Body Condition and Feeding Ecology of Kuskokwim River Chum Salmon (*Oncorhynchus keta*) During Freshwater Outmigration

Julie M. Meka[1]

Christian E. Zimmerman[1]

Ron A. Heintz[2]

Shiway W. Wang[1]

[1]US Geological Survey
Alaska Science Center
1011 E. Tudor Rd.
Anchorage, AK 99503

[2]NOAA Fisheries
Auke Bay Laboratory
11305 Glacier Highway
Juneau, AK 99801

ABSTRACT

The marine environment has been identified as the primary influence to salmon survival, however, freshwater and estuarine habitat use during early life history are also considered critical stages influencing ocean survival. Research on the freshwater early life history of chum salmon (*Oncorhynchus keta*) in the Kuskokwim watershed is currently nonexistent. We explored capture methods of under-ice sampling before and during spring break-up to evaluate ways to estimate outmigration timing of chum salmon fry in the Kuskokwim watershed. Spawning populations of summer chum salmon have been documented at over 900 km in the Kuskokwim drainage, as well as populations spawning close to the ocean. Investigations into the feeding ecology and energy reserves of chum salmon fry originating from the upper Kuskokwim River (> 900 km from estuary) and the Kwethluk River (< 200 km from estuary) may help scientists gain an understanding of the factors relating to migration distance that can influence survival during the smolt transition from freshwater to estuarine life history stages.

INTRODUCTION

Chum salmon are an important environmental, cultural, and economic resource in the Kuskokwim River. Declines in chum salmon runs, since 1998, have resulted in closure and restriction of subsistence and commercial fisheries in the Kuskokwim and other western Alaska Rivers. Because of these declines, Kuskokwim River chum salmon were designated as a "stock of concern" by the Alaska Department of Fish and Game (ADFG) in 2000 based on challenges maintaining projected harvest and escapement levels (Burkey et al. 2000). The causes of declines in salmon runs to western Alaska are unknown. Identification of sources of mortality and mortality schedules are needed to evaluate reasons for declines in salmon runs. Many scientists attribute the declines to unknown factors in the marine environment such as the effects of climate change (Farley et al. 2003; Beamish et al. 2000; Beamish and Mahnken 2001), while it is also recognized that high mortality during early life history stages in transitional environments among freshwater, estuarine, and marine habitats may be influencing the numbers of returning salmon (Parker 1968; Peterman 1978; Holtby et al. 1990; Friedland et al. 1996; Beamish and Mahnken 2001). Unfortunately, little is known about the juvenile life stages of salmon in western Alaska Rivers. In the Kuskokwim River, for example, no work has focused on juvenile salmon and, therefore, nothing is known about the timing or duration of migration by juvenile salmon in the Kuskokwim River. Similarly, little is known about juvenile salmon in other western Alaska Rivers, such as the Yukon River, so it is difficult to apply findings from other rivers. Because the early life history of salmon populations in western Alaska is poorly understood, it is difficult to develop or test hypotheses concerning population regulation and the role of environmental variation or change. In this report, we describe a pilot study to test methods of capture and analyze the energetics and feeding ecology of Kuskokwim River chum salmon fry during spring outmigration.

Downstream Migration

The variability in spawning habitat requirements, migration timing, and upstream migration distance for adult chum salmon likely influence the life history strategies of their offspring. For example, chum salmon typically spawn in rivers and streams relatively close to the estuary (< 100 km), however rivers in the Arctic-Yukon-

Kuskokwim (AYK) region such as the Yukon and Kuskokwim Rivers have documented spawning populations up to 2800 km and greater than 1000 km, respectively, from the ocean (Milligan et al. 1986; Beacham et al. 1988; S. Gilk, ADFG, personal communication). Late-maturing chum salmon, or fall-run chum salmon, generally spawn at greater distances upstream and enter rivers with greater energy reserves necessary for long distance migration compared with early-maturing salmon, or summer-run chum, which generally spawn closer to salt water (Beacham et al. 1988). However, spawning populations of summer chum salmon have been documented at over 1500 km and 900 km in the Yukon and Kuskokwim drainages, respectively, as well as populations spawning less than 50 km from the ocean (Loftus and Lenon 1977; Clark and Molyneaux 2003). Research on Kuskokwim River chum salmon has been conducted on adults only, therefore, there is an information gap regarding knowledge of outmigration timing of chum salmon fry in relation to outmigration distance and the life history strategy (i.e. summer- or fall-run) of the parent stocks.

The duration of freshwater residency and outmigration timing of chum salmon fry in western Alaskan rivers has not been thoroughly examined. Studies in the Chena River (summer chum) and Tanana River (fall chum) found outmigration timing of fry to be similar (mid-April to June) despite the 2-3 month difference in spawn timing between populations (Finn et al. 1998). Similar results were found for summer and fall chum salmon stocks in the Fraser River (Beacham and Murray 1986). Other studies have estimated outmigration timing of chum salmon fry from the Noatak and Yukon rivers to begin prior to ice-out and potentially lasting through August (Merritt and Raymond 1983; Martin et al. 1986; Thorsteinson et al. 1989), indicating a high variability in outmigration timing may exist among watersheds in western Alaska. Although the results from these studies indicate outmigration begins prior to ice-out, the logistical challenges of under-ice sampling in remote locations have prevented thorough outmigration monitoring in western Alaska rivers. The commencement of outmigration has been demonstrated to be triggered by environmental factors such as temperature (Koski 1975), high flow rates (Quinn and Groot 1984), and light intensity (Mikulich and Gavrenkov 1986; Salo 1991), and migration timing may be more variable in longer streams and rivers. Annual variations in environmental conditions such as flow rates, temperatures, and ice break-up

may be a source of variability in fry outmigration timing (Koski 1975; Martin et al. 1986), yet the relationship between migration timing and survival has not been investigated. Differences in the timing of salmon smolts entering the estuary may influence survival rates by exposure to varying predator and prey communities. Prey availability and abundance as juvenile chum salmon enter estuarine and marine habitats are important factors determining the growth potential and therefore mortality rates of juvenile chum salmon (Mason 1974; Healey 1982; Salo 1991).

Body condition and feeding ecology of chum salmon fry

The transition from the freshwater to marine phase is a critical period of high mortality in the life history of salmonids (Pearcy 1992). Mortality rates in chum salmon initially following ocean entry may range as high as 38-49% per day in Puget Sound (Bax 1983), and 3-25% per day in coastal waters off the coast of Japan (Fukuwaka and Suzuki 2002). There is evidence for size-selective pressure for predation of chum fry in freshwater and estuarine environments; larger chum fry have a survival advantage over smaller fry and may be able to spend less time feeding in nearshore habitats where predation is high (Parker 1971; Beall 1972; Healy 1982; Pearcy 1989; Holtby et al. 1990; Friedland et al. 1996). Because the metabolic costs of migration and maintenance are key energetic constraints on the production and survival of juvenile chum salmon migrating through estuaries and the nearshore environment (Wissmar and Simenstad 1988), it is important to understand the energetics of juvenile salmon as they make the transition from freshwater to saltwater.

Lipid content in emerging fry has been linked to survival during freshwater migration and likely plays an important role in fish emerging far from the estuary (Saddler et al. 1970). Feeding before and/or during outmigration has been documented for chum fry from rivers in Asia, Japan, Russia, British Columbia, Oregon, Washington, and Alaska, and may be particularly important for chum fry making long distance downstream migrations (Sparrow 1968; Loftus and Lenon 1977; Merritt and Raymond 1983; Martin et al. 1986; Salo 1991). For example, summer- or fall-run chum salmon fry making long distance downstream migrations would presumably need to feed during outmigration to increase chances of survival by becoming larger, and variations in the lipid content observed in emerging fry may be an adaptation to cope with different

migration distances. Investigations into the feeding ecology and energy reserves of Kuskokwim River chum salmon fry originating from rivers close or far from estuarine environments may help scientists gain an understanding of the factors relating to migration distance that can influence survival between the freshwater and estuarine life history stages.

Our goal was to conduct a pilot study to evaluate methods of capture of Kuskokwim River chum salmon fry before and during spring ice break-up in order to document outmigration timing and examine variations in feeding ecology, size, and body condition in fry emerging close or far from the ocean and originating from summer- or fall-run parent stocks. The objectives of this study were to: 1) explore sampling methods to capture chum salmon fry before spring break-up under the ice and after ice-out to document outmigration timing, 2) describe the feeding ecology of chum salmon fry emerging close or far from the estuary through stomach content analysis, 3) estimate the body condition of chum salmon fry emerging close or far from the estuary through length-weight analysis, caloric content, lipid class analysis, and fatty acid composition, and 4) estimate the body condition of chum salmon fry entering the estuary through analysis of caloric content, lipid class analysis and fatty acid composition.

Chapter 1: Capture Methods of Chum Salmon Fry from Under Ice and after Ice-out

1.1 Abstract

The goal of this study was to explore capture methods of under-ice sampling before and during spring break-up to evaluate ways to estimate outmigration timing of chum salmon fry in the Kuskokwim watershed. Results from previous studies indicate outmigration begins prior to ice-out, however, logistical challenges of under-ice sampling in remote locations have prevented thorough outmigration monitoring rivers in western Alaska.

1.2 Introduction

High mortality during early life history stages in transitional environments among freshwater, estuarine, and marine habitats may be influencing the numbers of returning salmon (Parker 1968; Peterman 1978; Holtby et al. 1990; Friedland et al. 1996; Beamish and Mahnken 2001). Unfortunately, the juvenile life history stages of salmon in western Alaska rivers have not been well studied. The timing of chum salmon fry outmigration is unknown in the Kuskokwim River, which has suffered in declines of chum salmon runs, and makes it difficult to develop or test hypotheses concerning population regulation and the effect of environmental variation on populations.

The objective of this study was to test capture methods by sampling chum salmon fry before spring break-up under ice and after ice-out to document outmigration timing. Exploring capture methods in the spring is essential in determining outmigration timing of chum salmon fry in locations throughout the Kuskokwim watershed.

1.3 Materials and Methods

1.3.1 Study sites & sample collection

Two river sampling sites were chosen to represent chum salmon fry emerging from rivers close or far from the Kuskokwim estuary. The Kwethluk River is located within the Yukon Delta National Wildlife Refuge in the lower Kuskokwim basin. The sampling location was an existing weir site approximately 88 rkm upstream from the

Kuskokwim River and 190 rkm from the southern tip of Eek Island (Figure 1.1). The Takotna River begins approximately 835 rkm upstream from the tip of Eek Island and sampling locations included the Takotna main stem, Fourth of July Creek, and Big Creek (Figure 1.2). Fry were also sampled from the freshwater plume in the lower Kuskokwim basin approximately 16 rkm upstream from the southern tip of Eek Island to represent juvenile chum salmon as they prepare for saltwater transition (Figure 1.1).

Chum salmon fry were captured using small mesh seines (10 feet in length, 1/8 inch mesh), fyke net traps, and small dip nets before and during ice break-up in April and May, 2004, on the Kwethluk and Takotna rivers. Fyke net traps were set up in depths of approximately 3 feet and were fished for 10-minute increments, seines were fished in 2-3 feet of water for 1-minute increments, and small dip nets captured fry along the banks on an opportunistic basis. The amount of time fyke nets and seines were fished was determined by the amount of debris flowing down the river; fyke nets fished over 10 minutes and seines fished over 1 minute filled up with excessive amounts of debris. The Kwethluk River was sampled following spring ice break-up. The main stem was ice-free but river banks were covered with ice. The Takotna River and its tributaries were sampled using fyke net traps before spring break-up and seines following ice-out. Excessive amounts of debris following ice-out made fyke net sampling on the Takotna ineffective. Before ice-out, fyke net traps were set up with the trap frame mounted on the ice and the net lowered through a hole in the ice and fished for 30-minute increments. Juvenile chum salmon nearing the Kuskokwim Bay estuary were captured using a modified Kvichak surface net on June 1, 2004. All captured fish were frozen whole immediately in liquid nitrogen tanks in the field, and subsequently at -80^{o}C in the laboratory until further analysis.

An additional set of samples was collected from Auke Creek and the Ladd Macaulay Hatchery in southeast Alaska. These samples served as archetypes representing newly emerged, fed, and starved fry. The emerged fry were collected on April 8, 2004 as they migrated through the weir on Auke Creek. The weir is located within 2 rkm of the river mouth above the tidal zone. The maximum outmigration distance by fry in the system is less than 10 rkm and the majority of chum salmon spawn directly above the weir. Fry were assumed to have emerged within the 24-hour period preceding capture. The fed and

starved fry were captured at the weir and transferred to the hatchery where they were cultured in freshwater for 30 days and then in saltwater for an additional 21 days. The fed fry were fed with commercial food during culture, but they only appeared to feed actively after the introduction of saltwater. Starved fry were starved throughout the culture period. Size, proximate composition, lipid class, and fatty acid composition analyses of Kuskokwim River and Auke Bay samples were conducted at the National Oceanic and Atmospheric Administration (NOAA) Auke Bay Laboratory in Juneau. The small size of the fry required that samples for protein, lipid, and fatty acid content be composites from multiple numbers of individuals. Quality assurance of composite samples was determined from reference samples included in each batch of samples.

1.4 Results

On April 22-25, 2004, 214 chum salmon fry were captured on the Kwethluk River (fyke net n=95; seine n=82; dip nets n=37). Additional species captured were pink salmon (seine n=9), coho salmon (fyke net n=2; seine n=4; dip net n =1), chinook salmon (seine n=1), whitefish (fyke net n=1), sculpin (seine n=10), and lamprey (fyke net n=1; seine n=10). Although fyke nets captured more chum salmon, it was difficult to separate fish from the amount of debris captured in the net and the nets in general were difficult to handle because of high flows and large pieces of ice moving through the river. Seines were easier to handle and captured a more diverse sample of fishes. The average water temperature was 0.43^{o}C.

On May 4-11, 2004, 172 chum salmon fry were captured with small mesh seines on the Takotna River following ice-out. Additional species captured were not recorded. No fish were captured with fyke net traps before ice-out in April and early May.

Chapter 2: Feeding Ecology of Chum Salmon Fry during Freshwater Outmigration Using Stomach Content Analysis

2.1 Abstract

The feeding ecology of chum salmon fry during outmigration in the Kuskokwim River watershed has not previously been studied. These data may reveal potential variations in life history strategies between summer-run and fall-run chum salmon fry. We described the diet of chum salmon fry at three different locations in the Kuskokwim River watershed through stomach content analysis.

2.1 Introduction

Chum salmon are an important environmental, cultural, and economic resource in the Kuskokwim River. Chum salmon runs have been declining since 1998 which have resulted in closure and restriction of subsistence and commercial fisheries in the Kuskokwim and other western Alaska rivers. Because of these declines, Kuskokwim River chum salmon were designated as a "stock of concern" by the Alaska Department of Fish and Game (ADFG) in 2000 based on challenges maintaining projected harvest and escapement levels (Burkey et al. 2000). The causes of these declines of salmon in western Alaska are currently unknown. In order to determine the causes of these declines, sources of mortality and mortality schedules need to be identified. Many scientists attribute the declines to unknown factors in the marine environment such as the effects of climate change (Farley et al. 2003; Beamish et al. 2000; Beamish and Mahnken 2001), while it is also recognized that high mortality during early life history stages in transitional environments among freshwater, estuarine, and marine habitats may be influencing the numbers of returning salmon (Parker 1968; Peterman 1978; Holtby et al. 1990; Friedland et al. 1996; Beamish and Mahnken 2001). Currently, little is known about the juvenile life stages of salmon in western Alaska Rivers. In particular, the timing or duration of migration by juvenile salmon in the Kuskokwim River is poorly understood. As a result, it is difficult to develop or test hypotheses concerning population regulation and the role of environmental variation or change in these areas.

Important factors determining the growth potential and therefore mortality rates of juvenile chum salmon include prey availability and prey abundance upon entering estuarine and marine habitats (Mason 1974; Healey 1982; Salo 1992). The objective of this study was to describe the feeding ecology of chum salmon fry emerging close or far from the estuary through stomach content analysis.

