Morbidity/Mortality Effects of Sea Lice on Juvenile Salmon Workshop

When: Wednesday, 18th November, 2009
Who: Five leading experts in the area and an invited audience of informed scientists
Where: Sauder Industries Policy Room (Room number 2270) at Simon Fraser University Harbour Centre, 515 West Hastings Street, Vancouver, BC
Sponsors: Coastal Alliance for Aquaculture Reform and Marine Harvest Canada

Background:

The issue of sea lice impacts on wild juvenile salmon is a concern to both Marine Harvest Canada (MHC) and the Coastal Alliance for Aquaculture Reform (CAAR). Despite recent studies in the Broughton Archipelago (BA) and other areas, questions remain regarding the morbidity-mortality (M&M) effects of lice on juvenile salmon. Under conditions of a dialogue framework, both MHC and CAAR have agreed to collaboratively strive for a common understanding of the morbidity-mortality effects of lice on wild juvenile salmon.

The terms of reference (ToR 1 of the CAAR-MHC framework agreement) for this study have been finalized by both parties. Potential research was identified in this ToR, and a workshop of experts was chosen as a key first step to help synthesize our collective scientific state of knowledge on the morbidity-mortality effects of lice, and to assess future research directions.

With the help of Crawford Revie, CAAR and MHC began identifying experts to help with this issue. A workshop is planned for November 18th 2009 in Vancouver. The Broughton and its pink salmon will be the primary focus of the workshop, but it is expected that the experts may also consider potential effects of lice on other salmon.

The objectives of the workshop include:

- To review recent research covering what we know about effects of lice on juvenile salmon, including mortality, morbidity, behavioural, and sub-lethal effects: Are there real and as yet unresolved discrepancies in lice effects reported in recent studies (e.g. size-related risk thresholds)?
- To provide views on potential impacts as they relate to species of lice (*Lepeophtheirus* and *Caligus*), lice demographics (age, stage, intensity, prevalence, etc.), or size and species of fish;
- To provide advice on how to measure risk that takes into consideration both morbidity and mortality factors;
- To provide advice on what future studies (if any) it might be appropriate for CAAR and MHC to give priority to as they seek to extend their research under ToR 1.

Programme:

8:30 am
Coffee and 'registration'

9:00 – 9:15 am
Introduction and plan for the day

9:15 – 10:00 am
Sea lice - physiological and ecological effects on wild salmonids in Europe
Dr. Bengt Finstad, *Senior Research Scientist, Norwegian Institute for Nature Research (NINA), Trondheim, Norway*

10:00 – 11:00 am
Sea lice on juvenile pink salmon: Holey terrors or just a drag?
Dr. Tony Farrell, *Professor and Research Chair in Sustainable Aquaculture, University of British Columbia, Vancouver, Canada*

11:00 – 11:30 am
Coffee

11:30 am – 12:30 pm
The early development of resistance to mortality associated with *Lepeophtheirus salmonis* in juvenile pink salmon
Dr. Simon Jones, *Research Scientist, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, Canada*

12:30 – 1:30 pm
Lunch

1:30 – 2:15 pm
Sea lice parasitism increases predation vulnerability of juvenile Pacific salmon
Dr. Larry Dill, *Professor Emeritus, Evolutionary and Behavioural Ecology Research Group, Dept of Biological Sciences, Simon Fraser University, Vancouver, Canada*

2:15 – 3:15 pm
Models for morbidity/mortality studies
Dr. Martin Krkošek, *NSERC Postdoctoral Fellow, School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA*

3:15 – 3:45 pm
Coffee

3:45 – 5:00 pm
Final Discussions

Welcome and Introductions (Notes taken from Audio tapes hereafter):

Welcome by Crawford Review (University of PEI) - meeting Chair. Round meeting introductions of participants:

Crawford Revie – University of PEI
Sonja Saksida – BC Centre for Aquatic Health Sciences
Sharon DeDominicis – Marine Harvest Canada (MHC)
Diane Morrison – MHC
John Reynolds – SFU
Brad Boyce – MHC
Brent Hargreaves – Fisheries and Oceans Canada
Tony Farrell – UBC
Bengt Finstad – Norwegian Institute for Nature Research
Brendan Connors – SFU
Dario Stucchi – Fisheries and Oceans Canada
Mike Sackville – UBC
Marty Krkosek – University of Washington
Simon Jones - Fisheries and Oceans Canada
Allen Gottesfeld – Skeena Fisheries Commission
Stan Proboszcz– Watershed Watch and Coastal Alliance for Aquaculture Reform (CAAR)
Craig Orr – Watershed Watch and CAAR
George Gettinby – University of Strathclyde, Scotland
Larry Dill – SFU

Opening Comments

Crawford Revie's objectives:

1. Get clear understanding within our collective fields of interest in terms of key research questions on morbidity and mortality.
2. Highlight areas of major contention/debate/uncertainty and whether we can highlight some of the kinds of research to fill those gaps.

Craig Orr – Sponsor objectives:

CAAR and MHC need help to move closer to a common understanding of effects of sea lice. CAAR and MHC have been talking since 2004 to get together on issues of mutual concern around salmon farm impacts and perceptions around impacts. First major program: increase transparency in sealice monitoring trying to resolve our differences around the interpretation of the science around interactions between farmed and wild salmon. A Terms of Reference (TOR) was drawn up around several areas of interest including source of lice and morbidity & mortality effects of lice on juvenile wild salmon.

Some specific TOR objectives include:

1. In fry ranging from 1g to less than 10g in size - what is the relationship between mortality as a function of fry size and the intensity of sea lice infection by stage on those fry?
2. In fry ranging from 1g to less than 10g in size - what behaviour and physiological changes including morbidity and mortality occur as a function of the intensity by stage of sea lice infection?

CAAR's objectives for the meeting today:

1. To review research on effects of lice on juvenile salmon, including mortality, morbidity, behavioural and sub-lethal effects. We have not been able to marry these three things very well in terms of effects on fish – hope to do that today because we know they have to be considered together and not in isolation. Are there real unresolved discrepancies in lice effects reported in recent studies e.g. size related risk thresholds on fish? Mainly we have been talking about BA and pink salmon but looking to think broader today to include sockeye etc. We have been looking mainly at individual impacts, mostly at *Leps*, looking at physiology side, some behavioural issues and we need to bring them together a bit more.
2. Provide views on potential impacts as related to species of lice (*Leps* vs *Caligus*, lice demographics, age, stage, intensity, prevalence etc.) and species and size of fish.
3. Provide advice on how to measure risk to wild fish from lice, taking into account M&M factors, ideally at both an individual and a population level.
4. Provide advice on what future studies, if any, it might be appropriate to give priority to; where are the gaps and what can we avoid repeating?

Overall outcome would be some kind of synthesis on what we can say about the effects of sea lice on juvenile fish.

Sharon DeDominicis (MHC – sponsor): I agree with what Craig said – we are looking to assess current state of knowledge and are striving for common understanding of effects of sea lice.

Presentations:

(Note: text in red italics taken from workshop program)

1. Sea lice - physiological and ecological effects on wild salmonids in Europe

Dr. Bengt Finstad

Senior Research Scientist, Norwegian Institute for Nature Research (NINA), Trondheim, Norway

In this presentation a summary of the physiological effects of sea lice on salmonids (Sea trout, Arctic char and Atlantic salmon) in Europe will be given. Further, results from the national wild fish monitoring programme on the effects of sea lice on wild salmonids in Norway will be presented and an overview of the effects of sea lice on wild fish populations will be discussed. A presentation of the fish farming activity in Norway will

also be presented and the potential of spread of sea lice from fish farms to wild fish will be given.

This presentation will provide a review from European perspective with regards to salmon lice, physiological effects and effect on fish in the wild and also will look at the fish farming industry in Norway and show some recent results from monitoring in fish farms throughout Norwegian coast.

Basic background levels for natural infection levels for sealice - in 70-80s in Norway there were very few hosts available for salmon lice in the winter period (Atlantic salmon feeding in open ocean, sea trout and Arctic char mostly in fresh water) and infection pressure in fjord systems at this time was quite low and were mostly derived from ascending Atlantic adult salmon. Peaks on wild fish were in late summer and approx. 4-8 lice per fish in late autumn. There were also some sea lice peaks observed due to crowding before fish migrate up the rivers. Fish farming led to a change in infection system for salmon lice. Some broad calculations undertaken show that the wild fish stock in Norway now is at 202,000 tonnes, that is about 490,000 salmonids with a weight of 4.1 kilos. The total number of farmed salmonids in the sea now is 800,000 tonnes about 220 million salmonids. The farmed salmon stock is about 400 times greater than wild salmon stock in fjord systems. Central west coast of Norway had most density of farms in 2008.

Sources of sea lice include escapees, salmon farms, wild fish, environmental factors such as salinity and temperature leading to infected copepodid and infections on the host smolts.

Physiology of host:
Since the early 90s we have done experiments on infecting wild fish (Arctic char, Atlantic salmon and sea trout at all stages) in tanks with sea lice larvae hatched from egg strings, copepodid, lice cultures. We have followed life cycle from 0 to 40 days up to adult lice and we see on Atlantic salmon that the cortisol levels on non-infested fish is quite low and when infested with sea lice the cortisol levels are increasing and are seen to increase for the whole duration of the infestation period. The physiological capacity in non-infested fish is normal and the fish that are infested with larvae and developed to adult lice we see that the physiological capacity is reduced – they have lots more regulatory problems. We also see that when infected, the health status of the fish decreases – if you look at lymphocytes as part of the leucocytes. Of fish we see that they are normal in uninfected fish and infected fish have decreased immune capacity during infestation phase.

We have also done infestation studies on sea trout (30-70g post smolts) with different lice levels from 10, 30 to 50 lice per fish through whole lice cycle and following Atlantic salmon response we see that highest infested fish have poorest osmo-regulatory capacity and control fish are behaving well in sea water.

Q: How many lice did you have to expose fish to get to those lice levels?
A: there was a 65% survival rate in copepodids so if you increase the number of copepodids regarding the mortality you could calculate the lice levels.

Also see the same high stress cortisol levels in highest infected fish.

From sea trout experiment we have done statistical calculations and we see that with about 13 sea lice, trout from 30-70g are getting physiological problems when lice levels exceed 13 – we see it from plasma cortisol levels.

Wagner 2008 shows that problems with clinical infection are increasing on salmon (20-50g smolts) with >10 lice. A number of studies on the effect of water quality on the infestation of fish have also been done – control fish exposed to sea lice where mortality is low. If fish are experiencing high aluminum (from 15-40 micrograms) and poor water quality (pH 5.8) or low (<10 micrograms) and episodic aluminum, fish are more susceptible for sea lice infestation. The water quality pre history of the fish is very important for fish getting infested by sea lice.

Q: is the kinetic speed of fish mortality the same i.e. do the fish die in the same time frame but at a higher level if they've previously been exposed to aluminum?
Ans: Fish were dying randomly throughout the experiment [can't hear rest of audio].

Experiment exposed them for 10-15 days in fresh water, in different water qualities (clean water altered in the lab to change aluminum and pH levels) and then exposed them to sea water and infested them with sea lice (2.3 copepodids per gram of fish weight) and we saw that the mortality increased in the fish that were in poor water quality.

Q: In the absence of sea lice challenge is there a difference in mortality explained by poor water quality? If there is no challenge, does the poor water quality give rise to fish mortality too or is it just exacerbated by sea lice?
Ans: they can make their way out to sea from fresh water but if they've been [incomplete].

Have also looked at effect of sea lice on maturation of Arctic char: - larger fish (0.3-1 kilogram) placed in fresh water first, then exposed to sea lice (0.15, 0.07 lice per gram of fish weight and control), then back to fresh water for spawning. We see that Arctic char mortality is increasing in those with the highest lice load, similar to response seen in salmon and sea trout. There is also a delay in relations in fish with highest lice. The sea lice infestation has also reduced the number of spawning females, several months after infestation. Other studies have shown that the offspring from higher infested fish show lower survival rates. Similar serious problems with reproduction seen in other studies on Atlantic salmon and sea trout.

We also see a similar trend with fecundity: control fish have highest testosterone levels. Highest infestation group have significantly lower testosterone.

Norwegian study by Ince [?] On effect of sea lice on swimming capacity in adult salmon (approx. 1.6 kilos): We see that where lice load increases from 0.1 to 0.12 lice per gram of fish weight the swimming capacity is reduced.

From studies on fish farms on Western part of Norway - production is 800,000 tonnes and 220 million fish from approx. 650+ farms. Have calculated the production of lice eggs produced from farmed fish in Norway from 2000 and 2009. A previous paper in 2001 showed the lice levels produced from farmed fish in Norway was approx. 40 billion sealice eggs in May during the migration period for fish in 2000 – data collected and calculated by the National Fisheries Directorate – website called www.lusedata.no (mandatory reporting by industry of more or less raw data since approx. 2003). In Norway there has been an increase of lice egg production of approx. 42% in water column in all areas since 2000.

