

AN EXPANSION OF NONLETHAL TOOLS FOR USE ON
JUVENILE PALLID STURGEON IN THE UPPER
BASIN OF THE MISSOURI RIVER

by

Matea Asahi Djokic

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

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2020

©COPYRIGHT

by

Matea Asahi Djokic

2020

All Rights Reserved

ACKNOWLEDGEMENTS

I thank the technicians, undergrads, and grad students that I have had the privilege of working with for the support they have given me throughout the progression of my thesis. I would like to thank Joshua Heishman for being an awesome technician and for spending long days/nights helping me sample and care for my fish. I would like to thank Taylor Preul for always being willing to proofread and edit my work, being my sounding board, and always cheering me on.

I thank my coadvisors Dr. Christine Verhille and Kevin Kappenman and my other committee member, Dr. Christopher Guy, for the mentorship, support, and feedback that they have given me during my time at Montana State University.

I thank my collaborators at the US Fish and Wildlife Service, Ryan Wilson and Rob Holm, and at Montana Fish, Wildlife, and Parks, Tyler Haddix, John Hunziker, and Zach Shattuck, for providing support throughout my project, helping me collect samples, and making fieldwork fun. I thank everyone at the US Fish and Wildlife Service Bozeman Fish Technology Center, Drs. Gibson Gaylord, Molly Webb, and Wendy Sealey and Jason Ilgen, Matt Toner, Cal Fraser, Jon Gilleen, Zach Conley, and Jason Frost for the training and help they have provided throughout my project. I thank Western Area Power Administration for the funding to make my project possible. I thank MSU Statistical Counseling and Research Services and Dr. Kezia Manlove from Utah State University for statistical support and the MSU Writing Center and Erin Strickland for feedback on the writing of my thesis. Finally, I would like to thank my loving and wonderful family and friends for supporting me throughout this process.

TABLE OF CONTENTS

1. INTRODUCTION TO THESIS	1
Overview of Thesis	9
References	12
2. INVESTIGATIONS AND IMPLICATIONS OF BLOOD BIOCHEMISTRY, ENERGETIC RESERVES, AND VISUAL ASSESSMENTS TO ASSESS HATCHERY-REARED JUVENILE PALLID STURGEON HEALTH	15
Contributions of Authors and Co-Authors	15
Manuscript Information Page	16
Abstract	17
Introduction	18
Methods	23
Study System and Animals	23
Blood Collection for Biochemical Reference Interval Determination	24
Temperature and Feed Manipulation Treatments	24
Physiology and Performance Metrics	26
Proximate Analysis and Blood Biochemistry Laboratory Protocols	29
Proximate Analysis	29
Blood Biochemistry	30
Data Analysis	31
Reference Intervals	31
Principal Component Analysis	33
Linear Regression	35
Moribund Fish	36
Results	36
Blood Biochemical Reference Intervals for Hatchery-reared Juvenile Pallid Sturgeon	36
Relationship Between Physiological Variables and Recent Growth	38
Relationship Between Composite Variables and Percent Growth	44
Physiological Values of Moribund Fish	46
Effects of Feed and Temperature Treatments on Principal Component Scores of Pallid Sturgeon	49
Discussion	49
Expected Blood Analyte Concentrations in Hatchery-reared Juvenile Pallid Sturgeon	49
Relationship Between Physiological Variables and Percent Growth In Pallid Sturgeon	51
Reference Ranges and Post-manipulation Values of Abnormal Fish	54
Summary Conclusion	55

TABLE OF CONTENTS

References.....	58
3. AN ASSESSMENT OF NON-LETHAL TOOLS TO ESTIMATE ENERGETIC RESERVES IN JUVENILE PALLID STURGEON (<i>SCAPHIRHYNCHUS ALBUS</i>).....	64
Contributions of Authors and Co-Authors.....	64
Manuscript Information Page	65
Abstract	66
Introduction.....	67
Methods.....	71
Study System and Animals	71
Fatmeter Measurements	74
Proximate Analysis	77
Data Analysis	78
Results.....	82
Population Energetic Reserves, Mass, Fork Length, and Relative Condition.....	82
Relationships Between Fatmeter Readings and Whole-body Energetic Reserves	82
Discussion	88
References.....	94
4. CONCLUSION TO THESIS	99
References.....	104
APPENDICES	105
APPENDIX A: Blood Analytes Table Outlining Function and Ranges In Sturgeon Species.....	106
APPENDIX B: Experimental Groups Scores on Principal Component 1 And Principal Component 2.....	113
REFERENCES CITED.....	116

LIST OF TABLES

Table	Page
2.1. Percentage of post-manipulation hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) whose values for a given blood biochemical analyte fell outside the detectable range of the VetAxcel blood chemistry analyzer.....	33
2.2. Descriptive statistics of metric measurements for juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) reared under typical hatchery conditions and sampled for baseline measurements included in reference interval determination where relative condition is as defined specifically for pallid sturgeon in the Missouri River Basin (Shuman et al. 2011).....	37
2.3. Blood biochemical reference intervals for hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>), located at Bozeman Fish Technology Center where min is the minimum value measured across all fish and max is the maximum value measured across all fish. Reference intervals were determined by parametric methods when possible and robust methods were used when assumptions of parametric methods were not met (robust determination indicated by *). Analytes are represented by abbreviated terms where TP is plasma total protein, NA is plasma sodium, CL is plasma chloride, MG is plasma magnesium, K is plasma potassium, PHOS is plasma phosphorous, CA is plasma calcium, CHOL is plasma cholesterol, ALB is plasma albumin, GLU is plasma glucose, ALP is plasma alkaline phosphatase, LDH is plasma lactate dehydrogenase, and HB is whole-blood hemoglobin	38
2.4. Descriptive statistics of metric measurements, energetic reserves (whole-body energy and lipid content determined by proximate analysis), and percent growth for hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) subjected to experimental feed and water temperature manipulations aimed at inducing variation in energetic reserves and chronic stress levels in the population	39

LIST OF TABLES CONTINUED

Table	Page
2.5. Comparisons of physiological variables included in the first three principal components for moribund hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) with the reference range (Ref Range; determined on pre-experimental fish) and post-manipulation average (within one standard deviation (SD) of the average determined on post-experimental fish). MF1 is Moribund Fish 1, MF2 is Moribund Fish 2, VHA is visual health assessment, WB is whole body, HB is hemoglobin, ALB is albumin, CA is calcium, TP is total protein, LDH is lactate dehydrogenase, ALP is alkaline phosphatase, GLU is glucose, CHOL is cholesterol PHOS is phosphorous, MG is magnesium, Kn is relative condition (Shuman et al. 2011), and NA is sodium.	48
3.1. List of fish species for which Distell Fatmeter readings have been tested on to predict whole-body lipid content and whole-body energy content with R-squared values and references	69
3.2. Descriptive statistics of energetic reserves (whole-body energy and whole-body lipid content determined through proximate analyses), relative condition (Kn), fork length, and mass in a population of hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) (n = 126 experienced water temperature and diet manipulations over the course of four months; n = 6 were reared under typical hatchery conditions) where SD is standard deviation.....	82
3.3. Akaike's Information Criteria (AICc) scores and coefficients of determination (R^2) from single linear regressions in which natural logarithmically transformed (ln) Fatmeter readings from individual positions and averages of multiple positions significantly contributed to a model predicting whole-body lipid and energy content of hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>). Abbreviations are as follows: ln is natural log transformed, UM is Upper Medial Fatmeter measurement, UA is Upper Anterior Fatmeter measurement, UP is Upper Posterior Fatmeter measurement, and avg is the average of multiple Fatmeter measurements.	83

LIST OF TABLES CONTINUED

Table	Page
3.4. Akaike's Information Criteria (AICc) scores and coefficients of determination (R^2) from competitive (models within 2 AICc values of $AICc_{min}$) multiple linear regressions (MLR) and individual variables in those MLR models for hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) in estimating whole-body energy and lipid content where Kn is relative condition, FL is fork length, UA is Upper Anterior Fatmeter measurement, UM is Upper Medial Fatmeter measurement and ln is natural logarithmically transformed (ln), and avg is an average of multiple Fatmeter measurement sites.....	88

LIST OF FIGURES

Figure	Page
1.1. Map (USFWS 2014) denoting the pallid sturgeon (<i>Scaphirhynchus albus</i>) management units: Great Plains Management Unit (GPMU), Central Lowlands Management Unit (CLMU), Interior Highlands Management Unit (IHMU), and Coastal Plains Management Unit (CPMU) as defined by the Revised Recovery Plan for the Pallid Sturgeon (USFWS 2014). The upper basin of the Missouri River is part of the GPMU from Great Falls, MT to Gavins Point Dam, SD.....	2
1.2. Pallid sturgeon (<i>Scaphirhynchus albus</i>) mean fork length at age (mm), grouped by year-classes, in segment 4, a subsection of the upper Missouri River basin (Wilson et al. 2017)	4
2.1. Correlation matrix of a subset of the physiological variables included in principal component analysis where TP is total protein, NA is sodium, CL is chloride, MG is magnesium, CA is calcium, ALB is albumin, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) across a hatchery experiment with varying feed and water temperature manipulations. See Table 3 for axis units associated with each variable	40
2.2. Correlation matrix of a subset of the physiological variables included in principal component analysis where K is total potassium, CHOL is cholesterol, GLU is glucose, ALP is alkaline phosphatase, LDH is lactate dehydrogenase, VHA is visual health assessment, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) across a hatchery experiment with varying feed and water temperature manipulations. See Table 3 for axis units associated with each variable.....	41

LIST OF FIGURES CONTINUED

Figure	Page
2.3. Correlation matrix of a subset of the physiological variables included in principal component analysis where HB is hemoglobin, Lipid is whole-body lipid content, Energy is whole-body energy content, Protein is whole-body protein content, Kn is relative condition, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) across a hatchery experiment with varying feed and water temperature manipulations. See Table 3 for axis units associated with each variable	42
2.4. Percentage of variance accounted for by the first three principal components (PCs), as determined through principal component analysis, that account for the majority of variance (54.2%) in the hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) dataset of fish reared at nine combinations of feed and water temperature treatments for four months.....	43
2.5. Correlations between physiological variables and the first three principal components (PCs) determined through principal component analysis on a population of hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) reared under various feed and water temperature treatments at Bozeman Fish Technology Center to induce variation in energetic reserves and chronic stress levels in the population. Physiological variables are represented by abbreviated terms where VHA is visual health assessment, Kn is relative condition, Protein is whole-body protein, Energy is whole-body energy, Lipid is whole-body lipid, HB is whole-blood hemoglobin, LDH is plasma lactate dehydrogenase, ALP is plasma alkaline phosphatase, GLU is plasma glucose, ALB is plasma albumin, CHOL is plasma cholesterol, CA is plasma calcium, PHOS is plasma phosphorus, K is plasma potassium, MG is plasma magnesium, CL is plasma chloride, NA is plasma sodium, and TP is plasma total protein. Bars in black indicate variables that are considered to strongly correlate (positively or negatively) relative to other variables with PCs while bars in gray do not strongly correlate	45

LIST OF FIGURES CONTINUED

Figure	Page
2.6. Linear regression of percent growth calculated across the experimental period on principal component one (PC1) and principal component two (PC2) of juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) exposed to nine different combinations of feed and water temperature manipulations for a four-month period aimed at inducing variation in energetic reserves and chronic stress levels at the Bozeman Fish Technology Center.....	46
3.1. Outline of side-view and abdominal view of pallid sturgeon (<i>Scaphirhynchus albus</i>) indicating twelve total preliminary measurement sites of the Distell Fatmeter to assess the whole-body energy content and whole-body lipid content of hatchery-reared juvenile pallid sturgeon in a pilot study. A) Preliminary measurement sites on adult fish included three measurement sites dorsal and ventral of the scutes and three on the abdomen. B) Preliminary measurement sites on small juvenile fish included one site dorsal and ventral of the scutes and one on the abdomen.....	76
3.2. Outline of side-view of finalized measurement sites for the Distell Fatmeter used to assess the whole-body energy content and whole-body lipid content of hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) in the manipulation study. A) For fish large enough to fit two distinct anterior and posterior measurement sites, three finalized measurement sites were identified: Upper Anterior (UA), Upper Posterior (UP), and an overlapping medial site, Upper Medial overlap (Umol). B) Finalized Upper Medial (UM) measurement site on fish that were too small to fit multiple overlapping measurement sites).....	77
3.3. Best fit multiple linear regression models predicting (A) whole-body lipid and (B) whole-body energy content in hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) across all sizes of fish, using only medial Fatmeter measurements, included in the study where Kn is relative condition, lnMass is natural log transformed mass, and the colored gradient represents energy content of individuals where blue is low and red is high.....	85

LIST OF FIGURES CONTINUED

Figure	Page
3.4. Best fit multiple linear regression models predicting (A) whole-body lipid and (B) whole-body energy content in hatchery-reared juvenile pallid sturgeon (<i>Scaphirhynchus albus</i>) across only fish large enough to fit multiple Fatmeter measurement sites included in the study where lnUA is natural log transformed Fatmeter readings at the Upper Anterior site, lnMass is natural log transformed mass, and the colored gradient represents energy content of individuals where blue is low red is high	87

ABSTRACT

Pallid sturgeon (*Scaphirhynchus albus*) are an endangered species endemic to the Missouri and Mississippi drainages in North America. Mitigation activities including conservation spawning and stocking programs were implemented to prevent extinction of the species. Monitoring of growth, survival, and condition are conducted annually to determine efficacy of managerial efforts to recover pallid sturgeon. My thesis aimed to establish a knowledge base of juvenile pallid sturgeon physiological bioindicators that can supplement field monitoring of growth, size, and condition measurements. Ultimately, I aimed to resolve mechanisms underlying population fluctuations (e.g. growth rate) caused by environmental changes or management actions. Fisheries managers have observed reduced growth rates of recently stocked hatchery origin pallid sturgeon (HOPS) relative to earlier cohorts. Based on high survival rates of historic stocked HOPS, some fisheries biologists hypothesize that growth rates of recent HOPS cohorts reflect compensatory density dependence in growth caused by overstocking. However, size, growth, and condition metrics commonly tracked on wild-captured sturgeon cannot assess underlying mechanisms driving trends in population growth rate. The purpose of this hatchery-based research was to explore nonlethal physiological measurements to expand the toolset available to managers to assess the status of physiological processes within HOPS that may reflect ecosystem effects on these fish. I investigated a suite of physiological bioindicators that could reflect key physiological processes (nutrition, tissue damage, and chronic stress) expected to respond to common ecosystem stressors. I determined reference intervals for 13 blood biochemical analytes on a population of healthy hatchery-reared juvenile pallid sturgeon ($n = 41$). Inter-individual variation in blood biochemical analytes and five additional physiological variables were analyzed to determine composite variables predictive of growth. To investigate nonlethal tools to quantify whole-body energetic reserves of juvenile pallid sturgeon, I determined best-fit predictive models to estimate whole-body energy ($df = 33$; $R^2 = 0.40$; $p < 0.001$) and lipid ($df = 33$, $R^2 = 0.45$, $p < 0.001$) content using Distell Fatmeter and mass measurements. Research presented in this thesis aims to provide a preliminary understanding of expected ranges of various physiological bioindicators and methods for nonlethally measuring these bioindicators in hatchery-reared juvenile pallid sturgeon.

INTRODUCTION TO THESIS

The pallid sturgeon (*Scaphirhynchus albus*) is an endangered species whose decline is attributed to the culmination of several anthropogenic factors throughout the native range of the Missouri and Mississippi rivers in North America (USFWS 1990). In response to the decline of the species, hatchery-origin pallid sturgeon (HOPS) propagation and stocking have occurred since 1998. Generally, the toolset managers use in annual pallid sturgeon population monitoring assessments is limited to observations and subsequent calculations such as relative condition (Kn), length, mass, and growth rate. These tools have the potential to elucidate correlative relationships between pallid sturgeon status and environmental change but are not able to reveal the mechanisms underlying these changes. Additionally, managers generally require accumulation of size, relative condition, and growth data over multiple years to detect trends in a population, causing a lag in time before biologists can assess and respond to environmental effects on individuals or populations. Therefore, with common tools, managers are limited in their ability to assess mechanisms underlying pallid sturgeon responses to ecosystem changes and management restoration efforts (e.g., pallid sturgeon stocking) as those responses are occurring within the pallid sturgeon population. My thesis investigates non-lethal tools that could be applied by fisheries biologists to supplement their current toolset to elucidate underlying physiological mechanisms that may be driving individual responses to environmental change.

There are four Management Units in the Missouri and Mississippi Rivers designated for the management of pallid sturgeon (Figure 1.1) (USFWS, 2014). For my

thesis research, I focused on questions regarding the population of juvenile pallid sturgeon in the upper basin of the Missouri River (UBMR), part of the Great Plains Management Unit (Figure 1.1).

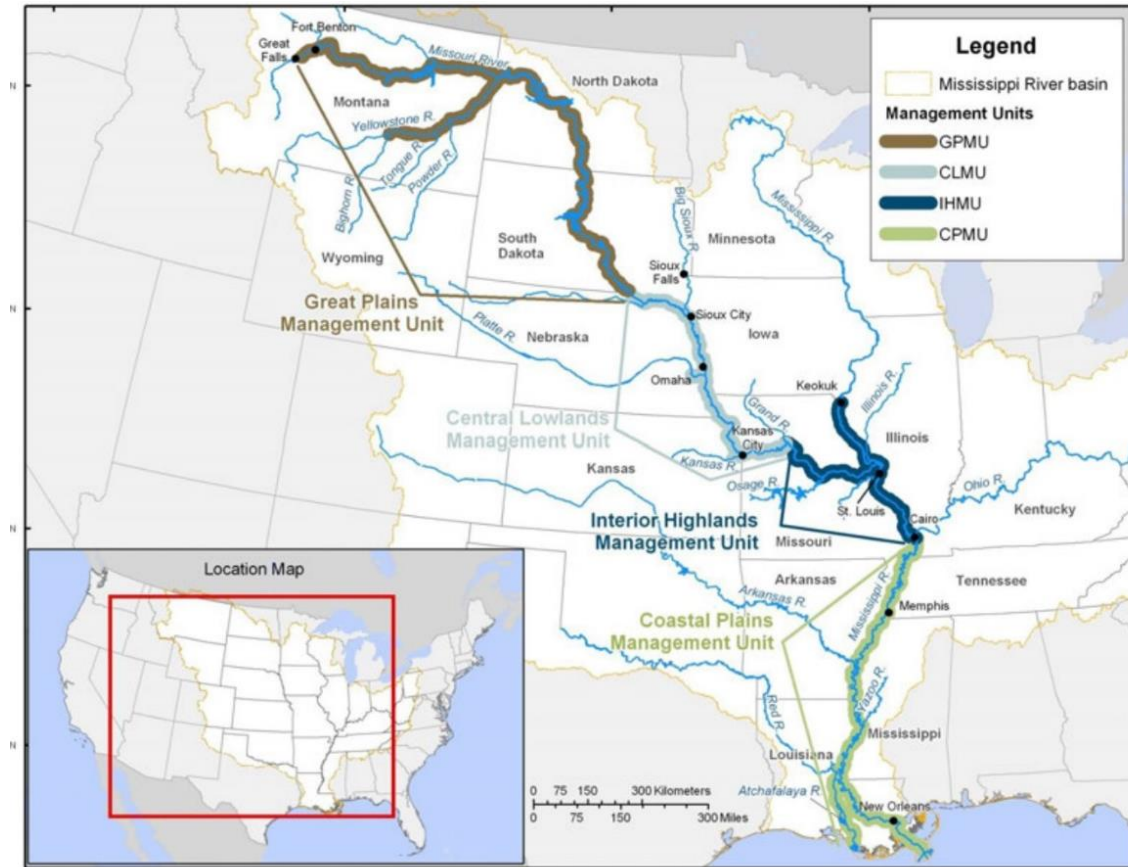


Figure 1.1. Map (USFWS 2014) denoting the pallid sturgeon (*Scaphirhynchus albus*) management units: Great Plains Management Unit (GPMU), Central Lowlands Management Unit (CLMU), Interior Highlands Management Unit (IHMU), and Coastal Plains Management Unit (CPMU) as defined by the Revised Recovery Plan for the Pallid Sturgeon (USFWS 2014). The upper basin of the Missouri River is part of the GPMU and ranges from Great Falls, MT to Gavins Point Dam, SD.

Pallid sturgeon propagation and stocking occurs to sustain different age classes in the ecosystem and prevent extinction until managers can define issues surrounding

species survival and recruitment (UBPSPC 2005). Initial stocking efforts began in 1998 and became annual occurrences in the UBMR in 2002 (USFWS 2008). The Upper Basin Pallid Sturgeon Propagation Plan (UBPSPP) revised policies put forward by the Pallid Sturgeon Propagation/Genetics Plan with the information gathered in the following decade, specifically for use in the UBMR (UBPSPC 2005). Since 1998, approximately 300,000 HOPS have been stocked into the UBMR (Rotella 2017) and stocking continues today (USFWS 2018).

A recent study revealed relatively high rates of survival among stocked HOPS (Rotella 2017). For example, in 2016 approximately 72% of fish classified in the age-5 to age-10-year classes (stocked from 2006 to 2011) were estimated to survive (Rotella 2017). Stocking, at reduced numbers of fish with the goal to increase genetic diversity, and monitoring of HOPS survival has continued despite not knowing the carrying capacity of pallid sturgeon in the UBMR.

Fisheries managers have observed a decline in growth rates of recently stocked pallid sturgeon in the UBMR during routine monitoring. For example, in a subsection of the UBMR, annual population assessments have recorded reduced growth rates in more recently stocked year-classes of pallid sturgeon compared to their earlier stocked counterparts (Wilson et al. 2017). The 2002 year-class of stocked HOPS exhibited a higher average growth rate than the 2006-2009 year-classes that were, on average, 191 mm smaller than the 2002 year-class at age 10 (Wilson et al. 2017; Figure 1.2).

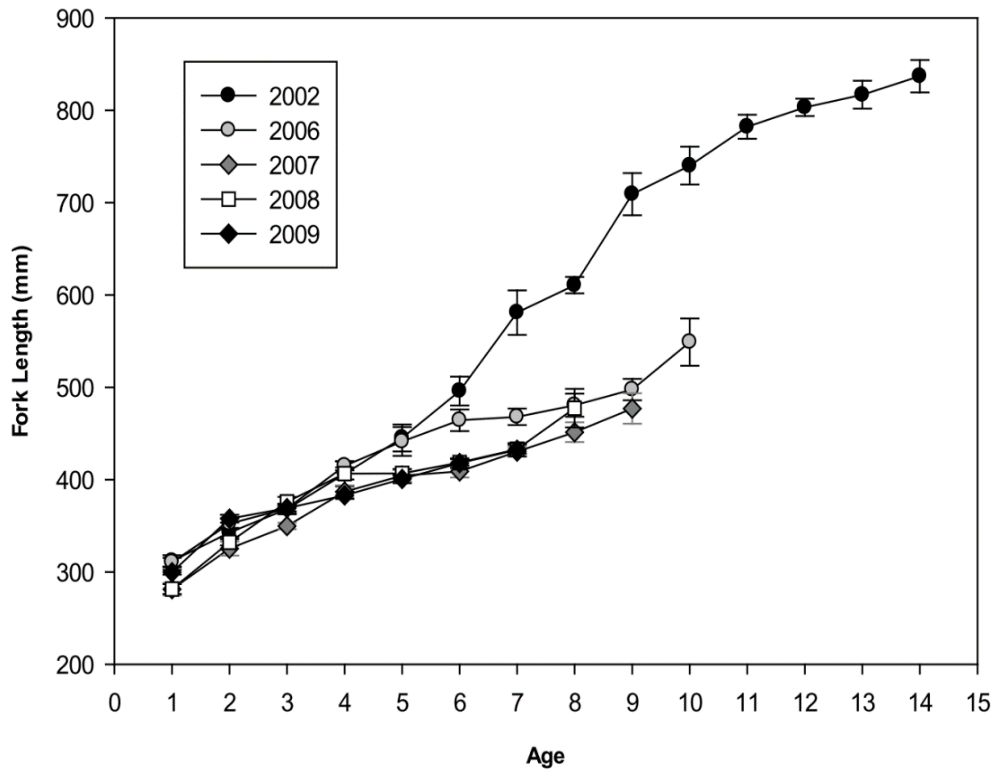


Figure 1.2. Pallid sturgeon (*Scaphirhynchus albus*) mean fork length (mm) at age, grouped by year-classes, in segment 4, a subsection of the upper Missouri River basin (Wilson et al. 2017).

Changes in competition for food and habitat resources resulting from changes in stocking of juvenile pallid sturgeon may be two factors influencing the difference in growth rates observed between earlier and later stocked HOPS. Intraspecific competition for food sources may prevent pallid sturgeon in the wild from meeting the energetic demands necessary to achieve growth. Previous studies have shown that growth rate generally decreases with increased stocking density of hatchery-reared great sturgeon (*Huso huso*) (Rafatnezhad et al. 2008) and hatchery-reared Amur sturgeon (*Acipenser schrenckii*) (Li et al. 2012). These data are concurrent with a hypothesis that pallid sturgeon growth rate may be affected by density-dependent competition; however, this

has not been assessed in the wild. Additionally, growth rates of sustainable wild juvenile pallid sturgeon populations have never been determined, making interpretation of these observed trends in growth rate unclear. An expanded toolset that includes physiological measurements, such as blood biochemistry, body composition measurements, and assessment of visual appearance, can help fisheries biologists understand the mechanisms underlying responses of individual HOPS to environmental changes and management decisions.

Conservation physiology is a scientific field that applies physiological approaches to understanding the effects of environmental factors on organisms, populations, and ecosystems (Seebacher and Franklin 2012). Many of the patterns detected between environmental changes and population changes are based on correlational inference rather than understanding of the underlying mechanism driving population responses. Ecosystem effects on populations are filtered through responses of physiological processes at the individual level. Thus, monitoring the status of physiological processes within individuals can be informative of the mechanisms driving overall population responses to habitat change. Conservation physiology approaches focus on identifying causal mechanisms driving organismal responses to their environment through assessing physiological status of individuals to inform management decisions (Wikelski and Cooke 2006; Seebacher and Franklin, 2012). Physiological measurements have become important for managers to promptly perceive the effects of management actions (e.g., stocking) on individuals of at-risk species (Wikelski and Cooke 2006). Thus, physiological approaches may provide fisheries biologists with causal mechanisms

behind HOPS response to management efforts and environmental change as well as allow managers to observe these effects in real time, therefore, providing a better-informed path for management decisions for the conservation of pallid sturgeon.

Several physiological variables, including blood biochemistry, can be applied to identify mechanistic links between ecosystem conditions and health of individuals that length, mass, and relative condition measurements are less informative of. Blood biochemical variables can provide information about nutritional status, tissue damage, stress, organ function, metabolic disturbances, and disease (Wagner and Congleton 2004; Ferri et al. 2011; Sepúlveda et al. 2012). Therefore, comparisons of analyte values sampled from individual fish to typical ranges of these analytes may allow fisheries biologists to make inferences on health of the fish. Furthermore, blood biochemical variables that correlate have been applied as indicators of specific areas of health including nutritional status, chronic stress, and tissue damage in salmonids (Wagner and Congleton 2004). Equipping managers with the knowledge to link specific analytes with specific areas of health could allow for interpretation of the physiological process impacted by management actions and ecosystem changes and, thus, improve identification of the mechanisms underlying population level responses of pallid sturgeon to these conditions. By expanding this approach to incorporate other physiological variables, such as whole-body composition and a visual health assessment, I propose to develop a tool for managers to assess nutritional status, tissue damage, and chronic stress of pallid sturgeon.

