States, then [they] would say that” (personal communication, Joel Bader, U.S. Fish and Wildlife Service, 8/14/13).

So, with respect to consultation outside the FDA, our findings indicate the FDA indeed consulted with several outside agencies on their environmental assessment, and the consultation was formal enough to warrant the sending of two signed letters from the heads of outside agencies agreeing they were consulted. And both agencies agreed they would become more involved in the future if ABT applies for permission to raise AAS within the U.S.

4.2.2 Labeling Issues:

After speaking with several legislative assistants and other public interest groups, we found that people like to know what they are eating. This brings forth an issue of labeling GM food. Should we label the AAS meat as GM for consumers? Daniel Sousa, NOAA Sea Grant Fellow for Congressman Mike Thompson, D-CA, said: “Labeling GE salmon is a good idea
because it gives consumers the right to choose what they are putting on their dinner tables and feeding their families” (personal communication, 8/13/13). As discussed earlier, the FDA has not made official comments on the labeling of the AAS, although the federal government currently does not require it, and the data shows that AAS is very similar to WT salmon in nutritional values. Although not required to do so by law, ABT indicates they plan on labeling it anyway according to Henry Clifford who states: “Any statements to the effect that ABT’s product will not be labeled GM salmon are false. However, since AAS fish are equivalent to WT fish, we are not required to carry an identifying label” (personal communication, Henry Clifford, ABT, 7/1/13). Not surprisingly, consumer groups are in favor of requiring the labeling. For example, Colin O’Neil of the Center for Food Safety said “if the FDA does approve AAS, it must be labeled” (personal communication, 8/5/13). Although several bills requiring the labeling of GM foods have been introduced in Congress, all of them remain in committee and have not been brought to a vote (discussed in more detail below).

4.2.3 Food Safety

One of the main concerns raised by the public is whether AAS fish are safe for human consumption. The FDA’s GFI 187 procedure is designed to determine whether GM meat is safe to eat, and to find any potential risks caused by the DNA construct. Not surprisingly, consumer groups argue the fish is not safe for consumption. For example, Colin O’Neil of the Center for Food Safety indicated “there is not enough data to say that [AAS meat] is ok for humans to eat” (personal communication, 8/5/13). However, our research indicates that a substantial amount of the data submitted to the VMAC focused on food safety, including analyses showing that the chemical and hormonal composition of the AAS is equal to WT salmon, and that any
morphological alterations seen in AAS result more from the triploid state (of any fish) and are not specific to AAS. In August of 2011, the Center for Food Safety (CFS) submitted additional comments to the FDA stating that “the data provided to the public on food safety is altogether deficient given that the FDA has had 10 years to review the product. The study on morphological changes in AAS fish only involved 12 fish, and the allergenicity study only six fertile and six sterile fish. The FDA only looked at data for 6 fish in its allergy assessment. That’s clearly not a big enough sample set. This company boasts about having the most studied fish in the world and have been studying this for 2 decades. Well, I really don’t think that’s the case, if they can only bring 6 fish to the table” (personal communication, Tim Schwab, Food & Water Watch, 6/20/13). Schwab mentions that the answer is to have more research on this topic, not to just commercialize the process and see what happens. Table 29 on page-102 of the VMAC Briefing Material (2010) validates the CFS and FWW argument that 6 transgenic fertile fish and 6 transgenic triploid fish were analyzed for allergenecity measurements. However, the Chinook GH produced in AAS is not a known allergen, and the VMAC concluded that AAS salmon pose no additional allergenic risk relative to WT Atlantic salmon (FDA VMAC, 2010, page-106).

Moreover, there appears to be no medical consensus about what level of an allergen actually constitutes a public risk (Goodman et al., 2008; VanEenennaam and Muir, 2011), so without such studies being performed evaluating the AAS allergenicity potentials remains very difficult. CFS argues this is a small study and that a larger study should be done, and perhaps this is true. The FDA announced that their approval would require post-market reviews, but the CFS claims these would be impossible without consistent and mandatory GM labeling (CFS, Additional Comments, 8/1/11), otherwise how would consumers know whether they are eating GM-fish to help report any observed allergenic episodes.
4.2.4 Setting a New Legal Precedence:

Some critics of GM foods think the real danger of the FDA approving ABT’s application is it would set a legal precedent for other companies to apply for FDA approval for their GM animals, greatly increasing the number of applications and their likelihood of approval. Thus, even if ABT’s current application is highly restrictive (proposing to sell sterile eggs to foreign vendors who will raise the fish and ship the meat back to the U.S. for consumption) other later applications may not attempt to be so restrictive. For example, Eric Hoffman, Biotech Policy Campaigner at Friends of the Earth, said, “AquaBounty has genetically engineered tilapia and trout [besides salmon]. There are also genetically engineered cows, goats, and chicken. Everyone is just waiting, and if the FDA approved this fish it is going to open up a flood gate” (Link TV, 2011). This concern has also been voiced by Food and Water Watch who stated that “Biotech companies are pushing transgenic fish hard to further the use and creation of transgenic animals. GE salmon is the sacrificial lamb for the biotech industry” (personal communication, Tim Schwab, Food and Water Watch, Washington, DC, 6/20/13).

In response to these assertions, on their website AquaBounty indicates that its only aim is to make aquaculture more environmentally friendly, more efficient, and profitable (ABT, 2013). However, the fact that ABT’s application has been under review for almost two decades is hurting ABT financially. According to the CEO of ABT himself, Ronald L. Stotish “[the regulatory delay is] threatening our [ABT’s] very survival. We only have enough money to survive until January 2013” (Perrone, 2012). ABT has spent $67 million in developing the AAS since it started in the early 1990s (Perrone, 2012). So, to say that ABT has a lot riding on FDA approval sooner rather than later is an understatement.
Whether subsequent applications to the FDA will be as restrictive as ABT’s remains to be seen, but the FDA approval process has already set a precedent for biotech scientists. Many researchers are changing their plans to avoid being stuck in regulatory limbo in the United States, and in fear are discouraged from investing in animal biotechnology. For example, researchers at the University of California, Davis have already transferred an experimental herd of genetically engineered goats that produce protein-enriched milk to Brazil, due to concerns about delays at the FDA (Perrone, 2012). With more researchers moving their work to other countries, this could put the United States at a competitive disadvantage when countries like China are currently pouring in millions into this field (Perrone, 2012).

However, it is still important to note that even if AAS is approved by the FDA and it sets a precedent, other applications will still have to be individually approved by the FDA. If AAS receives FDA approval, this does not mean that all GM organisms will automatically be approved thereafter. Furthermore, “if AquaBounty wants to expand to other [production] facilities in the future they would need to apply for another FDA approval” (personal communication, Eric Hallerman, Virginia Tech, 7/14/13).

4.2.5 New Legislation:

Our interviews with legislators and public interest groups found that many agree that “We need new laws. Congress must pass new laws that tell federal agencies how to regulate genetic engineering. There is no current law about how they regulate GE animals [for consumption] and this AAS is kind of the guinea pig, to see what they are doing and how to do it for future GE animals” (personal communication, Colin O’Neil, Center for Food Safety, 8-5-13). Many legislators and public interest groups believe that as science has progressed our laws and
regulations have not kept up. Therefore, new laws need to be introduced to ensure regulative balance. Several federal bills regarding GM food have been introduced into the House of Representatives and the Senate (Table-4).

