# ENERGY ACQUISITION AND RETENTION BY JUVENILE AND ADULT PADDLEFISH IN RELATION TO AGE, GROWTH, AND REARING CONDITIONS

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## AUTHORIZATION TO SUBMIT THESIS

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## ABSTRACT

Studies were conducted in 2011 and 2012 on paddlefish stocks from North Dakota, Montana, and Oklahoma to develop a better understanding of paddlefish physiological based on energy content. Lipid accumulation and storage in juvenile paddlefish in relation to age, stock, tissue type, year, and an index of growth using RNA/DNA ratios were pursued in Chapter 2. An emphasis on growth and a relatively large rostrum size in age-0 and age-1 fish may be adaptive in avoiding predation while accruing necessary energy reserves for overwintering. Chapter 3 provides reference data for proximate composition of 3 tissue types from wild adult paddlefish, and investigates lipid accumulation and storage in adult paddlefish in relation to tissue type within individual fish, between sexes, and between two different stocks. Life history, growing season, and metabolism may help explain observed differences in lipid concentrations. Chapter 4 concerns suitability of alternate methods of estimating energy density in juvenile and adult paddlefish.

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# **CHAPTER 1.** BACKGROUND INFORMATION.

## ABSTRACT

In an effort to better understand recruitment and life history strategies of paddlefish, investigations of energy storage and allocation were initiated on juveniles and adults involving populations in three states, Montana, North Dakota, and Oklahoma. Chapter 1 provides background information on the use of energy data in fisheries, as well as a description of source populations used in this study. The objectives of this study were to: (1) characterize energy density in age-0 and age-1 paddlefish by age, stock origin, tissue type, year, and growth rate (Chapter 2), (2) characterize lipid content in various tissues from adult male and female paddlefish in relation to tissue type, stock origin, body length, weight, age, gonad weights and GFB weights (Chapter 3), and (3) evaluate the use of fish condition (plumpness) and a microwave-based fat-meter to estimate energy density in juvenile and adult paddlefish tissues (Chapter 4).

## INTRODUCTION

Energy reserves play a number of important roles in the life history of fishes (Jönsson and Jönsson 2005). The physiological capacity of a fish to derive, store, and utilize energy influences growth, survival, migration, maturation, reproduction, longevity, and ultimately fitness (Hurst and Conover 2003).

Fish can store energy in a variety of forms, including protein, carbohydrate, and lipid. Although protein contains less than half the caloric density of lipid, in fish, poikilothermy and an efficient mechanism for elimination of nitrogenous byproducts allow fish to utilize protein

for energy (Silva and Andersen 1994). For example, many salmonids depend heavily upon protein from muscle tissue for energy during spawning migrations (Mommsen et al. 1980; Penney and Moffitt 2013) Carbohydrates are of limited value to fish as energy as most fish have a poor ability to metabolize glucose (Brett and Groves 1979). Lipid is the most efficient form of energy storage, and the most important for meeting long-term energy demands (Adams 1999; Anthony 2000; Tocher 2003).

Lipid is stored and utilized from diverse tissues and locations within the body, including liver, muscle, abdomen, viscera, and gonads (Adams 1999). Lipid storage location can be influenced by numerous factors including species (Spitz et al. 2010), size (Sogard and Spencer 2004), age (Anthony et al. 2000), sex (Basade 2000), and behavior (Biro et al. 2005; Columbier et al. 2007). Lipids stored in different locations may be used to meet different organismal needs, including migration, and are used differently according to specific physiological requirements of a particular species. For example, in Norway, migrating arctic char *Salvelinus alpinus* use more lipids from muscle than from other locations (Jobling et al. 1998), while migrating American shad in the Connecticut river use lipid preferentially from skin and sub-dermal fat before depleting muscle tissue (Leonard and McCormick 1999).

Because the allocation of energy to lipid storage is shaped by an organism's specific adaptations (Sheridan 1994), identifying lipid storage patterns can be a powerful tool for understanding life history. Measures of tissue lipid have been successfully used to quantify energy content and dynamics in both juvenile as well as adult fish during periods of growth (Jacobs 2011), overwintering (Jonas et al. 1996; Huntingford et al. 2001; Hurst and Conover 2003), reproduction (Hendry et al. 2000), aestivation (Pusey 1990), and migration (Crossin and Hinch 2005). Lipid content has been used to investigate how energy allocation changes

between growth and storage over time in juvenile fish, including sablefish *Anoplopoma fimbria* (Sogard and Spencer 2004), muskellunge *Esox masquinongy* (Jonas et al. 1996), and largemouth bass *Micropterus salmonoides* (Jacobs et al. 2012). Lipid values have also been successfully used to investigate trade-offs between storage and reproduction in adult fish of many species including striped bass *Morone saxitilis* (Hurst and Conover 2003), sockeye salmon *Oncorhynchus nerk*a (Hendry et al. 2000), longnose gar *Lepisosteus osseus* (Johnson and Noltie 1997), and golden mahseer *Tor putitora* (Yasmeen et al. 2000). In addition, measures of lipid have been used to evaluate condition in response to population size and environmental conditions (Rand 1994), and to compare nutritional quality of forage (Spitz 2010).

Proximate analysis and adiabatic bomb calorimetry are accepted methods of assessing lipid and total energy content in fish (Craig et al. 1978; Hendry et al. 2000). Proximate analysis, divides a substance into four broad categories-- lipid, protein, water, and carbohydrate—and is used globally as an indicator of relative nutritional quality of fish (Rand 1994) and fish products (Decker 1991). Proximate results are expressed as percentages of wet or dry weight. Total energy levels in fish tissues can be measured with calorimetry, which measures heat of oxidation during combustion to determine units of energy (calories, kilocalories or joules) per unit mass (usually grams) wet or dry weight. Because lipid and protein are the primary components of energy in fish, total energy of tissues is estimated from proximate analysis with commonly accepted caloric equivalents: 4.6 kcal/g for protein and 9.8 kcal/g for lipid. Types of tissues commonly evaluated in fish studies include whole fish samples, as well as specific tissues such as muscle (Mohamed and Al-Sabahi 2014), viscera (Hendry et al. 2000), or gonads (Neves and Brayton 1982).

As a result of the high cost and time associated with preparation and analysis of tissues, other faster, less expensive methods to estimate lipid in fish have been explored. Many researchers have included the use of coarse condition indices as a surrogate indicator for lipid (Hartman and Brandt 1995; Jonas et al. 1996; Wuenschel et al. 2006). Kaufman et al. (2007) reported the use of several new methodologies to measure energy content in fish, including bioelectrical impedance (Cox and Hartman 2005) and a low energy microwave probe (Crossin and Hinch 2005). Protocols for using bioelectrical impedance are still being developed (Pothoven et al. 2008; Hafs and Hartman 2011), but recent studies have successfully used a microwave probe (Distell Fish Fatmeter) to assess lipid content of muscle tissue in migrating American Shad *Alosa sapidissima* (Mann et al. 2010, 2011).

Little is known about the energy allocation in North American paddlefish *Polyodon spathula,* a large, ancient, migratory, long-lived Acipenseriform species that inhabits large rivers in temperate regions of North America (Gengerke 1986; Scarnecchia et al. 2007; Bettoli et al. 2009). A comparative study of the Yellowstone-Sakakawea stock (hereafter SAK; spawning in the Yellowstone and Missouri rivers, rearing in Lake Sakakawea, North Dakota) with that of the Grand Lake stock (hereafter GL; spawning in the Neosho River, rearing in Grand Lake, Oklahoma) indicated that the more northerly stock (SAK), which was under a much lower metabolic demand, stored more gonadal fat reserves (as indicated by the weight of gonadal fat attached to the gonads (gonadal fat bodies or GFBs; Scarnecchia et al. 2007) and had a much longer lifespan than the more southerly GL stock, (Scarnecchia et al. 2011). Both stocks, however, have shown highly variable, episodic recruitment, resulting in highly variable year classes contributing to fisheries (Scarnecchia et al. 2011; 2014). Year class strength is likely influenced by overwinter survival of young paddlefish, as well as

subsequent survival, recruitment, and reproductive success of adults. All of these processes may be influenced by energy reserves.

In an effort to better understand recruitment and life history strategies of paddlefish, investigations of energy storage and allocation were initiated on juveniles and adults involving populations in three states. Because of their longevity, paddlefish are a good candidate for studying long-term energy dynamics (accumulation and depletion) and allocation in relation to age, size, and maturation state. Although lipid content in captive paddlefish has been quantified and reported, (Decker et al. 1991), no studies have been conducted on free ranging wild fish. The study reported here was designed to gain a better understanding of the physiological states of paddlefish based on lipid reserves, and to pursue the potential for using energy measures as a tool for understanding allocation of energy during different life history stages.

The objectives of this study were to: (1) characterize energy density in age-0 and age-1 paddlefish by age, stock origin, tissue type, year, and growth rate (Chapter 2), (2) characterize lipid content in various tissues from adult male and female paddlefish in relation to tissue type, stock origin, body length, weight, age, gonad weights and GFB weights (Chapter 3), and (3) evaluate the use of fish condition (plumpness) and a microwave-based fatmeter to estimate energy density in juvenile and adult paddlefish tissues (Chapter 4).

### *Study site and Source Populations*

Paddlefish were obtained from four distinct locations: the FP stock of the Missouri River upriver of Fort Peck Dam, the SAK stock of eastern Montana and western North Dakota, and hatchery paddlefish raised at Garrison Dam National Fish Hatchery (GD) located

in North Dakota, just downriver of Lake Sakakawea, and the stock of Grand Lake Oklahoma (GL; Figure 1). Garrison Dam National fish hatchery periodically produces paddlefish marked with coded wire tags (CWT) used to supplement natural production and management information for the SAK stock (Scarnecchia et al. 2008).

The SAK and FP stocks of Montana and North Dakota can be characterized as long-lived (commonly 30-40 years), with extended periods of low metabolic activity during extended winter months. The GL Stock is comparatively shorter-lived (typically 20 years), without the extended periods of low metabolic activity of the more northerly stocks (Scarnecchia et al. 2011). For all three stocks, immature paddlefish and those between spawns rely almost exclusively on reservoir rearing, and exhibit a life history that is adfluvial, with rearing in lentic (reservoir) habitats and spawning in riverine habitat upriver of the reservoirs.

The FP stock, the northwestern most of the three, rears in Fort Peck Reservoir, completed in 1940 with the closure of Fort Peck Dam to provide flood control, hydropower, and irrigation. After its 10 year filling process (from 1937 -1947), the reservoir inundated approximately 215 km of the upper Missouri River basin, and had an area of 97,100 ha, and a shoreline of 2,575 km (West et al. 1987). Since the mid 1960's (the period for which extant age classes of paddlefish exist), the reservoir pool has varied between 670 m above sea level (asl) and 686 m asl (with full pool at 685 m asl), and tends to be drawn down in the fall and

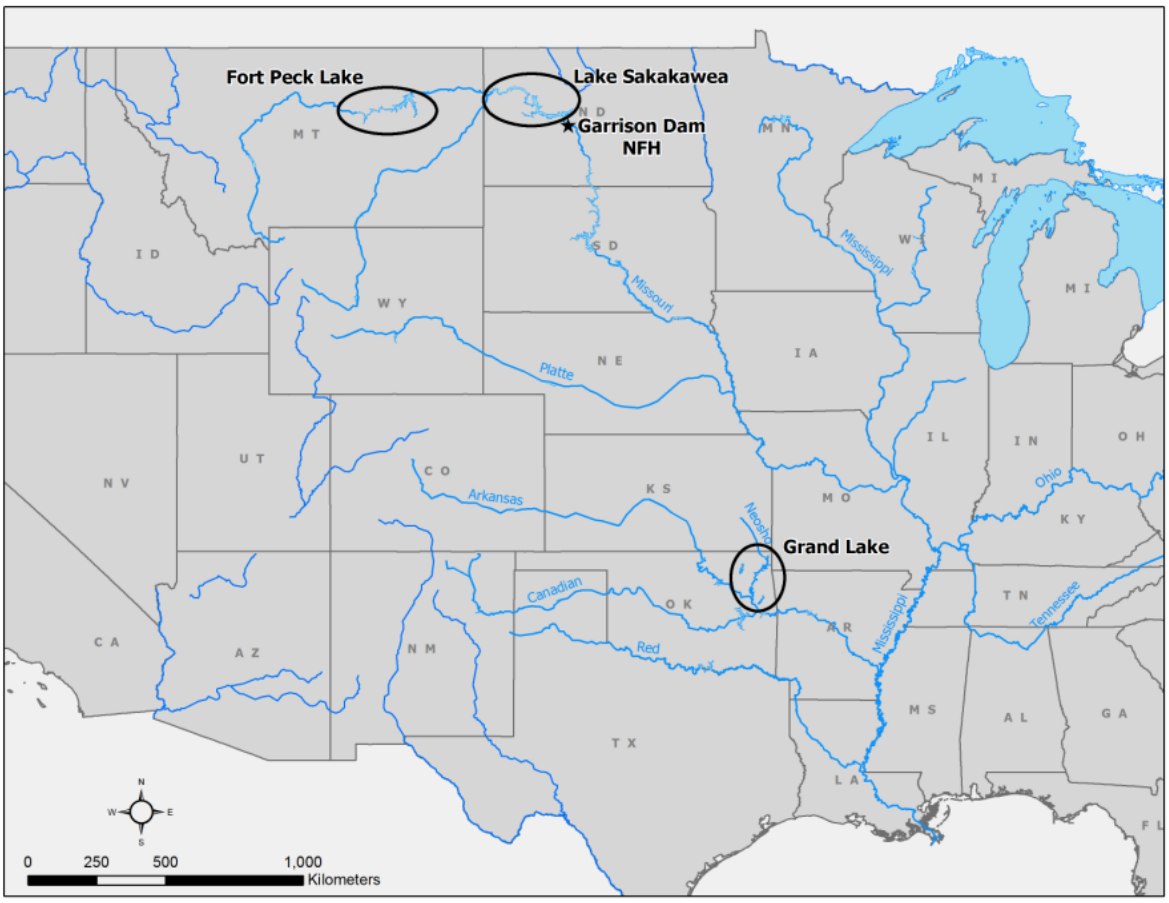


Figure 1. Origins of paddlefish used in this study.

winter (Bowersox 2004). With the construction of Fort Peck, paddlefish above the dam were isolated from downriver fish that were likely once part of the same stock. Fish that historically migrated large distances between rearing and spawning areas (Wilson and McKinley 2004), have since been restricted to the reservoir and river above the dam.

The SAK stock rears in Lake Sakakawea, located in the western half of North Dakota, also on the Missouri River. Garrison Dam, impounding the reservoir, was closed in December, 1953, followed by a 13-year filling period, which inundated approximately 286

km of river. At an elevation of 554 m asl, the area covered by the reservoir is 124,239 ha, with an approximate shoreline of 2,120 km. The reservoir level has fluctuated by as much as 8 m since filling depending on drought and flood conditions (Scarnecchia et al. 2014).

GD fish are the result of broodstock obtained by gillnetting in spring from the Yellowstone and Missouri rivers above Lake Sakakawea. Broodstock are spawned, and young hatched and reared in ponds at the hatchery, which is located directly below Garrison Dam, near the town of Riverdale, ND. Fish are stocked in September into Lake Sakakawea.

The GL stock rears in Grand Lake (21,000 Ha; completed 1940) and spawns in the Neosho and Spring rivers, which both enter the reservoir at the headwaters at Twin Bridges State Park, Ottawa County, Oklahoma. (Scarnecchia et al. 2013).

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**CHAPTER 2.** ENERGY ACQUISITION AND RETENTION BY AGE-0 AND AGE-1 PADDLEFISH IN RELATION TO SIZE, GROWTH, AND REARING CONDITIONS IN TWO GREAT PLAINS RESERVOIRS AND HATCHERY PONDS.

## ABSTRACT

Studies were conducted on 2011 and 2012 age-0 and age-1 paddlefish from Fort Peck (FP) Reservoir, Lake Sakakawea (SAK), and Garrison Dam National Fish Hatchery (GD) rearing ponds to investigate lipid accumulation and storage in juvenile paddlefish in relation to age (age-0, age-1), stock (FP, SAK, GD), tissue type (whole body (Whole) and eviscerated (Evisc)), year (2011, 2012), and an index of growth using RNA/DNA ratios. Age-0 lipid values were distributed into 2 groups (high and low lipid), with fish in the high group showing higher lipids with increasing length, but fish in the low group showing proportionately lower lipids with increasing length. SAK Age-1 fish from 2012 had less lipids than age 0-fish of the same cohort in 2011, but more than the following cohort of age-0 fish in 2012. GD fish had higher lipids and a different tissue profile than FP and SAK fish. Age-0 fish lipid differed by year, with 2011 fish having higher lipids than 2012 fish. No trend was found between RNA/DNA ratio and lipid content. Age-based differences suggest a split allocation between growth and lipid storage for juvenile paddlefish, with growth being the highest initial priority and emphasis on energy storage occurring at a larger size, later in life. Differences in lipid allocation between stocks indicate that allocation is influenced by hatchery/wild rearing conditions. Differences within and between year classes are consistent with field evidence of a strong 2011 year class, and indicate that paddlefish may allocate energy to both body growth and lipid reserves, and that allocation differs among years. The

lack of a relationship between RNA/DNA ratio and lipid does not support a physiologically exclusive allocation strategy between growth and lipid. Evidence from this and other studies suggests rather that an emphasis on growth, some energy storage, and a large rostrum size in relation to overall fish length in age-0 and age-1 fish, may be adaptive in avoiding predation while accruing necessary energy reserves for overwintering. This study also provides reference information regarding proximate composition of wild and hatchery origin juvenile paddlefish.

## INTRODUCTION

Characterization of energy acquisition and use in a fish species or stock is fundamental to understanding its life history, recruitment, and population dynamics. Within a fish species or stock, energy acquisition and use (primarily in the form of lipids) differ with age and condition, as well as with external environmental conditions and selective pressures (Adams 1999; Olsen 1999). As individual fish develop from exogenous feeding through senescence, energy is allocated differentially into maintenance, somatic growth, storage, maturation, and reproduction (Adams 1999; Scarnecchia et al. 2007; Hilton et al. 2008). In the early stages of life, larval fish are highly susceptible to starvation and predation (Osse et al. 1997; Hunter 1981; Estevez et al. 2000). After the larval stage, energy is allocated to growth of tissues such as bone, cartilage, and muscle (Craig 1978) in order to increase size and speed, thus avoiding predation (Post and Parkinson 2001; Biro et al. 2005; Wuenschel et al. 2006). Little energy is stored as lipids (Sogard and Spencer 2004). Later in ontogeny, in response to seasonal variations in environmental conditions, energy can be stored as lipid, and also with sexual maturation, utilized in gonadal maturity. After a period of prime reproduction (Scarnecchia

et al. 2007), senescence can lead to an additional reallocation of energy to storage and maintenance (Tedesco et al. 2008). Because of the link between food availability, growth, and energy acquisition, energy allocation is thus related to, and is likely a driver of many life history events (Jorgensen and Fiksen 2006; Scarnecchia et al 2007). The timing of these events is in turn dependent on age specific environmental stressors and selection pressures.

Energy storage may be useful at different life stages in order to supplement metabolic needs during periods of low metabolism or reduced food availability (Biro et al. 2005; Jacobs et al. 2012). Long term energy storage primarily involves accumulation of lipids at various locations within the body, including white muscle (Adams 1999), red muscle, and within the abdomen (Sheridan 1988). Reliance on stored energy reserves tends to increase in northern climates, where fish are adapted to lower temperatures and longer periods of reduced food availability. For example, capelin *Mallotus villosus* feeding at high latitudes on lipid rich zooplankton exhibit higher lipid levels than their more southerly counterparts (Anthony et al. 2000; Tocher 2003). Schultz and Conover (1997) reported similar latitudinal variation in energy allocation in Atlantic silverside *Menidia menidia*.

Inter-annual differences in age-0 fish survival and recruitment may be affected by inter-annual differences in their energy accumulation and storage. In particular, overwinter survival may be related to late-summer growth and energy reserves going into winter (Adams 1999; Post and Parkinson 2001). For example, Thompson et al. (1991) found that lipid reserves affected survival of age-0 Colorado pikeminnow *Ptychocheilus lucius*, and Jacobs et al. (2012) reported that age-0 largemouth bass *Micropterus salmoides,* switched allocation from growth to energy storage as winter approached. Because lipid contains roughly twice the energy per unit mass compared to protein, it is the most efficient form of energy, and the

most energetically valuable form that can be stored by small, young fish. However, in terms of first year survival and recruitment success, energy storage at a young age involves a potentially critical trade-off with growth. Storing energy may come at the expense of a smaller total length and potentially increased susceptibility to predation (Wuenshel et al. 2006). How fish manage this trade-off in different populations may be indicative of selection pressures limiting recruitment that are geographically specific. Because tradeoffs between growth and energy acquisition can take place over relatively short periods of time, changes in short term growth rates may reflect the onset of such changes.

In evaluating energy storage and growth tradeoffs, two potentially useful approaches are proximate analysis for energy storage and RNA/DNA ratios (Bulow 1987) for short term growth in fish. Proximate analysis, which categorizes a substance into components-- lipid, protein, water, and carbohydrate-- has been used globally as an indicator of relative nutritional quality of fish feeds, forage species, (Rand 1994), fish tissues (Hendry et al. 2000), and fish products (Decker 1991). Because fish from different locations may have different hatch dates, traditional measures of growth such as length may be insufficient for comparing juvenile fish. Nucleic acid quantitation techniques such as RNA/DNA ratio have been used extensively for assessing larval and juvenile fish growth (Buckley and Bulow 1987) because DNA levels in a cell are constant, but RNA levels fluctuate with protein synthesis, thus enabling the ratio of RNA to DNA in tissues to be useful in assessing recent growth rates.

Little is known about energy allocation or the selection pressures that drive it in juvenile paddlefish *Polyodon spathula*. This ancient species (Grande and Bemis 1991) has has been impacted by alteration of much of its large river habitat from impoundment and channelization (Sparrowe 1986; Gerken and Paukert 2009). In addition, loss of spawning

habitat and an altered flow regime have reduced reproductive success and recruitment in many populations. Northern and southern stocks have been shown to exhibit highly episodic recruitment (Scarnecchia et al. 2011). The causes of the highly differential success in year class strength are thought to be related to river discharges and reservoir levels (higher water levels and trophic upsurge; Scarnecchia et al. 2009), although the exact mechanisms are not well studied. The highly inconsistent recruitment complicates harvest management (Scarnecchia et al. 2014).

From a physiological perspective, differences in annual paddlefish reproductive success and recruitment may be affected by inter-annual differences in energy accumulation and storage**.** Understanding energy use, allocation and storage in paddlefish thus has practical management applications, including insight into early (age-0 and age-1) life history and survival, use in predicting year-class strength,, evaluation of optimal hatchery stocking practices, and evaluation of reservoir productivity (e.g. higher paddlefish lipid storage in more productive reservoir conditions, Scarnecchia et al. 2009). Paddlefish stocks associated with Lake Sakakawea,North Dakota (the SAK stock), and Fort Peck Lake, Montana (the FP stock), two Missouri River main stem reservoirs, show variable and often weak recruitment. Although wild stock management is still promulgated, hatchery- rearing of fish to advanced age-0 stage has been conducted at Garrison Dam National Fish Hatchery (GD) as a contingency management tool. Little information is also available on the relation between energy density and juvenile growth in wild paddlefish. Information on energy reserves in relation to growth (as indicated by RNA/DNA ratios) would be useful as baseline data to compare with other stocks and species or reservoir productivity changes caused by reservoir aging (Scarnecchia et al. 2009) and climate change. For effective management of these two

stocks and others, the underlying factors that lead to differential age-0 survival and recruitment, perhaps involving energy acquisition, use, and associated food web factors (e.g., competition, predation; Scarnecchia et al. 2014), need to be much better understood.

