EFFECTS OF FISH WHEELS ON FALL CHUM SALMON (Oncorhynchus keta): NON-ESTERIFIED FATTY ACIDS AND PLASMA INDICES OF STRESS

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A

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Abstract

The effect of tagging and capture on plasma concentrations of cortisol, glucose, lactate, chloride, and non-esterified fatty acids (NEFA) in chum salmon was investigated. Adult chum salmon were captured in August-September 2000 and 2001 with a fish wheel on the lower Kantishna River. Tagged and untagged fish were subsequently captured on the lower Toklat River and sampled for blood. Tagged fish (males and females) at the Toklat recovery fish wheels had lower ($P < 0.04$) plasma cortisol concentrations than untagged fish. Glucose concentrations were lower ($P = 0.03$) in tagged than untagged males but did not differ between tagged and untagged females. Lactate and chloride concentrations did not differ between tagged and untagged fish. Tagged chum salmon captured at the Toklat River recovery wheels had lower concentrations of NEFA ($P = 0.02$). Taken together, these results suggest there is a metabolic cost from capture and tagging using fish wheels.

Table of Contents

List of Figures

Page

List of Appendices

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General Introduction

The Yukon River drainage is the largest in Alaska (854,700 km^2), comprising nearly one-third the area of the entire state. Five species of Pacific salmon return to the Yukon River and tributaries and are utilized in subsistence, personal use, commercial and sport fisheries. Chum salmon return to the Yukon River in genetically distinct summer and fall runs (Crane et al. 2001). Summer chum salmon enter the Yukon River in early May, and fall chum salmon in mid July. The Tanana River is the largest tributary of the Yukon River. It flows northwest through a broad alluvial valley for approximately 700 km to the Yukon River at Tanana Village, draining an area of 115,250 km^2. The Tanana River drainage is a major producer of Yukon River fall chum salmon (*Oncorhynchus keta)* and contributes significantly to various in-river fisheries. Migration of fall chum salmon in the Tanana River drainage generally peaks around mid-September and continues into early October. Spawning takes place from mid-October through November, primarily in areas where upwelling ground water prevents freezing (Figure1). The Toklat River, a tributary to the Kantishna River, where part of this study was conducted, is an example of one of these areas and is one of the most productive fall chum spawning sites in the Tanana River drainage (Vania et al. 2002).

Fish wheels have been commonly used as a method of estimating run strength and timing of salmon migrations in the Yukon River drainage since 1981. Fifteen fish wheels were used in 2001 to monitor salmon runs in the Yukon River drainage by the Alaska Department of Fish and Game (ADF&G), the United States Fish and Wildlife Service, (USFWS), and the Canada Department of Fisheries and Oceans (CDFO). There has

been concern in recent years that fish wheels used to assess run strength and abundance through mark-recapture studies may be causing delayed mortality of fall chum salmon (Underwood et al. 2002). Fall chum salmon migrate approximately 1,300 km from the mouth of the Yukon to the Kantishna River. Pacific salmon do not feed during migration and dedicate energy to migration and gonad development. Because they are fasting and endogenous energy resources are limited, stress from capture and tagging have the potential to decrease energy reserves and condition of these fish.

Chapter I: Stress effects from capture and tagging on plasma concentrations of cortisol, glucose, lactate and chloride in fall chum salmon.

Introduction

The definition of stress varies according to the investigator. Barton and Iwama (1991) define stress as a response reaction by a fish to a stimulus that alters the homeostatic state of the fish. The stress response, as defined by Barton et al. (1985), is the difference between the mean resting concentrations plasma cortisol and the mean concentration of cortisol one hour after handling stress. A stimulus from the environment when severe enough, it is called a "stressor". Total stress load is determined by the cumulative effects of prior stressors. The stress response is adaptive and does not necessarily affect survival if the duration of the stressor is short. However, fish exposed to chronic stress divert energy reserves to cope with the stimuli. Diverted energy affects the long-term health of a fish (for reviews see Adams 1990; Wedemeyer et al. 1990; Barton and Iwama 1991; Barton 1997; and Barton et al. 2002).

The stress response can be initiated in fish that are subjected to catch-and-release, captured for population estimation (using gill nets, hoop traps or fish wheels), or handling, sorting, grading, transport. Other factors that cause stress in fish include poor water quality (Norris et al. 1999; Leblond 2001) unsuitable or abrupt changes in temperature (Mather et al. 1986), or metal contaminated water (Pickering and Pottinger 1987; Roche and Boge 1996; Brodeur et al. 1998; Norris et al. 1999). Stress from tagging and handling has been well documented in various studies of salmonids and other fishes. Measures of plasma cortisol and other hematological indicators (glucose, lactate

and chloride) have been useful as indicators of stress in fishes (Barton and Schreck 1987; Carragher and Sumpter 1990; Hunn and Greer 1991; Barton and Zitzow 1995; Roche and Boge 1996; Davis and Schreck 1997; Sharpe et al. 1998; Stouthart et al. 1998; Barton 2000).

Stress responses in fish are divided into primary, secondary, and tertiary responses (reviewed by Barton 2002). Primary stress responses include rapid changes, such as the release of catacholamines (epinephrine) from chromaffin cells located on the surface of the kidney after exposure to a stressor (Mazeaud et al. 1997). Also included in the primary stress response is the release of corticotropin releasing factor (CRF), which causes the release of endrenocortocotropin hormone (ACTH) via the hypothalamic-pituitary-interrenal axis, which stimulates the release of corticosteriod hormones from interrenal cells.

Secondary stress responses associated with cortisol include a reduction in glycogen reserves, high concentrations of circulating glucose (Mazeaud et al 1997), and lactate (Turenen et al. 1994), respectively termed hyperglycemia and hyperlacticemia. Mediated by epinephrine, stress can increase gill permeability and thus leads to osmoregulatory disturbance (hyporchlororemia) ionic imbalance (Barton and Schreck 1987; Wedemeyer et al. 1990; Barton and Iwama 1991) and an increase in oxygen consumption and transport (Barton and Schreck 1987; Davis and Schreck 1997). Secondary stress effects are associated with mobilization of lipid reserves, including non-esterified fatty acids (Mommsen et al. 1999; Jobling 1994). Other secondary stress

effects include changes in the number of white blood cells and in blood flow (Jobling 1994; Wedemeyer et al. 1990).