**Table-4: Current Legislation Regarding Transgenic Fish**

<table>
<thead>
<tr>
<th>Bill No.</th>
<th>Name</th>
<th>Purpose</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR 584</td>
<td>To amend the Federal Food, Drug, and Cosmetic Act to require labeling of genetically engineered fish.</td>
<td>To amend the Federal Food, Drug, and Cosmetic Act to require the labeling of genetically engineered fish.</td>
<td>Introduced in the House on 2/6/13 by Representative Don Young (R-AK). On 2/8/13 referred to the Subcommittee on Health, where it remains under consideration.</td>
</tr>
<tr>
<td>S 248</td>
<td>A bill to amend the Federal Food, Drug, and Cosmetic Act to require labeling of genetically engineered fish.</td>
<td>A bill to amend the Federal Food, Drug, and Cosmetic Act to require the labeling of genetically engineered fish.</td>
<td>Introduced to the Senate by Senator Mark Begich (D-AK) on 2/7/13. On the same day it was referred to the Committee on Health, Education, Labor, and Pensions, where it remains under consideration.</td>
</tr>
<tr>
<td>S 246</td>
<td>Pegasus Act</td>
<td>To prevent the escapement of genetically altered salmon in the United States, and for other purposes.</td>
<td>Introduced in the Senate on 2/7/13 by Senator Mark Begich (D-AK). Then it was referred to the Committee on Commerce, Science, and Transportation, where it remains under consideration. Also, it was introduced to Congress on 4/23/13 by Representatives Don Young (R-AK), Mike Thompson (D-CA), and Jared Huffman (D-CA).</td>
</tr>
<tr>
<td>HR 1699</td>
<td>Genetically Engineered Food Right-to-Know Act</td>
<td>To establish a consistent and enforceable standard for labeling of foods produced using genetic engineering, including fish, thereby providing consumers with knowledge of how their food is produced.</td>
<td>Introduced in the House on 4/24/13 by Representative Peter DeFazio (D-OR). Referred to subcommittee on Energy, Commerce, and Health where it remains under consideration.</td>
</tr>
</tbody>
</table>

Bill S 248 was introduced to the Senate by Senator Mark Begich of Alaska. Our IQP group was introduced to this bill through an interview with Bob King, a legislative assistant to Senator Mark Begich. As we were discussing the types of action the Senator would take if AAS was approved, Mr. King mentioned the legislation that had been introduced in order to keep our nation safe and keep us from any harmful impacts AAS might have. Senator Begich also
introduced the Pegasus Act to help prevent escapement in case the AAS is approved for growth in the U.S. in the future. Bill HR 584, the house equivalent of S 248, was introduced by Representative Don Young of Alaska. Senator Begich and Representative Young have been working together to introduce new legislation for our safety. Together, these bills show that our legislators are looking to the future, and also looking at the bigger picture. They want to ensure that the government is doing all it can to inform the consumer and to avoid environmental and economic risks from GM foods. In the interest of full public disclosure, and to support any future post-consumer studies on individuals who have consumed AAS meat, we support the passing of these GM food bills.

4.3 Environmental Issues

4.3.1 Escape Risk

As discussed previously, ABT’s grow-out facility in Panama includes multiple barriers designed to prevent AAS fish escaping into and surviving in the wild (see Table-2 and Figure-7). The FDA environmental assessment for AAS concluded that the likelihood of escape, establishment, and spread of AAS is infinitesimally small due to redundant containment measures, such as: biographical, geographic/geophysical, physic-chemical, and physical measures (FDA EA, 2010). “ABT will use 21 physical containment barriers (screens, filters, nets, boxes), with a minimum of 11 barriers in sequence” (personal communication, Henry Clifford, ABT, 7/1/13). Many scientists agree with ABT that their containment measures are sufficient. For example, Eric Hallerman, a researcher at Virginia Tech, thinks there are sufficient redundant screening and barrier placements to prevent any environmental issues in terms of ABT’s inland tanks (personal communication, Eric Hallerman, 7/14/13). Eric Nelson from the EPA shared a
similar sentiment saying “I think that land-based culture operations are the best way to prevent the unintentional release of transgenic fish into the wild” (personal communication, Eric Nelson, Boston EPA, 6/26/13). Because land-based tanks are more expensive than ocean cages, obviously ABT has gone to great expense to attempt to prevent AAS escape, and some scientists argue their plan is state-of-the-art.

On the other hand, in spite of ABT’s proposed safeguards, some stakeholders were concerned that no system can guarantee zero fish escapes. Escapes from coastal, open-net facilities (more risky than ABT’s) have been well documented (Table 5), although this data does not directly apply to ABT’s in-land facility.

Table-5: Global Salmon Escape Events (mostly Atlantic salmon).

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>EVENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Brunswick, Canada (October 2005)</td>
<td>The Atlantic Salmon Federation claims that farmed Atlantic salmon escapes from the Bay of Fundy accounted for more than 80% of salmon returns in 2005 in the St. Croix and Magaguadavic Rivers. Only nine wild salmon have returned to the Magaguadavic as of 10/13/2005, which, as recently as the 1980s, supported runs of 800 or more wild salmon annually.</td>
</tr>
<tr>
<td>Cook Inlet, Alaska (July 2006)</td>
<td>An Atlantic salmon caught in a set net in Cook Inlet, Alaska that had been marked (via thermal technique) was eventually traced to the Rochester, Washington (Scatter Creek) Atlantic salmon hatchery (currently owned by American Gold Seafood). Scatter Creek fish are eventually transferred to marine net pens in Puget Sound, and it is thought that the Cook Inlet fish escaped in May 2006 from a net pen during transfer to a barge.</td>
</tr>
<tr>
<td>Campbell River, BC, Canada (November 2007)</td>
<td>Closed containment aquaculture is seen as a way to reduce environmental impacts of customary marine aquaculture. However, closed containment systems were shown to be unable to contain escapes when in November 2007, two Chinook salmon escaped from the Middle Bay Sustainable Aquaculture Institute’s facility (<a href="http://www.sustainable-aquaculture.ca">http://www.sustainable-aquaculture.ca</a>). The closed containment system failed when storm activity snapped off a waste trap, allowing the salmon to escape.</td>
</tr>
<tr>
<td>Norway (2007)</td>
<td>Aquaculture industry giant, Marine Harvest, estimates that escapes from its Norway operations resulted in 935,000 salmon and trout escaping in 2006 and 407,000 in 2007.</td>
</tr>
<tr>
<td>Scotland (2006, 2007)</td>
<td>The Scottish Salmon Producers Organization (SSPO) reports over 70 million smolts were put to sea at various times during 2005 and 2007, and “breaches of containment where fish were known to have escaped during 2007 corresponded to approximately 0.2% [or 140,000 fish] of the total amount of farmed salmon in Scottish waters during this period. [Also in 2007, the Scottish based Salmon Farm Protest Group, claimed more than one million salmon and trout escaped or died in 2005 in Scottish fish farms].</td>
</tr>
<tr>
<td>Chile (2008/2009)</td>
<td>In Chile, on December 31, 2008, 198,000 farmed salmon (species not known) escaped from salmon cages owned by Mainstream Chile (owned by CERMAQ). More than half were reportedly recaptured.</td>
</tr>
</tbody>
</table>

Although “[t]here has been considerable improvement on controlling escape [from fish pens] . . . as long as we are working in open net pens, escapes are inevitable” (Ian Fleming, Department of Ocean Sciences, University of Newfoundland, 8/13/13). Similarly, Eric Hallerman of Virginia Tech worries that “Escapes [from floating net pens] on the order of 1% are routine” (personal communication, 7/14/13). With respect to the number of escapees, some stakeholders commented on the improved technology for preventing escapes in view of the expanding industry: “The percentage of fish escaping has decreased over time, but at the same time the size of the aquaculture industry has increased, so in essence we still have as many escapes now as we did probably back 10-15 years ago” (personal communication, Ian Fleming, 8/13/13).

Obviously escape data observed for ocean pens does not apply to ABT’s proposed inland tanks. “The data on escapees only applies to open ocean pens” (personal communication, Henry Clifford, ABT, 7/1/13). Escapes from inland facilities, however, have been documented. For example, in 2007 two Chinook salmon escaped from a closed containment facility in Campbell Rive, British Columbia following storm damage (Table 5). As discussed previously, ABT and other proponents of GE fish argue that even in the unlikely event that AAS fish were to escape from the closed-containment facility in Panama they would likely soon die downstream in the relatively warm waters where they would be unable to feed above 28°C, would likely be unable to breed given their likely triploid state, and even if fertile the hybrids themselves likely would be sterile.

Currently, the farmed salmon industry has consolidated, and over half of cultured salmon is produced by only five companies with operations in Canada, the United Kingdom, Chile, the United States, and Norway. Escapes from those operation facilities are well documented with
hundreds of thousands of fish escaping in single events. The example in Campbell River, BC, Canada, shows how even closed containment systems are not immune to escapes (Phillips, 2009). Data shown in the Phillips reference (2009) also document that some escaped fish have the ability to migrate, live, and breed in the wild years post-escape, but these fish were not AAS fish, they were not escaping into waters which downstream would be fatal to them, and they were surrounded by WT fish of the same species. Thus, although sea cages show a much greater chance of escape, escapes from closed systems indeed can sometimes occur. Coupled with the fact that roughly 1-2% of each AAS egg batch will be fertile (see 2.6.6, Triploid Fish), means that scientists should at least consider the likelihood of potential hybridization with any local fish, and whether such hybrids themselves will be sterile (discussed in detail below).