The objective of this study was to investigate lipid accumulation and storage in age-0 and age-1 paddlefish in relation to age, stock, tissue type, year, and growth. This study describes and compares lipid content in paddlefish from three different sources (SAK wild stock, FP wild stock, and Garrison Dam Hatchery-reared fish of SAK stock (GD)). Depending on availability of fish of each source and age, five questions were posed: (1) Do lipid values differ between age-0 and age-1 fish?; (2) Do lipid values differ among fish of different stocks?; (3) Are there differences in lipid storage between tissue types (whole and eviscerated) in juvenile paddlefish?; (4) Is there a difference in lipids between brood years of age-0 fish?; and (5) Is there a significant trend between growth rate and whole body lipids that can be detected with RNA/DNA ratios? I hypothesized that there would be differences in tissue lipid storage by age stock, tissue, and year, and that fish with higher RNA/DNA ratios would have lower lipid levels. In addition to the hypothesis testing, the establishment of effective protocols and baseline data was designed to provide a basis for future annual monitoring and evaluation of the paddlefish stocks.

## METHODS

### *Sample and Data Collection*

Although target sample sizes were set for each stock and year, actual sample sizes obtained were constrained by availability for the wild fish from the SAK and FP stocks. Age-0 and age-1 paddlefish were obtained from Fort Peck Reservoir (FP stock) and Lake

Sakakawea (SAK stock) from late July through mid-August in 2011 and 2012 as part of annual age-0 abundance and distribution surveys. Fish were collected with long-handled dip nets during random searches following the methods described by Fredericks and Scarnecchia (1997).

During 2011 and 2012, 250 juvenile paddlefish were collected: FP 2011 age-0 (*N*=33), FP 2012 age-1 (*N*=3), SAK 2011 age-0 (*N*=73), SAK 2012 age-0 (*N*=29), SAK 2012 age-1 (*N*=21). Samples were also obtained from 2011 age-0 hatchery-reared fish (*N*=91) that had been hatched and reared at GD (SAK origin) for release into Lake Sakakawea (Table 1).

All fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222), and measured (TL, mm) and weighed (g) in the field. There were 31 fish (13 from FP in 2011, four from SAK, four from GD in 2011, and 10 from SAK in 2012) that were collected for stomach content analysis for a separate project, and as a result, I removed the remaining viscera and saved the eviscerated carcasses in order to compare lipid values between two tissue types: whole body and eviscerated fish (Table 1).

### *Proximate analysis*

Samples of tissue were analyzed using standard methods (AOCS 2005, AOAC 2005) for proximate analysis. Briefly, moisture content was estimated by subtraction after drying for 21 hours at 70$^{o}$ C, and ash was determined on dried material by loss on ignition after 4 hours at 550$^{o}$ C in a muffle furnace. Lipid and total energy were obtained from dried tissues at the University of Idaho Fish Culture Experiment Station in Hagerman, Idaho with an ANKOM lipid extraction instrument (Macedon, New York, model XT-15.) . Lipid extraction was completed following established protocols (AOCS 2005), and fish were processed for

total energy (in calories) using a Parr Instruments calorimeter (Moline, Illinois, model 6300). Calorimetry samples were reprocessed if duplicates differed by 100 calories. The calorimeter was calibrated at the beginning of each day with benzoic acid tablets, with a required relative standard deviation of 0.2% between days of operation. Protein was estimated by subtraction (Hendry et al. 2000). Tissue types that were analyzed were whole body and eviscerated. All samples were analyzed in duplicate, and mean relative percent difference of lipid duplicates was 0.09 (± 0.96). Inter-assay coefficients of variation between batches were calculated using a control sample of dry animal feed and were 0.05 for moisture, 0.06 for lipid, and 0.13 for ash.

Fish size and procedural difficulties affected whether a particular fish was able to undergo all procedures associated with proximate analysis. A minimum amount of tissue was required to perform all analyses, and this amount corresponded with fish approximately 170 mm in total length. In the case of small fish, as well as when procedures needed to be repeated to maintain quality control, there was sometimes not enough sample left over to complete all the steps of proximate analysis. For these reasons, there is sometimes a discrepancy between samples collected and sample sizes used for analyses (Tables 1, 2). In all, 224 (183 whole body, 39 eviscerated) of 250 fish samples collected underwent all proximate procedures (Table 2).

### *Growth*

Collected fish were euthanized, so repeated measures on individual fish were not possible. A 5-mm muscle plug was removed from 29 age-0 SAK fish, 21 age-1 SAK, and three age-0 FP fish in 2012, to estimate RNA/DNA ratios. The plug was placed in a 2.0 ml vial of RNAlater (Qiagen, Inc) and stored on ice until it could be frozen at -80$^{o}$ C. Individual

fish were placed in labeled sealable bags, and stored in a cooler on wet ice until a -20$^{o}$ C freezer was available. Sample plugs were frozen as soon as possible, and shipped on dry ice to the Ted Stevens Marine Research Institute in Juneau, Alaska, for RNA/DNA quantification (Buckley and Bulow 1987). The RNA/DNA ratios from juvenile fish were calculated from white muscle (Buckley and Bulow 1987; Bulow 1971) using a flourometric protocol described in Caldarone et al. (2001). This technique extracts RNA and DNA from tissues and quantifies the amount of each with a fluorescence microplate reader. Because RNA level in cells fluctuates with protein synthesis, but DNA levels remain constant, the ratio between them can be used as an index of growth (Thorgaard and Disney, 1990).

### *Statistical Analysis*

To compare lipid values of fish between different ages (0 and 1) of 2012 SAK fish (which was the only group for which we had sufficient sample sizes for a within-year comparison), a Wilcoxon test was used. To evaluate differences among stocks (FP, SAK, and GD), a Kruskal-Wallis test of ranks of lipid values was used, followed by a *post hoc* Tukey-Kramer test of individual mean ranks. Non-parametric tests were used because the distribution of lipids was not normal.

To compare lipid between tissue types (Whole, Eviscerated), within each stock (FP, GD, SAK), a Wilcoxon test was used for 2 group comparisons. To compare lipid levels in age-0 fish between years, a Wilcoxon test was applied to SAK age-0 fish collected in 2011 ($N$= 47) and 2012 ($N$=20). This comparison was limited to SAK fish because it was the only stock from which we were able to collect age-0 fish during both years of the study.

In addition, to evaluate the relationship between RNA/DNA ratio and lipid content of 29 2012 age-0 and 20 age-1 fish from SAK, I used linear regression. Although RNA/DNA values were normally distributed and similar between age-0 and age-1 fish, lipid values were different. As a result, I performed regression with just age-0 fish, followed by just age-1 fish. All statistical tests were evaluated for significance at $\alpha = 0.05$

In order to further explore the significance of an observed bimodal distribution of age-0 fish, fish were divided into high lipid (> four %) and low lipid (< four %) groups and additional analysis was performed, including linear regression of lipid and total length for 2011 age-0 fish, and 2012 age-0 and age-1 fish. Statistical analyses were completed using SAS (SAS 2013) and Excel (Microsoft 2007).

## RESULTS

Lipid values for all groups of 2011 age-0 fish were bi-modally distributed with a break at approximately four percent (Figure 1). SAK 2012 age-0 fish did not exhibit this bimodal distribution; however their lipid values were within the lower ranges of the 2011 age-0 fish (Table 2).

Lipids differed significantly by age for the 2012 SAK paddlefish. I rejected the null hypothesis that there was no difference in lipid between ages (Wilcoxon test, $P< 0.0001$). Age-0 fish had significantly lower levels of lipid than age-1 fish (Figure 2). Lipid values of 2011 age-0 fish differed significantly by stock.

I also rejected the null hypothesis that lipids did not differ among stocks (Kruskal-Wallis test, $P= 0.0492$). GD fish had significantly higher lipid than YS fish, but not FP fish

(Figure 3). The GD fish were also larger (ANOVA, $P< 0.0001$), although there was no difference in length or lipid between FP and SAK fish.

Lipid values of 2011 age-0 FP fish did not differ significantly by tissue (Wilcoxon, $P= 0.6381$). For FP fish, I failed to reject the null hypothesis that there were no differences between tissues (Figure 4). Lipid values of 2011 GD age-0 fish differed significantly by tissue (Wilcoxon, $P= 0.0039$). For GD fish, I rejected the null hypothesis that there were not differences between tissues. GD eviscerated (Evisc) fish had higher lipid than GD Whole fish (Figure 5). Lipid values of 2011 age-0 SAK fish did not differ significantly by tissue (Wilcoxon, $P= 0.3254$). I failed to reject the null hypothesis that there were not differences between tissues (Figure 6). Lipid values of 2012 age-0 SAK fish did not differ significantly by tissue (Wilcoxon test, P=0.2679). I failed to reject the null hypothesis that there were not differences between tissues (Figure 7).

Lipid values of age-0 SAK fish were significantly different by year (Wilcoxon test, $P< 0.0001$). I rejected the null hypothesis that there were not differences between years. Lipid values of 2011 SAK age-0 fish were much higher than lipid of 2012 age-0 fish (Figure 8).

RNA/DNA ratios did not relate significantly with lipid values for any of the groups tested ($R^2< 0.17$, $P> 0.53$). For all fish, age-0 fish, and age-1 fish, I failed to reject the null hypothesis that RNA/DNA ratios were not related to lipid levels (Figure 9). In addition, no significant difference was found between RNA/DNA ratios of SAK age-0 and age-1 fish in 2012 (Wilcoxon test, $P= 0.65$).

The relationships between length and lipid were not significant for 2011 age-0 fish sorted into high and low lipid categories (Table 4), with the exception of GD high lipid fish

($R^2$=.404, $P$< 0.0001) (Figure 10b). The 2011 age-0 fish displayed a similar trend as that of 2012 age-0 fish, which did not show a significant relationship between length and lipid ($R^2$=0.0259, $P$= 0.4979) (Figure 11). The strongest relationship between length and lipid was for 2012 age-1 SAK fish, which displayed a strong positive trend between the two variables ($R^2$=0.5044, $P$= 0.0007) (Figure 12).

Table 1. Total number of 2011 and 2012 FP, GD, and SAK age-0 and age-1 paddlefish samples collected (by total and tissue types) (whole body (Whole) and eviscerated carcasses (Evisc)) and measured for lipids and available for use in analyses. Collected denotes the number of fish that were collected, and lipid denotes the number of samples of that type that were processed for proximate constituents.

| Group | Total collected | Whole collected | Whole lipid | Evisc collected | Evisc lipid |
|---|---|---|---|---|---|
| FP11-0 | 33 | 20 | 17 | 13 | 11 |
| FP12-1 | 3 | 3 | 3 | 0 | 0 |
| GD11-0 | 91 | 86 | 77 | 4 | 4 |
| SAK11-0 | 73 | 69 | 47 | 4 | 4 |
| SAK12-0 | 29 | 20 | 20 | 9 | 9 |
| SAK12-1 | 21 | 20 | 19 | 1 | 1 |

Table 2. Proximate composition (% ± SD) of age-0 and age-1 whole body paddlefish from FP, GD, and SAK stocks collected during 2011 and 2012 that were processed for proximate constituents. Categories are denoted with Stock/Year-Age.

| Stock, Year, and Age | N | Lipid % (SD) | Water % (SD) | Ash % (SD) | Protein % (SD) | Cal (SD) |
|---|---|---|---|---|---|---|
| FP/11-0 | 17 | 4.96 (2.66) | 87.37 (.76) | 0.110 (.001) | 6.18 (1.39) | 5313.85 (143.27) |
| FP/12-1 | 3 | 2.36 (1.31) | 85.50 (1.66) | 0.132 (.017) | 11.70(.17) | 4939.38 (374.78) |
| GD/11-0 | 77 | 5.51 (2.86) | 86.94 (2.18) | 0.128 (.018) | 7.43 (1.77) | 4792.14 (291.17) |
| SAK/11-0 | 47 | 4.03 (2.39) | 86.68 (1.46) | 0.104 (.026) | 9.60 (2.06) | 4790.36 (214.27) |
| SAK/12-0 | 20 | 0.85 (.17) | 88.45 (.57) | 0.137 (.009) | 10.65 (.48) | 4613.49 (93.0) |
| SAK/12-1 | 19 | 2.60 (1.28) | 84.68 (1.80) | 0.128 (.017) | 12.62 (1.14) | 4978.25 (298.19) |

Table 3. Parameter estimates for high and low lipid groups of 2011 age-0 fish from FP, GD, and SAK.

| Group | *N* | Length | Intercept | $R^2$ | *P* |
|---|---|---|---|---|---|
| FP low | 9 | 0.0068 | 1.0986 | 0.0652 | 0.5414 |
| FP high | 9 | 0.0042 | 6.5033 | 0.0089 | 0.8098 |
| GD low | 16 | 0.0025 | 0.23 | 0.0778 | 0.2954 |
| GD high | 61 | 0.0271 | -1.182 | 0.404 | <.0001 |
| SAK low | 14 | 0.0044 | 1.2125 | 0.0453 | 0.4648 |
| SAK high | 18 | 0.0169 | 2.6478 | 0.1412 | 0.1219 |

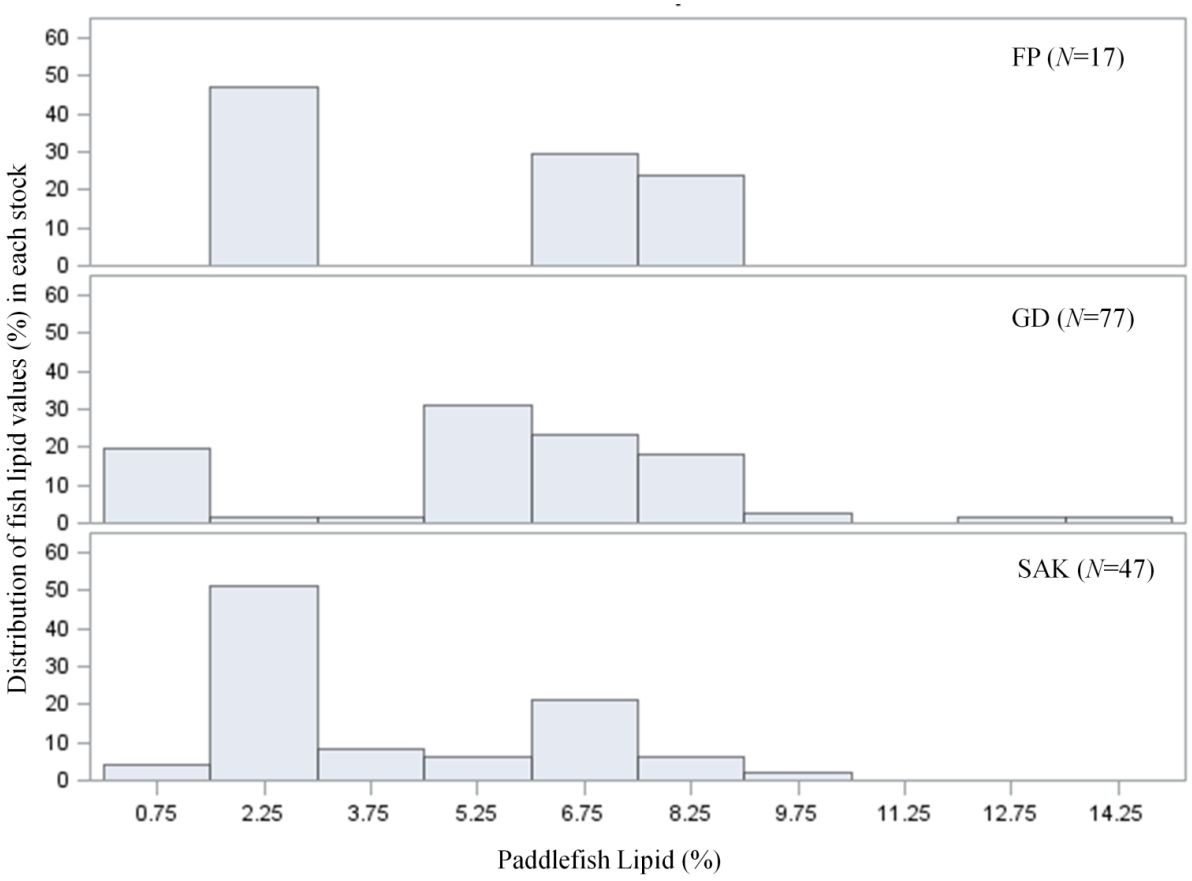


Figure 1. Distribution of lipid (%) of 2011 FP, GD, and SAK age-0 paddlefish showing bi-modal distribution.

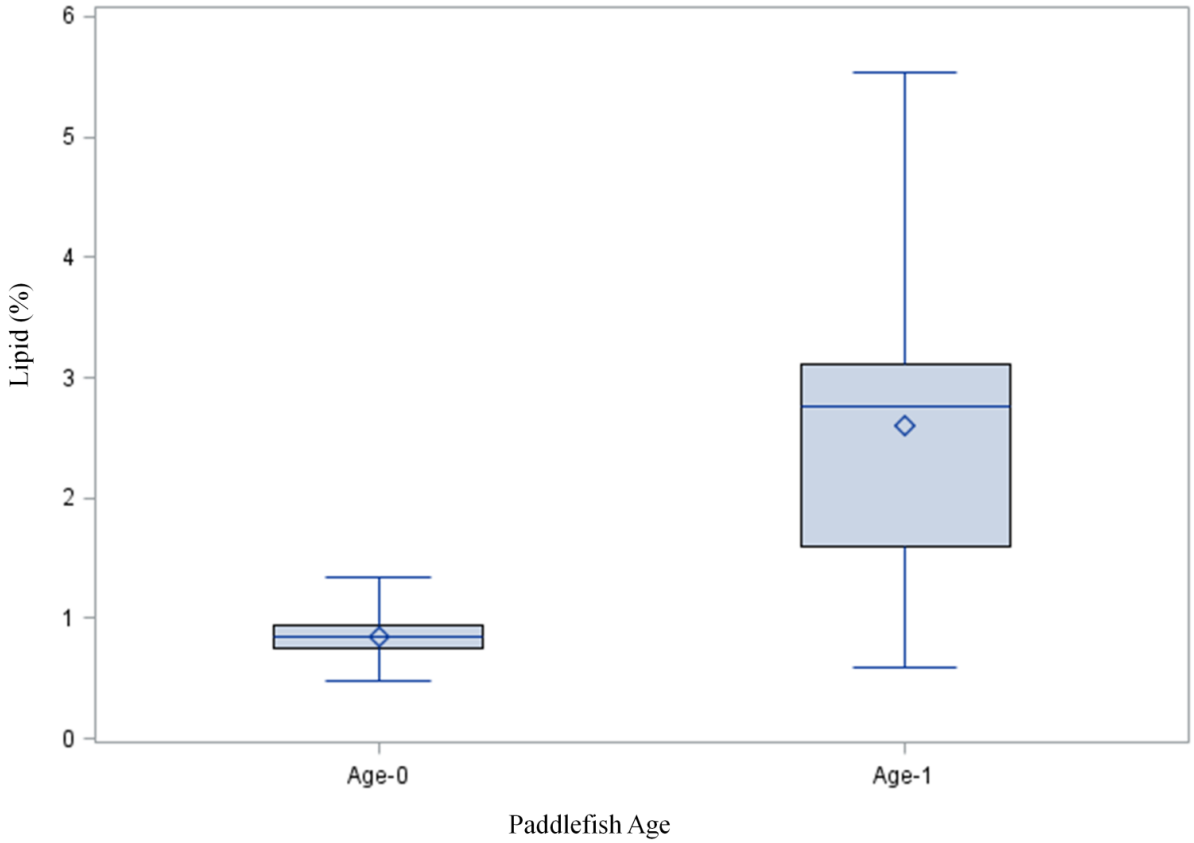


Figure 2. Distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) of 2012 SAK age-0 (*N*=20) and age-1 (*N*=19) paddlefish (Wilcoxon test, *P*< 0.001).

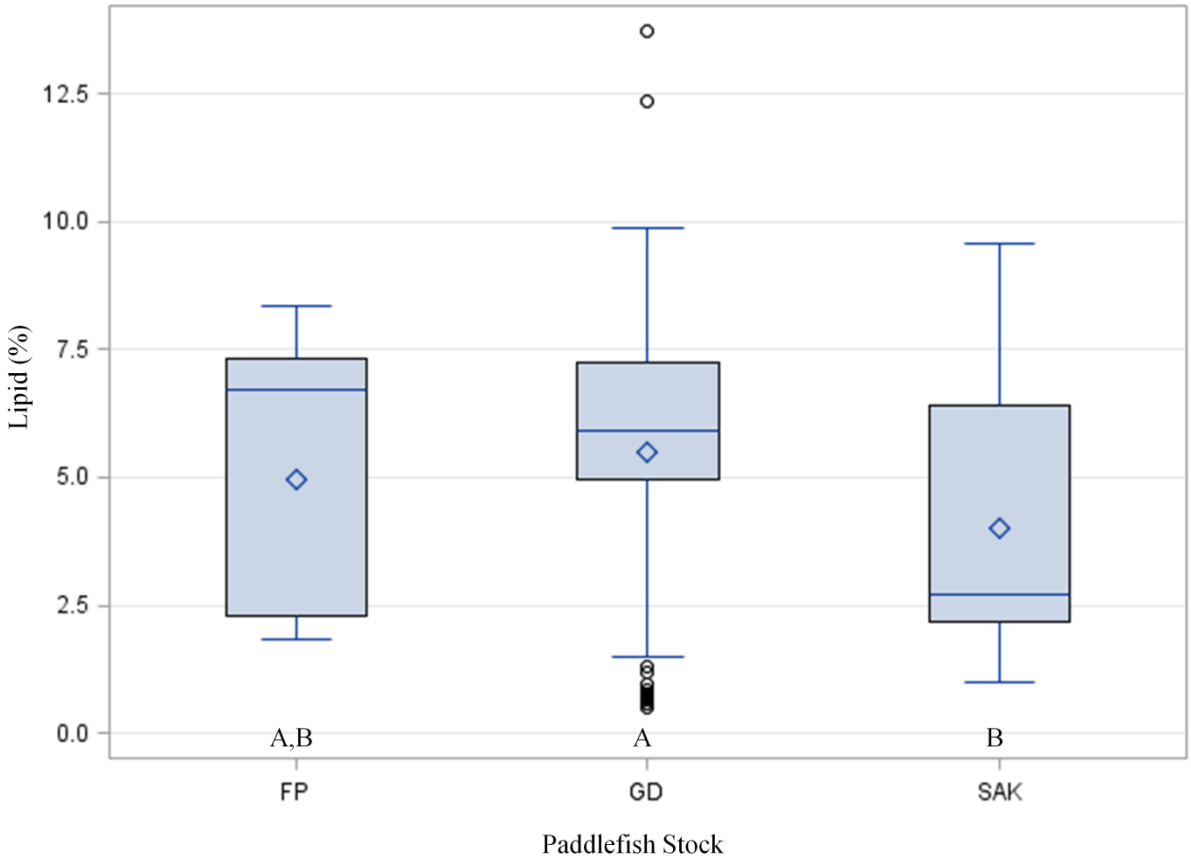


Figure 3. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2011 age-0 paddlefish from Fort Peck (FP) (*N*=17) Garrison Dam (GD) (*N*=77), and Sakakawea (SAK) (*N*=47) (Kruskal-Wallis test, *P*= 0.0492). Comparisons significant at the α = 0.05 level (Tukey-Kramer test) are indicated by different letters.