Recent data studied from 2007, 2008 and 2009 shows: in 2009 lice levels increasing to extremely high levels in nearly all counties except off the Arctic and a couple of other areas. One problem area is that we are facing lice resistance to SLICE or althomax/betamax in some farms. We see a 3 fold increase in Norway compared to 2008 and 2007.

Q: how do you know SLICE is not working?
Ans: vets are sampling in different counties and using PCR for looking at resistance. First we saw that sea lice were not responding to SLICE and now we are seeing same effect with other medicines too.
Q: were there any significant differences in environmental conditions in 2009 over 2007-2008?
Ans: All profiles are on the website, but 2009 was quite a warm summer. Treatment trigger from September onwards is 1 female lice.

Wild fish monitoring
West coast of Ireland - Paddy Gargan's work shows that the nearer to the fish farm you get, the higher the lice load and mean abundance of lice are also increasing as is mortality in the fish. Also shows that closer to the farm it is mostly chalimus larvae on fish showing that they have been recently infected.

West coast of Scotland - Butler shows that sea lice abundance on wild fish is higher closer to the farms and lice loads are lower further away from farm.

Q: data quite old. Any more recent studies?
Ans: yes, reference Michael Penston.

Norway's National sea lice program - Norway has a national sea lice monitoring program which has been ongoing since 1990. Norway has 29 national salmon fjords where there are no fish farming activities to protect the wild stock. Also have a monitoring program with gillnets, sentinel cages, trawling after smolts, release of smolts protected and not protected against sea lice and bag net ring stations on returning salmon.

Sentinel cages throughout example national salmon fjord, gillnet for sea trout and trawling for Atlantic salmon. At innermost location – low fish farm activity, brackish water; middle area and outer area (both high farm activity and similar salinity). Lice load on the fish are increasing from inner most to outer most part of the fjord. The mean intensity on seatrout is quite high at approx. 55 sea lice on sea trout (approx. 100g). Also have some *Caligus* on the fish too.

Also trawled for 14 days and captured Atlantic salmon smolts with abundance of 32 lice per fish and sea trout had about 75 lice per fish – lethal levels are met and are exceeded.

Also had smolts in sentinel cages and released them in different sequences and in different segments of fjord system. Use the fish as indicators for sea lice – results show that sea lice levels increase from inner most to outer most part of the fjord. Results show that even in cages the lice numbers are approaching lethal levels on fish. Good method for monitoring sea lice infestation in Norway.

Q: Issues with predation?
A: No, the fish are quite protected in this environment (2m below water surface for ~2 weeks).

Farm free/national protected fjord - Intensity on wild sea trout (42-150 g) in farm free fjord from first period 1-2 = 0. At end Period 2 (late summer): low levels in inner most area but at the outer most and just outside fjord, lice levels begin to increase. Salinity levels are >25 pp thousand. Trawling results over 15 years in this fjord system show that abundance levels are low on wild sea trout – very different to the previous fjord results above. Sentinel cage results also show lice levels are quite low.

Q: when they reach the outer edge of the protected fjord how close is the nearest farm?
Ans: It would take about 1 week to reach it.
Q; do you have any idea of any cage effect on the infection?
Ans: trying to look at the different mesh sizes because it certainly will be a barrier for sea lice larvae to come into the cage.

Modelling to show effect of currents and sea lice larvae
Population effects of sea lice larvae on smolts: have done several releases of tagged fish (3000 salmon smolts). Some were protected from sea lice with a bath treatment for 16 weeks. Recapture rates show that protected fish have a better rate than unprotected

fish. Another paper by Paddy Gargan shows that fish treated with SLICE have a much higher recapture rate than control fish.

Conclusions

To conclude, we have good data on host physiology for all the salmonoid species. We have good data from fish farms (all data on lusedata.com) – this is to be recommended because open dialogue with farmers means there is no hiding of data. The quality of counting can vary but no hiding that lice levels are high and they are doing everything they can to decrease the lice levels. We have progress on mathematical modeling and hydrography. For wild fish monitoring we started monitoring in 1991 and have an annual report and we have increased surveillance and regulating the national salmon fjords. As in all research, there are problems with financing a good overview of what the fish farming industry are doing and to use the wild fish as the target for success, not the success in treatments. We have seen in some areas that this has succeeded. We don't have a lot of trawl data but in 2008 in the example fjord with fish farms in, it was calculated that about 50% mortality of smolts. National sea lice monitoring shows small improvements from 2008-2009.

Synchronized delousing and collaboration between industry partners and wild fish interests are very important to eliminate the problem.

Recommendations

Suggested measures to reduce/eliminate the problem:

1. reduce production volumes
2. remove farms from heavily loaded areas
3. lower delousing limits
4. coordinate delousing
5. strong national program for wild fish very important to determine what farms are doing. Aim is to keep below 10-13 louse per fish on wild fish.

Q: looking at a lot juvenile out migrating, is there any information on returning fish?
A: yes, looking at this too and we see that lice levels are about 10-15 on adult female fish and returns/population size is reduced.

2. Sea lice on juvenile pink salmon: Holey terrors or just a drag?

Dr. Tony Farrell
Professor and Research Chair in Sustainable Aquaculture, University of British Columbia, Vancouver, Canada

Sea lice are likely to have sub-lethal physiological effects on juvenile pink salmon at a lower sea lice density and well before lethality is manifest. The research presented test the ideas that sea lice are (a) "holey terrors" by creating an ionoregulatory disturbance by breaching the protective barrier of the skin with their feeding and attachment activity; and/or (b) "drag artists" by slowing the swimming performance of fish due to a

surface area effect on the fish. These ideas are tested in the context of pink salmon of variable body mass (0.2 to 3 g) and sea lice developmental stage and numbers (0 to 3). Incidental information on mortality will also be presented.

Background

Mike Sackville was doing the experiments over the last 2 years. The focus of the talk is on Glendale juvenile pink salmon (on even years, 90% of pink salmon in the BA come from Glendale spawning channel region, in odd years approx. 40% come from Glendale) and *Leps*. Challenge for research: we wanted to do controlled (dose dependent) coarse effects sub-lethal experiments. 'Holey terror' effect is to do with the fact that the lice are eating the skin off of the fish, which is a protective barrier for ions. The 'drag' effect is that they cause physical drag and reduce the maximum swim ability. We have seen some nice depth profiles and think sentinel cages are the way to go in terms of sea lice monitoring, but we do need to understand a bit more about the biology of the pink salmon. Will wrap up with conclusions.

The lice moves from a pin head (copepodid) to half penny size (mature adult). The size is important because in BA it takes between 1-2 months, depending on temp, to go from copepodid to mature adult. The BA area is about the size of Strathcona Park, approx. 26 sites in total with about 50% operational at any one time – total production here is approx. equivalent to one fjord in Norway.

Glendale juvenile pink salmon

Glendale pink salmon were focused on because on even years, 90% of pink salmon in the BA come from Glendale spawning channel region, in odd years approx. 40% come from Glendale region. The life cycle of pink salmon is phenomenal, they pop out of the gravel and go straight down stream, they get into saltwater so fast that we think they almost aren't ready to enter salt water. Once they get out there, they grow very quickly – they can double their mass in a month and they are doing this at a time when the lice are developing. Need to consider these two dynamics occurring at the same time.

Challenges of working in the BA – no labs. MHC gave group a fallowed farm for students to live and work (set up lab) in for 3 months in 2008. Used 2 approaches to analysing infected fish.

Approach 1: to control the infection so we knew exactly what their infection history was. Took as small as possible Glendale fish out of the river, allowed them to grow in lab in sea water. Infected them 1 week after being entered into sea water. Intention to put 1-3 lice on each fish and follow them through growth cycle. Put them in a bath with 150 copepodids per litre for 4 hours and ended up with groups of 1-10 lice per fish – around a typical farm would expect 6 orders of magnitude lower density of copepodids than that. The exposure regime was not near natural and would really hammer the small fish. Tested and sampled fish over 28 days as both fish and lice grew.

Approach 2: ended up not having enough of Approach 1 fish (did not have enough sea lice to culture and place on our fish) so we went to wild fish with unknown infection histories. Used seine to catch larger fish (later in the experiment period). Aimed to get fish with 1-4 lice and we tested and sampled fish after holding for just a week or less. Sampled about 5,000 wild fish and 90% of them had no sea lice – 10% prevalence. Typically there was only 1 louse per infected fish. The average intensity of all these (after culling some of the fish with no lice to increase it) was about 1.2, and therefore, very little possibility of estimating effects of high lice loads. The challenge was to keep the lice on the fish. There was a progressive loss of sea lice, depending on how long they were held (wild – up to 7 days, cultured fish – up to 20 days) and whether they were wild or cultured fish. Lice were reported only if they were on the fish at the time of testing, they may have had more lice previously but this is not reported.

Controlled infections produced hundreds of fish with 4-10 lice. Research mandate was to only look at up to 3. After 15 days very few of these fish actually had 3 lice. Planned to look at increase in fish weight from 0.3g to 3.0g and to look at lice intensity over that time. Also did some experiments in West Van in 2007. Central question: is a louse on a pink salmon one too many? Asked Alex Morton on Pacific Salmon Forum call how many lice should we be worried about and she said one lice per baby salmon.

We were specifically interested in the sub-lethal effects. Two main hypotheses:
1) *The 'holey terror' effect*: a fish that's sitting in sea water has a lower salt concentration than the salt water so by diffusion NaCl will tend to move into the animal across the gills or skin and water will tend to leave the animal. The fish doesn't like that as it tends to have its ion levels set already so it has to compensate against sea water effects by drinking sea water and takes in more salts and excretes them out across the gill with an enzyme. We can't sample plasma on a 0.25g fish but we can measure total body ions. We take a baby fish, grind it up and measure the number of ions in it. If they've gone up we can measure an increase. When you put a louse on it creates holes in the fish and the prediction is that this hole increases the flux of salt in and the water flux out, therefore whole body ion concentration should increase with sea lice infections and it should get worse as sea lice get bigger and as you add more lice onto the fish the problems should be worst for the smallest fish because the relative size per louse is greater per surface area on a small fish and eventually they should die of ionic imbalance. The principle of ionic imbalance leading to death has been well established by studies on the effects of acid rain.
2) *the 'drag' effect*: by pulling around a louse the maximum swimming ability should decrease, gets worse as the louse gets bigger and it's the biggest problem for the smallest fish.

Holey Terror effect
Colin Burke had done some studies on juvenile coho moving out of sea water – if you move coho into seawater prematurely and they haven't fully smoltified they get excess ions in their bodies as shown by the plasma sodium being elevated. If you do a swim

challenge with excess ions they don't do as well. So reduced swimming capacity could be due to one of these two reasons.

Incidental observations on mortality: controlled infections with over 400 fish, fish were separated by lice load into a 1, 2, 3 (low load) and >3 (high load) bins. Experiments were performed on low load fish up to day 15 and we discovered that the fish were losing lice. High load fish were used to finish off experiments after day 15 because these had also lost lice and were down in the 1,2,3 load level. Did not keep records of mortalities in control infected fish and didn't keep track of mortality in high load fish until after day 15. 339 low load fish that started off with 1-3 lice. No mortality before day 12 and there were 8 total mortalities of the total 395 fish which is 2.5% mortality by end of experiment. 3 fish died with one lice on, 5 fish with 2-3 lice on so that's not a dose response effect. Less than 0.5g fish and 17 of them with high loads died after day 15 and had 4 or 10 lice on them. At low loads mortality didn't seem to be an issue. Not the focus of our study but there was definitely something going on mortality wise on fish of this size (0.5g) and at lice loads >3 that we should be concerned about.

In control fish, you add one louse and in growth stage C3-C4 you begin to see an effect in terms of body ions increasing. Add more lice (2-3) the effects of increasing body ions are seen earlier. A dose response effect is not seen between 2 and 3 probably because a threshold is reached and body ions can't go any higher or they would probably die. Couldn't get to the point where the fish continued to have 2-3 lice to see what would happen because the fish dropped lice at that time.

In the larger, ocean caught fish we don't know history but they had at least 1 louse per fish on them. We see no effect. What we do see is a body size effect, if you look at range of body sizes over time moving from entry to salt water from fresh water, to being in salt water for several months you see that the total body ions go down.

We suggest that juvenile pink salmon are struggling to maintain ion homeostatis until they reach 0.7-1g (getting to this mass involves doubling or tripling their weight). Holey terror predictions were: we are going to damage the skin, and see an increase in ions. We did see this happen and there was a bigger problem where there were more lice and where the fish were smaller. The size threshold seems to be ~0.7g – below this we see effects, above this not so much. A 2.5% mortality rate (may be background) is the best estimate but the Leps were shedding off even the smallest fish. Lots of studies show that pink salmon get rid of *Leps*, and we didn't have a control – in summary – very few mortalities and they were hammered with *Leps* when they were very small.

