



Habitat use and population structure of the shoal chub (*Macrhybopsis hyostoma*) in the upper Mississippi River basin

Sarah Gaughan · Kirk Steffensen · Guoqing Lu

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Abstract Anthropogenic alterations of river systems may have a profound effect on native fish community and habitat use; however, it's difficult to understand the extent of these impacts without establishing well-defined habitat preferences. We investigated the Shoal chub, *Macrhybopsis hyostoma*, a native obligate river species from nine sampling locations in the upper Mississippi River Basin (UMRB). Field surveys demonstrated that overall Shoal chubs preferred tributaries, yet this was statistically significant only for gravid females. Diet analysis and comparative morphology suggested that the Shoal chub is insectivorous and prefer benthic habitats. Our analysis of habitat use suggested that juvenile Shoal chubs preferred sand substrate and adults preferred medium to large gravel. Shoal chubs developed more melanophores as they aged, which is a likely an adaptation to their habitat shifts. The field survey identified possible sites where spawning was occurring and may be important for future conservation efforts for the Shoal chub. In addition, we conducted population genomic analysis of

Shoal chub samples collected from the streams in three Midwest states (Illinois, Missouri, and Nebraska) and found low genetic diversity among the chubs that raises a concern in conservation. This preliminary study provides insights into further investigation of the impact caused by stream habitat alteration on native species and into the conservation of Shoal chubs in the UMRB.

Keywords *Macrhybopsis hyostoma* · Habitat use · Population structure

Introduction

The Mississippi River watershed, including the Missouri River tributary, has undergone dramatic ecological changes in the past century (Hrabik et al. 2015). The Upper Mississippi River Basin (UMRB) is becoming a highly regulated and degraded ecosystem due to human activities such as channelization, the construction of dams, and the removal of natural formations and agricultural discharge (Weitzell et al. 2003). This high degree of the natural hydrological regime in the UMRB may have negatively affected fish habitat use and population structure.

Macrhybopsis chubs are a representative genus of the chub clade consisting of small-bodied fishes that are typically obligate river species in the Mississippi River (Galat et al. 2005). There are twelve species within this genus, and recent studies have determined that this genera is taxonomically complex (Eisenhour 2004; Gilbert et al. 2017). The most morphologically diverse

S. Gaughan · G. Lu (✉)
Department of Biology, University of Nebraska Omaha, Omaha,
NE 68182, USA
e-mail: glu3@unomaha.edu

S. Gaughan
Nebraska Cooperative Fish and Wildlife Research Unit and
School of Natural Resources, University of Nebraska, Lincoln, NE
68583, USA

K. Steffensen
Nebraska Game and Parks Commission, Lincoln, NE 68503, USA

fish in this genus is the Shoal chub. Shoal chubs are very small minnows with a streamlined body that's dorso-ventrally flattened and a rounded snout overhanging the subterminal mouth. They have small upward gazing eyes with smooth scales and a complete lateral line. The caudal fin possesses a white line along the ventral margin (Fig. 1). Populations in the east of the Mississippi River appear to show little variation, in marked contrast to those in western drainages where a large percentage of individuals possess a secondary pair of maxillary barbels (Eisenhour 2004; Gilbert et al. 2017). In the Arkansas and Red River Basins members of this genus have demonstrated introgressive hybridization with other members of the genus (particularly between *M. tetranema* and *M. hyostoma*) which may explain some of the morphological variation. This variation is also hypothesized to be a result of a combination of pre-Pleistocene evolutionary processes, together with subsequent long-term instability and changes in stream-drainage patterns and flow regimes related to periodic advances and retreats of the Pleistocene ice sheets (Mayden 1985; Wiley and Mayden 1985; Cross et al. 1986; Gilbert et al. 2017). This study focused on the upper Mississippi River Basin (Nebraska, Illinois, and Missouri) in order to prevent including hybrids in the analysis.

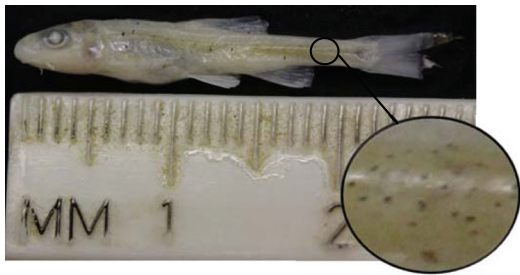
Within the upper Mississippi River Basin there are only four species of *Macrhybopsis* chubs including, the Shoal chub, *M. hyostoma*, the Sturgeon chub, *M. gelida*, the Sicklefing chub, *M. meeki*, and the Silver chub, *M. storeriana*. Recently, population reductions exceeding 70% for all *Macrhybopsis* chubs have been observed within the upper Mississippi River Basin (Dynesius and Nilsson 1994; Hesse 1994; Steffensen et al. 2014). Two other *Macrhybopsis* species, the Sturgeon chub, *M. gelida*, and the Sicklefing chub, *M. meeki*, have been listed as threatened or endangered throughout much of their historical range (Rahel and Thel 2004) and are currently petitioned to be listed federally endangered. The Silver chub is currently listed as vulnerable in the upper Mississippi River Basin, and is considered a species of Special Concern throughout parts of Canada (Hesse 1994; Mandrak and Holm 2001; Steffensen et al. 2014). The construction of six dams and reservoirs on the mainstem river converted riverine habitat to lentic systems which has been hypothesized as a potential cause for the dramatic population reductions south of Gavins Point Dam (Service 2001). Population declines in this region may suggest these populations are under

strong selection pressures and warrant conservation concern as *Macrhybopsis* chubs serve as key food chain species during the juvenile and adult stages for the endangered pallid sturgeon *Scaphirhynchus albus*, particularly the Shoal chub (Gerrity et al. 2006; Herman et al. 2008).

