EXAMINING THE USE OF LIPID ANALYSIS
AS AN INDICATOR OF FISH HABITAT CONDITION
AND OVERWINTER SURVIVAL OF CHINOOK SALMON
IN LARGE RIVERS OF CENTRAL BRITISH COLUMBIA

by

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ABSTRACT

Lipid and triglyceride levels have the potential to be indicators of the status of individuals and populations of fish because they may affect overwinter survival and energy allocation strategies. Identification of (1) seasonal lipid levels in juvenile fish, and (2) the relationship between fish lipid levels and fish habitat may provide managers with a tool for monitoring the quality of fish habitat in rivers. I assessed seasonal and spatial variation in lipid and triglyceride levels for young-of-the-year stream-type chinook salmon (*Oncorhynchus tshawytscha*) throughout central British Columbia, Canada. I found triglyceride levels were highest in fish from the Bridge River, a flow-regulated river, with maximum levels occurring in November. I related triglyceride levels to environmental variables, and found a significant positive relationship between triglyceride levels and food availability. My research supports the potential for using triglyceride analysis as an evaluative tool in freshwater monitoring programs.

Keywords: Chinook salmon, freshwater ecology, lipids, triglycerides, monitoring, fish habitat

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INTRODUCTION

Effective habitat monitoring

Freshwater fish habitat is continually affected by human activities. Inside

watershed boundaries, activities such as forestry (Hartman et al. 1996), mining (Brown et

al. 1998), hydroelectric power generation (Dauble et al. 2003), and land development

(Wang et al. 2000) can alter fish habitat, resulting in changes to water quality, spawning

and rearing habitat, cover, and/or fish passage. In response to these activities, fish habitat

monitoring programs have been set up across North America with a goal to monitor and

evaluate the response of fish populations to habitat alterations and restoration practices

(Roni et al. 2002).

Monitoring fish abundance in rivers is an on-going challenge for managers.

Abundance can be estimated using a variety of techniques, including electrofishing

surveys (Reynolds 1983; Roni and Fayram 2000), snorkel surveys (Roni and Fayram

2000), mark-recapture studies (Wydoski and Emery 1983), or rotary screw traps during

out-migration (Thedinga et al. 1994). However, obtaining precise abundance estimates is

often difficult. Consequently, abundance estimates are often complemented with metrics

that evaluate habitat quality such as macroinvertebrate abundance (Resh et al. 1996;

Bailey et al. 1998) and physical habitat features (Maddock 1999). However, these

indirect measures are quite expensive to monitor. For instance, BC Hydro spends

approximately $60,000 a year to monitor benthic and drifting invertebrates in the Bridge

River (P.S. Higgins, BC Hydro, Burnaby, B.C., personal communication). Furthermore,

these complementary monitoring techniques are difficult to implement in large rivers and only provide indirect measures of fish population status. Thus, if less expensive methods that can provide similar or superior quality of information are available, their use is certainly worth pursuing.

Biomolecules as monitoring tools

Recently a new approach has emerged that uses metrics of individual fish performance based on biological macromolecules as a means to make more direct inferences regarding the health or fitness of fish populations (Adams 1999). Biological macromolecules, such as lipids and proteins, are believed to be effective measures of individual fitness and condition (Adams 1999). These individual-based parameters may be a more direct means to evaluate the health or fitness of populations than traditionally used population-based measures (e.g., population abundance) because they have better statistical properties (larger "signal to noise ratio") and because these parameters reflect the mechanisms that underlie change at the population level (Osenberg et al. 1994).

Lipids

The four major classes of biological macromolecules are lipids, proteins, carbohydrates, and nucleic acids, all of which are essential components of all living organisms (Horton et al. 1996). Lipids have widely varied structures and biological functions, are important in the formation of biological membranes, waxes, vitamins, hormones, and steroids, and act as storage molecules for metabolic energy. Fatty acids are the simplest type of lipid and several other lipids contain, or are derived from, fatty acids (e.g., storage lipids and membrane lipids, Horton et al. 1996).

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Fatty acids are an important source of metabolic energy. Due to their unique structure, oxidation of fatty acids yields more energy ($\sim$37 kJ·g^{-1}) than oxidation of proteins ($\sim$16 kJ·g^{-1}) or carbohydrates ($\sim$16 kJ·g^{-1}) (Horton et al. 1996). Most fatty acids are stored as neutral lipids called triglycerides (also known as triacylglycerols) where each triglyceride molecule is composed of a glycerol backbone and three fatty acid side chains. Triglycerides are hydrophobic; thus, they can be stored in cells in an anhydrous form, which is an efficient form of energy storage. Depending on the fish species, triglycerides can be stored in various tissues including muscle, liver, subdermal tissue, and the mesenteries. However in salmonids, triglycerides are primarily stored in the adipose tissues within the visceral cavity in association with the mesentery of the gut and the gonads (Henderson and Tocher 1987). Although triglycerides can be synthesized in the liver, most stored energy is of exogenous origin because the lipid content of the diet is generally high (Champe et al. 2005).

Lipids as a monitoring tool

Lipids are important to fish health and play an important role in (1) overwinter survival, (2) energy allocation strategies, (3) reproductive performance, (4) early life history strategies, and (5) environmental stress response (Adams 1999). As with all animals, triglycerides are the primary form of storage lipid in all fish (Sheridan 1988) making them also the primary form of stored energy (Higgins and Talbot 1985). These molecules are readily mobilized during periods of food deprivation (Henderson and Tocher 1987; Jobling et al. 1998) and upon exposure to environmental stress (Henderson and Tocher 1987).

Fishes overwintering in rivers or lakes in temperate or Arctic regions are exposed to extended periods of very low water temperatures, low primary productivity, and low food abundance throughout the winter months. For stream-dwelling salmonids, wintertime is considered a critical period for survival (Cunjak and Powers 1987). In mid-to late-summer, juvenile salmonids begin to prepare themselves for this food-limiting period by increasing their lipid stores (Bailey 1975). During the wintertime, fish can then depend on their energy reserves, in combination with a reduced rate of feeding, to sustain themselves until springtime.

Several studies provide evidence to suggest juvenile salmonids must maintain a critical minimum lipid level in order to survive. Mortality resulting from lipid depletion below a critical minimum level has also been presented as depletion below a fixed proportion of pre-winter levels, referred to as the 50 % rule (Kleiber 1961). Adams (1999) clarifies the 50 % rule as the point at which a starved fish depletes the usable portion of its body energy (mainly triglycerides), leaving only phospholipids, protein, and small amounts of carbohydrates. Starvation-induced mortality likely occurs once all triglycerides have been exhausted (as well as any proteins and carbohydrates) and fish begin to use phospholipids as an energy source (Adams 1999). This is because the breakdown of cell membranes, which are comprised of phospholipids, has severe biochemical and physiological consequences.

Winter-induced mortality has been examined using starvation studies. Under simulated winter conditions, Biro et al. (2004) found whole-body lipid levels for young-of-the-year rainbow trout (*Oncorhynchus mykiss*) depleted gradually over time, resulting in death when lipid concentration fell below 1 % of body weight. Cunjak (1988) collected

brook trout (*Salvelinus fontinalis*) and brown trout parr (*Salmo trutta*) seasonally over a three year period and reported the lowest lipid levels occurred in the winter, with levels being depleted to between 2 and 4 % of body weight (compared with spring values of up to 8 %). For young-of-the-year chinook salmon (*O. tshawytscha*) collected during the wintertime, Beckman et al. (2000) reported lipid levels were depleted down to between 2 and 3.5 % of body weight (compared with summer values of 5-8 %).

Seasonal changes in lipid levels in juvenile salmonids have been documented in many other studies as well. For juvenile Atlantic salmon (*Salmo salar*) and brown trout collected from a temperate river in Norway, whole-body lipid levels declined greatly over the winter period but were replenished rapidly in the spring (Berg and Bremset 1998). From September through April, the greatest decline in lipid levels was observed in parr (age-1) of both species, where levels declined by 50-68 %. Over the same period, lipid levels declined for young-of-the-year Atlantic salmon and brown trout by 44-50 %. For both species, lipid depletion was most rapid from September to December, while the decrease from December to April was less severe. Sutton et al. (2000) reported similar seasonal changes in whole-body lipids of Atlantic salmon parr collected from Northeast Brook located on the southeast coast of Newfoundland, Canada. Here, lipid depletion was most rapid from August to November, but continued to decline through April, though at a lesser rate. Fish collected in the spring (June) were considerably fatter than post-winter lean fish (April).

Individual preparedness for the overwinter period likely depends on food availability, a reflection of habitat conditions, as well as foraging strategies and energy allocation strategies. Juvenile fish allocate assimilated energy towards three main

functional processes: growth, metabolism, and lipid storage (Adams 1999). Successful

overwinter survival depends on a fish's ability to allocate a necessary proportion of food-

derived energy towards lipid (triglyceride) storage in order to accumulate sufficient

energy reserves to sustain itself through this food-limiting period. However, an

individual's energy allocation strategy and ability to accumulate enough energy stores

before the onset of winter is also influenced by foraging strategies. When foraging,

juvenile salmon face the risk of being preyed upon and this risk varies considerably on a

seasonal, daily, or even minute-to-minute basis. There is a trade-off for stream-dwelling

fish between energy acquisition strategies and predator avoidance (Lima and Dill 1990).

Juvenile salmon often seek refuge from predators by concealing themselves within

physical habitat features (e.g., rocky substrate, woody debris), turbid waters, or darkness.

Individual fish must then make decisions about when to venture from cover in order to

forage, and these decisions can have major impacts on individual fitness and ultimately

population dynamics (Walters and Juanes 1993).

Research objectives

The purpose of my research was first to evaluate the feasibility of using

triglyceride levels as a non-abundance-based indicator of fish-population and habitat

health, and second, to explore some of the key features of this indicator, including

variability across fish sizes, seasons, and spatial locations. Several researchers have

acknowledged the importance of triglycerides in overwinter survival (e.g., Adams 1999;

Finstad et al. 2004); however, to date there are no studies which have tested the use of

triglycerides as an evaluative tool in monitoring programs. My study is the first to

directly monitor temporal and spatial changes in fish triglyceride levels and to explore the

potential for using triglycerides as a monitoring tool for habitat quality and overwinter survival. Until quite recently, measuring triglyceride levels could only be carried out using specialized techniques such as high-pressure liquid chromatography (HPLC) or thin layer chromatography (TLC) (e.g., Jobling et al. 1998; Heintz et al. 2004), which likely contributed to the lack of research on seasonal variability in fish triglycerides. However, Weber et al. (2003) developed a new and less complicated procedure for determining triglyceride mass in a lipid sample. This new technique, which uses a spectrophotometer to measure triglyceride levels, increases the feasibility of including measures of triglycerides in fish habitat monitoring programs.

Monitoring programs are usually set up to survey fish populations that are potentially (or have been) affected by disturbances. In order to ascertain whether triglycerides represent a useful, complementary addition to existing habitat and abundance monitoring programs, it is first necessary to understand the basic aspects of triglyceride dynamics in wild fish populations. The study design for this research considered both temporal and spatial variability in triglyceride levels of young-of-the-year chinook salmon. I examined seasonal changes in triglyceride levels from young-of-the-year chinook salmon from the Bridge River, a tributary of the Fraser River, British Columbia, Canada. This involved intensive sampling carried out over a two-year period. The Bridge River is a flow-regulated river on which BC Hydro (a crown corporation primarily responsible for generation and distribution of electricity in British Columbia) has been involved in fish habitat monitoring since the 1990s. Currently there is a long-term experimental flow release program in place aimed at assessing the direct impacts of flows on fish under different flow treatments. Salmonid biomass is currently used as the

primary indicator for evaluating population response to each flow treatment. Along with surveys for biomass estimates, ancillary habitat information such as invertebrate abundance is also collected. This makes the Bridge River an ideal site for examining seasonal dynamics in triglyceride levels, as well as exploring the value of using individual-based measures of fish performance and condition as complementary, non-abundance-based indicators of population and habitat health. For example, if a given experimental flow regime affects food availability, which is difficult to measure directly, this could be reflected in the triglyceride levels of the juvenile chinook salmon.

I was also interested in whether the dynamics of fish lipids vary according to fish habitat. Therefore, sampling on the Bridge River occurred at two locations, each with a different set of habitat conditions. At both sites I also sampled environmental variables, such as water quality and food availability, in order to determine whether I could relate observed differences in lipid dynamics between reaches to measurable differences in stream habitats.

A spatial component was also included in this study, allowing for comparisons between triglyceride levels observed in young-of-the-year chinook salmon from the Bridge River and those from neighbouring non-flow-regulated rivers. Ten additional sites were chosen throughout the Fraser River basin, and these sites were sampled three times over the period of a year, providing a temporal and spatial "snapshot" of chinook salmon triglyceride levels. This comparative approach is analogous to the reference-condition approach used by freshwater biologists, whereby impacted and non-impacted sites are compared using biotic indices derived from metrics of benthic macroinvertebrate community structure (Resh et al. 1996; Bailey et al. 1998). I also collected metrics of

water quality and food availability at the Bridge River sites and all reference sites to determine whether these commonly collected habitat variables could explain some of the observed among-site differences in triglyceride dynamics. This approach allowed me to further understand the relation between fish size and triglyceride levels and the range of natural variability of this indicator across space and time.

METHODS

Study areas

Bridge River

Intensive seasonal monitoring of triglyceride levels in young-of-the-year chinook salmon was conducted on the Bridge River located in the Lillooet Land District in the Southern Interior of British Columbia, at 50°45' latitude and 121°56' longitude. This river flows southeast from the snowfields of the Coastal Mountains into the Fraser River just above the town of Lillooet. The Bridge River is a flow-regulated system in which anadromous salmon are restricted to the 42 kilometre section of river located below the Terzaghi Dam (Figure 1). This river is a significant salmonid producing river and provides spawning and rearing habitat for juvenile chinook and coho salmon (*O. kisutch*), juvenile pink (*O. gorbuscha*) and sockeye salmon (*O. nerka*), juvenile steelhead trout, resident rainbow trout, and bull trout (*Salvelinus confluentus*). Resident mountain whitefish (*Prosopium williamsoni*) are also found in this river (for more details see Higgins and Bradford 1996 and Bradford and Higgins 2001).

I sampled two sites on the Bridge River, referred to as the upper reach and lower reach. Although channel substrate is similar throughout the river, consisting largely of boulders (>25 cm in diameter) and large cobbles (10-25 cm in diameter) (Higgins and Bradford 1996), each reach has a unique discharge regime (Figure 2) resulting in considerable differences in fish habitat conditions (see velocity profiles in Bradford and

Higgins 2001). Flow in the upper reach, which extends 15 km downstream of the Terzaghi Dam, is influenced directly by scheduled flow releases from the dam, whereas flow in the lower reach, a 28 km section, is influenced by both releases from the dam and inflow from the adjoining Yalakom River. The upper reach is characterized by low stable flows, with discharge ranging from 2.0-4.7 $m^3 \cdot s^{-1}$, whereas flows in the lower reach are much more variable and range from 3.2-21.2 $m^3 \cdot s^{-1}$.

Fraser River sites

The 10 reference sites were located throughout the Fraser River basin (Figure 3). Criteria for selecting sites included presence of stream-type juvenile chinook salmon, large first-order tributaries to the Fraser River (or sites within the Fraser River mainstem), low-gradient rivers, sites known to have significant chinook salmon spawning runs, and year-round access.

Sampling protocol

I sampled young-of-the-year chinook salmon, benthic macroinvertebrates, and water chemistry from both reaches of the Bridge River between June 2002 and February 2004. There were 5 sampling events in 2002 (June, July, August, September and November), 7 sampling events in 2003 (January, April, June, July, August, September, November), and 1 sampling event in 2004 (February). Thus, the cohort of salmon that was spawned in 2001 was sampled from June 2002 through April 2003, and the cohort of salmon that was spawned in 2002 was sampled from June 2003 through February 2004. A similar sampling protocol for young-of-the-year chinook salmon, benthic macroinvertebrates, and water chemistry was carried out at each of the 10 reference sites

in August and November 2003 and in April 2004. Sampling of the reference sites was thus limited to the 2002 cohort.

For each sampling event, I collected up to 20 young-of-the-year chinook salmon from each site using a backpack electroshocker or a beach seine. Fork length (±1 mm) and weight (±0.1 g) were determined in the field for all fish. Euthanized fish were placed in individual plastic bags and immediately frozen on dry ice. Upon returning from each sampling trip, I transferred fish to a -80 °C freezer. I examined food availability at each site using density estimates of benthic macroinvertebrates. From each site I collected three replicate benthic invertebrate samples from gravel and cobble surfaces using a 0.1 m^2 Hess (substrate) sampler. These samples were passed through a 500 μm sieve and then counted to produce an overall estimate of invertebrate density (number·m^{-2} or No.·m^{-2}). Invertebrate taxonomy was not considered for density estimates. Finally, I collected water chemistry data from each site to provide information on water quality and primary productivity. Field measurements of conductivity (μS·cm^{-1}), temperature (°C), alkalinity (ppm), turbidity (NTU), and pH were collected during each sampling trip, along with water samples for water chemistry analysis. The Cultus Lake Laboratory (Fisheries and Oceans Canada) carried out water chemistry analyses, providing measures of nitrate (μg·L^{-1}), ammonia (μg·L^{-1}), total phosphorous (μg·L^{-1}), total dissolved phosphorous (μg·L^{-1}) and soluble reactive phosphorous (μg·L^{-1}). Laboratory methods are outlined in Stephens and Brandstaetter (1983). I also obtained monthly discharge (m^3·s^{-1}) data for each site from the Water Survey of Canada.

In my analysis, I concentrated on measures that can directly affect primary productivity, as well as food availability and stream habitat conditions for juvenile

salmonids. Specifically I considered discharge, temperature (which directly influences dissolved oxygen levels and can have a deleterious effect of fish when too high), conductivity, alkalinity and pH (which interact to affect ion concentrations as well as the solubility and availability of nutrients), and total nitrogen and soluble reactive phosphorous (which often limit productivity in freshwater ecosystems).

Monitoring on the Bridge River takes place at 7 different locations and two of these locations coincide with the upper and lower reach locations sampled during my research. Consequently, additional fish community and fish habitat information was available for both Bridge River sites. BC Hydro conducts salmonid surveys at each site, producing estimates of average length and weight for young-of-the-year chinook and coho salmon as well as for ages 0-3 rainbow trout. All salmonid populations were sampled monthly from July-December 2002 and from May-December 2003. The density of juvenile salmonids ($g \cdot 100m^{-2}$) is estimated in the fall of each year using an extensive electrofishing survey.

BC Hydro samples aquatic macroinvertebrates throughout the Bridge River to examine invertebrate community structure and food availability for stream-dwelling salmonids. Drifting invertebrates (number$\cdot m^{-3}$ or No.m^{-3}) are sampled at both the upper reach and lower reach over a 24 hour period using a Mundie drift sampler (Mundie 1964). Six drift nets are placed at each site. Drift nets are emptied every 4 hours and the collected invertebrates are rinsed through a 500 μm sieve and placed in labelled vials with formalin for taxonomic identification. All invertebrate samples are identified to the level of taxonomic family using a dissecting microscope. BC Hydro also collects water

chemistry data from all sites. Thus, rather than collecting additional water samples, I used the Bridge River results provided by BC Hydro for my analyses.