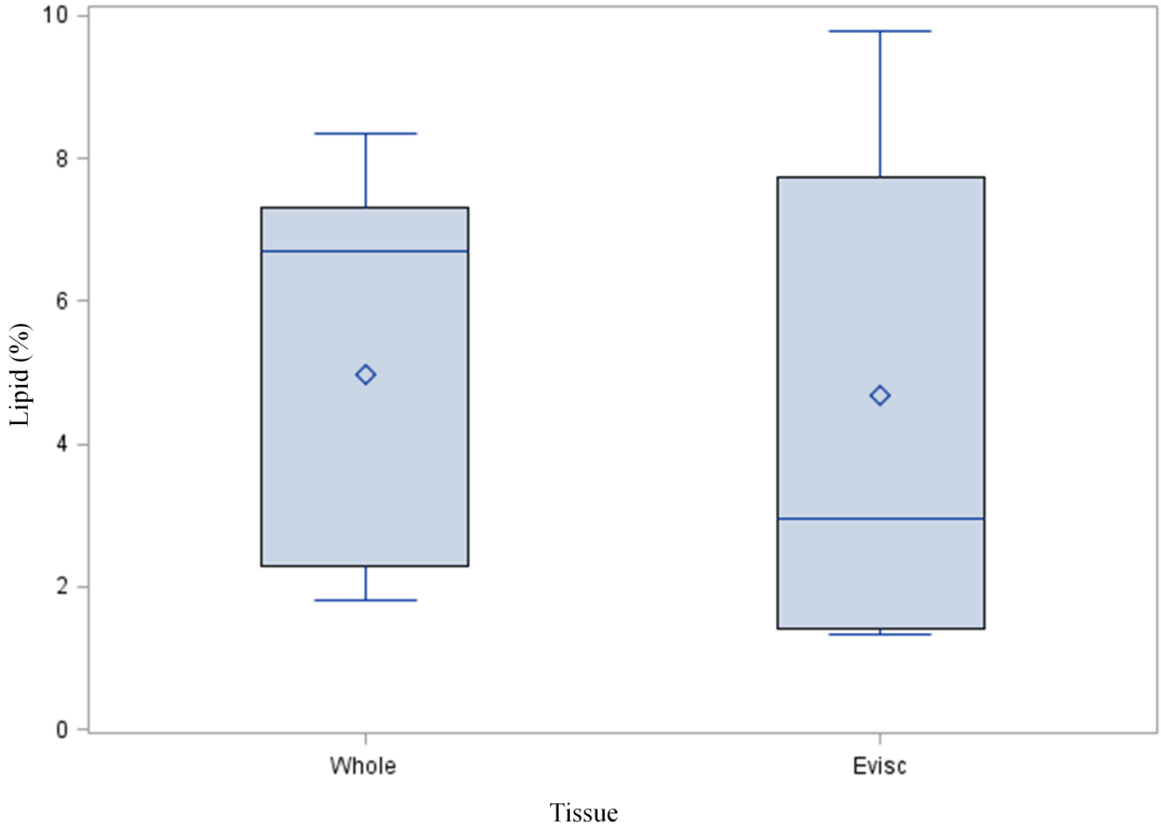


Figure 4. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2011 FP age-0 paddlefish by tissue type. Tissue types are whole body (Whole) (*N*=17) and eviscerated (Evisc) (*N*=11) (Wilcoxon test, *P*= 0.6381).

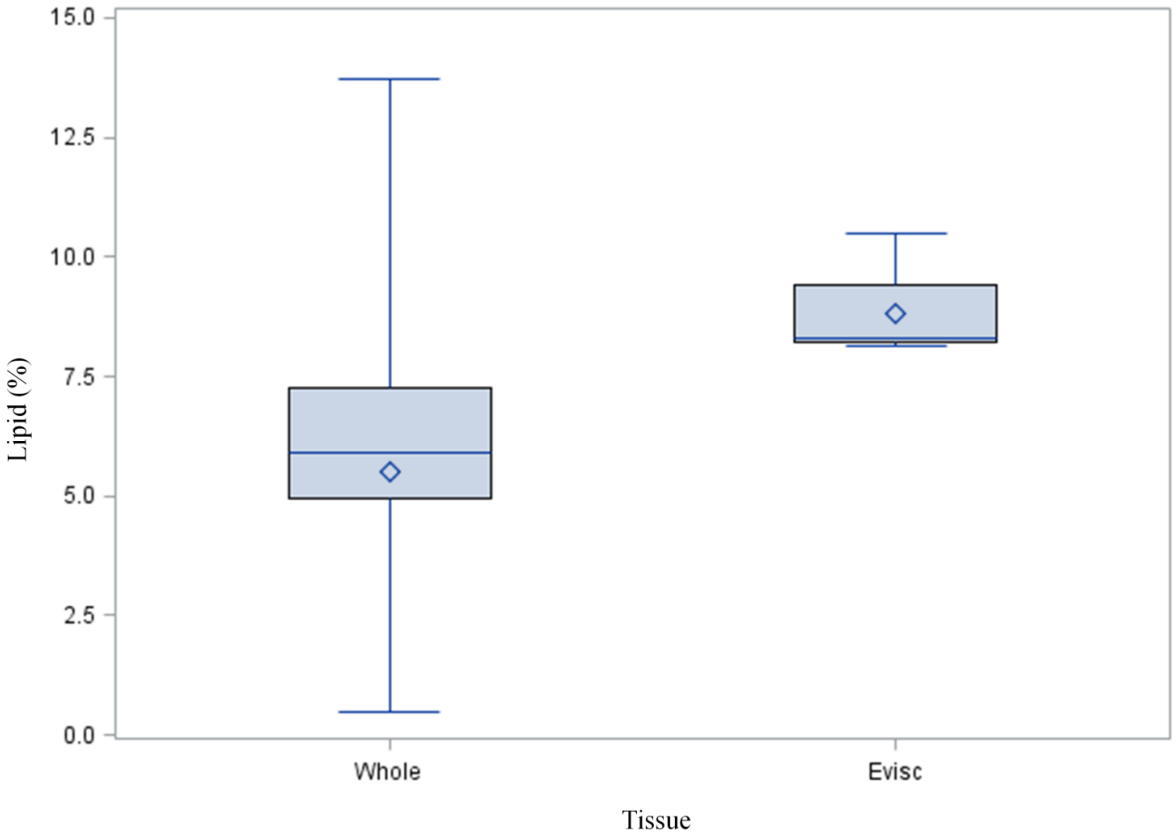


Figure 5. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2011 age-0 GD paddlefish by tissue type. Tissue types are whole body (Whole) (*N*=77) and eviscerated (Evisc) (*N*=4) (Wilcoxon test, *P*= 0.0039).

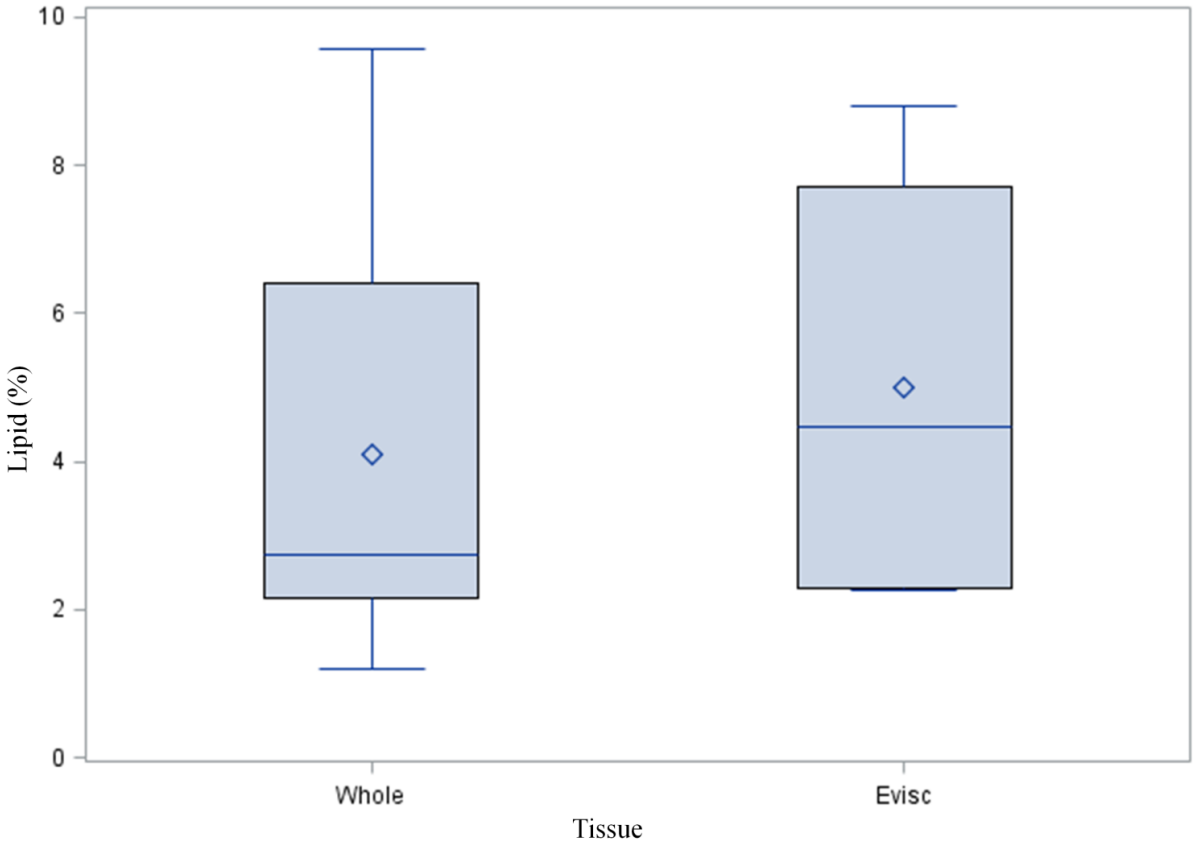


Figure 6. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2011 age-0 SAK paddlefish by tissue type. Tissue types are whole body (Whole) (*N*=47) and eviscerated (Evisc) (*N*=4) (Wilcoxon test, *P*= 0.3254).

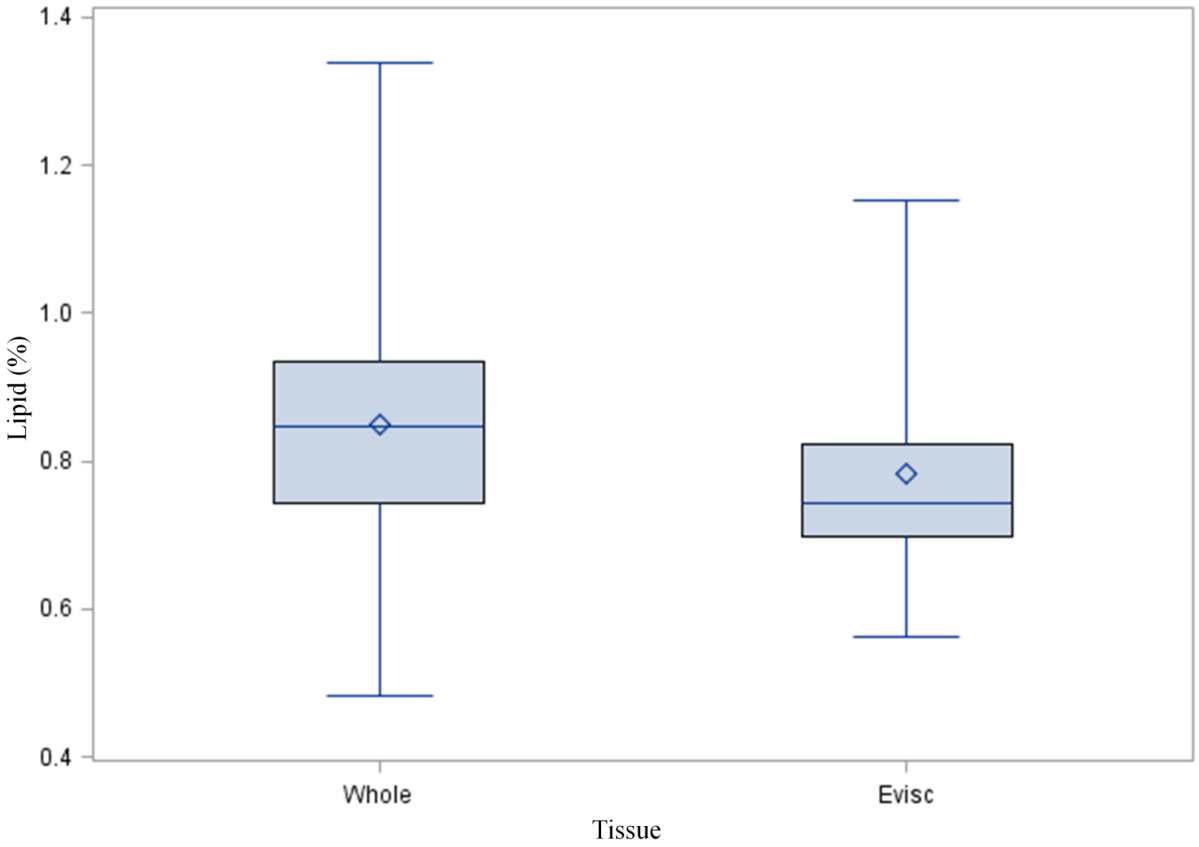


Figure 7. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2012 age-0 SAK paddlefish by tissue type. Groups are whole body (Whole) (*N*=20) and eviscerated (Evisc) (*N*=9) (Wilcoxon test, *P*= 0.2679).

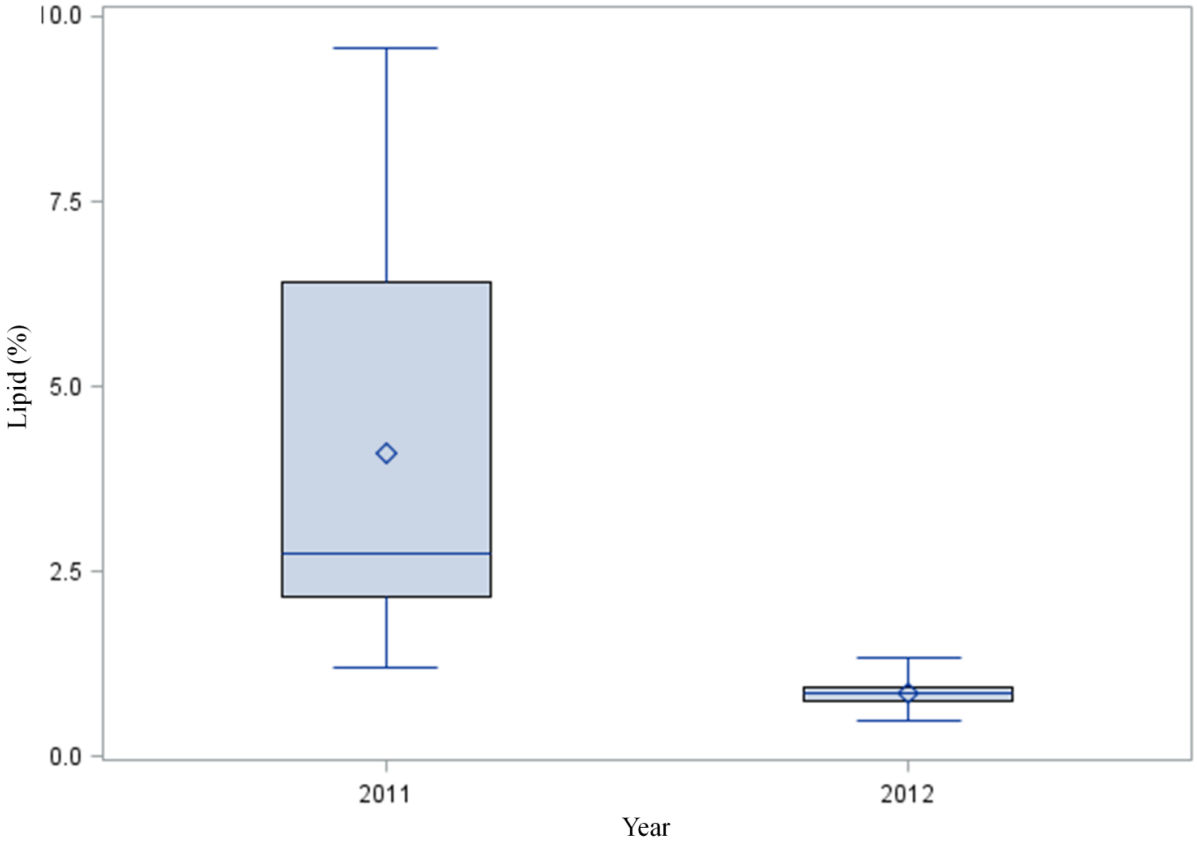


Figure 8. Box plot distribution of lipid (%) (including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values) in 2011 SAK (*N*=47) and 2012 SAK age-0 (*N*=20) whole body paddlefish by year. Groups are by year. (Wilcoxon test, $P < 0.0001$).

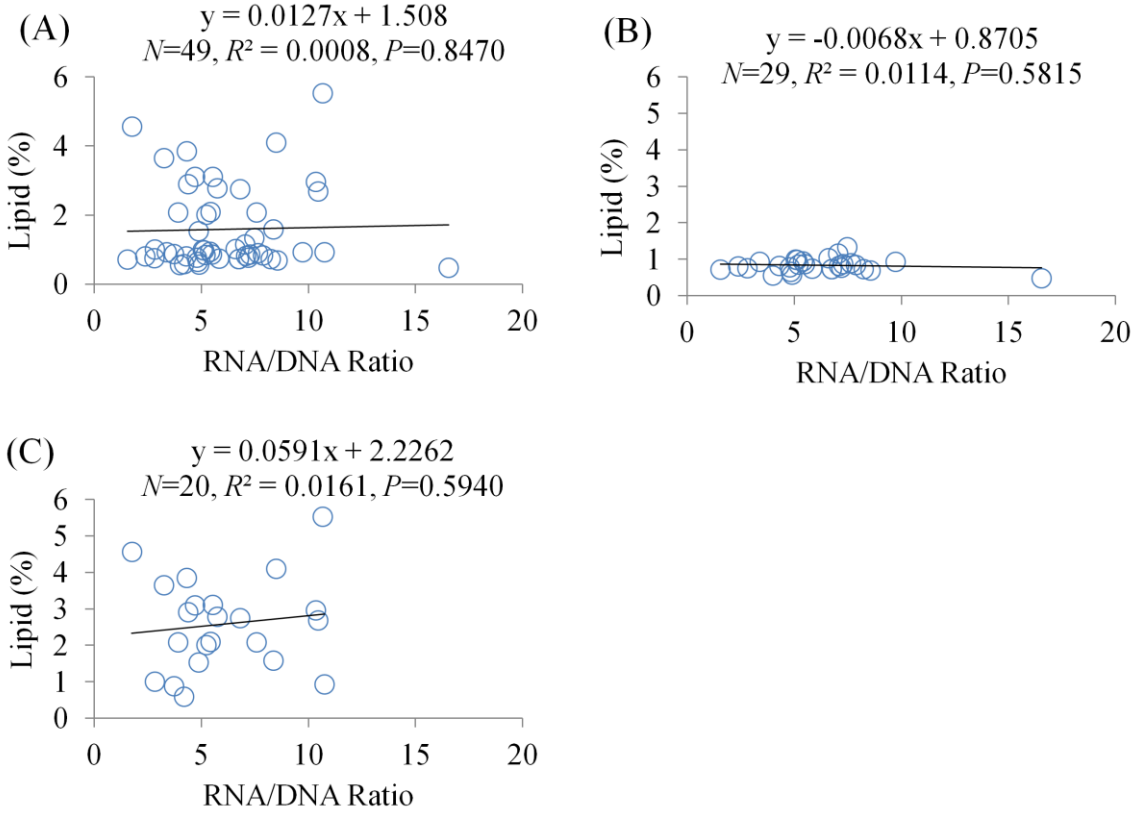


Figure 9. Least-squares linear regressions of lipid (%) vs. RNA/DNA ratio of 2012 age-0 (*N*=29) and age-1 SAK (*N*=20) paddlefish. Graphs include all 2012 age-0 and age-1 fish (A), only age-0 fish (B), and only age-1 fish (C).

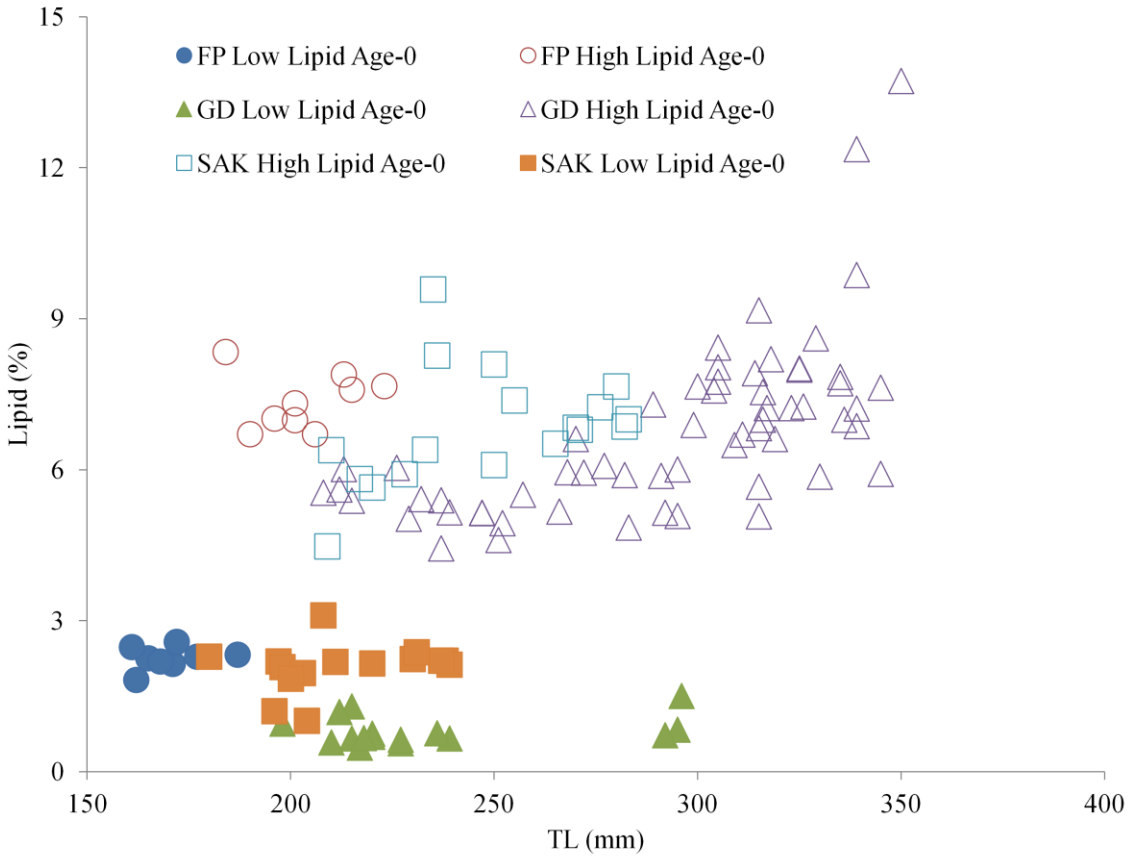


Figure 10a. Relationships of lipid (%) with total length (TL) of SAK, FP, and GD 2011 age-0 paddlefish classified into low lipid (<4%) and high lipid (>4%) groups. None of the groups were significantly correlated with length except SAK high lipid. Regression parameters for all groups are listed in Table 3.