Tertiary stress responses involve whole animal performance and includes changes at the ecosystem level through perturbations in energy flow through trophic levels which may alter species composition (reviewed by Barton et al. 2002). Tertiary stress responses associated with cortisol include changes in condition, general health and swimming capacity, changes in feeding and aggression (Wedemeyer et al. 1990), reduction in circulating concentrations of vitellogenin (Carragher 1989; Carragher and Sumpter 1990; Schreck 2000), and decline in growth rates (Carragher et al. 1989). Cortisol is also involved in immuno-suppression, and even low concentrations of circulating cortisol have been shown to cause decreased disease resistance in a number of fish species (Maule et al. 1987; Carragher and Sumpter 1990; Jobling 1994; Brodeur et al. 1998).

Cortisol is the primary glucocorticoid hormone in teleost fishes (Evans 1993). Produced by kidney interrenal cells, cortisol promotes the synthesis, storage and regulation of glucose, glycogen, proteins, regulates the deposition of fat, increases metabolic rate, and increases blood fatty acid concentrations. Cortisol release is controlled by negative feedback where ACTH production from the pituitary is inhibited in the presence of high concentrations of cortisol. The series of reactions and responses to signals are referred to as the hypothalamic-pituitary-interrenal axis (HPI axis) (reviewed by Donaldson 1981). Cortisol may also prevent the re-esterification of NEFA's (Mazeaud et al.1997). The initial elevation of plasma glucose mobilized via the action of epinephrine is derived from glycogenolysis in the liver. Cortisol leads to high glucose

concentrations in plasma through gluconeogenisis from endogenous glycerol and protein substrates. Cortisol also acts to reduce the uptake of glucose and amino acids by muscle and peripheral tissue while amino acids are released by muscle cells (Ekert 1997). Metabolic changes from stress can be quantified by measuring variations in plasma cortisol, glucose, lactate and chloride. These variations are useful measures of fish stress because they have been found to change according to the amount and duration of stress in various fish species.

Metabolic clearance rate (MCR) is an important factor to consider when measuring stress in fish. Metabolic clearance rate is the rate at which a hormone is removed from circulation and may be dependent on the presence of binding proteins and tissue receptors. In addition, uptake and catabolism of cortisol may change in stressed fish (reviewed by Wedemeyer et al. 1990). Metabolic clearance rate can vary in salmonids depending on maturity, nutritional state, salinity and species (Mommsen 1999); (Gamperl et al. 1994). Plasma MCR from chronically stressed fish differs from that of fish that have not experienced the same level of stress. Although stress events may increase the number of cortisol receptors (Fagerlund et al. 1981), chronic exposure to stress may lead to down-regulation of cortisol receptors (Jobling 1994).

There is little information concerning possible stress caused by mark-recapture projects involving fish wheels. Stress induced by capture and tagging may be manifested in the effects described above. The primary objective of this study was to determine if capture and tagging in fish wheels have a measurable effect on the physiological

condition of chum salmon. Specifically, it was predicted that capture and tagging with fish wheels would result in elevated plasma cortisol, glucose, and lactate concentrations.

Study Area

The Kantishna River is a tributary of the Tanana River and is a typical turbid interior Alaska river. Its tributaries include Birch Creek, McKinley, Bearpaw and Toklat Rivers all of which have headwaters that originate in Denali National Park. The Toklat River is the largest tributary of the Kantishna River and originates in the ice fields of the Alaska Range near Mount Pendleton. It is a glacial river with turbid, silt-laden water and braided, gravel channels. Chum salmon are tagged on the lower Kantishna River (64 ° 44. 361′ north latitude and 149 ° 59. 690′ west longitude) at approximately 5 km upstream of where the Kantishna River joins the Tanana River. Tagged fish are recovered fish wheels 114 km upstream on the lower Toklat River (N 64 ° 23. 805′, W 150 ° 17. 768′) and 139 km upstream on the upper Kantishna River (N 64 ° 10. 425′, W 150 ° 38. 741′) near the mouth of the Bearpaw River (Figure 1).

Methods

Fish capture

Fish wheels were equipped with baskets measuring 2.5-3 m in width with a dip capacity of approximately 4 m deep and a live box measuring 1.3 m^3 constructed of spruce poles and one-half inch plywood and attached to the offshore side of the fish wheel. Fish leads, ranging from 2 to 5 m in length, were installed shoreward as needed, depending on the distance of the fish wheel from the riverbank. These served as a weir to keep fish from escaping between the shore and the fish wheel.

Fall chum salmon were captured in August and September 2000 in a fish wheel on the lower Kantishna River (1,300 river km). All healthy salmon (based on

appearance) were tagged with spaghetti tags (Floy Inc., Seattle WA.) and released. Blood samples were collected at this location from untagged male and female fish. In addition, samples were collected from tagged fish (fish that had been captured and tagged at the lower Kantishna fish wheel) and untagged fish (control samples) at two fish wheels used for tag recovery on the lower Toklat River (1,414 river km; Figure 1.)

