

Project Title: Examining drivers of temporal variation in hybridization outcomes in bigheaded carp in the UMR.

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Location of Activities: UMR, Iowa/Illinois/Missouri

Statement of Need: Bigheaded carp continue increasing abundance and distribution in the Mississippi River Basin (MRB). Hybridization between Silver Carp and Bighead Carp in the MRB was first identified from distinctive hybrid gill raker morphology in 2005 and confirmed shortly after using molecular markers in 2006 (reviewed in Lu et al. 2020). Still, the genetic and ecological mechanisms underlying the extensive hybridization between bigheaded carp only seen outside their native range remain elusive (Lamer et al. 2010, Lamer et al. 2014, Lamer et al. 2019). The production of new genetic combinations through hybrid backcrossing, characteristic of a hybrid swarm, also hinders our ability to assess patterns in introgressive hybridization in the UMR solely relying on the presence or absence of an intermediate feeding morphology (see Lu et al. 2020 for an illustration of the gill raker morphology observed in early hybrids, Marian et al. 1986). Yet, the extent of hybridization (i.e., as the proportion of individuals of the parental species, first-generation hybrids, back-crosses, or later-generation hybrids) varies across environmental contexts (Lamer et al. 2014, Mandeville et al. 2015, Mandeville et al. 2019) and the question whether the extent of bigheaded carp hybridization remains unchanged over time warrants further attention (Lamer et al. 2019).

Hybridization is expected to dampen population growth of closely related species that co-occur in the same habitat and are ecologically distinct, if hybrid individuals contribute fewer offspring to the next generation relative to the individuals of the parental species (i.e., lower hybrid fitness scenario, Irwin and Schluter 2022). Since population declines can be driven solely by lower hybrid performance relative to non-hybridized bigheaded carp individuals, we need to assess the extent to which introgressive hybridization varies across individuals in order to understand recent past and future population dynamics. Past hybridization events can come with associated costs in terms of fitness disadvantage (Lamer et al. 2019) or, conversely, confer new genetic variation, providing invasive species with greater potential for adaptation to new conditions (Barton 2001, Taylor and Larson 2019). Indeed, the invasion success of bigheaded carp could be partly explained by positively selected genomic features associated with environmental adaptation or invasion-related traits only seen in hybrids (Wang et al. 2016, Wang et al. 2020). In addition, Coulter et al. (2020) proposed that the adaptive potential of unique genetic combinations in hybrids may facilitate the establishment and range expansion of two invasive carp species through individual differences in movement patterns. Whether poorly tuned energetic trade-offs between body growth and reproduction only found in hybrids (Lamer et al. 2019) could offset the unique benefits gained by a greater adaptive potential (Wang et al. 2020, Coulter et al. 2020) is still unknown.

To overcome the issue of stealth hybrid backcrosses and later-generation hybrids lacking diagnostic morphologies (Lamer et al. 2010), advances in genomic technologies and associated developments in computing power now offer the opportunity to measure the extent of introgressive hybridization at a population level with an unparalleled degree of genomic resolution. High throughput sequencing technologies provide large numbers of genetic loci to quantify the number of individuals of each of the parental species (over 90% parental species ancestry), and in those hybrids (less than 90% parental species ancestry) assess both the extent of introgression (as the proportion of Bighead Carp ancestry in hybrid Silver Carp or vice versa, i.e., q) and the proportion of loci in an individual's genome that are estimated to have ancestry from both parental species, thus allowing for the distinction of F1 hybrids from later generation hybrid crosses (i.e., interspecific ancestry Q , Gompert et al. 2014).

For this project, we will take advantage of fin and tissue samples to examine the extent of introgressive hybridization across bigheaded carp harvested by commercial anglers and: **1)** assess the extent to which introgressive hybridization varies temporally in the UMR relative to other areas; and **2)** examine potential environmental drivers of temporal variation in hybridization outcomes. We will also rely on genomic data (restriction-site associated DNA or RAD sequencing data) collected by Lamer et al. (2014) which was used for the above-mentioned studies (e.g., Lamer et al. 2019, Coulter et al. 2020) and expand this existing data set with the 2022 samples that we collected by sequencing these using the same methodology to ensure data compatibility between the two studies (Lamer et al. 2014).

An updated estimate of hybridization can inform current demographic models guiding population control strategies across locations in the Mississippi River Basin and help clarify potential links between differential harvest of parental species across locations and hybridization dynamics over time. Quantification of hybridization along with recruitment dynamics may be relevant in explaining declines in the relative abundances of parental species across locations and to screen for potential genomic features found in hybrids that could in turn, facilitate invasion success. For instance, the different prevalence of potentially risky mobile individuals among hybrids, as documented by Coulter et al. (2020, 2022) should be considered in where individuals are harvested and in barrier design. Information on variable individual outcomes of hybridization can be then paired with knowledge on the species-specific responses to acoustic barriers to foresee potential changes in barrier permeability over time (Murchy et al. 2017, Wilson et al. 2021). Moreover, failure to consider potential differences in the ability to tolerate harmful toxins of hybrids relative to the parental species (Rach et al. 2008) could potentially prevent the success of management strategies involving chemical measures.

This research will help guide ongoing efforts to remove invasive carp populations and prevent them from further expanding their geographic range. The results of this research will be made publicly available in different formats specifically designed to reach varied audiences.

Evidence on how hybridization could influence population will inform our understanding on the population growth dynamics of Silver Carp. A clear understanding of all factors influencing population growth may demonstrate the need for further harvest and population control of Silver carp in the UMR. Additionally, this study will update current understanding of hybrid prevalence and drivers of temporal variation in the extent of introgressive hybridization. This knowledge is

key in explaining variability in the invasion success of bigheaded carp across locations in their invaded range.