Lipid extractions

Gravimetric determination of total body lipids

Eight fish from each sampling event were selected for total body lipid analysis. Fish were selected to span the range of sizes found at each site. I used a chloroform/ methanol extraction procedure to isolate total lipids from each sample, in accordance with the method of Bligh and Dyer (1959). A sample size of eight fish was chosen because the laboratory analysis used a lengthy procedure and a sample size of eight fish allowed me to complete the lipid extractions for one sampling event in one day. As well, this sample size fit within the budget constraints of this project; analysis costs were ~$40 per sample. At some sites, winter sampling conditions made it difficult to locate fish. In these cases, the sample size for lipid extraction was less than eight fish. For sample sizes, see the Appendix. Total lipid mass is reported as grams of lipid per fish. For calculations, see the Appendix.

Spectrophotometric determination of triglycerides

I used the chloroform/methanol lipid extracts obtained from individual fish for the subsequent triglyceride assay. Spectrophotometric determination of triglyceride mass involves the use of a clinical kit (Sigma, St. Louis, MO, U.S.A.) for serum triglycerides. Weber et al. (2003) modified the serum method (McGowan et al. 1983) for use in fish. I modified this procedure further to reduce intra-sample variability (see Appendix).

Triglyceride mass is reported as grams of triglyceride per fish. For calculations, also see the Appendix.

Glyceride molecules can have one, two, or three fatty acid side chains, referred to as mono-, di-, and tri-glycerides, respectively. The spectrophotometric method of glyceride determination cannot differentiate between the three types, but I assumed that all detected molecules are triglycerides. There is some basis for this assumption. Heintz et al. (2004) used HPLC to show that that mono- and di-glyceride molecules are absent from lipid samples produced using a chloroform/methanol procedure similar to the Bligh and Dyer (1959) method I used.

Data analyses

Fish condition

The analysis of body constituents (e.g., lipids, proteins) is often based on the assumption that the constituents vary isometrically with body size so that metrics such as percent of total body weight composed of that constituent are appropriate to use. However, if the relation between a constituent and body size is nonlinear, such simple metrics will be biased, and the bias will depend on the size of the fish. Jobling (1994) states that as fish age they deposit disproportionately increasing amounts of body fat and that the percent body fat of a larger, older fish is usually higher than in rapidly growing young fish. The relation between fish weight and lipid mass is therefore allometric – the rate of lipid accumulation in fish increases with fish weight.

The effects of varying fish size must therefore be accounted for in any comparison of lipid levels in young-of-the-year chinook salmon sampled at different

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times and locations. I examined the underlying relations between length and weight and between weight and either total lipid mass or triglyceride mass. In cases with nonlinear relations, I fit a polynomial regression to the data using least-squares method (Fox 2002).

For each individual fish, I calculated the deviation between each observation and the best-fit regression ("residuals"). For each sampling event I then calculated the average of residuals across all sampled individuals and a corresponding standard error. Means of these residual lipid and triglyceride values were then used as weight-independent metrics of lipid and triglyceride mass in subsequent time series analyses. A similar approach was proposed by Sutton et al. (2000), who developed residual indices from the least-squares regressions between fish length and physiological measures such as fat weight. They concluded that residual indices are a useful alternative to more traditional condition indices (e.g., percent body weight) when the underlying assumptions of linearity are not valid.

Statistical analyses

For comparisons *within* the Bridge River, I used analysis of variance (ANOVA) to test for significant effects of month, reach, cohort, and all interactions on the lipid and triglyceride residuals. When comparing triglyceride residuals *between* the Bridge River sites and the reference sites, I used a nested ANOVA to test for significant effects of month and sampling site, nested within treatments (Bridge River vs. reference site). For all tests, the level of statistical significance was set at $\alpha = 0.05$.

Environmental data

Invertebrate data

Invertebrate density (No.·m^{-2}), collected using the Hess sampler, was compared across all sampling events to determine whether observed differences in lipid levels could be attributed to changes in food availability. To reduce the influence of outliers, densities from replicate invertebrate samples were summarized using a geometric mean (G):

(1) $\qquad G = \left[\prod_{i=1}^{n} a_i \right]^{1/n}$

where a_i denotes density in replicate sample i; densities of zero were assigned a value of 1. The addition of a 1 in place of a zero density will not materially affect the calculation of G because the mean invertebrate density values are all large (median value: 97·m^{-2}).

Using the invertebrate drift data collected on the Bridge River by BC Hydro, I also calculated the total invertebrate drift rate (No.·m^{-3}), as well as the drift rate of Chironomidae (No.·m^{-3}), Simuliidae (No.·m^{-3}) and EPT (No.·m^{-3}), where EPT is the sum of Ephemeroptera, Plecoptera and Trichoptera, a biotic index that identifies presence/absence of pollution-sensitive invertebrates (Lenat 1988). These rates were also compared across sampling events (Bridge River only) to determine whether seasonal differences in triglyceride levels could be attributed to food availability. The drift rate was defined as:

(2) $\qquad drift\ rate = \dfrac{N}{t \cdot W \cdot H \cdot V}$

where N is the number of invertebrates per sample, t is the time the sampler was in the stream, W is the sampler's width, H is the mean height of the water column in the

sampler's mouth, and V is the mean water velocity at the sampler's mouth (Hauer and Lamberti 1996).

Statistical analyses

For the Bridge River sites, I used ANOVA to determine whether reach-level differences in environmental variables could explain the observed reach-level differences in lipid and triglyceride residuals. I tested for significant effects of reach, cohort, and reach x cohort interactions using the monthly samples of total nitrogen, SRP, conductivity, alkalinity, pH, invertebrate drift rate, and temperature as response variables. I also compared annual discharge profiles for the upper and lower reaches of the Bridge River (Figure 2), in order to determine whether differences in discharge levels could explain the observed reach-level differences in lipid and triglyceride residuals.

For the main sampling events (August and November 2003, spring 2004: Bridge River February 2004 and Fraser River April 2004 data combined), I compared invertebrate density (collected using the Hess sampler), water quality indicators, temperature and discharge to the lipid and triglyceride residuals among all sites (Bridge River and reference sites) using Pearson correlation coefficients to determine whether these variables could explain the observed spatial and temporal differences in lipid and triglyceride residuals.

RESULTS

Seasonal changes in fish weight, total lipid mass, and triglyceride mass in young-of-the-year chinook salmon were observed at all sites. I first present results from the Bridge River followed by those from the reference sites.

Bridge River

Fish weight

Substantial seasonal variation in the wet weight of young-of-the-year chinook salmon was observed for both reaches (Figure 4). Fish from the upper reach were consistently larger than fish from the lower reach ($F_{1,1692} = 418.8$, $P < 0.0001$). Fish from the 2002 cohort were larger at the onset of winter (November-December) than fish from the 2001 cohort (month x cohort interaction: $F_{5,1692} = 16.9$, $P < 0.0001$). This was the case for both the upper and lower reaches (reach x month x cohort interaction: $F_{5,1692} = 3.0$, $P = 0.01$). Analyses of fish weight were restricted to data collected from June-December by BC Hydro.

Total lipid mass and triglyceride mass

Seasonal variation also occurred in measures of lipid mass (Figure 5) and triglyceride mass (Figure 6). In the upper reach, lipid mass of the 2001 cohort increased steadily from June through September and decreased from September to November. In contrast, for the 2002 cohort in the upper reach and both cohorts in the lower reach lipid

mass decreased from June to July, followed by an increase through November, the month

in which the maximum mean lipid mass occurred (Figure 5). For the total lipid mass data,

the effects of reach ($F_{1,137}$ = 8.5, P = 0.004) and month ($F_{1,137}$ = 9.3, P < 0.0001) were

both significant. However, the effects of cohort were not significant ($F_{1,137}$ = 0.1, P >

0.05). Very similar seasonal patterns to those for lipid mass were also observed in

measures of triglyceride mass (Figure 6). Once again, the effects of reach ($F_{1,137}$ = 9.2, P

= 0.003) and month ($F_{1,137}$ = 9.1, P < 0.0001) were significant. However, the effects of

cohort were not significant ($F_{1,137}$ = 0.1, P > 0.05).

Allometry of body size and lipids

The general relationship between either total lipid mass or triglyceride mass and

fish weight is both nonlinear and allometric (Figures 7 and 8). I combined fish weight and

total lipid mass data from all Bridge River sampling events and fit these data with a

polynomial regression using a least-squares method (Figure 7). I used the same procedure

for the fish weight and triglyceride mass data (Figure 8). Both analyses involved

choosing a polynomial degree parameter and a "smoothing parameter" to determine how

much of the data to include for each local polynomial fit (Cleveland 1979). In both

models I used a first degree polynomial and a smoothing parameter of 0.75. I explored

smoothing parameters ranging from 0.05 to 0.95 and selected a value of 0.75 based on

visual inspection of the fitted model.

Seasonal variability in total lipids and triglycerides

Lipid residuals

For each sampling event I calculated the mean and standard error of the weight-specific residuals in lipid levels from the best-fit function (Figure 7) and interpreted these as the "size-corrected" lipid status of fish relative to the overall average across all fish. Within the upper and lower reaches, the mean of the residuals for each sampling period displayed similar seasonal patterns across cohorts (Figure 9). For the growing season (June through November) mean total lipid residuals were higher in the upper reach than the lower reach (reach x month interaction: $F_{4,137} = 4.1$, $P = 0.004$) and higher for the 2002 cohort than the 2001 cohort ($F_{1,137} = 33.9$, $P < 0.0001$). That is, the upper reach fish were fatter relative to the lower reach fish. The difference between cohorts was greatest for the upper reach (reach x cohort interaction: $F_{1,137} = 4.2$, $P = 0.04$). In that reach, the mean lipid residuals from the 2002 cohort remained above-average over the entire growing season and the maximum lipid residuals occurred in November (Figure 9a). In the lower reach, maximum lipid residuals in both cohorts occurred in June and in November (2002 cohort only). These patterns are similar to the patterns of maximum mean total lipid mass depicted in the raw data (Figure 5).

Triglyceride residuals

Seasonal patterns in triglyceride residuals (Figure 10) were similar to the seasonal patterns observed in lipid residuals (Figure 9). For the growing season (June through November) mean triglyceride residuals were higher in the upper reach than the lower reach ($F_{1,137} = 4.4$, $P = 0.04$) and higher and consistently above average for the 2002 cohort compared to the 2001 cohort ($F_{1,137} = 8.6$, $P = 0.004$). In both cohorts, lower reach

fish achieved maximum triglyceride accumulation levels by November, two months later than fish from the upper reach (Figure 10). There was a significant reach x month interaction ($F_{4,137} = 4.1$, $P = 0.004$), highlighting that the seasonal patterns differed between reaches. Unlike the situation for lipid residuals, the reach x cohort interaction was not significant for the triglyceride residuals ($F_{1,137} = 1.32$, $P > 0.05$).

Proportion of triglyceride mass per total lipid mass

The proportion of triglyceride mass in the total lipid mass increases asymptotically with fish weight (Figure 11). I described this relation with the model:

$$(3) \quad y = a\,(1 - \exp(-k\,(x - c)))$$

where y is the proportion of triglyceride mass per total lipid mass, x is fish weight, a is the asymptote, k is the decay constant and c is the directional shift in the x-axis. I estimated parameters a, k, and c by fitting equation 3 to the data using a nonlinear least-squares estimator. Newly emergent chinook salmon fry have a mean weight ranging from 0.42 to 0.59 g (Beacham and Murray 1989). The shift parameter was used to allow for a minimum fry size, ensuring the model was not forced through the origin. This method resulted in an R^2 value of 0.48 and parameter values that were all significantly greater than zero: $a = 0.51$ (SE $= 0.03$, $P < 0.001$, $N = 157$), $k = 0.44$ (SE $= 0.11$, $P < 0.001$, $N = 157$), and $c = 0.35$ (SE $= 0.31$, $P = 0.26$, $N = 157$). Note that this analysis was restricted to chinook salmon collected in June – November. The proportion of triglyceride per total lipid for a given size of chinook salmon is highly variable, which contributed to the low R^2 value (Figure 11). However, there is no visible pattern in the residual plot (Figure 12) indicating that the model specification is appropriate (Weisberg 1985).

The plateau in Figure 11 indicates that there is a maximum ratio of stored triglycerides to total lipids (approximately 0.5) for young-of-the-year chinook salmon. The shape of this saturation curve is independent of reach and sampling month – a more detailed investigation revealed that the data from both reaches and from all sampling periods were distributed throughout the graph (not shown).

Membrane lipids

I calculated membrane lipid mass for each fish in order to determine whether the observed changes in total lipid mass were largely driven by changes in triglyceride accumulation. I calculated membrane lipid mass as:

(4)   *membrane lipid mass (g) = total lipid mass (g) – triglyceride mass (g)*

I use the term membrane lipid mass to refer to the mass of all non-triglyceride lipids, the majority of which are cell phospholipids (Voet et al. 1999). However, this measure may also include a small amount of lipophilic proteins (Barnes and Blackstock 1973).

The relationship between fish weight and membrane lipid mass is nonlinear and allometric, similar to the relationship between fish weight and total lipid mass (Figure 7). I calculated the residuals from the best-fit function, which was a local polynomial regression. The time series of mean membrane lipid residuals do not generally show any strong seasonal trends (Figure 13). However, the ANOVA revealed a significant effect of month ($F_{4,137} = 3.4$, $P = 0.01$) and cohort ($F_{1,137} = 43.9$, $P < 0.0001$), and significant interactions for reach x month ($F_{4,137} = 6.5$, $P < 0.0001$), reach x cohort ($F_{1,137} = 5.4$, $P = 0.02$), and month x cohort ($F_{4,137} = 9.2$, $P < 0.0001$). Significant effects in the ANOVA were mainly due to the November samples when there was greater variability in the data because the fish were larger and spanned a wider range of size than in earlier samples.

Nonetheless, the time series of mean membrane lipid residuals (Figure 13) does not resemble that of total lipid residuals (Figure 9) or triglyceride residuals (Figure 10).

Fraser River reference sites

Fish weight, total lipid mass, and triglyceride mass

Mean fish weight increased from August 2003 through November 2003 for the Bridge River (both reaches) and all 10 reference sites (Table 1). From November 2003 to April 2004 mean fish weight increased at 7 sites (Chilko, Quesnel, McGregor, Clearwater and Nicola rivers, and the Fraser River mainstem sites at Stoner and Tete-Jaune Cache) and decreased at 4 sites (Bridge River- both reaches, Little Chilcotin and Cottonwood rivers). No fish were collected from the Nechako River in April 2004. Seasonal patterns in total lipid mass and triglyceride mass were similar to those observed in fish weight. As expected from the relationships between weight and lipids as well as triglycerides shown in the Bridge River fish (Figures 7 and 8), the largest mean fish weight, total lipid mass, and triglyceride mass over all sampling periods came from the Nechako River (November 2003) and Nicola River (April 2004) (Table 1).

Seasonal and spatial variability in triglyceride residuals

At the reference sites, there was a general downward trend in mean triglyceride residuals from summer through late fall and spring (Figure 14). Significant heterogeneity occurred among sites within the treatment groups ($F_{10,175} = 3.68$, $P = 0.0002$), but despite this result, variability within each treatment group (Bridge River vs. reference sites) is smaller than variability between the groups. Overall, the Bridge River residuals were greater than the reference sites ($F_{1,10} = 6.4$, $P = 0.03$; analysis restricted to August and

November samples). Bridge River residuals were similar to the reference sites in August, but were significantly higher in November (treatment x month: $F_{1,175} = 19.6$, $P < 0.0001$).

Lipids and environmental variables

Bridge River sites

I examined the environmental data on the Bridge River (Table 2) to determine whether any of these variables could explain why in both years fish from the lower reach had slower growth and delayed accumulation of lipids and triglycerides compared to fish from the upper reach. Comparisons among lipids and environmental variables were limited to July-September because it is during these months that habitat conditions are likely to have influenced lipid accumulation rates. There were significant reach-level differences in conductivity ($F_{1,8} = 29.2$, $P = 0.0006$) and alkalinity ($F_1 = 84.2$, $P < 0.0001$). However, year, or year x reach interactions, were not significant ($P > 0.05$). Conductivity and alkalinity, considered indicators of stream productivity, were higher in samples from the lower reach (Table 2), but lipid and triglyceride levels were higher in the upper reach (Figures 9 and 10). Thus, conductivity and alkalinity are unlikely to have a significant influence on seasonal lipid accumulation in fish.

There were no significant differences in total nitrogen, SRP, pH, invertebrate drift rates or temperature ($P > 0.05$). During the months of July, August, and September there was little variation in mean monthly water temperature and pH at both reaches of the Bridge River (Table 2). Although total nitrogen, SRP, and invertebrate drift rates were highly variable, there were no consistent patterns across months or between sites that could explain the lower weight (Figure 4) or lower lipid and triglyceride levels (Figures 9

and 10) observed in fish from the lower reach. Note the total nitrogen and SRP values were substantially higher in September 2003 than for all other months: total nitrogen was up to 19 times higher in September than July and SRP was up to times 5 times higher in September than July. Similarly, invertebrate and chironomid drift rates were highest in September 2003. It is important to recognize that the environmental data available for these comparisons are limited both by the frequency of data collection (1 x month) and by the duration of the sampling period (July - September). These limitations may have influenced the detection of significant differences. Incidentally, the annual discharge profiles from the Bridge River indicate that discharge levels are considerably higher in the lower reach than in the upper reach, and this is particularly evident from April though September (Figure 2).

Comparisons across all sites

Data from the Fraser River reference sites were combined with data from the upper and lower reaches of the Bridge River, for a total of twelve sites, and correlation analysis was used to examine relations between both residual indices (total lipids and triglycerides) and all environmental variables collected in the field on each sampling trip (Tables 3-5). Although significant correlations were present between the residual terms and several environmental variables, the only one that was significant over all seasons (and therefore providing the strongest evidence) was the positive correlation between triglyceride residuals and invertebrate density: August 2003, $r = 0.64$, $P = 0.02$; November 2003, $r = 0.64$, $P = 0.03$; and spring 2004, $r = 0.70$, $P = 0.02$ (Figure 15).

DISCUSSION

Lipid dynamics in wild salmonid populations

This study is the first to directly monitor temporal and spatial changes in total lipid and triglyceride levels in wild stream-rearing salmonid populations. It is also the first study to attempt to relate variability in lipid measures to fish habitat condition, using environmental variables such as discharge, food availability, and water chemistry. I compared across sites and seasons the residuals of triglyceride levels from the best-fit function relating these levels to fish weight. This method, which was proposed by Sutton et al. (2000), allowed me to make comparisons without the confounding effects of varying fish size. Although the dynamics of storage lipids in stream-rearing salmonids is still a new area of study, my research supports the potential for using triglyceride analysis as an additional evaluative tool in freshwater monitoring programs.

