PAAR Project Proposal 2010/11

1. Research Priority:

This research proposal is relevant to the PAAR 2010/2011 directed call t**o conduct laboratory studies to describe the susceptibility of juvenile pacific salmon to sea lice infection.** The proposed research builds upon expertise and resources developed at the Pacific Biological Station to study interactions between juvenile salmon and the salmon louse, *Lepeophtheirus salmonis*.

2. Region:

DFO Pacific Region will lead this project.

3. Title:

The effects of single and repeat *Lepeophtheirus salmonis* infections on the health of juvenile Pacific salmon.

4. Project Overview:

There is evidence that different species of Pacific salmon differ in their susceptibility to infection with *L. salmonis* under laboratory conditions. For example, pink and coho salmon have been shown to be less susceptible to infections of *L. salmonis* when compared to chinook or chum salmon (Johnson and Albright, 1992a,b; Jones *et al.* 2007). The susceptibility of juvenile sockeye salmon to *L. salmonis* has never been examined in a controlled laboratory study.

Numerous laboratory studies have demonstrated the adverse effects to juvenile salmonids of a single pulse exposure to *L. salmonis* (Bjorn and Finstad 1998, Finstad *et al.* 2000, Fast *et al.* 2002). However, Krkosek *et al.* (2009) recently suggested that single pulses of infection are a poor model for studying susceptibility to *L. salmonis* in the wild where infections are normally acquired over time. In this proposal we are seeking funds to conduct a 3 year program in which we will:

1) Determine the susceptibility of juvenile sockeye salmon to infection with *L. salmonis.*

2) Determine the lethal infection level of *L. salmonis* on juvenile sockeye salmon.

3) To investigate whether previous infection with *L. salmonis* has an impact of the susceptibility and the physiological response of juvenile, coho, chum, pink and sockeye salmon to infection.

5. Project Leaders:

Stewart Johnson, PhD., Research Scientist/ Section Head, Aquatic Animal Health Section, Salmon and Freshwater Ecosystem Division, Pacific Biological Station, Nanaimo, British Columbia, V9T 6N7, Tel: 250-756-7077, Email: Stewart.Johnson@dfo-mpo.gc.ca

Dr. Johnson has published 41 scientific articles and given numerous invited lectures on various aspects of the biology of sea lice and their effects on their hosts.

Simon Jones, PhD., Research Scientist, Aquatic Animal Health Section, Salmon and Freshwater Ecosystem Division, Pacific Biological Station, Nanaimo, British Columbia, V9T 6N7, Tel: 250-729-8351, Email: Simon.Jones@dfo-mpo.gc.ca

Dr. Jones is a recognised expert in the parasites of marine and freshwater fishes. He has published over 70 scientific articles including 17 in the last 4 years on sea lice biology.

6. Project Team Members:

Technician (EG-4), shared time (50/50) with PARR sockeye marine surveillance program
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7. Project Team Collaborators

8. Project Rationale and Objectives

Rationale

The Fraser River is the largest producer of salmon in Canada. Of its five salmon species, sockeye (*Oncorhynchus nerka*) is most commercially valued, the second most numerically dominant and comprised exclusively of wild stocks (e.g. hatcheries are not involved with their production). Over a dozen large stocks spawn throughout the watershed in locales ranging from 100 to 1200 km from the ocean. In 2009, the abundance of adult sockeye returning to spawn was less than ten percent of the preseason forecast. A suggestion has been made that infections of post-smolt sockeye with sea lice arising from salmon farms contributed to the poor return in 2009. Similar concerns have been expressed for juvenile chum and coho salmon. There are insufficient data on the relative susceptibility of the juvenile stage of these species to infection to enable us to understand what if any risk sea lice from salmon farms pose to these fish.

In addition the validity of past laboratory studies that have used single pulse infections with *L. salmonis* to study effects on the host have been called into question.

The studies that we have proposed here address both of these issues.

Objectives

We will investigate susceptibility of juvenile salmon to infection with *L. salmonis* and establish an infection threshold for death under controlled laboratory conditions using protocol developed earlier for juvenile pink and chum salmon (Jones *et al.* 2007). We will conduct laboratory experiments to examine the possibility that single exposures to *L. salmonis* do not provide an accurate measure of the effects on juvenile salmon. To this end we will determine if previous infection with *L. salmonis* has an effect on subsequent susceptibility to infection and associated physiological and immunological responses.

