Freshwater nursery ecosystem linkages to juvenile Fraser River sockeye salmon condition: Exploring predictors of inter-stage effects on survival across diverse nursery ecosystems

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Introduction

Freshwater and early-marine survival variations may be a significant influence on recent variability in Fraser River sockeye salmon returns (Bradford 1995; Selbie et al 2010). Fry and smolt size can elicit inter-stage influences on post-smolt sockeye survival (Ricker 1959, Koenings et al. 1993, Henderson & Cass 1993, Bradford et al. 2001), with growth varying greatly within (i.e. density-dependence) and amongst nursery lakes (i.e. habitat capacity) (Hume et al 1996; Shortreed et al 2001; Cox-Rogers et al 2004). Previous work at Shuswap lake from 2011-2013 explored the relationships between lake ecology and juvenile sockeye growth, condition, and survival across varying salmon densities. In order to further examine the role that nursery lake productivity has on juvenile Sockeye growth and condition, we collected similar data in 2014 and 2015 from multiple lakes, which were selected to reflect a gradient of food web resiliency and carrying capacity for sockeye salmon.

Specifically, we examined several condition metrics including size, condition factor, energetic status (lipids, storage lipids), and osmoregulatory preparedness among smolts leaving Chilko, Cultus, Chilliwack, and Shuswap lakes. Sockeye smolts were collected during the spring outmigration of 2014 from Chilko, Cultus, and Chilliwack lakes, and analyzed in conjunction with new, recently-collected, and historical limnological data, and archived juvenile sockeye samples (fry and smolts). Further smolt sampling in Chilliwack Lake will be conducted in 2015 to pair with the limnology program. The project includes an inter-lake eco-physiological and limnological comparison, and a focussed retrospective analysis on Chilko Lake during the 2007-2013 period, which coincided with an order of magnitude variation in adult sockeye escapement (2007-2012 range: 0.22 - 2.46 M adults), and detailed limnological research conducted by DFO (2009-2012). It is anticipated that an expanded eco-physiological and freshwater food web investigation across these diverse stocks will significantly improve our understanding of the freshwater factors influencing intra- and inter-stage survival for sockeye salmon in the Fraser River.

Study Sites

Chilko Lake

Chilko Lake is a coldwater, ultra-oligotrophic sub-alpine Sockeye Salmon nursery lake, located on the eastern margin of the Coast Mountain Range (51°20'N, 124°05'W). Total surface area of Chilko Lake is 184.5km^2. While a long-term, major contributor to overall Fraser River Sockeye Salmon production, in 2012, Chilko Lake received 246,602 spawners (90,758 EFS).

Chilliwack Lake

Chilliwack Lake is a relatively cold, oligotrophic lake located in the eastern Fraser Valley (49° 03'N, 121° 25'W) with a surface area of 11.9 km^2.

Chilliwack Lake serves as the nursery lake for stocks that spawn in Dolly Varden Creek and in Chilliwack Lake. Recorded escapement estimates to Chilliwack Lake have shown substantial variability (Grant et al. 2011). In 2012, a record escapement returned to the lake with 126,164 total Sockeye (78,823 EFS).

Cultus Lake

Cultus Lake is a relatively small lake (6.3 km^2), located in southwestern B.C. (49° 03'N, 121° 59'W). Cultus Lake is relatively steep sided with limited littoral area, and a mean depth of 31 m and maximum depth of 44 m. The Cultus Lake drainage basin is substantially developed with residential, agricultural and recreational use. Cultus Lake is the most productive Sockeye Salmon nursery lake (as measured by photosynthetic reates) studied in the Fraser Basin (Shortreed et al. 2001). Cultus Lake is the nursery lake for a population of Sockeye Salmon that are listed under COSEWIC (Committee on the Status of Endangered Wildlife in Canada). The Cultus Sockeye stock has been the subject of numerous recovery efforts including hatchery supplementation and predator control. The effectiveness of this program on the recovery of the wild stock is uncertain.