Effects of body size

In both sampling years, fish from the upper reach of the Bridge River were larger than fish from the lower reach (Figure 4) and several studies suggest that there is a positive relationship between fish size and overwinter survival rate. Does this mean fish from the upper reach have a higher probability of surviving the overwinter period, relative to lower reach fish? There are four conflicting theories that address this issue. The first simply states that overwinter mortality is a size-dependent phenomenon, with

smaller fish experiencing higher levels of mortality than larger fish (Toneys and Coble 1979; Shuter et al. 1980; Hutchings 1994).

The second theory builds on the first, explaining that overwinter mortality is size-dependent, but is based, in part, on allometric properties of metabolism. For example, in an experiment with young-of-the-year yellow perch (*Perca flavescens*), Post and Evans (1989) concluded that smaller fish tend to have lower energy stores and relatively higher metabolism, resulting in higher winter mortality than in large fish because the small fish are unable to meet basic energy demands. In such cases, during periods of low food availability, smaller fish use a higher proportion of their lipid stores to maintain basal metabolic rates than do larger fish (Shuter et al. 1980; Schmidt-Nielsen 1984).

The third theory describes overwinter mortality as the outcome of the interplay of fish size and water temperature. During cold winters, larger fish, which have lower basal metabolic rates, can endure longer periods of limited food intake and starvation than smaller fish under the same conditions (Berg and Bremset 1998). During warmer winters, metabolic rates and energy demands increase and this may switch the survival advantage to smaller fish within the same young-of-the-year cohort (Schultz and Conover 1997). Connolly and Petersen (2003) tested for the effects of fish size and winter water temperatures on lipid content and survival of young-of-the-year steelhead trout and suggest that cold winters could deplete the energy stores of small fish by the end of winter (i.e., large size would be more advantageous) and that exceptionally mild winters could deplete the energy stores of large fish (i.e., small size would be more advantageous). Consequently, the relative harshness or mildness of a given winter will determine whether it is more advantageous to be small or large.

The final theory links winter-induced mortality to levels of actual stored energy rather than body size. Throughout three consecutive winters, Finstad et al. (2004) examined somatic energy levels, calculated by summing the caloric value of extracted lipids and proteins, in 2- and 3-year-old Atlantic salmon parr from a Norwegian river located at 70 °N. They observed a change in the energy distribution of 2- and 3-year-old Atlantic salmon during wintertime and linked this to selective removal of low-energy individuals from the population.

The question still remains whether larger fish size and the earlier timing of maximum triglyceride levels I observed in the upper reach compared to the lower reach, results in a higher probability of overwinter survival. The short answer is "it depends". Simply monitoring fish size cannot provide sufficient information to estimate overwinter survival in stream-rearing salmonids. As evidenced by the aforementioned studies, overwinter survival is likely a function of a combination of fish size, size-specific metabolic allometries, habitat conditions, and overall level of stored energy (triglycerides) at the onset of winter. In order to determine whether the reach-level differences observed on the Bridge River have an influence on winter survival rates, further research should be directed towards monitoring triglyceride levels as well as survival rates in juvenile chinook salmon throughout the winter period at both the upper and lower reaches.

Energy allocation strategies

Ideally, energy allocation strategies (e.g., strategies for allocating energy between somatic growth and energy storage) should be investigated by calculating and summing the caloric energy content of the lipid, carbohydrate, and protein portions of each fish,

using bomb calorimetry (Berg and Bremset 1998; Trudel et al. 2005). This step was not carried out in my research, and instead I have used an indirect method to evaluate the energy allocation strategies used by young-of-the-year chinook salmon from the Bridge River. For each fish, I calculated the proportion of triglyceride mass per total lipid mass and found this ratio to increase with fish weight to a mean maximum of approximately 0.5 (Figure 11). The plateau of this saturation curve indicates that there is a maximum ratio of stored triglycerides to total lipids that young-of-the-year chinook salmon from the Bridge River will achieve. This maximum ratio is either the result of a physiological limitation in the ability of fish to store more than half of their lipids as triglycerides, or that this level of stored triglycerides is generally enough to sustain overwintering juveniles in this system. By comparison, Jobling et al. (1998) examined lipid composition in immature Arctic charr (prior to sea residence) and found the ratio of triglycerides to total lipids was 0.57 in males and 0.59 in females. Even though these Arctic charr are considerably larger than the chinook salmon used for lipid analysis in my study (~400-600 g here vs. 1.2-12.1 g), the ratios of triglycerides to total lipids were similar to the values I observed on the Bridge River. Thus, the plateau of the saturation curve is likely the result of a physiological limitation. However, as far as I am aware, there are no studies further investigating this apparent limitation.

Smaller fish allocate a higher proportion of their food-derived energy towards storage, relative to larger fish, because smaller fish need to build up their triglyceride stores before concentrating on somatic growth. Roche-Mayzaud et al. (1998) monitored growth and triglyceride levels in brook charr fry over a 1-month period (beginning 5 weeks post-hatching) and found fish length increased over the experimental period and

triglyceride levels decreased, even though food was readily available. Furthermore, Roche-Mayzaud et al. (1998) found no evidence of lipid synthesis during the developmental stages considered. In order to grow in length, these fry would have needed to convert both their stored triglycerides and any triglycerides obtained in their diet into energy for somatic growth. Consequently, by June, my first sampling month, young-of-the-year chinook salmon from the Bridge River are likely to have very low triglyceride stores, making it necessary for them to use the summer months to build these levels back up towards the maximum ratio. As the smallest fish increase in body weight, the proportion of food-derived energy allocated towards triglyceride storage becomes limited by the apparent allometry of the proportional relationship (Figure 11). As these fish get larger, their total stored triglyceride mass also grows and maintains a triglyceride-to-total-lipid ratio of 0.5.

Young-of-the-year chinook salmon from the Bridge River appear to use an energy allocation strategy that focuses preferentially on accumulating triglyceride stores during the spring and summer, and then once the ratio of triglyceride mass to total lipid mass is maximized, they switch to a strategy that attempts to maximize growth while maintaining stored energy levels as a fixed fraction of fish size. This strategy differs from the energy allocation strategy proposed by Post and Parkinson (2001) who hypothesized that age-0 rainbow trout must first attain a minimum body size before they begin to allocate energy towards storage for winter use. Thus, the optimal energy allocation strategy for an individual fish depends on the point at which a lipid-maximization strategy becomes more beneficial than a growth-maximization strategy. This point depends on individual growth rates (Post and Parkinson 2001). It is also possible that energy allocation

strategies differ by species or location or both. Sogard and Spencer (2004) found energy

allocation strategies in juvenile sablefish (*Anoplopoma fimbria*) to be size-dependent.

They found that smaller juveniles allocate relatively more energy towards growth and

less towards lipid storage than larger juveniles.

Seasonal dynamics

Seasonal patterns in triglyceride residuals are unique to each reach of the Bridge

River (Figure 10). I found a significant difference in residual patterns between reaches

and a significant interaction between reaches and months. Fish in the upper reach

maintain about average triglyceride levels through the spring and summer and become

fatter by September (Figure 10a), whereas fish in the lower reach are fairly lean

throughout the entire growing season (June-November), becoming fatter in November

(Figure 10b).

In my study, the time series of mean membrane lipid residuals (Figure 13) does

not resemble the time series of total lipid residuals or triglyceride residuals (Figures 9 and

10). This implies that the monthly changes I observed in total lipid levels are largely due

to changes in the triglyceride portion of the total lipids, and not to changes in the

structural or membrane lipids. Consequently, when monitoring lipid levels, researchers

should focus on measures of triglycerides, rather than whole-body lipids. Changes in

triglyceride levels are a direct reflection of available stored energy and this measure may

be more sensitive to a fish's response to environmental changes.

There are considerable differences in fish habitat between reaches of the Bridge

River due largely to differences in stream flow. Consequently, fish from each reach have

probably developed habitat-specific triglyceride accumulation strategies (movement

between reaches is unlikely). I compared environmental data from each reach to determine whether measurable differences in fish habitat could explain why fish from the lower reach have slower growth and a delayed triglyceride accumulation strategy. However, I found seasonal differences in discharge to be the only measure that could help explain this consistent difference. Throughout the year (over all dates sampled by BC Hydro), discharge levels in the lower reach are higher than levels in the upper reach (Figure 2). These high discharge rates could negatively affect the foraging abilities of stream-rearing chinook salmon resulting in reduced food-intake rates, leading to lower mean weight and a strategy of delaying triglyceride accumulation.

Although there was variability in the water chemistry data among months and reaches, there did not appear to be any consistent trend which could help explain the lower mean weight and triglyceride levels of the fish from the lower reach. In September 2003 there was a large pulse in the total nitrogen and SRP data, likely a response to the presence of pink salmon (*O. gorbuscha*) spawning throughout the river. Every two-years adult pink salmon move up through the Fraser River to spawn. These decaying spawners are considered an important source of marine-derived nutrients (nitrogen and phosphorous) in freshwater ecosystems (Johnston et al. 2004). I observed large numbers of pink salmon spawning in the Bridge River during the fall of 2003, which likely explains the elevated levels of total nitrogen and SRP relative to September 2002 (Table 2). High invertebrate densities observed in September 2003 may have also been linked to the presence of salmon carcasses.

Total nitrogen and SRP responded almost instantaneously to the presence of marine-derived nutrients, which means they are very sensitive to changes within the

environment. In my study, water chemistry data were collected and compared on a monthly basis. However, this might not be an appropriate time scale to determine whether relationships exist between these metrics and triglyceride storage levels. Cattaneo and Prairie (1995) examined temporal variability in water chemistry measures (including conductivity and alkalinity) to determine the minimum sample size necessary to describe stream chemistry in the summer, while maintaining a standard error of the mean of 20% or less. When measuring conductivity and alkalinity, Cattaneo and Prairie (1995) recommend a sample size of 1-3 and 1, respectively. However, the Bridge River conductivity values are considerably higher than those reported in Cattaneo and Prairie (1995) (71-170 vs. 22-73) and the recommended sample size for conductivity should have been higher (2-27 samples) in order to account for variability in these measures. Thus, the inability to detect significant differences in environmental variables across sites, sampling events, and cohorts may, in part, be related to small sample sizes.

Foraging strategies

Although energy allocation strategies influence whether fish have stored enough energy to sustain themselves during the winter, accumulating sufficient stored energy is largely influenced by foraging strategies. Previous research with juvenile stream-rearing chinook salmon from the Bridge River suggests that habitat conditions are likely to affect the trade-off between risky daytime foraging and less efficient, but safer, nighttime foraging (Bradford and Higgins 2001). Differences in fish foraging strategies between the upper and lower reaches may also be contributing to the observed differences in mean weight and stored triglyceride levels.

Overwinter survival and critical minimum lipid levels

In the months leading up to winter, the decision to allocate energy towards somatic growth versus energy storage has important implications for first-year survival and overall fitness (Walters and Juanes 1993; Post et al. 1997). Fish that enter the winter season with enhanced energy stores are more likely to survive (Post and Evans 1989; Shuter and Post 1990; Johnson and Evans 1991; Schultz and Conover 1997). If winter arrives early for the Bridge River, chinook salmon fry rearing in the lower reach could face a higher probability of winter-induced mortality because they may not have accumulated sufficient energy stores to sustain them until spring. Estimating survival or mortality rates is a complicated task, and direct measurement of winter mortality rates in wild riverine populations is a monumental undertaking. Seasonal monitoring of juvenile salmonid triglyceride storage is a practical alternative, the results of which may be useful to make inferences about overwinter survival.

Fish must maintain a critical minimum lipid level in order to survive (Hoar 1983). Biro et al. (2004) found that during simulated winter conditions, age-0 rainbow trout gradually deplete lipid reserves to a minimum critical lipid level after which death occurs. In small juvenile trout (mean weight = 0.41 g) the critical level was 0.0099 g lipid·g fish weight^{-1}, and in large juvenile trout (mean weight = 0.75 g) the critical level was 0.0081 g lipid·g fish weight^{-1}, equivalent to 0.8 to 1 % of body weight. Over all months sampled on the Bridge River, the minimum membrane lipid level was 0.0089 g lipid·g fish weight^{-1}, which is very similar to the critical minimum lipid levels reported for small and large juvenile rainbow trout (Biro et al. 2004). This suggests that the critical minimum lipid levels reported by Biro et al. (2004) represent the point when these fish have converted

all their stored triglycerides into energy thus mortality is imminent. Although I did not find fish with total lipid levels lower than the minimum critical levels reported by Biro et al. (2004), I expect that at some point during the winter season fish with critically low lipid levels were present and succumbed to starvation-induced winter mortality.

Rather than comparing grams of lipid per gram of fish weight among studies, it is perhaps easier and more informative to compare the ratio of triglycerides to total lipids for similar-sized fish between studies. I do not know the maximum ratio of triglycerides to total lipids that fish can accumulate, although I speculate that the ratio is 0.5 for juvenile chinook salmon. I presume that as the ratio existing prior to winter declines, the probability of mortality over the winter increases. Even though metabolizing membrane lipids (phospholipids) during starvation will provide some minimal energy (Wilkens 1967; Love 1980), breakdown of cell membranes has severe biochemical and physiological consequences that will ultimately lead to death (Adams 1999).

Berg and Bremset (1998) examined seasonal changes in whole-body lipids of young riverine Atlantic salmon and brown trout and although they did not measure seasonal changes in triglyceride levels, they did report a 44-50 % decline in mean total lipid levels (which include triglycerides) over the winter period. If the maximum ratio of triglycerides to total lipids for juvenile salmonids is ~0.5 and fish convert stored triglycerides into energy before all other types of lipids, the observed 44-50 % decline in mean total lipid levels likely represents depletion of almost all stored triglycerides for these juvenile Atlantic salmon and brown trout.

Spatial dynamics

Triglyceride residuals at the 10 reference sites located within the Fraser River basin were at a maximum in the summer and declined throughout the winter, reaching minimum levels by springtime (Figure 14). Because fish from the Bridge River continued to accumulate triglycerides until at least September, it is likely that at some point between August and November, fish from the reference sites had peak levels of triglycerides; my sampling schedule may not have captured this. Between treatments (Bridge River vs. reference sites), the effects of month were significant, and the greatest difference between the Bridge River and the reference sites appeared in November when triglyceride levels were well above average at the Bridge River (positive residuals) and average or below average at most other sites (Figure 14). Fish from the reference sites may have already begun to depend on their lipid reserves for energy by November.

Comparing the time series of triglyceride residuals across all sites showed that fish from the Bridge River achieved and maintained higher maximum stored triglyceride levels than fish from all other Fraser River stocks examined (Figure 14). Both the Bridge River and all of the reference sites are large first-order tributaries to the Fraser River (or sites within the mainstem Fraser River). However, fish habitat conditions do vary considerably among sites. Healthy fish habitat is a function of many interrelated environmental variables and although it is not possible to measure "fish habitat condition", we can measure the environmental variables that we believe are important for successful rearing.

On a large spatial scale, triglyceride residuals appear to be more closely related to invertebrate density than all other environmental variables collected. Invertebrate density

is the culmination of the many factors that affect productivity and ultimately fish production (Rosenfeld et al. 2005). There are many biological reasons why environmental variables may be correlated with triglyceride levels during one season and not during another. However, in order to explore whether any of these environmental variables could consistently explain triglyceride levels in fish, I focussed on correlations that were significant across all seasons. I found a significant correlation for all sites between triglyceride residuals and invertebrate density (Figure 15). Future research should involve further investigation into this relationship.

The invertebrate density estimates for the Bridge River upper reach are considerably higher than the estimates for most of the other sites, and this is particularly evident in November and in the spring. Throughout the year, hypolimnetic reservoir water is continually discharged into the upper reach of the Bridge River. This has significantly reduced ice cover in the upper reach of the Bridge River (M.J. Bradford, DFO, Burnaby, BC, personal communication) and even though the upper reach sampling site is located 15 km downstream of the dam, it is probable that reservoir discharge is affecting fish overwintering in the upper reach. Regulated rivers (tailwaters) generally have warmer winter temperatures than non-regulated rivers, which can prevent surface ice formation (Cunjak et al. 1987) and in some cases maintain water temperatures closer to temperatures which are optimal for metabolism and growth for trout (Ward and Sanford 1979). Cunjak et al. (1987) found the absence of surface ice and the presence of warmer water allowed trout to see and consume drifting aquatic invertebrates during winter. It seems likely that favourable conditions for invertebrate production have lead to higher triglyceride levels in the Bridge River than most other streams.

Implications for monitoring programs

My research is the first study to attempt to establish a link between triglyceride levels and fish habitat conditions. When comparisons were made across a large spatial scale (across all sites), I found that for all seasons there was a positive correlation between invertebrate density and triglyceride levels. Although I was unable to identify a similar statistical relationship between triglyceride levels and habitat measures on a smaller scale (within the Bridge River growing season), I did find annual discharge profiles to be considerably different between reaches. My findings support the need for more research into the use of triglyceride analysis in fish habitat monitoring programs.

Before incorporating triglyceride analysis into stream habitat monitoring programs, it is first necessary to establish a more concrete relationship between triglyceride levels and overwinter survival rates. Once this link is established, evaluation of triglycerides could potentially benefit many types of fish habitat monitoring programs. For example, storage lipid dynamics may be useful in helping managers to evaluate fish health and rearing success in streams and rivers, either by helping to quantify a population response to habitat impacts or changes in food availability, or by collecting baseline data for future comparisons. Lipid analysis can also be used in the evaluation of marine fish populations. For example, lipid levels have been used to assess energy levels during spawning migration for mature and spent Atlantic salmon (Jonsson and Jonsson 2003) and mature sockeye salmon (Kiessling et al. 2004) as well as to evaluate reproductive potential in Atlantic cod (*Gadus morhua*) (Marshall et al. 1999).

Research directions for the future

Seasonal monitoring of juvenile salmonid triglyceride levels is a practical approach for gathering information to link stream habitat conditions and juvenile salmonid health and survival rates. Results from this study provide a solid basis to motivate future research in this area. Before I could evaluate the potential for using triglycerides as a monitoring tool for fish habitat condition and overwinter survival, I needed to examine both seasonal and spatial variability in triglyceride levels in order to capture the range of variability in unimpacted systems. By using residuals in triglycerides from the relationship between fish weight and triglyceride mass, I accounted for the effect of fish weight on triglyceride levels before comparing these levels across sites and seasons. Although the need for size-independent indices of fish condition has been recognized in other studies (e.g., Jobling 1994; Sutton et al. 2000; Post and Parkinson 2001), this study is the first to consider the use of a weight-corrected metric of triglyceride levels (triglyceride residuals) as an evaluative tool in freshwater habitat monitoring programs. This approach provided baseline data describing seasonal changes in triglyceride levels throughout the Fraser River basin which could then be used for comparisons with sites affected by human activities, such as the Bridge River.

