Testimony of

Dr. Ronald L. Stotish

President & CEO

AquaBounty Technologies, Inc.

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1.0 Introduction

AquaBounty is seeking FDA approval for a genetically modified Atlantic salmon with enhanced growth characteristics. The enhanced growth phenotype enhances the economics of land-based production of Atlantic salmon, overcoming many of the practical and environmental issues associated with conventional sea cage aquaculture of this species. The United States currently imports approximately 300,000 metric tons of Atlantic salmon each year from a variety of foreign producing countries, but produces less than 17,000 metric tons from aquaculture. The ability to produce Atlantic salmon in land based aquaculture systems in the US could reduce our dependence upon foreign sources, and create a US based industry with the accompanying jobs and economic development opportunities. The availability of a fresh and desirable Atlantic salmon product closer to US consumers would also reduce the sizeable "carbon footprint" associated with transport of large volumes of this food over great distances as is the current practice. Lastly, the cultivation of Atlantic salmon would not likely impact the wild caught Alaskan salmon fishery market as this product is well positioned both with respect to brand and price. The current wild Alaskan salmon catch has been stable at approximately 300,000 tons per year, with approximately 60 % of this product exported to Japan, China and other overseas markets; the remaining Alaskan wild caught salmon satisfies approximately 26 % of the total market demand for salmon in the US, and is a well differentiated marketed product. Interestingly, in the management of the Alaskan wild caught fisheries, five billion smolts are released into the Pacific Ocean each year from Alaskan hatcheries (Alaska Fish & Wildlife).

AquAdvantage Salmon is a genetically engineered (GE) Atlantic salmon with a rapid-growth phenotype that has been developed over the past 15 years. The genetic modification comprises one copy of a salmon growth hormone transgene that is stably integrated at a specific site in the genome in a line of Atlantic salmon. Triploid *AquAdvantage* Salmon eggs for are produced in a manner that results in the culture of an all-female population of reproductively sterile fish that are otherwise substantially equivalent to farmed Atlantic salmon. The monosex nature of the population derives from the use of a breeding strategy that is 100% effective; and the induction of triploidy, which renders the animal reproductively incapable, is achieved using a validated method that is more than 99% effective at commercial scale. The product is intended for the contained, land-based culture of Atlantic salmon for commercial sale and human consumption under the following specific conditions: production of eyed-eggs in Canada; shipment of eyed-eggs to Panama; grow-out and processing of fish in Panama; and, shipment of table-ready, processed fish to the United States for retail sale.

Assessment of the potential risks to the environment from *AquAdvantage* Salmon involves consideration of the likelihood and consequences of the fish escaping, becoming established in the environment, and spreading to other areas. If the likelihood of these events, which are analogous to “exposure” in the traditional risk assessment paradigm, is zero or close to zero, it is reasonable to conclude that the consequences of these events, which are analogous to the “effects,” are not of concern. In other words, if there is no exposure, there is no risk. The likelihood of escape, establishment, and spread of *AquAdvantage* Salmon is effectively zero due to redundant containment measures, including physical, physicochemical, geographic/geophysical, and biological measures that are being implemented at the sites of egg production and grow-out. The combination of these various methods results in a very high degree of control. Physical containment measures include multiple mechanical barriers to prevent escape (e.g., screens, filters, etc.). A strong management operations plan ensures that these containment measures are reliably implemented. Geographical and geophysical containment is provided by the location of the egg production and grow-out sites: the environment surrounding the egg-production site in Canada is inhospitable to early-life stages of Atlantic salmon due to high salinity; and, the environment downstream of the grow-out site in Panama is inhospitable to all life stages of Atlantic salmon due to high water temperatures, poor habitat, and physical barriers (e.g., several hydro-electric facilities). Biological containment is accomplished through the grow-out of all-female triploid (sterile) fish, which significantly reduces the risk of transgene propagation in the environment. The domesticated nature and lack of competitive fitness in the wild relative to native fish also constitutes a formidable barrier to survival and spread in the wild.

In summary, production and rearing of *AquAdvantage* Salmon will involve simultaneous, multiple, and redundant containment strategies of various types that serve to adequately mitigate the environmental risk. These measures consist of producing triploid, all-female salmon that will be reared in a land-based aquaculture system itself possessed of redundant physical containment measures engineered and managed to confine the fish to the culture systems. Furthermore, the facilities are located in geographical areas that are highly unfavorable to the survival, establishment and spread of *AquAdvantage* Salmon, should there be an escape. Consequently, the environmental risk associated with the production and grow-out of *AquAdvantage* Salmon under the conditions described is as low as can be reasonably expected.

2.0 Product and Production

2.1 Product Definition.

The *AquAdvantage* Salmon to be sold into commerce is a triploid Atlantic salmon bearing a single copy of a stably integrated transgene (termed opAFP-GHc2) at a specific location in the genome (the α-locus) in a specific line of salmon (the EO-1α line). The product subject to regulatory approval is an eyed-egg produced in Canada and delivered to Panama for grow-out to market size and processing, pursuant to retail sale in the United States. The opAFP-GHc2 transgene is a recombinant DNA construct comprising the coding sequence from a Chinook salmon growth hormone gene and regulatory sequences (the switches that turn on the growth hormone gene) from the gene encoding the ocean pout anti-freeze protein. The founder animal from which the *AquAdvantage* line derives was a transgenic female (EO-1) generated by injecting the transgene into the fertilized eggs of wild Atlantic salmon. Two rapidly growing transgenic progeny were selected for further development. The breeding of eight subsequent generations has led to the establishment of an *AquAdvantage* Salmon line (EO-1α) which bears a single copy of the integrated transgene. The broodstock used in spawning of *AquAdvantage* Salmon are homozygous females (i.e., having two copies of the transgene) that have been phenotypically sex-reversed for breeding purposes. These so-called neomales are bred with non-transgenic female Atlantic salmon to produce eggs containing a single-copy of the transgene. The fertilized eggs resulting from the cross are pressure-shocked to induce triploidy, a process which renders the fish sterile. Therefore, the salmon deriving from these eggs are females incapable of reproduction. The fish that develop from these eggs have an enhanced growth rate compared to non-transgenic Atlantic salmon.

In evaluating potential environmental risk associated with the construct itself, three specific elements of genetic engineering were taken into consideration: the selection of genes and promoters from fish; the removal of antibiotic resistance genes; and, the avoidance of viral vectors and transposons. The *AquAdvantage* construct employs a salmon growth hormone gene and a fish-derived promoter from the ocean pout. The use of an all-fish gene transfer cassette suitable for gene transfer in other fish avoids issues with genes and genetic materials from other groups of organism (Du *et al*., 1992a). The vector used to prepare the *AquAdvantage* construct was a bacterial plasmid called pUC18. Because the plasmid was purified from the transgene prior to injection into the salmon eggs, no bacterial genes were introduced into the genome of *AquAdvantage* salmon. Viral vectors and transposons were not used in the *AquAdvantage* construct to improve transgene integration efficiency. The absence of viral vectors and transposons eliminates a major mechanism for unexpected movement of genetic material within the genome of the GE fish or transfer to other unrelated species.

2.2 Technical Details and Logistics of Commercial Production

2.2.1 Development of *AquAdvantage* broodstock

In order to produce *AquAdvantage* broodstock, eggs from *AquAdvantage* females with two copies of the transgene are subjected to gynogenesis, an established reproductive method that generates an all-female population. These female fish are then sex-reversed to produce neomales. Neomales are genetic females (thus possessing no Y chromosome) that produce sperm, and produce only female progeny when crossed with a female. These *AquAdvantage* (neomale) broodstock are reared to sexual maturity and bred with nontransgenic females to produce 100% female offspring. All broodstock and egg production takes place at the production facility in Prince Edward Island (PEI).

2.2.2 Maintenance of *AquAdvantage* Broodstock for Commercial Manufacture:

Subsequent generations of *AquAdvantage* broodstock can be derived from existing neomales with two copies of the transgene by using the milt from those animals to fertilize eggs from females with two copies of the transgene. The offspring are sex-reversed, graded, tagged, and genotype confirmed prior to their use as *AquAdvantage* broodstock.

2.2.3 Production of *AquAdvantage* Eyed-Eggs for Commercial Sale

The *AquAdvantage* neomales are bred with non-transgenic females to produce fertilized egg populations that are 100% *AquAdvantage* females with a single copy of the transgene. Triploidy in the eggs is then induced by pressure shock to render the animal sterile. The eyed-eggs will be incubated for at least 325 deg-days, at which time batch-wise sampling will be done to confirm the successful induction of triploidy via flow cytometry (FACS) prior to quality control (QC) approval for commercial sale. The eggs will then be transferred to the approved grow out site in Panama. The production plan is defined in **Figure 1**.