Methods

Fish Collections 2013-2015

Fish samples were collected at Chilko, Chilliwack, and Cultus lakes for condition analysis using either seining nets set from the shore, trawling nets towed through the lake, or at fences installed at outlets to enumerate out-migrating smolts. Outmigrating smolts were collected from the outlet of Chilliwack Lake in the spring of 2014 and will be collected again in the spring of 2015 using beach seines set along the shore. Collections in 2014 were conducted weekly starting April 22 and ending May 14. Offshore fry were also collected at Chilliwack Lake in 2013 and 2014 using a closable mid-water trawl net with an opening measuring 3m in width by 7m in depth (for details, see Enzenhofer and Hume 1989). Trawls were made at locations and depths where fish targets were identified using hydroacoustic equipment. Smolts from Chilko Lake were collected weekly from the enumeration fence located at the outlet from April 23 to May 13. Smolts were collected from the Cultus Lake smolt enumeration fence from May 12-15. Limited smolt collections were conducted on this stock due to its status as endangered under COSEWIC. A proportion of fishes caught were stored at -80 ^{o}C for the assessment of energetic status by the determination total body lipids and storage lipids (triglycerides). To date, 80% (239 of 299, Table 1) of samples collected have been analyzed for total lipid, and extracts have been stored for future triglyceride assays.

Lipid determination

Whole fish were removed from ultra-cold storage and allowed to thaw at room temperature before weight and length measurements were taken. Fish were then coarsely chopped with a scalpel and placed in 50ml Nalgene polypropylene copolymer (PPCO) centrifuge tubes with two steel grinding beads and ground at 1700 rpm for two minute periods until homogenized with a SPEX SamplePrep 2010 Geno/Grinder (SPEX, 15 Liberty St., Metuchen, NJ).

Total lipids were extracted from the whole body homogenate using the chloroform-methanol procedure developed by Bligh and Dyer (1959) with some modifications. With a limited amount of tissue available, sample and solvent volumes were reduced to allow for replication without pooling samples. The solvent ratio was maintained at 1:1:0.48 parts methanol, chloroform and water, respectively. The dilution (tissue to non-water solvent) was 20 times, or 0.2 g wet tissue to 4 mL total solvent. The SPEX Geno/Grinder was used to blend homogenate, solvents and water before filtration and biphasic separation of the lipid-chloroform layer from the water-methanol layer. A known volume of lipid-chloroform was pipetted to a weigh dish, the chloroform evaporated, and the remaining lipid weighed using a 4-place balance.

Triglycerides

Whole body triglycerides levels were determined using a commercially available colorimetric assay kit from Cayman Chemical (Item No. 10010303; Cayman Chemical Company, Ann Arbor, MI). We employed methods from Weber et al. (2003) who modified methods developed for serum triglycerides (McGowan et al. 1983). We chose Cayman Chemical over Sigma assay kits (used by Weber et al. 2003) based on cost. The assay itself does not differ, and uses the enzymatic hydrolysis of the triglycerides by lipase to glycerol and free fatty acids. The glycerol determined by this assay may be any combination of mono-, di-, or triglycerides and cannot be differentiated between. However, triglycerides are the primary storage lipid in fishes (Hendersen and Tocher, 1987; Sheridan 1988), and Iverson et al. (2001) showed through thin layer chromatography that triglycerides were the primary component of the lipid extract from the Bligh and Dyer method.

Chloroform-lipid extracts (100ul reserved from lipid extraction procedure) were removed from ultra-cold storage and dried under nitrogen. Samples, standards and a blank (dried chloroform) were then reconstituted in isopropanol and assayed in duplicate according to the assay protocol supplied with the kit. Average absorbance was read over the spectrum range of 530-550 nm using a FLUOstart Omega multimode microplate reader. Absorbance was corrected by subtracting the average absorbance of the blank from all samples and standards. The concentration of triglycerides was then calculated using the following equation:

$$\text{Triglycerides (mg/dL)} = [(\text{corrected absorbance}) - (\text{y-intercept})] \times \text{slope}^{-1}$$

Triglyceride concentration was then converted to mass in the following calculation. Values were expressed as triolein, as it is comprised of a glycerol and three units of the fatty acid oleic acid, and oleic acid is the most common fatty acid in triglycerides (Weber et al. 2003).

$$\text{Triglyceride (g)} = \text{triglyceride (mg/dL)} \times 0.0113\ \text{(mmol/L)} \times \text{recovered chloroform (mL)} \times 885.5\ \text{(g/mol)} \times \text{dilution} \times 10^{-6}$$