To establish a relationship between triglyceride levels and overwinter survival rates seasonal monitoring of triglyceride levels in juvenile chinook salmon could be combined with a tagging study to monitor overwinter survival. The main logistical problem with this type of study design is that currently triglyceride analysis involves using the whole fish. Thus, even though fish could be tagged before the winter and recaptured in the spring, it wouldn't be possible to directly relate pre-winter triglyceride

levels to overwinter survival. An alternate approach to using the whole fish for triglyceride analysis would be to collect a tissue plug from the adipose tissue of each tagged fish at the end of the growing season, and then perform whole body triglyceride analysis on the tagged fish upon recapture in the spring. If a relationship can be established between the triglyceride level of an adipose tissue plug and the whole body triglyceride level, then inferences relating pre-winter triglyceride levels and overwinter survival rates could be made. Non-invasive techniques, such as the handheld microwave energy metre used by Crossin and Hinch (2005) to estimate whole body lipid and energy concentrations in adult Pacific salmon, could also be considered for use on juvenile chinook salmon.

Limitations

Time constraints and budget can limit the feasibility of including triglyceride analysis in fish habitat monitoring programs. Time limitations may affect observational studies more than experimental studies. In an observational study, for example, one that compares triglyceride levels from lake-dwelling fish at different latitudes, it would be necessary to sample repeatedly throughout the year over several years in order to accurately capture the effects of season and latitude on triglyceride levels. However, in an experimental study, such as evaluating the impacts of food availability on survival rates in a laboratory, temporal sampling requirements are less of an issue because the key comparisons are between experimental and control treatments.

Because budgets are always limited for monitoring programs, it is important to consider how the project budget will influence the number of possible samples and the corresponding implications for statistical power, before including triglyceride analysis as

an evaluative tool in a monitoring program. For instance, if the project budget were to limit the sample size to 5 fish per site (for each sampling event), it would be necessary to conduct a pilot study to examine the range of natural variability within each site in order to determine via power analysis the number of samples required to detect significant differences between sampling events for a given true effect size. If the power analysis revealed that a sample size of 5 fish (or whatever the constraint was) was too small to detect a specified important deviation from the null hypothesis, then the use of triglyceride analysis should be discouraged.

Currently, BC Hydro spends approximately $60,000 per year to monitor benthic and drifting invertebrate communities throughout the Bridge River (P.S. Higgins, pers. comm.). This does not include costs associated with sampling fish populations. In comparison, the per-fish cost for total lipid and triglyceride analysis is about $40, totalling approximately $4,000 per year (following the Bridge River sampling protocol outlined used in my research). Even if the sampling intensity were doubled, bringing the additional costs to $8,000, the addition of triglyceride analysis to the monitoring program may provide managers with more useful and relevant information from which they could evaluate the response of fish populations to changes in stream flow. Additional research could also be directed towards identifying relationships between triglyceride levels and invertebrate densities. If there appears to be a high degree of correlation between these metrics, managers may decide to reduce or remove the costly evaluation of invertebrate drift communities, which would in turn reduce yearly monitoring costs.

Finally, if the fish habitat monitoring program has a spatial component, as was the case in my research, the cost of collecting samples and carrying out triglyceride analysis for each additional reference site will substantially increase project costs.

Guidelines for monitoring triglycerides in freshwater habitats

Here are suggested guidelines for including triglyceride analysis in fish habitat monitoring programs.

1. <u>Determine whether triglyceride analysis is an appropriate monitoring tool</u>. The decision to include triglyceride analysis as an index for habitat monitoring programs should depend on the overall objectives of the program. For instance, if the program aims to evaluate long-term effects of human disturbances on a stream-dwelling salmonids, then monitoring triglyceride levels can give an indication of how juvenile fish respond to habitat alterations when compared with pre-disturbance levels or neighbouring unimpacted populations. Triglyceride analysis would not be appropriate for short-term monitoring projects or projects with considerable budget constraints.

2. <u>Focus on triglyceride levels</u>. Because triglycerides are the primary form of storage lipid in all fish, monitoring programs should monitor triglyceride levels, rather than simply examining total lipid levels.

3. <u>Collect baseline triglyceride data.</u> Triglyceride levels vary both seasonally and annually, as evidenced by this study. Thus, it is important to collect sufficient baseline data from the river(s) and reaches of interest in order to capture the range of natural variability for each system. I recommend collecting baseline data for a minimum of two years.

4. Consider seasonal variability. Triglyceride levels in juvenile stream-dwelling salmonids vary seasonally. To start, fish should be sampled monthly for the duration of the freshwater life-stage to account for seasonal variability in triglyceride levels. Monthly sampling will allow identification of when fish achieve maximum and minimum levels of triglyceride storage. After analyzing the baseline data, it may be possible to restrict future sampling to a 3- or 4- month period, focusing on the time period of most interest.

5. Consider allometric relationships. The relationship between fish weight and triglyceride mass is likely allometric and larger fish may have proportionally higher triglyceride levels. When selecting a subset of the fish for triglyceride analysis, choose fish to represent the range of sizes present at each sampling site. Carefully consider the underlying relationship between fish weight and triglyceride levels and if it is nonlinear, make comparisons based on triglyceride residuals from that underlying relationship.

6. Collect baseline habitat data. There is likely a relationship between habitat conditions and triglyceride levels. Collect complementary habitat data, such as benthic macroinvertebrates and water chemistry, to determine whether correlations exist between triglyceride levels and measures of food availability and/or water quality.

7. Consider overwinter survival. Pair the monitoring program with a tagging program to help determine whether there is a direct relationship between pre-winter triglyceride levels and the probability of surviving the winter period. Zabel et al. (2005) used PIT-tags to mark juvenile chinook salmon and steelhead trout in order to examine survival

during seaward-migration. The method outlined in Zabel et al. (2005) could be used to study overwinter survival in juvenile chinook salmon from the Bridge River.

8. Decision-making. Consider how the resulting information may be used in decision making. For instance, with the experimental flow release program on the Bridge River, decision rules could define a minimum acceptable pre-winter triglyceride level. Thus, if fish collected in November are found with triglyceride levels below this minimum acceptable level, then managers may determine the current experimental flow to be inappropriate for maintaining a healthy fish population.

Management recommendations for the Bridge River

As of 2005, BC Hydro completed the first stage of the "Bridge River experimental flow release program". In June 2000, continuous flow release from the Terzaghi Dam began with an annual average discharge of 3 cms. A two-year delay between initial flow release and sampling for lipid and triglyceride levels was planned in order to allow sufficient time for invertebrate colonization in the upper reach. The second stage of the experimental flow release program is scheduled to begin in 2006. It will have an annual average discharge of 1 cms, and a 4 or 5-year duration.

In light of my results, I recommend that managers incorporate monitoring of triglyceride levels in young-of-the-year chinook salmon into the "Bridge River experimental flow release program". Currently, salmonid biomass is used as the primary measure for evaluating population response to each flow treatment. However, the level of uncertainty associated with each estimate of biomass will change with each stage of flow release (due to difficulties with access), making comparisons difficult between stages of the flow release plan. Such uncertainty in biomass estimates at two times creates even

larger uncertainly in estimates of overwinter survival rates. Triglyceride analysis should not replace estimates of salmonid biomass or any other habitat variable; instead it could serve as a complementary metric to help managers evaluate population response to changes in fish habitat resulting from changes in stream flow.

Research priorities for the "Bridge River experimental flow release program" focus on evaluating the factors that contribute to overwinter survival for juvenile salmonids. The shared opinion among scientists involved in the Bridge River project is that in the fall, the stream habitat below the Terzaghi Dam is already at maximum capacity in terms of number of rearing salmonids (M.J. Bradford, pers. comm.). Thus, a possible management objective for this river is to maximize survival rates during the winter period, which will enhance smolt out-migration in the spring. Incorporating the evaluation of triglyceride levels into the Bridge River monthly monitoring program, paired with a tagging program to monitor overwinter survival, may give managers an alternate estimate of how well prepared young-of-the-year chinook salmon are for the onset of winter, as indirectly reflected by food availability and habitat quality through proportional triglyceride levels.

I recommend that the Bridge River monitoring program be revised as follows:

1. Continue collecting monthly length and weight data for all salmonid species at all sites.

2. Continue annual electrofishing and snorkel surveys to estimate population abundances of all salmonid species.

3. Begin collecting fish for lipid and triglyceride analysis once a given stage of experimental flow has been in place for 2 years. This will ensure sufficient time has

elapsed for primary and secondary productivity to reach a new level of production (M.J. Bradford, pers. comm.). Data should be collected in a manner that facilitates comparisons among years.

- Seasonal sampling at both reaches: July, August, September, October, November, December, February, March (weather- and access- dependent).

- Conduct a power analysis that utilizes the know variability in triglyceride levels, an effect size of interest and a specified alpha value, to estimate the sample size for each site. The effect size will depend on the management objectives of the study.

- Measure fish lengths and weights and store fish on dry ice.

- Continue to collect benthic and drifting invertebrates (identified to Family level) and attempt to evaluate the value of this information. If possible, determine whether it is feasible to phase out the intensive sampling of aquatic macroinvertebrates (identification to Family level), and replace it with estimates of benthic macroinvertebrate density. The latter is the approach I used when sampling invertebrates across all 12 sites, and it was the measure most highly correlated with triglyceride levels.

- Increase the number of water chemistry samples per month using the steps outlined in Cattaneo and Prairie (1995).

- Follow the analytical steps outlined in Methods. Use a weight-corrected metric of triglycerides (e.g., mean triglyceride residuals) for comparisons between sites and seasons.

- Continue to explore relationships between all environmental variables and triglyceride levels.

- Introduce a tagging study to attempt to quantify the relationship between pre-winter triglyceride levels and overwinter survival in juvenile chinook salmon.

For long-term monitoring studies, such as the "Bridge River experimental flow release program", the merits of using triglyceride analysis, intensive invertebrate sampling, and/or water chemistry sampling need to be considered to determine which metrics can potentially provide more insight into overwinter survival rates and population-level responses to habitat alterations.

TABLES

Table 1. Mean fish weight, total lipid mass, and triglyceride mass for young-of-the-year chinook salmon collected in (a) August 2003, (b) November 2003, and (c) spring 2004, from 12 sites throughout the Fraser River basin. (Bridge River February 2004 and Fraser River April 2004 data combined.)

(a)

Site	August 2003		
	Fish weight mean ± SEM (N)	Total lipids mean ± SEM (N)	Triglycerides mean ± SEM (N)
Bridge River - Upper reach	5.29 ± 0.30 (53)	0.123 ± 0.029 (8)	0.057 ± 0.016 (8)
Bridge River - Lower reach	2.77 ± 0.18 (91)	0.057 ± 0.011 (9)	0.013 ± 0.004 (9)
Little Chilcotin River	2.93 ± 0.24 (20)	0.101 ± 0.021 (8)	0.045 ± 0.011 (8)
Chilko River	2.74 ± 0.14 (20)	0.064 ± 0.013 (8)	0.021 ± 0.006 (8)
Quesnel River	1.75 ± 0.17 (20)	0.049 ± 0.007 (8)	0.019 ± 0.004 (8)
Cottonwood River	2.47 ± 0.12 (17)	0.058 ± 0.009 (8)	0.025 ± 0.005 (8)
Fraser River at Stoner	3.43 ± 0.20 (19)	0.067 ± 0.012 (8)	0.027 ± 0.006 (8)
Nechako River	5.91 ± 0.37 (12)	0.178 ± 0.029 (8)	0.091 ± 0.016 (8)
McGregor River	2.18 ± 0.24 (10)	0.034 ± 0.006 (8)	0.008 ± 0.002 (8)
Fraser River at Tete-Jaune Cache	1.48 ± 0.16 (20)	0.024 ± 0.004 (8)	0.004 ± 0.001 (8)
Clearwater River	2.01 ± 0.15 (20)	0.041 ± 0.006 (8)	0.012 ± 0.003 (8)
Nicola River	2.44 ± 0.15 (20)	0.074 ± 0.011 (8)	0.033 ± 0.005 (8)

(b)

Site	November 2003		
	Fish weight mean ± SEM (N)	Total lipids mean ± SEM (N)	Triglycerides mean ± SEM (N)
Bridge River - Upper reach	9.20 ± 0.81 (25)	0.275 ± 0.072 (8)	0.111 ± 0.033 (8)
Bridge River - Lower reach	6.59 ± 0.37 (46)	0.144 ± 0.032 (8)	0.072 ± 0.018 (8)
Little Chilcotin River	5.04 ± 0.30 (18)	0.164 ± 0.040 (8)	0.071 ± 0.026 (8)
Chilko River	3.17 ± 0.21 (20)	0.077 ± 0.012 (8)	0.029 ± 0.006 (8)
Quesnel River	3.06 ± 0.39 (19)	0.070 ± 0.020 (8)	0.020 ± 0.010 (8)
Cottonwood River	3.74 ± 0.21 (20)	0.077 ± 0.019 (8)	0.027 ± 0.010 (8)
Fraser River at Stoner	4.43 ± 0.48 (12)	0.121 ± 0.046 (8)	0.040 ± 0.024 (8)
Nechako River	10.28 ± 1.92 (4)	0.431 ± 0.133 (4)	0.189 ± 0.053 (4)
McGregor River	3.48 ± 0.37 (13)	0.069 ± 0.010 (8)	0.019 ± 0.003 (8)
Fraser River at Tete-Jaune Cache	2.35 ± 0.27 (11)	0.055 ± 0.010 (8)	0.019 ± 0.004 (8)
Clearwater River	3.29 ± 0.22 (19)	0.045 ± 0.008 (8)	0.006 ± 0.003 (8)
Nicola River	4.32 ± 0.24 (12)	0.131 ± 0.015 (8)	0.051 ± 0.007 (8)

(c)

	Spring 2004		
Site	Fish weight mean ± SEM (*N*)	Total lipids mean ± SEM (*N*)	Triglycerides mean ± SEM (*N*)
Bridge River - Upper reach	8.11 ± 0.68 (14)	0.332 ± 0.045 (8)	0.140 ± 0.024 (8)
Bridge River - Lower reach	4.98 ± 0.67 (4)	0.156 ± 0.027 (4)	0.068 ± 0.011 (4)
Little Chilcotin River	4.34 ± 0.32 (5)	0.044 ± 0.002 (5)	0.006 ± 0.001 (5)
Chilko River	4.08 ± 0.43 (5)	0.117 ± 0.018 (5)	0.044 ± 0.008 (5)
Quesnel River	5.88 ± 0.34 (6)	0.092 ± 0.004 (6)	0.016 ± 0.003 (6)
Cottonwood River	3.02 ± 0.15 (6)	0.055 ± 0.005 (6)	0.016 ± 0.002 (6)
Fraser River at Stoner	8.08 ± 1.15 (6)	0.156 ± 0.020 (5)	0.052 ± 0.010 (5)
Nechako River			
McGregor River	4.62 ± 0.78 (5)	0.095 ± 0.021 (5)	0.025 ± 0.007 (5)
Fraser River at Tete-Jaune Cache	5.20 ± 2.40 (2)	0.067 ± 0.026 (2)	0.020 ± 0.006 (2)
Clearwater River	5.76 ± 0.34 (14)	0.096 ± 0.010 (10)	0.030 ± 0.005 (10)
Nicola River	11.53 ± 2.99 (4)	0.467 ± 0.165 (4)	0.203 ± 0.074 (4)

Table 2. Environmental variables collected from the Bridge River in July, August and September. Units are: total nitrogen ($\mu g \cdot L^{-1}$), soluble reactive phosphorous (SRP, $\mu g \cdot L^{-1}$), conductivity ($\mu S \cdot cm^{-1}$), alkalinity (ppm), temperature (°C), discharge ($m^3 \cdot s^{-1}$), drifting invertebrate/ Chironomidae/ Simuliidae/ EPT density (No.$\cdot m^{-3}$), and total salmonid biomass (g·100 m^{-2}).

	2002			2003		
	Jul	Aug	Sep	Jul	Aug	Sep
Bridge River - Upper reach						
Measures of primary productivity						
Total nitrogen	14.0	15.7	8.3	8.4	5.9	138.3
SRP	4.9	4.4	2.1	2.5	3.6	14.4
Conductivity	125.0	113.0	71.0	118.0	103.0	94.0
Alkalinity	105.4	89.9	78.0	93.8	81.1	72.8
pH	8.1	8.2	8.1	8.2	8.4	8.1
Measures of secondary productivity						
Invertebrate density	553	437	341	206	226	1359
Chironomidae density	141	297	232	46	157	982
Simuliidae density	60	7	22	32	7	124
EPT density	45	35	31	37	20	135
Temperature	11.9	12.3	11.7	12.2	12.5	12.0
Discharge	3.9	3.8	2.9	3.9	3.9	2.9
Total salmonid biomass			632			478
Bridge River - Lower reach						
Measures of primary productivity						
Total nitrogen	9.2	11.0	15.2	8.4	18.0	165.3
SRP	3.6	2.4	4.3	2.1	2.9	12.8
Conductivity	148.0	160.0	151.0	149.0	157.0	170.0
Alkalinity	132.8	138.4	134.2	130.2	136.7	140.8
pH	8.2	8.2	8.2	8.3	8.2	8.0
Measures of secondary productivity						
Invertebrate density	675	293	368	219	134	893
Chironomidae density	213	136	207	85	60	525
Simuliidae density	27	12	3	9	6	35
EPT density	111	92	103	49	44	232
Temperature	13.0	12.6	10.9	12.2	12.2	11.0
Discharge	12.1	9.3	6.3	11.9	8.8	6.0
Total salmonid biomass			249			387

Table 3. Correlation matrix for August 2003 used to examine the degree of relatedness among environmental variables, total lipid residuals, and triglyceride residuals for young-of-the-year chinook salmon (Bridge River and Fraser River data combined).

	Total nitrogen	SRP	Conductivity	Alkalinity	pH	Invertebrate density	Temperature	Discharge	Lipid residuals	Triglyceride residuals
Total nitrogen										
SRP	0.08									
Conductivity	0.04	0.24								
Alkalinity	0.20	0.21	0.88							
pH	0.30	0.49	0.58	0.53						
Invertebrate density	-0.19	0.37	0.18	0.29	0.30					
Temperature	-0.21	0.39	0.13	-0.11	0.14	-0.09				
Discharge	0.23	-0.18	-0.21	-0.19	-0.03	-0.41	0.05			
Lipid residuals	-0.35	0.44	0.33	0.31	0.28	0.57	0.54	-0.62		
Triglyceride residuals	-0.59	0.42	0.16	0.05	0.34	0.64	0.47	-0.51	0.86	
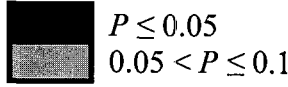
Legend: $P \leq 0.05$ (black); $0.05 < P \leq 0.1$ (grey)

Table 4. Correlation matrix for November 2003 used to examine the degree of relatedness among environmental variables, total lipid residuals, and triglyceride residuals for young-of-the-year chinook salmon (Bridge River and Fraser River data combined).

