

Thiamine and Lipid Utilization in Fasting Chinook Salmon

Dale C. Honeyfield¹, Amber K. Peters², and Michael L. Jones²

¹U.S. Geological Survey, Northern Appalachian Research Laboratory,
176 Straight Run Road, Wellsboro, PA 16901, USA

²Department of Fisheries and Wildlife, Michigan State University,
13 Natural Resources Bldg., East Lansing, MI 48824, USA

Honeyfield, D.C., A.K. Peters, and M.L. Jones. 2016. Thiamine and lipid utilization in fasting Chinook salmon. N. Pac. Anadr. Fish Comm. Bull. 6: 13–19. doi:10.23849/npafcb6/13.19.

Abstract: A laboratory study was conducted to estimate utilization rates of thiamine (vitamin B₁) and lipid in whole fish, muscle, and liver tissues of fasting Chinook salmon (*Oncorhynchus tshawytscha*). The experiment was conducted with Chinook salmon held at 5°C over a period of 150 days to simulate fasting during migration or overwintering. Chinook salmon body length and wet weight did not change ($p > 0.05$) over the course of the study; however, significant declines were observed in muscle thiamine ($p < 0.01$) and lipid ($p < 0.01$). There was an inverse relationship between lipid and water content. Under the experimental conditions with no strenuous swimming, thiamine utilization rates reported are conservative estimates and were found to be 5.3–6.8 pmol·g⁻¹·day⁻¹ in muscle tissue and ~110 pmol·g⁻¹·day⁻¹ in liver tissue over the first 100 days. Fasting lipid depletion rates in whole fish were calculated to be 0.14–0.16%·d⁻¹. Muscle lipid decline rate (0.13%·day⁻¹) over the first 100 days was similar to whole fish lipid loss, however, muscle lipid utilization was slower (0.04%·day⁻¹) over the last 50 days. During periods of fasting, Chinook salmon deplete bodily reserves of both thiamine and lipid which may have consequences for successful spawning migration and overwinter survival.

Keywords: overwinter, spawning migration, metabolic energy, vitamin B₁

INTRODUCTION

Body composition of Chinook salmon, *Oncorhynchus tshawytscha*, is dynamic over its life cycle. Chinook and other anadromous salmon species expend a significant amount of energy during their spawning migration and do not actively feed (Dickhoff et al. 1997). Overwinter lipid and thiamine (vitamin B₁) content in Lake Michigan Chinook salmon have been shown to decrease and tissue water content to increase (Peters et al. 2007; Honeyfield et al. 2008). Chinook have been reported to catabolize 95–99% of their muscle lipid and 73–86% of their visceral lipid to complete their spawning migration up the Columbia River (Mesa and Magie 2006). These changes in lipid composition show a pattern of increasing energy reserves in summer when food is plentiful, and declining reserves over winter or during spawning migration. While seasonal variation in lipids has been reported across a number of species (Jonas et al. 1996; Dawson and Grimm 1980; Craig et al. 2000; Madenjian et al. 2000; Pedersen and Hislop 2001; Brown and Murphy 2004; Vollenweider et al. 2011), there is little or no comparable information available for body stores of thiamine.

Lipid is a form of energy reserve whereas thiamine is necessary to convert lipid subunits of fatty acid carbon into

ATP (Harper 1973). Production of metabolic energy from fatty acyl triglycerides, the primary storage form of lipid, begins with beta-oxidation. This pathway reduces fatty acid carbon chains into two carbon fragments (acetyl-CoA). Acetyl-CoA is incorporated into a Krebs cycle intermediate and continues through the cycle passing through a thiamine-requiring enzyme, α -ketoglutarate dehydrogenase. Without thiamine, which is converted to the active co-factor, thiamine pyrophosphate, the Krebs cycle stops and the production of ATP ceases (Depeint et al. 2006). Thiamine loss occurs through normal metabolic turnover with the degradation of thiamine pyrophosphate to thiamine monophosphate (Gubler 1991). Thiamine is also used as an antioxidant (Portari et al. 2008) in addition to its primary metabolic role, thus oxidative processes and stress can lead to additional thiamine loss (Lundstrom et al. 1999; Vuori and Nikinmaa 2007; Vuori et al. 2008). In summary, production of metabolic energy requires a source of energy (lipid) and a metabolic process that utilizes thiamine.