For production details, see the briefing packet prepared by US FDA (Food and Drug Administration Center for Veterinary Medicine, 2010, p 51-60).

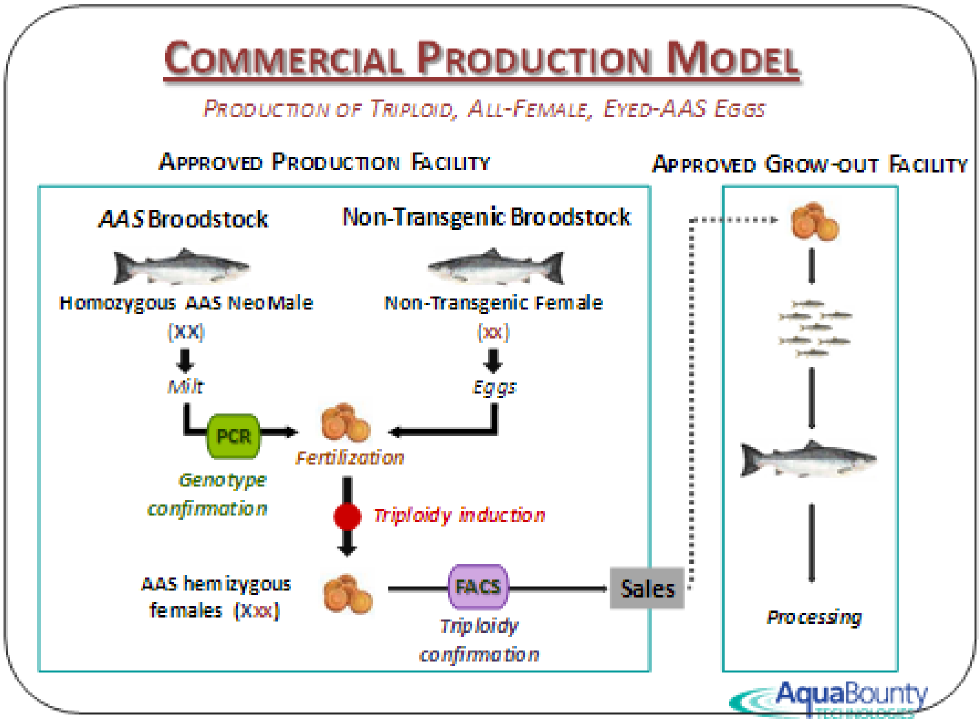

Figure 1. Production plan for *AquAdvantage* Salmon.

3.0 Environmental Risk

The environmental assessment of *AquAdvantage* Salmon has incorporated an ecological risk assessment approach, modified for the consideration of GE organisms as described by the National Research Council (NRC, 2002). Ecological risk assessment "*evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors*" (U.S. Environmental Protection Agency, 1992). Inherent in this definition is that both exposure and effects are required components of risk, i.e., Risk = Exposure x Effects. Muir (2004) has presented a modification of this concept for the risk assessment of GE organisms, wherein exposure comprises two parts: 1) the probability of the organism escaping into the wild, dispersing and becoming feral; and, 2) the ability of the transgene to spread into the wild population once it has been introduced by an escaped animal. These two parts condense the five steps identified by the NRC (2002) and concisely express the two requirements for the existence of ecological risk: both exposure and effects. Without either, there can be no risk. Redundant measures can be taken to ensure that the probability of escape and establishment of *AquAdvantage* Salmon, and of the *AquAdvantage* transgene spreading, is so remote that it is essentially zero. With essentially zero exposure, the risk is essentially zero.

No single containment measure can be assured of 100% effectiveness. Therefore, optimum containment can be achieved by the simultaneous deployment in series of a number of independent containment measures. Three to five separate measures have been recommended (ABRAC, 1995). The NRC (2002) recommended the simultaneous use of multiple, redundant containment strategies for GE fish. By combining containment measures with different strengths, attributes and modes-of-action, the compromise of aggregate containment by the failure of a single measure becomes increasingly unlikely. GE fish are considered to pose little risk to native populations if they are adequately contained (Mair *et al*., 2007).

The major difference between *AquAdvantage* Salmon and their non-GE counterparts is an increased rate-of-growth that is most evident during their first year of life. Muir (2004) has observed that the environmental risk of GE fish results from a chain of events: escape, followed by spread, followed by harm, such that the weakest link defines the upper-limit of risk. If the probabilities of any of the links can be shown to be close to zero, it is not necessary to quantify all of the risks.

A number of questions are pertinent when considering the environmental hazards of GE salmon (Muir, 2004; Kapuscinski *et al*., 2007):

- Are GE salmon able to escape into the environment?
- If an accidental escape occurred, could GE salmon survive in the surrounding environment and compete with wild salmon (and escaped domestic nontransgenic salmon), or otherwise impact natural or ecological resources of global importance?
- Could the rDNA construct be transmitted to wild salmon, escaped non-GE domesticated salmon, or other species?
- Could GE salmon breed successfully with populations of wild salmon (and escaped domesticated non-GE salmon)?
- Could the offspring resulting from these hypothetical matings adversely affect the population of Atlantic salmon or other ecological resources of global importance?

These questions are important because populations of wild Atlantic salmon are in decline. The potential hazards addressed in this document center on the likelihood and consequences of *AquAdvantage* Salmon escaping, becoming established in the environment, and spreading to other areas.

3.1 Likelihood of Escape

For *AquAdvantage* Salmon, both the production of eyed-eggs and the grow-out of the fish are conducted in land-based facilities with redundant physical barriers designed to prevent escape. In general, fish are among the groups of organisms with a high degree of mobility and significant capacity to escape captivity and become feral (NRC, 2002). They can be highly mobile if the aquatic environment is sufficiently hospitable. The use of land based facilities and concurrent containment measures can reduce the potential of escape to a small fraction of 1%.

3.2 Likelihood of Establishment

The risk assessment paradigm involves the integration of the probability of exposure with the probability of harm resulting from exposure. In evaluating the environmental concerns associated with GE organisms, the National Research Council stated that exposure must be more than just release or escape for a GE organism to constitute a hazard; rather the GE organism must spread into the community (NRC, 2002). The NRC (2002) thus defined exposure as the establishment of a GE organism in the community, and identified the following three variables as important in determining the likelihood of establishment: (1) the effect of the transgene on the fitness of the animal within the ecosystem into which it is released; (2) the ability of the GE animal to escape and disperse into diverse communities; and, (3) the stability and resiliency of the receiving community. The components of fitness include all attributes of the organism's phenotype that affect survival and reproduction. For example, a transgene could increase the organisms' adaptation to a wider range of environmental conditions or allow it to obtain nutrition from previously indigestible sources. A stable receiving community has an ecological structure and function that is able to return to the initial equilibrium following a perturbation; resiliency is a measure of how fast that equilibrium is re-attained (Pimm, 1984). The overall concern is a product of these three variables, not the sum; thus if the risk of any one of the variables is negligible, the overall concerns would be very low (NRC, 2002). In order for escapees to survive and proliferate, the accessible ecosystem must meet their needs for food, habitat, and environmental cues for reproduction. In addition to grow-out sites with all-female and >99% sterile salmon, escapee *AquAdvantage* Salmon would demonstrate life history characteristics associated with enhanced growth that would reduce survival in natural environments, and have demonstrated deficiencies in spawning behavior and securing mates.

As Kapuscinski and Brister (2001) have noted, even if the escaped fish were sterile, a type of pseudo-establishment could occur if successive waves of large numbers entered the environment, with each wave replacing the former as it dies off. This scenario implies frequent release of large numbers, which will not be pertinent to either the egg production or grow-out sites for *AquAdvantage* Salmon due to the multiple redundant containment measures employed.

It should be noted that intentional efforts to re-establish Atlantic salmon in their native habitats have been largely unsuccessful, inclusive of programs targeting Prince Edward Island and Lake Ontario, efforts in the latter case have been unsuccessful despite more than 100 years of attempting to do so. Moreover, farmed Atlantic salmon have not established themselves successfully in the wilds of North America (Council on Environmental Quality, 2001), despite the fact that they are reared in ocean pens on both coasts. *AquAdvantage* Salmon have no obvious life history advantages to suggest they would be any more invasive than conventional farmed Atlantic salmon.