Where 0.0013 (mmol/L) is the conversion of triglycerides from mg/dL to mmol/L, 885.5 (g/mol) is the molecular weight of triolein, recovered chloroform is the volume retained during lipid extraction, and the dilution factor has no units

Juvenile Sockeye Salmon Diet

Sockeye diet was examined in fish collected from Chillwack, Chilko, Cultus lakes. Stomach contents from up to 20 *O. nerka* per sample event were examined. In cases where

stomach samples were collected from fish captured in trawls, we attempted to minimize bias caused by different digestion rates of prey, by only sampling fish captured in trawls made within 3 h after the onset of darkness (approximate start of the most intensive feeding period). Samples consisting of the contents of up to ten pooled stomachs were subsampled with a Folsom plankton splitter and enumerated with a computerized video measuring system (MacLellan et al. 1993). Relative volume of prey types in the 12 stomachs and an index of stomach fullness expressed as a percentage by volume were estimated using a technique modified from Hellawell and Abel (1971).

Limnology

A full suite of physical, chemical, and biological limnological variables were collected in Chilliwack and Cultus lakes in 2014. Limnological data were collected monthly from May to October (n=6/yr). Each month, 1 limnological station was sampled in Chilliwack and Cultus lakes. The methods were similar to those used on many other limnological investigations of B.C. Sockeye lakes (i.e. Shortreed et al. 2001), but those pertaining to the data analyzed here are described below.

All water sampling took place between 0800 and 1100 h (PST). We used an opaque, 8-L Van Dorn bottle to collect all water samples. Integrated water samples (water mixed from 4-5 depths within each interval) were collected from within the euphotic zone (depth of light penetration to 1%). During lake stratification, separate integrated water samples were retrieved from the epilimnion and from the bottom of the epilimnion to the bottom of the euphotic zone. A hypolimnetic sample was collected from a depth of 50 m. All collected water was processed (filtered, frozen, or preserved) within 3 h of collection. A Li-Cor data logger (model LI-250A) equipped with a model LI-193SA spherical quantum sensor was used to measure photosynthetic photon flux density (PPFD) (400-700 nm) and determine euphotic zone depths.

In situ phytoplankton photosynthetic rates (PR) were estimated using standard techniques of ^{14}C uptake in light and dark bottles (Shortreed et al. 2001). PR was determined at 7 discrete depths from the surface to below the euphotic zone. At each depth, three 125-mL glass bottles (2 light and 1 dark) were filled, inoculated with a ^{14}C-bicarbonate stock solution, and incubated for 1.5-2 h at the original sampling depth. Incubations commenced between 0900 and 1100 h (PST). At each sampling date three scintillation vials containing 0.5 mL of 0.2 N NaOH were also inoculated with the ^{14}C-bicarbonate solution for later determination of radioactivity. Post-incubation, the bottles were placed in light-proof boxes and transported to the field laboratory where samples were filtered onto 25-mm diameter AMD glass fiber filters (0.45 µm pore size) <2 h after the incubations stopped. Post-filtration, the samples were placed in scintillation vials containing 0.5 mL of 0.5 N HCl and lids were left off the vials for 6-8 h to evolve unattenuated ^{14}C from each sample. 10 mL of Scintiverse II (Fisher Scientific) was added to each scintillation vial and the activities were determined using a Beckman Coulter LS6500 liquid scintillation counter. A quench series composed of the same scintillation cocktail and filters used for sampling was used to determine counting efficiency. The equation of Strickland and Parsons (1972) was used to calculate hourly PR. Daily PR (mg $C \cdot m^{-2} \cdot d^{-1}$) was estimated

using sunlight data collected with Li-Cor LI-1400 data loggers and Li-Cor 190SA quantum sensors at the Cultus Lake Salmon Research Laboratory (Cultus) and the Chilliwack River Hatchery (Chilliwack).