2.3 Materials and Methods

A total of 23 and 22 juvenile chum salmon were collected from the Kwethluk and Takotna Rivers, respectively for stomach analyses. All fish were frozen and shipped to Anchorage for analysis. In the lab, the samples were allowed to thaw and stomachs were removed from each fish.

2.4 Results

Chum salmon fry from both the Kwethluk and Takotna Rivers had fed prior to capture. Eight fry collected from the Kwethluk River had fed and five fry from the Takotna River had fed. Adult dipterans were the dominant prey item. One fry from the Takotna River had fed on Ephemoptera nymphs.

Chapter 3: Body Condition of Chum Salmon Fry during Freshwater Outmigration

3.1 Abstract

Recent unexplained declines of chum salmon (*Oncorhynchus keta*) in the Kuskokwim River have prompted studies to determine what factors influence survival and growth during the early life history of this species in western Alaska. Upon emergence, Kuskokwim River chum salmon migrate varying distances (50 to more than 900 km) to estuarine habitat in Kuskokwim Bay. The factors affecting chum salmon survival during this critical life history stage are poorly understood. Energy reserves at the onset of migration, feeding rates, and migration distance likely interact to influence growth and survival of chum salmon during freshwater outmigration. In this study, we estimated the body condition of chum salmon fry emerging close (Kwethluk River) or far (Takotna River tributaries) from the Kuskokwim Bay estuary and also of fry entering the estuary. We used length-weight analysis, proximate composition analysis, lipid class analysis, and fatty acid composition analysis to evaluate the overall body condition of chum salmon fry. In general, the body condition was significantly different among fry from Kuskokwim Bay, Takotna and Kwethluk Rivers. The percent of lipid energy was significantly lower in the Kuskokwim Bay fry compared to river fry. Fry from the Kwethluk and Takotna rivers also had higher lipid levels than the Kuskokwim Bay fry, indicating the river fry had greater amounts of energy in storage. Our results show that fry migrating downstream lost lipid between emergence and saltwater entry. These results also suggest that the river fry are actively foraging and storing the ingested energy in contrast to Kuskokwim Bay fish that appear to be converting ingested lipid into energy for growth. However, the fatty acid analysis revealed high amounts of omega-3 marine-derived fatty acids in river fish which may be a product from provisioning by adult females rather than from active foraging. These results imply that marine conditions are likely to have a strong influence on the salmon fry survival because the amount of energy supplied to emergent fry will depend directly on the amount of lipids the females lay down in their eggs.

3.2 Introduction

The transition from the freshwater to marine phase is a critical period of high mortality in the life history of salmonids (Pearcy 1992). Mortality rates in chum salmon initially following ocean entry may range as high as 38 – 49% per day in Puget Sound (Bax 1983) and 3-25% per day in coastal waters off the coast of Japan (Fukuwaka and Suzuki 2002). There is evidence for size-selective pressure for predation of chum fry in freshwater and estuarine environments; larger chum fry have a survival advantage over smaller fry and may be able to spend less time feeding in nearshore habitats where predation is high (Parker 1971; Beall 1972; Healy 1982; Pearcy 1989; Holtby et al. 1990; Friedland et al. 1996). Because the metabolic costs of migration and maintenance are key energetic constraints on the production and survival of juvenile chum salmon migrating through estuaries and the nearshore environment (Wissmar and Simenstad 1988), it is important to understand the energetics of juvenile salmon as they make the transition from freshwater to saltwater.

Lipid content in emerging fry has been linked to survival during freshwater migration and likely plays an important role in fish emerging far from the estuary (Saddler et al. 1970). Feeding before and/or during outmigration has been documented for chum fry from rivers in Asia, Japan, Russia, British Columbia, Oregon, Washington, and Alaska, and may be particularly important for chum fry making long distance downstream migrations (Sparrow 1968; Loftus and Lenon 1977; Merritt and Raymond 1983; Martin et al. 1986; Salo 1991). For example, summer- or fall-run chum salmon fry making long distance downstream migrations would presumably need to feed during outmigration to increase chances of survival by becoming larger, and variations in the lipid content observed in emerging fry may be an adaptation to cope with different migration distances. Investigations into the feeding ecology and energy reserves of Kuskokwim River chum salmon fry originating from rivers close or far from estuarine environments may help scientists gain an understanding of the factors relating to migration distance that can influence survival between the freshwater and estuarine life history stages.

Recent unexplained declines of chum salmon (*Oncorhynchus keta*) in the Kuskokwim River have prompted studies to determine what factors influence survival and growth during the early life history of this species in western Alaska. Upon emergence, Kuskokwim River chum salmon migrate varying distances (50 to more than 900 km) to estuarine habitat in Kuskokwim Bay. Mortality during freshwater outmigration may influence the abundance of returning adults; however, the factors affecting chum salmon survival during this critical life history stage are poorly understood. Energy reserves at the onset of migration, feeding rates, and migration distance likely interact to influence growth and survival of chum salmon during freshwater outmigration.

The goal of this study was to 1) evaluate the body condition of chum salmon fry undergoing short-distance (Kwethluk River, less than 200 km) and long-distance (Takotna River, greater than 900 km) migrations in the Kuskokwim River watershed; and 2) describe the body condition and size of chum fry as they enter estuarine habitat in Kuskokwim Bay. Relating body condition to migration distance will provide insight on how salmon fry compensate energetically to survive long or short distance migrations. Body size and post migration energy reserves available to salmon fry likely influence survival during the transition from freshwater to estuarine habitat. There are little data describing the nutritional status of emergent chum salmon fry and the time since emergence in the Kwethluk and Takotna River samples is not known. Therefore, we also conducted a captive feeding study using newly emerged chum salmon from Auke Creek, Alaska and chum salmon fry with known feeding histories. These latter samples are presented as a standard against which the Kuskokwim Basin samples can be compared.

3.3 Materials and Methods

3.3.1 Sample collection: wild salmon

Juvenile chum salmon in the Kuskokwim drainage were collected from three locations in April, May and June 2004: Kuskokwim Bay (n = 123), Takotna (n = 140) and Kwethluk Rivers (n = 140). The Takotna River is located approximately 900 km from the Bay while the Kwethluk River is only 200 km from the Bay. Immediately after collection, the fish were frozen in liquid nitrogen and shipped frozen to the Auke Bay

Lab (Juneau, Alaska) where they were stored at -80ºC until processed. Fish were measured for fork length and wet mass and combined into composite samples weighing 2.9 to 6.6g (Tables 3.1, 3.2). There was a wide variation in the size of the Kuskokwim Bay fish; therefore in addition to the randomly selected composites of Kuskokwim Bay fish, three size classes were also analyzed. The three size classes, referred to here as Small (≤34 mm), Medium (35 – 42 mm) and Large (≥ 43 mm) were identified by dividing the length frequency distribution into thirds and sampling fish from each of these subdivisions. There were 6 Small composites, 3 medium and 5 large. Composites consisted of 2 to 7 fish and ranged between 2.4 and 3.6 g. After grouping fish into size classes composite samples were made by randomly selecting individuals from a common size class (Table 3.2).

3.3.2 Sample collection: standards

Juvenile chum salmon were collected from Auke Creek, Alaska and the Ladd Macaulay Hatchery in Juneau, Alaska. There were 3 composited samples of emergent fry from Auke Creek comprising 5 fish each and weighing 1.5 to 1.6 g. There were 3 composite samples of fed fish, each comprising 3 to 4 fish and weighing between 1.7 and 2.3 g. There were only 2 composites of starved fish, each comprised of 4 individuals. These composites ranged between 1.0 and 1.1 g. These samples are provided as standards representing newly emerged, fed and starved fry, hereafter referred to as Emerged, Fed and Starved fry, respectively. The Emerged fry were collected on April 8, 2004 as they migrated through a weir on Auke Creek. Chum salmon spawning beds are located immediately upstream of the weir and fry were assumed to have emerged within the 24 hour period preceding capture. Fed and Starved fry were transferred from the Ladd Macaulay Hatchery to the Auke Bay lab after emergence where they were cultured in fresh water for 30 days and then in salt water for an additional 21 days. The Fed fry were fed with commercial fish food during culture, but they only appeared to feed actively after the introduction of saltwater. The Starved fry were not fed any food throughout the culture period. While these fry are from different locations they represent fish from the same hatchery stock.

3.3.3 Proximate composition analysis

Lipids were extracted from samples with a Dionex Accelerated Solvent Extractor (ASE) 200 using a modification of Folch's method as outlined by Christie (1982). Wet sample homogenate was mixed with a drying agent (Hydromatrix) and masticating agent (sand) and loaded into ASE cells. Samples were extracted using a 2:1 (v:v) chloroform:methanol solvent at 1200 psi and 120 C. Following extraction, the filtrate was washed to remove coextractables with a 0.88% KCl solution followed by a solution of 1:1 (v:v) methanol:deionized water, both in a volume equal to 25% of the extract volume. Excess solvent was evaporated using a Yamato BM400 water bath to reduce the sample to 1 ml. Percent lipid was calculated gravimetrically by sacrificing 0.5 ml of lipid-solvent solution and evaporating the solvent to dryness. Quality assurance samples extracted with each batch of 17 samples included: 1) a blank, 2) a replicate of one of the batch samples, and 3) a reference sample of herring homogenate previously characterized for proximate composition.

Protein content was determined indirectly by multiplying the nitrogen content of each sample by a conversion factor of 6.06 (Leco Instruction Manual 2001; Craig et al. 1978). Nitrogen content was measured with a LECO model FP528 nitrogen analyzer following the Dumas method (Association of Official Analytical Chemists, 2002). Samples of dried and crushed homogenate (about 0.1 g) were wrapped in foil and the excess air squeezed out. Samples were then combusted in a chamber at 850 ºC and the expelled nitrogenous gases quantified. Samples were replicated and reanalyzed if the standard deviation of the replicates exceeded 1. Quality assurance samples included with each batch of 17 samples were: 1) a blank reference sample of pure cane sugar, 2) a reference walleye pollock homogenate calibrated to a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM), and 3) a NIST SRM 1546. Additionally, the instrument is calibrated daily with EDTA samples.

Moisture and ash content was measured with a Leco Thermogravimetric Analyzer. Samples were placed in ceramic crucibles and heated to a temperature of 135 ºC until a constant mass was achieved from which moisture content was calculated. Immediately following, the temperature was increased to 600 ºC and maintained until a constant mass was achieved from which ash content was calculated. Quality assurance

samples for moisture and ash analyses included with each run were blanks and reference pollock homogenate calibrated to the National Institute of Standards and Technology Standard Reference Material number 1946 (SRM1946). The total energy content (kJ) of each sample was calculated as the sum of the energy content contributed by total-body lipid and protein proximate fractions using the equation (Brett 1995):

$$\text{Energy Content (kJ)} = \text{Lipid Content (g)} \cdot 36.43 \text{ kJ g}^{-1} + \text{Protein Content (g)} \cdot 20.10 \text{ kJ g}^{-1}$$

Total lipid content was estimated as the product of percent lipid and sample weight, total protein content was similarly estimated. Total energy content was standardized to energy density (kJ g^{-1} sample mass) to account for differing masses of composite samples. The proportional contribution of lipid and protein energy to total energy was also calculated to examine differences in energy allocation by chum salmon fry among sites.

3.3.4 Lipid class composition analysis

Lipid class composition was determined by high performance liquid chromatography (HPLC) with a Hewlett Packard HP1050 solvent pump equipped with a 3 μm Phenomenex SphereClone 100x 4.60 mm 3μ column. Separated classes were detected using a Sedex 55 evaporative light scattering detector. Concentrations of the classes were quantified against 4-point calibration curves normalized to an internal standard 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (PDME). A solvent gradient is used to separate classes with differing polarity, beginning with 99:1 Iso-Octane:tetrahydrofuran (99:1 v:v) followed by mixing with Isopropanol IPA:Chloroform (4:1) and IPA:Water (1:1). Calibration standards included representative compounds from each of six lipid classes: wax and sterol esters (WE), sterols, traicylglycerols (TAG), mono-acylglycerols, free fatty acids, phosphatidylcholine (PC), and phosphatidylethanolamine (PE). The representative compounds were myristyl myristate (WE), cholesterol, triolein (TAG), mono-olein, fatty acid 21:0, bovine phosphatidylcholine (PC), and bovine phosphatidylethanolamine (PE), respectively. Wax and sterol esters are considered a single class because they co-elute. The samples are injected onto the column with a 1:1 mixture of hexane:chloroform using a Gilson autoinjector. Uncalibrated peaks were quantitated using the monolein calibration curve.

Samples were processed in batches of 17 along with a reference sample, a duplicated sample, hexane blank, a method blank and a set of two calibration standards. The herring tissue used as the reference sample has been examined repeatedly by the Auke Bay Laboratory. The duplicated sample provides a measure of analytical repeatability, the method blank sample identifies the presence of interfering lipids acquired during extraction and the hexane blank indicates column cleanliness. The lipids from the reference sample, sample duplicate and method blank are extracted at the same time lipids were extracted from the chum salmon tissues (see Proximate Composition Analysis for lipid extraction methods).

3.3.5 Fatty acid analysis

Fatty acids comprising the purified lipid in each sample were transesterified to fatty acid methyl esters (FAMEs), separated by gas chromatography, and quantified by mass spectrometry. Approximately 300 μg of lipid was combined in a glass tube with hexane, 0.5 N sulfuric acid in methanol, and fatty acid surrogate spikes (19:0 and 23:0). The mixture was incubated at 50 ºC overnight, briefly cooled, and mixed vigorously with a 5% sodium chloride solution. This was followed by two liquid-liquid extractions with hexane in which the non-polar phases were retained and combined. Transesterification reactions were quenched and neutralized using an aqueous 2% potassium bicarbonate solution, followed by a final liquid-liquid extraction. Residual water was removed from the hexane extract by passing it through a sodium sulfate drying column. The eluant was collected and evaporated under vacuum at 40 ºC to a final volume of approximately 1ml, and a FAME internal standard (21:0) was added.

Spiked FAMEs were analyzed on a Varian CP3800 gas chromatograph equipped with a 100 m Varian CP Select for FAME cyanopropyl-bonded fused silica column operating under a ramped temperature program. Separated fatty acids were detected with a Varian Saturn model 2200 mass spectrometer operating in selective ion storage mode. Fatty acid concentrations were determined using five-point calibration curves for each FAME and internal standard recovery, as well as duplicate and reference sample spectra were used for QA evaluation. Data collection was made for batches of 15-20 samples, including a reference sample consisting of the National Institute for Standards and

Technology SRM1946. Concentrations observed for SRM1946 in each batch were typically within 25% of the certified values. The coefficient of variation for duplicate analyses performed within a batch was generally less than 10%. The estimated total fatty acid content of method blanks was less than 10% that of the lowest estimate for samples in a batch.

3.3.6 Statistical analysis: lengths and weights

Lengths and weights of individual fish were examined to determine if there were differences in body size among groups. Initially, one-way ANOVAs were performed on the length and weight data with group as the main factor. However, obvious size differences among the groups were detected. Consequently length specific masses were evaluated by ANCOVA. Length specific mass provides a measure of body condition and is hereafter referred to as condition factor. In order to examine length specific mass the slopes of the length weight relations were initially examined to determine if they differed by examining the interaction term in the following model:

$$\text{Weight (g)} = \text{Groups} + \text{Length (mm)} + \text{LengthXGroup} \qquad \text{(Eq. 3.1)}$$

Weights were transformed by their natural logarithms in order to linearize the relationships, but all data are reported as untransformed values. The condition factors of groups with similar slopes ($\forall > 0.05$) were compared after removing the interaction term.

3.3.7 Statistical analysis: proximate composition

Energy density, percent lipid, protein and the proportion of total energy allocated to lipid were compared among groups using one-way analysis of variance (ANOVA). The percent energy allocated to lipid was estimated as the percentage of total energy represented by total lipid. Because there are only two energy compartments, lipid and protein, differences in the lipid compartment mean that there are complementary differences in the protein component. The small size of the fry required that samples for lipid and protein content be pooled from multiple numbers of individuals. Consequently it was not possible to control for fish size in the ANOVAs. Differences among means were determined using Tukey's multiple comparison test. The assumption of

homogeneity of variances was examined by Levene's test prior to the ANOVAs and appropriate transformations were made. All results are presented as untransformed numbers.