	Total nitrogen	SRP	Conductivity	Alkalinity	pH	Invertebrate density	Temperature	Discharge	Lipid residuals	Triglyceride residuals
Total nitrogen										
SRP	-0.35									
Conductivity	0.51	0.49								
Alkalinity	0.37	0.6	0.88							
pH	0.06	-0.84	-0.56	-0.63						
Invertebrate density	-0.22	0.64	0.30	0.41	-0.40					
Temperature	-0.40	-0.21	-0.63	-0.45	0.36	0.09				
Discharge	0.19	-0.16	0.00	-0.02	0.17	-0.57	0.22			
Lipid residuals	-0.12	0.02	0.20	0.11	0.22	0.46	-0.32	-0.40		
Triglyceride residuals	-0.22	0.18	0.17	0.15	0.10	0.64	-0.22	-0.48	0.93	
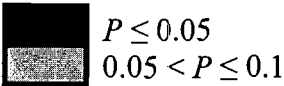
$P \leq 0.05$

$0.05 < P \leq 0.1$

Table 5. Correlation matrix for spring 2004 used to examine the degree of relatedness among environmental variables, total lipid residuals, and triglyceride residuals for young-of-the-year chinook salmon. (Bridge River February 2004 and Fraser River April 2004 data combined).

	Total nitrogen	SRP	Conductivity	Alkalinity	pH	Invertebrate density	Temperature	Discharge	Lipid residuals
Total nitrogen									
SRP	NA								
Conductivity	NA	NA							
Alkalinity	NA	NA	0.95						
pH	NA	NA	-0.10	-0.12					
Invertebrate density	NA	NA	-0.28	-0.29	0.65				
Temperature	NA	NA	0.72	0.55	0.00	0.09			
Discharge	NA	NA	0.08	0.20	-0.06	-0.43	-0.21		
Lipid residuals	NA	NA	-0.72	-0.79	0.12	0.59	-0.54	-0.19	
Triglyceride residuals	NA	NA	-0.63	-0.73	0.15	0.70	-0.19	-0.53	0.88

$P \leq 0.05$

$0.05 < P \leq 0.1$

FIGURES

Figure 1. The Bridge River, a first-order tributary to the Fraser River.
The stars represent sampling locations for the upper and lower reaches. From
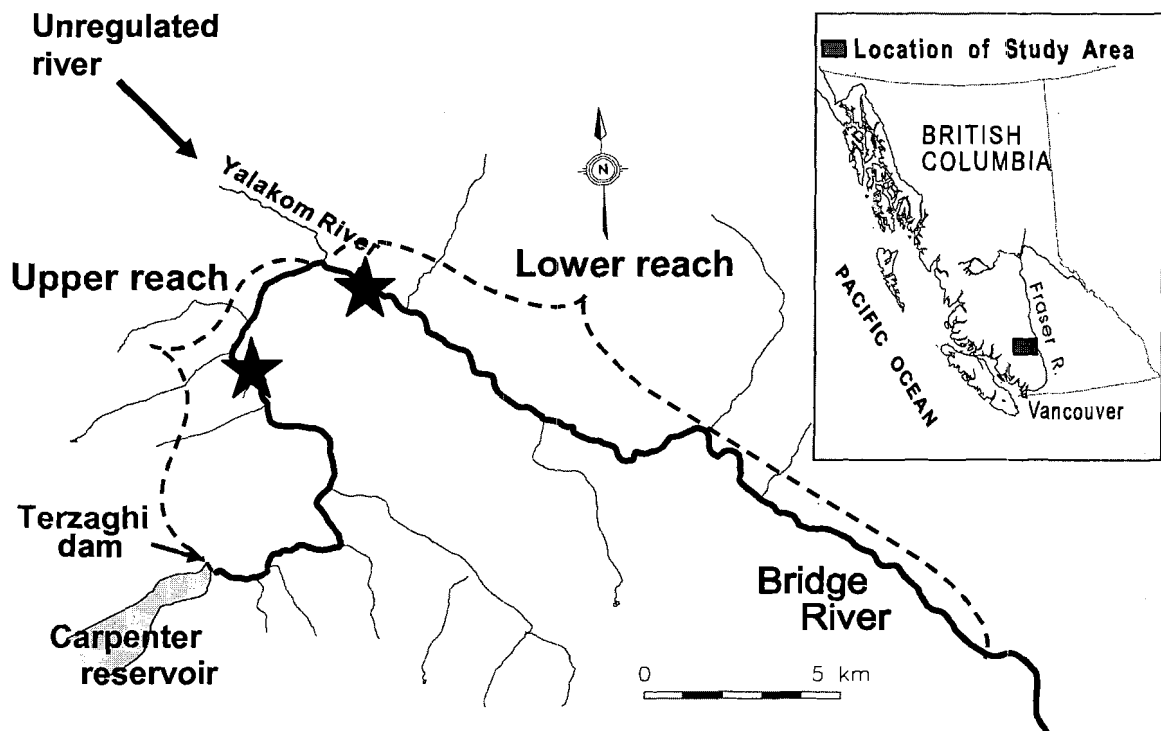Higgins and Bradford 1996, adapted with permission.

Figure 2. Discharge profiles for the (a) upper and (b) lower reaches of the Bridge River. Solid lines: 2002, dashed lines: 2003.
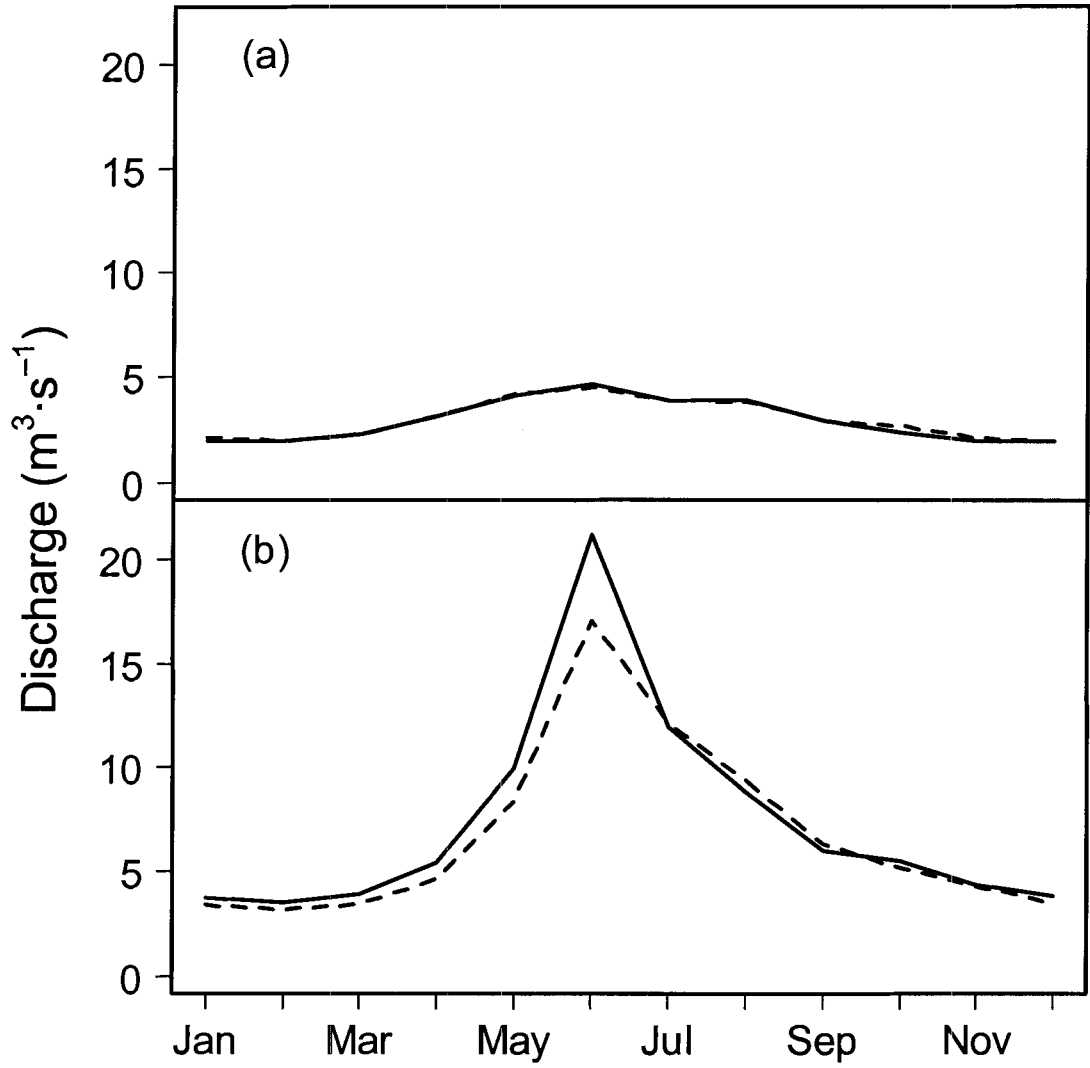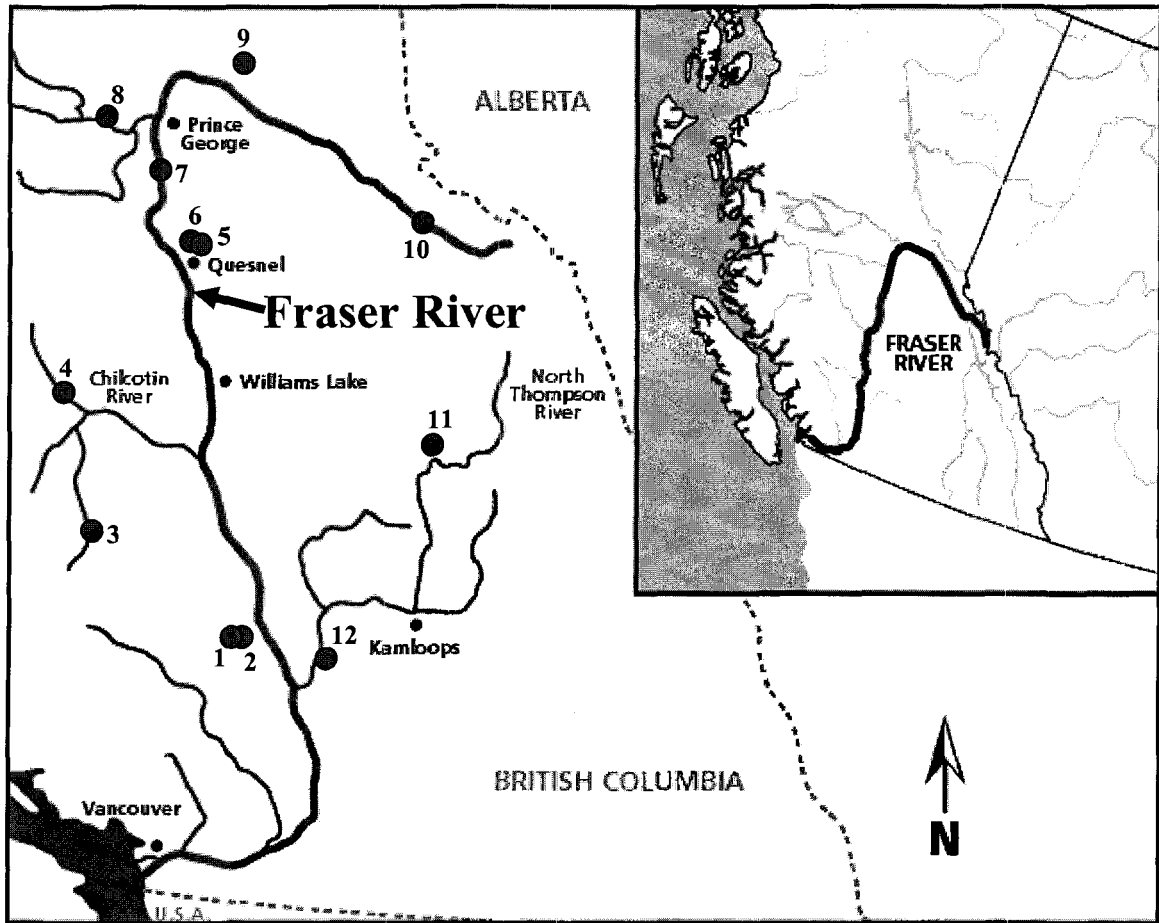
Figure 3. Location of sampling sites within the Fraser River basin.
1: Bridge River upper reach, 2: Bridge River lower reach. 3: Chilko River, 4: Little Chilcotin River, 5: Quesnel River, 6: Cottonwood River, 7: Fraser River at Stoner, 8: Nechako River, 9: McGregor River, 10: Fraser River at Tete-Jaune Cache, 11: Clearwater River, 12: Nicola River.

From: The Canadian Heritage Rivers System, http://www.chrs.ca/Rivers/Fraser/Fraser-M_e.htm, adapted with permission.