3.3 Likelihood of Spread

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The spread of GE fish would depend upon how many escaped and survived, their characteristics, and their reproductive potential. For example, highly domesticated fish may be ill-equipped to persist in the wild due to the effects of captivity, such as poor adaptation, reliance on artificial diets, and rearing at a high stocking density (Kapuscinski *et al*., 2007). The reproductive potential of escapees is based upon their survival rate and fertility, and environmental conditions affecting reproduction in the affected ecosystem.

3.4 Consequences of Potential Escape, Establishment, and Spread

There are numerous factors, both genetic and environmental, that can influence the ability of *AquAdvantage* Salmon to affect the environment should they escape, survive and spread; these factors may have positive or negative impacts, which are further complicated by their mutual interaction. However, per the analogy of Muir (2004), it is not necessary to quantify the consequences (or harm, or effects) if the probability leading to the harm (the exposure) is zero or close to zero. The environmental risk posed by GE organisms is similar to that of introduced species. As discussed by Kapuscinski and Hallerman (1991), ecological impacts of GE individuals would be related to their fitness, interactions with other organisms, role in ecosystem processes, or potential for dispersal and persistence. With respect to their interactions with other organisms, *AquAdvantage* Salmon would be expected to occupy the same ecological niche as wild and domesticated Atlantic salmon, and compete for food, shelter, and other resources. As will be described later, because *AquAdvantage* Salmon are cultured as sterile females, they will be unable to reproduce. Finally, the potential for dispersal and persistence of *AquAdvantage* Salmon is very low due to the multiple redundant biological, physical, geographical and geophysical containment measures, as well as likely reduced ability to survive in natural ecosystems and reduced reproductive capacity. The scale and frequency of introductions of GE fish into a particular environment would have a large influence on the potential ecological risk. Any introductions would have to include a critical mass to allow survival of natural mortality, and would have to be of sufficient frequency and occur in the proper season to allow for establishment. Kapuscinski and Hallerman (1991) have stated:

> "*Although surprising outcomes cannot be ruled out a priori, low ecological risk may be a reasonable conclusion in situations where phenotypic and ecological attributes of transgenic individuals raise concerns, but the scale and frequency of their introductions are so small that their chances of becoming established in the natural setting are extremely low*."

4.0 Mitigation of Environmental Risk

It is not necessary to quantify the consequences of the escape, establishment and spread of GE salmon if the probability of escape leading to the exposure (i.e., establishment and spread) is zero or close to zero. Therefore, the use of measures to ensure that the exposure is effectively zero is considered the best means of reducing the risk. Measures for containment of *AquAdvantage* Salmon preventing exposure are discussed in this section. It is difficult to guarantee that 100% containment can be achieved by any single method. Thus, several different methods are used simultaneously to provide redundancy and ensure that the likelihood for escape for GE salmon is as close to zero as can be reasonably expected. These measures are: biological containment, physical containment, geographical/geophysical containment, and life history associated barriers of *AquAdvantage* Salmon to invasiveness.

4.1 Biological Containment

Biological containment can serve as a barrier by either a) preventing any possibility of reproduction at the site, thus avoiding risk of escape of gametes, embryos, or larval stages, or b) significantly reducing the possibility of reproduction or survival of the GE organisms in the unlikely event of an escape.

4.1.1 Induction of Triploidy

Triploidy as a process is commonly applied to make fish sterile, and is used commercially in aquaculture. For example, triploidy is used to produce sterile rainbow trout for aquaculture purposes by the leading supplier of trout eggs in the world, TroutLodge (an Idaho based salmonid genetics company; http://www.troutlodge.com/index.cfm?pageID=9C4DCE84-3048-7B4D-A93C4B67EECD271F). Additionally, all grass carp sold commercially in the United States are rendered triploid and sterile, a program monitored by the Fish and Wildlife Service (http://www.fws.gov/warmsprings/FishHealth/frgrscrp.html). Triploidy has two fundamental effects on fish physiology (Benfey 2001): 1) the size of the cells increases to accommodate the extra genetic material, but the number of cells decreases so that triploids are no larger overall than diploids; and, 2) gametogenesis and gonadal development is so severely impaired that triploids are sterile. Other than their sterility, a comprehensive review of the literature conducted by Benfey (1999) reveals little difference between triploids and diploids on a whole-animal level.

AquaBounty uses triploidy to produce sterile *AquAdvantage* Salmon. One of the most important means of biological containment is the sterility of the fish. Thus, even if some *AquAdvantage* Salmon were to escape the grow-out facility and survive in the environment, and find a compatible male even though the cultured populations is all-female, they would not be able to reproduce if triploid. The induction of triploidy is the only accepted method currently available for sterilizing fish on a commercial scale. AquaBounty uses this method on all eyed-eggs destined for commercial production, achieving an induction of triploidy on a commercial scale of 99.8% (Food and Drug Administration Center for Veterinary Medicine 2010, p 56-57). This is significantly greater than the 95% minimum level of induction of triploidy recommended by FDA (Food and Drug Administration Center for Veterinary Medicine 2010, p 50).

Although the reproductive potential of triploid escaped *AquAdvantage* Salmon would be essentially nil, the method used to induce triploidy to eliminate reproductive risk is not perfect. A small proportion of *AquAdvantage* Salmon may remain reproductively capable, since the induction process, albeit greater than 99% effective on average, is not 100% in all cases. Of countervailing benefit is the fact that the production of all-female populations of *AquAdvantage* Salmon can be accomplished with 100% efficiency, since the process of gynogenesis offers that guarantee based upon reproductive biology.

4.1.2 All-Female Populations.

The commercial deployment of all-female populations has obvious advantages in reducing risk of environmental impact and establishment of feral populations (Beardmore *et al* 2001, Devlin *et al* 2006). If all-female fish are cultivated in areas where species with which they can interbreed are absent, then establishment of feral populations is impossible. *AquAdvantage* Salmon will be cultivated as 100% female populations in the highlands of Panama, which support no native salmonids. This prevents the establishment of feral populations in all escape scenarios. Production of 100% female populations of Atlantic salmon is a well described process that has been practiced for almost 30 years (Johnstone and Youngson 1984; Johnstone and MacLachlan 1994).

In summary, the combination of triploidy with the production of all females, is considered the most reliable for biological containment (Donaldson and Devlin, 1996). As stated by Mair *et al*. (2007)

> "*The production of all-female triploids combines the benefit of almost-guaranteed sterility of any escapees with the reduced risk of disruption of spawning in natural populations that might arise with triploid males*." Arai (2001) has stated "*All female triploids can be used for effective biological containment of transgenic fish, so as to protect wild populations from contamination with genetically modified fish*."

Taken together, for commercial production systems like the one in Panama, the combination of 100% of the AquAdvantage salmon being female and at least 99.8% of the fish being sterile, plus locating grow-out in areas where no native reproductively compatible salmonids exist, makes the chance of escapee salmon establishing a feral population effectively zero. Nevertheless, physical containment in the grow-out facilities has been taken very seriously to mitigate the risk of escape.

4.2 Physical Containment

Physical containment refers to measures implemented on-site, such as the use of mechanical devices, either stationary or moving (e.g., tanks, screens, filters, covers, nets, etc.), or the use of lethal temperatures or chemicals to prevent uncontrolled escape. An important component of physical containment is the implementation of Standard Operating Procedures (SOPs) to ensure that proper procedures and use of devices are followed (Mair *et al*., 2007). Security measures are also needed to prevent unauthorized access, control movement of authorized personnel, and prevent access by predators.

The potential for accidental escape could derive from any of the following components of the water system: influent water and makeup water; effluent and draw-down water; and, waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning (ABRAC, 1995). In addition, it is important that all equipment that comes in contact with live GE animals is properly cleaned and drained after each use. The physical containment measures are described below for both the sites of egg production (Prince Edward Island) and grow-out (Panama).