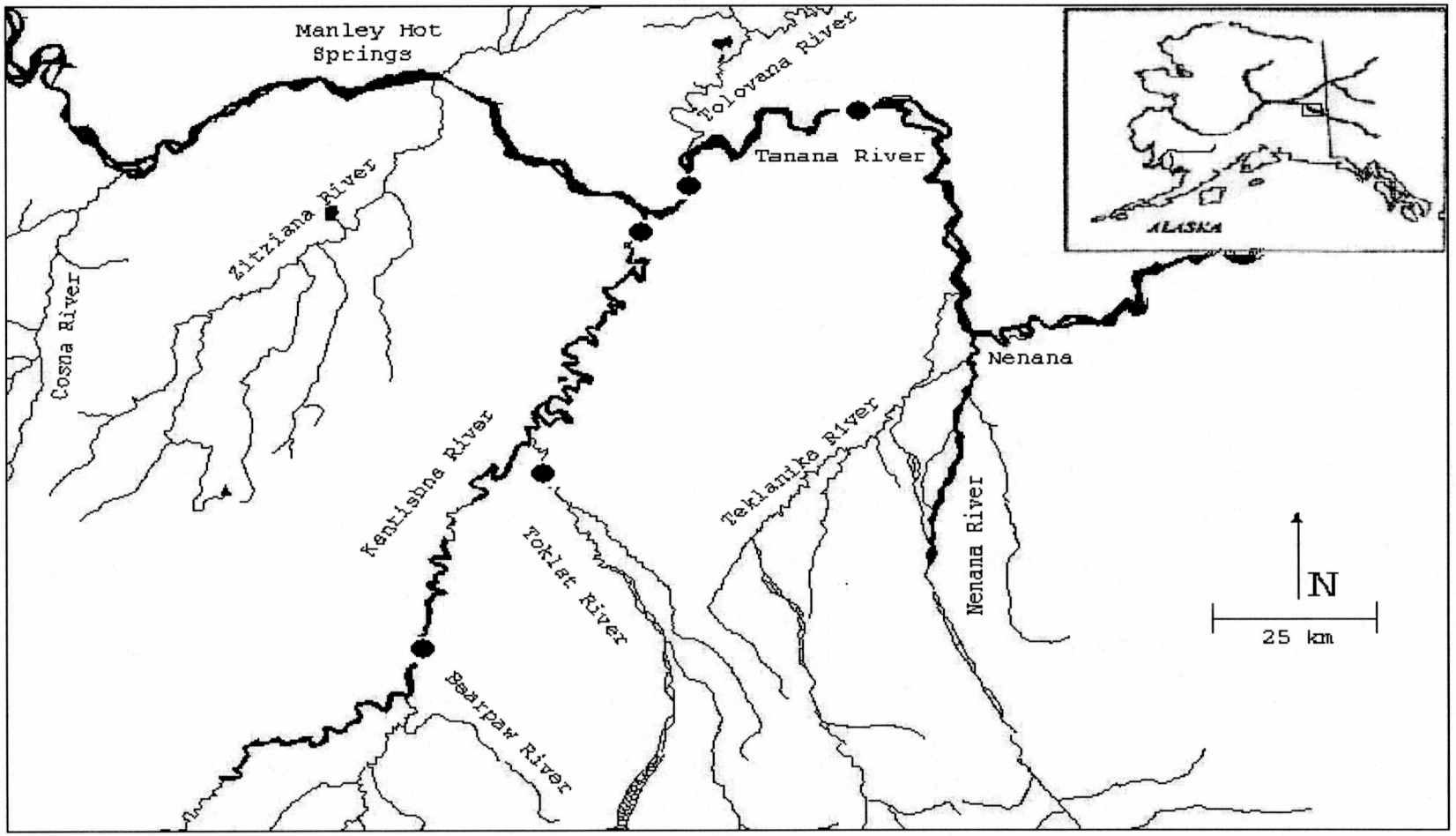

Figure 1. Location of tag deployment and recovery fish wheels (black circle) operated in the Tanana and Kantishna Rivers Alaska, 2000 and 2001. Samples were not collected at the upper Kantishna wheel in 2000.

Blood collection

Salmon were anesthetized in a 1.5 x 0.5 meter fiberglass tub using a 30 mg/l clove oil/ethanol solution (R. Brown, United States Fish and Wildlife Service personal communication). After fish rolled on their sides (approximately 3 minutes), blood samples were collected from the dorsal vasculature of adult chum salmon with a 21-gauge 3 ml 21-gauge 1.5-inch (0.8mm x 40 mm) luer lock syringe. The barrel of the syringe had been rinsed with a 4% solution of sodium heparin. The needle was inserted through the dorsal musculature, placed against the vertebra, and retracted a sufficient distance to allow blood flow into the syringe. Slight gradual pressure was applied to the syringe to draw blood. Approximately 1 ml of blood was collected from each fish. The needle was removed to prevent lyses of blood cells and samples were transferred to 1.5 - ml micro centrifuge tubes and refrigerated. Approximately one hour post-collection, samples were centrifuged at approximately 1000 x gravity (*g)* and plasma was drawn off via pipette and transferred to clean micro centrifuge tubes and frozen. Samples were maintained at – 45 ^{o} C until analyzed.

Analysis of cortisol, glucose, lactate and chloride

Cortisol was measured using a [^{3}H]-radio immunoassay (Foster and Dunn 1974) modified and verified for use with salmonid plasma (Redding et al. 1984), and samples were read with a liquid scintillation counter. Plasma glucose was determined colorimetrically using ortho-toluidine reagent (Wedemeyer et al. 1990) and read at 635 nm with a spectrophotometer. Plasma lactate was measured enzymatically using a commercial kit (Sigma Diagnostics Kit No. 735). Color change was read at 540 nm using

a spectrophotometer. Plasma chloride was measured using a Corning Model 925 Chloride Analyzer. Samples sizes in figures vary because more untagged fish were sampled than tagged and some samples (glucose, lactate and chloride) did not contain enough plasma for analysis.

Statistical analysis

Data are reported as mean ± standard error (*SE*). Significant differences between tagged and untagged chum salmon were determined by a one tailed *t*-test. Where non-normally distributed, a Man-Whitney rank sum test was used. Differences were considered significant at the $\alpha = 0.05$ probability.

Results

Plasma samples collected at the Kantishna River tag deployment wheel indicated no significant difference between female and male chum salmon in concentration of any of the stress indicators ($P > 0.05$) (Appendix A). Samples collected at the Toklat River recovery wheels had lower cortisol concentration in male chum ($P = < 0.05$; males, 90.5. ± 10.9; females, 205.3 ± 17.5), lower glucose concentration in females ($P = < 0.05$; females, 85.5 ± 5.4; males, 125.6 ± 5.0, Figure 2), lower lactate concentration in males ($P = < 0.009$; males, 19.5. ± 1.9; females, 24.3 ± 2.1), and lower chloride concentration in males ($P = < 0.03$; males, 119.7. ± 1.1; females, 123.3 ± 1.1, Figure 3). Tagged chum salmon had lower cortisol concentration than untagged salmon. ($P = 0.04$; tagged, 114.9 ± 17.9; untagged, 166.3 ± 16.7; Figure 2). Concentrations of glucose, chloride, and lactate did not significantly differ between tagged and untagged salmon ($P > 0.05$) with

the exception that tagged males had lower plasma glucose concentration than untagged males ($P = 0.03$; tagged, 115.3 ± 5.4; untagged, 136.1 ± 7.7; Figure 3).