A study published in the Proceedings of National Academy of Sciences showed that the characteristics of GH-fish alter depending on their environment (Sundstrom et al., 2007). They analyzed GH salmon under a variety of growth conditions and concluded that the fish show phenotypic plasticity depending on their environment. Devlin states, “[Because of this], ‘it is clear that forecasting ecological consequences for transgenic organisms reared and assessed in simple laboratory facilities could be inaccurate’” (Sundstrom et al., 2007). Although this may be true, the VMAC data indicates that the rapid growth observed with AAS only occurs in cultured conditions where the AAS are fed to satiety, and does not occur in the wild, so although growth plasticity may indeed occur, if AAS fish escape before reaching full size the lack of constant feeding would render them closer to WT size.

4.3.2 Environmental Assessments

When discussing the environmental assessments of AAS, the two main issues we identified in our interviews were the consumer groups worries that the FDA’s environmental
assessment was not extensive enough, and fear of a Trojan gene effect in which the transgene would spread from AAS fish to WT fish by hybridization with escaped AAS with local fish. The Trojan gene effect is discussed in a later section. With respect to the lack of extensive review, for example Tim Schwab of the Food and Water Watch indicated he believes that the FDA did not meaningfully consult with other agencies that have the necessary environmental information and expertise that FDA lacks. He asserts that “if these fish are able to escape, they could harm the environment” (Tim Schwab, personal communication, 6-20-13). However, as discussed previously, our research identified two signed letters by the directors of two federal agencies (FWS and NMFS) (that all stakeholders agreed held appropriate expertise for performing environmental assessments) indicating their agencies were indeed formally consulted by the FDA on several different occasions over 4 months of evaluations. Bob King, Assistant to Senator Mark Begich (D-AK) noted that the “National Marine and Fisheries Services (NMFS) is qualified to do so” (personal communication, Bob King, Assistant to Senator Mark Begich, D-AK, 7/22/13). Moreover, the FWS and NMFS in their signed letters indicated they acknowledge they will be consulted again in more detail if ABT ever proposes in the future producing AAS within the U.S.

4.3.3 Proposed Culture Location

ABT proposes to culture their AAS in an FDA-approved facility in Panama (FDA EA, 2010). When Henry Clifford of ABT was asked why Panama was chosen as the location he said, “Panama was selected because it has a combination of ideal growing conditions for salmonids and access to good quality water (in its highlands), ideal environmental containment barriers, a long history of aquaculture, and visionary government that is pro-biotech and welcomes innovations in agriculture. Panama also has in place a regulatory framework for overseeing R&D and commercial production of genetically modified plants and animals.” (personal communication, Henry Clifford, Marketing Director, AquaBounty Technologies, 8/2/13)
However, other stakeholders have other opinions on why Panama was chosen. “This is going to be a facility that is going to be run in Panama, and I have no doubts [that] other countries will simply take the FDA’s stamp of approval as meaning that it is [simply] safe to do this. It is true that other countries do that... the FDA can be seen as a stamp of approval in a lot of countries including Canada.” “I think that the [non-U.S.] producers are going to raise the AAS however they wish.” (personal communication, Johns Hopkins University, scientist, Center for Sustainability, 7/2/13)

The severe Panama weather could also help increase fish escapes relative to other in-land tank locations. Panama is known to have heavy rain and storms annually. For example, in August of 2008 an event happened that ABT claims would be unlikely in its physical plan. That month, heavy rain from a storm caused a tank’s water inlet system to fail during the night, “[A]ll the fish were lost [killed]. It was intended that the fish, subject to regulatory approval, would be marketed during the first half of 2009” (LeVaux, 2013). During that particular August, Panama saw 36 inches of rain, and in August 2010 over 61 inches of rain fell (LeVaux, 2013). Although in this instance the AAS fish did not escape, they were killed, a place with consistent flooding does not appear to be the safest location to cultivate transgenic salmon that ideally would have a zero percent escape rate.

4.3.4 Distribution

Currently, the only distribution of AAS fish being sought by ABT is the authorization to sell the AAS eggs, which according to Henry Clifford “will be sold only to qualified and FDA pre-approved fish farmers.” He also states that AquaBounty is not “in the business of farming salmon to commercial market size,” and that “this [farming] falls to their prospective customers
in the fish farming industry” (personal communication, Henry Clifford, 7/1/13). Similarly, Joel Bader of the U.S. Fish & Wildlife Service noted that “the FDA plan is for ABT to produce AAS fish overseas, and then send dead, eviscerated (gutted) fish meat back into the U.S. for human consumption.” This also limits the involvement of the FWS, so when asked whether the application has been officially reviewed by FWS, they responded that “FWS has no authority over whether a human consumes this thing, we don’t deal with dead animals, we deal with live animals. So we have no official say in ABT’s application. If we felt that a dead animal was going to affect U.S. Atlantic salmon, then we would have said so” (personal communication, Joel Bader, U.S. Fish and Wildlife Service, 8/14/13). This may be true, but the FWS was indeed formally consulted for the FDA’s environmental assessment, as described previously.

With respect to ABT potentially in the future producing AAS in the U.S., presentations by Ronald Stotish, the CEO of AquaBounty, indicated an intention to perhaps bring some AAS production into the United States in the future. Thus, Joel Bader was under the impression that “they may initially simply bring in fillets and let people consume those and see if there is a market, and afterwards, if there is a market bring in live fish and move that direction” (personal communication, Joel Bader, U.S. Fish and Wildlife Service, 8-14-13). Bader went on to say that one issue with the current approval procedures is that environmental organizations cannot officially proceed until after the FDA has concluded its approval on human consumption. “The FDA first has to make a decision one way or another on humans consuming [AAS], and then we make a decision at that point on whether we let a live genetically engineered salmon into the U.S.”. Bader believes that ABT earlier made an attempt to produce AAS within the U.S., “while these initial requests were made by AquaBounty, it’s like taking the cart before the horse. The FDA has to make a decision first, and then they [AquaBounty] have to issue us paper work. So
essentially, we didn’t say no, we didn’t say anything on it because we are not the first ones to make the decision. There is essentially nothing we could do and it’s premature” (personal communication, Joel Bader, U.S. Fish and Wildlife Service, 8/14/13). Thus, environmental regulators have limited input about likely impacts on the U.S. environment if the application does not include production within the U.S. The FWS would come stronger into play if ABT in the future applied for such permission. If the company were to pursue raising AAS fish within our borders in the future, they would have to obtain the necessary permission not only for setting up the necessary aquaculture structures, but an additional FDA approval process including new limitations and permits would have to be obtained due to the Atlantic salmon’s status as a federally endangered species. As a result, the state and federal views and regulations would likely vary greatly based on local wildlife risk, especially with consideration of natal streams for wild salmon populations.

4.4 Ecological Issues

4.4.1 Trojan Gene Effect:

The Trojan gene effect describes the potential movement of a transgene through a WT population of fish during cross mating. This effect was originally defined by Muir and Howard (1999) with transgenic medaka fish where the authors showed the movement of the transgene into WT populations under conditions where GM males had a mating advantage over the WT males while the GM offspring (of either sex) were at a disadvantage relative to WT. Theoretically, such conflicts in fitness could drive both GM and WT populations to extinction, obviously getting the attention of environmentalists and consumers relative to AAS fish. In 2001, the theory was confirmed in transgenic salmon grown in cages (Hedrick, 2001). In 2002, the
theoretical model was extended to include a quantitative risk assessment (Muir and Howard, 2002). Howard et al. (2004) studying GM medaka fish showed that GM males have a mating advantage over wild-type males, and included the data into their mathematical model based on six major variables affecting survival fitness (juvenile and adult viability, age at sexual maturity, male and female fertility, male mating advantage). Their model showed that the transgene is expected to spread into WT populations due to the mating advantage, leading to medaka population extinction due to the viability disadvantage.