Fry from Kuskokwim Bay were variable in size, consequently we stratified some of the Kuskokwim Bay samples by size to examine size effects on energy density, percent lipid, protein and the proportion of total energy allocated to energy structure. Fish were arbitrarily sorted into size classes by first measuring the lengths of all fish in the sample and designating those below 34 mm as "small" and those above 43 mm as large. Fish with intermediate lengths are designated as "medium". Similar analyses to those for groups were performed on the fish sorted by size. The analyses by group described above were initially performed with samples of Kuskokwim Bay fish that were randomly drawn from the population.

3.3.8 Statistical analysis: lipid class composition

Lipid class composition was evaluated for a subset of the samples, 9 from Kuskokwim Bay, 15 from the Takotna River, and 7 from the Kwethluk River and all the Fed and Starved fish samples from Auke Creek. There were 3 composited samples of emergent fry from Auke Creek comprising 5 fish each and weighing 1.5 to 1.6 g. There were 3 composite samples of fed fish, each comprising 3 to 4 fish and weighing between 1.7 and 2.3 g. There were only 2 composites of starved fish, each comprised of 4 individuals. These composites ranged between 1.0 and 1.1 g. Results of the analyses were compared by one-way ANOVA where location was the main factor. Post-hoc comparisons of the means relied on Tukey's HSD with $\alpha = 0.05$. Response variables included wax and cholesterol esters (WE+CE), and triacylglycerols (TAG) to test the hypothesis that fry from Kuskokwim Bay had the greatest amounts of lipid in storage. We also used ANOVA to determine if fish from the different locations had the same amount of their energy allocated to storage. The proportion allocated to storage was calculated as:

$$\%Estorage = \frac{wt \times \%lipid \times \left(\%TAG + \%\left(WE + CE\right)\right) \times 36.43}{wt \times energydensity} \quad \text{(Eq. 3.2)}$$

The constant, 36.43, is the number of kJoules of energy per gram lipid in fish tissue (Brett 1995). In addition, the proportion of lipid found as fatty acids and the ratio of

PC/PE were also tested because these values can indicate sample quality. All ANOVAS used fish from all sampling locations, but only the 95% confidence intervals for the Kuskokwim basin fry are depicted in figures, point estimates of vouchers are provided for comparison.

3.3.9 Statistical analysis: fatty acid composition

Statistical analysis of chum salmon fatty acid compositions were aimed at determining if there were differences in composition among the groups and identifying which groups were most similar. ANOVA and MANOVA were used to detect differences in composition among the groups. One-way ANOVAs were performed for each fatty acid with group as the main factor to identify which fatty acids varied among groups. Only 30 of the 36 fatty acids we examined were tested, because three of the fatty acids were undetected in all groups. The relative concentrations of the 30 detected fatty acids were further transformed using Aitchison's approach to compositional data (Aitchison 2003):

$$FA_i^{'} = \frac{\log(FA_i)}{\log(18:1n-9)} \qquad \text{(Eq 3.3.)}$$

where $FA_i^{'}$ is the transformed value of the ith fatty acid (FA_i) after normalizing to the relative concentration of 18:1n-9. The transformation prevented testing of 18:1n-9, consequently the untransformed relative concentration of this fatty acid was examined by ANOVA. Homogeneity of variance testing, using Levene's test, indicated that only the Kuskokwim Bay, Takotna and Kwethluk River samples met the assumptions underlying the ANOVA and were therefore the only samples tested. The one-way MANOVA used the set of 30 transformed fatty acids representing the Kuskokwim Bay, Takotna, and Kwethluk samples.

To visualize differences detected by analysis of the fatty acid compositions a principle component analysis (PCA) model was constructed using the entire data set. Only the first three components were retained and the component scores for each sample were plotted in the three dimensional scatter plot.

The differences identified by the MANOVA were further resolved by fitting the data to a series of PCA models and evaluating the residual error. Two component models were constructed for each of the Kukokwim, Takotna and Kwethluk groups. A 95 percent confidence interval for the model centroid was determined from equation 3.4:

$$0 \pm \sqrt{s_{t_a}^2 \times F_{2,N-2,\alpha} \times 2\left(N^2 - 1\right) \Big/ \left(N\left(N - 2\right)\right)} \qquad \text{(Eq. 3.4)}$$

Where $s_{t_a}^2$ is the variance of the scores for the a[th] component and N is the number of samples in the group. Note that the principle components model is constructed from the correlation matrix so that the centroid mean is set to 0. The first two eigenvectors were used to predicted the locations of all of the unmodeled samples. Those samples whose locations fell outside the 95% confidence interval for a group centroid were considered to have a different fatty acid composition than that described by the group's mean. In addition, the residual error between the observed and modeled value was considered for each of the modeled samples. Dividing the residual standard deviation by the pooled standard deviation for the modeled group estimated the distance between the sample and the centroid mean in units of standard deviation. Moreover, the ratio has an approximate F distribution so the probability of membership in the centroid could be calculated. The size of the Takotna group (Table 3.3) limited the number of fatty acids that could be included in the principle component models, so a subset of 15 fatty acids was used. Fatty acids selected for the analysis all had observed concentrations greater than zero and represented those fatty acids with the greatest concentrations. These included the following fatty acids: 14:0, 16:0, 16:1n-7, 18:0, 18:1n-11, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:1n-9 + -11 (concentrations of fatty acids 20:1n-9 and 20:1n-11 combined because they co-eluted), 20:4n-6, 20:5n-3, 24:1n-9, 22:5n-3 and 22:6n-3. Combined, these fatty acids also accounted for more than 95% of the total recovered mass of fatty acids. Their concentrations were re-expressed as the percent of the unit sum for the subset and transformed as in equation 3.3 for statistical analyses.

3.4 Results

3.4.1 Lengths and weights

Lengths of the sampled fish depended on whether or not they had been feeding ($P < 0.001$). The Fed and Kuskokwim Bay fry were the longest fish sampled ($P < 0.005$) (Figure 3.1). The Fed fry were longer than the Emerged and Starved fry, which represented the same southeast Alaskan stock. Similarly, the Kuskokwim Bay fry were longer than either the Kwethluk or Takotna River fry. In addition, the southeast Alaskan fish were longer than their Kuskokwim analogs: Emergent and Starved fry were significantly longer than the Kwethluk and Takotna River fry, and the Fed fry were significantly longer than the Kuskokwim Bay fry.

Similar to length, the heaviest fish were those that were feeding (Figure 3.1). The heaviest fish were the Fed and Kuskokwim Bay fry ($P < 0.003$). Despite the differences in length, the Kuskokwim Bay and Fed fry did not differ in weight ($P = 0.206$). Similarly, there were no detectable differences among the average weights of the recently emerged or starved fry ($P > 0.976$).

Condition factors of feeding fry were significantly higher than those of unfed fry. Initially, the ANCOVA revealed a significant interaction ($P < 0.001$) between group and length on weight, indicating a significant difference among the slopes of weight and length among groups. Examination of the slopes for each group demonstrated that those of the Fed fry and Kuskokwim Bay fry were significantly steeper than the remaining groups (Figure 3.2). Therefore it was not possible to directly compare their length specific masses to those of the remaining groups. Removing the Fed and Kuskokwim Bay fry from the data set resulted in no interaction between group and length on weight ($P = 0.308$), but a significant effect of group on mass specific weights ($P < 0.001$). Pairwise comparisons revealed that fry from the Takotna River had higher condition than the Emerged, Starved or Kwethluk fry ($P < 0.007$). The Starved fish had lower condition than either the Emerged or Kwethluk Fry ($P < 0.006$).

3.4.2 Proximate composition analysis

Comparison of the proximate compositions of Emerged, Fed and Starved fry indicate that feeding fry increased their allocation of energy towards storage of lipids (Figure 3.3). The mean percent lipid was significantly greater in Fed fry than either the

Emerged or Starved fry ($P = 0.002$), but there were no differences in mean percent protein ($P = 0.710$). In addition, moisture contents did not differ ($P = 0.525$). Despite differences in lipid content, there were no detectable differences in their mean energy densities (mass specific energy content, $P= 0.203$). This likely resulted from the relatively low contributions of lipid to total body mass for all groups. However, the significantly larger percent lipid in the Fed fry resulted in lipid contributing a significantly greater number of kilojoules to the total energy content of the Fed fry ($P = 0.013$) compared with the Emerged fry. Lipid contributed an average 30.0% to total energy in Fed fry compared with 21.5 and 24.1% in the Starved and Emerged fry, respectively. In contrast, no difference in the proportion of energy allocated to lipid was observed between Fed and Starved fry ($P > 0.05$) suggesting that starving fry used energy proportionate to their initial allocations.

The proximate compositions and energy densities of the Kuskokwim watershed fry differed significantly among sampling locations ($P < 0.002$, Figure 3.4), but did not follow the pattern observed in the collections of Emerged, Starved and Fed fry. Rather than increasing in lipid content after feeding, fry from the Kuskokwim drainage reduced their lipid content as they moved seaward. Kwethluk River fry had the highest lipid content ($P < 0.018$) averaging 3.8% compared with 3.3% and 2.1% for fry from the Takotna River and Kuskokwim Bay, respectively. Consistent with the relatively high lipid content, fry from the Kwethluk River had significantly less moisture ($P < 0.001$) than fry from either the Takotna or Kuskokwim Bay. The mean percent protein was highest among Kuskokwim Bay fry (14.9%), followed by Kwethluk (14.1%) and Takotna River (13.4%) fry. As with the percent lipid, each of these groups differed from the remaining two ($P < 0.018$).

In contrast to the Emerged, Starved and Fed groups, fry from the Kuskokwim River drainage had the highest energy density shortly after they emerged. The relatively high lipid contents of the upstream fish meant that they had higher mean energy densities than the Kuskokwim Bay fry. Energy density was greater among Kwethluk River fry (4.2 kJ/g) than either those from the Takotna River (3.9 kJ/g) or Kuskokwim Bay (3.8 kJ/g) ($P < 0.008$, Figure 3.4). However, no difference in energy density was detected between the Takotna River and Kuskokwim Bay fry ($P = 0.139$). In contrast to the Fed fry, the Kuskokwim Bay fry allocated more energy towards growth than storage. Lipid provided

the largest contribution to total energy content in the upstream fry compared to Kuskokwim Bay fry (Figure 3.5). On average, lipid contributed 32.2% and 31.0% of the total energy of Kwethluk and Takotna River fry, respectively ($P = 0.997$). In contrast, a significantly lower amount (20.1%) of the total energy of the Kuskokwim fry was derived from lipid ($P < 0.001$). Consequently, protein contributed significantly more to the total energy of the Kuskokwim Bay fry than the Kwethluk and Takotna River fry.

Fry in Kuskokwim Bay had the same energy densities and energy allocations, regardless of size. Initially, fry in Kuskokwim Bay were arbitrarily divided into three size classes after examining the length frequency distribution of all the sampled fish. The fish in the small, medium and large size classes had average fork lengths of 36, 42 and 46 mm, respectively. Fish from each of these classes had similar energy densities ($P > 0.106$), averaging 3.67 kJ/g. Similarly, the contribution of lipid to the total energy in these fish was the same among size classes ($P > 0.120$), averaging 19.4%.

3.4.3 Lipid class composition

Analytical results indicated that the calibrated compounds generally accounted for more than 87% of the observed peak area. The uncalibrated peaks likely represent structural elements such as cerebrosides, diphosphatidylglycerol, phosphatyidylglycerol, phosphotidylinositol, sphingomyelin and lyso-PC. Both calibrated and uncalibrated peaks varied by less than 20% in the duplicated samples and method blanks appeared clean. There was wide variation between batches when the reference materials were compared, which likely results from degradation of the reference material between runs. This is further indicated by the high degree of consistency (< 20% variation) in the results of the calibration standards included with each batch of samples.

Levels of free fatty acids (FFA) indicate little evidence of hydrolyzation of the samples during storage. FFA levels varied significantly among locations ($P = 0.003$, Figure 3.6a), with the highest levels observed among fry in Kuskokwim Bay, accounting for nearly 50% of their lipid. This was significantly higher than the amount observed in Kwethluk fry ($P = 0.005$), but not Takotna River fry. While FFA levels near 50% might indicate a significant degree of hydrolyzation during processing and shipping, these levels were consistent with those of Fed fry, which were frozen at –80˚C within an hour of sampling. It is therefore more likely that the similarity between the Fed and

Kuskokwim Bay fry indicates fry are actively feeding and rapidly converting ingested lipids to energy.

Similarly, comparison of the PC/PE ratio indicates that oxidation of the samples during collection and storage was minimal. PC/PE ratios varied among the locations ($P < 0.001$, Figure 3.6b), with the highest ratio observed among the Kwethluk fry. Their average PC/PE ratio was 3.8, which was significantly greater ($P = 0.005$) than that of the Takotna fry, (PC/PE = 2.7). Both of these were greater than that of the Kuskokwim fry, 2.0 ($P < 0.005$). Ratios less than 2.0 often suggest evidence of oxidation of PC, however only the Emergent fry had an average ratio less than 2.0, and these were frozen to –80 ˚C within an hour of collection in the field, providing little time for oxidation. Increased levels of PE relative to PC are common among fish exposed to warm water (Hochachka and Somero 2002). Rainbow trout acclimated to 5 ˚C and transferred to 20 ˚C increased their PC/PE ratios from less than 2.5 to greater than 3.0 over the course of 10 days (Hazel and Carpenter 1985). Chum salmon fry collected from the upriver sites likely did not experience temperatures as high as 20 ˚C. Consequently, the reason for elevated PC/PE ratios among the Kwethluk and Takotna River fry is unknown.

The lipid content of fish correlated with the TAG content ($r^2 = 0.873$, Figure 3.7). Fish from the Kwethluk and Taktona Rivers had the highest levels of TAG averaging 16 to 17% of the total lipid ($P < 0.001$, Figure 3.6c). In contrast, fish from Kuskokwim Bay averaged less than 1% TAG. The high levels of TAG in the Kwethluk and Takotna River fish were similar to those of the Fed fish ($P > 0.05$), which averaged 13%. In addition, the Kwethluk and Takotna River fish had significantly greater amounts of TAG than those of the Emerged fry from Auke Creek, which averaged slightly more than 1% TAG. No TAG was detected in the Starved fry.

In addition to TAG, wax and cholesterol esters also represent a potential energy store for which Kwethluk and Takotna River fry had significantly elevated levels ($P < 0.001$). Takotna fry had the highest levels of wax and cholesterol esters with average levels (4.0% of total lipid) that were higher than those of the Kwethluk River or fry in Kuskokwim Bay. Similarly, the Kwethluk fry (2.8%) had significantly higher levels than those from Kuskokwim Bay (1.4%). It was difficult to make comparisons with the

Emerged, Starved and Fed fry samples, because only 2 to 3 samples were collected, which led to high variability in the estimated means.

Increased levels of TAG and wax and cholesterol esters in Kwethluk and Takotna River fry meant that they had greater amounts of energy in storage (Figure 3.8). Both groups had approximately 5% of their energy in storage in contrast to the Kuskokwim Bay fry who averaged 0.1% stored energy, a value that was significantly less than the upriver fry ($P = 0.005$). Low levels of energy allocated to storage among fry in Kuskokwim Bay was consistent among all size classes ($P = 0.567$), with the Small, Medium and Large fry all with less than 1.0% of their energy in storage.

The high levels of energy in storage likely resulted from feeding by the Kwethluk and Takotna fry as demonstrated by the comparison between the Emerged and Fed fry. The former averaged 0.3% of their energy in storage at emergence, nearly an order of magnitude less than the 4.0% observed in the fry that had been fed.

