Influence of diet and environmental variation on physiological responses of juvenile pallid sturgeon (*Scaphirhynchus albus*)

By

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

Influence of diet and environmental variation on physiological responses of juvenile pallid sturgeon (*Scaphirhynchus albus*)

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Shortly after the pallid sturgeon *Scaphirhynchus albus* was listed as federally endangered in 1990, a recovery plan was drafted to outline efforts to restore the pallid sturgeon throughout its' native range in the Missouri and middle and lower Mississippi rivers. The plan set forth a number of recovery efforts such as habitat restoration, population assessment, a comprehensive sturgeon research program and a propagation program to mitigate the decline of pallid sturgeon. Stocking of hatchery raised pallid sturgeon has been an important short-term recovery effort for the prevention of local extirpations. Thus, producing healthy pallid sturgeon, maximizing genetic diversity, and preserving locally adapted populations are critical components of the pallid sturgeon propagation program. In this study, I compared feeding, growth and energy status of juvenile pallid sturgeon fed a commercial or invertebrate diet and explore whether pallid sturgeon exhibit physiological adaptations based on recent, genetic stock structure analysis.

To compare growth, food consumption, energy status, and routine metabolic rates of pallid sturgeon fed formulated and natural diets, I conducted a laboratory study using twenty-four hatchery reared pallid sturgeon. Pallid sturgeon were fed a daily ration of either commercial pellets (1 mm, slow sinking; 45% protein, 19% fat), or chironomid larvae for 5 weeks. Pallid sturgeon fed chironomid larvae had faster average growth (0.5 g/d) than fish fed a formulated diet (0.06 g/d). Moreover, mean metabolic rate was significantly higher (82%) among fish fed a natural diet, likely owing to increased cellular metabolism associated with higher growth rates. Energy density was significantly higher among sturgeon fed chironomids (3.7 kJ/g wet weight) compared to those fed commercial diets (0.73 kJ/g wet weight), despite lower energy density (86%) of chironomid prey. In contrast, the hepatosomatic index (HSI) was significantly higher (44%) in fish fed formulated feed, indicative of a high lipid diet. Natural feeds resulted in superior growth and condition than formulated diets. Use of a natural diet prior to stocking may maximize growth, energy stores and post-stocking survival for hatchery reared pallid sturgeon.

To determine whether pallid sturgeon are locally adapted to regional environmental conditions, I conducted a common-environment experiment using individuals from two genetically distinct source populations. I measured absolute growth, food consumption, feed conversion efficiency and routine metabolic rate of pallid sturgeon from the upper basin (UB; Montana, North Dakota, South Dakota) and middle basin (MB; Gavins Point Dam, SD to Kansas City, MO) of

the Missouri River at 10, 14, 18, 22, 26 and 30°C. Upper basin pallid sturgeon exhibited faster growth at temperatures greater than 18°C. Feed conversion efficiency by UB sturgeon was significantly greater than MB sturgeon at 18 and 22°C. Upper basin pallid sturgeon had higher consumption rates at 22, 26 and 30°C (32.3%, 15.2%, and 15.2%, respectively) and greater metabolic rates (20.4%) at 18°C than MB sturgeon. Pallid sturgeon from the UB exhibited faster growth, greater consumption rates and greater metabolic rates than MB sturgeon, at temperatures greater than 18°C. These results indicate that UB sturgeon have developed a higher capacity for growth at warmer temperatures. Rapid growth by UB fish could potentially be a compensatory adaptation to contracted growing seasons typically experienced in northern latitudes. Previous pallid sturgeon microsatellite analysis indicated a pattern of genetic stock structure that follows a latitudinal pattern, similar to our results of increased growth and consumption by UB fish. Understanding the presence and pattern of local adaptations in broadly distributed species is important for preservation of such adaptations as a means to conserve genetic diversity.

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CHAPTER 1. INTRODUCTION

Introduction

The pallid sturgeon *Scaphirhynchus albus* is a large, riverine fish native to the Missouri, and the middle and lower Mississippi rivers that was listed as federally endangered in 1990 (Dryer and Sandoval 1993). Pallid sturgeon are well suited to riverine life and characterized by a broad, dorsoventrally flattened snout, a long, slender caudal peduncle and broad pectoral fins (Kallemeyn 1983). Pallid sturgeon can be differentiated from the closely related shovelnose sturgeon *S. platorhynchus* by the absence of bony scutes along the belly, and by the position and relationship of barbels on the rostrum (Kallemeyn 1983). Main channel areas in the Mississippi and Missouri rivers with swift water velocity (0.65 - 0.78 m/sec) are preferred habitats for pallid sturgeon (Bramblett and White 2001, Hurley et al. 2004, Gerrity et. al 2008). A sub-terminal, protrusible mouth and four sensory barbels allow for efficient detection and capture of benthic prey in turbid riverine environments. Pallid sturgeon feed primarily on aquatic invertebrates and/or fish depending on life stage (Gerrity et. al 2006, Hoover et. al 2006, Wanner et. al 2007, and Grohs et. al 2009). Maximum attainable size for pallid sturgeon is about 40 kg in weight and up to 2 m in length (Shuman et al. 2006). Similar to many sturgeon species, age at sexual maturity for pallid sturgeon is delayed. Male pallid sturgeon reach maturity at 7 to 9 years, whereas females reach maturity at 15 to 20 years and require several years for eggs to mature (Dryer and Sandoval 1993, Keenlyne and Jenkins 1993,

DeLonay et. al 2009). Larval pallid sturgeon passively drift downstream from the hatch site for 11-17 days post hatch, and settle out of the water column to transition into the benthic life stage (Kynard et. al 2007, Braaten et al. 2008). Drifting larvae may travel as far as 245-530 km, depending on water velocity and temperature (Braaten et al. 2008).

Pallid sturgeon are vulnerable to rapid environmental changes because of their unique life history. Late (i.e., older) sexual maturity and relatively large lag times between generations can be problematic for a species that faces habitat alterations in 67% of its historic range (Hesse 1987). Additionally, larval pallid sturgeon require large sections of free-flowing river to complete the drift stage of development (Braaten et al. 2008). Habitat alteration is believed to be the principal cause of the declining pallid sturgeon population (Dryer and Sandoval 1993). Construction of six dams on the main stem Missouri River between 1937 and 1966 has fragmented and reduced available habitat, altered temperature and flow regimes and reduced turbidity and sediment transport. Extensive channelization of the middle and lower Missouri and Mississippi rivers has also altered flow and homogenized once diverse riverine habitat. Large dams act as barriers to upstream spawning migrations, alter spawning habitat and change flow regimes that can affect drifting larvae and restrict life cycle requirements of pallid sturgeon (Keenlyne 1989, USFWS 2007). Other threats to pallid sturgeon include hybridization with the sympatric shovelnose sturgeon, environmental contaminants, and incidental harvest by commercial fisheries (Carlson et al.

1985, Ruelle and Keenlyne 1992, Dryer and Sandoval 1993, Bettoli et.al 2009). In September 2010, the U.S. Fish and Wildlife Service (USFWS) listed shovelnose sturgeon as threatened due to similarity of appearance (SOA) to pallid sturgeon, because of documented failures to differentiate the two species by commercial fishermen (USFWS 2010). The SOA provision of the endangered species act will protect pallid sturgeon from incidental harvest by commercial shovelnose fisheries where the two specie's range overlaps.

The USFWS developed a recovery plan in 1993 to address issues that are attributed to the listing of pallid sturgeon (Dryer and Sandoval 1993). Restoration efforts outlined in the recovery plan include habitat restoration, population assessment and augmentation, and a comprehensive sturgeon research program designed to assess factors affecting reproduction, recruitment, habitat use and population dynamics of pallid sturgeon. Six recovery priority management areas (RPMAs) that encompassed the historic range of pallid sturgeon were identified in the recovery plan as areas with remaining suitable habitat where recovery actions would be most beneficial to the species (Dryer and Sandoval 1993). In 2006, the Pallid Sturgeon Recovery Team redefined the RPMA's into four management units (MUs) based on genetic stock structure data and pallid sturgeon biogeography (USFWS 2008). The current MUs are: 1) Great Plains Management Unit (GPMU) including the Missouri River from the great falls of the Missouri River in Montana to Fort Randall Dam, South Dakota, 2) Central Lowlands Management Unit (CLMU) that extends from Fort Randall

Dam, South Dakota to the confluence of the Grand and Missouri rivers in Missouri, 3) Interior Highlands Management unit (IHMU), from the Missouri River at the confluence of the Grand River to the confluence of the Missouri and Mississippi rivers as well as the Mississippi River at Keokuk, Iowa to the confluence of the Ohio and Mississippi rivers and 4) Coastal Plains Management Unit (CPMU) including the Mississippi River from the confluence of the Ohio and Mississippi rivers to the Gulf of Mexico and the Atchafalaya River distributary. Wild pallid sturgeon populations in the GPMU are currently believed to be small (as few as 136) remnant populations of old adults, indicative of recruitment failure (Kallemeyn 1983, Duffy et al. 1996, UBPSRW 2004). Data suggests that the portion of the CLMU from Fort Randall dam to Gavin's Point Dam does not support a naturally reproducing population, with only two large adult fish captured during the past 9 years (Shuman et al. 2011). Researchers have documented a small wild population of pallid sturgeon in the CLMU downstream of Gavins Point dam and in the IHMU, with documentation of apparent spawning migrations and congregations of adults (Duffy et. al 1996, DeLonay et al. 2010). These MUs received the most attention for recovery effort implementation in terms of shallow water habitat construction and a flow modification plan at Gavins Point Dam to simulate a spring rise (USFWS 2007). The portion of the IHMU from the confluence of the Missouri and Mississippi rivers downstream to the Ohio River confluence and the CPMU support higher numbers of pallid sturgeon than other MUs, and some larval pallid sturgeon have been collected (Hrabik et al. 2007,

USFWS 2007). Restoration of flood plain and side channel connectivity have been implemented in the middle and lower Mississippi River (USFWS 2007). The Atchafalaya River portion of the CPMU may contain as many as 2,750 – 4,100 pallid sturgeon, and also has an increased incidence of hybridization between pallid and shovelnose sturgeon (Duffy et. al 1996, Tranah et. al 2004). Although some MUs have documented reproduction, recruitment has been limited, indicating occurrence of a recruitment bottleneck (USFWS 2007). The pallid sturgeon recovery plan recommended a propagation program as a short-term recovery effort to supplement declining wild populations until critical habitat is restored and populations are self-sustaining (USFWS 2008). Population augmentation is especially important in the upper basin of the Missouri River (GPMU, portions of CLMU), where reproduction has not been documented for over 30 years (USFWS 2007, Bergman et al. 2008).

The first pallid sturgeon propagation plan was implemented in 1992, and updated in 2005 to include the most recent data and knowledge on production practices (USFWS 2005). An essential part of the propagation plan was to develop a captive broodstock program at the USFWS Gavin's Point National Fish Hatchery, Yankton, SD to preserve a source of gametes if wild origin pallid sturgeon were to become no longer reproductively viable or available (USFWS 2005). Currently, wild adults are collected from the GP, CL and IHMUs in fall (August-November) and spring (April-May) and transported to the hatchery for artificial spawning. A factorial mating design (multiple males crossed with one

female) is used to create half-sibling family groups. The current propagation plan is designed to capture and conserve as much of the wild pallid sturgeon genome as possible by creating unique crosses to maximize genetic diversity (USFWS 2005). Most progeny are reared to an advanced fingerling size (~225 mm fork length), tagged with a passive integrated transponder (PIT) tag and visible implant elastomer and then stocked, at age-1, into the corresponding MU according to yearly stocking quotas. Stocking efforts are primarily used to establish multiple year classes within the Missouri River in hopes that future recruitment by hatchery progeny will supplement existing wild populations to ensure persistence of the species while habitat is restored (USFWS 2008).

Up to 68,000 pallid sturgeon have been stocked annually into the Missouri River since the establishment of the propagation program since 1992 (USFWS 2007). Until adequate habitat improvements can be implemented to restore critical habitat, (e.g. spawning and rearing habitat) population augmentation will be used as a short term recovery effort to ensure the persistence of the species (USFWS 2008). Development of pallid sturgeon culture methods has been relatively recent (mid 1990's), compared to other sturgeon species that have been cultivated since the 1950's and 1960's (Burtzev 2007). Early methods for spawning pallid sturgeon were based on protocols developed for white sturgeon (*Acipenser transmontanus*) and were met with limited success (USFWS 2005). Although pallid sturgeon propagation and augmentation efforts have improved since 1992, knowledge gaps still exist. Currently, little is known about nutritional

needs of pallid sturgeon at all life stages. Although the current local broodstock collection and range-wide stocking plan are based on MU's that are delineated using information from genetic stock structure analysis, we have a limited understanding of whether this apparent stock structure influences local adaptations in pallid sturgeon populations.

To address these questions, I designed two laboratory experiments that allowed me to 1) compare feeding, growth and energy status of juvenile pallid sturgeon fed a commercial or invertebrate diet (Chapter 2) and, 2) determine whether pallid sturgeon show evidence of regional physiological adaptations based on genetic stock structure analysis (Chapter 3).

References

Bergman, H.L., A.M. Boetler, K. Parady, C. Flemming, T. Keevin, D.C. Latka, C. Korschgen, D.L. Galat, T. Hill, G. Jordan, S. Krentz, W. Nelson-Stastny, M. Olson, G.E. Mestl, K. Rouse, and J. Berkley. 2008. Research needs and management strategies for pallid sturgeon recovery. Proceedings of 31 July-2 August 2007 workshop, St Louis, Missouri. Final report to the U.S. Army Corps of Engineers. William D. Ruckelshaus Institute of Environment and Natural Resources, University of Wyoming, Laramie.

Bettoli, P.W., M. Casto-Yerty, G.D. Scholten, and E.J. Heist. 2009. Bycatch of the endangered pallid sturgeon (*Scaphirhynchus albus*) in a common fishery for shovelnose sturgeon (*Scaphirhynchus platorynchus*). Journal of Applied Ichthyology 25:1-4.

Braaten, P.J., D.B. Fuller, L.D. Holte, R.D. Lott, W. Viste, T.F. Brandt, and R. G. Legare. 2008. Drift dynamics of larval pallid sturgeon and shovelnose sturgeon in a natural side channel of the upper Missouri River, Montana. North American Journal of Fisheries Management 28:808-826.

Bramblett, R.G., ad R.G. White. 2001. Habitat use and movements of pallid and shovelnose sturgeon in the Yellowstone and Missouri rivers in Montana and North Dakota. Transactions of the American Fisheries Society 130:1006-1025.

Burtzev, I.A. 1999. The history of global sturgeon aquaculture. Journal of Applied Ichthyology 15:325.