Chinook salmon are predominantly piscivorous and often forage on clupeid species (Daly et al. 2009, 2010). Chinook were introduced into the Great Lakes in the 1960s and became an important part of the sport fishery (Tanner and Tody 2002). These fish were brought in to control an

overabundance of alewife, *Alosa pseudoharengus*, a clupeid that dominated the forage base. Many clupeid species are known to contain thiaminase, an enzyme that destroys thiamine (NRC 1983). Reproductive failure in Lake Michigan salmonids including Chinook salmon has been linked to foraging heavily on alewife (Marcquenski and Brown 1997; Fitzsimons et al. 1999; Brown et al. 2005b; Wolgamood et al. 2005). Consumption of alewife greater than 20–25% of the diet leads to thiamine deficiency and mortality (Brown et al. 2005a; Honeyfield et al. 2005). Although thiamine has been reported to decrease overwinter in Lake Michigan Chinook (Honeyfield et al. 2008), it is not known if the decrease in thiamine was the result of thiaminase from the consumption of an occasional alewife or from basal metabolism utilizing thiamine. The objective of the reported work was to document changes in Chinook salmon tissue thiamine and lipid content in animals fasted for 150 days.

METHODS

Chinook salmon with an average length of 35.3 cm, average weight of 428 g, and age of approximately 1 year were included in the study. The fish were obtained from the Salmon River Hatchery in Altmar, New York, and were similar in size to the small-sized fish category in Peters et al. (2007). Fish were reared from eggs collected from Lake Ontario Chinook salmon stocks spawned at the hatchery. The fish were transported from New York to Wellsboro, Pennsylvania (USGS, Northern Appalachian Research Laboratory), and were divided equally into two insulated rectangular tanks (51 cm x 58 cm x 305 cm) with continuous flowing fresh well water (11.4 L·min⁻¹, 9°C, 8–9 ppm dissolved oxygen). Tanks were covered with one-inch thick polystyrene insulation board during the study to limit heat gain from the ambient laboratory temperature. Fish were held in the labo-

ratory and fed commercial feed for two months prior to the experiment. Forty-eight hours before the start of the study, feed was withdrawn. During the 150-day study, no feed was offered to the fish. Water temperature was lowered incrementally over a five-day period from 9°C to < 5°C and held at 4.5–4.8°C for the duration of the study to simulate winter conditions. Fish were monitored daily throughout the study and fish mortality recorded.

Chemical analysis was conducted on fish collected on days 0 (n = 8), 100 (n = 8) and 150 (n = 10) of the study. Starting body weight and length were recorded for all fish (n = 27) on day 0. Body weight and length were also collected on day 100 and 150. Fish were randomly selected, killed by cervical dislocation and immediately processed. A dorsal muscle sample (5–10 g) and the entire liver were collected. Liver and intestinal tract were removed and weighed. For whole fish lipid and water content, intestinal tract and carcass minus the liver were mixed and processed as described in Peters et al. (2007). Muscle, liver and ground whole fish were stored frozen at -80°C until analysis. The muscle sample removed for lipid and thiamine analysis constituted approximately 1–2% of the total body weight. Whole fish lipid and water content reported were not adjusted for the muscle and liver tissue removed.

Muscle and liver thiamine were determined using the method of Brown et al. (1998). The chromatography (HPLC) method separated thiamine pyrophosphate, thiamine monophosphate, and free or unphosphorylated thiamine which were quantified using commercially available authentic standards. Duplicate analysis was conducted and data are reported from duplicates with < 10% difference. Water content or dry matter analysis was conducted on whole fish and muscle samples from each fish (AOAC 1995). The Soxtec method was used to determine lipid content (Soxtec System HT6; Tecator, Sweden) (AOAC 1995).