4.2.1 Panama Grow Out

There is only one proposed FDA approved site for commercial growout of AquAdvantage Salmon anywhere in the world, a site in the highlands of Panama. The site is located more than 100 km from the Pacific Ocean, at an elevation of approximately 1800 meters. The site is equipped with a total of 21 individual containment measures, which maintain the salmon in confinement (Table 1; Draft EA for *AquAdvantage* Salmon, CVM, 2010). Physical containment to prevent the escape of fish at the grow-out facility is provided by the use of screens wherever water flows out of the system. There are a minimum of 11 sequential physical barriers in place between the fish tanks and the nearest natural body of water (a river), confining *AAS* to the site; seven of these barriers are positioned posterior to the outflow from the grow-out tanks. In addition, netting prevents the fish from being actively removed from containment by predators or passively removed in the event of any overflow of the water level. The multiple, redundant containment measures consist of tanks, screens, filters, stand-pipes, containment boxes, netting, and sedimentation ponds (Figure 2; Draft EA for AquAdvantage Salmon, CVM, 2010), making it virtually impossible for the salmon to leave the confines of the culture system and enter the environment.

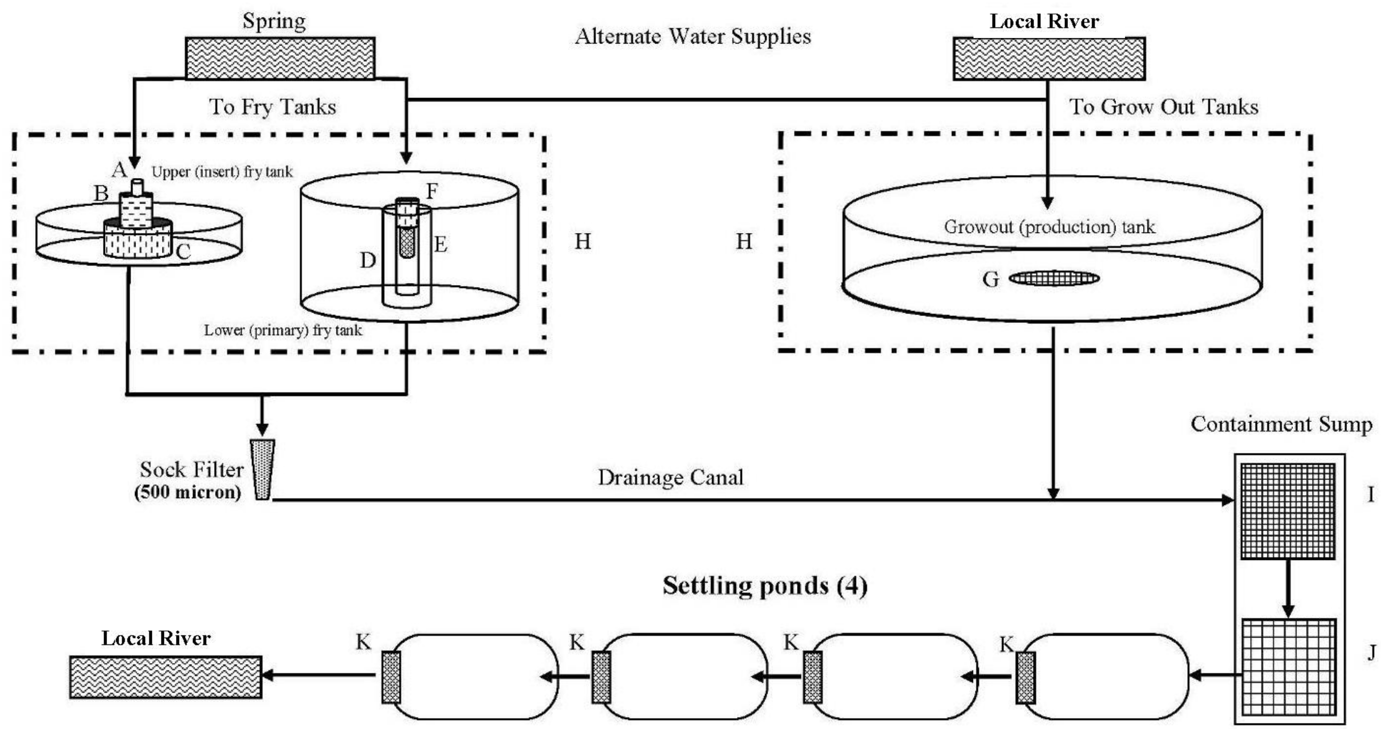
Drainage from the fish tanks must pass through rigid metal screening sized to block migration of even the smallest fish in the population. The effluent from the tanks enters the drainage canal where it flows through a second concrete containment sump equipped with a 12 mm steel screen-plate, anchored in such a way that all water passing through the sump is screened. Distal to the sump, the water flows into a sequential series of four settling ponds, each of which is equipped with a 12 mm rigid-metallic outlet screen on which a secondary, variable-gauge screen is placed to facilitate flow, while maintaining exclusion of fish as they increase in size from fry to market size.

Table 1. Key Components of Physical Containment Measures at the Grow-Out Facility

Purpose	Feature or Component
Primary containment	
To prevent escape from fry tanks via water	Center standpipe cut below tank rim to ensure water level is always below rim Netting stretched taut over top of tank to prevent fish from escaping even if tank was overflowing
	Collar-sleeve screens inserted into top of standpipes to prevent fish from entering standpipe by swimming
	Metal screen inside 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
	Porous 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
To prevent escape from the grow-out tanks via water	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
	Porous 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 by avian predators	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
Secondary containment	
To prevent escape from fry tanks into drains	Sock filter (500 μm) on the terminal end of the only drain pipe receiving effluent from the fry tanks
To prevent escape from grow-out tanks into drains	Sealed metal cage (affixed to ground) through which all effluent from grow-out tanks must pass before entering drain canal
To prevent escaped fish from passing through the drain canal to the sedimentation ponds	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 from one sedimentation pond to another	Rigid metal screens on the outlet of each pond
To prevent escaped fish from entering the river from the drain canal	Four sedimentation ponds in series, each with its own outlet screen
Tertiary and Quaternary containment	
To prevent unauthorized personnel from entering the fish rearing area	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

Figure 2. Schematic Summary of Containment Measures at the Grow-Out Facility



A	Interior Stand Pipe
B	Interior Stand Pipe Screen (1.0, 1.5 mm)
C	Exterior Stand Pipe Screen (1.5 mm)
D	Exterior Stand Pipe
E	Basket Screen (3, 6 mm)
F	Top Screen (3, 6, 12 mm)
G	Slotted Drainage Plate (0.9 cm)
H	Security Fence
I	Screen Basket (6 mm)
J	Screen Plate (12 mm)
K	Outlet Screen (12 mm)

The fry tanks and building containing them, as well as the outdoor grow-out tanks, are covered with netting to prevent avian predation and “jumpers” (i.e., fish that escape confinement by jumping out of the tank). In particular, the grow-out tanks are sealed horizontally and vertically inside a cage comprised of netting supported by a rigid structure. Escape from the tanks by jumping, or removal of fish by avian predators, is impossible. Security is provided by surrounding the fish tanks with netting and fencing topped with barbed wire to deter human or animal intrusion.

The facilities at this site are secured as follows:

- The site is located in a remote, highland area with very limited access.
- Entry onto the site requires passage via a securely gated footbridge that crosses a river, and is the only pedestrian access to the site.
- Culture facilities are enclosed by an 8-foot security fence topped with barbed wire.
- Entrance gates are securely locked and the area is protected by dogs.
- A private residence adjacent to the property provides for additional surveillance by management living on-site.

In summary, a minimum of 11 sequential physical barriers (total of 21) are in place between the fish tanks and the nearest body of water, confining the salmon to the site; seven of these barriers are installed following outflow from the grow-out tanks. In addition, netting prevents the fish from being actively removed from containment by predators or passively removed in the event of any overflow of the water level.

An additional level of physical containment is provided by several downstream hydro-electric plants, which also serve to prevent passage of any escaped fish to downstream riverine areas or the Pacific Ocean

4.2.1.1 Thermal Containment Barriers – Panama

In addition to the numerous physical containment barriers in place at the Panama growout site, there also exists a powerful natural, geographic, thermal barrier that would effectively prevent AquAdvantage Salmon from migrating from the growout site to the Pacific Ocean. Stead and Laird (2002) have cited the upper lethal temperature for salmon as being 23ºC. Water temperature measurements recorded for the rivers leading from the aquaculture project to the Pacific Ocean (Table 2; Draft EA for AquAdvantage Salmon, CVM, 2010) amply demonstrate that any escaped salmon attempting to migrate downstream towards the Pacific Ocean would inevitably encounter lethal water temperatures, preventing the fish from reaching the ocean.