But with respect to AAS fish, it was not known whether their large size would create conditions where the GM males actually have a mating advantage over WT males (which would enable the Trojan effect). Medaka fish breed daily, while Atlantic salmon breed near the end of their lives, so it was not clear whether the Trojan effect would apply to salmon. Robert Devlin (Fisheries Ocean Canada, West Vancouver) developed an Excel model that Richard Howard of Purdue University applied to salmon, “You still get a [theoretical] Trojan gene type of effect with a salmon-like history. Using Devlin’s model in my courses, it’s easy to get a Trojan gene effect for the same conditions of males having a reproductive advantage but the young not surviving as well as the transgenic” (personal communication, Richard Howard, Purdue University, 8/13/13). Thus, the Purdue team’s data indicates that theoretically a Trojan gene effect could apply to salmon, if the AAS salmon actually showed a reduced mating success.

So, several fish experts, including Eric Hallerman, Ewen McLean, Ian Fleming, and Garth Fletcher, secured a large grant to assess AAS mating behavior. Their data concluded that AAS in fact show a great mating reduction relative to WT salmon, which would make a Trojan effect impossible with respect to AAS fish (Moreau et al., 2011; Moreau and Fleming, 2012).
Their data also backs that up of Robert Devlin’s lab who showed that MT-GH-salmon are reproductively out-competed by WT salmon (Bessey et al., 2004; Fitzpatrick et al., 2011).

In 2013, the Fleming research group extended their research to show that AAS can hybridize with brown trout (Oke et al., 2013), but extensive earlier work showed that salmon and brown trout hybrids are sterile (Galbreath and Thorgaard, 1994; 1995; 1997), which would again negate a Trojan gene effect. Consumer groups often cite these studies as evidence that AAS fish can indeed hybridize with either WT salmon or brown trout, but do not point out that the conclusions actually demonstrate that the AAS or hybrids show no fitness advantages relative to WT, negating the possibility of a Trojan effect.

When interviewed for this IQP, Ian Fleming agreed that the survivorship of the GH-fish offspring is reduced: “I think the survivorship [of GH-salmon] would be lower than wild fish, assuming that the escaped fish would be fertile” (personal communication, Ian Fleming, Department of Ocean Sciences, University of Newfoundland, 8/13/13).

The fact that AAS are capable of hybridizing with brown trout led us to investigate whether brown trout might exist in Panama’s highland streams. ABT indicates there are no brown trout in Panama. The brown trout (*Salmo trutta*) is native to Europe, northern Africa, and western Asia (Dorofeeva, 1998); but it has also been widely introduced for aquaculture and recreational fishing, so it is now found in streams, lakes, and coastal areas worldwide. It is an aggressive species, and has been listed as one of the world’s 100 worst invasive species (ISSG, 2013). Brown trout are blamed for declines in native fish populations, for example in the Great Lakes and in California, especially for other salmonids, through predation, displacement, and food competition (CABI, 2010; Fuller et al., 2012). Brown trout normally prefer cold water habitats, with a preferred temperature range between 4.9 to 10.8 °C (Dorofeeva, 1998), which is
why it is most common in the northern Atlantic ocean, North Sea, White Sea, Baltic Sea, Iceland, northernmost rivers of Great Britain and Scandinavia, Lake Geneva basin, upper Danube and Volga drainages, although it now exists worldwide due to aquaculture practices. It can live at higher temperatures than most other trout, and this is probably why they were chosen for aquaculture. They are a successful and aggressive species who are permanent residents in most of the regions they have been released (Dorofeeva, 1998). The VMAC report (2010, page 123) indicates that the 15°C water near the facility in fact contains rainbow trout, but does not specifically mention brown trout. Since brown trout prefer very cool water, like AAS, they likely would not do well in the 26-28°C warm waters downstream from the ABT grow out facility, which is near the 28°C incipient lethal level (the temperature that would kill the fish after 7 days) for Atlantic salmon (Elliott, 1991), assuming the lethal temperature for brown trout is similar. So, the river area downstream from ABT’s Panama facility, likely contains no brown trout, but due to human introduction they might exist in Panama’s highlands. The point likely is moot, if the AAS and brown trout hybrids are sterile, and negate a Trojan or Invasion Risk scenario.

4.4.2 Invasion Risk:

Invasion risk is similar to the Trojan gene effect, except the transgenic offspring survive as well as the WT salmon. Invasion risk occurs when an invaded population of normal fish become transformed through generations into an entirely transgenic fish. The transgene spreads into the WT population if the GM males show a mating advantage relative to WT males and if the transgenic offspring are fertile. If those two fitness assumptions apply, the GM fish eventually replaces the WT population. In the invasion risk case, “The transgenic fish are
capable of breeding, but they survive as well as the normal fish and they have a mating advantage and they take over the population and they become fully transgenic. You’ve now introduced an exotic species into that area that may differ in growth patterns and growth forms, which could play a very different ecological role with its competitors, its prey, species that prey upon it. This could have dire consequences and drive other species to extinct. Unfortunately, that type of risk is possible, however it’s something you can’t test in the lab” (personal communication, Richard Howard, Purdue University 8/13/13). However, based on the data discussed above showing that AAS males do not show a mating advantage over WT males, the invasion risk scenario does not apply to AAS. In addition, because AAS and brown trout offspring are not fertile, this further negates an invasion risk scenario with brown trout.

4.5 Economic/ Sustainability Issues

Some scientists argue that salmon aquaculture is not sustainable. They argue that salmon aquaculture will not relieve the pressure on WT populations because salmon are near the top of the ocean food chain and require on average three pounds of food to produce one pound of meat. “Do we really want to rely on a non-sustainable supply of fish [from one company]?” (personal communication, Bob King, 7/22/13). “Salmon are the wolves of the sea, and we already know that it is not sustainable to farm wolves, so why are we farming wolves in water? About ¼ of all fish harvested in the ocean [each year] are used to feed farm raised fish” (personal communication, fish ecologist, Johns Hopkins University, 7/2/13). In agreement with the idea that farming the top of a food chain is not a good idea, Roz Naylor, a leading scholar on the subject at Stanford University's Center for Environmental Science and Policy said that
"Aquaculture's current heavy reliance on wild fish for feed carries substantial ecological risks" (Stier, 2007).

Some individuals believe there is no need for ABT’s AAS right now. “I think the salmon industry is doing just fine without it, and I don’t think that the consumers are asking for transgenic fish” (personal communication, fish ecologist, Johns Hopkins University, 7/2/13). Though this opinion was not voiced by all our interviewees, the World Wildlife Fund shares this view saying, “Farming of fish, shrimp, and shellfish is often viewed, and marketed, as a way to take pressure off wild fisheries. But some types of aquaculture are actually increasing pressure on several already threatened marine species” (Rangeley, 2013). Specifically, wild Atlantic salmon populations have plummeted over the past three decades, while farmed salmon has increased 55-fold, putting added pressure on the remaining wild populations (Rangeley, 2013). This opinion is also held by the EPA (EPA, 2001).

AquaBounty indicates that “the proposed methods of AAS culture are more environmentally friendly and sustainable than traditional sea cage farming of salmon, and that aquaculture is reducing the pressure on already threatened wild fisheries by helping to meet the growing demand for seafood” (personal Communication, Henry Clifford, 7/1/13). However, other consumer groups are concerned that this is not the case, and that this method is not sustainable, saying that “there is no great benefit from farming these salmon” (personal Communication, Tim Schwab, Food and Water Watch, 6/20/13).

Some consumers argue that ABT should consider not only the ecological effects of a potential AAS escape, but its economic impact. “They do not take into account the….economic impact that would arise. The economic impact would be huge! There would be contamination of the wild salmon market and strong impact on the salmon industry because the FDA has already
states that there would not be a required labeling for these GE salmon. People might boycott all salmon because they wouldn’t know if it was GE or not” (personal communication, Colin O’Neil, Center for Food Safety, 8-5-13).