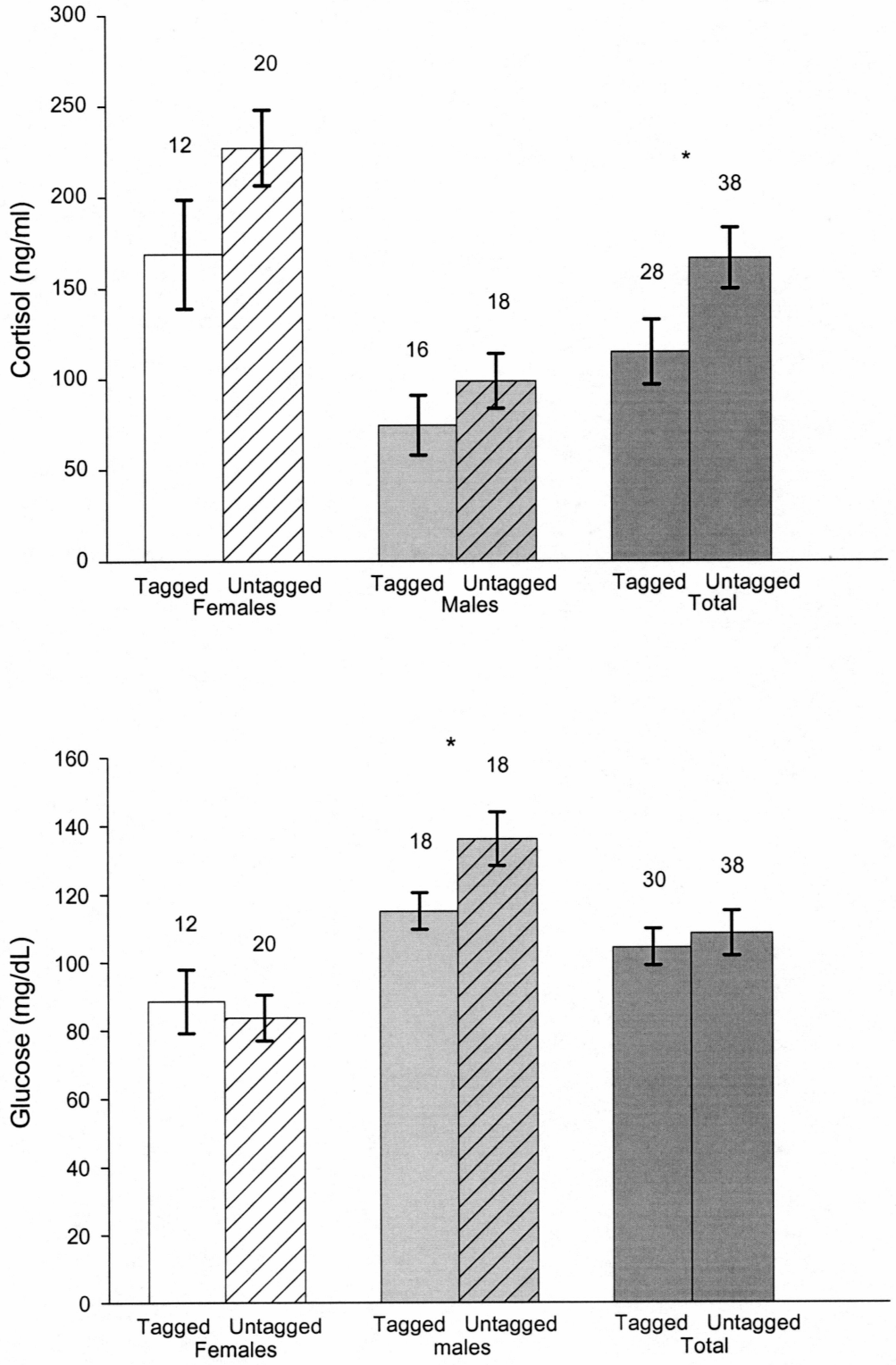

Figure 2. Mean concentrations (± *SE*) of plasma cortisol and glucose in chum salmon at the Toklat River fish wheels, Alaska, 2000. Total tagged chum salmon had lower cortisol concentration than untagged salmon ($P = 0.04$), (above). Glucose values were lower in tagged males ($P = 0.03$). Sample sizes are above bars. The asterisk indicates a significant difference.

