Fisheries and Oceans Canada | Pêches et Océans Canada

Aquaculture Collaborative Research and Development Program (ACRDP)

APPLICATION FORM

1. INDIVIDUAL OR ORGANIZATION INFORMATION

INDIVIDUAL OR ORGANIZATION FULL LEGAL NAME	IN OPERATION SINCE	INCORPORATED
Creative Salmon Ltd	1990	☐ FED ☒PROV ☐N/A

ADDRESS	CITY	PROVINCE	POSTAL CODE
REDACTED	REDAC	REDA	REDACT

TELEPHONE #	FAX #	E -MAIL ADDRESS
REDACTED	REDACTED	REDACTED

HEAD OFFICE ADDRESS (IF NOT SAME)	CITY	PROVINCE	POSTAL CODE

Number of employees: 1-4 _____ 5-19 _____ 20-49 _____ 50-100 ___X___ 100+ _____

PLEASE INDICATE TYPE OF OPERATION	FOR THE PROJECT, INDICATE
☒ Marine Finfish ☐ Shellfish ☐ Freshwater Finfish ☐ Seaweed ☐ Association ☐ Other (please specify) ☐ Commercial ☐ Developmental	Species involved:__chinook salmon ☒ Commercial ☐ Developmental

PROJECT MANAGER CONTACT (NAME)	TELEPHONE #	FAX #	E-MAIL
Karia Kaukinen	250-759-8358		Karia.kaukinen@dfo-mpo.gc.ca

2. PROJECT INFORMATION

Project Title: **Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon**

A. The following should be submitted with this application form:

- A proposal giving the details of the project as outlined on the Proposal Guideline sheet.
- Budget details (provide details of each line item)
- Project budget summary and summary by fiscal year (see budget form).

B. This project addresses what: national priority **Optimal fish health**

regional priority **Industry environmental performance**

C. Other sources of funds related to this project (if applicable, provide details in the proposal)

1. 2. 3.

I hereby declare that all of the above information contained within this application and proposal is correct and that if found to be otherwise, I agree to withdraw my organisation's participation from the Aquaculture Collaborative Research and Development Program.

Project proponent Signature	Please Print Name CLEARLY	Title	Date

DFO Project Lead Signature	Please Print Name CLEARLY	Email address	Date

DFO-ACRDP Use only

Project ID Number	Date Submitted	Region

PROTECTED 'B' (COMMERCIAL CONFIDENTIAL) WHEN COMPLETED

Canada

Aquaculture Collaborative Research and Development Program (ACRDP)

PROPOSAL GUIDELINES

Please submit a proposal giving the following details:

1. Project title: **Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon**

2. Name, address and position of project manager

Karia Kaukinen
Bi-02 in the Molecular Genetics Laboratory
Pacific Biological Station
3190 Hammond Bay Rd
Nanaimo, BC V9T 6N7
250-759-8358

3. Description of project work team and required qualifications for key positions (with names, addresses, titles, and CV's where available; maximum length 4 pages per team member)

Kristi Miller, PhD
Head, Molecular Genetics, PBS
Genomics lead, genomics data analysis and interpretation

Karia Kaukinen, MSc
BI-02 Molecular Genetics, PBS
Project manager, Genomics laboratory research, genomics data analysis

Sonja Saksida, DVM, MSc
Veterinarian and Epidemiologist
Center for Aquatic Animal Health Sciences
871A Island Highway, Campbell River BC V9W 5B1
Fish Health expertise, industry liaison, epidemiology expertise

Barb Cannon -
Fish Health Manager - Creative Salmon
Tofino BC
Industry Partner

4. Project problem / rationale (maximum length ½ page)

Over the past seven years mortalities of Chinook salmon farmed in the Tofino inlet have been observed with unique clinical presentation. The salmon present with mild to severe yellow discolouration of the skin (jaundice). This is most evident on the abdomen and around the eyes. These fish also have very pale gills indicating anemia. Internal signs include pale livers and often the stomachs of the fish are empty indicating the fish have not eaten for a number of days

although the overall condition of the fish is good. Grossly the other organs appear unaffected. The clinical presentation is very different from Marine Anemia syndrome, another Chinook salmon disease, which typically presents with splenomegaly, renomegaly, and anemia (Kent and Poppe 1998).