3.4.4 Fatty acid composition

Twenty-nine fatty acids were detected in the Kuskokwim Bay, Kwethluk and Takotna River samples while 33 fatty acids were detected in the Emerged, Fed and Starved samples (Tables 3.3, 3.4, respectively). Concentrations are reported as a percent of the total fatty acids observed. A MANOVA revealed significant differences in the fatty acid compositions of the Kuskokwim Bay, Kwethluk, and Takotna River fish (Wilk's $\lambda = .001$, $P < 0.001$). The one-way ANOVAs indicated that these differences were greatest between the Kuskokwim Bay and upriver fish. Concentrations of each of the 30 fatty acids considered differed between the Kuskokwim Bay and upriver fish ($P < 0.001$). In general, concentrations of the saturated fatty acids, 18-carbon n-6 and n-3 fatty acids, 20:4n-6 and 22:6n-3 were higher among Kuskokwim Bay fish than in the upriver fish (Table 3.3). Upriver fish had greater concentrations of monounsaturated fatty acids such as 18:1n-9, which comprised as much as 25% of the total fatty acids observed. Differences in the 18-carbon n-6 and n-3 fatty acids were most extreme for the n-6 fatty acids, so that n-3/n-6 ratios averaged 4.58 for the Kuskokwim Bay fish in contrast to 12.4 and 13.2 for the Takotna and Kwethluk fish, respectively. Kwethluk and Takotna River fish differed significantly in their concentrations of 18-carbon n-6 and n-3 fatty acids, 18:1n-9 and 22:6n-3 ($P < 0.001$). The values of the 18-carbon n-6 and n-3 fatty acids

were higher for the Kwethluk River fish so that they were intermediate in value between those of Kuskokwim Bay and the Takotna River. Fatty cid 18:1n-9 was highest for fish from the Takotna River.

Emerged, Fed and Starved fish also had differing fatty acid compositions (Table 3.4). Similar to differences between the Kuskokwim and upriver fish, the Emerged and Fed fish differed ($P = 0.004$) in their n-3/n-6 ratios with mean values of 13.7 and 3.2, respectively. The Starved fish were intermediate with a mean ratio of 7.5. The high ratio observed among the Emerged fish resulted from relatively large concentrations of 22:6n-3 and 20:5n-3, and combined these accounted for 42% of the total fatty acid content in the Emerged fish. In contrast, values observed in the Fed fish averaged 29%. In contrast to the comparison between upriver and Kuskokwim Bay fish, the Emerged fish had lower levels of monounsaturated fatty acids and higher levels of saturated fatty acids than the Fed fish.

The Small, Medium and Large groups had compositions generally consistent with that of the Kuskokwim Bay group (Table 3.5), but there was some evidence of size-related changes in composition. All four groups had similar amounts of saturated and monounsaturated fatty acids, with average amounts ranging from 18.8 to 21.2% and 21.0 to 24.9%, respectively. The relative amounts of n-3 and n-6 fatty acids appeared to vary with size. The Small group had an average n-3/n-6 ratio equal to 6.1, Medium averaged 5.5 and Large averaged 4.7. As fish size increased n-3 concentrations declined and n6 concentrations increased. For example, 18:2n-6 averaged 3.7%, 4.3% and 5.3% in the Small, Medium and Large groups, respectively. In contrast, 22:6n-3 declined from an average 34.0% among the Small group to 32.7% among the Medium and 31.8% among the Large group.

The differences in composition were apparent after plotting the first three component scores for each sample from the initial principle component analysis (Figure 3.9). The first three components accounted for more than 97% of the variation in the data set and the model appeared to separate the groups into three clusters. The first component separated the Kuskokwim Bay, Large, Medium, and Small groups from the Takotna, Kwethluk, Emerged, Starved, and Fed groups. Except for 18:1n-7, all of the fatty acids contributed equally to this separation. The second component, accounting for an

additional 11.1% of the error, separated the Fed and Starved groups from the remaining groups. Fatty acids 18:1n-11 and 18:2n-6 accounted for most of this separation. The third component accounted for 3.3% of the error and increased separation between the Starved and Kuskokwim Bay fish. The remaining components accounted for less than 2% of the error.

Principle component models constructed to examine the separation between the groups confirmed the differences between the upriver fish and the Kuskokwim group. All of the samples in the Kuskokwim Bay group fell within the group's 95% confidence interval, though two of the samples had unusually large errors (Figure 3.10). All of the Kuskokwim samples were within 1.6 standard deviations of the centroid mean. Similarly, all of the fish in the Small, Medium, and Large groups fell within 1.3 standard deviations of the Kuskokwim Bay centroid. In contrast, none of the Emerged, Fed, Starved, Kwethluk, or Takotna River samples fell any closer to the Kuskokwim centroid than 3.6 standard deviations. Consequently, the probability of any of these samples being included in the Kuskokwim group was extremely low ($P < 0.001$). Comparison of the Kwethluk and Takotna principle component models revealed a high degree of overlap between the fatty acid compositions of the Takotna and Kwethluk groups (Figure 3.11). All of the Kwethluk samples and five of the 15 Takotna samples were described by the mean fatty acid composition of the Kwethluk samples. In contrast, none of the remaining groups were adequately described by the Kwethluk models. Similarly, the mean centroid derived from the Takotna model described all of the Kwethluk samples and 11 of the 14 Takotna samples (Figure 3.12). Residual error for the remaining groups exceeded 3.2 standard deviations, yielding a low probability of membership ($P < 0.001$).

While none of the samples from the Emerged, Fed, or Starved groups fit any of the group models, the fatty acid compositions of the Fed and Starved groups were most similar to the Kuskokwim Bay group (Table 3.6). The distances between the Fed and Starved fish samples and the Kuskokwim Bay centroid were consistently lower than the distances to either of the upriver centroids (Table 3.6). This indicates that the model for the Kuskokwim Bay group was best at describing the fatty acid compositions of the Fed and Starved groups. In contrast, the Emerged samples fit all three models equally (Table 3.6), with distances ranging between 3.2 and 4.2 standard deviations.

3.5 Discussion

3.5.1 Size and energy allocation

These data indicate fry migrating down the Kuskokwim River lost lipid between the emergence and saltwater entry, while fry that were held and fed increased in lipid content. The lipid content of fish sampled in Kuskokwim Bay was only about 50 to 60% that of fry from the Kwethluk and Takotna Rivers. Consequently, Kuskokwim Bay fry had diminished energy densities. This is apparently the result of greater allocations of energy towards protein as previously reported by Azuma et al. (1998). Lipid has nearly twice the energy per unit mass than protein, but protein is denser. Consequently, Kuskokwim Bay fry had greater amounts of calorie-reduced but heavier protein in their tissues. In contrast, Fed fry increased their lipid content. This suggests that the relatively high quality food and frequent feeding experienced by these fish allowed them to maximize growth while simultaneously storing energy.

Additionally, these data suggest estimates of the proportion of energy allocated to lipid during estuarine residence may predict future survival of wild chum salmon. Fluctuations in the energetic contribution of lipid result from fluctuations in energy stored as TAG. Phospholipids also contribute to lipid energy, but these are used primarily as structural elements and their levels generally do not fluctuate with changes in total energy (Nomura et al. 2000). Increasing amounts of lipid during estuarine residence may therefore signal that growth is maximized and surplus energy is being allocated to storage.

It is not known if any of the fish sampled from Kuskokwim Bay emerged in the Takotna or Kwethluk basins. Consequently, the conclusions drawn for Kuskokwim Bay fish may be confounded by the presence of fish in various nutritional states. For example, increased lipid content of fish with maximal growth rates may be masked if samples include fish with reduced growth rates and depleted lipid. Our use of randomly pooled samples may have exacerbated this problem by mixing fish with different nutritional states. However our observations of no differences in the energy density or allocation among the different size classes of fish sampled in Kuskokwim Bay suggest this is not the case. Furthermore the analysis indicates that all the fish in Kuskokwim Bay allocated energy the same way, regardless of size.

Juvenile fish foraging in estuaries require rapid growth in order to avoid predators (Parker 1968). In addition, rapid growth ensures a relatively large mass in winter, which lowers metabolic cost and reduces the probability of starvation (Nomura et al. 2000 Beamish et al. 2004). Therefore, we expect fry rearing in estuaries to store relatively little lipid in favor of maximizing tissue growth and protein synthesis as indicated by the relatively high protein content of the Kuskokwim Bay fry. Varnavsky et al. (1992) reported increasing growth rates among coho and pink salmon as they emigrated seaward from the Paratunka River and through an associated estuary. Comparisons between the Fed and Kuskokwim Bay fry suggest that energy storage may occur if fry obtain sufficient energy to maximize growth. However, there are no data describing the growth rates of the chum from either location. Furthermore, allocation of energy to lipid in the Fed fry may be an artifact of captive culture in small containers devoid of predators. The relationship between growth and energy storage in juvenile fish might therefore provide insight into processes that regulate recruitment in the early marine stage.

The relatively high lipid content of the upstream fish is an energy supply that could be used as fry learn to forage during the first days following emergence. In Atlantic salmon this energy reserve is developed between hatching and emergence by catabolizing protein (Berg et al. 2001). Evidence that the high lipid levels in the Kwethluk and Takotna fry (> 3%) represent energy provisions for their downstream migration is offered by the relatively low (2.1%) lipid content of the Emerged fry who need to migrate less than 1 km to reach salt water. However, this explanation does not account for the relatively low lipid content of the Takotna River fry relative to that of the Kwethluk river. Takotna River fry may be actively feeding with concomitant decreases in lipid content. This is also consistent with their increased length specific mass. Takotna River fry have a much longer distance to reach sea water and therefore may require feeding in order to secure sufficient energy to complete emigration.

A possible alternative explanation for the decreased lipid content of the Takotna River fry is that their mothers had less energy to allocate to eggs than Kwethluk females. The Takotna adults must migrate at least 700 km more than the Kwethluk chums. This difference could impose a restriction on the amount of energy available to eggs, if females arrive at the mouth of the Kuskokwim with similar amounts of energy reserves.

We are unaware of any data describing the energy content of adult chum salmon in the Kuskokwim basin. However, Crossin et al. (2004) reported that adult sockeye salmon migrating entering the Fraser River varied in mass specific energy content according to the length and difficulty of their migration, but arrived on the spawning grounds with similar amounts.

Comparisons with starved fry should be made with caution, because they may have been feeding during the culture period. Starved and Fed fry shared the same water supply and were separated by a screen. Therefore, Starved fry may have been able to obtain food particles not consumed by the Fed fry. This is further suggested by the absence of differences in size and proximate composition between them and the Emerged fry. However, Starved fry did have lower length specific mass than the Emerged fry, suggesting a loss of condition. If this resulted from low rations or starvation, then it is apparent that these fish lost energy from the protein and lipid compartments in proportion to their initial composition.

3.5.2 Lipid class composition

Previously we reported that fry from the Kwethluk and Takotna Rivers had higher lipid levels than those of Kuskokwim Bay. These data are consistent with those and suggest that the Kwethluk and Takotna River fry are actively foraging and storing the ingested energy in contrast to the Kuskokwim Bay fry who appear to be converting ingested lipid into energy. The low level of TAG in the Emergent fry indicates that fry in the Kwethluk and Takotna must be acquiring energy. The previously noted reduction in the lipid content of the Takotna River fry is due to a reduction in the amount of TAG as shown in Figure 3.6. If these upriver fry are actively foraging and storing energy, then reduced lipid in the Takotna fry may indicate either poor foraging conditions or less time spent foraging than the Kwethluk fry.

Previously we noted that the lipid content of chum rearing in estuaries may provide a measure of their future success. Data from the Fed fry demonstrate that chum fry in saltwater are capable of storing energy, however the conditions were clearly artificial. Wild fry will be faced with uncertain food supplies, greater spatial ranges associated with foraging and the risk of predation. All of these constraints are likely to reduce the ability of fry to store energy. Therefore, a more sensitive measure of the

condition or nutritional status of estuarine fry may be their growth rates. This is further demonstrated by the observation that there was no difference in the amount of energy stored by the Kuskokwim Bay fry with respect to size.

3.5.3 Fatty acid composition

Comparison of the upriver and Kuskokwim Bay groups revealed differences that describe the extremes of a continuum with emergent fry at one end and foraging fry on the other. The relative concentrations of the included fatty acids differed between the upriver and Kuskokwim Bay groups. These differences were clearly resolved by the principle components analyses. This conclusion is reinforced by the inability of either of the upriver models to adequately describe the Small, Medium, and Large groups while they fit the Kuskokwim Bay model with minimal residual error. The difficulty resolving compositional differences among the two upriver groups suggests that despite evidence of differences in the relative concentrations of specific fatty acids, their compositions are more similar to each other than to the Kuskokwim Bay group.

Shifts in the fatty acid compositions of diadromous species are well-described and believed to result from differences in the availability of essential fatty acids at the base of the fresh and saltwater food webs. Marine alga are rich sources of 20- and 22-carbon polyunsaturated fatty acids such 20:5n-3 and 22:6n-3 (Sargent et al.1995). In contrast, essential fatty acids produced by vascular plants and green alga comprise 18:2n-6 and 18:3n-3. Essential fatty acids cannot be synthesized by animals and predators, such as salmonids, must obtain them from their diet. A result of these differences is that freshwater resident salmonids have essential fatty acid compositions with relatively high amounts of 18:2n-6 and 18:3n-3. In contrast, smolts and other marine resident forms tend to have fatty acid compositions with relatively high concentrations of 20:5n-3 and 22:6n-3 (Lovern 1934). These changes are often summarized using n-3/n-6 ratios, with marine species tending to have higher n-3/n-6 ratios than freshwater species (Henderson and Tocher 1987).

The fish from the Takotna and Kwethluk groups had relatively high n-3/n-6 ratios, suggestive of marine-type fatty acid compositions. The ratios observed in the two upriver groups were consistent with those reported for marine species such as rainbow smelt, walleye pollock, and Pacific herring (Iverson et al. 2002). In contrast, the ratio

observed for the Kuskokwim Bay fish were nearer to 3.3, the value reported for masu salmon prior to smolting (Ota 1976). Further evidence of a marine-type fatty acid composition among the upriver groups is indicated by the relatively high values of n-11 fatty acids. The data reported here combined the estimates for 20:1n-9 and 20:1n-11 as well as those of 22:1n-9 and 22:1n-11. However, peak areas for the n-11 isomers were generally much greater for upriver fish than those from Kuskokwim Bay. The n-11 isomers are primarily produced as fatty alcohols in the wax esters of marine calanoid copepods (Saito and Kotani 2000) and are generally unknown in fresh water.

It is likely that the marine-type composition in the upriver fry was derived from maternally derived yolk lipids. The upriver fish were likely collected much closer in time to their emergence date than those found in Kuskokwim Bay, therefore probably have more residual yolk lipids than those that have had more time to forage. Adult female chum salmon migrating upstream do not feed, consequently their lipids are derived from marine sources. Provisioning of yolk lipids occurred during the upstream migration of sockeye salmon in the Fraser River (Ballantyne et al. 1996), however most of this provisioning appeared to affect energy substrates. High n-3/n-6 ratios (i.e. > 17.0) were reported for wild Chinook salmon alevins in Robertson Creek and the Qualicum River in British Columbia (Ashton et al. 1993). Previous studies describing the fatty acid composition of chum and pink salmon ovaries collected at freshwater entry indicate that n-3/n-6 ratios averaged 19.8 and 18.5 for pink and chum salmon, respectively (Heintz unpublished data). Thus, the presence of relatively large amounts of n-3 fatty acids in the upriver fry more likely reflects the composition of maternally provided lipids than those acquired in the diet.

Contrary to the conclusion that the upriver fish are newly emerged is the lack of fit between the Emerged group and the upriver groups, which might suggest evidence of feeding by the upriver groups. However, the Takotna, Kwethluk and Emerged groups had the lowest average 18:2n-6 content, which is diet-derived. Moreover, the n-3/n-6 ratios among the Emerged, Kwethluk, and Takotna groups were the highest observed among all the groups and there appeared to be a relationship between the n-3/n-6 ratio and the distance required to migrate. Emerged fish from Auke Creek, Alaska only need to migrate a few hundred meters to reach the estuary in contrast to the Kwethluk and

Takotna groups. The relatively high value for the n-3/n-6 ratio observed in the Emerged group likely reflects the relatively high phospholipid content. These membrane components accounted for more than 40% of the total lipid in contrast to the Kwethluk and Takotna groups which averaged less than 26% phospholipid. This higher amount of phospholipid in the Emerged fish tissues likely accounts for the higher n-3/n-6 ratio in these fish (Kreps et al. 1969). In contrast, the Takotna and Kwethluk groups had greater amounts of monounstaturated and saturated fatty acids, which are primarily used as an energy source, consistent with their longer migration distance. Thus the differences in fatty acid composition between the Emerged and upriver groups is more likely to be due to stock-related differences in migration distance than feeding behavior.