Table 1. Tissue weight and percentage water and lipid in Chinook salmon fasted for 150 days.

| Variable | Days after fasting | | | | | | | | | p |
|--------------------------|--------------------|-------------------|-----|----------|-------------------|-----|----------|-------------------|-----|---------|
| | 0 Days | | | 100 Days | | | 150 Days | | | |
| | n | Mean | SE | n | Mean | SE | n | Mean | SE | |
| Liver weight, g | 8 | 5.6 ^a | 0.8 | 8 | 3.6 ^b | 0.3 | 10 | 3.6 ^b | 0.5 | < 0.05 |
| Viscera weight, g | 8 | 20.7 ^a | 2.7 | 8 | 14.3 ^b | 1.2 | 10 | 14.2 ^b | 0.9 | < 0.05 |
| Whole Fish | | | | | | | | | | |
| Water, % | 8 | 73.4 ^a | 0.4 | 7 | 78.7 ^b | 0.7 | 10 | 81.0 ^c | 0.5 | < 0.001 |
| Lipid, % DM ¹ | 8 | 29.1 ^a | 1.9 | 8 | 14.4 ^b | 1.5 | 10 | 11.9 ^b | 2.6 | < 0.001 |
| Muscle | | | | | | | | | | |
| Water, % | 8 | 75.8 ^a | 0.7 | 7 | 79.3 ^b | 0.4 | 10 | 81.1 ^c | 0.4 | < 0.001 |
| Lipid, % DM | 8 | 18.3 ^a | 1.6 | 7 | 5.7 ^b | 0.9 | 10 | 3.5 ^b | 0.7 | < 0.001 |

¹DM, lipid as percent of the dry matter.

^{abc}Means with similar superscripts are not significantly different.