Body composition (whole-body energy and whole-body lipid content) is indicative of nutritional status and impacts successful navigation of ecologically relevant life events such as overwintering (Deslauriers et al. 2018) and reproduction (Young et al. 2006). However, whole-body energy and whole-body lipid content are typically measured through lethal means, which is not a viable approach for extensive tracking in wild-captured pallid sturgeon. Thus, it would be valuable to investigate a non-lethal alternative to estimate whole-body lipid and energy content in juvenile pallid sturgeon. The Distell Fatmeter (Distell, West Lothian, Scotland, UK) is a hand-held device that uses a microwave sensor to non-destructively determine the lipid content of a sample, which makes this device a non-lethal alternative to proximate analysis. Recently, the Distell Fatmeter has been validated to estimate whole-body energetic reserves in several fish taxa (Colt and Shearer 2001; Vogt et al. 2002; Crossin and Hinch 2005; Bayse et al. 2018). However, to my knowledge, the Fatmeter has not been validated in any sturgeon species. Therefore, the Fatmeter may be a promising tool capable of equipping fisheries biologists with the ability to non-lethally estimate energetic reserves in pallid sturgeon.

Visual assessments are noninvasive and can reveal important physiological information about an individual such as presence of disease (Huizinga et al. 1979), physical deformities, or pollutant exposure (Khan 2003). For example, skin pigmentation is used as a metric by managers to assess the acute stress level of wild-caught pallid sturgeon in the field (R. Wilson, personal communication, 2018). Although the relationship between skin redness and stress is generally unexplored in fish, it is likely a result of increased vasodilation as, in humans, vasodilation occurs to meet an increased

metabolic demand in response to stress (Momen et al. 2009). Elevated metabolic demands are maintained during chronic stress in sturgeon (Lankford et al. 2005) and, therefore, skin redness may be a metric of chronic stress in sturgeon when measured prior to the onset of acute redness. Visual assessments can also be applied to quantify deformities such as fin curl (Deslauriers et al. 2016) or injuries such as fin erosion (Kindschi 1987) that may hinder the survival of hatchery-reared pallid sturgeon in the wild (Oldenburg et al. 2011).

Combining visual assessments with quantified blood biochemical parameters and energetic reserves may provide an informative suite of tools capable of aiding fisheries biologists to monitor impacts of habitat change and management actions on HOPS. However, typical ranges and physiological implications of these variables are not well studied in juvenile pallid sturgeon. Thus, exploring blood biochemistry, energetic reserves, and visual health assessments may form indicators of pallid sturgeon health, expanding the toolset available to biologists to assess the status and health of the species while also filling a gap in the literature. To address this information gap, I performed a comprehensive study to determine how blood biochemistry, energetic reserves, and visual health relate to each other and to performance metrics (i.e., percent growth) in a population of hatchery-reared juvenile pallid sturgeon with varying health statuses induced by long-term feed and water temperature treatments. Feed and water temperature treatments were used to induce variation in energetic reserves and chronic stress levels as they are ecologically relevant challenges that fish would likely experience

in the wild due to seasonal fluctuations in water temperature and food availability (i.e., overwintering, competition for limited food resources).

Overview of Thesis

In order to expand the tools available to fisheries biologists to assess the status of HOPS in the UBM, my goal was to investigate the following question: Are there non-lethal physiological measurements that can be applied by managers to identify the mechanisms underlying effects of ecosystem conditions and management actions on HOPS populations? To address this question, I identified groups of physiological variables that correlate among juvenile pallid sturgeon and how they can predictively model recent growth (Chapter 2). Then, in Chapter 3, I validated a tool to nonlethally quantify one of the important, but lethally determined, variables identified in Chapter 2 for juvenile pallid sturgeon.

Chapter 2 describes a hatchery study approach in which I isolated a suite of physiological variables for application to juvenile pallid sturgeon. The objectives of this study were 1) to define ranges of physiological variables expected to be observed in pallid sturgeon reared under typical hatchery conditions and how they correlate in a population of fish with varying energetic reserves and chronic stress levels induced by feed and water temperature manipulation and 2) to determine the relationship between the physiological variables in Objective 1 and an ecologically relevant performance metric (i.e., growth rate). I predicted that the majority of the variance in my dataset would be accounted for by three composite variables consisting of physiological measures that correlate with one another. Additionally, I expected that these composite variables would

each reflect one of three indicators of health: nutritional indicator, chronic stress indicator, and a tissue damage indicator and that a model made up of these components would predict recent growth of the hatchery population. Defining health indicators and relating them to an ecologically relevant performance variable is a novel approach and, to the best of my knowledge, has not been investigated in any sturgeon species.

In order to investigate the composite variables in my hypothesis, I used principal component analysis (PCA). The variables included in the PCA were 13 blood biochemical analytes, whole-body protein, lipid, and energy content, and a visual health inspection. In Chapter 2, I showed that whole-body energetic reserves, relative to the other measured variables, were important to the ability of a composite variable to explain the variation among the experimental fish. However, whole-body energetic reserves are determined by lethal means, so I investigated non-lethal alternatives to estimate whole-body energetic reserves in Chapter 3.

In Chapter 3, I describe my efforts to test the Distell Fatmeter as a non-lethal alternative to estimating the whole-body energetic reserves of juvenile pallid sturgeon. The objective of the study described in this chapter was to develop a best-fit model to estimate whole-body energetic reserves of juvenile pallid sturgeon using Fatmeter readings and common non-lethal measurements including relative condition, mass, and fork length as predictor variables. Based on previous research on other taxa of fish (Crossin and Hinch 2005; Colt and Shearer 2001), I predicted that the Distell Fatmeter would be informative to estimating energetic reserves of juvenile pallid sturgeon.

Chapter 4 is the conclusion of my thesis that summarizes the main results and conclusions from Chapters 2 and 3. Chapter 4 also discusses the potential implications of the findings of this research on pallid sturgeon literature and management.

References

- Bayse, S.M., Regish, A.M., McCormick, S.D. 2018. Proximate composition, lipid utilization and validation of a non-lethal method to determine lipid content in migrating American shad *Alosa sapidissima*. *Journal of Fish Biology*, 92: 1832-1848.
- Colt, J., Shearer, K.D. 2001. Evaluation of the use of the Torry Fatmeter to Non-lethally Estimate Lipid in Adult Salmon. U.S. Army Corps of Engineers. 75 pp.
- Crossin, G.T., Hinch, S.G. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. *Transactions of the American Fisheries Society*, 134(1): 184-191.
- Deslauriers, D., Johnston, R., Chipps, S.R. 2016. Effect of Morphological Fin-Curl on the Swimming Performance and Station-Holding Ability of Juvenile Shovelnose Sturgeon. *Journal of Fish and Wildlife Management*, 7(1): 198-204.
- Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., Anderson, W.G. 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs. *Environmental Biol Fish*, 101: 623-637.
- Ferri, J., Popovic, N.T., Coz-Rakovac, R., Beer-Ljubic, B., Strunjak-Perovic, I., Skeljo, F., Jadan, M., Petric, M., Barisic, J., Simpraga, M., Stanic, R. 2011. The effect of artificial feed on blood biochemistry profile and liver histology of wild saddled bream, *Oblada melanura (spretidae)*. *Marine Environmental Research*, 71: 218-224.
- Huizinga, H. W., G. W. Esch, and T. C. Hazen. 1979. Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides (Lacepede)*. *Journal of Fish Diseases* 2(4):263–277.
- Khan, R.A. 2003. Health of flatfish from localities in Placentia Bay, Newfoundland, contaminated with petroleum and PCBs. *Environmental Contamination and Toxicology*, 44: 485-492.

- Kindschi, G.A. 1987. Method of Quantifying Degree of Fin Erosion. *The Progressive Fish-Culturist*, 49(4): 314-315.
- Li, D., Liu, Z., Xie, C. 2012. Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, *Acipenser schrenckii*. *Fish Physiology and Biochemistry*, 38(2): 511-520.
- Oldenburg, E.W., Guy, C.S., Cureton, E.S., Webb, M.A.H., Gardner, W.M. 2011. Effects of acclimation on poststocking dispersal and physiological condition of age-1 pallid sturgeon. *Journal of Applied Ichthyology*, 27: 436-443.
- Rafatnezhad, S., Falahatkar, B., Gilani, M.H.T. 2008. Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. *Aquaculture Research*, 39: 1506-1513.
- Rotella, J. 2017. Upper Basin Pallid Sturgeon Survival Estimation Project 2017 Update.
- Seebacher, F., Franklin, C.E. 2012. Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. *Philosophical Transaction of the Royal Society B*, 367: 1607-1614.
- Sepúlveda, M.S., Sutton, T.M., Patrick, H.K., Amberg, J.J. 2012. Blood chemistry values for shovelnose and lake sturgeon. *Journal of Aquatic Animal Health*, 24(3): 135-140.
- UBPSPC [Upper Basin Pallid Sturgeon Propagation Committee]. 2005. Upper basin pallid sturgeon propagation plan.
- USFWS [United States Fish and Wildlife Service]. 1990. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Pallid Sturgeon. *Federal Register*, 55(173): 36641-36647.
- USFWS [United States Fish and Wildlife Service]. 2008. Pallid Sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. Billings, Montana.

- USFWS [U.S. Fish and Wildlife Service]. 2014. Revised Recovery Plan for the Pallid Sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Denver, Colorado. 115 pp.
- USFWS [U.S. Fish and Wildlife Service]. 2018. Revised Pallid Sturgeon *Scaphirhynchus albus* Range-wide Stocking Plan. Denver, CO. 44 pp.
- Vogt, A., Gormley, R., Downey, G., Somers, J. 2002. A Comparison of Selected Rapid Methods for Fat Measurement in Fresh Herring (*Clupea harengus*). Journal of Food Composition and Analysis, 15: 205-215.
- Wagner, T., Congleton, J.L. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences, 61: 1066-1074.
- Wikelski, M., Cooke, S.J. 2006. Conservation physiology. Trends in Ecology and Evolution, 21(2): 38-46.
- Wilson, R., S. Hultberg, and Z. Sandness. 2017. 2016 Annual Report Pallid Sturgeon Population Assessment and Associated Fish Community Monitoring for the Missouri River : Segment 4. U.S. Fish and Wildlife Service, Bismarck, North Dakota.
- Young, J. L., S. G. Hinch, S. J. Cooke, G. T. Crossin, D. A. Patterson, A. P. Farrell, G. van der Kraak, A. G. Lotto, A. Lister, M. C. Healey, and K. K. English. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 63(5):1067–1077.

INVESTIGATIONS AND IMPLICATIONS OF BLOOD BIOCHEMISTRY,
ENERGETIC RESERVES, AND VISUAL ASSESSMENTS TO ASSESS
HATCHERY-REARED JUVENILE PALLID STURGEON HEALTH

Contributions of Authors and Co-Authors

Manuscript in Chapter 2

Author: Matea A. Djokic

Contributions: Implemented the study, collected and analyzed data, and wrote the manuscript

Co-Author: Kevin Kappenman

Contributions: Obtained funding, collected data, conceived the study design, discussed the implications of the results, and provided guidance on the manuscript.

Co-Author: Joshua Heishman

Contributions: Collected data and provided guidance on the manuscript.

Co-Author: Kezia Manlove

Contributions: Provided guidance on the statistical analyses in the manuscript.

Co-Author: Christine Verhille

Contributions: Obtained funding, collected data, conceived the study design, discussed the implications of the results, and provided guidance on the manuscript.

Manuscript Information Page

Matea A. Djokic, Kevin Kappenman, Joshua Heishman, Kezia Manlove, Christine

Verhille

Status of Manuscript:

- Prepared for submission to a peer-reviewed journal
- Officially submitted to a peer-reviewed journal
- Accepted by a peer-reviewed journal
- Published in a peer-reviewed journal

ABSTRACT

Unexplained declines in growth rates of hatchery origin pallid sturgeon (HOPS; *Scaphirhynchus albus*) within Upper Missouri River Basin habitats have emphasized the need to develop physiological biomarkers that can help to identify the mechanisms underlying ecosystem and management effects on HOPS populations. Blood biochemistry, energetic stores, and visual assessments are physiological measures that can be applied as bioindicators of direct impacts of the ecosystem on the physiological processes within individual fish. As ecosystem effects on populations are filtered through individual physiological responses, these physiological bioindicators can elucidate the causal mechanisms underlying population fluctuations and reduce reliance on non-causal correlational observations. However, in pallid sturgeon, many of these measures have gone unexplored and are not well understood. This study described physiological variables indicative of nutrition-, chronic stress-, and tissue damage- related physiological processes in HOPS reared under typical hatchery conditions as well as manipulated conditions aimed at maximizing the variation in these physiological processes within the population. Expected reference ranges for 13 blood biochemical analytes in juvenile pallid sturgeon ($n = 41$) reared under typical hatchery conditions were determined. The same blood biochemical analytes and an additional five physiological variables were also quantified in the post-manipulation population of sturgeon. Principal component analysis was then performed in the post-manipulation dataset and identified three principal components (PCs) that explained a cumulative 54% of the variation in the dataset. Multiple linear regression revealed that the first two of these PCs accounted for 45% of the variation in growth that occurred within the experimental population during the four-month rearing condition manipulation ($df = 124$; $R^2 = 0.454$; $AIC = 1207.8$; $p < 0.001$). Thus, the physiological variables making up these first two PCs have the potential of application as bioindicators of recent growth in wild-captured HOPS. Although findings must be validated on wild-captured HOPS before application, the physiological bioindicators identified here have the potential to inform fisheries biologists on the causal mechanisms underlying population responses to ecosystem or management changes. This approach could allow rapid management responses and reduce reliance on correlational evidence.

Introduction

The pallid sturgeon (*Scaphirhynchus albus*) is a long-lived, endangered species endemic to the Missouri and Mississippi rivers in North America (USFWS 1990). Extensive conservation measures have been implemented through the Pallid Sturgeon Recovery Plan (USFWS 2014) to prevent the species from becoming extinct. Mitigation and conservation activities are conducted together to track the efficacy of recovery efforts. Recent growth trends observed through ongoing monitoring efforts have biologists concerned that density dependence in HOPS growth (Rose et al. 2001) may be occurring in the upper basin of the Missouri River (UBMR), part of the Great Plains Management Unit (USFWS 2014; Rotella 2017). Density-dependent effects on population growth act at the individual fish level through altered physiological processes. The obvious physiological processes predicted to be impacted by high population densities include down regulation of processes such as fat deposition or skeletal growth associated with reduced food consumption, which later manifests as reduced growth. However, high population densities may also impact growth indirectly through conspecific competitive interactions that activate stress pathways causing diversion of energy away from growth-related processes, resulting in low food conversion efficiency and reduced growth (Li et al. 2012). Monitoring the status of physiological processes within individual HOPS can elucidate the mechanisms underlying impacts of ecosystem conditions on population level growth, survival, and relative condition. Therefore, this study aims to identify and characterize key physiological indicators of nutrition, chronic stress, and tissue damage that could help

fisheries biologists assess how ecosystem conditions, such as the potential competition for limited nutritional resources, could be affecting individual HOPS.

Pallid sturgeon were declared endangered under the Endangered Species Act (ESA) in 1990 due to anthropogenic factors such as the effects of habitat degradation and commercial harvesting on populations throughout their range (USFWS 1990). Several efforts including the Pallid Sturgeon Recovery Plan (USFWS 1993) and the Upper Basin Pallid Sturgeon Propagation Plan (UBPSPP 2005) were implemented to monitor the range-wide status of pallid sturgeon and guide mitigation efforts to avoid extinction of the species. Monitoring efforts include mass and length measurements of captured fish with associated estimates of survival, relative condition, and growth. Mitigation efforts include augmentation of the wild populations through propagation and hatchery origin pallid sturgeon (HOPS) stocking throughout the range of the species (USFWS 2018; Range-wide Pallid Sturgeon Propagation Committee 2019).

A survival estimation of stocked HOPS in the upper basin of the Missouri River (UBMR) revealed relatively high rates of survival among HOPS (Rotella 2017). For example, in 2016 approximately 72% of fish classified in the age-5 to age-10-year classes (stocked from 2006 to 2011) were estimated to have survived (Rotella 2017). In segment 4, a subsection of the UBMR, recent analysis of annual population assessment data has revealed slower growth rates in the 2006-2009 year-classes compared to the 2002 year-class of HOPS (Wilson et al. 2017). Insufficient information on ideal growth rates for wild juvenile pallid sturgeon make interpretations of this trend uncertain.

One hypothesized explanation of reduced growth in more recently stocked HOPS cohorts is that increasing competition for food resources, as successful stocking efforts have increased the density of juvenile pallid sturgeon in the river, may be limiting HOPS growth (Braaten et al. 2009). Intraspecific competition among year-classes may have led to detrimental effects on the health of pallid sturgeon populations in the wild, raising concerns that UBMR habitats are being overstocked (USFWS 2008). An assessment of the physiological status of HOPS captured from the ecosystem could help to resolve the mechanism underlying these growth trends and better guide management responses as ecosystem impacts on populations are filtered through individual physiological responses. Before this approach could be attempted, however, an improved understanding of bioindicators of healthy pallid sturgeon and how these indicators fluctuate with ecosystem stressors is required

Blood biochemistry, whole-body energetic reserves, and visual health can serve as bioindicators to track fish health (Ferri et al. 2011; Sepúlveda et al. 2012; Adams 1999) responses to ecosystem conditions because they can provide information about nutritional status, tissue damage, and chronic stress. Blood biochemical variables that correlate together have been applied as indices of energetic reserves (e.g., cholesterol, triglycerides, alkaline phosphatase, calcium, and protein), stress (e.g., sodium, chloride, glucose, cortisol), and tissue damage (e.g., creatine kinase, alanine aminotransferase, lactate dehydrogenase, and aspartate aminotransferase) in migrating salmonids (Wagner and Congleton 2004), but I am aware of no similar attempts to define indices for sturgeon. Understanding typical ranges of blood biochemical analytes and how they

correlate among juvenile pallid sturgeon may allow fisheries biologists to identify bioindicators of fluctuations in physiological processes related to nutrition, tissue damage, and chronic stress.

Whole-body energy and lipid content of fish can predict the capacity of fishes to perform ecologically important life processes. These physiological variables are important to cellular homeostasis, and metabolism (Adams 1999). They also limit fish survival during periods of prey shortages (Deslauriers et al. 2018).

Inspection of the visual appearance of fish (here on referred to as visual inspection), including fin condition, skeletal deformities, and wounds, serves as a non-invasive assessment method that, when combined with other methods, can be indicative of tissue damage in a variety of fish species and reveal physical deformities that may negatively affect the ability of a fish to perform ecologically important actions. For example, fin curl has been a hinderance to post-stocking success of pallid sturgeon released into the wild (Oldenburg et al. 2011) and skin pigmentation is used as a metric by managers to assess the stress level of wild-caught pallid sturgeon in the field (R. Wilson, personal communication, 2018).

Therefore, identification of pallid sturgeon physiological bioindicators of nutrition, tissue damage, and chronic stress can equip managers to monitor ecosystem impacts on pallid sturgeon and make more informed management decisions for the benefit of the population. However, typical ranges of these variables and physiological implications of values outside of these ranges are not well studied in juvenile pallid sturgeon. To address this information gap, I performed a study to determine how blood

biochemistry, energetic reserves, and visual health relate to each other and growth in a population of hatchery-reared juvenile pallid sturgeon with a broad range of energetic reserves and levels of stress induced by chronic feed and water temperature treatments. Feed and water temperature treatments were used as they are ecologically relevant challenges that fish would typically experience in the wild because of seasonal fluctuations in water temperature and food availability (i.e., overwintering, competition for limited food resources).

Growth rate is an important metric to assess the ability of fish to perform in the wild, as minimum size requirements are often necessary to recruit to different life stages and for survival. For example, smaller fish are typically at higher risk of predation, thus, recruiting to larger size classes will reduce the predation risk of an individual (Gadomski and Parsley 2005), therefore, increasing their chance of survival. Additionally, size facilitates a dietary shift to piscivory in pallid sturgeon (Grohs et al. 2009), and a minimum size threshold is necessary for pallid sturgeon to reach sexual maturity (Holmquist et al. 2019). It has been shown that environmental factors such as diet ration, water temperature (Kappenman et al. 2009; Chipps et al. 2010), and population density (Rafatnezhad et al. 2008; Li et al. 2012) affect growth rate in sturgeon. However, the physiological processes that link the interactions of a fish with its ecosystem and resultant growth are complicated and not fully understood (Sumpter 1992). Therefore, identifying physiological variables correlated with growth, determined in a laboratory experiment, may reveal relationships that can be applied to wild-captured pallid sturgeon.

My objectives were 1) to define ranges of physiological variables expected to be observed in pallid sturgeon reared under typical hatchery conditions and how they correlate in a population of fish with varying energetic reserves and chronic stress levels induced by four months of feed and water temperature manipulation and 2) to determine the relationship between the physiological variables in Objective 1 and an ecologically relevant performance metric (i.e., growth rate).

Methods

In order to develop a suite of physiological variables to track aspects of health in juvenile pallid sturgeon, physiological measurements were measured after a four-month water temperature and feed ration experimental manipulation period. This manipulation period was aimed at maximizing the variation in overall health of a hatchery-reared population and not directly at testing for temperature and feed effects on fish physiology. The suite of variables was based on blood biochemical parameters, whole-body energetic reserves, a visual health assessment, and performance metrics.

Study System and Animals

Hatchery-born juvenile pallid sturgeon ($n = 135$) were reared at the Bozeman Fish Technology Center (BFTC) in Bozeman, MT for all manipulations necessary to develop a suite of physiological variables related to various aspects of health. The fish originated from two distinct spawning events in 2017 at Gavins Point National Fish Hatchery in Yankton, South Dakota and were reared according to the parameters recommended by the Upper Basin Pallid Sturgeon Propagation Plan (UBPSPC 2005) until September 2018

when fish were moved to 75L rectangular flow-through tanks with flows of 2L/minute for tank acclimation. During the two-month acclimation period, fish were reared at $18^{\circ}\text{C} \pm 1^{\circ}\text{C}$ on a mix of cold and warm spring water and fed Extruded Trout (now called Classic Trout; Skretting, Tooele, UT, USA) sinking feed pellets until satiation. After acclimation to experimental tanks and before implementation of feed and water temperature treatments, preliminary sampling of blood plasma and measurements of mass (g) and length (FL; mm) were performed as fish were re-allocated among the same tanks in groups of five.

Blood Collection for Biochemical Reference Interval Determination

In November 2018, as fish were re-allocated among tanks for experimental manipulations, plasma was sampled from 117 fish to serve as baseline assessments to determine reference intervals of blood biochemistry variables on fish reared within the parameters recommended by the Upper Basin Pallid Sturgeon Propagation Plan (UBPSPC 2005). Fish were approximately 18 months old at sampling time.

Temperature and Feed Manipulation Treatments

Groups of fish were subjected to nine combinations of water temperature and feed ration conditions to create a comprehensive range in energetic status, tissue damage, and chronic stress level within a cohort of juvenile pallid sturgeon before performing final measurements. The four-month experimental manipulation period began in November 2018 and ended in March 2019. Each of the 27 tanks were randomly assigned one of three water temperature conditions and one of three feed ration conditions such that three

replicate tanks of five fish ($n = 15$ fish) were assigned to each of the nine possible combined water temperature and feed ration conditions: low temperature, low feed (LTLF); low temperature, moderate feed (LTMF); low temperature, high feed (LTHF); moderate temperature, low feed (MTLF); moderate temperature, moderate feed (MTMF); moderate temperature, high feed (MTHF); high temperature, low feed (HTLF); high temperature, moderate feed (HTMF); high temperature, high feed (HTHF).

Water temperature treatments were determined with the objectives of achieving stressfully low temperatures, optimal or moderate temperatures, and stressfully high temperatures while avoiding mortalities. Targeted water temperatures included a low temperature of 12°C [approximately $11.7^{\circ}\text{C} \pm$ one standard deviation (SD) 0.32°C], a moderate temperature of 18°C [approximately $17.8^{\circ}\text{C} \pm$ SD 0.24°C], and a high temperature that increased from 25°C to 28°C [approximately $24.6^{\circ}\text{C} \pm$ SD 1.02°C at the beginning and $27.2^{\circ}\text{C} \pm$ SD 0.79°C at the end] during the experimental manipulation period. Rearing water temperatures were determined based on reduced probability of juvenile pallid sturgeon survival as temperatures increase to 28°C and reduced or no growth in fish reared at 12°C (K. Kappenman, personal communication, July 30, 2018). To avoid high temperature-related mass mortalities, following the acclimation period, water temperatures for the high temperature rearing groups were gradually increased to 25°C , where they were stable for approximately two months. Water temperatures were gradually increased to 27°C , where they were held for one month, and, finally, temperatures were raised to 28°C , where they were maintained for the final month.

Feed rations were determined with the objectives of inducing a loss in mass on average of fish in low feed treatments, slow growth on average of fish in moderate feed treatments, and rapid growth on average of fish in high feed treatments (without the overaccumulation of food that may cause water-quality issues within experimental tanks). Feed rations were determined based on previous work that showed age-0 pallid sturgeon at 17.8°C and 20.0°C exhibited low to negative growth rates when reared on feed rations of 1.0% of mean fish weight per fish per day (Oldenburg et al. 2011). The same study reported improved growth in pallid sturgeon reared on 2.5% of mean fish weight per fish per day (Oldenburg et al. 2011). In my study, initial daily feed ration treatments were set at 3.5% of tank biomass per day (high), 2.5% of tank biomass per day (moderate), and 1.0% of tank biomass per day (low). Feed rations were adjusted three times on a tank-specific basis based on daily observations of food accumulation in rearing tanks and specific growth rates (SGRs) averaged across all individuals in a given tank. Specific growth rate was calculated approximately monthly following inventories of fish mass and FL and at the end of the experiment using the equation:

$$\text{SGR} = (\ln(m_2) - \ln(m_1)) / (t_2 - t_1) \times 100,$$

where m_1 is mass at the most recent inventory (prior to present sampling day), m_2 is mass at present sampling day, t_1 is day of the most recent inventory, and t_2 is day of the present sampling day (Ricker 1979).

Physiology and Performance Metrics

At the end of the four-month rearing period, fish were randomly sampled for blood, visual health, and proximate composition after fasting for six days; fish were

approximately 22 months old at this sampling time period. Tank water temperatures were increased to 18°C two days after the six-day fasting period began. To reach the target water temperature of 18°C, water temperature of 12°C treatment tanks was increased by 1°C per day and water temperature of 28°C treatment tanks was decreased by 2°C per day. Therefore, all physiological measurements were performed on fish with empty gastrointestinal tracts and at a common water temperature to prevent confounding effects of temperature or feed on physiological measures. Fish were euthanized at the end of the six days of fasting and after physiological measurements were collected.

Fish were moved from their experimental rearing tanks to a holding tank for sampling on day six of sampling. Fish were promptly removed from the holding tank, visual assessment criteria were recorded, and blood was immediately drawn from the caudal vein into a heparinized syringe. Visual assessments and blood measurements were conducted first to prevent any confounding effects of acute stress on the measurements. Fish were then immersed in a solution of MS-222 (tricaine methanesulfonate, Syndel (formerly Western Chemical), Ferndale, WA, USA; 250mg/L water dosage) for euthanization. Measurements for FL, mass, and Fatmeter (see Chapter 3) were recorded after euthanization. After the completion of all sampling, excess moisture was removed from the fish with a dry towel and the fish was frozen in a sealed bag until proximate analyses could be conducted to determine fish body composition.

Visual assessment criteria assessed stress coloration, gill, eye, wound, and fin condition on a 1 to 5 rating scale for each variable with 1 being worst and 5 being best. These scales were developed based on a hypothetical worst and best appearance that

could potentially occur and not the observed range within the experimental population, for example, none of the experimental fish were scored at value of 1 for eyes.

Assessments of each feature were summed to quantify a visual health assessment (VHA).