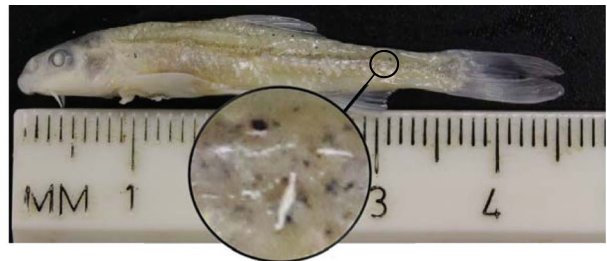
One of the major impediments to conservation efforts has been determining habitat preferences. Previous studies have described a wide variety of habitats that *Macrhybopsis* chubs can utilize ranging from sandy substrate with clear water with moderate currents (Klutho 1983; Luttrell et al. 2002) to deep turbid water with gravel substrate (Starrett 1950; Jones 1997; Eisenhour 2004; Rahel and Thel 2004). Some of the variation in this literature may be due to these preferences changing in relation to age or gender (Starrett 1950; Jones 1997; Eisenhour 2004; Rahel and Thel 2004). The broad continuum of habitats described in the literature makes identifying critical habitats difficult as well as targeting particularly susceptible populations of these species (Galat et al. 2005). There is an accepted ecological premise that an organism's habitat provides the template for trait adaptation and over time these organisms evolve under these habitat parameters (Southwood 1977; Townsend and Hildrew 1994). Based on this premise ecologists can use morphological characteristics to infer what types of habitats an organism would utilize (Fulton et al. 2001; Irschick et al. 2005; Collar et al. 2010; Colombo et al. 2016). In order to prevent continuing population declines it is imperative to refine key life history parameters for all *Macrhybopsis* chubs within the upper Mississippi River Basin, and furthermore, it is particularly crucial to understand how the Shoal chub utilizes particular habitats at different life stages to restore historic population level and because they are a key dietary component of the endangered pallid sturgeon.

The other major impediment for conservation efforts is determining a species' intrinsic genetic resources. Data from previous studies have demonstrated that populations with reduced genetic diversity often experience reduced growth and increased extinction rates (Keller and Waller 2002). Many genera of native upper Mississippi River Basin fishes, including *Macrhybopsis* chubs, may possess limited genetic resources due to historic glaciation events or adaptation to historical river conditions, such as those demonstrated in other North American fish (Harris and Taylor 2010; Hrabik et al. 2015). Cost and technological limitations

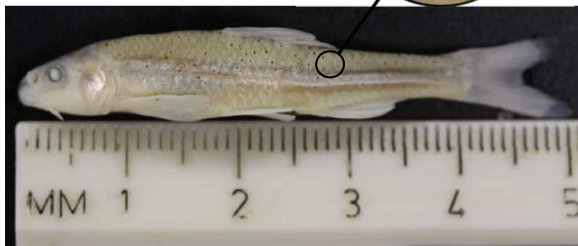
a. Juvenile



b. Male



c. Female



d. Gravid Female

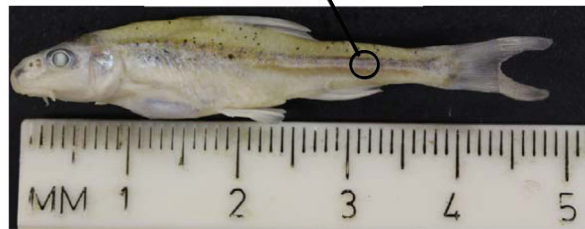


Fig. 1 Shoal chubs at different life-history stages. a. Juvenile collected from Pool 20, Mississippi River, Iowa side north of the Des Moines River confluence (Site I); b. Two year old male collected from Segment 8 of the Missouri River (Site D); c. Two year old female collected from Segment 9 of the Missouri River (Site E);

d. Gravid female collected from the Loup River (Site C). Each circle represents a portion of the specimen's skin magnified 40x so the melanophores can be clearly seen. All adult Shoal chubs, regardless of sex, possess more melanophores than juveniles and their melanophores are larger

have historically restricted these kinds of assessments, however, such evolutionary consequences can now be addressed at a nuclear genome scale with the advent and advances of next-generation sequencing technology (Luikart et al. 2003; Li et al. 2008; Hohenlohe et al. 2010). Genotyping by sequencing (GBS) is a highly multiplexed, low cost system which requires less handling, fewer PCR and purification steps to generate large numbers of single nucleotide polymorphisms for population studies (Davey and Blaxter 2010; He et al. 2014) which quantifies the degree of genetic diversity within the population.

This study attempts to describe habitat preferences for the Shoal chub using morphological and dietary analysis and explore the population structure of the Shoal chub throughout the upper part of the Mississippi River to determine the number of populations that are present and determine the amount of genetic diversity. The degree of genetic diversity along with morphological and habitat use studies, may offer new management strategies for the Shoal chub.

Methods

Sampling was conducted from September 2013 through August 2015 at nine sites, five tributary and four mainstem sites, throughout the upper Mississippi River Basin (Table 1) based on historical ranges and previous field experience. A total of 234 Shoal chubs were collected from these nine sites.