Carlson, D.M., W.L. Pflieger, L. Trial and P.S. Haverland. 1985. Distribution, biology and hybridization of *Scaphirhynchus albus* and *S. platorynchus* in the Missouri and Mississippi rivers. Environmental Biology of Fishes 14:51-59.

DeLonay, A.J., R.B. Jacobson, D.M. Papoulias, D.G. Simpkins, M.L. Wildhaber, J.M. Reuter, T.W. Bonnot, K.A. Chojnacki, C.E. Korshgen, G.E. Mestl, and M.J. Mac. 2009. Ecological requirements for pallid sturgeon reproduction and recruitment in the Lower Missouri River: A research synthesis 2005-08: U.S. Geological Survey scientific investigations report 2009-5201.

DeLonay, A.J., R. B. Jacobson, D. M Papoulias, M.L. Wildhaber, K.A. Chojnacki, E.K. Pherigo, C.L. Bergthold, and G.E. Mestl. 2010. Ecological requirements for pallid sturgeon reproduction and recruitment in the Lower

Missouri River: Annual report 2009. U.S. Geological Survey open-file report 2010-1215.

Dryer, M.P., and A.J. Sandoval. 1993. Pallid sturgeon recovery plan. Bismarck, North Dakota.

Duffy, W. G., C.R. Berry and K.D. Keenlyne. 1996. The pallid sturgeon biology and annotated bibliography through 1994. South Dakota Cooperative Fish and Wildlife Research Unit Technical Bulletin 5.

Hesse, L.W. 1987. Taming the wild Missouri River: What has it cost? Fisheries 12:2-9.

Hrabik, R.A., D.P. Herzog, D.E. Ostendorf, and M.D. Petersen. 2007. Larvae provide first evidence of successful reproduction by pallid sturgeon, *Scaphirhynchus albus*, in the Mississippi River. Journal of Applied Ichthyology 23:436-443.

Hurley, K.L., R.J. Sheehan, R. C. Heidinger, P.S. Wills and B. Clevenstine. 2004. Habitat use by middle Mississippi River pallid sturgeon. Transactions of the American Fisheries Society 133:1033-1041.

Gerrity, P.C., C.S. Guy, and W. M. Gardner. 2006. Juvenile pallid sturgeon are piscivorous: A call for conserving native cyprinids. Transactions of the American Fisheries Society 135:604-609.

Gerrity, P.C., C.S. Guy and W.M. Gardner. 2009. Habitat use of juvenile pallid sturgeon and shovelnose sturgeon with implications for water-level

management in a downstream reservoir. North American Journal of Fisheries Management 28:832-843.

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 25:48-53.

Hoover, J.J. S.G. George, K.J. Killgore. 2006. Diet of shovelnose sturgeon and pallid sturgeon in the free-flowing Mississippi River. Journal of Applied Ichthyology 23:494-499.

Kallemeyn, L.W. 1983. Status of the pallid sturgeon (*Scaphirhynchus albus*). Fisheries 8(1):3-9.

Keenlyne, K.D. 1989. A report on the pallid sturgeon. U.S. Fish and Wildlife Service, Pierre, South Dakota.

Keenlyne, K.D., and L.G. Jenkins. 1993. Age at sexual maturity of the pallid sturgeon. Transactions of the American Fisheries Society 122:393-396.

Kynard, B., E. Parker, D. Pugh, T. Parker. 2007. Use of laboratory studies to develop a dispersal model for Missouri River pallid sturgeon early life intervals. Journal of Applied Ichthyology 23:365-374.

Ruelle, R., and K.D. Keenlyne. 1992. Contaminants in Missouri River sturgeon. U.S. Fish and Wildlife Service, Pierre, SD.

Shuman, D.A., D.W. Willis, and S.C. Krentz. 2006. Application of a length-categorization system for pallid sturgeon (*Scaphirhynchus albus*). Journal of Freshwater Ecology 21:71-76.

Shuman, D.A., R.A. Klumb, R.H. Wilson, M.E. Jaeger, T. Haddix. W.M. Gardner, W.J. Doyle, P.T. Horner, M. Ruggles, K.D. Steffensen, S. Stukel and G.A. Wanner. 2011. Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. Journal of Applied Ichthyology 27: 269-281.

Tranah, G., D.E. Campton and B. May. 2004. Genetic evidence for hybridization of pallid and shovelnose sturgeon. Journal of Heredity 95:474-480.

UBPSRW (Upper Basin Pallid Sturgeon Recovery Workgroup). 2005. Upper basin pallid sturgeon work group 2004 annual report. Montana Fish, Wildlife and Parks.

USFWS (U.S. Fish and Wildlife Service). 2005. Upper basin pallid sturgeon propagation plan. Bismarck, North Dakota.

USFWS. 2007. Pallid sturgeon (*Scaphirhynchus albus*) 5-year review summary and evaluation. Billings, Montana.

USFWS. 2008. Pallid sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. Billings, Montana.

USFWS. 2010. Endangered and threatened wildlife and plants; threatened status for shovelnose sturgeon under the similarity of appearance provisions of the endangered species act. Federal Register 75 no. 169 pp. 53598-53605.

Wanner, G.A., D.A. Shuman, and D.W. Willis. 2007. Food habits of juvenile pallid sturgeon and adult shovelnose sturgeon in the Missouri River

downstream of Fort Randall Dam, South Dakota. Journal of Freshwater Ecology 22:81-92.

CHAPTER 2. GROWTH, FOOD CONSUMPTION AND ENERGY STATUS OF JUVENILE PALLID STURGEON FED A COMMERCIAL OR INVERTEBRATE DIET

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Introduction

Sturgeon (Acipenseriformes) are considered one of the most endangered group of fishes in the world, with many species listed as threatened or endangered through much of their range (Birstein 1993; Van Winkle *et al*. 2002; Moyle and Cech 2003). Harvest, habitat loss, pollution and habitat fragmentation (i.e., migration barriers) have been attributed to the decline of sturgeon species worldwide (Birstein *et al.* 1997). The pallid sturgeon *Scaphirhynchus albus* is a federally endangered species that occurs in the Missouri and lower Mississippi rivers (Dryer and Sandoval 1993). Shortly after the pallid sturgeon was placed on the endangered species list in 1990, a recovery plan was developed to address population declines that led to the listing (Dryer and Sandoval 1993). Recovery efforts have largely been targeted at habitat restoration, population assessment, captive propagation, and a comprehensive sturgeon research program (Dryer and Sandoval 1993).

Efforts to mitigate declines of imperiled fish populations often rely on captive propagation and stocking programs in recovery efforts (Williamson 2001; Chebanov *et al.* 2002; Secor *et al*. 2002; Smith *et al.* 2002). The U.S. Fish and

Wildlife Service (USFWS), for example, currently maintains 28 federal hatcheries involved in recovery efforts of threatened or endangered species (Hartman and Preston *et al.* 2001). Population augmentation has served an important role in pallid sturgeon recovery efforts, particularly in the upper Missouri River basin (e.g. Montana, North Dakota, and South Dakota) where there has been little evidence of natural reproduction by pallid sturgeon (Bergman *et al*. 2008). Until 2009, the current propagation program has focused on maximizing fish production to meet basin-wide stocking quotas for the propagation program, which has produced approximately 936,413 pallid sturgeon for stocking between 1992 and 2007 (Krentz *et al.* 2005; Smith and Brannen 2010).

Production of hatchery reared fish can be constrained by space, costs and labor requirements that account for 60-80% of total costs (Westers 2001). Growing the largest fish possible in a short time frame can be difficult to achieve while attempting to minimize production costs. Pallid sturgeon are generally grown to a length of about 250 mm fork length (FL) prior to stocking in the Missouri River. Sturgeon smaller than 250 mm are not usually stocked because fish this size are difficult to tag using passive integrated transponders (PIT tags), and valuable recapture information can be lost. Because of these rearing requirements, it takes 10-15 months to produce pallid sturgeon to the desired stocking length which leaves hatchery managers with the task of rearing pallids almost twice as long as most other stocked fish species (Hart *et al*. 1996;

Summerfelt *et al*. 1996). While stocking of large fish increases post-stocking survival, it also leads to an increase in production costs.

Use of commercially formulated diets in hatchery settings can reduce labor and feed costs associated with fish production. These diets are inexpensive, readily available, easy to store, require no preparation and can be administered through the use of automatic feeders (Barrows and Hardy 2001). Most hatcheries involved in pallid sturgeon propagation rely on commercial diets that contain fish meal and are designed primarily for salmonid fishes (>40% protein and >10% fat). However, little is known about dietary requirements of juvenile pallid sturgeon.

The composition of digestive enzymes can vary among fishes depending on the types of prey they naturally consume, suggesting an evolutionary adaptation to natural diets (Jobling 1995; Horn 1998). Post-stocking assessments of juvenile pallid sturgeon (<400 mm FL) have shown that in the wild, they consume primarily aquatic invertebrates, especially Chironomidae and Empheroptera taxa (Grohs *et al*. 2009). Insectivorous fish produce high levels of chitinase to break down exoskeletons, therefore one would expect juvenile pallid sturgeon digestive tracts to be better adapted to process invertebrate diets over fish-based diets (Jobling 1995). Nonetheless, it is unclear if growth and energy reserves among pallid sturgeon differ between fish fed commercial or invertebrate diets because comparative studies of these food types are lacking.

Understanding the influence of commercial diets on pallid sturgeon growth, metabolism and energy stores has important implications for the propagation program, given the extended rearing phase and the need to augment an endangered population with healthy fish. A number of studies have shown that natural diet items such as small fish prey or aquatic invertebrates outperform formulated diets by enhancing growth and (or) survival of cultured fishes such as white sturgeon (*Acipenser transmontanus*), coho salmon (*Oncorhynchus kisutch*), and walleye (*Sander vitreus;* Buddington and Doroshov 1984; Lindberg and Doroshov 1986; Barrows and Hardy 2001). Survival of hatchery raised pallid sturgeon is highest (with greatest uncertainty) 1-2 years post-stocking (Hadley and Rotella 2009; Steffensen *et. al* 2010). In this study, I compare growth, food consumption, routine metabolism, and energy status of juvenile pallid sturgeon fed commercial or invertebrate diets over a five week period and discuss potential implications for propagation and post-stocking success.

Methods

Juvenile pallid sturgeon (n=24) were obtained from the USFWS Garrison Dam National Fish Hatchery, Riverdale ND on 14 April, 2010. Fish were individually placed in 24 insulated, 114-liter glass aquaria. The aquaria were connected to a re-circulating system with a biofiltration tank, and a sand filter. Water flow was kept constant to individual aquaria and temperature loggers (n=4; Tidbit, Onset Computer Corporation, Bourne, Massachusetts) were placed in the

system and hourly water temperature was recorded. Fish were maintained on a natural photoperiod with natural lighting from laboratory windows. Fish were acclimated to the recirculation system for approximately two weeks prior to the experiment. During the acclimation period, 12 fish were chosen at random and slowly, over approximately 8 to 10 days, switched from a commercial pellet diet (1mm sinking feed; Nelsons and Sons Inc.; 45% protein and 19% fat guaranteed analysis) to a diet of chironomid larvae. Frozen chironomids were obtained from a commercial dealer (Hikari USA Inc. Hayward, CA) and thawed prior to being offered to fish. Pallid sturgeon in both diet groups were fed a daily *ad-libitum* ration.

Feeding and growth

After the two week acclimation period, I measured feeding and growth of juvenile pallid sturgeon fed chironomid or commercial diets for five weeks. Fish were measured to fork length (FL; mm) and weighed (to the nearest 0.1g) at 7 day intervals. Differences in both length and weight were compared using a repeated measures analysis of variance (ANOVA) with diet and time as grouping factors. To evaluate significant interactions (diet*time), I used a post-hoc two-sample t-test with a Bonfferoni correction ($\alpha = 0.01$).

Fish assigned to each feeding group were fed a pre-weighed *ad-libitum* ration of the respective diet twice a day with uneaten food recovered daily to measure consumption. Chironomids were blotted and weighed wet to the nearest 0.1 g, and pellets were air-dried for at least 24 hours after siphoning and

weighed to the nearest 0.1 g. Daily food consumption (g day^{-1}) was calculated for each fish as the difference between the amount of food fed and that recovered from the aquaria 24 hours later. I measured recovery of pellets and chironomids in tanks without fish to account for leaching and breakdown of food items. Five grams of either chironomids or pellets were introduced into fishless aquaria (three replicates per food item) and recovered from the tank 24 hours later. A recovery ratio was calculated as the amount recovered divided by the initial amount fed, and averaged for each food type. I recovered frozen chironomids at a rate of 68.4 % of initial amount fed, and 63.3 % for pellets.

The energy content (kJ g^{-1}) of each prey type was determined using bomb calorimetry (1108 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, Illinois). I oven-dried (60°C) five samples of each food type to a constant weight and calculated a ratio of dry weight:wet weight for each food type to adjust for differences in water content. Daily food consumption (g d^{-1}) was multiplied by the wet weight energy content of each prey type (kJ g^{-1} wet wt) to yield daily consumption (kJ d^{-1}). Because feeding rate varies with body size for pallid sturgeon (Chipps, S. unpublished data), I adjusted daily consumption to account for variability in fish weight. To account for allometric variability among fish, I estimated daily energy intake (DEI) using the equation

$$DEI = (kJ\ d^{-1})/W^{(0.674)}$$

where W is wet weight of fish in g (Chipps, S. unpublished data). I compared mean DEI values using repeated measures ANOVA with diet and time as

grouping factors. A post-hoc two-sample t-test with a Bonfferoni correction factor ($\alpha = 0.01$) was used to evaluate significant interactions between diet and time. I also compared feed conversion efficiency (CE) using the equation

$$CE = 100 \times \text{total wet weight gained} / \text{dry weight of feed offered}$$

Conversion efficiency was calculated for each food type and compared between feeding groups using a two-sample t-test ($\alpha = 0.05$).

