INFORME TÉCNICO FINANCIERO

CONSULTORÍA ESPECIALIZADA

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EMPRESA: AQUA GEN CHILE S.A.

PUERTO VARAS, AGOSTO 2010

A. ANTECEDENTES

Aqua Gen Noruega A.S., es una empresa de selección genética con más de tres décadas de operación, que desarrolla, produce y entrega material genético a la industria acuícola. A través de su mercado orientado a la investigación y desarrollo, Aqua Gen ha logrado una posición de liderazgo como proveedor de ovas de salmón del atlántico y de trucha arcoiris.y de

En el año 2000 comienza el trabajo de Aqua Gen Chile S.A., ocho años más tarde construyen el Primer Centro de Alta Tecnología para la producción de ovas de salmónidos en Chile.

La empresa se ha destacado desde sus orígenes por su capacidad de innovación, tanto en el desarrollo de Investigación como de mejores prácticas. En este marco que Aqua Gen Chile decidió contratar un experto internacional en reproducción de salmónidos, el Dr. Jim Powell. El trabajo desarrollado privilegió el análisis de las actuales prácticas, capacidades técnicas, infraestructura y capital Humano, de manera de evaluar la viabilidad y conveniencia de incorporar un nuevo proceso de desove en mar o en su defecto mejorar el actual procedimiento.

La consultoría se desarrolló en dos viajes. El primero desde el 25 de abril al 3 de junio para luego retomar desde el 26 de mayo al 3 de junio. Entre ambos viajes y posterior al último, el consultor trabajó desde Canadá en el análisis de la Información y la preparación de Informes.

La primera etapa de la consultoría se centró en la evaluación de las capacidades de las instalaciones de Aqua Gen en su piscicultura de Comau y el centro de Chesque. También se realizaron Talleres de trabajo con el equipo de profesionales de Aqua Gen, con el objeto de evaluar sus competencias y definir en conjunto también los puntos críticos en los procedimientos que actualmente ejecutan. Para finalizar se realizaron dos capacitaciones con diferentes contenidos. El Primer Informe del Consultor incluye también propuestas de entrenamiento enfocadas a mejorar las capacidades del Capital Humano de Aqua Gen en el mediano plazo.

En el segundo viaje, las actividades se concentraron en el ensayo de un desove en mar, buscando evaluar su eficiencia. Para ello se ejecutó una prueba en el centro de mar de la empresa ubicado en la Bahía Pumalín.

B. DESCRIPCIÓN DE LAS ACTIVIDADES

1. VISITAS A TERRENO

Esta actividad involucró visitas a los centros productivos que la empresa posee en COMAU y CHESQUE. En estas visitas se levantó la información necesaria para diagnosticar las capacidades actuales de la empresa.
También se definieron los aspectos fisiológicos y productivos claves en la reproducción de salmónidos, considerando la experiencia chilena y las exigencias de sus mercados.

El consultor analizó las instalaciones de la empresa tanto en su estructura física como en la ejecución de procedimientos. Las conclusiones fueron dirigidas de manera de identificar puntos críticos y sus alternativas de solución. Se describieron cada una de las etapas críticas que pudieran influir tanto en la bioseguridad del proceso como en su eficiencia productiva.

2. TALLERES GRUPALES

Una vez concluidas las visitas, se ejecutaron talleres de trabajo para analizar en equipo las observaciones del Consultor de manera de identificar brechas tecnológicas y sus posibles soluciones para asegurar el éxito en la implementación de los nuevos procesos. La participación del staff técnico de la empresa fue relevante en esta actividad para asegurar que

los resultados de la Consultoría estén alineados con la realidad local y que su implementación sea efectiva. La metodología consistió en la ejecución de tablas redondas en torno a los puntos críticos identificados previamente por el Consultor.

Detalles y conclusiones de ambas actividades se incluyen en el Informe entregado por el Consultor, el cual se adjunta como Anexo Nº1.

3. REUNIÓN DE TRABAJO ENTRE EL CONSULTOR Y LA GERENCIA DE AQUA GEN CHILE

En esta actividad la discusión se centró en las siguientes preguntas:

1. ¿Qué hacer si la demanda de ovas supera la capacidad de Comau?

2. ¿Qué es más necesario a corto plazo?, ¿Mayor entrenamiento o desarrollo de nuevos recursos?.

3. ¿Cómo el holding puede apoyar la consolidación de Aqua Gen Chile?.

En los aspectos en que Fidelis Aquaculture Management puede colaborar, se definió un borrador de Acuerdo de Cooperación entre ambas empresas.

4. CAPACITACIÓN

Durante la ejecución de la Consultoría se desarrollaron varias actividades de capacitación. En el primer viaje, se realizaron entrenamientos prácticos en las instalaciones de COMAU, los que involucraron los siguientes temas:

a) Test para la determinación del porcentaje de fertilización. Esta técnica involucra la observación a través de microscopía, de una muestra de ovas.

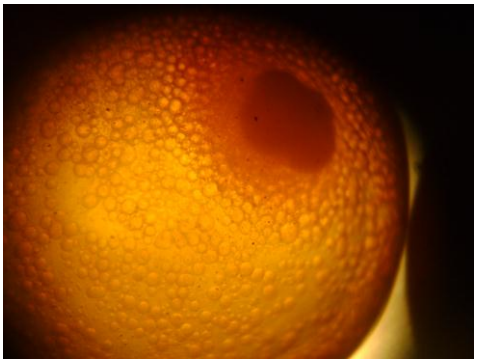

Foto Nº1: observación de ova 4 células al microscopio.

b) Cateterización de machos.

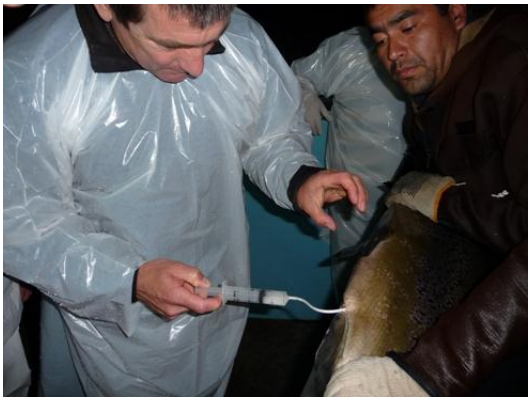

Foto Nº2: extracción de semen mediante cateterización

5. ANÁLISIS DE LA INFORMACIÓN RECOPILADA.

Este trabajo el Consultor lo realizó en Canadá, el Informe se adjunta como Anexo Nº1. De este trabajo se obtiene también un protocolo para la ejecución de la prueba de desove en mar. Este documento incluye indicaciones para:

a) Bioseguridad.

b) Chequeo de maduración.

c) Metodología de extracción de gametos.

d) Almacenamiento y transporte de gametos.

e) Desinfección del material genético.

f) Screening.

g) Disposición de mortalidad.

h) Limpieza.

i) Consideraciones de cultivo para un óptimo desove.

Por otra parte, en el Informe mencionado, el Consultor sugiere temáticas relevantes para la capacitación del staff de Aqua Gen Chile. Según su juicio, deberían realizarse las siguientes actividades de capacitación:

i) Pasantía en British Columbia. Entrenamiento en instalaciones canadienses en épocas de desove. Con ello podrían conocer las prácticas de empresas que ya tienen experiencia en este procedimiento.

ii) Curso con expertos internacionales en manejo de machos para el aumento de la calidad del esperma.

Las razones y beneficios de ambas se detallen en el Anexo nº1.

6. EJECUCIÓN DE PRUEBA PILOTO

A fines de mayo el Consultor vuelve a Chile para completar la Consultoría, mediante la ejecución de la prueba piloto, nuevas capacitaciones y protocolos de trabajo, definición de Acuerdo de Cooperación y Difusión de Resultados.