Sampling protocol

Water velocity, turbidity, and depth were measured with a water velocity meter (Marsh-McBirney Flo-Mate™, Frederick, MD), a turbidity meter (Hach 2100P Portable Turbidimeter) and a meter stick or boat mount sounder, respectively. Substrate composition samples were collected using a glass jar when the bottom of the sampling site could be reached, or by pipe dredge for water depths exceeding 1.5 m. Particles were classified according to the Wentworth scale (Wentworth 1922).

The type of gear used to collect fish was dictated by accessibility and depth. A 3.66 m wall seine with 6-mm mesh was used to sample the Elkhorn and Loup Rivers, Sites B and C respectively, because sites B and C were shallow enough to allow wading. A benthic 4.9-m otter trawl was actively towed downstream at Sites A, D, and E following the protocols of the Pallid Sturgeon Population Assessment Program by the U.S. Fish and Wildlife Service, Missouri River Fish and Wildlife Conservation Office (Bismarck, ND) and the Nebraska Game and Parks Commission (Welker and Drobish 2010; Welker and Drobish 2011; Steffensen et al. 2014). A bottom trawl was used to sample Sites E-I by the Missouri Department of Conservation, Southeast Regional Office and Jim Lamer at the Kibbe Field Station (Jim Lamer pers. comm.). This bottom trawl consisted of two-seam, 4.8-m slingshot balloon trawls (TRL16BC, Memphis Net and Twine Co., Inc., or the equivalent). The body of the trawl was made of No. 9 nylon with stretch mesh 18 mm in diameter. The cod end was made of No. 18 nylon with stretch mesh 18 mm in diameter. The cod end contained a 1.8-m liner consisting of 3 mm Ace-type nylon mesh. Floats were spaced every 0.91 m along the headrope, and a 4.8-mm steel chain was tied to the footrope. The trawl was equipped with 37-cm-high by 75-cm-long iron “V” doors (otter boards) (Bartels et al. 2003, 2004). Specimens were preserved in 100% ethanol solution for further investigations.

Relative abundance

Catch per unit effort was used to assess the habitat usage. Catch per unit effort was calculated as the total number of fish in relation to the area (length of the gear \times number of meters trawled) at each collection site (Hahn et al. 2007; Welker and Drobish 2010). These catch per unit efforts were then standardized using multigear mean standardization (Gibson-Reinemer et al. 2016). Mean standardized catch of species (i)(j) ($MSC_{ij} = \frac{C_{ij}}{\bar{C}_j}$) / $\left(\frac{\bar{C}_j}{e}\right)$ where (C_{ij}/e) is the CPUE of species (i) in observation (j) and \bar{C}_j/e is the mean total catch per unit effort (Gibson-Reinemer et al. 2016). Chi square analysis was used to determine the statistical significance for standardized catch per unit effort in relation to system type and substrate type.

Morphological analysis

Each specimen was photographed with a Canon EOS Rebel SL1 digital camera with a Canon EFS 60 mm f/2.8 Macro USM. Each melanophore on the lateral side of each specimen was visually counted and measured using GIMP (Gimp 2008). The number of melanophores were visually counted and measured with a microscopic scale. To ensure accuracy, the total number of melanophores was counted until the same number was acquired three separate times for each fish. Melanophores were classified as small (smaller than 0.15 mm in diameter), medium (between 0.15–1.5 mm) or large (greater than 1.5 mm).

Fish were aged to determine how morphological features were affected by age class. Six cycloid scales were removed between the lateral line and the dorsal fin and then placed on a clear plastic slide with ridges down. Each slide was sandwiched between two more pieces of plastic and run through a roller press. Age was determined by counting the number of annuli as described by Schneider (Schneider 2000). Life stage was assigned to each specimen using the scale given in Table 2.

Gut contents are a significant indicator of habitat resource use (Starrett 1950). Gut contents were visually identified to a genus level to determine the individual components of each species' diet using reference texts (Merritt and Cummins 1978; Wiggins 1977; Borror et al. 1989). The frequency of occurrence for each prey item was calculated as the number of stomachs in which each item occurs and expressed as a total number of stomachs examined using the following equation:

Frequency of Occurrence (O_i) = J_i/P , where J_i is number of fish containing prey i and P is the number of fish with food in their stomach. The frequency of occurrence (%F) of each dietary item provides the most robust and interpretable measure of diet composition (Baker et al. 2014).

The gender of each specimen was determined by macroscopic examination of the gonads under a light microscope at 10x magnification. The gonadosomatic index was used to evaluate sampling sites as potential breeding grounds. Eggs were removed using forceps and individually counted under a light microscope. Each gravid female was weighed on a digital scale before and after egg removal and egg weight was calculated from subtracting the fish's weight without eggs from the total fish's weight, including eggs. The gonadosomatic index was calculated by dividing the

Table 1 Sampling sites throughout the upper Mississippi River Basin, with detailed location names, geographic coordinates and habitat parameters for each collection site