Some politicians agree with the CFS arguments. Speaking for Sen. Begich, Bob King said, “Senator Begich is interested in the ‘big picture’ of sustainability, and if we approve the AAS fish, do we really want to rely on a ‘non-sustainable supply of fish’” (personal communication, Bob King for Senator Mark Begich, D-AK, 7/22/13). It would only be a single company (ABT) providing stocks for these fish, which could have a major effect on the economics of the salmon industry and the farmers involved, especially with the additional issue of shipping from as far away as Panama. While current aquaculture fish are shipped to the United States from Canada or China, for this new business model to survive the company would eventually need to bring the GE fish they are producing much closer to the major markets. This would potentially include moving AAS production into the U.S., which as mentioned before would spark another long series of approval measures and a more extensive environmental evaluation.
5.0 CONCLUSIONS & RECOMMENDATIONS

Technical Issues

An initial problem encountered in our project was the difference between the DNA constructs used to make various transgenic GH-fish, which made direct comparisons of the refereed studies somewhat difficult, and likely resulted in some confusion for consumer groups trying to determine whether growth hormone (GH) is elevated in ABT’s fish. During transgenesis, the choice of gene promoter is important because it dictates where and when the GH is produced (expressed) in the fish. Much of the original transgenic fish literature focused on describing metallothionein (MT) GH-fish (that strongly elevate GH in the serum and muscle), while later studies focused extensively on ABT’s AquAdvantage Salmon (AAS) that uses an ocean pout (op) promoter isolated from an antifreeze protein to drive expression of a Chinook salmon GH gene. Although several consumer groups argue that relatively little data is publically available on AAS fish, we found the refereed literature on AAS to be quite extensive, and conclude they are among the best characterized of all types of transgenic fish.

With respect to technical issues, in addition to consumer concerns about AAS’ elevated GH levels, they also denied that AAS really grow faster, worried they showed elevated levels of other hormones (especially insulin-like growth factor-1, IGF-1, known to cause cancer so is related to food safety), and had concerns about AAS’ potential reduced pituitary gland sizes (which could affect fish behavior).
Elevated GH Levels?

With respect to GH levels, although some MT-GH-fish show GH levels almost 40 times that of wild-type (WT) salmon, several published studies and the data submitted to the FDA VMAC using op-GH fish (AAS) showed that their GH levels do not appear to exceed that of WT salmon. We found that the format for the data submitted to the VMAC has created much confusion with consumer groups. Although the numerical values for AAS GH appear to be slightly higher than WT fish (consumer group arguments), the difference is not statistically significant (ABT’s argument), so both sides are partially correct in their heated arguments. Moreover, by eliminating GH values in the VMAC tables that were beneath the limits of detection of the assay (were not numerically accurate at such low levels), only the values above the limits of detection were included in the tables, the numerical means was somewhat elevated. So, if consumer groups take their data only from the published tables, they will conclude that much fewer samples were analyzed than actually were.

With no elevation in GH, the mechanism for how the AAS grow faster remains speculative for now, both in the literature and in the VMAC data, that the op promoter promoter drives GH expression at low levels in a broad array of tissues in close proximity to GH receptors, providing more efficient growth. Thus, the consumer worries that elevated GH levels in AAS might reduce the size of the pituitary gland (in a negative feedback loop) is not a problem, and the lack of GH elevation backs up ABT’s claim of no changes in pituitary size. We recommend that ABT more fully investigate the mechanism for the rapid growth in AAS to help gain consumer acceptance of how these fish work.
Do AAS Really Grow Faster?

Some consumer groups interviewed for our project continue to dispute ABT’s claims of rapid growth rates for AAS. However, our findings indicate that in spite of their complaints, the accelerated growth of op-GH-salmon has been thoroughly documented in the refereed literature, from the very first creation of the AAS fish (who described the accelerated growth) (Du et al., 1992), to later published experiments (e.g., Cook et al., 2000; Deitch et al., 2006; Oke et al., 2013), and also the FDA’s in the Environmental Assessment (2010, page-20) where they showed the mean body weight of ASS (261.00 ± 60.93 g) was significantly greater (p < 0.0001) than WT diploid salmon (72.63 ± 17.80 g) grown under the same culture conditions.

Elevated Levels of IGF-1?

The first argument is a major one, as IGF-1 is a hormone known to cause cancer. As discussed in our Results section, the FDA’s Environmental Assessment and VMAC briefing data indicated that AAS fish do not show statistically relevant elevations in any hormone tested, including IGF-1, estradiol, 11-keto-testosterone, T3, or T4. However, the consumer groups still argue that the means for IGF-1 is elevated in AAS and that only 6 AAS fish were evaluated for hormone levels. ABT claimed that 30 AAS fish were evaluated (and the IGF-1 mean was not elevated). Our investigation shows that both sides of the argument are partially correct. As discussed in our Results section, the hormone table in the publically available VMAC data shows IGF-1 levels for only 6 AAS fish (validating the consumer group’s point), but the report text indicates that the FDA-approved protocol requires any values below the lower limits of detection to be excluded from the table. The IGF-1 levels for 24 AAS fish were beneath the lower limits of detection, so indeed 30 AAS fish were evaluated by ABT (validating ABT’s claim). Although the AAS average IGF-1 levels appeared to be slightly elevated (10.26 ng/ml for AAS versus 7.34
ng/ml for non-GM siblings) (consumer claims), the apparent increase was not statistically significant (see NS not-significant, in the table) (validating ABT’s claim). When the 24 non-included samples were included in the calculation of the means, the AAS IGF-1 mean was actually lower than the non-GM salmon. To prevent confusion in the future, we recommend including all numerical values in the calculations shown in the highly visible data tables (the tables cited most often by consumer groups), and if it is critical to also show the means using only those values above the limits of detection to do so side-by-side next to the full sample set. And importantly, even if the IGF-1 levels in AAS had been elevated, our research determined that the amount of IGF-1 naturally produced in the human digestive tract daily greatly exceeds (by 100-fold) the amount that would be consumed daily eating AAS meat (see Results section). So our findings indicate that the extensive consumer worries about IGF-1 likely are not valid.

_Elevated Pituitary Gland Size?_

The argument on pituitary gland size reduction was relatively easy to solve. MT-GH-fish show strongly elevated levels of GH in the serum and muscle, and this negatively affects the pituitary gland. GH is normally synthesized, stored, and secreted by the pituitary gland. Animals (or humans) that receive GH injections frequently show reduced pituitary gland sizes and reduced GH content within the gland, perhaps because what was being provided by that gland is now provided exogenously. But AAS fish do not show the strong elevations in GH seen in MT-GH-fish (discussed earlier), and the VMAC data show no alterations in pituitary gland size in AAS. So, this consumer argument may have arisen from the readings of the MT-GH-fish literature with the assumption that it would automatically apply to AAS.
Food Safety

With respect to food safety, we identified three main areas of consumer concern: 1) potential hormone alterations (especially GH and IGF-1) (which were discussed previously), 2) potential increased allergenecity of AAS meat, and 3) potential problems eating meat from fish with increased morphological problems. Some consumer groups argue that the FDA did not study food safety issues sufficiently, however the VMAC briefing data quite extensively focused on food safety issues.

Hormone Levels and Food Safety

The GH and IGF-1 levels were discussed previously as likely not being elevated, and are thus likely not a problem. In addition, our interviews with fish experts indicated that even if Chinook GH were elevated and ingested, most of the GH we ingest daily (including Chinook salmon GH) is degraded in the digestive system, and even if any were absorbed intact into the circulatory system, it would not bind human GH receptors and would not be biologically active. And as discussed previously, even if IGF-1 levels were increased, the levels are vastly less than what humans naturally produce daily in our own digestive systems.

Increased Allergenecity?

With respect to allergenecity, the consumer groups argue that the FDA’s data only included 12 fish, and in this case we agree. As discussed in the Results section, the VMAC Briefing data shows that 6 transgenic fertile fish (diploid) and 6 transgenic triploid fish were analyzed for allergenecity measurements. So, perhaps more fish should have been analyzed. However, Chinook GH is not a known allergen, and even if it were there appears to be no
medical consensus about what level of allergen actually constitutes a medical risk, so without such studies being performed evaluating AAS allergenicity is difficult. The FDA indicates that if they approve AAS meat for consumption, they intend to perform post-market reviews with consumers who have ingested the meat to identify any allergenicity problems relative to WT salmon meat (many consumers are allergic to salmon meat), but we agree with consumer group complaints that such consumers would be hard to identify if AAS meat is not labeled as GM food, otherwise how would the consumer know what he/she is ingesting (discussed below).

