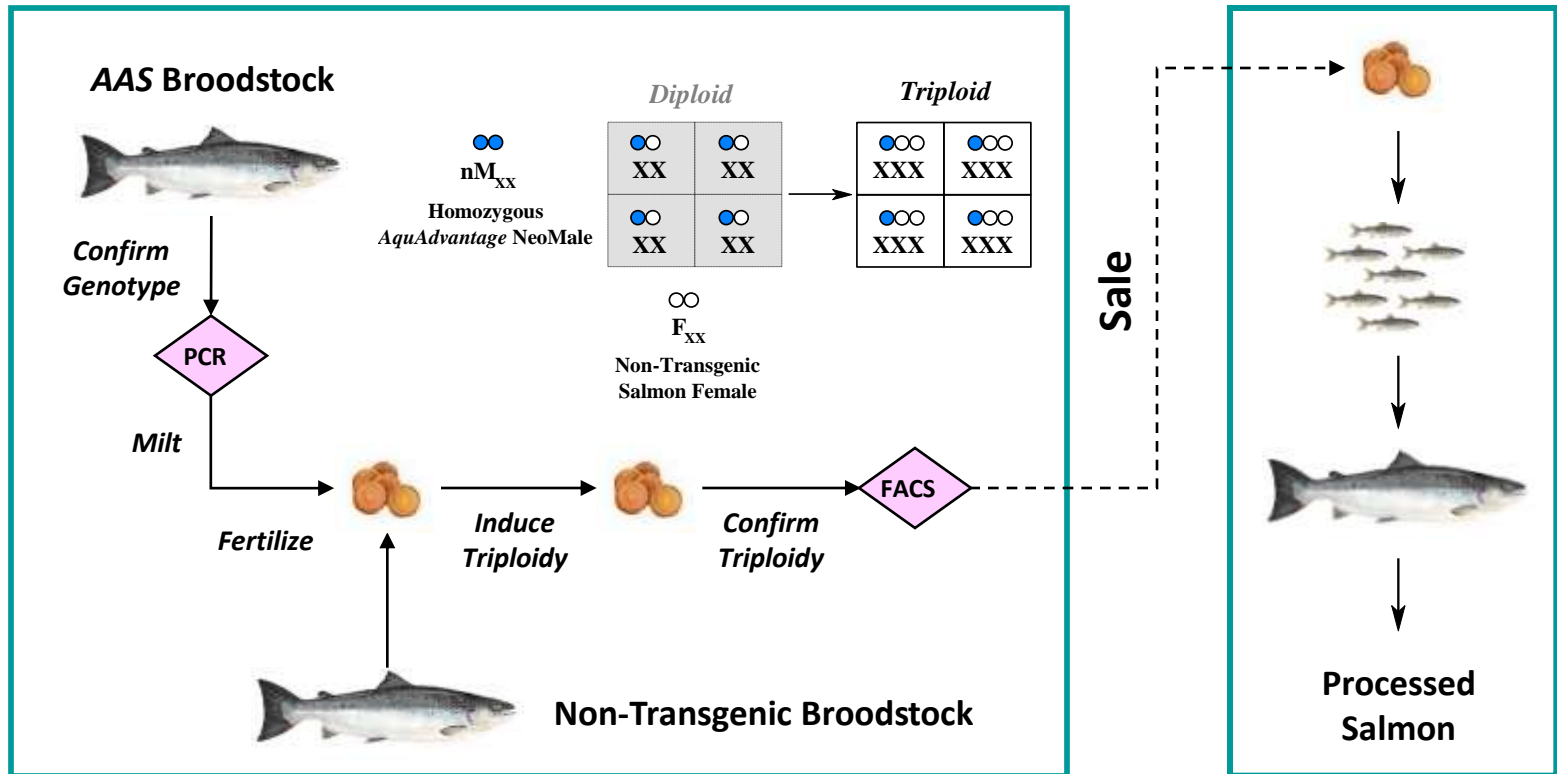




**Biosafety, Biosecurity, and Biocontainment in Aquaculture**  
Ronald L. Stotish, AquaBounty Technologies

# AAS Commercial Production

## Production of Triploid, Monosex, Eyed-AAS Eggs



# Validation of the Conditions for the Induction of Triploidy in AquAdvantage™ Salmon

Study 1: Validation using a 0.75 L pressure chamber

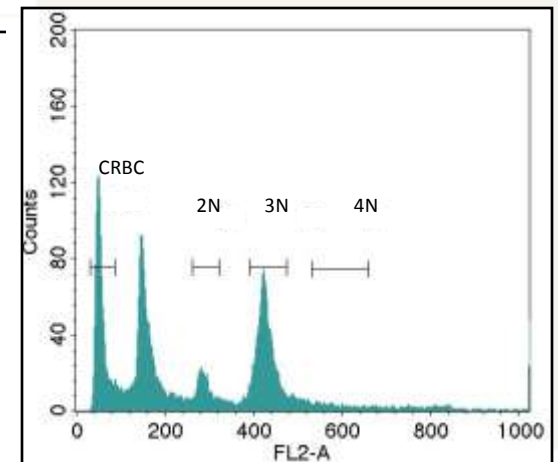
Intra-Cross Replicates					
% Triploid (lower CI)					
Cross	1	2	3	4	Mean
<b>A</b>	100 (99.2)	99.7 (98.6)	100 (99.2)	100 (99.2)	<b>99.9 (99.7)</b>
<b>B</b>	99.4 (98.2)	100 (99.2)	100 (99.2)	99.4 (98.2)	<b>99.7 (99.4)</b>
<b>C</b>	100 (99.2)	98.9 (97.4)	100 (99.2)	100 (99.2)	<b>99.7 (99.4)</b>
<b>D</b>	100 (99.2)	99.4 (98.2)	100 (99.2)	100 (99.2)	<b>99.9 (99.6)</b>
<b>E</b>	99.7 (98.7)	100 (99.2)	100 (99.2)	100 (99.2)	<b>99.7 (99.7)</b>

- overall average of 99.8% triploid (lower CI=99.7)

# Validation of the Conditions for the Induction of Triploidy in AquAdvantage™ Salmon

Study 2: Validation using a 3 L pressure chamber