Figure 4. Time series of fish weight for the period of May 2002 to February 2004. Solid line and solid circles represent the 2001 cohort; broken line and open circles represent the 2002 cohort. Data include all fish sampled for this project and fish sampled by BC Hydro (including those not used for subsequent lipid extractions). Error bars represent ± 1 S.E. of the estimated values; mean sample size for each month was N = 60 fish. (a) Bridge River – upper reach; (b) Bridge River – lower reach. Note that sampling did not occur in October or March.
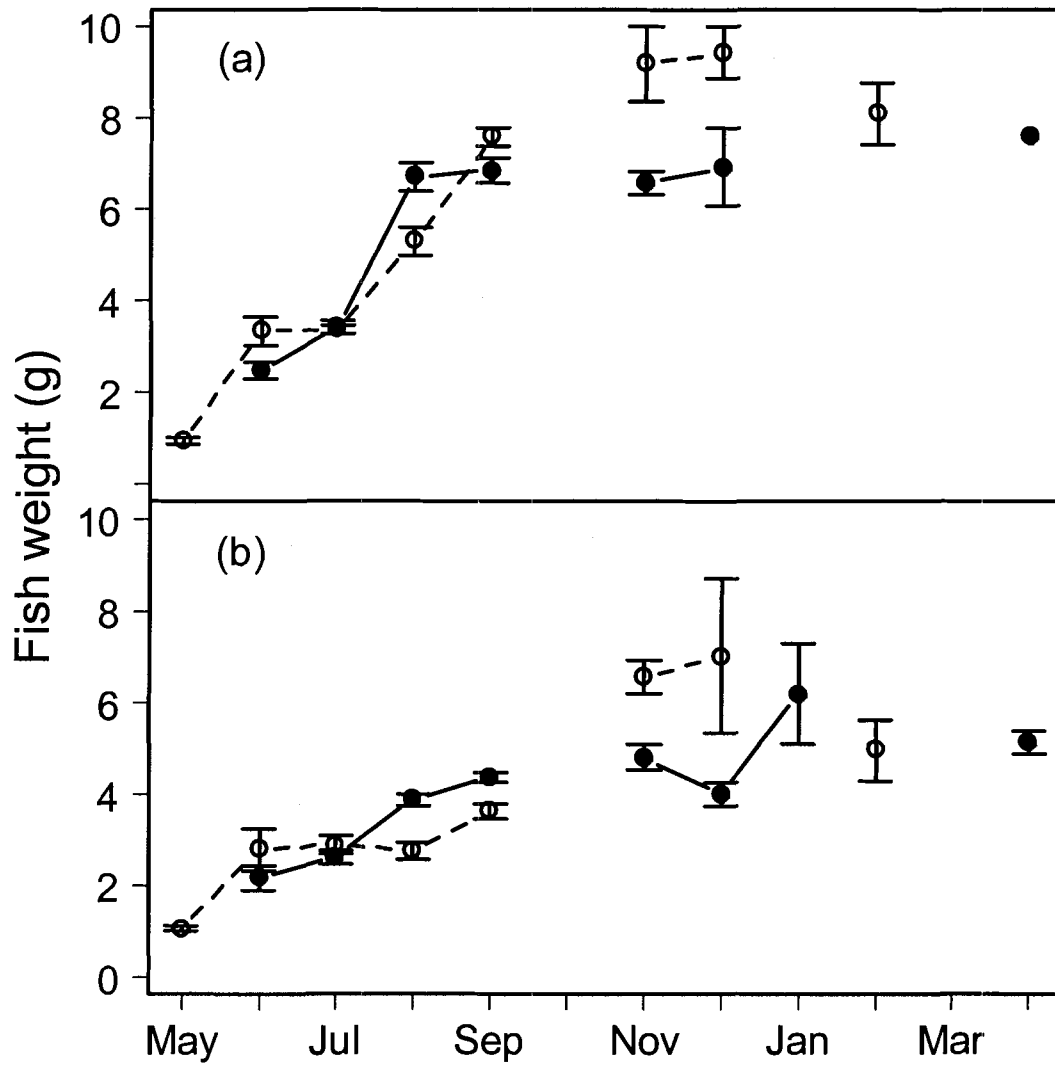
Figure 5. Time series of total lipid mass for the period of June 2002 to February 2004. Solid line and solid circles represent the 2001 cohort; broken line and open circles represent the 2002 cohort. These data are a subset of fish collected in the field. I selected up to eight fish from each sampling event for total lipid extraction. Fish were chosen to span the range of sizes observed. Error bars represent ± 1 S.E. of the estimated values. (a) Bridge River – upper reach; (b) Bridge River – lower reach. Note that sampling did not occur in October, December or March.
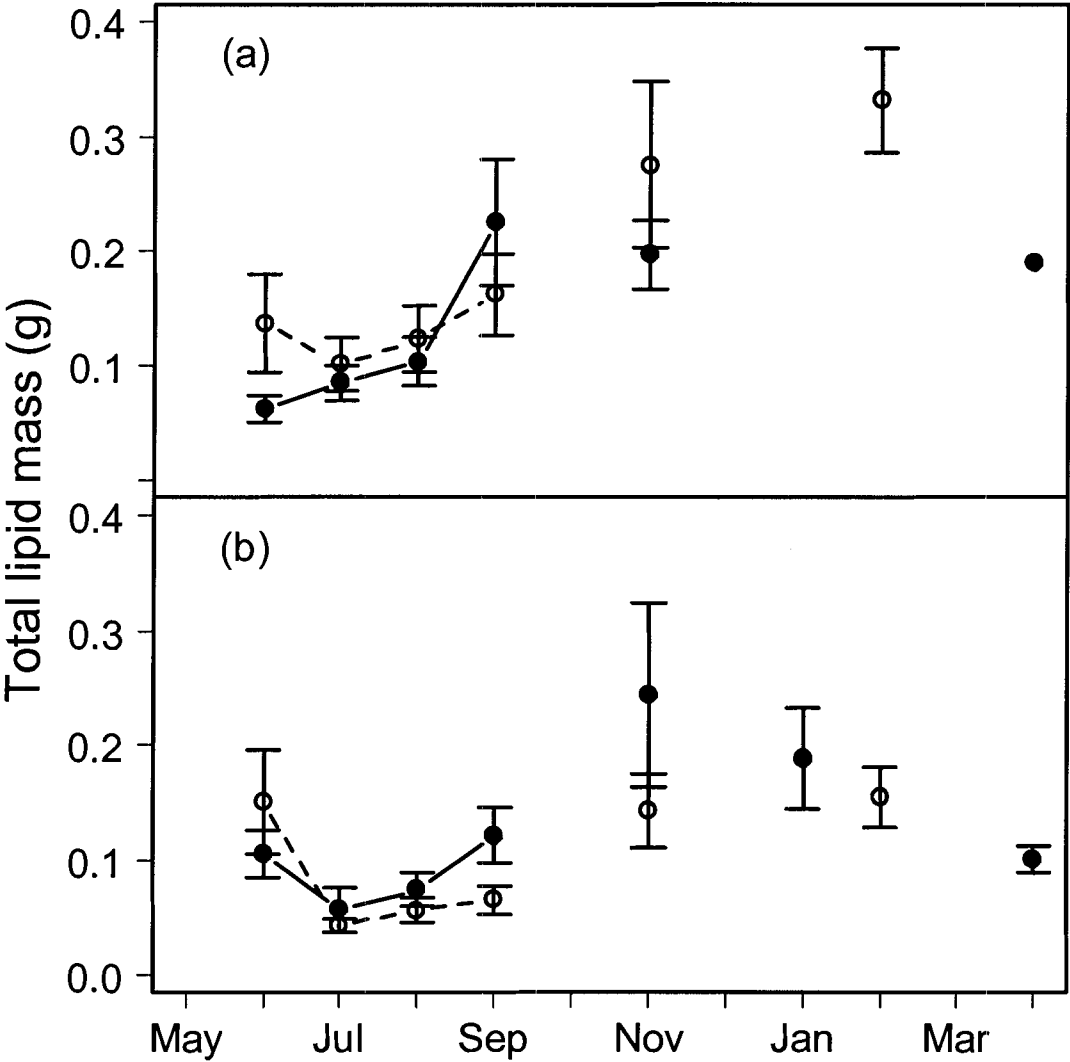
Figure 6. Time series of triglyceride mass for the period of June 2002 to February 2004. Solid line and solid circles represents the 2001 cohort; broken line and open circles represents the 2002 cohort. These are the same individuals as in Figure 5. Error bars represent ± 1 S.E. of the estimated values. (a) Bridge River – upper reach; (b) Bridge River – lower reach. Note that sampling did not occur in October, December or March.
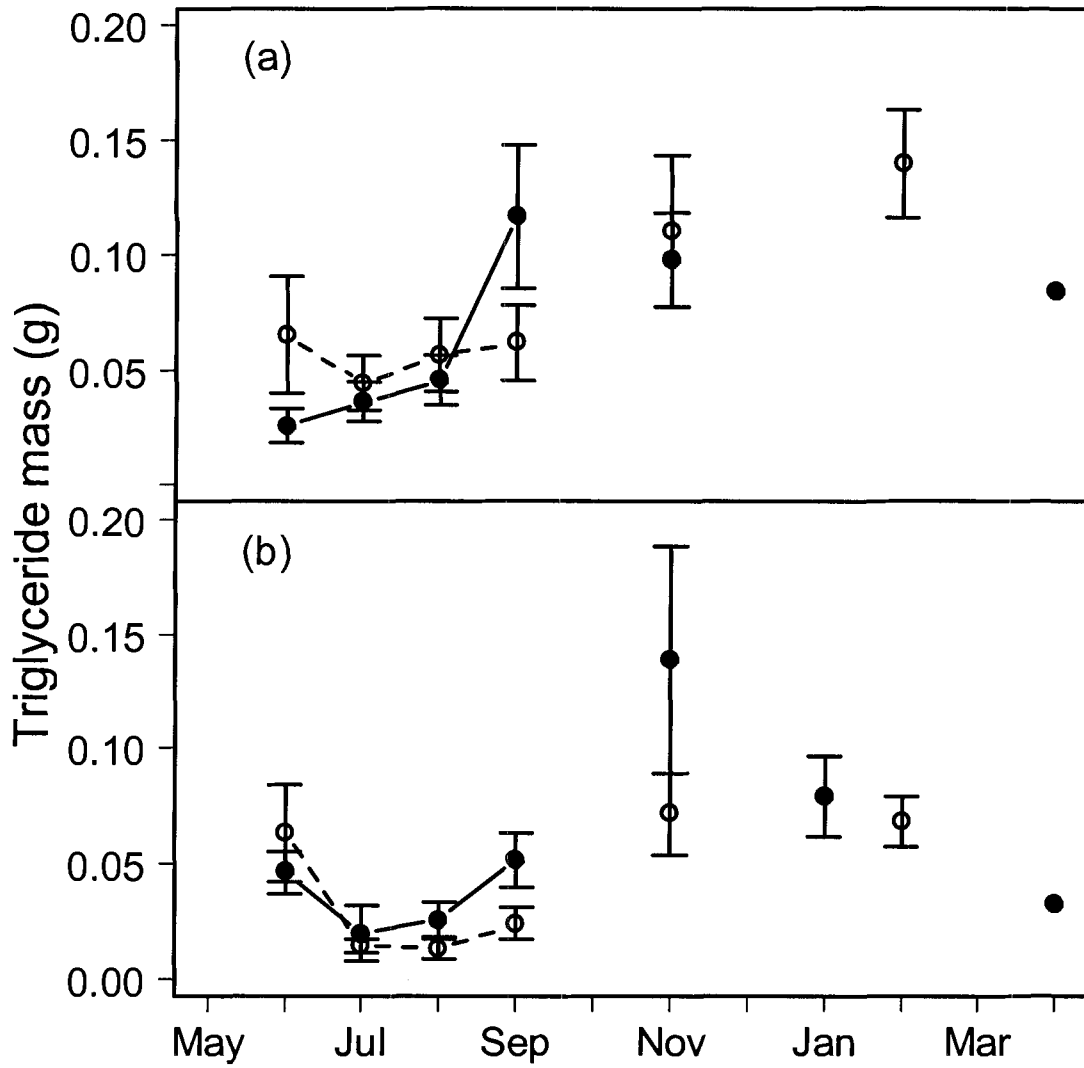
Figure 7. The nonlinear relationship between fish weight and total lipid mass for young-of-the-year chinook salmon for the period of June 2002 to February 2004. This relationship is best described by a polynomial regression (solid line fit to the data by least squares).
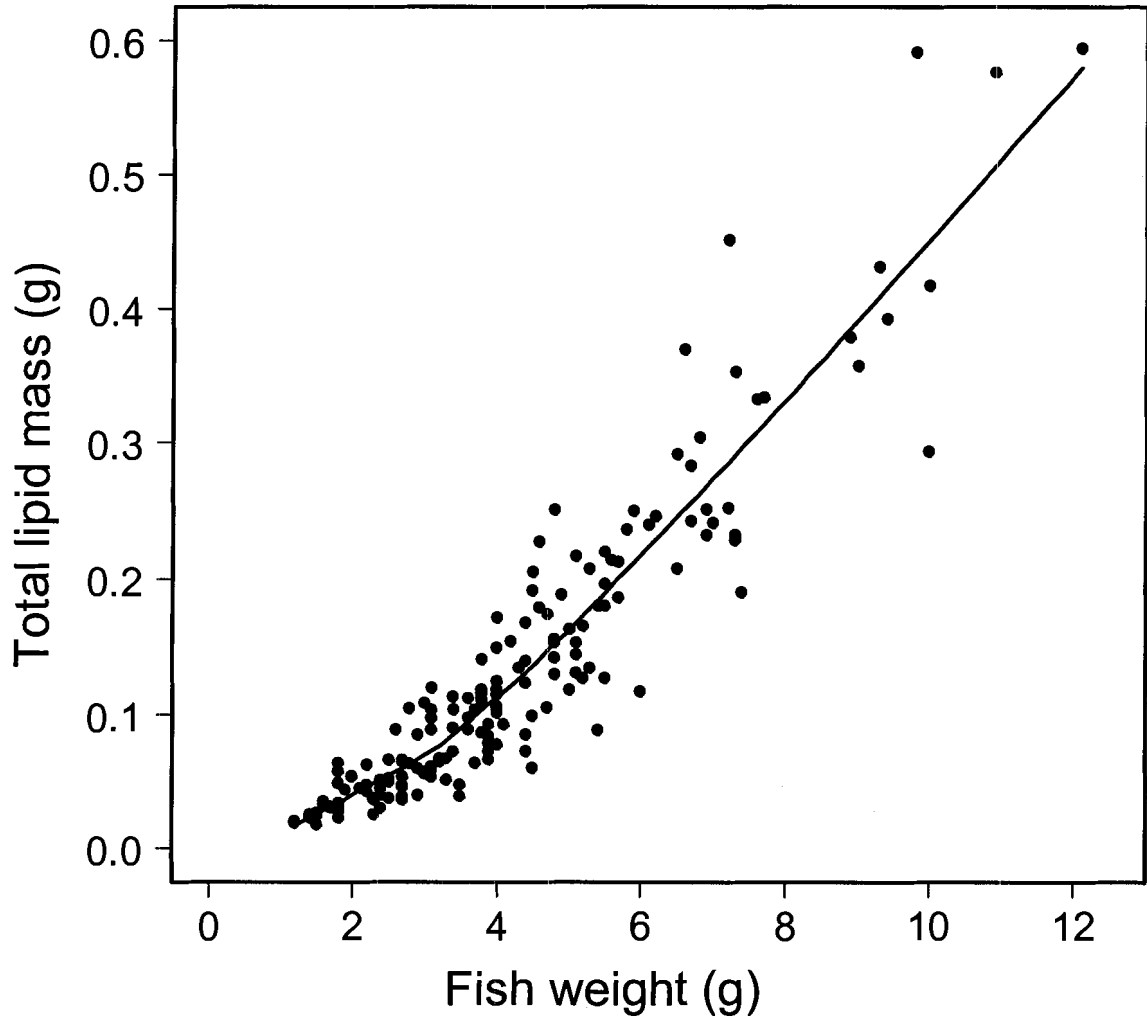
Figure 8. The nonlinear relationship between fish weight and triglyceride mass for young-of-the-year chinook salmon for the period of June 2002 to February 2004. This relationship is best described by a polynomial regression (solid line fit to the data by least squares).
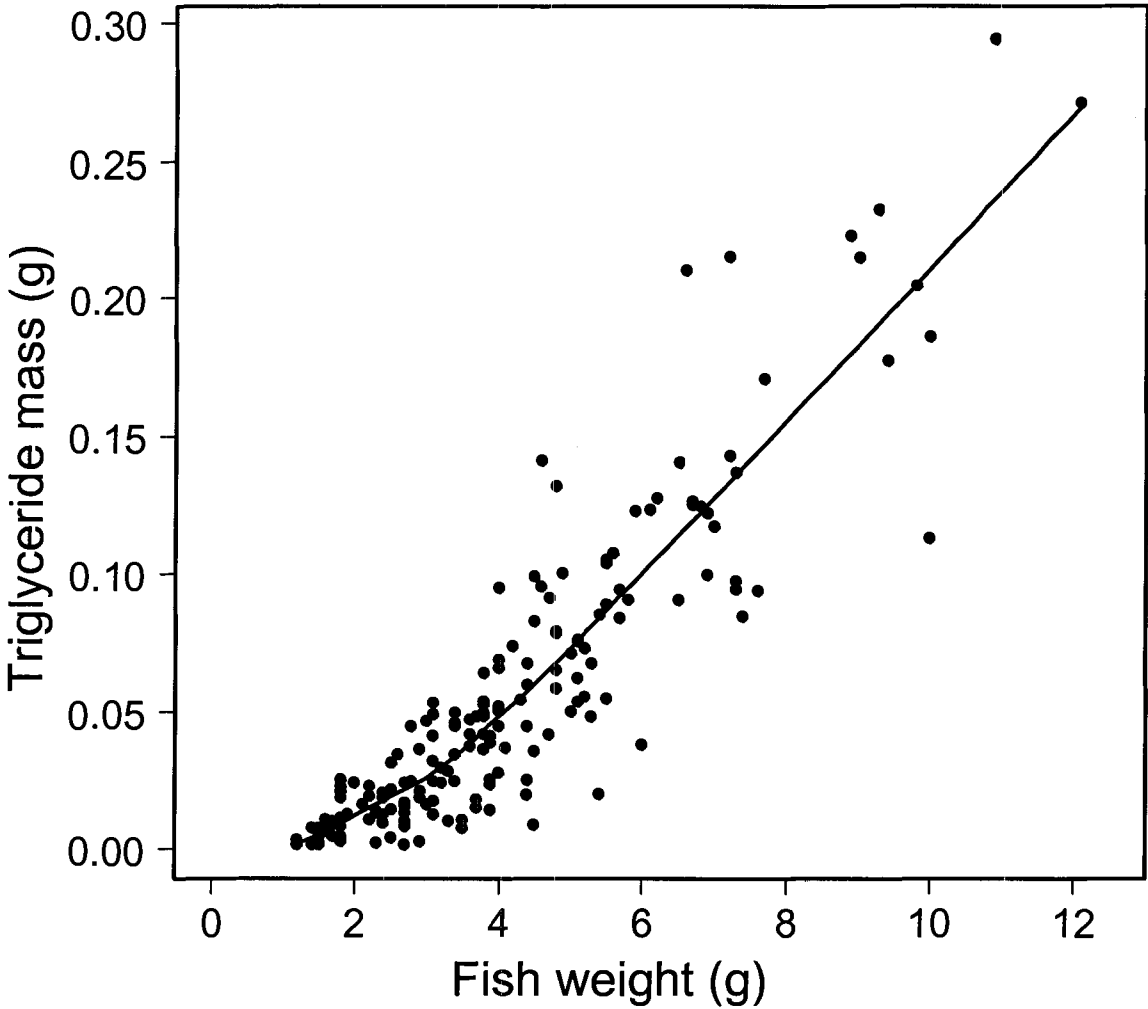
Figure 9. Time series of total lipid residuals obtained from a polynomial regression curve fit to the fish weight and total lipid mass data. Solid line and solid circles represents the 2001 cohort; broken line and open circles represents the 2002 cohort. Horizontal dotted lines indicate average lipid levels across all fish and all sampling periods. (a) Upper reach and (b) Lower reach. Note that sampling did not occur in October, December, or March.