*Increased Morphological Problems?*

Although some consumer groups pointed out that AAS fish have increased morphological problems relative to WT fish (discussed below), the same problems are seen in all triploid fish (even when not GM), so this likely results from the triploid nature of AAS not their genetic modification.

With no difference in composition of AAS meat chemicals or hormones relative to WT fish, no documented increase in allergenicity, and no known problems eating triploidy meat, we agree with most scientists that likely the AAS meat is safe for human consumption. But due to the novel nature of AAS being the world’s first GM animal food, we agree with the FDA to perform extensive post-consumer interviews to monitor potential problems.

*Fish Triploidy*

With respect to the pressure treatment process that ABT will use to sterilize its fish, we identified two main consumer concerns: 1) the fact that the process is not totally effective
leaving some AAS fertile (which might impact the environment in the event of an escape), and 2) potential morphological problems.

**Triploidy Effectiveness**

With respect to sterility effectiveness, most scientists interviewed for our project believe that triploidy is the most effective current method for inducing sterility, and is in fact the current industry practice. So, ABT’s choice of pressure treatment appears to be state-of-the-art. All parties, including ABT, agree that based on data obtained with medium-sized batches of eggs (up to about 60,000), approximately 2-5% of the fish remain fertile, so it becomes important to consider the potential effect of a fertile AAS escape on the environment (discussed later). All parties also agree that we currently lack data on the percent effectiveness on larger batches of eggs (in the hundreds of thousands) as is proposed in Panama, so we agree with the FDA’s requirement that ABT monitor the percent effectiveness of all egg batches produced in Panama and discard any batch in excess of 5% fertility. Although some interviewees brought up the possibility of re-diploidization of the triploid fish (which would produce fertile fish from initially sterile fish), the published literature indicates that this process has been identified only in oysters, so it does not likely occur in AAS fish.

**Triploidy Morphological Changes?**

With respect to morphological changes, as discussed in the Results section, the triploidy method of sterilization can cause jaw erosions, fin lesions, heart abnormalities, focal inflammation, fin abnormalities, and can have weakened immune systems, but the deformations
appear in all kinds of triploid fish (even when not GM), so likely do not result from AAS’ transgene.

**Regulatory Issues**

Regulatory issues were major consumer concerns in this project, including whether the FDA review process was thorough, whether GM-fish should be labeled for the consumer, and whether an FDA-approval would open the flood-gates for future approvals of other GE animals.

*The Review as a NADA*

With respect to the FDA review process itself, it has been one of the longest in FDA history (currently in its 18th year and still counting) as this agency considers the complexities of whether to approve the world’s first transgenic animal food. Because the U.S. currently has no legislation specifically regulating transgenic animals, the FDA under the Federal Food, Drug, and Cosmetic Act (FFDCA) is reviewing ABT’s application as a new animal drug application (NADA). Despite some consumer claims that the FDA *chose* to review the application as a NADA to avoid doing a thorough review, the FDA was *required* to do so under the FFDCA guidelines, Section 512 which states that if an article (here cDNA) is intended to alter the structure or function of the animal it is considered a new animal drug, so all rDNA constructs that change the characteristics of an animal qualify as a new “drug”. But by reviewing the application as a NADA, some legislators and consumers believe the review process is more intended for drugs *given* to animals than reviewing GM animals themselves, and argue that new legislation should be introduced that is specific for GM animals. We agree that doing so might enhance the credibility of the entire review process.
Review Transparency?

With respect to transparency, although several of the consumer group interviews pointed to a general lack of transparency in the FDA’s review process, our research indicates that the review had at least two unprecedented steps towards transparency initiated by the FDA in recognition that this would be the first GM animal for consumption. The first was the FDA’s unprecedented requirement for the formation of a committee of scientific experts and veterinarians (the Veterinary Medicine Advisory Committee, VMAC) to review ABT’s submitted data, and the FDA required the VMAC to hold a public meeting after its review to present its findings and receive public comments. And second, although not required to do so, two weeks in advance of the September 19-20, 2010 public meeting, ABT made an unprecedented effort for transparency by making its 172-page VMAC briefing package (FDA VMAC, 2010) and 84-page environmental assessment (FDA EA, 2010) publically available for comment.

Was the Review Rigorous Enough?

With respect to whether the FDA environmental assessment was rigorous enough, some consumer groups and politicians argued that the FDA does not have the expertise to rigorously assess environmental impact of transgenic animals. Several respondents pointed to the Fish and Wildlife Services (FWS) or the National Oceanic and Atmospheric Administration (NOAA) as appropriate agencies containing the relevant expertise to conduct and environmental assessment. However, our research (see Results section) identified publically available documents showing those exact two agencies were indeed formally consulted by the FDA in their review. The
NMFS and FWS were provided with the environmental assessment, VMAC briefing material, and FONSI, and concurred with the FDA’s finding of no effect (signed letters from the heads of both of these agencies are publically available in the FDA Environmental Assessment (2012, Appendix D.1). Moreover, a publically available document (FDA EA, 2012, page-100) states that the FDA in 2002 hosted a meeting to exchange information and discuss environmental risk assessment issues for the AAS fish, and the 45 meeting participants included representatives from several FDA offices (the FDA’s Center for Veterinary Medicine, Center for Food Safety and Applied Nutrition, Office of the Commissioner, and Office of International Programs), and several additional agencies outside the FDA, including the National Marine Fisheries Service, U.S. Fish and Wildlife Service, Canadian Food Inspection Agency, Environment Canada, Canada’s Department of Fisheries and Oceans, Australia’s Department of Primary Industries, Academics from Harvard University, Southampton University, and the University of New Brunswick. Our interview with one FWS scientist indicated that he thought the FDA consultation with the FWS was both formal and informal (conversation about experiments over lunches). Some consumer groups pointed out that the FDA was not required to follow the advice of their consulting agencies, however the signed letters included in the Environmental Assessment indicated they agreed with the no impact conclusion of the FDA, and they made no further recommendations at this time. So, with respect to consultation outside the FDA, our findings indicate the FDA indeed consulted with several outside agencies on their environmental assessment, and the consultation was formal enough to warrant the sending of two signed letters from the heads of outside agencies agreeing they were consulted. And both agencies agreed they would become more involved in the future if ABT applies for permission to raise AAS within the U.S.
GM Food Labeling Issues

With respect to GM food labeling issues, several consumer groups and legislators pointed out that it currently is not a requirement to label GM food, and that labeling AAS would be a good idea because it gives consumers the right to choose what they are putting on their dinner tables. Many legislators and public interest groups believe that as science has progressed our laws and regulations have not kept up, therefore we need new laws introduced to ensure regulative balance. Although not required to do so by law, ABT indicates they plan on labeling AAS as GM food anyway. Our Results section described several bills that have been introduced in the Senate and House requiring the labeling of GM foods, but all of them remain in committee and have not been brought to a vote. In the interest of full public disclosure, and to support any future post-consumer studies on individuals who have consumed AAS meat, we support the passing of these GM food bills.

New Legal Precedence?

Some consumer groups interviewed for our project worried that FDA approval of ABT’s application would set a new legal precedent that would make the approval of subsequent applications easier. They worry this would “open a flood gate” greatly increasing the number of applications and their likelihood of approval. However, several regulators interviewed noted that even if ABT in the future applies for permission to raise GM fish within the U.S., the application will still have to undergo approval again, and this time it would have to pass the muster of the Endangered Animal Welfare Act because native salmon are considered an endangered species. ABT would not only have to undergo an additional federal FDA approval process, but at the
location site would also have to obtain the state’s permission for setting up the necessary aquaculture structures based on that state’s own regulations, which in the U.S. vary greatly based on local wildlife risk. So, despite consumer worries, any future applications would not be “automatically approved”.

**Environmental Issues**

Environmental issues were a strong concern with many consumers and legislators interviewed for our project. These stakeholders were concerned whether ABT’s physical barriers can really prevent an AAS escape, whether any escaped AAS fish will be fertile (discussed previously), whether escaped fish will really be killed in the warm waters downstream, whether AAS can cross-hybridize with other fish, whether the transgene will be spread to other WT fish, and whether WT fish populations will be affected.

