TYPE AND TIMING OF PHOSPHOROUS ADDITION FOR COHO SALMON AND STEELHEAD TROUT PRODUCTION

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Applied Science

in

The Faculty of Graduate Studies

(Chemical and Biological Engineering)

The University of British Columbia

April 2006

ABSTRACT

In-stream fertilization is recognized as an effective rehabilitation strategy for increasing fish production in nutrient poor streams. Past research has focused on timings and concentration levels, and short term trials. Several alternative management schemes are possible, but only a limited number have been field-tested. Sixteen nutrient replacement schemes were developed based on an imitation of historical nutrient inputs such as inorganic and organic components of fish carcass, leaves and background hydrology and geology. Further, six schemes (3 schemes one year and 3 others the next year, see below) were field tested over one year using nine artificial sub-alpine stream channels that received natural background water from a nearby spawning channel and two *Oncorhynchus* species, namely endangered coho salmon (*O. kisutch*) and steelhead trout (*O. mykiss*). Schemes tested include: 1) summer inorganic fertilizer addition to reach 3 µg/L SRP, 2) organic fertilizer to reach 3 µg/L SRP, 3) no fertilizer addition, 4) organic fertilizer addition in the summer to reach 3 µg/L SRP, 5) organic fertilizer in the fall to reach 3 µg/L SRP and 6) no fertilizer addition. Background SRP conditions in schemes 1-3 were nil in the summer and above phosphorous growth saturating conditions due to upstream salmon carcass decomposition (SCD) in the fall , and in schemes 4-6, they were approximately phosphorous growth saturating in the summer and fall (due to SCD that fall and the previous fall). Schemes 1 and 6 were pseudo-replicates and their results were compared to investigate the effects on the response variables of having a variable amount of inorganic phosphorous (either from fertilizer or natural sources) available in the summer and dissolved phosphorous from SCD available in the fall. Juvenile fish length, weight, fat

stores, and over-winter biomass were the primary response variables. Food web response to phosphorous inputs including algal standing biomass, stream macroinvertebrate numbers and biomass, and resident fatty acid profiles were investigated to elucidate the results. In general, the benefits were not universal in terms of fish species. Steelhead trout benefited the most from in-stream phosphorous augmentation. Year 1, steelhead trout over-winter sizes under schemes 1 and 2 were significantly larger than under scheme 3 ($p<0.0001$). Lipid levels were highest year one in steelhead trout under scheme 1 during the summer and under scheme 2 and 3 after winter. Coho salmon size years 1 and 2 was significantly larger after summer under treatments 1 and 4 than under the others conducted during the same time period, but the size advantage did not persist over winter. Total lipid content in coho salmon year 1 followed a similar trend. Year 2 after summer steelhead trout size obtained under scheme 4 was larger than obtained under scheme 5 and 6, but like year 1 coho salmon, this advantage did not persist over winter. Scheme 1 and 6 the pseudo replicates did not produce statistically similar results. Scheme 1 that received a higher input concentration of SRP during the fall than scheme 6 produced more after winter biomass. As well, in the summer coho salmon grew faster under scheme 6, than under scheme 1. This can be attributed to SRP being available for a longer time period in scheme 6. These results illustrate that future research should be conducted to investigate how concentration and duration of phosphorous inputs as well as source and timing can be manipulated to mimic historical stream phosphorous inputs and produce increased benefits to freshwater resident juvenile salmonids. More tests on other schemes and species compositions could lead to improved management practices and higher fish yields

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DEFINITIONS

Age-0: juvenile salmonids that hatched in the spring and have not spent a winter in the system

Age-1: juvenile salmonids that hatched the previous spring and have spent one complete year in the system

Poikilotherm – an animal whose body temperature fluctuates with that of the environment

Common forms of phosphorous in water and sediment:

Dissolved phosphorous: consists of dissolved inorganic phosphorous, dissolved hydrolysable phosphorous and dissolved organic phosphorous. It is all of the phosphorous present in the filtrate of a sample filtered through a phosphorous-free filter of 0.45 microns pore size.

Dissolved inorganic phosphorus: This form of phosphorus is available to algae and other aquatic plants for growth and is referred to as orthophosphate or soluble reactive phosphorus (SRP). It is derived from allochthonous sources, rock weathering, particulate inorganic phosphorous, and dissolved inorganic phosphorous derived from organic material by excretion or decomposition, and the dissolution of other phosphate deposits including fertilizers containing magnesium phosphate. Orthophosphate levels do not equal SRP levels due to analytical differences in measurement techniques.

Dissolved organic phosphorus: This form of phosphorus is excreted by animals and zooplankton or is contained within algae. Through the action of decomposers, it can be converted into dissolved inorganic phosphorus. Tied up in algae, it can be past onto zooplankton and other grazers after digestion.

Particulate phosphorous: Particulate inorganic phosphorous: when oxygen is present, dissolved inorganic phosphorous reacts with iron and calcium to form insoluble compounds. These compounds can be bound up in the sediments. They can be converted back into soluble forms if oxygen is not present.

Particulate organic phosphorous: this phosphorous is contained within live or dead tissues. It exists as a phosphate molecule associated with a carbon containing molecule. It can be degraded into both dissolved organic and inorganic phosphorous. Some types of organisms can utilize it directly after consuming tissue. Much of the particulate organic phosphorous within aquatic systems ends up in the sediments.

Phosphorous fertilizer treatments used in this study:

Inorganic slow release fertilizer: solid magnesium phosphate. The aim of this treatment is to stimulate the primary production in phosphorous-limited streams by providing phosphorous at concentrations less than the freshwater aesthetic US federal SRP concentration (see below).

Organic slow release phosphorous fertilizer: fish bone meal product. This fertilizer contains particulate organic phosphorous, and the treatment was developed to achieve a similar aim as above. Unlike the inorganic fertilizer, the phosphorous within the organic fertilizer can also be passed directly to animals through direct consumption of the fertilizer (see definition of particulate organic phosphorous).

Other definitions relating to phosphorous:

Freshwater aesthetics US federal phosphorous criteria: 0.1 mg/L SRP in streams or flowing water systems not discharging into lakes or reservoirs to control algae growth

Redfield ratio impact: aquatic systems may be phosphorous limited when nitrogen concentrations are high and the Redfield ratio (N:P) 16-23:1.

Nil: phosphorous status for highly oligotrophic streams, indicated by a dissolved inorganic phosphorous concentration less than 1 μg/L.

ACKNOWLEDGMENTS

Funding for this project was provided by the Province of British Columbia Ministry of Environment (MOE) and the National Science and Engineering Research Council of Canada. Generous in-kind support was also provided by the United States Department of Commerce National Oceanic and Atmospheric Administration (NOAA) and the British Columbia Conservation Foundation (BCCF). Thank-you to the funding agencies involved in this research, without your support it would not have been possible.

I would like to extend a special thank-you to my supervisor, Dr. Royann Petrell. Your guidance and advice throughout this entire process has been much appreciated. Thank-you to my committee members: Dr. Ken Ashley with MOE, and Dr. Sue Baldwin and Dr. Ken Hall with the University of British Columbia. Thanks also to Ron Johnson with NOAA for all of your input and help with the fatty acid analysis.

I would like to thank everyone who came out to volunteer and the staff from BCCF and MOE who assisted in field data collection. In particular, thanks to Sheldon Reddekopp, Greg Wilson, and Emma-Jane Johnson who provided invaluable assistance in the field as well as comic relief and Friday afternoon tailgate parties. I cannot tell you how much I appreciate the support and entertainment.

Finally, a heart felt thanks to my family and friends. You all had a very big hand in the completion of this thesis. A special thanks to Laura Siberry for all your help with editing the manuscript. Thank-you to Wendy Schmidt for all the venting sessions over coffee and the afternoon mountain bike rides – you did a great job keeping me sane. Finally, thanks to Aaron Thompson, and my mum and dad. You guys managed to love me even when I was crazy.

Chapter 1: Introduction

Since the turn of the nineteenth century, Pacific salmon continue to be an important economic resource and social icon for coastal British Columbia. The state of Pacific salmon stocks has been the focus of public concern and scientific research for decades. Stocks have been in decline due to historical logging practices, hydropower generation, over-fishing, and other human activities (Litchatowich et al., 1999) as well as recent climate change (Beamish et al., 1999). More recently, the ecological importance of salmon as a source of carbon and nutrients for freshwater and nearby terrestrial ecosystems has been recognized (Larkin and Slaney, 1997). This ecological role comes from the fact that most Pacific salmon (*Oncorhynchus spp.*) are anadromous. In other words, they begin life in streams as eggs that hatch to become fry. Most species rear for a time as juveniles in freshwater, before migrating out to sea and continuing to grow and mature in the ocean. Once sexual maturity is reached, the adult salmon return to their natal streams to spawn and die, thus beginning the cycle again. Upon dying, nutrients are released into stream environments and are then used to directly and indirectly feed young salmon. Unfortunately, as salmon populations decrease, so does their ability to indirectly nurture their young.

Due to fewer adult salmon returning to coastal streams to spawn and die, marine derived nutrients within the system have decreased (Larkin and Slaney, 1997). The changes in nutrient availability affect the timing and composition of macroinvertebrate communities (a vital food source for juvenile salmonids) (Chaloner and Wipfli, 2002). With fewer nutrients available and a decrease in the productivity of stream ecosystems, the emerging fry and resident juvenile salmon have a decreased access to food.

The overall objective of this research is to use food web theory and experimentation to investigate the effects of timing and source of stream phosphorous inputs on the over-winter length, weight and lipid stores of freshwater resident juvenile salmonids.

The second chapter presents a review and a case study used to outline the possible sources of historical inputs of phosphorous and investigate how the source and timing of phosphorous inputs affects the salmon stream food web. The subsequent chapters present the findings of two years of field trials developed to test some of the ideas resulting from the second chapter. Finally, conclusions based on the theoretical review and field trials about the influence of phosphorous source and timing on the juvenile salmonids: coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) are presented in the last chapter. Each chapter is written in paper format as per the guidelines for manuscripts submitted to the Transactions of the American Fisheries Society.

Chapter 2: Literature Review and Case Study: Timing of Nutrient Inputs in Oligotrophic Streams and Effects on Energy Transfer and Fish Productivity

Summary: Due to several natural factors and anthropogenic impacts, many streams are nutrient poor, and this negatively affects fish productivity. For such systems, in-stream fertilization, as it directly and indirectly increases food production, is an effective rehabilitation strategy for increasing fish production. What is unclear is when to add the nutrients to mimic historical inputs and to maximize benefit for multiple species. Herein is an analysis of the workability of and the potential benefits arising from various fertilization schemes that imitate the timings of natural in-stream input. Used as an example system is a low gradient (<3%) stream containing a mixed species population of resident coho salmon and steelhead trout juveniles located in a non-urban/non-agricultural high precipitation watershed in the Pacific Northwest. Food web theory and available literature were utilized to carry out the analysis. The work was used to develop a framework for future experiments and fisheries rehabilitation management. Based on the food web theory and previous experimental evidence applied to this particular case study, juvenile salmonid growth rate and survivability can be increased with fertilization schemes that mimic natural inputs of phosphorous and prolong the period through which the phosphorous is available in the streams. More field investigations need to be conducted to determine if the source of phosphorous, either inorganic, organic or a combination of both, will impact the magnitude of the benefit to multiple species of resident freshwater Pacific salmon.

2.1 Introduction

In many parts of the world, streams in non-urban and non-agricultural watersheds are characterized by low concentrations of essential nutrients such as nitrogen and phosphorous, which are the building blocks in the synthesis of complex bio-molecules such as DNA, RNA, amino acids and lipids. In the coastal Pacific Northwest there are many such streams, and this is unfortunate, given that streams are vital for endangered fish populations such as the anadromous steelhead trout and coho salmon. For the coho such systems found at higher elevation have become primary refuges due to the pollution or draining of the generally preferred lower elevation systems. Artificial methods for increasing the nutrients in nutrient poor systems have shown potential for enhancing fish production. These methods should, however, imitate natural inputs so as to reduce the likelihood of negative impacts.

Streams can be nutrient poor for many reasons. Firstly, certain types of geologies in combination with high precipitation can produce nutrient poor watersheds. For example, while variations do occur, in the Pacific Northwest the bedrock consists primarily of granite that is low in mineral nitrogen and phosphorous. It is overlaid by a thin layer of topsoil. These low-nutrient conditions combined with high dilution due to high annual precipitation create low levels of dissolved nutrients within a stream. Nutrient poor conditions can also result from a lack of leaf litter, anthropogenic conditions, low-sedimentation rates from upstream sources or riparian zones, and a decline in anadromous fish returns.

The deposit of leaf litter from surrounding riparian vegetation, in particular the seasonal input of red alder (*Alnus rubra*) leaves, represents a substantial input of nitrogen

and phosphorous rich organic matter to watersheds dominated by this tree (Volk et al., 2003). Nutrients are cycled within a stream ecosystem during the natural processes of life, death and decay of fish, macroinvertebrates, microbes, algae and fungus.

Anthropogenic processes such as dam construction, forestry practices, over-fishing and habitat destruction have led to the decrease in available nitrogen and phosphorous in stream ecosystems. The construction of water retention dams causes the creation of large stagnant lakes and pools. This leads to the settling out of suspended particles upstream of the dam and causes low-nutrient status in downstream waters. Logging of coastal watersheds causes an initial increase in sedimentation from the newly exposed drainage basin and an increase in the suspended nutrient status of a stream, but once the thin layer of topsoil is eroded, there is a period of nutrient scarcity as the decrease in riparian vegetation leads to a decrease in allochthonous organic matter inputs (Bilby and Bisson, 1992). The historical practice of harvesting the riparian forest contributed to a decrease in leaf litter transportation into nearby stream systems and a decrease in available nutrients.

In the Pacific Northwest, the return and subsequent death of spawning salmon represents a large source of marine derived nitrogen and phosphorous for freshwater stream ecosystems (Gresh et al., 2000, Larkin and Slaney, 1997, Schoonmaker et al., 2003, Stockner and Ashley, 2003). Unfortunately, over-fishing and habitat destruction have decreased the numbers of returning spawning salmon such that there are insufficient quantities to support the nutrient requirements of the system (Cederholm et al., 2000, Larkin and Slaney, 1997). Historical cannery records, historical run estimates and current catch statistics were used to estimate the decline in salmon returns (Gresh et al., 2000,

and Schoonmaker et al., 2003). Pacific salmon biomass has been estimated to have declined to either 5-7 % of historic escapement levels (Gresh et al., 2000), or 15 % of historical escapement biomass (Schoonmaker et al., 2003) depending on the predictive model used for estimation. Both of these studies indicate that the decline in Pacific salmon biomass becomes more pronounced farther south in the range. Salmon stocks declined due to historical logging practices, hydropower generation, over-fishing and other human activities (Litchatowich et al., 1999) as well as recent climate change (Beamish et al., 1999). This decline in biomass directly affects nutrient levels in stream systems.

Different Pacific salmon species hatch and rear for a varying length of time in freshwater. After the freshwater residence, the salmon migrate to the ocean to mature. Once they are sexually mature, the salmon return to their natal streams to spawn and die. Some species such as steelhead trout are able to return to the ocean after spawning and some can live to spawn again, but the majority of *Oncorhynchus* species are semelparous and die after a single spawning event. At the time the spawning migration occurs, salmon consist of approximately 95 % marine derived biomass (Larkin and Slaney, 1997). In streams that support a large run, the annual fall influx of spawning salmon represents a large input of marine derived nitrogen and phosphorous. In streams that have historically supported large runs but no longer have the spawning returns, the loss of the marine derived nutrients represents a large net loss of available nutrients within the system. Gresh et al. (2000) estimated that the contribution of Pacific salmon returns to phosphorous within streams along the Washington coast has dropped from a historical 31,000 kg to 1,000 kg currently.

With decreasing nutrients available within coastal stream systems, rehabilitation techniques focusing on the active input of inorganic and organic fertilizers to increase productivity are receiving considerable attention (Bilby et al., 1998, Mundie et al., 1991, Sterling et al., 2000, Stockner ed., 2003, Wipfli et al., 2004). Nutrients are applied in different ways. One possibility is nutrients in the form of inorganic fertilizer are applied such that nutrients are in the system during the summer growing period (June-September) to increase autotrophic production and increase the available forage for resident fish populations. Applied in this way, inorganic fertilization imitates a nutrient shadow from a large fall input of spawning salmon (described in detail later), an input of sediment from upstream, or a transfer of nutrients from the hyporheic zone. Studies focusing on the use of this technique have shown a marked increase in periphyton standing biomass, macroinvertebrate standing biomass, and resident fish growth, size-at-smolt and spawning return in fertilized rivers (Johnston et al., 1990, Slaney et al., 2003, Wilson et al., 2003).

A second possibility is that nutrients in the form of organic fertilizer or fish carcasses are applied in the fall to mimic the return of spawning salmon. After the addition of either pink salmon carcasses or carcass analogs resident fish growth and lipid storage increases (Wipfli et al., 2003 and 2004).

Both of these nutrient addition schemes have provided benefits to fish. To date they have not been tested against each other, against other possible schemes nor using multiple possibly competing fish species. So, questions remain as to: 1) when and how often to add the nutrients, 2) which combination (either inorganic or organic or both) as a

mimic of historical inputs is most beneficial to fisheries, and 3) how to maximize benefit for multiple species.

To address these questions, within this chapter, the roles that different types of natural phosphorous inputs have in energy flow and fish production are analysed. Next, how best to imitate natural inputs using fertilizer and/or carcasses is discussed. Finally, a case study is presented to illustrate how fish in a stream might benefit after fertilization. A review of available literature was used to examine the fertilization schemes that mimic the natural inorganic and organic nutrient status of streams, and evaluate their potential benefits for the resident multi-species fish populations. Pacific salmon are an economically, socially and ecologically important fish to the Pacific Northwest. This case study is focused on juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) because these species are at risk, they reside often together, and of the anadromous Pacific salmon, they remain the longest in streams. Conceivably, due to the long resident time, coho and steelhead would benefit the most from freshwater fertilization activities. For this study, the target nutrient of interest is phosphorous because many of the streams in the Pacific Northwest are either phosphorous limited, or co-limited with phosphorous and nitrogen.

2.2 Phosphorous and nutrient flow within a salmonid stream

The analysis of the role of phosphorous within a stream starts with the phosphorous cycle. Phosphorous is the most important growth limiting element in freshwater ecosystems due to the relatively small amounts available in water and phosphorous' major role in biological metabolism (Schwedt, 1996, Wetzel, 2001). Total

phosphorous in streams is partitioned into two fractions: particulate and dissolved. Particulate phosphorous can be further subdivided into the phosphorous contained in living organisms, phosphorous in mineral rock and soil, and phosphorous associated with dead organic matter. Dissolved phosphorous is usually partitioned into orthophosphate (Soluble Reactive Phosphate: PO_4^{3-}), organic colloids and phosphate esters (Wetzel, 2001). Soluble Reactive Phosphate (SRP) in the water column can be removed from the water and taken up by autotrophic algae or can be removed through adsorption to the benthic substrate (Wetzel, 2001). Phosphorous associated with particulate organic matter is taken up by heterotrophic microbes. Phosphorous is cycled through the stream ecosystem by excretion and uptake of organic and inorganic, particulate and dissolved forms by living stream biota. Phosphorous can also be released by the death and decay of dead organisms (Wetzel, 2001). See Figure 2.1 for a summary of the phosphorous cycle in stream ecosystems.

IP: Inorganic Phosphorous
DIP: Dissolved Inorganic Phosphorous
PIP: Particulate Inorganic Phosphorous
POP: Particulate Organic Phosophorous
DOP: Dissolved Organic Phosphorous

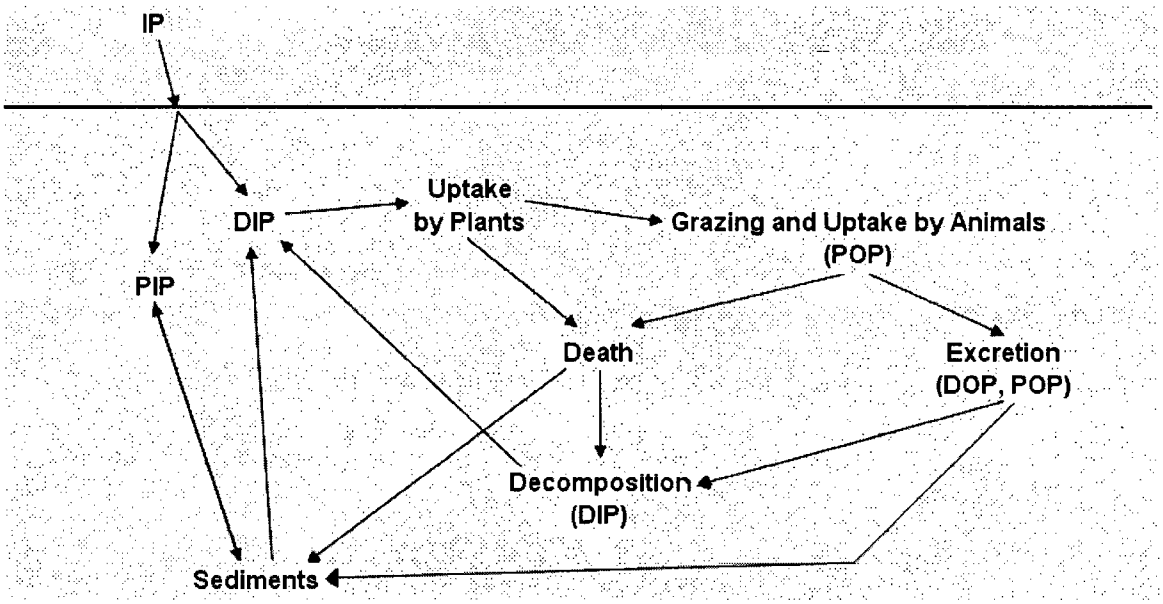


Figure 2.1: The Stream Phosphorous Cycle

The phosphorous cycle alone does not explain the importance of phosphorous inputs and timings in salmonid streams. There is a complex interaction in stream ecosystems between biotic and abiotic factors. The stream food web represents the complex important biotic interaction between nutrients, food source and consumer. The connection between trophic levels is influenced by physical characteristics such as size of the stream, hydrology and habitat elements that can change the distribution, density and potential for interactions between consumers and consumed (Hullar and Vestal, 1989). The food web is also influenced by chemical disturbances such as acidification and changes in nutrient availability (Hildrew, 1992).

The basis of the stream food web is the biofilm consisting of algae and other microorganisms on any submerged because it is the site of a complex interaction between

photosynthetic organisms and heterotrophic fungi, bacteria and micro- and meiofauna. Its formation and demise affect higher trophic levels including fish stocks.

Biofilms form on almost any surface that is exposed to water. The substrate that a biofilm develops on can be porous or non-porous. It can be inert (eg. rock) or can provide an energy source for the colonizing organisms (eg. carcass material, leaf litter) (Wilderer and Characklis, 1989). The flow regime of the stream and thickness of the biofilm impacts the mass transfer between the bulk fluid and the biofilm and within the biofilm itself. Under turbulent flow conditions, the laminar boundary layer decreases, and mass transfer between the bulk fluid and the biofilm occurs at a faster rate than under completely laminar flows (Granet, 1996, Wilderer and Characklis, 1989). Bothwell showed that the specific growth rate of lotic periphytic diatoms is saturated at phosphorous levels of 0.3-0.6 μgP/L (1985), but SRP levels that saturate peak aerial biomass are two orders of magnitude higher (at temperatures <10°C) (1989). This is because, as the algal accumulation increases, the growth and accumulation of the biofilm exhibits a shift from cellular Monod kinetics to community diffusion limited kinetics (Bothwell, 1989). United States Environmental Protection Agency (USEPA) water quality criteria states that phosphorous should not exceed 0.05 mg/L if streams discharge into lakes, and 0.1 mg/L in streams not discharging into lakes to control nuisance algal growth (USEPA, 1986). Surface waters maintained at 0.01- 0.03 mg/L of total phosphorous tend to remain uncontaminated with algal blooms (USEPA, 1986). Bothwell (1988) using continuous K_2PO_4 additions within experimental troughs was able to show that light has little effect on benthic algal growth under nutrient limited growth. That is, growth is not necessarily higher when more sunlight is available. He also provided

evidence that growth under phosphorous limitations was linear with temperature between 0 and 20°C, and algal growth was measurable at temperatures near zero (Bothwell, 1988).

The Hillebrand et al. (2002) investigated the effects of nutrient levels on biomass and community structure of biofilms in a freshwater lake in Sweden. They found that increasing the nutrient availability through fertilizer addition, with adequate light available, increased the biomass of algae, and that algae generally dominated the biofilm assemblage. Macro-consumers, such as macroinvertebrate larvae, fed preferentially on algae, and the presence of these consumers had a negative effect on algae and a positive effect on heterotrophic components of the biofilm. Bott and Borchardt (1999) examined the consumption rates of protozoa, bacteria and diatoms by meiofauna in the sediments of a Pennsylvanian creek. Of the multiple meiofauna groups encountered, chironomids contributed the greatest percentage of biomass in all experiments. Their results suggest that meiofauna densities are lowest during the winter, and that the meiofauna diet shifts seasonally between diatoms in summer and protozoa and bacteria in winter.

An important function of biofilms is the cycling of nutrients and bioconversion within an ecosystem. Stream detrivores, fungi and bacteria contribute to the breakdown of organic material within the system, such as leaf litter that falls into the streams and spawning salmon carcasses that occur in salmon bearing streams in the fall of each year. Leaf litter, including riparian Red Alder (*Alnus rubra*), which dominates many riparian zones in the Pacific Northwest as a result of clear-cut logging, can provide a substantial input of nutrients such as phosphorous and nitrogen (Volk et al., 2003). In watersheds dominated by Alder, dissolved phosphorous levels in the fall during leaf deposition were 6.8 µg/L compared to 4.5 µg/L of coniferous dominated riparian zones (Volk et al.,

2003). In watersheds with a low return of spawning salmon, this represents a significant input of nutrients. Hieber and Gessner (2002) examined the relative contribution of fungus, bacteria and macroinvertebrate shredders to the breakdown of leaf packs in a third order reach of the Steina River in the Black Forest of Germany. The leaf packs were rapidly colonized by fungi, bacteria and macroinvertebrates. The results of the study suggest that fungus contributes more to the breakdown of leaf material than bacteria, based on biomass data, but that fungi, bacteria and macroinvertebrate shredders all contribute to the rate of decomposition of the leaf material.

Salmon carcasses impact nutrient status in streams. In an early paper, Richely et al. (1975) presents data from a two-year field trial on Kokanee salmon (*O. nerka*) decomposition that shows periphyton biomass, heterotrophic activity and nutrient concentrations were only greater downstream than upstream during a peak carcass year. Wold and Hershey (1999) used clay substrate as a stream rock surrogate upstream and immediately downstream of decomposing salmon to measure biofilm accrual and stable isotope signatures. Their results suggest that biofilm growth is increased downstream of decomposing salmon, and the stable isotope analysis suggests that fish-derived nitrogen was incorporated into periphyton and the total biofilm.

Parmenter and Lamarra (1991) investigated the decomposition rates of steelhead trout in a freshwater marsh. They demonstrated that carrion decomposition followed a three-stage pattern. Initially, the carcasses exhibited a rapid leaching of salts (K, and Na). The microbial activity increased rapidly and carcass N, P, and S were depleted with most of the decomposition occurring in the first sixty days of the experiment. Finally, the bone material decomposed at a much slower rate, dominated by physical breakdown and

chemical dissolution. In this study, no aquatic insects were found feeding on the carcass material. In contrast, Chaloner et al. (2002) found that several macroinvertebrate taxa colonized the carcasses of pink salmon in south-eastern Alaskan streams. As with the study performed by Parmenter and Lamarra (1991), the carcasses initially underwent a rapid decomposition rate that decreased over time. See section 2.5 for a more detailed discussion of these studies and the accompanying carcass decomposition rates.

Invertebrates act as a carbon and nutrient retention site in streams by providing a link between primary producers and predators in the upper trophic levels. Macroinvertebrates feed on the complex communities existing in biofilm (Malmqvist, 2002) and directly on fish carcasses (Chaloner et al., 2002, Wipfli et al., 1998). Fish depend heavily on macroinvertebrates as a food source (Bilby and Bisson, 1992, Malmqvist, 2002, Williams et al., 2003, Wipfli, 1997).

Insects are abundant and diverse in freshwater habitats. Insects use the full spectrum of available food sources, and insects from the same genera can be members of different functional feeding groups (Wiggins, 1996). It is useful, therefore, when examining a particular food web, to look at classifying macroinvertebrates based on their functional feeding group. Functional feeding groups include shredders, collectors, scrapers and predators (Cummins and Klug, 1979). The different functional feeding groups inhabit different trophic levels. Shredders, collectors (filterers and gatherers) and scrapers are classified as detritivores and herbivores; predators are classified as carnivores (Cummins and Merritt, 1996).

Macroinvertebrates have evolved a large variety of morphological adaptations to enable them to colonize and thrive within the many different habitat niches in a stream

(Wallace and Anderson, 1996). A broad range of microhabitats exist within a stream due to the variations of flow regimes and the influence of flow characteristics on substrate structure and complexity. Insects exist as skaters, divers, swimmers, clingers, sprawlers or burrowers depending on the microhabitat they inhabit and the method of feeding they employ (Cummins and Merritt, 1996). The habits of the insects within the different functional feeding groups also affect the likelihood that the particular insect will be found in the stream drift (Cummins and Merritt, 1996, Humphries, 2002).

Macroinvertebrates have developed a broad range of life histories to have access to seasonally available food and avoid competition between and predation from individuals. Timing of lifecycle stages is a response to temperature, oxygen, light levels and food availability (Wallace and Anderson, 1996). Georgian and Wallace (1983) investigated the seasonal population dynamics of insects belonging to the scraper functional feeding group in the southern Appalachians. Of the approximately five species studied, one had a bivoltine pattern (ie. summer and winter cohorts) and the remaining species displayed a univoltine pattern. The research suggests that the lifecycles of the species in the system have developed in such a way so that the population peaks for each the species of macroinvertebrates from the same functional feeding groups occurred during separate times of the year and did not overlap by much. The lack of direct overlapping reduces competition for food resources within a functional feeding group.

Macroinvertebrates exhibit a positive response to increased levels of nutrients. Studies in artificial stream channels and natural stream systems have shown that the presence of salmon carcasses (Chaloner et al., 2002, Chaloner and Wipfli, 2002, Wipfli et al., 1998) and organic fertilizer (Wipfli et al., 2004) in the fall increases the biomass and

standing stock of stream macroinvertebrates. The carcass and organic fertilizer represents a vector of nutrient and macro-molecule input that can be consumed directly by collector and shredder macroinvertebrates. Studies in artificial and natural stream systems have shown that increasing inorganic phosphorous inputs with active fertilization during the summer growing season will also increase macroinvertebrate biomass and standing stock (Mundie et al., 1991, Quamme and Slaney, 2003, Wilson et al., 2003).

During their freshwater residency, coho and steelhead fry feed extensively on macroinvertebrates. Chironomidae, Diptera, Trichoptera and Coleoptera are found most abundantly in the juveniles' diet (Higgs et al., 1995). Freshwater yearlings feed more heavily on smaller fish, as well as Diptera, Ephemeroptera and other insects found in the drift (Higgs et al., 1995). Macroinvertebrates generally have a lipid content of between 10-20 % of the total insect dry weight (Hansen et al., 1985). Fry of both coho salmon and steelhead trout species require lipid energy content of 6-8 % of the total digestible energy found in their diet during freshwater residency (Higgs et al., 1995). Consequently, the energy requirements for juvenile salmonids can be met by sufficient numbers of the available freshwater macroinvertebrate larvae. There will be more on lipid requirements in a later section.

In food webs containing species that compete for similar food items, energy flow becomes more complex. For example, both coho and steelhead fry feed on drifting macroinvertebrates, although each one inhabits different freshwater niches. Coho fry rear in slower moving water mostly pools and low gradient side channel habitat, whereas steelhead fry prefer fast flowing, boulder reaches (Bond et al., 1988, Rosenfeld et al., 2000, Quinn, 2005). In a case such as this, where two species share a common resource

(macroinvertebrate drift) and the population sizes are limited by the availability of this resource, it can be expected that any increase in the availability of the resource will decrease the likelihood and intensity of the species' interaction (Danielson, 1991). Increasing phosphorous inputs into a stream, thereby increasing the availability of prey for juvenile salmonids, should lessen the potential for competition between species.

Mature salmon returning to spawn represent a nutrient and macro-molecule input into stream systems that juvenile residents can feed on and utilize directly. Carcasses provide a direct feed benefit for juvenile salmonids residing in stream reaches where spawning occurs (Wipfli et al., 1998). Bilby et al. (1998) studied the stomach contents of juvenile salmonids in two streams in southwestern Washington after adding salmon carcasses from a nearby hatchery. The results indicate that when carcasses were available in the stream, steelhead and coho fry fed preferentially on adult salmon flesh and eggs over macroinvertebrates.