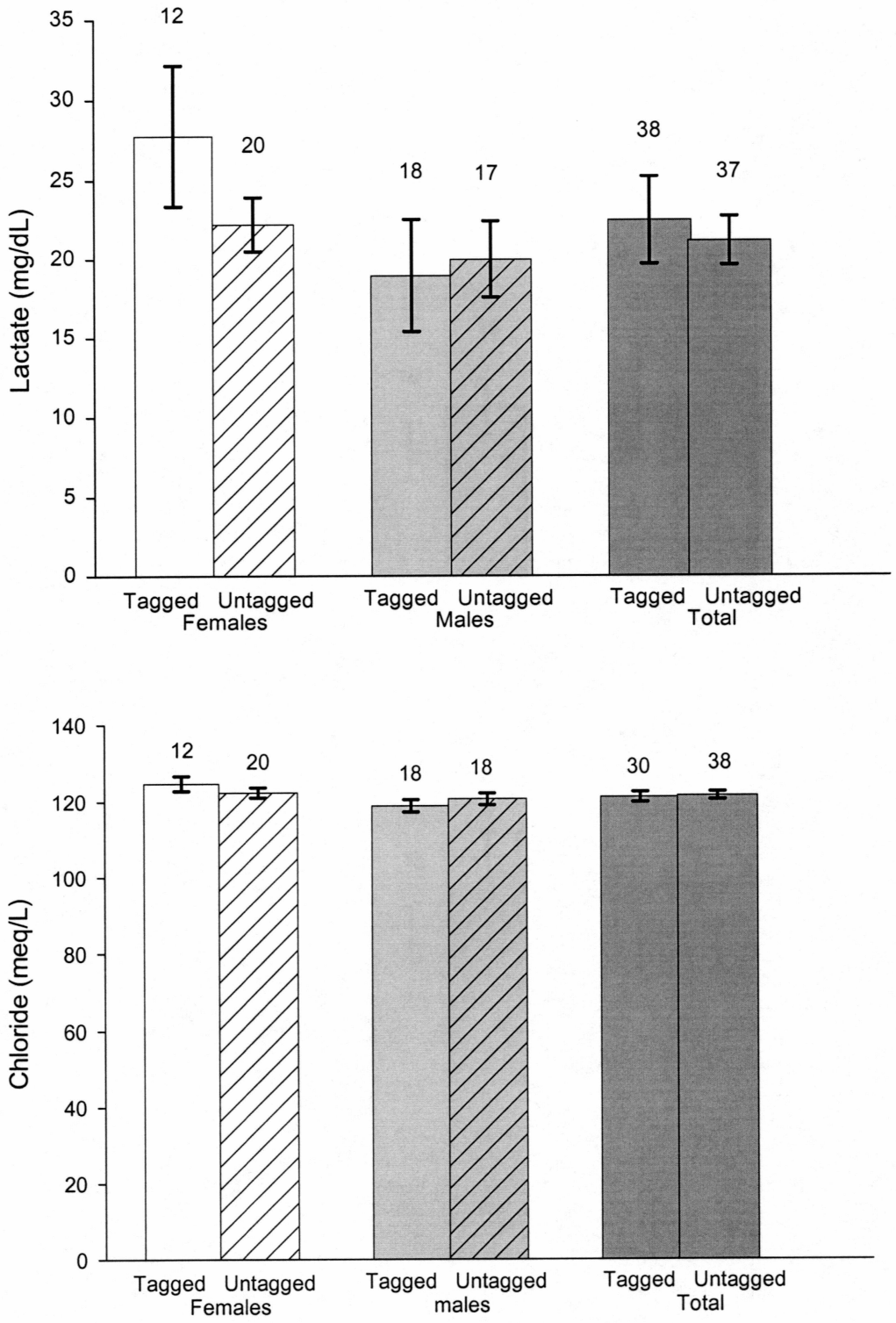

Figure 3. Mean concentrations (± *SE*) of plasma lactate and chloride in chum salmon at the Toklat River fish wheels Alaska, 2000. Plasma lactate (above) and chloride (below) concentrations did not significantly differ between tagged and untagged fish. Sample sizes are above bars.

Discussion

Exposure to a stressor typically leads to an increase in circulating concentrations of cortisol. If the duration of the stimulus is short, plasma concentrations of cortisol rapidly return to normal concentrations (Carragher and Sumpter 1990). However, chronic stress may cause down-regulation of interrenal cells inhibiting their ability to secrete cortisol (Bradford et al. 1992), and negative feedback mechanisms within the HPI axis lead to the suppression of secretion of cortisol (for review see Sumpter 1997). Stress events can increase the number of cortisol receptors but can also reduce the sensitivity of cortisol receptors in the interrenal tissue in response to chronic stress (Jobling 1994; Pottinger et al. 2000; Fagerlund et al 1981), which leads to reduced secretion of cortisol. Lower cortisol concentration of tagged Toklat River chum salmon may indicate fish captured in a fish wheel held in a live box, and tagged may lose the ability to respond to stress, which would be indicated by decrease in plasma cortisol concentration.

There are large differences in physiological demands associated with migration and gonadal development between male and female sockeye salmon (Idler and Bitners 1959; Ballantyne et al. 1996). Similar to the Ballantyne (1996) study, there were large differences in cortisol concentration between sexes at the Toklat River recovery wheels (Figure 2). These results suggest a difference in the magnitude of the stress response between male and female chum salmon. High cortisol concentration in female chum salmon at the Toklat Recovery wheel may indicate a greater metabolic load of gonad development through cortisol mediated lipid and somatic tissue catabolism.

Results of the current study indicated male chum salmon had a higher concentration of plasma glucose than females. Although mean glucose values were different between sexes, lower glucose concentrations (through stress related reduction in cortisol secretion) in tagged males suggest they are affected by capture and tagging. Supporting the results of this study, Beckman et al. (2000) found that juvenile chinook salmon had depleted liver glycogen and lipid reserves at a dam bypass, suggesting that increased stress causes an increase in metabolism necessary to support migration through the bypass.

In summary, plasma cortisol concentration significantly differed between tagged untagged chum salmon indicated by lower cortisol concentration in tagged fish at the Toklat River fish wheels. Although interrenal tissue function was not measured in this study, a reduction in cortisol secretion can occur through a reduction in the number and sensitivity of interrenal cortisol receptors (down regulation) through the HPI axis. This may explain reduced cortisol values through chronic stress in tagged chum salmon captured at the Toklat River recovery fish wheels. The stress indicators glucose, lactate and chloride did not significantly differ with the exception of lower glucose values in tagged male chum salmon.

Chapter II: Reduction in plasma non-esterified fatty acid concentration in fall chum salmon through capture and tagging using fish wheels.

Introduction

Fall chum salmon migrating to the Kantishna River drainage have limited energy stores for use in gonadal development and migration. Migrating salmon also use carbohydrates as an energy source; however, lipids are the main source of fuel for migration and gonadal development (Black and Skinner 1986). Non-esterified fatty acids (NEFA), the metabolically active form of plasma fatty acids, are the primary source of fuel for migrating salmon and have been used in quantifying available energy of migrating salmon (Ballantyne et al 1996). Like other species of Pacific salmon, Toklat River fall chum salmon migrate long distances and store large quantities of lipids that are catabolized during migration.

Several studies have described the change in body condition of migrating salmon. Takahashi et al. (1985) documented adult chum salmon lipid content decreased significantly during migration. Migration is energetically expensive because in addition to the cost of continuous swimming, chum salmon are making the physiological changes necessary for spawning. Idler and Bitners (1958) found migrating salmon undergo major development of gonads during migration and due to the high metabolic cost of egg production, females dedicate substantially more energy than males to gonadal formation through mobilization of both lipids and muscle protein. These developmental changes include gluconeogenisis from proteins, depletion of glycogen reserves in muscle and liver

(French et al. 1983; Hatano et al 1989) and large changes in the concentration of blood glucose and lipids (Peterson and Emmerson 1977).

Migrating fall chum salmon travel more 1300 km to natal streams and require NEFA to power muscles. Like glucose, NEFA is a readily available source of energy for metabolism (Larson and Fänge 1977; Zammit and Newsholm 1979; McKinley et al. 1993; Ballantyne et al. 1996) and may be directly utilized from muscle without release to blood plasma (Larsson and Fänge 1997). Ballantyne et al. (1996) used NEFA profiles of migrating sockeye salmon (*Oncorhynchus nerka*), found there were sex specific differences in migrating sockeye salmon and documented the importance of NEFA as fuel to sustain swimming during migration. The focus of this study was to determine if capture in fish wheels and tagging is manifested in a change in plasma NEFA in tagged chum salmon recaptured at the recovery fish wheels on the Toklat River.