Project Objectives:

1. Assess the extent to which introgressive hybridization varies temporally in the UMR and examine potential environmental drivers of temporal variation in hybridization outcomes.

Project Highlights:

Recently available genomic resources facilitate unprecedented screening for potential genomic features found in hybrids that could facilitate or hamper invasion success in the MRB. We extracted DNA from 480 bigheaded carp (Silver or Bighead Carp or hybrids; fin clips), achieving DNA quality and yield thresholds for genomic library preparation and the high throughput sequencing needed for genomic screening. We processed and analyzed the resulting whole genome sequencing data using Iowa State University's NOVA high performance computing cluster to quantify the proportion of Bighead Carp ancestry in hybrid Silver Carp or vice versa (i.e., extent of introgressive hybridization or introgression). Continued analysis will provide further quantification of interannual dynamics in hybridization outcomes with the potential to partly explain observed shifts in the relative abundances of bigheaded carps near an invasion front. Examination of lesser-studied mechanisms (e.g., introgressive hybridization) predicted also to influence annual variability in recruitment will inform ongoing population control strategies, including harvest efforts in the Mississippi River Basin.

Methods: We measured total length, body weight, and gonad weight of 505 Silver Carp, 16 Bighead Carp, and 5 hybrids (identified by the presence of twisted gill rakers, external features) caught between October and December 2022 in pools 16-19.

We extracted DNA from fin clips using the Qiagen's DNeasy Blood and Tissue kit (Qiagen GmbH, Hilden, German) at the DNA facility at Iowa State University. Due to initially low DNA yields (<20ng/ul), we used solid-phase reversible immobilization (SPRI; AMPure XP kit, Beckman Coulter) paramagnetic beads for the purification of nucleic acids. We followed the NEBNext Ultra II DNA FS library prep Kit for Illumina (E7805L) using NEBNext Multiplex Oligos for Illumina (E6609S). Samples were then sequenced in One full S4 NovaSeq 6000 150-cycle PE flow cell as individual lanes.

We analyzed the resulting whole genome sequencing data using Iowa State University's NOVA cluster, a High-Performance Computing system. Raw sequencing data involved 150 bp-long reads produced 20,000,000,000 reads for a 7X depth of coverage (mean ~48,000,000 sequences per individual). We subsequently checked sequence quality using FastQC (version 0.11.9) and MultiQC (version 1.19; Ewels et al. 2016) and clipped sequencing adapters using cutadapt (version 5.0; Martin 2011). After conducting standard data processing, we aligned resulting DNA reads to the Bighead Carp genome from GenBank (access number: GCA_019925145.1), which was the bigheaded carp reference genome with the highest quality (i.e., N50: 33,747,976; 32 sequences, average sequence length: 26,782,686) and the lowest number of sequences to meet reference quality standards for downstream analyses. To align sequences to the reference genome, we used the Grene Pipe (Czech and M Exposito-Alonso 2022), and to conduct various

population genomic analyses, we followed the loco-pipeline (Zhou et al. 2024) both of which are bioinformatic analyses integrated within the workflow management system Snakemake (Köster et al. 2012). We explored the correlation between the studentized residuals (our proxy for year class strength) and the extent of introgressive hybridization. We calculated the extent of introgressive hybridization using Ohana admixture analysis (Cheng et al. 2019) as implemented using the loco-pipeline (Zhou et al. 2024), but we plan to implement other available metrics of hybridization (e.g. Entropy). We calculated the studentized residuals for the data subset including only individuals with an inferred early life environment downstream of LD 19 (n=205; see Component 5 for more details on how this was calculated). We executed analyses in R version 4.2.2, (R Core Team 2024) and R Studio (R Studio 2024) using the “tidyverse” environment (Wickham et al. 2019).

Project Activities:

As part of the population genomic analyses, we are exploring our genomic data using a dimensionality-reducing principal components analysis (PCA; **Figure 53**) and a model-based clustering (e.g., admixture analysis Ohana, Cheng et al. 2019; Zhou et al. 2024; and Entropy, Shastri et al. 2021; using custom scripts).

Our genomic ancestry assignment analyses are still ongoing. Preliminary results were presented at the 2024 American Fisheries Society Annual Meeting. Once current analyses are completed, we will contrast our preliminary results with metrics of introgressive hybridization calculated using Entropy with year-class strength (thus also with those environmental variables correlated with yearclass strength, i.e., temperature-related variables; see Component 5).

New allelic combinations resulting from introgressive hybridization could confer greater adaptation potential in new environments if exchanged alleles are associated with ecologically relevant phenotypic traits (e.g., gill raker morphology). If this is the case, hybrid phenotypes could differ in feeding performance due to foraging trade-offs. Hybrids may then have lower performance than either of the parental species at their optimal-sized food particles. If the size-distribution of prey items in a degraded environment is skewed towards intermediate-sized prey items, hybrids with an intermediate gill-raker morphology may outperform either of the parental species in feeding rates. Quantification of this instance of human-mediated hybridization may help refine our understanding of the observed declines of either of the bigheaded carp species across locations and to screen for potential genomic features found in hybrids that facilitate invasion success and adaptation to changing habitats. Because of the interspecific differences that have been documented between Bighead and Silver carp for traits with management relevance such as movement, diet, and reproductive success, understanding hybridization may allow managers to factor the consequences of ongoing hybridization into management strategies.

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