GILL HEALTH
WORKSHOP
Feb 9th & 10th Campbell River BC

ABSTRACT
By understanding the fundamental structure and function of the gill, culturists and fish health practitioners view a window of individual and herd health. This workshop reviews the structure and function of the gill architecture, physiological operation, changes in the parr-smolt transition and the indicators of optimal animal health.

Sponsors: DFO ACRDP & BC Salmon Farmers Association
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Contents
Introduction........................................................................................................................................1
Workshop Objectives..........................................................................................................................1
Goals..................................................................................................................................................1
Knowledge Gaps and Research Directions .......................................................................................1
Gill Health in Cultured Salmon: Current Knowledge & Future Directions Workshop ......................4
Day 1: Freshwater February 9, 2017 ..................................................................................................4
Campbell River, BC..........................................................................................................................4
Introductions & Welcome - Moderator – Jim Powell (BCCAHS)...........................................................4
The Multifunctional Fish Gill: Plasticity & Compromise - Mike Sackville (UBC)...............................4
  Questions........................................................................................................................................6
Gill health in freshwater populations of wild & cultured salmonids in BC - Simon Jones (DFO) .........7
  Questions........................................................................................................................................9
Gill health: challenges in freshwater - Hamish Rodger (Fish Vet Group) .......................................10
  Questions......................................................................................................................................13
Skretting ARC Gill R&D: development of a functional diet against Amoebic Gill Disease (AGD) - Julia Mullins (Skretting) ........................................................................................................14
  Questions.....................................................................................................................................15
Salmon gill poxvirus (SGPV) characterisation, Atlantic salmon susceptibility & initial survey in farmed & wild salmon - Nellie Gagné (DFO) .........................................................................................16
  Questions.....................................................................................................................................16
SmoltVision – a new welfare indicator - Siri Vike (Pharmaq) .............................................................17
  Mike Ness – Sales rep for PHARMAQ in BC ...............................................................................18
  Questions.....................................................................................................................................19
BC Experience – Jim Powell .............................................................................................................19
Panel Discussion - Moderator & Presenters (Simon, Mike, Hamish, Siri, Nellie, Mike, Julia) ..........20
Gill Health in Cultured Salmon: Current Knowledge & Future Directions Workshop ....................23
Day 2: Saltwater February 10, 2017 ................................................................................................23
Campbell River, BC........................................................................................................................23
Introductions & Welcome - Moderator – Jim Powell (BC CAHS)......................................................23
The Multifunctional Fish Gill: Plasticity & Compromise - SW ion regulation and smoltification - Mike Sackville (UBC) ......................................................................................................................23
  Pink Salmon and Sea Lice...........................................................................................................24
  Questions.....................................................................................................................................25
Gill health in marine populations of wild & cultured salmonids in BC - Simon Jones (DFO) .........26
  Mark Sheppard: Loma ..............................................................................................................28
Gill health: challenges for marine salmonids - Hamish Rodger (Fish Vet Group) .................................................................29
Questions.................................................................................................................................31
SmoltVision – a new welfare indicator – Siri Vike (Pharmaq).................................................................32
Skretting ARC Gill R&D: development of a functional diet against Amoebic Gill Disease (AGD) - Julia Mullins (Skretting) .................................................................................................................................32
Questions.................................................................................................................................32
Neoparamoeba perurans: Global relationships & environmental detection - Jessica Johnson-McKinnon (U of Tasmania) .................................................................................................................................33
Questions.................................................................................................................................33
History & Current Status of Gill Disease in Farmed Atlantic Salmon in the Atlantic Region - Leighanne Hawkins (Cooke Aqua) .................................................................................................................................35
Questions.................................................................................................................................35
Panel Discussion - Moderator & Presenters (Hamish, Mike, Leighanne, Julia, Siri, Jessica): Knowledge Gaps and Research Directions .................................................................................................................................36
Introduction
The gills of fish serve a multiplicity of functions that support the overall health of the organism. They are the active interface between the external and internal environments of the fish. Gills not only serve as barriers to particle movement between environments, they also serve as the main organ indicator of whole animal health that is visible to the fish culturist. By understanding the fundamental structure and function of the gill, culturists and fish health practitioners view a window of individual and herd health. This workshop reviews the structure and function of the gill architecture, physiological operation, changes in the parr-smolt transition and the indicators of optimal animal health. Over both days, the assemblies will also review the local existing and emergent pathogens and effectors now present in BC fresh and salt waters. Local and international experts will discuss the indicators of optimal gill health, illustrate the state of the art for care and prevention of pathogens and harmful agents, identify knowledge gaps and recommend research opportunities for further work.

Workshop Objectives
1. To review the current knowledge of gill structure and function in fresh- and sea-water, observation, detection and prevention of gill disorders in order to mitigate losses to production, and steps to promote optimal health.
2. At the end of the workshop there will be a clear understanding of the future needs for research, identification of the key participants and a pathway forward to investigate and eliminate the knowledge gaps identified.

Research opportunities will be identified and reported.

Goals
The proposed workshop fits within the goals of the ACRDP as described below:

- **Improve the competitiveness and sustainability of the Canadian aquaculture industry:** By learning about gill structure and function, current issues, detection, and treatment options, workshop participants will take away information to help better inform their decisions and guide their practices in their production facilities, thus increasing their competitiveness and sustainability in the industry.

- **Increase collaborative research between the department and industry:** One of the goals of the workshop is to identify knowledge gaps and future directions for research in gill health. With key speakers and participants attending from both DFO and industry, as well as a moderated panel discussion each day, the opportunity to present ideas for new collaborations will exist.
• **Facilitate the process of technology transfer and knowledge mobilization:** The workshop report will document details provided over the two days of technical information and moderated discussions. This report will be shared and publicized with the intent to increase gill health related knowledge within the industry, identify knowledge gaps, and future directions for research.

• **Increase scientific capacity of the Canadian aquaculture industry for essential aquaculture research and development:** With a broader audience of both freshwater and saltwater culturists and fish health practitioners, a greater opportunity will exist for new discussions and ideas to be exchanged. The inclusion of international perspectives on gill health and identification of knowledge gaps and new research opportunities will contribute to fostering new research collaborations, thereby supporting ACRDP’s key goal of increasing the scientific capacity of the Canadian aquaculture industry.

**Objectives:** The ACRDP’s primary objective is to serve to increase the level of collaborative research and development activities between the aquaculture industry and Fisheries and Oceans Canada. The proposed gill health workshop will include moderated panel discussions to help prioritize the needs of industry, which will create opportunities for government researchers to assist industry through collaborations.

**Priorities:** The proposed workshop will include information about disease detection and surveillance, causative agents affecting the gills, and health management practices – therefore it fits with the ACRDP’s objective of Optimal Fish Health. The workshop will also touch on ecological/environmental conditions and impacts on aquaculture under the Environmental Performance objective.

The proposed workshop also fits well with the 2016-17 National Research Priorities for Marine Finfish - Health Management, Management and Control of Pests and Pathogens, and Environmental Impacts - from the environment to aquaculture.
Knowledge Gaps and Research Directions

Panel Participants:

Dr. Hamish Rodger, Fish Vet Group  
Dr. Leighanne Hawking, Cooke Aquaculture  
Siri Vike, Pharmaq Analytical  
Dr. Jullia Mullins, Skretting Norway  
Dr. Nellie Gagne, DFO  
Dr. Simon Jones, DFO  
Jessica Johnson-Mackinnon, U Tasmania  
Mike Sackville, UBC

Moderator: Dr. Jim Powell, BC CAHS  
Recorder: Tina Podlasly, BC CAHS

Freshwater, Feb 9th, 2017

1. Keep track and identify emergent gill health issues, such as pox virus.
2. Define the role that RAS rearing has on gill health in smolts. RAS is a uniform environment to grow a big fish with ATPase and SW tolerance. It will encounter a heterogeneous environment in SW. Are fish up to it?
3. Evaluate the need for a SW conditioning site on land to expose fish to SW conditions before entry. (Stemming from 3 above.)
4. Determine how runts and poor performers emerge.
5. Photoperiod is used to advance smolts. What role does ‘pushing’ fish have on gill development and post smolt pathogen resistance at the gill/environment interface? (Gill integrity/quality.)
6. Evaluate the need for a gill health monitoring programme for industry.