Unit	No. Triploid	No. Not Triploid		% Triploid (lower CI)
		Diploid	Other	
1	475	2	0	99.6 (98.7)
2	475	0	0	100 (99.4)
3	471	0	4	98.5 (98.1)
4	473	1	1	99.6 (98.7)
5	475	0	0	100 (99.4)



- overall average of 99.5% triploid (lower CI= 99.0)

# *Validation of the Conditions for the Induction of Triploidy in AquAdvantage™ Salmon*

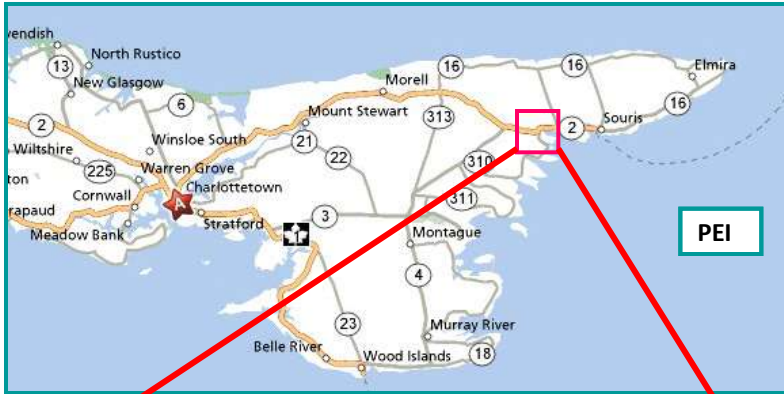
## Study 3: Validation of 7 L pressure chambers

Cross	No. Triploid	No. Not Triploid		% Triploid
		Diploid	Other	
1	199	0	1	99.5
2	200	0	0	100
3	200	0	0	100
4	200	0	0	100
5	199	1	0	99.5

Cross	No. Triploid	No. Not Triploid		% Triploid
		Diploid	Other	
6	200	0	0	100
7	200	0	0	100
8	199	0	1	99.5
9	199	0	1	99.5
10	200	0	0	100

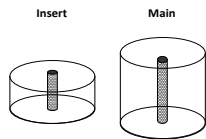
- overall average of 99.8% triploid

# Bay Fortune Facility

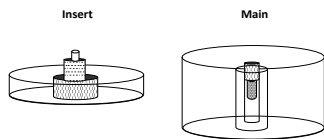


- ◆ 9k ft<sup>2</sup> hatchery & 2k ft<sup>2</sup> lab-office
- ◆ Approved commercial by EC
- ◆ Routine inspection program
- ◆ Capable of 100 % RAS
- ◆ Broodstock / Research facility