An assessment was adapted from Matsche (2011) regarding stress coloration and, in the current study, a score of 5 indicates no presence of redness on the skin and a score of 1 indicates that there was redness on the skin across the entire length of the body. An assessment of eye condition was adapted from Adams et al. (1993) and in the current study, a score of 5 indicates both eyes were present and there was no apparent damage to either eye while a score of 1 indicates that the fish was missing both eyes. An assessment of gill condition was adapted from Adams et al. (1993) and in the current study, a score of 5 indicated that gills were deeply red, and no erosion was present while a score of 1 indicated gills with gray coloration and extreme erosion. An assessment of wounds was adapted from the skin assessment in Adams et al. (1993) but was modified to include broken bones (e.g. tail or spine). In the current study, a score of 5 indicated that there was an absence of wounds or lesions across the body and a score of 1 indicated excessive lesions across the body or extreme wounds such as a broken spine or severed tail.

Finally, the scale for fin condition was adapted from a combination of the fin curl scale in Deslauriers et al. (2016) and fin erosion scale in Kindschi (1987) but was modified to include pelvic fin condition as well. In the present study, a score of 5 indicated an absence of fin curl on all fins and no erosion present on any fins while a score of 1 indicated extreme fin curl on all pelvic and pectoral fins and extreme fin erosion. The scores of each variable were combined to give the overall score on the VHA for an

individual fish where the highest VHA score possible was 25 and the lowest possible was 5.

Blood was measured for hemoglobin, then centrifuged to collect plasma for plasma biochemistry. Hemoglobin was immediately measured on a small subsample of whole blood using a HemoCue Hb 501+ (HemoCue America, Brea, CA, USA) as per instructions of the manufacturer. The rest of the blood was transferred from the syringe into a microtube and centrifuged (5 minutes at 2,960 g) using a portable Tomy Multispin Centrifuge (Amuza Inc, San Diego, CA, USA). After the blood was separated, plasma was transferred into a new microtube and temporarily stored in a -20°C freezer until transfer to a -80°C freezer within three days.

Proximate Analysis and Blood Biochemistry Laboratory Protocols

Proximate Analysis: Whole-body energy, whole-body lipid, and whole-body protein content were determined through proximate analysis of pallid sturgeon carcasses. Carcasses were thawed, cut into small pieces and homogenized in an industrial meat grinder (Hobart Corporation, Troy, OH, USA). In order to further homogenize and shelf stabilize samples, ground samples were freeze dried in a LabConco lyphilizer (LabConco Corporation, Kansas City, MO, USA) and ground again.

The whole-carcass freeze-dried homogenized samples were stored in sealed tubes in a -20°C freezer until the samples were subsampled to measure whole-body lipid, whole-body energy, and whole-body protein content. Whole-body energy, lipid, and protein content were quantified on subsamples of the freeze-dried homogenized samples. Whole-body lipid content was determined through the ether extraction method using an

Ankom XT10 Extractor (Ankom Technology, Macedon, NY, USA) (AOAC, 2012).

Whole-body energy content was determined through the bomb calorimetry technique as described in Glover et al (2010), using a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL, USA). Whole-body protein content was determined on a Leco TruSpec N nitrogen determinator (LECO Corporation, St. Joseph, Michigan, USA) by the Dumas method with a nitrogen conversion factor of 6.25 (AOAC, 2012).

Blood biochemistry: A total of 19 plasma analytes related to nutrition, chronic stress, or tissue damage were identified for consideration in my assessment of physiological measurements. A thorough explanation of the hypothesized contribution of each analyte to nutritional status, chronic stress, or tissue damage is described in Appendix A. In summary, plasma total protein (TP), cholesterol (CHOL), calcium (CA), alkaline phosphatase (ALP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CREAT), phosphorous (PHOS), and hemoglobin (HB) were hypothesized to be indicators of nutrition. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT) were hypothesized to be indicators of tissue damage. Finally, glucose (GLU), chloride (CL), sodium (NA), magnesium (MG), and potassium (K) were hypothesized to be indicators of stress. Most analytes were measured using the Vet Axcel Clinical Chemistry System (Alfa Wasserman, West Caldwell, NJ, USA) according to Alfa Wasserman instructions whereas HB was measured using the HemoCue Hb 501+ as previously described earlier in the methods.

Halfway through processing of post-manipulation plasma samples, I grew concerned that lipemic samples may interfere with the efficacy of the analyzer to carry out analyses. From this time point on, frozen plasma samples (remaining post-manipulation samples and all baseline samples) were thawed and centrifuged in a Sorvall ST16R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) as described in Dimeski and Jones (2011) to separate out the lipid layer and prevent potential interference effects from lipemia. Infranatant was separated from the lipid layer and processed in the Vet Axcel Clinical Chemistry System (here onwards referred to as the analyzer). Because infranatant needed to be removed from the lipid layer prior to analysis, approximately 500 μ L of sample was necessary for lipid layer removal and to carry out assays on all 19 analytes measured by the chemistry analyzer described above. A comparison of analyte concentrations pre and post-centrifugation revealed that there were no statistical differences between the reported concentrations of analytes measured in the experiment before and after centrifugation ($n = 25$; data not shown). Therefore, I do not suspect an effect of centrifugation to exist on analyte concentrations (of those described in the methods) of post-manipulation fish analyzed before centrifugation occurred compared to post-manipulation fish analyzed after centrifugation occurred.

Data Analysis

Reference Intervals: Reference intervals were determined on baseline blood samples collected prior to the implementation of feed and water temperature manipulations. These intervals established baseline blood biochemical values on a population of fish reared under typical hatchery conditions.

To determine reference intervals on the hatchery population of fish, the recommended American Society for Veterinary Clinical Pathology (ASVCP) methods were followed (Friedrichs et al. 2012). Prior to determining reference intervals, a Shapiro-Wilk test was used to confirm whether the data fit a normal distribution. When an analyte distribution was not normal, data for that analyte were plotted to determine the distribution type and transformed accordingly (i.e., cube root or natural log) to achieve normality. When normality was not achieved, the reference interval was determined using the robust method through the `robust()` function in the `referenceIntervals` R package (Finnegan 2014) without removal of outliers. When normality was achieved, outliers were detected using the parametric Horn's algorithm using Tukey's interquartile fences (Horn and Pesce 2003) and subsequently removed. After the removal of outliers, the reference interval was determined using the parametric `refLimit()` function in the `referenceIntervals` R package (Finnegan 2014). For reference intervals derived from both parametric and robust methods, 90% confidence intervals were calculated around the upper and lower reference limits. Resultant intervals for all transformed data were then back transformed to the original units of the analyte.

Due to limitations of the analyzer, trustworthy reference intervals could not be calculated for six analytes. A reference interval for a specific analyte was determined untrustworthy when values determined for the reference interval fell outside the detection range of the analyzer as those values were not verifiable and were potentially skewing reference intervals. Therefore, analyses for reference intervals and post-experimental blood biochemistry were performed on only 13 plasma analytes.

Principal Component Analysis: Principal components analysis (PCA) was performed using the `prcomp()` function in R (R Core Team 2019) on 18 physiological metrics adjusted to address assumptions, requirements, and susceptibility of PCA to outliers.

The variables included in PCA were: VHA, relative condition (Kn; Shuman et al. 2011), whole-body lipid, energy, and protein content, and the 13 blood biochemical analyte values obtained from blood samples collected during the final handling event of post-manipulation fish. The six eliminated variables scored outside of the limit of detection of the machine >30% of the time for post-manipulation fish and would have likely skewed PCA results (Table 2.1). Thus, the PCA included the following plasma analytes: total protein (TP), cholesterol (CHOL), calcium (CA), alkaline phosphatase (ALP), albumin (ALB), phosphorous (PHOS), lactate dehydrogenase (LDH), glucose (GLU), chloride (CL), sodium (NA), magnesium (MG), potassium (K), and hemoglobin (HB) as well as whole-body proximate composition (i.e. whole-body energy, lipid, and protein content), Kn, and visual health assessment (VHA).

Table 2.1. Percentage of post-manipulation hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) whose values for a given blood biochemical analyte fell outside the detectable range of the VetAxcel blood chemistry analyzer.

Analyte	Detectable Range	Percent Post-Manipulation Fish Outside of Limit of Detection
Gamma-glutamyl Transferase (U/L)	7 – 950	81%
Creatine Kinase (U/L)	11 – 3223	48%
Alanine aminotransferase (U/L)	4 – 669	31%
Aspartate Aminotransferase (U/L)	4 – 588	35%
Creatinine (mg/dL)	.33 – 25	72%
Blood Urea Nitrogen (mg/dL)	3 – 100	100%

Of the initial 135 fish in the experiment, eight fish were removed from the dataset prior to PCA because they were either suspected to be outliers (PCA is sensitive to and largely influenced by outliers) or because measurements on those individuals were not available bringing the sample size to 127 fish. Two of these fish determined as outliers had become moribund before the end of the experimental manipulation period, so they were considered explained biological outliers. For a third eliminated fish, many of the blood biochemistry variables were unexplainably reported as errors or below the limit of detection of the analyzer, potentially indicating an issue with sample quality. There were no blood biochemical data for two fish that died during the experiment, before blood could be collected and, therefore, they were not included in PCA. Additionally, plasma volumes collected from three fish were too low to meet the 500 μ l requirement to analyze all blood biochemical variables and were, therefore, not analyzed.

Prior to determination of Principal components (PCs) using PCA, missing values for blood biochemical variables were imputed in the dataset and all variables were standardized to account for scale differences between variables measured in the study. To account for any missing values in the dataset (due to machine error or experimenter error), missing data were imputed using the regularized iterative PCA algorithm by the `imputePCA()` function in R from the `missMDA` package (Josse and Husson 2016). The data were analyzed for PCA by the `prcomp()` function in R (R Core Team 2019) and, because the analytes were of different scale, were standardized using the `scale` argument within `prcomp()` as recommended by Everitt and Hothorn (2011). Determined PCs and

associated explained variance were included in the output of the `prcomp()` function used for PCA.

After PCs were defined by PCA, I further investigated all PCs that individually accounted for >10% of the variation in the dataset and cumulatively accounted for >50% of the total variation in the dataset. For each PC, strongly contributing variables were determined based on the loading score of each individual variable relative to the loading score of the other variables contributing to that PC. Specifically, all variables within a specific PC that achieved a loading score greater than 50% of the highest contributing variable's loading score were determined to be strongly contributing variables.

Linear Regression: Simple (SLR) and multiple (MLR) linear regressions were applied to identify relationships between PCs and recent percent growth in mass. A separate simple linear regression (SLR) was used to determine the relationship between each PC and percent growth. Percent growth was calculated using the equation:

$$\text{Percent growth} = (m_1 - m_0/m_0) \times 100$$

where m_1 is mass (g) at final sampling and m_0 is mass (g) at initial sampling. An alpha level of 0.05 was used to identify significant relationships between PCs and percent growth. After PCs that significantly contributed to SLRs were identified, multiple linear regression (MLR) was applied to determine if a combination of these PCs would better predict percent growth in the hatchery population of fish. Any PCs that were not significant at an alpha level of 0.05 were eliminated from consideration. Finally, the best fit model to predict percent growth was identified by comparing separate models for all possible combinations of the remaining PCs using Akaike's Information Criteria with a

correction for small sample sizes (AICc). For all candidate models, Δ_i was determined with the following equation:

$$\Delta_i = \text{AICc}_i - \text{AICc}_{\min}$$

where AICc_{\min} is the lowest AICc value of investigated models and AICc_i are the values of other models (as described in Burnham and Anderson 2004). Models with $\Delta_i \leq 2$ were selected as competitive for the best model fit.

Moribund Fish: Five fish died during the progression of the experimental manipulation period. Two of these fish were found dead on arrival and, therefore, blood was not harvested for analysis. Three fish were moribund and were sampled for blood, but insufficient plasma volume was collected from one of these fish to run the suite of blood biochemical variables. Therefore, the full suite of physiological variables was measured on the remaining two moribund fish. Measurements on these fish were compared with reference intervals and post-experimental values to provide insight into potential physiological mechanisms underlying morbidity of each separate moribund fish. All moribund fish were euthanized by MS-222 after the collection of blood was completed. Because of the moribund state of the fish during sampling, moribund fish were considered biological outliers and were not included in PCA because PCA is highly sensitive to the influence of outliers.

Results

Blood Biochemical Reference Intervals for Hatchery-reared Juvenile Pallid Sturgeon

Reference intervals were determined using plasma samples on baseline fish (i.e., collected prior to initiation of feed and water temperature treatments on juvenile pallid sturgeon reared under typical hatchery conditions - see methods for pre-treatment rearing conditions) only that had minimal variation in metric measurements (Table 2.2). As the small fish size and non-lethal sampling prevented collection of the required 500 μ L blood volume for biochemical measurements from many (i.e., 76) fish, plasma samples from only a subsample of 41 pre-treatment fish were used for reference interval determination.

Table 2.2 Descriptive statistics of metric measurements for juvenile pallid sturgeon (*Scaphirhynchus albus*) reared under typical hatchery conditions and sampled for baseline measurements included in reference interval determination where relative condition is as defined specifically for pallid sturgeon in the Missouri River Basin (Shuman et al. 2011).

Parameter	Mean \pm Standard Deviation	Minimum	Maximum
Mass (g)	83.3 \pm 21.4	53.7	160.8
Fork Length (mm)	289 \pm 20	245	340
Relative Condition (Kn)	1.2 \pm 0.12	0.94	1.63

In total, reference intervals were determined by robust and parametric methods on 13 blood biochemical analytes: TP, NA, CL, MG, K, PHOS, CA, CHOL, ALB, GLU, ALP, LDH, and HB (Table 2.3) and were generally similar to previously reported ranges in other species of sturgeon (Appendix A). Reference intervals were not determined for GGT, CK, creatinine, BUN, ALT, and AST because the values fell outside the detectable range of the machine. These analytes were removed from all further analyses.

Table 2.3. Thirteen blood biochemical reference intervals for hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*), located at Bozeman Fish Technology Center where min is the minimum value measured across all fish and max is the maximum value measured across all fish. Reference intervals were determined on all analytes that fell within range of the chemistry analyzer by parametric methods when possible and robust methods were used when assumptions of parametric methods were not met (robust determination indicated by *). Analytes are represented by abbreviated terms where TP is plasma total protein, NA is plasma sodium, CL is plasma chloride, MG is plasma magnesium, K is plasma potassium, PHOS is plasma phosphorous, CA is plasma calcium, CHOL is plasma cholesterol, ALB is plasma albumin, GLU is plasma glucose, ALP is plasma alkaline phosphatase, LDH is plasma lactate dehydrogenase, and HB is whole-blood hemoglobin.

Analyte	N	Mean	Min	Max	Reference Interval	Lower Limit	Upper Limit
TP (g/dL)	37	3.0	2.4	3.5	2.4 – 3.5	2.3 – 2.6	3.4 – 3.6
NA (mmol/L)	41	120.8	107.2	128.5	107.5 – 128.4	103 – 112	127 – 129
CL (mmol/L)	41	105.5	88.5	113.2	90.4 – 113.5	84 – 96	113 – 114
MG* (mg/dL)	41	1.6	1.3	2.3	1.2 – 2.1	1.1 – 2.1	7.3 – 10.4
K* (mmol/L)	39	2.85	2.55	3.40	2.02 – 3.74	1.58 – 2.40	3.65 – 3.87
PHOS (mg/dL)	40	11.4	8.2	15.8	8.2 – 14.9	7.5 – 9.0	14.1 – 15.6
CA* (mg/dL)	39	8.7	7.1	18.0	5.0 – 12.3	4.3 – 5.7	10.7 – 14.5
CHOL (mg/dL)	40	127	59	207	64 – 199	49 – 79	184 – 214
ALB (g/dL)	36	1.1	0.8	1.4	0.8 – 1.4	0.7 – 0.9	1.4 – 1.5
GLU (mg/dL)	37	63	43	82	46 – 82	42 – 50	78 – 86
ALP (U/L)	41	121	48	201	52 – 203	35 – 69	187 – 221
LDH* (U/L)	41	210	92	666	90 – 434	69 – 113	353 – 549
HB (g/dL)	34	5.7	4.7	6.4	4.9 – 6.5	4.7 – 5.0	6.2 – 6.6

Relationship Between Physiological Variables and Recent Growth

The metric measurements, energetic reserves, and percent growth for individuals included in PCA are included in Table 2.4. Feed and water temperature treatments induced a wide range of variation in the energetic reserves and growth rates of the population. Whole-body lipid content varied from 0 to 11.3% and whole-body energy

content varied from 358 to 1930 calories per gram of fish and percent growth rate varied from -35.3 to 149.5% (Table 2.4).

Table 2.4. Descriptive statistics of metric measurements, energetic reserves (whole-body energy and lipid content determined by proximate analysis), and percent growth for hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) subjected to experimental feed and water temperature manipulations aimed at inducing variation in energetic reserves and chronic stress levels in the population.

	Mean \pm Standard Deviation	Minimum	Maximum
Mass (g)	97.5 \pm 38.1	40	213.6
Fork Length (mm)	314.6 \pm 34.9	240	415
Relative Condition (Kn)	1 \pm 0.11	0.65	1.32
Whole-body Lipid (%)	4.6 \pm 2.4	0.0	11.3
Whole-body Energy (cal/g)	1089.3 \pm 299.5	358.0	1930.0
Percent Growth (%)	13.33 \pm 37.17	-35.28	149.45

Several of the physiological variables measured in this study correlated (as defined by Schober et al. 2018) with one another and with the recorded recent percent growth of hatchery-reared juvenile pallid sturgeon. Specifically, percent growth moderately, strongly, or very strongly correlated positively with TP, CA, ALB, and whole-body lipid and energy content and negatively with CHOL (Figure 2.1; Figure 2.2; Figure 2.3). Each of the blood biochemistry variables that strongly correlated with recent percent growth were previously determined to be related to nutrition (Appendix A).

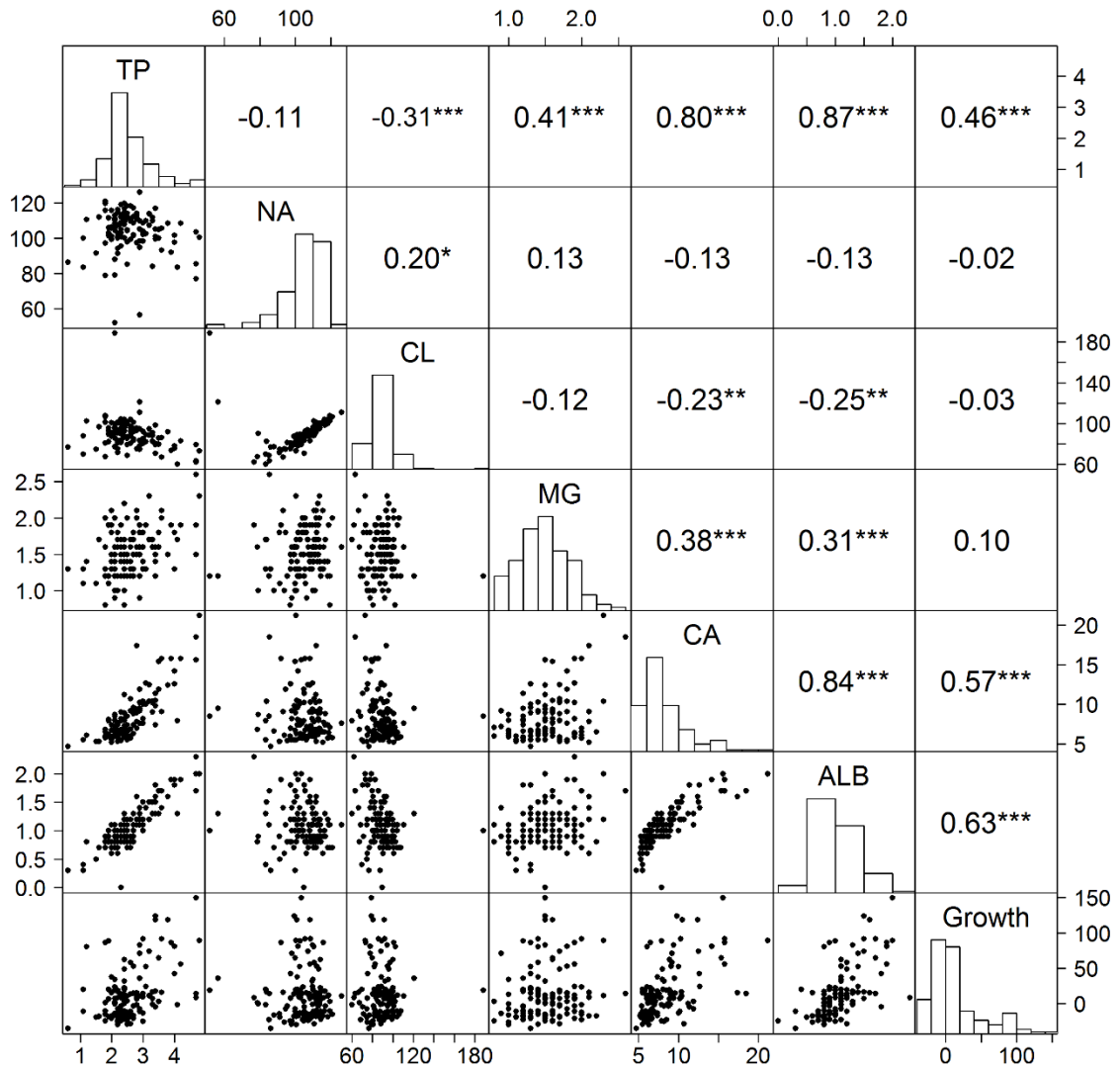


Figure 2.1. Correlation matrix of a subset of the physiological variables included in principal component analysis where TP is total protein, NA is sodium, CL is chloride, MG is magnesium, CA is calcium, ALB is albumin, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) across a hatchery experiment with varying feed and water temperature manipulations. See Table 2.3 for axis units associated with each variable.

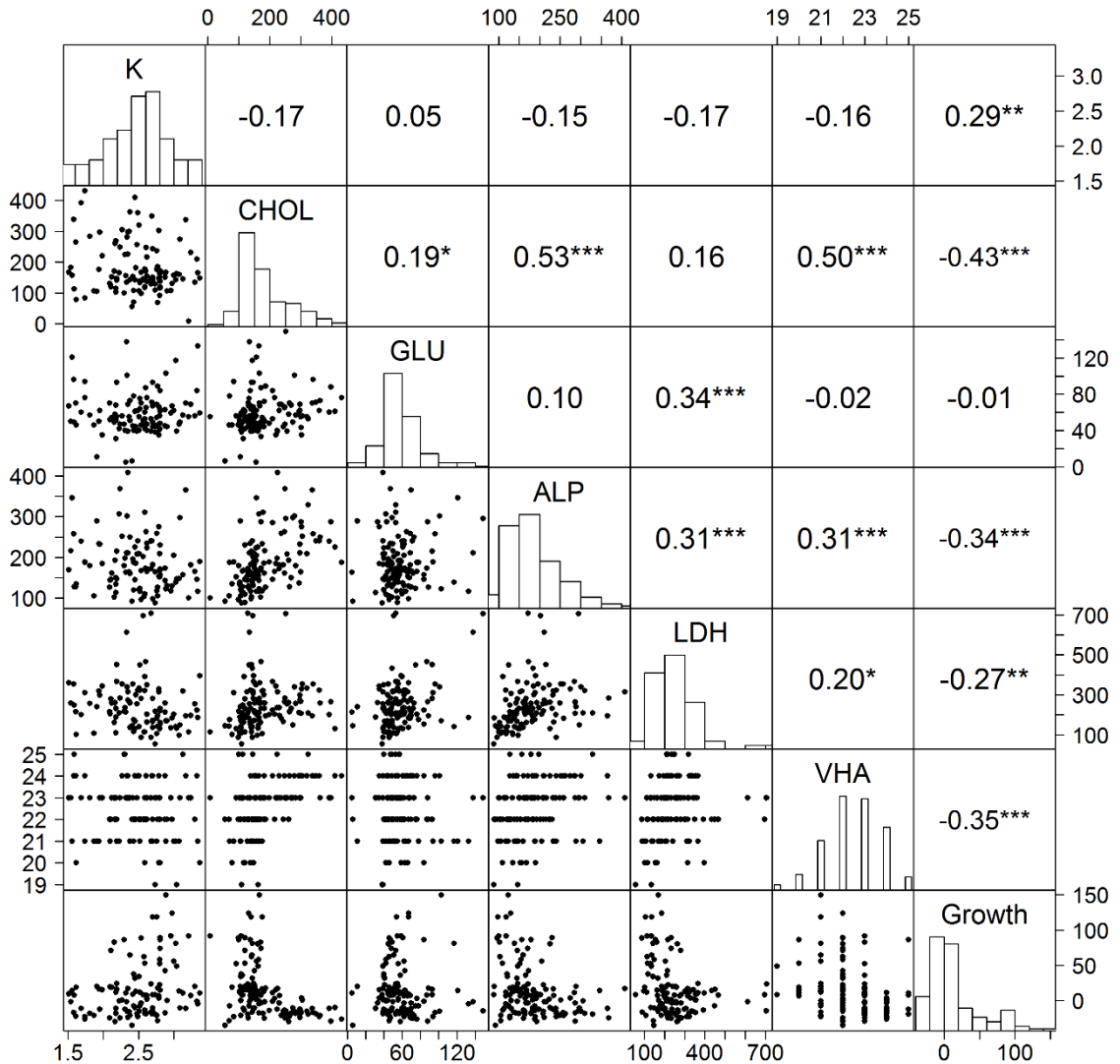


Figure 2.2. Correlation matrix of a subset of the physiological variables included in principal component analysis where K is total potassium, CHOL is cholesterol, GLU is glucose, ALP is alkaline phosphatase, LDH is lactate dehydrogenase, VHA is visual health assessment, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) across a hatchery experiment with varying feed and water temperature manipulations. See Table 2.3 for axis units associated with each variable.