Histological examination has found severe liver and kidney damage (hydropic degeneration). The proposed etiology includes a pathogen or exposure to a negative environmental influence (hereafter referred to as an undefined toxin). Repeated testing using traditional diagnostic tests have been unable to identify a pathogen. Tests including classical bacterial culture, viral cell culture, PCR, blood assessment and histopathology have yielded negative results for pathogens including *Renibacterium salmoninarum* (BKD), *Listinella sp* (vibriosis), VHSV, IHNV, ISAV, VEN, EIBS, Loma *salmonae*, and *Nucleospora salmonis* (marine anemia).

Little is known of the epidemiology of the condition. It affects fish that have been in sea water for greater than 6 months and therefore is not considered related to smolt quality. There appears to be a seasonal pattern to this condition with clinical signs and mortalities observed late fall/early winter (December) spiking in the winter and apparently resolving by early summer. This condition is most typically observed at the farm site operated by Creative Salmon that contains the greatest freshwater influence, which is one reason to suspect an environmental effect may be at play, although it is sometimes observed at relatively lower incidence levels at other farm sites. It has been seen in most of the generations stocked at the freshwater-influenced farm even though the company operates single year class sites with a fallow period before re-stocking. At the most affected farm most often one or two pens of fish are severely affected, however the condition may be seen in many of the pens. The mortalities levels in most heavily affected pens typically would be several folds higher than the other pens on the farm. For example in January 2011, the single affected pen (of an 8 pen site) disproportionately made up 35% of the mortalities grossly examined. Of the mortalities examined from this single pen over 77% of the fish examined exhibited jaundice. Total mortality attributed to this condition has not been fully assessed although at certain times of the year it can be as significant as other diseases.

Currently there are no tools available to manage the problem. A better understanding of the epidemiology and etiology would enable us to develop these tools.

5. Project objectives (maximum length ½ page)

Our main objective is to apply functional genomics technology to gain a better understanding of the factors that may underlie the poorly understood jaundice-related disease experienced by farmed Chinook salmon in Tofino. Of most import, Creative Salmon managers need to know whether this disease is more likely the result of an infectious agent or environmental conditions. If the genomic signature indicates that an infective agent is likely involved, they will pursue the identification of this agent through 454 sequencing of affected tissues in a follow up study. If the genomic signature is more likely associated with environmental conditions at this farm site, e.g. low salinity, toxicants, or other factors, follow-up studies could be performed to assess the most likely environmental mechanisms. In either case, knowledge of the mechanisms leading to this disease will potentially provide managers with tools to track, predict, and/or potentially mitigate the impact of this disease in future.

Our other objective is to improve our understanding of the epidemiology of the condition – why is the condition more prevalent at one farm as compared to the others in the same area, or in some pens and not others? This would include examining the mortality pattern, and determining overall mortality attributed the condition, and the environmental factors associated with it.

6. Description of work and experimental protocol (maximum length 2 pages)

Fish on Creative Salmon's farms are regularly screened for health, and the presence of jaundice is one of the metrics that is tracked bi-weekly in each netpen at each site. While mild jaundice can occur to some degree at all sites, jaundice-related mortality is predominant at the farm with the greatest freshwater influence. Despite extensive histological study, we still do not understand whether the jaundice may result from an infective agent or may be a manifestation of environmental conditions, possibly relating to low salinities, in the winter, or a combination of both. Hence, we need to design an experiment that considers both possibilities.

The molecular genetics laboratory has successfully used microarray approaches on wild fish to assess unknown factors associated with poor performance (e.g. Miller et al. 2010) and transcriptional responses to shifting environments (Miller et al. 2009; Evans et al. In Review). We have also conducted microarray studies of host response to disease (Miller et al. 2007; IHNV) and have conducted studies with Peter Ross at IOS on the influence of toxicant exposure on immune response to Vibrio (data still in preparation for publication). Moreover, we have been working closely with a bioinformatics group at UBC, lead by Dr. Paul Pavlidis, on the development of meta-analysis tools to identify correlated profiles among microarray experiments. Hence, we have the experience and expertise to undertake this exploratory study. We will use similar experimental designs and approaches as we have used in previous studies (e.g. Miller et al. 2009) whereby we apply balanced replication across each biological variable, or treatment, of interest. Here, treatments are not really treatments, as one would define in a controlled laboratory study, but rather biologically meaningful entities, like sample sites, disease state, and life-history stage.