Study Area

The Kantishna River is a tributary of the Tanana River and is a typical turbid interior Alaska River. Its tributaries include Birch Creek, McKinley, Bearpaw and Toklat Rivers all of which headwaters originate in Denali National Park. The Toklat River is the largest tributary of the Kantishna River and originates in the ice fields of the Alaska Range near Mount Pendleton. It is a glacial river with turbid, silty water and braided, gravel channels. Fish are tagged on the lower Kantishna River (64 ° 44. 361′ north latitude and 149 ° 59. 690′ west longitude), approximately 5 km upstream of where the Kantishna River joins the Tanana River. Tags are recovered at recovery fish wheels 114 km upstream on the lower Toklat River (N 64 ° 23. 805′, W 150 ° 17. 768′) and 139 km

upstream on the upper Kantishna River (N 64 ° 10. 425′, W 150 ° 38. 741′) near the mouth of the Bearpaw River (Figure 1).

Methods

Fish capture

Fish wheels were equipped with baskets measuring 2.5-3 m in width with a dip capacity of approximately 4 m deep and a live box measuring 1.3 m^3 constructed of spruce poles and one-half inch plywood and attached to the offshore side of the fish wheel. Fish leads, ranging from 2 to 5 m in length, were installed shoreward as needed, depending on the distance of the fish wheel from the riverbank. These served as a weir to keep fish from escaping between the shore and the fish wheel.

Fall chum salmon were captured in August and September 2001 in a fish wheel on the lower Kantishna River (1,300 river km). All healthy salmon (based on appearance) were tagged with spaghetti tags (Floy Inc., Seattle WA.) and released. Blood samples were collected at this location from untagged male and female fish. In addition, samples were collected from tagged fish (fish that had been captured and tagged at the lower Kantishna fish wheel) and untagged fish (control samples) at two fish wheels used for tag recovery on the lower Toklat River (1,414 river km; Figure 1.)

Blood collection

Salmon were anesthetized in a 1.5 x 0.5 meter fiberglass tub using a 30 mg/l clove oil/ethanol solution (R. Brown, United States Fish and Wildlife Service personal communication). After fish rolled on their sides (approximately 3 minutes), blood samples were collected from the dorsal vasculature of adult chum salmon with a 21-

gauge 3 ml 21-gauge 1.5 inch (0.8mm x 40 mm) luer lock syringe. The barrel of the syringe had been rinsed with a 4% solution of EDTA (di-sodium salt of ethelyne-diamine tetra acetic acid). The needle was inserted through the dorsal musculature, placed against the vertebra, and retracted a sufficient distance to allow blood flow into the syringe. Slight gradual pressure was applied to the syringe to draw blood. Approximately 1 ml of blood was collected from each fish. The needle was removed to prevent lyses of blood cells and samples were transferred to 1.5 - ml micro centrifuge tubes and refrigerated. Approximately one hour post-collection, samples were centrifuged at approximately 1000 *g* and plasma was drawn off via pipette and transferred to clean micro centrifuge tubes and frozen. Samples were maintained at – 45 ^{o} C until analyzed.

Non-esterified fatty acid assays

Blood plasma samples were processed in the laboratory using an enzymatic procedure (Wako diagnostics ® NEFA C, ACS-ACOD kit) that produces a purple colored adduct and measured colorimetrically using a micro-plate reader at 550 nanometers. The micro-plate reader measures optical density (OD). NEFA values were converted from OD to nmoles/ml. For the purpose of the analysis, concentrations of NEFA below the minimum detection limit, (2.3 ng/ml) of the assay were assigned 0 values.

Statistical analysis

Non-esterified fatty acid concentrations in tagged and untagged chum salmon were analyzed by a two tailed *t*-test (95% CI) and a Mann-Whitney rank sum test was

used when assumptions of normality were not met. Differences were considered significant at the $\alpha = 0.05$ probability.

Results

Plasma samples collected at the lower Kantishna River wheel did not significantly differ in NEFA concentration between sexes ($P = 0.94$). However, female chum salmon differed significantly from male chum salmon captured at the Toklat River fish wheels ($P < 0.05$; female, 288.0 ± 26.2; males, 410.0 ± 26.8; Figure 4). Tagged chum salmon differed significantly from untagged chum salmon captured at the Toklat River fish wheels ($P = 0.02$; tagged, 296.8 ± 28.1; untagged, 385.0 ± 26.0; Figure 4).