To sum up the complex biotic and abiotic factors that influence the stream food web: autotrophic algae use the sun's energy, dissolved carbon dioxide and nutrients within the water column to synthesize complex bio-molecules such as lipids and proteins that in turn cycle through the upper trophic levels: from heterotrophic bacteria and protozoa, through the functional feeding groups of macroinvertebrates, finally to resident juvenile salmonids such as coho and steelhead. Another source of complex bio-molecules is the lateral transfer of biomass from spawned out salmon carcasses and deposited salmon eggs directly to macroinvertebrates and resident juvenile salmonids. From this food web interaction, it is apparent that the timing of occurrence of dissolved nutrients within the water column and the timing of the presence of organic nutrients and bio-

molecule (such as lipid) sources within the stream system will affect the response of the food web and the growth of the species of interest: juvenile coho salmon and steelhead trout.

2.3 Energy flow in a salmonid stream

As was discussed in detail in the previous section, stream primary production can be increased by increasing the available dissolved phosphorous, and there will be a subsequent increase in production at higher trophic levels. However, the flow of energy between plants and animals is highly variable and poorly understood (Brett and Muller-Navarra, 1997).

All living organisms need an energy store to provide fuel for the constant, essential biochemistry of life and to provide a source of energy that can be utilized when necessary for different physiological requirements, including the development of reproductive organs (Gurr et al., 2002, Jonsson and Jonsson, 1998). As a carnivorous fish, Pacific salmon species do not ingest large quantities of carbohydrates; instead, they obtain energy from dietary protein and lipids (Watanabe, 1982). The carbohydrate energy requirement is met by the protein and lipid levels found in prey species, and salmonids are able to form glucose through gluconeogenesis from dietary glucogenic amino acids, lactate and glycerol (Higgs et al., 1995). Optimal growth occurs when the relationship of digestible protein to non-protein energy (namely lipid) and overall digestible energy is such that there is sufficient dietary lipid energy to spare dietary protein for growth (Higgs et al., 1995). A major biological function of lipids is to provide a storage site for excess dietary energy. Beyond an energy storage site, lipids have other important biological

functions. Lipid derivatives act as structural cell membrane molecules and also function as precursors to hormones.

As well as affecting the quantity of primary production and available prey in the aquatic food web, nutrient availability also appears to affect the quality of food. In aquaculture, increasing the polyunsaturated fatty acid content in feed has been found to be critical for high feed conversion rates, which lead to high growth rates in juvenile organisms (Brett and Muller-Navarra, 1997). In the wild, essential fatty acids are not so easy to obtain and depend on the available prey.

2.3.1 Lipid concepts

Lipids are a class of chemical compounds that are grouped together based on the commonality of their insolubility in water and solubility in non-aqueous solvent. The term describes a wide ranging, heterogeneous group of fatty substances including fatty acids (Gurr et al., 2002).

Fatty acids are made up of a hydrocarbon chain with a terminal carboxylic acid group. They are classified as saturated or unsaturated, referring to the presence of carbon to carbon double bonds within the hydrocarbon chain. Saturated fatty acids have no double bonds, whereas monounsaturated fatty acids have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds.

Unsaturated fatty acids occur preferentially in the cis configuration, and have 120° bend in their carbon chain not present in straight-chain, saturated counterparts. The unsaturated, cis form is less thermodynamically stable than the saturated chain, and consequently has a lower melting point. This has important implications for membrane fluidity, particularly in fish, which are poikilotherms and therefore, must regulate

membrane fatty acid composition in response to changes in environmental temperature (Gurr et al., 2002, Greene and Selivonchick, 1987).

Fatty acid nomenclature indicates the number of carbons in the chain length and the number of double bonds, separated by a colon. The location of the double bonds can be indicated by a Δ (delta) followed by the position given in superscript, with C1 located at the carboxyl carbon. For example, an 18 carbon fatty acid with two double bonds at carbons 9 and 12 would be written: $18:2(\Delta^{9,12})$. Multiple double bonds are nearly always separated by a methylene group (eg. $-CH=CH-CH_2-CH=CH-$) (Nelson and Cox 2000). Another method of denoting the position of the double bonds is to designate the carbon farthest from the carboxylic end as the ω (omega) carbon and count the double bond methylene groups from the non-carboxylic end. The same fatty acid described above would then be labelled: C18:2ω-6. For the purpose of this publication, the older ω nomenclature will be used as the ω-3 and ω-6 families of fatty acids are of biological interest.

Lipids are made up of different fatty acids complexed to a variety of other compounds. Lipids can be grouped into two major classes based on whether they are neutral or polar compounds.

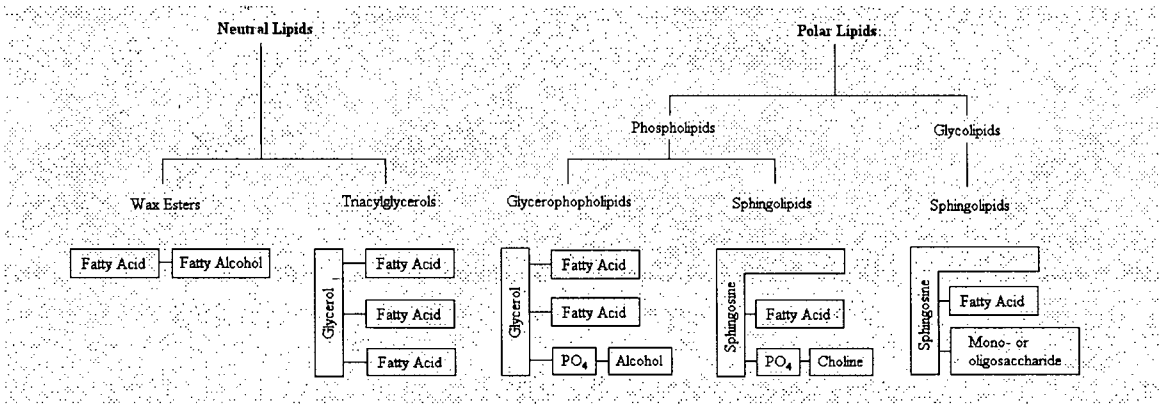


Figure 2.2: Major classes of neutral and polar lipids (based on Nelson and Cox 2000)

Neutral lipids generally act as energy storage sites, and polar lipids act as structural membrane molecules. A class of neutral lipids, triacylglycerols (TAG) are the major component of fat and oil, and represent a major storage site for energy. The oxidation of TAG produces over twice the amount of energy per weight basis than the oxidation of carbohydrates (Nelson and Cox, 2000). TAG consists of three fatty acids linked to a glycerol backbone through ester linkages. The individual fatty acids are not randomly distributed among the three positions, but exhibit a stereo-specificity (Gurr et al., 2002). In a review of available research, Henderson and Trocher (1987) concluded that in general, for wild fish, monounsaturates>saturates>polyunsaturates within TAG.

TAG is stored when the energy requirements of the organism are exceeded by the dietary energy available. Due to the hydrophobic nature of the lipid molecules, the storage of energy as TAG results in a very dense, compact, dehydrated form that provides more energy and less bulk than a glycogen counterpart (Gurr et al., 2002). The cells responsible for the storage of fat are the adipocytes. These cells can swell well beyond their original size depending on the amount of lipid available in the diet. When there is excess lipid supplied in the dietary intake, TAG is transported to these cells via

lipoproteins in the bloodstream. From these cells, TAG can be mobilized and transported out of the cell to fulfill energy requirements elsewhere within the organism.

Biological membranes exist as a double layer of lipids referred to as the lipid bi-layer. The polar lipids are arranged in sheets such that the hydrophobic hydrocarbon chains are oriented away from water. These structural lipids prevent the uncontrolled passage of polar molecules and ions across the cellular membrane. The numerous possible combinations of polar head groups and saturated and unsaturated fatty acid derivatives in the molecules create enormous diversity in the structural lipids present in the cell structure. For fish, membrane molecule composition changes as environmental temperature changes. As temperatures decrease, the phospholipids within the membrane are found to have decreased in saturation to increase membrane fluidity (Greene and Selivonchick, 1987, Henderson and Trocher, 1987). Fish modify phospholipids through a change in the acyl chain, the fatty acid or the phospholipids class (Greene, 1990).

2.3.2 Lipid biochemistry

Fatty acid biosynthesis can be divided into two classes of reactions: de novo and elongation reactions. During de novo reactions, a small 2C precursor is gradually elongated in 2C sections to give rise to 16C or 18C chains. The precursor, acetyl-CoA, is supplied by pyruvate metabolism in animals, and ultimately from photosynthesis in plants. The overall reaction in the synthesis of palmitate is:

$$CH_3CO\text{-}CoA + 7HOOCCH_2CO\text{-}CoA + 14NADPH + 14H^+ \Leftrightarrow$$

$$CH_3(CH_2)_{14}COOH + 7CO_2 + 8CoASH + 14NADP^+ + 6H_2O$$

During elongation reactions, long chain fatty acids are formed in reactions catalyzed by elongases, which lengthen fatty acids produced through the de novo reactions, or

originating in the organism's diet. Acyl-CoA is involved as a primer, malonyl-CoA is the 2C unit donor and NADPH is the reducing coenzyme. An important function of elongases is the transformation of essential dietary fatty acids to longer chain PUFA.

Biosynthesis of unsaturated fatty acids is done by oxidative desaturation. Double bonds are inserted directly into the precursor, preformed fatty acid, with O_2 and NADH as cofactors. Plants introduce the double bond between the existing double bond and the terminal methyl group, and animals insert the double bond between the existing double bond and the carboxyl group. For plants the precursor to higher PUFA is oleate (C18:1ω-9). This is used to form linoleate (C18:2ω-6) which is used to form linolenate (C18:3ω-3). Oleic acid gives rise to the ω-9, linoleic acid to the ω-6 and linolenic acid to the ω-3 families of fatty acids. Like most animals, fish are not able to synthesize linoleic or linolenic acids and must obtain these fatty acids from dietary sources. They are able to desaturate and elongate the ω-9, ω-6, and ω-3 families (Greene 1990, Henderson and Trocher 1987, Watanabe 1982).

2.3.3 Dietary lipids and essential fatty acids

Dietary lipids provide two major functions: they act as an energy source and a source of essential fatty acids. The consumption of high energy fatty acids provides a readily available source of fuel for the biochemistry of life. TAG provides a major source of metabolic energy through β-oxidation of the constituent fatty acids.

Essential fatty acids (EFA) are those fatty acids that cannot be synthesized by the animal directly, and must be obtained from the diet. Linoleic and α- Linolenic acids must be supplied by the diet, and once inside the cells, may be further elongated and desaturated into longer chain PUFA. Autotrophic organisms such as plants and algae are

able to synthesize EFA using the carbon available from photosynthesis, and these complex macromolecules transfer through a food web to higher trophic levels. EFA provide two distinct roles within an organism. PUFA are major constituents of structural lipids; therefore, a deficiency of EFA in the diet creates changes in cell membrane permeability and food conversion efficiency (Gurr et al., 2002). Another very important biological role of EFA is to act as the precursors to eicosanoids. Eicosanoids are important regulators of many cellular and tissue functions including the growth hormone, blood flow, respiration and biological signalling within the organism (Nelson and Cox, 2000). Eicosanoids are produced locally in a series of reactions that are catalyzed by specific phospholipases that remove the precursor PUFA from the membrane phospholipids and transfer them to the enzymes of eicosanoid biosynthesis. These PUFA must then be replaced in the membrane by new fatty acids of dietary origin.

2.3.4 Implications for lipids in salmonid food webs

The PUFA that are necessary in the diets of higher organisms originate through biosynthesis and storage in algal cells. Green algae and diatoms are important producers of PUFA, including essential linoleic and α-linolenic (Napolitano, 1999). Accumulation of fatty acids occurs in algal phospholipids, glycolipids and TAG, and the fatty acid profiles of the different populations of algae depend heavily on the environmental conditions under which they are grown (Napolitano, 1999, Brett and Muller-Navarra, 1997, Reitan et al., 1994, Harrison et al., 1990, Ben-Amotz et al., 1985).

Nutrient availability is a factor that affects the growth and storage of fatty acids in algae. Increasing the total lipid and in particular, the PUFA content of algae will result in a higher food quality for subsequent consumers (Brett and Muller-Navarra, 1997). Reitan

et al. (1994) found in marine algae that the percentage of total PUFA decreases and the percentage of saturated and monounsaturated fatty acids increases as nutrient limitations increase. This difference is due mostly to the changes in ω-3 contents. The study performed by Ben-Amotz et al. (1985) with freshwater algae showed that a decrease in nutrient availability caused an increase in percent lipid storage but a decrease in growth rate. Harrison et al. (1990) found that phosphorous starvation exhibited a species specific response. *Isochrysis* and *Chaetoceros* showed no change in C20:5ω-3 (EPA), an important PUFA in the ω-3 family. In contrast, phosphorous-starved *Thalassiosira* showed a marked decrease in EPA, which is not entirely surprising given that these long chain PUFA are found most commonly in the membrane phospholipids.

Bio-molecules that have been synthesized by algae are transferred through the food web. Lipids provide a storage site for EFA in the phospholipids and energy in the TAG. Juvenile salmonids need PUFA from the ω-3 and ω-6 families. Like most higher order species, salmonids cannot synthesize PUFA de novo. In particular, dietary C18:2ω-6 and C18:3ω-3 are elongated and desaturated to longer chain C20:4ω-6, C20:5ω-3 (EPA) and C22:6ω-3 (DHA). Although salmonids can convert linolenic acid (C18:3ω-3) to EPA and DHA, the conversion appears to be inefficient and juveniles will grow better when provided with a direct source of EPA and DHA (Brett and Muller-Navarra, 1997).

Fatty acids are incorporated into fish tissues that serve a variety of functions within the organism. Changes in fatty acid composition of the diet have a varying effect on the tissues of the fish. Fatty acid profiles will depend on the tissue of interest, the age of the fish and its environment. Mjaatvatten et al. (1998) noted that the fatty acid profile of the brain tissue remains essentially unchanged after the first year of life, regardless of

changes in diet. This suggests that fish have a mechanism to guard against changes in the more specialized tissues. Typically, storage lipids (i.e. TAG) will most likely show the greatest response to variations in dietary fatty acids. In salmonids, it has been observed that muscle tissue acts as an important lipid storage site and, as a result, changes significantly with changes in the diet and physiology of the fish (Mjaatvatten et al., 1998).

Life history stages change the composition of fish fatty acids and the interaction between protein growth and fatty acid storage. In brown trout, typically, as the age of the fish increases, the amount of fatty acids stored as lipids also increases (Jonsson and Jonsson, 1998). Initially, surplus fatty acids consumed are allocated to the protein synthesis and growth of the fish. As the age of the individual increases, fat is stored to combat periods of starvation and to provide energy for physiological processes such as smoltification. The results from the study performed by Jonsson and Jonsson (1998) also suggest that in mature anadromous versus freshwater individuals, the anadromous fish had higher lipid stores in the somatic tissue.

Post and Parkinson (2001) investigated the allocation of energy between growth and lipid storage in rainbow trout reared in lakes in south-central British Columbia. The results of the research suggest that higher growth rates in juvenile salmonids were linked to higher percentage of lipids stored in body tissues. Slower growing fish allocate excess energy to promote somatic growth at the expense of lipid storage. This appears logical, as smaller, slower growing individuals are more susceptible to size-dependant mortality during the growing season, and a small increase in size leads to a large increase in

survival. Once winter food shortages become prevalent, the smaller fish with smaller lipid stores are then more at risk of starvation (Adams, 1999).

For fish in colder climates, such as Pacific salmon that experience a shorter summer growing period and a marked period of food scarcity during winter months, over-winter survival is an important constriction in freshwater survival (Olsen, 1998). Over-winter survival is size dependant. Smaller fish experience relatively higher metabolic rates and smaller lipid stores to utilize during periods of starvation (Adams, 1999). As a result, they use proportionally more of their lipid stores during the winter and have the potential to deplete their resources beyond the viable limit. Mortality from winter starvation becomes more important at higher latitudes as the length of the growing season decreases (Adams, 1998).

2.4 Phosphorous addition: when and how to add phosphorous for maximum benefits for mixed species of juvenile salmonids

From food web theory, the active addition of phosphorous, either in inorganic or organic form, to phosphorous limited streams will increase the biofilm and macroinvertebrate production. This in turn will increase the food availability to resident juvenile salmonids. Benefits from enhanced food availability and quality can include an increase in growth rate and lipid storage. This contributes to higher over-winter survival, increases in size-at-smolt and the potential for improved ocean survival.

An important benefit of fertilization to juvenile salmonids is an increase in growth rates. Growth occurs when dietary energy intake is in excess of the fishes' expenditures on standard metabolism and normal spontaneous movement (Higgs et al., 1995). The

rearing temperature and the size of the fish are major factors affecting standard metabolism and growth. Chemical reaction rate is influenced by the temperature under which the reaction is taking place. An increase in temperature within the acceptable temperature/growth range, generally manifests as an increase in reaction rate. Since fish are poikilotherms, the environmental temperature dictates the rate of biochemical reactions and increasing environmental temperature increases standard metabolism expenditures and the rate at which new tissue can be synthesized (growth).

Sullivan et al. (2000) developed a bioenergetics model to describe the gain in weight during the summer growth period for age-0 coho and steelhead in natural stream systems based on the average daily temperature and species specific food consumption capacities. Based on the predictive model and field data obtained for age-0 coho in a Pacific Northwest stream (Sullivan et al., 2000) apparent food consumption was below the maximum predicted level (consumption for feeding to satiation) for most of the summer growing period with a sharp difference in the later summer time period. The difference in these values indicates that there is a food limitation within this system. In a stream such as this, an examination of the nutrient levels, biofilm and macroinvertebrate production, would help to identify if fertilization is a viable option for increasing the food availability. This could potentially have a large impact on the growth rate of resident juvenile coho. In the same system during the same time period, data on cutthroat trout were used as a surrogate for steelhead and it appeared as though the trout were feeding at near maximum potential consumption. The authors contend that this may be due to the fact that cutthroat (and steelhead) emerge later than coho and are smaller in later summer,

so satisfying the maximum consumption rate based on grams of prey per gram of body mass per day is less demanding (Sullivan et al., 2000).

As was discussed in the previous section, another benefit for juvenile resident salmonids with an increased growth rate is an increase in the storage of lipid to ward against periods of food scarcity and in preparation for the drop in stream temperatures during the winter (Adams, 1998, Jonsson and Jonsson, 1998, Post and Parkinson, 2001).

Results from experiments using inorganic fertilization in the summer growing period or carcass placement to mimic a returning salmon run, indicate steelhead and coho both benefited despite the different freshwater habitat niches that these two species inhabit. Wilson et al. (2003) found that active addition of inorganic fertilizer during the summer growing period in two oligotrophic streams in south coastal British Columbia increased the density of resident rainbow (*O. mykiss*) and size and condition of young-of-the-year juvenile steelhead (*O. mykiss*). The use of inorganic fertilization during the summer growth period in combination with restorative stream habitat structures placed in the Keogh River in south coastal British Columbia showed an increase in steelhead parr densities and size-at-age of steelhead juveniles in autumn, even over habitat structures or enrichment used exclusively (Ward et al., 2003). The work on the Keogh River also demonstrated an increase in steelhead and coho smolt yield during rehabilitation treatments. Bilby et al. (1998) found that both young-of-the-year coho and steelhead densities increased in two streams in southwestern Washington following the addition of salmon carcasses. During the period where carcass material was available to resident juvenile salmonids, the condition factor of both species also showed an increase over a carcass-free control. Research conducted using juvenile coho salmon in constructed

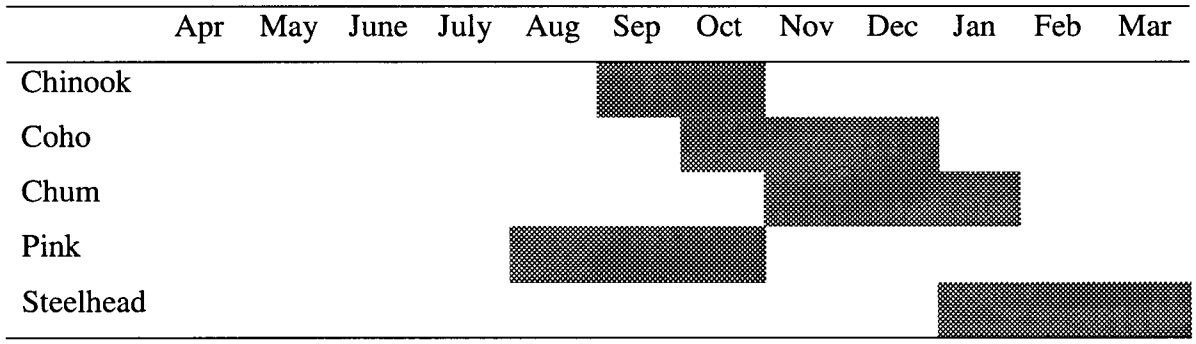
stream mesocosms in southeastern Alaska suggests that using carcass enrichment or carcass analogs will increase the condition factor, production and percentage of lipid of residents (Wipfli et al., 2004). In summary from literature, using the two fertilization schemes (inorganic in the summer or organic in the fall) appears to benefit both coho and steelhead to varying degrees. It remains to be seen which scheme will maximize the benefit to both species. The following case study will be used to elucidate the importance of form and timings of phosphorous inputs.

2.5 Case study: managing phosphorous levels in a coho salmon and steelhead trout stream in the Pacific Northwest with historical inputs of pink, chum and Chinook salmon spawning runs

Historically, in the Pacific Northwest, the return of spawning salmon predominantly in the fall marks a large influx of nutrients and complex bio-molecules to the system. Even during historical runs, the returns of Pacific salmon exhibit dominant years, depending on the area of interest, brood year and survival from emergence to sexual maturity. To narrow the area of interest, if we look at the province of British Columbia, according to Gresh et al. (2000) the historical input of salmon biomass returning to spawn is estimated to range from 122,940,000 kg to 263,442,000 kg. Using a wet weight basis, the phosphorous input from these historical runs can be estimated at 430,290 kg to 922,047 kg annually (0.35 % P from Larkin and Slaney, 1997). Currently the estimated return of Pacific salmon to the province of British Columbia is 59,312,000 kg, or 207,592 kg of phosphorous.

Spawning salmon return to the streams throughout the fall, beginning in late summer and continuing through the winter. For a system of off-channel habitat such as the Chilliwack River, a tributary of the Fraser River on the south coast of British Columbia that supports populations of chinook (*O. tshawytscha*), coho (*O. kisutch*), chum (*O. keta*), pink (*O. gorbuscha*) salmon and steelhead trout (*O. mykiss*), the timing of spawning within the watershed could be expected to extend from July through the end of March. The following table describes the theoretical peak returns for the Chilliwack River system as reviewed in Pacific Salmon Life Histories (Ed. Groot and Margolis, 2003) and based on the Fisheries and Oceans Canada, Pacific Scientific Advice Review Committee, Stock Status Reports.

Table 2.1: Timing of peak salmon spawning abundance in a south coastal British Columbian watershed



In the case of the Chilliwack River system, with an odd-year dominant pink salmon run and a vibrant chum and coho return, the input of spawning salmon is most prevalent from late August through the end of December.

The high historical annual input of phosphorous from spawning Pacific salmon had the potential to influence dissolved inorganic phosphorous levels in the stream systems through the spring and summer growing period. Chaloner et al. (2002) conducted a shorter term study of the decomposition of pink salmon (*O. gorbuscha*) in artificial and

natural streams in south-eastern Alaska. The mass loss from the carcasses was estimated using a single exponential decomposition model. Based on the results from the study, the percentage of mass remaining is described as follows:

$$y=87.16e^{-0.033t}$$

(where y is the percentage of carcass mass remaining, and t is the time in days that the carcass has been in the stream.) Based on this model, less than 0.03% of the carcass material would be present in the stream during the summer growth period. However, this decomposition rate was determined during a short-term study and could potentially over-estimate the rate of decomposition of recalcitrant elements such as magnesium and phosphorous, which are bound primarily in bone and scale tissues. Parmenter and Lamarra (1991) investigated the decomposition of steelhead trout (*O. mykiss*) in a freshwater marsh. The study was conducted from July through the winter and subsequent freezing of the marsh to the spring thaw the following May. The decomposition rate was determined using a double-exponential decomposition where the first term describes the rapid decomposition of the flesh and internal organs, and the second term describes the slower decomposition of the recalcitrant minerals such as the phosphorous-rich bone material. The fraction of mass remaining is described as follows:

$$y=0.74e^{-0.061t}+0.26e^{-0.002t}$$

(where y is the fraction of carcass mass remaining and t is the number of days the carcass has been in the system.) If we assume that peak spawning occurs in September/October, and the summer growth period starts at the decline of the hydrograph in June, there are approximately 240 days for the carcass to decompose. Based on the decomposition model described above, this leaves 15 % of the carcass material still available in the streams at

the beginning of the summer growth period. During the study conducted by Parmenter and Lamarra, between July and May, only 60 % of the carcass phosphorous had decomposed and the remaining 40 % was contained in the bone and scale fraction of the remaining carcass. It could be assumed that of the 15 % of carcass material theoretically left in the streams at the start of the summer growing period, a large portion would be bone material, which is a source of slow leaching phosphorous.

Phosphorous from carcass material can be retained within the stream system in the biomass of algae and macroinvertebrates (Richey et al., 1975). Stream periphyton have cellular growth rates that can be measured in hours and algal biomass will show a response to changes in limiting nutrient conditions, in this case phosphorous, within a very short period of time (approximately 2-3 days) (Bothwell, 1989, Harrison et al., 1990). Macroinvertebrates will also act as a nutrient retention site for marine derived nutrients as they feed directly on the carcass material, or benefit from the increase in biofilm productivity. Shredder and collector macroinvertebrate growth rates, abundance and standing biomass have been shown to increase in the presence of decomposing salmon carcasses (Chaloner et al., 2002, Chaloner and Wipfli, 2002). The increase in growth rates and the response to increases in nutrient availability as standing biomass of macroinvertebrates can be detected in 3-4 weeks (Chaloner and Wipfli, 2002).

The response of resident juvenile salmonids to inputs of salmon carcasses is similar to both macroinvertebrates and algae in that instantaneous growth rates increase and standing biomass increases (Wipfli et al., 2003, Wipfli et al., 2004). Experiments conducted to investigate the response of juvenile salmonids to increases in nutrients and

macromolecules are conducted over longer time periods (approximately 2 months) to allow sufficient time for changes in growth to become apparent.

Another potential retention mechanism for spawning salmon nutrients within a continuously flushing stream system is nutrient cycling in the hyporheic zone. O'Keefe and Edwards (2003) investigated the possibility that the hyporheic zone of groundwater could act as a possible transient storage site for marine derived nutrients. The results of the study suggest that during spawning activity in nearby stream channels, the transfer of marine derived soluble reactive phosphate to the riparian vegetation occurs readily along the flow path within the hyporheic groundwater zone. The authors identify a need to investigate further how long phosphorous is stored within the hyporheic zone and how long it takes to travel the length of the flow path and re-enter the stream system. They predict that in years where there are sufficient numbers of spawning salmon such that their consumption by terrestrial and aquatic consumers is saturated, many salmon will decompose in the stream and the hyporheic transfer and subsequent storage may be an ecologically significant storage of marine derived nitrogen and phosphorous.

Besides spawning salmon in the fall; spring snow melt, runoff, and freshet events represent a vector for the transportation of phosphorous entering streams in sediment and debris from riparian zones. The following figure, Figure 2.2, shows the daily average discharge in cubic meters per second (cms) from the Chilliwack River from Environment Canada's gauging station above Slesse Creek 1963-2002. From the figure, it can be seen that the melting of the snow pack causes a swell in the hydrograph during May and June. During the initial annual increase in flow within the system, sediment load and turbidity increases, and the level of dissolved and particulate phosphorous within the water column

increases as the river is flushed. Because this initially increases the turbidity, which decreases the ultra-violet penetration during a time of relatively low water temperatures, primary productivity is low and this phosphorous is transported from the system without being incorporated into the food web. As the temperatures and light levels increase, primary productivity increases and phosphorous in the water column is incorporated into the complex bio-molecules at the autotrophic level of the food web.

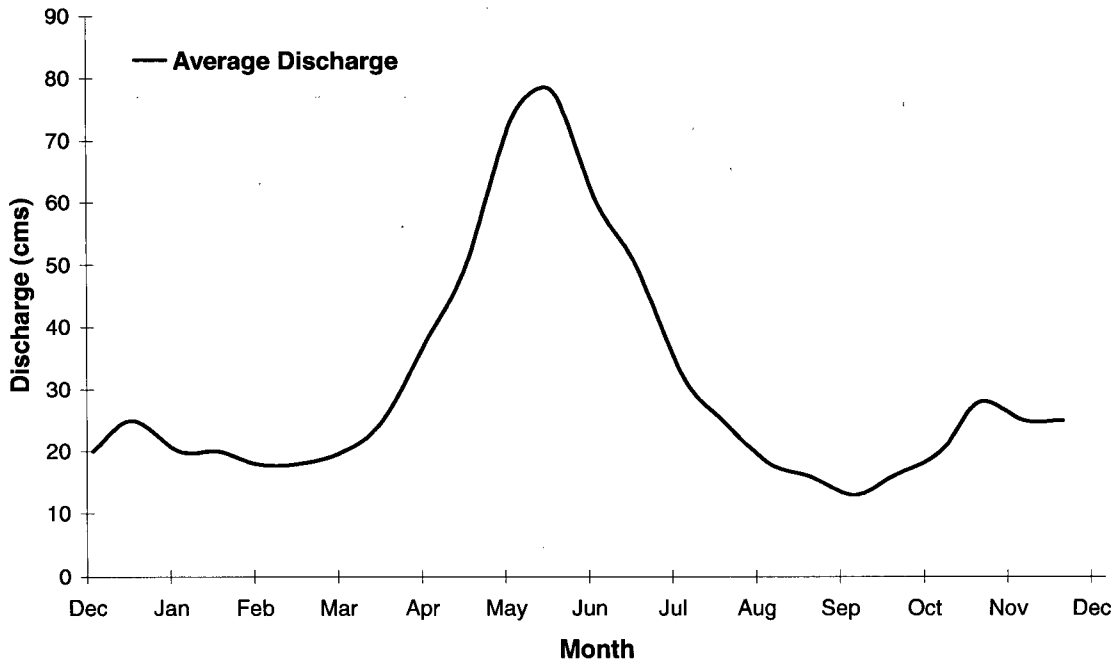


Figure 2.3: Average daily discharge 1963-2002 in cubic metres per second (cms) from Environment Canada gauging station on Chilliwack River above Slesse Creek

Given this hydrograph and the associated increases and decreases in phosphorous associated with increased sediment load and flushing, as well as the input of salmon carcasses described in the earlier table, one could predict that the historical average daily levels of phosphorous in a south coastal stream system similar to the Chilliwack River in British Columbia (which supports a viable spawning return of pink, coho, and chum

salmon) would approximate Figure 2.3. A large peak of total phosphorous would occur in the fall, corresponding with the return and subsequent death of the spawning Pacific salmon. During the spring freshet, phosphorous would be transported through the system, but a residual nutrient shadow of the Pacific salmon carcasses would occur through the summer growing season.

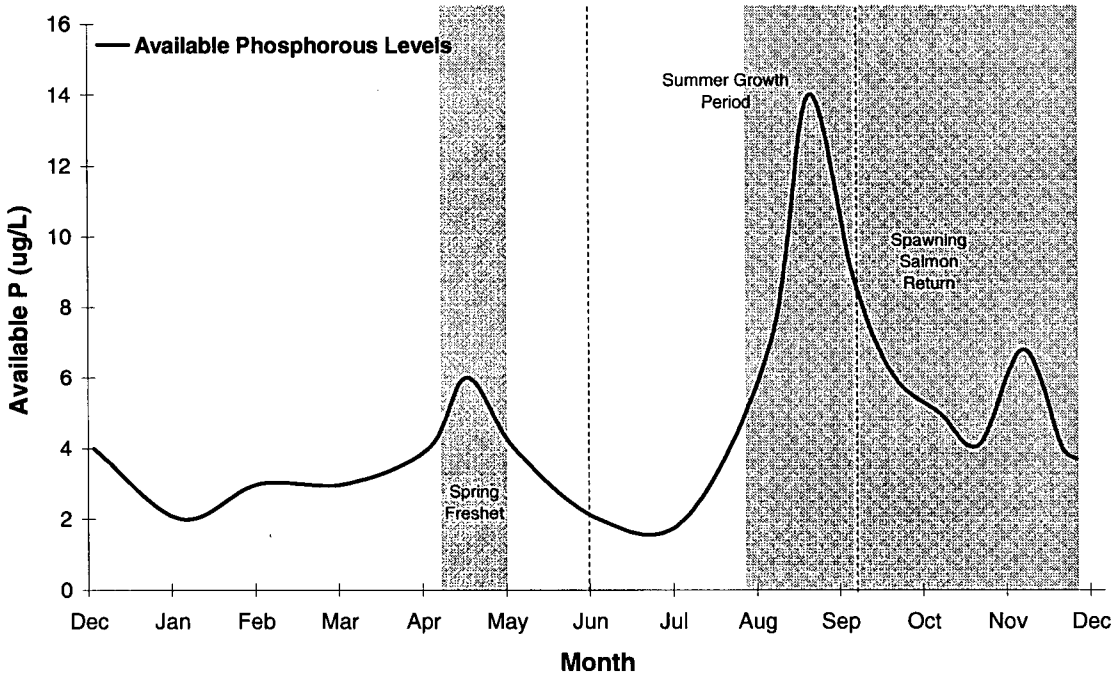


Figure 2.4: Theoretical historical average daily phosphorous levels of a salmon supporting stream in south coastal British Columbia. Based on an historical run of pink salmon in the Chilliwack River (see Appendix A for more details)

With a decrease in the return of spawning salmon to Pacific coastal watersheds, the peak of total phosphorous in the stream system will decrease and the influence of carcass phosphorous during the summer growing period will decline. Figure 2.4 illustrates the decline in available phosphorous in a stream system with a low returning population of chum and coho salmon. The contrast between the two figures illustrates the

deficit that needs to be overcome to return coastal streams to historical levels of productivity and increase juvenile salmon production in this important juvenile habitat.