Respiration

I used intermittent flow respirometry to quantify routine respiration rate (R) of juvenile pallid sturgeon (Steffensen 1989). Individual fish were placed into 4 L plastic respirometry chambers and acclimated to the respirometer for at least 12 hours prior to trials. Food was withheld for at least 48 hours prior to trials to minimize effects of specific dynamic action (i.e. digestive costs) on respiration rates. Seven blank chambers were used as a control to account for microbial respiration (Cech 1990). Oxygen consumption was measured using NeoFox oxygen probes and NeoFox Software (Ocean Optics Inc, Dundedin, Florida). Dissolved oxygen (D.O.) concentration (mg L^{-1}) was recorded every ten seconds until a measurable decline was detected. Dissolved oxygen levels were monitored, and trials were terminated if D.O. dropped below 6 mg L^{-1}. Trial duration was dependent upon fish size and lasted between 17 and 84 m. Three replicates were performed on individual fish, and mean oxygen depletion was calculated as:

$$\text{Mg } O_2 \text{ hour}^{-1} = [([\text{initial } O_2] - [\text{final } O_2]) * \text{volume respirometer-D}] / \text{time}$$

for each replicate, where D equals displacement by fish, assuming 1 mL water equals 1 g of fish (Cech 1990). Mean specific metabolic rate (R) was calculated as mean g O_2 d^{-1} for each fish and was adjusted to account for allometry using the equation:

$$ADJR = (g\ O_2\ d^{-1})/W^{0.85}$$

where W is wet weight of fish in g (Klumb, R. unpublished data). The ADJR was compared between feeding groups using a two-sample t-test ($\alpha = 0.05$).

Energy status

I calculated relative condition factor (K_n) of pallid sturgeon fed chironomids or pellets at the end of the five week feeding trial using the equation:

$$K_n = W/W'$$

where W is the observed weight of the fish and W' is length-specific mean weight for a fish predicted by a weight-length regression equation (Murphy and Willis 1992). The weight-length regression equation for pallid sturgeon captured from the Missouri and Mississippi rivers is:

$$\log_{10}W = -6.378 + 3.357\log_{10}L$$

where W is the predicted weight and L is fork length (Keenlyne and Evenson 1993). I used a two-sample t-test to compare mean K_n between feeding groups ($\alpha = 0.05$).

After completion of the respirometry trials, all fish were euthanized using an overdose (approximately 0.5 g L^{-1}) of MS-222 (Finquel ®, Argent Chemical Laboratories, Redmond, Washington). Fish were weighed wet to the nearest 0.1

g, and the liver was excised and weighed wet to the nearest 0.1 g. I calculated the hepatosomatic index (HSI) for each fish as:

$$HSI = (\text{Liver wt/body wt}) * 100$$

and compared mean HSI between feeding groups using a two-sample t-test (α = 0.05).

Whole body energy density (kJ g^{-1} wet weight) of pallid sturgeon was determined using oxygen bomb calorimetry (1108 Parr Oxygen Bomb Calorimeter). Six pallid sturgeon from each feeding group were randomly selected for analysis. I compared mean energy density between feeding groups using a two-sample t-test (α = 0.05).

Whole body proximate composition analysis was performed on the same subset of pallid sturgeon chosen for energy density analysis (n = 12). Whole fish were dried (60°C) to a constant weight and homogenized. Homogenized fish were then combined into one sample according to the respective feeding group (i.e. pellet-fed or chironomid-fed), because of the minimum sample weight required for analysis. Samples were sent to the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories for processing where they were analyzed for crude protein (g kg^{-1}) and crude fat (g kg^{-1}). Protein content (in the form of total nitrogen) was determined by combustion analysis, using protocols from the Association of Official Analytical Chemists (Horwitz 2006). Crude protein (CP) was reported as 6.25 x nitrogen value (Horwitz 2006). Crude fat (CF) was determined by ether extraction of lipids using the Folch method

(Folch *et al*. 1957). Proximate composition analysis was also performed on samples of each food type according to the methods described above.

Results

Feeding and growth

Water temperatures over the 5 week period ranged from 18.7 (SE = 0.05) to 25.6 °C (SE = 0.04), but never varied more than 2.1 °C among tanks. Mean initial weights of pallid sturgeon did not differ between feeding groups (P = 0.08).

After four weeks, chironomid-fed fish were significantly longer than pellet fed fish (Figure 2-1). Mean fork length (FL) of pallid sturgeon varied significantly by diet*time interaction (Table 2-1). Chironomid-fed fish grew an average of 1.13 mm d^{-1}, whereas pellet-fed fish grew an average of 0.23 mm d^{-1} over the 5 week feeding trial. On the average, chironomid fed pallid sturgeon grew 54.3 mm compared to only 10.8 mm for the pellet-fed fish over the five week period. Similarly, chironomid-fed fish were significantly heavier than pellet-fed fish after week 4, having gained nearly twice as much mass as pellet-fed fish by the end of the experiment (Figure 2-2). Weight of pallid sturgeon varied significantly by diet*time interaction (Table 2-1). Chironomid-fed fish gained an average of 0.5 g d^{-1}, whereas pellet-fed fish had an average daily growth of 0.06 g d^{-1}.

Mean energy density of chironomids was significantly lower than pellets (Table 2-2). Pallid sturgeon fed chironomids consumed less than those fed pellets during the 5 week period. Daily energy intake varied significantly by diet,

but not by time or diet*time interaction, indicating consistent consumption throughout the experiment for each feeding group (Table 2-2). Pallid sturgeon on a chironomid diet consumed 0.59 (SE = 0.008) (kJ d^{-1})/$wt^{(0.674)}$ on average with daily consumption of chironomids varying between 0.19 to 0.69 (kJ d^{-1})/$wt^{(0.674)}$. Pallid sturgeon on the pellet diet consumed 1.36 (SE = 0.06) (kJ d^{-1})/$wt^{(0.674)}$ on average, and varied between 0 to 6.07 (kJ d^{-1})/$wt^{(0.674)}$. Energy consumption for pallid sturgeon on the chironomid diet was less variable (CV = 0.216) than consumption by pallid sturgeon on the pellet diet (CV = 0.703). Pallid sturgeon fed chironomids had higher mean CE than those fed pellets (Table 2-2). Similar to consumption rate, feed conversion efficiency for chironomid-fish was less variable (CV = 0.73) than for pellet-fed fish (CV = 1.87).

Respiration

Temperatures during respiration trials averaged 22.4 °C (± 0.2 SE). Respiration by chironomid-fed fish was measured at an average temperature of 22.2°C (± 0.27 SE), similar to 22.7°C (± 0.27 SE) for pellet-fed fish (P = 0.22). Pallid sturgeon fed chironomids had higher ADJR than those fed pellets (Table 2-2). Chironomid-fed fish had an adjusted R that ranged from 0.00417 to 0.01011 (g O_2 d^{-1})/$wt^{0.85}$, and pellet-fed fish had an adjusted R that ranged from 0.00523 to 0.00825 (g O_2 d^{-1})/$wt^{0.85}$. Chironomid-fed fish had less variable respiration rates (CV = 0.15) than pellet-fed fish (CV = 0.26).

Energy status

Pallid sturgeon fed chironomids had higher mean K_n than those fed pellets (Table 2-2). Relative condition factor of chironomid-fed pallid sturgeon was less variable (CV = 0.049) than sturgeon fed pellets (CV = 0.097). Pallid sturgeon fed chironomids had a lower HSI than those fed pellets (Table 2-2). The hepatosomatic index of chironomid-fed pallid sturgeon ranged from 1.31 to 1.75% and from 1.0 to 3.41% for pellet-fed fish. Hepatosomatic index for pellet-fed fish was more variable (CV = 0.23) than chironomid-fed fish (CV = 0.1).

Chironomid-fed fish had a significantly higher energy density than pellet-fed fish (Table 2-2). Energy density of pallid sturgeon on the chironomid diet ranged from 2.48 to 6.65 kJ g^{-1}, with a mean energy density of 3.7 kJ g^{-1} of wet weight. Energy density of pallid sturgeon on the pellet diet ranged from 0.38 to 1.02 kJ g^{-1}, with a mean energy density of 0.73 kJ $g^{-1}$of wet weight. Energy density of chironomid-fed fish was more variable (CV = 0.41) than pellet-fed fish (CV = 0.35). Pallid sturgeon fed chironomids contained more crude protein and less crude fat than those fed pellet diets (Table 2-3). Pellet food was approximately 4.42 g kg^{-1} CP and 1.26 g kg^{-1} CF, which yields a protein:energy (P:E) ratio of 0.48. Frozen chironomids contained approximately 0.47 g kg^{-1} CP and 0.03 g kg^{-1} CF, which yields a P:E ratio of 0.34.

Discussion

The chironomid diet resulted in superior growth and condition of pallid sturgeon, compared to the commercial diet. Fish fed chironomids had faster

growth, higher energy stores, and higher CE. Food consumption by fish fed commercial diets was approximately twice that of chironomid- fed fish. Nonetheless, chironomid-fed fish grew faster and were almost twice as heavy and higher K_n at the end of the experiment. Faster growth and superior performance of chironomid-fed fish is most likely due to higher CE of chironomids by pallid sturgeon. Although the pellet fed fish were ingesting more calories, the food source may not be compatible with the sturgeon digestive system, as supported by low CE. The type of protein supplied in a diet may be the most important factor in somatic growth in fish (Stuart and Hung 1989; Jobling 1994). Although the pellet diet contained approximately 44% protein, which is near the optimal percent protein required for white sturgeon (Moore *et al*. 1988), growth rate for pellet-fed pallid sturgeon was much lower than for chironomid-fed fish. My results imply that the type of protein used in the pellet diet was not optimal for pallid sturgeon growth.

Juvenile pallid sturgeon are known to be insectivores (Hoover *et al.* 2006; Wanner *et al*. 2007; Grohs *et al.* 2009), and chironomids may contain a more appropriate type of protein that is compatible with pallid sturgeon digestive systems. Concentrations of micronutrients (vitamins and minerals) in chironomids may differ from those in commercial diets, which could potentially affect CE. In a related study, growth response of Atlantic sturgeon (*A. oxyrinchus*) was linked to the quality of nutrients (e.g. vitamins A, B, D, E, choline, zinc, etc.) in the diet (King 2004). Moreover, bioavailability of nutrients

may be reduced by interactions with binding agents, or other ingredients in formulated feed, which could potentially reduce absorption (Jobling 1994).

Natural diets have been shown to outperform commercial diets in a number of cultured fish species such as walleye, larval coho salmon and white sturgeon (Lindberg and Doroshov 1986; Barrows and Hardy 2001). My results demonstrate that a natural diet of chironomid larvae outperformed a pellet diet in pallid sturgeon. Natural diets may be superior to commercial diets over a wide range of species because of differences in feeding strategies. Although formulated diets are fortified with nutrients and vitamins, digestibility and uptake may differ among fish taxa. Nutrient transport systems in the digestive tract are related to the natural diet of a species, and may be an evolutionary adaptation (Jobling 1995). For example, secretion of digestion enzymes has been shown to differ among fishes in different feeding groups (Horn 1998). Insectivorous fishes have high levels of chitinase to break down exoskeletons, whereas piscivorous fishes have high levels of pepsin to break down proteins (Jobling 1995). Incomplete digestion of formulated diets from a lack of proper digestive enzymes, or improper nutrient transport may contribute to sub-optimal growth of pallid sturgeon reared on pelleted food.

Protein:energy ratios are important to incorporate into formulated feeds for specific fish species to maximize growth, CE, and energy stores (NRC 1993; Tibbetts *et al.* 2005). Hernandez et al. (2001) found that higher growth rates in sharp snout sea bream (*Diplodus puntazzo*) were associated with diets with

lower P:E ratios. An optimal P:E ratio has not been determined for pallid sturgeon, however our results indicate that pallid sturgeon performed better at a lower P:E ratio (0.34 versus 0.48).

Respiration is important for aerobic conversion of food to energy in fish (Cech 1990) Chironomid fed fish exhibited faster growth, and assimilated energy more efficiently which may be why they exhibited an elevated metabolic rate. Metabolic rates of growing animals that are well fed have been found to be higher than animals that have been undernourished (Jobling 1994). For example, largemouth bass (*Micropterus salmoides*) of similar size fed maintenance rations had 63% lower metabolism than those fed *ad-libitum* rations (Ranney 2008). Metabolic rate increases as fish digest food items and synthesize new tissue. The low metabolic rate of pellet-fed fish indicates that poor digestion and little somatic growth occurred with this diet.

Cultured fishes often have high levels of liver lipids, especially those on formulated feeds (Serrano *et al*. 1992; Brown *et a*l.1993; Flood *et al*. 1996). While lipids are an important form of energy for fish, an excess of lipids in a diet may be detrimental to health. The liver is important for lipid and carbohydrate storage, gonad development, immune function, bile production, and acts as a primary site for biotransformation of chemicals such as nutrients, steroids, and contaminants (Bruslé and Gonzàlez i Anadon 1996; Flood *et al*. 1996). Formulated feeds with excessive lipids can cause liver damage (hepatic lipoid disease) in some cultured fish species, and substantial accumulation of lipids in

the liver may be responsible for declines in health or sub-optimal growth (Flood *et al*. 1996; Tibbetts *et al.* 2005). Siberian sturgeon fed high lipid diets deposit lipid in the liver and digestive tract, rather than in muscle tissue (Médale *et al.* 1991). Pallid sturgeon fed a commercial diet had HSI values twice that of chironomid fed fish, which may indicate increased deposition of lipid in the liver. Since I did not examine liver lipid or liver enzymes during our experiment, it is unknown whether liver function was impaired in fish fed the commercial diet. Fatty livers (indicated by a high HSI value) may indicate inefficient utilization of dietary energy (Tibbetts *et al.* 2005), which may explain why pallid sturgeon fed commercial diets with higher HSI levels had slower growth and lower CE than those fed chironomids. If liver function was impaired in fish fed commercial diets, releasing hatchery reared pallid sturgeon with enlarged livers may have detrimental effects on growth and development of those fish in the wild.

Insufficient knowledge of dietary needs of pallid sturgeon could affect recovery efforts, specifically population augmentation. Recently there has been a reduction in stocking quotas for the upper Missouri River basin and the pallid sturgeon stocking program has begun to shift from large scale hatchery production to focus on a conservation genetics based approach. Using this approach, hatcheries would rear a smaller number of fish to larger sizes to ensure higher post-stocking survival, while maximizing genetic diversity. With fewer numbers of fish being released into the Missouri river system, pallid sturgeon propagation efforts should focus on rearing large, healthy fish for

stocking, as well as maintaining healthy broodstock populations. High survival and growth of stocked pallid sturgeon is dependent upon health and performance of fish during rearing. The particular commercial diet used in this study is approximately 1/5 the cost of frozen chironomids, about \$1.79 (USD) kg^{-1} of fish (\$0.01 g^{-1}), compared to \$46.40 kg^{-1} of fish (\$0.05 g^{-1}). While it may not be economically feasible to rear pallid sturgeon entirely on natural diets, it may be beneficial to alter feeding regimes from formulated feeds to natural feeds prior to stocking. Faster growth rates, higher K_n and higher CE obtained by using a diet of frozen chironomids may reduce grow-out time for hatchery reared pallid sturgeon, and may also abet the increased cost of feed. Acclimating pallid sturgeon to natural prey items prior to stocking may be advantageous for greater post-stocking survival and growth.