In Years 1 and 2 the following specific objectives will be achieved:

1) The susceptibility of juvenile sockeye and coho salmon to infection with *L. salmonis* will be determined.
2) The lethal infection level of juvenile sockeye and coho salmon with *L. salmonis* will be determined

3) The effects of previous exposure to *L. salmonis* on susceptibility to infection and the physiological and immunological responses to infection will be determined for sockeye and coho salmon.

In Year 3 the following specific objectives will be achieved:

1) The susceptibility of juvenile chum salmon to infection with *L. salmonis* will be determined.
2) The lethal infection level of juvenile chum salmon with *L. salmonis* will be determined
3) The effects of previous exposure to *L. salmonis* on susceptibility to infection and the physiological and immunological responses to infection will be determined for pink and chum salmon.

9. Innovation and Linkages

This area of study was identified as a priority in the 2010-2011 PARR call for proposals. These data will enable us to better understand how infection with *L. salmonis* affects the early juvenile stages of Pacific salmon. Data from these studies is extremely important with respect to: our understanding of the biological significant of sea lice levels reported on wild juvenile salmon. These data will also improve our ability to understand the validity of past modelling efforts to determine the effects of sea lice from salmon farms on wild salmonids.

This study complements long-term research that was conducted by Drs. Hargreaves and Jones on the abundance of sea lice on juvenile salmonids in the Broughton Archipelago, and Dr. Beamish on juvenile salmonids in the Strait of Georgia and on the Central Coast. It also builds upon laboratory studies on the susceptibility of pink and chum salmon to infection conducted by Dr. Jones. In addition this information can be applied to aid in the planning and development sea lice management strategies for the BC Aquaculture Industry.

10. Project Description and Methodology:

Under laboratory conditions we will determine the susceptibility of juvenile Pacific salmon to infection with *L. salmonis* as well as determine the lethal level of infection for this species. In addition we will determine if previous infection with *L. salmonis* has a subsequent effect on the susceptibility and physiological/immunological responses of juvenile coho, chum, pink and sockeye salmon.

Methods:

1.0 Wild Fish Stocks:

Juvenile Pacific salmon will be obtained from Federal Salmon Enhancement facilities and reared in freshwater or saltwater in the research aquarium at the Pacific Biological Station (PBS), Nanaimo. Sub-samples of all incoming fish will be screened for infectious diseases using standard protocols and husbandry methods will adhere to all protocols established and maintained by the Animal Care Committee at PBS. Methods for rearing juvenile salmon for the purposes of exposure to infective *L. salmonis* larvae have previously been published (Jones *et al.* 2006, 2007, 2008).

2.0 Collection and Culture of *L. salmonis*

Gravid female *L. salmonis* will be collected from farmed and wild salmonids. Transportation to the laboratory and incubation of the egg strings will follow the methods described in Jones *et al.* (2006).

3.0 Experimental Design

3.1 Susceptibility of Juvenile Pacific Salmon (Coho, Chum and Sockeye) to Infection with *L. salmonis*.

Challenge trials will be conducted on juvenile salmon following the methods described in Jones *et al.* (2007). In addition to obtaining data on *L. salmonis* we will also obtain samples from all fish for cortisol analysis and use in gene expression studies. Cortisol levels will be determined by ELISA. Real Time PCR will be used to examine genes that have been previously identified as being important in the interactions between salmonids and *L. salmonis* (Jones *et al.* 2007). Sockeye studies will be conducted in 2010-2011, coho studies in 2011-2012 and chum studies in 2012-2013.

3.2 Lethal Threshold of Infection for Juvenile Pacific Salmon (Coho, Chum and Sockeye)

The lethal level of infection for juvenile salmon will be determined using the methods described in Jones and Hargreaves (2009). Sockeye studies will be conducted in 2010-2011. coho studies in 2011-2012 and chum studies in 2012-2013.

3.3 Effects of Previous Exposure on Susceptibility, Physiological and Immune Responses

Juvenile coho, chum, pink and sockeye salmon will be used in these studies. Laboratory challenge trials will be conducted using methods similar to those described in Jones *et al*. (2006) with the following exceptions. Fish will be divided into replicate groups designated as control, single infection and repeat infection groups. The repeat infection group will be initially infected with low levels of *L. salmonis* and the numbers of *L. salmonis*, distribution on the body and developmental stage present will be determined a 7 and 14 days post initial-infection (DPI). On day 15 both the single infection and the repeat infection group will be infected with low number of *L. salmonis* samples will be taken at 7, 14 and 21 days post re-infection (DPRI). The control group will be manipulated and sampled in exactly the same way as the infection groups with exception that they will not be exposed to *L. salmonis*.