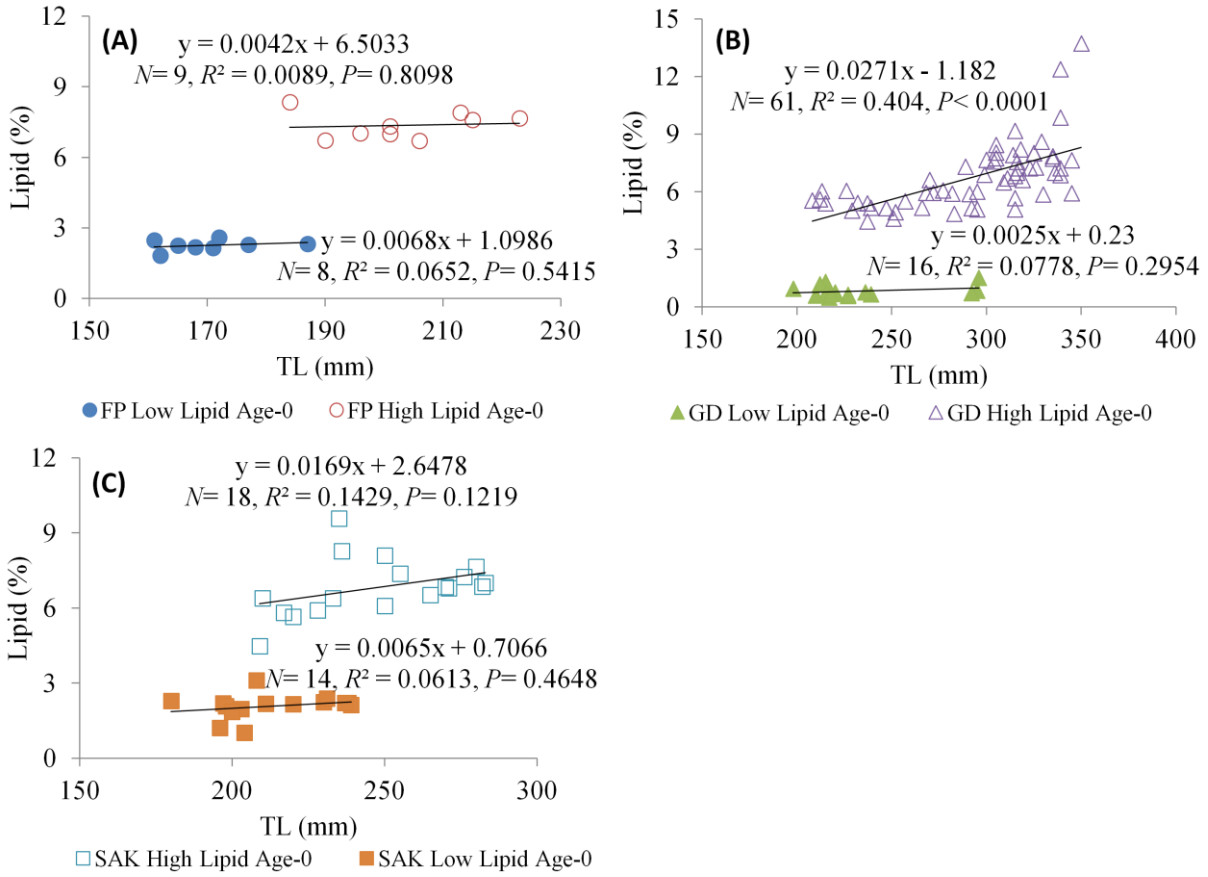


Figure 10b. Relationships of lipid (%) with total length (TL) of SAK, FP, and GD 2011 age-0 paddlefish classified into low lipid (<4%) and high lipid (>4%) groups. Panels are (A) FP low and high lipid fish, (B) GD low and high lipid fish, (C) SAK low and high lipid fish.

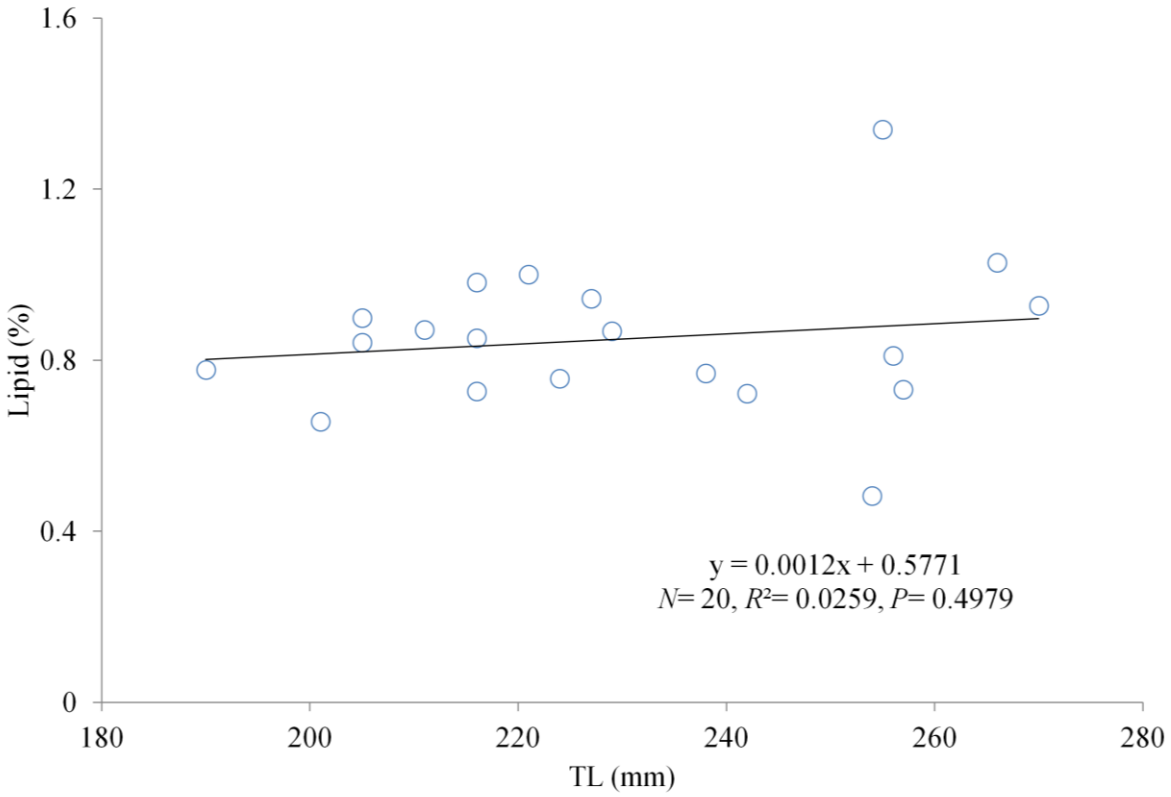


Figure 11. Relationship of lipid (%) with total length (TL) of 2012 SAK age-0 paddlefish.

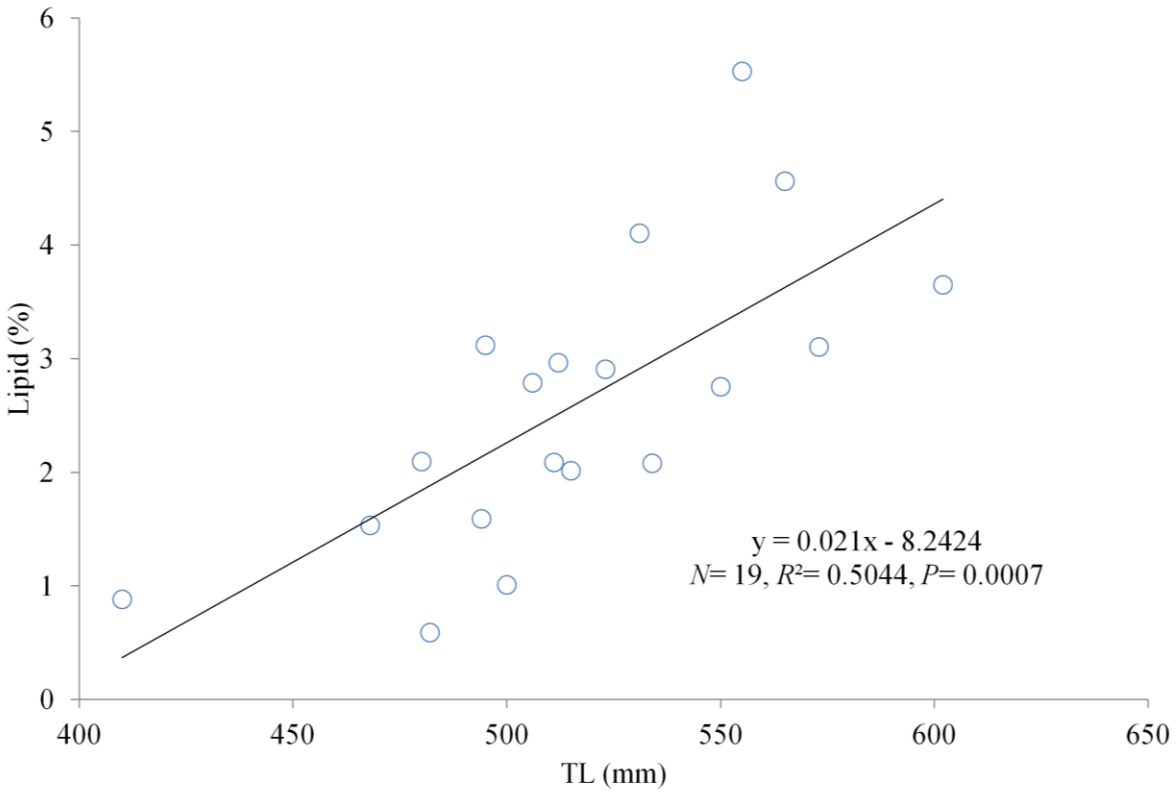


Figure 12. Relationship of lipid (%) with total length (TL) of SAK 2012 age-1 paddlefish.

## DISCUSSION

The significantly higher lipid content in SAK age-0 paddlefish in 2011 over samples in 2012 (Figure 8, Table 2) provides evidence of substantial inter-annual differences in lipid storage during the first summer of rearing, prior to winter. These differences may influence overwinter survival. Hurst and Conover (2003) linked inter-annual variation in fish size and lipid content to overwinter survival of age-0 striped bass. Thompson et al. (1991) reported that both fish length and lipid level influenced over-winter survival of age-0 Colorado pikeminnow. Overwinter survival may also depend on inter-specific interactions. Garvey et al. (1998) found that age-0 largemouth bass that were smaller, with lower energy values, suffered greater overwinter mortality only in the presence of predation.

In explaining these inter-annual differences in lipid content of age-0 paddlefish (Figure 8), the role of variations in inter-annual ecological conditions, and their potential effects on recruitment, should not be overlooked. In 2011, the year of higher lipid levels, spring and summer discharge in the Yellowstone River were at record highs and reservoir levels were also as high as could be maintained in an effort to reduce downriver flooding (Maximuk and Nadolski 2012). Higher river discharges, as well as reservoir water levels and associated trophic upsurge, have been implicated as influencing growth and year class strength in paddlefish in general (Alexander and McDonough 1983; Russell 1986) and the SAK stock in particular (Scarnecchia et al. 1996, 2009). Strong recruitment success (i.e., survival to at least age-1) in the SAK stock has been characterized as episodic, and was documented only two years since 1990: in 1995 and 2011, that were both high flow and high reservoir level years (Scarnecchia et al. 2014). During 2012, age-1 fish were also abundant at Fort Peck, also indicating a strong 2011 year class in that reservoir. Conversely, age-0 fish were nearly absent during index counts in both Lake Sakakawea and Fort Peck reservoir in 2012 (Figure 16). The few age-0 fish found in Lake Sakakawea had low lipids, and results of transect sampling from 2013 indicated few age-1 fish compared to age-2 fish. Lower lipid levels seen in age-0 fish in 2012 (Table 1), and their comparatively low numbers, could reflect poor condition from a number of possible causes beyond the scope of this study, including lower reservoir productivity or density-dependent foraging competition due to habitat saturation from the large 2011 year class.

The higher lipids in age-0 SAK fish in 2011 over 2012 indicated that paddlefish allocated energy to both body growth and lipid reserves, and allocation differed between years. A split allocation between growth and lipids is what would be expected to be selected

for in a strongly temperate climate, where some overwinter reserves may be adaptive (Hurst 2007). For example, Mogensen and Post (2012) found that overwinter survival of age-0 rainbow trout *Oncorhychus mykiss* in British Columbia was dependent upon environmentally driven allocation between growth and lipid storage.

The lower lipid reserves in age-1 fish late in their second summer than in age-0 of that year class for the previous year (Table 2) was consistent with the idea that, at least after their first winter, rapid growth in length also remains a highly adaptive use of energy for age-1 fish. The hypothesis of growth prioritization over lipid storage gains support from Thompson (1934) who evaluated relative growth proportions between the paddlefish body and rostrum. Thompson found that fish approximately 200-500 mm total length have the longest rostrum length relative to total length, with rostrum length often exceeding the length of the rest of the fish. Comparing data from Thompson (1934) with that from the present study of 2012 SAK age-0 (the only group of fish for which we had rostrum lengths) showed that rostrum length ratios of SAK fish match those measured 80 years earlier (Table 1, Appendix 1; Figure 13). Using the combined data to plot rostrum length as a percentage of total length demonstrates that the proportional rostrum length of the paddlefish increases from hatching through approximately 400 mm (which was about the dividing line between age-0 and age-1 fish in this study), and then decreases proportionately throughout life (Table 1, Appendix 1; Figures 14, 15). This relationship supports the notion that for age-0 fish and into age-1, the highest priority is growth, including growth of a relatively large rostrum. From an evolutionary perspective, this prioritization favors fish that reach a large length, and appear as large as possible, as soon as possible, thereby escaping excessive predation. One cost and outcome of

this prioritization is reduced lipid storage. Energy storage can occur prior to winter in years when habitat conditions allow it, as in 2011 (Table 2).

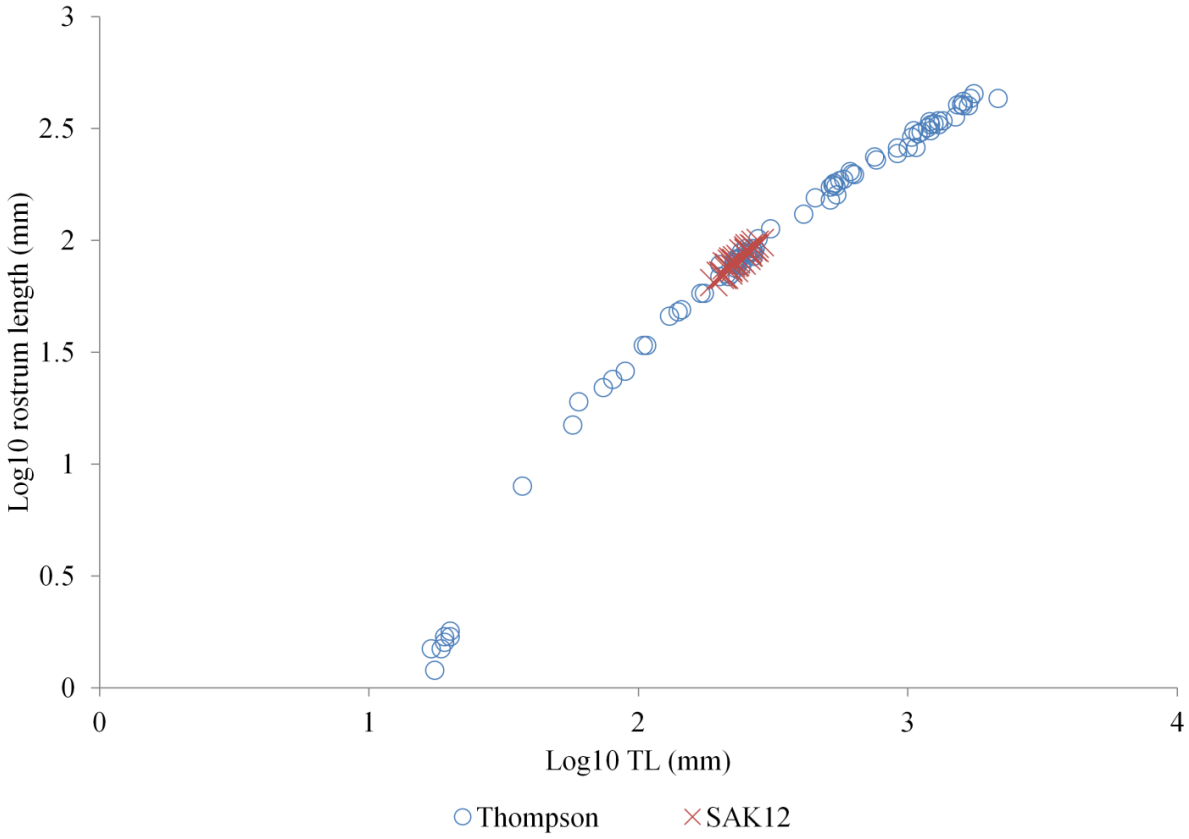


Figure 13. Relationship between rostrum length and total length of paddlefish from Thompson (1934) (*N*=87) and age-0 fish (*N*=29) sampled in Lake Sakakawea in 2012.

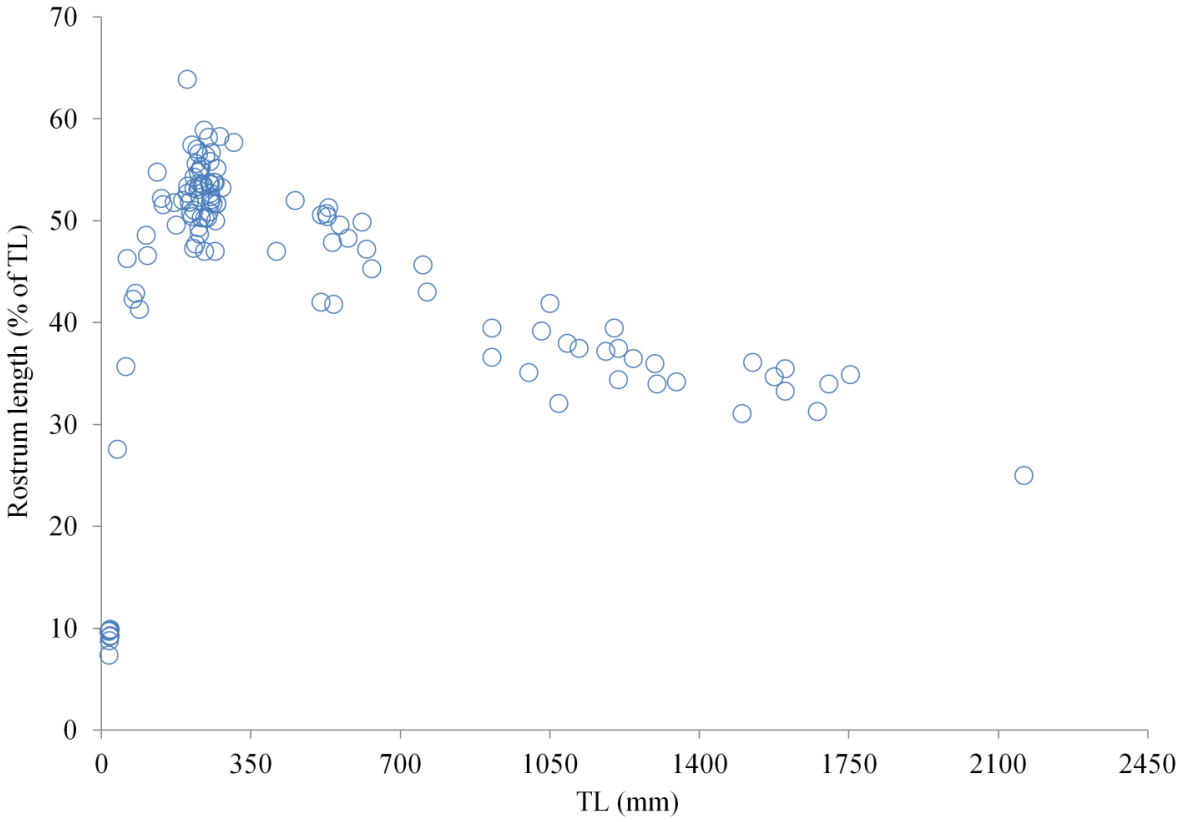


Figure 14. Relationship between rostrum length (% of TL) and total length (mm) of paddlefish from Thompson (1934) (*N*=87) and age-0 fish (*N*=29) sampled in Lake Sakakawea in 2012.

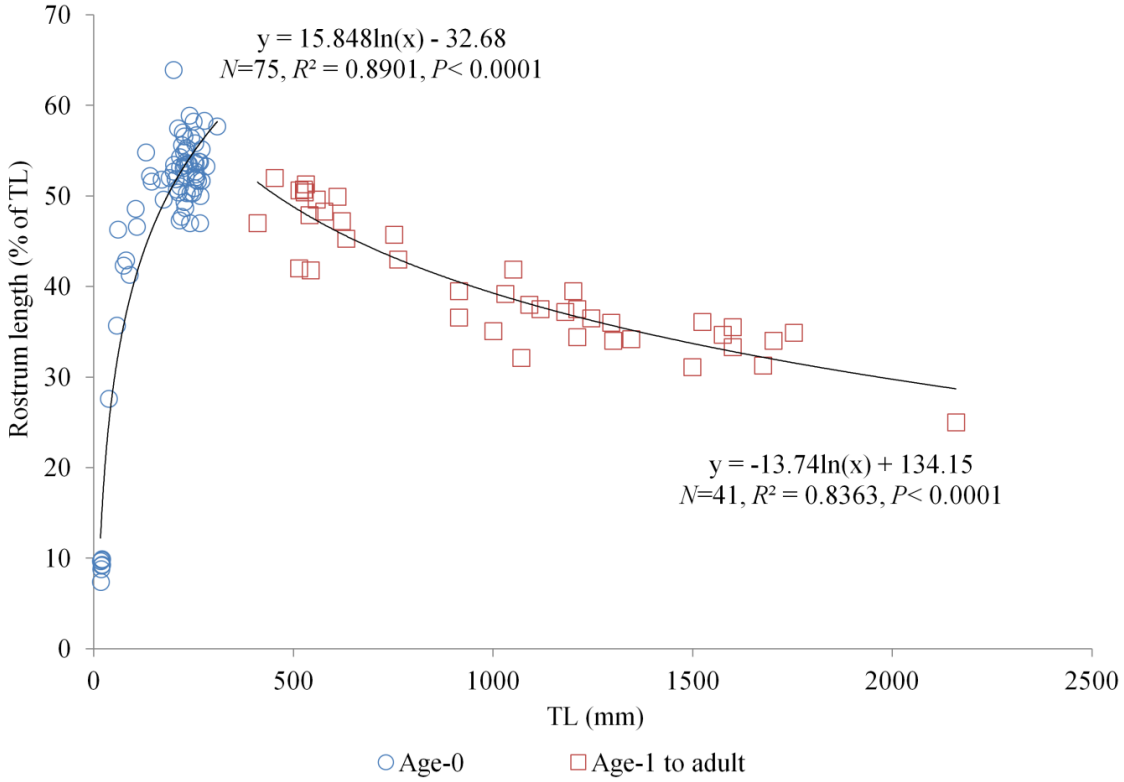


Figure 15. Relationship between rostrum length (% of TL) and total length (mm) of paddlefish from Thompson (1934) (*N*=87) and age-0 fish (*N*=29) sampled in Lake Sakakawea in 2012, divided into two groups (Thompson and SAK Age-0 fish, Thompson Age-1 to adult fish).

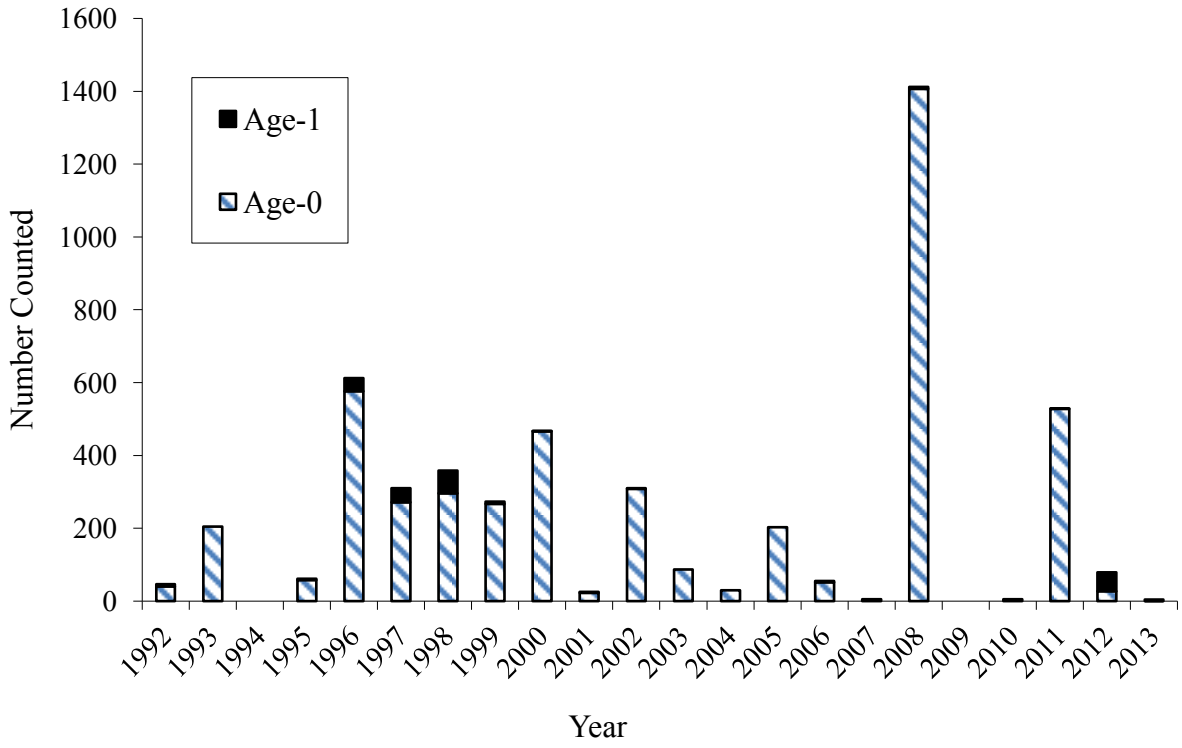


Figure 16. Number of Age-0 and Age-1 paddlefish counted during annual late summer visual index counts from 1992-2013 in Lake Sakakawea, Montana (North Dakota Game and Fish Department, unpublished data).