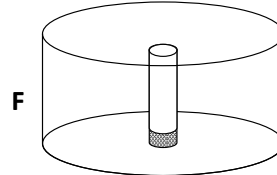




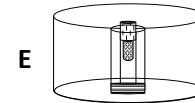
**A, B & D**  
0.8m



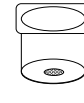
**C**  
0.8m



**F**  
12.7mm



**E**  
3.2mm



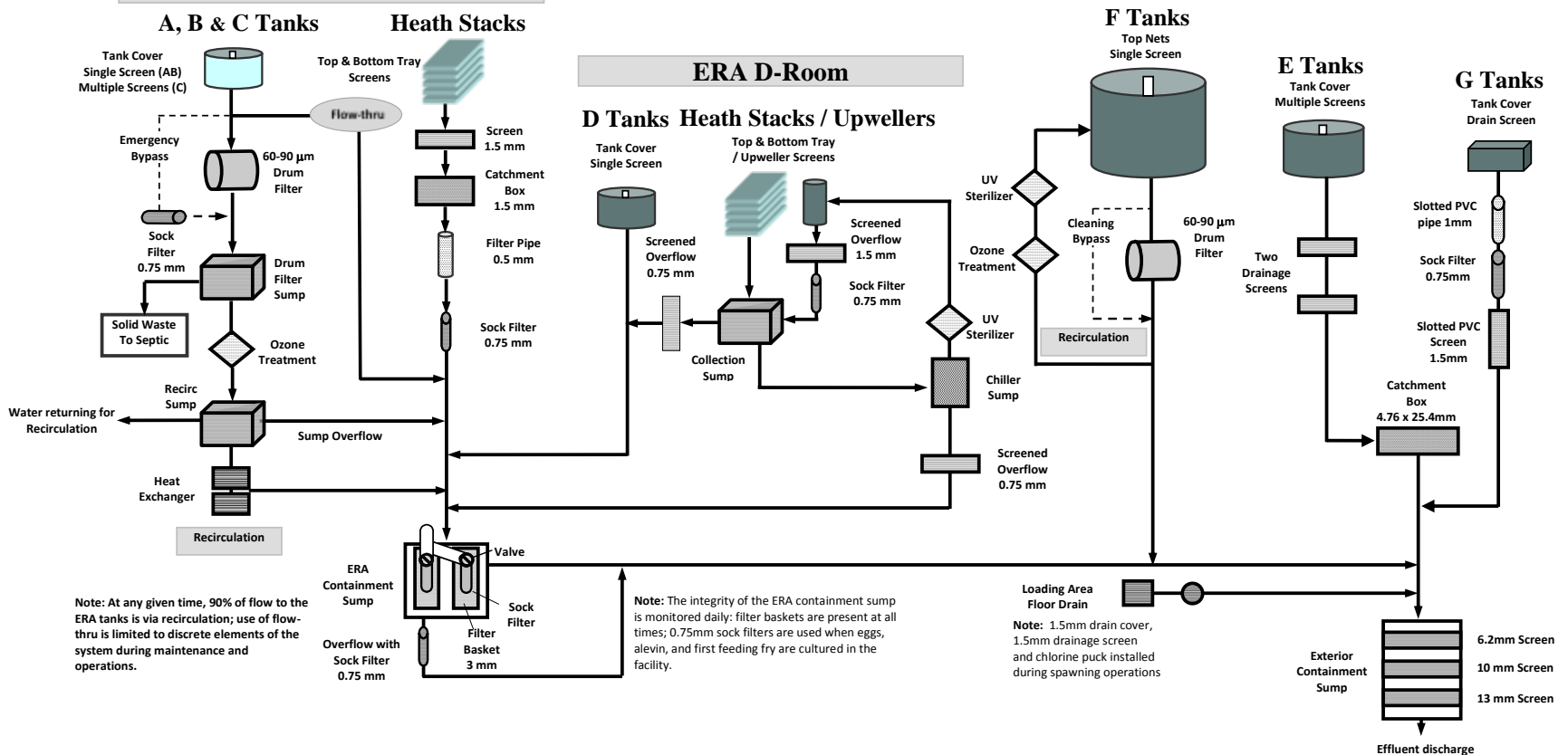
**G**  
1.6mm

## Early-Rearing Area (ERA)

## Grow-Out Area (GOA)

### Main ERA

### ERA D-Room





## Heath Stacks ERA / Nursery







# Containment Sumps



**Inspection and Service Form for Hatchery Effluent Screens and Containment Equipment  
in the ERA**

Date: \_\_\_\_\_

Life Stage Present: Eggs and Yolk Sac Fry <input type="checkbox"/> YES <input type="checkbox"/> NO				
Equipment Location & Description	Yes	N/A	Comments	Initials
PVC effluent 1.5 mm screening (2) and filter pipe on heath stacks are clean and in place				
0.75 mm sock filters on upwellers effluent are clean and in place				
0.75 mm sock filters on cold water incubation overflows are clean and in place				
Effluent screen on standpipes within A, B, and D tanks are of appropriate size.				
Life Stage Present: Feeding Fry to Smolt + <input type="checkbox"/> YES <input type="checkbox"/> NO				
Equipment Location & Description	Yes	N/A	Comments	Initials
Effluent screen on standpipes within tanks are of appropriate size.				
Tank covers in place on ERA tanks where appropriate				
Overflow screens on tanks are clean and in place				
Floor drains covered				
Sock filter of appropriate size clean and in place on drum filter overflow				
Drum filter in operation				
Waste flows on all tanks containing fry 2g and less directed to recirculation				