Approach: If an infective agent is involved, it is important to determine whether it is present in smolts used to stock the farm sites, or whether it likely emanates from the marine environment. As such, our study will include smolts used to stock net pens in 2011 (Treatment 1; note that the sample size of smolts would only detect positives that affected 10% or more of fish, so it is not an exhaustive, definitive assessment of the role of freshwater). To control for the environment, we will contrast apparently healthy (no evidence of jaundice) fish a farm that is not substantively affected by jaundice (Treatment 2) with healthiest fish that can be obtained sampled from the affected freshwater-influenced farm (Treatment 3). To obtain healthy fish, we will chum fish from netpens that have been shown to have the lowest levels of jaundice and conduct histological analyses to ascertain their state of health. Fish that were collected in this manner but deemed to be positive for, but not dying of, jaundice at the time of collection will also be included in the analysis to control for the effects of morbidity (Treatment 4). Finally, moribund fish with clear evidence of jaundice will be included in the study (Treatment 5). Multiple tissues from all collected fish will be examined histologically.

Head and Posterior kidney, spleen, liver, heart, muscle, gill, and blood of 10-15 fish will be collected for each "treatment" category.

I. Smolts
II. Healthy fish from unaffected farm at mouth of the inlet
III. Healthy fish from affected farm
IV. Fish with histological signature of jaundice but not moribund at affected farm
V. Moribund fish with histological signature of jaundice at affected farm

In anticipation of this study, some of the samples of moribund and jaundiced fish were collected during a mortality event at the end of February 2011. Additional samples of jaundiced but not moribund and healthy fish from both farm sites will be collected in early March, along with additional moribund and jaundiced fish, if still present. Smolts will be collected in April before they are put to sea.

The microarray study will be performed on cDNA from liver tissue, as liver is one of the most severely affected organs and is also the primary tissue for detoxification. We will keep the remaining tissues for potential future study. In a balanced experimental design, twelve biological replicates (individuals) will be included for each of the five treatments, with the total study comprising 60 arrays. The Salmonid Agilent 4x44K oligonucleotide arrays developed through the cGRASP program by Ben Koop's laboratory at University of Victoria will be used in the study, with approaches similar to those we have used in other studies (e.g. Miller et al 2009, 2011). A reference sample comprised of liver cDNA from all of the individuals used in the study (labeled with Alexa555) will be hybridized along with the experimental sample (labeled with Alexa647) on each array. After slide quality assessment and Lowess normalization, arrays will be statistically analysed using both supervised (multifactorial ANOVA with posthoc testing via Mann Whitney U t-test) and unsupervised (Principle Component Analysis) approaches to identify transcriptional differences among treatments and the main physiological trajectories in the data. Functional analysis of the biological processes over-represented among the differentially regulated genes will be determined using the programs DAVID (http://niaid.abcc.ncifcrf.gov/) and PathwayStudio (Ariadne Genomics). Further information on the functional role of the most significantly differentially regulated genes will be gleaned from the protein literature mining website ihop (http://www.ihop-net.org/UniPub/iHOP/).

Many salmon diseases have already been characterized by microarrays (e.g. Miller et al. 2007, Rise et al. 2004, Morrison et al. 2006, Baerwald et al. 2008), including the infectious diseases ISAV, IHNV, VHSV, *Aeromonas salmonicida*, Amoebic gill disease, salmon louse, and others. A wealth of microarray studies also exist for toxicant responses in fish (e.g. Finn et al. 2007, Tilton et al. 2006, Hook et al. 2006) for a diverse array of chemicals, including PBDE (flame retardant), endocrine disrupting compounds, heavy metals, PCB's, and others. In our own research, we also have data from both wild fish and controlled laboratory studies that assesses responses to shifting salinities and temperature, and we have assessed wild salmon in the ocean at the same time of the year as the fish from this study (the first winter at sea), some even from the west coast. We will use these studies as a backdrop to assess the possible correlation of signatures emanating from jaundiced Chinook salmon with signatures associated with pathogenic disease, toxicant exposure and other environmental stressors. To do this, we will use the recently develop meta-analysis program for microarray data GEMMA

(http://www.chibi.ubc.ca/maintenance.html), developed from our colleague and collaborator on our wild salmon studies, Paul Pavlidis. The literature mining software available within PathwayStudio will be similarly applied.

Because the farm site most affected by the jaundice-related disease also contains the lowest salinities of all the farm sites, we will additionally address the potential role that osmoregulatory dysfunction may play in the manifestation of this disease. We will assess the osmoregulatory state of fish in each treatment through quantitative PCR of gill cDNA for the isoforms of Na^+ K^+-ATPase, cold-inducible RNA binding protein, prolactin, and growth hormone. We will also determine osmolality and ion concentrations in blood plasma, which will indicate whether fish are able to maintain homeostasis in their gills.