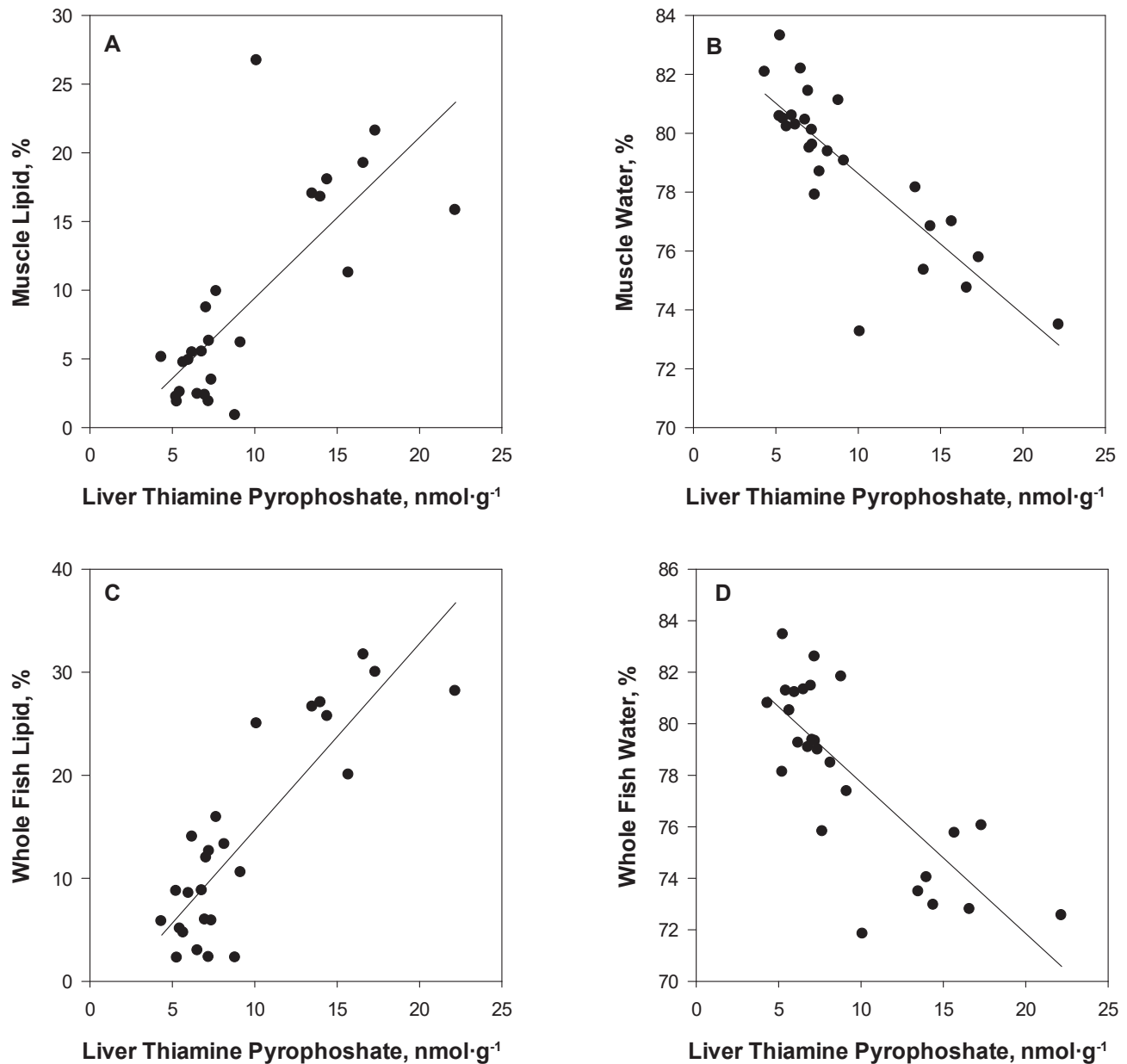


Fig. 1. Relationship between tissue thiamine pyrophosphate and percent muscle and whole body lipid and water in experimental fish held at 5°C for 150 days.

Data Analysis

Statistical analyses were completed using R statistical software package (R Development Core Team 2013). Analysis of Variance was used to test for significant differences among sampling periods (days 0, 100, 150) for fish length, weight, liver and viscera weight, water and lipid levels in muscle tissue and the whole fish, and thiamine levels in muscle and liver. A Tukey “Honest Significant Difference” test was used for a post-hoc comparison among means.

RESULTS

Whole body weight and length did not change during the course of the study. Average, \pm SE, and starting fish length and weight (34.9 ± 0.5 cm; 469 ± 29 g) were not significantly different between fish examined after fasting for 100 days (35.1 ± 0.7 cm; 409 ± 24 g) or 150 days (35.1 ± 0.5 cm; 409 ± 21 g). Liver and viscera weights were significantly lower at days 100 and 150 compared to day 0 (Table 1). Percent water in whole fish and muscle increased substantially over time while percent lipid decreased in a corresponding fashion (Table 1; Fig. 1). There were significant differenc-

es among all three time periods for percent water. On day 131, one fish was found dead from unknown causes. All other fish remained active displaying normal fish behavior through day 150 of the study when all remaining fish were killed and tissues collected for chemical analysis.

Total thiamine decreased over the course of the study in muscle and liver tissue (Fig. 2). Thiamine levels in liver tissue were significantly lower by the end of the study, with no apparent difference between day 100 and day 150 (Table 2). Changes in muscle thiamine were not as great as for the liver, with day 0 different from day 150 but no significant difference between day 0 and day 100 or day 100 and day 150 (Table 2). The loss of liver thiamine occurred predominately during the first 100 days with little or no loss during the last 50 days. Thiamine pyrophosphate levels were correlated with lower lipid levels in muscle ($R^2 = 0.79$; Fig 1) and whole fish ($R^2 = 0.68$). Thiamine pyrophosphate was negatively correlated with muscle water ($R^2 = -0.78$) and whole fish water ($R^2 = -0.80$) content. Free thiamine was low in both muscle and liver and was not different across time.

DISCUSSION

Chinook salmon fasted over the 150-day study showed depleted thiamine and lipid stores in both muscle and liver tissues. Additionally, a strong relationship between liver thiamine pyrophosphate (TPP), the active co-factor in thiamine-requiring enzymes in the Krebs cycle, and whole body lipid ($R^2 = 0.68$) and muscle lipid ($R^2 = 0.79$) was found (Fig. 1a, c). Utilization of lipid during fasting is considered common knowledge (Hendry and Berg 1999; Hendry and Beall 2004; Mann et al. 2009), but this is the first data to suggest that significant thiamine utilization is co-occurring with lipid catabolism during fasting. Mann et al. (2009) reported that 95% of Chinook lipid was utilized by fish that successfully spawned, but only 65% lipid utilization was found in fish that did not reach their spawning sites. These authors

suggested that lack of lipid was not the cause of death and that disease, water temperature, or other factors should be investigated for the cause of death. Both lipid and thiamine are required to produce metabolic energy and thiamine deficiency has been linked to immune dysfunction (Ottinger et al. 2012, 2014). Cooke et al. (2006) reported no difference in lipid content of successful and unsuccessful spawning sockeye salmon (*O. nerka*) in the Fraser River, Canada. However, it's not clear whether low thiamine was involved in Fraser River sockeye because fish were dying prior to ascending the river with low body stores of lipid. Although there are many reasons unsuccessful fish may die prior to spawning, possible explanations include limited lipid stores, or limited lipid utilization and disease as a result of thiamine deficiency. Therefore, the importance of adequate lipid and thiamine should not be overlooked in salmon that have not successfully reached their natal spawning areas.