One louse is sufficient to disrupt the fish with sublethal effects where the fish is small. As the fish gets bigger need 2 or more lice to see sublethal effects.

Drag effect

Experiment was to look at 400 fish in a swim tunnel in less than 3 months to see if there was a drag effect. The typical way to test for max swimming performance is by using a test developed by Roley Brett years ago – a U_{crit} swim test. But it takes a long time to run, a shortened version takes ~2hrs. Test was adapted to accelerate testing period to about 10-20 mins length, with 6 fish tested per day.

Control fish tested on day 0 and day 28 – on average same swim performance.
In beginning with copepodids on (C1 stage) no difference seen, as you move into C3-C4 stages we notice an effect on swimming when you get to a louse stage big enough. When you go from 1 to 2 to 3 lice, there is only an additive effect at this particular stage. If you've got drag this can't be the case so this is suggesting that this is not necessarily a drag effect.

If you look at body ions you see them decreasing as the fish get bigger, when lice infect them you get ion regulatory disturbance both in swum fish and unswum fish and we see a decrease in swimming performance. We don't think it's a drag effect, we think the effect on swimming performance is mediated through elevation of body ions. The impact on U_{crit} is predicted to increase as sea lice get bigger and with more lice. With bigger fish, relative swimming speed goes down. With up to 3 lice if you are bigger than 0.4g no impact on swim speed observed. So, 1 louse disturbs the baby fish but probably not through a direct drag effect – more an ion regulatory issue. But when you get bigger you're not seeing a swimming or an ion regulatory effect with 1 to 3 pre-adult so the sub-lethal effects for bigger fish moves way out to beyond 0.5 g.

What were juvenile pink preferences?
The fish have to encounter the lice – this needs to be considered in 3 dimensions. If you have a sea farm cage 30m deep and lice are coming out at 30m we need to know if they're moving up or down 30m. It doesn't matter how many eggs are coming out, there is no impact unless there is exposure.

Experiment: using a 10m long column with different openings to different interconnected sections at different depths, put 50 fish into the middle section and drop it in the water. After 3 hrs, close sections off, pull out of water and count number of fish in each section (1m, 2m, down to 10m).

The 0.5g fish like the top 1-2m (83% of fish caught in there in daytime and 17% of fish caught in there in night time). Repeated over time; at 3 weeks they start to explore more depth. In 1g ocean caught fish they are exploring far more of the water column, going down to the full 9m. Tests were done in a particularly rough weather period. The fish tend to move deeper if the water surface is rough. Not sure if this is an artefact of the monitoring columns or a real thing – Marty Krkosek thinks it's real. No significant change in either temperature or salinity was observed with depth therefore fishes response was probably a phototactic one.

Overall conclusions:

- One louse (C3-C4) can disrupt swimming performance and ionic regulation in fish of 0.5-0.7g but this response is not observed in larger pinks. Three lice are disastrous for the baby fish.
- Can replicate the same thresholds from the compensatory response (the gill enzyme that is used to get rid of the salts).
- Very few pinks died with up to 3 lice attached after 28 day period. They grow rapidly and become more tolerant with increasing size (up to 28 days – more study needed into adult lifecycle).
- Need to be accurate in using and reporting data.

We have observed a doubling of mass in this first month of the 0.2g fish. Didn't have a scale that worked on floating dock.

Q: In controlling the infection levels at the start of the experiment the fish lice loads ranged from 0 to approx. 10. How much of these infection levels can be attributed to louse effects as opposed to louse vulnerability effects?
Ans: this study didn't come close to looking at this. Separate study required to define vulnerability.

Q: What do you think the average survival rate is from a copepodid to an adult?
Ans: Less than 25%. When we put highs in high bin we had at least 4 and they were dropping down to 1.
Marty thinks it's really low – approx 1%.

Q: With respect to thresholds, what do you think would happen if fish were being continuously exposed to copepodids?
Ans: Read Marty's model. He tells me the concept of fallowing at critical stages is worthwhile. Best precaution is to let fish get bigger before they get exposed to lice.

Q: How did you hold fish after infection? Were the holding conditions optimum? Did they have access to lots of lots of food, etc?
Ans: Looked at data from West Van last year. Overall density in this experiment was less, they were fed regularly so conditions were probably fairly optimal.

The early development of resistance to mortality associated with Lepeophtheirus salmonis in juvenile pink salmon

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Pink salmon juveniles grow rapidly after entering the marine environment. A series of experiments showed that above a threshold of approximately 1g, exposure to sea lice failed to cause mortality and juvenile pink salmon rejected most parasites. This resistance was associated with skin maturity and inflammatory capacity. Significant

mortality was observed only within smallest size class (~0.3g) pink salmon following exposure to a sufficiently high challenge. Extrapolation to infections observed on wild juvenile pink salmon provides insight into population-level effects.

DFO has been at this lab exposure work since 2003. As with any parasite, it is clear that there is a consequence to the host and the louse does cause disease in their host. The severity of the disease is dependent on a number of factors. We need to understand the role of the intensity of the number of parasites on the fish, the developmental stage of the parasites, the host species (there is tremendous variation among salmon species and how susceptible they are to infection), the age of the host and host condition (nutritional history, stress etc).

Effects range widely. Disease can be sub-lethal up to including lethal levels of infection. Motile stages are a lot more virulent than non-motile stages. Atlantic salmon and sea trout seem to be more susceptible than Pacific salmon. Susceptibility of post smolts appears to be greater than adults especially from studies done in Atlantic Ocean. Not very much data is available on the effects of age of Pacific salmon. Susceptibility to lice infection is increased in stressed salmon e.g. Chinook salmon implanted with cortisol. A lice infection may lead to other bacterial, fungus or virus infections in the host – the pathways through which this occurs are just beginning to be explored.

Pacific salmon appear to be somewhat resistant to lice infection but there are always circumstances that show the contrary; e.g., adult sockeye salmon in Alberni Inlet where water levels were very low, water temperatures high and oxygen levels were low. Under these conditions adult sockeye were extremely susceptible to effects of salmon lice (lesions exposing musculature etc.).

Our work is mainly interested in the evidence that supports whether or not salmon lice cause disease in juvenile Pacific salmon in Western Canada. European experience is very clear that there is an association between elevated sea lice numbers on stocks of salmon and sea trout in some areas that support salmon farming. In many of these cases in Norway, Ireland and Scotland the levels of infestation are higher than what lab studies have demonstrated to be lethal so it's not only an association with salmon farming but it's an association with levels that can potentially have an effect on an individual fish. Extrapolation from these pieces of information to make conclusions on population level effects is still unclear – evidence is still not compelling, but we need to look more at how we interpret individual level effects in a population context.

In 2003 in BC there were concerns that sea lice (particularly *Lep salmonis* from farmed salmon) were causing an adverse impact in pink salmon populations. At that time we were working in a virtual vacuum in terms of the availability of data and understanding of susceptibility of pinks, chum and other Pacific salmon. We were very dependent on the kind of information coming from Scotland and Norway. This information was used to begin to understand what was going on in BC. It was also identified through research

that was being undertaken in Scotland that there were some genetic differences between salmon lice in the Atlantic and Pacific Ocean. DFO undertook a study a few years ago where they sequenced the genetics of a number of Pacific salmon lice (mitochondrial genome of salmon lice from Pacific) and compared that to what was already available from salmon lice in the Atlantic. On average, found that the DNA sequence was about 10% different from a similar sequence from the Atlantic *Leps*. A 10% difference is in many taxa sufficient to justify the status of these groups as being distinct species. We don't really understand the significance of this 10%. Maybe they are distinct species? More work needs to be undertaken but we do need to understand different attributes such as 1) virulence/the capacity to cause disease - how that is similar/different between the two types of sea lice, and 2) the ability to respond to drugs or develop resistance to drugs. **Context:** we are working in an environment where there is virtually no data on susceptibility of Pacific salmon juveniles to salmon lice and the salmon louse that we are working with is probably a distinct species from the parts of the world where we're getting our information from.

When the research program first began in 2004 we recognized the need to build capacity in the lab for exposing fish to salmon lice infection in a reproducible manner to eliminate random effects and to allow us to record how they were responding. One of our program objectives was to do this in a comparative way, that is, to not only look at pink salmon and their response, but to compare chum salmon and their response to Atlantic salmon, etc. Underlying theme is to try and understand relative susceptibilities. Once we had established the differential susceptibilities between species we wanted to propose mechanisms of resistance. We were interested in documenting the onset and development of resistance (focused on pink salmon for this work). We also made some attempts to estimate lethal thresholds.

Summary of thinking:
We began collecting quite simple data: abundance of lice on fish that had been exposed (followed known exposure methods from Europe mainly). It became very clear early on when we exposed juvenile pink and chum to levels of lice that other labs had been using (in the order of 10, 20, 50 lice per fish) it was extremely rare that we found lice on the fish after 7 days. Over time we developed exposure protocols that involved much higher levels of exposure. 'Low' levels of exposure in the study worked out at approx. 243 copepodids per fish. After 7 days on fish of approx. 20g this resulted in approx. 1 louse per fish, but this increased over time. Where exposure was 'high' at 735 per fish, after 7 days this resulted in approx. 15 lice per fish. You see a loss of lice in pink salmon (by 14 days significantly reduced level of lice on pink salmon) that you don't see in chum (numbers are very significant after 2-3 weeks of infection and eventually fall off).

Q: is this because lice or fish are dying?
A: there were no fish mortalities recorded. The lice are coming off, we assume they are dying but we're not finding them on the fish.

Q: Did you notice higher levels of infection in larger sizes of fish?
A: Didn't look specifically at this but didn't notice a trend in this way. The range of fish in the study from 3 to 70g and within that range we didn't see signs that size affected success of the infection.

A: Stages of development were 21 and 28 days. Water temp 9-10 degrees in a full saline environment. 50:50 adults to preadults and virtually all adults by 28 days.

Q: were adults producing eggs?
A: No. When study was ended at 28 days we did not find uvitreous females being produced at that time.

Q: did you see a sex distribution between 21-28 days?
A: Don't recall seeing that.

In the same experiment we took blood samples from pinks and chums side by side. We were reporting on the 'low' and 'high' levels of exposure. In pinks after 21-28 days the hematocrit or red blood cell volume is not significantly different either after a low or high level of exposure. In chum, which retained much higher levels of lice than pink, we saw significant reduction of hematocrit after 21 but not after 28 days. After high level of exposure we saw significant reduction of hematocrit after 7 and 14 days on chum.
No difference in cortisol in pink salmon but we saw chum salmon cortisol levels spike after 21 days of low exposure, but cortisol returned to control levels after 28 days – this ties in with when numbers of lice were beginning to fall off chum salmon at 28 days and stress is reduced.

Also measured expression of genes in these fish that were related to inflammatory responses. One of genes measured was Interlucan 8 which is an early mediator of cell migration into inflammatory lesions. We compared pink salmon that were exposed or not exposed and found that iolate? expression was very significant in early exposure. No evidence of it later on. In chum salmon we did see iolatic expression relative to controls but only later on during infection and when they were losing lice. Early loss of lice at day 7 in pinks and later loss of lice in day 21 and 28 in chum. Iolate expression seems to coincide with these observations.

Other mediators include tumour necrosis factor. Very significant expression later on in pink salmon and no significant expression in chum salmon.

In a similar study, size matched Atlantic salmon were added to equation. Exposed to copepodids in a similar way to previous study. Results are pretty similar but Atlantic and chum fall into the highly susceptible category in terms of their ability to retain lice infections and in pink salmon see a very significant loss of lice. Pink salmon seem to have an ability to respond quickly in an inflammatory way and somehow this rapid

inflammatory response (tumour necrosis factor) seems to be able to induce the rejection of lice from the fish quite quickly.

Have done some microarray analyses. Microarrays are ways in which we can measure lots of different genes and we compared the expression of lots of different genes in Atlantic, chum and pink salmon 7 and 14 days after they were exposed. The genes were either in the skin or the kidney of the fish and genes either increased or decreased in their function. Across species and depending on the time that you sample you see very significant differences in genes related to structural and immunological processes being turned on or off. The point really being that salmon are not just salmon, there are very profound differences in how each of these species are able to respond to infections. It is important that we understand that in a developmental context i.e. how do these processes change in younger and older juvenile fish and therefore what are the impacts of salmon lice on these individuals and populations of these fish.