We will evaluate health records collected and environmental data collected for the last seven years for the farm where the jaundice is most prevalent. Similar data will also be collected from one other farm where jaundice has not been observed or has been observed at very low prevalence. Health data is collected twice a week over the duration that the fish are at sea (~18-20mos). Environmental data (temperature, salinity and dissolved oxygen) is collected on a daily basis. The data will encompass 3 different generations stocked on the farm. These records will be used to estimate prevalence and describe the pattern of the disease both temporally (i.e. time of onset, age of onset, duration of disease, environmental profile) and spatially (difference in prevalence between years, between pens, between farm sites). This will be the first epidemiological analysis of this condition.

References:

Baerwald MR, Welsh AB, Hedrick RP, May B. 2008Discovery of genes implicated in whirling disease infection and resistance in rainbow trout using genome-wide expression profiling. *BMC genomics*;9:37.

Evans, TG, E Hammill, K Kaukinen, AD Schulze, DA Patterson, KK English, JMR Curtis, KM Miller. Transcriptomis of environmental acclimation and survival in wild adult Pacific sockeye salmon (Oncorhynchus nerka) during spawning migration. Molecular Ecology: In Review.

Finne EF, Cooper GA, Koop BF, Hylland K, Tollefsen KE. 2007. Toxicogenomic responses in rainbow trout (Oncorhynchus. *Aquatic Toxicology* 81:293-303.

Hook, SE. AD Skillman, JS Small, IR Schultz. 2006. Gene expression patterns in rainbow trout, Oncorhynchus mykiss, exposed to a suite of model toxicants. *Aquatic Toxicology* 77: 372-385.

Kent, M.L. and T.T. Poppe. *Diseases of seawater netpen-reared salmonids* Pacific Biological Station Press, Nanaimo, British Columbia, Canada (1998:) 138 pp.

Miller, K.M., Li, S, Kaukinen, K.H., Ginther, N., Hammill, E., Curtis, J.M.R., Patterson, D.A., Sierocinski, T., Donnison, L., Pavlidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., and Farrell, A.P. Genomic signatures predict migration and spawning failure in wild Canadian salmon. *Science*: 331: 214-218.

Miller, KM, AD Schulze, N Ginther, S Li, DA Patterson, AP Farrell, SG Hinch. 2009. Salmon Spawning Migration: Metabolic Shifts and Environmental Triggers. Comp. Biochem Physiol D 4: 75-89.

Miller, K.M., G. Traxler, K.H. Kaukinen, S. Li, J. Richard and N. Ginther. 2007. A cDNA microarray study of Atlantic salmon (*Salmo salar*) response to Infectious Hematopoietic Necrosis (IHN) virus. *Aquaculture* 272 (Supplement 1): S217-S237.

Morrison RN, Cooper Ga, Koop BF, et al. 2006Transcriptome profiling the gills of amoebic gill disease (AGD)-affected Atlantic salmon (Salmo salar L.): a role for tumor suppressor p53 in AGD pathogenesis? *Physiological genomics* 26(1):15-34.

Canada

Rise ML, Jones SR, Brown GD, et al. 2004Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage and hematopoietic kidney response to Piscirickettsia salmonis infection. *Physiological genomics* 20(1):21-35.

Tilton* SC, Givan† Sa, Pereira‡,§ CB, Bailey*,‡ GS, Williams*,‡ DE. 2006Toxicogenomic profiling of the hepatic tumor promoters indole-3-carbinol, 17beta-estradiol and beta-naphthoflavone in rainbow trout. *Toxicological* 90(1):61-72.

7. Description of how this project meets the goals, objectives and priorities of the program (maximum length 1 page)

This project meets three of the four goals of the ACRDP program. It provides **improved competitiveness of the Canadian aquaculture industry** by providing scientific information that may be used to manage or mitigate disease impacts on aquaculture production. It provides **increased collaboration between the department and industry on scientific research and development** by using genomic technology and expertise available within the department to help resolve issues impacting the industry. It also **increases the scientific capacity for essential aquaculture research** by employing a technology that has yet to be fully realized by the salmon farming community in BC.

The research project addresses priority research within two of the broad research and development objectives: **Optimal fish health** and **Industry environmental performance**. By assessing the potential involvement of pathogen-driven versus environmentally-induced factors in the jaundice-associated disease, the project assesses the potential role of biological **causative agents** associated with disease and the **influence of the environment**. Moreover the findings of this project could impact **health management** strategies and lead to better tools for **disease surveillance and detection**.