At each PR depth, water was also collected for pH and alkalinity determinations in 125-mL glass bottles. Within 4 h of collection, a Cole-Parmer Digi-Sense pH meter (Model 5986-10) and Ross combination electrode were used to determine pH and total alkalinity (mg $CaCO_3$/L) according to the standard potentiometric method of APHA (1998). Dissolved inorganic carbon (DIC) concentrations, used in PR estimation, were calculated indirectly from pH, temperature, total dissolved solids and bicarbonate alkalinity.

Surface area of the lake was determined by digitizing the lake shoreline from 1:50,000 topographic maps using Oziexplorer GIS software. Seasonal averages of data from each station were calculated as time-weighted means of data obtained from April to November. Seasonal average PR was computed by assuming PR was zero on May 1 and October 31. Means for limnological station in Chilko Lake were calculated. Whole-lake averages were calculated by weighting the value for station in Chilko Lake by the proportion of the lake each represented.

At each station in 2014, replicate zooplankton samples were collected with a 160-µm mesh Wisconsin net (mouth area =0.05 m^2) hauled vertically to the surface from 30 m. All samples were placed in 125-mL plastic bottles and preserved in a sucrose-buffered 4% formalin solution (Haney and Hall 1973). Zooplankton were later counted (rotifers and nauplii were not counted), identified to genus or species (Pennak 1978; Balcer et al. 1984), and measured with a computerized video measuring system (MacLellan et al. 1993). Measurement of body length was carried out as described by Koenings et al. (1987). Zooplankton biomass (milligrams dry weight) was calculated with species-specific length-weight regressions adapted from Bird and Prairie (1985), Culver et al. (1985), Stemberger and Gilbert (1987), and Yan and Mackie (1987).

Preliminary Results

Juvenile Sockeye condition

Sampling at additional lifestages for Chilliwack Lake Sockeye showed an expected decrease in lipid values from fall fry to smolts, as fish would have consumed some portion of their reserves over the winter months (Figure 1). Interestingly, fall fry in 2013 (BY 2012; dominant year) had lipid values approximately twice those of fall fry in the subsequent (BY 2013) year. This was apparent despite the fact that there were nearly seven times the number of fry in the lake in 2013 (n = 1,791,942) than there was in 2014 (n = 245,378). The lower lipid value for fry in 2014 may be an indication of a carry-over effect from the higher density year.

Lipid content in outmigrating smolts from Chilliwack and Chilko lakes showed some variation over the course of the migration, with earlier timed migrants generally having higher lipid content than later timed migrants (Figures 2 and 3). Similar changes in lipid values were also previously observed in out-migrating smolts from Shuswap Lake in 2012 (BY 2010).

Mean percent body lipid content of outmigrating Chilko smolts were higher than smolts from Chilliwack, which could be representative of their longer migration (Figure 4). Outmigrating

Cultus smolts have the highest lipid content (5.1%), but it should be noted that only 50% of samples collected have been analyzed, and that these fish were of hatchery origin.

Initial analyses of 2-year old smolts leaving Chilko show lower lipid content than the 1-year old smolts (table 1), however adjustments have not been made for the larger size of the 2 year olds. High lipid content in lake rearing juveniles (2012 brood year) in the fall of 2013 in Chilliwack Lake indicates preparedness for overwintering, however it is concerning that the corresponding collection of lake rearing juveniles in the fall of 2014 (2013 brood year) is significantly lower, which raises the possibility of low overwinter survival.

Next Steps

Currently we are still waiting for results to come back from several fish (e.g. isotopes, diet analysis, fish size, osmoregulatory preparedness, etc.) and limnological analyses (i.e. zooplankton, etc.). Once available, these results will be integrated into the current dataset for a more holistic analysis, which should allow us to draw conclusions for the final report.

Lipid content for the remaining 60 samples from 2014 will be complete by March 15st 2015. The corresponding triglyceride assays will be run immediately following the completion of the lipid extractions. Planning and preparation for collections at Chilliwack Lake in April-May of 2015 have been initiated.

Table 1: Summary of 2014 juvenile sockeye collections by location, processed to date.