Figure 10. Time series of triglyceride residuals obtained from a polynomial regression curve fit to the fish weight and triglyceride mass data. Solid line and solid circles represents the 2001 cohort; broken line and open circles represents the 2002 cohort. Horizontal dotted lines indicate average triglyceride levels across all fish and all sampling periods. a) Upper reach and b) Lower reach. Note that sampling did not occur in October, December, or March.
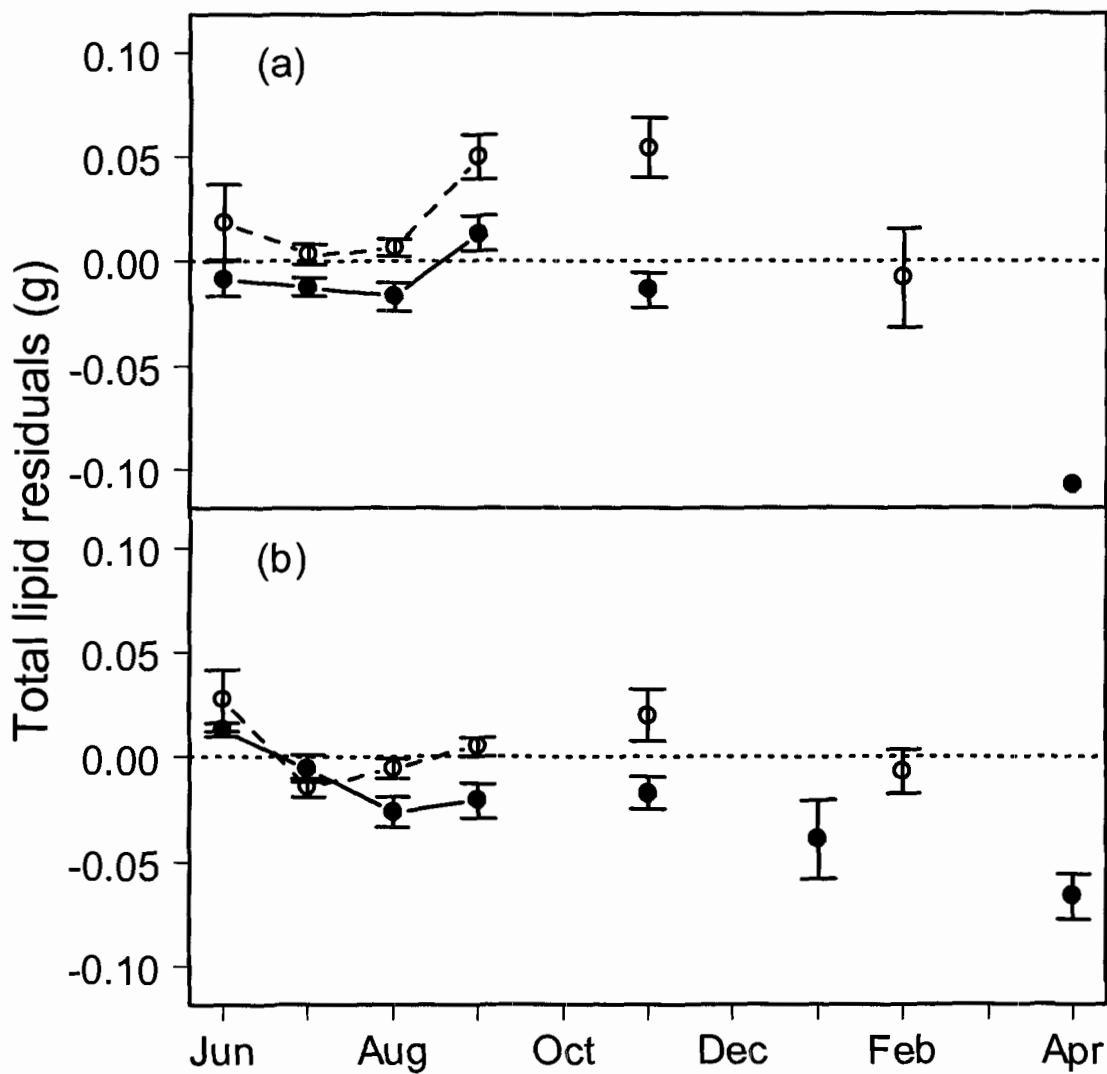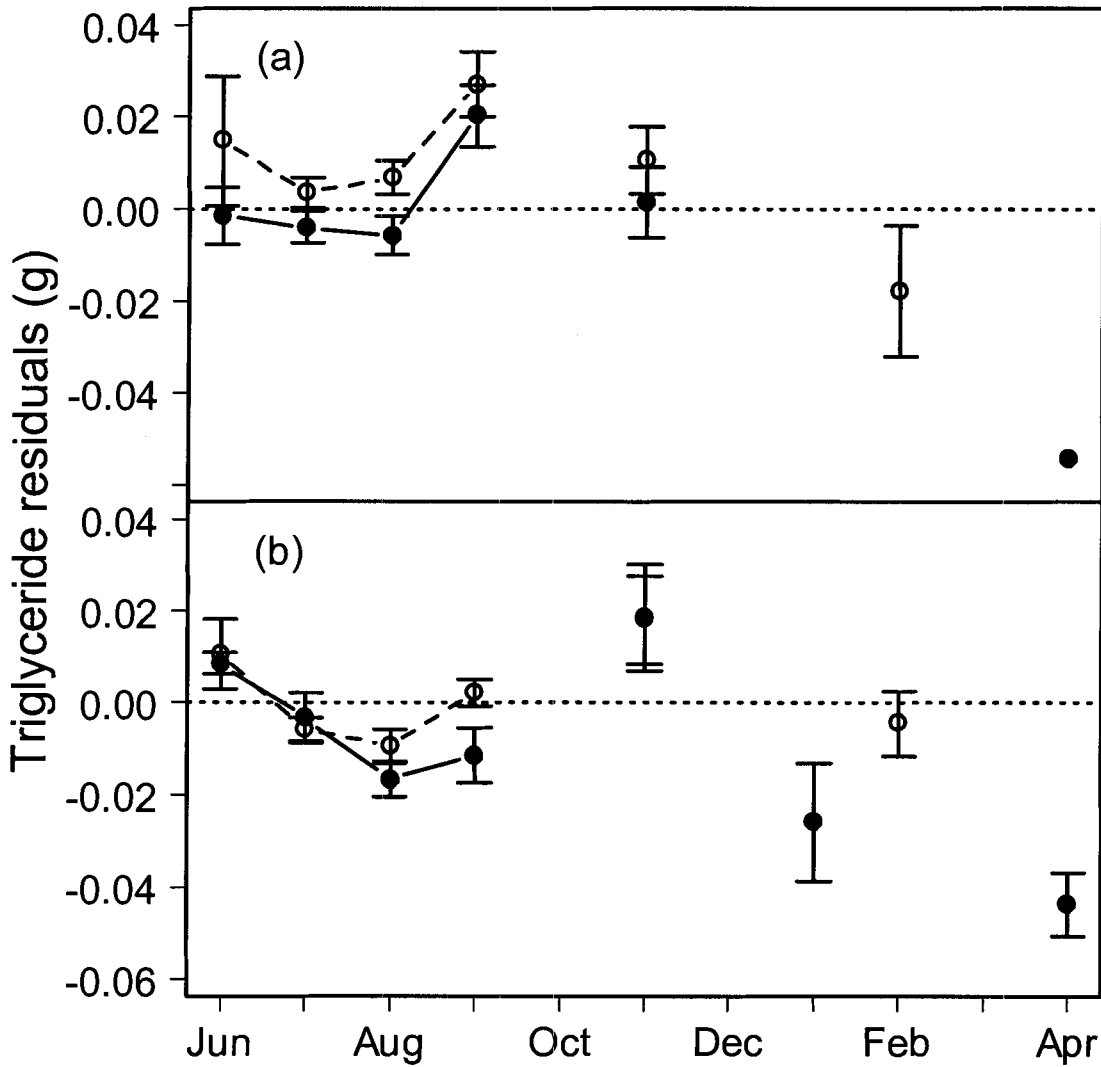
Figure 11. Relationship between fish weight and the proportion of triglycerides per total lipid mass for young-of-the-year chinook salmon. Analysis includes fish collected in the growing season (June 2002 to November 2002; June 2003 to November 2003). Each data point represents one fish. The solid line represents a nonlinear regression fit to the data using least-squares on equation 3.
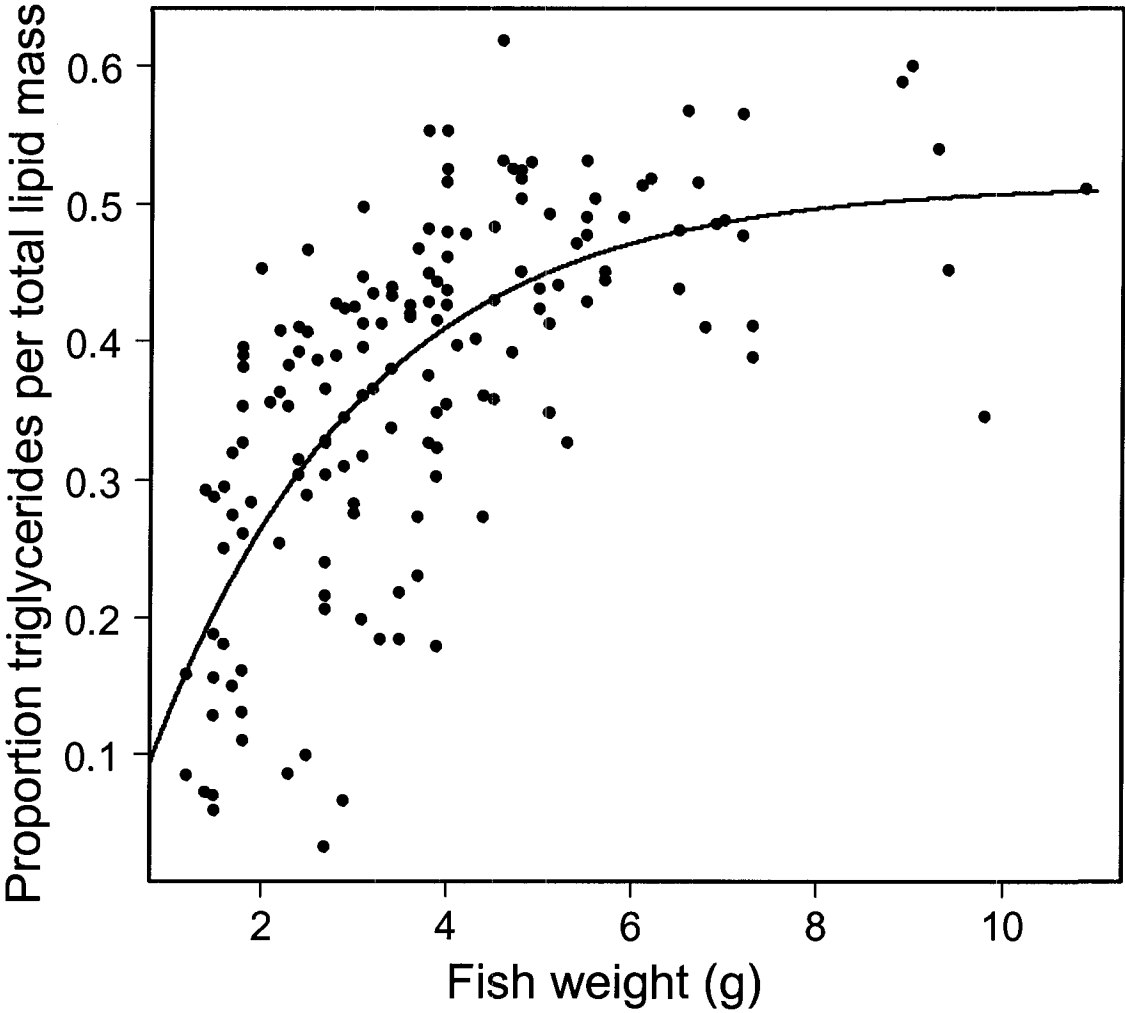
Figure 12. Residuals from the proportional relationship between triglyceride mass and total lipid mass plotted against fish weight. Residuals correspond to the data in Figure 11.
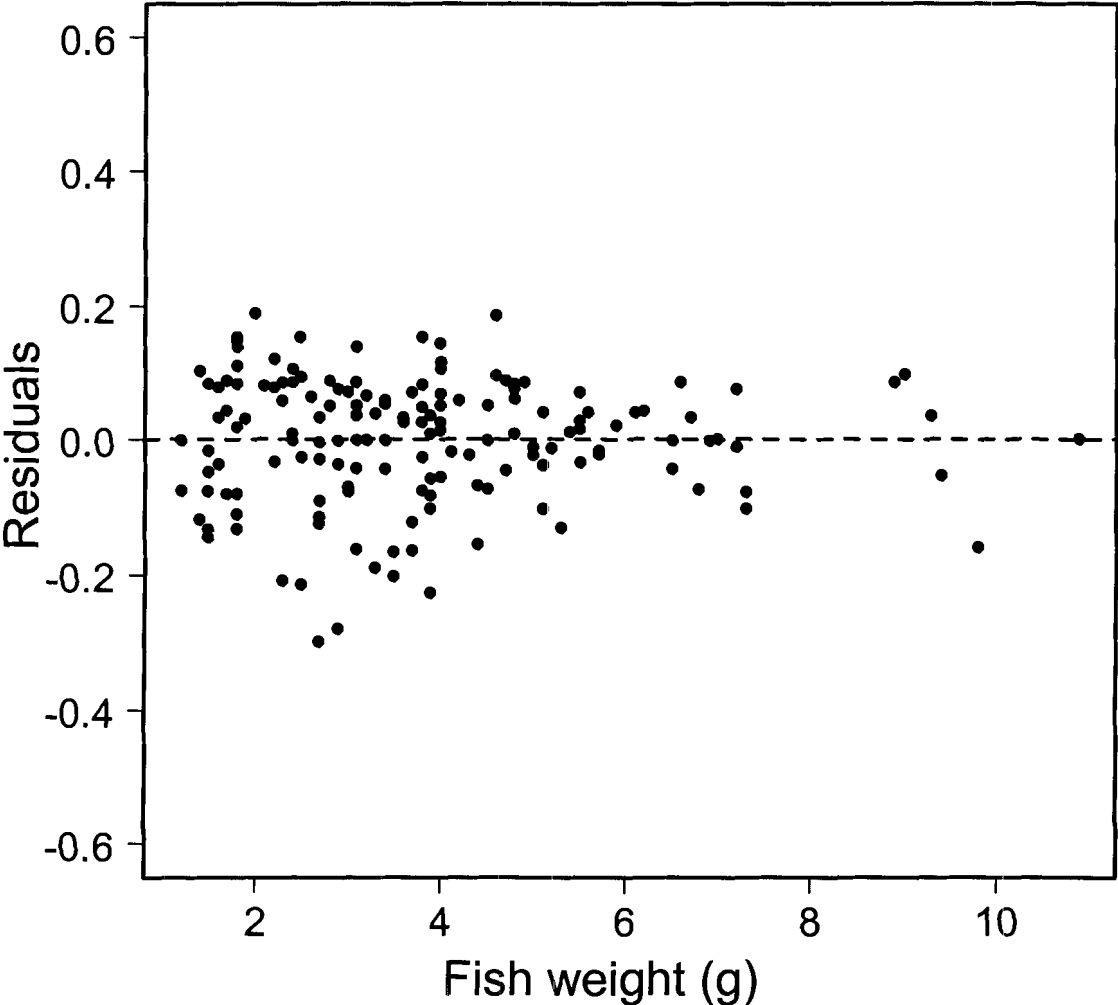
Figure 13. Time series of mean membrane lipid residuals. Residuals were obtained from a polynomial regression fit to the fish weight and membrane lipid mass data. To facilitate comparisons, the y-axis for this figure is on the same scale as data in Figure 9. Solid line and solid circles represents the 2001 cohort; broken line and open circles represents the 2002 cohort. Error bars represent ± 1 S.E. a) Bridge River – upper reach; (b) Bridge River – lower reach.
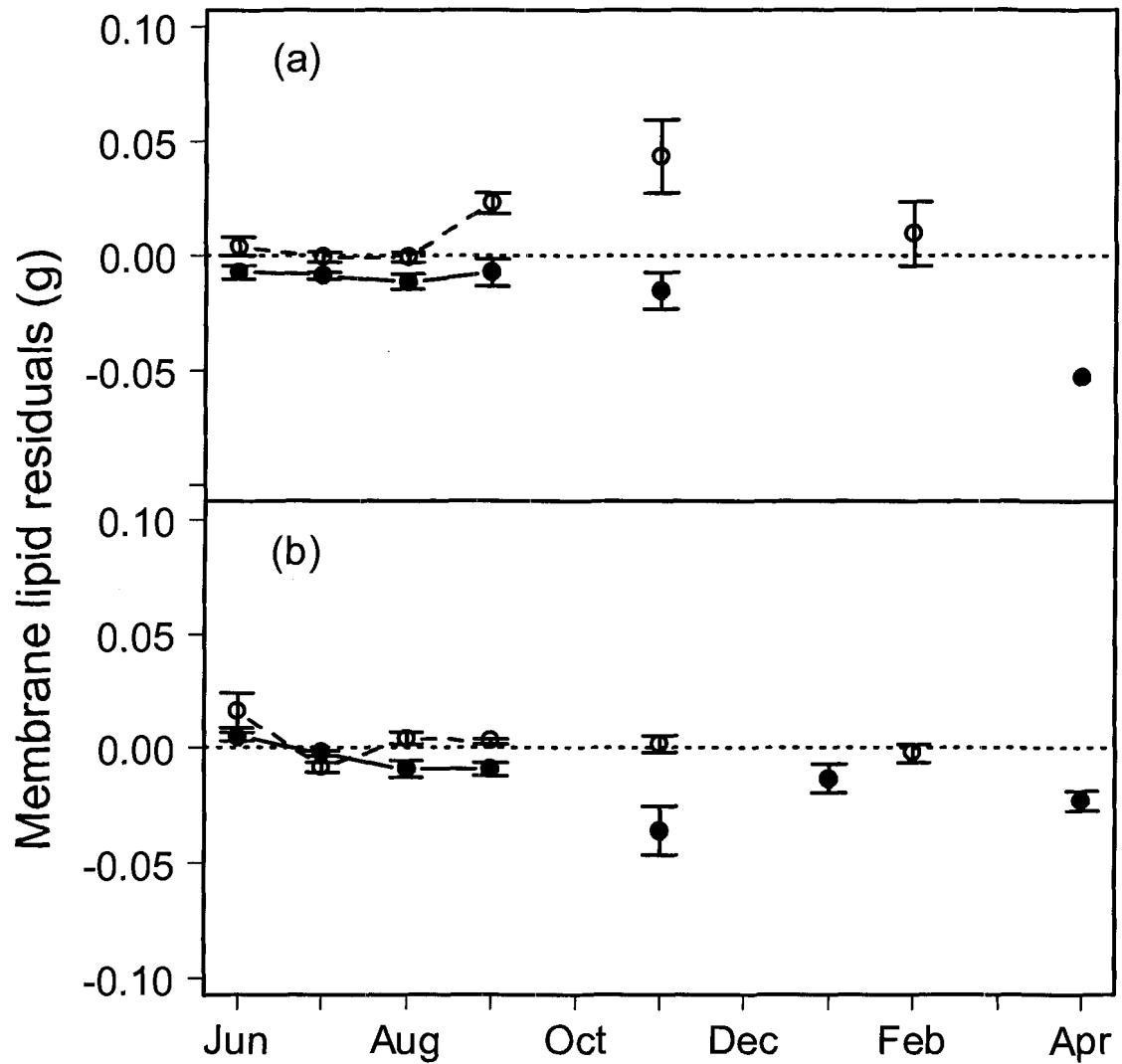
Figure 14. Triglyceride residuals from all sites at three sampling periods: August and November 2003 and April 2004. For comparison with reference sites, solid line and solid circles represent the Bridge River upper reach (2002 cohort) and broken line and open circles represent the Bridge River lower reach (2002 cohort). Thick black lines represent reference sites, which are (a) Little Chilcotin River, (b) Chilko River, (c) Quesnel River, (d) Cottonwood River, (e) Fraser River at Stoner, (f) Nechako River, (g) McGregor River, (h) Fraser River at Tete-Jaune Cache, (i) Clearwater River, (j) Nicola River.
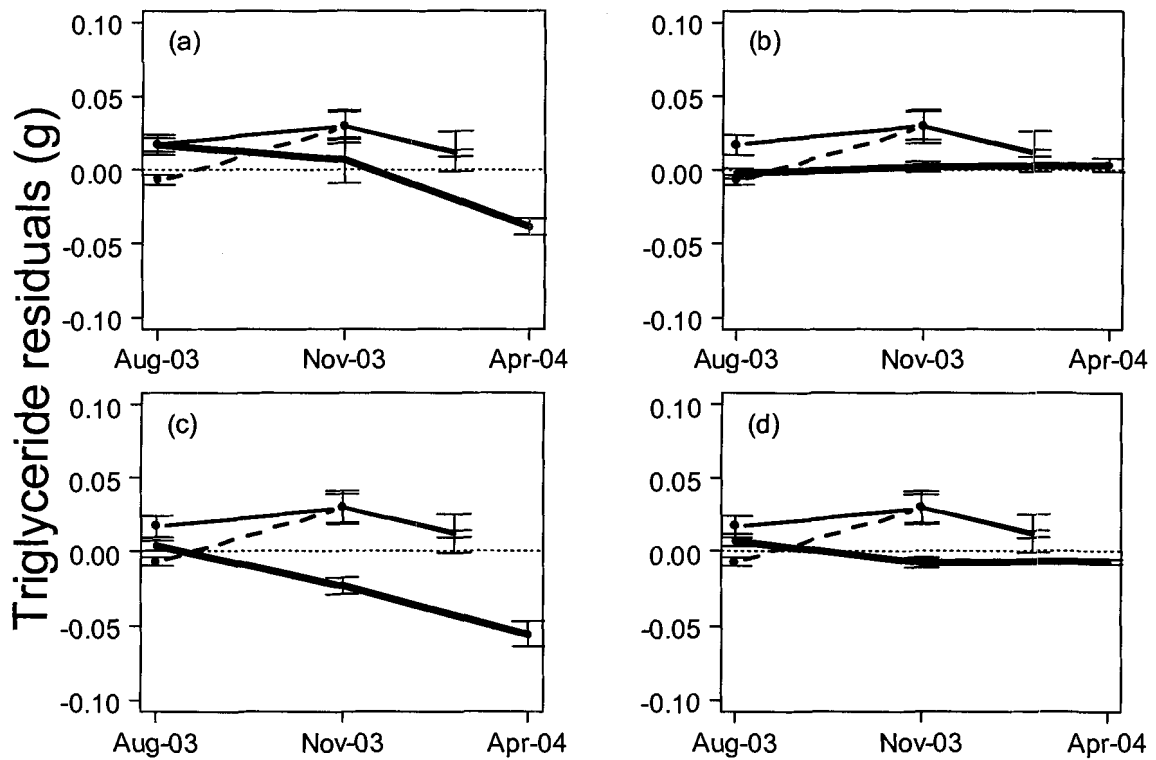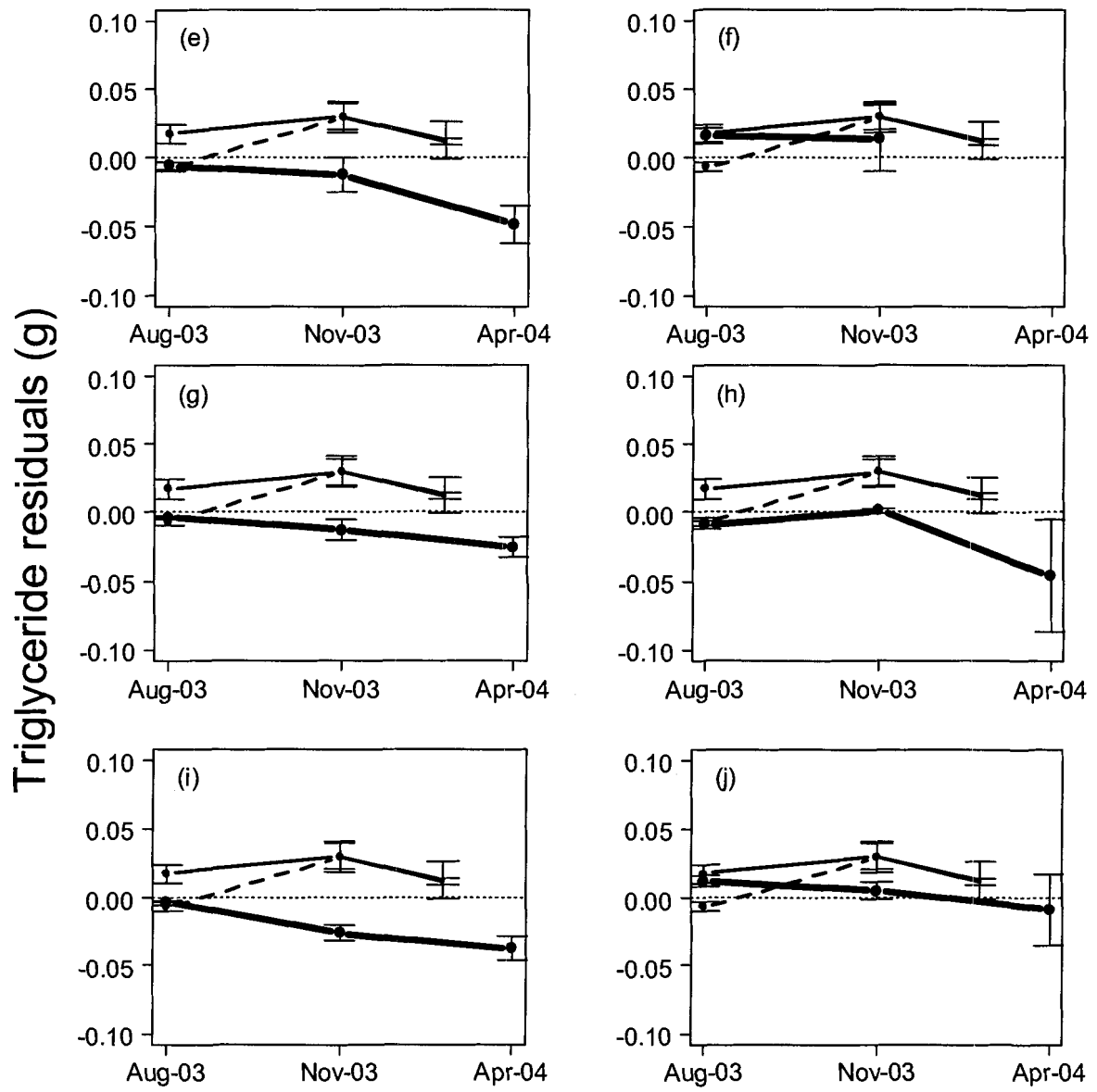
Figure 15. Correlations of invertebrate density and triglyceride residuals for (a) August 2003 (b) November 2003 and (c) spring 2004.
Numbered labels represent individual sites: 1=Bridge River- Lower reach; 2=Bridge River- Upper reach, 3=Little Chilcotin River, 4=Chilko River, 5=Quesnel River, 6=Cottonwood River, 7=Fraser River at Stoner, 8=Nechako River, 9=McGregor River, 10=Fraser River at Tete-Jaune Cache, 11=Clearwater River, 12=Nicola River. Missing data: Fraser River at Stoner in November 2003 and the Nechako River in April 2004. Bridge River February 2004, and Fraser River April 2004 data combined.
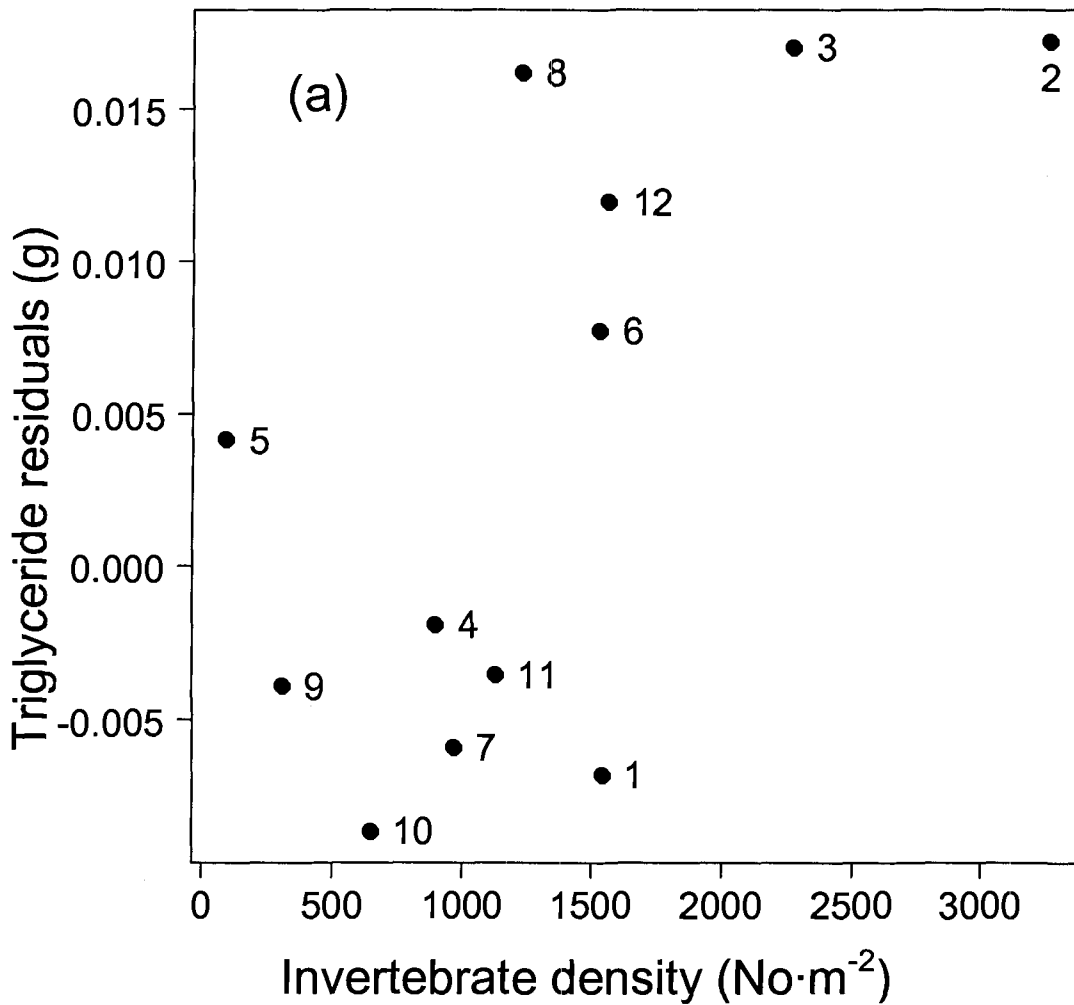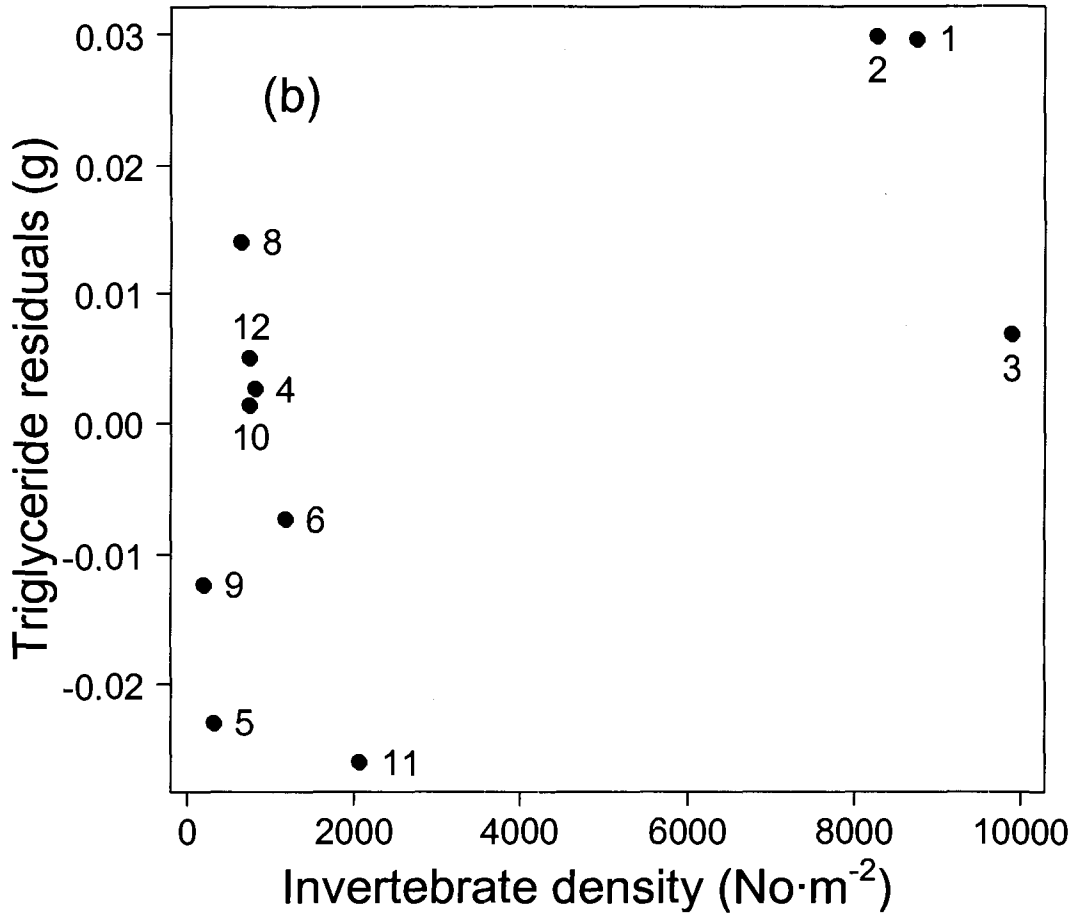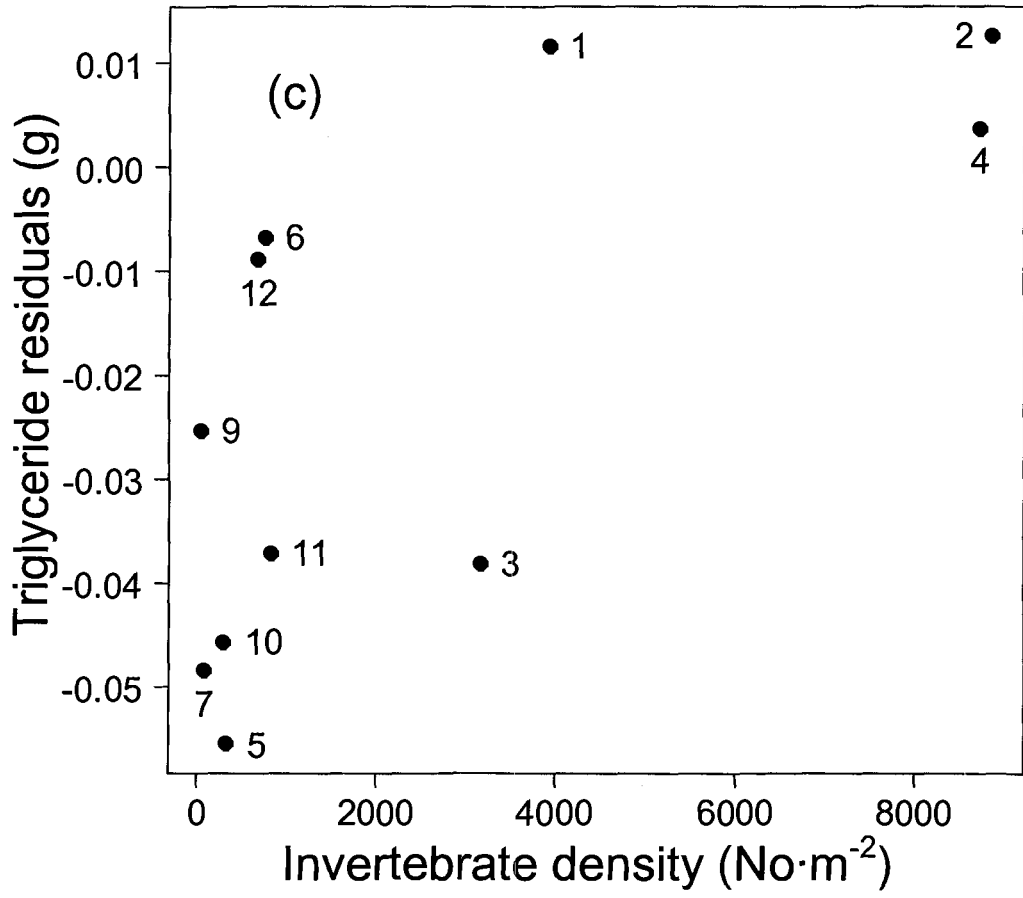
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APPENDIX