Table 2. Air & Water Temperatures in the Rivers Leading from the Growout Facility to the Pacific Ocean *

		Temp (°C)	
Point	**Elev** (m)	**Air**	**Water**
1	13	28.9	26.4
2	91	31.9	28.1
3	250	29.4	26.0
4	347	28.6	25.8
5	649	24.3	22.6
6	995	21.6	19.3
7	1024	21.6	19.0
8	1086	21.7	20.7
9	1278	20.7	18.8
10	1792	17.2	15.1
11	1850	18.1	15.8

* Abbreviations: ***Elev***, elevation; ***Temp***, temperature.

An additional temperature related barrier to migration and survival that is present at the Panama growout location is the lack of suitable temperatures required by Atlantic salmon for spawning and egg incubation. The ideal water temperature for incubating Atlantic salmon eggs is 8° C, and temperatures in excess of 12° C result in low hatchability and viability (Stead & Laird, 2002). Based on water temperature data from the nearby river (Table 2), it is evident that ambient water temperatures in the river would not allow for spawning or hatching of eggs produced from escaped *AquAdvantage* salmon (ignoring for purposed of discussion, that the AquAdvantage salmon are sterile and all-female).

4.2.2 PEI Production

There is only one proposed approved site for the production of *AquAdvantage* Salmon eyed-eggs, the land-based, freshwater aquaculture facility on Prince Edward Island (PEI) owned and operated by AquaBounty, which comprises a main building, storage facility, and ancillary enclosures for operational structures that are secured as follows:

- ***Perimeter security:*** Approximately 1590 linear feet of galvanized chain-link fence of commercial quality surrounds the property, inclusive of freshwater well-heads, back-up generators, liquid oxygen containment, and the storage facility. A service entry adjacent to the storage building remains secured by a double-swing, chain-link gate except when service access to the property is required. A roll-away, chain-link gate spanning the main entry to the property, which is adjacent to the main building, is secured during non-business hours. At night, the entire perimeter remains well-lit.

- ***Outside entries:*** Windows on the lower-level of the main building are barred, and all exterior steel-doors on the main and storage buildings are dead-bolted. Entry into the main building requires a key or intercom-interrogation and remote unlocking by facility staff. Within the main building, access to the first-floor aquaculture facility is further protected by a cipher-locked, interior entry.

- ***Security monitoring:*** Eight motion-activated security cameras are positioned for maximum surveillance of the property immediately surrounding the main building. These cameras are in continuous operation and automatically capture digital images that are stored for later retrieval. Magnetic door-contacts and interior motion-detectors deployed throughout the main building, storage facility, and out-buildings comprise a network of zones that are monitored by a commercial security service.

- ***Water supply & pump-house:*** The primary well and pumping facilities (one primary, two back-ups) that supply the aquaculture facility are securely enclosed in a steel containment structure.

- ***Remote notification of status:*** Environmental alarms indicating emergent change in operational conditions (e.g., water level, dissolved oxygen (***DO***) content), and security alarms indicating suspected intrusion during non-working hours, are conveyed by the security service to senior facility staff via numeric page; in addition, direct telephone contact with the facility manager or other on-call staff is pursued until successfully made, so that clear communication of the event occurs and proper and immediate response is managed.

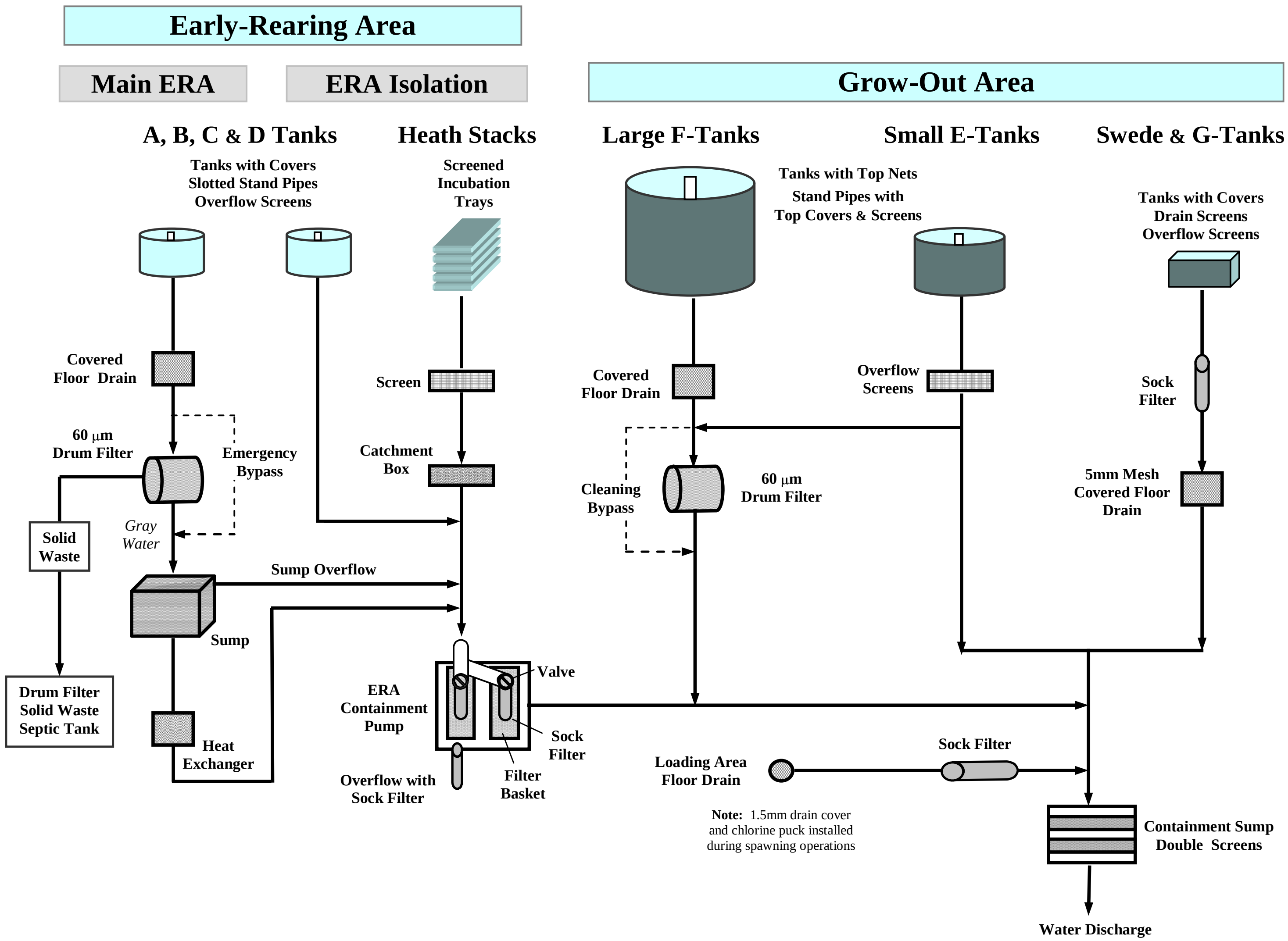
- ***Additional security:*** AquaBounty may employ professional security personnel to remain on-site during non-business hours as conditions warrant. In addition to their direct surveillance of the property, these personnel would have access to the central, security-monitoring system in the main building, but would not have access to the facility at-large, which would remain locked-down and subject to the network of electronic sensors and motion-activated cameras comprising that system. An apartment in the main building provides for additional surveillance by staff living on-site.

A number of measures have been implemented to provide physical containment of the GE salmon at the Prince Edward Island facility. In general, means of physical containment comprise entrapment of animals at the immediate source of housing for cultivation (i.e., via tank covers or nets), and redundancy in screening and filtration of water flows into which fish could gain access. These containment measures function at different as well as multiple levels of the containment strategy. Key components of the system are described in great detail in Aqua Bounty Protocols. The measures are summarized in Table 3 and a schematic is provided in Figure 3. Inspections for various purposes over the past 10 years have resulted in the facility having been: (1) deemed compliant with containment practice and licensed to conduct research on GE fish under applicable Canadian regulations; and (2) classified as an acceptable manufacturing establishment and judged as having no significant environmental impact by FDA.