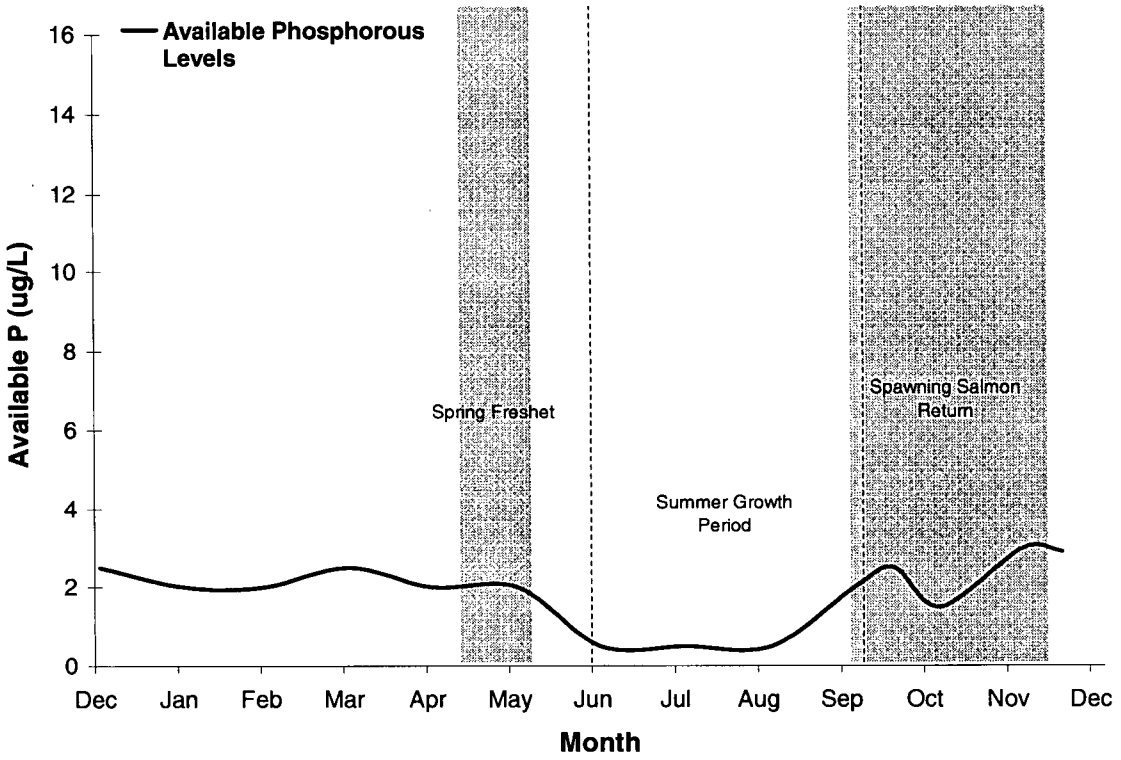


Figure 2.5: Current average daily phosphorous levels of a salmon supporting stream in south coastal British Columbia with low returns of chum, chinook, coho and steelhead based on the Chilliwack River (British Columbia Ministry of Environment unpublished data)

There are four possible options for phosphorous nutrient status in streams: Nil, Inorganic, Organic and Inorganic/Organic. There may be little or no organic or dissolved inorganic phosphorous present (<1µg/L of soluble reactive phosphate), such as a system characterized by having no spawning salmon present, a granitic bedrock and high precipitation (Nil). A system may have dissolved inorganic phosphorous present in the water column from sediment, a naturally occurring deposit of mineral phosphorous such as volcanic rock, or the spawning and decomposition of salmon upstream (Inorg). With a small return of spawning salmon or an input of organic material from riparian zones, a

stream may experience a scheme where there is a localized organic phosphorous containing solid present within the stream system (Org). For a system with a large return of salmon there may be organic solid containing phosphorous within the system as well as dissolved inorganic phosphorous present through the decomposition of the organic material (Inorg+Org). Based on those four possible phosphorous conditions and considering two times of interest for phosphorous status (summer growing period and fall spawning period), there are 16 potential combinations for fertilization options. The following table shows all possible combinations:

Table 2.2: Possible phosphorous status during the summer growing period and the fall spawning period (Org: Organic phosphorous, Inorg: Inorganic phosphorous)

Summer	Fall
Nil	Nil
Nil	Org
Nil	Inorg
Nil	Inorg+Org
Inorg	Nil
Inorg	Inorg
Inorg	Org
Inorg	Inorg+Org
Org	Nil
Org	Inorg
Org	Org
Org	Inorg+Org
Inorg+Org	Nil
Inorg+Org	Inorg
Inorg+Org	Org
Inorg+Org	Inorg+Org

Based on the figures shown previously that illustrate the discrepancy between the historical levels of phosphorous and the current available phosphorous within stream watersheds and the 16 possible combinations that exist for the two timings of interest, fertilizer treatments can be created that best mimic the natural inputs of phosphorous to a

stream system. For a watershed such as the Chilliwack River, where the system historically supported large runs of Pacific salmon including a spring run of steelhead trout, it could be theorized that the ideal scheme to try and emulate for fertilizer addition would be an Inorg+Org scheme through both the summer and the fall, or an Inorg summer and Org fall. According to food web theory, both of these schemes address the need for inorganic dissolved phosphorous in the water column during the summer growth period such that complex bio-molecules will be synthesized at the autotrophic level and have sufficient time to transfer through the trophic levels to be consumed by coho and steelhead juveniles before the stream temperatures drop and growth slows over winter. Both schemes also address the importance of having an organic source of nutrients in the fall with all of the associated complex bio-molecules available for direct transfer to the upper trophic levels, including juvenile coho and steelhead in the period before the temperatures drop over winter. The question of whether the Inorg+Org scheme for both the summer and fall, where phosphorous is available in both dissolved and organic form through the entire growing and spawning season, produces the greatest benefit for resident juveniles, or if the Inorg summer and Org fall nutrient scheme is sufficient will need to be addressed in future research in order to identify where fertilization activities should be focused to produce the best results with limited stream rehabilitation funds.

2.6 Conclusion

With the decline in returning spawning Pacific salmon to coastal watersheds and the increasing discrepancy between historical nutrient levels and the current nutrient status, nutrient replacement strategies for coastal streams in the Pacific Northwest are

garnering considerable attention as an effective way to rehabilitate stream habitats. Active nutrient addition schemes that mimic the historical, natural nutrient status have the potential to beneficially impact resident freshwater fish populations, including resident juvenile salmonids such as coho salmon and steelhead trout. Food web theory shows that increasing dissolved nutrient levels and autotrophic productivity in the summer growth period will subsequently increase food availability and quality to resident juvenile salmonids, as will increasing the availability of organic carcass material in the fall. Questions still exist as to which of the potential stream fertilization schemes will provide the maximum benefit to multiple species; these questions must be answered in future field studies.

Chapter 3: Effect of Artificial Summer and Fall Additions of Phosphorous on Fry Growth, Lipid Content and Biomass in Artificial Stream Channels Containing Coho Salmon and Steelhead Trout in South Coastal British Columbia

I: Fertilization during a year with a heavy fall salmon run that followed a year with a small fall salmon spawning run

Summary: Salmonid populations have declined in most of the world. In some cases, early in-stream rearing conditions are poor due to anthropogenic impacts. In the case of low nutrient conditions, in-stream fertilization has been carried out and found to increase fish yields. The effect of timings and type of phosphorous inputs on fish production in a phosphorous limited stream is not clear, but in general it can be said that an imitation of the historical in-stream nutrient inputs and fish rearing conditions would be the most natural and possibly the most beneficial for fish.

Herein are the results of a set of experiments designed to imitate different natural phosphorous inputs and compare the resulting after-winter fry biomass and growth. The three artificial phosphorous inputs are: 1) slow release inorganic phosphorous fertilizer throughout the summer, 2) slow release organic phosphorous fertilizer in the fall, and 3) no artificial phosphorous fertilizer addition. The background SRP level in the stream system was < 1 µg/L (considered nil) during the summer and > 4 µg/L (not considered to be limiting) in the fall after a large salmon run upstream. The fall salmon run provided SRP and possibly a small amount of debris containing organic phosphorous. The three treatments are 1: **Inorg**/Inorg, 2: Nil/Inorg+**Org**, and 3: Nil/Inorg, where the bold type indicates fertilizer inputs and regular type indicates the background conditions existing in the stream channels.

In addition to biomass and growth, fish lipid levels, algal standing biomass as chlorophyll a, benthic macroinvertebrate biomass and water chemistry were monitored to discern the overlaying casual mechanisms that affect fry biomass and growth.

After winter, steelhead trout (*Oncorhynchus mykiss*) in treatment 1 (**Inorg**/Inorg) and 2 (Nil/Inorg+**Org**) were longer and had a greater weight than those in treatment 3 (Nil/Inorg) ($p<0.0001$). Coho salmon (*O. kisutch*) showed no difference in size after winter for any of the three treatments ($p=0.5010$).

The age-0 steelhead in treatment 1 are significantly larger than those steelhead in treatments 2 or 3 after the summer growth period ($p<0.0001$). This is supported by the significantly greater standing biomass of both algae (expressed as chlorophyll a) and benthic macroinvertebrates during summer sampling events.

The average weight of age-0 steelhead increased significantly over the summer growing period in all treatments. During the fall, only the steelhead from treatment 2 showed a significant increase in percent lipid stores over winter ($p=0.0005$). These results suggest that having a fall source of organic phosphorous available within a non-limiting phosphorous system provides for a direct feed mechanism for salmonids.

In summary having both inorganic phosphorous available for a longer period of time (summer through fall) and having an organic source of nutrients available in combination with inorganic dissolved phosphorous in the fall will produce similar measurable increases in length and weight accumulation in juvenile steelhead over-winter, as compared to having inorganic nutrients only in the fall. Increases in nutrient availability did not appear to produce the same benefits for coho.

3.1 Introduction

From the turn of the nineteenth century, Pacific salmon (*Oncorhynchus* species) have been an important economic resource for coastal British Columbia. More recently, the ecological importance of these fish has been recognized as well. As fewer adult salmon return to coastal streams to spawn and die, there is a decrease in marine derived nutrients within the system (Larkin and Slaney, 1997). With fewer nutrients available and a decrease in the productivity of stream ecosystems, the emerging fry and resident juvenile salmon have a decreased access to food.

There are numerous possible natural inputs of phosphorous into a stream system. These include dissolved phosphorous in groundwater, leaf and terrestrial macroinvertebrate input, sediment transfer, excretions from resident fish populations, and returning anadromous fish spawners (Stockner et al., 2000). Due to the underlying geology of the region and high annual precipitation, coastal streams in British Columbia and in the greater Pacific Northwest are characterized as oligotrophic (nutrient poor). The annual return of spawning Pacific salmon in the fall provides a large influx of phosphorous, nitrogen, and many other biological macro- and micro-molecules to these systems. Marine phosphorous is held within the streams as biomass of algae, macroinvertebrates, and resident fish populations.

As was discussed in the previous chapter, there are four possible options for phosphorous nutrient status in streams. There may be no detectable organic or dissolved inorganic phosphorous present (Nil), as is the case in most oligotrophic streams. Dissolved inorganic phosphorous may be present in the water column (Inorg) due to

inputs from groundwater or a spring nutrient shadow of a large spawning salmon run that occurred the previous fall. Organic solid containing phosphorous may be present within the stream system (Org), such as is present during small fall spawning events. Finally, there may be organic solid containing phosphorous within the system as well as a dissolved inorganic phosphorous present (Inorg+Org), which is typically found during large spawning events. Based on those four possible phosphorous conditions and considering two potential times for phosphorous input (summer growing period and fall spawning period), there are sixteen potential combinations for treatment options (as is shown in Table 2.2).

Fertilization schemes to manage the deficit between historical and current levels of nutrients available in streams have received considerable attention in recent decades (Bilby et al., 1998, Heintz et al., 2004, Johnston et al., 1990, Slaney et al., 2003, Wilson et al., 2003, Wipfli et al., 2003, Wipfli et al., 2004). However, the current state of practice and of research does not address the issue of which nutrient replacement strategies, as outlined in Table 2.2 will produce the largest benefits to the resident fish populations. Following the reasoning developed in the previous chapter, treatment schemes to be used for nutrient replacement activities should be based on stream food web theory and the historical inputs of phosphorous to the system in question.

Currently, organic and inorganic fertilizers are used to increase productivity within nutrient poor streams. Firstly, beginning in the early 1980s, there has been considerable research in British Columbia investigating the short term and long term response of streams and lakes to the addition of inorganic fertilizer during the summer growing season. This strategy has been shown to increase primary production and

stimulate the synthesis of complex, biologically important macro- and micro-molecules within the aquatic food web, thereby increasing food availability and juvenile salmonid size and smolt production (Johnston et al., 1990, Slaney et al., 2003, Wilson et al., 2003). Secondly, in other areas, the research focus has been on adding organic material (fertilizer) - often salmon carcasses from near-by watersheds, ground and pelletized hatchery spawned carcasses, or Pollock bone meal (this study)- in the fall to mimic the natural input of nutrients and biological molecules from spawning salmon in order to investigate the effects on juvenile salmonid size, diet and lipid stores (Bilby et al., 1998, Heintz et al., 2004, Wipfli et al., 2003, Wipfli et al., 2004).

The objective of this study was to investigate the effects of timing and source of phosphorous inputs into the stream systems and the response of juvenile salmon length, weight, and lipid stores (indicating energy transfer). Not all possible natural sources of phosphorous inputs were examined due to a limited number of research channels.

The three treatments chosen for this experimental plan were devised to consider naturally occurring fluctuations in nutrient levels in the Chilliwack River system and available constructed stream channels (to be described in detail later). As well the experimental plan was developed to represent both inorganic and organic fertilization schemes (as discussed previously). The controlled system in a natural setting permitted a near imitation of indigenous phosphorous inputs and control of some key variables. The habitat available at the research site mimics the complex natural habitats found in streams throughout the Pacific Northwest, with native substrate and riparian vegetation, pool/riffle sequences, and sheltering overhang. The site receives water from a nearby off-channel habitat of the Chilliwack River with naturally occurring phosphorous loads. This

research focused on three phosphorous inputs that would yield different in-stream phosphorous conditions, namely: 1) inorganic phosphorous present in the summer and fall, 2) no phosphorous present in the summer and inorganic and organic phosphorous present in the fall, and 3) no phosphorous present in the summer and inorganic phosphorous present in the fall.

Two resident species of juvenile Pacific salmon coho salmon (*O. kisutch*) and steelhead trout (*O. mykiss*), were the test organisms. They introduced another level of complexity because of the potential for multi-species interaction and different reactions to changing phosphorous inputs.

It was hypothesized that changes in phosphorous timing and source (either inorganic or organic) will affect resident fish size, growth, and lipid stores. It is unclear whether inorganic inputs of phosphorous will have sufficient time to cycle through the food web and influence fish populations when compared to the direct input of organic phosphorous containing material available for fish consumption. It is also unclear whether multi-species interactions will cause differing responses in coho and steelhead based on the changing nutrient status in streams. The response of lower trophic levels of the stream food web (algae and macroinvertebrates) and the fatty acid profiles of the salmonids are investigated and used to explain the underlying mechanisms influencing the primary variables of fish length, weight, and lipid stores.

3.2 Materials and methods

3.2.1 Experimental plan

The three phosphorous treatments were carried out simultaneously and in triplicate starting June 2003 and ending April 2004. During this time, fish were stocked, and their growth and numbers monitored through electrofishing events in June, September, and November 2003 and finally in April 2004. Water chemistry and temperature was monitored every two weeks from June to November 2003. Periphyton was sampled every 5-7 days during fertilization activities (June-August and September-November). Benthic macroinvertebrates were sampled in single sampling events in August and November to coincide with fish sampling events. As well, fish lipid levels from a random sampling of fish removed during the electrofishing events were analyzed. See Table 3.1 for timing details on sampling activities and fertilizer additions. The primary variables of interest were fish length, weight, lipid content, growth and total biomass in each treatment over-winter. Secondary variables of algal biomass as chlorophyll a, macroinvertebrate biomass per treatment, and fish fatty acid profiles are used to explain and verify results. Following are the details pertaining to the site, phosphorous input, stocking, monitoring, and statistical plan.

Table 3.1: Timeline of fertilization applications and sampling activities conducted at the Centennial Channel, research channels 2003-2004

Activity	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Inorganic fertilizer added		x										
Organic fertilizer added					x							
Coho stocked	x		–									
Steelhead stocked		x										
Water chem. (14 days)		x	x	x	x	x	x					
Water chem. (monthly)								x	x	x	x	x
Chloro a (5-7 days)		x	x	x	x	x	x					
Replace chloro a plates					x							
Macroinvertebrate				x			x					
Electrofishing	x				x		x					x
Spawning upstream					x	x	x	x				

3.2.2 Site description

The research facility (site) was located adjacent to the Centennial channel, an off-channel habitat of the Chilliwack River. The site consisted of nine identical, parallel, constructed stream channels. Each constructed channel was approximately 80 m long and 1 m wide and had 24 riffles (depth: 0.07 m) and 23 pools (depth: 0.62 m) alternating along its length. This represents good habitat for both steelhead and coho with coho preferring slow moving pools and steelhead being found mostly in higher gradient riffle habitat (Bond et al., 1988, Rosenfeld et al., 2000, Quinn, 2005).

Water diverted from the Chilliwack River into the Centennial channel was controlled via two weir gates located at the entrance to the off-channel habitat. This protected the system from gross variations in stream flow. Water was diverted from the Centennial channel upstream of the site via an inlet channel. The upstream water inlet at

the Centennial channel had re-bar positioned vertically at regular intervals to prevent adult fish from entering the study area. During the fall spawning event, the upstream and downstream re-bar fish fences were augmented with a 1.25 inch mesh to prevent large pieces of carcass material from upstream spawning activity from intruding into the study area. The flow into the site was split uniformly into the nine research channels via a distribution manifold designed by Rheal Finnegan. The flow of water into each channel was nearly constant at 0.03 m^3/sec. Minor adjustments to the flow into each channel were controlled by an aluminium plate at its inlet. The distribution manifold located at the upstream end of the nine channels had screens (mesh size approximately 0.5 cm) in place to prevent juvenile fish from migrating into the channels from upstream. The downstream end of each channel was equipped with a trap box to prevent uncontrolled juvenile escapement from and adult migration into the study area. Water diverted into the system from the Centennial channel was returned to the Centennial channel downstream of the study area.

3.2.3 Phosphorous inputs

For this research project, three treatments were used to investigate the effects of timing of artificial inorganic and organic nutrient addition in a system that is highly phosphorous limited in the summer, but that has a large influx of nutrients in the fall due to upstream spawning activities during a large fall run of Pink salmon (*O. kisutch*). The three treatments are: 1) slow-release inorganic phosphorous fertilizer added throughout the summer, 2) no phosphorous fertilizer addition in the summer and slow-release organic phosphorous containing fertilizer added in the fall, and 3) no fertilizer additions. Treatment 1, solid slow-release inorganic phosphorous was added in June and in

September naturally occurring dissolved inorganic phosphorous entered the research channels from spawning activities in the source water channel (**Inorg**/Inorg). Treatment 2 represented a phosphorous deficient system (<1 µg/L ortho-phosphate in the water column, with no concentrated organic inputs) during the summer growing season, which received both organic solid phosphorous, from an organic fertilizer, and the same naturally occurring inorganic phosphorous as in treatment 1 in September (Nil/Inorg+**Org**). Treatment 3 was a phosphorous deficient system in the summer growing period that received the same naturally occurring inorganic phosphorous as in treatments 1 and 2 in September (Nil/Inorg). The three treatments represented two possible vectors of fatty acid input into a stream system: the stimulation of synthesis of complex polyunsaturated fatty acids at the autotrophic level using inorganic nutrient addition, and the direct input of fatty acids and nutrients using an organic fertilizer.

The organic phosphorous input was an organic fertilizer that is a waste product from the Alaskan pollock (*Theragra chalcogramma*) processing industry. Pollock, without fillets, were ground, dried, and pressed into logs for ease of handling. Based on a 90% dry weight, the product contains 7.69±0.34 % phosphorous and 17.3±0.5 % total phosphate (as P_2O_5) with a fertilizer N:P:K ratio label of 6:9:0.5. This organic fertilizer has a significant amount of protein and lipid at 44.2±2.7 % and 7.5±1.5 % respectively. The pollock bone meal logs also contain minerals such as magnesium, potassium, sodium, iron, manganese, and zinc, which are important for balanced nutrition (Johnson et al., 2003). The product has generated considerable attention both in British Columbia and across the border into the United States as a potential cost effective, slow-release fertilizer (Johnson et al., 2003).

The inorganic fertilizer used in this study is a 7:40:0 (N:P:K) magnesium phosphate slow-release fertilizer from Lesco Inc. The product has 12 % magnesium, 7 % nitrogen, and 40 % phosphate by weight in the form $MgNH_4PO_4 \bullet H_2O$ (Sterling and Ashley, 2003).

Fertilizer loading rates were determined based on practices used for nutrient replacement strategies in British Columbia. Fertilizer application is constrained by government regulations and public perception. For the purposes of this research project, the target concentration used is high enough to saturate the specific growth rate of diatoms, which occurs at 3-4 µg/L SRP (Bothwell, 1985 but is one third the value that causes nuisance concentrations of algae (Ashley and Stockner, 2003). Phosphorous inputs from spawning activity, similar to what occurred upstream of the study site, are not constrained by either government regulations or public perception, and the loading rates were a natural mimic of conditions in a stream. As such, the fertilizer loading rates used for this research could be considered a partial mimic of historical inputs due to the limitations imposed.

For each production cycle, six of the nine stream channels in the study site were treated with fertilizer, as described below, and three remained untreated. Stream channels were randomly selected for treatment to avoid bias.

Fertilizer treatments were standardized based on phosphorous loading. Phosphorous loading is based on the target dissolved phosphorous level (background and treatment) of 3 µg/L SRP (Bothwell, 1985). Bothwell (1985) found that phosphorous limitations begins at approx 3-4 SRP for temperature greater than 10°C. This level is considered to be equal to 0.3-0.6 µg/L phosphate (Note: SRP does not equal phosphate).

The target level of 3 µg/L saturated the growth rate found for benthic diatoms studied by Bothwell (1988) in outdoor artificial streams in Thompson BC and should produce a measurable response in periphyton communities. The value is one third of the concentration at which excessive algal growth has the potential to cause problems such as decreased dissolved oxygen and toxic excretions (Ashley and Slaney, 1997).

The inorganic fertilizer phosphorous loading rate was determined using equations developed by Ashley and Slaney (1997), as follows:

$$P\ (kg) = Q*t*[P]$$

$$\text{Total } P_2O_5\ (kg) = P^*\ \%\ P_2O_5 /\ \%\ P$$

$$\text{Fertilizer } (kg) = \text{Total } P_2O_5 * P_2O_5 \text{ ratio of fertilizer}$$

Where P is the phosphorous loading, Q is discharge of the stream, t is the length of the fertilizer release period and [P] is the concentration of phosphate to be added to the stream. Total P_2O_5 is the total phosphate added to the stream, and % P_2O_5/ % P is the percent phosphate in the fertilizer divided by the percent phosphorous in the fertilizer. The mass of fertilizer to be added in my experiment is then calculated assuming the P_2O_5 ratio of fertilizer is the percent value of P_2O_5 in the fertilizer divided by 100.

The background Soluble Reactive Phosphate (SRP) was based on 2002 water chemistry data and determined to be 1 µg/L. Based on that background level, a discharge of 0.03 m^3/s, and a target concentration of 3 µg/L SRP, the phosphorous added to the system was calculated to be 0.47 kg. Using slow release fertilizer 40 %P_2O_5 and 17.5 %P with a release period, t, of 90 days, the mass of fertilizer needed per each channel being treated was calculated to be 2.7 kg. The mass of organic pollock fertilizer was

standardized with the inorganic fertilizer based on phosphorous loading. To determine the mass of organic material to be added to the study channels, a fertilizer ratio of 6-9-0.5 and a value of 17.6 $\%P_2O_5$ and 7.69 %P on a 90 % dry weight basis was used (Johnson et al., 2003). Based on those values, the mass of organic fertilizer needed per treated channel was 6.1 kg.

During the initial fertilizer treatment in June 2003, an inorganic, slow release fertilizer was distributed in the first riffle section of three of the channels. During the September 2003 fertilization event, pollock bone meal pellets were distributed within the first two riffle sections of three of the stream channels to fulfill the phosphorous loading value for fertilizer application.

3.2.4 Sample collection and processing

3.2.4.1 Water chemistry

During the summer, the three of the unfertilized channels and the three treated channels (from the June fertilizer addition) had water quality samples collected from the third, eleventh, and twenty-third pools. During the fall, all nine channels had water samples collected from the third, eleventh, and twenty-third pools. Water samples were collected twice monthly during the summer and fall fertilizer release periods. The water samples were sent to Phillips Analytical (now Maxxam Analytical) and analyzed for nitrate and nitrite nitrogen (detection limit: 2 µg/L, EPA method 353.2 (USEPA, 1993)), ammonia nitrogen (detection limit: 5 µg/L, standard method SM-4500MH3 (Eaton et al., 1995)), soluble reactive phosphate (detection limit: 1 µg/L, standard method SM 4500, (Eaton et al., 1995)), total dissolved phosphate (detection limit: 2 µg/L, standard method SM 4500, (Eaton et al., 1995)), total phosphate (detection limit: 2 µg/L, standard method

SM 4500, (Eaton et al., 1995)), and pH (detection limit: 0.1, standard method SM 4500H+B, (Eaton et al., 1995)).

Water chemistry samples were collected in sterile wide-mouth plastic bottles. Bottles were rinsed three times with stream water to further minimize the potential for contamination. Low level nutrient samples were collected and filtered in the field using a 60 mL syringe equipped with a 0.45 µm disposable filter. The syringe was rinsed three times with distilled water and three times with stream water before each sample collection. The filter was rinsed through with approximately three filter volumes, which were then used to rinse the collection bottle. Syringes were cleaned with 10 % HCl solution and distilled water between sampling events. Unfiltered samples were collected by removing the bottle's cap and inverting the sample bottle in the pool. Once the sample bottle was completely submerged, it was returned to an upright orientation and allowed to fill. This ensured the sampled water was sampled from the well-mixed portion of the pool and not unduly biased by the surface condition.

3.2.4.2 Chlorophyll a sampling

Two open cell, florist styrofoam algal plates were placed at the third, twelfth and twenty-third riffles in the treated and the untreated channels. Styrofoam substrate, in a sheet measuring approximately 20 cm x 20 cm, was attached to Plexiglas and cinderblock bases with nuts and bolts and nylon ties. The bases were submerged in the study riffles flush with the natural substrate. The styrofoam substrate was sampled every 5-7 days for a period of 6-8 weeks to measure algal accrual curves during the summer growing period, and for a period of 6-8 weeks during the fall to confirm the accrual of algal biomass during the autumn spawning runs. The foam substrate was sampled using a pill bottle as a

hole-punch with a known area; the samples were sent to Phillips Analytical Laboratory (now Maxxam Analytical) for analysis of chlorophyll a (detection limit: 0.3 mg/m^2, standard method SM-10200 H, (Eaton et al., 1995)). The styrofoam substrate was replaced at the beginning of fertilizer addition activities in June and September.

3.2.4.3 Benthic macroinvertebrate sampling

Benthic macroinvertebrates were sampled in the last riffle section of each stream channel. Three benthic invertebrate baskets with clean, in situ substrate were placed in the study area and the baskets were left for greater than six weeks for colonization by macroinvertebrates. Basket samples were collected using a 400 µm mesh Surber sampler, as follows. The Surber sampler was placed around the benthic sample basket with the net immediately downstream and the large rocks from the sample area were washed in the stream flow such that the organic material was collected in the net. The smaller gravel was placed in a bucket and rinsed six times with stream water until the decant ran clear. The decant from each rinse was strained through the Surber sampler net. After sampling, the baskets were replaced.

The contents of the three baskets were identified to family by Mike Stamford (Aquatic Ecologist, British Columbia Conservation Foundation) as follows: each sample was washed through a series of NITEX bolt-cloth sieves. Samples were washed through a 1.3 mm sieve and the substrate that did not pass through was sorted and specimens removed for identification and enumeration. The small specimens and substrate that washed through the large sieve (micro portion) was caught and washed on a 250 µm sieve and split into proportions using a plankton splitter. The size of the sub-sample (i.e., 1/4, 1/16, 1/64, etc., of the total micro volume) depended on the number of specimens

present, but a minimum of 100 specimens were removed for taxonomy and enumeration. Estimates of the counts and biomass for taxa present were then obtained by multiplying by the inverse of the proportions processed. Macroinvertebrate biomass estimates were obtained by drying the sorted samples at 60° C until the samples reached a constant weight. The micro and macro portions of the sample were analysed separately for biomass estimates.

3.2.4.4 Juvenile coho and steelhead stocking and sampling

Prior to the start of the experiment in 2003, the screens in the upstream distribution manifold that prevent fish migration into the research site were removed and the channels were colonized by resident juvenile coho salmon and steelhead trout. The number of fish in the channels was determined by electrofishing by certified personnel, and the occupants of each channel were identified to species and age class. Juvenile fish that had not over-wintered in the stream were classified as age-0, and fish that had over-wintered once in the system were classified as age-1. The channels underwent a three pass electroshock for removal. Lengths and weights were measured, and fish were stored in large containers onsite in the stream until the completion of the electrofishing event. The upstream control structure was then modified to prevent fish from migrating out of the treatment area.

Stocking density was determined based on a literature review by Sandercock (1991). The density of coho within the channels that represents a good density for average to good food availability is 75-145 coho per channel, assuming a survivability of 69 % and an area of 80 m^2 for each channel. For the purposes of this experiment a

stocking density of 100 coho fry per channel was chosen based on the presumed productivity of the channels. The fish were randomly redistributed through the nine channels at a density of 100 coho per channel, such that each channel was uniform in species community composition and fish density.

Due to the low numbers of steelhead trout within the research channels, all resident steelhead were removed and the channels were re-populated with swim-up fry from the Chilliwack River Hatchery. Swim-up fry are juveniles that have hatched and absorbed their yolk sack but have not yet had their first feeding. Steelhead fry were placed at a density of 100 fry per channel. Fish were randomly redistributed through the nine channels based on size class and species, such that each channel was uniform in species community composition and fish density.

Fish were sampled four times over the course of the experimental period: at the start of the experiment before the addition of the fertilizer treatment, at the end of the summer growing period before the addition of the fall fertilizer treatment, in the fall before water temperatures dropped below 4° C, and the final time at the completion of the experiment in the spring once the water temperatures warmed above 7° C. This interval was deemed sufficient to minimize stress to wild fish. The fish biomass in the channels was estimated using closed-site two-pass electrofishing estimation. Fish length and weight measurements were recorded. After processing, fish were redistributed in their channel of origin.

3.2.5 Lipid analysis

At each of the fish measuring events, five age-0 coho and five age-0 steelhead were randomly selected from each channel and euthanized using clove oil dissolved in

10 % ethanol. These fish were immediately placed on dry ice (solid carbon dioxide) and flash frozen in the field. After field collection, the fish remained on dry ice and were transported to Ronald B Johnson at the Northwest Fisheries Science Center, National Marine Fisheries Service, in Seattle, Washington for analysis. The samples were stored at -80° C until preparation for lipid analysis. Only age-0 fish were sampled for lipid analysis due to the low number of age-1 coho in each treatment.

Samples were freeze dried for approximately 24 hours and the whole fish was ground finely using a hand-held coffee grinder set to espresso grind. Samples were ground with approximately 125 mL of dry ice to prevent loss of lipid due to increased temperatures from friction. The samples were further freeze dried for 48 hours. Samples were individually stored in airtight vials and placed in a conventional freezer (-20° C) until final analysis.

Whole body percent lipid content of the juvenile coho and steelhead was determined by supercritical hexane extraction based on the method developed by Johnson and Barnett (2003). Gas chromatographic determination of the fatty acid profiles was conducted by AOAC Official Method 991.39: Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters.

3.2.6 Statistical analysis

Statistical analysis for water chemistry and algae standing biomass was conducted using the SAS Release 8.2 Analysis of Variance (ANOVA) procedure with a two-factor factorial design (treatment, sampling period) and the Student-Newman-Keuls (SNK) test to test for significance between means.

Statistical analysis for fish length, weight and percent lipid data was conducted using the SAS Release 8.2 General Linear Model procedure with a three-factor factorial (fertilizer treatment, species, sampling period) split-plot design. Comparisons between treatments for length and weight data were done using the Least Squares Means procedure and Bonferroni's critical difference adjusted for multiple comparisons such that the critical p-value is 0.0007. Percent lipid data was transformed using an arsine transformation before the analysis was conducted.