Site ID	Location name	Geographic coordinates	System type	Water depth (m)	Water velocity (m/s)	Turbidity (NTU)	Water temperature (°F)	Substrate type
A	Missouri River, directly below confluence with the Yellowstone River	47° 58' 39.612"N 103° 59' 5.82"W	Mainstem	0.98	0.58	333.61	60.93	Sand
B	Elkhorn River (Davis Site)	41° 12' 01.9"N 96° 17' 57.3"W	Tributary	1.05	0.42	46.35	76	Medium Gravel
C	Loup River (Pawnee Park, Columbus, NE)	41° 25' 02.9"N 97° 22' 11.6"W	Tributary	1.04	0.38	127	81.8	Medium Gravel
D	Missouri River (Segment 8)	42° 19' 07.8"N 96° 22' 41.6"W	Mainstem	2.33	0.57	63.43	71.52	Medium-Large Gravel
E	Missouri River (Segment 9)	40° 59' 55.3"N 95° 51' 53.9"W	Mainstem	2.69	0.50	141.11	70.34	Medium-Large Gravel
F	Cape Girardeau, MO-Marquette Island	37° 16' 48.76" N 89° 31' 13.43" W	Tributary	2.69	0.46	190	72	Sand
G	Cape Girardeau, MO-Marquette Island Side Channel	37° 16' 57.56" N 89° 30' 52.16 W	Tributary	2.69	0.46	190	71	Sand
H	Cape Girardeau, Picyune Chute	37° 19' 47.32"N 89° 28' 44.87"W	Tributary	4.57	0.35	132	71	Sand
I	Pool 20, Mississippi River, Iowa side North of the Des Moines River confluence	40° 23' 00.79" N 91° 24' 49.89"W	Mainstem	7.62	0.50	19 cm	68	Sand

weight of the eggs by the total weight of the fish (Devlaming et al. 1982; Hassanin et al. 2002).

Melanophore analyses

To better predict how total melanophore abundance fluctuates in relation to age and environmental variables, fourteen linear models were constructed which explored the effects of total length, sex, age class, turbidity, current velocity, water temperature and water depth with the total number of melanophores that were present, including a global model with all the covariates. Models were constructed based on the four continuous covariates for evidence of collinearity with pairwise Pearson's correlation coefficients. If two variables had $|r| \geq 0.75$ they were not included in the same model. The total number of melanophores, number of small melanophores, number of medium melanophores and the number of large melanophores were logarithmically transformed based on the pairs plot. Total length was standardized using the formula: $\text{standardized total length} = (\text{total length} - \text{mean total length}) / (\text{standard deviation of the total length})$. AICc and calculated Akaike weights were used to compare models and determine the optimal model. All analyses were carried out in R version 3.1.3 (Team 2014). Non-linear models were run using the package *mgcv* (Wood and Wood 2015). Graphs were created using *ggplot2* (Wickham et al. 2013). Regression analysis was conducted using the package *rpart* (Therneau et al. 2010).

Table 2 Life stages of the Shoal chub with corresponding characters

Life stage	Description
Juvenile	Total length was less than 37 mm, corresponded to a year class of 0 and their reproductive structures were too immature to visually identify.
Adult	Total length exceeded 37 mm, corresponded to a year class of 1+ and their reproductive structures could be visually identified.
Male	Testis reddish-white, however, no milt produced under pressure. Testis occupies about 2/3rds of ventral cavity.
Female	Ovary has a reddish tint. Eggs are clearly discernible, opaque. Ovary occupies about 2/3rds of ventral cavity.
Gravid female	Sexual organs fill ventral cavity. Eggs completely round, some already translucent and ripe.

Population genetic structure

Nuclear genomic DNA of forty-eight Shoal chubs were extracted and purified from fin tissue using the Qiagen DNeasy Blood and Tissue Kit for Genotyping by Sequencing (GBS). PCR free libraries were constructed with a custom Illumina protocol by performing a double digest of 100 ng DNA with PstI-HF and MspI. The sheared DNA was isolated with magnetic beads and re-quantified. The barcoded libraries were constructed from 1 ng of DNA and sequenced on an Illumina NextSeq500 at the USGS Leetown Science Facility. Four libraries with twelve individuals from Sites (C, D, G and I; Loup, Missouri River-Segment 8, MO-Marquette Island Side Channel, IL respectively) per library were constructed. Pooled individuals were identified with unique 9-bp barcodes. All specimens used for genetic analysis are part of the ichthyology collection at the University of Nebraska State Museum (Z-2019-02).

Reads were trimmed and aligned using CLC Genomics Workbench (CLC bio) where only one ambiguous base was allowed. Before trimming, quality scores are converted to an error probability ($p = 10^{-(Q/10)}$, Q is quality score) and during trimming the error rate had to be smaller 0.03 to maintain high quality within the reads. Reads that were shorter than 40 bp were discarded. Over 65% of all the reads had a Phred score of 35 and over 50% of the total reads had a GC content between 40 and 50%.

Single nucleotide polymorphisms (SNPs) were identified using the *Stacks* software, which utilizes a maximum likelihood statistical model to identify loci de novo (Catchen et al. 2011). The *Populations* program within *Stacks* was used to calculate population genetic statistics, including genetic diversity, heterozygosity and F_{ST} .

Three locations were selected to determine the genetic structure of the Shoal chub within the upper MRB. One location, from Segment 8 of the Missouri River, had to be removed due to a low number of reads following sequencing. The first population contained eleven samples from the sample site in Illinois (Site I), the second population contained twelve specimens from the sample site from the Loup River in Nebraska (Site C) and the third population was defined as twelve specimens collected in Missouri (Site G). A total of 2,696,647 reads from thirty-five specimens were used for downstream analysis. All raw reads were deposited at the National Center for Biotechnology Information (BioProject ID PRJNA516905).