Previa a la ejecución de la prueba en mar, se volvieron a visitar las instalaciones en COMAU, donde se chequearon temas relativos con el proceso de maduración vía inducción hormonal y se realizó un nuevo seminario centrado en el Manejo de reproductores para la disminución del estrés.

Luego de COMAU, el consultor y parte del staff de Aqua Gen se trasladaron a PUMALÍN, centro donde la empresa tiene sus reproductores. Las primeras actividades tuvieron relación con la inspección de los peces y la infraestructura disponible.

Una vez concluida la inspección, se realizó el screening de los reproductores y se definió el lugar de la prueba piloto, en un sitio protegido e históricamente libre de enfermedades.

Foto Nº3: Selección de reproductores

Foto Nº4: Reproductor listo para desove.

Mayores detalles de estas actividades y las temáticas abordadas se encuentran en el 2º Informe entregado por el Consultor, el cual se adjunta como Anexo Nº2.

Luego de la ejecución de la prueba, se realizaron 3 nuevos seminarios en los que se abordaron los siguientes tópicos:

a) Reproducción y endocrinología.

b) Técnicas para el desove en mar.

c) Estrés y Reproducción.

En todos los casos el objetivo fue definir en qué aspectos debe Aqua Gen reforzar sus capacidades de manera de mejorar su performance.

7. DISEÑO DE NUEVOS PROCESOS

Para la definición de los nuevos procesos, el Consultor realizó un nuevo seminario que cubrió los siguientes tópicos:

- Efectos de salinidad
- Efectos de la temperatura
- Condiciones de mantenimiento
- Manejo
- Calibración
- Inducción a la maduración
- Extracción de gónadas
- Manejo de ovas y transporte
- Recolección del semen

A partir de esta actividad, la empresa desarrollará sus nuevos protocolos. En el Anexo Nº2 se encuentran más detalles de la temática abordada.

8 - DIFUSIÓN DE RESULTADOS

Para la difusión de los resultados se realizó un Seminario en el hotel Diego de Almagro de Puerto Montt. Fueron invitados clientes de la empresa, representantes sectoriales y de la CORFO. Durante el Seminario expusieron el Dr. Jim Powell y el Gerente de Producción de Aqua Gen Chile, Joaquín García. Como Anexo Nº3 de este informe se adjunta un reportaje del evento.

C. CONCLUSIONES DE LA EMPRESA: PRINCIPALES ASPECTOS A DESTACAR Y BENEFICIOS DE LA CONSULTORÍA.

Las principales mejoras que la empresa ha podido apreciar en el corto plazo de detallan a continuación, divididas por cada centro productivo visitado.

PUMALIN:

- ⇨ Se desarrolló un nuevo protocolo de fotoperiodo para peces que podrían ser desovados en el mar tanto en su temporada normal como un fuera de temporada. Con esto se busca **optimizar el uso de agua dulce** en pisciculturas, con su consecuente **ahorro en logística y disminución del stress** de los peces previo al transporte de los peces vía marítima y terrestre y al desove. En suma se obtiene una mejora en los rendimientos dado por **mayores sobrevivencia de los reproductores y mejor calidad de semen.**

- ⇨ Se diseña un programa especial y adecuado para el mantenimiento y manejo de los reproductores de tres inviernos en Pumalín. Donde se incorporan técnicas de anestesiado, palpaje y manejos en general.

- ⇨ **Manejo del stress en reproductores en mar** a través del conocimiento de la **fisiología** asociada al stress y la respuesta en los peces. Con esto se obtiene una nueva herramienta que es una **tabla de manejo/efecto/riesgo**, usada a la hora de programar cualquier manejo.

- ⇨ Se levanta un esquema mejorado de **condiciones para el transporte de peces**, lo cual optimiza las **variables fisiológicas**, identifica los **monitoreos** más adecuados y asegura **mejoras en la sobre vida posterior.**

CHESQUE:

- ⇨ Uno de los principales aspectos revisados en este sitio de agua dulce que mantenía reproductores a desove, fue cambios en el uso y estrategia de aplicación de implantes hormonales (Ovaplant) en los peces. Se generó un **protocolo** que nos permitió **optimizar el uso de los machos implantados** y lograr el **máximo potencial de obtención de semen** previo al sacrificio de estos, se aumentò los días entre la aplicación del implante y el desove o sacrificio de los machos, además de mejorar la planificación al desove, **se obtuvo en promedio un 30% más de semen por cada macho,** esto significó el poder incrementar la posibilidad de cruza para lotes de hembras con machos de excepcional valor genético.

- ⇨ Sobre el uso del semen, también se generó una estrategia distinta para el transporte del semen en jeringas de 20 ml. Se hicieron varias pruebas al fin de las cuales se pudo definir las mejoras en el proceso respecto de la viabilidad del semen después de 24 horas (necesarias entre desove/screening/resultado/fecundación). **Se optimizaron el manejo término y sistemas de transporte del semen.**

- ⇨ Un tema que se revisó y parte de las recomendaciones fueron relacionadas con el

transporte de gametos y la T° asociada. Esto fue tema de los talleres y finalmente se definió dentro del protocolo, realizar los desoves de noche, usar camiones con control de T°, pero sin hielo dentro de las cajas.

COMAU:

- ⇨ Revisión general del protocolo de desove y fertilización de gametos, con recomendaciones y observaciones del proceso, de las cuales se pueden destacar una **mejora en el proceso de uso de la cantidad de semen necesaria para fertilizar una ova**. La recomendación fue disminuir el volumen a un valor que depende de la motilidad máxima medible del semen a utiliza, la cual se mide segundos desde su activación y que se ha calculado. No obstante, se aplicó la estrategia solo cuando existió falta de semen, los resultados de sobrevivencia de esos lotes en particular fueron los mismos que el resto, como conclusión, si se puede disminuir la cantidad de semen en la fertilización, esto también genera una mejora en el proceso de fertilización al lograr una mayor eficiencia y optimización en el uso de machos con valor genético excepcional.

- ⇨ Se trabajó sobre la **interpretación temprana de fecundidad en Ovas** después de la fertilización, el protocolo que nos entregó nos ayudó a obtener esta interpretación pasadas 24-48 horas post fecundación, utilizando la lectura de 4 células. Esto se traduce en una posibilidad de tener resultados o una estimación final más "temprano", lo que es un indicador usado para tomar decisiones referentes al protocolo utilizado en desove-fecundación-desinfección. etc. Se obtuvo una mejora en el proceso de evaluación de los resultados de las etapas mencionadas, al evaluar y **tomar decisiones de mejor manera en caso de ver que los porcentajes obtenidos de 24 horas post fecundación no son los esperados.**

- ⇨ Fotoperíodo en agua dulce, en referencia a este tema nos entregó una presentación con diversas posibilidades y nos explicó la fisiología involucrada con estos diversos manejos. **Sin duda la aplicación de estas estrategias será uno de los aspectos más importantes en lo que a reproducción en agua dulce se refiere, si bien no se aplicarán los protocolos entregados en el 100% de los peces, se usarán en parte de ellos para evaluar resultados y posibilidad de reemplazo/combinación o complementar a futuro con nuestros protocolos de manejo de fotoperiodo. Esto seguramente será materia de futuros trabajos conjuntos con Fidelis Aquaculture.**

Por otra parte, la Consultoría permitió identificar posibilidades de cooperación al mediano y largo plazo, las cuales se están analizando y coordinando entre la empresa beneficiaria y Fidelis Aquaculture Management Ltd.

ANEXO Nº1: PRIMER REPORTE DE ACTIVIDADES, MAYO 2010.

Draft Report for AquaGen Chile

Corfo Project

May 2010

Introduction

The following report is prepared to comply with the stipulations regarding Corfo support of Technology Training and Transfer for maximizing eyed egg production at AquaGen Chile.

1) Field visits

Details of activities and physical description of Comau and Chesque Alto hatcheries

Comau

The Comau facility is structured into three distinct parts: Incubation, Rearing and Administration. Each of the areas is a designated biosecure zone with several sub-zones occurring in the fish culture areas.