Total biomass was calculated as a way to express mortality as it considered consumption by predation. Graphical display of total biomass data was performed using Honestly Significant Difference (HSD) with unequal sample size (Andrews et al., 1980, Steel and Torrie, 1960). Means with non-overlapping error bars (±HSD) can be considered significant ($p<0.05$). For the total biomass estimate, data in one replicate in treatment 1 in the over-winter sampling period had to be corrected. This channel experience a flow interruption in October and the channel went partially dry due to a blockage of the inlet structure during the spawning event in near-by Centennial Channel. The standard deviation of the uncorrected average for treatment 1 is ±424 g, which is approximately double the next largest value of ±236 g. As a result, the value for the channel was corrected such that the standard deviation of the inorganic treatment was equal to ±236 g. The data point was corrected, as opposed to completely removed, because of the loss of statistical power that would result from its removal would render any statistical analysis useless.

3.3 Results

This section begins with a description of the water quality and temperature at the research site over the experimental period, followed by a general description of the aquatic ecosystem consisting of algal and macroinvertebrate conditions. The response of the juvenile salmonids to the variation in phosphorous input source and timing are then presented. Raw data is presented in the attached data CD, Appendix D.

3.3.1 Treatment channel conditions

Water temperature in the research channels ranged from 9° C-16° C during the summer (June-September) and averaged at 13° C; in the fall (September-November), the range was 14° C-6° C with an average of 10° C; and winter temperatures (November-April) ranged 4° C-7° C and the average was 5° C.

Nitrogen was not limited at any sampling event (see Figure 3.1). The N:P ratio remained above 20 for sampling events in the summer growing period. Streams with an N:P ratio >23 are considered to be phosphorous limited (Wetzel, 2001). The addition of phosphorous fertilizer to oligotrophic streams and subsequent stimulation of primary productivity has the potential to decrease the concentration of available nitrogen, which creates a situation where the system becomes nitrogen limited (Wilson et al., 2003). The results from the water chemistry monitoring indicate that this did not occur during the summer growing period. There was no observed decrease in nitrogen in channels that were exposed to increased levels of available phosphorous through fertilization. Nitrogen increased sharply in the fall with the influx of spawning Pink salmon (*O. gorbuscha*) into the nearby Centennial channel. Channels treated with fertilizer showed no significant

elevation in nitrogen concentration compared to untreated channels (see Appendix B for more details on water chemistry and periphyton data).

The research site was phosphorous limited during the summer growing season with a SRP concentration <1 µg/L. Treatment 1 channels, despite being treated with an inorganic fertilizer with a target dissolved SRP of 3 µg/L showed no elevated phosphorous levels compared to the other unfertilized channels. Periphyton sampling did, however, show that the inorganic fertilizer was effective at promoting algal growth suggesting that all of the phosphorous from the fertilizer was likely assimilated (see next section). Bothwell (1989) showed that as periphyton biomass increases, more phosphorous had to be available (up to 28 µg/L) in order to maintain dissolved levels and growth. During the fall spawning event, the SRP concentration in all treatment channels increased dramatically to be considered phosphorous saturated (Bothwell, 1985) (see Figure 3.2).

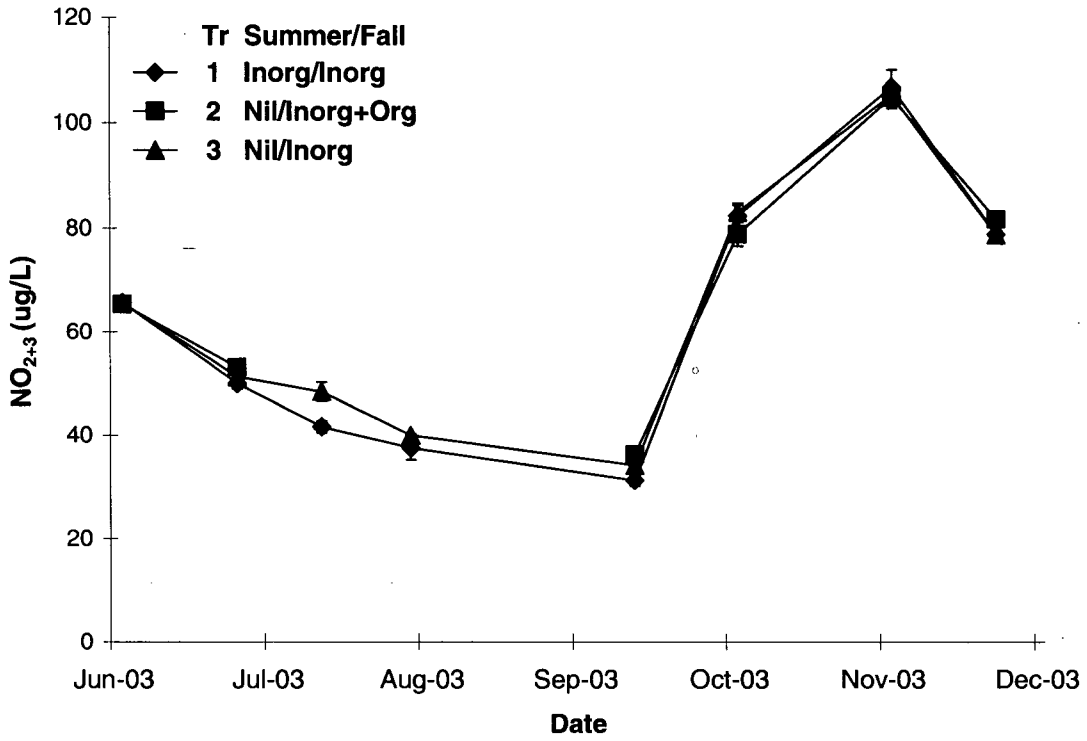


Figure 3.1: Nitrate and nitrite nitrogen concentrations (±SE) in the research channels

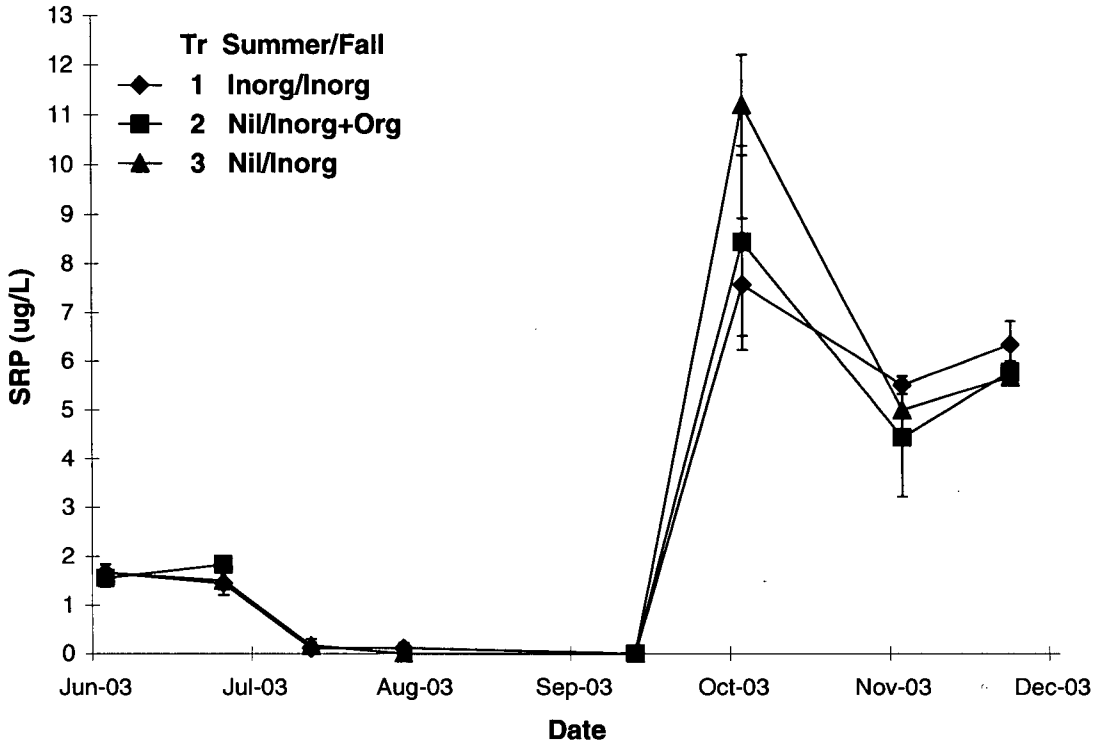


Figure 3.2: Soluble Reactive Phosphate (SRP) concentrations (±SE) in the research channels

3.3.1.1 Periphyton biomass within treatment channels

During the summer period (June-September) periphyton standing biomass in treatment 1 (**Inorg**/Inorg) channels was significantly greater than the nil treatments (treatment 2 and treatment 3) (SNK $p<0.05$). This suggests that nutrients applied during the summer treatment were taken up by primary producers in the system and shows the effectiveness of the fertilizer application. The periphyton standing biomass in the inorganic channels peaked in early August, before sloughing occurred (see Figure 3.3).

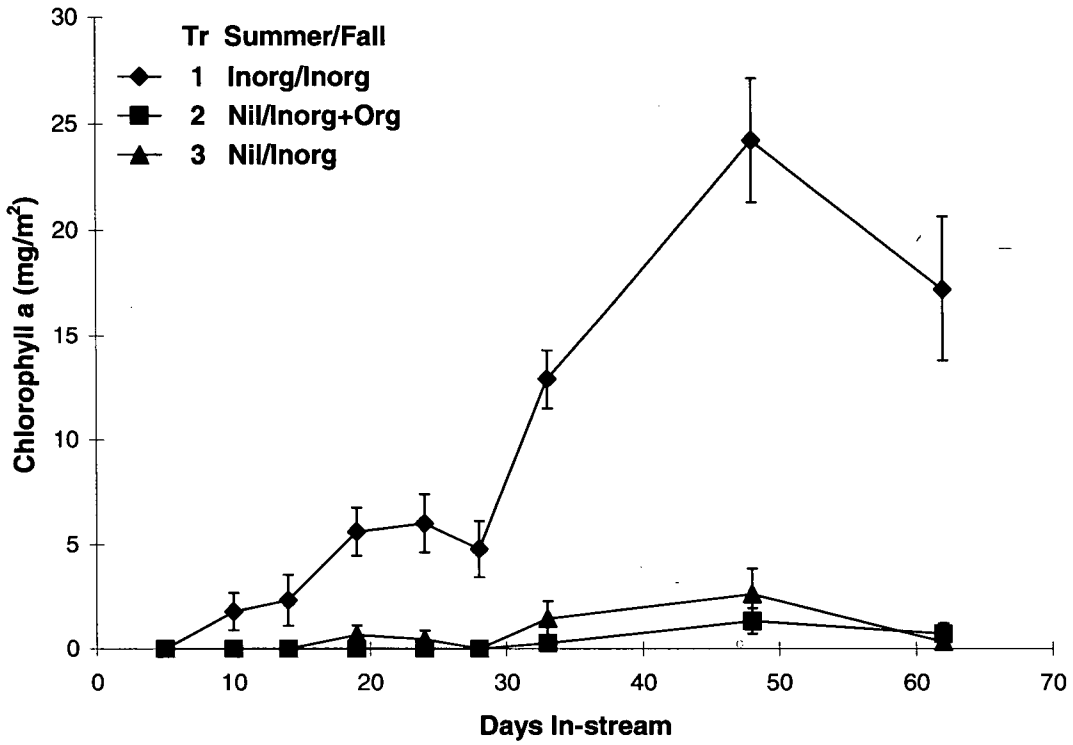


Figure 3.3: Chlorophyll *a* concentrations (±SE) in the three treatments during the summer (June-September)

During the fall period (September to November) periphyton standing biomass did not differ statistically between the three treatments (see Appendix B for more detail).

3.3.1.2 Benthic macroinvertebrates in the treatment channels

There was no treatment effect on the number of individuals of the important juvenile salmonid macroinvertebrate prey Chironomidae during either of the sampling periods (summer and fall). For Baetidae, another important juvenile salmonid macroinvertebrate prey species, there were more individuals in treatment 1 than treatments 2 or 3 during the summer sampling event (p=0.02). During the fall sampling period, the number of Baetidae in treatment 1 (**Inorg**/Inorg) was lower than in treatment 2 (Nil/Inorg+**Org**) (p=0.02) and treatment 3 (Nil/Inorg) (p=0.05) (see Figure 3.4 and Figure 3.5).

During the summer benthic macroinvertebrate sampling event, the biomass of large (>1250 µm) macroinvertebrates was greater in the inorganically fertilized treatment 1 than the unfertilized treatments 2 and 3 (p=0.04) (see Figure 3.6). The biomass of large macroinvertebrates in treatment 3 was greater than the biomass in treatment 1 (p=0.02) and treatment 2 (p=0.01) during the fall benthic macroinvertebrate sampling event. The biomass of large macroinvertebrates in treatment 1 did not differ significantly from that in treatment 2. The biomass of small (<1250 µm) macroinvertebrates did not differ significantly among treatments in either sampling event (see Appendix C for details).

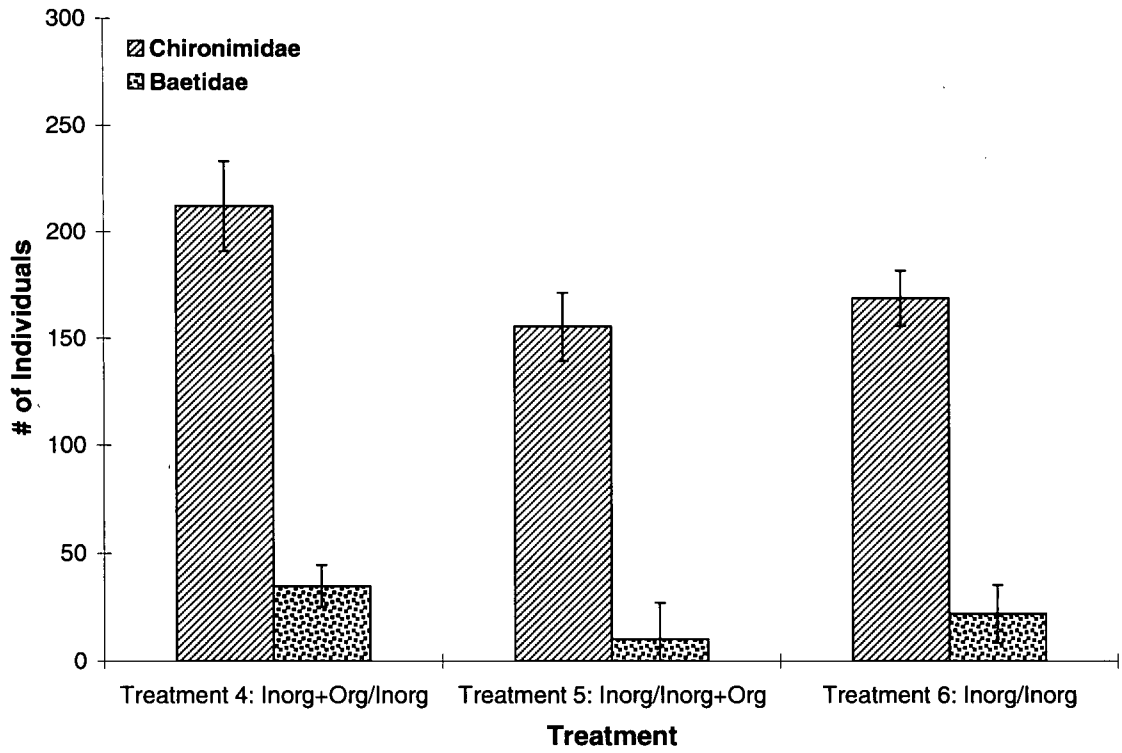


Figure 3.4: Mean ± (SE). Number of Chironomidae and Baetidae during the summer benthic macroinvertebrate sampling period.

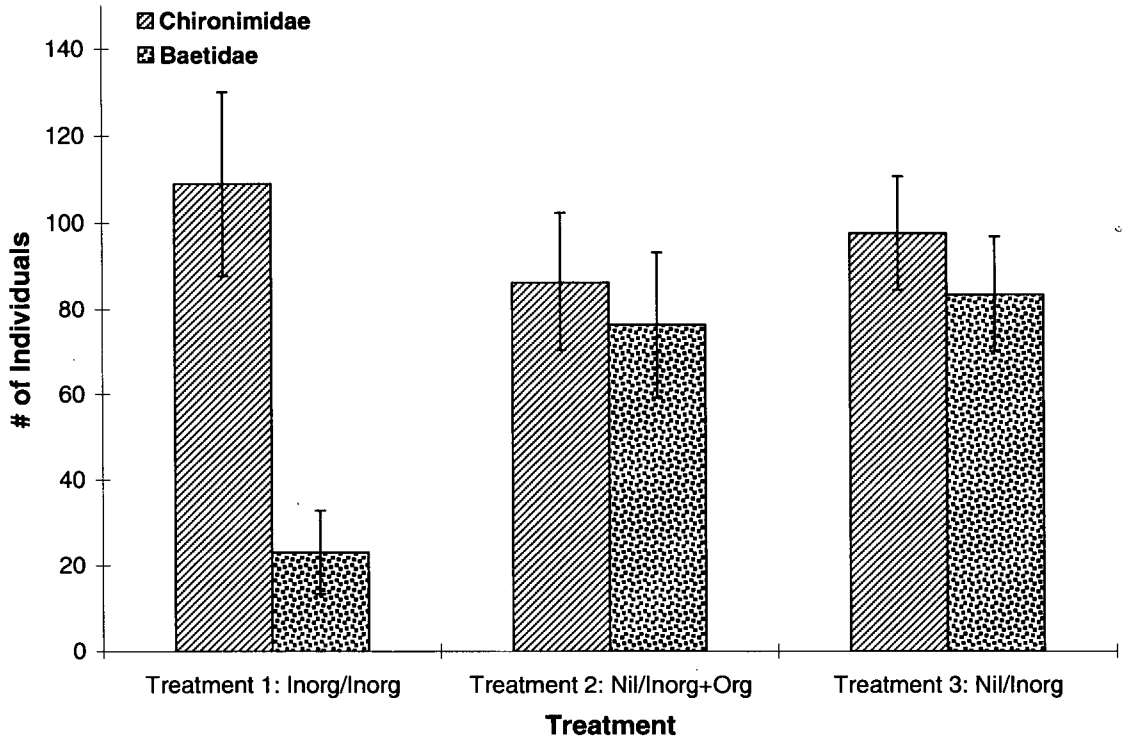


Figure 3.5: Mean ± (SE). Number of Chironomidae and Baetidae during the fall benthic macroinvertebrate sampling period.

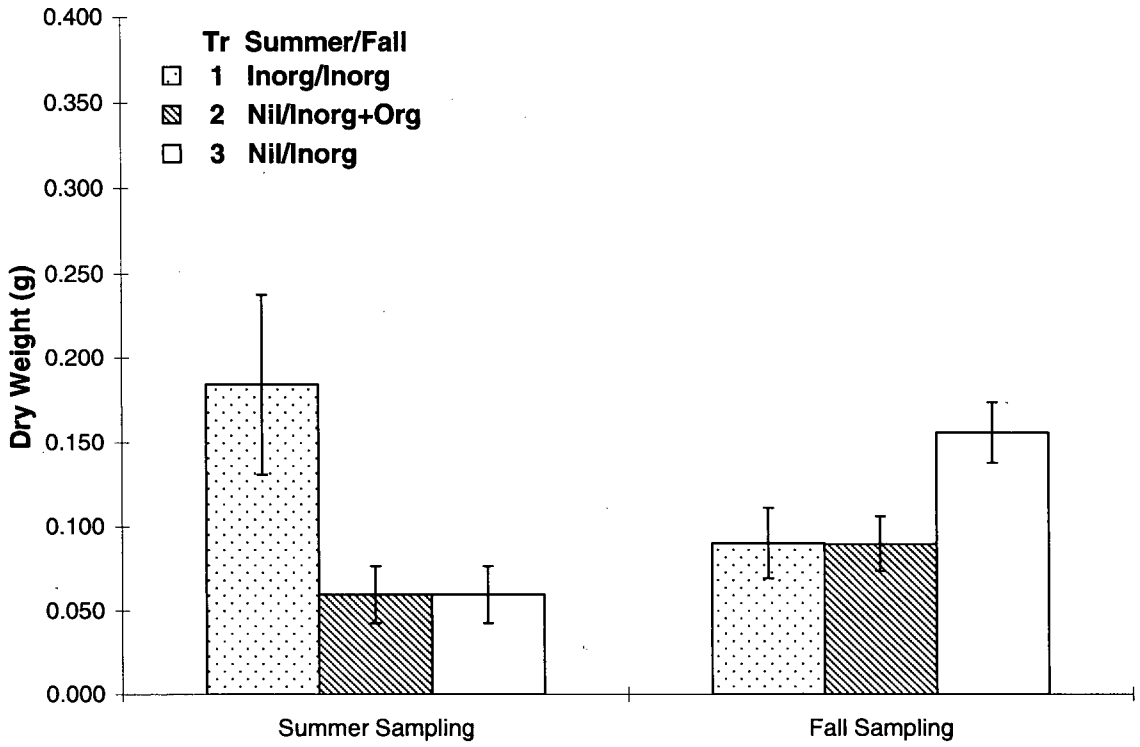


Figure 3.6: Mean ± (SE). Macroinvertebrate biomass (>1250 µm, dry weight basis) during the two sampling events in the three treatments

3.3.2 Fish responses to nutrients

3.3.2.1 Steelhead trout

At the electrofishing event in September, the steelhead in treatment 1 channels treated with inorganic fertilizer during the summer growing period had a longer fork length and greater weight than the steelhead in the unfertilized treatment 2 and 3 channels ($p<0.0001$) (see Table 3.2 for average length, weight and percent lipid values for steelhead from each treatment during each sampling event).

In the November sampling event, the steelhead in treatment 1 channels were significantly larger in both length and weight than those in treatment ($p<0.0001$). For the same sampling event, the steelhead in treatment 1 were significantly longer than those in treatment 2 ($p<0.0001$), but they did not weight significantly more ($p=0.0798$). The

steelhead in treatment 3 and treatment 2 were not significantly different in the November sampling event in either weight or length (p=0.0066).

After over-wintering in the system, the average weights of the steelhead in treatment 1 and treatment 2 were both significantly larger than those in treatment 3 (p<0.0001) but were not significantly different from each other (p=0.0087). The length data among the three treatments was not significantly different.

Comparing the steelhead in each individual treatment through the time series shows that the average length fish in all treatments increased over all sampling periods (p<0.0001). The steelhead in all treatments also gained a significant amount of weight over the summer growth period (p_{Tr1}<0.0001, p_{Tr2}<0.0001, p_{Tr3}=0.0002). Only the steelhead in treatment 2 gained significant weight between the September and November sampling events (p_{Tr1}=0.0066, p_{Tr2}<0.0001, p_{Tr3}=0.0839). The average weight of steelhead in all treatments increased significantly over-winter (all p<0.0001).

For the September sampling event, steelhead in the treatment 1 channels had a higher percent lipid than those in the unfertilized treatments 2 and 3 (p=0.0001). (See Table 3.2 for the average percent lipid values for the three treatments.)

In the November sampling event, the steelhead in all treatments had similar percent lipid stores. During the after-winter sampling event in April, the steelhead in treatment 2 had significantly higher percent lipid than the steelhead in treatment 1 (p<0.0001) but not than the steelhead in treatment 3 (p=0.0173).

Comparing the lipid stores over the time series for steelhead show that the average value for percent lipid dropped over the fall period (September-November) for steelhead in treatment 1 (p<0.0001). The steelhead from treatment 2 showed a significant

increase in percent lipid stores over winter (p=0.0005). There was no significant difference between time periods for steelhead in treatment 3. See Appendix D for a complete list of statistical results for the length, weight and percent lipid stores from steelhead in all treatments at all time periods.

Table 3.2: Results summary for steelhead trout length, weight and percent lipid. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SEM (mm)	Treatment		Sampling Time			
	Summer	Fall	June	September	November	April
1	**Inorg**	Inorg	28 ± 3(12)	72 ± 1^{a}(96)*	82 ± 1^{a}(142)*	92 ± 1^{a}(104)*
2	Nil	Inorg+**Org**	28 ± 3(12)	62±1^{b}(180)*	75 ± 1^{b}(104)*	91 ± 1^{a}(90)*
3	Nil	Inorg	28 ± 3(12)		71 ± 1^{b}(99)*	86 ± 1^{b}(74)*
Weight ± SEM (g)	Treatment		Sampling Time			
	Summer	Fall	June	September	November	April
1	**Inorg**	Inorg	0.1(12)	5.1 ± 0.3^{a}(96)*	6.2 ± 0.3^{a}(142)	11.0 ± 0.3^{a}(104)*
2	Nil	Inorg+**Org**	0.1(12)	3.5±0.3^{b}(180)*	5.5 ± 0.3^{ab}(104)*	9.8 ± 0.3^{a}(90)*
3	Nil	Inorg	0.1(12)		4.3 ± 0.3^{b}(99)	7.8 ± 0.4^{b}(74)*
% Lipid ± SEM (dry wt basis)	Treatment		Sampling Time			
	Summer	Fall	June	September	November	April
1	**Inorg**	Inorg	-	18.3 ±0.8^{a}(7)	12.6 ±0.8(7)*	13.8 ± 0.5^{a}(18)
2	Nil	Inorg+**Org**	-	13.4±1.1^{b}(10)	13.8 ±0.9(5)	17.8 ± 0.5^{b}(16)*
3	Nil	Inorg	-		14.2 ±0.9(5)	15.6 ±0.5^{ab}(18)

The average fatty acid profiles (wt %) for steelhead from the three treatments during the September, November, and April sampling events are summarized in Table 3.3. Statistically significant results within sampling events are indicated by a superscript, with different letters signifying significantly different means. For steelhead in the September sampling event, arachadonic acid (C20:4 ω-6, AA) and docohexanoic acid (C22:6 ω-3, DHA) in the fish from the channels fertilized with inorganic fertilizer (treatment 1) were significantly lower than in the fish from the channels that did not have phosphorous present (treatments 2 and 3). The sum of the ω-3 and ω-6 polyunsaturated fatty acids in fish from each treatment did not differ among treatments.

Table 3.3: Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	September Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.5±0.1	1.7±0.5	
16:0	19.3±0.3	19.2±0.5	
18:0	6.6±0.2[a]	7.4±0.1[b]	
22:0	0.5±0.2	0.2±0.1	
16:1	8.2±0.6	7.6±1.2	
18:1 ω-9	11.3±1.8	12.3±1.4	
18:1 ω-11	5.1±0.6	5.4±0.4	
20:1	0.2±0.1	0.2±0.1	
24:1	0.2±0.0	0.4±0.1	
18:2 ω-6	5.6±0.4	5.3±0.3	
18:3 ω-3	10.2±1.4	7.8±0.9	
18:4 ω-3	1.9±0.4[a]	0.9±0.1[b]	
20:2 ω-6	0.4±0.0	0.5±0.1	
20:3 ω-3	0.6±0.2	0.5±0.1	
20:3 ω-6	0.3±0.0	0.3±0.1	
20:4 ω-3	0.6±0.1	0.7±0.0	
20:4 ω-6	1.5±0.2[a]	2.6±0.3[b]	
20:5 ω-3	5.7±0.4	6.4±0.5	
22:4 ω-6	0.3±0.1	0.4±0.1	
22:5 ω-3	1.3±0.1[a]	1.6±0.1[b]	
22:6 ω-3	7.2±0.5[a]	10.4±0.8[b]	
Σ ω-3	27.4±1.1	28.3±0.9	
Σ ω-6	8.1±0.5	9.0±0.8	

Table 3.3 (continued): Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	November Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.5±0.5	2.3±0.1	2.2±0.1
16:0	19.7±1.2	18.3±0.1	18.5±0.1
18:0	6.7±0.3	6.4±0.2	6.2±0.1
22:0	0.1±0.0	ND	ND
16:1	8.2±0.6	9.4±1.3	9.9±0.3
18:1 ω-9	12.2±0.9	10.4±0.6	11.1±0.3
18:1 ω-11	6.0±0.7	6.5±0.6	7.4±0.2
20:1	0.5±0.1	0.7±0.1	0.4±0.0
24:1	0.5±0.1	0.3±0.1	0.3±0.1
18:2 ω-6	4.6±0.7	4.4±0.0	4.0±0.3
18:3 ω-3	8.0±1.4	7.1±0.5	7.9±0.1
18:4 ω-3	1.0±0.2	0.9±0.1	0.9±0.0
20:2 ω-6	0.3±0.1	0.6±0.1	0.3±0.1
20:3 ω-3	0.5±0.1	0.5±0.0	0.5±0.0
20:3 ω-6	0.2±0.1	0.4±0.1	0.3±0.1
20:4 ω-3	0.6 ± 0.1^{a}	0.7 ± 0.0^{ab}	0.8 ± 0.0^{b}
20:4 ω-6	2.0±0.2	2.7±0.5	2.0±0.1
20:5 ω-3	5.5±0.6	7.2±0.4	7.0±0.2
22:4 ω-6	0.3±0.1	0.4±0.2	0.2±0.1
22:5 ω-3	1.5±0.2	1.8±0.1	1.7±0.1
22:6 ω-3	9.4±0.6	9.9±1.2	9.5±0.5
Σ ω-3	26.3±1.5	27.9±0.5	28.3±0.9
Σ ω-6	7.4±1.0	8.3±0.9	6.8±0.7

Table 3.3 (completed): Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	April Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.4±0.1	2.8±0.7	2.7±0.1
16:0	20.6±0.3	22.3±3.1	22.1±1.6
18:0	6.1±0.2	6.6±1.0	6.8±0.4
22:0	ND	ND	ND
16:1	9.4 ± 0.3^{a}	12.4 ± 1.3^{b}	11.6 ± 0.3^{b}
18:1 ω-9	13.8 ± 0.3^{a}	15.7 ± 0.6^{b}	15.4 ± 0.6^{b}
18:1 ω-11	7.9±0.2	9.6±1.2	8.8±0.7
20:1	0.5±0.0	0.6±0.1	0.6±0.1
24:1	0.2±0.1	0.2±0.1	0.2±0.1
18:2 ω-6	3.6±0.1	2.4±0.8	2.8±0.5
18:3 ω-3	6.8±0.4	4.6±1.7	4.5±0.8
18:4 ω-3	0.6±0.0	0.4±0.2	0.5±0.1
20:2 ω-6	0.1±0.0	0.1±0.1	0.1±0.1
20:3 ω-3	0.5±0.0	0.2±0.2	0.2±0.1
20:3 ω-6	0.2±0.1	0.1±0.1	0.1±0.1
20:4 ω-3	0.7±0.1	0.4±0.2	0.5±0.1
20:4 ω-6	1.6±0.1	1.1±0.7	1.3±0.1
20:5 ω-3	6.2±0.3	4.6±1.6	5.3±0.6
22:4 ω-6	0.1±0.1	0.1±0.1	0.1±0.1
22:5 ω-3	1.3±0.1	1.0±0.3	1.1±0.1
22:6 ω-3	8.0±0.5	4.3±2.4	5.7±0.5
Σ ω-3	23.9±0.6	15.4±6.3	17.7±2.2
Σ ω-6	5.6±0.2	3.8±1.7	4.4±0.6

3.3.2.2 Coho salmon

3.3.2.2.1 Age-0 coho salmon

At the electrofishing events in September (p=0.0002) and November (p<0.0001), the age-0 coho in treatment 1 had a longer fork length than the age-0 coho in treatments 2 and 3. (See Table 3.4 for average length, weight and percent lipid values for age-0 coho from each treatment during each sampling event.) During the September sampling event, there was no significant difference in weight of age-0 coho in any of the three treatments (p=0.0239). In November, however, the age-0 coho in treatment 1 had a significantly greater average weight than those in treatment 3 (p=0.0005), but not than those in treatment 2 (p=0.0086). Age-0 coho were similar in both length and weight during the after-winter sampling event in April (p=0.5010).

Comparing the average length of age-0 coho in each treatment over time, it can be seen that all age-0 coho had significant increases in length over each time period (all p<0.0001). The age-0 coho in treatments 1 and 2 showed significant weight gains over all sampling events (all p<0.0001), whereas the average weight of age-0 coho in treatment 3 showed significant increase over the summer growth period and after winter (p<0.0001) but not during the fall period (p=0.0016).

For the September sampling event, age-0 coho in treatment 1 had a higher percent lipid than those in treatments 2 and 3 (p=0.0004). In all other sampling events, age-0 coho did not have significantly different percent lipid values in any of the three treatments. (See Table 3.4 for average values.)

The average percent lipid stores in age-0 coho in treatment 1 show a significant decrease over winter (p<0.0001). For all other sampling periods, in all treatments, there is

no significant changes in percent lipid stores over time. See Appendix D for a complete listing of the statistical analysis and results for age-0 coho in all treatments over all time periods.