The present work shows that during fasting, there is a loss of thiamine due to normal metabolic processes without considering metabolic cost of work such as rigorous migratory swimming. Migration and overwintering are a non-feeding time when prey items either with or without thiaminase are not being ingested. Chinook salmon do not harbor thiaminase (NRC 1983). Therefore, these results isolate the basal metabolic turnover of body stores of thiamine from thiaminase degradation or other demands for thiamine, such as swimming. If Chinook have reduced energy (lipid) stores, reduced thiamine, or both, these fish will be less likely to successfully reproduce. Low thiamine has previously been shown to limit salmonid spawning migration (Fitzsimons et al. 2005; Ketola et al. 2005, 2009). Liver thiamine loss plateaued between 100 and 150 days whereas the muscle thiamine loss was continuous over the 150-day period. Thiamine is important in the liver because the liver processes dietary carbohydrates, proteins, fats, vitamins and minerals, is vital for ridding the body of toxins and microbes, and produces ATP. The liver plays a vital role in maintaining the body's metabolic balance. Our data suggest there is a meta-

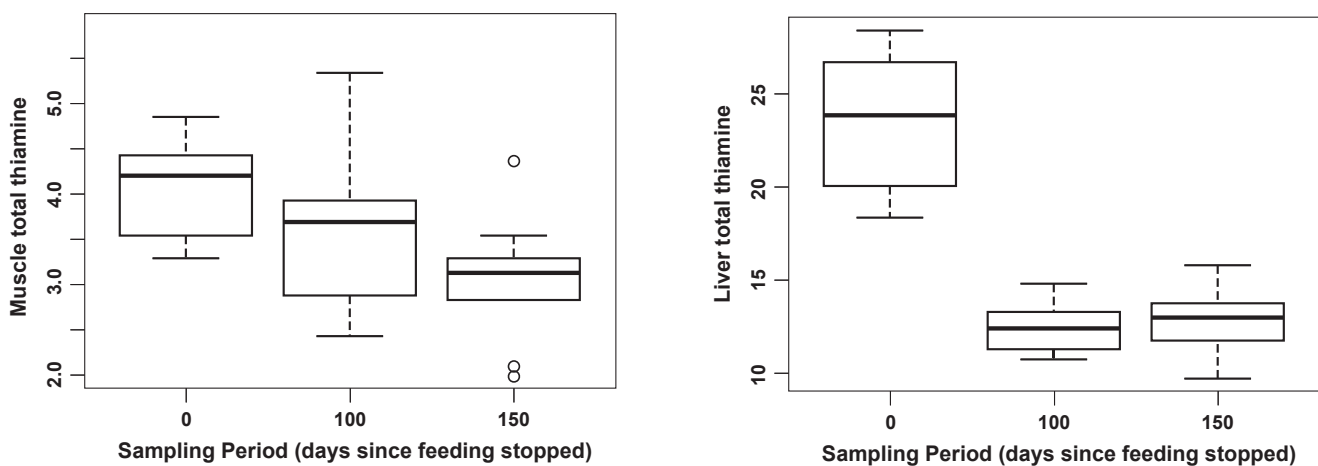


Fig. 2. Muscle and liver total thiamine (nmol·g⁻¹ wet weight) in Chinook fasted for 0, 100 and 150 days.