Table 3. Key Components of Physical Containment at the Production Facility

Purpose	Feature or Component
Primary containment	
To prevent escape through rearing unit or incubator water overflow	Perforated metal screens on tank bottoms
	Screens on stand pipes, top and bottom (where appropriate for size of fish to be contained)
	Incubator tray screens
To prevent escape over the side of a tank or incubator	Screened tank overflows Cover nets Jump fences Tank covers Incubator tray screens
To prevent downstream passage of newly fertilized eggs and/or gametes	Chemically lethal environment (chlorine puck) in spawning area drain
	Perforated metal drain cover in spawning area
	Closed septic system
Secondary containment	
To prevent entry of fish into drains	Floor drain covers, solid or mesh
	Incubator-stack catchment box
	Waste de-watering sieve box
To prevent downstream passage of fish within the drains	Barrier screens within drains
	Drum filter
Tertiary and Quaternary containment	
To prevent downstream passage of fish within the drains	Barrier screens within drains of various sizes & locations
	Double screens within the sump
	Mesh filter on drum-filter gray water
	Heat exchanger
Waste treatment	
Sock filters, containment screens, basket-sieve for straining waste material from the ERA tanks	
Chlorine kill solution (5 mL Javex containing 0.52 grams sodium hypochlorite per liter of water)	
Chlorine pucks	

Figure 3. Schematic Summary of Containment Measures at the Production Facility



Hatchery-reared Atlantic salmon do inhabit the ocean waters surrounding PEI, although they are not known to frequent the area near the egg production site. Thus, the local environment does provide suitable habitat for at least some life stages during part of the year. The climate is temperate, with warm summers and cold winters. Open waters in proximity to the production facility are saline. Salmon eggs and fry are adapted to freshwater conditions and would be adversely affected by escape into the local estuarine environment. The extreme temperature conditions during the winter months at this location would be lethal to salmonids of all developmental stages. During the remainder of the year, the local environment would not be inhospitable to escaped smolt, juvenile or adult GE salmon, which have adapted to salt water and could survive. Escapees would face considerable environmental impediments to survival, one clear indication being the substantial failure of intentional efforts to re-establish Atlantic salmon in their native habitat. In fact, as noted by the Council on Environmental Quality and Office of Science and Technology Policy (*CEQ-OSTP*), farmed Atlantic salmon have not established themselves successfully in the wilds of North America (CEQ-OSTP, 2001), despite the fact that they are reared commercially on both coasts.

In 15 years of operation, there has never been a documented escape from the PEI facility.

4.2.3 Containment Infrastructure Management

The containment measures described above for the sites of egg production and grow-out include physical measures (e.g., screens, covers, filters), as well as physico-chemical measures (e.g., chlorine) and environmental tolerances (e.g., temperature). In addition, a strong operations management plan is in place at both sites, comprising policies and procedures that meet the recommendations for an integrated confinement system for GE organisms (Kapuscinski, 2005), as summarized in Table 4. All of these factors mean that the likelihood of even a single *AquAdvantage* Salmon escaping into the wild is extremely low.

AquaBounty will comply with these same standards of effectively zero risk of establishment of feral escapee salmon populations for every facility that produces *AquAdvantage* Salmon. To further mitigate risk, AquaBounty has no plans to sell eyed-eggs to any grow-out facility with drainage to native Atlantic salmon habitat.

For additional prospective grow out facilities for AquaAdvantage Salmon, the same rigorous management and containment strategies will be employed, consistent with the terms of the NADA provisions for conditions of use. Candidate sites will be the subject of an Environmental Assessment and preapproval inspection by CVM, and additional inspections to assure compliance with the terms of the NADA. The administrative device CVM has indicated it will use for this process is the Supplemental New Animal Drug Application, or S-NADA. This is analogous to the long standing FDA process used to approve alternate drug manufacturing facilities or changes in facilities. The regulation of the grow-out sites for AquAdvantage Salmon will therefore be more rigorous than the regulation of any production site for any food animal.

Table 4. Implementation of an Integrated Confinement System for *AquAdvantage* Salmon (From: Kapuscinski, 2005).

	Use at Production & Grow-Out	
Recommended element	**PEI Egg Production**	**Panama Grow-Out**
Commitment by top management	✓	✓
Written plan for implementing backup measures in case of failure, including documentation, monitoring, and remediation	✓	✓
Training of employees	✓	✓
Dedication of permanent staff to maintain continuity	✓	✓
Use of standard operating procedures for implementing redundant confinement measures	✓	✓
Periodic audits by an independent agency	✓	✓
Periodic internal review and adjustment to allow adaptive modifications	✓	✓
Reporting to an appropriate regulatory body	✓	✓

5.0 Invasiveness

A final barrier to establishment and spread of feral *AquAdvantage* Salmon populations is the potential invasiveness of GH transgenic salmon. The extent to which the genetic construct can spread into wild populations would depend on the fitness of transgenic individuals in the receiving environment, which may vary along a continuum featuring high fitness at one end - leading to the fixation of the transgene, and low fitness at the other end - leading to its elimination within a few generations (Muir and Howard 1999). If the salmon are highly effective at adapting to and competing in natural ecosystems, they may persist for long periods of time in the environment. This increases the chance for encounter with suitable mates for reproduction and establishing a reproductive population. If the transgenic fish do not adapt well to the natural environment, the risk of invasiveness is low and the transgene will likely be lost from the wild population. Additionally, in modeling the invasiveness of a hypothetical escape of transgenic fish populations, a hypothesis known as the Trojan Gene Hypothesis has been advanced (Muir and Howard, 1999). Under this hypothesis, it was calculated that escaped transgenic fish could theoretically drive a native population to extinction within as little as 40 generations. This hypothesis could be true only if the transgenic fish enjoyed an advantage in competing for mates (based on color for example), but experienced a disadvantage in overall fitness (so were unable to survive in the wild well) (Muir and Howard, 1999). As will be explained below, all indications are that *AquAdvantage* Salmon are poorly adapted for life in the wild, are remarkably ineffective in securing mates, and that the transgenic fish would not be invasive, but would rather more likely be selected against and eliminated from wild populations.

5.1 Life History Constraints that Reduce Invasiveness

The main distinguishing feature of *AquAdvantage* Salmon is rapid growth, where growth rate is a composite of many physiological factors. *AquAdvantage* Salmon have metabolic traits that also appear in other fast-growing Atlantic salmon or in fish that have been treated with time-release GH implants (Johnsson and Bjornson, 2001). Metabolic rates influence the components of the overall energy budget for an individual; the components of the energy budget in turn influence an individual's impact on nutrient and energy flows and on other organisms. The unique attributes of the GE fish appear to be an increase in the scale of trait expression commensurate with the increase in growth rate when food is available, and the allocation of energy to current growth at the expense of stored reserves (Cook *et al*., 2000b).

GH increases metabolic activity through several channels: lipid breakdown and mobilization are improved and energy more immediately deployed for maintenance or growth; protein synthesis is enhanced, providing the essential material for faster additions to body mass; mineral uptake is enhanced promoting skeletal development and longer, leaner fish; and, feeding efficiency (feed conversion ratio, or FCR) is improved (Bjornsson, 1997). The cost to the animal is higher oxygen need due to increased digestive demand and anabolic protein synthesis, and the need for increased feed availability. In early-generation relatives of *AquAdvantage* Salmon (hereinafter "*AquAdvantage* relatives"), feed consumption was 2.1-2.6 times higher than in non-transgenic controls; during starvation, transgenics depleted body protein, dry matter, lipids, and energy more quickly that controls, and had lower initial energy reserves (Cook *et al*., 2000a,b). Routine oxygen uptake in these fish was 1.7 times that of controls, including the higher 'heat increment' associated with digestion (Stevens *et al*., 1998); and, oxygen consumption under activity was 1.6 times the non-transgenic rate, further increasing with effort (Stevens and Sutterlin, 1999). Although these *AquAdvantage* relatives demonstrated an ability to reduce their metabolic rate in response to starvation, their higher metabolic effect and lower initial energy reserves suggest that they would be unlikely to grow rapidly or survive outside of culture conditions (Hallerman *et al*., 2007). The increased requirement for oxygen exhibited by *AquAdvantage* relatives (Abrahams and Sutterlin, 1999; Cook *et al*., 2000a; Cook *et al*., 2000b; Deitch *et al*., 2006) would engender a reduced tolerance for diminished oxygen content in general, and a reduced capacity for survival when DO content is critically low, compared to their non-transgenic counterparts in the wild. In experiments with *AquAdvantage* relatives, oxygen uptake was independent of oxygen concentration above 10 mg/L, but started to decrease at about 6 mg/L DO in transgenic fish versus 4 mg/L in control fish (Stevens *et al*., 1998). Under conditions of oxygen saturation, transgenics are not at a disadvantage compared to controls, since oxygen demand is readily satisfied. Oxygen saturation is rarely encountered in natural environments.