Table 3.4: Results summary for age-0 coho salmon length, weight and percent lipid. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SEM (mm)	Treatment		Sampling Time			
	Summer	Fall	May	September	November	April
1	**Inorg**	Inorg	42 ± 1(143)	65 ± 1^{a}(241)*	72 ± 1^{a}(348)*	88 ± 1(165)*
2	Nil	Inorg+**Org**	43 ± 1(139)	62 ± 1^{b}(557)*	68 ± 1^{b}(300)*	87 ± 1(162)*
3	Nil	Inorg	43 ± 1(136)		66 ± 1^{b}(352)*	85 ± 1(129)*
Weight ± SEM (g)	Treatment		Sampling Time			
	Summer	Fall	May	September	November	April
1	**Inorg**	Inorg	0.9 ± 0.3(143)	3.5 ± 0.2(241)*	4.6 ± 0.2^{a}(348)*	8.2 ± 0.2(165)*
2	Nil	Inorg+**Org**	1.0 ± 0.3(139)	2.9 ± 0.2(557)*	4.0 ± 0.2^{ab}(300)*	8.5 ± 0.2(162)*
3	Nil	Inorg	1.0 ± 0.3(136)		3.8 ± 0.2^{b}(352)	7.6 ± 0.3(129)*
% Lipid ± SEM (dry wt basis)	Treatment		Sampling Time			
	Summer	Fall	May	September	November	April
1	**Inorg**	Inorg	-	15.9 ± 0.6^{a}(14)	13.2 ±0.5(15)	9.8 ± 0.5(18)*
2	Nil	Inorg+**Org**	-	13.7±0.6^{b}(27)	12.6±0.6(16)	11.0 ± 0.5(17)
3	Nil	Inorg	-		11.6 ±0.6(14)	10.5 ± 0.5(18)

The average fatty acid profiles (wt %) for age-0 coho from the three treatments during the September, November, and April sampling events are summarized in Table 3.5. Statistically significant results within sampling events are indicated by a superscript. During both the September and November sampling events, arachadonic acid (C20:4 ω-6, AA) in age-0 coho was significantly lower in treatment 1 than in treatment 2 or treatment 3. The AA in age-0 coho in treatment 2 and those in treatment 3 were not significantly different from each other. During the November sampling event, the age-0 coho in treatment 1 had significantly lower eicopentanoic acid (C20:5 ω-3, EPA) than those in either treatment 2 or 3. The EPA in the age-0 coho in the latter two treatments was not significantly different. In the same sampling event, the docohexanoic acid (C22:6 ω-3, DHA) content in age-0 coho in treatment 1 was significantly lower than in age-0 coho in treatment 3, whereas the DHA in age-0 coho in treatment 2 was not different from those in either treatments 1 or 3. There was no treatment effect on the sum of the ω-3 polyunsaturated fatty acids (PUFA) content in the age-0 coho. In the September sampling event the age-0 coho in treatment 1 had less total ω-6 PUFA than the age-0 coho in treatments 2 and 3. In November, the sum of the ω-6 PUFA in age-0 coho was lower in treatment 1compared to treatment 3. At the same sampling event, the ω-6 PUFA in the age-0 coho from treatment 2 was not statistically different from those in either treatments 1 or 3.

Table 3.5: Fatty acid composition of juvenile coho salmon from the three treatments during three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	September Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.5±0.1		2.7±0.9
16:0	19.1±0.0		17.8±0.3
18:0	6.3±0.1[a]		7.1±0.2[b]
22:0	0.2±0.0		0.2±0.1
16:1	7.3±0.3[a]		5.3±0.4[b]
18:1 ω-9	15.5±0.7[a]		19.9±1.4[b]
18:1 ω-11	4.7±0.1		4.0±0.3
20:1	0.2±0.0		0.3±0.1
24:1	0.3±0.1		0.4±0.1
18:2 ω-6	6.0±0.1		6.4±0.3
18:3 ω-3	8.7±0.6[a]		5.6±0.4[b]
18:4 ω-3	2.4±0.3[a]		1.7±0.2[b]
20:2 ω-6	0.2±0.1		0.5±0.2
20:3 ω-3	0.3±0.1[a]		0.2±0.0[b]
20:3 ω-6	0.2±0.1[a]		0.3±0.0[b]
20:4 ω-3	0.4±0.1		0.3±0.0
20:4 ω-6	1.6±0.1[a]		2.6±0.3[b]
20:5 ω-3	5.2±0.2		4.8±0.4
22:4 ω-6	0.3±0.0		0.4±0.1
22:5 ω-3	1.4±0.0[a]		1.6±0.1[b]
22:6 ω-3	7.5±0.3		8.8±0.8
Σ ω-3	25.8±0.8		23.1±1.4
Σ ω-6	8.3±0.2[a]		10.2±0.6[b]

Table 3.5 (continued): Fatty acid composition of juvenile coho salmon from the three treatments during three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	November Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.4±0.1	3.4±1.3	1.9±0.1
16:0	18.6±0.9^{a}	17.3±0.6^{b}	17.8±0.2^{b}
18:0	6.5±0.3	6.3±0.2	6.5±0.1
22:0	0.2±0.1	ND	ND
16:1	7.1±0.6^{a}	6.8±0.4^{ab}	6.0±0.5^{b}
18:1 ω-9	15.6±0.9	14.4±1.0	15.3±0.5
18:1 ω-11	4.9±0.6	4.8±0.2	4.8±0.2
20:1	0.6±0.1^{a}	0.8±0.1^{ab}	1.1±0.2^{b}
24:1	0.4±0.1^{a}	0.5±0.0^{ab}	0.6±0.1^{b}
18:2 ω-6	5.0±0.7	4.9±0.4	4.6±0.2
18:3 ω-3	7.3±0.8^{a}	5.5±0.2^{b}	4.7±0.3^{b}
18:4 ω-3	1.7±0.2^{a}	1.4±0.1^{b}	1.2±0.1^{b}
20:2 ω-6	0.2±0.0^{a}	0.5±0.1^{b}	0.5±0.1^{b}
20:3 ω-3	0.1±0.1	0.1±0.1	ND
20:3 ω-6	0.4±0.1	0.4±0.0	0.5±0.1
20:4 ω-3	0.3±0.0	0.3±0.0	0.4±0.0
20:4 ω-6	2.0±0.2^{a}	2.9±0.1^{b}	3.1±0.4^{b}
20:5 ω-3	5.1±0.3^{a}	5.8±0.4^{b}	6.0±0.2^{b}
22:4 ω-6	0.2±0.1^{a}	0.5±0.1^{b}	0.5±0.2^{b}
22:5 ω-3	1.6±0.2^{a}	1.8±0.1^{ab}	2.0±0.1^{b}
22:6 ω-3	9.1±0.6^{a}	10.6±0.8^{ab}	12.5±0.8^{b}
Σ ω-3	25.2±1.8	25.5±1.0	26.8±1.0
Σ ω-6	7.7±0.8^{a}	9.2±0.5^{ab}	9.2±0.7^{b}

Table 3.5 (completed): Fatty acid composition of juvenile coho salmon from the three treatments during three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	April Sampling Period		
Fatty Acid	**Inorg** Inorg	Nil Inorg+**Org**	Nil Inorg
14:0	2.4±0.2^{ab}	2.7±0.6^{a}	2.3±0.1^{b}
16:0	22.6±2.0	21.6±2.6	21.3±1.4
18:0	7.3±0.7^{a}	6.9±0.8^{ab}	6.4±0.3^{b}
22:0	0.1±0.1	0.4±0.3	ND
16:1	7.2±0.7	8.3±2.2	8.3±0.8
18:1 ω-9	14.1±0.5	13.9±1.7	14.7±0.1
18:1 ω-11	6.5±0.7	7.2±1.4	6.9±0.6
20:1	1.0±0.1	1.0±0.6	0.6±0.2
24:1	0.4±0.0	0.4±0.3	0.4±0.0
18:2 ω-6	2.9±0.5	2.5±0.5	3.1±0.2
18:3 ω-3	4.6±0.9	4.2±1.2	4.3±0.5
18:4 ω-3	0.8±0.3	0.9±0.3	0.9±0.2
20:2 ω-6	0.1±0.0	0.1±0.1	0.1±0.1
20:3 ω-3	0.1±0.0	ND	0.1±0.0
20:3 ω-6	0.1±0.1	0.1±0.1	0.1±0.1
20:4 ω-3	0.3±0.1	0.3±0.1	0.2±0.1
20:4 ω-6	1.7±0.3	1.7±0.6	2.0±0.2
20:5 ω-3	6.1±0.9	6.1±2.1	6.7±0.2
22:4 ω-6	0.2±0.1	0.1±0.1	0.1±0.0
22:5 ω-3	1.7±0.3	1.7±0.6	1.8±0.1
22:6 ω-3	8.8±1.7	8.0±3.4	8.8±1.1
Σ ω-3	22.3±4.1	21.2±7.5	22.8±1.7
Σ ω-6	5.1±1.0	4.4±1.2	5.4±0.5

3.3.2.2.2 Age-1 coho salmon

Age-1 coho did not have significantly different length or weight values for any of the sampling events conducted during the research (see Table 3.6 following for the average length and weight values for age-1 coho in each treatment for each sampling event).

Comparing age-1 coho over time indicates that the average value for length and weight of age-1 coho in all treatments increased over the summer (all $p<0.0001$). There were no significant differences between the average lengths or weights of age-1 coho in any treatment over the fall period. There was a significant increase in the average weight of age-1 coho after winter in treatments 2 and 3, but not the average weight of those in treatment 1 ($p_{Tr1}=0.0032$, $p_{Tr2}=0.0002$, $p_{Tr3}<0.0001$). See Appendix D for a complete listing of the statistical analysis and results for age-1 coho in all treatments over all time periods.

Table 3.6: Results summary for age-1 coho length and weight. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SEM (mm)	Treatment		Sampling Time			
	Summer	Fall	May	September	November	April
1	**Inorg**	Inorg	83 ± 1(56)	97 ± 2(43)*	101 ± 1(48)	111 ± 3(43)*
2	Nil	Inorg+**Org**	81 ± 1(60)	95 ± 2(89)*	101 ± 2(28)	111 ± 4(26)
3	Nil	Inorg	83 ± 1(62)		102 ± 2(33)	112 ± 3(26)
Weight ± SEM (g)	Treatment		Sampling Time			
	Summer	Fall	May	September	November	April
1	**Inorg**	Inorg	7.7 ± 0.4(56)	10.7 ± 0.5(43)*	11.8 ± 0.4(48)	14.5 ± 0.8(43)
2	Nil	Inorg+**Org**	7.1 ± 0.4(60)	9.5 ± 0.5(89)*	11.1 ± 0.6(28)	16.5 ± 1.2(26)*
3	Nil	Inorg	7.0 ± 0.4(62)		11.2 ± 0.5(33)	16.0 ± 0.8(26)*

3.3.3 Total Biomass

The total biomass of coho and steelhead in treatment 1 during the November sampling event is significantly higher than that in treatment 2 but not in treatment 3. At no other sampling event is there a significant difference in total biomass of steelhead and coho among treatments (see Figure 3.7 for details).

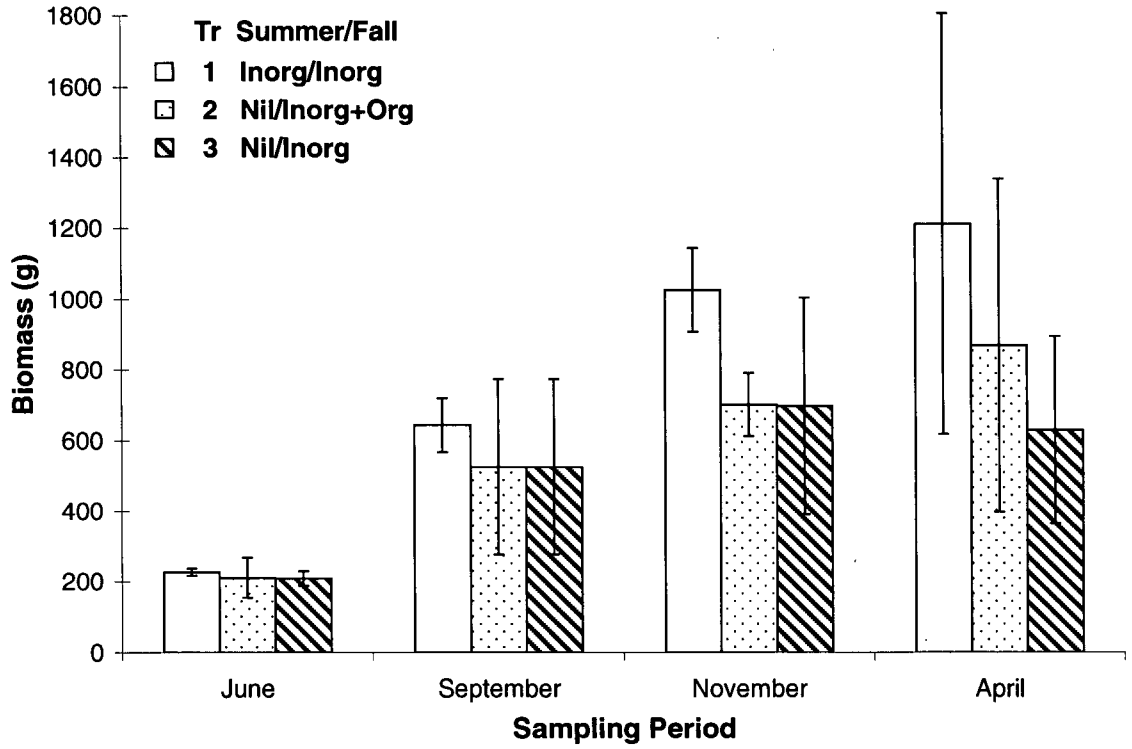


Figure 3.7: Total biomass of coho and steelhead in three treatments (±HSD)

3.4 Discussion

This research represents one side by side test of previous state-of-the-art nutrient replacement strategies (namely summer inorganic fertilization and fall organic fertilization) in a system considered to be P-limited in the summer. There was a high salmon run in the fall that contributed saturating levels of dissolved nutrients into all the

treatment channels. This influx of fall nutrients unfortunately did not permit an adequate test of a fall organic fertilization system (that would have occurred if the waters were nutrient poor in the fall. It did however show the importance of additional input beyond what had been considered adequate. This importance and the overall importance of all the results are discussed within this section.

In the presence of increased nutrients, either inorganic in the summer or organic in the fall, steelhead fry increased in length and weight. Thus, by increasing inorganic, dissolved phosphorous during the summer, the bottom-up effects within the stream food web are stimulated. Periphyton accrual is stimulated along with benthic macro-invertebrate production, thereby increasing food availability and the growth of resident fish populations (Mundie et al., 1991, Perrin et al., 1987, Quamme and Slaney, 2003, Slaney et al., 2003, Ward et al., 2003, Wilson et al., 2003, Wold and Hershey, 1999). Having an organic nutrient source available in the fall, in conjunction with a dissolved inorganic phosphorous source, can increase fish length and weight gains in freshwater ecosystems. Resident fish populations can feed directly on the organic material and likely benefit from indirect bottom-up effects as well (Bilby et al., 1998, Wipfli et al., 2004 and1998, Wipfli et al., 2003).

For steelhead, the approximately 90-day summer treatment period – when dissolved nutrients are available – was sufficient to produce a significant increase in juvenile length and weight. During the fall – when temperatures are dropping and fish are partitioning resources for storage and growth (Jonsson and Jonsson, 1998, Post and Parkinson, 2001) – the approximately 75 day interval between the addition of organic material and the evaluation of the resident population was sufficient to allow these

steelhead to accumulate mass equal to those fish exposed to inorganic phosphorous in the summer and fall, but was not sufficient to allow them to accumulate enough mass to surpass those fish exposed to inorganic phosphate in the fall only.

The results of this study show that having inorganic nutrients available for a longer period of time, through the summer and fall, is more effective for increasing bottom-up effects within the food web and for maintaining juvenile steelhead's size over-winter than having an input of inorganic nutrients only in the fall. The required time lag is shorter when organic phosphorous sources are used in the fall, since these can be consumed by juvenile steelhead and directly incorporated into fish tissue. As well, the presence of dissolved inorganic phosphorous resulting from the decomposition of the organic material stimulates the bottom-up effects of the stream food web.

More research needs to be conducted to investigate the benefits of having an organic source of phosphorous available through the summer growing period. In the future, work should be done to consider whether a source of organic material in conjunction with dissolved inorganic phosphorous during the summer growth period will increase the over-winter benefits seen in steelhead that are exposed to inorganic phosphorous throughout the summer and fall.

After the summer growing period, steelhead in the channels with inorganic phosphorous available had a higher storage of lipid than those steelhead in channels with no phosphorous present. This result suggests that the increase in available dissolved phosphorous translated into an increase in available forage for juvenile steelhead such that there was an energy surplus. This excess energy could be diverted from growth and allotted towards early storage as lipid. During the fall, there was no difference in lipid

stores among treatments, which indicates that the steelhead in the summer inorganic fertilized channels may have metabolized their lipid stores to maintain high growth rates.

After over-wintering in the system, steelhead in the channels with organic fertilizer available through the fall in conjunction with inorganic phosphorous had a higher percent lipid than the fish in channels with inorganic phosphorous available in the summer and fall. The percent lipid stores of steelhead in the treatment with no detectable dissolved inorganic phosphorous through the summer and only inorganic phosphorous available in the fall were not different from the lipid stores of steelhead in the other treatments. The decrease in lipid storage expressed as percent lipid between September and the following April in the treatments with long term availability of dissolved inorganic nutrients is consistent with previous work conducted on Atlantic salmon (*Salmo salar*) (Higgins and Talbot, 1985). It has been shown that age-0 Atlantic salmon preparing to smolt in the spring will establish this life-history pattern the previous fall, and that the continued feed and growth observed during the winter is fixed based on that internal mechanism (Metcalfe et al., 1988). For the steelhead in this treatment, maintenance or increases in size over-winter may be explained by the development of an early migrant life-history such that the metabolism of lipid stores is undertaken to shelter protein, which is then allocated to somatic growth and smolt transformation (Metcalfe et al., 1988, Morgan et al., 2000, Sheridan et al., 1983).

More difficult to explain is the increase in lipid stores over-winter in the treatments with organic phosphorous available in the fall. Metcalfe et al. (2002) studied Atlantic salmon that had experienced controlled periods of starvation and re-feeding during either summer or fall. Their results demonstrated that periods of food scarcity in

the summer and subsequent re-feeding will cause fish to increase compensatory skeletal growth as well as to increase lipid stores, whereas a similar food deprivation in the fall will lead to increases in lipid stores at the expense of skeletal growth. In this instance, steelhead from those treatments with the lower nutrient levels and food availability during the summer growth period and increases in the fall may be exhibiting compensatory growth through the fall and into the winter months. It has been suggested that while increases in body size will translate into decreases in size-dependant mortality and predation, there are costs to compensatory growth including growth abnormalities and decreases in swimming fitness (reviewed in Ali et al., 2003). The studies conducted to evaluate the potential for and effects of compensatory growth were undertaken in an aquaculture setting. More work should be conducted to investigate the long term effects of subjecting juvenile salmonids in natural settings to extended periods of food deprivation and food re-availability.

For the age-0 and age-1 coho salmon, the increases in nutrient availability, either as inorganic dissolved phosphorous in the summer or through the addition of organic fertilizer in the fall, did not produce a detectable response in average length, weight, or percent lipid over-winter. Since coho have a slower specific growth rate than steelhead, the time required for a significant divergence in average weight and size between populations is potentially greater (Sullivan et al., 2000). The length and weight of age-1 coho was not different among any of the three treatments for any of the sampling periods, suggesting that age-1 coho do not respond vigorously to increases in food availability during their second summer or fall. Age-0 coho salmon exhibited an afterwinter mortility of nearly 50% while steelhead mortality was much lower. Age-1 coho salmon

and steelhead were the largest of the fish, and may have consumed the smaller age-0 coho salmon.

As was discussed earlier, the decrease in lipid stores for coho in all treatments from September through April is consistent with similar findings in Atlantic salmon (Higgins and Talbot, 1985) and brown trout (*Salmo trutta*) (Jonsson and Jonsson, 1998). Values of percent lipid content for both coho and steelhead in all treatments for all time periods is above the minimum viable level of 9.1 % lipid (dry weight) based on 78 % moisture content (Jonsson and Jonsson, 1998, Post and Parkinson, 2001).

Fatty acid profiles for both coho and steelhead are consistent with fish feeding on a primarily freshwater macroinvertebrate diet. Chironomidae larvae have been found to have high levels of C20:4 and C20:5 fatty acids, 1.2-3.7 % and 6.3-15.1 % respectively, along with high levels of C18:3 fatty acids, 5.2-9.1 %, depending on the species (Hanson et al., 1985). The differences among the treatments in this experiment can be explained by the variation found in major prey species.

The input of organic material in the fall did not appear to change the fatty acid profiles of the fish in that treatment. This is reasonable considering the small amount of lipid that was added with the fertilizer used (6.1 kg fertilizer with 7.5 % lipid). Heintz et al. (2004) studied the effects of having high densities of organic material (large pieces of pink salmon carcass) on the fatty acid profiles of small numbers of coho in a trough study. Their findings indicate that having a source of organic material with a high content of ω-3 fatty acids available for direct consumption increased the ω-3 content of the resident juveniles by approximately 3-fold. This result, however, is hard to compare to

the results of this study considering the loading rate used by Heintz et al. (2004) is approximately 1.75 juvenile coho per pink salmon carcass.

This study shows that the synthesis of phosphorous addition strategies currently in use throughout the Pacific Northwest – namely, using inorganic phosphorous in the summer and organic phosphorous in the fall – is effective and should be investigated further in order to produce the most beneficial nutrient replacement schemes for fish.

3.5 Conclusions

For both coho and steelhead, there was no difference in after-winter length or weight between treatment 1 inorganic phosphorous present during the duration of the study (with sufficient time for the nutrients to be incorporated into the food web), and treatment 2 organic and inorganic phosphorous available only during the fall spawning period (with the possibility of direct transfer of organic macromolecules to the steelhead). Both of these treatments were more beneficial to steelhead than treatment 3 with dissolved inorganic phosphorous present only in the fall. These findings suggest that either of these two nutrient replacement schemes will produce measurable benefits for steelhead. The lack of after-winter response in coho may suggest that steelhead respond more favourably when exposed to increased food levels in situations of food shortage, or that steelhead simply out-compete coho for food. Further investigation could determine whether other combinations of timing and organic phosphorous loading will produce increased benefits to coho and steelhead.

Chapter 4: Effect of Artificial Summer and Fall Additions of Phosphorous on Fry Growth, Lipid Content and Biomass in Artificial Stream Channels Containing Coho Salmon and Steelhead Trout in South Coastal British Columbia

II. Fertilization during a year without a large fall salmon run that followed a year with a large fall salmon run and comparison of two years of experimentation

Summary: Salmonid populations have declined in most of the world. In some cases, early in-stream rearing conditions are poor due to anthropogenic impacts. For nutrient poor systems, in-stream fertilization has been found to increase fish yields. The effects of varying the timings and source of phosphorous inputs on fish production in phosphorous limited streams are not clear, but an imitation of the historical in-stream nutrient inputs would potentially benefit fish the most; it would also be the most natural approach.

Herein are the results of a set of experiments designed to imitate different natural phosphorous inputs and compare the resulting after-winter fry biomass and growth. This work was undertaken to continue to develop the nutrient replacement schemes addressed in chapter 2 and expand on the strategies presented in chapter 3. The three artificial phosphorous inputs were: 1) slow-release organic phosphorous fertilizer in the summer, 2) slow-release organic phosphorous fertilizer in the fall, and 3) no artificial phosphorous inputs. The background SRP level was between <1 µg/L and 3 µg/L (considered to be below phosphorous saturation) during the summer growth period and between 2-4 µg/L (considered to be just phosphorous saturated) during the small fall spawning run. The three treatments were labelled: 1) Inorg+**Org**/Inorg, 2) Inorg/Inorg+**Org**, and 3) Inorg/Inorg. Where the bold type face indicates artificial fertilizer inputs and the normal type indicates background sources of phosphorous.

In addition to biomass and growth, fish lipid levels, algal standing biomass as chlorophyll a, benthic macroinvertebrate biomass, and water chemistry were monitored to discern the overlying casual mechanisms that affect fry biomass and growth.

Results indicate that having an organic source of phosphorous in either the summer or the fall in a system with constantly detectable dissolved inorganic phosphorous through the summer and fall did not increase the size of resident juvenile steelhead trout (*Oncorhynchus mykiss*) or coho salmon (*O. kisutch*) over-winter when compared to a system with only dissolved inorganic phosphorous available over the same time period. The average length and weight of age-0 coho and steelhead were shown to have increased significantly over the length of the study for all treatments.

Data indicates that there is a higher standing biomass of macroinvertebrates over the summer growing period when compared to the fall period in all treatments ($p<0.05$). This drop in available prey coincides with a drop in percent lipid stores of age-0 steelhead. The average percent lipid stores of steelhead in all treatments decreased significantly over-winter suggesting that these fish were metabolizing fat to survive over-winter.

It appears that the concentration of inorganic nutrients in the fall is an important consideration for over-winter production in the stream. In two treatments differing only by the amount of SRP in the fall, the treatment with the highest SRP in the fall produced the highest after winter biomass and fall total fish numbers ($p<0.05$). This finding illustrates that more investigation needs to be conducted into not only the source and timing of phosphorous inputs but also the loading concentrations, particularly in the fall.

4.1 Introduction

From the turn of the nineteenth century, Pacific salmon (*Oncorhynchus* species) have been an important economic resource for coastal British Columbia. More recently, the ecological importance of these fish has been recognized as well. As fewer adult salmon return to coastal streams to spawn and die, there is a decrease in marine derived nutrients within the system (Larkin and Slaney, 1997). With fewer nutrients available and a decrease in the productivity of stream ecosystems, the emerging fry and resident juvenile salmon have a decreased access to food.

As was discussed in the previous chapters, there are numerous possible natural inputs of phosphorous into a stream system, including groundwater, sediment transfer, leaf litter and terrestrial influxes, natural fish excretions, and the return, death, and decomposition of returning anadromous fish (Stockner et al., 2000). Many coastal streams in British Columbia and in the greater Pacific Northwest are characterized as oligotrophic (nutrient poor), and the annual return of spawning Pacific salmon (*Oncorhynchus* species) in the fall has the potential to provide a large influx of phosphorous, nitrogen, and many other biological macro- and micro-molecules to these systems. Marine phosphorous is held within the streams as biomass of algae, macroinvertebrates, and resident fish populations.

As previously discussed, there are four possible options for phosphorous nutrient status in streams. There may be no detectable organic or dissolved inorganic phosphorous present (Nil), as is the case in most oligotrophic streams. Dissolved inorganic phosphorous may be present in the water column (Inorg) due to inputs from groundwater or a spring nutrient shadow of a large spawning salmon run that occurred the previous

fall. Organic solid containing phosphorous may be present within the stream system (Org), such as is present during small fall spawning events. Finally, there may be organic solid containing phosphorous within the system as well as a dissolved inorganic phosphorous present (Inorg+Org), which is typically found during large spawning events. Based on those four possible phosphorous conditions and considering two potential times for phosphorous input (summer growing period and fall spawning period), there are sixteen potential combinations for treatment options, as is shown in Table 2.2.

Fertilization schemes to manage the deficit between historical and current levels of nutrients available in streams have received considerable attention in recent decades (Bilby et al., 1998, Heintz et al., 2004, Johnston et al., 1990, Slaney et al., 2003, Wilson et al., 2003, Wipfli et al., 2003, Wipfli et al., 2004). The current state of practice and of research, however, does not address the issue of which nutrient replacement strategies (as outlined in Table 2.2) will produce the largest benefits to the resident fish populations. Following the reasoning developed in chapter 2, treatment schemes to be used for nutrient replacement activities should be based on stream food web theory and the historical inputs of phosphorous to the system in question.

The study presented in the previous chapter examined three of the fertilization schemes outlined in Table 2.2 during a year having a large input of inorganic phosphorous from a large salmon spawning run upstream. From that work, the results indicate that having an inorganic source of nutrients through both the summer growing period and fall spawning period, or having an inorganic and organic source of nutrients in the fall during the spawning period, is more effective at increasing resident juvenile steelhead length and weight over-winter than just having a source of inorganic nutrients

in the fall. More fertilization schemes are available for testing and questions linger about the benefit of having an organic source available in concert with inorganic nutrients through the summer growing period and fall spawning period. Is this the most beneficial time to add organics to a stream system to increase fry growth and survival, or is it more beneficial to add inorganic nutrients through the summer growth period and augment the inorganic nutrients with organic fertilizer through the fall spawning period?

The objective of this study was to investigate the effects of timing and source of phosphorous inputs into the stream systems and the response of juvenile salmon length, weight, lipid stores and fatty acid profiles during a year with summer and fall background SRP levels close to saturation. In addition, results were compared to data obtained the previous year. During that year, summer background SRP levels were close to nil, while fall levels were above saturation due to a heavy fall salmon run. The choice of the three treatments used in this study were devised to consider the naturally occurring fluctuations in nutrient levels in the Chilliwack River system and available constructed stream channels and carry-on from previous work conducted at the research site described in chapter 3. The controlled system in a natural setting permitted a near imitation of indigenous phosphorous inputs and control of some key variables. The habitat available at the research site mimics the complex natural habitats found in streams throughout the Pacific Northwest, with native substrate and riparian vegetation, pool/riffle sequences, and sheltering overhang. The site receives water from a nearby off-channel habitat of the Chilliwack River with naturally occurring phosphorous loads. As in the previous chapter, this research focused on three phosphorous inputs that would yield diverse in-stream phosphorous conditions. The three treatments are: 4) inorganic and organic phosphorous

in the summer and dissolved inorganic phosphorous in the fall, 5) inorganic phosphorous in the summer and inorganic and organic phosphorous in the fall, and 6) inorganic phosphorous in the summer and fall. Numbers 4-6 are used, so that the data from treatments (1-3) from the previous year can be compared. Data in treatments 1 and 6 were compared as they both represented a system with the same P type in the water and assumedly sufficient (although different concentrations) levels of that type in both the summer and fall.

Two resident species of juvenile Pacific salmon coho salmon (*O. kisutch*) and steelhead trout (*O. mykiss*) were the test organisms. They introduced another level of complexity because of the potential for multi-species interaction and different reactions to changing phosphorous inputs.

It is hypothesized that changing phosphorous timing and source, either inorganic or organic, will change resident fish size, growth, and lipid stores within the treatments. It is unclear whether inorganic inputs of phosphorous will have sufficient time to cycle through the food web and influence fish populations when compared to the direct input of organic phosphorous containing material available for fish consumption, or if multi-species interactions will cause differing responses to changing nutrient status in streams.

4.2 Materials and methods

Experiments conducted through June 2004 and ending in April 2005 were carried out in a manner similar to the experiments conducted the year before (June 2003 to April 2004, described in chapter 3). Differences between the treatment years are highlighted in the following section.

4.2.1 Experimental plan

The three phosphorous treatments were carried out simultaneously and in triplicate, starting June 2004 and ending April 2005. During this time, fish were stocked, and their growth and numbers monitored through electrofishing events in September and November 2004 and finally in April 2005. Water chemistry and temperature were monitored every two weeks from June to November 2004, and then monthly through the winter (December-April, 2005). Periphyton was sampled every 5-7 days during fertilization activities (June-August, and September-November). Benthic macroinvertebrates were sampled in single sampling events in August and November to coincide with fish sampling events. As well, fish lipid levels were analyzed from a random sampling of fish that were removed during the electrofishing events (see Table 4.1 for timing details on sampling activities and fertilizer additions). The primary variables of interest were fish length, weight, growth, and total biomass in each treatment over-winter. Secondary variables of lipid content, lipids, algal biomass as chlorophyll a, macroinvertebrate biomass per treatment, and fish fatty acid profiles are used to explain and verify results. Details pertaining to site, phosphorous input, stocking, monitoring, and statistical plan follow.

Table 4.1: Timeline of fertilization applications and sampling activities conducted at the Centennial channel research channels 2004-2005

Activity	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Organic fertilizer added			x			x							
Coho stocked		x											
Steelhead stocked			x										
Water chem. (14 days)			x	x	x	x	x	x					
Water chem. (monthly)									x	x	x	x	x
Chloro a (5-7 days)			x	x	x	x	x	x					
Replace chloro a plates						x							
Macroinvertebrate					x			x					
Electrofishing	x					x		x					x
Spawning upstream						x	x	x	x				

4.2.2 Site description

The research facility (site) was located adjacent to the Centennial channel, an off-channel habitat of the Chilliwack River. The site consists of nine identical, parallel, constructed stream channels. Each constructed channel was approximately 80 m long and 1 m wide and had 24 riffles (depth: 0.07 m) and 23 pools (depth: 0.62 m) alternating along its length (see section 3.2.2 for a further description of the research facility).

4.2.3 Phosphorous inputs

For this research project, three treatments were used to investigate the effects of timing of inorganic and organic nutrient addition in a system containing summer SRP levels exceeding 1 µg/L (below phosphorous saturation) and fall levels between 2-4 µg/L (near phosphorous saturation). The treatments were as follows: 4) organic phosphorous-containing fertilizer applied in the summer, 5) organic phosphorous-containing fertilizer applied in the fall, and 6) no fertilizer addition. In all treatments, there was dissolved

inorganic phosphorous during the summer growing period from the nutrient shadow most likely caused by the large number of spawned out pink salmon carcasses that were deposited in the upstream Centennial channel the previous fall. All treatments experienced a dissolved inorganic phosphorous input in September, resulting from a small spawning run of Chum salmon (*O. keta*) in the upstream Centennial channel. In treatment 4, organic phosphorous in the form of organic fertilizer was added to the channels in June along with the dissolved inorganic phosphorous nutrient shadow through the summer and the naturally occurring input of inorganic phosphorous from upstream spawning activities beginning in September (Inorg+**Org**/Inorg). For treatment 5, the same phosphorous shadow occurred during the summer growing season followed by the addition of organic fertilizer combined with inorganic phosphorous in the water column from upstream spawning activities beginning in September (Inorg/Inorg+**Org**). Treatment 6 was the inorganic phosphorous shadow during the summer growing season and the inorganic phosphorous starting in September (Inorg/Inorg). This treatment follows the same phosphorous type and timing as treatment 1 carried out the previous year and discussed in the previous chapter, except that the concentrations of phosphorous are distinct. The three treatments represent a portion of the larger available treatment options based on Table 2.2 in chapter 2, and they represent two possible vectors of fatty acid input into a stream system: stimulation of synthesis of complex polyunsaturated fatty acids at the autotrophic level using inorganic nutrient addition, and the direct input of fatty acids and nutrients using an organic fertilizer.