Seawater Feb 10th, 2017

1. Develop a universal scoring method to track gill health. It should fit in with other countries’ methods.
2. Explore the effect of in situ net cleaning on gill health. (Spin off is chain reaction of gill insults post environmental event.)
3. Examine the risk of secondary or piggy-back infections from progressive scores of gill damage. What disease comes first or takes over after gill damage? What is the effect of damaged gills on downstream fish health and welfare?
4. ID local amoebae species, strains.
5. Phyto/zooplankton monitoring for a predictive model in the light of climate change using gill scores as a metric.
Introductions & Welcome - Moderator – Jim Powell (BCCAHS)

1. Recognition of ACRDP
2. Sponsors – Thank you very much
3. Objectives
   a. Action plan for today
4. Gill Health
   a. Health fish
   b. Fundamental aspect of their evolutionary class
   c. More than for gas exchange
   d. Window into fish health
5. Format
   a. Local national and international experts
6. Housekeeping
Proceedings on the website afterwards

The Multifunctional Fish Gill: Plasticity & Compromise - Mike Sackville (UBC)

1. Thank you very much for being here.
2. General overview of gill structure and function
3. Three take aways:
   a. Multifunctional organ
      i. Not just for breathing
      ii. Gas exchange, ammonia excretion, ion osmoregulation & acid base balance
   b. Effectiveness depends on the structure and
   c. Plasticity structural change can enhance regulation
4. Basic gill structure
   a. Blood vessels with specialized epithelia
   b. Blood from heart goes thru the gills
   c. Three main structures
      i. Four Arches (robust, thick, cartilaginous, filaments more delicate, lamellae sheet
         lake capillary beds, single layer epithelial cells thick)
d. Most of the action happens at filaments and lamellae
   i. Blood space, pillar cells, pavement cells 90% SA, ionocytes (< 10% surface)
5. Gills move stuff in and move stuff out (gases and ions between water and blood)
6. Structural changes to enhance gas exchange compromise ion regulation and vice versa
   osmorespiratory compromise
7. Gasses diffuse freely across the gills
   a. Fick equation (Area, Thickness, partial pressure gradient, diffusion Constant)
   b. E.g. 200 g salmon has 6000 cm2 lamellae area
      i. Lamellae area is 5x greater
      ii. Lamellae 5x thinner than filaments an 35x thinner than the skin
      iii. Lamellae 25x more effective for gill capacity
      iv. At exercise 100% perfusion, at rest 60%
   c. Mackerel 100 cm2/g vs Atlantic 30 cm2/g vs toad fish 15 cm2/g; i.e.: the lifestyle is
      reflected in gill surface area; pelagic vs. demersal vs. benthic.
8. Short term morphological plasticity
   a. Scale-less carp as the model for chronic hypoxia
   b. Control vs 12 h, vs 24 h vs recovery
   c. Changes are striking
      i. Lamellar area increased and diffusion distance halved
      ii. Exposing salmonids to different conditions, you will see differences in lamellar
         recruitment
9. Partial pressure – countercurrent flow
   a. Similar to heat pump in your house
   b. Water (100% saturation) and blood (5% saturation) in opposite directions
   c. Complete transfer of oxygen (impressive extraction efficiency) 80%
10. Gas exchange summary
    a. Lamellae are the dominant structure for gas exchange
11. Ion /osmoregulation in FW
    a. FW 1 mOsm, plasma 300 mOsm
    b. Actively excrete water (copious, dilute urine)
    c. Actively take up ions from the water and occurs at the ionocytes (10% of the gill surface
       area (cuboid in shape, many mitochondria, mostly on the filaments, some on lamellae
       in FW)
12. Ion uptake in the FW gill
    a. Two general models/subtypes
    b. Sodium and chloride uptake
    c. Sodium from FW, through the membrane – N⁺-K⁺-ATPase (NKA)
       i. Potassium back into the blood
       ii. Proton H ATPase (active process)
          1. Negatively changes relative to the outside
    d. Chloride uptake
       i. Cl⁻ – NKA and NHE (piggy back on the NKA) exchange one sodium for a proton
1. Accumulation of bicarbonate in the cell

ii. AE1

e. Na/K ATPase is the primary driver of ion regulation in virtually all ionocytes

13. Ionoregulatory plasticity in FW

a. Vancouver (soft water) vs Hamilton (hard water) 10 fold difference in abumdnace

i. Possible solutions

1. Modify existing machinery....


a. Control, 2 weeks in soft water (dramatic counter current increase to maintain ion balance and doubling of fractional gill area occupied by ionocytes, doubling of mean diffusion distance)

b. Diffusive capacity is halved!!

c. 30% increase in ventilation to maintain routine O2 uptake

d. Morphological plasticity to enhance ion regulation.

15. Summary

a. Ionocytes are the dominant site of ion reg

b. Basolateral Na K ATPase is the primary driver for virtually all ion related pathways

c. Ionocyte proliferation to enhance ionoreg compromises the diffusive capacity

d. The gill is a dynamic osmoregulatory organ that mediates exchange between the internal and external environments.

e. Structure.

Questions:

16. How does pH affect the ionocytes?

a. Really acidic water can compromise the gill epithelial integrity

b. Epithelial integrity (calcium is a really important component)

c. Fluctuation of pH can destabilize delicate electrochemical gradients (direction and magnitude) Huge impacts on how fish ion regulate

17. How does it affect the metabolic cost?

a. Measuring metabolic cost is definitely more challenging.

18. What is the pH range of a recirc system? 7.2

a. Vietnam work - Basa (catfish) pH of 5 is when things get rough (below 6), rely more on the kidney to handle ion reg over gill.

19. What is the metabolic cost of ion regulation in relation to growth?

a. Work is being done currently at UBC lab recirc system -

i. Quantify baseline metabolic growth. There are optimal salinities for growth and may also be life stage dependent.

20. Low pH and High pH

a. Interior (Rainbows) high pH, did not look at gill morphology, more concerned with the effects of ammonia - is a tricky compound, gas as NH3, gas phase is more detrimental for fish. High 9s and the 10s increases toxicity of NH3. High pH becomes a problem and may be related to waste extrusion
21. Fish under exercise (cardiac output is up) high O2 exchange, how does the gill regulate to the right point, without leaking sodium, potassium and calcium out?
   a. Pillar cells play an important role. – Potentially endocrine roll.
   b. Have to vaccinate the fish, driving up pCO2 fact remains it is a stressful event. Does the gill recover really quickly (get rid of drug, rebalance ions, O2)?
      i. Impressive function: calcium concentration (hard water - lots of ions in recirc) gills do not have to do much for ion regulation in recirc, flow thru is very soft water and devoid of ions – more metabolic cost.
      ii. How does it recover from high output?
         a. Make it as less stressful as possible, can reduce the amount of excitement (keep blood flow down), will decrease the passive flux. Greater leak, when pushed to the max, a resting fish has a lot less leak in terms of perfusion (gill not being pushed to the max).

22. Optimal pH for maintaining
   a. pH 7.8 (blood) May vary dependent on the species and life stages. General rule of thumb to keep water conditions as close to the natural conditions as possible that is where they want to be.

23. Recirc systems, we provide variation versus the constant steady state is that an issue for them?
   a. Makes sense to me that fish that see more environments will be better prepared to deal with those drops, cannot say for sure until doing experiment.

24. Carp and hypoxia, salmon do we know how quickly that occurs,
   a. There must be something for rainbow trout. There are superstars – physiological capacity, salmonids are not that impressive. Salmonid hypoxia tolerance is not known for that. Morphological change - will take a look and get back to you.

25. Link structure to function
   a. Examples of plasticity and compromise

Gill health in freshwater populations of wild & cultured salmonids in BC - Simon Jones (DFO)
1. Aquatic Animal Health at PBS
   a. Fed regulations, Fish Health Program and Diagnostic Services, Virology, Molecular Diagnostics, Marine Parasitology, Histology, shellfish Health
      i. Marine Parasitology Program
         1. Kudoa, Gill disorders, Probiotics, Sea Lice
2. Salmon Enhancement Program (SEP)
   a. Salmon Production 300 M fish produced /yr
   b. 23 major enhancement hatcheries an spawning channels
   c. 19 contracted community and F hatcheries
   d. Habitat restoration (60+ projects /yr
   e. Community involvement 350 volunteer projects
f. SEP hatcheries and spawning channels produce 15% of BC FN, recreational and commercial harvests

g. 90M contribution to Canada’s economy and over 1500 person years of employment annually

h. Over 10000 volunteers

i.

3. SEP fish Health Program

   a. Annual caseload 200 – 250 annually
      i. Stock loss, broodstock screening, DFO hatchery pre-release pathogen screening
      ii. 20%: ITC / ACC pre-transfer screening, research stocks diagnostic support, wild fish kill investigations

   b. Diagnostic test capabilities
      i. Focus on culture base..

   c. Overview of diagnostics findings (1971 to present)
      i. Health checks or management of salmon
      ii. Pacific salmon (except steelhead) always fell under Prov Gov.
      iii. Typical BKD (mainly chinook, then coho), ichthyobodo (Coho then Chinook), trichodina salmincola, trichophyra, ichthyophthirius, epistylis

4. Case studies

   a. Sockeye spawning channels in BC (6)
      i. Skeena (Fulton 115k, Pinkut 58k)
      ii. Fraser River (Nadina 20k, Weaver 40k, Horsefly 23k, Gates 20k)
      iii. Gill Health concerns include Ichthyophthirius, Parvicapsula
      iv. Pre-spawn mortality (25 – 40%) 2001 to 2013
         1. Possible causation (escapement size, temperature, migration timing
         2. Epidemiological multi-factorial analysis presently underway

   b. Quinsam River Pink Salmon
      i. Genetically distinct odd and even year populations
      ii. Strict 2 year life history
      iii. Fry migrate to ocean immediately following emergence
      iv. Very sensitive to ocean conditions (one shot chance)
      v. Track over time the escapement (variable) in the last 10 years a spike (odd and even years) recent high levels of productivity
      vi. Last year quite low
      vii. Myxosporidian and microsporidian parasites
      viii. Normal and hyperplastic gill lesions (lamellar fusion) looks reminiscent of AGD
      ix. Interested in understanding environmental effects vs biological processes.
         1. Loma salmonae (MGD) 2009 and 2013 and tetra(PKD) parasites in gill tissue
         2. Correlations (fork length vs intensity of the infection – no correlations)
         3. Temperature range (slightly higher in more recent years)
            a. Quinsam vs Campbell River (August to Sept) Beaman et al. 1999
b. Relationship do not support nature vs lab

4. Correlations between infection and environmental parameters Megan (VIU Student)
   a. Unable to find a statistical relationship (temp, pop abundance, fish size)
   b. We need to look harder at pink salmon are exposed to infection upon return to FW assumption and pink salmon are naïve to the infection assumption.
   c. Gill health as an indicator of changing FW habitats (climate change, impoundment effects)
   d. Federal and non-profit organisations – reviewed
   e. Landlocked and anadromous populations (rainbow trout/steelhead, kokanee, cutthroat trout, pacific salmon)