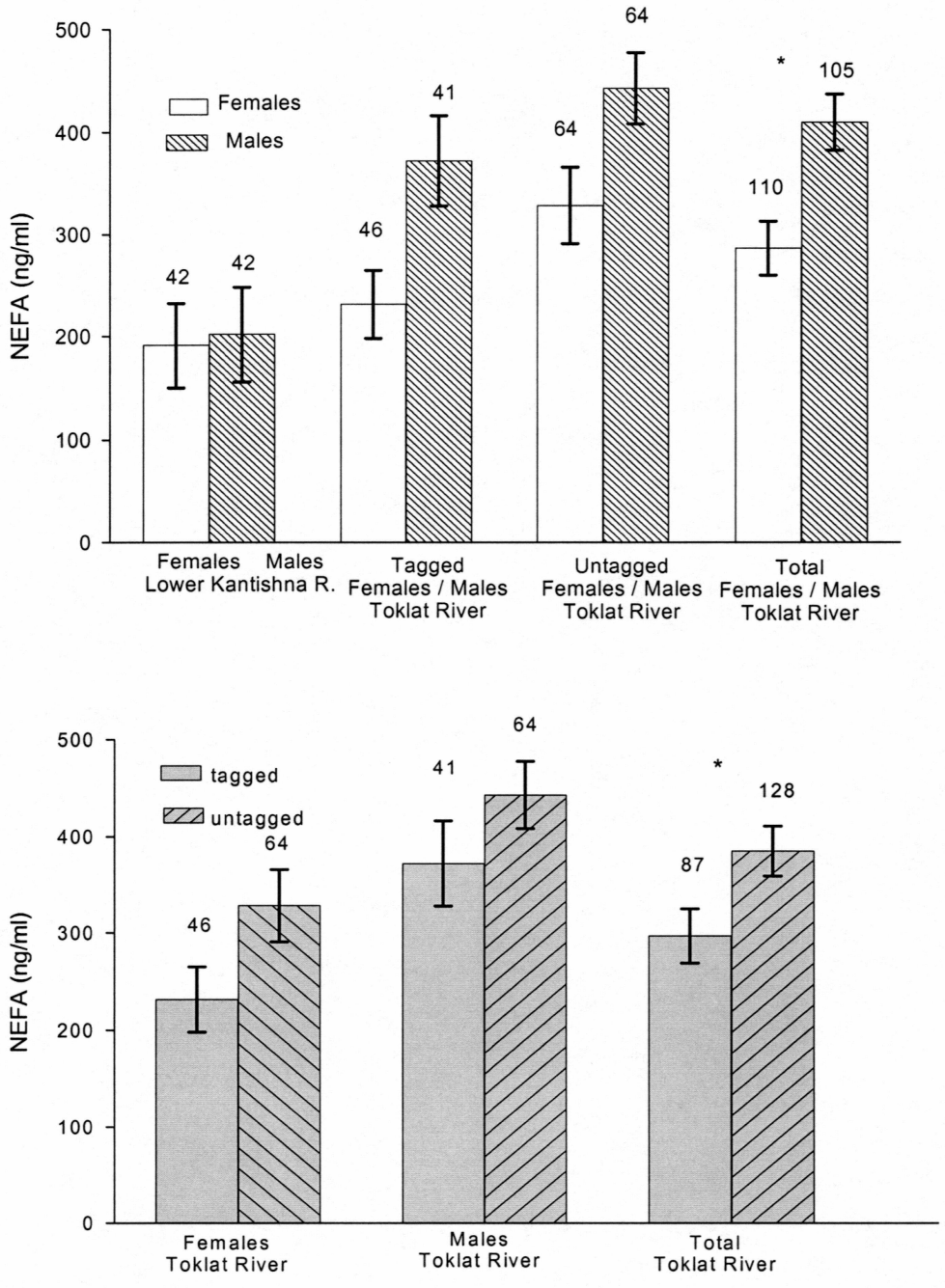

Figure 4. Mean concentrations (± *SE*) of plasma NEFA in chum salmon from the lower Kantishna River fish wheel and the Toklat River fish wheels Alaska, 2001. NEFA concentration did not significantly differ between males and females at the lower Kantishna River fish wheel but significantly differed between males and females at the Toklat River (above) and total tagged and untagged at the Toklat River fish wheels (below). Sample sizes are above bars. The asterisk indicates a significant difference.

Discussion

The results of this study verify that fall chum salmon captured in fish wheels are affected by capture and handling, which is manifested in a reduction of plasma NEFA in tagged chum salmon. The increase in NEFA observed from the lower Kantishna River to the Toklat River is similar to results of other studies on migrating chum salmon. Ando et al. (1985) found that plasma fatty acid concentrations in migrating chum salmon gradually increased during spawning migration and found that fatty acids of muscle lipid were used for energy of upstream migration and gonadal maturation. An increase in plasma NEFA concentration also occurred in bass (*Dicentrarcus labrax*) when mobilized from adipose tissue stores of tryacylglycerols from starvation (Zammit and Newsholme 1979). In addition, Saddler and Cardwell (1971) found extensive mobilization of fatty acids in tagged pink salmon (*Oncorhynchus gorbuscha*) and differential mortality between tagged and untagged pink salmon. He hypothesized that mortalities in tagged pink salmon were due to high stress from tagging, which was a combination of capture, handling anesthesia and tagging

In summary, the results of the current study verify that capture using fish wheel and tagging has a measurable effect on the physiology of migrating fall chum salmon. Whether these effects result in delayed mortality is beyond the scope of this study and a subject of further investigation. However, large numbers of migrating chum salmon are affected annually by fish wheel capture and handling in mark-recapture studies. To what extent stress is occurring should be examined by collecting samples from projects that

handle large numbers of salmon annually to determine if effects are present in these stocks. For example, the United States Fish and Wildlife Service (USFWS) has documented that the probability of recapture of tagged chum salmon on the Yukon River decreases as fish travel upstream, suggesting that delayed mortality may be occurring (Underwood et al. 2002). In addition, they note the probability of recapture of tagged chum salmon decrease as a function of time fish are held in a live box.

Fish wheels are among the most feasible and practical methods to assess run strength and abundance in the Yukon River drainage. Because using fish wheels for mark-recapture studies is important for management of subsistence, personal use and commercial fisheries, fishery managers should continue to strive to minimize handling time. Fishery managers will be able minimize the impact of mark recapture studies on salmon stocks in the Yukon River drainage with the application of new handling methods and monitoring stress indices through physiology.

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Appendix A. Concentrations (± *SE)* of cortisol, glucose, lactate and chloride in the plasma of chum salmon captured at a fish on the lower Kantishna River, Alaska in August and September, 2000. Males and females did not significantly differ in any plasma variable measured. [a]

Variable	Females	SE	n	Males	SE	n	*P*
Cortisol (ng/mL)	108.2	18.0	18	60.1	10.9	22	0.08
Glucose (mg/dL)	111.6	6.5	18	121.8	5.5	21	0.24
Lactate (mg/dL)	33.7	2.4	18	32.5	3.0	21	0.77
Chloride (meq/L)	123.9	1.3	17	122.4	1.4	19	0.43

[a] *t* -test assuming unequal variances (95% CI)

Appendix B. Concentrations of plasma cortisol and glucose of tagged and untagged chum salmon from the Toklat River fish wheels, Alaska 2000.