References

Barrows, F.T., & Hardy, R.W. (2001) 'Nutrition and feeding'. In: *Fish Hatchery Management* (Wedemeyer, G.A. ed.), 2nd edn, pp. 483-558. American Fisheries Society, Bethesda, MD, USA.

Bergman, H.L., Boetler, A.M., Parady, K., Flemming, C., Keevin, T., Latka, D.C., Korschgen, C., Galat, D.L., Hill, T., Jordan, G., Krentz, S., Nelson-Stastny, W., Olson, M., Mestl, G.E., Rouse, K., & Berkley, J. (2008) *Research needs and management strategies for pallid sturgeon recovery*. Proceedings of 31 July-2 August 2007 workshop, St Louis, Missouri. Final report to the U.S. Army Corps of Engineers. William D. Ruckelshaus

Institute of Environment and Natural Resources, University of Wyoming, Laramie, WY, USA.

Birstein, V. J. (1993) Surgeons and paddlefishes: threatened fishes in need of conservation. *Cons. Biol*, 7, 773-787.

Birstein, V.J., Bemis, W.E., & Waldman, J.R. (1997) The threatened status of acipenseriform species: a summary. *Environ. Biol. Of Fish*, 48, 427-435.

Brown, P.B., Griffin, M.E., & White, M.R. (1993) Experimental and practical diet evaluations with juvenile hybrid striped bass. *J. World Aquacult. Soc*, 24, 80-89.

Bruslé, J., & Gonzàlez i Anadon, G. (1996) 'The structure and function of fish liver'. In: *Fish Morphology*: *Horizon of New Research* (Dutta, H.M., & Datta-Mushi, J.S. eds), pp. 77-94, A.A. Balkema, Brookfield, VT, USA.

Buddington, R.K., & Doroshov, S.I. (1984) Feeding trials with hatchery produced white sturgeon juveniles (*Acipenser transmontanus*). *Aquaculture*, 36, 237-243.

Burrows, R. E. (1969) The influence of fingerling quality on adult salmon survival. *Trans. Am. Fish. Soc*, 98, 777–784.

Cech, J.J. (1990) 'Respirometry'. In: *Methods for Fish Biology* (Schreck, C.B., & Moyle, P.B. eds.), pp 335-362. American Fisheries Society, Bethesda, MD, USA.

Chebanov, M.S., Karnaukhov, G.I., Galich, E.V., & Chmir, Y.N. (2002) Hatchery stock enhancement and conservation of sturgeon with an emphasis on the Azov Sea populations. *J. Appl. Ichthyol*, 18, 463-469.

Flood, L.P., Carvan, M.J., Jaeger, L., Busbee, D.L., Galatin, D.M. III., & Neill,W.H. (1996) Reduction in hepatic microsomal p-450 and related catalytic activity in farm-raised red drum. *J. Aquat. Anim. Health*, 8, 13-21.

Folch, J., Lees, M., & Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem,* 226, 497-509.

Grohs, K.L., Klumb, R.A., Chipps, S.R., & Wanner, G.A. (2009) Ontogenetic patterns in prey use by pallid sturgeon in the Missouri River, South Dakota and Nebraska. *J. Appl. Ichtyol,* 25, 48-53.

Hadley, G.L., & Rotella J.J. (2009) *Upper-basin pallid sturgeon survival estimation project*. Montana State University, Bozeman, MT, USA.

Hart, S.D., Garling, D.L., & Malison, J.A. (1996) *North Central Region Aquaculture Center Yellow Perch (Perca flavescens) Culture Guide*. NCRAC Culture Series, 103, Ames, IA, USA.

Hartman, K.J., & Preston, B. (2001) 'Stocking'. In: *Fish Hatchery Management* (Wedenmeyer, G.A. ed.), 2nd edn, pp 661-688. American Fisheries Society, Bethesda, MD, USA.

Hernández, M.D., Egea, M.A., Rueda, F.M., Aguado, F., Martínez, F.J., & García, B. (2001) Effects of commercial diets with different *P/E* ratios on

sharpsnout seabream (*Diplodus puntazzo*) growth and nutrient utilization. *Aquaculture*, 195, 321-329.

Hoover, J.J.; George, S.G.; Killgore, K.J., 2006: Diet of shovelnose sturgeon and pallid sturgeon in the free-flowing Mississippi River. *J. Appl. Ichthyol*. 23, 494-499.

Horn, M.H. (1998) 'Feeding and digestion'. In: *The Physiology of Fishes* (Evans, D.H. ed.), 2nd edn, pp43-64. CRC Press, Boca Raton, Florida, USA.

Horwitz, W, (2006) *Official Methods of Analysis of American Association of Analytical Chemists (AOAC) International*. 18th Ed. AOAC, Gaithersburg, MD, USA.

Hrabik, R.A., Herzog, D.P., Ostendorf, D.E., & Petersen, M.D. (2007) Larvae provide first evidence of successful reproduction by pallid sturgeon, *Scaphirhynchus albus*, in the Mississippi River. *J. Appl. Ichthyol*, 23, 436-443.

Jobling, M. (1994) *Fish Bioenergetics*. Chapman and Hall, London, UK, 309 pp.

Jobling, M., (1995) *Environmental Biology of Fishes*. Chapman and Hall, London, UK, 455 pp.

Keenlyne, K.D, & Evenson, P.D. (1993) Standard and relative weight for the pallid sturgeon, *Scaphirhynchus albus*. *Proc. South Dakota Acad. Sci.,* 72, 41-49.

King, K., (2004) Growth, survival and body composition of juvenile Atlantic sturgeon fed 5 commercial diets under hatchery conditions. *N. Am. J. Aquacult*, 66, 53-60.

Krentz, S., Holm, R., Bollig, H., Dean, J., Rhodes, M., Hendrix, D., Heidrich, G., & Krise, B. (2005) *Pallid sturgeon spawning and stocking summary report 1992-2004*. U.S. Fish and Wildlife Service, Bismarck, ND, USA.

Lindberg, J.C., & Doroshov, S.I. (1986) Effect of diet switch between natural and prepared foods on growth and survival of white sturgeon juveniles. *Trans. Am. Fish. Soc*, 115, 166-171.

Médale, F., Blanc, D., & Kaushik, S.J. (1991) Studies on the nutrition of Siberian sturgeon, *Acipenser baeri*. II. Utilization of dietary non-protein energy by sturgeon. *Aquaculture*, 93, 143-154.

Moore, B.J., Hung, S.S.O., & Medrano, J.F. (1988) Protein requirements of hatchery produced juvenile white sturgeon *Acipenser transmontanus*. *Aquaculture*, 71, 235-245.

Moyle, P.B., & Cech, J.J. (2003) *Fishes: An Introduction to Ichthyology*, 5th Edn. New Jersey, USA, 726 pp.

Murphy, B.R., & Willis, D.W. (1992) Proper distinction between relative weight and relative condition factor. *N. Am. J. Fish Manage.,* 12, 665-666.

National Research Council. (1993) Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Ranney, S.H. (2008) 'Influence of feeding history on metabolic rates in fishes: evidence for metabolic compensation in largemouth bass *Micropterus salmoides.'* Master's of Sience thesis. South Dakota State University, Brookings, SD, USA.

Secor, D.H., Anders, P.J., Van Winkle, W., & Dixon, D.A. (2002) 'Can we study sturgeons to extinction? What we do and don't know about the conservation of North American sturgeons,' *Biology, management, and protection of Sturgeon,* American Fisheries Society Symposium 28, St. Louis, Missouri, pp 3-10.

Serrano, J.A., Nematipour, G.R., & Galatin, D.M. III. (1992) Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. *Aquaculture*, 101, 283-291.

Singer, T.D., & Ballantyne, J.S. (2004) 'Sturgeon and paddlefish metabolism'. In: *Sturgeons and paddlefish of North America* (LeBrenton, G.T.O., Beamish, F.W.H, & McKinley, R.S. eds.), pp 167-194. Kluwer Academic Press, Dordrecht, Netherlands.

Smith, T.I.J., Collins, M.C., Post, W.C., & McCord, J.W. (2002) 'Stock enhancement of shortnose sturgeon: a case study'. *Biology, management, and protection of Sturgeon,* American Fisheries Society Symposium 28, St. Louis, Missouri, pp 31-44.

Steffensen, J.F. (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiol. Biochem*, 6, 49-59.

Steffensen, K.D., Powell, L.A., & Koch, J.D. (2010) Assessment of hatchery-reared pallid sturgeon survival in the lower Missouri River. *N. Am. J. Fish. Manage*, 30, 671-678.

Stuart, J.S., & Hung, S.S.O. (1989) Growth of juvenile white sturgeon (*Acipenser transmontanus*) fed different proteins. *Aquaculture*, 76, 303-316.

Summerfelt, R.C., Clouse, C.P., Harding, L.M., & Luzier, J.M. (1996) 'Walleye fingerling culture in drainable ponds'. In: *Walleye Culture Manual* (Summerfelt, R.C. ed.) pp. 89-108. NCRAC Culture Series, Ames, Iowa, USA.

Tibbetts, S.M., Lall, S.P., & Milley, J.E. (2005) Effects of dietary protein and lipid levels and DP DE−1 ratio on growth, feed utilization and hepatosomatic index of juvenile haddock, *Melanogrammus aeglefinus L*. *Aquacult. Nutr*, 11, 67-75.

USFWS (U.S. Fish and Wildlife Service). (1993) Recovery plan for the pallid sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Bismarck, ND, USA.

USFWS. (2005). Upper basin pallid sturgeon propagation plan. U.S. Fish and Wildlife Service Bismarck, North Dakota.

Wanner, G.A., Shuman, D.A., & Willis, D.W. (2007) Food habits of juvenile pallid sturgeon and adult shovelnose sturgeon in the Missouri River

downstream of Fort Randall Dam, South Dakota. *J. Freshwat. Ecol*, 22, 81-92.

Westers, H. (2001) 'Production'. In: *Fish hatchery management* (Wedenmeyer, G.A. ed.), 2nd edn, pp. 31-90. American Fisheries Society, Bethesda, MD, USA, pp 31-90.

Williamson, J.H. (2001) 'Broodstock management for imperiled and other fishes'. In: *Fish hatchery management* (Wedenmeyer, G.A. ed.), 2nd edn, pp. 397-482. American Fisheries Society, Bethesda, MD, USA.

Table 2-1. Repeated measures ANOVA results for differences in wet weight (g), length (mm, FL), and daily consumption (kJ d^{-1})/wt(0.674) for juvenile *Scaphirhynchus albus* fed a diet of either chironomid larvae or pellet food based on diet type, time, and diet*time interaction (α = 0.05).

Variable	Effect	d.f.	F-Value	Pr > F
Weight	Diet*time	4	9	<.0001
Length	Diet*time	3	5.65	0.0014
Consumption	Diet	1	47.27	<0.0001
	Time	4	1.03	0.4
	Diet*time	3	1.36	0.3

Table 2-2. Mean conversion efficiency (CE; %), food energy density (ED; kJ g^{-1}), whole body energy density (kJ g^{-1} wet weight), routine metabolic rate (R; (g O_2 d^{-1})/$wt^{0.85}$), relative condition factor (K_n) and hepatosomatic index (HSI), for juvenile *Scaphirhynchus albus* fed a diet of either chironomid larvae or pellet food. Standard error for means are shown along with results of two sample t-tests comparing values between chironomid and pellet fed pallid sturgeon.

	Pellet diet	**Chironomid diet**	**d.f.**	**T-statistic**	**P**
CE	13.8 ± 4.5	37.9 ± 4.6	67	3.72	< 0.001
Feed ED	9.2 ± 0.79	1.3 ± 0.03	6	-9.93	< 0.001
Whole body ED	0.73 ± 0.1	3.72 ± 0.62	10	4.73	< 0.001
Adjusted R	0.0065 ± 0.0004	0.0079 ± 0.0005	20	-9.93	< 0.001
K_n	0.87 ± 0.024	1.11 ± 0.015	22	-8.22	< 0.001
HSI	2.57 ± 0.6	1.45 ± 0.15	16	-5.49	< 0.001

Table 2-3. Moisture, crude protein, crude fat and ash (g kg^{-1}) of juvenile *Scaphirhychus albus* fed a diet of either chironomid larvae or pellet food (n = 1).

	Diet Type	
Composition	**Pellet**	**Chironomid**
Moisture	1.18	1.05
Crude protein	5.39	6.03
Crude fat	1.51	1.17
Ash	1.75	1.59

Figure 2-1. Mean fork length (mm) of chironomid-fed (open triangles) and pellet-fed (solid squares) juvenile *Scaphirhynchus albus* during five week feeding trials. Error bars represent 1 S.E and asterisks denote significant post-hoc t-test results ($p < 0.01$).

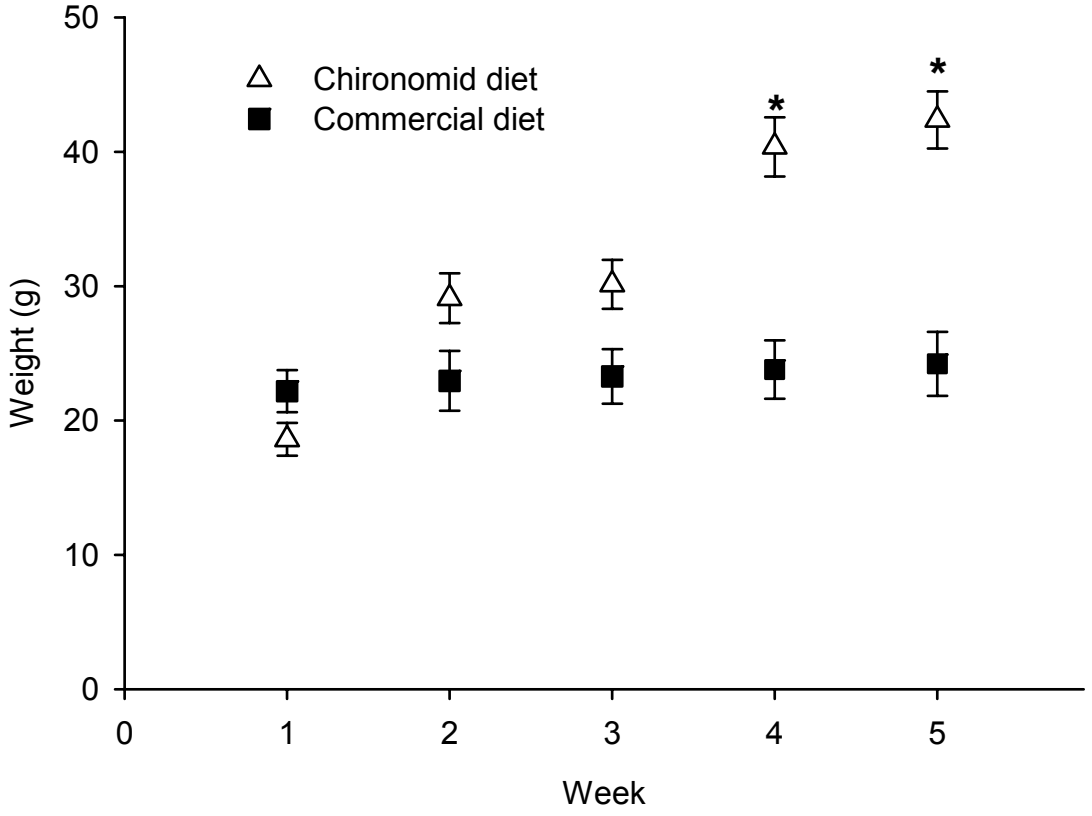

Figure 2-2. Mean weight (g) of chironomid-fed (open triangles) and pellet-fed (solid squares) juvenile *Scaphirhynchus albus* during five week feeding Error bars represent 1 S.E and asterisks denote significant post-hoc t-test results ($P < 0.01$).