In summary:

- *Lepeophtheirus salmonis* rejected from juvenile pink salmon ranging in size from 3 to 170g. This is significantly faster than from size matched Atlantic or chum salmon.
- Found that by reducing pink salmon diet (starving them) their capacity to reject lice remained significant even though their growth was stunted.
- In this size range of fish pink salmon avoided the clinical consequences of infection that we did observe in chum (e.g. reduced growth, reduced hematocrit and a stress response in chum).
- We believe that this significant earlier and more robust inflammatory reaction observed in pink salmon might be a mechanism by which these animals are able to reject the salmon lice.
- We still really need to know more about the effects of direct mortality associated with *Lep salmonis* among juvenile Pacific salmon i.e. what are the size related effects and what is the relative contribution of *Lep salmonis* to total mortality among juvenile pinks. We are seeing no evidence of mortality to date in this size range so perhaps we need to look more broadly at what are the potential contributors to mortality in wild populations.

Pink salmon are the smallest but the most abundant of the Pacific salmon. Their life history reflects this in that they have a 2 year life history: fish that leave the gravel and go to sea this year will return next year as adults to spawn. In streams that produce pink salmon we have this interesting process of odd and even year populations that are reproductively isolated from one another and may actually display quite different characteristics in some streams. In either case they are very small fish when they are entering the marine environment and coming in contact with diseases from the marine environment. Adaptations to compensate for this very small size include very rapid growth. We are very interested in the quality histological nature of the skin in animals of this size and track that skin development

during this time of weeks and possibly months after the fish enter the marine environment.

A micrograph of the skin of a 0.5g pink salmon after being in the marine environment for just a few days shows that it is architecturally simple (paper thin layer of epidermal cells) compared to a 2.5g fish that's been in marine waters for approx. 1 month where structure of skin is much more similar to adult salmon (very thick epidermis, mucous cells are abundant producing a protective mucous coat, the dermis is thicker and is producing scales). We think this is critical in understanding the transition from an animal that is more at risk to less at risk.

We conducted another experiment to understand the effects of the size of the pink salmon on the ability of that animal to resist infection. Used same approaches as described earlier of using reared pinks in the lab and we also took stocks of pink salmon from rotary screw traps in Glendale. Also took pinks from the Quinsam hatchery outside Campbell River. In the same year they were reared in the Pacific Biological Station and we sampled from those tanks at different times: very early on (fish approx. 0.3g); a few weeks later (fish approx. 0.7g); and a few weeks after that (Fish were approx. 2.4g). For each of these studies we exposed fish to 25, 50 or 100 copepodids per fish. Over a period of 5-6 weeks we monitored mortality in these fish; 6 days after exposure, the abundance of lice in the smallest group was about 4.5-5 lice per fish and over time we saw about 33% mortality. In the second group abundance was about 3.5-4 lice per fish and mortality rate was similar except for Quinsam fish which showed approx. 0% mortality. In the largest fish abundance was somewhat lower and no mortality was recorded.

Both Quinsam and Glendale fish responded in a very similar fashion (similar mortality curve) to this dose response study.

Here is evidence of this possible saturation effect where mortality after exposure to 50 and 100 fish really wasn't different at all and that pattern was repeated in both populations of fish. A 25 copepodid per fish exposure rate results in some mortality. Maximum mortality occurred with 50-100 lice per fish.

When is this mortality happening? Fish kept for 35 days. Mortality occurred between 10 and 26 days after exposure. No morts for last 10 days of trial. At end of study all of lice were adults but during the time of the mortalities 82% of the lice had not matured beyond the chalimus 4 stage (some were at earlier stages). This stage had not previously been reported to be pathogenic.

What were the attributes of the fish that died? 38 fish died and had a mean weight of 0.35g. The mean intensity was 4.7 lice/fish, on dead fish intensity ranged from 1 to 13/fish. Median density obtained using a bootstrap resampling process was 14.6 lice per gram (this allows us to compensate for range of sizes seen in fish and allows

comparisons to other literature) with confidence intervals ranging from 7.5 to 22.9. We chose the lower confidence interval arbitrarily as a threshold of lethal infection. What we said was – if you were a pink salmon weighing 0.7g or less and if you had an infection that was 7.5 lice per gram or higher then you would be at risk of direct mortality.
Q: Do you have data for distribution of lice on living fish?
A: Yes, after exposure all groups were sampled and the paper tabulates this data at 6, 12 and 26 days. During that same period these mortalities were occurring as well.

Q: During exposure fish were growing so what were their weights at 30-40 days?
A: 0.3g fish were about 1.5g at end of study. At approx. 25 days they were about 0.7g.

Like any laboratory study we have to define carefully the limits of our extrapolations. In this study we:

1) used healthy fish with no other infections (bacterial, viral or parasites)
2) applied a single dose exposure of sea lice (hit them with 100 or more lice at one time). In nature wild salmon are more probably exposed to ongoing or multiple exposures in their life history.
3) didn't measure a variety of sub-lethal effects so we are really only able to talk about direct lethal effects.
4) can say that death of juvenile salmon is not instantaneous. On average it took a little over 16 days for a heavily infected fish to die. What this means is that fish can be sampled in the field with levels of infection that exceed our threshold and can still be alive but on the trajectory to death.

Monitoring in the Broughton: DFO have been conducting surveillance efforts since 2003. Will talk specifically about data collected between 2005-2008/9. By using beach and purse seine gear we sampled juvenile pink and chum at over 100 sites as they migrated through the BA area. This is a very high density salmon farming area with approx. 15-20 active sites, producing on average up to 18,500 tonnes annually. Partitioned study area into geographic sections A,B,C.

We timed our samples every spring so that we would initially coincide with first wave of pink salmon entering the marine environment. Period 1 – March samples and so on monthly April, May, June and some years July. Data presented for 05, 06, 07 and 08. We see evidence that pink salmon size is increasing over the study period. Very dynamic system. First pinks enter system in March but they continue to enter the system well into May. We are most interested in small fish weighing 0.7g or less. A sizeable proportion of the fish sampled over each year falls into this category. 100% of fish caught in March fell into that category. In April virtually all were in this category some years and other years up to 50% of fish fell into this category. From year to year there is big variation in the rate at which the system is flooded with pink salmon. By May very few fish are in this risk category. By June and

July there are virtually no pink salmon falling into that category. March and April should be a key focus area where most of susceptible fish (0.7g or less) are occurring.

2004-2009 data from pinks and chum in BA. Surveys showed that approx. 60% of pink salmon were infected with *Leps salmonis*. In 2004 there was no March or April sampling but in months that we do have data it clear that 2004 was a particularly lousey year. It is also evident that the numbers have been declining ever since. In 05, 06 and 07 we see that there are levels of lice that begin with relatively low prevalence, peaks sometime during mid migration of the animals through the BA and then begins to decline. This low to high pattern is seen in all years but the magnitude of the increase is declining. In 2008 and 2009 our March samples were the first samples on which we saw no *Leps salmonis* on pinks - all of the fish are very small and none were infected. The pattern on chum is virtually identical in terms of the declining numbers. We did see a very small proportion of chum infected with *Leps* in that earlier time period in 2008 but not in 2009. The data indicates that over time there has been a very significant drop in numbers of lice on juvenile fish as they migrate through the BA. Part of the discussion of this group will likely be in explaining that.

Q: graphs show prevalence. Would you have seen anything different if you were showing abundance?
A: No.

Q: Assuming there is some sort of uniform density challenge, why do we not see susceptibility of the different species being reflected in the field data?
A: The overall abundance is similar over the years. Where you compare abundance or intensity on pink and chum within years you see that the intensity drops almost in a linear fashion – the lice numbers get lower and lower until the last sampling point where it's very hard to find lice on pink salmon and what you do find are virtually all adult lice at the end of the migration. What happens on chum salmon is that the numbers drop but the proportion of adult and new infections - if you compare early chalimus and motile stages you see a continuation of about equal numbers of both. The numbers are changing on pinks and chums as you go through the year but they are changing in a way that is quite different and reflects some of the findings we've seen in the lab. Somehow there's an opportunity for chum to become re-infected that doesn't appear to be happening on pinks.

Q: Can you comment on the geographical distribution of the 0.7g or less juveniles?
A: all of fish sampled in March are going to be between 0.3 and 0.7g no matter where we are in BA. Where we tend to see the numbers differing is that samples collected close to streams of origin e.g. closer to Glendale or Kingcome, etc. tend to be where we continue to see smaller fish as we sample later into the year.

Slide to bring together findings in lab with field lice counts: Lab studies determined a threshold of 7.5 lice per gram that when applied to fish that are equal to or <0.7g it is suggested that this might be a threshold for lethal infection that would be of some significance to pinks i.e. the risk of mortality directly associated with *Leps salmonis* is significant in the small size class fish when they are exposed to a sufficiently high level of salmon louse copepodid density. So the combination of small size and density of parasite challenge is key.

For each year and for the months within each year, what is the percent of all pink salmon that are <0.7g with infections that exceed that threshold? What percentage of the population we are catching appears to be at direct risk of mortality? The risk is enhanced in smaller size category and reduces as the animals increase in size. In 2005 ranging from approx. 8% of samples apparently being at risk declining to 0%. In 2006 the highest percentage was 1.1% and again declined to zero. In 2007 there was a prolonged outmigration of pinks into the BA and we see the risk was elevated in the middle of the migration period up to approx. 3% of fish. Since 2008 none of fish sampled fell into this risk category. Seems to show that the declining risk trend mirrors the declining level of parasite infection seen between 2005 and 2008 in pink and chum.

The questions we need to understand are what are the drivers behind this? This presentation does not have the answers to that.

Can summarize from the work that:

1) infections with *Leps salmonis* do pose a direct risk of mortality to small size class pink salmon. Lab studies suggest that if you are a pink salmon weighing 0.7g or less and if you are exposed to an infection density of greater than 7.5 lice per gram then you are at risk of direct mortality.
2) The smallest pink salmon are most abundant in the BA between March and May. This is an interesting time period because it coincides with the first 40 days at sea that Bob Parker 45 years ago estimated that pink salmon suffer 55-79% of their at sea mortality within that first 40 day period.
3) The total number of pink salmon juveniles at direct risk of *Leps salmonis* mortality declined from approx. 4.5% in all of the fish collected in 2005 to 0% in 2008 or in 2009. This coincides with a very clear decline in the abundance and prevalence of lice.
4) We are measuring direct mortality only in this study. There is a considerable body of evidence there that suggests that there will be mortalities that are not directly associated with the infection but are associated with indirect effects of the infection that are the result of the physiological or behavioural consequences of the infection. These data are not reflected in the presentation shown here and this is why the more conservative threshold of 7.5 lice per gram was used. There is need for more research to focus on the sub-lethal effects to provide a proper perspective of what the true risk is. This study shows that a relatively small

proportion of juvenile pink salmon appear to be at direct risk from salmon lice and that there is perhaps a need to be more holistic in how we understand the global effects that might be impacting survival of juvenile pink salmon during this period. If what Bob Parker said in 1968 that up to 77% of these fish are dying and we are seeing that only a 10% of these fish have salmon lice and a fraction, if any have infections that exceed a lethal threshold, then there are clearly other factors that are contributing to mortality in this species.

5) When you compare pink and chum salmon in lab studies, chum salmon appear to be far more susceptible to *Leps salmonis* than pink salmon. We don't have the data on chum salmon but suggest that there is a need to develop data on chum salmon given that they do develop clinical signs of infection and if we were to expose them to same lab studies as pinks we would expect to see significant mortalities as well.

Q. What would it look like if you doubled the weight of the fish from 0.7g to 1.4g? Would you still see similar patterns?
A: Yes. We did double the weights and we saw very similar patterns. In 2008 and 2009 it would still be zeros across the board.

Q: Can you do lab work on mortalities etc. and predict what is actually going to happen in the real world?
A: Always a challenge to put small lab studies into ecological context.

Q: We have 3 data points on adult pink returns for 2005, 2006, 2007. How does abundance of adult returns to BA compare to the risk exposures in this study?
A: don't have those numbers to determine a correlation at this point.

Brent Hargreaves mentioned that he has return numbers and some years you can see what might be considered a positive correlation, other years you do not. Trap you get into is looking at returns from just BA, if you look at bigger picture you see greater patterns of return from Alaska to Fraser River. This is not being driven by something happening in the BA, it's a much bigger thing where things are being driven in the North Pacific. This is only one component of the returns.

Much discussion on the importance of not looking at lice infection in isolation – taking a more holistic view. Ecological setting must be considered e.g. water quality.

Q: Do you think there is no mortality from lice in fish greater than 0.7g?
A: This study does not say there will be 'no' mortality but it is extremely unlikely from what we've seen in the lab setting. This setting is not natural though and does not account for other ecological factors such as metal levels in water etc. which can influence risk of mortality.

Q: did the study look for influence of infection on growth rates? e.g. does the inflammatory immune response seen in pinks come at a cost to growth?
A: chum salmon were significantly smaller at time if they had been exposed to infection. Pinks were not. So yes, a growth cost was observed in chum.