Despite capture in the Kuskokwim Bay estuary, the Kuskokwim Bay group had fatty acid compositions consistent with those of freshwater salmonids. This composition is characterized by relatively high concentrations of 18:3n-3, 18:2n-6, and 20:4n-6 along with low concentrations of 22:6n-3 (Saddler 1966). Fatty acids integrate recent feeding over a longer time span than that of stomach content analysis. The composition of the Kuskokwim Bay fish suggest foraging during their movement downriver. In addition, their capture within the freshwater plume in the estuary indicates continued feeding in freshwater. Evidence of foraging is further suggested by comparing the fatty acid compositions of the Small, Medium, and Large groups. As size increases, there is an increase in 18:2n-6 and a decrease in the n-3/n-6 ratios. Presumably, as fish forage they increase in size and acquire greater amounts of essential fatty acids derived from terrestrial plants and freshwater alga.

Consistent with the idea that Kuskokwim Bay fry were foraging is the apparent similarity in fatty acid composition with the Fed group. However, fry in the Fed group were collected from saltwater and fed a commercial diet. Consequently, it is unlikely that the Fed and Kuskokwim Bay groups were foraging on similar trophic levels or diets. In addition, the Starved group also fit the Kuskokwim Bay group model better than the upriver models. This likely results from the fact that the Starved fish were reared in the same tank, though upstream from the Fed group. Thus it is likely that some of the food provided to the Fed group was able to be consumed by the Starved fish resulting in the similarity between the Fed and Starved groups.

These data reveal two important energy sources that work to ensure the survival of juvenile chum salmon. Maternal provisioning provides juveniles with an important energy source that is apparently consumed shortly after emergence. The need to supply migrating fry with energy substrates places an obvious constraint on the productivity of upriver stocks. Ocean conditions are likely to have a much stronger influence on the productivity of upriver stocks because the amount of energy supplied to emergent fry will depend directly on the amount of energy passed to them by their maturing mothers. The second energy source that ensures the survival of juvenile chum is derived from riverine sources, and is likely employed as soon after emergence as possible. In the Kuskokwim River, dependence on this source apparently extends into the period of estuarine residence. This likely represents a boon to individuals that have consumed all their energy reserves during the downriver migration. Moreover, residence in the freshwater plume may offer reduced predation risk from marine predators. However, chum salmon in this phase have only small energy reserves. Consequently, reductions in the amount of availability of these riverine energy sources are likely to reduce survival in the rearing fry.

Table 3.1. Average range of fork length and wet mass of juvenile chum salmon collected from Kuskokwim Bay, and Kwethluk and Takotna Rivers.

	Date Collected	n	Fork length (mm)	Wet mass (g)
Kuskokwim Bay	1 June '04	123	42.1 (32-61)	0.688 (0.331-2.072)
Kwethluk R	23-25 April -04	113	33.4 (32-36)	0.327 (0.183-417)
Takotna R	1-11 May '04	140	34.0 (21-39)	0.316 (0.213-0.432)

[1]n = 113

Table 3.2. Number of pooled samples, number of fish pooled per sample, and average mass individuals used for pooling. Samples were collected from Kuskokwim Bay, and Kwethluk and Takotna Rivers for analysis of proximate, lipid class and fatty acid composition. Voucher specimens were collected from, Auke Creek in southeastern Alaska and a nearby hatchery.

	No. of samples	No. fish/sample	Average (1 s.e.) mass (g/individual)
Kuskokwim Bay	20	3-10	0.317 (0.011)
Kwethluk R	23	7-10	0.329 (0.019)
Takotna R	14	10-13	0.315 (0.004)
Kuskokwim Bay Size Stratified Samples			
Small	5	2-7	0.509 (0.011)
Medium	3	2-7	0.723 (0.018)
Large	5	2-7	0.964 (0.088)
Voucher Specimens			
Emerged	3	5	0.317 (0.011)
Starved	2	4	0.261 (0.003)
Fed	3	3-4	0.609 (0.089)

Table 3.3. Mean fatty acid compositions of juvenile chum salmon from Kuskokwim Bay, Kwethluk and Takotna Rivers. Sample sizes: Kuskokwim Bay 31, Kwethluk 23 and Takotna 14.

	Kuskokwim Bay	Kwethluk	Takotna
Saturated	18.81 ± 1.18	17.13 ± 1.06	16.75 ± 1.32
14:0	**1.63 ± 0.21**	2.29 ± 0.19	2.35 ± 0.16
15:0	**0.38 ± 0.05**	0.32 ± 0.09	0.29 ± 0.11
16:0	**11.28 ± 1.12**	10.00 ± 0.97	9.44 ± 1.28
17:0	**0.56 ± 0.05**	***0.33 ± 0.03***	0.28 ± 0.11
18:0	**5.50 ± 0.28**	4.11 ± 0.20	4.23 ± 0.15
20:0	**0.18 ± 0.03**	***0.05 ± 0.02***	0.05 ± 0.00
22:0	**0.09 ± 0.01**	***0.01 ± 0.02***	0.03 ± 0.01
24:0	**0.13 ± 0.03**	0.01 ± 0.02	0.04 ± 0.02
Monousaturated	**24.87 ± 3.58**	34.87 ± 1.66	37.45 ± 1.96
14:1n-5	- -	- -	- -
15:1n-5	**0.01 ± 0.02**	0.00 ± 0.01	0.04 ± 0.07
16:1n-7	4.66 ± 1.57	4.09 ± 0.30	4.10 ± 0.39
17:1n-7	**0.45 ± 0.09**	***0.50 ± 0.06***	0.38 ± 0.14
18:1n-11	**0.17 ± 0.06**	1.13 ± 0.22	1.28 ± 0.21
18:1n-9	**15.44 ± 2.22**	***23.80 ± 1.24***	25.89 ± 1.42
18:1n-7	**3.88 ± 0.51**	3.24 ± 0.11	3.56 ± 0.23
20:1n-9 + 20:1n-11	**0.40 ± 0.12**	1.15 ± 0.19	1.13 ± 0.13
22:1n-9 + 20:1n-11	**0.18 ± 0.05**	***0.31 ± 0.07***	0.29 ± 0.06
24:1n-9	**0.92 ± 0.22**	0.64 ± 0.14	0.77 ± 0.12
Polyunsaturated	51.56 ± 3.18	48.00 ± 1.87	45.80 ± 1.15
18:2n-6	**4.89 ± 1.08**	***1.11 ± 0.09***	1.08 ± 0.14
18:3n-6	**0.22 ± 0.05**	***0.05 ± 0.01***	0.03 ± 0.02
18:3n-3	**2.37 ± 0.39**	***0.78 ± 0.09***	0.70 ± 0.13
18:4n-3	**1.30 ± 0.45**	***1.08 ± 1.00***	0.70 ± 0.11
20:2n-6	**0.44 ± 0.06**	0.20 ± 0.02	0.20 ± 0.03
20:3n-6	**0.48 ± 0.12**	***0.14 ± 0.02***	0.13 ± 0.01
20:3n-3	**0.18 ± 0.02**	0.11 ± 0.01	0.09 ± 0.01
20:4n-6	**3.34 ± 0.34**	1.62 ± 0.14	1.67 ± 0.13
20:5n-3	**8.22 ± 0.76**	9.96 ± 0.71	9.96 ± 0.47
22:4n-6	**0.17 ± 0.02**	0.29 ± 0.03	0.31 ± 0.04
22:5n-3	**3.34 ± 0.49**	5.03 ± 0.38	5.43 ± 0.38
22:6n-3	**29.19 ± 2.98**	***27.64 ± 1.22***	25.42 ± 1.57
n-3	42.48 ± 3.73	44.60 ± 1.86	42.37 ± 1.25
n-6	9.07 ± 1.40	3.40 ± 0.19	3.43 ± 0.23
n-3/n-6	4.58 ± 1.00	13.15 ± 0.92	12.42 ± 1.00

[a] Values are mean mass percent ± 1 SD of the total fatty acids. Values in different fonts differ statistically ($P < 0.001$).

Table 3.4. Mean fatty acid compositions of the Emerged, Fed and Starved juvenile chum salmon groups. Values are mean mass percent ± 1 SD of the total fatty acids. Sample sizes: Emerged 3, Fed 3 and Starved 2.

	Emerged	Fed	Starved
Saturated	18.95 ± 0.59	17.86 ± 0.60	21.41 ± 1.61
14:0	1.19 ± 0.12	2.31 ± 0.21	1.19 ± 0.06
15:0	0.26 ± 0.02	0.29 ± 0.00	0.24 ± 0.00
16:0	12.44 ± 0.62	11.21 ± 0.65	14.75 ± 1.57
17:0	0.00 ± 0.00	0.27 ± 0.02	0.29 ± 0.02
18:0	4.93 ± 0.06	3.60 ± 0.08	4.77 ± 0.03
20:0	0.05 ± 0.00	0.08 ± 0.00	0.06 ± 0.00
22:0	0.03 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
24:0	0.05 ± 0.01	0.05 ± 0.00	0.07 ± 0.01
Monounsaturated	28.20 ± 0.80	39.01 ± 2.09	29.19 ± 0.37
15:1n-5	0.09 ± 0.06	0.00 ± 0.00	0.17 ± 0.10
16:1n-7	2.24 ± 0.05	3.74 ± 0.25	2.11 ± 0.11
17:1n-7	0.40 ± 0.01	0.26 ± 0.05	0.47 ± 0.06
18:1n-11	0.45 ± 0.03	0.29 ± 0.04	0.17 ± 0.06
18:1n-9	19.59 ± 0.60	27.90 ± 1.74	20.46 ± 0.67
18:1n-7	3.41 ± 0.09	3.51 ± 0.06	3.08 ± 0.02
20:1n-9 + 20:1n-11	0.16 ± 0.01	0.42 ± 0.01	0.19 ± 0.00
20:1n-11	0.16 ± 0.01	0.42 ± 0.01	0.19 ± 0.00
20:1n-9	0.52 ± 0.06	1.29 ± 0.02	0.79 ± 0.07
22:1n-9 + 20:1n-11	0.22 ± 0.03	0.82 ± 0.10	0.32 ± 0.02
22:1n-11	0.09 ± 0.01	0.55 ± 0.07	0.16 ± 0.02
22:1n-9	0.13 ± 0.04	0.27 ± 0.03	0.16 ± 0.00
24:1n-9	1.13 ± 0.12	0.78 ± 0.09	1.44 ± 0.26
Polyunsaturated	52.84 ± 0.61	43.13 ± 1.98	49.40 ± 1.98
18:2n-6	0.84 ± 0.06	7.18 ± 0.51	2.68 ± 0.20
18:3n-6	0.03 ± 0.00	0.15 ± 0.01	0.06 ± 0.01
18:3n-3	0.54 ± 0.06	1.17 ± 0.12	0.34 ± 0.04
18:4n-3	0.29 ± 0.02	0.89 ± 0.05	0.23 ± 0.00
20:2n-6	0.14 ± 0.02	0.51 ± 0.02	0.23 ± 0.03
20:3n-6	0.10 ± 0.00	0.42 ± 0.02	0.24 ± 0.00
20:3n-3	0.06 ± 0.00	0.11 ± 0.00	0.09 ± 0.01
20:4n-6	2.31 ± 0.20	1.62 ± 0.23	2.45 ± 0.32
20:5n-3	10.20 ± 0.04	5.87 ± 0.10	6.77 ± 0.31
22:4n-6	0.19 ± 0.02	0.26 ± 0.01	0.15 ± 0.00
22:5n-3	5.24 ± 0.29	1.87 ± 0.14	2.77 ± 0.12
22:6n-3	32.91 ± 0.35	23.11 ± 2.22	33.39 ± 1.52
n-3	49.24 ± 0.60	32.97 ± 2.25	43.60 ± 1.89
n-6	3.60 ± 0.21	10.16 ± 0.28	5.80 ± 0.09
n-3/n-6	13.69 ± 0.82	3.25 ± 0.31	7.51 ± 0.21

Table 3.5. Mean fatty acid compositions of Small (n=6), Medium (n=3) and Large (n=5) chum salmon fry collected in Kuskokwim Bay and pooled into composite samples. Values are mean mass percent ± 1 SD of the total fatty acids.

	Small	Large	Medium
Saturated	21.23 ± 0.74	19.58 ± 1.02	19.84 ± 0.94
14:0	1.62 ± 0.17	1.42 ± 0.12	1.57 ± 0.16
15:0	0.35 ± 0.03	0.31 ± 0.02	0.35 ± 0.04
16:0	12.59 ± 0.85	11.35 ± 0.79	11.45 ± 0.79
17:0	0.59 ± 0.06	0.57 ± 0.06	0.56 ± 0.10
18:0	5.69 ± 0.11	5.51 ± 0.27	5.51 ± 0.10
20:0	0.15 ± 0.01	0.18 ± 0.03	0.18 ± 0.02
22:0	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.00
24:0	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Monounsaturated	20.99 ± 2.11	23.45 ± 2.20	23.03 ± 1.35
15:1n-5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:1n-7	2.86 ± 0.54	4.73 ± 0.83	4.33 ± 0.44
17:1n-7	0.35 ± 0.04	0.35 ± 0.12	0.33 ± 0.11
18:1n-11	0.18 ± 0.09	0.13 ± 0.04	0.14 ± 0.04
18:1n-9c	12.64 ± 1.16	13.07 ± 1.06	13.15 ± 1.02
18:1n-7	3.44 ± 0.26	3.96 ± 0.33	3.91 ± 0.16
20:1n-9 + 20:1n-11	0.32 ± 0.10	0.33 ± 0.04	0.30 ± 0.03
20:1n-11	0.24 ± 0.07	0.24 ± 0.05	0.23 ± 0.02
20:1n-9	0.08 ± 0.04	0.09 ± 0.02	0.07 ± 0.01
22:1n-9 + 20:1n-11	0.13 ± 0.03	0.13 ± 0.00	0.16 ± 0.01
22:1n-11	0.08 ± 0.03	0.08 ± 0.01	0.11 ± 0.03
22:1n-9	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.02
24:1n-9	1.07 ± 0.21	0.76 ± 0.20	0.71 ± 0.07
Polyunsaturated	57.78 ± 2.34	56.96 ± 1.69	57.13 ± 1.73
18:2n-6c	3.73 ± 0.30	5.27 ± 1.68	4.31 ± 0.11
18:3n-6	0.13 ± 0.07	0.21 ± 0.03	0.19 ± 0.01
18:3n-3	2.11 ± 0.16	2.42 ± 0.17	2.42 ± 0.39
18:4n-3	1.39 ± 0.19	1.22 ± 0.47	1.36 ± 0.28
20:2n-6	0.45 ± 0.04	0.49 ± 0.03	0.40 ± 0.02
20:3n-6	0.32 ± 0.05	0.48 ± 0.12	0.39 ± 0.03
20:3n-3	0.17 ± 0.04	0.17 ± 0.01	0.16 ± 0.02
20:4n-6	3.30 ± 0.30	3.55 ± 0.21	3.35 ± 0.02
20:5n-3	8.69 ± 0.41	7.84 ± 0.71	8.16 ± 0.46
22:4n-6	0.17 ± 0.01	0.15 ± 0.03	0.16 ± 0.02
22:5n-3	3.32 ± 0.23	3.39 ± 0.34	3.51 ± 0.25
22:6n-3	33.97 ± 2.45	31.77 ± 1.80	32.72 ± 1.45
n-3	49.66 ± 2.45	46.81 ± 3.21	48.33 ± 1.69
n-6	8.12 ± 0.56	10.15 ± 1.52	8.79 ± 0.06
n-3/n-6	6.14 ± 0.60	4.71 ± 0.97	5.50 ± 0.17

Table 3.6. Distances between samples and group centroids. Values are in units of standard deviation from the centroid mean. Each sample represents a composite of several fish.