8. Detailed deliverables of project (must include final project report)

I. Collections of tissues from jaundiced and healthy fish that can be used for transcriptional studies and pathogen isolation and sequencing in future
II. Full functional genomics assessment of jaundiced fish, including the lists of genes and biological processes differentially regulated in response to the jaundice-associated disease and an assessment of the potential roles of pathogens versus environmental perturbations in eliciting the disease.
III. A list of genes that might be useful biomarkers to predict disease and stage disease progression
IV. Characterization of osmoregulatory state of fish at the two farm-sites through biomarker screening of gill tissue and plasma ion and osmolality levels
V. Epidemiological analysis of the disease prevalence at each of the farm sites over the past 7 years and environmental data
VI. Recommendations to industry on next step research to either identify an infective agent associated with the disease or to narrow down potential environmental factors involved.

VII. Manuscript to be published in a peer-reviewed journal describing results (will not be complete until after the project ends)
VIII. Final project report to ACRDP

9. Milestones and timelines

	Pre-study F	Pre-study M	A	M	J	J	A	S	O	N	D	J	F	M
Collect tissues from jaundiced and healthy fish														
Collect tissues from smolts														
Histological analysis of tissues														
Epidemiological research														
Extraction of RNA														
qPCR of osmoregulatory biomarkers														
Microarray Experiment														
Data analysis and interpretation														
Final Report														

10. Organisation profile (maximum length ½ page)

Creative Salmon, established in 1990, is one of only a few premium growers of Chinook salmon in Canada and the world. The company's six saltwater tenures are located in the traditional territory of the Tla-o-qui-aht First Nations. Creative Salmon and its staff are active and enthusiastic community partners.

Creative Salmon has a committed and dedicated workforce of approximately 55 full time staff – and these employees are crucial to the company's continued success. The company bases its production management on organic principles, including: a low density, low-stress growing environment that encourages fish health and welfare; close contact between staff fish culturists/farmers and the fish they raise; and husbandry practices and standards that grow healthy, high quality salmon with a small environmental footprint.

Creative Salmon produces over 1,500 metric tons per annum by operating an integrated production cycle. With painstaking control of quality from egg to harvest, the company has established an enviable reputation for high quality Chinook salmon, serving the most discerning clientele throughout Canada, the USA and Japan.

11. Partner(s) profile, including contact name and information (if applicable) (maximum length ½ page)

The BC Centre for Aquatic Health Sciences (BC CAHS) is a research facility designed to fill the void in marine health research capacity in British Columbia. It was established in 2005 in Campbell River BC. BC CAHS exists to advance understanding of British Columbia's aquatic resources by addressing issues of aquatic animal health and welfare, production and aquatic food safety, thereby facilitating the economic, social and environmental sustainability of British Columbia's aquatic based resource industries and increasing research and service capacity in rural and coastal communities. The strength of the facility has been the success that BC CAHS researchers have had in liaising with the appropriate government and academic researchers to facilitate research important for the environment and the continued sustainability of our aquatic based resource industries.

In addition to being the Executive Director and researcher for BC CAHS, Dr Sonja Saksida operates a private practice and has been providing veterinary services to Creative Salmon for the last 4 years.

Estimated budget – provide details of each budget item, a budget summary for each fiscal year, if applicable, and a total project budget summary.

Aquaculture Collaborative Research and Development Program (ACRDP)

Budget Summary by Fiscal Year__2011_____ (1 April – 31 March)

Please provide a budget for the total project with each fiscal year detailed on separate attached sheets. Details for each of the line items should also be documented on separate sheets.

Description	Industry Cash Contribution	Industry In-kind Contribution	ACRDP Contribution	DFO In-kind Contribution	Partner In-kind Contribution[1]	Total
Salary						
Scientist- Millar				4,000		4,000
Veterinarian-Saksida		3,200	5,600		1,600	10,400
Biologist	6,000		36,628			39,000
Technicians/Biologist		3,400				3,400
Post-Doc / Students						0
Divers		5,000				5,000
Sub-total						0
Equipment						0
Computer Equipment						0
Lab Equipment						0
Field Equipment						0
Other						0
Sub-Total						0
Material and Supplies						0
Lab		1,000	29,120			30,120
Field			510		150	460
Publication costs			500			400
Healthy Fish		1,500				0
Sub-Total						0
Travel						0
Field		2,100				2,100
Meetings			400			400
Conferences						0
Other						0
Sub-Total						0
Other						0
Administrative						0
Facilities						0
Other expenses						0
Sub-Total						0
Grand Total	6,000	16,200	72,758	4,000	1,750	95,280
% OF CONTRIBUTIONS	0.082	0.223				0.305