Location	Capture Method	Date	Mean	Stdev	Total Sample Size	Total Analyzed
Chilko River	Fence	23-Apr-14	3.4	1.1	20	20
		29-Apr-14	3.9	1.3	20	10
		07-May-14	3.3	1.2	20	10
		13-May-14	2.7	0.7	20	20
		2014 2YR	2.5	1.0	9	9
Chilliwack River	Beach Seine	22-Apr-14	2.9	1.1	20	20
		29-Apr-14	2.7	1.0	20	20
		06-May-14	2.2	0.8	20	10
		14-May-14	2.4	1.0	40	30
Chilliwack Lake	Trawl	22-Oct-13	6.8	1.5	20	20
		30-Apr-14	3.2	1.6	20	20
		13-May-14	2.8	1.4	20	10
		30-Jul-14	3.1	0.7	20	20
		20-Oct-14	3.5	1.1	15	10
Cultus (Sweltzer Cr)	Fence	12-May-13	5.1	1.3	15	10
		total			299	239

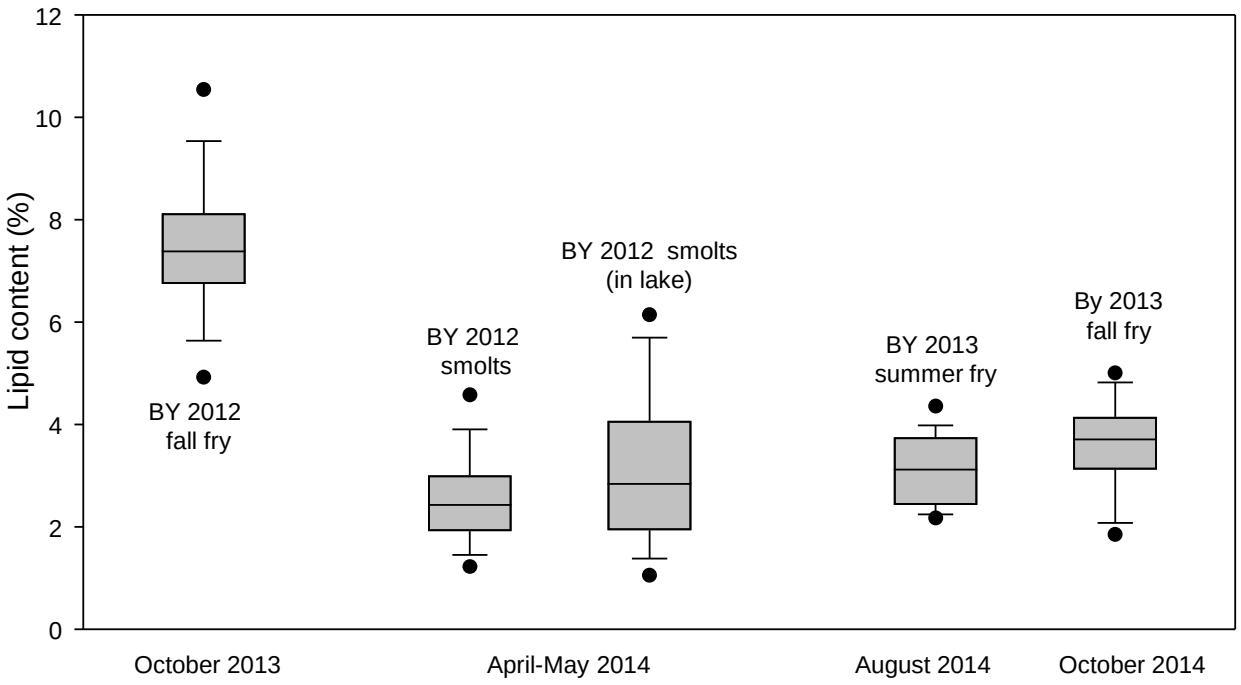

Figure 1. Lipid content (%) of Chilliwack Lake Sockeye fry and smolts sampled from late 2013 through late 2014.