The organic phosphorous input was an organic fertilizer, which is a waste product from the Alaskan pollock (*Theragra chalcogramma*) processing industry (see section

3.2.3 for more information on the composition and processing techniques for the organic fertilizer).

Similar to the previous experiment, fertilizer loading rates were determined based on practices used for nutrient replacement strategies in British Columbia. Fertilizer application is constrained by government regulations and public perception. For the purposes of this research project, the loading rate used is high enough to saturate diatom specific growth rate (0.3-0.6 μg/L phosphate) (Bothwell, 1988) but is one third the value which causes nuisance concentrations of algae (Ashley and Stockner, 2003). Phosphorous inputs from spawning activity, similar to what occurred upstream of the study site, are not constrained by either government regulations or public perception, and the loading rates were a natural mimic of conditions in a stream. As such, the fertilizer loading rates used for this research could be considered a partial mimic of historical inputs due to the limitations imposed.

Fertilizer treatments were standardized based on phosphorous loading (see section 3.2.3 for a detailed description of the calculation of organic fertilizer loading rates). Based on a target concentration of 3 μg/L SRP, the phosphorous to be added to the system was calculated to be 0.47 kg, and the mass of organic fertilizer needed per treated channel was 6.1 kg.

Six of the nine stream channels in the study site were treated with fertilizer as described below, and three remained untreated. Stream channels were randomly selected for treatment to avoid bias. During the initial fertilizer treatment in June 2004, and then in September 2004, pollock bone meal pellets were distributed within the first two riffle

sections of three of the stream channels to fulfill the phosphorous loading value for fertilizer application.

4.2.4 Sample collection and processing

4.2.4.1 Water chemistry

During the summer, three unfertilized channels and the three treated channels (from the June fertilizer addition) had water quality samples collected from the third pool (immediately downstream of the treatment). During the fall and winter, all nine channels had water sampled collected from the third pool. Water samples were collected twice monthly during the summer and fall fertilizer release periods. The water samples were sent to Phillips Analytical (now Maxxam Analytical) and analyzed for nitrate and nitrite nitrogen (detection limit: 2 µg/L, EPA method 353.2 (USEPA, 1993)), ammonia nitrogen (detection limit: 5 µg/L, standard method SM-4500MH3 (Eaton et al., 1995)), soluble reactive phosphate (detection limit: 1 µg/L, standard method SM 4500, (Eaton et al., 1995)), total dissolved phosphate (detection limit: 2 µg/L, standard method SM 4500, (Eaton et al., 1995)), total phosphate (detection limit: 2 µg/L, standard method SM 4500, (Eaton et al., 1995)), and pH (detection limit: 0.1, standard method SM 4500H+B, (Eaton et al., 1995)) (see section 3.2.3.1 for a detailed explanation of the collection method for water quality samples).

4.2.4.2 Chlorophyll a sampling

Two open cell, florist styrofoam algal plates were placed at the middle riffle in the treated and the untreated channels. Styrofoam substrate, in a sheet measuring approximately 20 cm x 20 cm, was attached to Plexiglas and cinderblock bases with nuts and bolts and plastic nylon ties. The bases were submerged in the study riffles flush with

the natural substrate. The styrofoam substrate was sampled every 5-7 days for a period of 6-8 weeks to measure algal accrual curves during the summer growing period and for a period of 6-8 weeks during the fall to confirm the accrual of algal biomass during the autumn spawning runs. The foam substrate was sampled using a pill bottle as a hole-punch with a known area, and samples were sent to Phillips Analytical Laboratory (now Maxxam Analytical) for analysis of chlorophyll a (detection limit: 0.3 mg/m^2, standard method SM-10200 H, (Eaton et al., 1995)). The styrofoam substrate was replaced at the beginning of fertilizer addition activities in June and September.

4.2.4.3 Benthic macroinvertebrate sampling

Benthic macroinvertebrates were sampled in the last riffle section of each stream channel. Three benthic invertebrate baskets with cleaned in-situ substrate were placed in the study area and the baskets were left for greater than six weeks for colonization by macroinvertebrates. Basket samples were collected using a 400µm mesh Surber sampler. The Surber sampler was placed around the benthic sample basket with the net immediately downstream and the large rocks from the sample were washed in the stream flow such that the organic material was collected in the net. The smaller gravel was placed in a bucket and rinsed six times with stream water until the decant ran clear. Decant was strained through the Surber sampler net. After sampling, the baskets were replaced.

The contents of the three baskets were identified to family by Mike Stamford (Aquatic Ecologist, British Columbia Conservation Foundation) (see section 3.2.4.3 for a detailed explanation of the procedure used to identify and enumerate the benthic macroinvertebrate samples).

4.2.4.4 Juvenile coho and steelhead stocking and sampling

Prior to the start of the experiment in 2003, the screens in the upstream distribution manifold that prevent fish migration into the research site were removed and the channels were colonized by resident coho salmon and steelhead trout. In 2004, due to the experimental activities of the year before, the screens upstream of the study area were not removed. As a result, the site needed to be stocked with age-0 coho and steelhead, which were obtained from the Chilliwack River Hatchery; the age-1 coho were resident in the treatment channels the previous year. Before the summer fertilizer addition, the number of fish in the channels was determined by electrofishing, and the occupants of each channel were identified to species and age class. Any resident age-1 steelhead were removed with minnow traps. The channels underwent a three pass electroshock for removal. The age-1 coho were processed for length and weight and stored in large containers onsite in the stream until the completion of the electrofishing event. At that time, the fish were redistributed randomly in the experimental channels (see section 3.2.4.4 for further information on stocking density). Fish were randomly redistributed through the nine channels based on size class and species such that each channel was uniform in species community composition and fish density.

Sampling of fish occurred four times to minimize stress to wild fish: once at the start of the experiment before the addition of the fertilizer treatment, a second time at the end of the summer growing period before the addition of the fall fertilizer treatment, a third time in the fall before water temperatures dropped below 4° C, and the final time at the completion of the experiment in the spring once the water temperatures warmed above 7° C.

The fish biomass in the channels was estimated using a two-pass estimation. Fish length and weight measurements were recorded, and five fish of each species per each channel were euthanized and preserved on dry ice for later lipid analysis. After processing, fish were redistributed in their channel of origin.

4.2.5 Lipid analysis

At each of the electrofishing events, five juvenile coho and five juvenile steelhead were randomly selected from each channel and euthanized using clove oil dissolved in 10% ethanol. These fish were immediately placed on dry ice (solid carbon dioxide) and flash frozen in the field. After field collection, the fish remained on dry ice and were transported to Ronald B Johnson at the Northwest Fisheries Science Center, National Marine Fisheries Service, in Seattle, Washington. The samples were stored at -80° C until preparation for lipid analysis (see section 4.2.5 for a detailed explanation on the processing and analysis of the fish lipid samples).

Whole body percent lipid content of the juvenile coho and steelhead was determined by supercritical hexane extraction, based on the method developed by Johnson and Barnett (2003). Gas chromatographic determination of the fatty acid profiles was conducted by AOAC Official Method 991.39: Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters.

4.2.6 Statistical analysis

Statistical analysis for water chemistry and algae standing biomass was conducted using the SAS Release 8.2 Analysis of Variance (ANOVA) procedure with a two-factor

factorial design (treatment, sampling period) and the Student-Newman-Keuls (SNK) test to test for significance between means.

Statistical analysis for fish length, weight and percent lipid data was conducted using the SAS Release 8.2 General Linear Model procedure with a three-factor factorial (fertilizer treatment, species, sampling period) split-plot design. Comparisons between treatments for length and weight data were done using the Least Squares Means procedure and Bonferroni's critical difference adjusted for multiple comparisons such that the critical p-value is 0.0007. Percent lipid data was transformed using an arsine transformation prior to analysis.

The graphical display of specific growth and total biomass data was performed using Honestly Significant Difference (HSD) with unequal sample size (Andrews et al., 1980, Steel and Torrie, 1960). Means with non-overlapping error bars (±HSD) can be considered significant ($p<0.05$).

4.3 Results

This section begins with a description of the water quality and temperature in the research channels through the experimental period, followed by an account of the aquatic ecosystem consisting of algal and benthic macroinvertebrate standing biomass. The response of juvenile salmonids to manipulations of the environmental phosphorous inputs is then presented. Finally, comparisons of growth rate and biomass between the two treatment years are presented.

4.3.1 Treatment channel conditions

Water temperature in the research channels ranged 9° C - 16° C during the summer (June – September) and averaged 13° C; the fall (September – November) range was 14° C - 6° C with 10° C as the average; during the winter, the water temperature ranged 2° C - 7° C (November – April) with an average of 5° C.

Nitrogen was not limited at any sampling event (see Figure 4.1). Channels treated with fertilizer showed no significant elevation in nitrogen concentration compared to untreated channels (see Appendix B for more details on water chemistry and periphyton data).

The soluble reactive phosphorous (SRP) concentrations remained above 1 µg/L for the majority of the sampling events during the summer growing period, although the channels with no organic nutrients added (treatment 5 and 6) were below that level for the sampling event in July. The treatment 4 channels treated with the organic fertilizer showed no significant elevated nutrient levels. During the fall spawning event, the SRP concentration in the channels increased but not dramatically (Figure 4.2). The spawning return in the fall of 2004 was considerably lower than the returns experienced the previous year; this is reflected in the significantly lower concentrations of SRP and nitrogen measured within the water column.

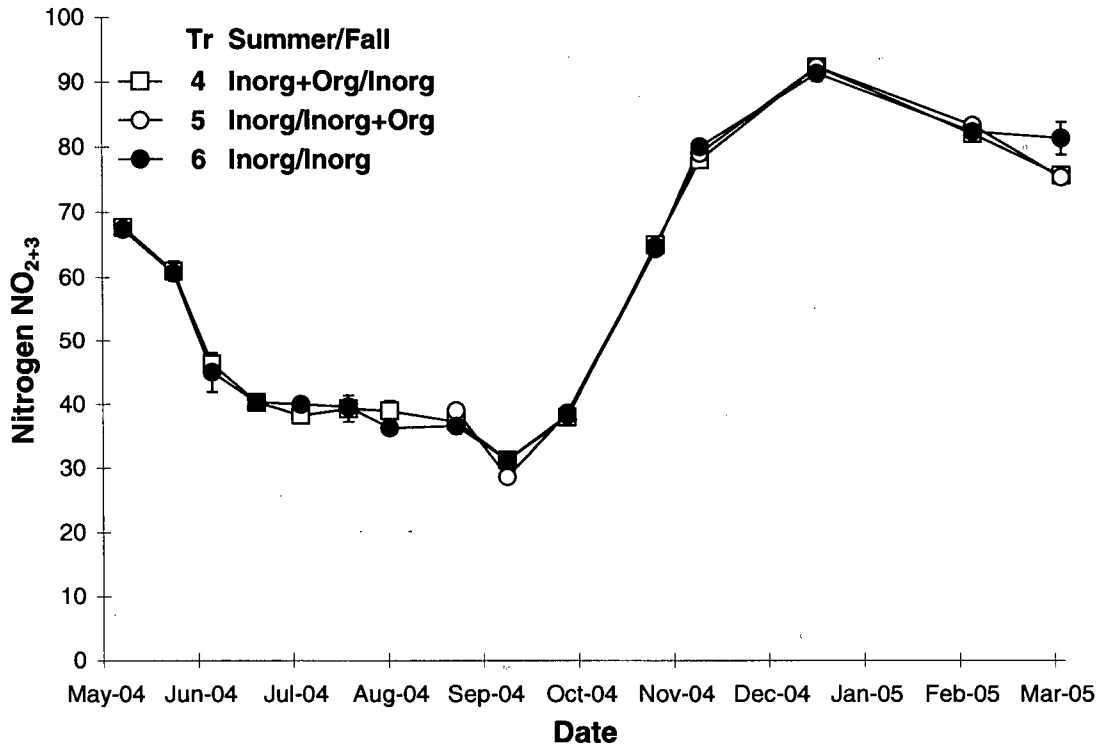


Figure 4.1: Nitrate and nitrite nitrogen concentrations (±SE) in the research channels

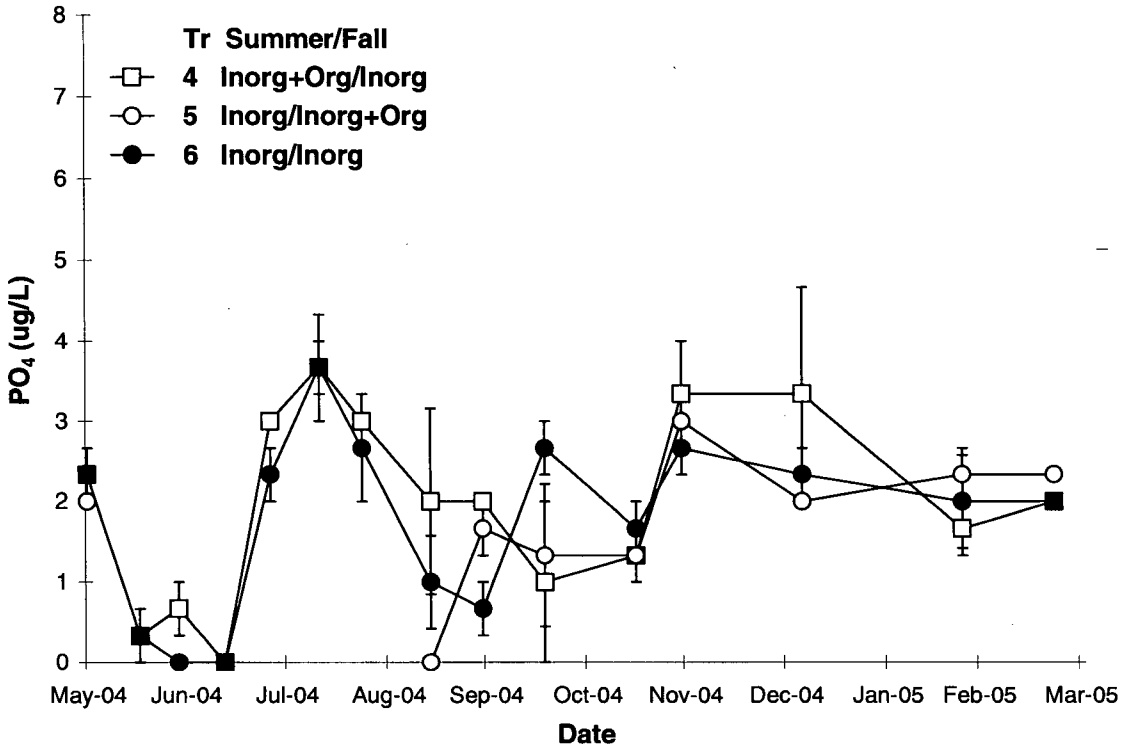


Figure 4.2: Soluble Reactive Phosphate (SRP) concentrations (±SE) in the research channels

4.3.1.1 Periphyton within treatment channels

During the summer period (June – September), periphyton standing biomass in treatment 4 (Inorg+Org/Inorg) channels was not significantly different than the inorganic treatments 5 and 6 (Figure 4.3). The periphyton standing biomass in all treatments was similar to treatment 1 from the previous study; this finding reflects that the algal response to inorganic nutrients in the second year was similar to the first, even considering that there was no detectable inorganic phosphorous in the fertilized channels the previous summer.

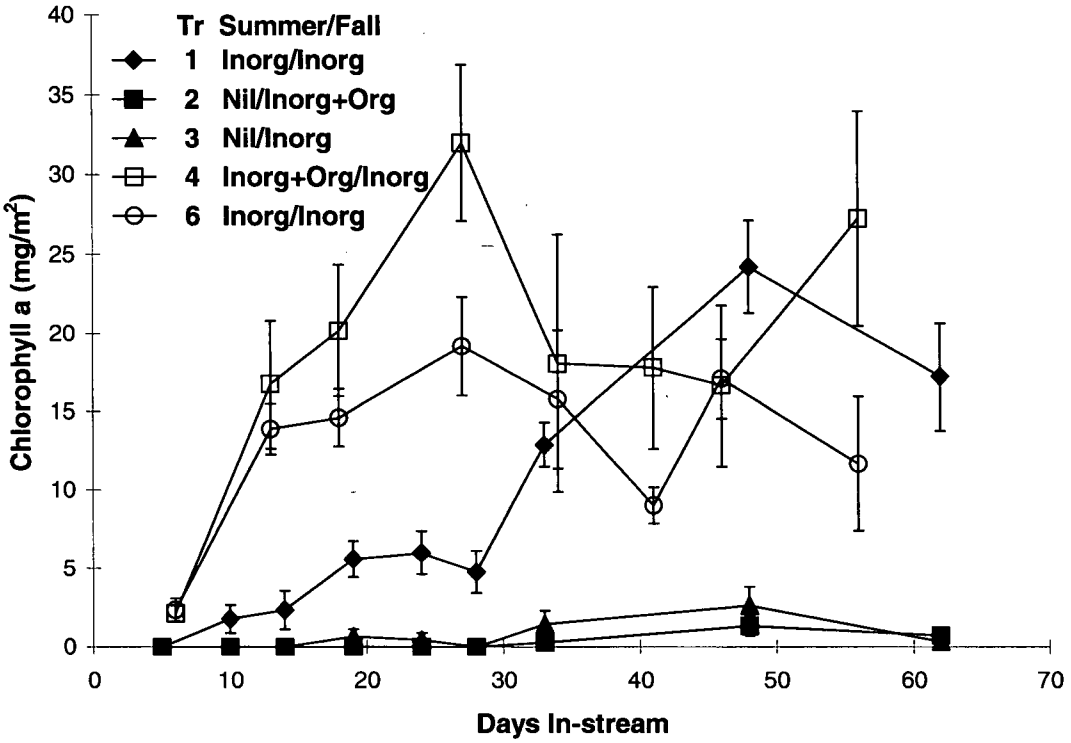


Figure 4.3: Chlorophyll *a* concentrations in the 2003 and 2004 treatments during the summer (June-September). Mean (±SE)

During the fall (September - November) and winter periods (January - April), the periphyton standing biomass did not differ statistically among the three treatments. The standing biomass of algae did not differ significantly among treatments conducted over both years during the fall sampling events (see Appendix B for more detail).

4.3.1.2 Benthic macroinvertebrate biomass within the treatment channels

There was no treatment effect on the number of individuals of two important juvenile salmonid macroinvertebrate prey Chironomidae and Baetidae during either the summer or fall sampling periods (see Appendix C for details). During the summer benthic macroinvertebrate sampling event, there was no difference in large (>1250 μm) macroinvertebrate biomass between the unfertilized treatments (treatments 5 and 6) and the organically fertilized treatment 4 (see Figure 4.4). The biomass of large

macroinvertebrates in treatment 4 was greater than the biomass in treatment 6 (p=0.01) during the fall benthic macroinvertebrate sampling event. The biomass of large macroinvertebrates in treatment 5 did not differ significantly from that in treatment 4 or treatment 6. The biomass of small (<1250 µm) macroinvertebrates did not differ significantly among treatments in either sampling event (see Appendix C for details).

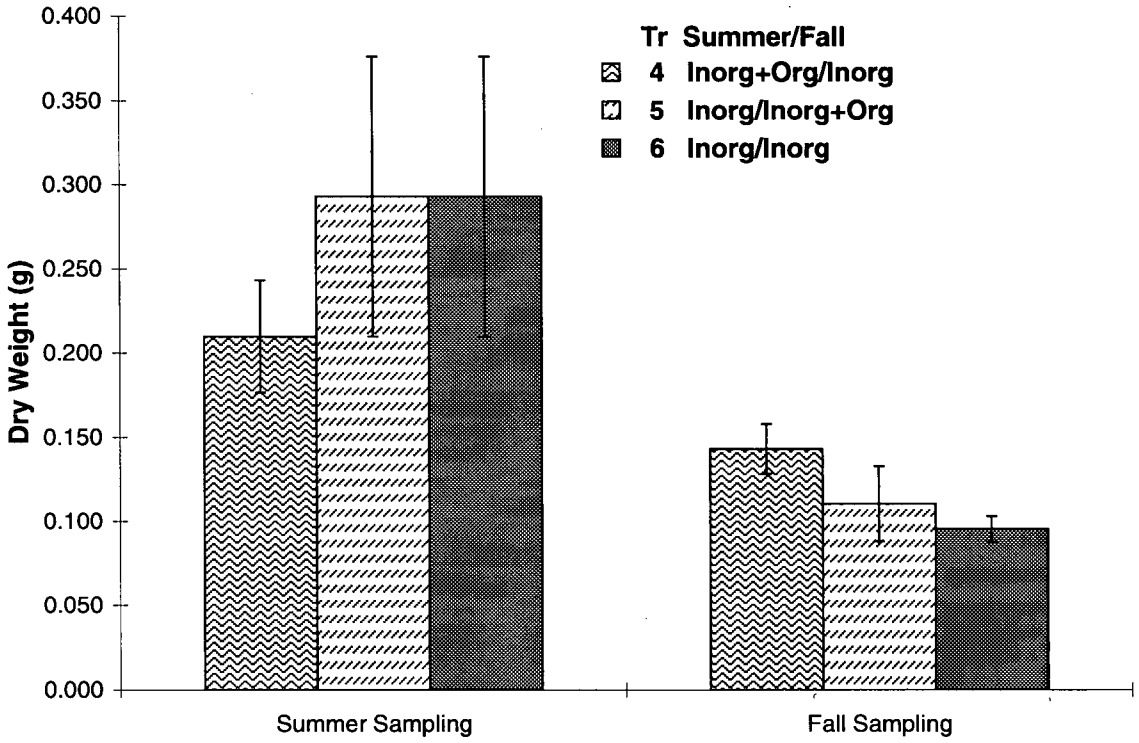


Figure 4.4: Mean (±SE). Macroinvertebrate biomass (>1250 µm, dry weight basis) during the two sampling events in the three

4.3.2 Fish response to nutrient inputs

4.3.2.1 Steelhead trout

Steelhead in the channels treated with organic fertilizer during the summer growing period (treatment 4) had a longer fork length (p<0.0001) and greater weight (p=0.0002) than the steelhead in channels with only inorganic nutrients present (treatment

5 and 6) (see Table 4.2 for average length, weight, and percent lipid values for steelhead trout from each treatment during each sampling event).

In November, the steelhead in treatment 4 channels remained longer than those in treatments 5 and 6 (p=0.0003 and p<0.0001). During this same sampling event, the fork length of the steelhead in treatments 5 and 6 did not differ significantly (p=0.4883). Furthermore, in November, the steelhead in treatment 4 continued to have greater weight than the steelhead in treatment 6 (p=0.0002), but the average weight of steelhead in treatment 5 was not significantly different from either treatments 4 or 6 (p=0.1121, p=0.0124).

There was no significant difference among the three treatments in terms of the length and weight of steelhead in the sampling period after winter. Also, there was no significant difference among treatments for steelhead percent lipid stores in any of the sampling periods.

Comparing the average length and weight values for steelhead in individual treatments between sampling events shows that steelhead in all treatments have significant length and weight increases over each time period (all p<0.0001). Another interesting result is that the average percent lipid content of steelhead in all treatments decreases significantly over winter (all p<0.0001).

See Appendix D for a complete listing of the statistical analysis and results for steelhead in all treatments over all time periods.

Table 4.2: Results summary for steelhead trout length, weight and percent lipid. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SE (mm)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	29 ± 2(16)	78 ± 1^{a}(76)*	86 ± 1^{a}(86)*	91 ± 1(40)*
5	Inorg	Inorg+**Org**	29 ± 2(17)	74 ± 1^{b}(135)*	82 ± 1^{b}(94)*	93 ± 1(45)*
6	Inorg	Inorg	28 ± 2(17)		80 ± 1^{b}(70)*	90 ± 1(42)*
Weight ± SE (g)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	0.1(16)	5.7 ± 0.2^{a}(76)*	7.4 ± 0.2^{a}(86)*	9.5 ± 0.3(40)*
5	Inorg	Inorg+**Org**	0.1(17)	4.7 ± 0.3^{b}(135)*	6.7 ± 0.2^{ab}(94)*	10.1 ± 0.2(45)*
6	Inorg	Inorg	0.1(17)		6.2 ± 0.2^{b}(70)*	9.7 ± 0.2(42)*
% Lipid ± SE (dry wt basis)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	-	22.4 ± 0.9(15)	18.7 ± 0.8(15)	9.8 ± 0.8(15)*
5	Inorg	Inorg+**Org**	-	20.5 ± 0.9(27)	16.8 ± 0.9(14)	9.6 ± 0.8(15)*
6	Inorg	Inorg	-		17.0 ± 0.8(16)	11.3 ± 0.8(15)*

The average fatty acid profiles (wt %) for steelhead in the three treatments during the September, November, and April sampling events are summarized in Table 4.3. Statistically significant results within a sampling event are indicated by a different superscript. For steelhead, there were no statistically significant differences between treatments for arachadonic acid (C20:4ω6), eicosapentaenoic acid (EPA, C20:5ω3), or docosahexaenoic acid (DHA, C22:6ω3) in any of the sampling periods. There was a higher percentage of the precursor fatty acid for EPA and DHA, linolenic acid (C18:3ω3), in the steelhead in treatment 4 in the September and November sampling periods. There was a lower percentage of docosapentaenoic acid (C22:5ω3, a second precursor to EPA and DHA) in the steelhead in treatment 4 in the November sampling period compared to the steelhead in treatments 5 and 6 ($p<0.05$). During the November sampling period, the sum of ω6 fatty acids was significantly larger in steelhead in treatment 6 than those in either treatments 4 or 5 ($p<0.05$). In April, the sum of ω6 fatty acids from steelhead in treatment 5 was higher than from those in treatment 4 ($p<0.05$) but neither was significantly different from the steelhead in treatment 6.

Table 4.3: Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	September Sampling Period		
Fatty Acid	Inorg+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	2.5±0.2	2.6±0.3	
16:0	22.1±0.9	21.8±0.9	
18:0	7.3±0.5	7.0±0.5	
22:0	0.2±0.1	0.2±0.1	
16:1	12.1±0.7	12.8±1.2	
18:1 ω-9	9.6±1.2	11.4±1.9	
18:1 ω-11	7.2±0.3	7.4±0.5	
20:1	0.3±0.1	0.2±0.1	
24:1	ND	0.1±0.1	
18:2 ω-6	4.7±0.3	4.7±0.5	
18:3 ω-3	9.3±0.8[a]	7.3±0.5[b]	
18:4 ω-3	0.9±0.2	0.7±0.1	
20:2 ω-6	0.2±0.1	0.1±0.1	
20:3 ω-3	0.6±0.0[a]	0.5±0.0[b]	
20:3 ω-6	0.2±0.1	0.2±0.1	
20:4 ω-3	0.6±0.1	0.6±0.0	
20:4 ω-6	1.0±0.2	1.2±0.2	
20:5 ω-3	5.8±0.5	6.0±0.4	
22:4 ω-6	ND	ND	
22:5 ω-3	1.2±0.1	1.4±0.2	
22:6 ω-3	4.4±0.4	4.9±0.9	
Σ ω-3	22.8±1.8	21.3±1.2	
Σ ω-6	6.1±0.5	6.1±0.5	

Table 4.3 (continued): Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	November Sampling Period		
Fatty Acid	Inorg+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	1.9±0.2	2.1±0.3	2.1±0.4
16:0	18.5±0.7	18.2±0.6	17.6±0.7
18:0	6.6±0.3	6.5±0.3	6.3±0.3
22:0	0.2±0.1	0.1±0.1	0.2±0.1
16:1	9.6±1.0	9.4±1.4	8.6±1.3
18:1 ω-9	10.5±1.3	11.2±2.1	11.1±1.7
18:1 ω-11	6.8±0.6	6.2±0.7	6.2±0.6
20:1	0.3±0.1	0.3±0.1	0.3±0.1
24:1	0.4±0.2[a]	0.3±0.1[ab]	0.1±0.1[b]
18:2 ω-6	5.2±0.3	5.3±0.8	5.3±0.4
18:3 ω-3	8.7±1.3[a]	7.1±0.7[b]	7.0±0.9[b]
18:4 ω-3	1.1±0.2	1.2±0.2	1.2±0.2
20:2 ω-6	0.4±0.1	0.4±0.1	0.5±0.2
20:3 ω-3	0.6±0.1[a]	0.5±0.1[b]	0.4±0.1[b]
20:3 ω-6	0.4±0.1	0.4±0.0	0.4±0.1
20:4 ω-3	0.7±0.1	0.6±0.2	0.6±0.1
20:4 ω-6	2.1±0.4	2.3±0.4	2.8±0.6
20:5 ω-3	6.5±0.4	7.3±0.8	6.8±0.6
22:4 ω-6	0.1±0.1	0.1±0.1	1.5±1.5
22:5 ω-3	1.7±0.1[a]	2.2±0.2[b]	2.1±0.2[b]
22:6 ω-3	7.1±0.8	8.2±0.6	8.4±1.2
Σ ω-3	26.3±1.8	27.1±1.4	26.5±1.6
Σ ω-6	8.3±0.8[a]	8.4±1.2[a]	10.6±1.9[b]

Table 4.3 (completed): Fatty acid profiles of juvenile steelhead trout from the three treatments during three sampling events. Values given as the average ± standard error of the mean. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$).

	April Sampling Period		
Fatty Acid	Inorg+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	1.6±0.2	1.4±0.1	1.6±0.2
16:0	19.0±0.7^{a}	17.2±0.5^{b}	18.0±0.6^{b}
18:0	5.7±0.4	5.5±0.3	5.7±0.3
22:0	ND	ND	ND
16:1	6.4±1.0	6.1±0.8	6.4±1.0
18:1 ω-9	9.8±0.7^{a}	11.3±1.2^{b}	10.3±0.9^{ab}
18:1 ω-11	5.5±0.5	5.6±0.5	5.6±0.6
20:1	0.2±0.1^{a}	0.4±0.0^{b}	0.3±0.1^{b}
24:1	0.4±0.1	0.4±0.2	0.3±0.1
18:2 ω-6	3.3±0.5	4.0±0.9	3.4±0.4
18:3 ω-3	4.9±0.8	5.1±0.7	5.2±1.1
18:4 ω-3	0.6±0.1^{a}	0.7±0.1^{a}	0.5±0.1^{b}
20:2 ω-6	0.3±0.1^{a}	0.5±0.1^{b}	0.5±0.1^{b}
20:3 ω-3	0.4±0.0	0.5±0.1	0.5±0.1
20:3 ω-6	0.4±0.0	0.5±0.1	0.5±0.1
20:4 ω-3	0.7±0.1	0.7±0.1	0.8±0.1
20:4 ω-6	3.1±0.4	3.4±0.3	3.3±0.5
20:5 ω-3	10.1±0.6	9.6±0.8	9.7±0.7
22:4 ω-6	0.2±0.1^{a}	0.4±0.1^{b}	0.3±0.1^{b}
22:5 ω-3	2.7±0.2	2.8±0.3	2.9±0.3
22:6 ω-3	15.9±2.1	14.9±2.7	14.8±2.7
Σ ω-3	35.3±1.8	34.3±3.1	34.4±2.4
Σ ω-6	7.3±0.6^{a}	8.8±0.9^{b}	8.0±0.6^{ab}

4.3.2.2 Coho salmon

4.3.2.2.1 Age-0 coho salmon

In September, age-0 coho in treatment 4 had a longer fork length ($p<0.0001$) but did not weight significantly more than the age-0 coho in treatment 5 or 6 ($p=0.0014$) (see Table 4.4 for average length, weight, and percent lipid values for age-0 coho from each treatment during each sampling event).

During the November sampling period, the age-0 coho in treatment 4 were longer ($p<0.0001$) and weighed more ($p=0.0002$) than the age-0 coho in treatment 5. Age-0 coho in treatment 6 had greater fork length ($p<0.0001$) but did not differ in weight ($p=0.0505$) compared to age-0 coho in treatment 5 in November. Treatment 4 and 6 age-0 coho were not statistically different.

There was no statistical difference among the three treatments after winter for age-0 coho length or weight. Also, there was no statistical difference among the three treatments for age-0 coho percent lipid stores during any of the sampling periods.

The average length of age-0 coho increases significantly in each treatment between all sampling periods (all $p<0.0001$). The average weights of age-0 coho increase significantly in all treatments over the summer growing period and over winter (all $p<0.0001$); however, there is no significant change in weight between September and November sampling events in any treatment ($p_{Tr4}=0.0174$, $p_{Tr5}=0.0037$, $p_{Tr6}=0.0016$). The age-0 coho in treatment 6 show the same trend as the steelhead in all treatments inasmuch as their average percent lipid store decreases over winter ($p=0.0002$). There are no other significant changes in percent lipid stores in age-0 coho in any treatment between sampling periods.