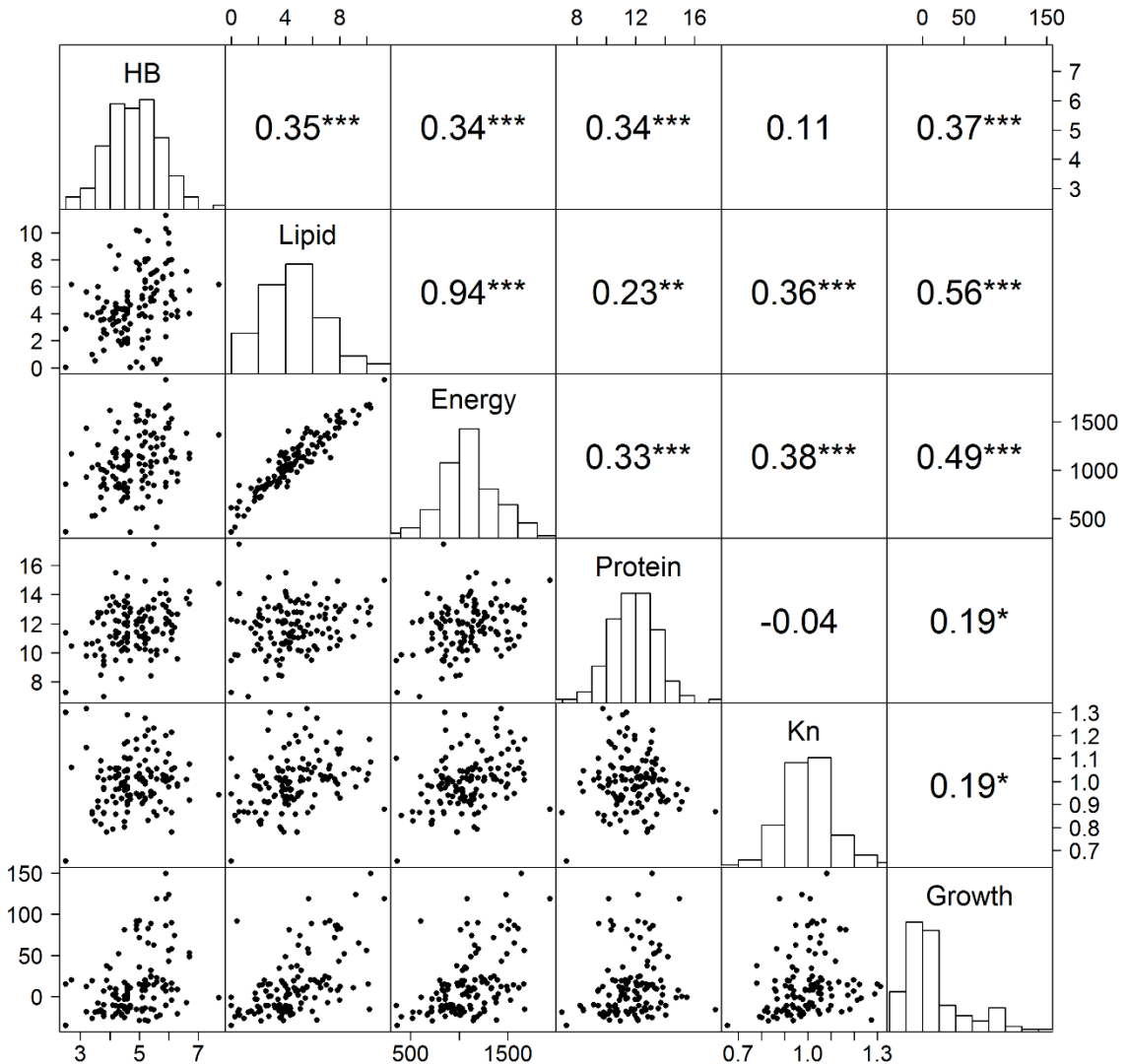


Figure 2.3. Correlation matrix of a subset of the physiological variables included in principal component analysis where HB is hemoglobin, Lipid is whole-body lipid content, Energy is whole-body energy content, Protein is whole-body protein content, Kn is relative condition, and Growth is percent growth recorded on hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) across a hatchery experiment with varying feed and water temperature manipulations. See Table 2.3 for axis units associated with each variable.

Three PCs accounting for the majority of the variation in the original dataset of physiological measurements on hatchery-reared juvenile pallid sturgeon were determined

through PCA from 18 physiological variables. These three principal components accounted for 54.2% of the variance in the dataset and each principal component had an eigenvalue above 0.7 and explained >10% of the variance in the dataset individually (Figure 2.4).

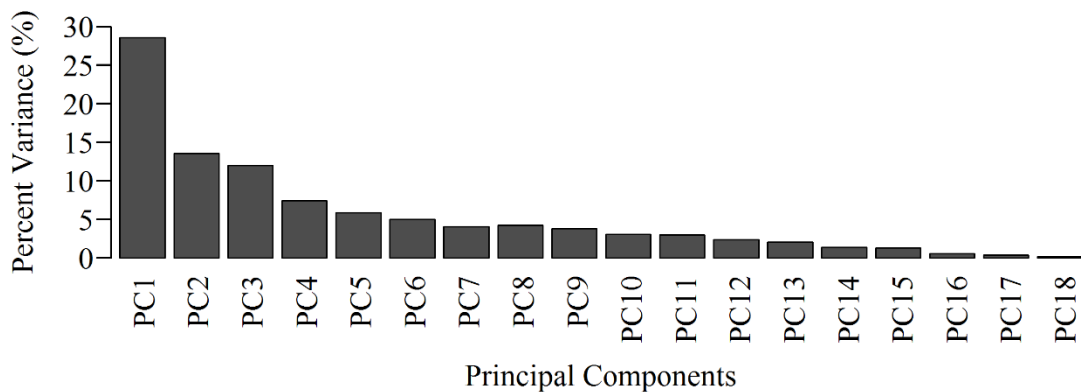


Figure 2.4: Percentage of variance accounted for by the first three principal components (PCs), as determined through principal component analysis, that account for the majority of variance (54.2%) in the hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) dataset of fish reared at nine combinations of feed and water temperature treatments for four months.

PC1 accounted for 28.6% of the variance in the dataset and was strongly correlated, relative to other variables, with whole-body protein, lipid, and energy content, whole-blood HB, plasma ALB, CA, TP, and visual health assessment (VHA) (Figure 2.5). On average, fish with high PC 1 scores had low values of whole-body protein, energy, and lipid content, whole-blood HB, and plasma TP, CA, and ALB and high values for VHA.

PC2 accounted for 13.6% of the variance in the dataset and was strongly correlated, relative to other variables, VHA and plasma LDH, ALP, GLU, CHOL, PHOS, MG, and TP (Figure 2.5). On average, fish with high PC 2 scores had high values of VHA and plasma LDH, ALP, GLU, CHOL, PHOS, MG, and TP.

PC3 accounted for 12.0% of the variance in the dataset and was strongly correlated, relative to other variables, with Kn and plasma PHOS, CL, and NA (Figure 2.5). On average, fish with high PC3 scores had high values of Kn and plasma PHOS, CL, and NA.

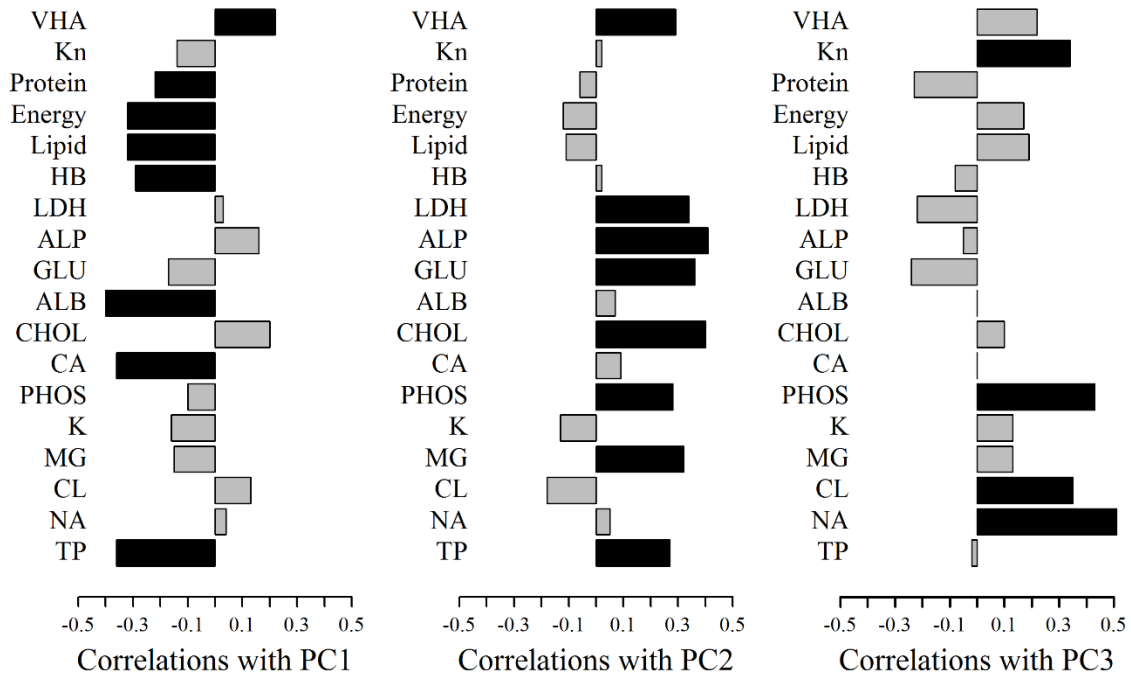


Figure 2.5. Correlations between physiological variables and the first three principal components (PCs) determined through principal component analysis on a population of hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) reared under various feed and water temperature treatments at Bozeman Fish Technology Center to induce variation in energetic reserves and chronic stress levels in the population. Physiological variables are represented by abbreviated terms where VHA is visual health assessment, Kn is relative condition, Protein is whole-body protein, Energy is whole-body energy, Lipid is whole-body lipid, HB is whole-blood hemoglobin, LDH is plasma lactate dehydrogenase, ALP is plasma alkaline phosphatase, GLU is plasma glucose, ALB is plasma albumin, CHOL is plasma cholesterol, CA is plasma calcium, PHOS is plasma phosphorus, K is plasma potassium, MG is plasma magnesium, CL is plasma chloride, NA is plasma sodium, and TP is plasma total protein. Bars in black indicate variables that are considered to strongly correlate (positively or negatively) relative to other variables with PCs while bars in grey do not strongly correlate.

Relationships Between Composite Variables and Percent Growth

There was a significant relationship between percent growth and PC1 ($p < 0.001$, $R^2 = 0.398$, $df = 125$), where PC1 accounted for 39.8% of the variation in percent growth (Figure 2.6). There was also a significant relationship between percent growth and PC2

($p < 0.01$, $R^2 = 0.052$, $df = 125$) where PC2 accounted for 5.2% of the variation in percent growth (Figure 2.6). There was no significant relationship between PC3 and percent growth ($p = 0.121$, $R^2 = 0.011$, $df = 125$). Together, in MLR, PC 1 and PC 2 accounted for 45.4% of the variation in the percent growth dataset ($p < 0.001$, $R^2 = 0.454$, $df = 124$) and, according to AICc scores, was a better fit model to predict percent growth than the SLR models that only included individual PCs.

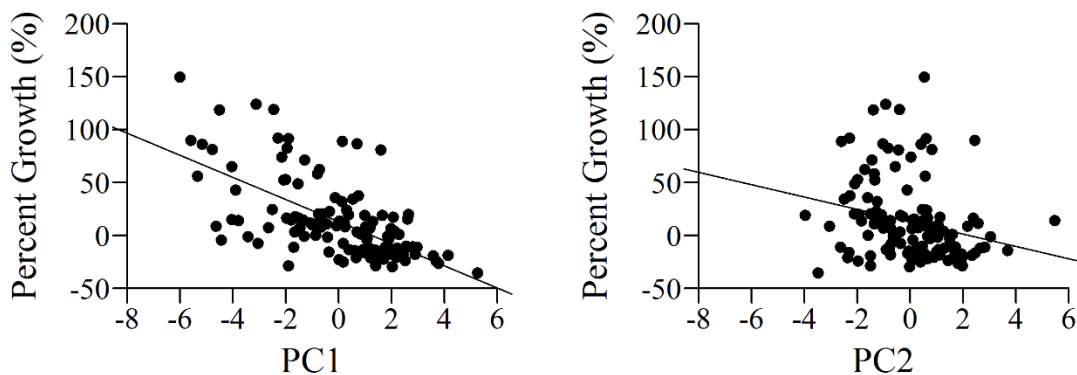


Figure 2.6. Linear regression of percent growth calculated across the experimental period on principal component one (PC1) and principal component two (PC2) of juvenile pallid sturgeon (*Scaphirhynchus albus*) exposed to nine different combinations of feed and water temperature manipulations for a four-month period aimed at inducing variation in energetic reserves and chronic stress levels at the Bozeman Fish Technology Center.

Physiological Values of Moribund Fish

The two moribund fish sampled during the study displayed blood biochemistry values that generally fell outside the ranges of reference intervals defined by baseline population sampling of the juvenile pallid sturgeon reared under typical hatchery conditions and the standard deviation of the average values of post-manipulation fish (Table 2.5). Notably, Moribund Fish 1 (MF1) and Moribund Fish 2 (MF2) both had the lowest score recorded for HB (1.3 g/dL) across both baseline and post-manipulation

population measurements. Additionally, MF1 had the lowest values of TP (0.5 g/dL) and ALB (0.3g/dL) recorded in the study as well as extremely low values for variables related to nutrition, including energetic reserves, compared to post-manipulation fish. However, MF2 had normal energetic reserves compared to post-manipulation fish but had the lowest recorded gill condition score and highest recorded value of plasma creatinine (0.74mg/dL; which made MF2 one of the only fish with a sufficient plasma creatinine concentration to fall within the detection range of the analyzer).

Table 2.5. Comparisons of physiological variables included in the first three principal components for moribund hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) with the reference range (Ref Range; determined on pre-experimental fish) and post-manipulation average (within one standard deviation (SD) of the average determined on post-experimental fish). MF1 is Moribund Fish 1, MF2 is Moribund Fish 2, VHA is visual health assessment, WB is whole-body, HB is hemoglobin, ALB is albumin, CA is calcium, TP is total protein, LDH is lactate dehydrogenase, ALP is alkaline phosphatase, GLU is glucose, CHOL is cholesterol PHOS is phosphorous, MG is magnesium, Kn is relative condition (Shuman et al. 2011), and NA is sodium.

Variable	Normal Fish			
	Ref Range	Post-manipulation Average \pm one SD	MF1	MF2
PC 1				
VHA	N/A	22.5 \pm 1.3	24	18
WB Protein (%)	N/A	11.8 \pm 1.6	11.2	10.4
WB Energy (cal/g)	N/A	1089.3 \pm 229.5	361.1	1121.8
WB Lipid (%)	N/A	4.7 \pm 2.4	0.5	5.1
HB (g/dL)	4.9 – 6.5	4.85 \pm 0.9	1.3	1.3
ALB (g/dL)	0.8 – 1.4	1.1 \pm 0.4	0.3	0.8
CA (mg/dL)	5.0 – 12.3	8.3 \pm 3.0	5.1	9
TP (g/dL)	2.4 – 3.5	2.6 \pm 0.7	0.5	1.6
PC 2				
VHA	N/A	22.5 \pm 1.3	24	18
HB (g/dL)	4.9 – 6.5	4.85 \pm 0.9	1.3	1.3
LDH (U/L)	90 – 434	247.0 \pm 116.3	150	372
ALP (U/L)	52 – 203	185.2 \pm 65.4	109	95
GLU (mg/dL)	46 – 82	59.1 \pm 22.3	1	34
CHOL (mg/dL)	64 – 199	183.1 \pm 81.5	50	45
PHOS (mg/dL)	8.2 – 14.9	7.4 \pm 1.5	5.3	13.3
MG (mg/dL)	1.2 – 2.1	1.5 \pm 0.3	1.1	1.5
TP (g/dL)	2.4 – 3.5	2.6 \pm 0.7	0.5	1.6
PC3				
Kn	N/A	1.0 \pm 0.1	0.9	1.3
PHOS (mg/dL)	8.2 – 14.9	7.4 \pm 1.5	5.3	13.3
NA (mmol/L)	107.5 – 128.4	104.6 \pm 11.4	73.5	97.8
TP (g/dL)	2.4 – 3.5	2.6 \pm 0.7	0.5	1.6

Effects of Feed and Temperature Treatments on Principal Component Scores of Pallid Sturgeon

Although it was not an initial objective in my study, I investigated the effect of water temperature and feed manipulations on PCA results. There was no significant difference in PC scores of individual fish among water temperature and feed manipulations (data not shown; Appendix C).

Discussion

Expected Blood Analyte Concentrations in Hatchery-reared Juvenile Pallid Sturgeon

Blood biochemical parameters are useful to provide information into the health of fishes (Simide 2018) and are important to the field of conservation physiology by elucidating the mechanisms behind organismal responses to environmental changes (Wikelski and Cooke 2006). However, comprehensive blood biochemical parameters are often lacking or nonexistent for rare and endangered species like the pallid sturgeon. Reference intervals were defined for 13 blood biochemical parameters on juvenile pallid sturgeon reared under typical hatchery conditions. Reference intervals defined in this study for plasma total protein (TP), cholesterol (CHOL), and glucose (GLU) were comparable to previously reported values for nonreproductive captive and wild-caught pallid sturgeon (DeLonay et al. 2016). Additionally, although creatinine (CREAT) values for samples collected in this study tended to fall below the lower limit of detection of the analyzer (<0.33 mg/dL), values observed for non-reproductive captive and wild-caught pallid sturgeon (<0.03 to 1 mg/dL) were similarly low (DeLonay et al. 2016). These similarities suggest that the hatchery measurements collected in this study are

relevant to wild-caught fish but further research into blood biochemistry of wild-caught pallid sturgeon would be beneficial to understanding whether there exists a difference in blood biochemistry of other analytes between the populations. The remaining analytes are compared to other (non-pallid) sturgeon species.

When compared to a range of blood biochemical parameters of other sturgeon species, reference intervals defined in this study were similar with a few exceptions. Reference intervals for plasma sodium (NA), chloride (CL), blood urea nitrogen (BUN), and alanine aminotransferase (ALT) were lower than the reported range of other sturgeon species while the reference interval for plasma lactate dehydrogenase (LDH) was higher than the reported range of other sturgeon species. Deviation in these five analytes from reported values in sturgeon literature could be linked to differences in rearing conditions and genus as most studies reporting blood biochemical ranges are limited to species of sturgeon in the *Huso* and *Acipenser* genera (Appendix A).

Due to limitations in the detection ranges of the analyzer used to determine the plasma analyte concentrations in this experiment, reference intervals were not calculated on six analytes. The VetAxcel chemistry analyzer frequently reported values above the limit of detection for aspartate aminotransferase (AST) and creatine kinase (CK) and below the limit of detection for gamma-glutamyl transferase (GGT), ALT, CREAT, and BUN. Future studies investigating these blood biochemical analytes would benefit from fine tuning assays to detect the specific range of values expected for these analytes in pallid sturgeon. This resulted in the final establishment of reference ranges for only 13

out of the 19 blood biochemical analytes (see Table 2.3 for a complete list of the analytes that reference ranges were defined on).

Relationship Between Physiological Variables and Percent Growth in Pallid Sturgeon

Variation in whole-body energetic reserves and relative condition was successfully induced with experimental manipulation of hatchery-reared juvenile pallid sturgeon in this study. Data reported for whole-body energy content (358 to 1930 cal/g) and Kn (0.65 to 1.32) exceeded the range reported by previously published findings in hatchery-reared juvenile pallid sturgeon. Data previously reported for whole-body energy content in juvenile pallid sturgeon fed artificial diets varied from 1.88 to 4.53 kJ/g (449 – 1083 cal/g) and Kn averaged 0.87 ± 0.02 (standard error) or 1.11 ± 0.01 , depending on diet (Meyer et al. 2016).

Although I initially hypothesized that PCA results would form principal components characterizing specific areas of health (i.e., nutrition, chronic stress, and tissue damage), I instead observed combinations of physiological variables indicative of multiple aspects of health within each PC that accounted for the most variance in the dataset. Although previous studies have been able to identify groups of physiologically related variables that correlated (Wagner and Congleton 2004), this finding is not surprising, considering several of the physiological variables quantified here can reflect multiple physiological processes. Depending on contextual circumstances, concentrations of hemoglobin (HB) can be indicative of iron deficiencies caused by poor nutrition (Witeska 2015), blood loss caused by internal or external tissue damage, or stress (Roche and Bogé 1995). Additionally, visual health assessment (VHA) can be

indicative of internal and external tissue damage (gill, eye, and wound condition) but also stress (stress coloration) and physical deformities (fin condition – presence of fin curl). Thus, several of the physiological variables observed in this research are important to diverse physiological processes and can be mechanistically linked to multiple areas of health (i.e., nutrition, tissue damage, and chronic stress).

In addition to including variables that may reflect multiple areas of health, limitations of the biochemistry analyzer to reliably capture only one of five indicators of tissue damage may also be contributing to the tendency of PCs to include variables from multiple areas of health. One tissue damage analyte (lactate dehydrogenase [LDH]) of the five-total identified as indicative of tissue damage was within the range of the analyzer and reflected generalized tissue damage. Whereas, some of the remaining analytes were characteristic of tissue damage to specific organs and tissues such as the liver, heart, and brain. Because only one tissue damage variable was included in PCA it may have limited the sensitivity of PCA to detect tissue damage induced in the population in specific organs or tissues, thus, potentially limiting the ability of PCA to form one PC specific to tissue damage.

Finally, although the water temperature treatments were meant to induce extreme chronic stress, the absence of a metric strongly characterized by chronic stress is suggestive that the temperature manipulations were not severe enough to induce extreme chronic stress. High and low water temperatures used for the temperature treatments were relatively conservative to prevent mortalities, which may have been too

conservative to induce the stress necessary to induce maximum variability in stress level in the population.

PC 1 (accounting for ~29% of variation in the total dataset) included nutritional indicators (whole-body protein, energy, lipid content and plasma ALB, TP, CA, and HB), but also VHA which can be indicative of stress or tissue damage in fish. Therefore, PC 1 may reflect a contrast between nutritional status and VHA scores (stress or tissue damage). In PC 2 (~14% of variation), several nutritional indicators (plasma ALP, CHOL, MG, and TP) as well as a tissue damage indicator (plasma LDH), a stress indicator (plasma GLU), and VHA correlate positively with PC 2. Finally, in PC 3 (~12% of variation), nutritional indicators (Kn and plasma PHOS) and stress indicators (plasma NA and CL) correlate positively with PC 3. Thus, although my hypothesis that each PC would form an indicator of a specific area of health was not validated, I did successfully identify groupings of physiological variables to further investigate predictors of juvenile pallid sturgeon ecological performance (i.e. growth).

PC1 and PC2, independently and in combination, were significantly related to percent growth of individuals subjected to nine combinations of different feed and water temperature treatments. The strongest predictor of growth was an MLR model included PC1 and PC2, which accounted for 46.8% of the variance in the percent growth dataset. From the approach utilized in the current study, I was able to predict almost half of the variation in growth over the course of four months in fish with varying chronic stress levels and energetic reserves with just one measurement time point. Whereas, traditional approaches to quantify growth rate typically do so on an annual basis as it is difficult to

recapture the same fish within one season. Thus, annual field sampling typically requires sampling at least two measurements over the course of two years, potentially creating lag time in identifying changes in growth in response to environmental factors. Although PC1 and PC2, in combination, provided insight into growth performance of a population of hatchery-reared juvenile pallid sturgeon, it is also important to note that a large amount of the variation in growth performance remained unexplained by the model.

Additionally, because whole-body energetic reserves are important to PC1 and are determined by lethal means, PC1 cannot be directly applied to HOPS and an investigation of nonlethal tools to estimate whole-body energetic reserves would be necessary to apply this approach to wild-captured HOPS.

Reference Ranges and Post-Manipulation Values of Abnormal Fish

Comparisons of the physiological measurements for two moribund fish (MF1 and MF2) in the study with determined reference intervals and average post-manipulation values can provide insight into the potential drivers of mortality and fish that may be at risk of extremely poor health. Additionally, moribund fish had values outside reference ranges or post manipulation averages of variables included in PC1, PC2, and PC3 meaning PCs successfully identified MF1 and MF2 as abnormal fish.

Although comparisons of MF1 and MF2 physiological variables with the non-moribund experimental fish can provide meaningful interpretations of these variables, these interpretations must be made with caution. It is important to note that blood biochemistry can change drastically as homeostatic physiological processes break down approaching death (Fevolden et al. 2003). Furthermore, the low sample size ($n = 2$) of

moribund fish, although they exhibited some similarities in blood biochemical values, do not provide a conclusive measure of expected physiological values in moribund pallid sturgeon.

Summary Conclusion

In an attempt to expand the toolset available to fisheries managers to assess health status of pallid sturgeon, I established a set of biochemical and physiological analytes that can quantitatively predict growth in juvenile pallid sturgeon using PCA while explaining nearly half of the variation in recent growth. I found that many of the physiological variables that contributed to the PCs that explained variation in percent growth were related to nutrition. Notably, whole-body energy and whole-body lipid content, which were both lethally determined, were important to PC1. In addition to nutritional variables, tissue damage, and chronic stress variables were also important to the PCs explaining percent growth.

Two shortcomings of this study include the lethal sampling requirement for some of the variables quantified here and limitations of the VetAxcel biochemical analyzer in quantifying some of the biochemical variables. The limitation of the biochemical analyzer prevented several of the analytes identified as potentially informative to juvenile pallid sturgeon health from being included in PCA as they fell outside the range of detection for the biochemical analyzer used in this study. Follow-up research would benefit from investigating alternative analytical methods to quantifying concentrations of these analytes in pallid sturgeon plasma. This would be especially informative for tissue damage, as a non-specific indicator (LDH) of tissue damage was the only tissue damage

analyte that was within range of the analyzer, whereas specific information on heart, brain, liver, and kidney damage was lost with analytes that were generally outside the detection range of the analyzer. As previously stated, whole-body proximate composition largely influenced PC1 which was included in the model predicting percent growth. Because proximate composition is determined through lethal means, it is not a viable method to assess wild-caught pallid sturgeon body composition, limiting application to management efforts. Therefore, it would be valuable to explore non-lethal alternatives to estimate whole-body energetic reserves in juvenile pallid sturgeon (see Chapter 3).

The results of this study identify and characterize biomarkers of physiological processes within juvenile pallid sturgeon that could be applied by fisheries managers to investigate hypotheses regarding the ecological mechanisms contributing to declines in growth rates of more recently stocked year-classes of pallid sturgeon. As environmental conditions filter through the physiological processes of individuals to impact populations, tracking physiological status of individuals can provide managers with a temporally sensitive approach to identifying effects of ecosystem changes and management actions on populations. Physiological tools available to pallid sturgeon managers have been limited due to a scarcity of knowledge of pallid sturgeon physiology. This study establishes reference ranges for blood biochemical analytes that can be applied as bioindicators of physiological processes related to nutrition, tissue damage, and chronic stress in HOPS. Comparisons of these bioindicators measured on wild-captured fish to findings described in this study could allow insight into the physiological processes of

individual fish that may be driving contemporary depressed growth in HOPS as well as greater population response to environmental changes. Additionally, the PCs defined in my study were able to explain 50% of the variation in recent growth, suggesting these bioindicators could be applied by managers to identify fluctuations in growth rate of pallid sturgeon in the wild without the lag time required to detect changes in growth with current measures. However, it is important to note that the PCs are not easily interpretable and do not indicate specific areas of health. Additionally, further investigation is necessary to determine the direct application of information from PCA in this study to wild-caught pallid sturgeon. Similarly, determining how blood biochemistry differs in juvenile wild-caught HOPS compared to juvenile hatchery-reared pallid sturgeon would expand the application of blood biochemistry to wild-caught individuals and further expand the toolset available to managers to assess the health of individual wild-caught HOPS. Finally, whole body energy and lipid content, which were determined through lethal approaches in this study, made important contributions to the PCs predicting recent growth. However, non-lethal approaches to quantifying these variables would be essential for wide application of these biomarkers to population monitoring. Therefore, in the third chapter of my thesis, I investigated the Distell Fatmeter (Distell, West Lothian, Scotland, UK) as a non-lethal tool for estimation of whole-body energy and lipid content.

References

- Adams, S.M. 1999. Ecological Role of Lipids in the Health and Success of Fish Populations. (p. 132-153). In: Arts M.T., Wainman B.C. (eds) Lipids in Freshwater Ecosystems. Springer, New York, NY.
- Adams, S.M., Bevelhimer, M.S., Greely, M.S., Levine, D.A., The, S.J. 1999. Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. *Environmental Toxicology and Chemistry*, 18(4): 628-640.
- Adams, S.M., Brown, A.M., Goede, R.W. 1993. A Quantitative Health Assessment Index for Rapid Evaluation of Fish Condition in the Field. *Transactions of the American Fisheries Society*, 122(1): 63-73.
- AOAC. 2012. Official Methods of Analysis of AOAC international. 19th edition. AOAC International, Gaithersburg, Maryland, USA.
- Braaten, P. J., D. B. Fuller, R. D. Lott, T. M. Haddix, L. D. Holte, R. H. Wilson, M. L. Bartron, J. A. Kalie, P. W. Dehaan, W. R. Ardren, R. J. Holm, and M. E. Jaeger. 2012. Natural growth and diet of known-age pallid sturgeon (*Scaphirhynchus albus*) early life stages in the upper Missouri River basin, Montana and North Dakota. *Journal of Applied Ichthyology* 28(4):496–504.
- Braaten, P.J., Fuller, D.B., Lott, R.D., Jordan, G.R. 2009. An estimate of the historic population size of adult pallid sturgeon in the upper Missouri River Basin, Montana and North Dakota. *Journal of Applied Ichthyology*, 25(Suppl. 2): 2-7.
- Burkhead, N. M. 2012. Extinction Rates in North American Freshwater Fishes, 1900–2010. *BioScience* 62(9):798–808.
- Burnham, K.P., Anderson, D.R. 2004. Multimodal inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research*, 33(2): 261-304.
- Chipps, S.R., Klumb, R.A., and Wright, E.B. 2010. Development and application of juvenile pallid sturgeon bioenergetics model: Final Report, South Dakota State Wildlife Grant Program, Brookings, South Dakota, Study T–24–R Study No. 2424, 40 p.