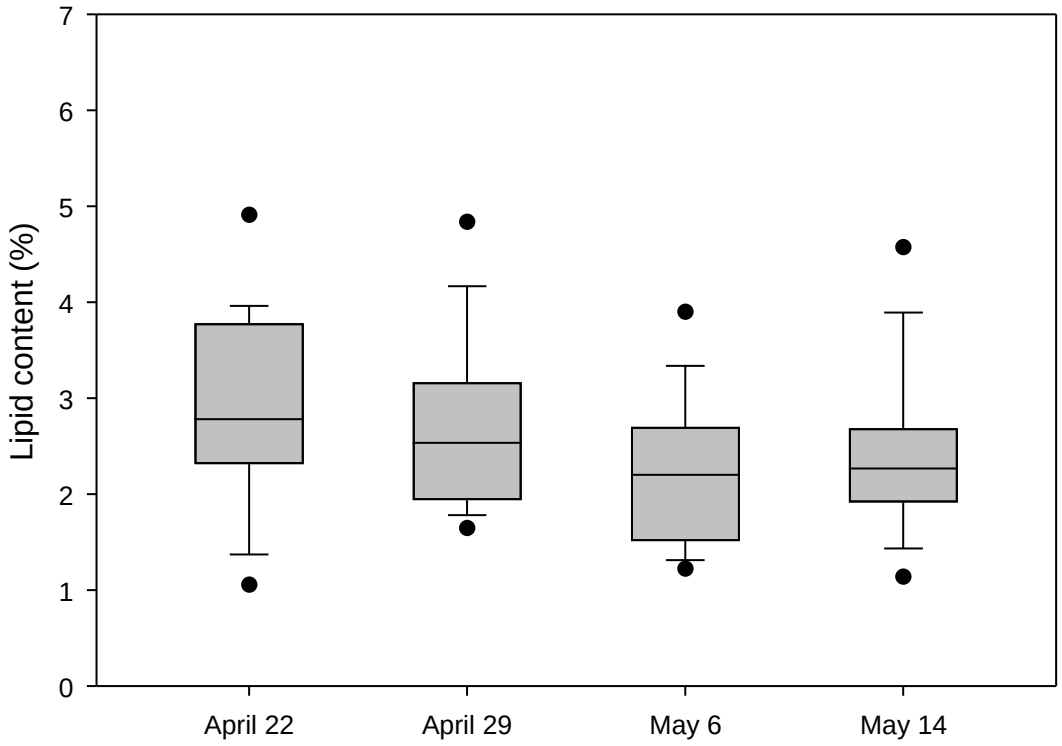

Figure 2. Lipid content (%) of Chilliwack Lake Sockeye smolts (BY 2012) captured at the lake outlet using seine nets in the spring of 2014.