5. BC FW Fisheries Society
   a. Independent, non-profit organization
      i. Fish culture, conservation, restoration, fisheries enhancement, public education, development and marketing of sports fishing in BC
      ii. 6 hatcheries (white sturgeon, eastern brook char, kokanee, cutthroat trout, rainbow trout, steelhead)
      iii. Fish Health Lab
      1. Diagnostic support for hatcheries, BKD, Flavobacterium psychrophilum

6. Conclusion and opportunities

Questions
   a. Do you know of any mortality in FW?
      i. Mortality elevated in years where high abundance of fish, high temps, low DOs, Loma may have contributed to respiratory concerns with the low DOs. Always many parameters.
   b. How about migrating fry?
      i. We have looked at migrating salmon (Strait of Georgia) Evidence of kidney infection – not sure if those pinks came from Quinsam
      ii. Can effect correlations on return if they do develop an immunity response. No evidence of Loma. (Pinks very small when they leave that system).
Gill health: challenges in freshwater - Hamish Rodger (Fish Vet Group)

1. Gill pathology terms
   a. Necrosis  cell is dying or has died (pyknosis, karyorrhexis, karyolysis)
      i. Focal (localized) (zooplankton, mineralisation)
      ii. Diffuse (harmful algae, toxins, parasites)
      iii. Irreversible for cell
   b. Apoptosis – programmed for cell death
   c. Gill reaction to injury – cellular swelling
      i. Cloudy swelling, hypertrophy, reversible
         1. Maladaptation to SW
         2. Osmoregulatory failure
         3. Exposure to irritants
   d. Gills very adaptive and plastic – can respond to changes
      i. Irregular surface
      ii. Apposition of lamellae
      iii. Hyperplasia
   e. Inflammation (like bronchitis to us)
      i. Acute (toxins, zooplankton, treatments) or chronic (fungal, persistent
         parasitism/irritation) produces mucus or chloride cell hyperplasia: PGD/I, EGCs
         increase
      ii. Vasodilation
   f. Proliferative cell reaction
      i. Due to bacteria or parasites, low virulence but high resistance
      ii. Heavily infiltrated with inflammatory cells
      iii. Granulomata (mature)
   g. Repair and recovery
      i. Replacement of dead or damaged cells occurs
      ii. Surface cells exfoliate
      iii. Clots (macrophages will digest)
      iv. Oedema within 2 - 3 days
      v. Gill disease is dynamic

2. Infectious gill disease
   a. Viruses
      i. SGPV  Salmon gill pox virus
         1. FW and SW environments
         2. Significant mortalities
         3. Distinct Histology and IHC
         4. Q PCR to confirm
         5. Minimise impact by cessation of feeding, increase in O2 and avoiding
            stress
7. NVI (Norwegian Veterinary Institute) confirmed positives by histology and PCR at 18 sites in 2015
8. 9/23 survey respondents ranked poxvirus 1st or 2nd most important disease in FW
9. Case from Scotland (pale patch on the gills)
10. Gjessing et al. 2015
   a. Virus widespread before mortality
   b. Ct trend increasing over disease progression
   c. Damages the gill surface and may allow other pathogens to enter
   d. Affects chloride and epithelial cells (smoltification issues)
   e. Can progress rapidly and result in high mortality during fry stage.
   f. SW sites, carrier fish may spread to naïve fish.
   g. May be coming in with the eggs – need to look at more thoroughly.
   h. What pathogen effects the fish first?

b. Bacteria
   i. Flavobacteria/Flexibacter sp – bacterial gill disease
      1. White patches in the gills
      2. Disease can be acute to chronic in impact
      3. Site specific
      4. Often flows flood/spate with high suspended solids/turbidity
      5. Chloramine T baths
      6. Oral antibiotics
      7. Improve/control environ conditions
      8. Rare in RAS systems
   ii. Candidatus brachchiomonas cysticola
      1. Epitheliocystic agent
      2. Distinct from chlamydia
      3. Not sure how it is transmitted
      4. Not cultured yet
      5. Hatcheries potentially can control with control of inflow (ozone and UV)
   iii. Candidatus Clavochlamydia salmonicola

   Paradox
   i. Often mixed infection with fungus
   ii. Costia
   iii. Ichthophthirius (white spot) can flare up dramatically within a week (same week annually)
   iv. FW gill amoebae
1. Nodular gill disease in rainbow trout
2. 9 species discovered
3. Treated with formalin
4. Biosecurity is so crucial in RAS systems

v. Desmozoon lepeophtherii (aka paranucleospora theridion)
   1. Hyperparasite of L. salmonis
   2. 1 – 10% of sea lice in some sites infested
   3. Affects egg development
   4. Confirmed in Atlantic salmon (pale gills, enlarged kidneys)
   5. Microsporidian parasite
   6. Infects white blood cells and endothelial, gill and skin cells
   7. Histology
   8. Not cultured
      b. High levels of the parasite correlation with gill score

   d. Fungi
      i. Saprolegnia
         1. Skin, gills and internal organs
         2. Biosecurity and hygiene
         3. Evidence that some strains/species primary pathogens
         4. Bronopol (& formalin)
         5. Salt
         6. Boric acid (LI ET AL. 2014)

3. Non-infectious gill disease
   a. Water quality (pH, TOC, metals (Al, Fe, Ag), high suspended solids, harmful algae, carbon dioxide, pollution)
   b. FW muscle larvae (Margaritifera margaritifera)
      i. Cause drastic proliferation of the gill tissue
      ii. Eliminated at some hatcheries with UV at inflow
   c. RAS
      i. Control of carbon dioxide is critical
      ii. Increased observation of mineralisation in kidneys gills and pseudobranch
      iii. pH, heavy metals, Nitrites, NH3
         1. low pH and aluminum
         iv. Be aware that fish may cope in FW but will struggle in SW
   d. Gill epithelial cell hypertrophy
      i. Volcanic ash in Chile was an issue
   e. Trauma
      i. Will repair but chronic effects may remain
      ii. Grading, pumping, transport, vaccination, etc.
      iii. Multiple micro haemorrhages
   f. Deformities with impacted gills
i. Opercular shortening/erosion
ii. Mandibular deformities
iii. Fish is unable to breathe properly (pump water over gills properly)
   1. Struggle when stressed
g. Seasonality of gill issues in Scotland
   i. Peak in mid to late summer,
   ii. Waterborne insult (harmful algae)
h. How to monitor gill health?
   i. Water quality
   ii. Physical exams
   iii. Routine fresh microscopy on site (weekly)
   iv. PCR screen for pathogens of concern
      1. May have problems when go to sea
   v. Histology of gills (monthly)

4. Gaps at the present time
   a. Epidemiology
   b. How to use molecular microbiology to apply effective control
   c. Isolation of some pathogens required (to establish significance, challenge models etc.)
   d. Pathogenesis – who’s on first?
      i. Multiple pathogens which may facilitate others to come in faster.
      ii. Differential diagnosis/case definitions
      iii. Show to treat or control
      iv. Transmission?
      v. Reservoirs?
e. Gill Health initiative (GHI) Annually Apr 27 – 28, 2017 mark.powell@nivano (Bergen Norway)

5. Fish Vet group – UK (global presence) started in 1995 as a vet practice in Scotland
   a. Now a part of Benchmark holdings
   b. Portland Maine
      i. Vet biosecurity, PCR, bacteriology histology, autogenous vaccine service, gill health training

6. FW gill health
   b. Scotland 73% of FVG clinical cases) FW histology cases featured gill pathology
   c. 27% of FW cases

Questions

d. Mineralization (how about low pH)?
   i. Not associated with low pH, but with High CO2.
e. No connection with water levels?
   i. Not established
f. Particular stain for mineral
   i. Varacosta – minerals are black
g. Can we say pox virus is a primary pathogen responsible for mortality?
   i. In FW environment, several cases where pox is the only pathogen observed. We do have cases where there are multiple pathogens. Histology is distinctive.

h. Delicate nature of the gill, can you comment on the need for optimal sampling processes?
   i. Methods used for killing the fish and taking the samples, can have a big impact on the results. Delay of more than 5 min, killing to fixative – will get artifacts.
   ii. Sampling dead fish? Widespread post mort change in histology
   iii. Proper sampling is very important for getting good results

i. Loma in Atlantics in Europe?
   i. Have not seen it in Atlantics. Rarely see it. Interested in Loma as it is a good model for desomoozen (Significant pathogen)

j. Did you see the same problem with soft water?
   i. I don’t think we have associated it. Could be investigated.

k. Mandibular deformities - Perhaps it is associated with nutrition
   i. Low phosphorus diet could be a factor, may not be available
   ii. Genetics could be a factor
   iii. Phosphorus deficiency

l. Deformities
   i. Observations include opercula (under 1/3 missing - could be grown back in FW in 1 – 2 months) else permanent deformity.

m. Could be a detriment for fish going to SW?
   i. Yes. – those fish could struggle with treatments

n. Pox outbreak in a recirc system – how to get rid of it
   i. Not aware of it in a recirc system, in flow thru, get it (clinical disease) for a period of weeks (annually) repeatedly at risk

o. Any example of pathogens that affect ATPase activity in the gills?
   i. Bath and formalin treatments could affect - I do not have any data. – Siri to cover in her presentation