Canada

Details of expenditures:

Salaries

Salary for ½ year of a BI-02 (Karia Kaukinen) in the molecular genetics laboratory who will be responsible for project management and reporting, RNA extractions, biomarker qPCR, microarray experiment, and preliminary data analysis provided by ACRDP. Karia's salary is $71,046 per year. ($71,046/2) *1.2 benefits = $42,628. The industry will provide a cash contribution for $6,000 of these salary dollars.

Dr. Miller will dedicate 3% of her time to the project, at an in kind cost of $4,000. Most of this time will be spent on data analysis and interpretation.

Dr Saksida will provide veterinary visits and help in sample collection for the project as well as conduct the epidemiological evaluation of the condition. Expense 13 days @$800/day
4 days @ $800/day for field and data collection to be covered By Creative Salmon. This expense will be covered by Creative Salmon.
7 days @ $800/day to conduct the epidemiological analysis funding which is requested from ACRDP.
4 (1/2 days) @ $400 = $1600 to attend meetings with DFO collaborators will be provided by BC CAHS as an in-kind contribution.

Fish Health Technician (Chris Dolphin)/ to provide assistance in field - 4 days @ $350/day = $1400 and Biologist (Barb Cannon) will be involved in data assembly for the epidemiological evaluation -3 days @ $500/day= $2,000 to assist in data collection. These people are employed by Creative Salmon and their time will be provided as in-kind contribution.
Total = $2,150

Commercial Contract Divers will be used to collect samples 5 days @ $1000/day = $5000. This will provided as an in-kind contribution from Creative Salmon

Equipment

Lab

Technology platforms and lab infrastructure are already in place in the **DFO Molecular Genetics Laboratory (MGL)** at the Pacific Biological Station for experimental microarray research. Dr. Miller is the Head of this laboratory, and in 2004, began developing a functional genomics laboratory for gene expression research. Current infrastructure for functional genomics research includes: Retsch MM301 mixer mill, Beckman Biomek NX^{P} Robot with a Span-8 Head and Integrated DTX 880 Plate Reader, TECAN HS 4800 Pro Hybridization Station with two extension units (24 slides/day capability and potential to expand to 4 extension units with 48 slide/day capability), Packard BioScience ScanArray Express Microarray Scanner, MJ Research PTC 100 PCR machines, Millipore MilliQ Biocel Water Purification System, ABI 7900HT Fast Real-Time PCR System and Integrated Carousel (384 well plate platform). The MGL is built for high throughput experimental research and application, and easily contains the capacity to carry out the proposed study. *No funding is being requested for use of this equipment.*

Field

Canada

Field supplies including formalin, histology cassettes, tools (forcep, scalpels, scissors etc) will be provided in kind by BC CAHS - value $150
Blood collection materials and RNAlater $510

Materials and Supplies

Creative salmon is donating 30 healthy fish to the project at a market value of $1,500. As the brains will be removed, these fish will not be saleable.

Lab

Histology 75*$32/sample = $2,400 Creative salmon will provide $1,000 as an in-kind contribution.
Plasma ion and osmolality will be conducted in David Patterson's lab. 60 samples * $12/sample = $720
Biomarker study 60 fish * 10 biomarkers (including housekeeping genes) * $5/biomarker = $3,000

Microarray Study: Each array costs a total (excluding labour) of $400 to run, which includes the slide costs ($160 per array within the 44K slide), RNA extractions, amplification, labeling, and hybridization costs, as well as a portion of the service contract on the Tecan hybridization robot and the Tecan robotic slide reader. There is also a built in 5% margin to accommodate slides that have to be re-run. Microarray study 60 fish * 400/array = $24,000

Publication costs

$500 will cover the cost of publishing a manuscript with one colour figure.

Travel

Collaborative Meetings travel CAHS-DFO $400 for 5 trips/year
Field Travel CAHS-Tofino for sample collections $1500 for 4 trips (600km @$.50/km per trip + accommodation (if required)+food), industry in-kind contribution
Boat Travel for sample collection $600 for 4 trips ($150/day), industry in-kind contribution.

1. If more than one partner, please provide details of contribution from each one.

Canada