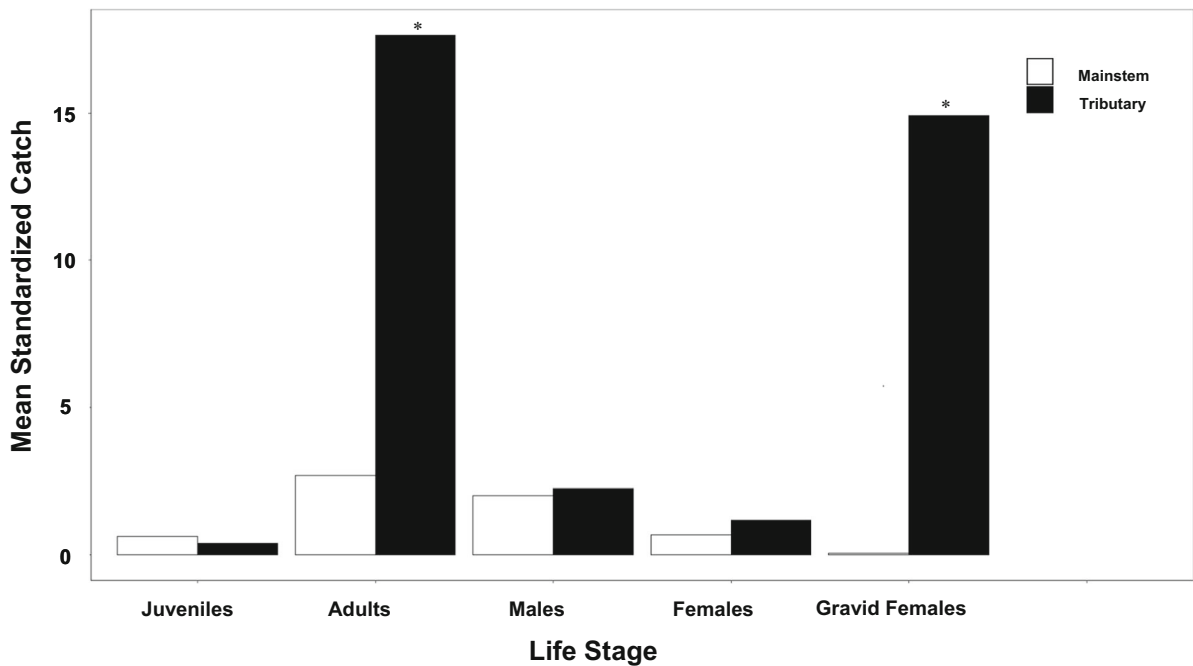
Table 3 Mean standardized catch for each Shoal chub life stage at each site. Detailed information about each site is available in Table 1

	Missouri River, directly below confluence with the Yellowstone River (Site A)	Elkhorn River (Davis Site) (Site B)	Loup River (Site C)	Missouri River (Segment 8) (Site D)	Missouri River (Segment 9) (Site E)	Marquette Island (Site F)	Marquette Island Side Channel (Site G)	Picayune Chute (Site H)	Pool 20 (Site I)
Juveniles	0	0	0	0	0	0	0	0.38	0.61
Adults	0	2.19	17.64	1.08	2.69	0.01	0.14	0	0
Males	0	0.73	2.26	0.75	2.02	0.01	0.12	0	0
Females	0	1.17	0.45	0.28	0.67	0	0.02	0	0
Gravid Females	0	0.29	14.93	0.05	0	0	0	0	0

The POPULATIONS program in *Stacks* was used to analyse the organization of the populations using multilocus genotypic information using output SNP data from across all GBS sites into a STRUCTURE-format file (Pritchard et al. 2000; Hubisz et al. 2009; Catchen et al. 2013). Due to computational limitations of handling many more than this number of loci in the current STRUCTURE application, we implemented a custom

Perl script to randomly choose 10,000 of these SNPs. STRUCTURE 2.3.4 (Pritchard et al. 2000; Hubisz et al. 2009; Catchen et al. 2013) was used to infer historical lineages through clustering of similar genotypes. The admixture model of STRUCTURE and the option of correlated allele frequencies between populations were used. For the entire population set K ranged from 1 to 3. The optimal K was determined using the deltaK method

Mean Standardized Catch of Shoal chubs at Various Life Stages in Relation to SystemType



Statistically significant preferences are denoted by *.

Fig. 2 The mean standardized catch of Shoal (MSCS) chubs at various life-history stages in relation to the system types. See Table 1 for system delineations and locations. Asterisk denotes significant differences

and visual inspection of the change in the $\ln P(D)$ of each model (Evanno et al. 2005). A burn-in of 100,000 steps followed by 1,000,000 additional Markov Chain Monte Carlo iterations were performed.

This same set of 10,000 SNPs from 251 nuclear loci created by *Stacks* was downloaded into *GenoDive* which calculated pairwise F_{ST} values for all population pairs (Meirmans and Van Tienderen 2004; Meirmans 2009). This was accompanied by 1000 randomization tests to determine if each F_{ST} value is different from zero utilizing a strict Bonferroni correction due to the multiple comparisons (Rice 1989; Catchen et al. 2013).