Reviewed by: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_

**Inspection and Service Form for Hatchery Effluent Screens and Containment Equipment  
in the Main GOA**

Date: \_\_\_\_\_

Life Stage Present: Eggs and Yolk Sac Fry <input type="checkbox"/> YES <input type="checkbox"/> NO				
Equipment Location & Description	Yes	N/A	Comments	Initials
PVC effluent 1.5 mm screening (2) or 0.75 mm socks on heath stacks are clean and in place				
0.75 mm sock filters on upwellers effluent are clean and in place				
0.75 mm sock filters on cold water incubation overflows are clean and in place				
1.5 mm steel plate is placed over floor drain in loading bay, 1.5 mm drainage screen in place, and chlorine puck installed				
Life Stage Present: Feeding Fry to Smolt <input type="checkbox"/> YES <input type="checkbox"/> NO				
Equipment Location & Description	Yes	N/A	Comments	Initials
Effluent screen on standpipes within F tanks are of appropriate size and standpipes are covered				
Tank covers in place				
Floor drains covered				
12 mm coated wire filters clean and in place on drum filter overflow				
Drum filters (2) in operation				
All Life Stages Present:				
Equipment Location & Description	Yes	N/A	Comments	Initials
ERA containment sump: 3mm stainless steel basket clean and in place				
ERA containment sump: 0.75 mm or 1.5mm socks (depending on life stage in ERA) clean and in place				
ERA containment sump: 0.75 mm screen on overflow clean and in place				
GOA containment sump: 6 mm, 10 mm, and 13 mm screens clean and in place				