Incubation

The incubation room is separate from the stripping room and the fertilization room. Ova and milt traffic is one way and is separated by physical barriers for reasons of biosecurity. However, the incubation room can be accessed by doors leading to service areas and thereby the outdoors with no biosecure entry points. This is also the route to the iodophore and isotonic water reservoirs.

Ova enter from the stripping room and are transferred to cleaned and disinfected plastic colanders. Ovarian fluid is drained from the eggs at this point and the ovarian fluid collected in a trough from later disinfection and disposal. At this point the eggs have not been refrigerated or cooled.

The eggs in colanders are rinsed with 8°C isotonic saline to remove latent ovarian fluid. Once drained, they are transferred to labelled plastic bags suspended in 20L plastic buckets. Up to 16ml of mixed milt is added to the eggs in each bucket and 1L of milt activation fluid is added; the mixture is mixed my hand for 30sec. The fertilized ova are left to stand for 3min and transferred to the disinfection table where 10L of 100ppm iodophore is added and the

mixture sits for 10 min. After disinfection the eggs are rinsed with 8°C incubation water and drained. The eggs are then passed through a half wall to the incubation room and placed in individual incubation jars. Water flow is set to 1.5lpm per jar.

Technical capacity

The process flow of the fertilization procedure capitalizes on an excellent floorplan to preserve biosecurity. Although not fully functional as a brood production facility (construction of the stripping area is incomplete), the technical capacity to expedite the fertilization process and preserve biosecurity is sufficient to maximize the process flow.

One aspect of improvement would be the addition of temperature control. Unfertilized (green) eggs have a 24h 'shelf life' whereby they can tolerate holding temperatures up to 9 or 10°C. In this case, transport from the hatchery is done at ambient temperatures and is prone to fluctuation. The green eggs are shipped in individual bags, overlaid with oxygen and packed into Styrofoam salmon fillet boxes. Ideally the boxes would be packed with reusable ice packs or freezer packs on which bubble wrap or paper separates the eggs in bags. In this way, the eggs remain at a constant low temperature throughout shipping.

The importance of keeping eggs cool centers around enzymatic degradation, bacterial growth and oxygen consumption. During the spawning process blood, urine, bile and other body fluids can be harvested along with the eggs and ovarian fluids. While ovarian fluid serves to keep the eggs in a high state of fertility, the other fluids may contain enzymes and chemicals that can decrease fertilization rate. Cooler temperatures during transport decrease the risk of degradation by lowering enzymatic function.

Bacterial growth in transported eggs can cause a decrease in egg quality and result in discarding eggs after screening results are in. As well, bacteria missed by screening may flourish and contaminate the incubation room if allowed to grow during the time from stripping to disinfection. Keeping temperatures cool during this period decreases the risk of bacterial growth in the ovarian fluid.

Low transport temperatures decrease egg oxygen demand. Although the majority of oxygen use in transported eggs is attributable to bacterial growth, this nonetheless decreases oxygen availability to the egg. Cool transport temperatures decreases metabolic oxygen

demand irrespective of the source of that demand.

For the above reasons, it is suggested that some method of keeping the transported eggs at 4°C be implemented. In addition, the stripping and fertilization rooms need to have temperature control installed to keep temperatures below 8°C and preferably 4-6°C. This temperature range is not a key factor when egg and milt quality is high, but can be a key factor when other conditions are not perfect.

AG has the necessary equipment to check milt quality with the use of a compound microscope. The addition of a 100X dissecting microscope would complement the milt- and egg-quality checking capability. The milt checking area was clean and orderly.

The practice of mixing milt during the fertilization process is not recommended. Sperm competition studies have repeatedly demonstrated that in pooled milt samples, the majority of the fertilization is completed by one male donor; the other males were superfluous to the objective. When viable milt is used in a 2:1 (female to male) ratio, genetic diversity of the progeny is sufficiently diverse and there is no waste of other milt. The commonly held concept is that inferior milt in a 2:1 cross would contribute to losses in egg fertility. Studies have shown that this is not the case and if milt motility is checked before use, fertilization rates will be assured. In reality, if poor, non-viable milt is used, the studies have shown that it is still capable of fertilizing the majority of eggs in some circumstances. From a customer standpoint, the progeny of many thousand eggs could be siblings which from a fish health and growth perspective is a risk.

On the issue of milt from macerated testis, this product is inferior to expressed milt or milt collected with a catheter. However, when milt is in scarce supply the practice may become necessary. The caution here is that proteolytic enzymes are often included with the supernatant and can damage the spermatozoa, especially if the preparation is left unrefrigerated. Spermatocytes, spermatids, inactive spermatozoa and mature spermatozoa are all present in testes homogenates. To compensate for the lack of motility in the preparation, more homogenate must be added to the fertilization container. The risk is that infertile spermatozoa may enter the egg germinal vesicle and activate the egg, causing an infertile egg. However, when milt supplies are low, the use of testicular macerate is a logical procedure.

Milt volume for fertilization is generally recognized as 1ml per litre of eggs. This is the 'Billard' standard of milt to egg ratio. However, polyspermy is not a problem in salmon and excessive milt becomes an issue if the dilution of milt is not sufficient to induce full activation of the sperm.

Technology utilized in the process

AG staff used the industry standards of best practices during fertilization, the above observations notwithstanding. The key equipment is at their disposal to address routine and non-routine procedures including quality control of the milt and eggs. The addition of supplied isotonic and iodophore solutions is above and beyond the normal procedures in most hatcheries.

Skill sets that AG possess to accomplish the goals

AG staff displayed a high level of competency and skill sets related to egg and milt handling, fertilization procedures, egg disinfection, primary incubation and biosecurity. In addition, staff were open to questions regarding their practices and eager to try new techniques. When presented with new approaches and techniques, the staff were adaptable, open-minded and willing to try.

Procedures that capitalize on the resources

The operating procedures for fertilization are on a level with best practices. The ability to move 350 sets of eggs through the fertilization/disinfection room in 6 hours or less speaks to the level of human and capital resources. With a few streamlining improvements to staff movements and small improvements to procedures, the process will become even more fluid.

Water quality

The water supply for the incubation side of the facility is from an adjacent river and is taken from the run of the river as a side-stream channel diversion. The water is diverted to an open channel by a manually set gate. Water then flows over a silt/debris screen and travels to a small settling are and then through a rotary drum filter. Water then flows through piping to the hatchery and through a series of UV lights before entering the incubation room. Water delivery to the incubation jars is via gravity feed from overhead pipes that act as

header reservoirs for the lines that feed the jars.

During the time of the site visit and evaluation, a storm came through and rainfall levels were extreme. Particulate matter was bypassing the sedimentation and filtration systems and clogging the ball valves that regulate water flow to the individual jars. It is unknown how long and how many incubation jars were affected, but the outcome of reduced or eliminated water flow to developing embryos is clear. Fortunately, staff were able to remedy the situation with improvised improvements to the incubation jars to restore water flow; an invention that was nothing short of brilliant. The staff are to be commended on their quick and effective actions.

Siltation of the eggs in incubation was evident for over 24h as the river rose. Siltation of eggs critically threatens egg survival in three ways: 1) the silt, primarily the clay portions of the silt 'smother' the eggs by restricting oxygen transfer to the developing embryo, 2) bacteria in the sediment can erode the egg shell and increase chances of bacterial infection from ubiquitous bacteria such as *Flavobacter* spp., and 3) bacterial load in the sediment uses oxygen that further exacerbates the smothering effect of the silt itself. Clearly, water quality is not sufficient for the operation of a safe and biosecure operation.

Part of the problem is that the UV sterilization system is incapacitated by particulate matter. It is apparent that at Comau, surface water supply is a biosecurity and physical risk to egg survival. It is also clear that the design of the water delivery system does not match the need for the facility. It is understood that measures are underway to remediate the situation; certainly technologies exist to address the issue.