- de Jong, P.E., Curhan, G.C. 2006. Screening, monitoring, and treatment of albuminuria: public health perspectives. *Frontiers in Nephrology*, 17: 2120-2126.
- DeLonay, A.J., Chojnacki, K.A., Jacobson, R.B., Albers, J.L., Braaten, P.J., Bulliner, E.A., Elliott, C.M., Erwin, S.O., Fuller, D.B., Haas, J.D., Ladd, H.L.A., Mestl, G.E., Papoulias, D.M., Wildhaber, M.L. 2016. Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River – A synthesis of science, 2005 to 2012: U.S. Geological Survey Scientific Investigations Report 2015-5145, 224 p.
- Deslauriers, D., Johnston, R., Chipps, S.R. 2016. Effect of Morphological Fin-Curl on the Swimming Performance and Station-Holding Ability of Juvenile Shovelnose Sturgeon. *Journal of Fish and Wildlife Management*, 7(1): 198-204.
- Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., Anderson, W.G. 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs. *Environmental Biol Fish*, 101: 623-637.
- Dimeski, G., Jones, B.W. 2011. Lipaemic samples: effective process for lipid reduction using high speed centrifugation compared with ultracentrifugation. *Biochemia Medica*, 22(2): 86-92.
- Everitt, B., Hothorn, T. 2011. *An Introduction to Applied Multivariate Analysis with R*. Springer, New York, New York, USA, pp. 15.
- Ferri, J., N. Topić Popović, R. Čož-Rakovac, B. Beer-Ljubić, I. Strunjak-Perović, F. Škeljo, M. Jadan, M. Petrić, J. Barišić, M. Šimpraga, and R. Stanić. 2011. The effect of artificial feed on blood biochemistry profile and liver histology of wild saddled bream, *Oblada melanura* (*Sparidae*). *Marine Environmental Research* 71(3):218–224.
- Fevolden, S. Røed, K.H., Fjalestad, K. 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. *Aquaculture*, 216: 67-76.
- Finnegan, D. 2014. referenceIntervals: Reference Intervals. R package version 1.1.1. <https://CRAN.R-project.org/package=referenceIntervals>.

- Friedrichs, K. R., K. E. Harr, K. P. Freeman, B. Szladovits, R. M. Walton, K. F. Barnhart, and J. Blanco-chavez. 2012. ASVCP reference interval guidelines : determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, 4:441–453.
- Gadomski, D.M., Parsley, M.J. 2005. Laboratory Studies on the Vulnerability of Young White Sturgeon to Predation. *North American Journal of Fisheries Management*. 25(2): 667-674.
- Glover, D.C., DeVries, D.R., Wright, R.A., Davis, D.A. 2010. Sample preparation techniques for determination of fish energy density via bomb calorimetry: An evaluation using largemouth bass. *Transaction of American Fisheries Society*, 139: 671-675.
- Grohs, K. L., R. A. Klumb, S. R. Chipps, and G. A. Wanner. 2009. Ontogenetic patterns in prey use by pallid sturgeon in the Missouri River, South Dakota and Nebraska. *Journal of Applied Ichthyology* 25(SUPPL. 2):48–53.
- Holmquist, L. M., C. S. Guy, A. Tews, and M. A. H. Webb. 2019. First maturity and spawning periodicity of hatchery-origin pallid sturgeon in the upper Missouri River above Fort Peck Reservoir, Montana. *Journal of Applied Ichthyology* 35(1):138–148.
- Horn, P. S., and A. J. Pesce. 2003. Reference intervals: An update. *Clinica Chimica Acta*, 334(1–2):5–23.
- Huizinga, H. W., G. W. Esch, and T. C. Hazen. 1979. Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacepede). *Journal of Fish Diseases* 2(4):263–277.
- Josse, J., Husson, F. 2016. missMDA: A package for handling missing values in multivariate data analysis. *Journal of Statistical Software*, 90(1): 1-31. Doi:10.18637/jss.v070.i01.
- Kappenman, K. M., Fraser, W. C., Toner, M., Dean, J., & Webb, M. A. H. 2009. Effect of temperature on growth, condition, and survival of juvenile shovelnose sturgeon. *Transactions of the American Fisheries Society*, 138, 927– 937.

- Kindschi, G.A. 1987. Method of Quantifying Degree of Fin Erosion. *The Progressive Fish-Culturist*, 49(4): 314-315.
- Li, D., Liu, Z., Xie, C. 2012. Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, *Acipenser shrenckii*. *Fish Physiology and Biochemistry*, 38: 511-520.
- Matsche, M.A. 2011. Evaluation of tricaine methanesulfonate (MS-222) as a surgical anesthetic for Atlantic Sturgeon *Acipenser oxyrinchus*. *Journal of Applied Ichthyology*, 27: 600-610.
- Meyer, H.A., Chipps, S.R., Graeb, B.D.S, Klumb, R.A. 2016. Growth, food consumption, and energy status of juvenile pallid sturgeon fed natural and artificial diets. *Journal of Fish and Wildlife Management*, 7(2): 388-396.
- Moyle, P.B., Cech, J.J. 2018. *Fishes: An introduction to ichthyology*. 3rd Impression. Pearson Education, Inc, New York, NY, USA.
- Oldenburg, E. W., C. S. Guy, E. S. Cureton, M. A. H. Webb, and W. M. Gardner. 2011. Effects of acclimation on poststocking dispersal and physiological condition of age-1 pallid sturgeon. *Journal of Applied Ichthyology* 27(2):436–443.
- Rafatnezhad, S., Falahatkar, B., Gilani, M.H.T. 2008. Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. *Aquaculture Research*, 39: 1506-1513.
- Range-wide Pallid Sturgeon Propagation Committee. 2019. Range-wide Pallid Sturgeon Propagation Plan. Denver, CO. 55 pp.
- Rcore Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Ricker, W.E. 1979. Growth rates and models. *Fish Physiology*, volume 8, bioenergetics and growth. Hoar, W.S., Randall, D.J., Brett, J.R. (Eds), Academic Press, Inc., New York, NY, USA, pp. 677-743.

- Roche, H., Bogé, G. 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Marine Environmental Research*, 41(1): 27-43.
- Rose, K. A., J. H. Cowan, K. O. Winemiller, R. A. Myers, and R. Hilborn. 2001. Compensatory density dependence in fish populations: Importance, controversy, understanding and prognosis. *Fish and Fisheries* 2(4):293–327.
- Rotella, J. 2017. Upper Basin Pallid Sturgeon Survival Estimation Project 2017 Update.
- Schober, P., Boer, C., Schwarte, L.A. 2018. Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia-Analgesia*, 126(5): 1763-1768.
- Sepúlveda, M. S., T. M. Sutton, H. K. Patrick, and J. J. Amberg. 2012. Blood chemistry values for shovelnose and lake sturgeon. *Journal of Aquatic Animal Health* 24(3):135–140.
- Shuman, D.A., Klumb, R.A., Wilson, R.H., Jaeger, M.E., Haddix, T., Gardner, W.M., Doyle, W.J., Horner, P.T., Ruggles, M., Steffensen, K.D., Wanner, G.A. 2011. Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. *Journal of Applied Ichthyology*, 27: 269 – 281.
- Simide, R. 2018. The blood indicators of Siberian sturgeon welfare. *Farming*, 2: 451-477.
- Sumpter, J.P. 1992. Control of growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 92: 299-320.
- UBPSPC [Upper Basin Pallid Sturgeon Propagation Committee]. 2005. Upper basin pallid sturgeon propagation plan.
- USFWS [United States Fish and Wildlife Service]. 1990. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Pallid Sturgeon. *Federal Register*, 55(173): 36641-36647.
- USFWS [United States Fish and Wildlife Service]. 1993. Pallid Sturgeon Recovery Plan. USFWS, Bismarck, North Dakota. 55 pp.

- USFWS [United States Fish and Wildlife Service]. 2008. Pallid Sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. U.S. Fish and Wildlife Service, Billings, Montana. Pp 71.
- USFWS [United States Fish and Wildlife Service]. 2014. Revised Recovery Plan for the Pallid Sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Denver, Colorado. 115 pp.
- USFWS [United States Fish and Wildlife Service]. 2018. Revised Pallid Sturgeon *Scaphirhynchus albus* Range-wide Stocking Plan. Denver, CO. 44 pp.
- Wagner, T., Congleton, J.L. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences, 61: 1066-1074.
- Webb, M.A.H., Allert, J.A., Kappenman, K.M., Marcos, J., Feist, G.W., Schreck, C.B., Shackleton, C.H. 2007. Identification of plasma glucocorticoids in pallid sturgeon in response to stress. General and Comparative Endocrinology, 154: 98-104.
- Wikelski, M., Cooke, S.J. 2006. Conservation physiology. Trends in Ecology and Evolution, 21(2): 38-46.
- Wilson, R., S. Hultberg, and Z. Sandness. 2017. 2016 Annual Report Pallid Sturgeon Population Assessment and Associated Fish Community Monitoring for the Missouri River: Segment 4. U.S. Fish and Wildlife Service, Bismarck, North Dakota.
- Witeska, M. Anemia in teleost fishes. Bulletin of European Association of Fish Pathologists, 35(4): 148-160.

AN ASSESSMENT OF THE DISTELL FATMETER FOR
USE IN JUVENILE PALLID STURGEON
(SCAPHIRHYNCHUS ALBUS)

Contributions of Authors and Co-Authors

Manuscript in Chapter 3

Author: Matea A. Djokic

Contributions: Implemented the study, collected and analyzed data, and wrote the manuscript

Co-Author: Kevin Kappenman

Contributions: Obtained funding, conceived the study design, collected data, discussed the implications of the results, and provided guidance on the manuscript.

Co-Author: Joshua Heishman

Contributions: Collected data and provided guidance on the manuscript.

Co-Author: T. Gibson Gaylord

Contributions: provided technical expertise on proximate analysis, discussed the implications of the results, provided guidance on the manuscript.

Co-Author: Christine Verhille

Contributions: Obtained funding, collected data, conceived the study design, discussed the implications of the results, and provided guidance on the manuscript.

Manuscript Information Page

Matea A. Djokic, Kevin Kappenman, Joshua Heishman, T. Gibson Gaylord, Christine Verhille

Transactions of the American Fisheries Society

Status of Manuscript:

- Prepared for submission to a peer-reviewed journal
- Officially submitted to a peer-reviewed journal
- Accepted by a peer-reviewed journal
- Published in a peer-reviewed journal

ABSTRACT

Energetic stores can be used as metrics of health in fish species; however, these metrics are traditionally quantified using lethal measures that depend on proven measurements of body condition. Validation of non-lethal alternatives to quantify energetic stores would facilitate the ability of managers to monitor nutritional and energetic status of wild-captured fish of conservation concern. Some fisheries biologists have hypothesized that declining growth rates in juvenile hatchery origin pallid sturgeon (HOPS; *Scaphirhynchus albus*) in the upper basin of the Missouri River is a density-dependent effect from intraspecific competition resulting from the potential overstocking of the ecosystem. However, a lack of information on historic sustainable growth rates of juvenile pallid sturgeon limits testing of this hypothesis. Accurate measurements of sturgeon energetic reserves could allow biologists to test whether growth trends reflect depleted reserves. The Distell Fatmeter, a handheld microwave energy meter, has been validated to estimate whole-body energy and lipid content in a wide range of fish taxa, but has been ineffective in some species, and never tested on sturgeon. Therefore, Fatmeter readings taken on a cohort of hatchery-reared juvenile pallid sturgeon, subjected to chronic rearing manipulations aimed at maximizing variation in energetic stores within the experimental population, were compared to energetic reserves determined lethally by proximate analysis. The Fatmeter alone did not effectively estimate energetic reserves in juvenile hatchery-reared pallid sturgeon. However, in the largest juveniles, a model including Fatmeter and mass measurements significantly predicted whole-body lipid ($p < 0.001$; $R^2 = 0.45$) and energy content ($p < 0.001$; $R^2 = 0.40$; $df = 33$) for hatchery-reared juvenile pallid sturgeon and explained nearly half of the variation in energetic reserves. This model explained more variation in energetic reserves than relative condition alone, which is the current metric most commonly interpreted to reflect juvenile HOPS energetic reserves. Therefore, although the amount of variation in juvenile hatchery-reared pallid sturgeon energetic reserves explained by models including Fatmeter measurements were lower than for other taxa, Fatmeter measurements can improve predictions of juvenile pallid sturgeon energetic reserves relative to currently tracked metrics.

Introduction

Energetic reserves, primarily driven by lipid stores, are crucial to physiological processes that govern growth, movement, reproduction, and long-term survival in fishes (Adams 1999; Cowey and Sargent 1979; Deslauriers et al. 2018). Energetic reserves have conventionally been quantified through lethal techniques. As lethal sampling is not a viable option for wild-caught rare and endangered fishes, non-lethal tools to estimate energetic reserves of these fish provide valuable additions to the metrics available to fisheries biologists. Microwave energy transmission measured with commercially available hand-held meters, like the Distell Fatmeter (Distell, West Lothian, Scotland, UK), provides a non-lethal alternative to estimate whole-body energy and whole-body lipid content of fish. The Fatmeter is a promising device that has been successfully applied to fish of several taxa (e.g., Bayse et al. 2018, Crossin and Hinch 2005; Schloesser and Fabrizio 2017), but, to my knowledge, the Fatmeter has not been evaluated on sturgeon species.

Whole-body energy and lipid content are important drivers of fish cellular homeostasis, metabolism (Adams 1999), and survival during periods of nutritional deprivation (Deslauriers et al. 2018). Lipids are an important energy reserve in fish because they possess a relatively high energetic density and play key roles in cellular metabolism and osmotic and electrolytic homeostasis (Adams 1999). In sturgeon, whole-body lipid content and, correspondingly, whole-body energy content has been shown to decrease with starvation (Furnè et al 2012; Hung et al. 1997; Hung & Lutes 1987; Deslauriers, et al. 2018) and restricted dietary lipid intake (Mohseni et al. 2007). Energy

density has been shown to predict laboratory-based overwinter starvation survival of lake sturgeon (Deslauriers et al. 2018) and spawning migration success of Pacific salmon (Young et al. 2006). Thus, whole-body energetic reserves can predict the capacity of fishes to carry out ecologically important life processes.

The concept of an index of length to weight is one of the most widely applied non-invasive tools to assess overall health status of fish. Relative condition (K_n) was developed to measure the deviation of an individual from the average weight for length of a population of fish (Le Cren 1951), making the measurement applicable for specific populations under investigation (Murphy and Willis 1992). With the concept of K_n , a weight-length equation was derived specifically for pallid sturgeon populations in the Missouri River basin and is commonly assessed during population monitoring (Shuman et al, 2011). However, K_n did not correlate with levels of whole-body energy in juvenile pallid sturgeon (Meyer et al. 2016). For these reasons, developing non-invasive tools to more accurately quantify whole-body energy content of fish may improve the ability of managers to better assess overall health status of fish.

The Fatmeter, which is a non-lethal alternative to lethal approaches to quantifying whole-body composition, may provide an option to expand on current metrics of health monitored for wild captured fishes. The Fatmeter uses a microwave sensor to non-destructively estimate the lipid content of a sample based on the well-established relationship between body lipids and moisture (Distell 2010). Although the Fatmeter has been validated to estimate whole-body energy and lipid content in a wide range of fish taxa, it has also been ineffective at estimating whole-body energy and lipid content in

some species of fish (Table 3.1). Thus, further investigation is necessary before the Fatmeter can be considered a promising tool to assess energetic reserves of rare and endangered species like members of the sturgeon family (*Acipenseridae*).

Table 3.1. List of fish species for which Distell Fatmeter readings have been tested on to predict whole-body lipid content and whole-body energy content with R-squared values and references.

Energetic Reserve	Species	R ²	Reference
Energy	Atlantic herring (<i>Clupea harengus harengus</i>)	0.71	Vogt et al. 2002;
Energy	Pacific Salmon	0.65 – 0.93	Colt and Shearer 2001; Crossin and Hinch 2005
Energy	Atlantic croakers (<i>Micropogonias undulates</i>)	0.58 – 0.62	Schloesser and Fabrizio 2017
Energy	Striped Bass (<i>Morone saxatilis</i>)	0.02 – 0.12	Schloesser and Fabrizio 2017
Energy	Summer Flounder (<i>Paralichthys dentatus</i>)	0.33 – 0.44	Schloesser and Fabrizio 2017
Energy	American shad (<i>Alosa sapidissima</i>)	0.72	Bayse et al. 2018
Lipid	Pacific Salmon	0.84 – 0.94	Crossin and Hinch 2005
Lipid	Atlantic croakers (<i>Micropogonias undulates</i>)	0.64 – 0.68	Schloesser and Fabrizio 2017
Lipid	Striped Bass (<i>Morone saxatilis</i>)	0.07 – 0.22	Schloesser and Fabrizio 2017
Lipid	Summer Flounder (<i>Paralichthys dentatus</i>)	0.39 – 0.41	Schloesser and Fabrizio 2017
Lipid	Steelhead Salmon (<i>Oncorhynchus mykiss</i>)	0.10	Hanson et al. 2010
Lipid	Smallmouth Bass (<i>Micropterus dolomieu</i>)	0.02	Mesa and Rose 2015
Lipid	Walleye (<i>Sander vitreus</i>)	0.17	Mesa and Rose 2015
Lipid	Channel Catfish (<i>Ictalurus punctatus</i>)	0.83	Mesa and Rose 2015

The pallid sturgeon (*Scaphirhynchus albus*) is an endangered sturgeon species experiencing limitations in recruitment (USFWS 2014) and early life growth (R. Wilson, personal communication, July 3, 2018) in the Missouri and Mississippi river systems. Validation of the Fatmeter for pallid sturgeon is expected to assist efforts of fisheries biologists to recover this protected fish. The pallid sturgeon was declared endangered under the Endangered Species Act (ESA) in 1990 (ESA 1973, as amended; 55 FR 36641, September 6, 1990) and subsequently hatchery production of pallid sturgeon for stocking has occurred since 1998 (UBPSPC 2005). Biologists suspect recent density-dependent effects on hatchery origin pallid sturgeon (HOPS) but lack the metrics necessary to evaluate these concerns. Fisheries biologists have observed a decline in growth rates of recently stocked HOPS relative to earlier cohorts (Wilson et al. 2017) leading some biologists hypothesize that this decline in growth rate is a density-dependent effect arising from intraspecific competition. This hypothesis is supported by evidence of high survival rates, high relative abundance of stocked HOPS (Rotella 2017), and reduced growth rates of subsequent year-classes (Wilson et al. 2017). However, a population-wide analysis of pallid sturgeon K_n in the upper basin of the Missouri River suggests a general increasing trend from 2005 to 2015 (Steffensen et al. 2017). This apparent contradiction between temporal trends in growth rate and K_n illustrates the importance of exploring metrics that more directly quantify energetic reserves of wild pallid sturgeon.

Thus, a non-invasive technique, like the Fatmeter, that can accurately estimate whole-body lipid content and energy content will expand the metrics available to fisheries biologists to investigate the health and energetic status of pallid sturgeon of the

Missouri River. The objective of this study was to validate the Fatmeter on juvenile pallid sturgeon and to develop a best-fit model to most effectively estimate whole-body energetic reserves of juvenile pallid sturgeon using common non-lethal measurements and Fatmeter readings as predictor variables in the model.

Methods

Study system and animals

A total of 142 hatchery-born juvenile pallid sturgeon were reared at the US Fish and Wildlife Service (USFWS) Bozeman Fish Technology Center (BFTC) in Bozeman, MT. Fish were approximately age two (FL (mm) = 240 to 415; mass (g) = 33.4 to 213.6; $K_n = 0.65$ to 1.32) when sampled. Of the 142 hatchery-born juvenile pallid sturgeon, 135 fish sampled at BFTC were exposed to water temperature-feed ration manipulations. These manipulations were aimed at maximizing variance in whole-body energetic reserves of the experimental population before sampling and were not intended for direct testing of temperature and feed effects on the experimental fish. The remaining seven fish were lethally sampled prior to the manipulative study to cull excess fish and achieve equal sample sizes of 15 fish per tank during the manipulative study. All 142 fish originated from two distinct spawning events in 2017 at USFWS Gavins Point National Fish Hatchery in Yankton, South Dakota and were reared within the parameters recommended by the Upper Basin Pallid Sturgeon Propagation Plan (UBPSPC 2005) until September 2018. In September 2018, these fish were moved for acclimation into rectangular flow-through experimental tanks and fish were fed Trout Extruded Sinking

feed pellets until water temperature and feed manipulations began in November 2018 (Skretting, Tooele, UT, USA).

To validate the Fatmeter as an accurate tool to estimate juvenile pallid sturgeon whole-body lipid content and whole-body energy content across a varying range of energetic reserves, groups of fish were reared under various water temperature and feed ration conditions. During the four-month rearing period, beginning in November 2018 and ending in March 2019, fish were reared in groups of five in 75L rectangular flow-through tanks with flows of 2L/minute. Each of the 27 tanks were randomly assigned one of three water temperature conditions and one of three feed ration conditions such that three replicate tanks of fish were assigned to each of the nine possible combined temperature and feed ration conditions.

Targeted water temperatures included a low temperature of 12°C [approximately 11.7°C ± standard deviation (SD) 0.32°C], a moderate temperature of 18°C [approximately 17.8°C ± SD 0.24°C], and a high temperature that increased from 25°C to 28°C [approximately 24.6°C ± SD 1.02°C at the beginning and 27.2°C ± SD 0.79°C at the end] during the rearing period. Water temperatures were held for four months and chosen based on published papers and unpublished work from a collaborator that suggests that the temperatures used in the study range between far below optimum to optimum conditions (Chipps et al. 2009; K. Kappenman, personal communication, July 30, 2018).

Initial daily feed ration treatments were set at high (3.5% of tank biomass per day), moderate (2.5% of tank biomass per day), and low (1% of tank biomass per day to

fasting). Feed rations were determined based on previous work that showed a range of growth rates (no growth to growth) in pallid sturgeon (Oldenburg et al. 2011). To create variation in energetic reserves, we had the following objectives for different diet ration groups: low ration treatment tanks experience a loss in fish mass on average; moderate ration treatment tanks experience slow gain in fish mass on average; high feed ration tanks experience rapid gain in mass on average and feed exceeds satiation (without accumulation of food that may cause water quality issues).

At the end of the four-month water temperature and feed manipulation period, all tanks of fish were fasted for six days before sampling and temperatures were brought to 18°C two days after fasting began. This allowed for Fatmeter measurements at a common water temperature for all fish and prevented impacts of gut contents on Fatmeter measurements. To reach the target 18°C, water temperature of 12°C treatment tanks was raised by 1°C per day and water temperature of 28°C treatment tanks was decreased by 2°C per day over the appropriate number of days preceding sampling.

During sampling, fish were euthanized using a lethal dose of MS-222 (tricaine methanesulfonate, Syndel (formerly Western Chemical), Ferndale, WA, USA; 250mg/L water dosage) and Fatmeter measurements were measured within two minutes of euthanization. Carcasses were immediately frozen and stored at -20°C until proximate analysis was performed. A companion study quantified blood biochemical responses to the experimental manipulations and required collection of ≤ 1 mL blood samples from fish before Fatmeter measurements, which was not expected to influence Fatmeter

measurements. Relative condition (Kn) was derived by the following weight-length equation (Shuman et al. 2011):

$$\text{Log}_{10}(\text{weight}) = -6.2561 + 3.2932 \times \text{Log}_{10}(\text{fork length})$$

Fatmeter measurements

Fatmeter measurements were measured using the RESEARCH-1 setting of the Distell Fatmeter that was preprogrammed to report the average of two readings for each measurement site. The Fatmeter User Manual provides recommended measurement sites in areas on, above, and below the lateral line for several species of fish but lacks guidance for any sturgeon species. Therefore, multiple preliminary measurement sites were tested on pallid sturgeon.

The locations for Fatmeter measurement sites (Figure 3.1) were determined in a pilot study that sampled preliminary measurement sites on adult and juvenile pallid sturgeon that were not further included in the Fatmeter study. The number of sites and locations investigated were restricted by fish size. A total of nine preliminary measurement sites were possible on large adult fish (Figure 3.1A; approximate FL (mm) = 650 to 900): three above lateral scutes, three below lateral scutes, and three on the abdomen. Three preliminary measurement sites were possible on small juvenile fish: one above lateral scutes, one below lateral scutes, and one on the abdomen (Figure 3.1B; approximate FL (mm) = 250 to 320). Coefficients of variation based on triplicate measurements for each site were calculated for 96 fish that were a separate population from the experimental population and were only used for coefficient of variation calculations (Figure 3.1A, [adults; n = 17]; Figure 3.1B, [juveniles; n = 79]) in a

preliminary study. Measurement sites which produced average coefficient of variation exceeding 10% were excluded (data not shown) to identify the finalized measurement sites.

Although I aimed to define multiple individual separate measurement sites, the size of fish included in the Fatmeter study limited the assignment of values to three measurements (including one overlapping measurement) on larger juvenile fish and a single measurement on smaller juvenile fish. The finalized measurement sites on fish large enough for multiple measurement sites were two distinct measurement sites Upper Anterior (UA) and Upper Posterior (UP) and one medial overlapping measurement, Upper Medial Overlapping (Umol) which overlapped with the UA and UP measurement sites (Figure 3.2A). On fish that were too small for multiple measurements, I defined a single medial measurement, Upper Medial (UM) (Figure 3.2B).