	Group Centroid		
	Kuskokwim Bay	**Kwethluk**	**Takotna**
Emergent	3.62	3.73	3.18
Emergent	3.66	3.76	3.21
Emergent	3.4	4.15	3.41
Fed	5.18	14.24	8.87
Fed	5.16	14.16	8.78
Fed	4.37	12.4	7.49
Starved	4.13	8.17	5.86
Starved	3.97	7.53	5.55

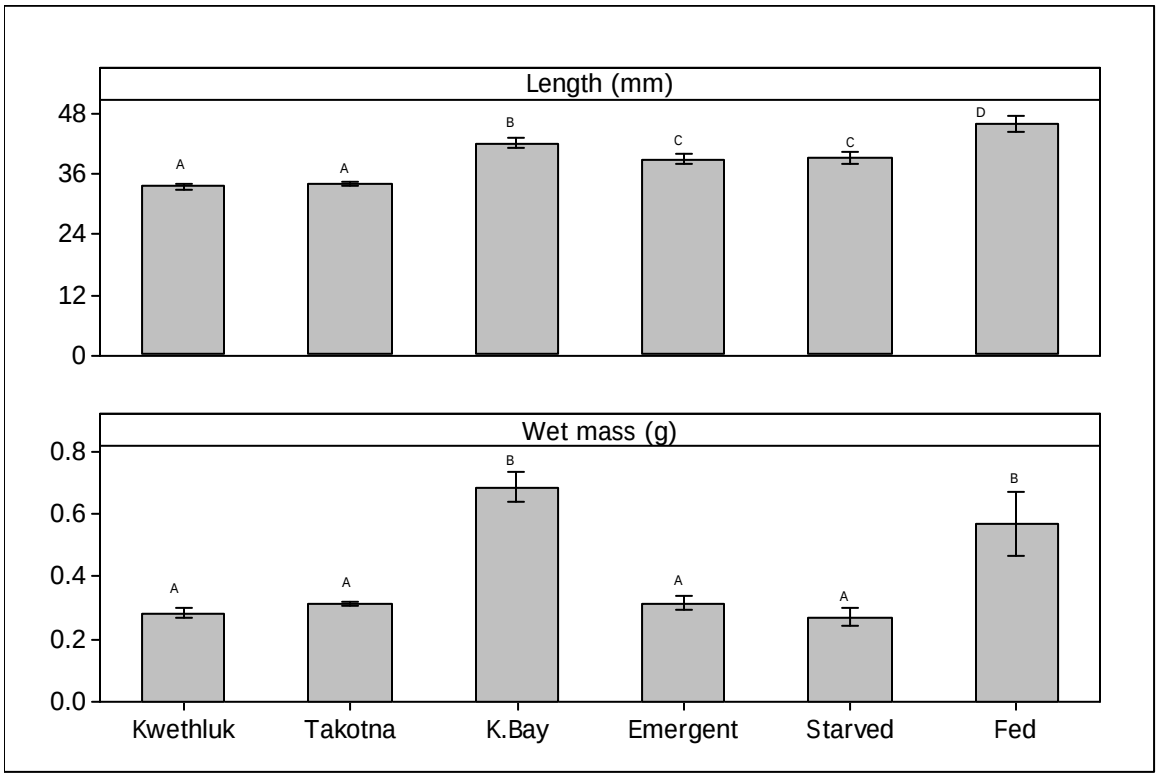

Figure 3.1. The 95% confidence intervals for lengths and weights of chum salmon fry. Emergent, Starved and Fed fish were collected from southeastern Alaska streams. Bars with common letters identify groups that do not differ significantly.

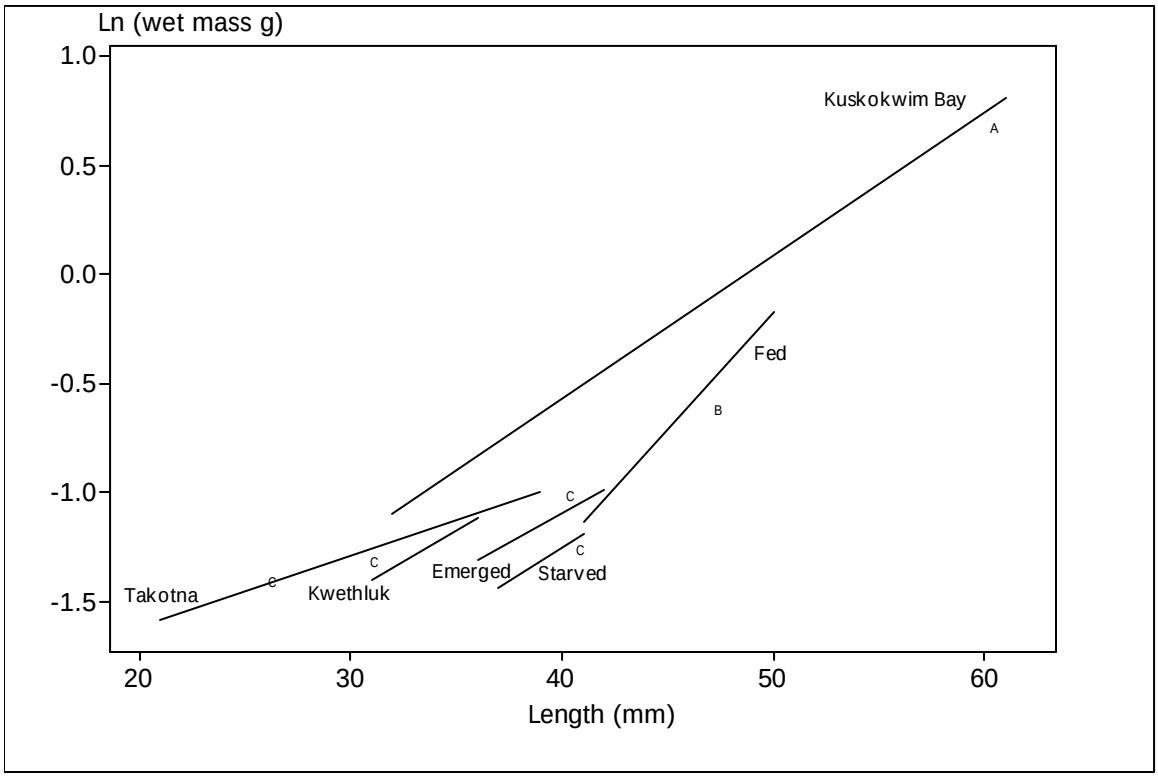

Figure 3.2. Slopes of regressions relating fork length and the natural log of wet mass for each of the groups studied. Slopes with common letters do not differ significantly.

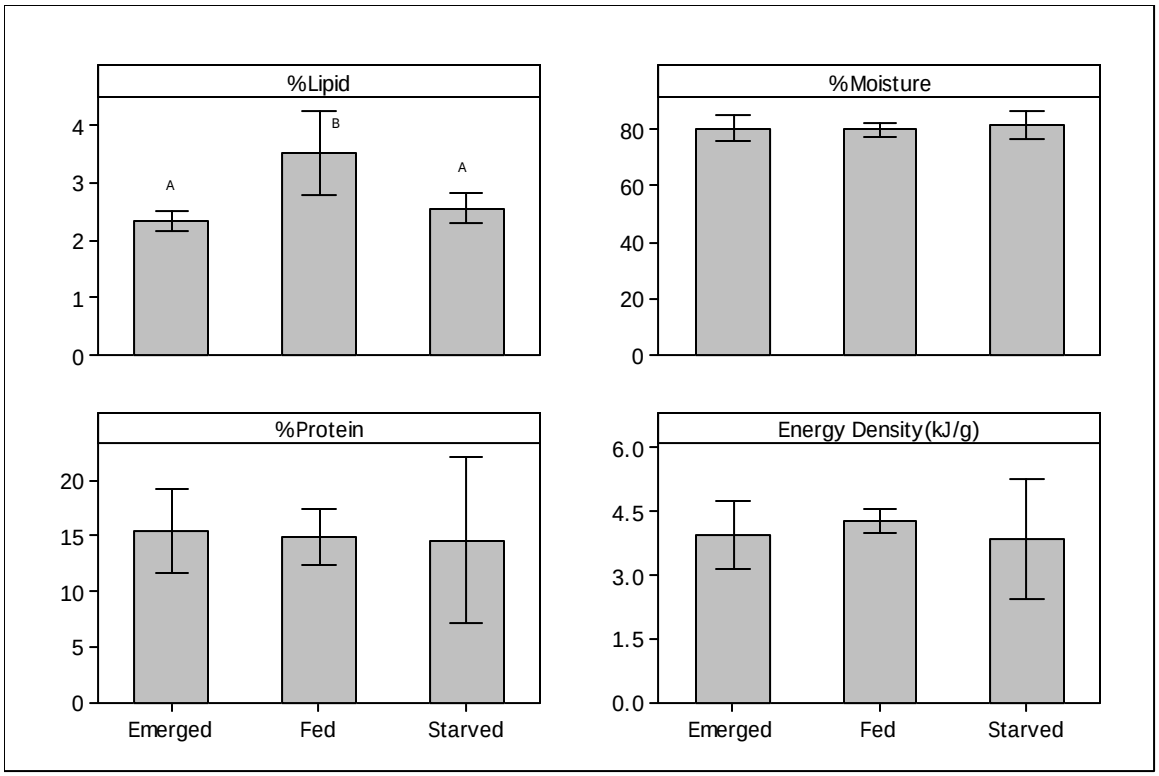

Figure 3.3. The 95% confidence intervals for the proximate composition and energy density of Emerged, Starved and Fed fry sampled in Juneau, AK. Bars with different letters have significantly different compositions.

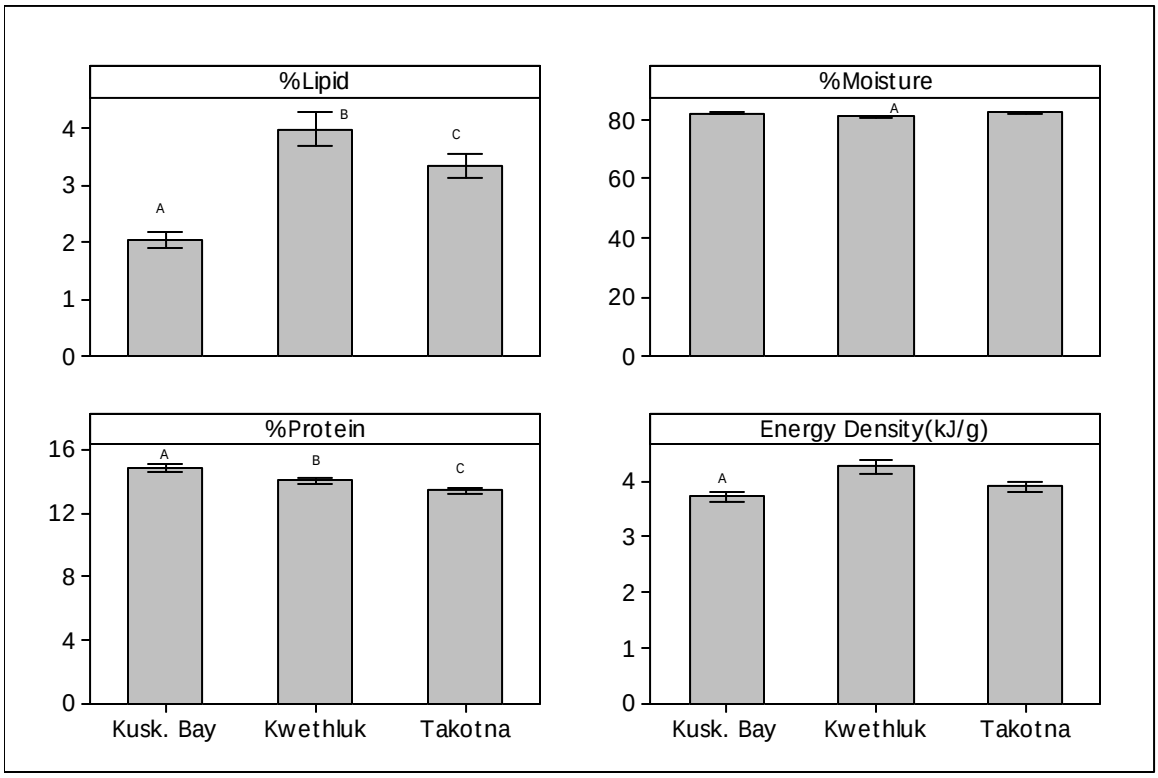

Figure 3.4. The 95% confidence intervals for the proximate composition and energy density of fry sampled from the Kuskokwim River drainage. Bars with different letters have significantly different compositions.

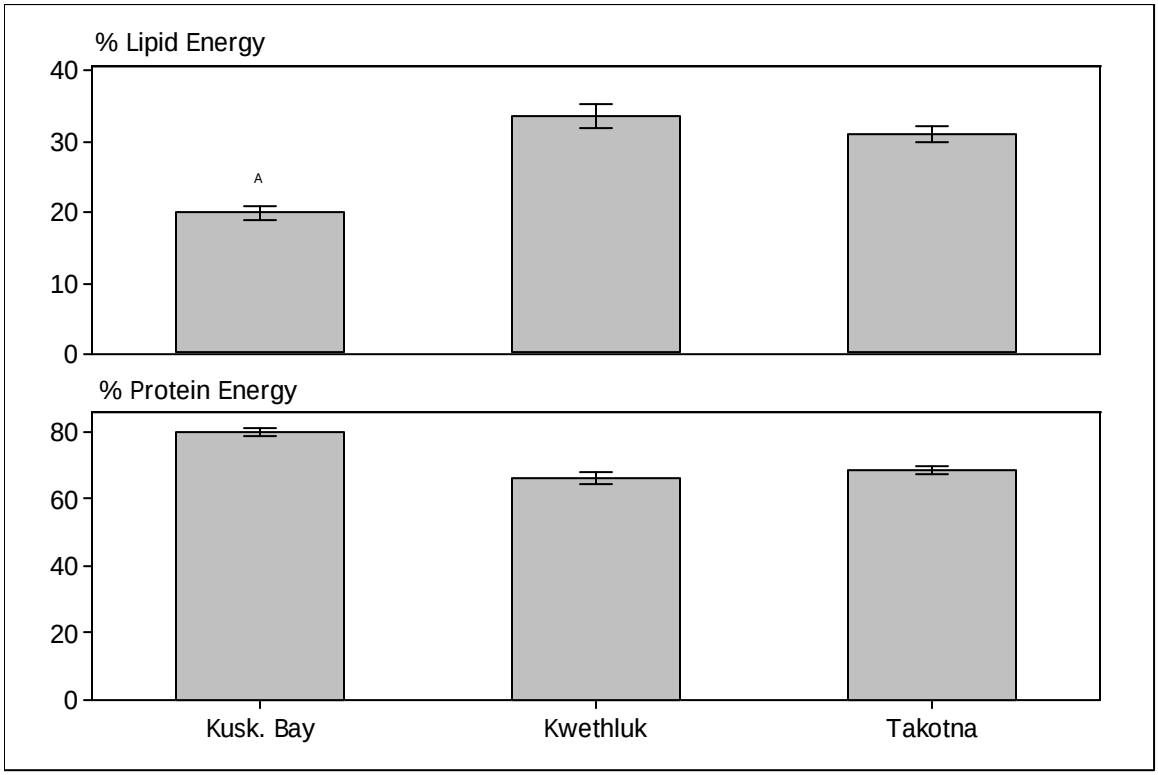

Figure 3.5. The 95% confidence intervals for the proportion of the total energy in fry allocated to lipid (top panel) and protein (lower panel). Bars with unique letters are significantly different from other bars. Protein was not tested, because the entire energy content is a function of lipid and protein.