A challenge to the facility water supply system will be when the supply of eggs is continuous throughout the year. In the summer, the river water source is drastically reduced, which from a delivery problem is a concern itself, but also poses physical threats through organic matter load and temperature profile in addition to bacterial load in slow-moving, warmer water. The AquaGen plan is to have spawning fish supplied throughout the year and if this is to be, water for incubation will likewise have to be secured throughout the year.

Rearing

The rearing side of the Comau facility is dedicated to the production of freshwater

broodstock. The need for full-cycle brood came from the ISA epidemic among seawater-held fish that sparked a demand for ISA-free eggs. There are two year classes and three lifestages of fish in recirculating tanks: Brood Years 2011 and 2012.

A subsection of the 2011 yearclass fish were exposed to a limited winter photoperiod and put on 24 hour light (LL) to help prevent grilsing (premature maturation), particularly in males. This is common practice in fish held in seawater net cages.

Two practices may not have been fully considered when this was implemented. Firstly, LL light needs to be implemented prior to the onset of a winter photoperiod to prevent any 'winter signal'. This winter signal is important in establishing the maturation cue for the subsequent spawning season. There must be no winter signal for there to be no grilsing. Secondly, fish held in freshwater for their complete lifecycle mature a year early, even if held on LL, although this modifies maturation time (see below).

The consequence of the small winter signal for the population served to be sufficient for about 40% of the existing male population to mature in the second summer, causing a large amount of precocious maturation. These fish were identified by ultrasound (ecography) and removed from the population. The remaining population remained predominantly female and limited the amount of available males for subsequent spawning. Although the winter signal was enough of a cue to induce male maturation, it was not of sufficient duration to induce female maturation and there were few if any female grilse. When the population was returned put on LL, maturation was arrested.

LL light will not permanently arrest maturation. The 'internal clock' of fish dictates and overrides the photoperiod cue and the fish will eventually mature albeit over one-half out of phase with the normal spawning period. It was unclear if the LL treatment was incidental or if second-season spawning was not anticipated from freshwater brood. However, a proportion of the 2011 year class that if held in seawater would spawn in May of 2011, is now in various states of partial maturation across all groups of the year class.

As it turns out, a proportion of each of the held groups will spawn over the next 4-8 months. This was apparent from visual identification and weight sample data conducted during the site visit. From a subsample of fish ultrasound, it became apparent that there were most

likely three distinct groups of maturing fish within the population: those 3-4 months; those 5-6 months and those around 8 months from spawning. This is consistent with the predictions based on published literature: LL will delay maturation time, but will not prevent it.

On the basis of the preliminary ultrasound information and visual evaluation of stock sub samples, a photoperiod and thermal regimen was developed to have the 2011 brood year spawn in four successive groups: September, December, March and May. By sorting for maturity and transferring the fish in each mature group to a photoperiod of 8L:16D and a temperature adjustment to 4°C from the current 9°C final maturation should be induced in the exposed fish. It is recommended that all fish be injected with GnRH implants to synchronize spawning times and capitalize on the limited milt availability. As a cautionary note, egg quality in these fish should not be expected to be comparable with 'naturally spawned' fish. The groups have been repeated handled and will have endured several 'maturational' effects to induce them to mature and spawn. The literature suggests that these treatments adversely affect egg quality. A margin of error may be applied to egg performance.

As a follow-up note, the maturation in the 2012 fish held in freshwater will not favour grilsing. To manipulate spawning date the fish will have to be placed on a photoperiod regime that is conducive to maturation cycles. This will mean the installation of individual photoperiod covers for many of the tanks in the main building. As well, for 'finishing' a group of tanks will need to be designated for final maturation and be kept at 4°C to provide the thermal cue prior to spawning. Infrastructure of the facility to apply photoperiod control will need to be installed.

Considerations

It is understood that the arrangement of fish in the recirculation rearing area was not intended for the current use and that circumstances dictated that the facility adapt to accommodate the ISA crisis. This notwithstanding, there are a few improvements that can be made to ensure production efficiencies.

Water quality

While sodium chloride is an acceptable addition to the water to help prevent and control

fungal infections, total water hardness in the Comau system is low. Hardness as calcium carbonate may need to be increased. A recirculation expert may need to be consulted, but hardness levels of up to150mg/l are common in freshwater broodstock sites. This level of hardness has positive effects on growth, maturation and fish health. Biofilters need elemental calcium as part of their nitrogen conversion process and calcium is essential in growth and maturation of the fish.

The addition of ozone to the fine-solid filtered water prior to the biofilter will increase water clarity. Tannins are a by-product of the recirculation process and while not harmful to the fish, affect water clarity. The addition of an ozone generator at the post-drum filter reservoir should clear up water colour.

Feeding

Feeding is currently done by hand. In tanks of the densities witnessed, hand feeding is inefficient and will result in a wide range in the size distribution of the fish. At high densities, feed penetration to the water column is at best a meter and not uniform over the surface of the water. This can result in a few fish having more feeding opportunity. This then results in fish that are 'fatty' and fish that may not receive their full nutritional compliment, something essential for broodfish. Automatic feeding, especially on a LL light regimen is recommended to ensure optimal feeding rates. Close contact with the fish and feeding response is still an essential part of fish culture and can only be monitored by personnel who hand feed. Most feeding programmes accommodate a percentage of daily ration fed by hand.

Maturing fish can be starved up to three months prior to spawning. Fish in the final stages of maturation do not need additional nutrients in the feed and will call upon body reserves to complete egg maturation. At the very least, feeding rations can be cut to minimal percentages. This ensures that 'silver' or non-maturing fish still in the population will receive a ration. Over-feeding or continued feeding of maturing fish serves to give a fat fish. This can interfere with egg harvesting and confounds fecundity calculations on a per kilo basis.

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pathogen transmission. Further, the pathway from feed storage to the tanks is outside which is an additional biosecurity risk. There are no biosecurity checkpoints or disinfection stations to preserve biosecurity. If feed is to be stored for ongrowing in the rearing side of the building, new feed storage facilities may be considered and relocated to preserve biosecurity.

Mortality disposal/necropsy area

The mortality disposal and necropsy area is adjacent to the fish production area and chemical storage. The mortality/necropsy area is also open to the elements and has a concrete floor with open-wire metal temporary fencing separating it from adjacent activities that include the transfer of feed from the storage area at the juvenile rearing section to the production side of the building. Access to the production area is unlimited from the mortality/necropsy area with only a footbath as a biosecurity measure.

This arrangement of the mortality/necropsy area in proximity to other fish culture areas and activities poses a significant and high biosecurity risk. Mortalities must be contained in a secured enclosure with limited access and a sanitation station at the entryway. Further, necropsy facilities need to be in enclosed, contained areas and fully biosecure. Both facilities need to be away from fish culture activities and access restricted. In no way must traffic be direct between either of these two and areas and fish culture areas. Ideally, both these areas would have steam-cleaning capability backed up with surface disinfection.

Administrative areas

Ideally, administrative areas would not be located between rearing areas and would not be the primary access point. The traffic to and from rearing areas is not controlled in a biosecure fashion. The opportunity to skip biosecurity protocol simply because staff 'are not going into the rearing areas – only to the office' is an opportunity for pathogen transfer. As well, janitorial and maintenance staff need to heed biosecurity protocols. By having direct primary access to the rearing areas from the office, the risk of biosecurity protocol breakdown is increased. It is understood that financial considerations were brought to bear in this decision to have centralized office areas in the current arrangement. Should economic conditions permit, relocation of the administrative areas may be considered. The arrangement at present is not alarming, however diligence is required to preserve biosecurity.

Chesque

Males

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Females

Female fish were sorted for maturation and awaiting stripping in the outdoor tanks. There were male fish in with the female fish and it was relayed that this is a standard practice for female fish holding conditions. The fish appeared in good condition and the handling of the fish was excellent.