Cortisol (ng/mL)				Glucose (mg/dL)			
tagged females	untagged females	tagged males	untagged males	tagged females	untagged females	tagged males	untagged males
315.13	180.99	22.26	84.31	67.66	140.71	119.49	122.70
203.49	231.56	31.15	118.97	59.31	73.14	85.21	163.64
137.98	250.50	231.98	171.14	62.55	98.02	148.26	203.65
226.34	165.14	44.48	22.96	23.36	105.10	90.46	136.81
136.82	318.28	68.51	2.30	120.32	29.23	99.02	100.95
243.82	355.35	43.72	55.06	84.70	70.21	129.49	114.85
336.95	144.39	137.49	82.67	97.78	70.70	119.46	80.46
199.74	336.95	30.88	107.17	112.17	43.12	103.63	167.06
23.83	319.31	12.79	53.67	90.22	69.48	118.10	131.93
74.32	273.61	35.82	183.86	148.03	94.61	80.10	129.25
28.22	406.93	139.36	172.21	101.68	62.55	91.52	96.31
99.87	48.71	89.51	129.42	94.61	118.02	120.71	140.22
	260.38	102.65	190.91		60.68	94.76	99.87
	240.16	4.45	79.07		71.19	153.64	177.30
	122.70	20.59	30.05		118.02	135.34	122.90
	168.45	174.41	51.62		81.29	134.12	171.93
	133.84		32.10		147.54	101.68	164.62
	142.37		210.29		71.19	147.30	126.07
	166.93				73.46		
	275.93				75.82		

Appendix C. Concentrations of plasma chloride and lactate of tagged and untagged chum salmon from the Toklat River fish wheels Alaska, 2000.

Chloride (meq/L)				Lactate (mg/dL)			
tagged females	untagged females	tagged males	untagged males	tagged females	untagged females	tagged males	untagged males
128	114	115	112	62.10	26.12	19.40	17.03
128	118	116	122	20.07	11.11	8.27	21.52
118	129	120	112	22.02	20.06	63.71	17.68
124	128	119	128	12.09	29.81	14.98	52.96
119	118	120	117	28.72	15.19	18.69	12.85
138	119	119	128	43.82	17.02	7.72	18.10
132	123	116	125	17.29	32.89	17.09	12.71
117	115	126	124	18.24	7.54	9.76	12.87
117	120	121	114	41.02	11.81	17.76	20.24
125	117	122	121	36.55	27.85	9.12	12.10
120	129	96	116	18.99	17.25	51.61	8.64
131	135	117	119	12.11	35.30	10.59	15.83
	123	122	117		18.57	25.07	26.40
	124	123	117		32.70	7.70	29.78
	118	128	136		20.27	7.87	17.99
	124	122	123		23.32	15.99	14.30
	131	124	120		19.75	13.95	31.60
	126	113			25.31	21.42	16.90
	123				27.13		
	113				25.24		

Appendix D. Concentrations of plasma NEFA of tagged and untagged chum salmon from the Toklat River fish wheels Alaska, 2001.

NEFA (ng/ml)			
tagged females	untagged females	tagged males	untagged males
0.00	0.00	499.59	0.00
815.15	0.00	997.52	17.40
222.96	0.00	276.24	23.85
265.99	0.00	325.41	45.39
239.35	0.00	501.64	51.85
409.43	0.00	265.99	60.51
261.89	22.56	356.15	89.77
30.34	40.59	954.49	128.06
143.04	61.08	397.13	141.53
434.02	89.77	583.60	155.83
753.94	90.03	286.48	174.75
164.43	91.49	669.66	177.32
432.30	97.07	470.90	179.93
157.94	120.76	292.63	186.07
0.00	122.55	481.15	192.22
179.90	124.85	0.00	206.91
89.62	125.35	809.00	223.98
505.64	126.65	403.66	225.01
32.87	149.19	794.66	243.45
270.21	151.24	111.76	249.55
424.95	161.49	641.63	249.60
0.00	163.84	337.22	265.99
27.15	193.26	190.54	306.97
0.00	193.99	19.41	311.07
0.94	200.42	103.62	319.27
383.68	206.84	196.34	329.51
336.93	208.61	0.00	329.51
280.18	228.57	895.19	335.66
616.10	232.75	274.75	354.10
185.06	233.20	578.98	360.25
71.02	237.30	809.30	413.53
831.54	255.74	306.30	441.78
150.61	257.29	81.88	453.70

Appendix D. Concentrations of plasma NEFA of tagged and untagged chum salmon from the Toklat River fish wheels Alaska, 2001 (Contd.)

NEFA (ng/ml)			
tagged females	untagged females	tagged males	untagged males
0.00	261.89	0.00	458.49
281.36	272.14	5.75	470.90
0.00	284.43	468.00	473.96
93.79	296.73	0.00	480.42
0.00	306.97	0.00	485.24
30.34	308.10	165.58	496.59
79.52	327.51	352.05	513.79
177.88	332.17	347.95	528.28
120.50	343.86		561.06
169.68	388.94		579.50
431.97	393.03		579.50
538.52	405.13		581.55
0.00	407.38		589.75
	413.53		593.85
	421.72		624.58
	421.72		636.88
	472.95		693.22
	479.12		702.45
	481.15		704.50
	513.93		710.65
	522.13		751.63
	524.18		765.97
	606.15		767.00
	761.87		775.40
	778.27		807.98
	811.14		864.33
	890.97		892.16
	1204.48		934.00
	1229.07		1007.77
	1452.42		1013.91
	505.64		1054.89

Appendix E. Concentrations of plasma NEFA from male and female chum salmon from the lower and upper Kantishna River fish wheels Alaska, 2001.

Lower Kantishna		Kantishna Recovery	
females	males	females	males
0.00	216.81	0.00	234.84
85.67	124.60	0.00	0.00
403.28	0.00	0.00	109.85
190.17	200.42	0.00	77.06
468.85	669.66	0.00	0.00
409.43	665.56	331.15	0.00
671.71	95.91	99.60	0.00
487.29	249.60	17.64	144.68
671.71	1030.31	435.66	17.64
0.00	466.80	193.86	0.00
423.77	872.52	0.00	54.52
397.13	386.89	95.50	0.00
858.18	960.64	0.00	
188.12	280.33	140.58	
1093.83	624.58	374.18	
0.00	704.50		
58.62	358.20		
5.34	0.00		
101.65	0.00		
77.06	0.00		
72.96	0.00		
0.00	0.00		
195.91	27.88		
0.00	0.00		
13.54	66.82		
249.19	0.00		
0.00	0.00		
0.00	0.00		
0.00	70.92		
56.57	0.00		
0.00	0.00		
0.00	0.00		
0.00	29.93		
0.00	208.21		
0.00	13.54		
0.00	0.00		
396.72	0.00		
0.00	0.00		
240.99	109.85		
216.40	5.34		
0.00	0.00		