Reviewed by: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_

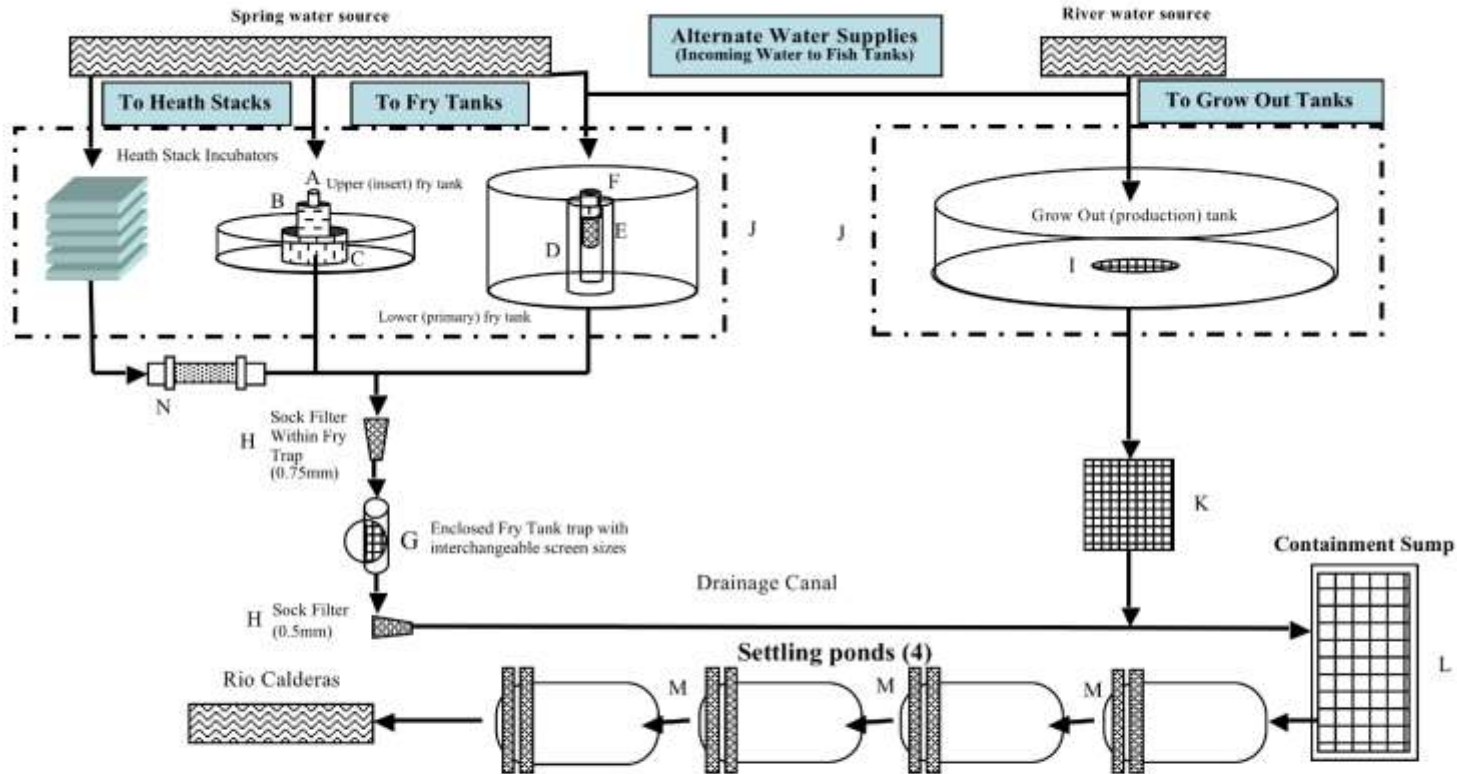


# Physical containment measures Panama





## AQUA BOUNTY PANAMA – Water Movement and Containment Measures



- |   |   |   |  |
|---|---|---|--|
| A | Interior stand-pipe (Figures 2a-2c)                                     | I | Slotted drain plate (0.9 mm) (Figure 2k)                   |
| B | Interior standpipe Screen (1.0, 1.5 mm) (Figures 2a-2d)                 | J | Security fence (Figure 2ab)                                |
| C | Exterior standpipe Screen (1.5 mm) (Figures 2a-2c)                      | K | Enclosed screen cage (6 mm) (Figures 2q, 2r)               |
| D | Exterior standpipe (Figures 2c-2g)                                      | L | Containment sump with screen plate (12 mm) (Figures 2s-2u) |
| E | Interior standpipe screen (1.0, 1.5 mm) (Figures 2d, 2f, 2g)            | M | Dual outlet screens (12, 6 mm) (Figure 2v)                 |
| F | Not shown: metal screen affixed to base of standpipe screen (Figure 2h) | N | Cartridge Filter 50µm                                      |
| G | Fry tank filter screen housing (0.5, 1.0, 3.0 mm) (Figures 5a-5e)       | ➔ | Indicates water flow and direction                         |
| H | Bag net filter sock (0.5 or 0.75 mm) (Figure 5f)                        |   |  |