Transport

The transport from the Pumalin seawater site to the Chesque hatchery can take up to 24h. some cursory investigations into transport conditions have produced a plan to investigate further the next year should the same set up for freshwater residency be used again. Ideally, the Pumalin fish would be transported to the Comau facility and allowed to undergo final maturation in the recirculation tanks.

A review of transport conditions and procedures has been tabled for later conversation after the spawning season is over. Transport standard operating procedures represent a positive set forward in fish health, welfare, biosecurity and productivity.

Egg take and screening

The process of egg stripping, disease screening and egg transport are of superior quality. The process has been streamlined and is very efficient. The stripping of a fish was accomplished in 45 – 60 seconds per fish, which is excellent in the present conditions. The only consideration for discussion is the disinfection of the steel shipping container floors and walls. It is understood that the current arrangement is a temporary one and the Comau

facility has addressed the issue.

The only concern of the Chesque facility is the location. If a long transport was not involved, the situation would be considered good to ideal.

2) Summary of technological group meetings

Identification of the technological gaps

In meetings with senior and junior staff members, round table discussion of the technological gaps mentioned above were discussed. In the majority of the cases, issues arose due to a change in emphasis for operational strategy. The shift in focus to freshwater brood holding appears to have had a ripple effect on other operations. These effects include both operational and capital adaptations which for the most part, were accommodated.

Technological gaps identified centered around:

- Transportation of adults and gametes
- Gamete storage
- Fertility and fertilization technique
- Water flows and water delivery
- Water quality
- Photoperiod control of spawning
- Feeding strategies and control
- Mortality/necropsy procedures

General approach to address these gaps

The approach to addressing the gaps was discussion and evaluation of capability/capacity within the current infrastructure and planning how modifications to current practices could be adapted. Further discussion lead to the identification of procedural amendments, training, capital improvements and technological adaptation. Each item of concern was evaluated and the appropriate plan of action discussed, urgency/need assigned and put on a timeline for implementation.

Resource availability

AquaGen has considerable resources within the Chile and Norwegian organizations. Support from the Norwegian operations could take place in exchange visits of staff. Key here is the

recognition of cultural approaches to problems and understanding local infrastructure.

For items of technical gaps, Fidelis Aquaculture Management can assist with acquiring technological solutions through networking and research. In addition Fidelis can arrange technology transfer visits with North American operations by AquaGen staff for further demonstration of technological opportunities. In effect, the international aspect of the global salmon aquaculture community can be accessed to help address the issues in front of AquaGen. Through industrial networking, Fidelis is fully capable of directing AquaGen to the most parsimonious route to technology access.

How off season travel benefits the project

Fidelis has clients worldwide. For example: in British Columbia, Canada, Fidelis has clients who also have freshwater broodstock operations and those who practice seawater spawning techniques introduced by Fidelis. If desired, an opportunity to visit operations in British Columbia exists during the Northern hemisphere salmon spawning season. This is off-season to the austral spawning season and presents an opportunity to view technological developments and practices with the notion of brining these ideas back to Chile in time for implementation for the approaching austral spawning season. Similarly, operators on the east Coast of Canada in New Brunswick have freshwater broodstock operations that can be viewed by AquaGen staff. Fidelis can arrange these meetings.

3) Meeting work with top management

Future development needs

Scope of capacity: if Comau cannot supply the need for AG development of all-year egg supply, then how?

Is more training required or the development of more resources?

From a high level standpoint, how can the partners fulfill the needs?

Ability to have individual tanks on individual photoperiods with access to changing water temps.

4) **Training**

Onsite training was delivered to the staff and management and involved:

- 24hr fertility test

A test to determine the percentage of fertilization in a subsample of eggs. The test involves clearing eggs with an acetic acid solution 24h after fertilization. The dissecting microscope is used to identify the blastodisc of the egg in the 4 or 8 cell stages.

- Catheritization of males to obtain pure milt
  Milt expressed manually from a male fish can contain contaminants such as bile, urine, water and fish slime. By using a catheter connected to a 25cc syringe, pure milt can be obtained. The catheter is inserted into the gonopore of the anaesthetized male fish and milt withdrawn with the syringe. The milt can then be transferred to a clean container, overlaid with oxygen and transported.

- Transport methods of milt
  Optimal transport methods of milt involve temperature, oxygen, agitation for mixing and avoidance of light. These conditions were discussed with staff along with the consequences of altering conditions.

- Optimal fertilization conditions: temp, milt dilution, activation, checking motility during the first 30 sec of fertilization
  Theoretical and practical methods of maximizing fertilization methods were discussed and demonstrated. Much discussion was focussed on standardization of protocols.

- Understanding ovulation and maturation of the egg from a physiological and endocrinological viewpoint
  A lecture with question and answer period was given to managers and staff. A general lecture on maturation endocrinology, practical applications and recent up-to-date information was given.

- Application of photoperiod to achieve delayed spawning
  Senior managers were engaged in options to advance/delay spawning times for the project fish. There were three sessions of instruction and planning associated with the topic including hands-on viewing of the fish.

5) SW Spawning training

A seminar introducing seawater spawning was given to staff and management. The seminar detailed the holding conditions necessary for optimal performance of the broodstock, the timelines for the procedures and techniques involved in obtaining high quality milt and ova. The process of seawater spawning involves different techniques for holding and handling the brood compared to freshwater spawning. Most notable is the monitoring of salinity and temperature prior to the spawning period. Staff were advised on the optimal holding conditions in the final growing period with particular attention to temperature profiles. As well, staff were instructed as to the thermal regimen during final maturation which is critical for good egg and milt quality. Of particular concern is ensuring the fish receive the thermal cue to initiate final maturation. Techniques and management tools were discussed to achieve the objectives.

Of further discussion was how to insure that the gamete quality is not compromised during seawater spawning. This is of particular concern because of the salinity of the holding conditions. Physiological aspects of this concern were discussed.

Finally, economic and logistical considerations of seawater spawning techniques were presented and discussed. The implications of transporting eggs versus spawners was of key concern as was the topic of disease transfer and biosecurity. Specific applications to the Pumalin site were discussed. Unfortunately, the previously mentioned storm prevented a site visit to the sea cages. A site visit is planned for the next visit.

It was general consensus that the training session prior to the seawater visit was of benefit. In retrospect staff felt that it was best to have the introductory workshop prior to the Pumalin site visit.

6) New protocol development and training

Spawning strategies for SW

After the site visit to the seawater site, a specific protocol for managing seawater spawning will be developed. There are several facets to seawater spawning that can be addressed through development of Standard Operating Procedures. These include:

- Biosecurity
- Maturity check and sorting

- Spawning: milt and egg extraction
- Gamete storage and transport
- Disinfection of transported ova
- Health check and screening
- Mortality disposal
- Cleaning.

There will also be ongoing monitoring of environmental conditions in the final year of growout. In addition to this, regular feed and feeding evaluation coupled with ultrasound will give information of maturity rate, fecundity, GSI and maturity status. These data are important to egg production estimates for marketing purposes.

Photoperiod control for RAS fish incorporating ultrasound in the process

As stated, the 2011 brood year will be the subject of an investigation to produce year-round egg supply. This will involve additional infrastructure improvements such as tank covers and lighting for photoperiod. As indicated, ultrasound will form a key technical component to selecting fish for immediate or future photoperiod and thermal exposure.

Information on the ultrasound survey of the 2011 brood year population was incomplete at the end of the first project period. While the skeleton of the proposed photoperiod project has been constructed, the final design of the project will nee the information from the ultrasound survey for complete the technical design.

The new and full technical protocol will be developed after the results of the 2011 brood year project is completed. For the present, sufficient discussion and planning has taken place to formulate interim technical protocols.

Training programme description

Further traincing on the key elements discussed above should continue. The essential physiological and technological elements have been discussed and trialed, however further development and training would be of benefit to managers and staff. The next level of training is the evaluation of results and, fine tuning the procedures and further development within the system. It is rare that optimal productivity results from a single, first time trial of a new process. Rather, it is only with familiarity and refinement that new processes and techniques are adapted for use as a routine procedure. Future training and technology

transfer plays a role in ongoing development by additional information, refining the process and assisting in skill development.