*Location and Proposed Physical Barriers Sufficient?*

With respect to ABT’s proposed physical barriers in Panama, most stakeholders interviewed agreed the barriers appeared to be state-of-the-art, but concluded that no facility can ensure a complete lack of escape. Our Results section described ABT’s grow-out facility in Panama and the multiple barriers designed to prevent AAS fish escaping into and surviving in the wild, including geographic/geophysical, physic-chemical, and physical measures. ABT will use land-based tanks with 21 physical containment barriers (screens, filters, nets, boxes), with a minimum of 11 barriers in sequence to prevent escapes. Because land-based tanks are more expensive than ocean cages, ABT has gone to increased expense to attempt to prevent AAS escape, and many scientists argue their plan is state-of-the-art. Our Results section documented
several fish escapes that occurred in the past from open ocean pens, and one documented escape from an inland tank, so we agree that likely no facility can guarantee a lack of escapes, and deem it important to consider potential consequences. Because 1-5% of the escaped fish likely will be fertile (discussed previously) the escape of a fertile AAS fish must be considered, whether such fish can hybridize with any other fish, and whether such hybrids themselves will be sterile.

Although some references in our Results documented that some escaped fish have the ability to migrate, live, and breed in the wild years post-escape, these fish were not AAS fish, they were not escaping into waters which downstream are likely fatal, and they were surrounded by WT fish of the same species.

The severe Panama weather may increase the percent escaped fish relative to other inland tank locations. For example, in August 2009 Panama saw 36 inches of rain, and in August 2010 over 61 inches fell (LeVaux, 2013). So, the tanks should be monitored closely when severe weather is expected, and escapes recorded and sent to the FDA.

*Thermal Barriers Sufficient?*

In the event of an AAS escape, ABT argues the relatively warm waters downstream from the Panama would kill the fish. In Panama, the fish-raising facility is located at a high altitude of approximately 5,000 feet, near a river that drains to the Pacific Ocean. The facility water is supplied by a nearby spring whose temperature is a relatively cold 15°C year round, which favors AAS growth (and likely was one of the reasons the site was chosen for salmon aquaculture). Any AAS escape would not be immediately be hindered by the ideal cold local highland water temperatures. But downstream, the river temperature rapidly increases to about 26-28°C as it approaches the Pacific Ocean, which is at (or near) the 28°C incipient lethal level for Atlantic
salmon (the temperature that would kill the fish after 7 days) (Elliott, 1991). In addition, Atlantic salmon do not feed at temperatures above \(\sim 23^\circ\text{C}\) (FDA EA, 2010, page 26), so the longer the fish remain in the warmer downstream water, the higher the chance of starvation. The presence of several downstream dams would also physically hinder the rapid migration of AAS from the fatal warm water to any colder ocean water further downstream, further increasing their chance of starving in the warmer water. Although it seems unlikely, it is not clear from the FDA VMAC Briefing report or the Environmental Assessment whether it is feasible for an escaped AAS fish to survive long enough in its migration through the fatal warm water to make it to the deeper cool ocean water further downstream. Would such a salmon be able to retain enough energy for such a swim while not feeding? We recommend testing this possibility with released WT salmon and monitoring their survival.

**Trojan Gene Effect Possibility?**

The Trojan gene effect describes the potential movement of a transgene through a WT fish population during cross-mating (hybridization). Consumers were worried this could occur with escaped AAS fish breeding with surrounding fish. Our Literature Review and Results sections documented the discovery of the Trojan gene effect, and how several experiments showed this transgenic gene movement into WT populations indeed occurs (in medaka fish) when the GM-male fish show *stronger* mating behavior relative to WT males. Theoretically, such conflicts in fitness could drive both GM and WT populations to extinction, obviously getting the attention of environmentalists and consumers relative to AAS fish.

With respect to AAS fish, published theoretical models showed that transgene movement would be *possible* if the conditions were right, but it was not initially known whether AAS rapid
growth actually creates conditions where the GM males have a mating advantage over WT males (which would enable the Trojan effect). So, several fish experts (including several interviewed for our IQP) (Eric Hallerman, Ewen McLean, Ian Fleming, and Garth Fletcher), secured a large grant to assess AAS mating behavior. Their data, which appeared in refereed journals, concluded that AAS in fact show a large mating reduction relative to WT salmon, making a Trojan effect impossible.

With respect to whether any local Panama fish can in theory hybridize with AAS, we discussed in the Literature Review and Results sections several refereed articles (in some cases the same articles investigating the Trojan effect) showing that AAS can hybridize with brown trout and with WT salmon, obviously gaining consumer attention. However, few consumer groups in our interviews pointed out that three refereed articles show that AAS and brown trout hybrids are sterile, which would further negate a Trojan gene effect. Our Results section discussed whether brown trout might exist in the Panama highlands, and concluded that it could if introduced there for aquaculture purposes (it has a worldwide aquaculture distribution), but the point likely is moot if the hybrids are sterile.

**Invasion Risk Possibility?**

Invasion Risk is similar to the Trojan gene effect, except the transgenic offspring survive as well as the WT salmon. Invasion Risk occurs when an invaded population of normal fish become transformed through generations into entirely transgenic fish. The transgene spreads into the WT population if the GM males show a mating advantage relative to WT males and if the transgenic offspring are fertile. If those two fitness assumptions apply, the GM fish eventually replaces the WT population, which could have dire consequences and drive other
species to extinct. The fact that such an effect is theoretically possible given the right mating behavior parameters obviously has the attention of consumer groups, but as discussed for the Trojan gene section, AAS males do not show a mating advantage over WT males, so the invasion risk scenario also does not apply to AAS. In addition, because AAS and brown trout offspring are not fertile, this further negates an invasion risk scenario. We conclude that the consumer worries about Trojan Gene Effect and Invasion Risk scenarios are likely unfounded for AAS fish.

**Economic/ Sustainability Issues**

Some scientists argue that salmon aquaculture is not sustainable long-term because salmon are near the top of the ocean food chain and require on average three pounds of food to produce only one pound of meat. Some of the consumers and politicians interviewed for our IQP pointed to experiments previously done with wolves showing that wolf farming is not sustainable without also culturing the animals lower down the food chain (the animals the wolves eat for their survival). Already about ¼ of all fish harvested from the ocean are used to feed cultured fish, so some scientists argue that salmon aquaculture (the top of an ocean food chain) will actually further deplete WT fish populations underneath salmon on the food chain. This finding is worrisome given that WT salmon populations have already plummeted over the past three decades, while farmed salmon has increased 55-fold. Perhaps we should be farming the fish underneath salmon on the food chain? If ABT’s application gets approved, and as salmon aquaculture continues to expand, likely it will soon become apparent to other companies that it is in their best financial interest to farm tadpoles or squid that salmon eat.
Overall Project Conclusion

Overall, we conclude that several disagreements have arisen between consumer groups and ABT regarding the FDA’s review of AAS fish, especially related to technical issues (such as the use of the ocean pout promoter and how it creates accelerated growth, potentially elevated GH levels, potentially elevated IGF-1 levels, potentially reduced pituitary gland sizes), food safety issues (such as potentially elevated IGF-1 levels and morphological changes associated with the triploidy phenotype), environmental issues (such as whether ABT’s proposed physical security barriers will actually prevent fish escape, whether AAS can hybridize with local fish, whether such hybrids are sterile, and what effects an escape would have on the environment), and sustainability issues (such as whether we should be culturing tadpoles and squid that salmon eat instead of salmon themselves). In some cases the arguments likely resulted from the format for the publically available data in the FDA Briefing document and Environmental Assessment tables that easily cause confusion about how many fish were tested in each assay (tested fish below the limits of detection are not shown in the tables but indeed were tested), and the apparently elevated means shown in the tables are easy for consumers to focus on without digging deeper to understand that the differences are not statistically significant. Throughout our conclusions section, we have provided several recommendations for resolving the disputes.
REFERENCES