Sample sizes for total lipid extractions

The target sample size for lipid extraction was eight fish per site, but in some cases, this was not possible (listed below).

Site	Sampling event	Sample size, N
Bridge River - Upper reach	Sep 2002	5
	Apr 2004	1
Bridge River - Lower reach	Nov 2002	3
	Jan 2003	2
	Apr 2003	6
	Jun 2003	12
	Aug 2003	9
	Sep 2003	9
	Feb 2004	4
Little Chilcotin River	Apr 2004	5
Chilko River	Apr 2004	5
Quesnel River	Apr 2004	6
Cottonwood River	Apr 2004	6
Fraser River at Stoner	Apr 2004	5
Nechako River	Nov 2003	4
	Apr 2004	0
McGregor River	Apr 2004	5
Fraser River at Tete-Jaune Cache	Apr 2004	2
Clearwater River	Apr 2004	10
Nicola River	Apr 2004	4

Calculations

The specific details of how authors have calculated metrics such as total lipid levels or triglyceride levels are rarely, if ever, included in the peer reviewed literature. Therefore, I included my calculations in this document to assist other researchers who wish to use these methods.

Total lipid mass was calculated as:

total lipid mass (g) = lipid mass per 1.0 mL (g·mL^{-1}) × recovered lipid volume (mL)

Triglyceride mass was calculated as:

triglyceride mass (g) = net glycerol (mg·mL^{-1}) × 1.13 (μmol·mg^{-1}) × dilution factor ×

recovered lipid volume (mL) × 885.5 (g·mol^{-1}) × 10^{-6} (mol·μmol^{-1})

where: 885.5 g·mol^{-1} is the molecular weight of triolein; 1.13 μmol·mg^{-1} is the reciprocal of the molecular weight of triolein (in units of μmol·mg^{-1}); and the dilution factor is unitless.

Modifications to spectrophotometric measurement of triglycerides

Whole-body triglyceride levels were determined using a modification of the methods outlined by Weber et al. (2003). The new methods are as follows:

1. Remove lipid samples from -80 °C freezer and leave to thaw for 10 minutes.

2. For each sample:

   a. Label one 13x100 mm test tube for each sample.
   b. Vortex the sample vial for 10 seconds.
   c. Using P10 pipette, add 20 µL (2x10 µL) of sample into labeled test tube.
   d. Place test tubes under N_2 for 30 minutes at 1 psi to evaporate residual chloroform from each sample.

3. Create a blank – pipette 20 µL chloroform/BHT into test tube and dry under N_2.

4. After drying, add 200 µL of isopropanol to each sample and blank (using P200 pipette). Cap and vortex each sample for 10-20 seconds until lipid residue is fully dissolved.

5. Each sample should reconstitute for at least an hour. Samples may be left overnight. Be consistent.

6. Remove Sigma Reagents A, B, and glycerol standard from fridge. Reconstitute using nano-pure water (ddH$_2$O) – add 40 mL ddH$_2$O to Reagent A and 10 mL ddH$_2$O to Reagent B. Invert Reagents slowly and make sure all powder has dissolved.

7. Make standards using serial dilution: note that the glycerol standard has a concentration of 2.5 mg·mL^{-1}. Vortex each standard thoroughly at each step. Keep standards on ice.

Standard	Concentration (mg·mL^{-1})	Instructions
S1	2.5	use directly from bottle
S2	1.25	100 µL glycerol standard + 100 µL isopropanol
S3	0.625	100 µL S2 + 100 µL isopropanol
S4	0.313	100 µL S3 + 100 µL isopropanol
S5	0.156	100 µL S4 + 100 µL isopropanol
S6	0.078	100 µL S5 + 100 µL isopropanol

8. Turn on microplate reader, open Soft Pro software and make/save your template in (see example below).

9. Vortex standards and blank – in order, add 10 μl of blank and standards S1-S6 to columns 1 and 2 of microplate (in duplicates).

10. Empty contents of Reagent A into plastic trough.

11. Add 180 μL of Reagent A to columns 3–12 only (using P200 multi-pipette).

12. Plate samples on top of Reagent A:
    a. Vortex sample 10 seconds.
    b. Using a P10 pipette, add 10 μL of sample into each well (in triplicates). Use a new pipette tip for each well.

13. Once all samples are plated, add 180 μL of Reagent A to columns 1 and 2.

14. Place microplate in microplate reader and set to shake for 5 minutes. Read samples at 540 nm - this is the free glycerol reading. Save your file.

15. Remove microplate and add 45 μL of Reagent B (using plastic trough and multi-pipette) to all wells.

16. Activate lower plate of SoftPro file. Return microplate to microplate reader and shake for 15 minutes, obtain second reading. This is your total glycerol reading. Save your file.

17. Return microplate to microplate reader and shake for additional 15 minutes, obtaining a third reading. Save file as version 2.

18. Compare results from second and third readings and use reading with highest values in subsequent triglyceride calculations.

19. Calculate triglyceride mass.

Template for spectrophotometer

The template required for the Soft Pro software should be set up as follows.

						Sample 27	Sample 28
						Sample 27	Sample 28
						Sample 27	Sample 28
		→					
		→					
Sample 1	Sample 2	Sample 3					
Sample 1	Sample 2	Sample 3					
Sample 1	Sample 2	Sample 3		→			
Blank	Standard S6	Standard S5	Standard S4	Standard S3	Standard S2	Standard S1	
Blank	Standard S6	Standard S5	Standard S4	Standard S3	Standard S2	Standard S1	