Skretting ARC Gill R&D: development of a functional diet against Amoebic Gill Disease (AGD) - Julia Mullins (Skretting)
1. Skretting (owned by Nutreco) Netherlands – Global
2. Experts in nutrition Health and Feed technology
3. Haemorrhagic smolt syndrome (Scotland and Norway mid 90s)
   a. Mostly in Spring and also in the fall
   b. Predictable annual outbreaks
   c. Non infectious
   d. Anaemia pale gills
   e. Widespread petechiation
   f. Ionic Feed – affects ion balance in the fish
i. Reduces HSS mortality (reduced by 29 – 44%), prevents HSS outbreak

g. Effects of feeding 2 different health regimes
i. HSMI (late 90s in Norway) – pale liver and heart, ascites and swollen spleen
ii. Epi, myocardial and red skeletal muscle inflammation and degeneration
iii. Causative agents include PRV
iv. HSMI trial design to assess the effect of different feeding regimes upon the development of HSMI pathology and blood chemistry parameters
   1. 50 salmon (80g each) per tank – 9 tanks
   2. Feed – Control, and two experimental diets
   3. 12 weeks feed
   4. PRV analysis – none positive for PRV before the trial
   5. 4 wpc, 6 w pc, 8 wpc (all three groups have the peak in inflammation, higher in control group), 10 wpc, 12wpc
   6. Liver enzymes – ALT and AST (better indicator progress of disease)
   7. Histology scoring – heart (epicardium and myocardium) 0 = normal appearance – 4 diffuse infiltration of inflammatory cells.
      a. First 6 weeks not much happens, higher scores start as of 8 weeks post challenge
   8. Histology scoring – muscle
      a. First 6 weeks not much happens, week 8 more severe muscle damage.
   9. Total score = combo of the 2; similar results
10. Conclusions both experimental diet feeding regimes reduce the peak and severity of inflammation at 8 wpc
11. Myogadial lesions were more pronounced that either epicardial or skeletal muscle lesions

h. IPN trial
i. Mortalities in day 8 and all tanks in day 11
ii. Experimental feed reduces mortality
iii. Survival rate 1.4x likely to be alive on day 25
iv. These diets are not available in Canada

i. Summary
i. Ionic feed prevents HSS outbreaks and reduces mortality associated with HSS

Questions
j. Mortality – does the ionic feed reduce the growth?
   i. Julia does not have the answer and can find out and get back
k. Blood to start the challenge, did it come from fish that were HSMI positive?
   i. Yes
l. Function of the ionic feed – the fact that it works
   i. Fragility of the red blood cells or vessels best guess; not aware of anyone looking at the different mechanisms.
   ii. Hamish - We did wonder of vitamin K – but no evidence.
Salmon gill poxvirus (SGPV) characterisation, Atlantic salmon susceptibility & initial survey in farmed & wild salmon - Nellie Gagné (DFO)

1. Proliferative gill inflammation: cell death, epithelial hyperplasia and circulatory disturbances
   a. Known to farmed Atlantic salmon in Norway since mid-80s.
   b. DNA large virus 241564 bp, FW and SW stages of the fish and targets the gills
   c. Apoptosis (strong indicator) in acute infection
   d. Fish are lethargic, show respiratory distress, and mortality
   e. Q PCR low Ct values appear associated with gill apoptosis
   f. Smoltification may increase severity
   g. A diagnostic challenge - hard to culture

2. Other poxviruses

3. Initial detection in New Brunswick SGPV
   a. Routine screening 2015, adult salmon enhancement hatchery
   b. Unknown virus isolated on CHSE cells
   c. Isolation and thigh throughput sequencing
   d. Mapping against Norwegian SGPV (80-90% similar)
   e. RT-qPCR developed against early transcription factor large subunit, matching the East coast SGPV
   f. Primary survey on the east coast – 2 year (NB and PEI)
      i. Broodstock, hatcheries, farms, enhancement hatcheries
      ii. Land hatcheries/Hatcheries suspected positives – CTs are high. Low level of infection.
      iii. Farmed salmon sea cages – lower suspected positives

4. In Vivo Challenges at St. Andrews Biological Station
   a. SW bath challenge and FW trials (Bath and IP challenges)
   b. Does not seem to be very infectious. Really difficult to study
   c. We would like to understand more about this virus.
   d. Or vial culture, is not a great virus to culture.

5. Conclusion
   a. Cultured SGPV on CHSE cells
   b. Not managed to transmit the virus in a challenge, the viral culture titer is low

Questions:

   c. Is a DNA virus?
      i. Yes
   d. Have you looked at other organs and tissues besides gills?
      i. Yes, very, very low anywhere else.
   e. Have you considered the wild and farmed fish compared to the Norwegian one?
      i. Sequences just back. Would like to do, in progress.
   f. Does the virus grow on any other cell lines besides CHSE?
      i. I don’t think so
g. How quickly did the Ct come up?
   i. Ct value is slow. Passaged several times and very low titer.
   ii. Would love someone to import our virus and try it.
   iii. Cell culture – living and growing – passing to the next set of wells, does not increase in number – not super growing fast or adapting or catching.
      1. Is it because the concentration is low? Ct values are high.
      2. Threshold value that Q PCR can pick up (High Ct value = low amount) is inverse.

h. Have you thought about doing some multiple pathogen challenges?
   i. Likely next one will be naturally infected fish. (challenge to be ongoing)
   ii. It looks infectious enough that we can detect...
   i. Have you done any histopathology on the positive samples?
      i. No, because there were not positives to test. Not for surveillance (only microbiology and cell culture in routine health screens).
      ii. We are lucky that we do not seem to have a problem with the virus. This work was conducted as it was found by accident.

SmoltVision – a new welfare indicator - Siri Vike (Pharmaq)
1. Norway always looking for better tools for fish health
2. PHARMAQ value chain driven by R&D
   a. Challenge – identify
4. Norway – more RAS and larger smolts
   a. Due to Sea Lice challenges
   b. Farmers want as short a time as possible in the sea
   a. Modified McCormick method; Golden standard and preferred method for results
   b. Does not tell the whole story.
6. Indicators used today
   a. SW challenge test (accurate)
   b. ATPase method (Enzyme)
   c. Gene Expression (measure on what will happen in the future) 5 – 10 days
7. Enzyme activity in the gills of wild salmon parr/fingerling (Green), smoltification Combo red and Green), smolt (red)
8. SmoltVision real-time RT-PCR analysis
   a. Three genetic markers: FW ATPase, SW ATPase and cofactor (housekeeping gene of the ionocyte)
   b. Increase in SW marker and cofactor to SW marker, Decrease in FW marker
   c. Can pick up the variation with SmoltVision.
   d. ATPase activity confirms SmoltVision results (17.9 grams)
9. Norway - +150 FW sites
   a. Baseline data is important.
10. Field data from 2016
   a. Relatively similar gene expression at different spots
   b. Variation in sampling (random selection) (sorted low) - hence recommend at least 20 fish sampled.
   c. Starving the fish (from salt feed) can interrupt results.
   d. Treatments for Costia can interrupt results.
11. SmoltVision is a new welfare indicator – can understand more.

Mike Ness – Sales rep for PHARMAQ in BC
1. Previous at Grieg Fish Health
2. Comparison trail outcome
   a. 28 day smoltification assessment (Cermaq)
   b. Three samplings 28, 14 and 3 days pre-transfer at 2 hatcheries
   c. Mid Dec to Mid Jan (results as of last week)
   d. Vaccinated in early Sept
   e. Winter photo period Lights on Dec 5th at both hatcheries
   f. Temperature (cold snap) affected one hatchery (2.5 degrees difference)
   g. Mortality in both groups low
3. Assessments and methodology
   a. Smolt index score – parr marks, silver coloration fin lines
   b. Blood chlorides
   c. ATPase
   d. SmoltVision
   e. 15 for analysis
   f. Randomly netted and bucketed from pop (5 at a time)
   g. No TMSATOAse and SmoltVision on small fish
4. Results – Hatchery 1
   a. ATPase
      i. Nice progression on the index – both labs say most of the fish are ready by second sampling, but fully ready by third sampling
   b. Chlorides
      i. May be overestimating readiness
   c. SmoltVision
      i. Good pattern over full time period indicating full ... through smoltification
      ii. Consistent message amount tests.
5. Results – Hatchery 2
   a. Mixed message for hatchery results with colder water.
      i. This is concerning. SmoltVision is telling us to wait.
6. SmoltVision and ATPase
   a. Natural Smolt Progression
7. Comparison –SmoltVision (patterns, not absolute values are important)
a. Sampling Point 2 at hatchery 1 is very similar to Point 3 at hatchery 2.
b. Smolt Index correlation

8. Conclusion
a. Assurance of progression in the smoltification process
b. Help ID when problems arise such as stalling of smoltification (cold water)
c. Chlorides
   i. Values seem to vary by facility/fish group
   ii. Not well correlated to smolt index
   iii. Tendency to overestimate readiness
d. McCormick ATPase
   i. Gold standard
e. Follow up
   i. 30 day non-smolt mortality from each group at sea site
   ii. Follow up on performance and behaviours thru production cycles
   iii. Look at other factors at sea that smoltification can be thought to affect

Questions
f. Armin
   i. Hatchery 1 had longer winter photoperiod (traditionally had problems with
      smoltification)
      1. Both groups started with the same values. Hatchery that displayed the
         normal progression (#1) did a lot better despite the traditionally
         worsening conditions of the other one. If extra 4 weeks made a
         difference? Not sure.

h. What do you use for Cofactor?
   i. Is a trade secret. Ionocytes (Chloride cells) there are some only producing SW.
      strong signal that this is going the right direction.

i. Have you thought about looking at the intestine as an indicator of smoltification?
   i. Not at this point but this is a very interesting area. We think we could
      distinguish between environmental impacts. We are in the beginning of learning
      with the intestine. Also started to work with Trout.