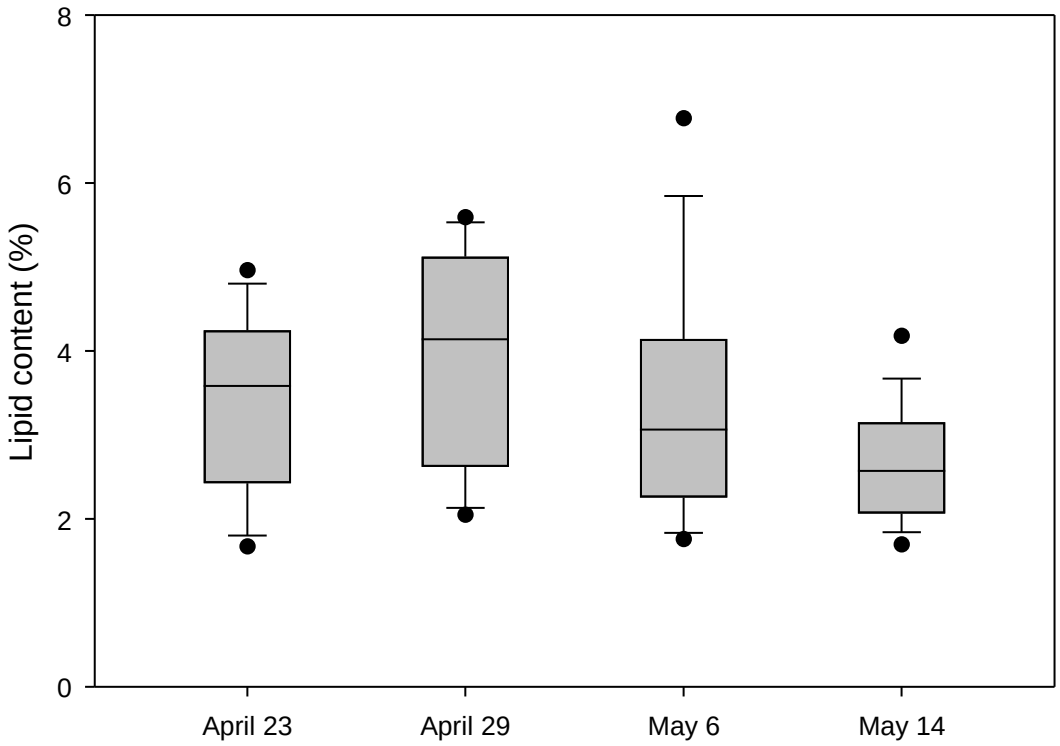

Figure 3. Lipid content (%) of Chilko Lake Sockeye smolts (BY 2012) captured at the smolt enumeration fence at the lake outlet in the spring of 2014.

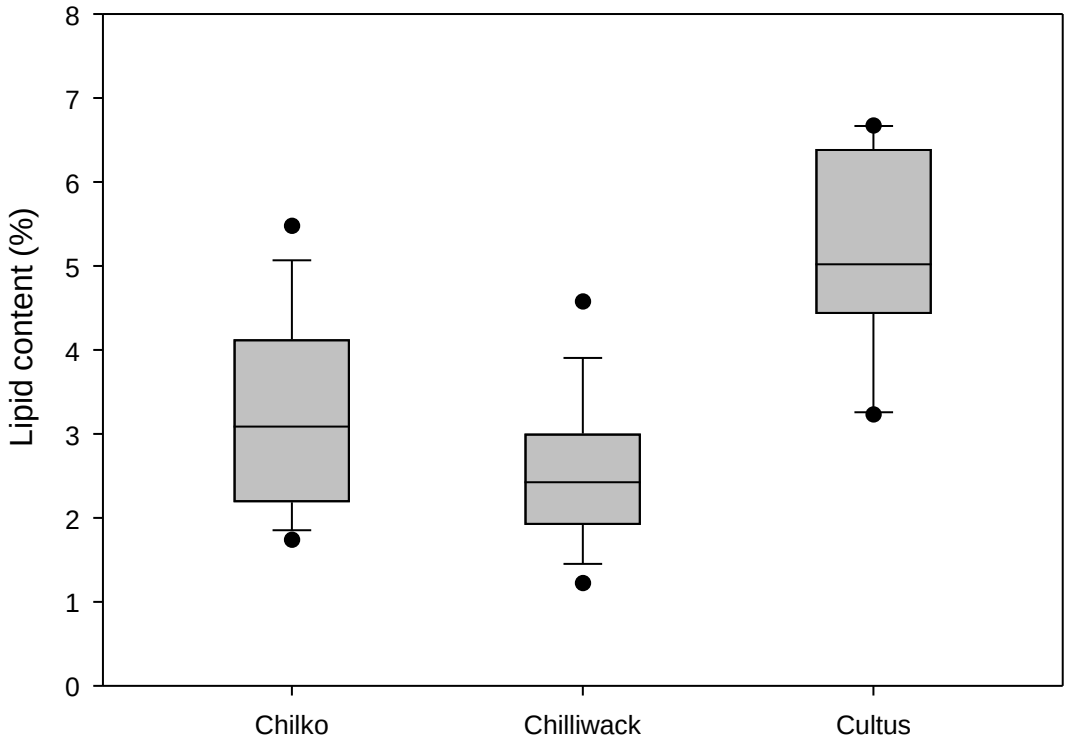

Figure 4. A comparison of lipid content (%) of Sockeye smolts (BY 2012) out-migrating from Chilko, Chilliwack, and Cultus lakes.

Project Budget Expenditures

Cost	Specifics	Initial Budget Estimate	Obligations and Costs Incurred to Date
Salary	Lab/field technicians, Employee Benefit Package (EBP)	$32,038	$28,538
Supplies/Materials	Field/lab consumables	$27,500	$24,486
Travel	Accommodation/meals/travel	$3,200	$3,200
Equipment repair/maintenance	Filed vehicles/vessels, analytical equipment	$2,400	$2,400
Subtotal		$65,138	$58,624
Total received to date			$58,624
PSC 10% holdback			$6,514
Forecasted account balance at March 31, 2015			$0