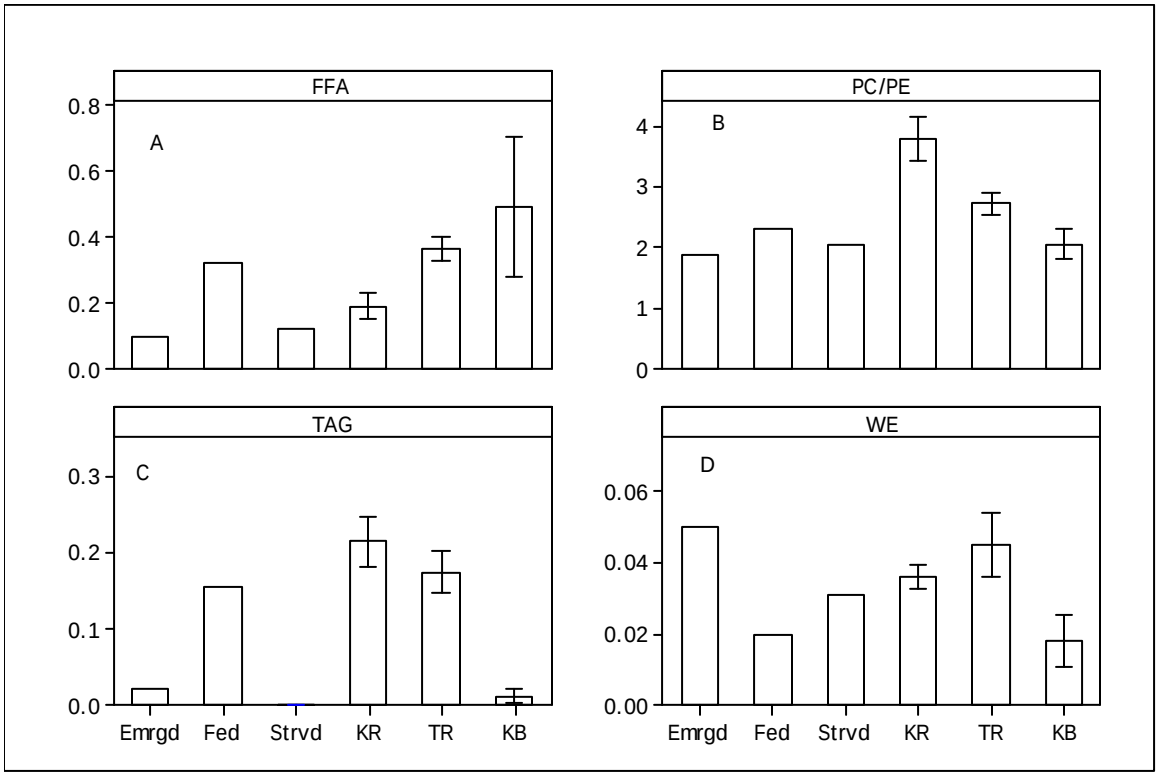

Figure 3.6. Average proportion of lipid allocated to a) free fatty acids, b) the ratio of phosphotidylchloine to phosphotidyethanolamine, c) triacylglycerols, and d) wax and cholesterol esters. Error bars reflect 95% confidence intervals; bars for Emrgd, Strvd and Fed are not shown because sample sizes were too small to calculate meaningful error estimates. Emrgd = Emerged, Strvd = Starved, KR = Kwethluk River, TR = Takotna River and KB = Kuskokwim Bay.

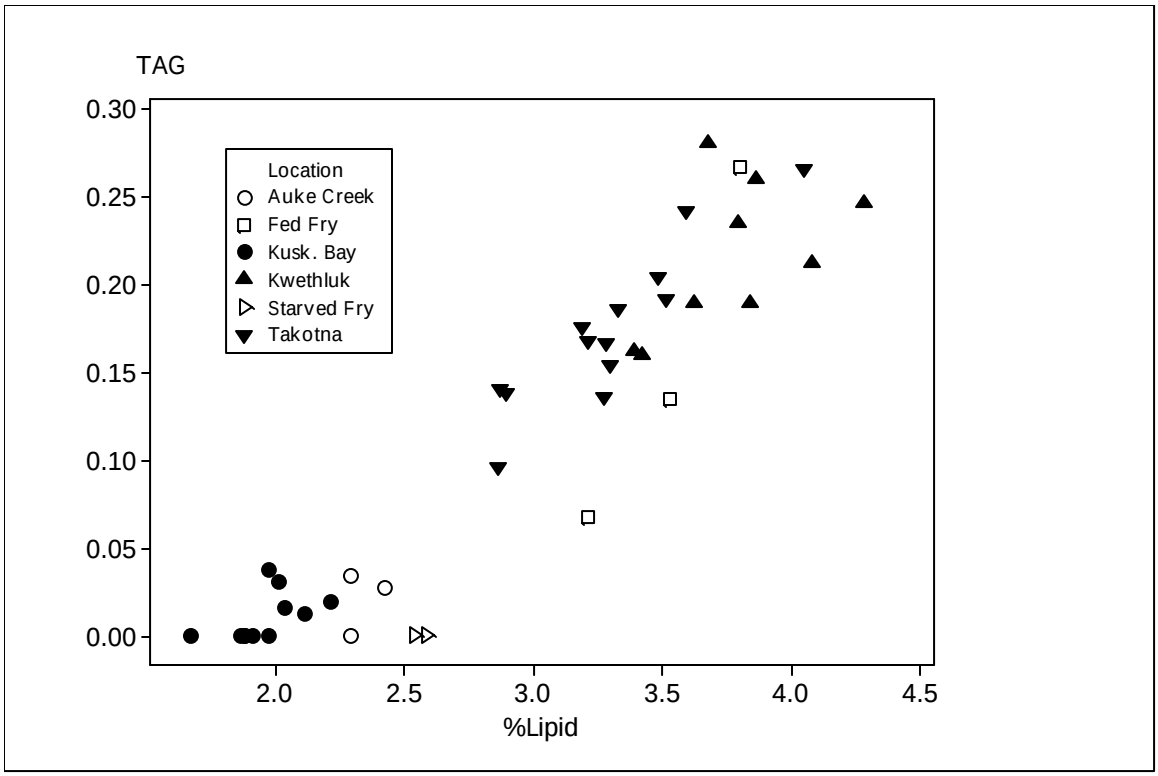

Figure 3.7. Relationship between lipid content of chum salmon fry and the proportion of that lipid allocated to triacylglycerol.

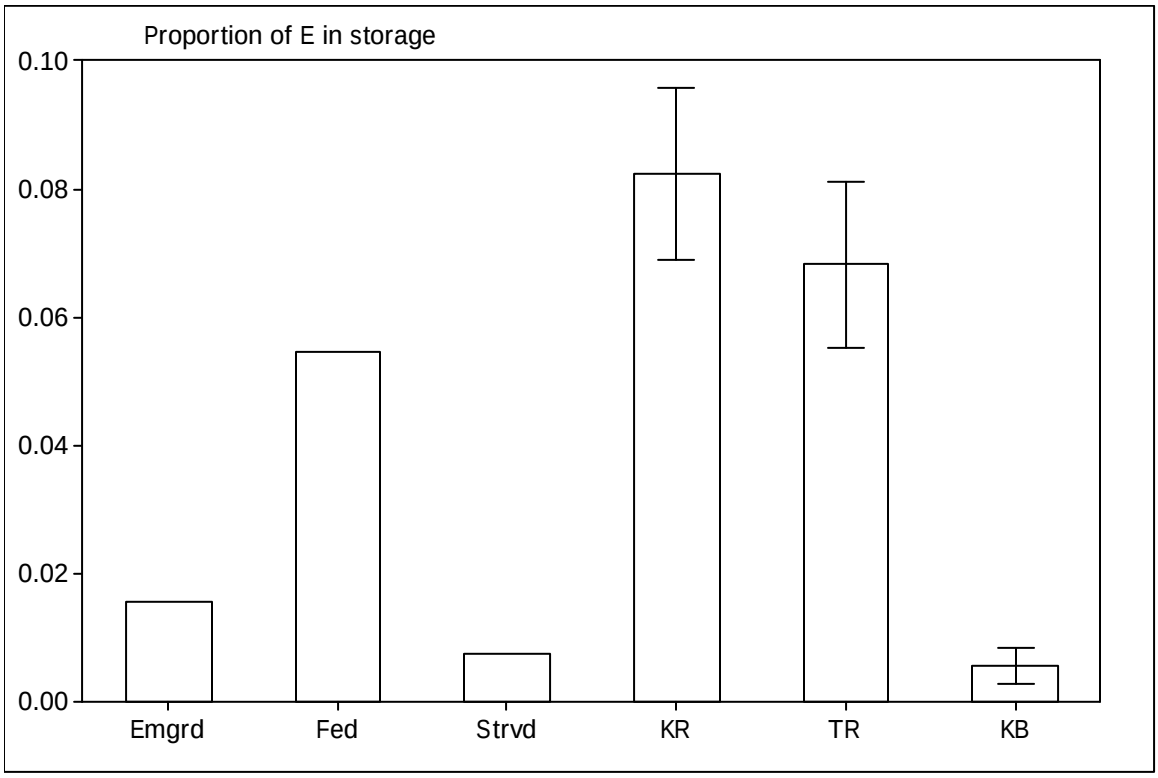

Figure 3.8. Average amount of energy allocated to storage (triacylglyercol and wax esters) in chum salmon fry. Ranges depict 95% confidence intervals. Emrgd = Emerged, Strvd = Starved, KR = Kwethluk River, TR = Takotna River and KB = Kuskokwim Bay.

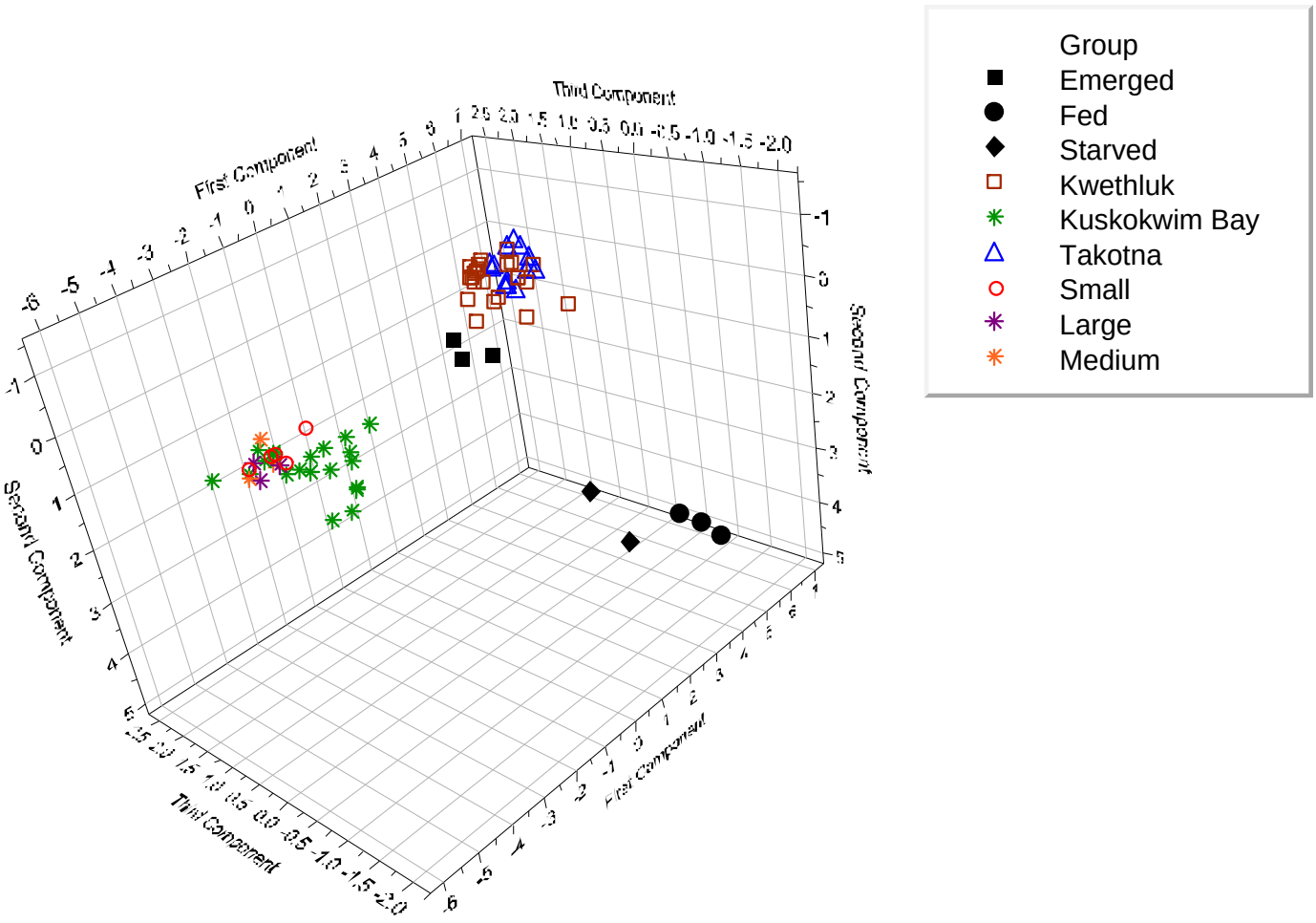

Figure 3.9. Principle components analysis for chum salmon samples from all groups combined.

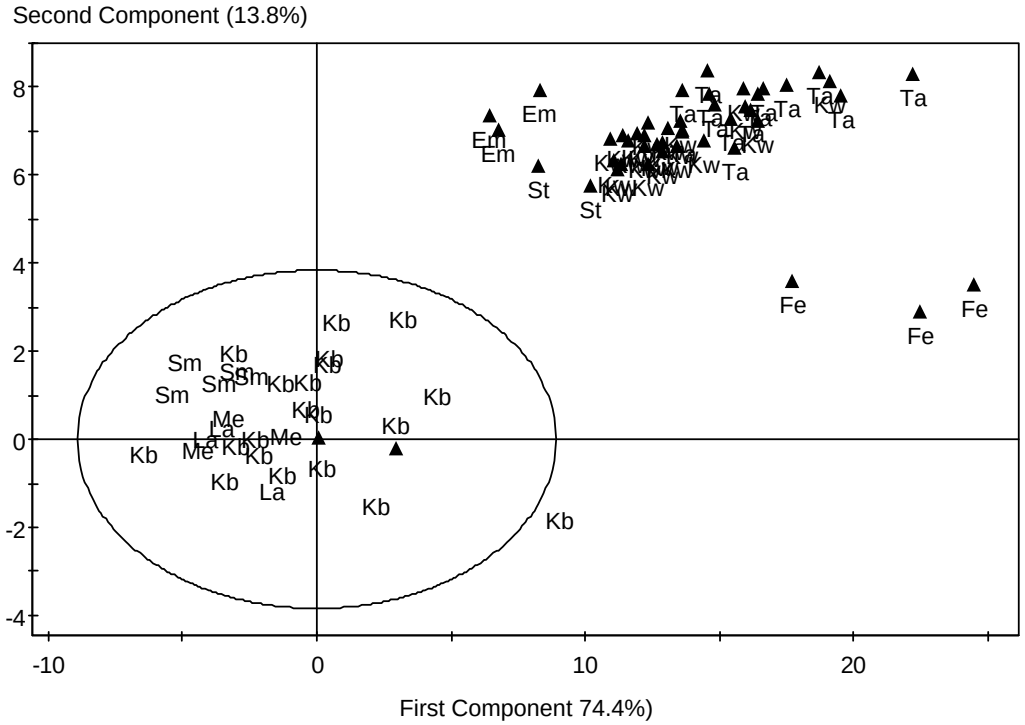

Figure 3.10. Principle component model for Kuskokwim Bay group and fitted locations other groups. Observations associated with triangles have residual error greater than 3.6 standard deviations from the average Kuskokwim composition, except for the two Kb observations which were within 2.0 standard deviations of the mean composition. The ellipse describes the 95% confidence interval for the Kuskokwim Bay mean.
Kb = Kuskokwim Bay; Sm = Small; Me = Medium; La = Large; Em = Emerged; St = Starved; Fe = Fed; Kw = Kwethluk; Ta = Takotna.