The need for food tends to increase the predation risk for GE fish. Abrahams and Sutterlin (1999) also demonstrated that *AquAdvantage* relatives would spend significantly more time feeding in the presence of a predator than non-transgenic salmon, indicating that they possess a higher tolerance for predation risk. The transgene confers a powerful stimulation of appetite in the presence of food and a larger capacity for food consumption in the presence of opportunity, even when predators are present. *AquAdvantage* relatives consumed approximately five times more food than same-age controls that were also size-matched by delaying the hatch time of the transgenics. In part, the consumption differential reflected the greater willingness of the transgenics to feed in the presence of a predator and, in part, a higher feeding motivation in transgenics, which were 60% more likely to be observed feeding at both the safe and the risky sites than were the controls (Abrahams and Sutterlin 1999). GH also increased appetite in various species of salmonids (Raven *et al*., 2006; Abrahams & Sutterlin, 1999; Devlin *et al*., 1999), which influences behavioral traits associated with feeding, foraging, and social competition. The availability of food also influences behavior. The difference in scale between GE and other fast-growing Atlantic salmon is less quantifiable for behavioral traits and further confounded by the effects of hatchery culture, particularly in acclimation to high rates of social interaction. Salmon form dominance hierarchies around foraging opportunities, and hatchery fish have more opportunities to reinforce their social status in confinement. In nature, social dominance is dampened by a resident advantage that generally deters other fish from evicting territory holders from home ground. It is estimated that at least a 25% difference in size is necessary to overcome the resident advantage (Metcalfe *et al*., 2003).

Changes in the morphology of the organism (e.g., size, shape & color) could alter species interactions (ABRAC, 1995); however, it should be noted that accelerated growth is not an assured outcome for GE salmon in nature. The rapid-growth phenotype is expressed only if supported by sufficient food, as has been shown in both transgenic Coho salmon (Devlin *et al*., 2004b; Sundström *et al*., 2007) and *AquAdvantage* relatives (Cook *et al*., 2000b). This is a function of both the productivity of the habitat and the density and behavior of competitors for the resource.

AquAdvantage Salmon are triploid fish, and triploidy may be another factor apart from transgenesis affecting environmental tolerance limits. Atkins and Benfey (2008) reported that triploids of Atlantic salmon had lower thermal optima than diploids, which could explain prior observations of mortality of other triploid salmonids (brown trout, brook trout, and rainbow trout) at chronically elevated, but sub-lethal, rearing temperatures. Data exist for a variety of species of fish to indicate that triploidy could be responsible for reduced survival of early-life stages and reduced survival and growth of later-life stages, particularly when environmental conditions are not optimal (Piferrer *et al*., 2009). Ocean migration studies in Ireland revealed that male triploids returned to their natal area in nearly the same proportions as diploids, whereas female triploids mostly did not (Wilkins *et al*., 2001). Similar results were found in another trial in which the return rate of triploid Atlantic salmon was substantially reduced (Cotter *et al*., 2000a).

5.2 Spawning and Reproduction

Changes in the age at maturation, fecundity, and sterility could alter population and community dynamics and interfere with the reproduction of related organisms (ABRAC, 1995). However, domesticated Atlantic salmon in general have markedly reduced spawning performance relative to wild fish (), and triploid females do not engage in spawning behavior.

Varying degrees of exposure to captive environments and domestication selection have been shown to affect the breeding behavior and success of adult salmonids negatively (Fleming and Gross 1993; Fleming *et al*. 1997; Berejikian *et al*. 2001a; Weir *et al*. 2004). Thus, the captive rearing environment appears to diminish the competitive and reproductive performance of salmonids, irrespective of genetic background (Berejikian *et al*. 1997, 2001a,b). As AquAdvantage salmon will be reared in intensive cultivation systems, a similar reduction in ability to compete for mates and survive outside of the culture environment is expected.

Age at maturation is a factor in estimating the risk of invasiveness of transgenic strains, with early maturation associated with increased invasiveness. If the transgenic fish mature before non-transgenic contemporaries, they have an increased opportunity for mating success. Atlantic salmon can mature as very young parr and sneak matings from larger fish, and if transgenic salmon matured more readily as parr, an increased risk of invasiveness could be prescribed. However, recent work (Moreau *et al* 2011c) clearly indicated that AquAdvantage salmon mature **later** than nontransgenics, with very little maturation as parr. The authors conclude that this characteristic reduces the risk of transgene invasion into a wild population.

Considering AquAdvantage Salmon specifically, recent research (Moreau *et al* 2011 b) indicates that transgenic AquAdvantage Salmon (whether adults or parr) are at a significant disadvantage competing for mates and contributing genetics to subsequent generations. When in competition, nontransgenic males dominated transgenic males in securing mates, participating in over 90% of spawning events. Transgenic parr were also at a disadvantage compared with nontransgenic parr. Taken together, this indicates that escapee transgenic salmon males would be at a significant disadvantage in securing mates in a wild environment, reducing invasive potential. Further, in simulated streambeds, there was no advantage to transgenesis in early life just after hatch in terms of feeding or aggression that might facilitate invasion of natural systems by transgenic salmon; the transgenic fry did not displace or out-compete nontransgenic fry (Moreau *et al* 2011a). The work with GH transgenic Atlantic salmon echoes similar work with GH transgenic Coho salmon (Fitzpatrick *et al* 2011), where researchers found that in competitive mating, transgenic salmon sired less than 6% of offspring. Milt harvested from transgenic males also contained fewer sperm that swam slower and for shorter durations than sperm from wild males (Fitzpatrick *et al* 2011). **Together, these findings suggest very limited potential for the transmission of transgenes from cultured GH transgenic salmon through natural mating should they escape from a contained culture facility into nature and reproductively interact with a local wild salmon strain. The additional redundant biological and physical containment provisions built into the production and grow-out of *AquAdvantage* Salmon product effectively eliminate any potential impact on the biological diversity or ecology of wild populations.**

5.3 Summary Comparison of Atlantic Salmon and *AquAdvantage* Salmon

Atlantic salmon display a wide range of characteristics and can adapt to a variety of conditions. *AquAdvantage* Salmon share many of these traits, the notable exception being their increased growth rate and the physiologic sequelae thereof (e.g., increased oxygen consumption).

Table 5 summarizes the observed differences between GH-transgenic salmonids and non-transgenic Atlantic salmon. In many cases, these differences were of greater magnitude under laboratory conditions than in a simulated natural environment. Consequently, not all of these differences may be expressed, or may be expressed to a lesser extent, in the wild.

None of these differences will lead to environmental impact unless *AquAdvantage* Salmon actually enter the environment. The likelihood of that happening is extremely remote.

Table 5. Differences between GE- and Non-transgenic Salmonids

Trait	Transgenic Relative to Non-transgenic
Metabolic rates	Increased metabolic rates Increased growth when food is available Reduced initial energy reserves Increased oxygen consumption
Tolerance of physical factors	Reduced tolerance to low oxygen availability Reduced thermal optimum range (effect of triploidy not GH)
Behavior (lab conditions)	Increased feeding motivation and reduced prey discrimination Reduced schooling tendency Reduced anti-predator response
Resource or substrate use	Increased utilization of lower quality food (lab conditions) Increased utilization of larger prey (potential)
Resistance to disease, parasites or predation	Reduced disease resistance Reduced anti-predator response, increased predation mortality
Reproduction	Accelerated growth to sexually-mature size Larger males can have a mating advantage
Life history	Accelerated growth to smolt-size Smoltification at higher temperatures and constant light

5.4 Comment on the Trojan Gene Hypothesis

Given the poor reproductive fitness of *AquAdvantage* Salmon, the Trojan Gene Hypothesis almost certainly does not apply to any escapees. The author of the Trojan gene hypothesis (Dr. Bill Muir) has weighed in on the applicability of this doomsday scenario, concluding emphatically that the Trojan Gene Hypothesis indeed does not apply to *AquAdvantage* Salmon, both in press releases (press release from Bill Muir; http://www.purdue.edu/newsroom/research/2011/story-print-deploy-layout_1_14241_14241.html) and the peer-reviewed scientific literature (Van Eenennaamm and Muir 2011). Quoting from Van Eenennaamm and Muir 2011, pg 708:

> **As a result, the Trojan gene effect would not be predicted to occur in the unlikely event AquAdvantage salmon did escape from confinement. Rather, selection over time would be expected to simply purge the transgene from any established population, suggesting a low probability of harm resulting from exposure to *AquAdvantage* Salmon.**

5.5 Ability to Breed with Pacific Salmon

It is a well established and documented fact that Atlantic salmon **cannot** reproduce or breed with any of the five species of Pacific salmon (Fisheries & Oceans Canada, 2005; Waknitz *et al*., 2002). Under controlled and protected laboratory conditions, where survival of hybrid offspring should be optimized, genetically viable hybrids between Atlantic and Pacific salmonid species have been impossible to produce (Waknitz *et al*., 2002). Therefore, in the unlikely event that *AquAdvantage* Salmon should breach the numerous redundant physical containment barriers that confine it to the culture system, and by some means find their way to the northern Pacific Ocean, they would be unable to mate or reproduce with native Pacific salmon.