Predation may be one important reason why the age-0 and age-1 paddlefish I studied allocate energy to growth in length. In Lake Sakakawea, age-0 paddlefish, and even some age-1 fish, have been shown to be highly vulnerable to predation from walleye *Sander vitreus*, sauger *Sander canadensis*, and other species (Mero et al. 1994; Parken and Scarnecchia 2002). The presence of these large predators may create a situation where overcoming predator gape limit by growing larger as rapidly as possible is beneficial within the constraints of overwinter survival their first year. Decker et al. (1991) reported extremely low levels of lipid in captive paddlefish that are comparable to my values for age-1 fish. Both SAK and FP

age-1 fish are from the brood year 2011, when environmental conditions resulted in an uncharacteristically strong year class. Age-1 fish were also abundant relative to age-0 fish in Fort Peck reservoir (D. Scarnecchia, Unpublished data). All the age-1 fish that we collected in both reservoirs appeared to be healthy and vigorous, so lower lipids in age-1 fish may be normal, and may reflect evolutionary prioritization of growth over storage during this stage of paddlefish development. Garvey et al. (1998) reported that age-0 largemouth bass overwinter survival was only lipid and size dependent under conditions of predation. If this relationship also applies to age-0 and age-1 paddlefish, then predation may be the factor that drives evolutionary prioritization patterns in allocation for both age 0 and age-1 fish. More substantial energy storage must wait for later in life, when the predation threat is reduced.

A second hypothesis, not readily testable, is that the low lipids in age-1 fish were the outcome of intra-specific competition from the strong 2011 year class, the strongest recorded since 1995 (Scarnecchia et al. 2014). In order to fully understand this potential tradeoff, lipids should be monitored consistently in age-0 fish in relation to growth and year class strength. More years of data will be needed to adequately assess effects of density on lipid reserves and energy allocation.

The overall significantly higher lipid levels of GD (i.e., hatchery-reared) fish (mean, 5.51) than in SAK fish (2011: mean = 4.03; 2012: mean =0.85) is consistent with the idea that the hatchery fish generally experience greater food availability, and expend less energy foraging. Sogard and Spencer (2004) reported that hatchery sablefish had much higher lipids than wild fish, and suggested that part of the reason was the higher energy requirement of foraging under wild conditions. GD fish were reared in artificially fertilized ponds, whereas FP and SAK fish consumed live feed and had to forage widely for it. In addition, GD fish

were raised to a larger size in a comparable period of time. The lack of both size and lipid differences in FP and SAK fish support the idea that greater length was a factor in higher GD lipid levels. It is unknown if this hatchery-reared versus wild difference occurs consistently, but this is beyond the scope of the current study. It would be advantageous to conduct additional studies that include other juvenile paddlefish populations.

Tissue comparisons suggested that there may be differences in the site of lipid storage in age-0 fish between populations. The lack of differences between tissue types in FP and SAK fish, compared with the differences exhibited by GD fish, may indicate that hatchery fish are storing more lipids in muscle, which accounts for most of the body (and a higher percentage of an eviscerated sample). Alternately, these differences in values may be a reflection of lipid depletion. In studies of juvenile white sturgeon, cited by Beamish and LeBreton (2004), lipid was more easily mobilized in response to low food availability (Beamish et al 1996), and was depleted from viscera before muscle (Hung et al. 1997). If this is the case in our study, higher percentages in the eviscerated carcasses of hatchery fish could be a reflection of higher lipid depletion in viscera. This could result from competition in a hatchery environment, or possibly lower feed conversion efficiency of lipid in hatchery-reared fish compared to wild fish feeding in a natural environment. Shi et al. (2013) found that juvenile paddlefish fed different feeds appeared to have different lipid conversion efficiencies. One limitation to this study was the inability to sample fish over time, and tissue lipids can change quickly in response to energetic demands. Another limitation to this study is the lack of the inclusion of water temperature data. Although Patterson et al. (2013) reported that water temperature did not affect paddlefish metabolism as strongly as body size, and less than for typical teleost fishes, Scarnecchia et al. (2011) suggested that the longer duration of

warmer water temperatures experienced by GL fish reduces the potential lifespan in this stock. Further studies should include temperature monitoring. It may be possible to do this with relatively inexpensive remote logging devices. Without further study, it is unknown if observed differences in lipid content reflect long term trends or if they are related to short term conditions.

The failure of RNA/DNA ratios to predict lipids lends no support to the hypothesis that lipid accumulation in juvenile paddlefish is related to the rate of protein synthesis. If the physiological ability to grow or to store lipids is exclusive, fish with higher lipid would show lower RNA/DNA, because fish would have to allocate energy to either growth or storage. Under this scenario, high growth rates would reflect less emphasis on lipid storage. This was not the case with age-0 and age-1 fish, despite a significant difference in lipid between the two groups. The inability of RNA/DNA ratio to detect a difference in growth rate could be due to several factors. One is that the technique measures protein synthesis only over a short time period (up to 1 week) prior to sampling. Existence and timing of a possible abrupt decision to store lipid in preparation to winter in juvenile paddlefish is unknown, and we only sampled fish once. It is possible that onset of a change in storage allocation from growth to storage does happen in age-0 fish, but occurs outside the window reflected in our sampling and analysis. The lack of differences in RNA/DNA between reservoirs in 2012, and the lack of a relationship between RNA/DNA and lipid indicate that differences in lipid storage between age-0 and age-1 fish are not a result of exclusive energy partitioning between growth and storage in larger fish. This may, however, be the case in smaller fish, as well as in years where resources are limited. Sogard and Spencer (2004) reported that although juvenile sablefish fed low rations exhibited a negative relationship between growth and lipid storage

(indicating an energetic trade-off), fish fed higher rations stored more lipid as they became larger. In this situation, larger fish under prime conditions have the ability to allocate to either storage or growth, but are able to capitalize on abundant energy and allocate successfully to both growth and storage concurrently. This idea is consistent with a cautious interpretation of the trends observed in 2011 age-0 high lipid fish. Although a positive relationship between lipid and length did not quite reach significance ($P = 0.1219$), age-0 SAK fish appeared to be intermediate between the responses observed in smaller FP fish and larger GD fish. The smallest fish, FP, showed no trend; the intermediate size fish, SAK, showed a trend just missing significance. The largest fish GD showed a positive trend (Figure 10b). The trend toward a positive relationship between total length and percent lipids in the high lipid groups as length increases, and no relationship between total length and lipids in the low lipid groups as length increases may reflect a tendency toward concurrent storage and growth as length increases (Figure 10a). The high SAK age-0 lipid group in 2011, the record water year, is on a positive track toward large size and higher lipids; prioritization of lipid storage and growth may not be mutually exclusive, but instead, larger fish may be more adept at both growth and storage. In contrast, the low lipid fish of 2011, and all of the age-0 fish in 2012 group, the lower water year, are trading off and can store lipids only at a potentially fatal cost of slow growth. The high lipid groups in 2011 would result in a stronger year classes of larger individual fish, the low lipid group in 2012 in a smaller year classes of smaller individual fish. A strongly positive feedback involving growth and adequate energy storage would thus be an optimal approach for survival in temperate areas, and that response would be selected for. The individual fish response, either a strongly positive feedback or a strongly negative feedback (Figure 10b), might also explain the boom or bust, episodic recruitment observed in this

species (Scarnecchia et al. 2014). In this scenario, for a strong cohort of recruits, there must ecological conditions, including upsurge (Scarnecchia et al. 2009) leading to a higher than typical number of positive feedback age-0 fish. A satisfactory confirmation of this pattern of positive feedback, as well as the exact mechanism for a potential positive vs negative feedback cannot be made clear unless fish are followed through a wider range of sizes from small age-0 to large age-1. Further study is needed into the relationship between growth, low and high lipid groups, lipid allocation in juvenile paddlefish as well as the existence and timing of allocation changes between growth and storage.

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# **CHAPTER 3.** ENERGY ACQUISITION AND RETENTION IN TISSUES IN SPAWNING ADULT PADDLEFISH

## ABSTRACT

Paddlefish *Polyodon spathula* were sampled in 2012 from the Yellowstone-Sakakawea, Montana and North Dakota (SAK); and the Grand Lake, Oklahoma (GL) stocks using proximate analysis to 1) provide reference information regarding the proximate composition of wild adult paddlefish tissues, and 2) to investigate lipid accumulation and storage in adult paddlefish in relation to tissue type (white muscle, red muscle, GFB) within individual fish, between the sexes, and between the two different stocks (SAK and GL) . Multiple regression models were also constructed using length, age, stock, sex, gonad weight, gonadal fat body (GFB) weight, and gonado-somatic index (GSI) for 3 tissue types: white muscle, red muscle, and GFB. White muscle was significantly correlated with both red muscle and GFB lipid, but red muscle lipid was not significantly correlated with GFB lipid. White muscle lipid content differed by stock and sex, with SAK fish, the more northerly stock, having higher lipid content than GL fish, the more southerly stock. Females also had higher white muscle and red muscle lipid content than males. In contrast, GFB lipid differed by sex but not stock, with females having lower lipid content than males. Life history differences, growing season, and the role of metabolism may help explain the differences in concentrations of lipids observed between stocks and between the sexes for all three tissues. This study suggests that different tissues may be used for different metabolic processes and that although metabolism likely strongly influences muscle tissue lipid, GFB lipid is probably linked more closely to reproductive demands associated with gonad development.

# INTRODUCTION

Understanding energy allocation in paddlefish *Polyodon spathula* requires consideration of species life history as well as life history differences among and within individual stocks. Among stocks, paddlefish show substantial variation in life history aspects such as growth, age at maturity, and lifespan (Russell 1986; Jennnings and Zigler 2009). Within stocks, paddlefish are sexually dimorphic, with females exhibiting a greater weight than males, later age at sexual maturity and often a longer period of gonadal recrudescence (i.e., more time between successive spawns; Scarnecchia et al. 2007).

Five stages of paddlefish life history were identified by Scarnecchia et al. (2007) in a stock inhabiting the Yellowstone River and Lake Sakakawea, Montana and North Dakota: (1) immature, (2) maturing, (3) adult- somatic growth and reproduction, (4) prime reproduction, and (5) senescence to death:

*"The five periods ... occur at different ages for each sex ... . During the first period (immature), fishes exhibit rapid somatic growth as well as accumulation of energy reserves in the form of gonadal fat bodies (GFBs) and other fat deposits. During the second period (maturing), somatic growth slows as production and stored energy reserves are diverted into reproduction. In the third period, fish are allocating energy to both somatic growth and reproduction. In the fourth, reproductive periodicity is typically close to two years for males and three years for female; the rate of gonadal recrudescence is at its maximum. Fish make shorter pre-spawning migrations upriver. In the fifth period (senescence to death), GSI (Gonadosomatic Index) of some of the oldest females decreases; the oldest males have fewer energy reserves and are long and lean. (p. 211)"*

The duration of these life stages is more compressed in paddlefish from more southern localities where total lifespan may be 20-25 years (e.g., Oklahoma) than farther north (e.g., Montana and North Dakota), where lifespan may be 50-60 years. The shorter lifespans were in this case associated with higher metabolic demands and smaller GFBs in the stock farther south (Scarnecchia et al. 2011). Females also deplete their GFBs more rapidly than males associated with higher costs of reproduction (Scarnecchia et al. 2007, 2011). As a cruising zooplantivore and a ram ventilator (Burggren and Bemis 1992), paddlefish have white muscle tissue and a considerable amount of red muscle outside of the white muscle core, next to the integument. Studies cited by Ackman (1980) indicated that red muscle in other fish species can have up to five times the lipids of white muscle. Paddlefish from different locations, different life stages, sexes, and different opportunities for growth are thus likely to exhibit different strategies for energy allocation. They may also store lipids differently by tissue type.

Knowledge of the way that energy is utilized throughout each of the life history phases of paddlefish would be helpful in their conservation. Long term monitoring of stage specific energy density (Wuenshel et al. 2006) in paddlefish may help describe effects of energy on life history events. Because energy allocation strategies in juvenile paddlefish may be fundamentally different from those of adults and by sex, (Wuenschel et al. 2006; Scarnecchia et al. 2011), energy density must be described in terms of sex and life-stage.

The Yellowstone-Sakakawea stock of North Dakota and Montana (hereafter SAK), and the Grand Lake stock of Oklahoma (hereafter GL) provide an opportunity to study lipid content in adult paddlefish in populations widely separated geographically that exhibit the

aforementioned life history differences. Investigations of how stock, sex, and tissue types (white muscle, red muscle, and gonadal fat body (GFB)) compare between these two widely separated populations may provide insight into factors that drive life history decisions in paddlefish under markedly different environmental conditions.

This study uses data from recreationally-caught adult fish in an attempt to explain variation in lipid content in male and female paddlefish by stock and by fish age. The objectives of this study were to 1) describe the relative importance of three distinct paddlefish tissues (white muscle, red muscle, and GFB) for lipid storage, 2) compare lipid content of these tissues between stocks and 3) examine the efficacy of using data collected from the fisheries (length, age, stock, sex, gonad weight, GFB weight, and gonado-somatic index (GSI)) to explain variability in lipid values of white muscle, red muscle, and GFBs. Hypothesese were that the three tissue lipid values are related to each other within individual fish, and that, consistent with results of Scarnecchia et al. (2007, 2011) values for all tissues will be sex and stock specific.

## METHODS

### *Data Collection*

Samples of white muscle, red muscle, and GFBs were collected during 2012 from adult fish harvested (snagged) by recreational fishermen. Because the fisheries capture almost exclusively adult migratory pre-spawning fish, and not all paddlefish spawn every year, at least in northern stocks (Scarnecchia et al. 2007) only that portion of the adult population was evaluated, not those fish between spawns remaining in reservoirs. Fish from the SAK stock were sampled for tissue at the fish cleaning station at the Confluence of the Missouri and

Yellowstone Rivers during May 2011, where the fish had been brought for processing (Scarnecchia et al. 2008). Fish from the GL stock were sampled for tissues at the paddlefish Research Center, at Grand Lake, near Miami, Oklahoma during April 2011. These collection times corresponded with the annual upriver spawning migrations for the respective populations.

White muscle tissue was collected immediately behind the head from 197 fish, 54 females and 83 males from SAK and, and 24 females and 36 males from GL. Red muscle was sampled only from the SAK stock, from 38 females and 36 males, with a fillet knife from the outer portion of the fillets during fish processing. Samples of GFB tissue were collected during existing GFB collection protocols (Scarnecchia et al. 2007). GFBs were collected from 164 fish, 59 females and 105 males from the SAK stock, and 41 males from the GL stock. Female fish from the GL stock were of nearly uniform age and were primarily prime spawners (Scarnecchia et al. 2007) with too little GFB material for use, so sample size for GL females was only one fish. Because some tissues contained up to approximately 90% water, at least 50 grams of tissue per sample was collected to ensure adequate dry sample volume. Samples were promptly frozen and transported on ice to freezers (-20 °C) at the University of Idaho.

Body length (front of eye to fork of caudal fin; BL, in mm), weight (WT), gonad weight (GWT), weight of GFBs, (FATWT), and gonado-somatic index (GSI) were collected on each fish. Dentaries were collected and ages assigned according to established methods (Scarnecchia et al. 2006). As of 2012, ages had been validated only through age -17.

*Laboratory processing and analysis*

In preparation for proximate analysis, frozen tissues of the three types (white muscle, red muscle, and GFB) were homogenized for 30-60 seconds with a NINJA™ blender, sealed in a centrifuge tube, and stored in a freezer until being transported to Hagerman, Idaho. Tissue samples underwent proximate analysis at the University of Idaho, Moscow, and the Fish Culture Experiment Station in Hagerman, Idaho. Ashing was completed in Moscow using AOAC method 938.08 (AOAC 2000). Moisture and lipid were determined using ANKOM method 5-04 (AOCS 2005). Protein was estimated by subtraction (Hendry et al. 2000). Lipid content of homogenized adult tissues was assessed in duplicate using the ANKOM method (AOCS 2005). The ANKOM used a solvent (heated petroleum ether) to extract lipid, and utilizes gravimetric loss to determine lipid content. This method returns a crude fat value, called crude because in addition to TAG's (triacylgylcerols), which are the primary component of storage energy (Adams 1999), it also likely extracts an insignificant fraction of structural lipids as well (Tocher 1993). Because ANKOM is an indirect method that removes and combines the lipid fraction of multiple samples, the remaining lipid fraction was unsuitable for further analysis. All procedures associated with proximate analysis included a control sample of homogenized pet food to evaluate homogeneity of run cycles. Relative percent difference (mean ± SD) for tissue duplicates were calculated for white muscle (1.56 ± 1.99), red muscle (2.93 ± 2.89) and GFB (1.55 ± 2.26). Inter-assay coefficient of variation was 0.06 for lipid extraction.

*Statistical Analysis*

Some of the lipid distributions were non-normally distributed. As a result, a non-parametric Kruskal-Wallis test was applied to lipid values for each tissue type via a one-way ANOVA on ranked lipid values, with a *post hoc* Tukey-Kramer test to identify differences in means due to stock and sex. A Wilcoxon test was used for comparisons for which there were only two groups.

Because of inherent life history differences between stocks and sexes, such as lifespan and age at maturity, proximate lipid values were combined with fishery data obtained from both fisheries to form a comprehensive multiple regression model including all stocks and sexes with lipid as the dependent variable and BL, WT, age, stock, sex, gonad weight (GWT), gonadal fat weight (FATWT), and GSI as independent variables for each tissue type. State and sex were coded with dummy variables (SAK = zero, GL = one, Male= zero, Female= one). Model selection was performed using Akaike's information criteria (AIC; Akaike 1974; Burnham and Anderson 2002), and the best fit model was used to compute parameter estimates for a multiple regression model. Variance inflation factor was calculated to check final models for collinearity, and diagnostic residual plots were used to check final regression models for homogeneity of variance (Ott and Longnecker 2010). If models violated assumptions of homogeneity of variance, transformations including natural logs for white meat lipid values, and squaring for GFB lipid values were used to meet regression assumptions. Expected vs. predicted values were used to evaluate model fit. If the multiple regressions identified significant explanatory variables, the signs of the parameter estimates were retained and assembled into a table in order to identify trends in regression results. For some fish collected, fishery data was unavailable, leading to some discrepancies between

sample sizes of fish collected, and those used for regressions and multiple comparisons (Tables 1, 2). The program SAS 9.3 (SAS Institute, 2013) was used for all statistical analyses, and all analyses were evaluated for significance at $\alpha$=.05.

## RESULTS

### *Proximate analysis*

White muscle lipid values were the lowest of the three tissues, with GL fish ranging from 4.99 – 6.74 %, and SAK fish ranging from 9.09 to 17.18 %. Red muscle lipid was higher, with SAK values ranging from 30.93 to 47.72%. The highest lipid values were found in GFBs, with SAK fish ranging from 79.79 to 89.08 %. and GL fish ranging from 91.72 to 92.17 % (Table 1). Additional reference information, such as moisture, ash, protein, and calorie values, is provided in Table 1.

### *White muscle*

Significant trends were found among some tissue types in lipid values of individual fish. There was a significant positive relationship between SAK white muscle lipid and red muscle lipid in individual fish ($R^2$=.40, $P$< 0.0001) (Figure 1). There was also a weaker negative relationship between white muscle lipid and GFB lipid ($R^2$= 0.07, $P$< 0.003) for SAK fish that did not exist for GL fish ($R^2$= 0.06, $P$= 0.181), and no trend between SAK red muscle lipid and GFB lipid ($R^2$= 0.02, $P$ =0.19) (Figure 1). I rejected the null hypothesis that tissue lipids are not related between white and red muscle for SAK, but failed to reject it for GL fish. I also rejected the null hypothesis that tissue lipids are not related for red muscle and

GFBs. I failed to reject the null hypothesis that tissue lipids are not related between red muscle and GFBs. Red muscle comparisons were limited to SAK fish.

Lipid values differed significantly by both location and sex (Kruskal-Wallis test, $P< 0.0001$). I rejected the null hypothesis that white muscle lipid values were the same among the groups. GL fish had significantly lower levels of white muscle lipid than SAK fish, and female fish from both stocks had significantly higher white muscle lipid than males (Figure 2).

Table 1. Proximate composition (% ± SD) of all tissues of 2011 spawning SAK and GL paddlefish. Groups are categorized by stock, sex (M, F, in parenthesis), and tissue WM (white muscle), RM (red muscle, and GFB (gonadal fat body). It was not possible to calculate SD for GL female GFB because sample size was one, so this is denoted by not applicable (na).

| Group | *N* | Lipid % (SD) | Water % (SD) | Ash % (SD) | Protein % (SD) |
|---|---|---|---|---|---|
| SAK (F) WM | 54 | 17.18 (6.67) | 6.49 (.53) | 0.014 (0.005) | 76.31 (6.24) |
| SAK (M) WM | 83 | 9.09 (4.29) | 7.1 (.35) | 0.015 (0.005) | 83.78 (3.98) |
| GL (F) WM | 24 | 4.99 (1.59) | 7.29 (.17) | 0.020 (0.005) | 87.69 (1.47) |
| GL (M) WM | 36 | 6.74 (2.74) | 7.36 (.27) | 0.017 (0.005) | 85.86 (2.51) |
| SAK (F) RM | 38 | 47.72 (10.19) | 4.06 (.78) | 0.044 (.101) | 48.20 (9.39) |
| SAK (M) RM | 36 | 30.93 (8.54) | 5.37 (.68) | 0.065 (.146) | 63.39 (7.86) |
| SAK (F) GFB | 59 | 79.79 (11.8) | 1.66 (1.0) | 0.002 (0.001) | 18.54 (10.8) |
| SAK (M) GFB | 105 | 89.08 (6.6) | .87 (.5) | 0.001 (0.001) | 10.04 (6.11) |
| GL (F) GFB | 1 | 92.17 (na) | .58 (na) | 0.001 (na) | 7.25 (na) |
| GL (M) GFB | 41 | 91.72 (2.4) | .61 (.19) | 0.0001 (0.008) | 7.67 (2.22) |

The best fit model for explaining variation in white muscle lipids was significant and included the terms WT, stock, and sex ($R^2$=.54, $P< 0.0001$) (Figure 3). The signs of the parameter estimates for the variables WT and sex were positive, while the sign of the parameter estimate

for stock was negative. I rejected the null hypotheses that the model lacked explanatory value with respect to lipid content.