CHAPTER 3. EVIDENCE FOR COUNTERGRADIENT VARIATION IN PALLID STURGEON PHYSIOLOGY AND IMPLICATIONS FOR CONSERVATION OF AN ENDANGERED SPECIES

This chapter has been prepared for submission as a manuscript to Conservation Biology and was co-authored with Dr. Steven R. Chipps, Dr. Brian D. S. Graeb, and Dr. Robert A. Klumb.

Introduction

Conservation of endangered species increasingly relies on the identification of evolutionarily significant units (ESU; e.g. distinct population segments, stocks, subspecies etc.) to develop and implement effective management policies (Moritz 1994; Waples 1995). Evolutionarily significant units can be defined as groups of species with distinct ecological, genetic and/or historical importance (Waples 1995). Often times, ESUs are categorized as populations or distinct population segments. In the wild, these populations experience selective pressures that influence the direction of evolutionary processes. Understanding the influences of selective pressures such as differential survival, fecundity or mating success and how they affect the evolution of a population is paramount in successful conservation and management of endangered species.

Populations in the wild are not homogenous units; rather they develop an internal structure based on interactions between the physical environment and the biology of the species (Nunney 2001; Shaklee & Currens 2003). Population structure is the pattern of differentiation of sub-populations within a larger,

broadly distributed population, also referred to as a metapopulation (or a population of populations; Kimura & Weiss 1964; Nunney 2001). Structure can manifest in one of two ways: first is genetic structure, which is defined as nonrandom distribution of genotypes in space and time, and second is proximity structure which are the intraspecific interactions that influence an individual's fitness (Nunney 2001). Genetic structuring can result from populations with restricted gene flow due to physical barriers, limited dispersal or temporal reproductive isolation. Population structure exerts substantial influence on how selective forces act on the evolutionary path of a species. In particular, genetic structuring can affect evolutionary processes such as local adaptation, coadaptation and even speciation (Nunney 2001). If a local population is genetically isolated, genotypes with high local fitness will be selected for, which will further enhance genetic structuring (Nunney 2001). Thus, maintenance of genetic variation, such as conservation of genetic stock structure, is a major concern in endangered species management.

Many traits in an individual are sensitive to the quality of the environment, and organisms can respond to variations in environmental conditions through phenotypic plasticity or long-term adaptations in traits (Mazer & Damuth 2001). Those selective forces can cause organisms to evolve specific traits that maximize fitness in their local environment (Kawecki & Ebert 2004). This process of evolving traits specific to local environmental conditions is known as local adaptation (Kawecki & Ebert 2004). Adaptive evolution is most likely to

occur in genetically structured populations, particularly in instances of isolation by distance (Wright 1951). Local adaptation in a population may lead to maintenance of genetic variation, which is important for endangered species as they typically have low levels of genetic variation (Nunney 2001). Thus, successful conservation of endangered species requires an understanding of local adaptation and the environmental factors that shape them

The pallid sturgeon (*Scaphirhynchus albus*) is a federally endangered, large riverine fish that has a broad geographic distribution throughout the Missouri, and the middle and lower Mississippi rivers (Dryer & Sandoval 1993). The Missouri River is one of the most highly altered systems in the world, with the pallid sturgeon facing habitat alterations in 67% of its range (Hesse 1987; Dryer & Sandoval 1993). Habitat alteration, environmental contaminants, overfishing and hybridization with sympatric shovelnose sturgeon (*S. platorhyncus*) are believed to be the main causes of decline (Dryer & Sandoval 1993). Population estimates suggest that as few as 6,000 to as many as 21,000 genetically pure pallid sturgeon still exist (Duffy et al 1996). Recent work using pallid sturgeon microsatellites suggests the presence of a distinct genetic stock structure (Schrey & Heist 2007). Current genetic stock structure analysis of pallid sturgeon identified three genetically distinct groups within the range of the species (Schrey & Heist 2007). This apparent stock structure suggests a stepping stone pattern (exchange of individuals between adjacent colonies only), and appears to follow a latitudinal trend with one distinct upper Missouri River

basin group and two less distinct lower basin groups (Kimura & Weiss 1964; Schrey & Heist 2007; USFWS 2008). The upper Missouri River basin (upper basin; UB) extends from the Great Falls of the Missouri River in Montana downstream to Gavins Point Dam, South Dakota (Fig. 3-1). The middle basin of the Missouri River (middle basin; MB) is defined as the Missouri River from Gavins Point Dam, South Dakota to Kansas City, Missouri (Fig. 3-1).

Species with a broad geographic distribution, such as the pallid sturgeon, are exposed to a large range of environmental conditions that vary latitudinally. For example, in the upper Missouri River (southeast South Dakota) optimal water temperatures for juvenile pallid sturgeon (25-28 °C) persist, on average, for about 2 weeks per year whereas in the lower Mississippi River, optimal feeding and growth temperatures can persist for over 3 months (Chipps et al. *In review*). Within the Missouri River, mean water temperature and number of growing degree days in the growing season for emerald shiner (*Notropis atherinoides*), sicklefin chub (*Macrhybopsis meeki*), freshwater drum (*Aplodinotus grunniens*), river carpsucker (*Carpiodes carpio*) and sauger (*Sander canadensis*) were 1.3 times greater in southern latitudes than northern latitudes (Braaten & Guy 2002). Such large, latitudinal variation in environmental conditions can give rise to countergradient variation (CGV) in fishes, where genetic and environmental influences on phenotype oppose one another (i.e., genotype adaptation according to latitudinal compensation; Conover & Schultz 1995). Evidence for CGV suggests that genetic capacity for growth increases with increasing latitude;

thus, individuals from more northern latitudes have higher metabolic rates than conspecifics from lower latitudes, at the same temperature (Conover 1990). This form of antagonistic selection appears counter-intuitive in that, the rate of most physiological processes (e.g. growth, metabolism) of ectotherms generally declines with temperature (Conover & Schultz 1995). However, patterns of CGV have been observed in a number of broadly distributed species such as lake sturgeon (*Acipenser fulvescens*), paddlefish (*Polyodon spathula*) Atlantic silverside (*Menidia menidia*), striped bass (*Morone saxatilis*), mummichog (*Fundulus heteroclitus*), green frog (*Rana clamitans*), gastropods, and marine invertebrates (see review by Conover & Schultz 1995; Power & McKinley 1997; Scarnecchia et. al 2011).

Morphological variation within pallid sturgeon populations follows a latitudinal trend similar to that reported for other fishes exhibiting CGV (Conover 1990). Body size, and outer barbell length increase with increasing latitude, while relative head and interrostral length decrease (Killgore et al. 2007; Murphy et al. 2007). Although evidence of latitudinal trends in morphology and genetic stock structure exist for pallid sturgeon, it is unclear whether these patterns are linked to local adaptations that might manifest as physiological differences along a latitudinal gradient. To address this question, I designed a common-environment experiment using juvenile pallid sturgeon from two distinct genetic groups (upper basin, UB and middle basin, MB of the Missouri River) to determine whether physiological attributes vary along a latitudinal gradient.

Upper basin pallid sturgeon are progeny from wild adults captured near the confluence of the Missouri and Yellowstone rivers in Montana (Fig. 3-1). Middle basin pallid sturgeon are progeny from wild adults captured in the Missouri River between Plattsmouth and Rulo, NE (Fig. 3-1). I hypothesized that feeding, growth and metabolic rates of pallid sturgeon will follow a pattern of CGV, where fish from northern latitudes (i.e., UB) exhibit faster rates than pallid sturgeon from lower latitudes (i.e., MB) when reared at similar water temperatures.

Methods

I obtained 48 juvenile pallid sturgeon from the U.S. Fish and Wildlife Service Gavin's Point National Fish Hatchery, Yankton, SD. Twenty four hatchery-raised juvenile pallid sturgeon, representing three families (8 fish/family), were obtained from parental stock captured near the confluence of the Yellowstone and Missouri rivers (UB; Fig. 3-1). Similarly, 24 fish representing three families were obtained from parental stock captured in the Missouri River between Plattsmouth and Rulo, NE (MB; Fig. 3-1). For the purpose of this study, I define family group as 1 female crossed with 1 male pallid sturgeon. Two fish (each from the same family) were randomly assigned to one of twenty-four, 114-liter glass aquaria. I clipped the pectoral fin of one fish from each tank to identify individual fish. The twenty-four aquaria were connected to a re-circulating system with a bio-filtration tank, and a sand filter. Water flow was kept constant to individual aquaria and temperature loggers (n=4; Tidbit, Onset Computer Corporation, Bourne, Massachusetts) were placed in the system to record hourly

water temperature. Fish were exposed to natural lighting (windows) in the laboratory and thus maintained on a natural photoperiod. Fish were held in the recirculation system for approximately four months prior to the experiment and fed a daily ration of chironomid larvae at 1-2% per day (i.e. maintenance ration). During the acclimation period, water temperatures varied with ambient laboratory temperatures and ranged between 17 to 20 °C.

Feeding and growth

I measured feeding and growth of UB and MB pallid sturgeon at 10, 14, 18, 22, 26 and 30°C beginning on 4 March to 18 July 2011. Test temperatures were selected to reflect seasonal changes in water temperatures in the Missouri/Mississippi River system. Water temperatures were controlled using a 1500 watt heater and (or) a 0.75 horsepower chiller unit and was increased or decreased at a rate of ~1^{0}C per day, until the desired test temperature was reached. Fish were then acclimated to the test temperature for at least seven days before starting 5-d feeding and growth trials.

Fish were fed a pre-weighed, *ad-libitum* ration of chironomids daily for five days. Uneaten food was siphoned daily from each aquarium and chironomids were blotted and weighed wet to the nearest 0.1 g. A pooled estimate of daily food consumption (2 fish/tank) was calculated as the difference between the amount of food fed and that recovered from the aquaria 24 hours later. To account for leaching and residual breakdown of food items, I measured recovery of chironomids in tanks without fish. Five grams of chironomids were introduced

into fishless aquaria (four to five replicates per temperature) and recovered from the tank 24 hours later. A recovery ratio for each temperature was calculated as the amount recovered divided by the initial amount fed (Table 3-1). I corrected daily food consumption estimates using the calculated recovery ratio corresponding to the test temperature.

Because feeding rate varies with body size, I adjusted daily consumption to account for variability in fish weight. In a related study, Chipps et al. (in review) reported that maximum feeding rate for juvenile pallid sturgeon varied with body size. Thus, to account for allometric variation, I adjusted daily consumption using the equation:

$$ADC = (g\ food/d)/W_1^{0.674} + W_2^{0.674}$$

where ADC is adjusted daily consumption, W_1 is the weight of fish 1 and W_2 is the weight of fish 2 in each tank. Weight of individual fish, measured at the beginning (day 1) and end (day 5) of each feeding trial, was used to estimate daily weight and to calculate mean, absolute growth rate (AGR, g/d) for individual fish as:

$$AGR = \text{total g body weight gained/number of days}$$

Information on feeding and growth rates were also used to calculate conversion efficiency (CE) as:

$$CE = 100 \times \text{total wet weight gained / dry weight of feed offered}$$

Respiration

I used intermittent flow respirometry to quantify routine metabolic rate of juvenile pallid sturgeon at 10, 14, 18, 22, 26 and 30°C (Steffensen 1989). Fish were acclimated to the desired test temperature for at least 14 days before a new respirometry trial began. Food was withheld for at least 48 hours prior to respirometry measurements to preclude effects of specific dynamic action (Beamish 1974). Individual fish were placed into 4 L plastic respirometry chambers and acclimated to the respirometer for at least 12 hours prior to trials to minimize handling stress. Four to five blank chambers were used as a control at each temperature to account for microbial respiration (M; Chech 1990). Oxygen consumption was measured using NeoFox oxygen probes and NeoFox Software (Ocean Optics, Dundedin, Florida). Dissolved oxygen (D.O.) concentration (mg/L) was recorded every ten seconds until a measurable decline was detected. Dissolved oxygen levels were monitored, and trials were terminated if D.O. dropped below 5 mg/L. Trial duration was dependent upon fish size and water temperature and ranged from 0.13 to 1.55 h. Three replicates were performed on individual fish, and oxygen consumption (R) was calculated as:

$$\text{Mg O2/hour} = [([\text{initial } O_2] - [\text{final } O_2])*\text{volume-D}] / \text{time}$$

where D = displacement by fish, assuming 1 mL water equals 1 g of fish (Cech 1990). Replicate measurements of R were averaged for each fish and then adjusted (ADJR) to account for allometry using the equation:

$$ADJR = (g\ O_2/h)/W^{0.85}$$

where W is wet weight of fish in g (Chipps et al. in review).

Statistical Analysis

I used analysis of variance (ANOVA) to compare mean weight, ADC, AGR, CE and ADJR among families from each basin (e.g. UB families 1, 2, and 3). I performed an ANOVA at each test temperature. Because no significant differences were found among families for any response variables, I pooled data (by basin) and used a repeated measures ANOVA to compare mean weight, ADC, AGR, CE and ADJR between UB and MB pallid sturgeon. Data expressed as proportions (i.e., CE and ADC) were transformed using an arcsine-square root transformation prior to statistical analysis (Zar 2010). In the repeated measures model, parental source (basin) and water temperature were used as grouping factors. Basin effects were evaluated by specifying nested terms using either tank(basin), which accounts for variability of individual fish or fish(basin) within a basin as the error term, depending on the response variable. Similarly, error terms for testing temperature and temperature*basin interactions were specified as either temperature*tank(basin) or temperature*fish(basin) effects (Cody & Smith 2006). If the interaction between temperature*basin was significant, a post-hoc two sample t-test with a Bonferroni-Holm step down correction was used to determine at which temperature(s) differences between basins occurred. All analyses were performed use the SAS statistical package (SAS 2009).