5.6 Resistance to Establishment in the Wild

In the past century, there have been numerous unsuccessful attempts in the United States and elsewhere to establish Atlantic salmon outside their native range via intentional introductions (Fisheries & Oceans Canada, 2005). At least 170 attempts to artificially introduce and establish populations of Atlantic salmon have been documented in 34 different states where Atlantic salmon were not native, including Washington, Oregon, and California. None of these efforts was successful (Waknitz *et al*., 2002). No reproduction by Atlantic salmon was verified after introductions of fertile, mixed sex populations of Atlantic salmon in the waters of these states.

The risk of anadromous Atlantic salmon establishing self-perpetuating populations anywhere outside their home range has been shown to be extremely remote, given that substantial and repeated efforts over the last 100 years have not produced a successful self-reproducing anadromous population anywhere in the world (Lever, 1996). In the Pacific Northwest, there have been no reports of self-sustaining populations resulting from deliberate or accidental Atlantic salmon introductions (Waknitz *et al*., 2002).

Given that escapee transgenic Atlantic salmon are likely to have diminished capacity to spawn successfully compared to wild type salmon, the risk of escapee AquAdvantage salmon establishing a feral population anywhere is very remote.

6.0 Conclusions

6.1 Escape, Establishment and Spread

The potential hazards addressed in this document center on the likelihood and consequences of *AquAdvantage* Salmon escaping, becoming established in the environment, and spreading to other areas. These hazards are addressed for the production of eyed-eggs and grow-out to market size fish. Because *AquAdvantage* Salmon is produced and grown out in secure facilities equipped with numerous redundant containment measures designed to prevent escape, the possibility that even one transgenic animal will enter the environment and survive is extremely remote. In addition, because *AquAdvantage* Salmon are produced to be triploid, all-female animals, the possibility of them reproducing in the wild is likewise extremely remote. The relatively poor reproductive fitness of *AquAdvantage* Salmon, as demonstrated in evaluations of breeding efficiency, clearly show that *AquAdvantage* Salmon fare poorly interacting with wild stocks. *AquAdvantage* Salmon are reproductively incompatible with almost all fish, in particular Pacific salmon. Finally, the inhospitable environmental conditions around the egg production and grow-out facilities further reduce the possibility of establishment and spread. In short, it is not reasonable to believe that *AquAdvantage* Salmon will have any impact on the environment by escaping, surviving and thriving in regional. This argument is reinforced by the historical fact that hundreds of worldwide attempts to intentionally introduce fertile mixed sex populations of Atlantic salmon in the wild have failed to establish self-sustaining populations.

6.2 Using Confinement Measures to Mitigate Risks

A key way to manage risks associated with the use of GE fish in aquaculture is through the application of confinement measures designed to minimize the likelihood of their causing harm to the environment (Kapuscinski, 2005). It is difficult to guarantee that 100% containment can be achieved by any single method. Thus, several different methods are used simultaneously to provide redundancy and ensure that it is highly unlikely that GE salmon can escape. These measures are: biological containment, physical containment (including physico-chemical containment and operations management), and geographical/geophysical containment.

The three primary aims of confinement cited by Mair *et al*., (2007) are listed below along with the measures used for production, grow-out, and disposal of *AquAdvantage* Salmon:

- Limit the organism: prevent the fish from entering and surviving in the receiving environment. *AquAdvantage* Salmon are prevented from entering the environment by the use of redundant physical and physico-chemical barriers at the sites of egg production and grow-out. They are further prevented from surviving in the receiving environment because of geographic and geophysical issues. The immediate environs of the Prince Edward Island facility are inhospitable to early-life stage salmon due to the salinity of the local waters. The environment downstream of the Panama site is inhospitable to all life-stages due to the high water temperatures, poor habitat, predation risk, and abundant physical barriers that diminish the likelihood of survival and establishment in the receiving stream. Atlantic salmon are not found in the tropical areas of Panama.

- Limit (trans)gene flow: prevent gene flow from the GE fish. Gene flow from *AquAdvantage* Salmon is prevented because the fish are triploid females incapable of reproduction, among themselves or with wild fish, should they escape and survive. For grow-out, species with which they could breed are not present in the surrounding environment.

- Limit transgenic trait expression. It is likely that the expression of the trait, not the transgene itself, poses the hazard. The enhanced growth rate of *AquAdvantage* Salmon is readily expressed under the optimum conditions provided in a commercial environment; however, in the wild, the absence of readily available food (to which they are accustomed) and consequent depletion of energy reserves decrease the likelihood of effective exploitation of their inherent growth capacity.

6.3 Redundant Mitigation Measures

Optimum containment is dependent upon the deployment of a number of independent measures in series. Biological, physical and geographical/geophysical means of containment will be used to mitigate the potential environmental risk of *AquAdvantage* Salmon. Each method has different strengths and weaknesses, but the combination results in a very high level of effectiveness. Biological containment includes the production of entirely female, triploid fish with essentially no capacity to breed with wild fish; in and of itself, this technique is considered very effective (Mair *et al*., 2007; Arai, 2001). Physical and physico-chemical means of containment comprise additional, multiple, and redundant measures in effect at the production and grow-out sites that will effectively prevent escape. The reliability of these measures is further ensured by adherence to a strong management operations plan that includes staff training, SOPs, and routine audits and inspections. In addition, geographical/geophysical containment is provided by the specific location of the aforementioned sites.

6.4 Summary of Ecological Risk Assessment

A report by the Ecological Society of America (ESA; Snow *et al*., 2005) has proposed six major environmental processes that may be associated with GE organisms. In Table 6, each of these processes and their theoretical ecological consequences, which remain largely undocumented to date, are presented vis-à-vis their prospective applicability to *AquAdvantage* Salmon.

Table 6. Risk of Environmental Impact of GE Organisms *

Process	Potential Ecological Consequence	Risk Associated with *AAS*
Persistence without cultivation	Transgenic organisms able to spread and maintain self-sustaining populations could disrupt biotic communities & ecosystems, leading to a loss of biological diversity.	*AAS* are all sterile females unable to reproduce; a self-sustaining population cannot be established. NO SIGNIFICANT RISK.
Interbreeding with related taxa	Incorporation of transgenes could result in greater invasiveness or loss of biodiversity, depending on particular transgenic trait and gene flow from generation to generation.	*AAS* are all sterile females unable to breed with wild Atlantic salmon or related taxa. NO SIGNIFICANT RISK.
Horizontal gene flow	Non-sexual gene transfer is common in some microbes but rare in plants & animals; ecological consequence would depend on particular transgenic trait and gene flow.	Integrated transgene in *AAS* is incapable of being passed thru non-sexual means. NO SIGNIFICANT RISK.
Change in viral disease	In virus-resistant transgenic organisms, genetic recombination could lead to increased virulence of viral disease and undesirable effects on natural hosts.	rDNA construct used for *AAS* had no viral component; this type of recombination is not possible. NO SIGNIFICANT RISK.
Non-target & indirect effects	Loss of biodiversity, altered community or ecosystem function, reduced biological pest control, reduced pollination, and altered soil carbon and nitrogen cycling.	*AAS* escape minimized by redundant containment; low probability of establishment due to poor fitness and reproductive incapacity; likelihood of further spread is nil. NO SIGNIFICANT RISK.
Evolution of resistance	Pesticide resistance leading to greater reliance on damaging chemicals or other controls for insects, weeds, and other pests.	Not applicable for fish. NO SIGNIFICANT RISK.

* Process and General Consequence information derives from Snow *et al*., 2005.

Conclusion: The production and grow-out of *AquAdvantage* Salmon under the conditions described in the USFDA NADA does not present a significant risk of adverse ecological effects.

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