Travel off season to BC

As stated above, a site visit and tour of BC production facilities has great benefit to AquaGen staff and managers. The opportunity to meet and talk to other experienced fish culture professionals cannot be underestimated in terms of technology transfer. For example, Marine Harvest Canada has constructed and new multi-million dollar state-of-the-art recirculation hatchery. Touring the new facility will assist with development of future developments at the Comau facility. In addition, AquaGen personnel can witness seawater spawning on the Marine Harvest or other sites. The timing of off-season travel permits adaptation and planning for use in the coming austral spawning season.

Bringing in a milt expert

In discussion with veterinary staff and management at the Comau facility, the issue of lower than average fertility in Chile led to conversations of milt quality. Studies to determine the root cause of the lower fertility rates involves experimentation of milt quality. To do this, experiments of full factorial mating design, manipulations of pre-maturation holding environments and biochemical analysis of males will need to be done. To ensure that the design and implementation of the work is of sound scientific rigour, it will be necessary to involve a team of international experts. While this may seem extravagant, a 10% increase in fertility rates would more than cover the cost of the investment and pay dividends for the years to come.

ANEXO Nº2: SEGUNDO REPORTE DE ACTIVIDADES, JUNIO 2010.

Draft Report for AquaGen Chile

Corfo Project

May 2010

Introduction

The following report is prepared to comply with the stipulations regarding Corfo support of Technology Training and Transfer for maximizing eyed egg production at AquaGen Chile.

7) Field visits

Details of activities and physical description of Comau and Chesque Alto hatcheries

Comau

The Comau facility is structured into three distinct parts: Incubation, Rearing and Administration. Each of the areas is a designated biosecure zone with several sub-zones occurring in the fish culture areas.

Incubation

The incubation room is separate from the stripping room and the fertilization room. Ova and milt traffic is one way and is separated by physical barriers for reasons of biosecurity. However, the incubation room can be accessed by doors leading to service areas and thereby the outdoors with no biosecure entry points. This is also the route to the iodophore and isotonic water reservoirs.

Ova enter from the stripping room and are transferred to cleaned and disinfected plastic colanders. Ovarian fluid is drained from the eggs at this point and the ovarian fluid collected in a trough from later disinfection and disposal. At this point the eggs have not been refrigerated or cooled.

The eggs in colanders are rinsed with 8°C isotonic saline to remove latent ovarian fluid. Once drained, they are transferred to labelled plastic bags suspended in 20L plastic buckets. Up to 16ml of mixed milt is added to the eggs in each bucket and 1L of milt activation fluid is added; the mixture is mixed my hand for 30sec. The fertilized ova are left

to stand for 3min and transferred to the disinfection table where 10L of 100ppm iodophore is added and the mixture sits for 10 min. After disinfection the eggs are rinsed with 8°C incubation water and drained. The eggs are then passed through a half wall to the incubation room and placed in individual incubation jars. Water flow is set to 1.5lpm per jar.

Technical capacity

The process flow of the fertilization procedure capitalizes on an excellent floorplan to preserve biosecurity. Although not fully functional as a brood production facility (construction of the stripping area is incomplete), the technical capacity to expedite the fertilization process and preserve biosecurity is sufficient to maximize the process flow.

One aspect of improvement would be the addition of temperature control. Unfertilized (green) eggs have a 24h 'shelf life' whereby they can tolerate holding temperatures up to 9 or 10°C. In this case, transport from the hatchery is done at ambient temperatures and is prone to fluctuation. The green eggs are shipped in individual bags, overlaid with oxygen and packed into Styrofoam salmon fillet boxes. Ideally the boxes would be packed with reusable ice packs or freezer packs on which bubble wrap or paper separates the eggs in bags. In this way, the eggs remain at a constant low temperature throughout shipping.

The importance of keeping eggs cool centers around enzymatic degradation, bacterial growth and oxygen consumption. During the spawning process blood, urine, bile and other body fluids can be harvested along with the eggs and ovarian fluids. While ovarian fluid serves to keep the eggs in a high state of fertility, the other fluids may contain enzymes and chemicals that can decrease fertilization rate. Cooler temperatures during transport decrease the risk of degradation by lowering enzymatic function.

Bacterial growth in transported eggs can cause a decrease in egg quality and result in discarding eggs after screening results are in. As well, bacteria missed by screening may flourish and contaminate the incubation room if allowed to grow during the time from stripping to disinfection. Keeping temperatures cool during this period decreases the risk of bacterial growth in the ovarian fluid.

Low transport temperatures decrease egg oxygen demand. Although the majority of oxygen use in transported eggs is attributable to bacterial growth, this nonetheless

decreases oxygen availability to the egg. Cool transport temperatures decreases metabolic oxygen demand irrespective of the source of that demand.

For the above reasons, it is suggested that some method of keeping the transported eggs at 4°C be implemented. In addition, the stripping and fertilization rooms need to have temperature control installed to keep temperatures below 8°C and preferably 4-6°C. This temperature range is not a key factor when egg and milt quality is high, but can be a key factor when other conditions are not perfect.

AG has the necessary equipment to check milt quality with the use of a compound microscope. The addition of a 100X dissecting microscope would complement the milt- and egg-quality checking capability. The milt checking area was clean and orderly.

The practice of mixing milt during the fertilization process is not recommended. Sperm competition studies have repeatedly demonstrated that in pooled milt samples, the majority of the fertilization is completed by one male donor; the other males were superfluous to the objective. When viable milt is used in a 2:1 (female to male) ratio, genetic diversity of the progeny is sufficiently diverse and there is no waste of other milt. The commonly held concept is that inferior milt in a 2:1 cross would contribute to losses in egg fertility. Studies have shown that this is not the case and if milt motility is checked before use, fertilization rates will be assured. In reality, if poor, non-viable milt is used, the studies have shown that it is still capable of fertilizing the majority of eggs in some circumstances. From a customer standpoint, the progeny of many thousand eggs could be siblings which from a fish health and growth perspective is a risk.

On the issue of milt from macerated testis, this product is inferior to expressed milt or milt collected with a catheter. However, when milt is in scarce supply the practice may become necessary. The caution here is that proteolytic enzymes are often included with the supernatant and can damage the spermatozoa, especially if the preparation is left unrefrigerated. Spermatocytes, spermatids, inactive spermatozoa and mature spermatozoa are all present in testes homogenates. To compensate for the lack of motility in the preparation, more homogenate must be added to the fertilization container. The risk is that infertile spermatozoa may enter the egg germinal vesicle and activate the egg, causing an infertile egg. However, when milt supplies are low, the use of testicular macerate is a logical procedure.

Milt volume for fertilization is generally recognized as 1ml per litre of eggs. This is the 'Billard' standard of milt to egg ratio. However, polyspermy is not a problem in salmon and excessive milt becomes an issue if the dilution of milt is not sufficient to induce full activation of the sperm.

Technology utilized in the process

AG staff used the industry standards of best practices during fertilization, the above observations notwithstanding. The key equipment is at their disposal to address routine and non-routine procedures including quality control of the milt and eggs. The addition of supplied isotonic and iodophore solutions is above and beyond the normal procedures in most hatcheries.

Skill sets that AG possess to accomplish the goals

AG staff displayed a high level of competency and skill sets related to egg and milt handling, fertilization procedures, egg disinfection, primary incubation and biosecurity. In addition, staff were open to questions regarding their practices and eager to try new techniques. When presented with new approaches and techniques, the staff were adaptable, open-minded and willing to try.

Procedures that capitalize on the resources

The operating procedures for fertilization are on a level with best practices. The ability to move 350 sets of eggs through the fertilization/disinfection room in 6 hours or less speaks to the level of human and capital resources. With a few streamlining improvements to staff movements and small improvements to procedures, the process will become even more fluid.