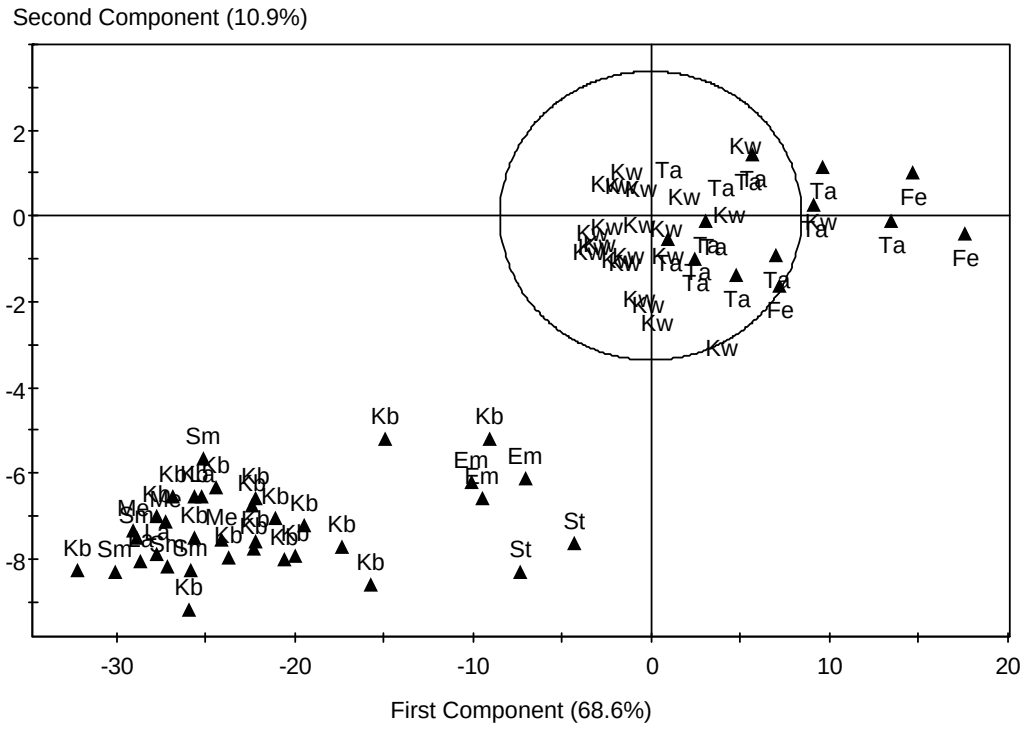

Figure 3.11. Principle components model for the Kwethluk River group showing fitted location of all samples. Observations associated with triangles are not adequately described by the model and have residual error greater than 3.7 standard deviations from the centroid mean. The ellipse describes the 95% confidence interval for the Kwethluk mean. Kb = Kuskokwim Bay; Sm = Small; Me = Medium; La = Large; Em = Emerged; St = Starved; Fe = Fed; Kw = Kwethluk; Ta = Takotna.

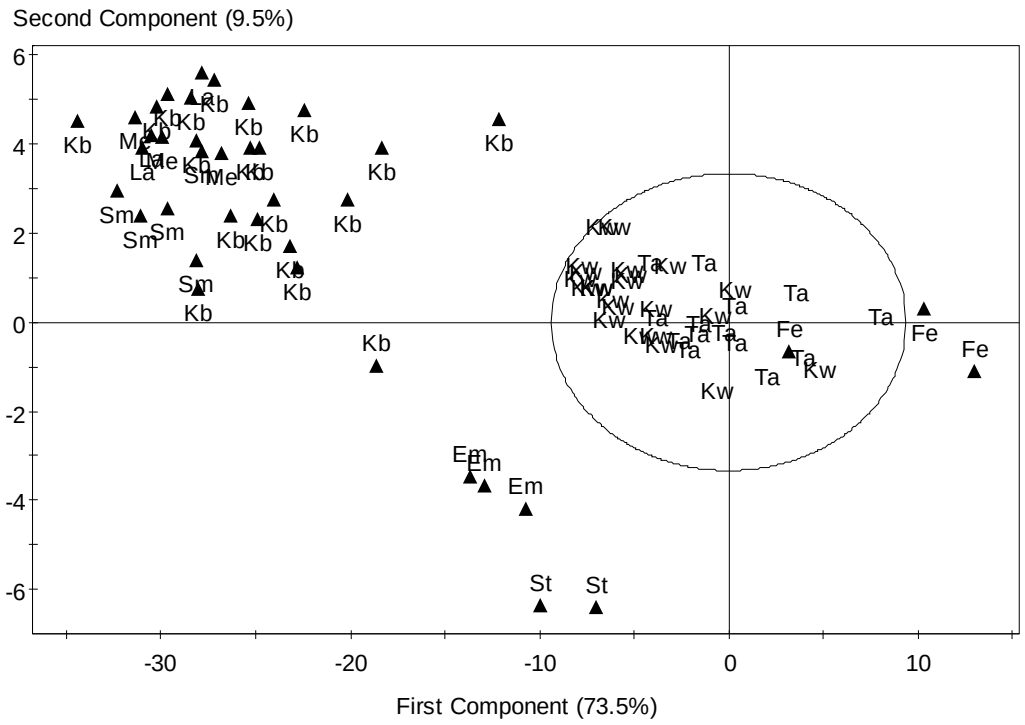

Figure 3.12. Principle components model for the Takotna River group with fitted location of all samples. *Observations associated with triangles are not adequately described by the model and have residual error greater than 3.0 standard deviations from the average Takotna composition, except for Kw samples, which are < 2.0 standard deviations away. The ellipse describes the 95% confidence interval for the Takotna mean. Kb = Kuskokwim Bay; Sm = Small; Me = Medium; La = Large; Em = Emerged; St = Starved; Fe = Fed; Kw = Kwethluk; Ta = Takotna.*

References

Association of Official Analytical Chemists (2002) *Official Methods of Analysis*. Association of Analytical Chemists. Washington, DC.

Aitchison J (2003) The statistical analysis of compositional data. Blackburn Press. Caldwell NJ, USA. 418 pp.

Ashton HG, Farkvan DO, March BE (1993) Fatty acid composition of lipids in the eggs and alevins from wild and cultured Chinook salmon (O*ncorhynchus tshawytscha*).Can J Aquat Sci 50:648-655

Azuma T, Yada T, Ueno Y, Iwata M (1998) Biochemical approach to assessing growth characteristics in salmonids. NPAC Bulletin 1:103-111

Ballantyne JS, Mercure F, Gerrits MF, VanDer Kraak G, McKinley S, Martens DW, Hinch SG, Diewert RE (1996) Plasma nonestrified fatty acid profiles in male and female sockeye salmon, *Onchorhyncus nerka*, during the spawning migration. Can J Fish Aquat Sci 53:1418-1426

Bax, N. J. 1983. Early marine mortality of marked juvenile chum salmon (*Oncorhynchus keta*) released into Hood Canal, Puget Sound, Washington, in 1980. Can. J. Fish. Aquat. Sci. 40:426-435.

Beacham, T. D. and C. B. Murray. 1986. Comparative developmental biology of chum salmon (*Oncorhynchus keta*) from the Fraser River, British Columbia. Can. J. Fish. Aquat. Sci. 43:252-262.

Beacham, T. D., C. B. Murray, and R. E. Withler. 1988. Age, morphology, developmental biology, and biochemical genetic variation of Yukon River fall

chum salmon, *Oncorhynchus keta*, and comparisons with British Columbia populations. Fishery Bulletin 86(4):663-674.

Beall, E. P. 1972. The use of predator-prey tests to assess the quality of chum salmon (*Oncorhynchus keta*) fry. Master's Thesis, University of Washington, Seattle. 105 pp.

Beamish, R. J., G. A. McFarlane, and J. R. King. 2000. Fisheries climatology: understanding decadal scale processes that naturally regulate British Columbia fish populations. Pages 94-139 *in* P. J. Harrison and T. R. Parsons, editors, Fisheries Oceanography: an Integrative Approach to Fisheries Ecology and Management. Blackwell Science, Oxford, UK.

Beamish, R. J. and C. Mahnken. 2001. A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. Progress in Oceanography 49:423-437.

Beamish RJ, Mahnken C, Neville CM (2004) Evidence that reduced early marine growth is associated with lower marine survival of coho salmon. Trans Am Fish Soc 133:26-33

Berg OK, Hendry AP, Svendsen B, Bech C, Arnekleiv JV, Lohrmann A (2001) Funct Ecol 15:13-23

Brett JR (1995) Energetics. *In* Physiological Ecology of Pacific Salmon (Groot C, Margolis L, Clarke WC eds.) UBC Press, University of British Columbia, Vancouver BC. p 3-63

Burkey, C. E., Jr., M. Coffing, D. B. Molyneaux, and P. Salomone. 2000. Kuskokwim River chum salmon stock status and development of management / action plan

options, report to the Alaska Board of Fisheries. ADFG, Commercial Fisheries Division, Regional Information Report No. 3A00-41, Anchorage.

Christie WW (1982) Lipid analysis: Isolation, Separation, Identification and Structural Analysis of Lipids. Second ed. Pergamon Press. New York.

Craig, JF, Kenley MJ, Talling JF (1978)Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxiation and proximate analysis. Freshwater Biol 8:585-590

Crossin GT, Hinch SG, Farrell AP, Higgs DA, Lotto AG, Oakes JD, Healy MC (2004) Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. J Fish Biol 65:788-810

Clark, K. J., and D. B. Molyneaux. 2003. Takotna River salmon studies and upper Kuskokwim River aerial surveys, 2002. ADFG Regional Information Report No. 3A03-10.

Farley, E. V., Jr., B. Wing, A. Middleton, J. Pohl, L. Hulbert, J. Moss, M. Trudel, E. Parks, T. Hamilton, C. Lagoudakis, and D. McCallum. 2003. 2002 Eastern Bering Sea (BASIS) coastal research (August-October 2002) on juvenile salmon. (NPAFC Doc. #) Auke Bay Laboratory, Alaska Fisheries Science Center, NMFS, NOAA, Juneau, Alaska. 25 pp.

Finn, J. E., E. E. Knudsen, R. Hander, S. Maclean, and A. Gryska. 1998. Estimating freshwater survival and environmental influences on the survival of several Yukon River chum salmon stocks: Progress report for 1996 and 1997 brood year. USGS, Alaska Science Center, Unpublished report, 100 p.

Friedland, K. D., R. E. Haas, and T. F. Sheehan. 1996. Post-smolt growth, maturation, and survival of two stocks of Atlantic salmon. Fishery Bulletin 94:654-663.

Fukuwaka, M. and T. Suzuki. 2002. Early sea mortality of mark-recaptured juvenile chum salmon in open coastal waters. J. Fish. Bio. 60:3-12.

Healy, M. C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. 39:952-957.

Henderson RJ, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. Prog Lipid Res 26:281-347

Holtby, L. B., B. C. Anderson, and R. K. Kadowaki. 1990. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 47:2181-2194.

Iverson SJ, Frost KJ, Lang SLC (2002) Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Mar Ecol Prog Ser 241:161-181

Koski, K. V. 1975. The survival and fitness of two stocks of chum salmon (*Oncorhynchus keta*) from egg deposition to emergence in a controlled-stream environment at Big Beef Creek. PhD Dissertation, University of Washington.

Kreps EM, Chebotareva MA, Akulin VN (1969) Fatty acid composition of brain and body phospholipids of the anadromous salmon, *Oncorhynchus nerka*, from fresh-water and marine habitat. Comp Biochem Physiol 31:419-430

Loftus, W. F. and H. L. Lenon. 1977. Food habits of the salmon smolts, *Oncorhynchus tshawytscha* and *O. keta*, from the Salcha River, Alaska. Trans. Am. Fish. Soc., 106(3):235-240.

Lovern JA (1934) Fat metabolism in fishes. V. The fat of the salmon in its young freshwater stages. Biochem J 28:1961-1963

Martin, D. J., D. R. Glass, C. J. Whitmus, C. A. Simenstad, D. A. Milward, E. C. Volk, M. L. Stevenson, P. Nunes, M. Savoie, and R. A. Grotefendt. 1986. Distribution, seasonal abundance, and feeding dependencies of juvenile salmon and non-salmonid fishes in the Yukon River Delta. NOAA OCSEAP Final Report 55 (1998):381-770.

Mason, J. C. 1974. Behavioral ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31:83-92.

Merritt, M. F. and J. A. Raymond. 1983. Early life history of chum salmon in the Noatak River and Kotzebue Sound. ADFG, Division of Fisheries Rehabilitation Enhancement and Development, Number 1.

Mikulich, L. V. and Y. I. Gavrenkov. 1986. Some biological properties and feeding of downstream migrating juvenile chum salmon, *Oncorhynchus keta*, from the Southern Maritime Territory. Voprosy Ikhtiologii, No. 4, pp. 610-618.

Milligan, P. A., W. O. Rublee, D. D. Cornett, and R. A. C. Johnston. 1986. The distribution and abundance of chum salmon (*Oncorhynchus keta*) in the upper Yukon River basin as determined by a radio-tagging and spaghetti tagging program: 1982-1983. Can. Tech. Rep. Fish. Aquat. Sci. 1351, 141 p.

Nomura T, Urawa S, Eueno Y (2000) Variations in muscle lipid content of high-seas chum and pink salmon in winter. NPAC Bulletin 2:347-352

Ota T (1977) Lipids of masu salmon – IV Changes of lipid composition and fatty acid composition in flesh lipids of juvenile masu salmon in the early stage to sea water life. Hoku Dai Suisan Iho 27(1):30-36

Parker, R. 1968. Marine mortality schedules of pink salmon of the Bella Coola River central British Columbia. J. Fish. Res. Board Can. 25:757-794.

Parker, R. R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia Inlet. J. Fish. Res. Board Can. 28:1503-1510.

Pearcy, W. G., C. D. Wilson, A. W. Chung, and J. W. Chapman. 1989. Residence times, distribution, and production of juvenile chum salmon, *Oncorhynchus keta*, in Netars Bay, Oregon. Fishery Bulletin 87:553-568.

Peterman, R. M. 1978. Testing for density dependent marine survival in Pacific salmonids. J. Fish. Res. Board Can. 35: 1434-1450.

Quinn, T. P. and C. Groot. 1984. The effect of water flow rate on bimodal orientation of juvenile chum salmon. Animal Behaviour 32(2):628-629.

Saddler JB, Lowry RR, Krueger HM, Tinsley IJ (1966) Distribution and identification of the fatty acids from the coho salmon *Oncorhynchus kisutch* (Walbaum). J Am Oil Chem Soc 43:321-324

Saito H, Kotani Y (2000) Lipids of four boreal species of calanoid copepods: origin of monoene fats of marine animals at higher trophic levels in the grazing food chain in the subarctic ocean ecosystem. Mar Chem 71:69-82

Salo, E. O. 1991. Life history of chum salmon (Oncorhynchus keta). Pages 231-309 *in* C. Groot and L. Margolis, editors. Pacific Salmon Life Histories. UBC Press, University of British Columbia, Vancouver, BC.

Sargent JR, Bell JG, Bell MV, Henderson RJ, Tocher DR (1995) Dietary origins and functions of long-chain (n-3) polyunsaturated fatty acids in marine fish. J Mar Biotechnol 3:26-28

Sparrow, R. A. H. 1968. A first report of chum salmon fry feeding in fresh water of British Columbia. J. Fish. Res. Board Can. 25(3):599-602.

Thorsteinson, L. K., P. R. Becker, and D. A. Hale. 1989. The Yukon Delta: a synthesis of information. NOAA Ocean Assessments Division Alaska Office, Anchorage, Alaska. Special Report MMS 89-0081, 89 pp.

Varnavsky VS, Kalinin SV, Kinas NM, Rostomova SS (1992) The early sea life of coho, *Oncorhynchus kisutch*, and pink salmon *O. gorbuscha*, as a period of completion of smoltification. Env Bio Fish 34:401-408

Wissmar, R. C. and C. A. Simenstad. 1988. Energetic constraints of juvenile chum salmon (*Oncorhynchus keta*) migrating in estuaries. Can. J. Fish. Aquat. Sci. 45:1555-1560.