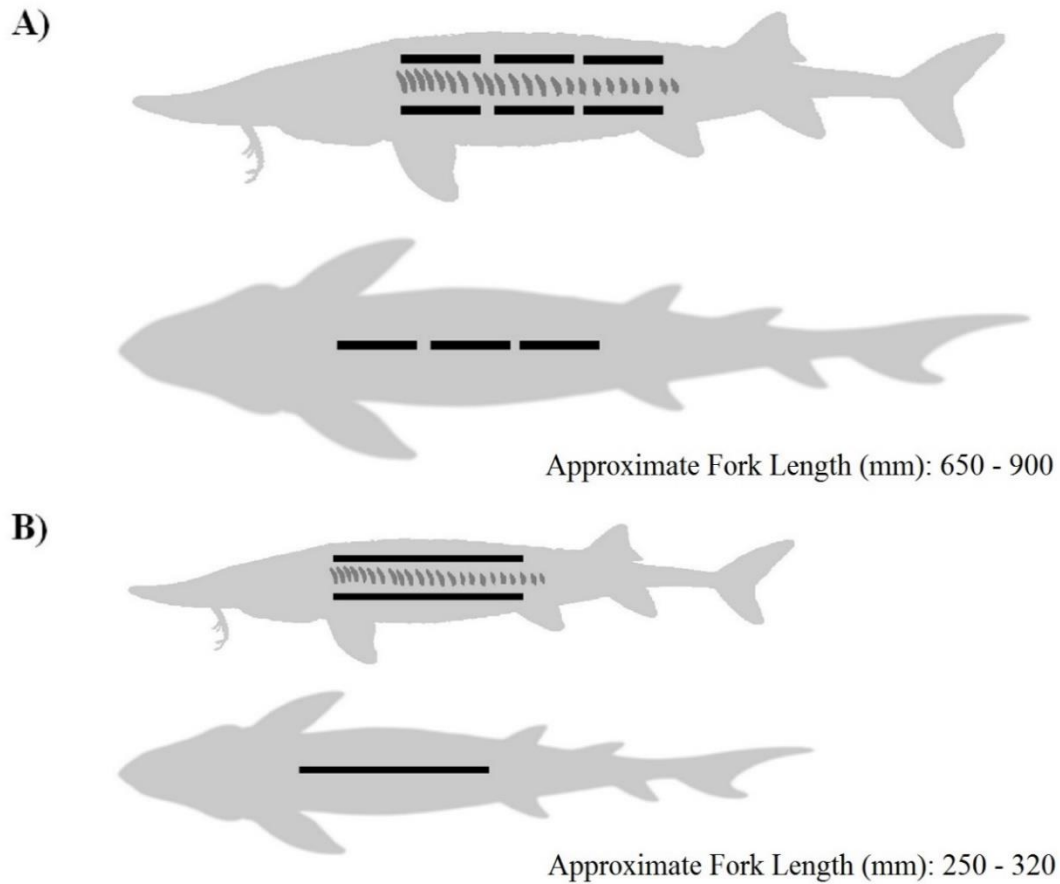


Figure 3.1. Outline of side-view and abdominal view of pallid sturgeon (*Scaphirhynchus albus*) indicating twelve total preliminary measurement sites of the Distell Fatmeter to assess the whole-body energy content and whole-body lipid content of hatchery-reared juvenile pallid sturgeon in a pilot study. A) Preliminary measurement sites on adult fish included three measurement sites dorsal and ventral of the scutes and three on the abdomen. B) Preliminary measurement sites on small juvenile fish included one site dorsal and ventral of the scutes and one on the abdomen.

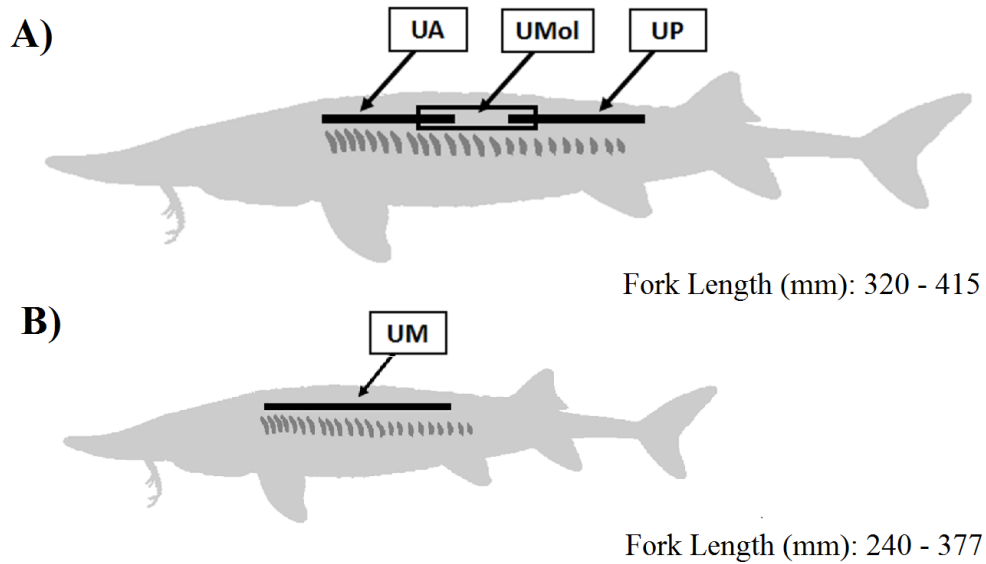


Figure 3.2. Outline of side-view of finalized measurement sites for the Distell Fatmeter used to assess the whole-body energy content and whole-body lipid content of hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) in the manipulation study. A) For fish large enough to fit two distinct anterior and posterior measurement sites, three finalized measurement sites were identified: Upper Anterior (UA), Upper Posterior (UP), and an overlapping medial site, Upper Medial overlap (Umol). B) Finalized Upper Medial (UM) measurement site on fish that were too small to fit multiple overlapping measurement sites).

Proximate analysis

Whole-body energy and whole-body lipid content were determined through proximate analysis of pallid sturgeon carcasses. Samples for proximate analysis were prepared in a step-wise process; carcasses were thawed, cut into small pieces and homogenized in an industrial meat grinder (Hobart Corporation, Troy, OH, USA). The ground samples were freeze dried in a LabConco lyphilizer (LabConco Corporation,

Kansas City, MO, USA) and ground again. Homogenized samples were stored in sealed tubes and subsampled to measure whole-body lipid and whole-body energy content.

Moisture content of wet whole-body samples was determined from sample mass before and after drying in a convection drying oven (Yamato Scientific Co, Ltd., Tokyo, Japan) (AOAC 2012). Whole-body lipid content was determined on freeze-dried samples through the ether extraction method using an Ankom XT10 Extractor (Ankom Technology, Macedon, NY, USA) (AOAC 2012). Whole-body energy content was determined on freeze-dried samples through the bomb calorimetry technique as described in Glover et al. (2010), using a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL, USA). The moisture content of wet whole-body samples was used to calculate the wet weight whole-body lipid and whole-body energy contents of individual fish from lipid and energy content determined on a freeze-dry basis.

Data analysis

Linear regression analysis was used to determine if Fatmeter readings alone or in combination with size and Kn (Shuman et al. 2011) measurements were predictive of whole-body energy and whole-body lipid content determined by proximate analysis. Linear regression occurred through two stages of modeling. For the first stage, Fatmeter measurements were investigated alone, without the addition of other predictor variables to determine if Fatmeter measurements alone could predict whole-body energy and whole-body lipid content. For the second stage of modeling, the best fit model of all possible combinations of Fatmeter measurements, mass, FL, and Kn was investigated to determine the best non-lethal estimate of whole-body energy and whole-body lipid

content. Of the 142 fish originating from Gavins Point, one fish was removed from analysis because the carcass was inadvertently not retained for proximate analysis. Another five fish were removed from the dataset before the analyses because experimenters were unable to achieve consistent Fatmeter readings on the fish. Four fish were removed from the analyses because Fatmeter measurements on these fish were recognized as outliers according to the generalized extreme Studentized deviate test (Rosner 1983). In total, 10 fish were removed from the original 142 fish and 132 were used in the final analysis. Consistent with previous publications (Colt and Shearer 2001; Crossin and Hinch 2005), Fatmeter readings followed an approximately logarithmic distribution and were, consequently, naturally log (ln) transformed. Natural log transformed Fatmeter measurements and whole-body energy and whole-body lipid content were compared in R (R Core Team 2019) using the `lm()` function.

To determine the one Fatmeter measurement site or combination of measurement sites that most effectively contributed to models predicting whole-body energy and whole-body lipid content, fish were divided into two groups based on the number of measurement sites achievable. Fatmeter readings of fish in each group were compared to proximate composition by simple linear regression (SLR). For most fish with FL greater than 330mm, multiple measurement sites were achievable; while for fish below 330mm, only one measurement site tended to be achievable. Therefore, the two groups used for predictive modelling included: large fish ($n = 36$; FL (mm) = 320 to 415; mass (g) = 98.3 to 213.6; $K_n = 0.78$ to 1.21) capable of three measurements sites and fish of all sizes ($n = 132$; FL (mm) = 250 to 415; mass (g) = 46.5 to 213.6; $K_n = 0.65$ to 1.32). For the large

fish, SLR was used to investigate the model coefficients of each Fatmeter site as well as averages of combinations of sites in relation to whole-body energy and whole-body lipid content. Additional analyses including all measurement sites was performed, but in this analysis, because the UM and Umol sites were the only shared sites across the size range, only middle measurements were compared to whole-body energy and lipid content.

To investigate the contributions of the independent variables, fork length (FL), mass, and relative condition factor (Kn) in addition to Fatmeter measurements in predicting whole-body lipid and whole-body energy content, multiple linear regression (MLR) was explored. To determine if any independent variables were linearly related within a model, multicollinearity was explored using a variance inflation factor (VIF) determined by the $VIF()$ function from the `regclass` package in R (Petrie 2020). In general, VIF is considered too large and to indicate collinearity between variables at a cutoff value between five to ten (Craney 2007). Although fork length and mass were very strongly correlated and contributed to multicollinearity in models with VIFs of greater than 100 when concurrently included, no other combination of variables significantly covaried. As a result, fork length and mass were not included concurrently in any models. Distributions of fork length and mass were positively skewed and were, thus, \ln transformed for multiple linear regression. Relative condition was normally distributed and, thus, not transformed.

The best model fit to predict whole-body energy and whole-body lipid content was identified based on Akaike's Information Criteria with a correction for small sample sizes (AICc), parsimony, and cross validation. An alpha level of 0.05 was used for all

analyses and any models including independent variables that were not significant at this alpha level were removed from consideration. Across all analyses, models investigated were compared using AICc using the following equation:

$$\Delta_i = \text{AICc}_i - \text{AICc}_{\min}$$

where AICc_{\min} is the lowest AICc value of all investigated models and AICc_i is the value of the i^{th} model (as described in Burnham and Anderson 2004). Models with $\Delta_i \leq 2$ were selected as competitive for the best model fit. If multiple models were within the $\Delta_i \leq 2$ cutoff, the most parsimonious model was picked as the best fit. The most parsimonious models were those with the lowest number of independent variables and measurement sites (i.e., a single measurement rather than averaged measurements across multiple measurement sites). If models were equally parsimonious (i.e. equal number of predictor variables and/or Fatmeter readings), models were compared by cross validation across 10,000 partitions to determine which model better performed in predicting whole-body energy or whole-body lipid content in an out-of-sample test. Training and test datasets were created from the total dataset of proximate analysis and Fatmeter values in each of the previously mentioned 10,000 partitions using the `createDataPartition()` function from the `caret` package in R (Kuhn 2019). Competing models were trained on the newly defined training dataset and tested on the newly defined test dataset. Then, root mean square errors (RMSEs) (Willmott et al. 1985) were compared between the two models across the 10,000 partitions and the model with lower RMSE values the majority of the time was selected as the best fit model.

Results

Population Energetic Reserves, Mass, Fork Length, and Relative Condition

The total population of fish used for linear regressions (i.e., after elimination of outliers and fish with inconsistent Fatmeter readings) exhibited a wide range of energetic reserves and Kn in the hatchery population of juvenile pallid sturgeon (n = 132; Table 3.2). For example, whole-body lipid ranged from 0 to 11.3%, which corresponded with Kn ranging from 0.654 to 1.32.

Table 3.2. Descriptive statistics of energetic reserves (whole-body energy and whole-body lipid content determined through proximate analyses), relative condition (Kn), fork length, and mass in a population of hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) (n = 126 experienced water temperature and diet manipulations over the course of four months; n = 6 were reared under typical hatchery conditions) where SD is standard deviation.

Measure	Mean	SD	Minimum	Maximum
Whole-body Energy (cal/g)	1092.2	296.2	358.0	1930.0
Whole-body Lipid (%)	4.7	2.35	0	11.3
Relative Condition (Kn)	1.01	0.120	0.654	1.32
Fork Length (mm)	314.3	34.3	250	415
Mass (g)	97.4	37.4	46.5	213.6

Relationships Between Fatmeter Readings and Whole-body Energetic Reserves

For SLR models using data collected from all fish to estimate whole-body lipid content (df = 130, p = 0.046, R² = 0.02; Table 3.3) and whole-body energy content (df = 130, p = 0.177, R² = 0.01; Table 3.3) only UM or Umol was included in the model and natural log transformed (ln) Fatmeter readings did not account for much of the variation.

However, for SLR models applying data collected from only the fish large enough to fit multiple Fatmeter measurement sites to estimate whole-body lipid content ($df = 34$, $R^2 = 0.13$ to 0.23 ; Table 3.3) and whole-body energy content ($df = 34$, $R^2 = 0.15$ to 0.21 ; Table 3.3), ln Fatmeter measurements explained up to ten times more variation compared to models developed with all fish and including only one measurement site. Because of the low amount of variation in whole-body energetic reserves explained by Fatmeter readings alone, the addition of Kn, fork length, and mass were included in MLR to determine models that best explain whole-body energetic reserves in juvenile pallid sturgeon.

Table 3.3. Akaike's Information Criteria (AICc) scores and coefficients of determination (R^2) from single linear regressions in which natural logarithmically transformed (ln) Fatmeter readings from individual positions and averages of multiple positions significantly contributed to a model predicting whole-body lipid and energy content of hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*). Abbreviations are as follows: ln is natural log transformed, UM is Upper Medial Fatmeter measurement, UA is Upper Anterior Fatmeter measurement, UP is Upper Posterior Fatmeter measurement, and avg is the average of multiple Fatmeter measurements.

Population	Fish Position	Lipid (%)		Energy (cal/g)	
		AICc	R^2	AICc	R^2
All Fish (240 – 415mm)	ln(UM)	602.1	0.02	1881.4	0.01
Large Fish (320 – 415mm)	ln(UM)	161.0	0.19	505.7	0.21
	ln(UA)	159.9	0.22	507.6	0.17
	ln(UP)	163.7	0.13	508.4	0.15
	ln(avg(UA, UM))	159.4	0.23	505.6	0.21
	ln(avg(UA, UP))	161.3	0.19	507.2	0.17
	ln(avg(UM, UP))	162.0	0.17	506.5	0.19
	ln(avg(UA, UM, UP))	160.7	0.20	506.1	0.20

Fatmeter readings did not contribute to best fit MLR models estimating whole-body energy and lipid content across all fish (the entire size range) measured in the study.

The best fit of these MLR models to predict both whole-body lipid content ($df = 129$, $p < 0.001$, $R^2 = 0.53$; Table 3.4; Figure 3.3A) and whole-body energy content ($df = 129$, $p < 0.001$, $R^2 = 0.48$; Table 3.4; Figure 3.3B) included K_n and \ln transformed mass as independent variables. Cross validation was used to determine the best fit models for both whole-body lipid and energy content because AICc and parsimony failed to separate the models. For whole-body lipid content, compared to the next competitive model, $K_n + \ln(\text{mass})$ performed better 81.3% of the time on out of sample prediction on a cross validation assessment. For whole-body energy content, compared to the next competitive model, $K_n + \ln(\text{mass})$ performed better 53.5% of the time on out of sample prediction on a cross validation assessment.

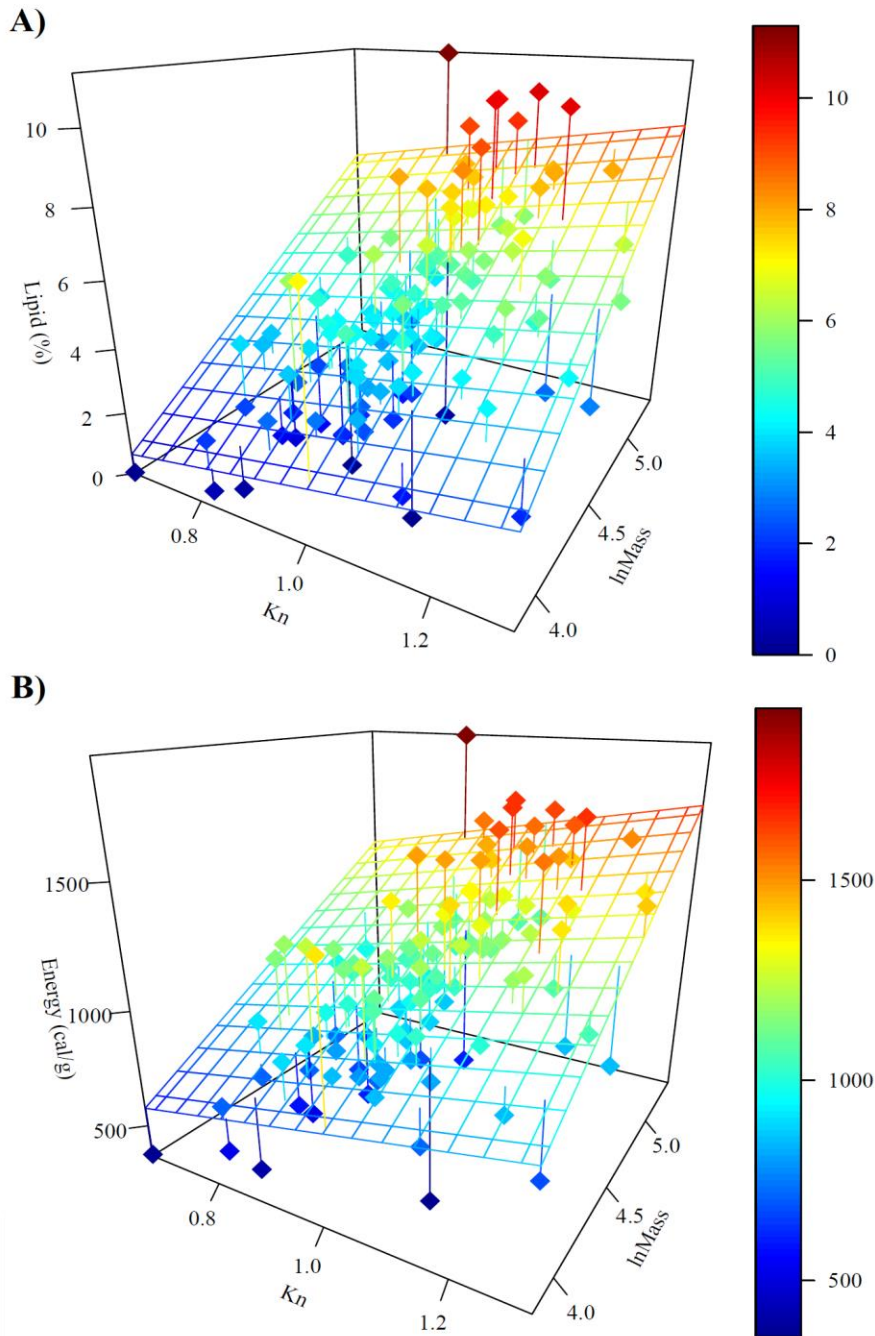


Figure 3.3. Best fit multiple linear regression models predicting (A) whole-body lipid and (B) whole-body energy content in hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) across all sizes of fish, using only medial Fatmeter measurements, included in the study where K_n is relative condition, $\ln \text{Mass}$ is natural log transformed mass, and the colored gradient represents energy content of individuals where blue is low and red is high.

There were multiple competitive MLR models fit to predict whole-body lipid content and whole-body energy content of the subset of large fish capable of UA, UM, and UP Fatmeter measures and both of the final best fit models included Fatmeter measurements (Table 3.4). The best fit model to predict whole-body lipid content across large fish was $\ln(\text{UA}) + \ln(\text{mass})$ ($df = 33$, $p < 0.001$, $R^2 = 0.45$; Table 3.4, Figure 3.4A). Multiple models predicting whole-body lipid content were equivalent according to AICc, so this best fit model was determined based on parsimony. For whole-body energy content, cross validation was used to determine the best fit model because two models were deemed equivalent based on AICc and parsimony. When compared to the model including Kn and $\ln(\text{FL})$, the model including $\ln(\text{UA})$ and $\ln(\text{Mass})$ performed better 66% of the time on out of sample prediction on a cross validation assessment in predicting whole-body energy and, therefore, $\ln(\text{UA}) + \ln(\text{Mass})$ was the best fit model to predict whole-body energy content of large fish that allowed consideration of three Fatmeter measurements sites ($df = 33$, $p < 0.001$, $R^2 = 0.40$; Table 3.4, Figure 3.4B).

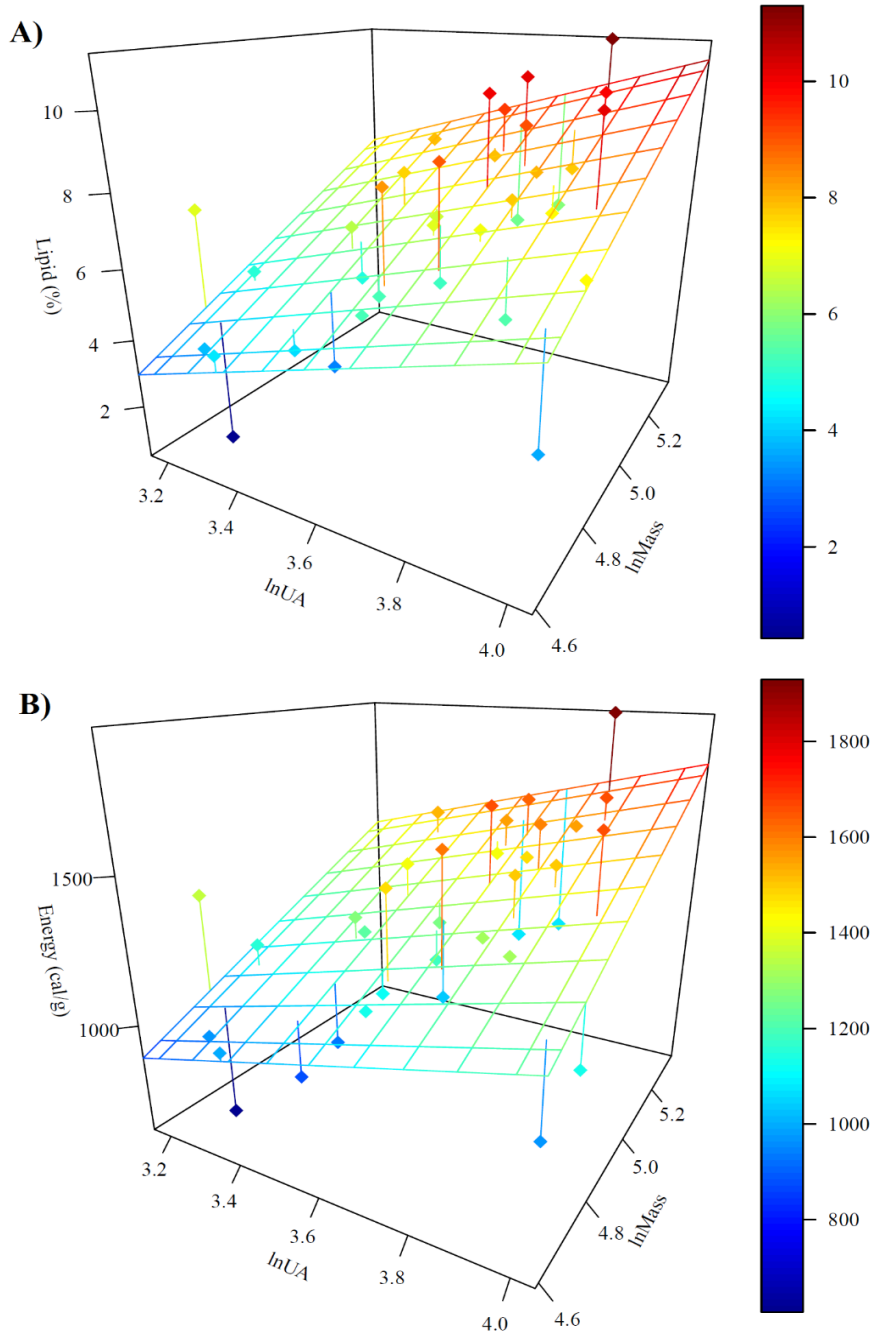


Figure 3.4. Best fit multiple linear regression models predicting (A) whole-body lipid and (B) whole-body energy content in hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) across only fish large enough to fit multiple Fatmeter measurement sites included in the study where $\ln UA$ is natural log transformed Fatmeter readings at the Upper Anterior site, $\ln Mass$ is natural log transformed mass, and the colored gradient represents energy content of individuals where blue is low and red is high.

Table 3.4. Akaike's Information Criteria (AICc) scores and coefficients of determination (R^2) from competitive (models within 2 AICc values of AICc_{min}) multiple linear regressions (MLR) and individual variables in those MLR models for hatchery-reared juvenile pallid sturgeon (*Scaphirhynchus albus*) in estimating whole-body energy and lipid content where Kn is relative condition, FL is fork length, UA is Upper Anterior Fatmeter measurement, UM is Upper Medial Fatmeter measurement and ln is natural logarithmically transformed (ln), and avg is an average of multiple Fatmeter measurement sites.

Population	Model/Predicting Variables	Lipid (%)		Energy (cal/g)	
		AICc	R ²	AICc	R ²
All Fish (240 – 415mm)	Kn + ln(Mass)	505.9	0.53	1797.1	0.48
	Kn + ln(FL)	507.0	0.53	1798.0	0.48
	Kn	592.6	0.09	1867.4	0.11
	lnMass	510.2	0.51	1803.7	0.45
	lnFL	540.1	0.39	1830.8	0.11
Large Fish (320 – 415mm)	ln(UA) + ln(Mass)	148.7	0.45	497.4	0.40
	ln(avg(UA, UM)) + ln(Mass)	150.2	0.43	497.2	0.40
	Kn + ln(FL)	153.7	0.37	499.1	0.37
	Kn	166.1	0.07	510.7	0.09
	lnMass	153.3	0.35	499.6	0.35
	lnFL	160.2	0.21	506.6	0.19

Discussion

The Fatmeter has been validated to estimate the energetic reserves of several species of teleost but this is the first investigation of the Fatmeter in any species in the family *Acipenseridae*. Fatmeter readings alone were not effective in predicting whole-body energy or whole-body lipid content but in combination with ln mass, Fatmeter readings formed the best fit model and explained nearly 50% of the variation of whole-body lipid and energy content. The limitations of Fatmeter measurements alone estimating energetic reserves in juvenile pallid sturgeon may be due to fat storage

patterns in sturgeon or the small size of fish included in the study. Fatmeter readings did not significantly differ between whole-body and gutted individual *Clupea harengus*, suggesting that Fatmeter estimates muscular lipid content and not lipid within the viscera (Davidson and Marshall 2010). In Acipenseriformes, gonad (Treanor et al. 2017), liver, and peritoneal lipid stores (Singer and Ballantyne 2004), tend to be high, potentially reducing the accuracy of the Fatmeter for this taxon. However, on the subset of large juvenile pallid sturgeon in the present study, ln transformed Fatmeter measurements (Umol) alone explained more variation than Fatmeter measurements (UM) on the total population of fish studied. This may indicate that Fatmeter measurements may have potential to explain a greater amount of variation in body composition of larger pallid sturgeon than the fish investigated in this study.

Fatmeter measurements alone were not effective in explaining variance in whole-body energetic reserves in juvenile pallid sturgeon. Across all sizes of fishes included in the analysis, Fatmeter readings accounted for only 1% to 2% of the variation observed in whole-body energy or whole-body lipid content. This is likely due to variation in performance of the Fatmeter across fish sizes as model performance with Fatmeter readings improved on the subset of larger juvenile fish investigated in the study where fork length ranged from 320 to 415mm. However, Fatmeter readings alone still only explained up to 23% of the variation observed in whole-body energetic reserves. This is consistent with findings for striped bass (*Morone saxatilis*; Schloesser and Fabrizio 2017), steelhead salmon (*Oncorhynchus mykiss*; Hanson et al. 2010), smallmouth bass (*Micropterus dolomieu*; Mesa and Rose 2015), and walleye (*Sander vitreus*; Mesa and

Rose 2015), in which Fatmeter measurements alone explained 2% to 22% of variation in energetic reserves. On the other hand, for Atlantic herring (*Clupea harengus harengus*; Vogt et al. 2002), Pacific salmon (Crossin and Hinch 2005), channel catfish (*Ictalurus punctatus*; Mesa and Rose 2015), and American shad (*Alosa sapidissima*; Bayse et al. 2018) the variation in energetic reserves explained by Fatmeter measurements was as high as 70% to 90%. The low levels of explained variation in juvenile pallid sturgeon energetic reserves by Fatmeter measurements is likely due to the inability of the Fatmeter to capture visceral stores.