Results

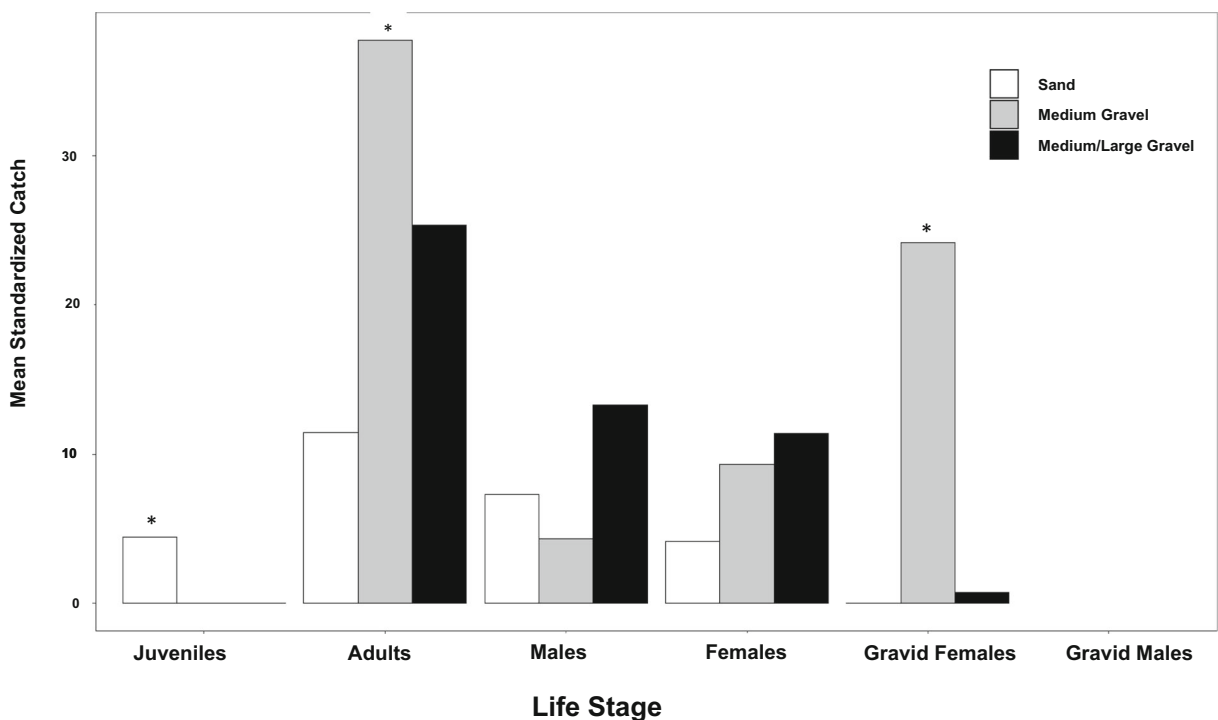
Overall, Shoal chubs preferred tributaries (p value = 0.002), particularly those with moderate current velocities (0.38–0.57 m/s) and relatively shallow water depth (1.04–2.69 m). The total lengths of specimens ranged from 27 to 57 mm. The site with the highest relative

abundance was the Loup River (MSC 17.65, Table 3). Although Shoal chubs preferred tributaries, different life stages had varying habitat preferences, particularly substrate preferences.

The total lengths of juvenile Shoal chubs were less than 37 mm (Table 2). The area with the highest mean standard catch (MSC) of juvenile Shoal chubs was Pool 20 of the Mississippi River in Illinois (MSC 0.61, Table 3). Overall, juvenile Shoal chubs did not demonstrate a statistically significant system preference ($p = 0.210$) (Fig. 2). Regardless of system type, however, juvenile Shoal chubs demonstrated a statistically significant preference for sand substrate ($p = 0.003$) (Fig. 3).

Specimens were considered ‘adults’ if their total length exceeded 37 mm, their age corresponded to a year class 1+ and had reproductive organs that could be visually identified as male or female (Table 2). Overall adult Shoal chubs preferred tributaries ($p = 0.001$). This preference may be skewed by gravid female’s significant preference for tributaries ($p = 0.010$) (Fig. 2). Neither adult males nor females demonstrated a

Mean Standardized Catch of Shoal chubs at Various Life Stages in Relation to Substrate Type



Statistically significant preferences are denoted by *.

Fig. 3 The mean standardized catch of Shoal chubs at various life stages in relation to substrate type. Asterisk denotes significant differences

significant preference for system type when their life stages were solely considered. All adult Shoal chubs preferred medium gravel significantly more ($p < 0.001$) (Fig. 3). Gravid females had total lengths ranging from 45 to 56 mm. The locations with the highest relative abundance of gravid female Shoal chubs (~80%) were collected in early June from the Loup River (MSC 17.65), suggesting Shoal chubs may use this area as a spawning site. Gravid females were also found in the Elkhorn in late September but only had a gonadosomatic index of 6–7%, indicating the specimens were collected at the end of the spawning season or display a bimodal or multi-modal spawning cycle. One other gravid female was collected from Segment 8 of the Missouri River in early July; however, it also had a relatively low gonadosomatic index (9%.) This may indicate that this portion of the mainstem river was unsuitable for spawning or spawning had already completed.

Chi square analysis suggested that adults preferred larger substrate than juveniles. The best linear model based on Akaike information criterion that explored the effects of total length, sex, age class, turbidity, current velocity, temperature and depth with the total number of melanophores that were present determined that there was an additive effect amongst total length and age

classes in relation to total melanophores present, AICc 267.60 (Table 4). The older Shoal chubs had more melanophores and a greater number of larger melanophores.

Shoal chubs ate primarily small dietary items, namely *Chironomidae* larvae (90%), regardless of age or collection site. The rest of the Shoal chub’s diet consisted of various dipteran body parts consisted of the remaining 10%. These body parts were too small for further identification.