In addition to obtaining data on *L. salmonis* we will also obtain samples from all fish for cortisol analysis and use in gene expression studies. Cortisol levels will be determined by ELISA. Real Time PCR will be used to examine genes that have been previously identified as being important in the interactions between salmonids and *L. salmonis*.

Coho and sockeye salmon will be studied in 2011-2012 and chum and pink salmon in 2012-2013.

11. Milestones

Year 1: Sockeye salmon susceptibility to infection and lethal levels of infection will be determined.

Year 2: Coho salmon susceptibility to infection and lethal levels of infection will be determined.
Effects of previous exposure on susceptibility to infection will be investigated for sockeye and coho salmon.

Year 3: Chum salmon susceptibility to infection and lethal levels of infection will be determined.

Effects of previous exposure on susceptibility to infection will be investigated for pink and chum salmon.

Project Challenges

There are significant challenges that need to be met during these research activities. It is critical that only healthy juvenile salmon are used in these trials as the use of compromised fish will have a dramatic effect on the results. With exception of coho salmon holding of other species of juvenile Pacific salmon is difficult and a large amount of time must be spent to ensure that they remain healthy. Obtaining sufficient numbers of sea lice to conduct challenge trials is also time consuming and often involves travel to various BC farm sites.

The methods that we are proposing to use have been used successfully to study the susceptibility of other species of salmon to infection with *L. salmonis*. This program will use the expertise and skills developed by staff members during these earlier studies. Both of the project leaders have published extensively on various aspects of sea lice biology and ecology. The technician we would use for this study has had a great deal of experience maintaining and working with juvenile Pacific salmon and conducting laboratory challenge trials with *L. salmonis*. However she is currently in a term position assigned to another project. The success and timeliness of this project will depend on access to full-time and appropriately trained technical support.

12. References

Bjørn P.A. and Finstad B. 1997. The physiological effects of salmon lice infection on sea trout smolts. Norwegian Journal of Freshwater Research 73:60-72.

Fast M.D. *et al.* 2002. , Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. Diseases of Aquatic Organisms 52:57-68.

Finstad B. *et al.* 2000. Laboratory and field investigations of salmon lice [*Lepeophtheirus salmonis* (Krøyer)] infestations on Atlantic salmon (*Salmo salar* L.) post-smolts. Aquaculture Research 31:795-803.

Johnson, S.C. and Albright L. J. 1992a. Comparative susceptibility and histopathology of the host response of naive Atlantic, chinook, and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms 14: 179-193.

Johnson, S.C. and Albright L. J. 1992b. The effects of cortisol implants on the susceptibility and histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms 14: 195-205.

Jones S.R.M. *et al.* 2006. Experimental infections with *Lepeophtheirus salmonis* (Kroyer) on threespine sticklebacks, *Gasterosteus aculeatus* L., and juvenile Pacific salmon, *Oncorhynchus* spp.
Journal of Fish Diseases 29: 489-495.

Jones, S.R.M. *et al.* 2007. Earlier rejection of *Lepeophtheirus salmonis* from pink salmon *Oncorhynchus gorbusca* compared with chum salmon *O. keta* associated with the expression of proinflammatory genes. Diseases of Aquatic Organisms 75: 229-238.

Jones S.R.M. and Hargreaves N. B. 2009. Infection threshold to estimate *Lepeophtheirus salmonis*-associated mortality among juvenile pink salmon. Diseases of Aquatic Organisms 84: 131-137.

Krkosek, M. *et al.* 2009. Sea lice and salmon population dynamics: effects of exposure time for migratory fish. Proceedings of the Royal Society B. 276: 2819-2828.

13. End Users and Outcomes:

Understanding how sea lice affect the health of juvenile salmon is very important for multiple stakeholders within Fisheries & Oceans Canada. For example, Aquaculture Management Directorate will be responsible for the regulation of B.C. aquaculture starting in December 2010. Understanding this issue will enable the development of regulations related to sea lice that work for the industry as well as reduce risk to wild salmonids. In addition, these data will improve our ability to understand the validity of past modelling efforts that have examined the effects of sea lice from salmon farms on wild salmonids.

14. Communication and Reporting

1. Yearly mid-term progress reports and a final reports of the results obtained from this research project will be submitted by the required deadlines using the templates that will be provided by DFO Science.
2. Manuscripts reporting the results of these studies will be submitted for primary publication.
3. Results will be communicated to the various stakeholder groups through public presentations and meetings.

15. Budget and Resources

See attached budget table.