Sea lice parasitism increases predation vulnerability of juvenile Pacific salmon

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Experimental evidence will be presented which demonstrates that parasitized juvenile pink and chum salmon are more vulnerable to predators than are unparasitized individuals. In addition data will be presented from a series of experiments which suggest the ways in which parasitism influences vulnerability through changes in behaviour and swimming performance.

There are two ways that parasites kill their hosts. Directly (as discussed in Simon's study) or indirectly via increased vulnerability to other mortality agents. This might be disease, breached defences (holes in skin), starvation due to reduced competitive ability and predation.

The focus of this talk is on studies around increased mortality rates on juvenile salmon due to predation. We are going to provide evidence that pink and chum juveniles are more vulnerable to predators and experimental evidence of possible reasons for this: 1) increased risk-taking behaviour, 2) altered schooling behaviour and 3) changes to swimming endurance.

Predator selection:
60 pairs of wild caught pink salmon (one of which was parasitized with mean number of almost all adult *Leps* of 2.3) were put into ocean enclosures with a single cut throat trout predator. The pairs were size matched within each trial and the average fork length was approx. 79mm.

In the 60 trial runs, the parasitized prey was caught first in 44 of them which is a highly significant result using a binomial test. The outcome was unaffected by small differences in lice intensity. Time to capture and strike per capture were the same for both types of prey. This is the experiment where many of the lice transferred from the pink salmon onto the cutthroat trout during handling (trophic transmission). The experiment shows evidence that the parasitized fish are more vulnerable; however, the setting is rather unnatural. In nature it's unlikely that a predator would be presented with two prey (one with lice, one without) to chose between.

In order to address this 'unnaturalness' we went to another experiment of putting larger groups of pink or chum juvenile salmon (200 in total varying between 40-80mm fork

length) with groups of bigger coho smolts in a large enclosure. Allowed them to interact for 36-48 hrs and then measured what their size and lice distribution looks like at the end of the trial. This was compared to the size and lice distribution in a sub-group of 100 (unhandled fish) caught in the original seine haul that the 200 also came from. 13 of these trials were run. 7 with pink, 6 with chum. In control trials instead of using the predator (coho smolts) the opposite species was used e.g. juvenile chum or pink.
At the end we measured the mean number of survivors (approx 81-82 out of 200) and counted lice on each of remaining fish.

Results: Significant selective predation observed on infected and smaller fish. There was a loss in lice during predation period i.e. there was less lice on fish at the end of the trial than at the beginning, but similar patterns were noted in the control trials. The decline in the predation trials was significantly related to initial lice abundance i.e. where initial lice abundance was high there was a greater decline in lice abundance during the predation trials, suggesting that lice and vulnerability are associated. There was also a detectable though weak relationship between the number of fish consumed during a trial and the average abundance of stage lice at the beginning of the trial i.e. the more lice there were at the beginning of the trial the greater number of fish were consumed during the trial. There also tends to be a shift upwards in the size distribution of the survivors relative to those that went in at the beginning – this has been found before in other studies, that there is size selective predation on smaller fish but what's novel about this study is the reduction of lice numbers (all lice stages) on the survivors relative to what they were at the beginning.

Conclusions: It seems that pink and chum juveniles are more vulnerable than their unparasitized con-specifics to predation by coho and cutthroat trout. This is true both in short-term experiments with paired fish and in longer terms experiments with populations of predators and prey interacts (which mimics field better).

Reasons for increased vulnerability?
Three experiments suggest reasons:

1. The experiment done by Paul Mages is about risk taking. He worked with pink salmon in range of 70-80 mm fork length and compared naturally infected to uninfected fish. Naturally infected fish are those which had 1 female *Lep* on them. Uninfected fish had no lice present and no evidence of prior infection (no scars, no predator teeth marks, etc.). Had 7 groups of each type to compare and within each group he had 33-41 fish. He was interested in testing the hypothesis that the infected fish would be more willing to take risks to access food than the uninfected fish. Test involved placing food in a central ring, shining bright light underneath ring (high risk zone), darker area outside this (lower risk) and fake kelp in the corner. Once a minimum of 60% of fish started feeding at the ring he dropped a heron model (heron head on a stick) into the water. It would fall and make a splash, the fish would scatter and he would measure the time it would take for 50% of the original feeders to come back after the simulated attack. The

infected fish returned three fold quicker in an average of 140 seconds (higher risk takers) than the uninfected fish (463.9 sec). That is an approx 5 minute difference in return time.

2. A study on schooling behaviour was done by two students in U.Vic. They took a group of 30 chum salmon matched for size; 1 was infected, 29 of them were uninfected. They let them swim in a shallow (keeping things to 2 dimensions) and circular (you could create a current where fish would school in) pool. Every 6 mins for 3 hours they would take a photo, 30 photos per trial, 16 trials. Fish mass varied substantially from 1-4.5g. From video analysis they would look for 3 things: 1) the nearest neighbour distance (from tip of nose to closest other fish in any direction): when zero they are schooling as closely with their neighbour as any of the other fish; when it's positive value they are further apart from neighbour than they would be if they were unparasitized. In smaller fish in particular they tend to be further away from neighbours than if they were unparasitized. This effect disappears completely for larger fish; 2), whether the parasitized fish occupied a central or peripheral position: for smaller fish there is a much higher likelihood that they are in periphery. This effect is absent or may even reverse as the fishes get larger; and 3) whether the parasitized fish was at the front or the back of the school: similar increased probability that parasitized fish will be found toward the back of the school and this effect disappears as fish get larger. **Overall findings:** parasitized fish are significantly more likely to be in the periphery, in the back of the school and have a greater nearest neighbour distance than unparasitized fish and these effects are reduced in larger fish. Fish at edges, back and further away from their neighbour are more likely to elicit an attack because they stand out from the rest or they are more likely to be captured because they are in a more unfavourable position relative to where the predator is attacking from.
3. Swimming endurance work done by Paul Mages in the Broughton. He was working with wild caught pink salmon with mean fork length of 55mm. He had naturally or experimentally infected fish and conducted prolonged swimming tests in swim tunnels in Echo Bay. He put fish in and gradually increased velocity to an initial velocity of 8.25 cm/s and every 5 mins thereafter he would jump velocity up by 2.75cm/sec until the fish failed – that is they couldn't swim anymore and had to rest against an electrified grid for more than 2 secs. Maximum velocity system could get to was just over 38 cm/sec. The study calculated Dmax which is maximum total distance swum by each fish prior to failure: the time swum at each stage during velocity increases multiplied by the velocity of each stage and summed for all stages swum. **Results for naturally infected fish:** 42 of each with a minimum of 1 *Lep*, mean of 1.31 and most were males and preadults and they also had chalimus scars. Uninfected fish had zero lice and no scars. There is significant effect seen. The median distance swum was very similar and although the trend is that the ones with lice drop out sooner, the difference is not significant. So, apparently naturally infected fish do not

reveal to us any effects of sea lice on swimming ability. **Results for experimentally infected fish:** sample size 37 pinks (55mm +/- 2mm fork length) broken into 4 categories: 13 with 1 adults female; 12 with 2; 9 with 3 and 3 with 4 (placed adult females manually onto fish in shallow water). He also had 17 controls who were sham infected. **Findings of survivors:** the median distance that fish swam were less than for control. Those with 1-3 adult female lice had similar performance, those with 4 lice had way poorer performance but unfortunately a very small sample size. As number of adult female lice increased the distances declined in a continuous fashion and this effect was highly significant and the effect was greater on smaller fish. This effect is possibly an underestimation of the effect of heavier lice loads on small fish. He set up 16 small fish with 4 female adult lice and 13 of them died in the space of 2 days before test began.

Concluding arguments:

- Altered schooling may make infected individuals more likely to be attacked and/or captured.
- Reduced swimming endurance may increase predation susceptibility if it makes infected individuals less likely to escape from a coursing predator such as a coho or cutthroat trout that chase them down repeatedly. Another potential implication is that they may also have a slower seaward migration, they may have to stop and feed and this may increase the time that they have to spend in coastal areas with predators longer than they would otherwise or they may have more difficulty feeding.
- Some of these negative effects e.g. a lower swimming endurance could be compensated for by example increased feeding to make up the energy deficit but what comes with that is an increase in risk of predation. The result is that pink and chum juveniles may suffer increased mortality from predators as suggested by enclosure experiments.

Caveats:

Pink and chum were used as more or less interchangeably in these experiments because we viewed them as ecological equivalents; however, the data suggest that physiologically they may be quite different so maybe this wasn't a fair assumption.

Naturally infected prey may well be a subsample of all the prey out there in that they are the ones most susceptible to parasitism or they may be the ones that are most susceptible to predation because for example they can't swim as fast or there may be other pre-existing reasons like for example disease. Conversely the ones we get out there with natural infestations are the survivors and maybe the ones that are most able to tolerate lice. So we don't know if we're measuring the survivors and fittest or those who were unlucky or those who for some pre-existing reason had more parasites. This is one of the problems of using naturally infected prey but experimentally infecting fish doesn't necessarily solve the problem either because not all the fish are equally likely to

become infected or survive that infection. What you end up getting is not necessarily a random sample of fish and what you get when you test a few weeks later is not likely a random sample, even those that got parasitized. Not sure how to deal with this very difficult problem.

Consequences and Implications:
We argue that studying mortality or morbidity in parasitized fish in lab tanks or in net pens may considerably underestimate true mortality rates in the wild. To understand consequences of infection on individuals, one has to look at these individuals in the context of their natural ecological community with all of the mortality agents that includes, especially predators. Mortality rates may be much higher than estimated to date than estimated by these other kinds of experiments. Consequences at the population level is the next step and that is covered by Marty's talk.

General discussion and a question around Parker's paper in 70's where bulk of pink declines were attributed to coho predation.

Larry: would caution referencing this paper too much because the population of the predators has probably changed significantly since then too.

Brent: you see huge variability from year to year depending on how big coho return is. An interesting observation in the DFO datasets – quite often the largest fish that we find are actually parasitized. Never really understood why this was but perhaps their swim speed is slowed down and it has been around longer but we are catching them mixed in with smaller, younger fish?

Craig – it would be useful at the end if we could agree on some research methodologies for better understanding some of these issues further. Has any further study been done on parasitized fish flashing/jumping and whether this behaviour attracts predators?

Larry: no further studies have been undertaken. Some of the video footage of predator interactions shows coho keying in on fish that are flashing. These behavioural changes seem to catch their attention more but no definitive evidence on this.

Models for morbidity/mortality studies
Dr Martin Krkošek
NSERC Postdoctoral Fellow, School of Aquatic and Fishery Sciences, University of Washington, Seattle, USA

In this talk the types of data that are available from observational/experimental sea lice infection trials will be discussed and a modeling framework for understanding the population dynamics of sea lice and juvenile salmon outlined. The models link data of sea lice abundance through time with data of salmon survival through time in order to

estimate important parameters such as sea lice survival and the rate of parasite induced host mortality associated with sea lice developmental stages.

How do you take Larry's results above and analyze them with a view of scaling from the view of individual to population? What does all this experimental work that we have done tell us about the population dynamics at the level of a cohort of juvenile salmon and then at a salmon population level?

Experiments: Using both experimentally infected and naturally infected fish. Data analysis methods are very similar.

Conceptual model: things we are going to think about in model include: average abundance of parasites per fish and number of juvenile salmon through time over the course of one of these experiments. There are also some things linking these: the average number of parasites per fish is related to the number of free living copepodids there are in the environment (this might be a constant number that we are exposing salmon to in bucket or it might be the number of larvae in the environment the fish are swimming through before we capture them). The larvae are in the environment and are attaching to and colonizing these fish. Some of the parasites die because they have their own intrinsic mortality and then some may also contribute to the death of their host and for this study we are going to assume that if their host dies, they die too.

In terms of the cohort of salmon – there are no births etc.; we are just looking at a group of juvenile salmon through time. There are some demographic rates/life history parameters that link them – these include: 1) transmission coefficient – the rate at which larvae can attach to a fish; 2) mortality rate of the parasites on the fish, independent of host survival and 3) mortality of the parasite that is related to host survival/death. Rate of parasite induced host mortality (3) is a really key parameter to understand when thinking about host-parasite population dynamics. This is a key focus of study - how to estimate this and how do to do statistical tests with it.

For juvenile pink salmon – they are dying and they are dying according to parasite induced host mortality but there may also be some natural (non sea lice related) mortality as well.

A. The juvenile salmon process.

Exponential growth model – very simple model with a birth and death rate. First off there is no birth rate – E = 0. Death related is to non sea lice sources and mortality related to parasites.

B. Model is a bit more complex for parasite growth and mortality rates. You can solve that, just like you can solve the exponential growth model. If you take initial population (initial number of fish used in experiment) size and set it equal to 1 (redefine as Q) you have a probability of surviving to some certain time point t.