The Shoal chub’s recent population declines, and dietary status make it an ideal representative of this genus to begin to explore population structure. Next generation sequencing yielded 105,433,437 nuclear sequences. The sequence lengths varied from 31 to 142 bp with a GC content of 42%. A total of 109,108,396 remained after CLC Genomics Workbench preliminary processed and trimmed the total number of reads. Over 50% of the total reads had a GC content between 40 and 50%. Ustacks utilized a total of 2,696,647 sequences to form 122,382 stacks. The *Stacks* program utilizes short-read sequences to assign identical short read sequences to a unique stack which is equivalent to a nuclear locus. The mean merged coverage depth was 21.2 and the maximum number of nuclear sequences present in a stack was 14,576. The minimum depth of coverage used

length where $STL = (\text{total length} - \text{mean total length}) / (\text{standard deviation of the total length})$

Table 4 General linear models used to explore how the total number of melanophores vary in relation to various life stages and environmental variables STL represents a standardized total

Model	AICc	k	Deltas	Weights
Total Number of Melanophores ~ Total Length + Age Class	267.60	4	0.00	1.00
Total Number of Melanophores ~ Total Length * Age Class	267.66	5	0.06	0.97
Total Number of Melanophores ~ Total Length + Age Class + Sex	270.72	7	3.12	0.21
Total Number of Melanophores ~ Total Length + Age Class + Sex + Turbidity + Current Velocity	271.66	9	4.06	0.13
Total Number of Melanophores ~ Total Length + Age Class + Sex + Turbidity	272.34	8	4.74	0.09
Total Number of Melanophores ~ Total Length * Age Class + Sex + Turbidity + Current Velocity + Water Depth + Substrate Type	275.63	14	8.03	0.02
Total Number of Melanophores ~ Total Length + Age Class + Sex + Turbidity + Current Velocity + Substrate Type	276.58	12	8.99	0.01
Total Number of Melanophores ~ Substrate Type	321.09	5	53.49	0.00
Total Number of Melanophores ~ Sex	328.96	5	61.36	0.00
Total Number of Melanophores ~ Age Class	337.39	3	69.79	0.00
Total Number of Melanophores ~ Water Depth	339.97	3	72.37	0.00
Total Number of Melanophores ~ Total Length	372.18	3	104.58	0.00
Total Number of Melanophores ~ Turbidity	377.76	3	110.16	0.00
Total Number of Melanophores ~ Current Velocity	377.84	3	110.24	0.00
Total Number of Melanophores ~ 1	391.70	2	124.10	0.00

Table 5 Summary of nuclear genetic statistics for all sampling sites for the Shoal chub, *M. hyostoma*.

Population	Number of Specimens	Private Sites	% Poly	P	Observed Heterozygosity	Observed Homozygosity	π	F_{IS}	
Variant Nucleotide Positions									
Illinois (Site I)	11	25,196	57,740	–	0.84	0.30	0.70	0.31	0.003
Loup River (Site C)	12	43,488	90,771	–	0.79	0.38	0.62	0.39	0.02
Missouri-Marquette Island Side Channel (Site G)	12	35,793	82,509	–	0.80	0.35	0.65	0.38	0.06
All Nucleotide Positions									
Illinois (Site I)	11	25,196	1.23E+08	0.05	0.999	3.07E-4	0.99	3.09 E-4	2.70E-06
Loup River (Site C)	12	43,488	1.62E+08	0.06	0.999	3.37E-4	0.99	3.49 E-4	2.19E-05
Missouri-Marquette Island Side Channel (Site G)	12	35,793	1.6E+08	0.05	0.999	3.16E-4	0.99	3.46 E-4	5.60E-05

These statistics have been split into polymorphic nucleotide positions (top, 'Variant positions'), as well as all nucleotide positions across all sites regardless of whether they are polymorphic or fixed (bottom, 'All positions'). These statistics include the average number of individuals genotyped at each locus (N), the number of variable sites unique to each population (Private), the number of polymorphic (top) or total (bottom) nucleotide sites across the data set (Sites), percentage of polymorphic loci (% poly), the average frequency of the major allele (P), the average observed heterozygosity per locus, the average observed homozygosity per locus, the average nucleotide diversity (π), and the average Wright's inbreeding coefficient (F_{IS})

to create a stack was three and the maximum distanced allowed between stacks was 2. The standard deviation for the coverage depth was 74.2.

For all loci that were polymorphic in at least one population in the entire data set, the average major allele frequency (P) ranged from 0.79 to 0.84 and the average observed heterozygosity ranged from 0.30 to 0.38. When considering all nucleotide positions, the values increase to 0.999 for the major allele frequency (P), and the observed average heterozygosity decreased to a range from $3.07e^{-4}$ – $3.37e^{-4}$. Genetic diversity is the best method to assess a species' capacity to respond to disturbance. Nucleotide diversity was 0.31, 0.39 and 0.38 with a standard error of 0.001 for all three populations, respectively. This reduction in genetic variation is particularly evident in the percentage of loci that are polymorphic at all loci which was 0.05 at Illinois (Site I) and the Marquette Island Side Channel (Site G) and 0.06 for the Loup River (Site C). The mean inbreeding coefficient (F_{IS}) was 0.003, 0.02 and 0.06 for samples from the site in Illinois (Site I), the Loup River (Site C) and the Marquette Island Side Channel (Site G), respectively (Table 5).

Pairwise comparisons of F_{ST} amongst these three populations reveals exceptionally low F_{ST} values at all populations indicating a high degree of gene flow amongst populations in the upper MRB. Using a numerical randomization approach of 10,000 randomly chosen SNPs the only pairwise F_{ST} values that were significantly different were the Illinois and Loup River populations at the $\alpha = 0.05$ level (Table 6). Although not statistically significant, the pairwise F_{ST} is lower for the Loup River and Missouri Marquette Island Side Channel collection sites (0.001) compared to the pairwise F_{ST} between Illinois and the Missouri Marquette Island Side Channel (0.013) collection sites.