Water quality

The water supply for the incubation side of the facility is from an adjacent river and is taken from the run of the river as a side-stream channel diversion. The water is diverted to an open channel by a manually set gate. Water then flows over a silt/debris screen and travels to a small settling are and then through a rotary drum filter. Water then flows through piping to the hatchery and through a series of UV lights before entering the incubation room. Water delivery to the incubation jars is via gravity feed from overhead pipes that act as header reservoirs for the lines that feed the jars.

During the time of the site visit and evaluation, a storm came through and rainfall levels were extreme. Particulate matter was bypassing the sedimentation and filtration systems and clogging the ball valves that regulate water flow to the individual jars. It is unknown how long and how many incubation jars were affected, but the outcome of reduced or eliminated water flow to developing embryos is clear. Fortunately, staff were able to remedy the situation with improvised improvements to the incubation jars to restore water flow; an invention that was nothing short of brilliant. The staff are to be commended on their quick and effective actions.

Siltation of the eggs in incubation was evident for over 24h as the river rose. Siltation of eggs critically threatens egg survival in three ways: 1) the silt, primarily the clay portions of the silt 'smother' the eggs by restricting oxygen transfer to the developing embryo, 2) bacteria in the sediment can erode the egg shell and increase chances of bacterial infection from ubiquitous bacteria such as *Flavobacter* spp., and 3) bacterial load in the sediment uses oxygen that further exacerbates the smothering effect of the silt itself. Clearly, water quality is not sufficient for the operation of a safe and biosecure operation.

Part of the problem is that the UV sterilization system is incapacitated by particulate matter. It is apparent that at Comau, surface water supply is a biosecurity and physical risk to egg survival. It is also clear that the design of the water delivery system does not match the need for the facility. It is understood that measures are underway to remediate the situation; certainly technologies exist to address the issue.

A challenge to the facility water supply system will be when the supply of eggs is continuous throughout the year. In the summer, the river water source is drastically reduced, which from a delivery problem is a concern itself, but also poses physical threats through organic matter load and temperature profile in addition to bacterial load in slow-moving, warmer water. The AquaGen plan is to have spawning fish supplied throughout the year and if this is to be, water for incubation will likewise have to be secured throughout the year.

Rearing

The rearing side of the Comau facility is dedicated to the production of freshwater broodstock. The need for full-cycle brood came from the ISA epidemic among seawater-

held fish that sparked a demand for ISA-free eggs. There are two year classes and three lifestages of fish in recirculating tanks: Brood Years 2011 and 2012.

A subsection of the 2011 yearclass fish were exposed to a limited winter photoperiod and put on 24 hour light (LL) to help prevent grilsing (premature maturation), particularly in males. This is common practice in fish held in seawater net cages.

Two practices may not have been fully considered when this was implemented. Firstly, LL light needs to be implemented prior to the onset of a winter photoperiod to prevent any 'winter signal'. This winter signal is important in establishing the maturation cue for the subsequent spawning season. There must be no winter signal for there to be no grilsing. Secondly, fish held in freshwater for their complete lifecycle mature a year early, even if held on LL, although this modifies maturation time (see below).

The consequence of the small winter signal for the population served to be sufficient for about 40% of the existing male population to mature in the second summer, causing a large amount of precocious maturation. These fish were identified by ultrasound (ecography) and removed from the population. The remaining population remained predominantly female and limited the amount of available males for subsequent spawning. Although the winter signal was enough of a cue to induce male maturation, it was not of sufficient duration to induce female maturation and there were few if any female grilse. When the population was returned put on LL, maturation was arrested.

LL light will not permanently arrest maturation. The 'internal clock' of fish dictates and overrides the photoperiod cue and the fish will eventually mature albeit over one-half out of phase with the normal spawning period. It was unclear if the LL treatment was incidental or if second-season spawning was not anticipated from freshwater brood. However, a proportion of the 2011 year class that if held in seawater would spawn in May of 2011, is now in various states of partial maturation across all groups of the year class.

As it turns out, a proportion of each of the held groups will spawn over the next 4-8 months. This was apparent from visual identification and weight sample data conducted during the site visit. From a subsample of fish ultrasound, it became apparent that there were most likely three distinct groups of maturing fish within the population: those 3-4 months; those 5-6 months and those around 8 months from spawning. This is consistent

with the predictions based on published literature: LL will delay maturation time, but will not prevent it.

On the basis of the preliminary ultrasound information and visual evaluation of stock sub samples, a photoperiod and thermal regimen was developed to have the 2011 brood year spawn in four successive groups: September, December, March and May. By sorting for maturity and transferring the fish in each mature group to a photoperiod of 8L:16D and a temperature adjustment to 4°C from the current 9°C final maturation should be induced in the exposed fish. It is recommended that all fish be injected with GnRH implants to synchronize spawning times and capitalize on the limited milt availability. As a cautionary note, egg quality in these fish should not be expected to be comparable with 'naturally spawned' fish. The groups have been repeated handled and will have endured several 'maturational' effects to induce them to mature and spawn. The literature suggests that these treatments adversely affect egg quality. A margin of error may be applied to egg performance.

As a follow-up note, the maturation in the 2012 fish held in freshwater will not favour grilsing. To manipulate spawning date the fish will have to be placed on a photoperiod regime that is conducive to maturation cycles. This will mean the installation of individual photoperiod covers for many of the tanks in the main building. As well, for 'finishing' a group of tanks will need to be designated for final maturation and be kept at 4°C to provide the thermal cue prior to spawning. Infrastructure of the facility to apply photoperiod control will need to be installed.

Considerations

It is understood that the arrangement of fish in the recirculation rearing area was not intended for the current use and that circumstances dictated that the facility adapt to accommodate the ISA crisis. This notwithstanding, there are a few improvements that can be made to ensure production efficiencies.

Water quality

While sodium chloride is an acceptable addition to the water to help prevent and control fungal infections, total water hardness in the Comau system is low. Hardness as calcium carbonate may need to be increased. A recirculation expert may need to be consulted, but hardness levels of up to150mg/l are common in freshwater broodstock sites. This level of

hardness has positive effects on growth, maturation and fish health. Biofilters need elemental calcium as part of their nitrogen conversion process and calcium is essential in growth and maturation of the fish.

The addition of ozone to the fine-solid filtered water prior to the biofilter will increase water clarity. Tannins are a by-product of the recirculation process and while not harmful to the fish, affect water clarity. The addition of an ozone generator at the post-drum filter reservoir should clear up water colour.

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Transport

The transport from the Pumalin seawater site to the Chesque hatchery can take up to 24h. some cursory investigations into transport conditions have produced a plan to investigate further the next year should the same set up for freshwater residency be used again. Ideally, the Pumalin fish would be transported to the Comau facility and allowed to undergo final maturation in the recirculation tanks.

A review of transport conditions and procedures has been tabled for later conversation after the spawning season is over. Transport standard operating procedures represent a positive set forward in fish health, welfare, biosecurity and productivity.

Egg take and screening

The process of egg stripping, disease screening and egg transport are of superior quality. The process has been streamlined and is very efficient. The stripping of a fish was accomplished in 45 – 60 seconds per fish, which is excellent in the present conditions. The only consideration for discussion is the disinfection of the steel shipping container floors and walls. It is understood that the current arrangement is a temporary one and the Comau facility has addressed the issue.

The only concern of the Chesque facility is the location. If a long transport was not involved, the situation would be considered good to ideal.

8) Summary of technological group meetings

Identification of the technological gaps

In meetings with senior and junior staff members, round table discussion of the technological gaps mentioned above were discussed. In the majority of the cases, issues arose due to a change in emphasis for operational strategy. The shift in focus to freshwater brood holding appears to have had a ripple effect on other operations. These effects include both operational and capital adaptations which for the most part, were accommodated.