Results

Feeding and Growth

Initial mean size of UB pallid sturgeon (49.3 g ± 1.26 SE) did not differ from mean size of MB sturgeon (48.7 g ± 2.62; P = 0.8). Mean size of UB fish increased significantly faster than that of MB fish (Table 3-2; Figure 3-2). By the end of the experiment, mean weight of UB sturgeon was 43% higher than that of MB sturgeon. Upper basin fish were significantly heavier than MB fish at temperatures greater than 22°C (Figure 3-2).

Daily consumption by UB pallid sturgeon was generally greater than that observed for MB fish, although the relationship between water temperature and food consumption rate varied by basin (i.e., interaction effect; Table 3-2). At water temperatures of 10 to 22°C, food consumption by UB fish was 23-25% greater than that of MB pallid sturgeon. As expected, daily consumption increased with water temperature and ranged from 0.07 to 0.71 [(g food/d)/$W_1^{0.674}$ + $W_2^{0.674}$] for UB and 0.03 to 0.69 [(g food/d)/$W_1^{0.674}$ + $W_2^{0.674}$] for MB fish at temperatures from 10 to 30 °C (Figure 3-3). I observed the highest consumption rate for both UB and MB pallid sturgeon at 26°C. Pairwise comparisons revealed that adjusted consumption (ADC) for UB pallid sturgeon was significantly greater at 10, 14, 22, and 30 °C compared to MB fish (Bonferroni adjustment, P<0.008; Figure 3-3).

Upper basin pallid sturgeon exhibited faster growth than MB sturgeon at temperatures greater than 18°C. At water temperatures of 10 and 14°C, growth

rate was similar between UB and MB pallid sturgeon (Figure 3-4). Absolute growth for UB pallid sturgeon was generally greater than that observed for MB fish, although the relationship between water temperature and growth rate varied by basin (i.e., interaction effect; Table 3-2). Absolute growth rate increased with water temperature and ranged from -1.15 to 2.6 g/d for UB and -0.5 to 1.88 g/d for MB fish at temperatures from 10 to 30 °C (Figure 3-4). Highest growth rates for both UB and MB pallid sturgeon occurred at 18°C, and were 1.64 and 0.75 g/d, respectively.

Upper basin pallid sturgeon had greater CE than MB pallid sturgeon at 18 and 22°C. Temperature*basin interaction had significant effects on mean CE of UB and MB pallid sturgeon (Table 3-2). Conversion efficiency peaked at 18°C and dropped precipitously at 22°C for UB and MB pallid sturgeon. However, UB CE decreased slightly between 22 and 30°C, while MB CE increased slightly between 22 and 30°C (Figure 3-5). Back-transformed, mean CE was greatest for UB sturgeon at 18°C (51.3 ± 0.048) and lowest (1.8 ± 0.008) at 10°C. Similarly, MB sturgeon had the greatest CE of 22.4 ± 0.009 % at 18°C, and the lowest (0.6 ± 0.005 %) at 10°C.

Respiration

Upper basin pallid sturgeon had greater ADJR than MB sturgeon at 18°C. Mean oxygen consumption rate by juvenile pallid sturgeon varied by basin*temperature interaction (Table 3-2). Mean adjusted R increased with water temperature for both MB and UB fish (Figure 3-6). Oxygen consumption

by UB pallid sturgeon was greatest at 30°C and lowest at 10°C. Similarly, mean adjusted R of MB pallid sturgeon was greatest at 30°C but was lowest at 14 C. Oxygen consumption for UB fish was similar to MB fish at water temperatures <18 °C and > 22 °C (Figure 3-6).

Discussion

Results from this study, along with current research on genetic population structure (Schrey & Heist 2007) presents evidence for adaptive differences between stocks of pallid sturgeon. Pallid sturgeon from the UB and MB of the Missouri River showed evidence of local adaptation in feeding, growth and respiration consistent with counter gradient variation documented in other species (Conover & Schultz 1995; Power & McKinley 1997; Scarnecchia et al. 2011). Greater capacity for growth in UB sturgeon is driven by higher food consumption, CE and higher metabolic rates at warmer temperatures. Our results are similar to those from other studies, where individuals from northern latitudes exhibit faster growth and higher metabolic rates than individuals from more southern latitudes (Conover 1990; Conover & Present 1990; Present & Conover 1992; Schultz et al. 1996; Galarowicz & Wahl 2003; Stillwell & Fox 2009). In a heterogeneous environment, physiological and behavioral responses to environmental variability could lead to the evolution of different stocks (Ihssen et. al 1981). The range of the pallid sturgeon encompasses 18° latitude, with a difference of ~10° latitude between the confluence of the Yellowstone and Missouri rivers (UB), and the Missouri River at Kansas City, MO (MB; Braaten &

Guy 2002). This difference in latitude indicates substantial variation in water temperatures experienced by UB and MB pallid sturgeon. Braaten and Guy (2002) found that the number of days in the growing season averaged 1.3 times greater in southern latitudes (Missouri and Mississippi river confluence) than northern latitudes (Missouri and Yellowstone river confluence). This range in temperature regime likely influences physiological responses of pallid sturgeon in the upper and middle basin of the Missouri River, which eventually lead to local adaptation.

Evolutionary energetics suggests that local adaptations driven by natural selection will tend to maximize physiological efficiencies of a population, if those efficiencies increase fitness (Lotka 1922; Dobzhansky 1970). A literature review of 109 studies on variation in body size of ectotherms found that 80% observed an increase in body size with decreasing temperatures (Atkinson 1994). A number of studies on CGV in natural populations of fishes cite size-selective winter mortality as a plausible explanation for faster growth in northern populations (Conover 1990; Conover & Present 1990; Present & Conover 1992; Schultz et al. 1996). Conover (1984) found that smaller fish were more susceptible to mortality from chronic exposure to low temperatures than larger fish. Hence, it is advantageous for juvenile fish in northern latitudes to maximize growth during a contracted growing season to enter winter with a large body size (Conover 1990; Conover & Present 1990). Little is known regarding over-winter survival of juvenile pallid sturgeon; however, faster growth, higher CE and

increased metabolic rate of UB pallid sturgeon suggests that sturgeon from northern latitudes have adapted the ability to grow more rapidly than MB fish, which would be advantageous for entering winter with a large body size. In addition to increased over winter survival, larger fish typically mature later in life, and exhibit greater reproductive success by means of higher fecundity (Stearns 1992). Greater reproductive success by larger fish may be evolutionarily advantageous in northern climates to counteract environmental instability that may cause erratic recruitment and year class success (Conover & Schultz 1995). Although size-selective winter mortality and increased reproductive success may be selective forces attributable to faster growth in UB pallid sturgeon, why then do MB pallid sturgeon exhibit slower growth rates at similar water temperatures?

One might expect that larger body size and faster growth would be selected for across all latitudes to increase fitness. Larger body size is advantageous because of a decreased risk of predation, maximized age-specific survival, and increased fecundity (Roff 1992). The CGV phenomenon suggests that there may be some factor(s) that favors smaller or intermediate body size at southern latitudes (Conover and Present 1990). In order to grow faster, UB pallid sturgeon have higher energy requirements than MB fish, as evidenced by higher consumption and metabolic rates. Higher energy requirements may be maladaptive in southern latitudes such as the Atchafalaya River, Lousiana where temperatures may reach well above the thermal optima for growth (~27°C for pallid sturgeon; Chipps et. al *In review*). On average, temperatures in the lower

Missouri River are 1.3 times greater than in the upper Missouri River (Braaten & Guy 2002). Knowledge of the underlying mechanisms influencing the pattern of CGV is poor; however evidence from this study and others suggests that a fitness trade-offs exist associated with increased growth at southern latitudes (Conover 1990; Conover & Present 1990; Present & Conover 1992; Schultz et. al 1996; Karl & Fischer 2008). Although a larger body size is advantageous for a variety of reasons, such as decreased risk of predation, and/or increased fecundity, there may be physiological constraints and ecological trade-offs associated with selection for growth rate in southern latitude fish (in this case MB pallid sturgeon) that are poorly understood (Present & Conover 1992; Sogard 1997). Selection for large body size as influenced by environmental variability may be adaptive, but body size may not be the target of selection, rather some cellular mechanism (e.g. endocrine mechanisms regulating period of growth) may be the underlying target (Davidowitz & Nijhout 2004; Karl & Fischer 2008).

Although mechanisms influencing differential feeding, growth and metabolism are poorly understood, this study is the first to document evidence for CGV in pallid sturgeon across their range. The presence of genetic stock structure and locally adapted populations suggests that selection is driven by differences in environmental conditions experienced by pallid sturgeon throughout their range (Kawecki & Ebert 2004). Other broadly distributed Acipenseriform species such as lake sturgeon and paddlefish show patterns of countergradient variation in growth rates, suggesting the presence of locally

adapted populations (Power & McKinley 1997; Scarnecchia et al. 2011). Local adaptations within populations are important, and thought to be a significant mechanism for maintaining genetic diversity (Hedrick et al 1976; Hedrick 1986). Indeed, inbreeding depression and maintenance of genetic diversity are of great concern for the conservation of endangered species and preserving local adaptations in genetically structured populations is crucial (Hedrick 2001). The persistence and viability of endangered fish populations are greatly affected by a population's adaptive potential; thus, recovery efforts should consider genetic variability in order to preserve local adaptations and mimic genotypes found in the wild (Anders 1998). Understanding the presence and magnitude of local adaptation across the range of a broadly distributed species, such as pallid sturgeon, can be useful for better defining geologically and ecologically meaningful management units for restoration efforts, such as habitat restoration or population augmentation.

Because many populations of endangered species have low genetic diversity, outbreeding depression is of major concern in developing conservation aquaculture programs (Anders 1998; Nunney 2001; Williamson 2001; Ireland et al. 2002). Mitigation efforts for imperiled fish species often use population augmentation programs as a short-term recovery strategy. Up to 68,000 juvenile pallid sturgeon have been stocked into the Missouri River annually, with the aim to prevent extirpation of the species (Hallerman 2003; USFWS 2005; USFWS 2007). Early stockings of pallid sturgeon have included translocations of

offspring from broodstock collected near the uppermost dam (Fort Peck Dam, MT) on the Missouri River, to below Gavin's Point Dam, some 800 river kilometers downstream (Schrey & Heist 2007). The current propagation program incorporates recent research on pallid sturgeon stock structure, and advises against stock transfers, which could reduce fitness of local populations (USFWS 2005; USFWS 2008). Currently, wild pallid sturgeon broodstock collected from the Missouri River are transported to the nearest state or federal hatchery for artificial spawning, and subsequent progeny are stocked into the management unit corresponding to parental origin. Our results strengthen the support for use of local broodstock to conserve local adaptations of pallid sturgeon, and reduce the potential for a genetic bottleneck. Because pallid sturgeon show significant genetic differentiation and evidence of local adaptation, use of local broodstock and scientifically sound breeding programs are important to minimize outbreeding depression between groups (Schrey & Heist 2007). Introduction of organisms with different physiological characteristics can negatively affect genetic integrity, growth and ultimately survival of a locally adapted population (Koppelman & Phillipp 1986). While endangered species recovery may require captive breeding programs, conservationists need to be well aware of the risks and benefits of augmentation programs, and how artificial propagation may potentially affect the process of natural selection on a population (George et al. 2009). Determination of an ESU for implementing conservation policies, such as population augmentation, requires an understanding of if and how populations

are locally adapted, and the underlying genetic stock structure. Natural resource managers must therefore consider the role of spatially structured and locally adapted populations in the delineation of management units, and implementation of recovery efforts in order to optimize restoration.

References

Anders, P.J. 1998. Conservation aquaculture and endangered species: can objective science prevail over risk anxiety? Fisheries, **23:**28-31.

Armour, C.L. 1991. Guidance for evaluating and recommending temperature regimes to protect fish. U.S. Fish and Wildlife Service Biological Report 90(22).

Atkinson, D. 1994. Temperature and organism size-a biological law for ectotherms? Advances in Ecology Research **25:**1-58.

Beamish, F.W.H. 1974. Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. Journal of the Fisheries Research Board of Cananda **31:**1763-1769.

Braaten, P.J., and C.S. Guy. 2002. Life history attributes of fishes along the latitudinal gradient of the Missouri River. Transactions of the American Fisheries Society **131:**931-945.

Cech, J.J., 1990. Respirometry. Pages 335-362 in C.B. Schreck, P.B. Moyle editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA.

Cody, R.P. and J.K. Smith. 2005. Applied statistics and the SAS programming language. 5th ed. Prentice Hall, Upper Saddle River, NJ.

Conover, D.O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. American Naturalist **123:**297-313.

Conover, D.O. 1990. The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. Transactions of the American Fisheries Society **119:**416-430.

Conover, D.O. and T.M.C. Present. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia, **83:**316-324.

Conover, D.O. and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in ecology and evolution **10:**248-252.

Davidowitz, G. and H.F. Nijhout. 2004. The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. Integrative and Comparative Biology **44:**443-449.

Dobzhansky, T. G. 1970. Genetics and the evolutionary process. Columbia University Press, New York, New York.

Dryer, M.P., and A.J. Sandoval. 1993. Pallid sturgeon recovery plan. U.S. Fish and Wildlife Service, Bismarck, North Dakota.

Duffy, W. G., C.R. Berry and K.D. Keenlyne. 1996. The pallid sturgeon biology and annotated bibliography through 1994. South Dakota Cooperative Fish

and Wildlife Research Unit Technical Bulletin 5, South Dakota State University, Brookings, South Dakota.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. Pages 1-98 in W.S. Hoar and J.D. Randall, editors. Fish physiology, volume VI. Academic Press, London, UK.

Galarowicz, T.L., and D.H. Wahl. 2003. Differences in growth, consumption, and metabolism among walleyes from different latitudes. Transactions of the American Fisheries Society **132:**425-437.

George, A.L., B.R. Kuhajda, J.D. Williams, M.A. Cantrell, P.L. Rakes and J.R. Shute. 2009. Guidelines for propagation and translocation for freshwater fish conservation. Fisheries **34:** 529-545.

Hallerman, E. M. 2003. Coadaptation and outbreeding depression. Pgs. 239-260 in E.M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Hedrick, P.W. 2001. Evolutionary conservation biology. Pages 371-384 in C.W. Fox, D.A. Roff and D. J. Fairbairn, editors. Evolutionary Ecology. Oxford University Press, New York, New York.