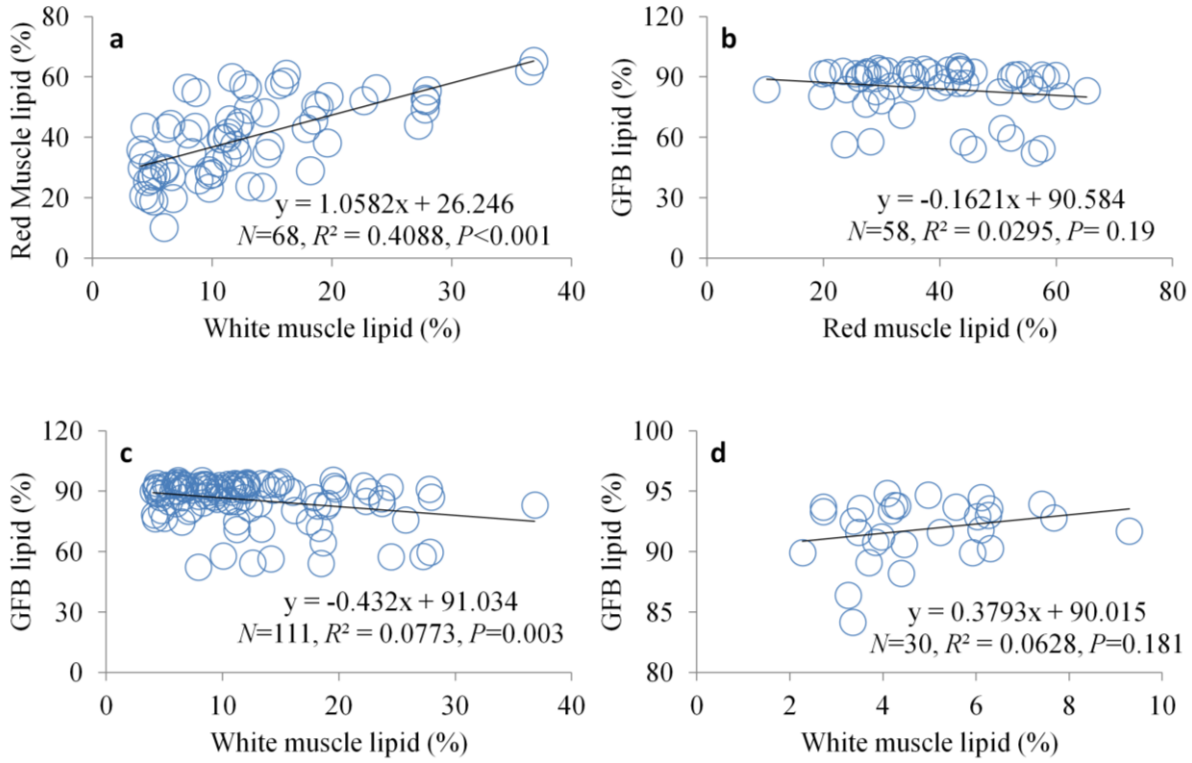


Figure 1. Relationships between tissue lipids (% wet weight) within individual 2012 adult SAK and GL paddlefish. Tissues are white muscle, red muscle and GFB. Panel a,b, and c are SAK, and d is GL.

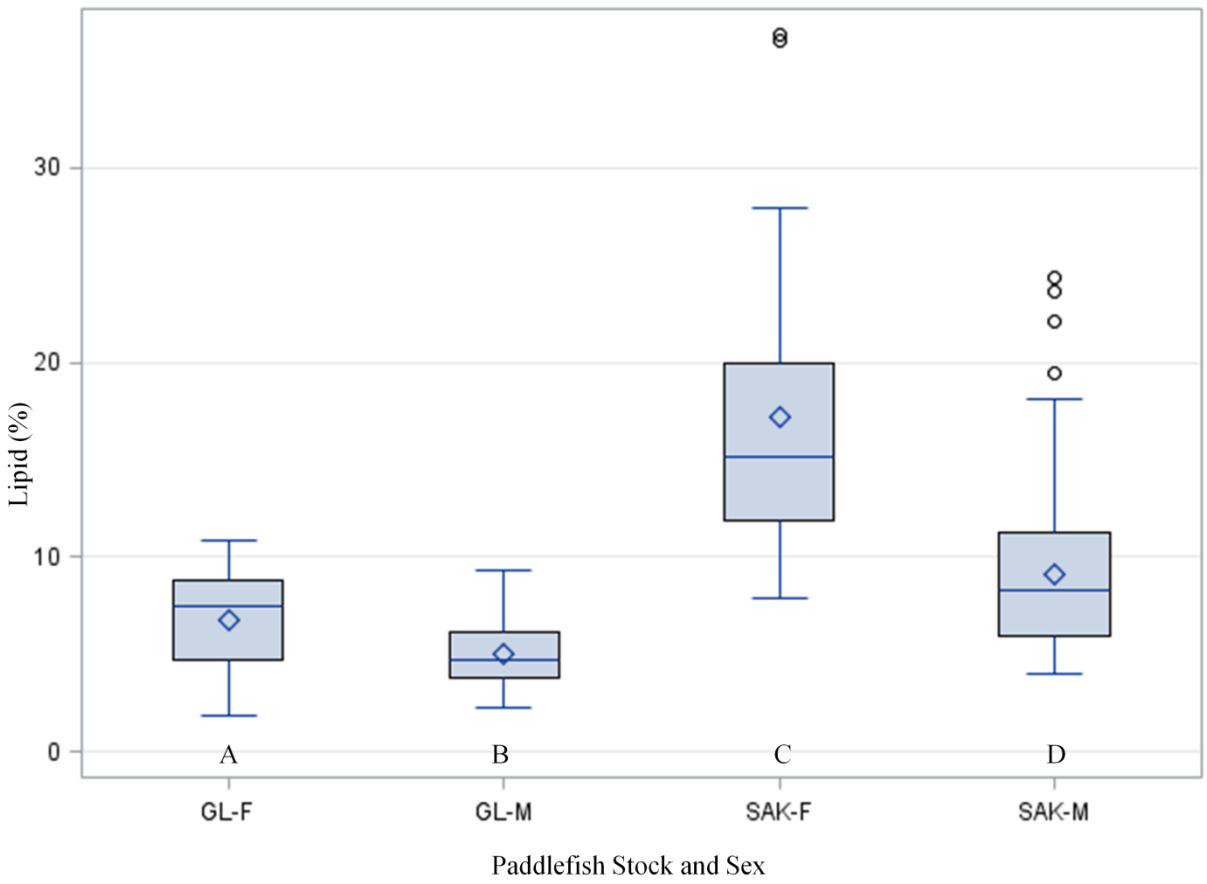


Figure 2. Box plot distribution of lipids (%) in 2012 adult GL and SAK paddlefish white muscle including mean (diamond) median (line), interquartile range (box), outliers, and minimum and maximum values). The four groups represented are GL female (GL-F), GL male (GL-M)), SAK female (SAK-F), and SAK male (SAK-M) (Kruskal-Wallis test, $P<$ 0.0001). Differences significant at $\alpha=0.05$ (Tukey-Kramer test) are indicated by different letters.

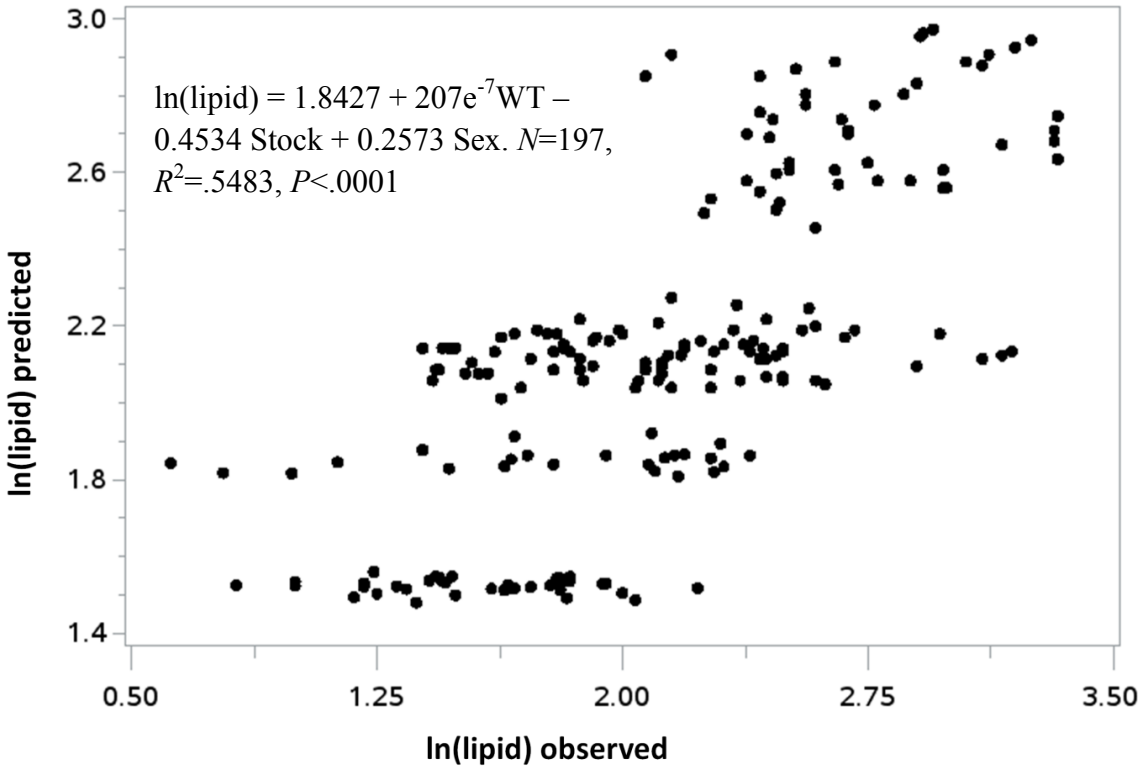


Figure 3. Predicted vs. observed values of ln(lipid) in 2012 SAK and GL adult paddlefish white muscle.

*Red Muscle*

Lipid values for SAK red muscle differed significantly by sex (Wilcoxon test, $P <$ .0001). I rejected the null hypothesis that red muscle lipid values were the same for the sexes, but was unable to test for differences among the stocks. Among SAK fish, females had significantly higher red muscle lipids than males (Figure 4).

All of the red muscle models significantly predicted lipid. The best fit model predicting red muscle lipids included the terms GWT and FATWT ($R^2= 0.55$, $P< 0.0001$) (Figure 5). As GWT or FATWT increased, lipid increased. Because red muscle samples did

not include GL fish, the model was a SAK stock specific model. For red muscle lipid, I rejected the null hypothesis that the models lacked explanatory value.

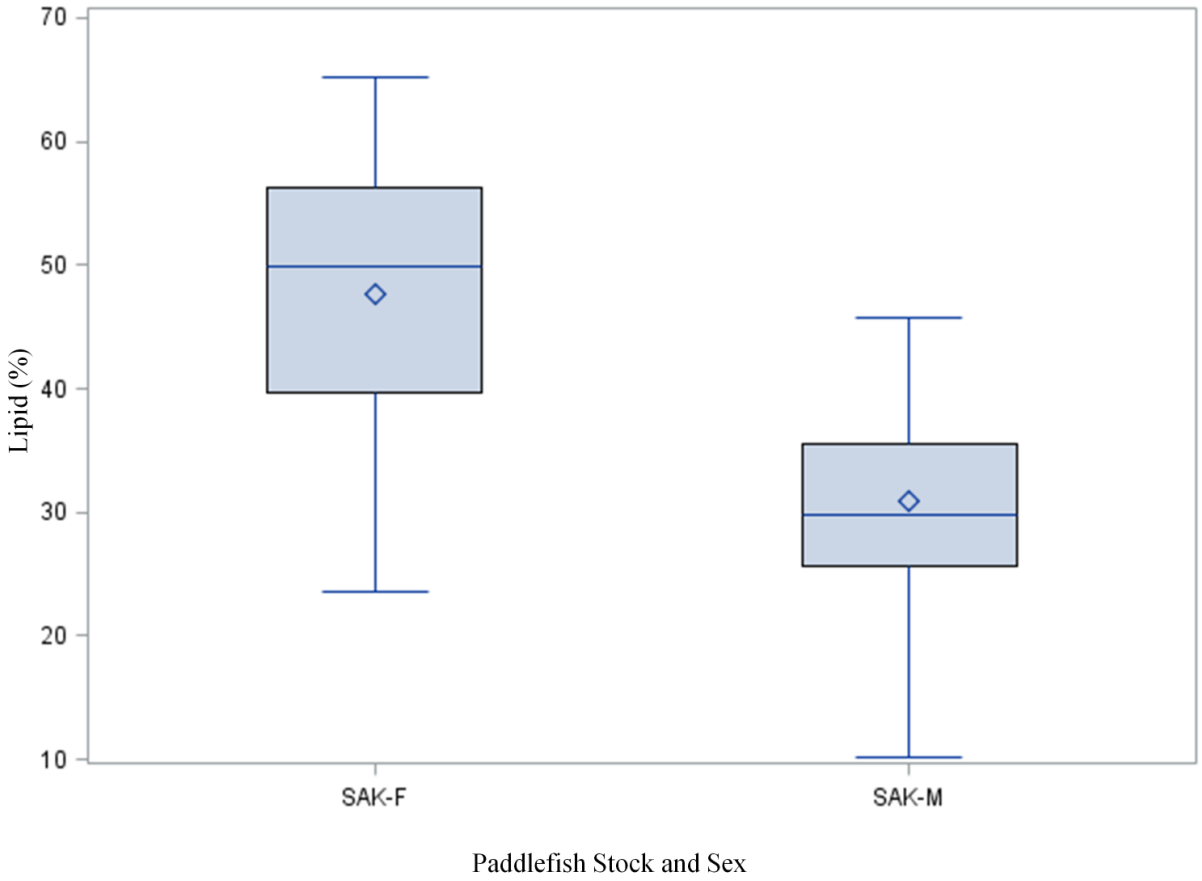


Figure 4. Box plot distribution of lipids (%) values for red muscle tissue from 2012 adult SAK paddlefish (including mean (diamond), median (line), interquartile range (box), outliers, and minimum and maximum values). Groups represented are SAK female (SAK-F) (*N*=38), and SAK male (SAK-M) (*N*=36) (Wilcoxon test, $P < 0.0001$).

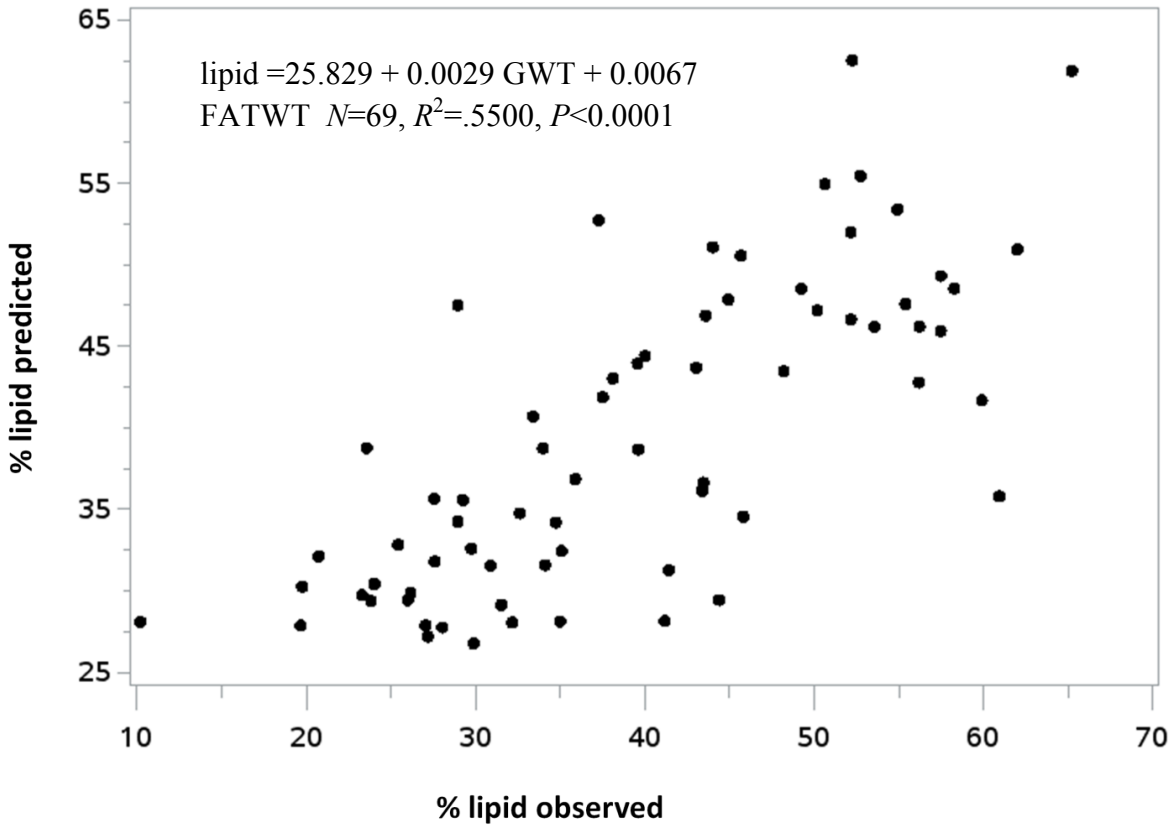


Figure 5. Predicted vs. observed values of % lipids in 2012 SAK adult paddlefish red muscle.

*GFB*

Lipid values of GFBs in SAK fish were significantly different by sex (Kruskal-Wallis test, $P < 0.0001$). I rejected the null hypothesis that GFB lipid values were the same between sexes. Among SAK fish, males had significantly higher lipids than females (Figures 6). Lipid values of males did not differ by stock (Wilcoxon test, $P < 0.0697$). I failed to reject the null hypothesis that GFB lipid values differed by stock.

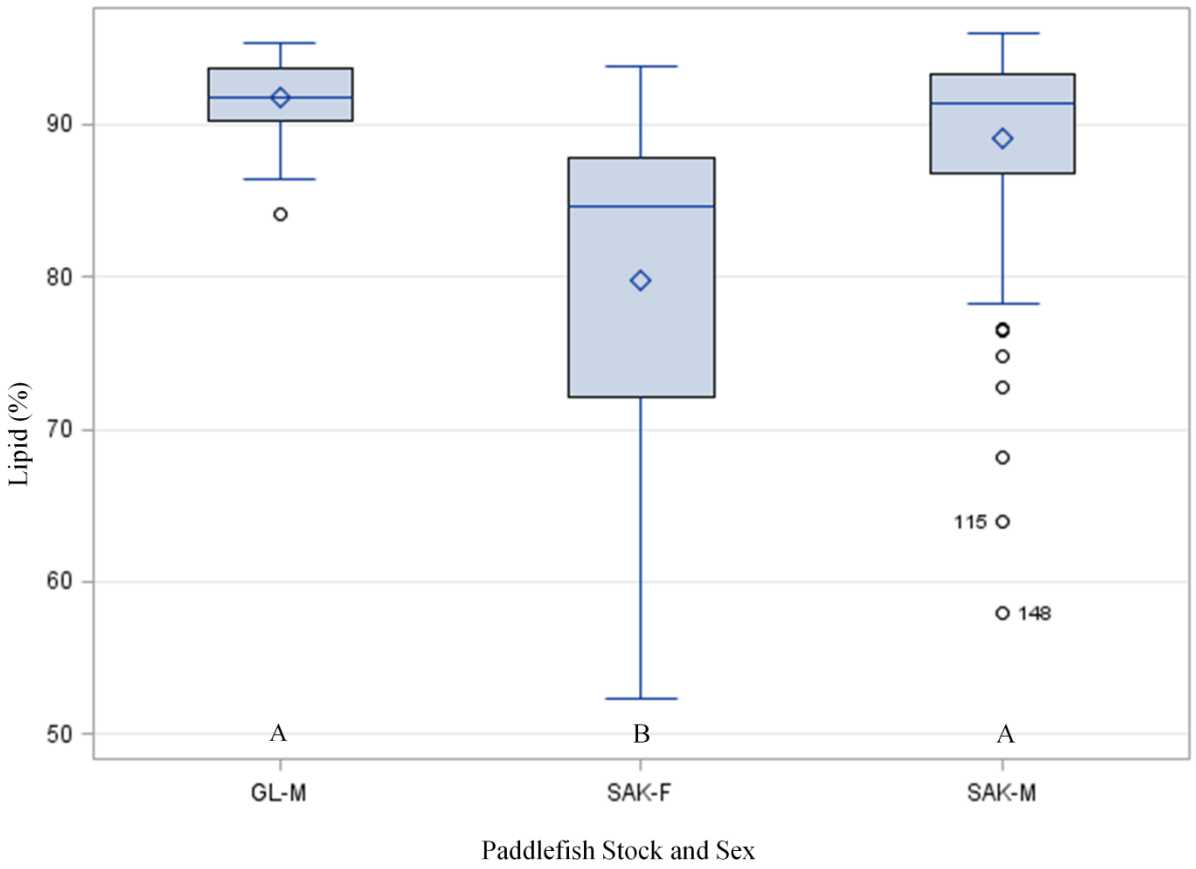


Figure 6. Box plot distribution of lipid (%) (including mean (diamond), median (line), interquartile range (box), outliers, and minimum and maximum values) in GFB tissue of 2012 GL and SAK adult paddlefish. The three groups represented are GL male (GL-M) (N=41), SAK female (SAK-F) (N=59), and SAK male (SAK-M) (N=105) (Kruskal-Wallis test, $P< 0.0001$). Differences significant at $\alpha= 0.05$ (Tukey-Kramer test) are indicated by different letters.

The best fit model for predicting GFB lipids was significant and included the terms BL, Stock, ln(FATWT), and GSI ($R^2$=.77, $P$< 0.0001) (Figure 7). Parameter estimates for the variables BL and GSI were negative, while parameter estimates for stock and ln(FATWT) were positive. For GFB lipids for all models, I rejected the null hypothesis that the models lacked explanatory value.

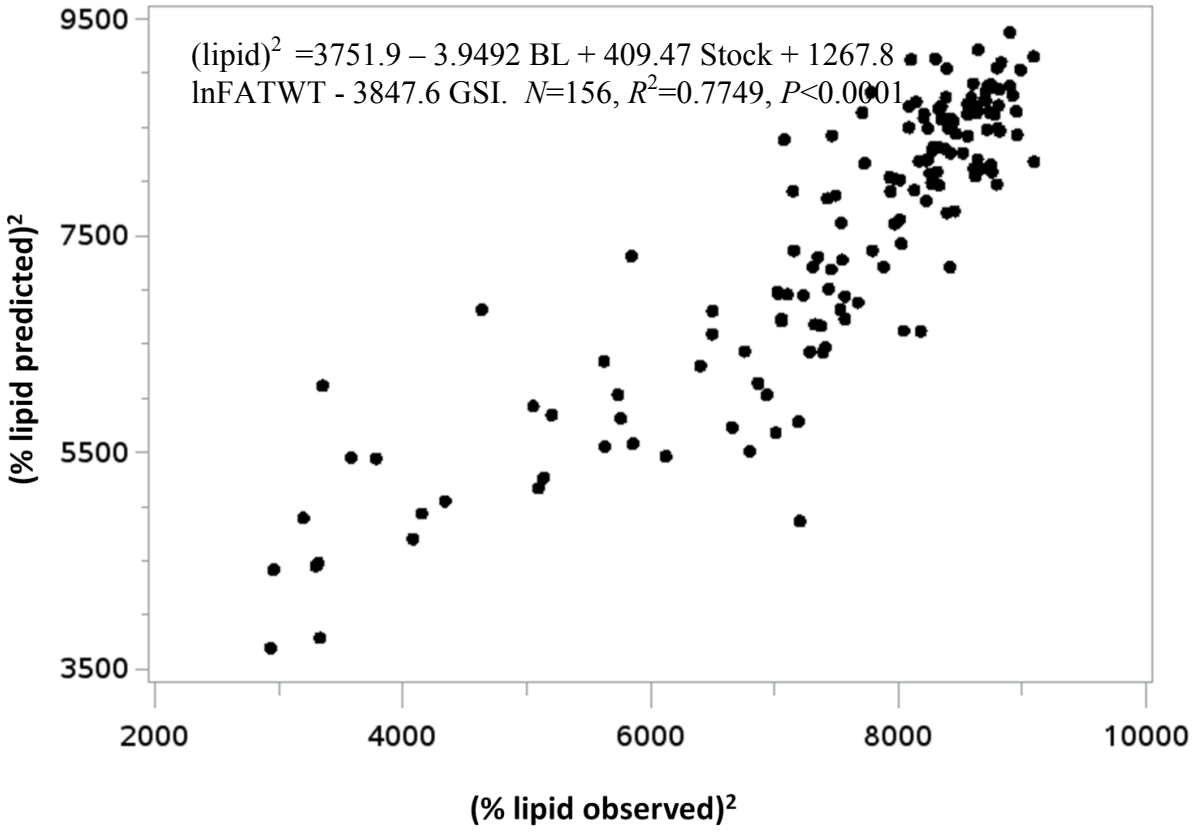


Figure 7. Predicted vs. observed values of $(lipid)^2$ in 2012 SAK and GL adult paddlefish GFBs.