Technological gaps identified centered around:

- Transportation of adults and gametes
- Gamete storage
- Fertility and fertilization technique
- Water flows and water delivery
- Water quality
- Photoperiod control of spawning
- Feeding strategies and control
- Mortality/necropsy procedures

General approach to address these gaps

The approach to addressing the gaps was discussion and evaluation of capability/capacity within the current infrastructure and planning how modifications to current practices could be adapted. Further discussion lead to the identification of procedural amendments, training, capital improvements and technological adaptation. Each item of concern was evaluated and the appropriate plan of action discussed, urgency/need assigned and put on a timeline for implementation.

Resource availability

AquaGen has considerable resources within the Chile and Norwegian organizations. Support from the Norwegian operations could take place in exchange visits of staff. Key

here is the recognition of cultural approaches to problems and understanding local infrastructure.

For items of technical gaps, Fidelis Aquaculture Management can assist with acquiring technological solutions through networking and research. In addition Fidelis can arrange technology transfer visits with North American operations by AquaGen staff for further demonstration of technological opportunities. In effect, the international aspect of the global salmon aquaculture community can be accessed to help address the issues in front of AquaGen. Through industrial networking, Fidelis is fully capable of directing AquaGen to the most parsimonious route to technology access.

How off season travel benefits the project

Fidelis has clients worldwide. For example: in British Columbia, Canada, Fidelis has clients who also have freshwater broodstock operations and those who practice seawater spawning techniques introduced by Fidelis. If desired, an opportunity to visit operations in British Columbia exists during the Northern hemisphere salmon spawning season. This is off-season to the austral spawning season and presents an opportunity to view technological developments and practices with the notion of brining these ideas back to Chile in time for implementation for the approaching austral spawning season. Similarly, operators on the east Coast of Canada in New Brunswick have freshwater broodstock operations that can be viewed by AquaGen staff. Fidelis can arrange these meetings.

9) Meeting work with top management

Future development needs

Scope of capacity: if Comau cannot supply the need for AG development of all-year egg supply, then how?

Is more training required or the development of more resources?

From a high level standpoint, how can the partners fulfill the needs?

Ability to have individual tanks on individual photoperiods with access to changing water temps.

10) **Training**

Onsite training was delivered to the staff and management and involved:

- 24hr fertility test

  A test to determine the percentage of fertilization in a subsample of eggs. The test involves clearing eggs with an acetic acid solution 24h after fertilization. The dissecting microscope is used to identify the blastodisc of the egg in the 4 or 8 cell stages.

- Catheritization of males to obtain pure milt

  Milt expressed manually from a male fish can contain contaminants such as bile, urine, water and fish slime. By using a catheter connected to a 25cc syringe, pure milt can be obtained. The catheter is inserted into the gonopore of the anaesthetized male fish and milt withdrawn with the syringe. The milt can then be transferred to a clean container, overlaid with oxygen and transported.

- Transport methods of milt

  Optimal transport methods of milt involve temperature, oxygen, agitation for mixing and avoidance of light. These conditions were discussed with staff along with the consequences of altering conditions.

- Optimal fertilization conditions: temp, milt dilution, activation, checking motility during the first 30 sec of fertilization

  Theoretical and practical methods of maximizing fertilization methods were discussed and demonstrated. Much discussion was focussed on standardization of protocols.

- Understanding ovulation and maturation of the egg from a physiological and endocrinological viewpoint

  A lecture with question and answer period was given to managers and staff. A general lecture on maturation endocrinology, practical applications and recent up-to-date information was given.

- Application of photoperiod to achieve delayed spawning

  Senior managers were engaged in options to advance/delay spawning times for the project fish. There were three sessions of instruction and planning associated with the topic including hands-on viewing of the fish.

11) SW Spawning training

A seminar introducing seawater spawning was given to staff and management. The seminar detailed the holding conditions necessary for optimal performance of the broodstock, the timelines for the procedures and techniques involved in obtaining high quality milt and ova. The process of seawater spawning involves different techniques for holding and handling the brood compared to freshwater spawning. Most notable is the monitoring of salinity and temperature prior to the spawning period. Staff were advised on the optimal holding conditions in the final growing period with particular attention to temperature profiles. As well, staff were instructed as to the thermal regimen during final maturation which is critical for good egg and milt quality. Of particular concern is ensuring the fish receive the thermal cue to initiate final maturation. Techniques and management tools were discussed to achieve the objectives.

Of further discussion was how to insure that the gamete quality is not compromised during seawater spawning. This is of particular concern because of the salinity of the holding conditions. Physiological aspects of this concern were discussed.

Finally, economic and logistical considerations of seawater spawning techniques were presented and discussed. The implications of transporting eggs versus spawners was of key concern as was the topic of disease transfer and biosecurity. Specific applications to the Pumalin site were discussed. Unfortunately, the previously mentioned storm prevented a site visit to the sea cages. A site visit is planned for the next visit.

It was general consensus that the training session prior to the seawater visit was of benefit. In retrospect staff felt that it was best to have the introductory workshop prior to the Pumalin site visit.

12) New protocol development and training

Spawning strategies for SW

After the site visit to the seawater site, a specific protocol for managing seawater spawning will be developed. There are several facets to seawater spawning that can be addressed through development of Standard Operating Procedures. These include:

- Biosecurity
- Maturity check and sorting

- Spawning: milt and egg extraction
- Gamete storage and transport
- Disinfection of transported ova
- Health check and screening
- Mortality disposal
- Cleaning.

There will also be ongoing monitoring of environmental conditions in the final year of growout. In addition to this, regular feed and feeding evaluation coupled with ultrasound will give information of maturity rate, fecundity, GSI and maturity status. These data are important to egg production estimates for marketing purposes.

Photoperiod control for RAS fish incorporating ultrasound in the process

As stated, the 2011 brood year will be the subject of an investigation to produce year-round egg supply. This will involve additional infrastructure improvements such as tank covers and lighting for photoperiod. As indicated, ultrasound will form a key technical component to selecting fish for immediate or future photoperiod and thermal exposure.

Information on the ultrasound survey of the 2011 brood year population was incomplete at the end of the first project period. While the skeleton of the proposed photoperiod project has been constructed, the final design of the project will nee the information from the ultrasound survey for complete the technical design.

The new and full technical protocol will be developed after the results of the 2011 brood year project is completed. For the present, sufficient discussion and planning has taken place to formulate interim technical protocols.

Training programme description

Further traincing on the key elements discussed above should continue. The essential physiological and technological elements have been discussed and trialed, however further development and training would be of benefit to managers and staff. The next level of training is the evaluation of results and, fine tuning the procedures and further development within the system. It is rare that optimal productivity results from a single, first time trial of a new process. Rather, it is only with familiarity and refinement that new processes and techniques are adapted for use as a routine procedure. Future training and

technology transfer plays a role in ongoing development by additional information, refining the process and assisting in skill development.

Travel off season to BC

As stated above, a site visit and tour of BC production facilities has great benefit to AquaGen staff and managers. The opportunity to meet and talk to other experienced fish culture professionals cannot be underestimated in terms of technology transfer. For example, Marine Harvest Canada has constructed and new multi-million dollar state-of-the-art recirculation hatchery. Touring the new facility will assist with development of future developments at the Comau facility. In addition, AquaGen personnel can witness seawater spawning on the Marine Harvest or other sites. The timing of off-season travel permits adaptation and planning for use in the coming austral spawning season.

Bringing in a milt expert

In discussion with veterinary staff and management at the Comau facility, the issue of lower than average fertility in Chile led to conversations of milt quality. Studies to determine the root cause of the lower fertility rates involves experimentation of milt quality. To do this, experiments of full factorial mating design, manipulations of pre-maturation holding environments and biochemical analysis of males will need to be done. To ensure that the design and implementation of the work is of sound scientific rigour, it will be necessary to involve a team of international experts. While this may seem extravagant, a 10% increase in fertility rates would more than cover the cost of the investment and pay dividends for the years to come.

ANEXO Nº3: REPORTAJE EN MUNDO ACUÍCOLA, JUNIO 2010.