Models that included measurements of size in addition to Fatmeter measurements were better able to predict whole-body energy and whole-body lipid content than those including Fatmeter measurements alone. Although, best fit models on fish across the full range of sizes (fork length range: 240 – 415mm) do not include Fatmeter measurements. For larger fish (fork length range: 320 – 415mm), the best fit model included Fatmeter measurements. Specifically, for larger fish, the best fit models to estimate whole-body energy and lipid content included $\ln(\text{UA})$ and $\ln(\text{mass})$ and explained 45% of variation in whole-body lipid and 40% of variation in whole-body energy. When these models were compared to the best fit model not including Fatmeter measurements (i.e., Kn and $\ln \text{FL}$), models with Fatmeter measurements explained nearly 10% more of the variation in whole-body lipid content, but only 3% more of the variation in whole-body energy content.

Models including Kn or Fatmeter measurements in addition to fork length or mass explained roughly half of the variance observed in whole-body energetic reserves of

juvenile pallid sturgeon. Across the entire size range of fish, the best fit model to estimate energetic reserves included Kn and mass while on a subset of large fish, the best fit model included Fatmeter readings and Mass. Together, these models explained between 40% and 53% of the variance in whole-body energetic reserves of juvenile pallid sturgeon. Although a large amount of variation in energetic reserves remained unexplained by models including the Fatmeter, these models were more effective at estimating the energetic reserves of juvenile pallid sturgeon than current metrics (i.e., Kn alone) that are commonly monitored in wild captured pallid sturgeon. Therefore, incorporation of Fatmeter measurements can improve the toolset available to managers to assess HOPS health.

In determining whether the Fatmeter is a tool worth investing in, managers should consider several factors. Models with Kn outperformed models with In Fatmeter measurements across a broad range of juvenile pallid sturgeon sizes but, when size was limited to the larger juveniles, Fatmeter became more informative to predict whole-body energy and whole-body lipid content. Therefore, managers should consider the size of targeted fish and their objectives to determine whether the Fatmeter is useful for their goals.

Further considerations include the cost of purchasing the Fatmeter and training requirements. At the time that I ordered the Distell Fatmeter, the total cost of the Fatmeter was almost \$7,000 including cost of international shipping and transit insurance. Additionally, training of field crews is required for consistent Fatmeter measurements. However, Kn is already routinely calculated from measurements

collected by fisheries managers when capturing pallid sturgeon in the field and requires no special training or expensive purchases. Therefore, utilizing Kn to estimate energetic reserves in juvenile pallid sturgeon is more time and cost efficient. Additionally, historically collected Kn values can be applied to the models derived here to estimate past whole-body energy and whole-body lipid levels in fish. Though, it is important to note that nearly 50% of the variation in energetic reserves remained unexplained with either Fatmeter or Kn modellings.

However, the increased importance of Fatmeter readings as an independent variable in models predicting whole-body energy and whole-body lipid content for larger juvenile fish suggests that the Fatmeter could be more effective on adult fish than the population investigated in this study. Therefore, further research is needed to determine the efficacy of the Fatmeter on a larger sized population of pallid sturgeon such as adults.

Physiological metrics, like energetic reserves estimated by the Fatmeter, have the potential to elucidate the underlying mechanisms behind population responses to management and environmental changes. Whole-body energy and whole-body lipid content are important for fish survival and change with environmental changes (i.e., water temperature, food availability, etc.) and, therefore, can provide managers with more immediate information regarding pallid sturgeon response to environmental changes and management efforts such as increased or decreased stocking of HOPS. Additionally, direct, lethal measurements of whole-body proximate composition were important to a suite of physiological variables that explained nearly half of the variance in recent growth of juvenile pallid sturgeon (see Chapter 2). In this study, the Fatmeter was investigated

as a potential approach for non-lethal quantification of whole-body energetic reserves. Although the Fatmeter explained only approximately 2% of the variance in whole-body energetic reserves of the total population of juvenile pallid sturgeon tested in this study, performance of the Fatmeter improved when applied to the largest juvenile pallid sturgeon in this study. Therefore, I recommend further investigation of the Fatmeter applied to larger sturgeon, including adult pallid sturgeon. However, there is also the potential that the Fatmeter is not suited for use in juvenile pallid sturgeon and, thus, alternative physiological measures to estimate energetic reserves should be investigated.

References

- Adams, S.M. 1999. Ecological Role of Lipids in the Health and Success of Fish Populations. (p. 132-153). In: Arts M.T., Wainman B.C. (eds) Lipids in Freshwater Ecosystems. Springer, New York, NY.
- AOAC. 2012. Official Methods of Analysis of AOAC international. 19th edition. AOAC International, Gaithersburg, Maryland, USA.
- Bayse, S.M., Regish, A.M., McCormick, S.D. 2018. Proximate composition, lipid utilization and validation of a non-lethal method to determine lipid content in migrating American shad *Alosa sapidissima*. *Journal of Fish Biology*, 92: 1832-1848.
- Burnham, K.P., Anderson, D.R. 2004. Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*, 33(2): 261 – 304. DOI: 10.1177/0049124104268644.
- Chipps, S.R., Klumb, R.A., Wright, E.B. 2009. Development and Application of Juvenile Pallid Sturgeon Bioenergetics Model: Final Report, South Dakota State Wildlife Grant Program, Brookings, South Dakota, Study T-24-R Study No. 2424, 40 p.
- Colt, J., Shearer, K.D. 2001. Evaluation of the use of the Torry Fatmeter to Non-lethally Estimate Lipid in Adult Salmon. U.S. Army Corps of Engineers. 75 pp.
- Cowey, C.B., Sargent, J.R. 1979. Nutrition. *Fish Physiology*, (8): 1-69.
- Crossin, G.T., Hinch, S.G. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. *Transactions of the American Fisheries Society*, 134(1): 184-191.
- Davidson, D., Marshall, C.T. 2010. Are morphometric indices accurate indicators of stored energy in herring *Clupea harengus*?. *Journal of Fish Biology*, 76: 913-929.
- Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., Anderson, W.G. 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*)

and its implications for conservation stocking programs. *Environmental Biol Fish*, 101: 623-637.

- Distell. 2010. Distell Fish Fatmeter: User manual. Fauldhouse, West Lothian, Scotland, UK.
- Froese, R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*, (22): 241–253.
- Furnè, M., Morales, A.E., Trenzado, C.E., Garcia-Gallego, M., Hidalgo, M.C., Domezain, A., Rus, A.S. 2012. The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. *Journal of Comparative Physiology B*, 182: 63-76.
- Glover, D.C., DeVries, D.R., Wright, R.A., Davis, D.A. 2010. Sample preparation techniques for determination of fish energy density via bomb calorimetry: An evaluation using largemouth bass. *Transaction of American Fisheries Society*, 139: 671-675.
- Hanson, K.C., Ostrand, K.G., Gannam, A.L., Ostrand, S.L. 2010. Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. *Transactions of the American Fisheries Society*, 139: 1733 – 1741.
- Hung, S.O., Liu, W., Li, H., Storebakken, T., Cui, Y. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 151: 357-363.
- Hung, S.S.O., Lutes, P.B. 1987. Optimum feeding rate of hatchery-produced juvenile white sturgeon (*Acipenser transmontanus*): at 20°C. *Aquaculture*, 65: 307-317.
- IUCN 2019. The IUCN Red List of Threatened Species. Version 2019-2. <http://www.iucnredlist.org>. Downloaded on 18 July 2019.
- Kuhn, M. Contributions from Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., Cooper, T., Mayer, Z., Kenkel, B., the R Core Team, Benesty, M., Lescarbeau, R., Ziem, A., Scrucca, L. Tang, Y., Candan, C., Hunt, T. 2019. *Caret*:

Classification and regression training. R package version 6.0-84. <https://CRAN.R-project.org/package=caret>.

- Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20(2): 201-219.
- Mesa, M.G., Rose, B.P. 2015. An assessment of morphometric indices, blood chemistry variables and an energy meter as indicators of the whole body lipid content in *Micropterus dolomieu*, *Sander vitreus* and *Ictalurus punctatus*. *Journal of Fish Biology*. 86: 755 – 764.
- Meyer, H.A., Chipps, S.R., Graeb, B.D.S., Klumb, R.A. 2016. Growth, food consumption, and energy status of juvenile pallid sturgeon fed natural and artificial diets. *Journal of Fish and Wildlife Management*, 7(2): 388-396.
- Mohseni, M., Sajjadi, M., Pourkazemi, M. 2007. Growth performance and body composition of sub-yearling Persian sturgeon, (*Acipenser persicus*, Borodin, 1897), fed different dietary protein and lipid levels. *Journal of Applied Ichthyology*, 23: 204-208.
- Murphy, B.R., Willis, D.W. 1992. Proper Distinction between Relative Weight and Relative Condition Factor. *North American Journal of Fisheries Management*, 12: 665-666.
- Oldenburg, E.W., Guy, C.S., Cureton, E.S., Webb, M.A.H., Gardner, W.M. 2011. Effects of acclimation on poststocking dispersal and physiological condition of age-1 pallid sturgeon. *Journal of Applied Ichthyology*, 27: 436-443.
- Petrie, A. 2020. Regclass: Tools for an Introductory Class in Regression and Modeling. R package version 1.6. <https://CRAN.R-project.org/package=regclass>
- Rcore Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rosner, B. 1983. Percentage Points for a Generalized ESD – Many Outlier Procedure. *Technometrics*, 25(2): 165 – 172.

- Rotella, J. (2017). Upper Basin Pallid Sturgeon Survival Estimation Project 2017 Update.
- Schloesser, R.W., Fabrizio, M.C. 2017. Condition Indices as Surrogates of Energy Density and Lipid Content in Juveniles of Three Fish Species. *Transactions of the American Fisheries Society*, 146: 1058-1069.
- Schober, P., Boer, C., Schwarte, L.A. 2018. Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia & Analgesia*, 126(5): 1763 – 1768. DOI: 10.1213.
- Shuman, D.A., Klumb, R.A., Wilson, R.H., Jaeger, M.E., Haddix, T., Gardner, W.M., Doyle, W.J., Horner, P.T., Ruggles, M., Steffensen, K.D., Wanner, G.A. 2011. Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. *Journal of Applied Ichthyology*, 27: 269 – 281.
- Singer, T.D., Ballantyne, J.S. 2004. Sturgeon and paddlefish metabolism. Pages 167-194 in LeBreton, G.T.O., Beamish, F.W.H., Mckinley, R.S., editors. *Sturgeons and Paddlefish of North America*. Kluwer Academic Publishers, New York, NY, USA.
- Steffensen, K.D., Mestl, G.E., Phelps, Q.E. 2017. Range-wide assessment of pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson, 1905) relative condition. *Journal of Applied Ichthyology*, 33: 13-21.
- Treanor, H.B., Miller, I.R., Halvorson, L.J., Van Eenennaam, J.P., Doroshov, S.I., Webb, M.A.H. 2017. Effect of dietary fat on adipocyte size in farmed age-2 white sturgeon (*Acipenser transmontanus*, Richardson, 1836). *Journal of Applied Ichthyology*, 34: 419-423.
- Upper Basin Pallid Sturgeon Propagation Committee (UBPSPC). 2005. Upper Basin Pallid Sturgeon Propagation Plan, 89 pp.
- USFWS [United States Fish and Wildlife Service]. 1990. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Pallid Sturgeon. *Federal Register*, 55(173): 36641-36647.
- U.S. Fish and Wildlife Service (USFWS). 2014. Revised recovery plan for the pallid sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Denver, Colorado, 115 pp.

- Vogt, A., Gormley, R., Downey, G., Somers, J. 2002. A Comparison of Selected Rapid Methods for Fat Measurement in Fresh Herring (*Clupea harengus*). *Journal of Food Composition and Analysis*, 15: 205-215.
- Willmott, C.J., Ackleson, S.G., Davis, R.E., Feddema, J.J., Klink, K.M., Legates, D.R., O'Donnell, J. Rowe, C.M. 1985. Statistics for the Evaluation and Comparison of Models. *Journal of Geophysical Research*, 90: 8995-9005.
- Wilson, R., S. Hultberg, and Z. Sandness. 2017. 2016 Annual Report Pallid Sturgeon Population Assessment and Associated Fish Community Monitoring for the Missouri River: Segment 4. U.S. Fish and Wildlife Service, Bismarck, North Dakota.
- Young, J.L., Hinch, S.G., Cooke, S.J., Crossin, G.T., Patterson, D.A., Farrell, A.P., Van Der Kraak, G., Lotto, A.G., Lister, A., Healey, M.C., English, K.K. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 1067-1077.

CONCLUSION TO THESIS

The overall objective of my thesis was to develop non-lethal tools for fisheries managers to assess the overall health status of wild-captured pallid sturgeon. In consequence, the sub-objectives of this thesis were 1) to define ranges of physiological variables expected to be observed in pallid sturgeon reared under typical hatchery conditions and how they correlate together in a population of fish with varying energetic reserves and chronic stress levels, then 2) to determine the relationship between the physiological variables characterized in Objective 1 and an ecologically relevant performance metric (i.e., growth rate) and 3) as whole-body energetic reserves, which were identified as important to the relationship between physiological variables and growth rate, are lethally determined, I aimed to develop a best-fit model to estimate whole-body energetic reserves of juvenile pallid sturgeon using non-lethal measurements, including Fatmeter readings, as predictor variables in the model. In Chapter 2, I determined reference intervals for 13 blood biochemical analytes and determined groups of physiological variables that correlated together among juvenile pallid sturgeon. I was then able to derive a model to predict recent growth of juvenile pallid sturgeon based on these groups of correlating physiological variables. In Chapter 3, I compared Fatmeter readings, relative condition (K_n), fork length (FL), and mass to whole-body energetic reserves lethally determined by proximate analysis in order to determine a best fit model to estimate energetic reserves in hatchery-reared juvenile pallid sturgeon.

In Chapter 2, I successfully characterized ranges for a suite of physiological variables related to nutrition, stress, and tissue damage in a population of juvenile pallid

sturgeon. I determined reference intervals for 13 blood biochemical analytes to characterize hatchery-reared juvenile pallid sturgeon under typical hatchery rearing conditions. Additionally, I characterized composite variables from the same 13 analytes and five more physiological variables that correlated together for juvenile pallid sturgeon reflecting a broad range of nutritional statuses after being reared under water temperature and diet ration conditions ranging from below optimal to above optimal.

Although this study did not support my hypothesis that PCA of physiological variables measured for juvenile pallid sturgeon reflecting a broad range of nutritional statuses would reveal three principal components defining three unique axes of health (nutrition, chronic stress, and tissue damage), I was able to show a relationship between PCs and percent growth of juvenile pallid sturgeon. The three PCs that accounted for most of the variance in our dataset did not separately reflect nutrition, chronic stress, and tissue damage, but each consisted of variables from multiple aspects of health. Each of the three PCs that were determined in this chapter were significantly related to percent growth of juvenile pallid sturgeon reflecting a broad range of nutritional statuses. Additionally, many of the physiological values of two moribund fish fell outside of the ranges described by the rest of the experimental population, suggesting that abnormal fish could be identified with variables included in the three PCs. Therefore, as all fish in this study were reared identically up to the initiation of the four month manipulation study, this study provided evidence that a suite of physiological variables can predict divergence in recent growth performance within four months of fish experiencing changes to their environment. Development of this approach for application to wild captured sturgeon

could reduce the lag time separating ecosystem changes and detection of their impacts on sturgeon. It is important to note that whole-body proximate composition contributed to the first PC that explained the most amount of variation in recent growth, but these are determined through lethal means. Nonlethal approaches to quantifying whole-body energetic reserves would be more broadly applicable to monitoring wild-captured pallid sturgeon.

In Chapter 3, I was able to show that, in juvenile pallid sturgeon exceeding 320mm fork length (FL), Fatmeter measurements in combination with commonly assessed size and relative condition measurements can predict whole-body energetic reserves. Across fish with FL ranging from 240 to 415mm, Fatmeter readings did not significantly contribute to explaining variation in whole-body energetic reserves. For these fish, the best fit model included Kn and $\ln(\text{Mass})$ in estimating whole-body lipid ($R^2 = .53$, $p < 0.001$, $df = 129$) and whole-body energy content ($R^2 = .48$, $p < 0.001$, $df = 129$). However, when focusing fish that were sufficiently large to allow multiple Fatmeter measurement sites (FL (mm): 320 to 415), Fatmeter readings contributed significantly to the multiple linear regression models and explained up to 8% more variance than models that did not include Fatmeter readings. In subset of large fish, the best fit model included Fatmeter readings (UA) and $\ln(\text{Mass})$ in estimating whole-body lipid ($R^2 = .45$, $p < 0.001$, $df = 33$) and whole-body energy content ($R^2 = .40$, $p < 0.001$, $df = 33$). Therefore, although the Fatmeter alone did not predict whole-body energetic reserves in hatchery-reared juvenile pallid sturgeon, for juvenile pallid sturgeon greater than FL 320mm, Fatmeter measurements in combination with size measurements did

account for a greater amount of variation in whole-body energetic reserves than size measurements alone.

In conclusion, I have filled important knowledge gaps regarding physiological measures of juvenile pallid sturgeon and provided alternative non-lethal tools that may be useful for managers to assess wild-caught pallid sturgeon. To my knowledge, this is the first study in pallid sturgeon to determine reference intervals for blood biochemical parameters, investigate the Fatmeter, and investigate how physiological variables correlate together to form composite variables and predict recent growth. The results of Chapter 2 provide ranges of physiological variables, including blood biochemistry, that measurements on wild-captured juvenile pallid sturgeon could be compared with to identify individuals displaying atypical nutrition-, tissue damage-, or chronic stress-related physiological processes. Although this study included a four-month manipulation of temperature and diet ration to provoke the maximum range expected of the measured physiological variables in juvenile pallid sturgeon, follow up monitoring on wild-captured fish is required to confirm applicability to wild-caught fish. As environmental impacts on fish populations are filtered through physiological responses at the individual fish level, successful application of these findings to wild-captured fish could allow fisheries biologists to rapidly identify causal effects of changes to the ecosystem or management activities on fish populations and reduce reliance of management decisions on correlational relationships. Application of nonlethal Fatmeter measurements to quantify whole-body energetic reserves may also be included in this approach, but the large amount of variation in whole-body energetic reserves unexplained by Fatmeter

measurements suggests further research into alternative nonlethal approaches to quantify whole-body energy reserves in juvenile pallid sturgeon is warranted. Thus, the studies in this thesis provide a conceptual foundation for future experiments to build on in creating a robust physiological assessment for use in wild-captured HOPS and other rare and endangered species.

References

- Davidson, D., Marshall, C.T. 2010. Are morphometric indices accurate indicators of stored energy in herring *Clupea harengus*?. *Journal of Fish Biology*, 76: 913-929.
- DeLonay, A.J., Chojnacki, K.A., Jacobson, R.B., Albers, J.L., Braaten, P.J., Bulliner, E.A., Elliott, C.M., Erwin, S.O., Fuller, D.B., Haas, J.D., Ladd, H.L.A., Mestl, G.E., Papoulias, D.M., Wildhaber, M.L. 2016. Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River – A synthesis of science, 2005 to 2012: U.S. Geological Survey Scientific Investigations Report 2015-5145, 224 p.
- Friedrichs, K. R., K. E. Harr, K. P. Freeman, B. Szlodovits, R. M. Walton, K. F. Barnhart, and J. Blanco-chavez. 2012. ASVCP reference interval guidelines : determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, 4:441–453.
- Shuman, D.A., Klumb, R.A., Wilson, R.H., Jaeger, M.E., Haddix, T., Gardner, W.M., Doyle, W.J., Horner, P.T., Ruggles, M., Steffensen, K.D., Wanner, G.A. 2011. Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. *Journal of Applied Ichthyology*, 27: 269 – 281.
- Simide, R. 2018. The blood indicators of Siberian sturgeon welfare. *Farming*, 2: 451-477.
- Skinner, J.P., Tuomi, P.A., Mellish, J.E. 2015. The influence of time in captivity, food intake and acute trauma on blood analytes of juvenile Steller sea lions, *Eumetopias jubatus*. *Conservation Physiology*, 3:1-12.
- Wagner, T., Congleton, J.L. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 1066-1074.
- Wikelski, M., Cooke, S.J. 2006. Conservation physiology. *Trends in Ecology and Evolution*, 21(2): 38-46.

APPENDICES

APPENDIX A

BLOOD ANALYTES TABLE OUTLINING FUNCTION AND RANGES IN
STURGEON SPECIES

Appendix A: Literature summary of blood biochemical analyte physiological significance and reported reference ranges in sturgeon species. The analytes are separated into groups of indicators of nutritional condition, tissue damage, and chronic stress level. Values and references based on pallid sturgeon (*Scaphirhynchus albus*) are indicated by (*).

Analyte	Function	Approximate Sturgeon Range	References
Nutritional Factor			
Total Protein (TP) (g/dL)	Plasma proteins are important to osmoregulation, immune defense, lipid transport, and blood clotting. Plasma protein concentrations are linked to changes in energetic reserves in fish. Total protein levels are influenced by increases and decreases in plasma albumin.	1.2 – 6.9; (1 – 9)*	Kroll and Elin 1985; Cataldi et al. 1998; Congleton and Wagner 2006; Knowles et al. 2006; Shi et al. 2006; Shahsavani et al. 2010; DiVincenti et al. 2013; Matsche et al. 2014; DeLonay et al. 2015*; Campbell et al. 2018
Cholesterol (CHOL) (mg/dL)	Cholesterol is important for the formation of cell membranes and the synthesis of steroids and hormones and, like triglycerides, is a nonpolar lipid that must be transported in plasma. Cholesterol in the membrane of cells regulates the transmembrane penetration of water. Cholesterol levels in the plasma are indicative of changes in energetic reserves in fish.	19.4 – 260; (25 – 336)*	Congleton and Wagner 2006; Knowles et al. 2006; Shi et al. 2006; Shahsavani et al. 2010; DeLonay et al. 2015*; Campbell et al. 2018; Moyle and Cech 2018
Calcium (CA) (mg/dL)	Calcium is integral to the transmission of nerve signals and the regulation of muscle contractions. Calcium also regulates the	1.1 – 36	Cataldi et al. 1998; Knowles et al 2006; Wagner and Congleton, 2006; Shahsavani

	uptake of water between cells. Reduced levels of calcium are indicative of extended periods of fasting in fish. Calcium binds to albumin, potentially at the same site as magnesium.		et al. 2010; DiVincenti et al. 2013; Matsche et al. 2014; Campbell et al. 2018; Moyle and Cech 2018
Alkaline Phosphate (ALP) (U/L)	ALP is important in skeletal development and for breaking down proteins. Concentrations of ALP respond to changes in food consumption in fish.	47 – 497	Hessle et al. 2002; Congleton and Wagner 2006; Knowles et al. 2006; Shi et al. 2006; Feng et al. 2011
Albumin (ALB) (g/dL)	Albumin is important for transporting fatty acids as well as other substances and contributes 80% of the colloid osmotic pressure and is important for maintaining blood volume. Albumin is highly soluble so large transport capacity for moving fatty acids and bilirubin. Albumin also maintains fluidity in vascular bed and is produced only in the liver so it can be indicative of liver health. Decreased levels of albumin can be indicative of starvation in fish. Albumin in combination with a family of other plasma proteins, globulins, make up total protein concentrations and albumin reflects the majority of total protein concentrations in the plasma. Albumin is a binding site for magnesium and calcium.	.8 – 1.7	Russell 1982; Rothschild et al. 1984; Kroll and Elin 1985; Knowles et al. 2006; Shahsavani et al. 2010; Sepúlveda et al. 2012; Matsche et al. 2014

Blood Urea Nitrogen (BUN) (mg/dL)	<p>BUN can be used in conjunction with creatine to determine the rate of protein catabolism. BUN can also be indicative of disrupted nitrogen excretion by the gills and increases when ammonia excretion is reduced.</p>	<p>13 – 17</p>	<p>Russell et al. 1982; Shahsavani et al. 2010</p>
Creatinine (CREAT) (mg/dL)	<p>Creatinine is a result of muscle metabolism, synthesized by the liver and transported to the blood for kidney excretion. It can be indicative of liver and kidney health (or disease/damage) and the rate of protein catabolism. Creatinine can also be indicative of muscle mass and renal function and may be better than the BUN:creatinine ratio at predicting renal functioning.</p>	<p><0.03 – 1.9; (<0.03 – 1)*</p>	<p>Baum et al. 1975; Russell et al. 1982; Knowles et al. 2006; Shi et al. 2006; Shahsavani et al. 2010; DeLonay et al. 2015*</p>
Phosphorous (PHOS) (mg/dL)	<p>Phosphorous is important for bones, scales, blood clotting, muscle function, nerve impulse transmission, osmoregulation, and cofactor during various enzymatic processes. Changes in phosphorous concentration indicate fluid imbalance or nutritional disease, poor growth, and bone mineralization.</p>	<p>5.1 – 13.5</p>	<p>Lovell 1989; Knowles et al. 2006; Shahsavani et al. 2010</p>
Hemoglobin (HB) (g/dL)	<p>Low HB values can reflect anemia in fishes and low iron levels due to malnutrition, starvation, bacterial infections, or</p>	<p>5 – 8.7</p>	<p>Roche and Boge 1996; Knowles et al. 2006; Matsche et al. 2014; Witeska 2015</p>