Table 2. Muscle and liver thiamine pyrophosphate (TPP), thiamine monophosphate (TP), free thiamine (T), and sum of three forms of thiamine (Total) concentrations (nmol·g⁻¹ wet weight) in Chinook salmon over the fasting period of 150 days at 5°C.

| Variable | Days after fasting | | | | | | | | | p |
|----------|--------------------|--------------------|------|----------|---------------------|------|----------|--------------------|------|---------|
| | 0 Days | | | 100 Days | | | 150 Days | | | |
| | n | Mean | SE | n | Mean | SE | n | Mean | SE | |
| Muscle | | | | | | | | | | |
| TPP | 8 | 3.35 ^a | 0.15 | 7 | 3.00 ^{abc} | 0.30 | 10 | 2.56 ^c | 0.20 | < 0.05 |
| TP | 8 | 0.65 ^a | 0.06 | 7 | 0.50 ^b | 0.05 | 10 | 0.45 ^b | 0.04 | < 0.05 |
| T | 8 | 0.05 | 0.01 | 7 | 0.03 | 0.00 | 10 | 0.03 | 0.00 | N.S. |
| Total | 8 | 4.06 ^{ab} | 0.19 | 7 | 3.58 ^{abc} | 0.38 | 10 | 3.04 ^c | 0.22 | < 0.05 |
| Liver | | | | | | | | | | |
| TPP | 8 | 14.10 ^a | 0.89 | 7 | 6.74 ^b | 0.51 | 9 | 6.84 ^b | 0.20 | < 0.001 |
| TP | 8 | 8.85 ^a | 1.07 | 7 | 5.29 ^b | 0.39 | 9 | 5.58 ^b | 0.31 | < 0.01 |
| T | 8 | 0.55 | 0.06 | 7 | 0.44 | 0.04 | 9 | 0.42 | 0.06 | N.S. |
| Total | 8 | 23.50 ^a | 1.37 | 7 | 12.47 ^b | 0.55 | 9 | 12.84 ^b | 0.57 | < 0.001 |

^{abc}Means with similar superscripts are not significantly different.

bolic priority for maintaining liver concentration of thiamine (Fig. 2) during the depletion process for important metabolic functions such as ATP production and the other processes mentioned above. Thiamine utilization in this study was calculated to be 5.3–6.8 pmol·g⁻¹·day⁻¹ in muscle and ~110 pmol·g⁻¹·day⁻¹ from 0–100 days in liver tissue.

In addition to thiamine, cumulative whole body dry matter and lipid declined significantly over the course of this study. Chinook salmon lost 21% and 30% of their whole body dry matter content after fasting for 100 and 150 days, respectively. Wet body weight, however, remained constant. A significant amount of dry matter loss was from lipid: 59% and 80% of whole body lipid after 100 and 150 days fasting, respectively. Lipid utilization rates in the present study were calculated to be 0.14–0.16%·day⁻¹ in whole body and 0.1–0.13%·day⁻¹ in muscle tissue. Mesa and Magie (2006) reported 95–99% depletion of Chinook muscle lipid and 73–86% visceral lipid stores in order to reach natal spawning areas on the Columbia River. Therefore, it appears that basal metabolic lipid utilization constitutes a measurable portion of the total energy cost of migration. This pattern would suggest that once spawning migration has begun, delays to a fish's up-stream movement could have consequences on reproductive success, especially in Yukon River Chinook salmon that travel up to 3200 km.

An inverse relationship between lipid and tissue water content was found in this study; as lipid decreased whole body water increased. The percentage of lipid lost from muscle (74%) was greater ($p < 0.05$) than from whole body (68%) after 100 days. At 150 days, muscle and whole body lipid losses were similar (85% vs. 86%). Comparable over-winter lipid losses have been reported in Lake Michigan Chinook (Peters et al. 2007) with losses of 75% lipid from muscle and 60% loss from whole body. The results suggest that overwinter lipid losses reported in small size

Lake Michigan Chinook salmon were similar to losses in laboratory Chinook fasted for 100 days. Because lipid and water are inversely related, data suggest that measuring muscle water could be an easy yet reliable way to estimate lipid stores in Chinook (Fig. 1b; Trudel et al. 2005).

Adequate stores of lipid and thiamine are needed for basic metabolic function and overall health. The significance of these findings on Chinook salmon reproduction has not been determined. Additional research is needed to determine thiamine and lipid utilization in migrating Chinook salmon. The present study demonstrated that fasting leads to a loss of tissue lipid and thiamine, which are both essential for health and reproductive success. This data may provide insight for some unexplained observations in migrating anadromous fish and in overwintering survival.

ACKNOWLEDGMENTS

We wish to acknowledge and thank the Great Lakes Fishery Trust for their help and gracious support of this project. We also wish to thank Stephanie Sweet for her excellent laboratory work on this project. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. federal government.

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