Table 2. Summary of signs of parameter estimates and corresponding $R^2$ values for multiple regression models developed with % lipid, body length (BL) weight (WT), age, stock (SAK, GL), sex (male, female), gonad weight (GWT), GFB weight (FATWT) and gonadosomatic index (GSI) from 2012 adult SAK and GL paddlefish. Groups represent tissues: white muscle (WM), red muscle (RM), and gonadal fat body (GFB).

| Figure | Group | *N* | BL | WT | AGE | STOCK | SEX | GWT | FATWT | GSI | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | WM | 197 | | + | | - | + | | | | 0.54 |
| 5 | RM | 69 | | | | | | + | + | | 0.55 |
| 7 | GFB | 156 | - | | | + | | | + | - | 0.77 |

## DISCUSSION

An important outcome of the current study is the establishment of reference data for lipid content in wild paddlefish tissues. My proximate results for white muscle lipid (4.99% to 17.18%) are much higher than the few other results reported in the literature for hatchery-reared fish. Decker et al. (2007) reported 1.53% for whole fillet, and 0.27% for white muscle; Simeanu et al. (2012) reported 2.45 - 3.96% for whole fillets. Their lower reported values than in my study are likely not as much due to rearing practices as due to differences in age and geographical origin of the paddlefish used in those studies. With regard to age, Decker et al. (2007) reported results for 17 month-old fish, and Simeanu (2012) reported results for paddlefish ages 0-3. Their results are comparable to lipid concentrations I reported in Chapter 1 (0.85 – 5.51%) using juvenile paddlefish of similar ages (0 and 1). The higher white muscle lipid values observed in larger, adult fish are compatible with an observation of LeBreton and Beamish (2004) for lake sturgeon, a similar-sized Acipenseriform fish, said to "undergo a disproportionate accumulation of lipid with age and growth with age". The overall pattern is consistent with the hypothesis that lipid accumulation increases once fish pass life stages where growth in length and apparent length are vital to escape predation. Lipids can then be

useful in preparation for reproduction and related activities. Capital breeders (Jonsson 1997) are organisms that utilize lipid stored earlier in life for reproductive demands later. Although the concept of capital breeding was developed for birds and reptiles, Jager et al. (2008) demonstrated that it applies to numerous fish species, especially those that are late maturing and slow growing. Paddlefish are a good example of this paradigm.

Proximate results for SAK female red muscle lipid obtained in this study (47.72%) are higher as than those reported by Gundersen and Pearson (1992) in commercially harvested female paddlefish in the Ohio River (22%). However, Ohio river fish had white muscle lipid at levels comparable to GL fish used in this study (Ohio 3.2%, GL 4.99%), and it is reasonable to expect that GL red muscle lipid values would be similar to Ohio river fish, which occur at similar latitudes. Decker (2007) reported slight differences between whole fillet and white muscle, and cited Ackman (1980) for red muscle values of other species, which were up to 5 times the value of white muscle. My proximate lipid results for GFBs were on the high end of those reported by Scarnecchia (2007), however, the lower values reported for older fish in that study probably reflected lipid content of collagen based connective tissue remaining from depleted GFBs. That tissue was not specifically excluded from proximate analysis in that study.

The results for adults are also consistent with the idea that geographical area as it relates to growing season may influence lipid concentrations. Life history adaptations of fish to temperate climates may help explain the higher white muscle lipid content observed in SAK fish than GL fish. Lou et al. (2000) reported a range of white muscle lipid of approximately 1-9 % for sub-adult and adult fish from Kentucky, with results comparable to those we obtained from the GL adults in Oklahoma. In contrast, lipids were significantly

higher in the more northerly SAK stock (9.09-17.18 %) (Table 1). Although effects of fish density cannot be ruled out in explaining the muscle lipid differences between the two stocks, the role of metabolism should not be overlooked. Huelett et al. (1995) found that body size, rather than temperature, was the primary factor affecting paddlefish metabolism. Scarnecchia et al. (2007) characterized the size differences of the two stocks in this study and suggested that fish from SAK are larger and have lower metabolic rates than those from GL. The smaller GL fish have a higher metabolic rate, which demands more energy, leaving less available for storage. The idea that size- influenced metabolism is a key factor in lipid storage gains support from the positive sign of the parameter estimate for weight in the multiple regression model for white muscle lipid. It is likely that higher lipids in northerly areas with shorter growing seasons may aid fish in maintenance during periods of lower food availability (Scarnecchia et al. 2009). The SAK fish are also subjected to longer periods of low water temperature (Scarnecchia et al. 2007, 2011) and lower food availability during winter, which is longer and more severe than that experienced by GL fish. In most fishes, white muscle lipid is a good surrogate of whole body lipid (Bulow 1971, Buckley 1987), so my results support the hypothesis that environmental factors such as latitude and climate affect lipid storage in paddlefish. Results from other species support this conclusion. Power and McKinley (1997) observed latitudinal differences in growth and condition in lake sturgeon *Acipenser fulvescens*, and Beamish et al. (1996) found seasonal variation in lipid content of lake sturgeon, with the lower lipid values following winter.

Another possible specific mechanism that may explain the relationship between lipid storage and environmental factors relates to the specifics of diet. The primary storage lipid in fish is triacylglycerols (TAGS), which form a specific class of fatty acids that most fishes do

not have the ability to manufacture (Adams 1999), and must acquire from their diet (Olsen 1999). Diet of SAK fish is likely to be higher in TAGs than that of GL fish, because many zooplankton species in colder climates are richer in TAGS than those in warmer climates (Golden 1999). This possibility could be investigated with a future study comparing lipid content of zooplankton from SAK and GL.

Life history differences and the role of metabolism may also help explain the differences in concentrations of lipid in females and males that were observed for all three tissues. If higher muscle lipid in females reflects higher overall body lipid, it is likely beneficial for female fish (which are larger than males at age) to store more lipid. Female fish likely have a higher initial reproductive investment than males; as a result of the volume and size of eggs they must produce (Tocher 2003). Higher lipid levels may help meet the energy demands of producing energy rich eggs during periods of low food availability. Since we sampled fish during the upriver migration, it is likely that males have not undergone the same level of parental investment at that time as the female fish. In addition, the energy that males have invested in migration is lost to the fish, while in females, the majority of energy invested is actually just transferred from GFBs to ovaries, with little net whole-body energy loss to the fish until egg deposition. Medford and Mackay (1978) found that in northern pike *Esox lucius*, mature ovaries contained more than 10 times the amount of lipid as mature testes. Large differences in gonad lipid may be reflected by muscle lipids. Sutharshiny et al. (2013) observed greater increases and decreases in muscle lipids of female double spotted queenfish *Scomberoides lysan* than in their male counterparts just prior to and after spawning. The notion that female paddlefish muscle lipids prior to spawning reflects increases in gonad

lipids gains support from the positive sign of the parameter estimate for the variable sex observed in the regression for white muscle in the current study.

The energetic demands association with ram ventilation may help explain observed differences in red muscle lipid between males and females. Because its primary energy source is lipid, red muscle is an important asset for the persistent swimming that a ram-ventilator requires (Sen 2005). This demand for constant movement may be more energetically costly for larger female fish, which respond by storing more lipid in red muscle. Alternately, at the time that we sampled, males may have expended more of their smaller depot of red muscle energy in migration, since they mature younger (Scarnecchia et al. 1996), spawn more often (Scarnecchia et al. 2007) , and may typically migrate earlier in the season (D. Scarnecchia, Unpublished data).

The temporal aspect of reproductive demands may help explain lower lipids in female GFBs, which may be due to the longer duration of physiological demands associated with reproduction. The theory of natural selection suggests that while overall reproductive energy expenditure in male and female organisms is similar, the investment of that energy is directed into different pathways, with females investing more in gamete production and males investing more in reproductive behavior (Fisher 1930). If this is the case for paddlefish, the primary energy expenditure of female fish is likely to be due to the physiological demand of reproduction, in which oocytes are maturing all winter; while in males, primary expenditure is likely behavioral and related to migration, which is experienced over a shorter time period in early spring.

Specificity of tissue storage and depletion pathways is another possibility in helping to explain the higher observed GFB lipid values in males than females. Paddlefish may use lipid from muscle differently than from GFBs. Mourente et al. (2002) found that female bluefin tuna *Thunnus thynnus* utilize lipids from the mesenteric perigonadal fat (the tuna equivalent of paddlefish GFBs) for gamete development, and energy from muscle for locomotion; this pattern is also plausible for the paddlefish, another ram ventilator. McPherson et al. (2011) found evidence for differentiated storage and depletion pathways in muscle vs. mesenteric fat in Atlantic herring *Clupea hurengus*. The hypothesis gains support from the different responses of muscle and GFB lipids in individual fish in this study, as well as the difference in parameters selected by the regression models for different tissue types, which suggest that lipid in different tissues may be used for different purposes. This study builds upon previous ideas in the literature regarding paddlefish lipid. Scarnecchia et al. (2007) suggested that lipid content was driven by metabolic differences between the SAK and GL stocks. However, the difference in the responses of muscle and GFB lipid to size metrics in the regression models, as well as the negative relationship between white muscle and GFB lipid in individual fish suggest that although size-induced metabolic rate may be the primary factor in muscle lipid, it may not be the primary driver for GFB lipid. The relationship between tissue specific lipid content and different metabolic demands in paddlefish is an area deserving further study.

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# **CHAPTER 4.** EVALUATION OF ALTERNATE METHODS OF ESTIMATING ENERGY IN JUVENILE AND ADULT PADDLEFISH (*Polyodon spathula*)

## ABSTRACT

Studies were conducted in 2011 and 2012 on age-0 and age-1 paddlefish *Polyodon spathula*, and in 2012 adult paddlefish from North Dakota to assess the efficacy of using Fulton's K to predict lipids in the age-0 and age-1 fish, and to evaluate the use of the Fish Fat-Meter™ for measuring lipid levels in the adult fish tissues (white muscle, red muscle and GFB). Fulton's K failed to predict percent lipid in 2011 age-0 fish, but significantly predicted lipid in 2012 age-0 and age-1 fish. Lipid concentrations in white muscle as measured by the Fat-meter were significantly related to lipid levels measured with proximate analysis for both adult male ($P< 0.0001$) and female ($P< 0.0001$) fish. The relationship for white muscle was closer for females ($R^2 = .5195$) than males ($R^2= 0.073$). The relationships between males and females were similar for red muscle (males $R^2= 0.3023$, $P< 0.0001$; females $R^2= 0.3474$, $P< 0.0001$). Lipid concentrations in GFBs as measured by the Fat-meter were significantly related to lipid levels measured with proximate analysis for both males ($P< 0.0001$) and females ($P< 0.0001$); the relationship was closer for males ($R^2= 0.7511$) than for females ($R^2= 0.6755$). These results indicate that in some years Fulton's K may be useful for predicting lipid in young juvenile fish, and that the Fat-meter may have application for measuring fat levels in adult paddlefish tissues.

## INTRODUCTION

Energetic changes in fish can be measured using traditional, coarse measures such as fish condition or plumpness, and by more sophisticated, direct biochemical measures such as energy content of fish tissues (Carlander 1969; Hartman and Brandt 1995; Jonas et al. 1996; Huntingford et al. 2001). Several researchers have cautioned about reliance on traditional measures of condition to assess energetic changes (Jonas et al. 1996; Kaufman et al. 2007). In many instances, the energetic factors that drive the different life stages may only be detectable by direct measures of energy content of fish tissues.

Proximate analysis and adiabatic bomb calorimetry are accepted methods of assessing lipid and total energy content in fish (Craig et al. 1978; Hendry et al. 2000). Proximate analysis has been used globally as an indicator of the relative nutritional quality of invertebrates, forage fishes, and fish products (Decker 1991; Hartman and Brandt 1995; Rand 1994). It has also been used to quantify overwinter energy losses in age-0 fish (Jonas et al. 1996).

Approaches for measuring energy density, including preparation of tissues and later lab analysis, have traditionally been expensive and time-consuming. Therefore, it would be desirable to develop and utilize faster methods to assess energy content in fish with little investment other than the initial cost of the equipment, and to incorporate the methods into traditional, ongoing fisheries sampling. Efficient monitoring of tissue energies during fieldwork would enable establishment of a database for future monitoring and evaluation. Logistical limitations related to determining energy have led to efforts to use easier to measure metrics, such as the aforementioned coarse condition indices (Hartman and Brandt 1995; Jonas et al. 1996; Post and Parkinson 2001; Wuenschel et al. 2006). Kaufman et al.

(2007) reported the use of several new methodologies to measure energy content in fish including bioelectrical impedance (Cox and Hartman 2005), and low energy microwave probe (Crossin and Hinch 2005). Protocols for using bioelectrical impedance are still being developed (Pothoven et al. 2008; Hafs and Hartman 2011), but recent studies have successfully used a microwave probe (Distell Fish Fatmeter™, Scotland, Uk) to assess lipid content of muscle tissue in American shad *Alosa sapidissima* (Mann et al. 2010, 2011).

The goal of this study is to evaluate the effectiveness of alternate methods of estimating lipid levels in juvenile and adult paddlefish from stocks in Montana and North Dakota. Stocks used for juvenile fish include the Fort Peck stock (FP) in Montana, the Yellowstone-Sakakawea stock (SAK) in North Dakota and Montana, and hatchery fish from the SAK stock raised at Garrison Dam National Fish Hatchery (GD). Adults evaluated include the SAK stock. Specifically, the objectives are to (1) assess the efficacy of using Fulton's K to predict lipids in age-0 and age-1 paddlefish and (2) to evaluate the use of the Fish Fat-Meter™ for measuring lipid levels in adult paddlefish tissue. If successful, these methods will enable rapid assessment of lipid content with data commonly collected during field surveys for age-0 fish and at adult fish cleaning and caviar processing stations in the three states.

## METHODS

I recorded total length (L) and weight (W) for all juvenile fish, and used this information to investigate if coarse measures of condition relate to energy and lipid values. I calculated Fulton's condition factor (K; Nash et al. 2006) for juvenile fish and compared it

with lipid and energy values with linear regression for 168 juvenile paddlefish (23 FP, 86 GD, 59 SAK). The condition factor is expressed as:

$$K = W/(L/10)^3 * 100$$

I also investigated the use of a low energy microwave probe (Distell fish Fat-meter model FFM-2) to assess lipid content in adult paddlefish muscle tissues and fat. The Fat-meter was calibrated by Distel, Inc., and I used settings 1 and 5 for my tissue samples. Three measurements were taken from each tissue sample with the Fat-meter for comparison with ANKOM values. The meter was used to approximate lipid levels for 135 white muscle samples of individual paddlefish (52 females and 83 males). The meter was also used for 73 red muscle samples (37 males and 36 females). Finally the meter comparison was applied to 152 GFB's (53 females and 99 males).

### *Statistical Analysis*

I compared Fulton's K with energy and lipid values via linear regression to assess the suitability of K as an index of lipid content in juvenile (age-0 and age-1 fish). I compared readings from the Fat-meter with results from proximate analysis via linear regression to compare methods for estimating energy content in tissues from adult paddlefish. In all comparisons, $\alpha = 0.05$ was required for significance.

## RESULTS

### *Condition Factor*

Fulton's K values for 2011 age-0 paddlefish ranged from 0.11 to 0.29 (mean, 0.21). Proximate lipid values ranged from 0.48 to 13.72 (mean, 4.362). Variability in proximate

lipid estimates was not predicted by Fulton's K in 2011 age-0 fish from any of the stocks (FP: $R^2$= 0.0972, $P$=0.1475; GD: $R^2$= .000001, $P$= 0.9914; SAK: $R^2$= 0.0494, $P$= 0.0906) (Figure 1). For 2011 age-0 fish, I failed to reject the null hypothesis that K had no relation to proximate analysis lipid values. However, for 2012, significant relationships were found between condition factor and proximate analysis lipid values for both age-0 fish ($R^2$ = 0.221, $P$= 0.0365) and age-1 fish ($R^2$ = 0.2992, $P$= 0.0154) from Lake Sakakawea (Figures 2, 3). For 2012 fish, I rejected the null hypothesis that K had no relation to proximate analysis lipid values.

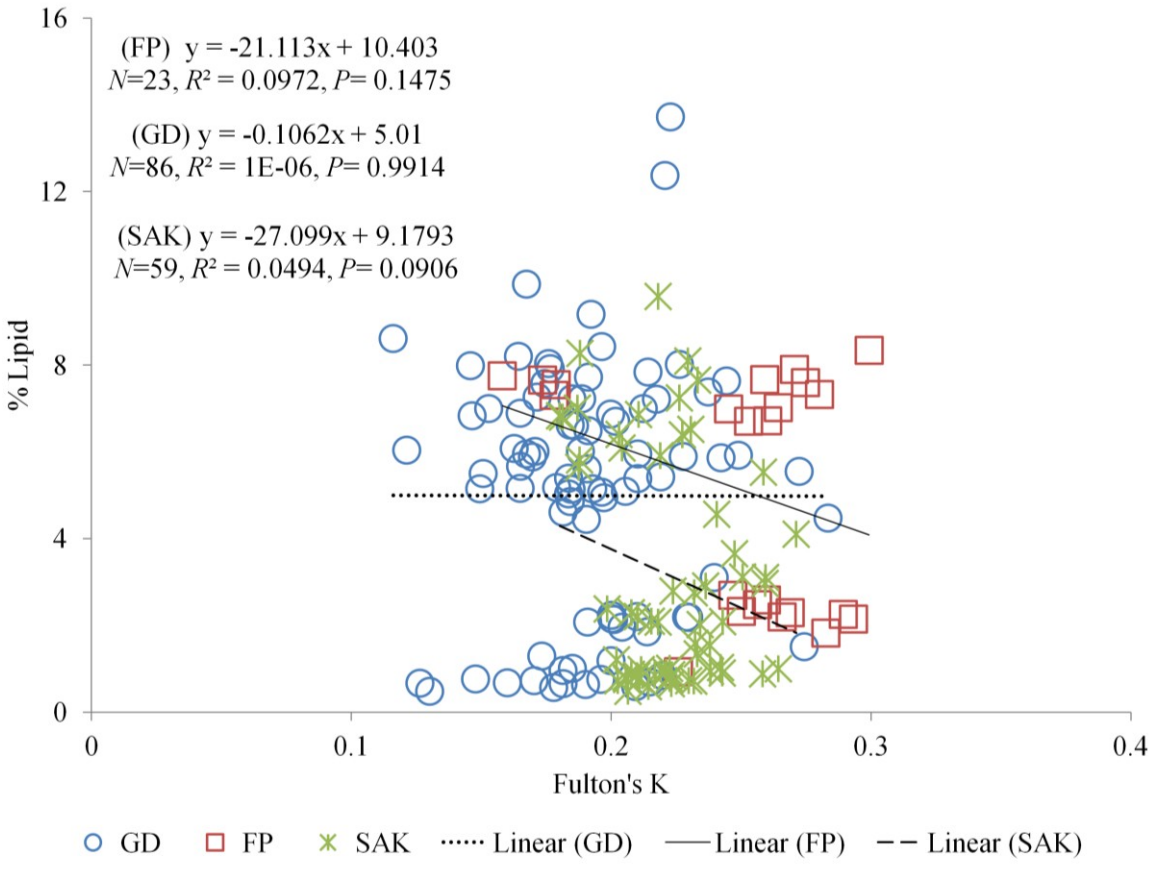


Figure 1. Relationships between Fulton's K and proximate whole body lipid values of 2011 age-0 FP, GD, and SAK paddlefish.

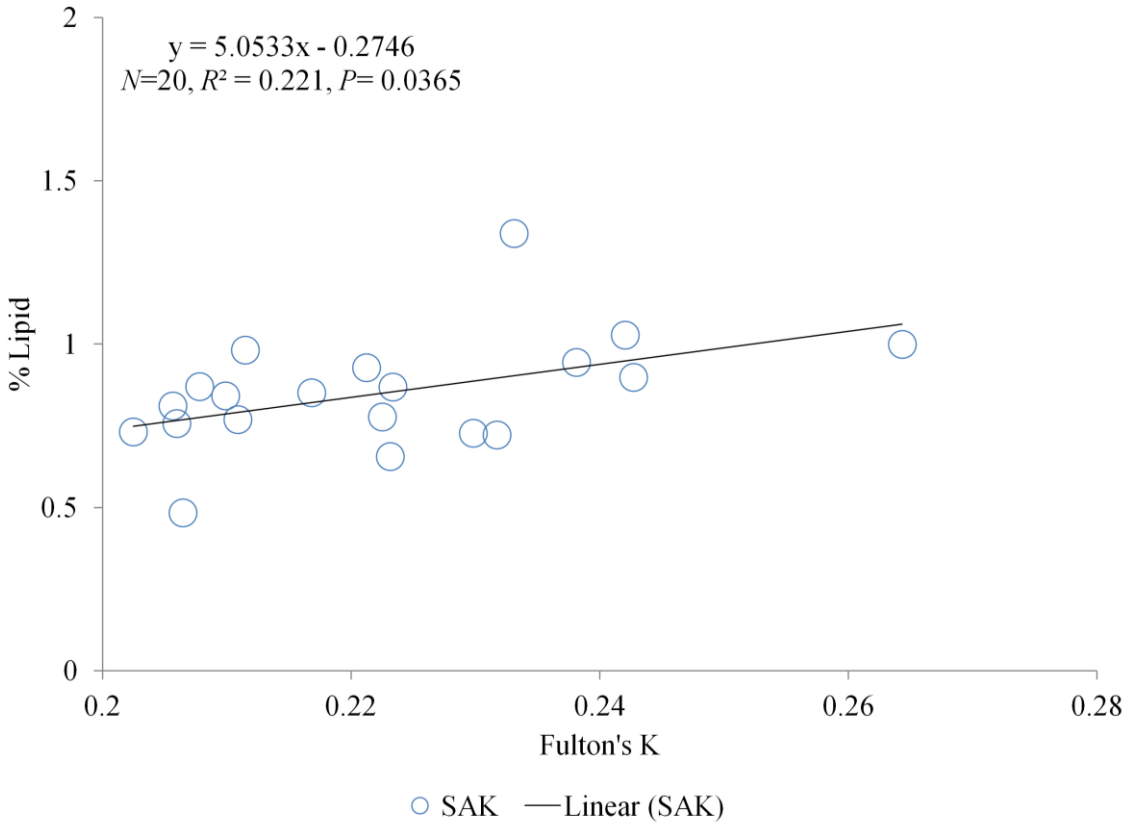


Figure 2. Relationship between Fulton's K and proximate whole body lipid values of 2012 age-0 SAK paddlefish.