Summary of steps involved in analysing these kinds of data:

1. We need a model for parasite population dynamics (all assumed to be *Leps* in the model) – we can estimate through maximum likelihood.
2. We need a model for host population dynamics – we can estimate through maximum likelihood.
3. You need 1 to do 2. But once we have this we can do some interesting things, e.g. what are the rates of parasite induced host mortality? Can construct confidence intervals and statistically test if they are different from zero. So, is there an impact on the survival of the host fish for that particular developmental stage? We might be interested in knowing if the impact of host survival changes with parasite stage – so, is alpha for an adult female different from alpha from chalimus 4 stage?

Things get more complicated if in the field your larval development rate is not constant, so not just placing fish into bucket with lice for 2 hours but you need to consider if your fish have been in the field for a couple of weeks exposed to larvae before you collected them. You need to model exposure to larvae and when you do that you also have to model the age distribution of lice. You have to move onto more complex partial differentiation models to do this.

Field trials: We caught approx 5000 juvenile salmon in one beach seine catch, randomly distributed them into approx 20 ocean enclosures with approx. 150 fish each. Over the course of 35 days we monitored abundance of sea lice and recorded survival of juvenile salmon through time. At each sampling point we would collect half the fish from one enclosure and would count lice on them and then let them go. At next sampling point would do same thing for remaining half then let them go and move on. Sampling size is decreasing over time but we need to do this to prevent stress and damage through handling and therefore bias. We get high resolution when the dynamics are fast and lice are developing quickly but it is staggered enough that we have fish long enough to observe development through the older stages.

Interesting outcomes:

1) lice have awful survival and 2) fish have exceptional survival (they are able to rid themselves of lice quickly and are robust to infections). Usually by day 20 the abundances of lice have dropped to near zero levels. 3) the average life span of a motile stage louse that comes out of this model was approx. 4 days. This is different to pink salmon in the wild and definitely different to results seen on other species of salmon that we have data from (where adult female lice have been seen to survive weeks to months). Not quite sure why results are such but one hypothesis is that when motiles are moving from one fish to another (in the water) they are possibly being eaten by juvenile salmon in the enclosures. Note: juveniles were fed every hour during daylight hours.

Had very few fish mortalities in the trials. Limitations: Alpha values might not be very good as motiles never survive past per-adult 1. Probably underestimating alpha.

Observations in early years 2001-2005 we saw 30-40 lice per fish. A school of fish could have 99% prevalence and you could actually see fish dying. How do we reconcile that with results we are finding? Hypothesis around differences between lab and reality:

1) that we're not getting exposure times right in experiments. A few hours or day and then put in an environment where they are no longer exposed to sea lice. We know from previous work that fish are migrating at approx. 1 km/day and are moving through a zone of elevated sea lice abundance approx 80 km long. That's a big difference in exposure time. In terms of the population dynamics it means the difference between an immigration and death process where you have continuous new lice attaching on to fish and dying through time as opposed to just a straight mortality process from some initial population size. When you model it with a very brief exposure time you can replicate the pattern you get out of the experiments and with the same parameter values and same abundance of L sustained through time, we can replicate the same patterns we see in the field. This indicates that there is good cause for further research in this area as what we are seeing may not be representative of what happens in the field. It might be the case that parameters change with exposure, maybe fish become more or less robust through time.

What else can we do with alpha values? We've got all data of juvenile salmon migrating through BA, we know how many lice they have on them, we know how fast approximately they are moving, we can then combine this lab model and stitch it together with model of salmon migration and dispersal of lice and estimate what the mortality is of juvenile salmon as they move through BA. It is a continuous process of integrating all of these processes as the fish are migrating out to sea and we can arrive at estimations of direct parasite host mortality as the fish migrate through the system. We can also look at conditions for persistence and extinction. We now have a model for juvenile salmon and sea lice population dynamics and the modelling for pink salmon population dynamics is also well developed so we can stitch these two things together and ask – how much mortality does it take when we add that mortality onto a Ricker model to change this population growth rate to something which allows persistence to one that leads to extinction? What are the numbers of lice in the environment and the duration of exposure that change the system from a regime of productivity to one of local extinction? We can show this as well.

So far everything shown has been direct parasite induced host mortality, we have not considered indirect effects. If 80% of these juvenile salmon are going to die anyway, likely due to predation by other fish, we need to be thinking how that predation interacts with infection and is this compensatory or not compensatory? Larry presented work on that and his experiments say that it's very likely that they are not independent and that there is an effect that might make the fish more prone to predation. We took a look at this from a theoretical point of view – looking at predator-prey population

dynamics. One way that the predator-prey population dynamics are represented is through type 2 functional response where you plot rate of predation per predator on host population versus the abundance of prey population. As the prey get increasingly abundant the predators get saturated, they are not limited by abundance but by how quickly they can process their prey. Intermediate to lower abundances of prey then there is an increasing relationship with predation rate on that host population. 2 parameters in this model 1) the rate of at which a predator captures an item of prey (gamma) and 2) handling time or length of time it takes predator to consume prey. We know from Larry's experiments that lice are probably making fish more prone to capture but modelling shows that the lice do not change the handling time (the time it is taking for predator to process prey once it is captured). What this means is that as you increase the parasite abundance it pushes the curve over to the left. In terms of the population dynamics this means that either a) lice are not affecting rate of predation on prey population but predators are selectively removing infected fish – predators are doing us a favour by reducing the sea lice population without effecting the prey population, or b) as juvenile salmon abundance gets lower the predator switches from doing us a favour to accelerating the decline of the salmon population. So, the subtlety in the dynamics of how lice might effect the predator-prey interaction is complicated and depends on lots of factors and in some situations may be leading to a benefit and in other cases leading to a demise.

Q & A: the time taken for a predator to capture, eat and digest prey depends on their relative size of a fry to a smolt, but in general in these studies the time ranged around 1 fish/day.

A plot over a parameter space of two things that we don't know very much about in terms of how it is scaled up to population dynamics - 1) the intensity of exposure of juvenile salmon to lice (beta times L), and 2) the strength of the effect of lice on the capture rate of the predator. If you go left to right on the plot you would expect to see the number of lice increase on juvenile salmon as the number of lice in the environment increases. As the predation regime is also increasing those predators are removing those parasites from the fish population. If we combine this plot with a Ricker model and also plot on the same axes the population growth rate (where zero is threshold between persistent and extinction, productive populations at the top and declining populations at the bottom), you have a situation where predators might be reducing sea lice population and simultaneously driving prey population down to extinction. This worried me because when we are looking a number of lice on juvenile salmon in the field we might be trying to relate the productivity of these populations relative to sea lice abundance on juveniles, but if we are not paying attention to what the predators are doing, we might be in a confused situation of few lice and declining populations and not understanding why that might be if there's a change in the predation regime. We haven't been paying attention to predators and we need to start thinking about it.

Q: Is the graph saying that if the true infection pressure is anything greater than 0.02, unless we've got a very unlikely scenario where the parasite induced predation is so low that it is non-existent, you would potentially have a problem?
A: You could. It depends on predators is the key message and there hasn't been enough attention on this aspect to date.

Closing conclusions:
We really need to be able to test these predictions and have an independent look at these processes. We talked earlier about how we can compare return abundances in the BA relative to changes in sea lice abundance - there are methods to do this but we need spatial comparisons to this right. One of our papers from a couple of years ago compared population dynamics of pink salmon from BA relative to an unexposed area to north. The reason we need these paired spatial comparisons is that there is a lot of environmental stochasticity here, a lot of variation which pink salmon are famous for. When it is a good year it tends to be a good year regionally and if it's a bad year it tends to be a bad year regionally. When we are looking for effects of sea lice on productivity of these populations we can't look at these in isolation as we are likely to mistake environmental stochasticity for effects of sea lice or something else. What we really need to do is look at the difference in productivity between paired populations and try to attribute differences to say management changes or changes in sea lice abundance.

Q: what happens if you run model with one day exposure versus continuous sea lice exposure?
A: graph on left is 1 day exposure. On right same model, same parameters, same number of sea lice present but they are there continuously over 80 days and the divergence in the model is seen on graph.

Q: It seems from data seen in BA that irrespective of the year there is a pattern of copepodid, early chalimus infection in March/April and development into mainly motiles by May and June. This seems less like continuous exposure but more of a changing exposure pattern through development.
A: From 2004, Knight inlet to Tribune data you do see that pattern because lice are aging over the season however, at the end of the season while population might have increased a bit in the motiles, we are still seeing quite high numbers of young stages there as well. I think we are seeing both things through time and we could tweak the model to represent this.

Q. based on Larry's data less than 3 motiles per fish therefore no increased risk of predation on that one, could I put those 2 sets of data together?
A: It does that.

Comment from Larry Dill: I have been critical of extrapolating from lab to field in past but even our field experiments are still in a rather unnatural environment, so we can see

effects but I think it would be dangerous to start talking about specific thresholds of 2 or 3 etc.

Q: what does return data look like in relation to what we know about exposure in the years since 2006?
A: After this fall we will have 3 years of replication across all these populations and it's arguable that this might be the minimum number of data to allow us to have confidence in doing some spatial comparisons, so it will be interesting to do that, but based on data from last two years things look a lot better. So, when sea lice numbers were high we had population growth rates that were low and negative and now that sea lice numbers have gone down because of management changes I think we are going to see something that may be positive or not different, but we need to do that analysis.

Q to Larry: is there a critical size for the pink and chum when they were no longer considered prey for the predator?
A: yes, don't know number offhand. Brent: predators can take up to 60% of their own body length as prey.

Q: So considering growth rates of both pinks and coho at what point would the pink salmon population no longer be considered 'prey'?
A: Brent - pinks are growing way faster than coho. It's usually by about early-mid June, July on North coast.

Marty presents slide: 3 surveys from early, mid and late season in 2004 for Knight Inlet to Tribune Channel showing all lice stages. Used spatial patterns in data to try and estimate how many lice are coming from natural and farmed sources. 2004 was the year with highest sea lice abundances (in the time that MK has been working on this topic). The slide also shows what things look like in 2009, same scale on axes, same study design, the only difference is the management regime.

Question from Stan to MHC: what has changed in management practices in this time that might account for these drops in sea lice levels?
A - Diane: in the last 2 years we have started treating earlier (December and January). We've had roughly same production levels for last 10 years.

MK: if you plot the time series of when treatments happen they have been happening earlier and earlier and you can see the number of lice on the farms declining after those treatments. I think this year was the first year where it was early enough that it bottomed out before the migration started and it stayed really low throughout the migration. I think the timing of treatment and early harvesting has been synchronised to wild salmon migrations and this looks like it might have worked for this year in question at least.
CR: Q to Brent and Simon – was 2004 data significantly different from other years?

Brent: we started collecting data early in 2003 but we didn't start sampling in 2004 until mid-May and so missed the early data; however, the lice levels encountered in 2004 were dramatically higher, even in mid May when sampling began so something very different was going on that year.

Comment from Sonja: difficult to look just at management practices alone, environmental factors such as water temperature and salinity vary from year to year because it is a dynamic outflow area and these can be important factors.

Brendan: It was mid May when the fresh water came in and production of lice eggs in 2008 shows quite a build up during the fall and late winter as expected. Following treatment there was a rapid reduction in egg production (70-80% reduction) just in time for outmigration. 2008 was the year that I think in the first survey where none of the pink were infected. Similar in 2009 so I think it was a combination of treatment and/or fallowing that really knocked down the source to very low levels.

Marty: these are the data asked about. They are complicated, but the grey and black lines show total number of lice in Tribune Channel and then time averaged to smooth them. The red region on each plot is the window for juvenile salmon migration and the vertical dotted lines are when treatment happened. You can see that treatment used to happen kind of late in outmigration and now it is happening before and the lice numbers have really bottomed out during outmigration.

Crawford: one of the reasons we're trying to get this CAMP initiative going between MHC and CAAR is to let people like Marty, myself, Brent and other scientists take a more thorough look at some of this data and to try and see whether we can find more of those causal associations we feel will help policy and management decisions in the next 5 years. It's nice when everything aligns but it would be nice to ensure that the particular policies that have been implemented are the reason for certain kinds of change so we know that is actually going to give us benefits going forward.

Sonja: the interesting thing about 2004 as shown on Marty's slide is that in all the other years, regardless of treatments, it looks like the lice numbers were really quite high before the fry outmigration. In 2004 starting at the outmigration, the numbers had dropped in the farms and they started almost immediately going up - it looks like a different pattern all together in 2004. It seems like it has shifted for some reason and that is what we were seeing – that there was a sudden spike in May June that didn't really make sense and it still doesn't make sense.

Brendan: 2004 is the only year where there were 3 SLICE treatments during the outmigration, is that right?