BC Experience – Jim Powell
1. FW Gill Health Issues – BC
2. Gyrodactylus – Salmon fluke
   a. Small monogenean ectoparasite (.5mm long)
   b. Attaches to skin and gills
c. Spreads quickly and is hard to manage.
  d. Treatment is salt or formalin

3. Costia ichthyobodo
   a. Life cycle is 24 h, encysts below 8 degrees C
   b. Surface water or ground water
   c. Signs are flashing/coughing/mucus
   d. Salt to 0.3%
   e. Formalin is the go to

4. BGD Flavobacterium psychrophylllum
   a. Enviro disease NH3
   b. Common in plug/flow raceways
   c. Hyperplasia, clubbing, necrosis
   d. Responds well to treatment salt, formalin, Aquaflor
   e. Re-infection common

5. Saprolegnia
   a. Gill mycosis
   b. Common on eggs and skin
   c. Wet mount to confirm
   d. Signs are cotton flashing coughing

Treatment is the same for all salt, formalin

Panel Discussion - Moderator & Presenters (Simon, Mike, Hamish, Siri, Nellie, Mike, Julia)

1. Chronic gill inflammation (low O2, anaerobic metabolism etc.) what do you do about that?
   Specifically in a net pen mitigation.
   a. Hamish - We have looked at ways to mitigate the effect of chronic Gill disease (repeated infections AGD). Becomes complicated. We have looked experimentally at non-steroidal anti-inflammatories in feed during pre-acute infection. – did not see any benefit. Looked at other compounds. At the moment, nothing available yet to help.
   b. What about corticosteroids? In other animals it is used, we have not tried it yet. Used to use as a stress test (old days). We have not tried aspirin. Hearsay that it did help the fish (tank with BGD).

2. What gills in trial?
   a. Cannot remember, but can get the info for you.

3. What is the pathophysiology of HSS? Diet?
   a. We don’t know what is causing it. Salt makes it go away.

4. Physiological Question regarding Mucus – goblet cells in the gill – specifically how increased mucus production in the gill will affect the physiology.
   a. Mike – does not know much about mucus at all. Immune response.
   b. Skretting is developing a diet against AGD. Mucus is one of those things that we looked at in more detail. Lysesime (antibacterial enzyme)
   c. Mucus is the first protective layer of the gills. Is it better to have thicker or thinner
mucus? There are lots of things that can affect the mucus in a beneficial way. (enzymes) Choosing what is good in the feed - ran several trials that resulted in thicker mucus on the fish.

d. If you have gills that are already compromised, and enviro with lower DO, if you can get those on board before stimulation occurs.

e. We did some quantitative histology image analysis, we can count the number of mucus cells and look at the depth of the mucus cells within the skin. With AGD count of mucus cells went up. Mucus hyperplasia. Area increase mid-infection. More cells but smaller at the end of the infection. Perimeter, respiration and osmoregulation. As the disease progressive, the perimeter area went down as well. Would like to look at in more detail.

f. Simon - Another angle, can also be applied to skin as well in our sea lice studies. Can see clear qualitative differences. Acidic neutral and basic predictable kinetics also occurs in the skin as well. Real value in a better understanding of biochemistry. Needs to be better worked out.

g. Simon - The mucus supports a very active and diverse microbial community. (We have looked at the gut) Relative proportion depending on the kind of feed that the fish were exposed to. We have not looked at whether the microbial community extends to the skin, gill and gut. And whether feed or manipulations affects the levels. (e.g.: does peroxide treatment make them more susceptible to something else)?

5. What stimulates the goblet cells and skin (parasites)? Acute form secretory (physical and or chemical) what do we know about those mechanisms right now?
   a. Can be mechanical or chemical (irritant?)
   b. Unutilized potential there. Rarely ever tried to use non-steroidal anti-inflammatory drugs to try to minimize the incubation...
   c. Land animals – reduces the consistency of the mucus.
   d. In terms of goblet cells stimulation – secretory (physical and or chemical) what do we know about those mechanisms right now?
      i. No Answer

6. Use of boric acid to treat Saprolegnia, treatment success, cost and safety of use in relation to formalin
   a. Hamish does not have any specific info on farming experience of boric acid being used, it was perceived as safe in comparison to formalin. Hamish can send the paper (dosages that were effective for eggs and fry.) 08 grams/litre?

7. In the SmoltVision test, what is the problem with using formalin?
   a. Siri - Cannot say in detail. It did have some effect on the gills and the smoltification process, not sure what was damaging the gills. Costia inconclusive. Any fungus needs to be looked into more.
   b. Understanding more about formalin use and its effects is something that needs to be looked into more.

8. Who’s first concept? Any work on the prevalence of coinfections? Gill Issues?
   a. We have done some work from Norway but there are different pathogens there compared to here. Siri recommends to start with what is prevalent and then follow up a
group testing all the pathogens over a longer period. It is possible to do.

b. Hamish - Really important to do longitudinal studies (sequential). If you do one spot sample, you would not know what that means.

c. (Checkerboard?) if you infect with this, you get this... better understanding of multipathogenic response.

d. We know that amoeba by itself is a primary pathogen that can cause disease by itself. Something may allow the fish to get infected with that. Exposing the fish

e. Simon - Harmful algae blooms will play a role in the toxicity. A matrix would benefit from context of environmental parameters that are going on at the same time?

9. Do you have any recommendations to keep an eye out in the future for any new drugs or technology, etc. that may be coming?

a. Nellie - Combo of treatments – In FW, a move towards recirc aquaculture is interesting as there are new conditions appearing as a result of new farming methods. Water quality and recirculants. When fish go to sea can be a challenge. Hopefully less infectious disease.

b. Keep an eye out for more challenges.

c. Stack environment where we are controlling all of the water quality conditions, PreSmolt Centre? To condition the gill prior to going in SW.

   i. Hatcheries with hyperoxygenization – smolts are more prone to IPN and virus infection in the sea. Is a risk factor.

d. Not looking at new drugs.

10. Siri - What about the possibility of SmoltVision to the predisposition of the smolt and the various diseases that they can get early on?

   a. Very interesting. We can do that for other species, but have not done for fish yet.

   b. Recurring theme – smolt quality and Tenacibaculum

   c. In vitro test. Genetic selection – Is there genetic markers that will make them more robust? Then could measure efficacy of the vaccine in the field as well.

11. Is there a special Diet for Saprolegnia susceptibility?

   a. No one is working on it but would be interesting to do. Comes down to what does industry need and to come up with a Top 10 list of conditions and the knowledge gaps.

12. Any future questions, please discuss with BC CAHS (via website or via BC CAHS staff)

_Notes recorded by Tina Podlasly, BC CAHS_
The Multifunctional Fish Gill: Plasticity & Compromise - SW ion regulation and smoltification - Mike Sackville (UBC)

1. Yesterday – gas exchange
   a. Gill is a multi-functional organ
      i. Ammonia excretion
      ii. Ion/osmoregulation
      iii. Acid-base balance
   b. Enviro challenges low O2
      i. Elevated CO2, ammonia, salinity, temp
   c. Lamellae are the dominant structure for gas exchange
   d. Morphological plasticity can alter diffusive capacity to meet fish needs on different timescales (acute, chronic, evolution)
   e. Ionocytes are the dominant site of ion regulation (mostly on filaments, but often on the lamellae in FW)
   f. Enhancement of gas exchange can incur an ionoregulatory burden by increasing passive ion flux
   g. Ion/osmoregulation in FW gill is permeable to water and ions
      i. Passive H2O gain, passive ion loss, active ion gain, active H2O lost

2. Ion regulation in SW
   a. Situation is completely reversed from FW
   b. Passive H2O loss, passive ion gain, active H2O uptake, (SW has ions, need to excrete them).
   c. Only one general cell type (SW gill)
      i. Na/K ATPase (NKA) – takes 3 Na and 2 K and combines them. Puts K back in the cell.
      ii. NKCC (enzyme) – take 1 Na, 1 K and 2 Cl from blood and moves them into the cell, buildup of Cl over time.
      iii. CFTR (channel) – removes the Cl from the cell into the SW
      iv. Boundary layer becomes negatively charges – NA from blood gets transferred to SW
      v. Apical crypt – Cl pumped out of the cell, can create the favourable electrical gradient
         1. In FW have microvillae (Katoh et al. 2003)
vi. Basolateral NKA is the primary driver of ion regulation.

3. Smoltification
   a. Preparation/transition from FW to SW
      i. Silvering, downstream migration, schooling change in buoyancy, ion/osmoregulation (FW – SW)
   b. FW to SW ionocyte transition
      1. FW - Smaller cells on filaments and lamellae
      2. SW-
   c. NKA activity increases in prep of SW entry (anadromous – Atlantic salmon)
   d. Increased NKA activity correlates with SW tolerance
   e. What is happening with the ionocytes during transition from FW to SW?
      i. (Richards et al., 2003) – looking at gene expression able to see a distinction
         1. These changes in gene expression were happening in every salmonid that was examined.
         2. Steve McCormick was able to make antibodies to see (SW cells developing on their own)
      ii. Proposed model for ionocyte transition (recently published)
         1. NKA may exchange 2K and H1 or 3Na.
   f. Do we see a reverse smoltification (return to FW)
      i. Pink salmon (fixed 2 year life cycle)
      ii. Measured isoform expression
      iii. Sept Oct = spawning
      iv. FW 0 – 25, SW no significant change
      v. Many queues to tell the fish to go back up the river. This may be one of the reasons why adult salmonids do not get back to the spawning grounds.