See Appendix D for a complete listing of the statistical analysis and results for age-0 coho in all treatments over all time periods.

Table 4.4: Results summary for age-0 coho salmon length, weight, and percent lipid. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SE (mm)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	32 ± 1(27)	63 ± 1^{a}(157)*	68 ± 1^{a}(218)*	81 ± 1(52)*
5	Inorg	Inorg+**Org**	32 ± 1(27)	59 ± 1^{b}(232)*	63 ± 1^{b}(221)*	80 ± 1(60)*
6	Inorg	Inorg	33 ± 1(27)		66 ± 1^{a}(174)*	80 ± 1(50)*
Weight ± SE (g)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	0.3 ± 0.3(27)	3.3 ± 0.2(157)*	3.9 ± 0.1^{a}(218)	7.0 ± 0.3(52)*
5	Inorg	Inorg+**Org**	0.3 ± 0.3(27)	2.6 ± 0.2(232)*	3.1 ± 0.1^{b}(221)	6.5 ± 0.2(60)*
6	Inorg	Inorg	0.3 ± 0.3(27)		3.5 ± 0.1^{ab}(174)	6.6 ± 0.3(50)*
% Lipid ± SE (dry wt basis)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	-	17.8 ± 1.1(9)	13.6 ± 1.0(10)	10.5 ± 0.8(15)
5	Inorg	Inorg+**Org**	-	17.0 ± 1.3(13)	12.3 ± 1.0(10)	10.8 ± 0.8(15)
6	Inorg	Inorg	-		12.2 ± 1.0(10)	9.7 ± 0.8(15)*

In coho, there were no statistically significant differences among the treatments for arachadonic acid (C20:4ω6), eicosapentaenoic acid (EPA, C20:5ω3), or docosahexaenoic acid (DHA, C22:6ω3) during any of the sampling periods. During the September sampling period, the percentage of the precursor fatty acid linolenic acid was larger in the coho from treatment 4 ($p<0.05$). The percentage of docosapentaenoic acid was lower in the coho in treatment 4 during the November sampling period compared to the coho in treatment 6 ($p<0.05$) but neither was significantly different from those fish in treatment 5.

Table 4.5: Fatty acid profiles of juvenile coho salmon from the three treatments during the three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	September Sampling Period		
Fatty Acid	Inorg+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	3.3±0.3	3.0±0.2	
16:0	21.2±0.6	20.8±0.8	
18:0	7.9±0.4	7.7±0.5	
22:0	0.2±0.1	0.2±0.1	
16:1	9.3±0.7	9.4±0.9	
18:1 ω-9	14.8±1.7	16.1±2.1	
18:1 ω-11	5.6±0.5	5.6±0.7	
20:1	0.3±0.1	0.2±0.1	
24:1	ND	0.1±0.1	
18:2 ω-6	6.3±0.6	6.8±1.0	
18:3 ω-3	6.7±0.5[a]	5.5±0.4[b]	
18:4 ω-3	1.8±0.4	1.7±0.2	
20:2 ω-6	0.2±0.1	0.1±0.1	
20:3 ω-3	0.2±0.1	0.1±0.1	
20:3 ω-6	0.1±0.1	0.1±0.1	
20:4 ω-3	0.2±0.1	0.1±0.1	
20:4 ω-6	1.3±0.1	1.5±0.2	
20:5 ω-3	4.1±0.1	4.3±0.5	
22:4 ω-6	ND	ND	
22:5 ω-3	1.2±0.1	1.2±0.2	
22:6 ω-3	4.4±0.4	5.3±0.6	
Σ ω-3	18.4±0.7	18.1±1.2	
Σ ω-6	8.0±0.7	8.5±1.0	

Table 4.5 (continued): Fatty acid profiles of juvenile coho salmon from the three treatments during the three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	November Sampling Period		
Fatty Acid	Inor+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	2.1±0.2	2.1±0.2	2.0±0.3
16:0	18.2±0.5	18.6±0.7	17.6±0.7
18:0	6.6±0.3	6.8±0.3	6.6±0.2
22:0	0.2±0.1	0.1±0.1	0.1±0.1
16:1	8.1±1.4	8.8±2.0	7.2±1.5
18:1 ω-9	13.6±3.1	11.9±2.2	12.4±1.3
18:1 ω-11	5.2±0.7	5.9±0.9	5.4±0.7
20:1	0.4±0.1	0.4±0.1	0.3±0.1
24:1	0.3±0.1	0.4±0.2	0.4±0.2
18:2 ω-6	5.9±0.9	5.4±0.5	5.2±0.5
18:3 ω-3	7.8±1.8	6.5±0.9	6.3±1.1
18:4 ω-3	1.5±0.4	1.1±0.2	1.2±0.2
20:2 ω-6	0.4±0.1	0.4±0.2	0.5±0.1
20:3 ω-3	0.4±0.1	0.4±0.1	0.4±0.1
20:3 ω-6	0.4±0.0	0.4±0.1	0.4±0.0
20:4 ω-3	0.5±0.2	0.6±0.1	0.5±0.1
20:4 ω-6	2.2±0.4	2.4±0.5	3.0±0.5
20:5 ω-3	5.7±0.8	6.9±0.7	6.7±0.6
22:4 ω-6	1.1±0.7	0.2±0.1	1.4±1.2
22:5 ω-3	1.7±0.2^{a}	2.0±0.2^{ab}	2.2±0.1^{b}
22:6 ω-3	7.3±1.0	8.9±1.6	10.2±2.3
Σ ω-3	25.0±2.4	26.3±1.9	27.5±2.0
Σ ω-6	9.7±2.1	8.8±1.2	10.3±1.8

Table 4.5 (completed): Fatty acid profiles of juvenile coho salmon from the three treatments during the three sampling events. These values do not total 100% because some minor fatty acids are not reported. Values in rows from a single sampling event with different superscripts (a,b) are significantly different (SNK, $p<0.05$)

	April Sampling Period		
Fatty Acid	Inorg+**Org** Inorg	Inorg Inorg+**Org**	Inorg Inorg
14:0	1.9±0.2	1.8±0.3	1.9±0.2
16:0	18.3±0.7	18.5±0.7	18.1±0.8
18:0	5.6±0.3[a]	5.2±0.2[b]	5.5±0.2[a]
22:0	ND	ND	ND
16:1	5.9±0.8	6.2±1.1	5.3±0.7
18:1 ω-9	9.9±0.9	10.1±0.7	9.5±0.7
18:1 ω-11	5.0±0.4	4.8±0.4	5.0±0.3
20:1	0.4±0.1	0.3±0.1	0.4±0.1
24:1	0.3±0.2	0.3±0.2	0.4±0.2
18:2 ω-6	3.4±0.5	3.4±0.6	3.3±0.7
18:3 ω-3	4.4±0.9	5.2±1.3	4.1±0.7
18:4 ω-3	1.1±0.3	1.2±0.3	1.0±0.2
20:2 ω-6	0.4±0.1	0.3±0.1	0.3±0.2
20:3 ω-3	0.3±0.1	0.4±0.1	0.3±0.1
20:3 ω-6	0.4±0.0[a]	0.2±0.1[b]	0.4±0.1[a]
20:4 ω-3	0.4±0.0	0.4±0.0	0.4±0.0
20:4 ω-6	3.5±0.4	3.1±0.5	3.7±0.4
20:5 ω-3	10.0±0.9	10.4±0.8	10.2±0.9
22:4 ω-6	0.4±0.1	0.3±0.1	0.4±0.1
22:5 ω-3	2.9±0.2	3.0±0.3	3.2±0.2
22:6 ω-3	15.5±1.9	15.5±2.7	16.3±1.9
Σ ω-3	34.6±1.7	36.0±2.1	35.5±1.9
Σ ω-6	8.2±0.7	7.4±0.6	8.2±1.0

4.3.2.2.2 Age-1 coho salmon

Age-1 coho did not have significantly different length or weight values for any of the sampling events conducted during the research (see Table 4.6 for average values of length and weight for age-1 coho from each treatment during each sampling event).

Age-1 coho in all treatments show a significant increase in length and weight through the summer growth period (weight: $p_{Tr4}<0.0001$, $p_{Tr5/6}=0.0002$; length: all $p<0.0001$). Only age-1 coho in treatment 5 show a significant increase in average length and weight over winter (both $p<0.0001$). There is no significant difference in length and weight values for age-1 coho over the fall sampling period in any treatment.

See Appendix D for a complete listing of the statistical analysis and results for age-1 coho in all treatments over all time periods.

Table 4.6: Results summary for age-1 coho length and weight. Values in columns from a single sampling event with different superscripts (a,b) are significantly different (Bonferroni critical difference $p<0.007$). Values in parenthesis are number of individuals included in the statistical analysis. Values in rows with an asterix (*) indicate significant difference between the average from the previous sampling period (Bonferroni critical difference $p<0.007$).

Length ± SE (mm)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	89 ± 1(150)	102 ± 3(9)*	102 ± 2(20)	101 ± 4(12)
5	Inorg	Inorg+**Org**	89 ± 1(150)	96 ± 2(28)*	97 ± 2(18)	113 ± 3(11)*
6	Inorg	Inorg	88 ± 1(150)		102 ± 2(10)	107 ± 4(6)
Weight ± SE (g)	Treatment		Sampling Period			
	Summer	Fall	June	September	November	April
4	Inorg+**Org**	Inorg	8.9 ± 0.1(150)	12.1 ± 0.6(9)*	11.0 ± 0.4(20)	11.1 ± 0.9(12)
5	Inorg	Inorg+**Org**	8.9 ± 0.1(150)	10.0 ± 0.5(28)*	9.5 ± 0.5(18)	13.1 ± 0.8(11)*
6	Inorg	Inorg	8.5 ± 0.1(150)		11.0 ± 0.6(10)	13.4 ± 0.9(6)

4.3.3 Data Comparisons between experimental years with and without heavy salmon runs

4.3.3.1 Steelhead specific growth rate

The specific growth rates of steelhead in treatments 1 and 6 are not significantly different between years for any sampling periods (see Figure 4.5). During the summer (June-September) sampling period, the specific growth rates of steelhead in treatments 2 and 3 are significantly lower than treatments 4, 5, and 6 (between years), but not significantly different from treatment 1 carried out the same sampling year. The specific growth rate for steelhead in treatment 1 in the first study is significantly lower than in treatment 4 in the following year, but not for treatments 5 and 6. In the fall (September-November), the specific growth rate of steelhead in treatment 2 is significantly greater when compared to treatments 3, 4, and 6.

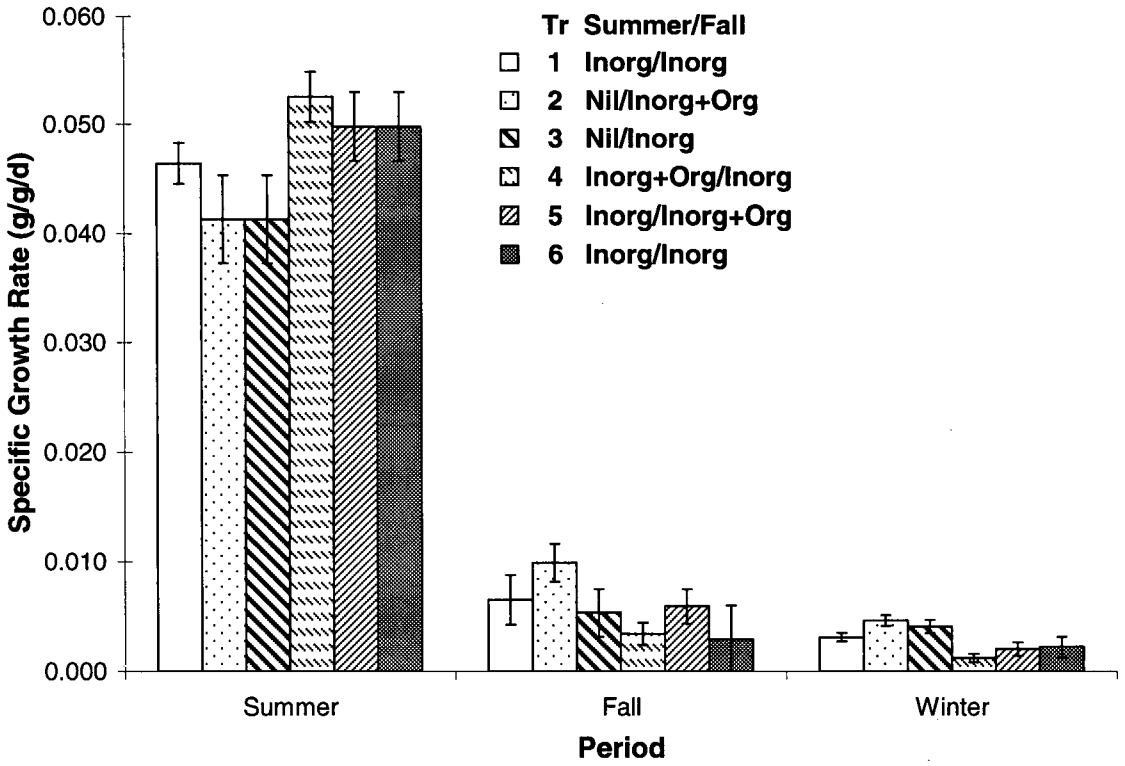


Figure 4.5: Steelhead specific growth rates from 2003/04 and 2004/05 study years (±HSD)

4.3.3.2Age-0 coho specific growth rate

Specific growth rates between years during the summer sampling period cannot be compared due to differences in the size of age-0 coho at the beginning of the sampling period. Specific growth rates are mass dependant such that smaller fish tend to grow more quickly. The age-0 coho specific growth rates in treatments 1 and 6 are equal in the fall and winter sampling events over the two years (see Figure 4.6). The age-0 coho specific growth rate in treatment 1 is significantly lower in the winter sampling period compared to that of treatment 2 conducted the same sampling year.

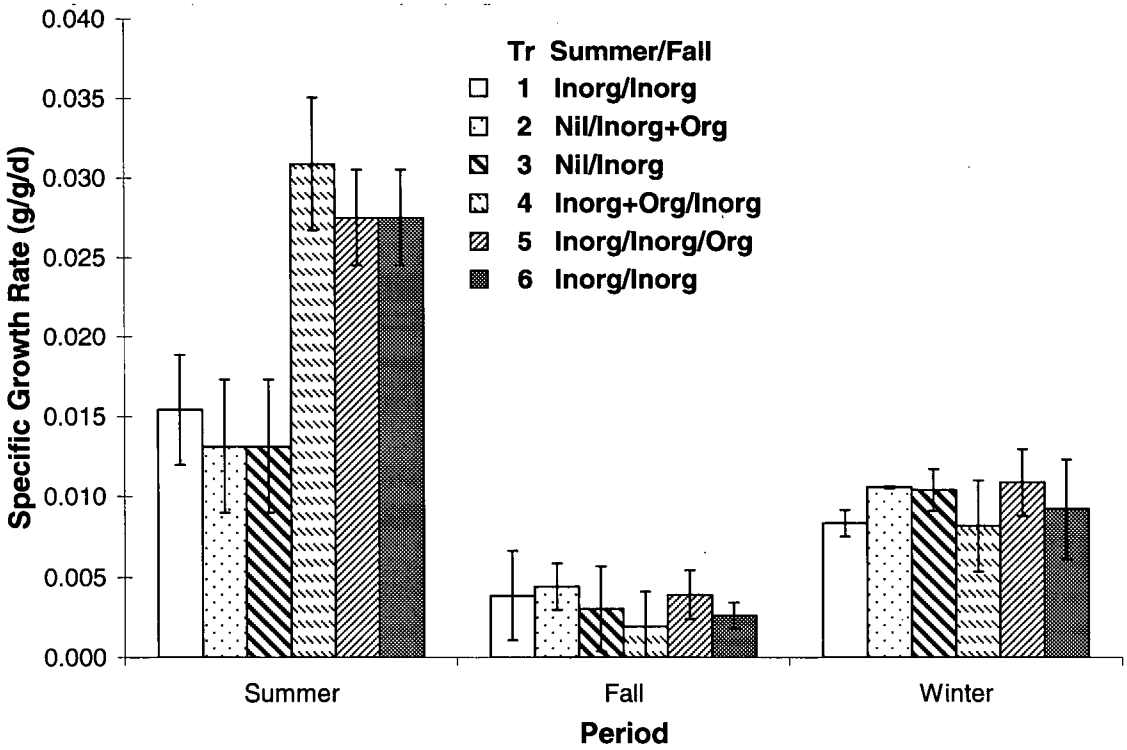


Figure 4.6: Coho specific growth rates from 2003/04 and 2004/05 study years. Mean (±HSD)

4.3.3.3 Total biomass and total numbers

Average total biomass of juvenile salmonids within the treatments in 2004 (treatments 4, 5, and 6) are not significantly different for any sampling period (see

Figure 4.7). Total biomass of juvenile salmonids in the treatments increases through the summer and fall, and then remains constant over-winter. In treatment 1, the total biomass of juvenile salmonids measured prior to the onset of winter (day 170, November) and after winter (day 336, April) is significantly higher than in treatment 6, even though the treatment (source and timing of phosphorous) is similar.

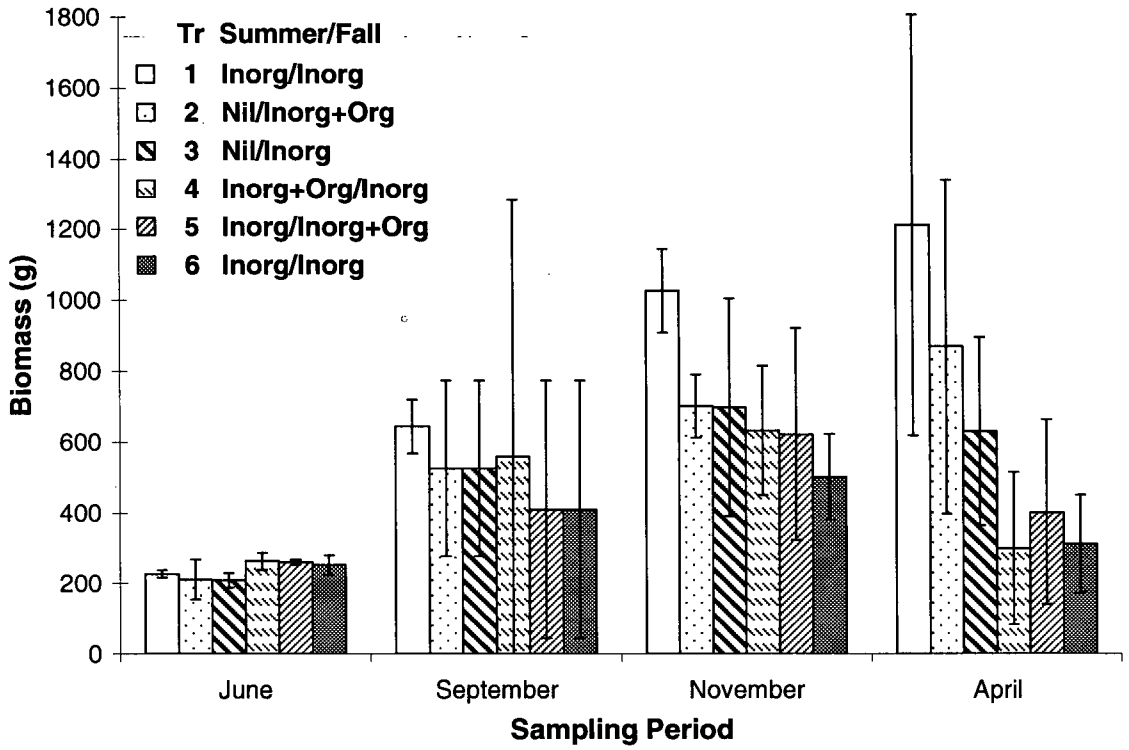


Figure 4.7:. Average total biomass of steelhead and coho from each treatment. Mean (±HSD)

Figure 4.8 shows the total number of fish in each channel for each treatment. Comparing the values and taking into account the honestly significant difference intervals, it is apparent that all treatments experienced a decline in population between June and September. The exception to this is treatment 2, where the variation in population numbers between channels within the treatment masks any potential drop in population. The number of fish in treatment 6 also decreases significantly between the

November and April sampling events. Another interesting comparison is between treatment 1 and treatment 6. The population of fish in treatment 1 was significantly greater than the population in treatment 6 in November. This illustrates the effect of nutrient concentration on survival of fish considering that the timing and type of phosphorous was similar for both treatments and the major difference was the concentration of available inorganic phosphorous. Figure 4.9 shows that the same trends extend to the individual species in each treatment.

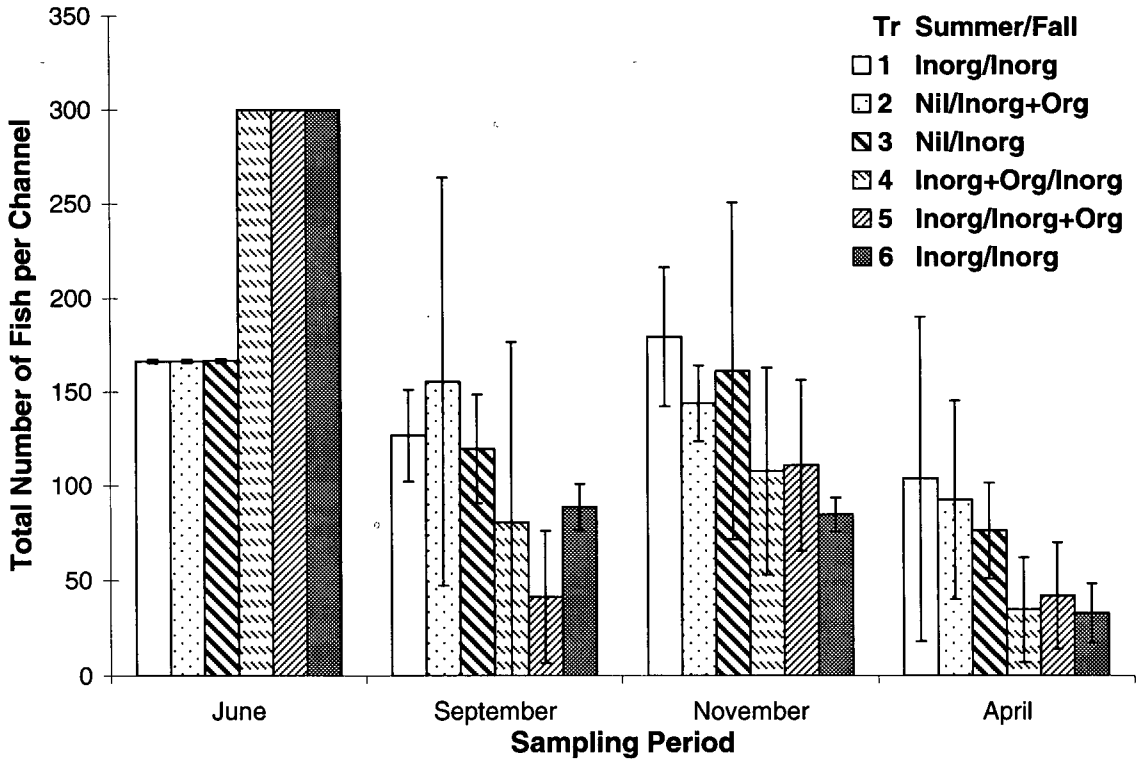


Figure 4.8: Average total number of steelhead and coho from each treatment. Mean (±HSD)

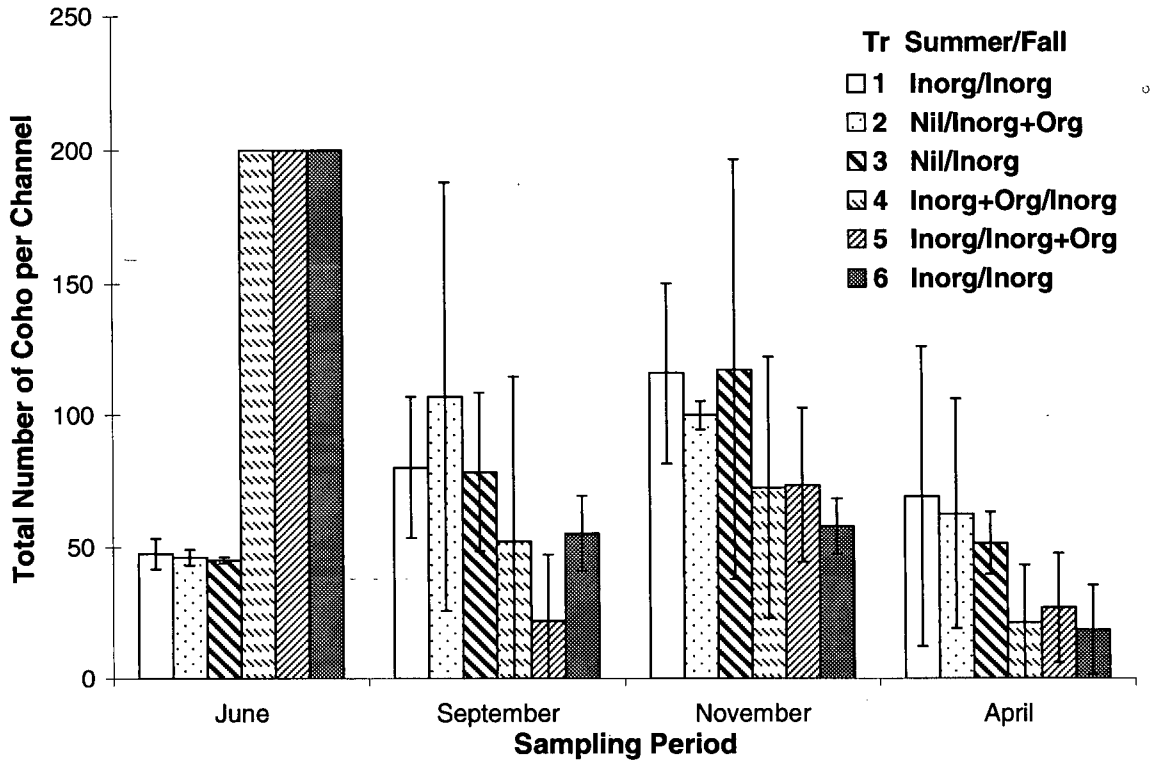


Figure 4.9: Average total number of coho from each treatment. Mean (±HSD)

4.4 Discussion

This research is a continuation of the ideas developed in the previous work described earlier in chapter 3. It is presented in an attempt to further quantify the benefits of possible nutrient management schemes and to elucidate the most effective method of nutrient replacement. The results of this study show that having an organic source of fertilizer available will stimulate the growth of juvenile resident steelhead, and to some extent, coho. Resident juveniles can feed directly on the organic material and benefit from an increase in available biological macro- and micro-molecules (Bilby et al., 1998, Wipfli et al., 2004, 1998, Wipfli et al., 2003). There was no algal or macroinvertebrate response to having increased organic material compared to inorganic nutrients alone, so it

can be assumed that benefits from increases in dissolved inorganic nutrients due to organic inputs were negligible and benefits to resident juveniles came primarily from the direct feed pathway. While there was no difference among treatments over-winter in steelhead average length and weight, having organic fertilizer available in the summer growing period produced a measurable response in steelhead length and weight over and above the response from having inorganic nutrients available. The difference remained through November but did not carry through over-winter. These results show that steelhead benefit from having a direct source of carbon and nutrients through the summer growing months and are able to increase their skeletal growth and lipid storage. The addition of organic fertilizer in the fall caused a catch-up effect such that the average weight of steelhead during the November sampling period (in those channels fertilized with the pollock bone-meal) was not statistically different from either the summer fertilized or inorganic only treatments. This increase in the steelhead from the fall organically fertilized channels did not follow through over-winter.

For age-0 coho, adding a source of organic nutrients during the summer growing period also translated into increased length gains but not increased weight compared to coho in treatments where inorganic nutrients were available. This suggests that there was sufficient energy surplus for skeletal growth but not enough for lipid storage. Coho in the channels that were fertilized with organic pollock bone-meal in the fall did not experience an increase in length or weight. These coho were statistically smaller than both other treatments, with lower weight than the coho in channels treated with organic fertilizer in the summer growing period. Considering that the steelhead in the same treatment did grow over the same time period, the lack of response in coho suggests that the steelhead

out competed or were able to prey upon the smaller coho. As juveniles enter their second year of freshwater residency, their diet shifts to piscivorous activity, and the effects of predation on smaller individuals increases (Higgs et al., 1995).

The storage of energy, expressed as percent lipid or lipid stores, for both steelhead and coho resident juvenile salmonids in all three treatments showed the highest levels in September and decreased through the November sampling period and over-winter. This is consistent with previous work conducted using Atlantic salmon (*Salmo salar*) (Higgins and Talbot, 1985) and suggests that the fish are depleting their energy stores during the period of low food availability. Average levels in all species at all sampling periods are above the minimum viable level, as outlined in the previous chapter.

For the channels fertilized with organic fertilizer over the summer growing period, the average specific growth rate of steelhead is 0.051 $gg^{-1}d^{-1}$. For the channels with dissolved inorganic phosphorous present in the system the average specific growth rate of steelhead is 0.050 $gg^{-1}d^{-1}$. Based on the average temperature in the system, the specific growth rate is higher than the 100% satiation growth rate 0.032 $gg^{-1}d^{-1}$ given for steelhead by Sullivan et al. (2000). This value is based on a 1 g weight, and the steelhead in the channel start at a smaller size, so they will theoretically be growing at an increased rate to compensate. The result does suggest that there is sufficient food to support the increase in growth and maintain a high level of feeding. For coho, the growth rates in the treatments are 0.031 $gg^{-1}d^{-1}$ and 0.028 $gg^{-1}d^{-1}$ for organically treated and inorganic only treatments respectively. The growth curves for coho given by Sullivan et al. (2000) list the growth rate at 100 % satiation at the average summer stream temperature as

0.025 $gg^{-1}d^{-1}$. This suggests that the coho in this experiment are also feeding in a system that is productive enough to support a high growth rate.

There was a significant difference in summer growth rates of coho between treatments 1 and 6. The coho in treatment 6 grew nearly twice as fast as treatment those in treatment 1. Initially, the coho in treatment 6 were smaller (0.3 g versus 1 g) and smaller fish grow faster. However, the growth rate differences between the treatments far exceeded the expected differences due to size (7.5% versus 7%) (Austreng et al., 1987). Nutrients and algae standing biomass in treatment 6 channels were at a high level for a longer period of time than that in treatment 6 (see Figures 3.2, 4.2 and 4.3), so it is likely that coho in systems with Steelhead trout require an extended summer time period of nutrients to achieved enhanced growth.

Specific growth rates drop in the fall and winter, although this is to be expected since the temperature regime is dropping and juvenile salmon are no longer in logarithmic growth but are allocating energy surplus to storage activities to prepare for the winter (Jonsson and Jonsson, 1998, Metcalfe et al., 2002, Post and Parkinson, 2001).

Results from average total biomass from all of the treatments show that for all treatments there is an increase in biomass through experiment through to the fall and that the total biomass over-winter does not change. This suggests that the growth observed in the channels through the winter could be explained by an increase in average mass and length due a reduction in smaller individuals from predation and/or over-winter mortality rather than a true indication of growth. The increase in biomass within the channels from the beginning of the experiment to the onset of winter indicates that individuals are growing and thereby increasing biomass and channel production.

Figures 4.5 to 4.9 present a comparison of all of the treatments during the two treatment years for biomass, growth and total numbers of fish. The results show that the treatments with inorganic nutrients through the summer and fall were not complete replicates because the number of fish, the total biomass of fish, and the growth rates of fish in treatments 1 and 6 were significantly different. The conditions within the treatment channels were the same for temperature and periphyton biomass, but the concentrations of inorganic nutrients were higher in the first year of treatment. This finding suggests that having higher concentrations of inorganic nutrients in the fall will affect the productivity of the stream over-winter, regardless of the autotrophic limiting conditions of low water temperatures and light levels. For inorganic nutrient replacement strategies in the summer, considerable research has been conducted to establish effective target levels of phosphorous (Ashley and Slaney, 1997, Ashley and Stockner, 2003, Bothwell, 1988, Mundie et al., 1991). This result, however, illustrates that more work should be conducted to establish target levels for fall applications of inorganic nutrients in conjunction with the density of organic material (carcass or organic fertilizer).

4.5 Conclusions

For both coho and steelhead, there was no difference in after-winter length or weight among treatments 4 inorganic and organic phosphorous present during the summer, treatment 5 dissolved inorganic phosphorous available in the summer and organic phosphorous in combination with inorganic phosphorous available during the fall, and treatment 6 inorganic phosphorous available for the duration of the study. Considering that treatment 6 is similar to treatment 1 of the previous year, and that

treatment 1 produced a measurable benefit to steelhead compared to a nutrient scheme where there was no phosphorous present in the summer and only inorganic phosphorous available in the fall, the results of this study suggests that any of the nutrient schemes presented herein will produce measurable benefits for steelhead. In the interests of cost efficiency, however, it may not be necessary to use fertilizer to provide sources of both inorganic and organic phosphorous directly. More investigation needs to be conducted to determine whether other nutrient treatment schemes will produce more benefits when compared to the presence of inorganic nutrients through both the summer and fall periods. There is a need to investigate further whether other combinations of timing and organic nutrient loading will produce increased results in coho and steelhead. Also, more investigation should be conducted into the concentrations, not just timing and source, of phosphorous inputs to mimic historical inputs into streams in the Pacific Northwest. There may also be a need to revisit pollution prevention regulations for carcass, organic fertilizer, and inorganic fertilizer addition as applied to nutrient replacement strategies.