As a further test of potential population structure, we analysed 10,000 randomly chosen SNPs using STRUCTURE. By examining the change in $\ln P(D)$ and using the deltaK approach of Evanno et al. (2005), we found that a model with $K = 2$ best fits the data which was supported by STRUCTURE's plot of posterior probabilities.

Pairwise F_{ST} and STRUCTURE analysis support the presence of two possible populations of Shoal chubs within the upper Mississippi River Basin. One population consists of the Loup River (Site C) and the Missouri Marquette Island Side Channel (Site G) collection sites

and the other population consists of the Illinois (Site I) collection site.

Discussion

This is the first study that demonstrated that Shoal chubs utilize various types of habitats, including different types of substrates, at different life-history stages. Overall, Shoal chubs preferred tributaries, but this was highly affected by the preference of gravid females. Juvenile Shoal chubs were prevalent in areas with sand substrate, and adults preferred medium gravel substrate. In addition, gravid females were more frequently found in sites with medium gravel. Melanophores may be one morphological feature that facilitates these fluctuating habitat preferences and may allow the Shoal chub to take advantage of a variety of habitats, particularly different types of substrate throughout their life history. Age classes and the total lengths of the Shoal chubs have an additive effect on the number of melanophores. As they age, the Shoal chubs can use larger substrate particles. Our results resolved a previous confusion about the habitat parameters described for the Shoal chub that is likely associated with the change of habitat preferences in different life-history stages.

At times large aggregations of individual Shoal chubs were found and such aggregations may serve as a means to avoid predation for juveniles or to maximize reproductive potential (Pitcher and Parrish 1993). One aggregation of juvenile Shoal chubs was collected from Pool 20 of the Mississippi River in Illinois. We found a proportion of the juvenile fish collected from Pool 20 in Illinois (~19%) infected with *Uvulifer* spp. ectoparasite. Previous studies have shown that anthropogenic disturbances such as dams may indirectly facilitate parasitism (Hernandez et al. 2007). Pool 20 had extensive damming which may increase the likelihood of

secondary fish host infection. The reduction of water velocity or the increase of the intermediate snail host as a result of damming could increase the probability that Shoal chubs would be exposed to *Uvulifer* spp.. Consequently, this parasite may increase the mortality of Shoal chubs by depleting their nutritional reserves and increasing the probability of being consumed by a predator or reducing the probability of survival during stressful periods (Barber et al. 2000; Pracheil and Muzzall 2010; Ferguson et al. 2011; Markle et al. 2014). Even a single ectoparasite may increase the mortality for larval or juvenile Shoal chub significantly (Grutter et al. 2010).

Life cycles including spawning migrations or the act of spawning rely on natural flow peaks by bringing reproductive adults together and maximizing habitat conditions for larval fish survival (Thomas 1988; Næsje et al. 1995; Hooper et al. 2005). Water redirection has previously affected native minnows by altering flow peaks and may affect spawning potential for the Shoal chub (O'Connor 2002; District and HDR Engineering 2015). We found a second aggregation of Shoal chubs at the Loup River, where 80% of the fish collected from the Loup River were gravid females. This section of the Loup River is under annual water redirection efforts. The water redirection that occurs along this stretch will lower water levels by as much as 75% which may remove valuable spawning areas or hinder recruitment potential for Shoal chubs.

Population genomic analysis revealed low heterozygosity within northern populations of the Shoal chubs. The low degree of heterozygosity displayed amongst upper Mississippi River Basin Shoal chub populations potentially reduces this species capacity to respond to anthropogenic disturbances, which may be responsible for the recent population reductions (Hoffmann and Hercus 2000). The population declines seen in the Shoal chub may eventually lead to inbreeding which would lower resistance to disease and environmental stress and could exacerbate the potential for extinction (Keller and Waller 2002). Southern populations of Shoal chubs have been observed hybridizing with *M. tetranema*, a southern sister species. These hybridization events may overcome the issues caused by low heterozygosity that may also explain why southern populations of the Shoal chub are not experiencing such dramatic population declines (Underwood et al. 2003). Future genetic studies should investigate the degree of divergence and genetic similarity between northern and

Table 6 Pairwise F_{ST} (lower left) and p -values (upper right) for random 251 nuclear loci for the shoal chub, *M. hyostoma*

	Illinois	Loup	Missouri-Marquette Island Side Channel
Illinois		0.026*	0.054
Loup	0.016		0.391
Missouri-Marquette Island Side Channel	0.013	0.001	

* $p < 0.05$

southern populations and explore how hybridization has impacted southern populations.

This study refined habitat preferences for Shoal chubs at various life-history stages, explored how melanophores may be related to habitat use, and identified two possible sites where large aggregations of Shoal chubs were present. Future studies should focus on the impact of these sites with juvenile survival, particularly in relation to infection rates of *Uvulifer* sp., and how water redirection affects reproductive efforts for this species in the Loup River. Future conservation efforts should focus on minimizing stressful environments, such as those disrupted by anthropogenic disturbances, which will likely decrease mean fitness that may continue to compromise the upper Mississippi River Basin's populations persistence (Kinnison and Hairston 2007; DiBattista et al. 2011).

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Compliance with ethical standards

Disclosure statement The authors report no conflict of interest. The authors are solely responsible for the content and writing of this manuscript.

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