4. Gill plasticity during smoltification

Pink Salmon and Sea Lice
   a. Issue (2007) - Fish farms may be exposing pinks to unnatural levels of sea lice.
   b. We wanted to determine safe and dangerous levels of lice load using ion levels as an indicator of effect.
   c. Fish could actively combat these disturbances.
   d. Sea lice – ectoparasites
      i. By poking holes in the fish could make them leakier
      ii. May increase passive hydro mineral disturbances
      iii. Predictions,
         1. Increasing infection load will increase compensation
         2. Beyond compensatory limits, ionic disruption induced.
   e. Pink salmon may be extra vulnerable – go to sea early
      i. Two - fold ionic disturbance after normal SW entry (2 months)
      ii. They may not be fully ready for SW, but they go anyway.
   f. Experiment Outline
      i. Examine lab infected and wild infected fish
      ii. Smallest, most vulnerable fish
      iii. Ecologically relevant infection
      iv. Measure Na, gill NKA activity and swim performance
   g. Broughton – MHC helped us out
i. Stayed at a farm site, mobile lab
h. River caught fish (0.2g), 1 week in SW lab, copepods infection, sort and hold
i. Also another study at the same time, studying salmon speed.
j. Lab infection – what happened with body sodium?
k. Gill NKA – rise over time (post infection)
i. By 15 days, there is an increase in NKA
l. Wild infected fish
i. Ocean caught (.5 to 3 g)
ii. Larger fish, louse infection was not very high. Sodium goes back down. 1 louse/fish)
iii. No difference from control fish
iv. At what body mass does this difference occur?
   1. By 0.5 gram we do not see an impact on blood sodium.
   2. Louse stage - no effect in fish larger than 0.7g.
m. UBC – Research – passionate and love to talk about it
i. There is a lot of diverse research there.
ii. River temp on migration (spawning adults and out-migrating juveniles)
iii. Downstream effects of hydro-electric dams (the bends) (super saturation of gasses what level becomes a problem for fish?)
iv. Wrasse – Optimal salinity for growth in closed containment aquaculture systems
v. High pH tolerance in Rainbows (published work Thomson et al.)

Questions

n. Why would there be 2 different isoforms of ATPase?
i. Don’t know – would be an exciting find. Could be changing the way that the sodium pump is believed to work. Perhaps they are doing different things. (thermodynamics?) consumes 30-60% very important enzyme. All animal life.
o. Has anyone looked at (landlocked e.g Kokanee, Atlantic salmon)?
i. Yes, Mike is aware that someone is working on it. Difficult on Salmonids because of the time frame.
ii. Species wide sequencing on landlocked fish in the interior.
p. Evolutionary perspective
i. Ice age, and duplication of the genome in salmonids are two theories; one of those isoforms could have changed and became adaptive to SW.
q. The filaments and the crypts are they formed in FW or during smoltification?
i. Don’t know. Good question. The filaments and the crypts, when they switch back – what happens?
ii. If you inject with growth hormone they become SW tolerant.
r. What are your concerns from a physiological perspective? What is a runt?
i. The non-smolt. Don’t know. Gill ATPase, gut function may not be ready for SW, Timing may be off, Kidney? Osmoregulatory standpoint – gill and gut are the major drivers.
s. Desmoltification in FW
i. Assume that the species has a lot of variability in when they go back to FW.
t. In the natural environment, smolting happens when they cross the threshold of 9-10 hours of photoperiod.
i. Photoperiod is one of the major drivers. Photoperiod is an important cue for seasonal migration. Whole life history depends on its seasonal timing. Water conditions, food availability. Everything is up and running and in time with their external environment. Causes endocrine changes.

u. What role is temp increase?
   i. Temperature will slow things down – external environmental cue - combo of photoperiod and temperature will get into a competent smolt signal.
   ii. SW diet can trigger changes as well. Some salmonids are able to make these changes quickly with little cues, others are slower. In the presence of salt in sea water, is a cue.
   iii. Pacific salmon – every population responds differently. Incredible amount of diversity there.

v. Coastal cutthroat - Euryhaline – fish that can go back and forth between FW and SW
   i. Tilapia, not sure if salmonids can do that. Contain both pathways simultaneously.

w. Growing in net pen, FW treatment for a few hours then back in the sea pen, what is the stress effect of that in compensation?
   i. Could look at that, short term exposure, depends on how are the fish behaving? The fish are happy afterwards. They are super resilient.
   ii. Jason is looking at that – FW re-entry. What happens when after they go to SW how long until they lose the ability to go back? Samples are in the freezer. Have not looked at yet. (Is such as short time, I would suspect that it is not an issue.)

x. Ahmed - We have different parameters that influence the smolt, we know that we have 2 isoforms, do we know the molecular mechanisms between the inducer and the expression of the isoforms?
   i. I don’t know. Possibly Steve McCormick (gene expression) or Trish Schulte (induction pathways in a lot of different systems of other fish). Not Mike’s area of research.


Gill health in marine populations of wild & cultured salmonids in BC- Simon Jones (DFO)

1. Intro to Salmon Aquaculture in BC
   a. 9 FH management zones
   b. 6000+ jobs, 92926 tonnes
   c. Enviro determinants of Gill health (Host-Pathogen- Environment)
      i. Gill integrity reflects optimal water quality (temp, salinity, dissolved O2, toxins, suspended solids, microbial infection)
      ii. Husbandry – density, handling, feeding

2. Oceanographic characteristics of coastal BC
   a. Peter Chandler (Victoria) FVCOM model domains in coastal BC
      i. Estuarine/wind, tidal, precipitation vs salinity
      ii. Domains – North coast (uniform Sal), strait of Georgia (low Sal), North Strait of Georgia (low Sal), West Coast VI (high Sal).
b. Seasonal Temp effects (Loma salmonae in cultured chinook salmon)
   i. Severe disease for gills, temp changes will influence.

c. Unusual warm conditions in late summer 2014
   i. NOAA – north pacific (the blob)
      1. Higher proportion of tropical fish in Gulf of Alaska
   ii. El Nino 2015
      1. Oct 2016 – return to more normal conditions
   iii. SST coastal BC – west coast warmer that east coast of VI

d. Neoparamoeba perurans and AGD in Atlantics BC
   i. 2014 – first report of N perurans and AGD in Canada
   ii. 2015 – PCR evidence of infection in 2 additional zones, Clinical AGD was reported in 1 additional zone
   iii. In original zone, total daily morts at sites with farm diagnosis of AGD increased from .1 to .29%.

e. Satellite imaging of plankton blooms VI 2014
   i. BC fish killing HAB species
      1. Heterosigma, chattonella, dictyocha, pseudoachat verruculosa
      2. HAD and low DO events – timing is important to capture. May be related. (Decomposition).

f. Jellyfish – scyphozoa
   i. Large blooms

g. Increasing awareness of gill health
   i. Occurrence of AGD and HAB raised the profile of gill health in the BC industry
   ii. Need for more systematic monitoring
   iii. Gill scoring systems established at farm level by industry and DFO (2014)
      1. Need for standardization of methods and score criteria
      2. Proliferative fill disease scoring (1- 5)

h. DFO Health Audit
   1. Random Quarterly checks (active sites)
   2. 300 fish/quarter
   3. Designed to allow farm level diagnosis based on history, mortality, clinical presentation and individual testing
   4. Gill scoring
   6. Gill score – regional scores (zones)

3. Advent of AGD and increased awareness of gill health
   a. Crypt production, not fundamentally different from how it appears elsewhere in the world
   b. Gill scoring 10 -5 (Clear to Heavy) Gross gill score system from (Taylor et al. 2009)

4. Description of new collaborative Gill Health research program
   a. 2 year collaboration, DFO, industry, BC MOA
   b. Marine reservoirs of infectious agents associated with proliferative gill disorders in farmed salmon
   c. Year 1 – 3 sites x 4 monthly samples
      i. Environmental data, gill histology, molecular assessment (N. perurans, D lepeophtherii, ich, SGPV
d. Year 2
   i. Increased # of sites, increased sampling frequency

e. Prelim data – histopathology
   i. Changes and resiliency and regenerative capacity of the gills to recover
   ii. Variable,
   iii. GLH – Hyperplasia was seen but does not say degree of it

5. Future directions
   a. Adopt a standardised gill health scoring system for use industry-wide and by regulators
   b. Integrate scoring system with histology and diagnosis data sets
   c. Coordinated industry wide collection and analysis of environmental data
   d. Role of changes in gill health as indicators (or predictors) of subsequent changes in
disease status. (interaction among sequential events - enviro and microbial).

Mark Sheppard: Loma
   e. Loma Branchitis - Clinical how it looks on the farm level
      i. Arises just before harvest. $.5 million/farm
      ii. Classic Lesions – Starts chronic then catastrophic cascade effect – fish die
         within hours
      iii. Massive outpouring of mucus.
   f. Another differential may be BKD
   g. Progression 24 – 48 hours afterwards. More and more mucus.
   h. Generally affects smolts that go into the water in June – when do the fish become
      infected, may not show Loma until the following year.
      i. Need epidemiology to investigate.
   i. Easy to diagnose via Gills and gram stains.
   j. To avoid large outbreaks - Management approach
      i. Early detection is key – no clinical indications until they start dying.
      ii. No realistic/effective commercial therapeutic
      iii. Monitor (for early detection)
      iv. Increase dive/pump frequency
      v. Top-seine the moribunds
      vi. Top seine harvests
Gill health: challenges for marine salmonids - Hamish Rodger (Fish Vet Group)

1. Global impact of marine gill disease in salmonid aquaculture is now equal to, or surpasses, that associated with sea lice.