# Multiple “in series” Filter Barriers

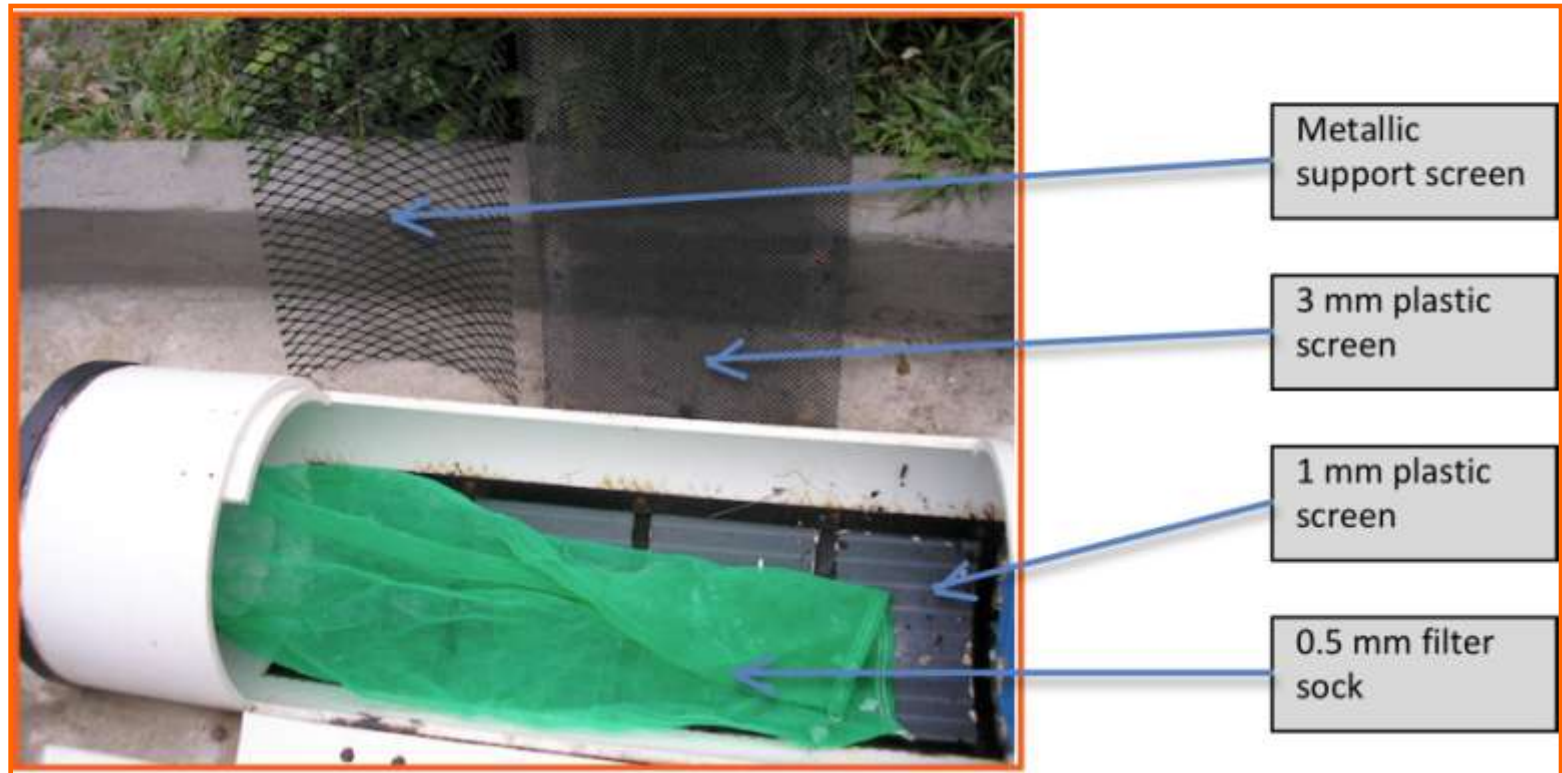


# Panama Fry Tanks





**Multiple, redundant containment elements – Can be customized in accordance with the life stage or size of the animal**



# Inspecting containment Appliances

Inspection and Service Form for Containment in the rearing area

Date: \_\_\_\_\_

Life Stage Present: Feeding Fry to Smolt + <input type="checkbox"/> YES <input type="checkbox"/> NO					
Equipment Location & Description	AM	PM	N/A	Comments	Initials
Effluent screen on standpipes within tanks are of appropriate size.					
Collar Sleeve of the appropriate size is in place at the top of the					
Tank covers in place on ERA tanks where appropriate					
Overflow screens on tanks are clean and in place					
Bag Net Filter (0.5mm mesh) at terminal end of drain pipe from fry					
Fixed screen in PVC housing for Bag Net Filter attached and clean.					
Secondary bag net filter (0.5mm mesh) from fry tank effluent pipe at the drainage canal is in place and					

Reviewed by: \_\_\_\_\_

Date Reviewed: \_\_\_\_\_



**Table 9. Implementation of an Integrated Confinement System  
for AquAdvantage Salmon \***

Recommended element	Egg Production & Grow-Out Sites	
	Egg Production	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 SOPs 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	✓	✓
* After Kapuscinski, 2005.		
** Written plan to be prepared in the event of an approval		

**Source: U.S. FDA Draft Environmental Assessment, 4 May 2012, p.75**

# Biosecurity and Biocontainment Systems

Thoughtful Design / Effective Operation

Devices and Procedures Life Stage Appropriate

Combining Biological and Physical Containment

Management Commitment

- Defined Procedures for Operation / Remediation

- Documentation

- Audit / Inspection

- Periodic Reporting

## A Continuous Process