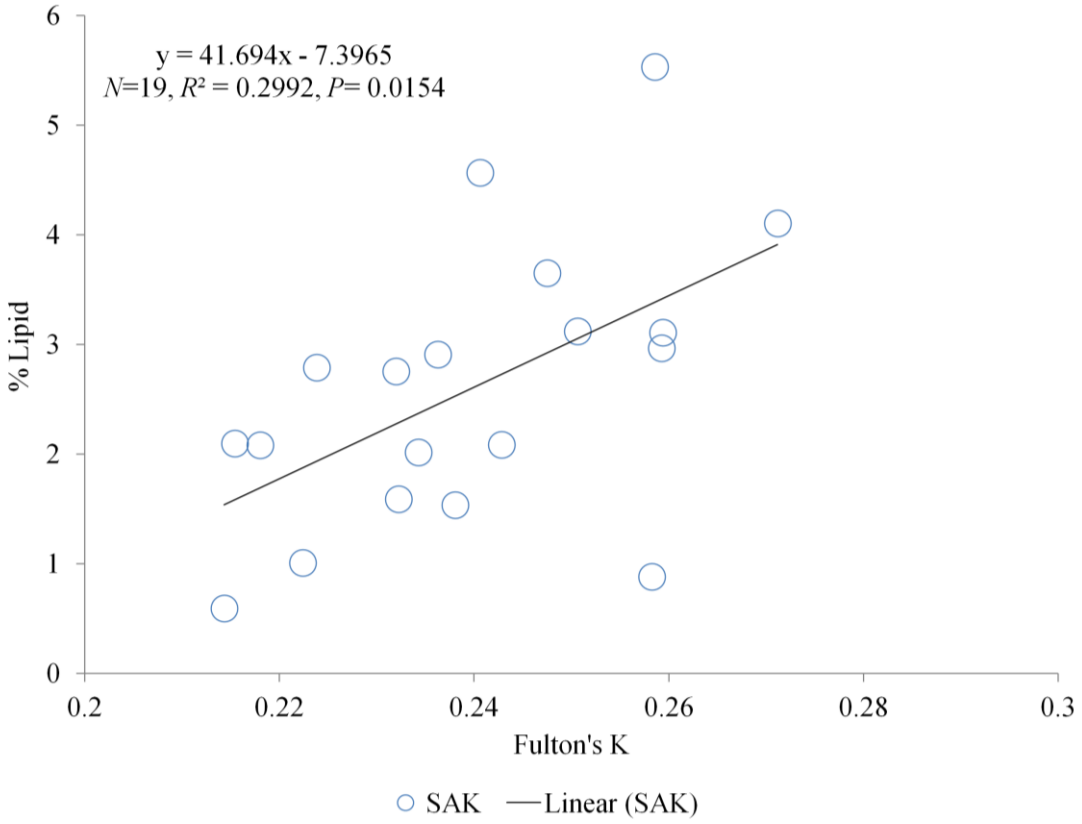


Figure 3. Relationship between Fulton's K and proximate whole body lipid values of 2012 age-1 SAK paddlefish.

*Fat-meter*

For white muscle, Fat-meter estimates of lipids were significantly related to proximate analysis results for both females ($R^{2=}$ 0.51, $P<$ 0.0001) and males ($R^{2}=$ 0.073, $P=$.0135). In the case of white muscle, I rejected the null hypothesis that the Fat-meter readings had no relation to proximate analysis lipid values. The two estimates of lipids were more closely related to each other for females than for males (Figure 4).

For red muscle, Fat-meter estimates of lipids were significantly related to proximate analysis results for both females ($R^{2=}$ 0.34, $P<$ 0.0001) and males ($R^{2}=$ 0.30, $P<$ 0.0001). In the case of red muscle, I rejected the null hypothesis that the Fat-meter readings had no

relation to proximate analysis lipid values. The two estimates of lipids were more closely related to each other for females than for males (Figure 5).

For GFBs, Fat-meter estimates of lipids were significantly related to proximate analysis results for both females ($R^{2=}$ 0.68, $P<$ 0.0001) and males ($R^2=$ 0.75, $P<$ 0.0001). In the case of GFB's, I rejected the null hypothesis that the Fat-meter readings had no relation to proximate analysis lipid values. The two estimates of lipids were more closely related to each other for GFB's than for any other tissues, and for males than for females (Figure 6).

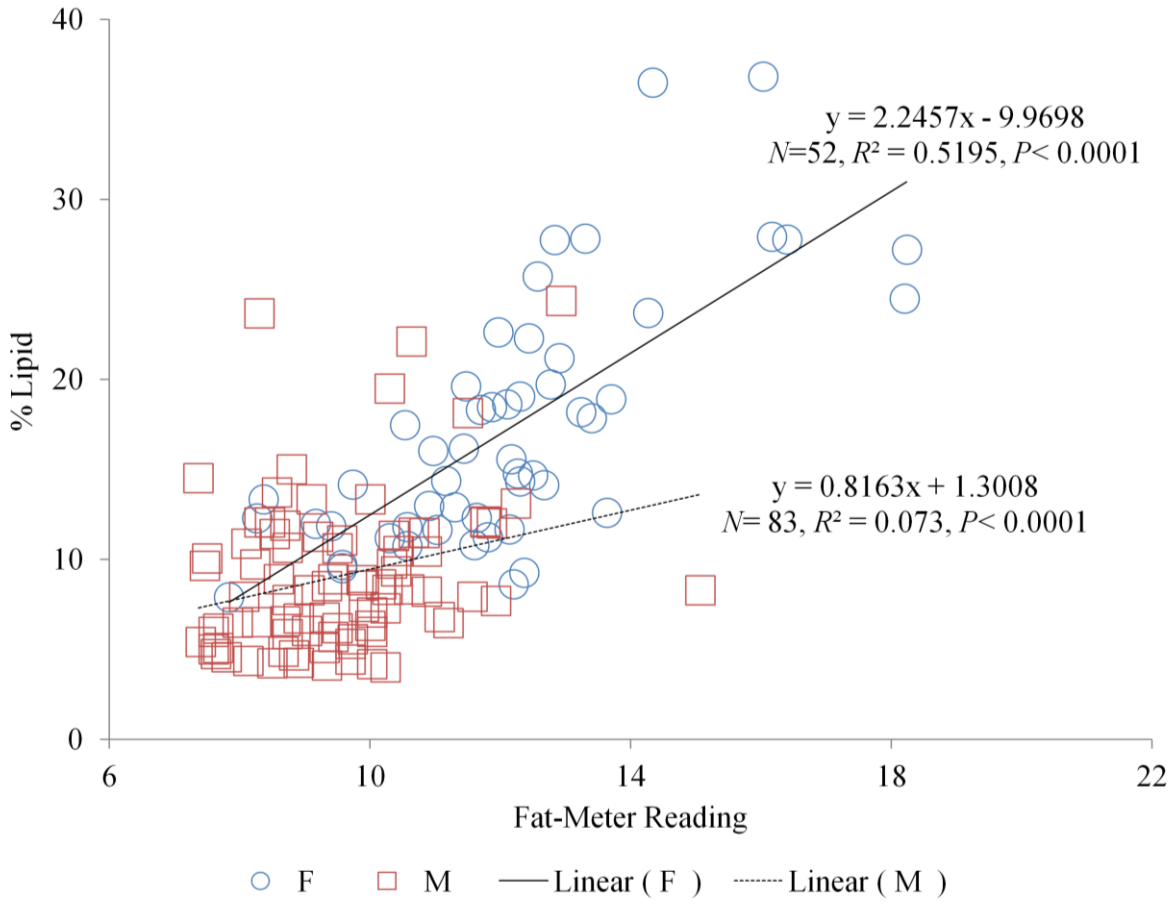


Figure 4. Relationship between Fat-meter readings and proximate lipid values by sex in white muscle of 2012 SAK paddlefish.

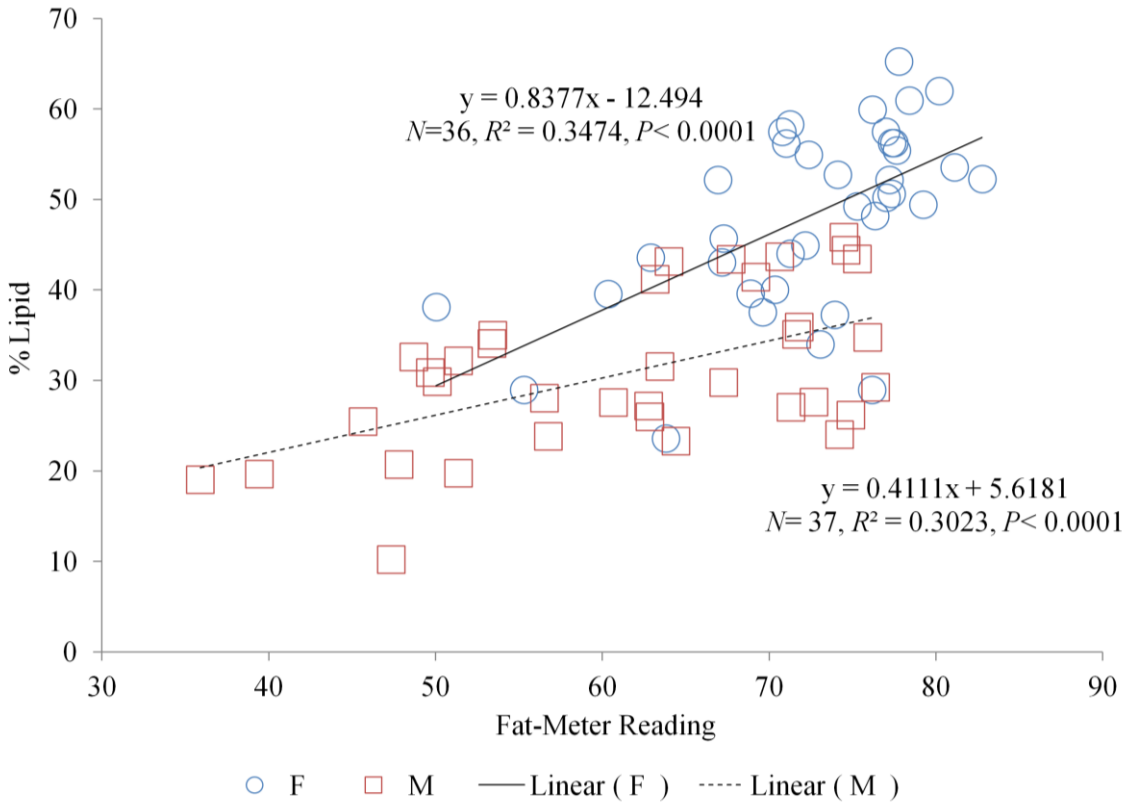


Figure 5. Relationship between Fat-meter readings and proximate lipid values of red muscle of 2012 SAK paddlefish.

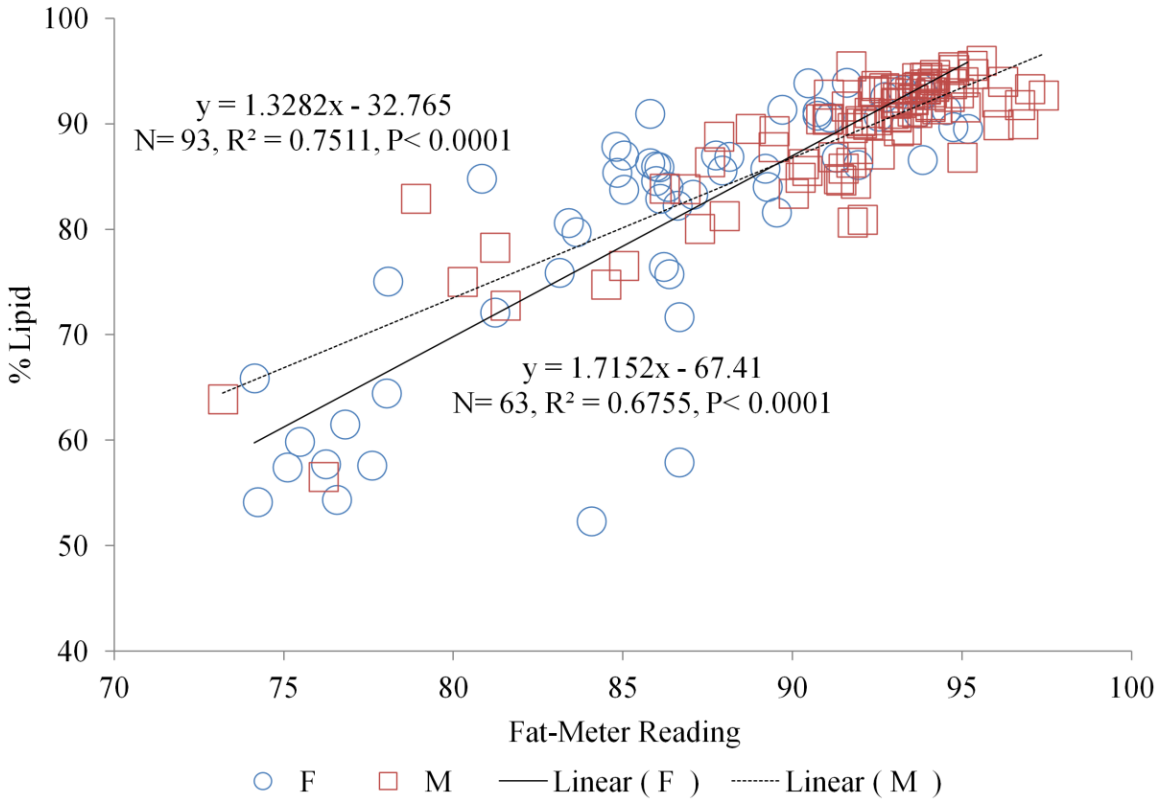


Figure 6. Relationship between Fat-meter readings and proximate lipid values of GFB's of 2012 SAK paddlefish.

## DISCUSSION

The lack of a close relationship between Fulton's condition factor and proximate analysis lipid estimates in age-0 and age-1 fish in 2011 indicates that caution should be applied when using conventional methods of measuring plumpness to estimate lipid content. This agrees with the findings of Jonas (1996) using juvenile muskellunge, who issued a similar caution. The differences between the relationship of condition to proximate results in 2011 and 2012 are likely due to the different distributions observed in lipid values in age-0 fish with respect to length between the years. In 2012, both age-0 fish and age-1 fish that we collected did not reflect the bimodal distribution of lipid values found in 2011 age-0 fish

(Figure 1). Because of the scale of lipid values observed in 2011, although high lipid fish often had more than double the amount of lipid as low lipid fish, the actual percentages were often just a few percent. It is understandable that such small differences are unlikely to result in measurable differences in condition factor. Although condition factor did appear to be significantly related to lipid content in 2012 fish, the potential existence of a high-low lipid scenario in any given year, as well as the inability of condition factor to resolve fine scale differences, suggests that it is risky to attempt to estimate lipid content with condition factor.

In explaining the differences in lipid in fish of similar condition, it may be tempting to suggest that these differences may be an early exhibition of sex-based differences observed in older adults. Although male and female adult paddlefish exhibit distinct lipid allocation strategies, different strategies in male and female fish are unlikely to be the cause of this high/low distribution. When young, both male and female paddlefish are under the same selection pressures (starvation and predation), and will not begin to sexually mature for many years, so there is likely no perceived benefit from sex based allocation differences at an early age. A more likely explanation concerns alternate survival trajectories among age-0 fish, with fish in the two groups representing those fish that will survive and those that will not. Fish with extremely low lipid levels may not be on a trajectory of survival. We did not see this split allocation pattern in 2012 age-0 or age-1 fish. This may be evidence that very low lipid levels is a density dependent response to higher age-0 fish abundance encountered in 2011.

Proximate analysis is widely accepted as a successful technique and is currently approved by both the AOAC and AOCS as an acceptable biochemical method of assessing lipid content (AOAC 2005; AOCS 2005). The performance of the Fat-meter has been extensively compared to biochemical results and found to be capable of good accuracy in

different fishes, including Atlantic salmon *Salmo salar* (Crossin and Hinch 2005), and Atlantic herring *Harengus harengus* (Kent 1990) . The past success of both of these techniques suggest that the failure of the Fat-meter lipid values to correlate with proximate analysis results for white muscle tissue in males could be due to several factors specific to this experiment, including calibration and measurement procedures. First, the lipid content in white muscle was higher than any reported in the literature heretofore. As this was exploratory work, I did not have a close reference value for wild adult paddlefish white muscle lipid content, and the calibration may have been set at the factory in a less effective range than what was encountered in our samples. The meter may also perform better at a higher fat range detection setting. This notion is supported by the observation that although male white muscle lipid values were more homogeneous than females, the meter's results were more closely correlated with proximate analysis results for females (for whom lipids ranged higher than for males). In addition, although product literature advises taking eight readings, I only took three, because the nature of sampling conditions at fish processing stations made eight readings logistically impractical. Another possible explanation concerns the nature of our sampling, which was concerned with lipid of individual fish. The Fat-meter was developed to be highly efficient primarily on batches of eight or more fish, and is known to exhibit a larger range of error (up to 27%) on individual fish (Kent 1990). The relationship between Fat-meter lipid values and proximate lipid values for red muscle tissue was slightly closer for male than for female fish. For both sexes, the relationship was the weakest of any of the tissue types that had significant results. Red muscle lipid had the largest range of any other tissue, which may have made finding a corresponding sensitivity setting more difficult. The nature of samples collected may also have impacted effectiveness. Samples cut from

fillets were very thin, and had to be rolled or folded to attempt to approach recommended thicknesses for fat-meter use. The resultant layering of tissue may have increased errors by introducing air space into the area measured. In contrast, the closer relationship between Fat-meter lipid values and proximate lipid values for GFB tissue demonstrated much better predictability than results seen with the other two tissue types. This closer relationship may have simply been an instance of correct factory calibration for the range of values that were encountered. We expected GFBs to have relatively high lipid levels, and the setting we used on the instrument was calibrated to be sensitive to high values. The instrument performed better on male fish than female fish, perhaps because male fish lipid values were more homogenous than female values.

Results suggest that the Fat-meter may be an acceptable instrument for measuring lipid levels in GFBs. For the muscle tissues, more study is necessary before the Fat-meter is an acceptable measuring instrument for use at paddlefish processing stations. In the time since this study was conducted, a new device (meat Fat-meter) has been developed that measures lipid in processed meat products. This device may be better suited to analysis of adult paddlefish tissues, where mechanical homogenization has the potential to make samples more uniform. Because this study has established reference values for paddlefish tissue lipid, calibration of Fat-meter devices should be facilitated.

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# Appendix 1

Information derived from Thompson's 1934 study of paddlefish rostrum length: Thompson, D. H. 1934. Relative growth of Polyodon. Natural History Survey of Illinois Biological Notes 2: 1-6

Table 1. Relative growth of rostrum in proportion to total length of 87 paddlefish from Thompson (1934) as well as 29 SAK 2012 paddlefish from this study.

| Source of fish | Total Length | Length of rostrum to front of eye | Length of body from front of eye | Rostrum as percent of body length |
|---|---|---|---|---|
| Thompson | 17 | 1.5 | 15.5 | 9.7 |
| | 17.5 | 1.2 | 16.3 | 7.4 |
| | 18.5 | 1.5 | 17 | 8.8 |
| | 19 | 1.6 | 17.4 | 9.2 |
| | 19 | 1.7 | 17.3 | 9.8 |
| | 20 | 1.7 | 18.3 | 9.3 |
| | 20 | 1.8 | 18.2 | 9.9 |
| | 37 | 8 | 29 | 27.6 |
| | 57 | 15 | 42 | 35.7 |
| | 60 | 19 | 41 | 46.3 |
| | 74 | 22 | 52 | 42.3 |
| | 80 | 24 | 56 | 42.9 |
| | 89 | 26 | 63 | 41.3 |
| | 104 | 34 | 70 | 48.6 |
| | 107 | 34 | 73 | 46.6 |
| | 130 | 46 | 84 | 54.8 |
| | 140 | 48 | 92 | 52.2 |
| | 144 | 49 | 95 | 51.6 |
| | 170 | 58 | 112 | 51.8 |
| | 175 | 58 | 117 | 49.6 |
| | 200 | 78 | 122 | 63.9 |
| | 200 | 69 | 131 | 52.7 |
| | 212 | 71 | 141 | 50.4 |
| | 215 | 69 | 146 | 47.3 |
| | 220 | 71 | 149 | 47.7 |
| | 225 | 78 | 147 | 53.1 |
| | 226 | 80 | 146 | 54.8 |
| | 227 | 82 | 145 | 56.6 |
| | 229 | 75 | 154 | 48.7 |
| | 230 | 79 | 151 | 52.3 |
| | 233 | 78 | 155 | 50.3 |
| | 233 | 83 | 150 | 55.3 |
| | 235 | 82 | 153 | 53.6 |

Table 1. (cont.)

| Source of fish | Total Length | Length of rostrum to front of eye | Length of body from front of eye | Rostrum as percent of body length |
|---|---|---|---|---|
| Thompson | 240 | 89 | 151 | 58.9 |
| | 241 | 77 | 164 | 47 |
| | 248 | 83 | 165 | 50.3 |
| | 250 | 92 | 158 | 58.2 |
| | 252 | 88 | 164 | 53.7 |
| | 255 | 87 | 168 | 51.8 |
| | 261 | 89 | 172 | 51.7 |
| | 263 | 92 | 171 | 53.8 |
| | 266 | 85 | 181 | 47 |
| | 267 | 89 | 178 | 50 |
| | 270 | 92 | 178 | 51.7 |
| | 277 | 102 | 175 | 58.3 |
| | 309 | 113 | 196 | 57.7 |
| | 410 | 131 | 279 | 47 |
| | 453 | 155 | 298 | 52 |
| | 514 | 152 | 362 | 42 |
| | 515 | 173 | 342 | 50.6 |
| | 526 | 177 | 349 | 50.7 |
| | 528 | 177 | 351 | 50.4 |
| | 531 | 180 | 351 | 51.3 |
| | 540 | 175 | 365 | 47.9 |
| | 543 | 160 | 383 | 41.8 |
| | 558 | 185 | 373 | 49.6 |
| | 577 | 188 | 389 | 48.3 |
| | 610 | 203 | 407 | 49.9 |
| | 621 | 199 | 422 | 47.2 |
| | 632 | 197 | 435 | 45.3 |
| | 752 | 236 | 516 | 45.7 |
| | 762 | 229 | 533 | 43 |
| | 914 | 259 | 655 | 39.5 |
| | 914 | 245 | 669 | 36.6 |
| | 1000 | 260 | 740 | 35.1 |
| | 1030 | 290 | 740 | 39.2 |

Table 1. (cont.)

| Source of fish | Total Length | Length of rostrum to front of eye | Length of body from front of eye | Rostrum as percent of body length |
|---|---|---|---|---|
| Thompson | 1050 | 310 | 740 | 41.9 |
| | 1070 | 260 | 810 | 32.1 |
| | 1090 | 300 | 790 | 38 |
| | 1118 | 305 | 813 | 37.5 |
| | 1180 | 320 | 860 | 37.2 |
| | 1200 | 340 | 860 | 39.5 |
| | 1210 | 330 | 880 | 37.5 |
| | 1210 | 310 | 900 | 34.4 |
| | 1245 | 333 | 912 | 36.5 |
| | 1295 | 343 | 952 | 36 |
| | 1300 | 330 | 970 | 34 |
| | 1346 | 343 | 1003 | 34.2 |
| | 1499 | 356 | 1143 | 31.1 |
| | 1524 | 404 | 1120 | 36.1 |
| | 1575 | 406 | 1169 | 34.7 |
| | 1600 | 400 | 1200 | 33.3 |
| | 1600 | 419 | 1181 | 35.5 |
| | 1676 | 400 | 1276 | 31.3 |
| | 1702 | 432 | 1270 | 34 |
| | 1753 | 454 | 1299 | 34.9 |
| | 2159 | 432 | 1727 | 25 |
| 2012 SAK | 244 | 88 | 156 | 56.4 |
| | 231 | 82 | 149 | 55 |
| | 242 | 81 | 161 | 50.3 |
| | 257 | 93 | 164 | 56.7 |
| | 205 | 70 | 135 | 51.9 |
| | 227 | 75 | 152 | 49.3 |
| | 257 | 88 | 169 | 52.1 |
| | 282 | 98 | 184 | 53.3 |
| | 229 | 80 | 149 | 53.7 |
| | 190 | 65 | 125 | 52 |
| | 256 | 88 | 168 | 52.4 |
| | 221 | 79 | 142 | 55.6 |
| | 216 | 76 | 140 | 54.3 |

Table 1. (cont.)

| Source of fish | Total Length | Length of rostrum to front of eye | Length of body from front of eye | Rostrum as percent of body length |
|---|---|---|---|---|
| 2012 SAK | 238 | 83 | 155 | 53.5 |
| | 216 | 75 | 141 | 53.2 |
| | 211 | 77 | 134 | 57.5 |
| | 252 | 85 | 167 | 50.9 |
| | 201 | 70 | 131 | 53.4 |
| | 270 | 92 | 178 | 51.7 |
| | 223 | 81 | 142 | 57 |
| | 270 | 96 | 174 | 55.2 |
| | 255 | 88 | 167 | 52.7 |
| | 266 | 93 | 173 | 53.8 |
| | 254 | 91 | 163 | 55.8 |
| | 216 | 73 | 143 | 51 |
| | 208 | 70 | 138 | 50.7 |
| | 255 | 89 | 166 | 53.6 |
| | 205 | 70 | 135 | 51.9 |
| | 227 | 79 | 148 | 53.4 |