2. Gill disease
   a. AGD
   b. PGD/I
   c. Harmful algae, zooplankton, biofouling cleaning
   d. Bleeding gill disease
   e. Complex gill disease (CGD)
   f. Impact (mortality) loss of growth, increased susceptible to other diseases, enviro/management changes

3. Pathogenesis of gill disease – evolving all the time. Cascade of things going on. – Who’s on first?

4. 10 key aspects for successful gill health
   a. Know the cause (HAB 200+), Harmful zooplankton (jellyfish), parasites, bacteria, viruses, (Chemical) mineralization in FW, will be an issue is SW
      i. Hydroids – discharged stinging cells (fish have live skin, Unlike humans 4-5 layers dead skin)
      ii. Net cleaning – makes fish vulnerable do to release of biofouling
      iii. Parasites (E.g. Desmozoon lepeophtherii)
         1. Study – gill scoring system strong gill score v. level of the pathogen
      iv. Bacteria (e.g. Candidatus branchiomonas cysticola, candidatus clavochlamydia salmonicola)
      v. Virus (SGPV)
      vi. Chemical (H2O2 – desolves quickly, Teflubenzuron, grading, pumping)

   b. Now the status of your gill health
      i. Impact from lice treatments,
      ii. Important pre-transfer
      iii. Rapid clinical changes with AGD, make the right decisions

   c. Monitor Gill Health
      i. Gross gill scores weekly (AGD and PGD scores)
      ii. Fresh gill smears
      iii. Histology & PCR (regular weekly or monthly basis)
      iv. Swabs are more sensitive than tissues (faster to get a result, non-lethal)
         1. PCR will give a warning 2 – 3 weeks pre gross signs
         2. Future – small handheld units/site (point of use)
      v. Presence of the pathogen is not equal to disease

   d. Pre-bath gill (& health ) assessment (Gross, histology, PCR, etc)

   e. Screen for other conditions (which impact FH)
      i. Jaw deformities

   f. Know when to treat (PGD, CGD or AGD)
      i. Get accurate diagnosis
      ii. Weekly monitoring important to see how it is changing
      iii. How is general health?
      iv. What are water parameters?
v. Availability/preparedness of FW or peroxide

g. Treatments and control of AGD
   i. FW baths (2-3 ours) <3ppt, softer is better
   ii. H2O2 use with caution
   iii. Treatments can lead to mortalities
      1. Stress
   h. Control O2 and CO2
      i. Superoxygenate
      ii. Treat at early stage, low scores
   i. Genetic selection for Gill health (AGD)
   j. Importance of accurate diagnosis
      i. Clinical history and signs
      ii. Water quality, plankton sampling and observation

5. Challenges
   a. Year round disease and many cases CGD
   b. Access to FW and equipment
   c. Increasing water temps
   d. Concomitant diseases
   e. Lack of alt treatments
   f. Economic and welfare impact
   g. Differential diagnosis

6. Summary
   a. Simple or complex, acute, chronic or multifactorial
   b. Histology is gold standard for many gill diseases and decision making
   c. 10 key aspect of successful health management
   d. See # 4 above.

7. Knowledge gaps
   a. AGD – not sure why it is starting to spread
   b. PGD/I not well defined
   c. CGD becoming more significant
      i. Epidemiology (risk factors, impact)
      ii. How to use molecular microbiology to apply effective control
      iii. Isolate the pathogens
      iv. Do pathogens interact?
      v. Biofouling and cleaning methods risk?
      vi. Why chronic disease?
      vii. Differential diagnosis/case
      viii. How to treat it
   d. Bleeding Gills – need more info
   e. Are increased treatments for lice leading to more gill issues?
   f. HABS, biofouling – what are the implications?

Gill pathology cases per month in Scotland (Chart)
Questions

1. O2 saturation question – what is acceptable? Under what circumstances?
   a. 200% saturation is not a good practice. Ideally 140% because when you stop the treatment, you get a big O2 crash – fish are very vulnerable for 20 min after treatment. Keep it up pre and post treatment.
2. Gas bubble formation in the vasculature (heart, brain) – any mortality associated with it?
   a. We have not seen gas bubble disease in salmon after a short period. (Long period may be vulnerable.) Larger fish, 2-3 kg, cannot treat them because of the acidosis that builds up in their bodies. (2 hours max treatment, smaller fish can handle 3 hours)
3. Format for treatments?
   a. Two methods - Well boat and tarp enclosure.
   b. Gill ventilation set up? Could but would be intensive.
4. Source of the gill pox virus – name in mind for genotyping in Europe to compare sequencing with Nellie?
   a. Oslo - Tang and Mona (NVI) genomics - horizontal or vertical transmission -has not been established yet.
   b. Canada – no pathological evidence in Canada. Are we dealing with a (PoxVirus) different strain of it? – may be a non-pathogenic strain of the PoxVirus. Norway has been unable to grow it yet in cell culture.
5. Smoltification Stress - Rash of Gill Disease during grow out – is there a time component to these events?
   a. There is a bit of a gap there. AGD can occur any time (3 weeks of fish at sea). CGD builds up over time (larger fish 3 – 4 kg+). In FW, we have not good info on PoxVirus in FW and then how they cope in SW. – no strong epidemiology evidence yet. Need the data.
6. Gene expression – defense response, has someone looked at severity of louse infestation?
   a. No not in particular. Scottish Salmon Producers have a good database – 5 years. Lice counts & gill scoring on the last few years. Analysis could be completed.
7. Hugh - Puget Sound 10 years ago, one of the first regions to have AGD. In the last 5 years, the problem has disappeared due to stocking from other sources. FW source of salmon has changed. Stock hangs low in the water. Stratification of Gill Amoeba
   a. Work just published - Diana Wright, Australia - where amoeba is in the water column. – seems to be concentrated on the surface layer.
   b. Role of Genetics may make fish more susceptible as well.
   c. Something changed in Scotland and Norway, may be temp related or other factors. Not clarified yet.
8. Anyone doing studies on biofouling & net cleaning?
   a. Yes, we did a few years ago. Exposure of fish. Mona (NOFIMA Norway) – challenge experiments hydroids and amoeba to see if they can get gill problems. Histopathology is similar after the exposure to hydroids (repeated exposures). Do not have the info yet as it is ongoing. Hamish can forward contact details.
9. Gill damage and handling, when you are in water, you are zero gravity, out of water, 1G, are we blowing the gills up on the inside? Mechanical damage of pumping?
   a. Hamish - Internal pressure and increased cardiac output. Likely contributing to gill hemorrhage.
SmoltVision – a new welfare indicator – Siri Vike (Pharmaq)

SEE NOTES FROM FW SESSION (same presentation)
Also Mike Ness

Questions

Wondering how much you expect cost? It is a PCR would be the same cost as qPCR.

Skretting ARC Gill R&D: development of a functional diet against Amoebic Gill Disease (AGD) - Julia Mullins (Skretting)

2. Skretting/Nutreco – global (Netherlands)
   a. ARC – 120 employees
   b. Experts in Nutrition, Health and Feed technology
3. AGD
   a. Global challenge
   b. Farmed (salmonids, turbot, sea bream, ayu) and wild fish (mackerel and lumpfish)
   c. No vaccines, no meds, chronic condition
4. In vitro testing
   a. Collab with institute of Parasitology, biology centre of the Academy of Sciences of the Czech Republic
   b. Paramoeba survival at 48 hr. post direct substance exposure (count live and dead)
      i. 4 different dilutions,
   c. 72 hrs. post exposure to mucus from fish fed substances
   d. In vitro results
      i. Significantly reduced survival of the amoeba
5. Quantitative histology
   a. Effects of diet – sampling, processing and analysing
   b. Standard gill measures (functional area of gills
6. In vivo testing
   a. Fish trials (U of Tasmania) collaboration
      i. Acclimation to tanks, SW 16 degrees C
      ii. Atlantic salmon smolts – 5 weeks
      iii. Up to 5 test diets
      iv. Up to 4 tanks/diet
      v. Challenge 1 – Diet A 27.1% survival over control
      vi. Challenge 2 – Diet A
      vii. Challenge 3 – Diet A 42.4% survival over control
      1. In line with vaccine trials (40 – 45%) in Australia
   b. Blood biochemistry
      i. CRP – Diet A lower
      ii. Lysozyme (measure of antibacterial activity) Diet A higher than control
      iii. Image analysis trial 3 – Diet A mortality lower
   c. Mucus assessment
      i. Mucus cell count & area – Diet A higher #s
      ii. Diet A = higher levels of mucus viscosity
      iii. Chilean field trail (after algae bloom)
1. Before Diet A, 3 weeks feeding Diet A, 6 weeks feeding Diet A (lowest gill score after 6 weeks)
2. Histology Score – (lowest score after 6 weeks)

7. Summary
   a. Reduced gill damage
   b. Increased AGD survival
   c. Norwegian recommendations
      i. Mid-June, 6 weeks Diet A, 4 weeks standard feed, 2 weeks Diet A

Questions
   d. Cannot say what the positive control. We measured viscosity at 12 degrees C. Can give out further details.
   e. How long was the infection load throughout the entire study maintained?
      i. Lower water level for several hours and decrease the flow, then return to normal levels after 3-4 hours. 1 or 2 doses to reach 500 cells/litre. Is a high dosage level.
      ii. Binary fission in the gills, replicating. Not constantly adding more amoeba to the water. Repeated infection AGD (Tasmania), there are lot of knowledge gaps in this area. We need to measure what is in the water right now.
   f. Field trials algae boom, what was the mortality rate?
      i. Do not have any of those details.
   g. In vitro testing with the Paramoeba, the field trials, was an Algae Bloom. The feed affects the gill health in a general way not specific to the amoeba.
      i. Would be good to try out in specific conditions.
   h. How do you know that if you do not track the mortality?
      i. Can get the data.
      i. Do you think that the diet will be available in Canada?
      i. Would be fantastic if it was available but is held up due to regulations. CFIA.