Brad: No, the three lines are three different farms that all got treated in mid May, early June because lice levels on those farms were negligible in February and March and they

spiked up in May in conjunction with outmigration. The decrease you see on the graph is probably due to a) harvesting and b) treatment of some of the larger fish that had higher numbers in the fall.

Sonja: all data shown in graph that treatment was occurring around the spike. This was also the case in 2004 but it just happened that the spike was later and coincided with outmigration.

Marty: I think it reflects a shift in what's driving the treatment. Before it may have been more reactionary to lice on farms and now it's more oriented to when juvenile salmon are migrating.

Sonja: if you ignore all of that and look at the pattern it's still the same. You're still treating at the spike and it could be a real spike or an artificial spike.

Marty: well it's always going to be a spike because lice numbers go down after you treat.

Crawford: we will never unpack everything here but it is important for us to unpack some of the mechanisms so that we know how to plan for these things going forward and what likely impact different kinds of strategies will have.

Diane: for myself as a manager I want to feel confident that what we are doing is the right thing. Is it having an effect or are there other things at play here or is this just serendipity? I would like to have more confidence and when I look at your models I am thinking, how would I use your model to predict or manage time treatments etc.

George: I think that was a question on where are you going with the model now? Is it making the model better, is it parameterizing it each year, is it using predictive model?

Marty: it's interesting to be looking at these data now and this represents an exciting modelling opportunity with some important applications and that is one thing that we want to do. And then we have a spatial transmission model where in the past we have considered the source of lice at the salmon farms to be at steady state and when you look at this you realise that this may not be the best assumption. So we are looking at relaxing that assumption, combining these data with the model which calculates the full temporal and spatial solution and fitting it to an entire season's outmigration. That is quite an ambitious model fitting exercise. We have quite a lot of it coded in MATLAB but we don't have it running efficiently enough to actually fit it to data yet.

In the end what we are hoping for is a quantitative framework that links these dynamics with the dynamics of juvenile fish migrating through the system with estimation of mortality.

End of Sessions/wrap-up discussion

Crawford: an additional comment from Craig on Marty's results.

Craig: since timing of SLICE treatments in 2004 engendered so much discussion in terms of the fact that they seemed to be after the migration, I just went back to one of my papers which looked at MHC web posted data from 2003-2004. I don't have the exact location of these treatments but there were 4 SLICE treatments on MHC farms in the Broughton before the end of Feb in 2004 but I'm not sure if they were in Fife-Tribune.

Brad: I can tell you that Sargeants and Humphrey were treated on May 14 in 2004 and Doctor Island was June 05/06. Wicklow and Glacier Falls were in later in June.

Craig: So the four treatments in Feb were farther away from Fife Tribune then?

Brad: Port Elizabeth rings a bell, possibly Swanson, Mid-Summer, Arrow Pass.

Crawford: this is an interesting exchange and I think one of the reasons why CAMP has been set up is because in the past people were trying to ascertain information from a distance or from the bits of information they could see posted on websites etc. and sometimes there was misinterpretation of data and one of the purposes of this initiative is to make all the data from 2003 to now available through a data sharing agreement to make sure we are all working with the same data sets and that questions around interpretation don't rise or fall in terms of people not getting access. I think we are making progress on this within the BAMP group but I don't want to focus on this now because it's not the purpose of this meeting but just to say that hopefully this will help to address some of the other issues going forward.

Do want to look back over the day and get everybody to put down 5 or 6 key things that we agree on and that may have some implications for policy and 3 or 4 things that there seems to be a fair amount of disagreement or ambivalence on or that requires additional research. Finally, what are some of the other gaps in our thinking and open ended questions that need to be answered through research?

Messages/things to agree on:

1. Tony: **pinks and Atlantics in terms of louse effects differ considerably and what follows from that is that Pacific species are highly likely to vary amongst themselves too.** A gap: does that continue over from host populations to parasite populations too? Maybe the parasite behaviour differs on different species, are there genetic differences, different propensity to resistance? Diane: when I look at different farms in different locations we do see different host-parasite relationship – it could be the regional environmental factors. What is best way to study this and is it important to do this? Simon: it probably wouldn't hurt to have some bioassays established on the West Coast for testing of medication efficacy.

2. Craig: **Studying morbidity/mortality of parasites on fish in lab tanks and/or net pens may considerably underestimate true mortality rates in the wild.** Crawford: you could conversely say, based on the kind of model Marty gave us that under certain assumptions you might also overestimate mortality. Perhaps we could more generally say that you shouldn't extrapolate in either direction? Craig: would like to hear what others have to say. I think Larry had some good points about this and we haven't fully tested Marty's model in terms of what the parasite issue really means. Brent: my experience is that I take lab and small container field results with a grain of salt. They can give you a lot of insight but there are a lot of things going on that are unnatural. If you change the size of the container in tests the results change again and again. Simon: some lab studies have shown repeatedly that there is a high degree of risk to Atlantic salmon and sea trout from salmon lice. When you take these studies and apply them to the field you find a strong level of support that lice are part of the process contributing to problems in wild salmon populations. In that case we've shown the opposite – that pink salmon under similar lab protocols we had tremendous difficulty in inducing mortality and in a comparative sense we see that pink salmon appear to be far more resistant than chum salmon are. If we are going to be cautious about applying lab studies to field situations, we have to be equally cautious about suggesting that they are going to be harmful in the wild when the potentially might not be. My suspicion is that we may not be properly estimating what the impacts are but we are starting to get a sense that we can make relative comparisons among species but what we are seeing so far is that pink salmon appear to be relatively resistant compared to chum salmon and certainly compared to the species investigated in Europe, in a relative sense we are beginning to see some trends. Larry: I don't think that we can say that. I think we should pay more attention to indirect effects. It may true in the laboratory but not in the field. In the field there may be something about the biology of chum which makes them less resistant, they grow faster, they get out of predator size class quicker so maybe the pinks are more vulnerable in the field despite the fact that their immunological mechanisms appear stronger? We need to spend more time considering these indirect interactions. A really important one is disease.
3. Tony: **I think we have been hearing that threshold number might be somewhere between 1 and 2?** CR: Whatever threshold number is, it needs to be linked to size of juvenile, the timing also seems to be important (i.e. there are critical times and shoulder times – March to May) where juveniles are outmigrating. Sonja: it also links to prey window – 65% of fish are out of predation window by June. Marty: one precautionary approach for setting the window and controlling all of the activities we don't fully understand is to consider timing of adult returns. Typically in the absence of farms, juveniles would not encounter big populations of lice until the adult pinks return in July (in BA). Setting the window around this timing would recreate closer to what happens naturally. Tony: that sets first boundary condition. The second boundary condition is: 1) what is happening on the farm and 2) is there an

output from the farm? And what is happening in the wild population? Maybe use a sentinel cage out there measure what the true flux is and would have to be tied to what is on the farm. Larry: what do you put in the sentinel cage? Atlantics or pinks? Tony: putting pink in there may not work. Mesh size would need to be really small and might clog up and lice might not even get in. Larry: If you put Atlantics in you've got the same problem we were talking about. Brent: Maybe we use sticklebacks – they are sponges for lice. Sonja: Simon showed an interesting piece of data today that I hadn't seen before where he had exposed Atlantics and had compared similar sized atlantics to chum and pinks and the Atlantic salmon actually had a higher abundance. If you want to use a sensitive species in the sentinel cage based on that data I would say that Atlantic salmon would be the right sentinel animal. Tony: it acts as a big sink, the problem with putting pink in there is that they could shed them off before we actually sample. Larry: it acts as a big sink but it doesn't allow you to estimate the parameter values. Tony: I'm not suggesting that it does. It is a surrogate for it if there is a high output. In toxicology they but these hexane bags in the Fraser river and the hexane just sucks all the lipophilics [?]. It doesn't tell you what the exposure risk is, it's just telling you what's there and if you get more in the hexane bag in one year or one month then it tells you that you've got more output into the system. What it does is give you a relative measure and if we can find a way to calibrate this to real stuff by linking that value to the monitoring efforts that would be phenomenal. Dario: we have some of the monthly monitoring data from the farms to make an estimate of the egg production from the farms but there is large uncertainty in those estimates because of egg viability coefficients, the salinity effects, mortality of the nauplii stages. Those are very uncertain parameters not least that we have monthly data and we don't know on a daily basis how much that production varies over that month, but we have begun to make those estimates of production from the farms that you can then try to tie to a sentinel cage or you can measure it directly with a sentinel cage and try to use that as a surrogate parameter. Tony: I think that still becomes another level of assumption that you have to make whereas if you've got lice actually making it onto a fish and that's it. For example, if we know that all the pink and the chum that move through the BA never make it down to 30m, what happens to the lice that are released in the daytime hours when the caged fish move down to the bottom of the farm cages and they are pouring out some proportion of that. I asked you about your model how you treated that 30m depth. Do you bring it up to the surface? Dario: we had to because there is some uncertainty about the vertical migration of these lice. The region studies indicate that there is diel migration yet Lewis and his column chamber studies couldn't find a preferred depth but there were criticisms that his study didn't run long enough so that experiment is being repeated in Norway next week. So we had two – we had passive behaviour and we had diel migration. You can start to focus in on those issues and identify them as research topics so you know what to do in your simulations when you are making your estimates. Tony: ok – just put the canary

out like the miners did years ago. Brent: I don't think we should discount the possibility of using stickleback for that. Practically they would be great – they are tough as nails, you can keep them in culture, you can move them back and forth from fresh to salt water really easily, all of our field monitoring results show that stickleback are way more heavily infected than pink and chum – they are almost an ideal host for these things at this early stage. Crawford: is that across all stages? Brent: we don't see adults on stickleback and I think that's because they eat them off each other. Simon: it probably doesn't matter that we don't see adult stages if we're only doing a 2 week exposure we are really measuring relative settlement of larvae. Crawford: one of the things I'm hearing is that DFO won't be undertaking the detailed level of wild fish monitoring that they have been doing in the past due to financial constraints and so if under CAMP/BAMP if DFO are involved with a scaled down level of wild monitoring perhaps we should look at placing some appropriately sited sentinel cages as a good way to get some additional data to plug in some of those gaps and unknown? Bengt: not disagreeing but we were talking about it during the break that each farm site should each have a sentinel cage to monitor production and the efficacy of delousing on the fish farms. Brent: I don't think there's any question that some monitoring has to continue but at what level is the question. I think I can say from the department's perspective that we expect industry to take that on as a responsibility eventually. Again, we don't know what that looks like just yet so one of the challenges to this or any other group is: how do you do that monitoring to get the data that everyone needs to feel comfortable that we are getting a good indication of what's going on? Is it sampling like we've been doing? Is it scaled down sampling? Is it sampling plus sentinel cages? It's a practical thing of cost and the data quality coming out of it so I think this whole group of people that are interested in this issue need to start thinking about what that should look like, not just in the BA but broader than that. Crawford: I am encouraged to hear that. I agree we have to have that discussion, but it seems to me that one of the outcomes of trying to identify where we still feel uncomfortable or where we still have some of these gaps will be through ongoing monitoring. I would be loathe to see you losing this kind of historical data set by switching to something totally different. I would hope there would be some level of effort to maintain compatibility with what happened in the past but I think if we can add some other dimension to the monitoring through something like sentinel cages so much the better because that might allow us to address some of these unknowns. Craig: I like the idea of the sentinel cages. We wanted to study with MHC earlier and try some experiments with sentinel cages and I think there's still some utility to that but the question still comes out from the objectives of this workshop – what is a safe level of output from the farms that we are monitoring on these fish, whatever we happen to use in sentinel cages, so that we don't have population level effects on wild fish and I understand that it varies by species but as a practical management question - what is a safe level? Is there a management threshold on wild fish that we