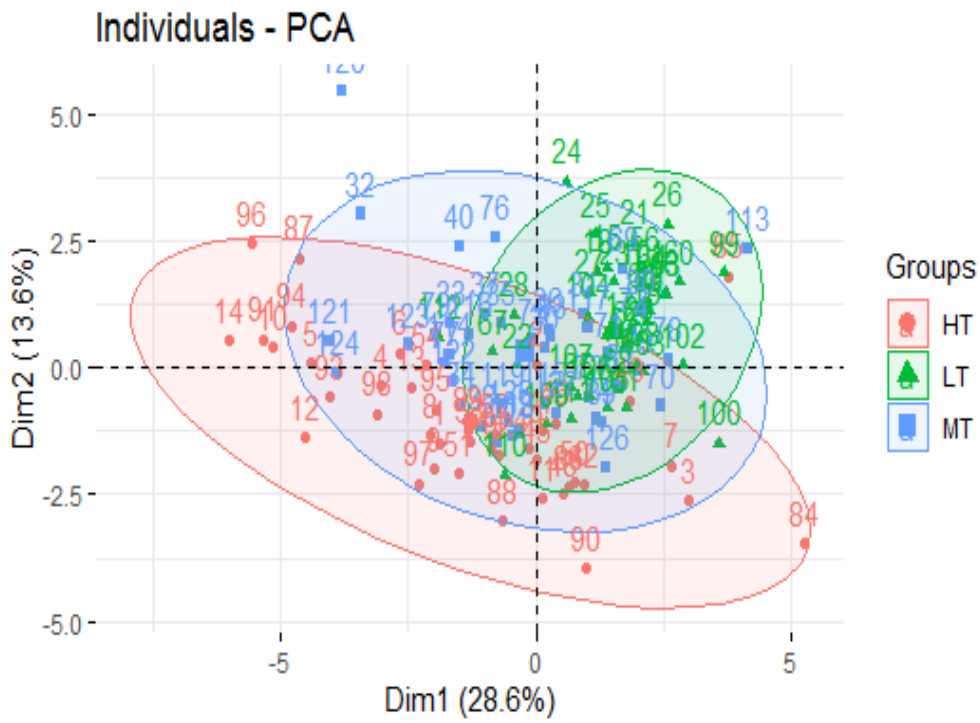
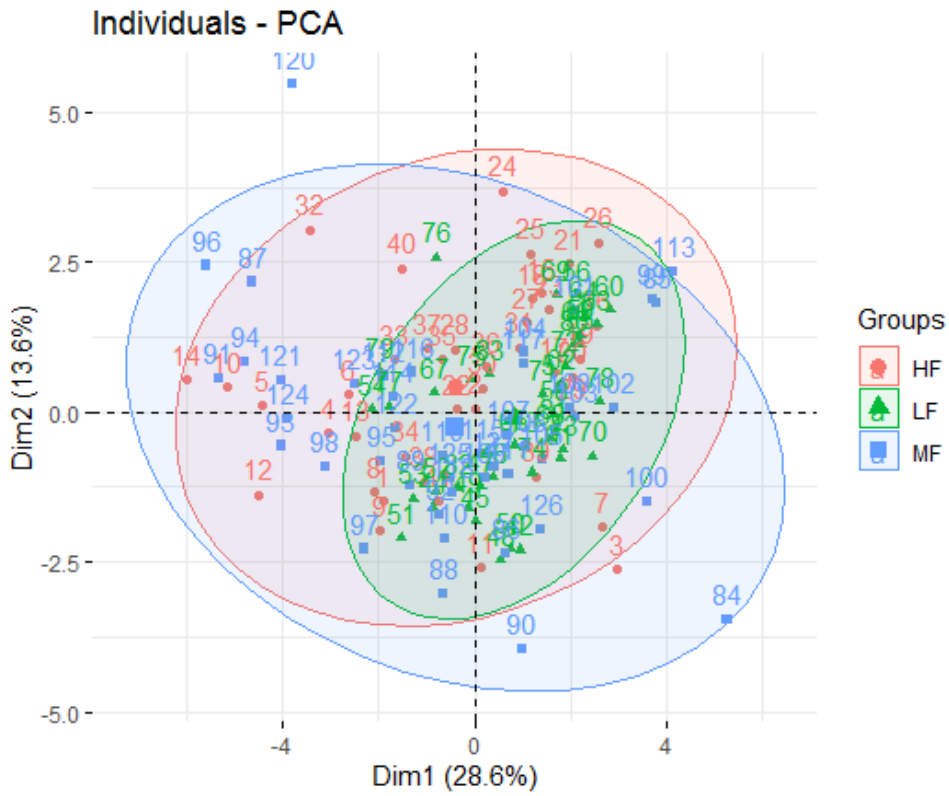
	parasites. HB levels are also involved in the secondary stress response and can be an indicator of stress in fish.		
Tissue Damage Factor			
Alanine Aminotransferase (ALT) (U/L)	ALT exists intracellularly within many fish tissues making this analyte an indicator of cell damage or death when they appear outside of tissue cells in the plasma.	21 – 203	Congleton and Wagner 2004; Shi et al. 2006; Feng et al. 2011
Aspartate Aminotransferase (AST) (U/L)	AST exists intracellularly within many fish tissues making this analyte an indicator of cell damage or death when they appear outside of tissue cells in the plasma (Congleton & Wagner, 2004).	90 – 956	Congleton and Wagner 2004; Knowles et al. 2006; Shi et al. 2006; Shahsavani et al. 2010; Feng et al. 2011; DiVincenti et al. 2013; Matsche et al. 2014
Creatine Kinase (CK) (U/L)	CK is located in muscular and heart tissues of fish, making this analyte an indicator of cell damage or death when they appear outside of tissue cells in the plasma. CK is also important for energy metabolism in tissues that contain CK like the heart or brain.	10 – 8974	Wyss and Kaddurah-Daouk 2000; Congleton and Wagner 2004; DiVincenti et al. 2013; Matsche et al. 2014
Lactate Dehydrogenase (LDH) (U/L)	LDH exist intracellularly within many fish tissues making this analyte an indicator of cell damage or death when they appear outside of tissue cells in the plasma and have stronger activity in the heart and show capacity to	633 – 4223	Congleton and Wagner 2004; Shi et al. 2006; Furne et al. 2009; Matsche et al. 2014

	utilize glucose anaerobically.		
Gamma-glutamyl Transferase (GGT) (U/L)	GGT is confined to tissues of the liver and is released when the liver is damaged or diseased or when bile ducts are obstructed and is, therefore, indicative of liver damage.	n/a	Russell 1982
Chronic Stress Factor			
Glucose (GLU) (mg/dL)	The liver increases the production of glucose during stress to match the increased energy demand resulting from stress as glucose is essential for cellular respiration. Elevated levels of plasma glucose indicate stress in pallid sturgeon.	0 – 184.7; 27 – 139*	Cataldi et al. 1998; Iwama 1998; Knowles et al. 2006; Shi et al. 2006; Webb et al. 2007*; Furnè et al. 2009; Shamsavani et al. 2010; DiVincenti et al. 2013; Matsche et al. 2014; DeLonay et al. 2015*; Campbell et al. 2018
Chloride (CL) (mmol/L)	Chloride is part of the secondary stress response in fish and is important for osmotic homeostasis. Chloride is also important for digestion as it is part of hydrochloric acid which is produced in the stomach. Elevated levels may be indicative of a stress response. Extreme changes may be indicative of failure to maintain osmotic homeostasis which may result from several stressors leading to death.	106 – 124	Lovell 1989; Cataldi et al. 1998; Barton 2002; Fevolden et al. 2003; Beyea et al. 2005; Knowles et al 2006; DiVincenti et al. 2013

Sodium (NA) (mmol/L)	Sodium is also part of the secondary stress response in fish. Important for osmotic homeostasis and pH regulation in fish. Extreme changes may be indicative of failure to maintain osmotic homeostasis which may result from several stressors leading to death.	124-155.5	Russell et al. 1982; Cataldi et al. 1998; Barton 2002; Fevolden et al. 2003; Knowles et al. 2006; Shavsavani et al. 2010; DiVincenti et al. 2013; Matsche et al. 2014
Magnesium (MG) (mg/dL)	Magnesium is important for hard tissue composition and for carbohydrate metabolism and protein synthesis. Magnesium is also necessary to maintain smooth muscle. Fish experiencing chronic stress or high metabolic demands may develop magnesium deficiency through diffusive loss across gills. Magnesium binds to albumin.	1.6 – 2.3	Lovell 1989; Bijvelds et al. 1998; Knowles et al. 2006
Potassium (K) (mmol/L)	Potassium is a cation that is required for glycolysis and is found in high concentrations in red blood cells. It is also important for nerve impulse transmissions. Extreme changes in potassium concentration may be indicative of failure to maintain osmotic homeostasis which may result from several stressors leading to death in fish.	2.4 – 3.9	Lovell 1982; Russell et al. 1982; Cataldi et al. 1998; Fevolden et al. 2003; Knowles et al. 2006; Shavsavani et al. 2010; DiVincenti et al. 2013; Matsche et al. 2014

APPENDIX B

EXPERIMENTAL GROUPS SCORES ON PRINCIPAL COMPONENT 1 AND
PRINCIPAL COMPONENT 2



Appendix C. Individuals (represented by numbered points) plotted based on their scores for principal component one (PC1) on the x-axis (Dim1) and principal component two (PC2) y-axis (Dim2) where PC1 explains 28.6% of the variation in the dataset and PC2 explains 13.5% of the variance in the dataset. The red color represents high temperature (HT) or high feed (HF), blue represents moderate temperature (MT) and moderate feed (MF), and green represents low temperature (LT) and low feed (LF). The colored ovals represent the 95% confidence intervals around the centroids of scores for the differing treatments.

REFERENCES CITED

- Adams, S.M. 1999. Ecological Role of Lipids in the Health and Success of Fish Populations. (p. 132-153). In: Arts M.T., Wainman B.C. (eds) Lipids in Freshwater Ecosystems. Springer, New York, NY.
- Adams, S.M., Bevelhimer, M.S., Greely, M.S., Levine, D.A., The, S.J. 1999. Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. *Environmental Toxicology and Chemistry*, 18(4): 628-640.
- Adams, S.M., Brown, A.M., Goede, R.W. 1993. A Quantitative Health Assessment Index for Rapid Evaluation of Fish Condition in the Field. *Transactions of the American Fisheries Society*, 122(1): 63-73.
- AOAC. 2012. Official Methods of Analysis of AOAC international. 19th edition. AOAC International, Gaithersburg, Maryland, USA.
- Bayse, S.M., Regish, A.M., McCormick, S.D. 2018. Proximate composition, lipid utilization and validation of a non-lethal method to determine lipid content in migrating American shad *Alosa sapidissima*. *Journal of Fish Biology*, 92: 1832-1848.
- Barton, B.A. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and comparative biology*, 42: 517-525.
- Baum, N., Dichoso, C.C., Carlton, C.E. 1975. Blood urea nitrogen and serum creatinine: Physiology and interpretations. *Urology*, 5(5): 583-588.
- Bijvelds, M.J.C., Van Der Velden, J.A., Kolar, Z.I., Flik, G. 1998. Magnesium transport in freshwater teleosts. *Journal of Experimental Biology*, 201: 1981-1990
- Braaten, P. J., Fuller, D.B., Lott, R.D., Haddix, T.M., Holte, L.D., Wilson, R.H., Bartron, M.L., Kalie, J.A., Dehaan, P.W., Ardren, W.R., Holm, R.J., Jaeger, M.E. 2012. Natural growth and diet of known-age pallid sturgeon (*Scaphirhynchus albus*) early life stages in the upper Missouri River basin, Montana and North Dakota. *Journal of Applied Ichthyology* 28(4):496–504.

- Braaten, P.J., Fuller, D.B., Lott, R.D., Jordan, G.R. 2009. An estimate of the historic population size of adult pallid sturgeon in the upper Missouri River Basin, Montana and North Dakota. *Journal of Applied Ichthyology*, 25(Suppl. 2): 2-7.
- Burkhead, N. M. 2012. Extinction Rates in North American Freshwater Fishes, 1900–2010. *BioScience* 62(9):798–808.
- Burnham, K.P., Anderson, D.R. 2004. Multimodal inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research*, 33(2): 261-304.
- Campbell, N.A., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V., Reece, J.B. 2018. *Biology*, 11th ed. Pearson Education Limited, Harlow, Essex, England.
- Cataldi, E., Di Marco, P., Mandich, A., Cataudella, S. 1998. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: *Acipenseriformes*): effects of temperature and stress. *Comparative Biochemistry and Physiology Part A*, 121: 351-354.
- Chipps, S.R., Klumb, R.A., and Wright, E.B. 2010. Development and application of juvenile pallid sturgeon bioenergetics model: Final Report, South Dakota State Wildlife Grant Program, Brookings, South Dakota, Study T–24–R Study No. 2424, 40 p.
- Colt, J., Shearer, K.D. 2001. Evaluation of the use of the Torry Fatmeter to Non-lethally Estimate Lipid in Adult Salmon. U.S. Army Corps of Engineers. 75 pp.
- Cowey, C.B., Sargent, J.R. 1979. Nutrition. *Fish Physiology*, (8): 1-69.
- Crossin, G.T., Hinch, S.G. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. *Transactions of the American Fisheries Society*, 134(1): 184-191.
- Davidson, D., Marshall, C.T. 2010. Are morphometric indices accurate indicators of stored energy in herring *Clupea harengus*?. *Journal of Fish Biology*, 76: 913-929.
- DeLonay, A.J., Chojnacki, K.A., Jacobson, R.B., Albers, J.L., Braaten, P.J., Bulliner, E.A., Elliott, C.M., Erwin, S.O., Fuller, D.B., Haas, J.D., Ladd, H.L.A., Mestl,

- G.E., Papoulias, D.M., Wildhaber, M.L. 2016. Ecological requirements for pallid sturgeon reproduction and recruitment in the Missouri River – A synthesis of science, 2005 to 2012: U.S. Geological Survey Scientific Investigations Report 2015-5145, 224 p.
- Deslauriers, D., Johnston, R., Chipps, S.R. 2016. Effect of Morphological Fin-Curl on the Swimming Performance and Station-Holding Ability of Juvenile Shovelnose Sturgeon. *Journal of Fish and Wildlife Management*, 7(1): 198-204.
- Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., Anderson, W.G. 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs. *Environmental Biol Fish*, 101: 623-637.
- Dimeski, G., Jones, B.W. 2011. Lipaemic samples: effective process for lipid reduction using high speed centrifugation compared with ultracentrifugation. *Biochimica Medica*, 22(2): 86-92.
- Distell. 2010. Distell Fish Fatmeter: User manual. Fauldhouse, West Lothian, Scotland, UK.
- DiVincenti, L., Priest, H., Walker, K.J., Wyatt, J.D., Dittman, D. 2013. Comparison of select hematology and serum chemistry analytes between wild-caught and aquarium-housed lake sturgeon (*Acipenser fulvescens*). *Journal of Zoo and Wildlife Medicine*, 44(4): 957-964.
- Everitt, B., Hothorn, T. 2011. *An Introduction to Applied Multivariate Analysis with R*. Springer, New York, New York, USA, pp. 15.
- Ferri, J., Popovic, N.T., Coz-Rakovac, R., Beer-Ljubic, B., Strunjak-Perovic, I., Skeljo, F., Jadan, M., Petric, M., Barisic, J., Simpraga, M., Stanic, R. 2011. The effect of artificial feed on blood biochemistry profile and liver histology of wild saddled bream, *Oblada melanura* (spridae). *Marine Environmental Research*, 71: 218-224.
- Feng, G., Zhuang, P., Zhang, L., Kynard, B., Shi, X., Duan, M., Liu, J., Huang, X. Effect of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon (*Acipenser baerii*). *Journal of Applied Ichthyology*, 27: 595-599.

- Fevolden, S. Røed, K.H., Fjalestad, K. 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. *Aquaculture*, 216: 67-76.
- Finnegan, D. 2014. referenceIntervals: Reference Intervals. R package version 1.1.1. <https://CRAN.R-project.org/package=referenceIntervals>.
- Friedrichs, K. R., K. E. Harr, K. P. Freeman, B. Szladovits, R. M. Walton, K. F. Barnhart, and J. Blanco-chavez. 2012. ASVCP reference interval guidelines : determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, 4:441–453.
- Froese, R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*, (22): 241–253.
- Furnè, M., Morales, A.E., Trenzado, C.E., Garcia-Gallego, M., Hidalgo, M.C., Domezain, A., Rus, A.S. 2012. The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. *Journal of Comparative Physiology B*, 182: 63-76.
- Furnè, M., Sanz, A., Garcia-Gallego, M., Hidalgo, M.C., Domezain, A., Domezian, J., Morales, A.E. 2009. Metabolic organization of the sturgeon *Acipenser naccarii* A comparative study with rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, 289: 161-166.
- Gadomski, D.M., Parsley, M.J. 2005. Laboratory Studies on the Vulnerability of Young White Sturgeon to Predation. *North American Journal of Fisheries Management*. 25(2): 667-674.
- Glover, D.C., DeVries, D.R., Wright, R.A., Davis, D.A. 2010. Sample preparation techniques for determination of fish energy density via bomb calorimetry: An evaluation using largemouth bass. *Transaction of American Fisheries Society*, 139: 671-675.
- Grohs, K. L., R. A. Klumb, S. R. Chipps, and G. A. Wanner. 2009. Ontogenetic patterns in prey use by pallid sturgeon in the Missouri River, South Dakota and Nebraska. *Journal of Applied Ichthyology* 25(SUPPL. 2):48–53.

- Hanson, K.C., Ostrand, K.G., Gannam, A.L., Ostrand, S.L. 2010. Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. *Transactions of the American Fisheries Society*, 139: 1733 – 1741.
- Hessle, L., Johnson, K.A., Anderson, C., Narisawa, S., Sali, A., Goding, J.W., Terkeltaub, R., Millan, J.L. 2002. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *PNAS*, 99(14): 9445-9449.
- Holmquist, L. M., C. S. Guy, A. Tews, and M. A. H. Webb. 2019. First maturity and spawning periodicity of hatchery-origin pallid sturgeon in the upper Missouri River above Fort Peck Reservoir, Montana. *Journal of Applied Ichthyology* 35(1):138–148.
- Horn, P. S., and A. J. Pesce. 2003. Reference intervals: An update. *Clinica Chimica Acta*, 334(1–2):5–23.
- Huizinga, H. W., G. W. Esch, and T. C. Hazen. 1979. Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterus salmoides* (Lacepede). *Journal of Fish Diseases* 2(4):263–277.
- Hung, S.O., Liu, W., Li, H., Storebakken, T., Cui, Y. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 151: 357-363.
- Hung, S.S.O., Lutes, P.B. 1987. Optimum feeding rate of hatchery-produced juvenile white sturgeon (*Acipenser transmontanus*): at 20°C. *Aquaculture*, 65: 307-317.
- IUCN 2019. The IUCN Red List of Threatened Species. Version 2019-2. <http://www.iucnredlist.org>. Downloaded on 18 July 2019.
- Iwama, G. 1998. Stress in fish. *Annals of the New York Academy of Science*
- Josse, J., Husson, F. 2016. missMDA: A package for handling missing values in multivariate data analysis. *Journal of Statistical Software*, 90(1): 1-31. Doi:10.18637/jss.v070.i01.

- Kappenman, K. M., Fraser, W. C., Toner, M., Dean, J., & Webb, M. A. H. 2009. Effect of temperature on growth, condition, and survival of juvenile shovelnose sturgeon. *Transactions of the American Fisheries Society*, 138, 927–937.
- Khan, R.A. 2003. Health of flatfish from localities in Placentia Bay, Newfoundland, contaminated with petroleum and PCBs. *Environmental Contamination and Toxicology*, 44: 485-492.
- Kindschi, G.A. 1987. Method of Quantifying Degree of Fin Erosion. *The Progressive Fish-Culturist*, 49(4): 314-315.
- Knowles, S., Hrubec, T.C., Smith, S.A., Bakal, R.S. 2006. Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). *Veterinary Clinical Pathology*, 35(4): 434-440.
- Kroll, M.H., Elin, R.J. 1985. Relationships between magnesium and protein concentrations in serum. *Clinical Chemistry*, 31(2): 244-246.
- Kuhn, M. Contributions from Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., Cooper, T., Mayer, Z., Kenkel, B., the R Core Team, Benesty, M., Lescarbeau, R., Ziem, A., Scrucca, L. Tang, Y., Candan, C., Hunt, T. 2019. caret: Classification and regression training. R package version 6.0-84. <https://CRAN.R-project.org/package=caret>.
- Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20(2): 201-219.
- Li, D., Liu, Z., Xie, C. 2012. Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, *Acipenser schrenckii*. *Fish Physiology and Biochemistry*, 38(2): 511-520.
- Lovell, T. 1989. *Nutrition and Feeding of Fish*. Springer Science + Business Media New York. New York, New York, USA.
- Matsche, M.A. 2011. Evaluation of tricaine methanesulfonate (MS-222) as a surgical anesthetic for Atlantic Sturgeon *Acipenser oxyrinchus*. *Journal of Applied Ichthyology*, 27: 600-610.

- Matsche, M.A., Arnold, J., Jenkins, E., Townsend, H., Rosemary, K. 2014. Determination of hematology and plasma chemistry reference intervals for 3 populations of captive Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) *Veterinary Clinical Patholog*, 43(3): 387-396.
- Mesa, M.G., Rose, B.P. 2015. An assessment of morphometric indices, blood chemistry variables and an energy meter as indicators of the whole body lipid content in *Micropterus dolomieu*, *Sander vitreus* and *Ictalurus punctatus*. *Journal of Fish Biology*. 86: 755 – 764.
- Meyer, H.A., Chipps, S.R., Graeb, B.D.S, Klumb, R.A. 2016. Growth, food consumption, and energy status of juvenile pallid sturgeon fed natural and artificial diets. *Journal of Fish and Wildlife Management*, 7(2): 388-396.
- Mohseni, M., Sajjadi, M., Pourkazemi, M. 2007. Growth performance and body composition of sub-yearling Persian sturgeon, (*Acipenser persicus*, Borodin, 1897), fed different dietary protein and lipid levels. *Journal of Applied Ichthyology*, 23: 204-208.
- Moyle, P.B., Cech, J.J. 2018. *Fishes: An introduction to ichthyology*. 3rd Impression. Pearson Education, Inc, New York, NY, USA.
- Murphy, B.R., Willis, D.W. 1992. Proper Distinction between Relative Weight and Relative Condition Factor. *North American Journal of Fisheries Management*, 12: 665-666.
- Oldenburg, E.W., Guy, C.S., Cureton, E.S., Webb, M.A.H., Gardner, W.M. 2011. Effects of acclimation on poststocking dispersal and physiological condition of age-1 pallid sturgeon. *Journal of Applied Ichthyology*, 27: 436-443.
- Petrie, A. 2020. regclass: Tools for an Introductory Class in Regression and Modeling. R package version 1.6. <https://CRAN.R-project.org/package=regclass>
- Rafatnezhad, S., Falahatkar, B., Gilani, M.H.T. 2008. Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. *Aquaculture Research*, 39: 1506-1513.

- Range-wide Pallid Sturgeon Propagation Committee. 2019. Range-wide Pallid Sturgeon Propagation Plan. Denver, CO. 55 pp.
- RCore Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Ricker, W.E. 1979. Growth rates and models. Fish Physiology, volume 8, bioenergetics and growth. Hoar, W.S., Randall, D.J., Brett, J.R. (Eds), Academic Press, Inc., New York, NY, USA, pp. 677-743.
- Roche, H., Bogé, G. 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. Marine Environmental Research, 41(1): 27-43.
- Rose, K. A., J. H. Cowan, K. O. Winemiller, R. A. Myers, and R. Hilborn. 2001. Compensatory density dependence in fish populations: Importance, controversy, understanding and prognosis. Fish and Fisheries 2(4):293–327.
- Rosner, B. 1983. Percentage Points for a Generalized ESD – Many Outlier Procedure. *Technometrics*, 25(2): 165 – 172.
- Rotella, J. 2017. Upper Basin Pallid Sturgeon Survival Estimation Project 2017 Update.
- Rothschild, M.A., Oratz, M., Schreiber, S.S. 1984. *In vivo* and *in vitro* regulation of albumin synthesis. (pp. 121-140). In Mariani, G. (Eds): Pathophysiology of plasma protein metabolism. Plenum Press, New York, NY, USA.
- Russell, N.J., Powell, G.M., Jones, J.G., Winterburn, P.J., Basford, J.M. 1982. Blood biochemistry. 1st Ed. Croom Helm Ltd, Provident House, Burrell Row, Beckenham, Kent, UK.
- Schloesser, R.W., Fabrizio, M.C. 2017. Condition Indices as Surrogates of Energy Density and Lipid Content in Juveniles of Three Fish Species. Transactions of the American Fisheries Society, 146: 1058-1069.

- Schober, P., Boer, C., Schwarte, L.A. 2018. Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia & Analgesia*, 126(5): 1763 – 1768. DOI: 10.1213.
- Seebacher, F., Franklin, C.E. 2012. Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. *Philosophical Transaction of the Royal Society B*, 367: 1607-1614.
- Sepúlveda, M.S., Sutton, T.M., Patrick, H.K., Amberg, J.J. 2012. Blood chemistry values for shovelnose and lake sturgeon. *Journal of Aquatic Animal Health*, 24(3): 135-140.
- Shahsavani, D., Kazerani, H.R., Kaveh, S., Gholipour-Kanani, H. 2010. Determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus* Pallas, 1771) during spring season. *Comparative Clinical Pathology*, 19: 57-61.
- Shuman, D.A., Klumb, R.A., Wilson, R.H., Jaeger, M.E., Haddix, T., Gardner, W.M., Doyle, W.J., Horner, P.T., Ruggles, M., Steffensen, K.D., Wanner, G.A. 2011. Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. *Journal of Applied Ichthyology*, 27: 269 – 281.
- Shi, X., Li, D., Zhuang, P., Nie, F., Long, L. 2006. Comparative blood biochemistry of Amur sturgeon, *Acipenser schrenckii*, and Chinese sturgeon, *Acipenser sinensis*. *Fish Physiology and Biochemistry*, 32: 63-66.
- Simide, R. 2018. The blood indicators of Siberian sturgeon welfare. *Farming*, 2: 451-477.
- Singer, T.D., Ballantyne, J.S. 2004. Sturgeon and paddlefish metabolism. Pages 167-194 in LeBreton, G.T.O., Beamish, F.W.H., Mckinley, R.S., editors. *Sturgeons and Paddlefish of North America*. Kluwer Academic Publishers, New York, NY, USA.
- Skinner, J.P., Tuomi, P.A., Mellish, J.E. 2015. The influence of time in captivity, food intake and acute trauma on blood analytes of juvenile Steller sea lions, *Eumetopias jubatus*. *Conservation Physiology*, 3:1-12.
- Steffensen, K.D., Mestl, G.E., Phelps, Q.E. 2017. Range-wide assessment of pallid sturgeon *Scaphirhynchus albus* (Forbes & Richardson, 1905) relative condition. *Journal of Applied Ichthyology*, 33: 13-21.

- Sumpter, J.P. 1992. Control of growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 92: 299-320.
- Treanor, H.B., Miller, I.R., Halvorson, L.J., Van Eenennaam, J.P., Doroshov, S.I., Webb, M.A.H. 2017. Effect of dietary fat on adipocyte size in farmed age-2 white sturgeon (*Acipenser transmontanus*, Richardson, 1836). *Journal of Applied Ichthyology*, 34: 419-423.
- UBPSPC [Upper Basin Pallid Sturgeon Propagation Committee]. 2005. Upper basin pallid sturgeon propagation plan, 89 pp.
- USFWS [United States Fish and Wildlife Service]. 1990. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Pallid Sturgeon. *Federal Register*, 55(173): 36641-36647.
- USFWS [United States Fish and Wildlife Service]. 1993. Pallid Sturgeon Recovery Plan. USFWS, Bismarck, North Dakota. 55 pp.
- USFWS [United States Fish and Wildlife Service]. 2008. Pallid Sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. Billings, Montana.
- USFWS [U.S. Fish and Wildlife Service]. 2014. Revised Recovery Plan for the Pallid Sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Denver, Colorado. 115 pp.
- USFWS [United States Fish and Wildlife Service]. 2018. Revised Pallid Sturgeon *Scaphirhynchus albus* Range-wide Stocking Plan. Denver, CO. 44 pp.
- Vogt, A., Gormley, R., Downey, G., Somers, J. 2002. A Comparison of Selected Rapid Methods for Fat Measurement in Fresh Herring (*Clupea harengus harengus*). *Journal of Food Composition and Analysis*, 15: 205-215.
- Wagner, T., Congleton, J.L. 2004. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 1066-1074.

- Webb, M.A.H., Allert, J.A., Kappenman, K.M., Marcos, J., Feist, G.W., Schreck, C.B., Shackleton, C.H. 2007. Identification of plasma glucocorticoids in pallid sturgeon in response to stress. *General and Comparative Endocrinology*, 154: 98-104.
- Wikelski, M., Cooke, S.J. 2006. Conservation physiology. *Trends in Ecology and Evolution*, 21(2): 38-46.
- Willmott, C.J., Ackleson, S.G., Davis, R.E., Feddema, J.J., Klink, K.M., Legates, D.R., O'Donnell, J., Rowe, C.M. 1985. Statistics for the Evaluation and Comparison of Models. *Journal of Geophysical Research*, 90: 8995-9005.
- Wilson, R., S. Hultberg, and Z. Sandness. 2017. 2016 Annual Report Pallid Sturgeon Population Assessment and Associated Fish Community Monitoring for the Missouri River : Segment 4. U.S. Fish and Wildlife Service, Bismarck, North Dakota.
- Witeska, M. Anemia in teleost fishes. 2015. *Bulletin of European Association of Fish Pathologists*, 35(4): 148-160.
- Wyss, M., Kaddurah-Daouk, R. 2000. Creatinine and creatinine metabolism. *Physiological Reviews*, 80(3): 1107-1213.
- Young, J. L., S. G. Hinch, S. J. Cooke, G. T. Crossin, D. A. Patterson, A. P. Farrell, G. van der Kraak, A. G. Lotto, A. Lister, M. C. Healey, and K. K. English. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 63(5):1067–1077.