Chapter 5: Conclusions and Future Work

The objective of this research was to use food web theory and experimentation to investigate the effects of timing and source of stream phosphorous inputs on the over-winter length, weight, and lipid stores of resident juvenile salmonids.

It was shown through the investigation into food web theory that in streams in the Pacific Northwest, which are demonstrated to be nutrient poor, management schemes to replace historical phosphorous inputs have the potential to increase resident fish size, lipid stores and consequently survival. The active addition of fertilizer to bridge the gap between current phosphorous inputs and historical types and timings of phosphorous inputs will increase the availability of forage for fish populations either indirectly through the bottom-up effects within the food web or directly through consumption of the fertilizer. In other areas of the world facing similar nutrient deficiencies, the management schemes developed in this thesis could be broadened and tailored to the specific food web needs and targeted fish benefits within the new system.

From the possibilities outlined in Table 2.2, field trials of five management schemes chosen for this research project showed that the response of coho salmon and steelhead trout fry was not universal for treatment or species. In particular, resident steelhead trout fry were shown to respond positively to having inorganic phosphorous through the summer and fall, and to having organic and inorganic phosphorous in the fall above what can be expected from steelhead fry in a stream with inorganic nutrients only available in the fall. For the same treatments, coho did not benefit in the over-winter sampling. These results answer the management question of whether inorganic fertilization in the summer or organic fertilization in the fall is more effective for

steelhead production. It also represents a comparison of the two management strategies and indicates that they are both effective at increasing steelhead production above an unfertilized stream.

To build upon the results obtained in the first year of field trials, the second year of experimentation focused on augmenting streams with inorganic phosphorous already available with an organic fertilizer either in the summer or the fall. The growth rate and size of juveniles in treatments with increased organic phosphorous displayed beneficial response during the period of application, but these increases did not necessarily carry through to benefits in over-winter size. This result suggests that in systems with adequate phosphorous available an increase in organic inputs, at the concentrations used here, will not produce significant after-winter benefits to juvenile salmonids.

There is a need for more work to be conducted to investigate the target concentrations necessary to produce measurable benefits to resident juveniles. It appears that concentration, not just be the timing and source of nutrients, is also important to juvenile production. To investigate the effects of concentration on fish production, experimentation could be set up such that the length of each channel is partitioned using nets or other fish barriers, and fertilizer (either organic or inorganic) could be added in a cumulative experiment. The concentration of available phosphorous would increase down the length of the channel. The Centennial channel, research channels are a state of the art research facility and there is considerable opportunity to investigate the short- and long-term effects of various nutrient conditions on varying species compositions in a controlled, but complex near-mimic of a natural stream system.

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Appendix A: Explanation for Figure 2.3

Assumptions:

1) Salmon decompose such that the mass fraction of carcass remaining at time, t, can be described by the equation from Parmenter and Lamarra (1991):

$$y=0.74e^{-0.061t}+0.26e^{-0.002t}$$

2) Salmon are 0.35 % phosphorous on a wet weight basis (Larkin and Slaney, 1997)
3) Background phosphorous levels are 2 µg/L
4) The concentration of available phosphorous is given as:

$$conc = -(m(t)-m(t_0))/(t*D) + background$$

Where t is the time the carcass has been in the stream, m is the mass of phosphorous in the carcass, D is the discharge of the stream (Chilliwack River), and background is the background phosphorous concentration in the stream

5) The available phosphorous in river water often increases by a factor of 2 fold during and immediately following increases in discharge from the early stages of snowmelt (Wetzel, 2001).
6) Historical input of salmon to British Columbia is 122, 940,000 kg (Gresh et al., 2000)
7) Percentage of total production for each of the Pacific Salmon species (Bigler et al., 1996):
    a. Chinook 3 %
    b. Coho 5 %
    c. Sockeye 14 %
    d. Chum 21 %
    e. Pink 57 %
8) Average size of mature salmon for each of the Pacific Salmon species (Bigler et al., 1996):
    a. Chinook 6.04 kg
    b. Coho 2.52 kg
    c. Sockeye 2.55 kg
    d. Chum 4.63 kg
    e. Pink 1.43 kg
9) Total production of fish returning to spawn for each of the Pacific Salmon species (in millions of kg):
    a. Chinook 3.69
    b. Coho 6.15
    c. Sockeye 17.22
    d. Chum 25.83
    e. Pink 70.11
10) Total number of fish returning to spawn for each of the Pacific Salmon species (in millions):
    a. Chinook 0.61
    b. Coho 2.44
    c. Sockeye 6.75
    d. Chum 5.58
    e. Pink 49.03

11) Based on the Pacific Scientific Advice Review Committee (PSARC) Stock Status Reports (SSR) produced by Fisheries and Oceans Canada, the Fraser River watershed contains approximately 50 % of the salmon escapement in British Columbia for chinook and coho. This estimate was used for pink and chum salmon as well.
12) Due to a lack of historical numbers available for the numbers of spawning salmon returning to the Fraser River and the proportion that spawn in the Chilliwack River, the proportion of pink salmon returning to the Chilliwack River were used as a surrogate for chinook, coho and chum salmon. According to the estimates for Pink salmon in 2003, approximately 20 million fish entered the Fraser River to spawn. Of that 2 million an estimated 70,000 returned to spawn in the Chilliwack (based on water chemistry results, personal communications with the Chilliwack River Hatchery staff and Fisheries and Oceans Canada Stock Status Reports). Based on those results, it was assumed that approximately 0.4 % of salmon that spawn in the Fraser River spawn in the Chilliwack River.
13) Based on the assumptions listed above, the historical numbers of spawning salmon in the Chilliwack River system are as follows:
    a. Chinook 1220
    b. Coho 4880
    c. Sockeye 1300
    d. Chum 11,160
    e. Pink 98,060

See the following Excel spreadsheet for calculations.

Calculations for peaks of phosphorous:
m(i) = t*D*conc*[1-(0.74e^(-0.061t)+0.26e^(-0.002t))]^(-1)

%P 0.35

Pink			Sept15-Dec1						
m (g)	t (d)	t(s)	D (m3/s)	D (L/s)	conc (ug/L)	()	m (kg)	carcass m(kg)	# of fish
447984	15	1296000	13	13000	12	0.548698	447.984	127995	89507
446366	30	2592000	16	16000	6.85	0.363565	446.3663	127533	89184
446000	45	3888000	20	20000	4.1	0.285166	446	127429	89111
448789	60	5184000	28	28000	2.32	0.249641	448.7889	128225	89668
442629	75	6480000	25	25000	2.1	0.231411	442.6291	126465	88437
			Sep-01						
Chinook									
25845	15	1296000	18	18000	0.5	0.548698	25.84523	7384	1223
Coho			Nov-01						
43075	15	1296000	20	20000	0.75	0.548698	43.07538	12307	4884
Chum			Dec1-Jan1						
193839	15	1296000	25	25000	2.7	0.548698	193.8392	55383	11962
193453	30	2592000	25	25000	1.9	0.363565	193.4525	55272	11938
184927	45	3888000	20	20000	1.7	0.285166	184.9268	52836	11412

Calculated values of available phosphorous:

01-Jan	01-Feb	01-Mar	01-Apr	01-May	15-May	01-Jun	01-Jul	
4	2	3	3	4	6	4	2	
01-Aug	01-Sep	15-Sep	01-Oct	15-Oct	01-Nov	15-Nov	01-Dec	15-Dec
2	7	14	8.85	6.1	5	4.1	6.8	4

Appendix B: Water chemistry and chlorophyll a data

The following Appendix present the water chemistry and chlorophyll a data collected for the experiment. The raw data is presented in its entirety in the attached data disc, Appendix D.

2003 Water Chemistry Data

nitrogen - total (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
4-Jun-03	104	9	3	104	11	4	106	5	2
27-Jun-03	90	5	2	97	5	2	100	8	3
14-Jul-03	80	5	2			0	86	8	3
1-Aug-03	68	4	1			0	80	12	4
15-Sep-03	97	9	3	66	12	4	87	17	6
6-Oct-03	454	19	6	463	11	4	460	18	6
6-Nov-03	220	58	19	290	30	10	228	57	19
27-Nov-03	176	41	14	246	279	93	307	386	129

nitrogen - NH_4 (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stden	sem
4-Jun-03	8	4	1	8	5	2	11	8	3
27-Jun-03	5	0	0	5	0	0	6	2	1
14-Jul-03	5	0	0			0	7	2	1
1-Aug-03	5	1	0			0	17	17	6
15-Sep-03	5	0	0	5	0	0	6	2	1
6-Oct-03	191	25	8	210	27	9	193	63	21
6-Nov-03	69	15	5	76	13	4	76	15	5
27-Nov-03	18	4	1	19	2	1	17	1	0

nitrogen - LL NO_{2+3} (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
4-Jun-03	66	1	0	65	2	1	66	3	1
27-Jun-03	50	3	1	53	3	1	51	4	1
14-Jul-03	42	3	1			0	48	6	2
1-Aug-03	38	7	2			0	40	1	0
15-Sep-03	31	3	1	36	1	0	34	2	1
6-Oct-03	82	6	2	79	7	2	83	5	2
6-Nov-03	107	10	3	105	6	2	105	6	2
27-Nov-03	79	1	0	82	3	1	79	1	0

2003 Water Chemistry Data Continued

phosphorus - LL PO_4 (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
4-Jun-03	2	1	0	2	1	0	2	1	0
27-Jun-03	1	1	0	2	0	0	2	1	0
14-Jul-03	0	0	0			0	0	0	0
1-Aug-03	0	0	0			0	0	0	0
15-Sep-03	0	0	0	0	0	0	0	0	0
6-Oct-03	8	4	1	8	6	2	11	3	1
6-Nov-03	6	1	0	4	4	1	5	1	0
27-Nov-03	6	2	1	6	1	0	6	1	0

phosphorus - total diss (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
4-Jun-03	2	0	0	2	0	0	2	0	0
27-Jun-03	3	1	0	3	2	1	2	0	0
14-Jul-03	4	3	1			0	2	0	0
1-Aug-03	2	0	0			0	2	0	0
15-Sep-03	2	0	0	2	0	0	2	0	0
6-Oct-03	14	8	3	15	10	3	15	10	3
6-Nov-03	17	2	1	18	2	1	14	5	2
27-Nov-03	4	1	0	5	2	1	3	1	0

phosphorus - total (ug/L)

	Inorg/Inorg			Nil/Inorg+Org			Nil/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
4-Jun-03	3	2	1	2	0	0	2	0	0
27-Jun-03	5	2	1	4	3	1	3	2	1
14-Jul-03	2	1	0			0	2	0	0
1-Aug-03	2	1	0			0	2	0	0
15-Sep-03	2	0	0	2	0	0	2	0	0
6-Oct-03	33	12	4	24	13	4	36	16	5
6-Nov-03	24	4	1	25	6	2	22	4	1
27-Nov-03	9	3	1	10	3	1	8	3	1

Cholophyll a data

(ug/(cm)^2) Nil/Inorg	Site			18-Jun-03	23-Jun-03	27-Jun-03	2-Jul-03	7-Jul-03	11-Jul-03
	Channel 1	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.70	0.80	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
	Channel 3	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
	Channel 8	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.50	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		Total	Mean	0.00	0.00	0.00	0.07	0.04	0.00
			Stdev	0.00	0.00	0.00	0.20	0.19	0.00
			Var	0.00	0.00	0.00	0.04	0.04	0.00

Cholophyll a data continued

(ug/(cm)^2) Inorg/Inorg	Site			18-Jun-03	23-Jun-03	27-Jun-03	2-Jul-03	7-Jul-03	11-Jul-03
	Channel 4	top	a	0.00	0.50	0.00	0.00	0.80	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	1.30	1.00	0.80	0.00
		bottom	a	0.00	0.00	0.00	0.50	0.00	0.00
			b	0.00	0.00	0.00	0.70	0.80	0.00
	Channel 5	top	a	0.00	0.00	0.00	0.60	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.60	0.00
			b	0.00	0.00	0.00	0.70	0.00	0.60
		bottom	a	0.00	0.00	0.00	0.70	0.80	0.60
			b	0.00	1.30	1.80	0.90	2.10	1.10
	Channel 9	top	a	0.00	0.00	0.00	0.00	0.00	0.50
			b	0.00	0.00	0.00	1.40	0.80	0.90
		middle	a	0.00	0.80	0.00	1.00	0.90	0.80
			b	0.00	0.00	0.00	1.50	0.90	1.80
		bottom	a	0.00	0.60	0.50	0.50	1.30	0.90
			b	0.00	0.00	0.60	0.60	1.00	1.40
		Total	Mean	0.00	0.18	0.23	0.56	0.60	0.48
			Stdev	0.00	0.37	0.52	0.49	0.58	0.57
			Var	0.00	0.14	0.27	0.24	0.34	0.33

Cholophyll a data continued

(ug/(cm)^2) Nil/Inorg+Org	Site			18-Jun-03	23-Jun-03	27-Jun-03	2-Jul-03	7-Jul-03	11-Jul-03
	Channel 2	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
	Channel 6	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
	Channel 7	top	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		middle	a		0.00	0.00	0.00	0.00	0.00
			b		0.00	0.00	0.00	0.00	0.00
		bottom	a	0.00	0.00	0.00	0.00	0.00	0.00
			b	0.00	0.00	0.00	0.00	0.00	0.00
		Total	Mean	0.00	0.00	0.00	0.00	0.00	0.00
			Stdev	0.00	0.00	0.00	0.00	0.00	0.00
			Var	0.00	0.00	0.00	0.00	0.00	0.00

Cholophyll a data continued

(ug/(cm)^2) Nil/Inorg	16-Jul-03	1-Aug-03	14-Aug-03	29-Sep-03	4-Oct-03	19-Oct-03	23-Oct-03	6-Nov-03	23-Nov-03
	0.00	0.00	0.00	2.00	4.70	2.01	3.96	5.98	5.42
	0.00	0.00	0.00	1.70	5.10	1.07	0.92	5.57	3.65
	0.00	0.00	0.00	1.50	6.90	1.62	0.19	5.49	7.80
	0.00	0.50	0.00	1.50	5.60	3.60	3.26	6.95	7.92
	1.30	0.00	0.00	0.90	5.60	4.21	0.18	7.28	6.46
	0.60	0.60	0.00	0.70	5.90	4.21	3.62	3.39	5.63
	0.00	0.00	0.00	1.90	4.20	2.69	3.69	3.98	4.68
	0.00	0.00	0.00	1.50	5.60	3.65	3.06	3.98	4.58
	0.00	0.50	0.00	3.10	5.80	5.98	0.00	5.69	5.03
	0.70	0.00	0.00	1.80	5.70	3.01	1.73	2.47	6.30
	0.00	0.00	0.00	1.10	4.50	2.21	0.00	6.34	7.58
	0.00	0.50	0.60	0.90	4.90	0.64	4.11	9.78	6.51
	0.00	0.00	0.00	2.00	6.00	3.46	1.73		8.08
	0.00	0.50	0.00	1.90	5.70	3.67	1.80		4.62
	0.00	0.00	0.00	1.10	4.90	2.32	2.51		1.79
	0.00	0.00	0.00	1.10	2.90	1.20	2.54		3.59
	0.00	0.00	0.00	1.50	5.20	3.51	3.70		4.35
	0.00	2.10	0.00	1.00	3.00	4.55	4.19		3.32
	0.14	0.26	0.03	1.51	5.12	2.98	2.29	5.58	5.41
	0.36	0.52	0.14	0.57	1.01	1.38	1.52	1.97	1.78
	0.13	0.27	0.02	0.33	1.02	1.91	2.30	3.89	3.18

Cholophyll a data continued

(ug/(cm)^2) Inorg/Inorg	16-Jul-03	1-Aug-03	14-Aug-03	29-Sep-03	4-Oct-03	19-Oct-03	23-Oct-03	6-Nov-03	23-Nov-03
	0.90	1.30	0.00	2.40	4.00	5.25	0.92	6.64	2.34
	0.00	0.70	0.80	0.90	3.30	3.14	0.89	6.17	4.69
	0.90	3.90	4.30	2.80	7.20	2.93	2.81	6.47	7.27
	1.50	3.30	2.30	2.50	7.00	2.38	3.11	2.91	6.35
	1.60	1.40	1.40	1.00	4.40	1.94	0.10	8.45	4.79
	0.80	2.60	1.10	1.50	5.00	2.79	2.89	3.01	4.79
	0.70	1.80	1.30	1.60	4.30	3.29	3.02	6.59	4.24
	1.10	1.30	0.00	1.70	3.80	4.47	4.05	4.36	3.72
	1.20	3.30	1.00	2.50	6.80	3.08	2.54	6.70	6.90
	1.70	1.90	0.80	1.40	4.40	3.11	0.00	5.44	6.23
	1.50	3.30	2.70	2.00	7.80	8.74	0.00	2.10	5.55
	2.30	3.30	0.70	1.80	7.10	20.90	3.66	4.95	7.76
	0.60	2.20	0.70	2.30	6.10	1.23	3.49		6.32
	1.30	3.60	0.00	1.00	3.90	3.42	4.01		4.30
	1.40	4.90	2.80	1.50	6.20	3.81	3.68		3.74
	2.30	2.30	2.80	1.60	3.70	3.64	4.56		3.81
	1.40	2.50	3.80	0.60	7.40	4.43	6.95		6.64
	2.00	0.00	4.50	1.60	7.30	4.55	6.06		9.17
	1.29	2.42	1.72	1.71	5.54	4.62	2.93	5.32	5.48
	0.59	1.24	1.46	0.62	1.58	4.37	1.97	1.90	1.73
	0.35	1.53	2.13	0.38	2.48	19.06	3.88	3.61	2.98

Cholophyll a data continued

(ug/(cm)^2) Nil/Inorg+Org	16-Jul-03	1-Aug-03	14-Aug-03	29-Sep-03	4-Oct-03	19-Oct-03	23-Oct-03	6-Nov-03	23-Nov-03
	0.00	0.70	0.00	2.20	4.80	1.34	4.01	3.18	5.29
	0.00	0.00	0.00	1.50	4.50	2.96	0.80	5.82	5.27
	0.00	0.00	0.80	2.80	6.70	5.19	3.56	2.18	5.88
	0.00	0.50	0.00	2.10	4.90	1.44	0.05	3.09	4.15
	0.00	0.00	0.00	1.60	5.20	4.50	0.88	1.78	1.35
	0.00	0.00	0.00	0.90	2.80	2.17	3.71	2.45	5.04
	0.00	0.70	0.00	1.70	7.10	0.77	2.24	6.85	6.89
	0.00	0.50	0.00	1.50	4.60	0.58	3.26	2.75	4.70
	0.00	0.00	0.00	3.50	5.90	3.91	3.55	6.42	6.93
	0.00	0.00	0.00	1.30	5.80	2.84	2.94	6.16	5.88
	0.00	0.00	0.50	1.10	4.30	3.11	3.56	5.68	6.24
	0.50	0.00	0.00	1.10	4.50	1.85	2.90	6.58	6.16
	0.00	0.00	0.00	1.90	5.90	2.33	2.89	5.95	5.89
	0.00	0.00	0.00	1.60	4.90	4.10	0.56	6.46	5.30
	0.00	0.00	0.00	1.50	5.50	3.31	2.93	1.14	3.90
	0.00	0.00	0.00	0.90	7.00	3.50	1.03	0.51	4.22
	0.00	0.00	0.00	1.50	6.00	3.89	4.48	1.64	5.07
	0.00	0.00	0.00	1.20	2.20	3.87	4.83	2.01	4.95
	0.03	0.13	0.07	1.66	5.14	2.87	2.68	3.93	5.17
	0.12	0.26	0.22	0.66	1.29	1.31	1.43	2.23	1.29
	0.01	0.07	0.05	0.44	1.67	1.72	2.04	4.99	1.66

2004 Water Chemistry Data

nitrogen - total (ug/L)

	Inorg+Org/Inorg			Inorg/Inorg+Org			Inorg/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
31-May-04	123	25	15	107	6	3	117	6	3
16-Jun-04	97	6	3				90	0	0
28-Jun-04	83	6	3				80	0	0
12-Jul-04	93	23	13				83	6	3
26-Jul-04	73	6	3				73	6	3
10-Aug-04	77	15	9				73	6	3
23-Aug-04	83	6	3				80	10	6
13-Sep-04	83	6	3	90	0	0	87	6	3
29-Sep-04	87	12	7	93	15	9	83	6	3
18-Oct-04	120	26	15	140	52	30	100	0	0
15-Nov-04	93	15	9	110	30	17	83	6	3
29-Nov-04	123	6	3	127	6	3	117	6	3
5-Jan-05	123	6	3	123	6	3	120	0	0

2004 Water Chemistry Data Continued

nitrogen - NH_4 (ug/L)

	Inorg+Org/Inorg			Inorg/Inorg+Org			Inorg/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stden	sem
31-May-04	0	0	0	3	5	3	0	0	0
16-Jun-04	13	6	3				9	3	2
28-Jun-04	2	3	2				2	3	2
12-Jul-04	0	0	0				2	3	2
26-Jul-04	4	3	2				7	3	2
10-Aug-04	0	0	0				2	4	2
23-Aug-04	0	0	0				0	0	0
13-Sep-04	3	3	2	2	3	2	5	5	3
29-Sep-04	2	3	2	0	0	0	0	0	0
18-Oct-04	2	3	2	6	1	1	2	3	2
15-Nov-04	2	3	2	6	5	3	3	3	2
29-Nov-04	0	0	0	0	0	0	7	8	5
5-Jan-05	0	0	0	0	0	0	0	0	0

2004 Water Chemistry Data Continued

nitrogen - LL NO_{2+3} (ug/L)

	Inorg+Org/Inorg			Inorg/Inorg+Org			Inorg/Inorg		
	mean	stdev	sem	mean	stdev	sem	mean	stdev	sem
31-May-04	68	1	0	67	1	0	67	1	0
16-Jun-04	61	3	2			0	61	1	1
28-Jun-04	46	1	0			0	45	5	3
12-Jul-04	40	1	1			0	40	1	1
26-Jul-04	38	1	0			0	40	0	0
10-Aug-04	39	4	2			0	40	1	0
23-Aug-04	39	3	2			0	36	2	1
13-Sep-04	37	2	1	39	1	1	37	2	1
29-Sep-04	31	2	1	29	2	1	31	1	1
18-Oct-04	38	0	0	39	1	0	38	2	1
15-Nov-04	65	1	1	65	1	0	64	1	1
29-Nov-04	78	2	1	79	2	1	80	1	1
5-Jan-05	92	1	1	92	1	1	91	2	1
23-Feb-05	82	1	0	83	1	1	82	1	1
23-Mar-05	76	1	0	75	1	1	81	4	3

2004 Chlorophyll a (ug/(cm)^2)		16-Jun-04	23-Jun-04	28-Jun-04	7-Jul-04	14-Jul-04	21-Jul-04	26-Jul-04	5-Aug-04
Channel 1									
	a								
	b								
Channel 2									
	a	0.17	0.77	0.81	2.03	5.76	2.94	4.00	3.46
	b	0.00	0.40	0.79	1.77	1.76	3.65	1.95	4.02
Channel 3									
	a								
	b								
Channel 4									
	a								
	b								
Channel 5									
	a	0.56	1.31	1.71	2.48	1.95	1.05	1.48	2.01
	b	0.18	1.85	1.76	0.61	0.18	1.02	0.76	0.26
Channel 6									
	a	0.31	2.93	3.15	3.69	0.24	0.60	0.79	4.78
	b	0.23	1.47	1.92	4.19	1.13	0.53	1.15	2.26
Channel 7									
	a	0.09	1.47	1.20	1.39	0.81	1.28	1.52	1.08
	b	0.11	1.50	1.64	2.04	3.35	0.91	2.11	2.82
Channel 8									
	a	0.13	0.68	0.66	2.45	1.75	0.59	2.59	0.25
	b	0.33	1.52	1.79	2.53	1.43	0.56	1.79	0.59
Channel 9									
	a	0.33	1.83	2.55	4.74	1.23	1.57	1.61	1.37
	b	0.23	2.64	2.88	2.77	0.71	1.38	0.49	0.46

2004 chlorophyll a (mg/(m)^2)

		16-Jun-04	23-Jun-04	28-Jun-04	7-Jul-04	14-Jul-04	21-Jul-04	26-Jul-04
Inorg+Org/Inorg	average	2	17	20	32	18	18	17
	stdev	1	10	10	12	20	13	13
	sem	0	4	4	5	8	5	5
Inorg/Inorg+Org	average							
	stdev							
	sem							
Inorg/Inorg	average	2	14	15	19	16	9	17
	stdev	2	4	4	8	11	3	6
	sem	1	2	2	3	4	1	3

2004 chlorophyll a (mg/(m)^2) cont

		5-Aug-04	29-Sep-04	13-Oct-04	18-Oct-04	25-Oct-04	10-Nov-04	15-Nov-04	29-Nov-04
Inorg+Org/Inorg	average	27	2	14	16	18	111	119	61
	stdev	17	0	7	7	7	70	130	15
	sem	7	0	3	3	3	29	53	6
Inorg/Inorg+Org	average		6	43	25	54	121	327	70
	stdev		3	30	13	12	75	314	36
	sem		1	12	5	5	31	128	15
Inorg/Inorg	average	12	2	26	13	14	74	286	61
	stdev	10	2	21	4	3	63	332	15
	sem	4	1	9	2	1	26	136	6

2004 Chlorophyll a (ug/(cm)^2) cont

		29-Sep-04	13-Oct-04	18-Oct-04	25-Oct-04	10-Nov-04	15-Nov-04	29-Nov-04
Channel 1								
	a	0.30	2.83	3.10	5.01	9.02	11.40	6.05
	b	0.22	9.80	2.35	5.93	10.40	6.48	12.10
Channel 2								
	a	0.15	1.02	1.35	1.56	19.20	5.87	4.67
	b	0.27	1.36	0.91	1.41	6.48	5.11	5.89
Channel 3								
	a	0.90	4.37	2.65	5.11	11.20	20.50	8.92
	b	0.70	4.26	4.44	5.78	6.91	15.50	7.43
Channel 4								
	a	0.44	0.75	1.01	3.59	7.85	57.80	1.39
	b	0.85	3.53	1.20	7.23	27.10	84.70	6.06
Channel 5								
	a	0.24	4.21	1.25	1.61	2.98	12.70	7.48
	b	0.13	0.42	1.21	1.28	4.02	82.00	6.61
Channel 6								
	a	0.16	0.48	1.42	1.44	18.20	37.90	7.35
	b	0.15	2.35	3.01	2.96	3.36	5.66	8.26
Channel 7								
	a	0.39	3.09	2.00	1.78	11.00	5.22	5.41
	b	0.07	5.71	1.56	1.28	6.80	4.62	4.29
Channel 8								
	a	0.10	0.69	0.79	1.31	1.59	8.63	4.78
	b	0.52	1.35	1.00	0.87	18.30	58.50	7.93
Channel 9								
	a	0.20	0.98	1.15	1.07	14.60	12.40	4.49
	b	0.23	2.11	1.49	2.11	4.95	4.74	5.73

Appendix C: Macroinvertebrate data

The following Appendix present the macroinvertebrate data collected for the experiment. The number of individuals in each sample and the dry weight of each sample is presented for the macro portions (>1250 µm) and micro portions (<1250 µm) separately. The graphs for the dry weights in each treatment of the micro portion of the samples are also presented below. See Appendix D, the attached data disc for the macroinvertebrate data in its entirety.

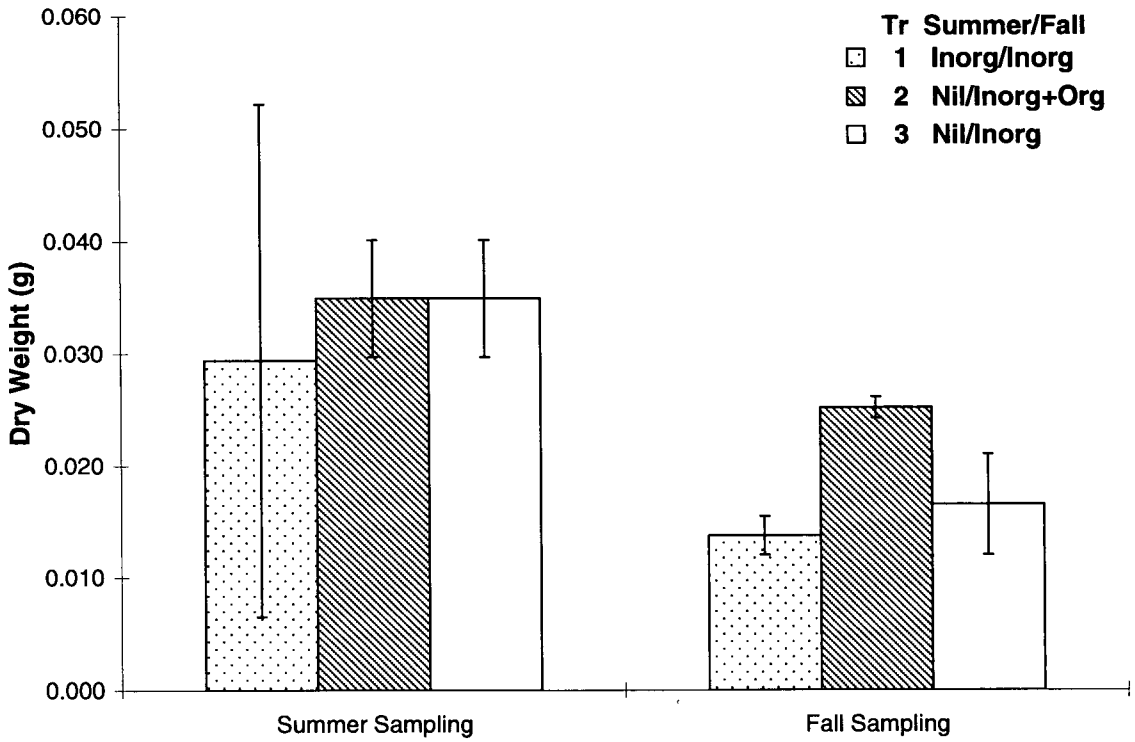

Figure C.1: Mean (±SE) Dry weight of small macroinvertebrates (<1250 µm) during the summer and fall macroinvertebrate sampling periods for treatments conducted in 2003/04

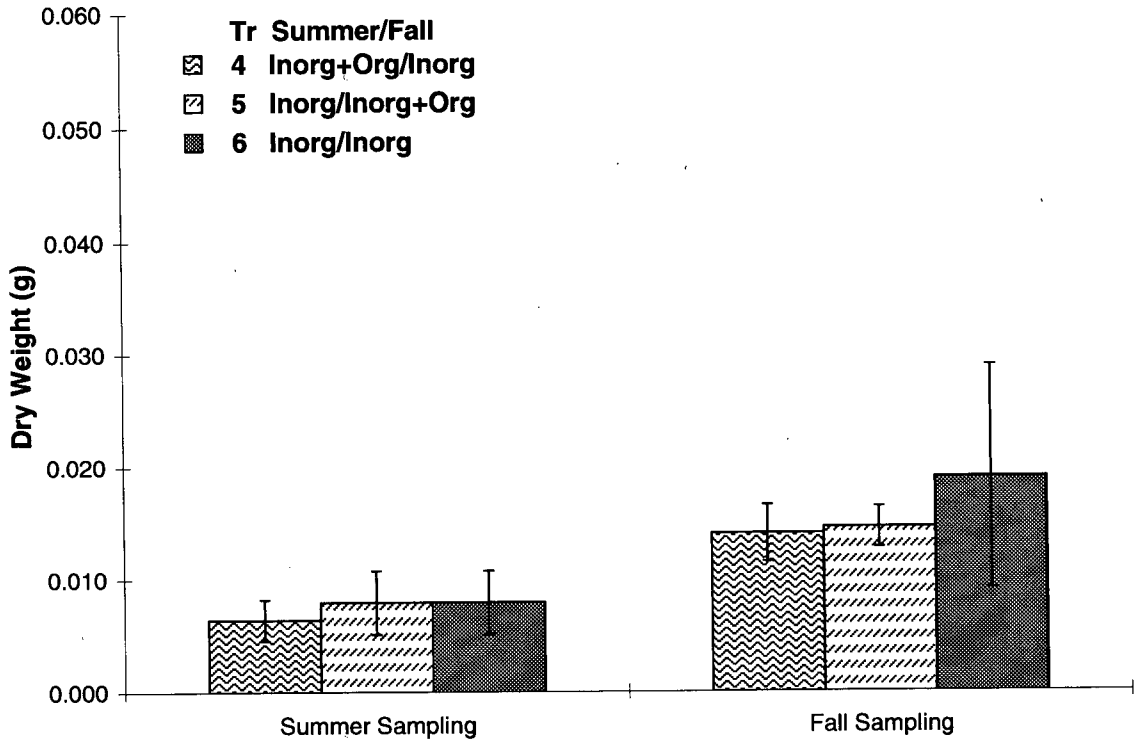

Figure C.2: Mean (±SE) Dry weight of small macroinvertebrates (<1250 µm) during the summer and fall macroinvertebrate sampling periods for treatments conducted in 2004/05