shouldn't be exceeding? I'm still kind of struggling with that and following on from that, is there more work that CAAR and MHC need to do or is it something that has already been done that we can rely on for those management questions to be answered? Brent: from my perspective, I think what we need is some way to either directly measure or index the sea lice on wild fish so back from that we can say well, what safe levels from various sources of those things, farms being one of them, maybe the only one I don't know. The sentinel cages feel like one possible tool in the toolkit because if I could somehow make a relationship between what we see in the continued wild sampling to what we see in the cages I think I would be much more interested in asking the farms to do some cage work than I am to let them go beach seining because it's difficult and it's dangerous and I'm not sure I can interpret the data from people that don't know what they're doing. I'm not saying that DFO is good at it but it takes time to develop all those skills. Craig: I like that idea but again, what's the safe output? Tony: I think where we're at is that things are looking promising. Whatever's happening and whatever's causing it and whatever seems to be working and whatever level that is, I mean Marty presented the graph with what I would call the pre-emptive strike of SLICE. Two years ago Dick Beamish was saying where are these lice coming from in December? The picture to me seems more clear, it's not that they're coming in September, it's that they're taking their time getting off adult fish in the fall and it's just that they're ramping up and so that to me may be an important point that comes out and so for the farms' perspective, if they can be pre-emptive that keeps it down and at least that's keeping the lid on it while we make these linkages. Diane: I want to make sure that we don't forget that there's the *Caligus* out there which we don't see on our farmed salmon in any kind of numbers but that is what we have in the past seen on the wild fish in the early monitoring where chalimus stages are *Caligus*. So, to have a safe number is a great target but is it a *Leps* safe number, is it a total lice number? We have to make sure this doesn't get lost in the discussion because there is another source of *Caligus* out there, not just farms. Brad: In terms of *Leps* on the farms, from MHC data the 08 and 09 data are almost mirror images for farm output in farm numbers. I understand the 08 and 09 data for wild fish are pretty similar with very low prevalence so are we reaching that minimum threshold where the farms apparently have very little impact on wild salmon? So have we got that minimum baseline output? Larry: I think the most important thing that was in Marty's presentation that people may not have understood totally or grasped the significance of, is the fact that there were areas in this parameter space where there could be virtually no lice on the fish and yet the population is going extinct because of predation mortality on liced fish and this brings me to a general statement that the answers are not always going to be intuitively obvious and the best way to address this very complicated, multi-dimensional system which has temporal and spatial components and different species, is going to have to be modelling and I think there has to be more emphasis put on modelling. Ideally it would be great if Marty one day had a

model which included Dario's model, a complete population model that he could give to managers and you could put in this year's salinity, this year's lice levels on the fish and say 'if I treat now what's the likely outcome to wild fish population'. So that would be an ideal product off the shelf and I think we ought to encourage further development of those kinds of models. Crawford: I know that this doesn't answer your question directly Craig but I think what I hear is that we seem to be moving in some of the right directions in terms of the policy changes that have happened already and we don't know if they're sufficient or not but the best way of finding out if they are sufficient or will be sufficient into the future under different assumptions about predation and/or efficacy of treatment, whatever the different parameters are, may well be to develop models in which we all have confidence such that we can put those kinds of things in and ask those kinds of questions. If that number moves from 2 up to 4, what are the implications or likely impacts? I think the answer seems to be that we don't have the tools or the knowledge yet to know exactly what that number is but the kinds of numbers that seem to be practically achieved within the farms over the last 2-3 years are ones that appear at least, and we've got to be very careful about not over-interpreting that data, they appear to be having a beneficial effect on the overall numbers that we are seeing in terms of wild and that's not negating the issues about predation and other things that might happen but without sitting back and becoming complacent we seem to have a way to move forward but we should be doing some of this additional sampling and we should potentially look at the sentinel cages and we should make sure that all of that data is coming into some sort of modelling framework that there are enough folks working in to get a model that we all believe in that and that allows us to make those sort of predictions moving forward. It might take us another 2-3 years to get there but it's probably the best solution. Brent: I think modelling is one component of the toolbox and we've sort of agreed to not talk about a particular species down south but if you look at that as a case sample, we've got a whole bunch of different stocks and for everyone of those things we've got up to 19 models and out success rate is 61% on predicting so we've been doing this a long, long time and there is almost no model imaginable that we haven't tried but the world is a complicated place for a salmon and no amount of modelling is ever going to figure out what's going to happen next year. Larry: But these are all individual models right? Brent: yes, but I am using this as a point that I think to try and capture what is going on in the BA is difficult because it's so complex that something different is happening every year. Keep loading information into the model and think that the model is going to give you the answer I think is a mistake. It's one tool, it's not the solution. George: there is a famous quotation in modelling 'all models are wrong but some are useful' – it's finding the useful ones. Marty: in terms of the modelling approach there are different components that it's missing and I think there are some components that we have a pretty good handle on and others that we can never hope to do really well. I think this kind of forecasting returns of salmon is something that

we're never going to be able to do really well because it's so stochastic, but some components of the modelling framework that we have developed for the Broughton like the source locations of lice, their dispersal and the impact on individual fish I think have come together a lot nicer than some of these other things. So I think there are different levels and different utilities. Brent: if I put up the picture of the adult returns for pink salmon for the odd and even years since the '50s and if you and anyone else in the room didn't know when salmon farms started there wouldn't be a single person in this room that could point to that graph and tell me where the salmon farms started so we are focusing on lice from salmon farms, we are learning a lot about that, we are plugging that into models but I defy you to tell me that either in the odd or even year where that happened. The pinks are not responding to that, there are other things driving it that are much bigger than that. Marty: I know but we have ways of partitioning out what we know and what we don't know between these deterministic and stochastic components and we can take what we know and make a prediction of what will happen and we can take what we don't know based on this random variation and put confidence intervals on that. So we have a way of characterising what we know and what we don't know and the confidence that we have in our predictions. Crawford: but isn't the point that you should neither blame aquaculture when we have bad years nor give them credit when we have good years like this because there are other things in the environment that are happening, but given that we know about potential impacts we should at least be using models to control where we can? Some of the things we can control, maybe it's even 90% of the variation that we can control so the model will never adequately show that and therefore there will always be that frustration but if we can do a better job of managing the things that we do have some control over then that's as much as we can expect. Brent: my concern is that what we're really interested in is the number of adults that come back and what I'm saying is that what happens in the BA is a very small part of that, whether it's fish farms or predation or whatever else it is that is driving it, we have to realise this and do the best job we can but I'm not convinced that if we don't apply substance X to them and see whether sea lice are actually down in the noise level of what is driving populations or if its really this big event, we won't know. We'll continue to do this for 20 years and our models will get better and better but we still won't have any idea what is affecting the returns of the adults. We've got to focus on the right questions and not just refine the sea lice piece down here which we know we're getting good because it might not be the driver at all. Marty: I disagree. I think that the analysis we have done with the escapement and catch data are pretty compelling that sea lice was part of the puzzle that has an impact on salmon productivity and it's something that we can do something about. So there are two components to the value: 1) I think we do have a pretty good understanding, and 2) it's something that is amenable to management as opposed to things like climate change or environmental variation. This is a deterministic component of this model which we can manipulate. Brent: I think

the test of our knowledge is to manipulate what you can do and make a prediction and see if it works and we haven't done that yet. Crawford: we are running up towards our deadline and we could discuss this for a long time. I take Brent's point that modelling is one element and shouldn't be the only thing that we do and in fact although the field data collection, sentinel cages etc. are useful for feeding into the model they are useful in their own rights as data sources to see how the picture develops over time. Dario: I want to touch on what we know about the modelling again. In the last couple of years (2008/2009) lice infestation levels have been low, at near background levels and in both those years output from the farms of lice has been managed to very low levels and the measure of output from the farms is not the level of gravid females on the farm but the output from the farm, so it's the concentration of females by the inventory of the farm. It may be coincidental but there has been a management approach where we can tie an action onto a farm to an outcome in the field, albeit only for a couple of years but we have some data now to compare so we should be going back and looking at some of the retrospective data e.g. 2004 to try and understand what was going on there so we can have more confidence in our management actions on farms in terms of reducing lice loads. Measuring infestation levels on the wild salmon has been one of the recommendations of the Pacific Salmon Forum as a target level to keep it below so I think we have some data to tie these together now – management action with outcomes. Craig: I hear what Brent is saying that there are larger drivers out there too and as conservationists we have to be concerned with doing what management actions we can to reduce local impacts on these fish and we are seeing that lice have local impacts, there's no question. Also want to say that these management actions like CAMP, are an enormous amount of work. We have negotiated CAMP and the monitoring with MHC for months and months and that's one small area on this coast. There are situations south of there where the lice levels seem to be much higher and we have much less certainty about what's happening especially since we're seeing *Caligus* appearing on those fish we're not supposed to be talking about, we're still trying to figure out what that means. That is a concern for CAAR and probably for MHC too. When we negotiated this Terms of Reference for Morbidity & Mortality in 2006 we were still talking about repeating these contentious barrel studies and doing other research like that so I just want to get a sense. I don't think it will be useful for us to go there, from the research I've seen here it seems like we are light years beyond that and it seems like we are looking at more of a synthesis and is there going to be more of an opportunity to get to this synthesis through modelling, through these kind of workshops. Would you recommend to CAAR and MHC that we abandon all these kind of ideas of repeating research and doing our own individual research or relying on what is being done by the folks in this room and then going towards the modelling and the monitoring – is that where we should be focusing- that's a question? And one last point: we still have the issue of SLICE. As conservationists we don't like this as a management action that we are going to be relying on in

the future and this is a concern. And recommendations on research, should we be focusing our effort on monitoring, setting up sentinel cages and working with people in this room? Or should we be doing other research? Crawford: just having come from the East coast if you raise the issue of SLICE with anyone, either from a farm or an environmental group over there, they would be saying please bring us back an efficacious product which has significant impact, but this is an aside topic. Craig is asking the group based on your research over the past number of years and what we've heard today, that really the best and most useful way for CAAR and MHC to move of this forward under the CAMP initiative, is to continue some of the key monitoring and perhaps change is slightly, support modelling exercises and any key studies that would help to fill in some of the gaps and parameters for those models. Would this be a good use of folks' time and resources to move our knowledge forward or are there some key studies that we should be repeating where folks feel very uncomfortable about our level of knowledge? Any views? Tony: I will speak to Craig's first point. I thought that by putting on pinks and non-Atlantics that Pacific salmon species might differ. Then we went onto host. It's the colour of the day. If we are happy with the data we've got on the pinks and we can regulate the pinks and people think that this is good enough for the west coast, then we don't have to do any more species, we don't have to do *Caligus* and that's it. But I think we would be remiss if we didn't consider the species effect, both of the host and the parasite stage. But when it comes to modelling I hear Larry and I hear Brent and I think it is part of the armoury. Marty and I have had this ongoing thing – I'm trying to remember his coefficients. He generates his coefficients by fitting the model to the data. If we're going to move to this modelling as being more important in the forecasting we need to validate those coefficients. Two of us disagree on that but we do agree that there is great difficulty in demonstrating them. To me the sentinel cages are a way to get to validating his one, particularly when you want to run the experiment where you allow a farm to let lice levels run up but it can't be in a critical area. The second one for Marty's model is the beta value – that is the transmission from a farm onto a fish. If we could demonstrate that in a real way which means tagging some lice on a farm, letting those lice go out and seeing what they come onto in terms of fish that would give the whole world tremendous confidence in whatever Larry has envisioned for Marty's model. I think you have to come at those models two ways. Craig: the other thing is that the sentinel cage will also help MHC (who are interested because I spoke to Clare about it) to know where there farms are getting their infections from and at what time of the year etc. Marty: I think you're right that getting a handle on this parameter values is really important and we need to calibrate and test them. Some of the values are really critical and I think the alpha value (the rate of parasite induced host mortality) is something that we do not have a good estimate for and we need more data to do this. There's some data in this room I think that could be used for this? We need estimates for that parameter, we also need different sea lice abundances, different fish sizes, different environmental

conditions that we really need to look closely at. Larry: and different lice stages. Brent: I really think we need to move on to the end study. We've done a lot of work trying to figure out pieces here, we still haven't addressed the question of do lice, whether from farms or elsewhere, and infections on juvenile salmon actually affect the number of fish that come back? That's the question we need answered. Let's get substance X, go into Glendale, mark 200,000 fish with tags, half of them are treated with substance X where we know sea lice won't have any effect on them for however many days the substance continues to work for (16 weeks?) let's tag another bunch at Doctors Island and let's look at the survival rates of those fish coming back. Is it down in the noise of the North Pacific effects on mortality or is it really a driver that we need to worry about and manage for the next 30 years coast wide? Let's figure it out, quit screwing around with lab studies to see if lice have an effect because maybe it doesn't matter. Even if they have an effect if it doesn't affect the results of the fish that come back let's forget it. Crawford: two responses – if you did your experiment under the current conditions that we happen to have managed to achieve assuming these conditions continue to be replicated on the farms and you get an answer, how does that answer help to inform you about the situation with the wilds had they been exposed to the levels of lice that were there in 2004/2005? It won't help answer that question. Brent: people in the room are saying that we think the low levels of lice in the BA right now are because of the farms. If people genuinely think that is the truth right now then let's turn it off, let's not treat for a few years or go back to the 3 level that they are required to do. The thing we are doing in the BA right now is an experiment, they are not required to do it, it's mainly for scientific reasons. Or pick other places to do this, do something where we can manipulate it so we can get the final result we want and not spend another 20 years doing lab, field studies and modelling that wont answer the question. Crawford: doing this on a small scale, in a controlled way might be a useful exercise, similar to what they did in Norway however it opens a whole other question of funding resources etc. We have run to the end of time and need to wrap now. Thank you to all the speakers and for everybody contributing etc.

Workshop end 5.05pm

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Audio tapes located at Watershed Watch office