Hedrick, P.W. 1986. Genetic polymorphisms in heterogeneous environments: a decade later. Annual Reviews in Ecology and Systematics **17:**535-66.

Hedrick, P.W., M.E. Ginevan and E.P. Ewing. 1976. Genetic polymorphisms in heterogeneous environments. Annual Reviews in Ecology and Systematics **7:**1-32.

Hesse, L.W. 1987. Taming the wild Missouri River: What has it cost? Fisheries **12:**2-9.

Ihssen, P.E., H. E. Booke, J.M. Casselman, J.M. McGlade, N.R. Payne, and F.M. Utter. 1981. Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences **38:**1838-1855.

Ireland, S.C., P.J. Anders, and J.T. Siple. 2002. Conservation aquaculture: an adaptive approach to prevent extinction of an endangered white sturgeon population. American Fisheries Society Symposium **28:**211-222.

Karl, I., and K. Fischer. 2008. Why get big in the cold? Towards a solution to a life-history puzzle. Oecologia **155:**215-225.

Kappenman, K.M., W.C. Fraser, M.T. Toner, J. Dean, and M.A.H. Webb. 2009. Effect of temperature on growth, condition and survival of juvenile shovelnose sturgeon. Transactions of the American Fisheries Society **138:**927-937.

Kawecki, T.J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters **7:**1225-1241.

Killgore, K.J., J.J. Hoover, S.G. George, B.R. Lewis, C.E. Murphy, and W.E. Lancaster. 2007. Distribution, relative abundance and movements of

pallid sturgeon in the free-flowing Mississippi River. Journal of Applied Ichthyology **23:**476-483.

Kimura, M. and G.H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics **49:**561-576.

Koppelman, J.B., and D.P. Phillipp. 1986. Genetic applications in muskellunge management. Pp 111-121 in G.E. Hall, editor. Managing Muskies. American Fisheries Society, Special publication 15, Bethesda, Maryland.

Mazer, S.J. and J. Damuth. 2001. Nature and causes of variation. Pages 3-15 in C.W. Fox, D.A. Roff and D. J. Fairbairn, editors. Evolutionary ecology. Oxford University Press, New York, New York.

Murphy, C.E., J.J. Hoover, S.G. George, and K.J. Killgore. 2007. Morphometric variation among river sturgeons (*Scaphirhynchus* spp.) of the Middle and Lower Mississippi River. Journal of Applied Ichthyology **23:**354-358.

Lotka, A.J. 1922. Contribution to the energetics of evolution. Proceedings of the National Academy of Sciences **8:**147-151.

Moritz, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology and Evolution **9:**373-375.

Nunney, L. 2001. Population structure. Pages 70-83 in C.W. Fox, D.A. Roff and D. J. Fairbairn, editors. Evolutionary ecology. Oxford University Press, New York, New York.

Power, M., and R.S. McKinley. 1997. Latitudinal variation in lake sturgeon size as related to the thermal opportunity for growth. Transactions of the American Fisheries Society **126:**549-558.

Present, T.M.C., and D.O. Conover. 1992. Physiological basis of latitudinal growth differences in *Menidia menidia*: variation in consumption or efficiency? Functional Ecology **6:**23-31.

Roff, D.A. 1992. The evolution of life histories: Theory and analysis. Chapman and Hall, New York.

Scarnecchia, D.L., B.D. Gordon, J.D. Schooley, L.F. Ryckman, B.J. Scmitz, S.E. Miller, and Y.Lim. 2011. Southern and northern great plains (United States) paddlefish stocks within frameworks of Acipernseriform life history and the metabolic theory of ecology. Reviews in Fisheries Science **19:**279-298.

Schrey, A.W., and E.J. Heist. 2007. Stock structure of pallid sturgeon analyzed with microsatellite loci. Journal of Applied Ichthyology **23:**297-303.

SAS. 2009. Version 9.2. Copyright, SAS Institute, Cary, North Carolina.

Schultz, E.T., K.E. Reynolds and D.O. Conover. 1996. Countergradient variation in growth among newly hatched *Fundulus heteroclitus*: geographic differences revealed by common-environment experiments. Functional Ecology **10:**366-374.

Shaklee, J.B. and K.P. Currens. 2003. Genetic stock identification and risk assessment. Pages 291-328 in E.M. Hallerman, editor. Population

genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Sogard, S.M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science **60:**1129-1157.

Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, Oxford, UK.

Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry **6:**49-59.

Stillwell, R.C., and C.W. Fox. 2009. Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. Oikos **118:**703-712.

USFWS (U.S. Fish and Wildlife Service). 2005. Upper basin pallid sturgeon propagation plan. U.S. Fish and Wildlife Service, Bismarck, North Dakota.

USFWS. 2007. Pallid sturgeon (*Scaphirhynchus albus*) 5-year review summary and evaluation. U.S. Fish and Wildlife Service Billings, Montana.

USFWS. 2008. Pallid sturgeon (*Scaphirhynchus albus*) range-wide stocking and augmentation plan. U.S. Fish and Wildlife Service Billings, Montana.

Waples, R.S. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. American Fisheries Society Symposium **1:**8-27.

Williamson, J.H. 2001. Broodstock management for imperiled and other fishes. Pages 397-482 in G.A. Wedemeyer, editor. Fish hatchery management, 2nd edition. Bethesda, Maryland.

Wright, S. 1951. Genetical structure of populations. Annals of Eugenics **15:**323-354.

Zar, J.H. 2010. Biostatistical analysis, 5th ed. Prentice Hall, Inc., Upper Saddle River, New Jersey.

Table 3-1. Mean recovery rate (%) of chironomids by wet weight (g) added to fishless aquaria at water temperatures from 10 to 30 °C.

Temperature (°C)	Recovery Rate (%)	Standard Error
10	33	0.026
14	32	0.057
18	30	0.016
22	42	0.05
26	23	0.107
30	15	0.019

Table 3-2. Results of repeated measures analysis of variance comparing mean weight, consumption, absolute growth rate (AGR), conversion efficiency (CE), and respiration between basins (upper Missouri versus middle Missouri river juvenile pallid sturgeon; n =12, n = 11 respectively) and water temperature (10 to 30 °C).

Variable	Effect	d.f.	F-value	p
Weight	basin*temperature	5	38.81	< 0.0001
Consumption	basin*temperature	5	2.6	0.0296
AGR	basin*temperature	5	5.3	0.0002
CE	basin*temperature	5	5.76	< 0.0001
Respiration	basin*temperature	5	2.29	0.0475

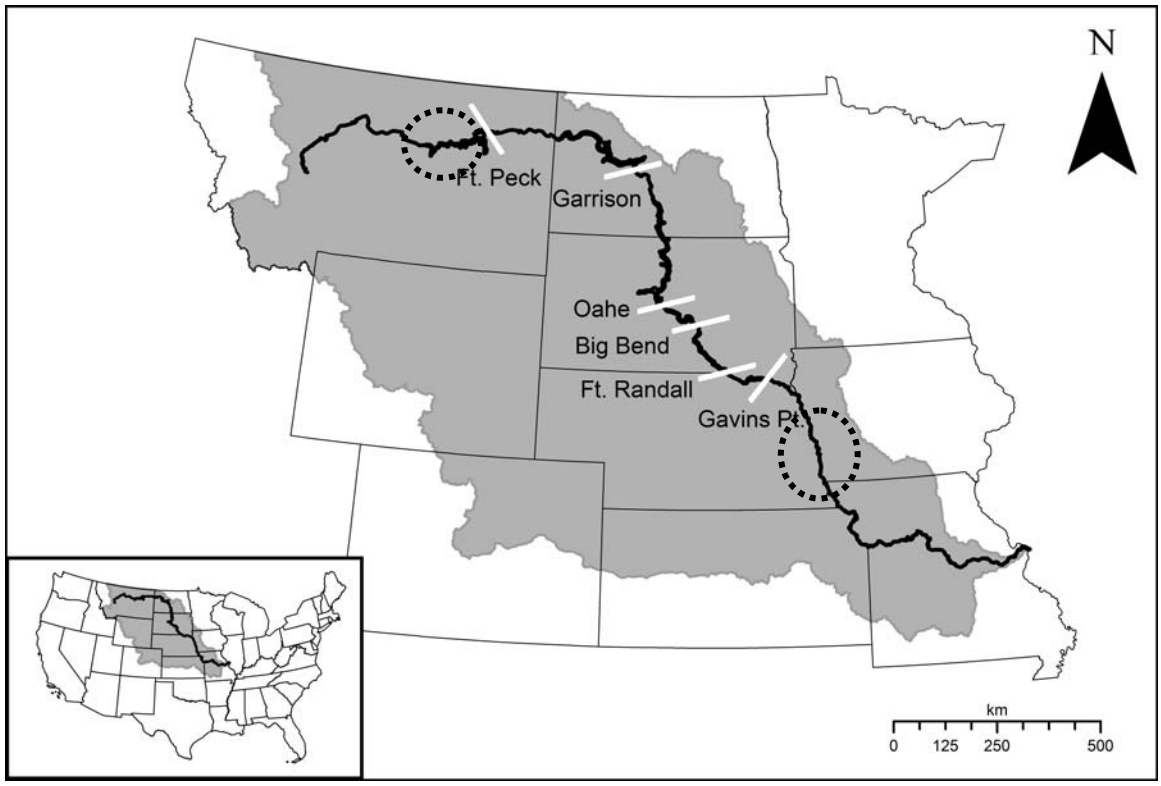

Figure 3-1. The Missouri River (solid black line) from Great Falls, MT to the Missouri-Mississippi River confluence, St. Louis, MO with locations of six mainstem dams (white lines). Dotted lines represent approximate capture locations of upper basin (Montana) and middle basin (Nebraska/Iowa) pallid sturgeon broodstock.

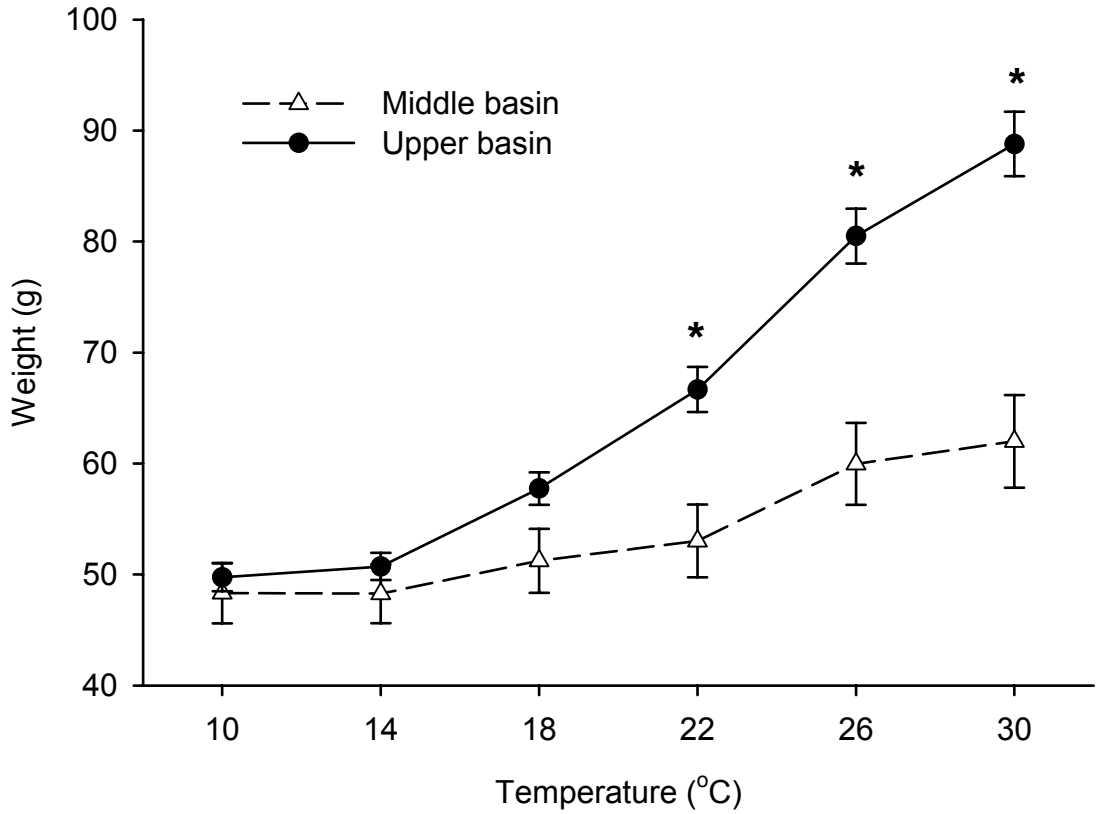

Figure 3-2. Mean weight (g) of middle basin (open triangle) and upper basin (black circle) pallid sturgeon at temperatures from 10-30°C. Mean weight at 14°C was extrapolated from data at 10 and 18°C due to sampling error. Error bars represent 1 S.E and asterisks denote significant post-hoc t-test results.

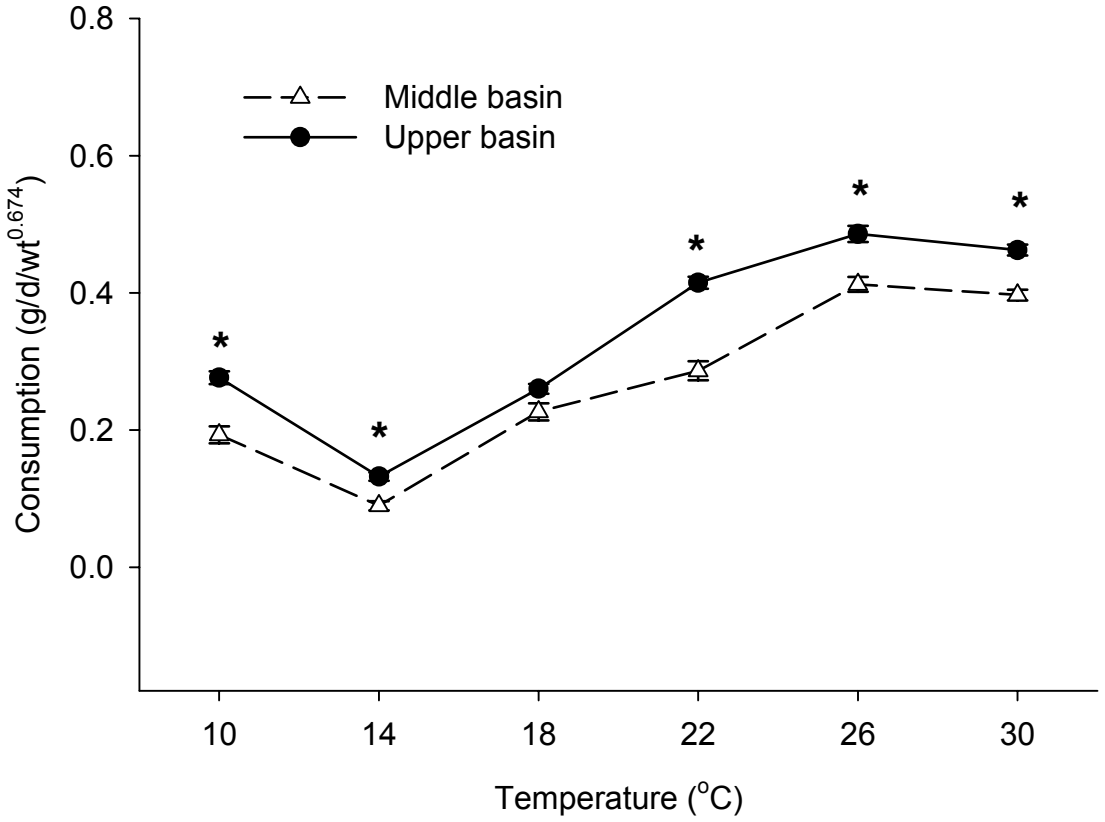

Figure 3-3. Back-transformed mean consumption (g/d/weight$^{0.674}$) of chironomid larvae by middle basin (open triangle) and upper basin (black circle) pallid sturgeon at temperatures from 10-30°C. Mean consumption at 14°C was calculated using extrapolated fish weights. Error bars represent 1 S.E. and asterisk denotes significant post-hoc t-test results.