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APPENDICES

APPENDIX A: SALMON GROWTH CYCLE

Atlantic salmon follow an anadromous life cycle, with part of their lives spent in salt water, and another part in fresh water. The adult fish contain chemotactic systems that lead them back to the same stream from which they hatched after spending as many as five winters at sea thousands of miles away. The salmon eggs laid and hatched in gravel stream hollows called redds, with about 9-20% of the fertilized eggs developing over the course of the winter to hatch in April. Newly hatched alevins remain in the redds for a few more weeks, fed by their residual yolk sacs until they are depleted and they are forced to fill their swim bladders. Once in this new phase they are referred to as fry and they begin to eat anything they can find, including salmon eggs. (FDA VMAC, 2010)

The salmon continue to grow until they begin to more closely resemble adult fish. Red spots and dark vertical stripes develop, serving as camouflage for the easily-preyed upon parr. If the environment is favorable, containing clean water and an abundant supply of food with few predators, the Parr can grow rapidly during the first summer. As they grow, they expand their territory to ensure enough resources are available. This stage of their life cycle lasts an average of two to three years before parr begin to migrate downstream to reach the ocean. (FDA VMAC, 2010)

The Salmon migration to the ocean requires physical changes to accommodate the switch from fresh to salt water. The changes are referred to as “smoltification”, and include changes in color and an elongation of the body. Once in the ocean, the Salmon continue to eat whatever is available, which depending on the food sources, can dictate the color of the flesh. During their
time in the ocean they reach sexual maturity, with black spots appearing on top of the salt water coloring developed during smoltification. From this point on, they are aged by the number of winters spent at sea, the longer they spend in the ocean the larger they get.

Once salmon return to their natal streams they see further physical changes. The males develop bolder pigments, becoming redder on the belly (or showing red spots), and the females developing a blue-black color. Both sexes become thicker in the body, losing some of the elongation as the females begin to carry eggs. In preparation for defending egg laying areas (redds), males develop teeth and a hooked lower jaw that is referred to as a ‘kype’. (FDA VMAC, 2010)
APPENDIX B: INITIAL LIST OF INTERVIEW QUESTIONS

General Introductory Questions:
1. Can you tell me a little more about your current position and how you became interested in the issue of transgenic fish?
2. Open-ended question: what do you see as some of the major remaining obstacles to the commercial introduction of transgenic fish?

The interviewee’s response to the opening questions was used to decide which follow-up questions best applied to this particular subject. For example, if the subject brought up the issue of food safety when eating GM meat, we followed-up by asking his/her opinion of the scientific literature arguing that salmon growth hormone is not biologically active in humans because it does not bind our growth hormone receptors.

Food Safety Issues:
1. Assessing whether any food safety obstacles remain for gaining FDA approval:
   a. In your opinion, do any food safety obstacles still stand in the way of FDA approval?
   b. How easily do you think these food safety obstacles can be addressed?
2. FDA regulator opinions on food safety issues:
   a. What is your opinion of the current proposed set of standards for marketing transgenic fish?
   b. AquaBounty Technologies first applied for FDA approval in 1995, and has been waiting for approval ever since (18 years). Why did this take so long, and why did the approval for the Flavr Savr GM tomato take only 3 years?
   c. During the public postings of the various drafts of the FDA guidelines, how did the FDA respond to public opinion as the drafts evolved?
3. Stakeholder opinions on food safety issues (public, grocers, etc.):
   a. Are you aware that transgenic fish may be nearing approval for human consumption in the U.S.?
   b. Public: Would you consider purchasing such fish? What, if anything, would make you more likely to consume such fish?
   c. Grocers: Would you consider stocking these fish in your store? Do you think they will sell?
4. Technical issues of food safety (academic and corporate scientists):
   a. Do you think that a sufficient amount of research has been performed on the issue of expressing extra extra growth hormone in food?
   b. What effect does eating fish high in growth hormone have on humans?
   c. What percent of the extra growth hormone gets denatured or degraded during food preparation?
d. If the extra growth hormone is ingested, is it inactivated (denatured or degraded) in the stomach so it has no physiological effect on us?

Environmental Issues:
1. Assessing whether any environmental obstacles remain for gaining FDA approval:
   a. In your opinion, do any environmental obstacles stand in the way of FDA approval?
   b. If so, how easily do you think these environmental obstacles can be solved?
2. EPA Regulator Opinions:
   a. Did any of your environmental concerns affect the FDA consideration process?
   b. Were your environmental issues of greater significance to the approval process than the food safety issues?
3. Stakeholder opinions on environmental issues (public, fishermen, ecologists, etc.):
   a. Do you think that escaped fish would be a problem to wild type fish, and why?
   b. Would your opinion change if the fish were proven to be sterile?
4. Technical issues of environmental safety (academic and corporate scientists):
   a. Do you think that escaped fish would be a problem with inland tanks?
   b. Do you think that escaped fish would be a problem with seaside tanks?
   c. How strong do you think the evidence is that AquaBounty’s fish are sterile and will remain sterile when mixed with wild type fish?
   d. What percent of the fish escape annually?
   e. Do you think that escaped fish might deplete the natural food supply for wild type fish?
   f. Do you think that diseases will spread more quickly in aquaculture populations?

Fish Biology (questions mostly for academic and corporate scientists):
1. What side effect does overexpressed growth hormone have on the fish, if any? Do you think more research should be performed in this area?
2. How strong is the evidence that AquaBounty’s fish are truly sterile and will remain that way?
3. How reliable is the trisomy method of sterility when performed on large fish populations? Should more research be done in this area?

Business Questions (questions mostly for corporate business executives):
1. What are the estimated annual costs of running these aquaculture facilities inland and shoreline?
2. Given the high costs of this type of research, can you make a profit selling only within the U.S.?
3 What are the estimated cost differences between raising transgenic salmon and raising wild type?
APPENDIX C: INTERVIEW PREAMBLE

We are a group of students from the Worcester Polytechnic Institute in Massachusetts, and for our research project we are conducting a series of interviews with various stakeholders to investigate concerns and conflicts surrounding the approval of transgenic fish for consumption in the United States.

Your participation in this interview is completely voluntary, and you may withdraw at any time. During this interview, we would like to record our conversation for later analysis. We will also be taking notes during the interview on key points. Is this okay with you?

Can we also have your permission to quote any comments or perspectives expressed during the interview? This information will be used for research purposes only, and we will give you an opportunity to review any materials we use prior to the completion of our final report. If the subject does not agree to be quoted, we will respond as follows: “Since you would not like to be quoted during this interview, we will make sure your responses are anonymous. No names or identifying information will appear in any of the project reports or publications.”

Your participation and assistance is greatly appreciated, and we thank you for taking the time to meet with us. If you are interested, we would be happy to provide you with a copy of our results at the conclusion of our study.
APPENDIX D: SAMPLE OF AMENDED INTERVIEW QUESTIONS

Regulatory:

1. Can you clarify the congressman’s/senator’s opinions on GE fish for human consumption and the approval of AAS by the FDA?
2. According to our research, the FDA did consult NOAA informally for their environmental assessment; do you think they should have been more on the forefront, a bigger part of the approval process?
3. What are some of the reasons congresswoman/man signed the letter to the FDA against the approval of GE salmon?

Environmental:

1. Do you think that escaped fish would be a problem with inland tanks?
2. What additional regulatory review is necessary to ensure transgenic fish are safe for public consumption?
3. Some forms of aquaculture can lead to public health risks due to the use of some chemicals and antibiotics; the concept of the aquaculture of transgenic fish comes with its own set of risks as well. Specifically, AquaBounty’s transgenic salmon. What is your opinion on transgenic aquaculture in general and specifically AquaBounty’s system of aquaculture/ plans for future expansion?
4. With aquaculture there is always the issue of escape; do you think escaped transgenic fish would cause greater problems to the natural fish population than regular escaped aquaculture fish?

Public Interest Groups:

1. Do you think that a sufficient amount of research has been performed on the issue of expressing the growth hormone in food?
2. What do you think would help the public be more accepting of GM food?

Technical:

1. The Aqua Advantage salmon (AAS) construct claims to not over express GH however it causes GH to be produced year round what side effect if any do you believe this would have on the fish?
2. Do you believe that AAS are safe for human consumption? If not what are your specific concerns?
3. Do AAS have an increased need for anything besides food such as oxygen and if so how will this affect the cost of raising them as well as their ability to survive in the wild (in the case of an escape)?
4. Do you think that if they escape there is a good chance of spreading the transgene?