Neoparamoeba perurans: Global relationships & environmental detection - Jessica Johnson-McKinnon (U of Tasmania)
1. Molecular biology parasitology point of view (POV)
2. Run briefly thru AGD
   a. Attachment – hyperplasia and fusion of lamellae – excess mucus production and white patches – respiratory distress and death
   b. Neoparamoeba perurans (based on the presence or absence of surface scales)
   c. Host specificity to finish
   d. Causative agent
      i. Neoparamoeba (morphologically similar across species and diverse within species and clones)
   e. Commercial impact of AGD in Tasmania
      i. AS $550 million/year
      ii. Primary health concern in Tasmania for AS
   f. Treatment
      i. FW bath, tarping and well boat
      ii. Multi treatments/season not 100% effective Expensive
g. Interested in the Why
   i. Molecular POV to find differences via location, global picture of what is happening
   ii. Sampled with MHC last year and globally

h. Geographical Comparison Methods
   i. Multilocus Sequencing Typing (MLST)
      1. Target one gene
      2. Australia, Canada US, Scotland and Ireland
      3. Looking at enviro factors pushing this forward
   ii. Random Amplified Polymorphic DNA (RAPD)
      1. Distinct banding pattern, in computer program, combine gel bands together. Phylogenetic tree
   iii. Gills, remove the amoeba from the gills,
   iv. Sediment – sand, gravel and mud/silt (potential reservoirs)
      1. Issues with volume, a lot of different compositions within geographical range
      2. A lot of organic matter & other PCR inhibitors
      3. Separation of cells or DNA from the sediment
   v. Detection in sediment
      1. Sieves (manual separation, larger volumes, time consuming and limited soil types)
      2. Kits all-in-one, all soil types, typically small volumes.
      3. Currently trying to come up with a better method to extract DNA from sediment
         a. 20 g sediment, 15 ml tube, buffer, incubate, spin down, DNA extraction and Q PCR detection
         b. Can currently use a sample volume of 20 grams – same day results
         c. Can detect less than one amoeba

3. Summary
   a. Appears that AG outbreaks are due to local type of N perurans becoming parasitic rather than the spread of a disease-causing type
   b. Experimental detection of M P in sediment is possible
   c. Creation of a larger volume sediment sampling protocol.

Questions
1. Multi method – strong grouping, Canada, US and Norway?
2. Is it well known and accepted?
   a. Is very preliminary. The analysis would benefit from getting more isolates from outbreaks. Please send my way! It is an interesting idea to see what is happening globally relationship between these outbreaks.
3. DNA extraction – CFIA is doing the same thing with benthic samples from riverbeds starting from a full mason jar, - we can discuss for a relatively low cost. (Nellie G understands the challenges that you are dealing with.)
History & Current Status of Gill Disease in Farmed Atlantic Salmon in the Atlantic Region - Leighanne Hawkins (Cooke Aqua)

1. East Coast – past 20 years Gill Disease
2. Gill disease categories
   a. Parasitic
      i. New Brunswick used to get a Dactylogyrus (formalin baths)
      ii. Costia, - poor water quality sites have been since abandoned
      iii. Glochidia (larval stage of the FW mussels) hang around for a long time.
   b. Deformities
      i. Inverted Gill Arch (Only during peak water temp)
   c. Bacterial
      i. BKD (in flow thru and recirc) treated with bath treatments
         1. Causes nodules and plaques in cases when heart is affected
      ii. BKD SW secondary to something else.
      iii. CLOs epitheliocystis FW facilities
      v. Acute damage – marked regions lamellae and filaments
   d. Algae and WQ
      i. Chaetoceros
      ii. Mortality graph – algae bloom – sharp rise in morts then recovery
   e. Proliferative/nodular lesions
      i. Do see mild usually in second year fish (market size fish)
      ii. Suspect in chronic gill irritation over time
      iii. Not thought to be related to any specific pathogen
   f. Gill surveillance
      i. Base on histology and gross pathology screen of routine moribund fish on a monthly basis
      ii. Lots of talk in recent years of AGD
      iii. We have not gross or histology evidence of AGD
      iv. Surveillance screen in 2014 and 2016
      v. Targeted moribunds on site. And at peak water temp (no positives for AGD)
         1. Found costia, Tenaci, Branchiomonas, paranucleospora theridion
         2. In 2016 added SGPV (found 1 pos)
         3. Tenaci found in FW fish (question the PCR)
      vi. Conclusions
         1. No evidence of AGD
         2. No histology evidence that agents detected are contributing to clinical disease except tenaci and branchio
         3. Conclusions from duplicate histology sent to Norway support our hypothesis that most of our gill disease is environmental
3. What can we do for the animals?
   a. Mort removal
   b. Clean nets – in situ washing vs changes (prefer changes)
   c. Gill disease peak same time as water temp peak
   d. Bath treatments
e. Feed withholding  
f. Supplemental O2  
g. Early detection/surveillance  

Questions  
h. Lobsters – do have an amoeba shell disease. Lobsters are moving north hence catches are high on the east coast. Water temps are up to 15.5 on the surface. (2 degrees higher than normally).  
   i. Compared with Puget sound, amoeba (were different) clinical signs.  
   i. Tenaci (which species is it?)  
      i. Never tried to isolate. Do have isolates that cause the face rot. Never tried to get Tenaci out of the gill. Something that needs to done.  
   j. Which PCR assay was used?  
      i. Ask Siri. She did the PCR. Siri will check.  
   k. Differences between East Coast and some of the work that Hamish presented, speculation or comments?  
      i. Hamish agrees with Leighanne, It is only a matter of time, things have changed and are constantly building up compared to 5 years ago.  
      ii. We do a lot of peroxide treatments for sea lice. Cutting down on our ability to even detect the amoeba.  

Panel Discussion - Moderator & Presenters (Hamish, Mike, Leighanne, Julia, Siri, Jessica): Knowledge Gaps and Research Directions  
1. Gill Health – where do you see the needs for research?  
2. Simon Jones would like a universal gill score program – Simon says do it once, do it proper.  
3. At one point, someone mentioned handheld PCR. When will they be available to purchase?  
   a. They are on the market for shrimp industry, and will be out later this year for finfish. At a significant price.  
   b. Good news to implement technology to use to get answers. ACRDP grant to try it out at no risk.  
4. So far we have talked about cultured fish – ocean eco-systems and the environment - Do we know anything about wild fish and AGD?  
   a. There has been a bit of work in Tasmania. Very low level of detection in mackerel in north Atlantic by PCR and cleaner fish when being farmed even before they have contact with salmon, can be affected naturally (lumpfish and wrasse).  
5. Ian Keith - What is unique about Tasmania, indigenous fish in farming bays. Would that be a place that one would suspect AGD? (northern Tasmania)  
   a. Siri – we are doing a screening of broodfish, lumpfish and wrasse (wild). We find the same pathogens.  
   b. Tasmania – the farmed salmonids were not native.  
   c. Temperature and salinity in Tasmania is ideal for the disease.  
6. What is the confounding factor? Higher temp and salinity in the summer, what will that interaction look like?  
   a. If farm staff could collect gills on a regular basis, Histological data. If we had lots of $$, could look at prevalence.
7. From the farm POV practicality, when we have an Algae program out there, zooplankton monitoring would be a huge job on the farm.

8. Oysters – Vibrio para agent of carrier is zooplankton.

9. Do we know if there is any trend with pathogen load and distance from shore?
   a. Hamish – we have some farms 4 miles off shore in Scotland (& they have AGD and Sea Lice)
   b. Siri - Closed or open? Either or – Closed is good if it cannot get inside.

10. Do we need a coordinated effort (comprehensive program)?
    a. How to do. According to DFO - a treatment or fish health event then you have to inform.

11. Surveillance (zooplankton in Europe), how is it organized?
    a. Zooplankton - Sporadic screening needs to be more frequent than monthly. Presently, they store weekly samples but need to be processed routinely if they have a problem. Resources to do are slim.

12. Net washing – project that Diane would be interested in. Could that be the cause of Gill Health changes? Hamish – what was their protocol in the field for a project that they had earlier.
    a. Also one of the risk factors during tenaci workshop.

13. Gill amoeba – there is a lot that we do not know about the lifecycle. Where it resides and what is the reservoir? Seems to be adapting to the fish. How to look at Virulence factors for amoeba.
    a. Jessica - At this point we do not know. There has not been a lot of genetic work that has moved forward, we are interested in pinpointing the virulence factors in the amoeba. It no longer causes the disease thru passage. A lot of work needs to be done. We have not got to the immune response yet.
    b. Siri - There is a project in Norway, University of Bergen and 3 big farmers to look for virulence in different strains. (to rate the virulence of the strains) 3 year project – 2 years left

14. Things to avoid in regards to gill health - is that well understood?
    a. Hamish - In Europe, there is a big gap in AGD. Some are high risk, others are always affected, what factors are risker.
    b. Standardized method of gill health scoring, may help towards that Risk Factor program.
       i. High salinity, Net washing – may be risk factors.
       ii. Jessica - Amoeba are very tolerant – in Tasmania, it would be helpful to look at differences in site. Southern (regular disease), Northern (never disease)

15. Ahmed - FW vs SW combo, to see if there is something that we can do to help the fish defend themselves up front? Differences in net pen scale of the analysis to the site or net pen. Go back to smoltification to identify any strain.
    a. (Hindcasting - This is what happened to figure out what came before.)

16. Hugh - Has anyone looked at the synthetic mucus to see if there is an improvement in gill health? Protective band aid to shield the gills a bit?
    a. (Mucus Layer on the outside) Skin was the target in those studies. Could be done to find those studies.

17. Opportunity to ask questions, send to BC CAHS. Will be noted as a part of ACRDP.

18. Mark Sheppard - Nonsteroidal anti-inflammatory drugs (Tylenol, aspirin). Does anyone know if anyone has done any kinetic work on whether fish can handle them?
    a. Hamish – one of the objects of some of our trials.