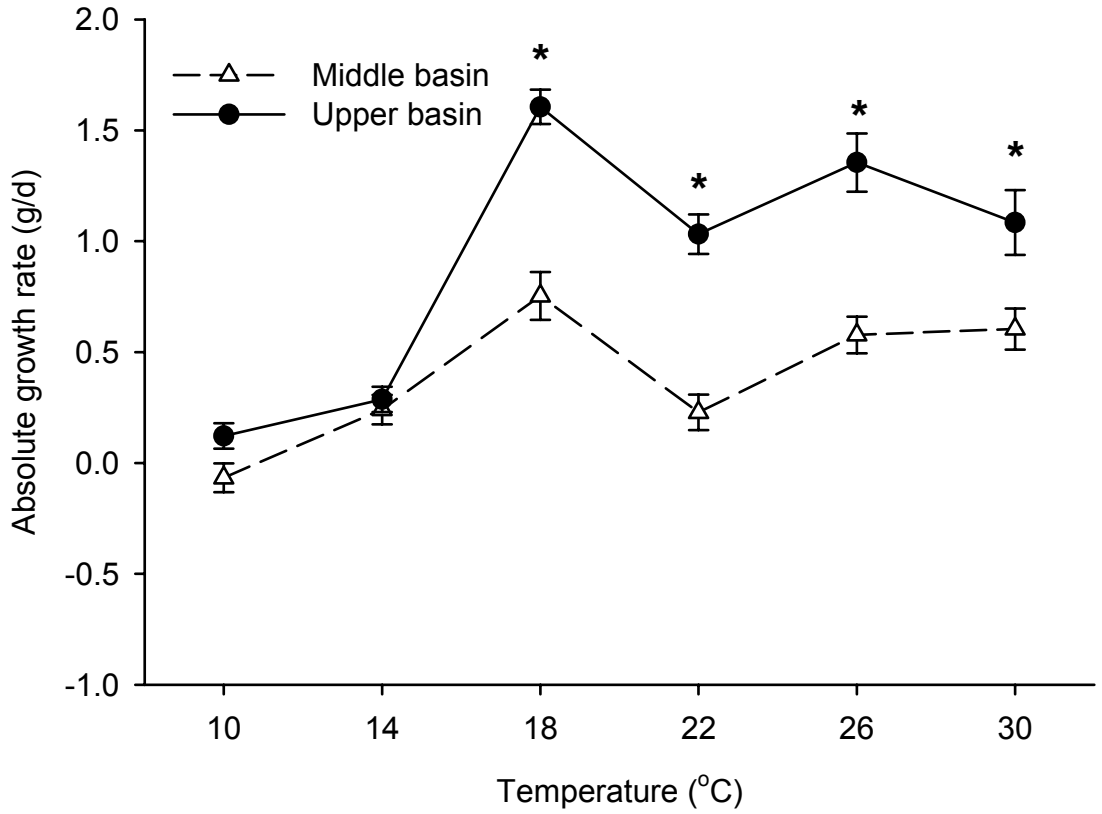

Figure 3-4. Mean absolute growth rate (g/d) of middle basin (open triangle) and upper basin (black circle) pallid sturgeon at temperatures from 10-30°C. Mean absolute growth rate at 14°C was calculated using extrapolated fish weights. Error bars represent 1 S.E. and asterisks denote significant post-hoc t-test results.

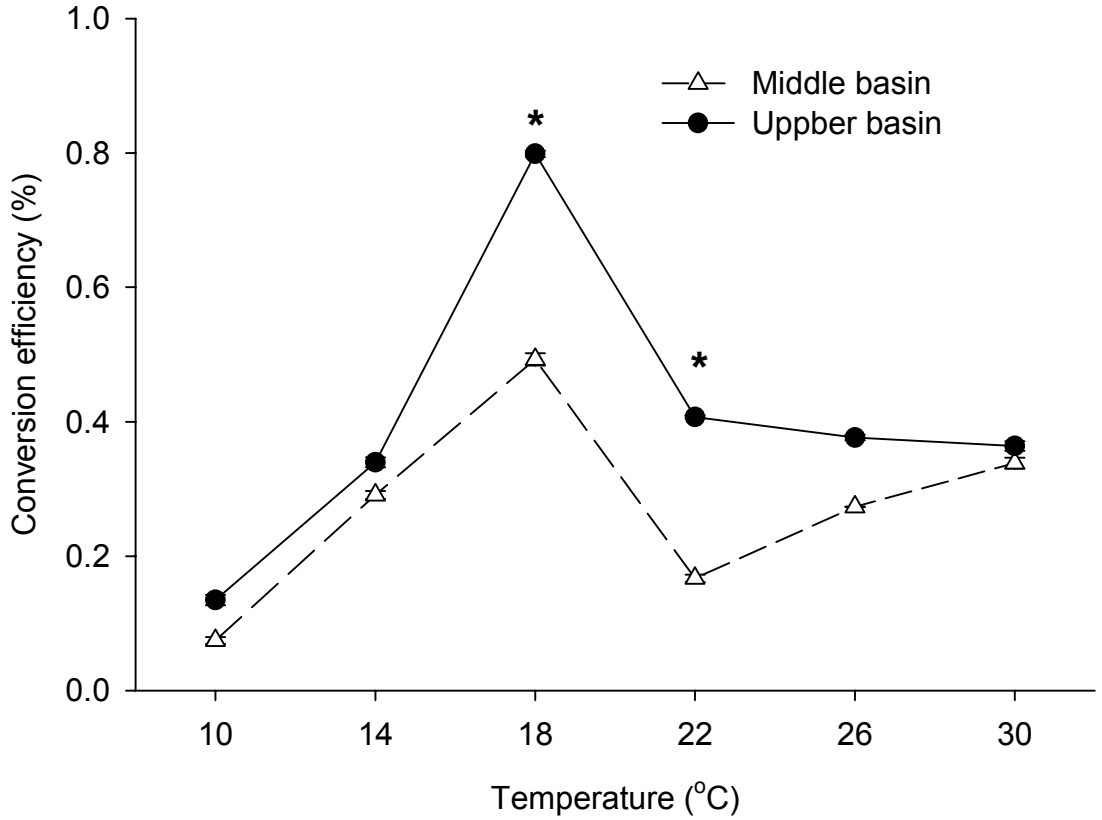

Figure 3-5. Back-transformed conversion efficiency (%) by middle basin (open triangle) and upper basin (black circle) pallid sturgeon at temperatures from 10-30°C. Mean conversion efficiency at 14°C was calculated using extrapolated fish weights. Error bars represent 1 S.E. and asterisks denote significant post-hoc t-test results.

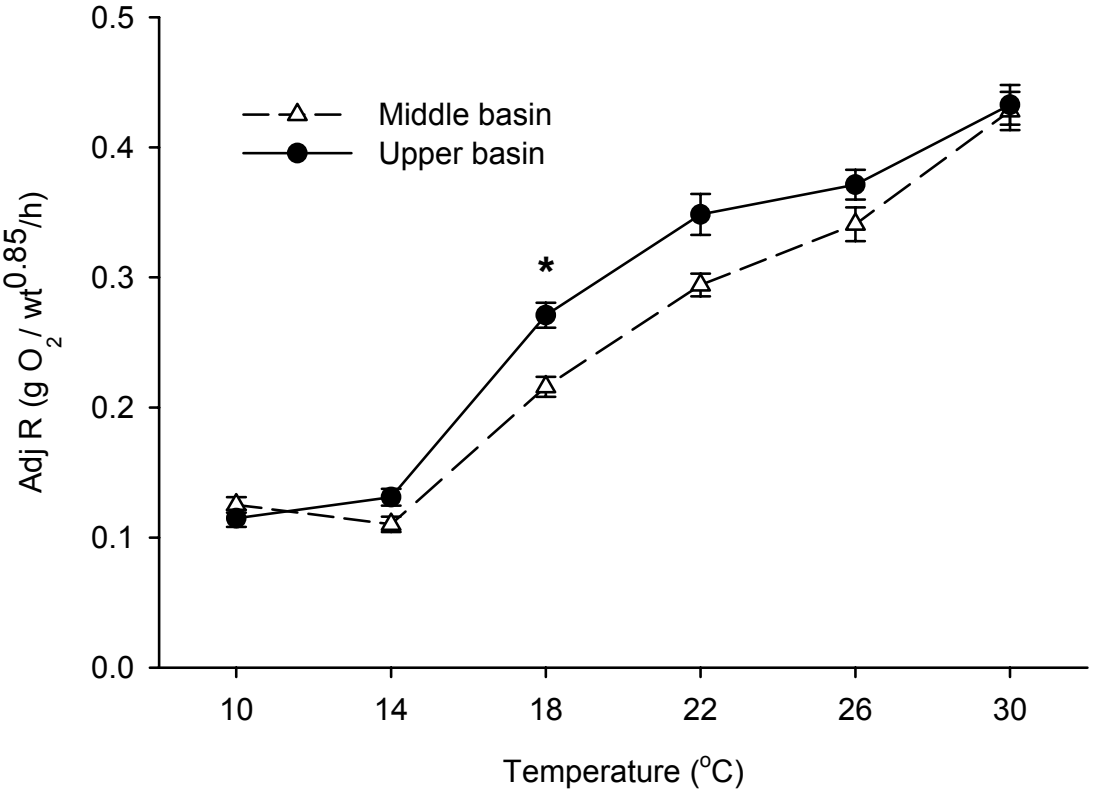

Figure 3-6. Mean adjusted routine metabolic rate (ADJR) of middle basin (open triangle) and upper basin (black circle) pallid sturgeon at temperatures from 10-30°C. Mean adjusted routine metabolic rate at 14°C was calculated using extrapolated fish weights. Error bars represent 1 S.E. and asterisk denotes significant post-hoc t-test results.

CHAPTER 4. SUMMARY AND RESEARCH NEEDS

Summary

My research improved our understanding of the effects of diet on pallid sturgeon physiology, as well as the presence and pattern of local adaptations. I demonstrated that pallid sturgeon fed a chironomid diet grew faster, had higher metabolic rates, lower HSI, and greater energy density than those fed a commercial diet. Moreover, pallid sturgeon on a chironomid diet assimilated energy more efficiently as evidenced by higher CE and whole body ED. Chironomid diets may be more compatible with pallid sturgeon digestive systems, as juvenile pallid sturgeon are known insectivores. Although rearing pallid sturgeon on chironomid larvae may not be economically feasible, it may be advantageous to convert fish from a commercial pellet diet to a chironomid diet shortly (e.g. 5-6 weeks) before stocking. After only five weeks, I observed faster growth of pallid sturgeon feeding on chironomids. Hatchery managers should focus on rearing large healthy fish for release in order to maximize post-stocking survival.

I provided evidence of local adaptations in pallid sturgeon physiology that follow a pattern of CGV. I hypothesized that sturgeon from the more northern latitudes (UB) would grow faster and have higher metabolic rates than fish from more southern latitudes (MB). Upper basin pallid sturgeon grew faster, had higher CE and metabolic rates than MB sturgeon at warm temperatures. My results indicate that UB pallid sturgeon grow more rapidly than MB fish when

reared at similar water temperatures, most likely as an adaptive response to a shorter growing seasons that typically occur in northern latitudes. Evidence of locally adapted populations has important implications for propagation and stocking of pallid sturgeon as a recovery strategy. Managers working towards the recovery of pallid sturgeon must be aware of the pattern and extent of locally adapted populations as a means of conserving genetic diversity.

Research Needs

1) I found that a chironomid diet was superior to a commercial diet for pallid sturgeon. Although this is one of the first diet comparison studies using pallid sturgeon, knowledge of nutrient requirements for pallid sturgeon remains poor. Future diet research should focus on using a variety of invertebrate diets, as well as other commercially prepared diets. Identifying the best available diet(s) for use in pallid sturgeon propagation will benefit hatchery managers by potentially increasing growth rates, survival rates and conversion efficiency.
2) I measured growth, feeding and energy status of pallid sturgeon fed a chironomid diet and a commercial diet. My results showed that the chironomid diet resulted in faster growth and higher CE than the commercial diet. Future diet performance research should focus on measuring swimming performance and liver enzymes to enhance our understanding of how diets may affect physical activity and liver function of pallid sturgeon.

3) Pallid sturgeon were fed a diet of chironomids or a commercial diet for 5 weeks during my study. After just five weeks, I saw marked differences in growth, hepatosomatic index and energy density. I suggest maintaining pallid sturgeon on a commercial diet for 6 weeks (or more) and then converting those fish to a chironomid diet to determine if/when liver condition, growth rates and energy assimilation improves. Hatchery managers would benefit from this information if pallid sturgeon were to be grown out on a commercial diet and converted to an invertebrate diet prior to stocking.
4) Using two genetically distinct groups of pallid sturgeon (UB and MB), I provided evidence supporting the presence of local adaptations consistent with CGV. Future research on the extent of local adaptations should include pallid sturgeon from the southern extent of their range, as fish from the lower Mississippi or Atchafalaya River were not included in this study. This information would enhance our understanding of the pattern and extent of locally adapted pallid sturgeon populations.