Developing an Invasive carp-based aquaculture feed to benefit Louisiana ecosystems and economies: Assessing carp body size, lipid content, and diet proxies of fish harvested in Louisiana and Illinois.

A Report Submitted to Louisiana Sea Grant

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Abstract: Non-native, Invasive carp pose a threat to Louisiana ecosystems as they can out compete local fisheries species and alter the structure of aquatic food webs. One solution is to increase the use of Invasive carp as feedstock, but this requires assessing the viability and quality of Invasive carp harvested in Louisiana as a protein and oil source for aquaculture feed. To inform this knowledge gap, our study analyzed the size, lipid content (a proxy for nutrient quality), and isotope values of invasive carp fish from sampling sites in Louisiana and Illinois. ANOVA analyses were conducted to measure statistically significant differences of the carp among the Louisiana and Illinois sites. We found differences in Louisiana and Illinois carp relative to body size, isotope values (i.e., diet and trophic level), but not lipid content. Larger Louisiana fish would be advantageous and will yield more aquaculture feed product per fish. However, differences in body size and diets among Invasive carp in Louisiana and Illinois have the potential to influence the bioaccumulation and trophic magnification of contaminants, respectively. Further investigation focused on nutrient and contaminant profiles will allow us to determine if and how body size and diet variation may impact the potential for Invasive carp from Louisiana to be used as aquaculture feed.

Introduction

The term "Invasive Carp" refers to four species of non-native fish that have become established in Louisiana waters: silver carp (*Hypopthalmichthys molitrix*), bighead carp (*H. nobilis*), black carp (*Mylopharyngodon piceus*) and grass carp (*Ctenopharyngodon Idella*; Fig.1). These species pose a threat to Louisiana ecosystems as they can out compete local fisheries species and alter the structure of aquatic food webs (Chick & Pegg 2001). Commercial harvest remains the most cost-effective control method of Invasive carp and may potentially benefit local economies. However, low prices have resulted in limited harvest in Louisiana. While there have been considerable efforts to promote human consumption of invasive carp through both recreational and commercial harvest (e.g., Varble & Secchi et al. 2013, Keevin & Garvey 2019), demand has not increased. This lack of demand means that both harvests and dockside prices remain low and invasive carp remain a threat to Louisiana's aquatic ecosystems and fisheries.

One solution is to increase the nonhuman consumption of Invasive carp as bait or animal feed (Li et al. 2021). For example, the aquaculture industry relies heavily on fishmeal and fish oil (sourced primarily from marine fish species such as menhaden and anchovies) as key ingredients in aquaculture feeds. Under the current rates of industry growth, availability cannot match projected demand, with the industry requiring an additional 37.4 million tons of aquafeed by the year 2025 (Hua et al., 2019). Companies across the United States are exploring the development of alternative aquafeed ingredients. One company, Nobilis Aqua LLC (Fort Collins, CO), has partnered with Dr. Stephanie Archer (Louisiana Universities Marine Consortium LUMCON) and Dr. Abigail Bockus (Bozeman Fish Technology Center) to develop and test a cost-competitive feed which incorporates Invasive carp fishmeal and fish oil into aquaculture feed for Channel Catfish (*Ictalurus punctatus*). However, before feed development or feeding trials can commence a first step will be to determine the viability and quality of Invasive carp harvested in Louisiana as a protein and lipid source for aquaculture feed.

Objectives

The overall objective of this project is to increase the demand for Invasive carp by assessing the viability and quality of Invasive carp harvested in Louisiana as a protein and oil source for aquaculture feed. Specifically, this UROP project component analyzed invasive body size, lipid content, and diet proxies at sites in Illinois and Louisiana as a first step towards feed development and feeding trials.

Methods

Fish collection

Fish were obtained from three sites along the Mississippi River. A site in Beardstown, IL, provides fish from the Upper Mississippi River Basin (Figure 1). Two sites along the Lower Mississippi River Basin (0101 and 0103) provide fish from Louisiana (Figure 2). Samples of white muscle were analyzed from six fish at each of the three study sites in each of four seasons (spring, summer, fall, winter).

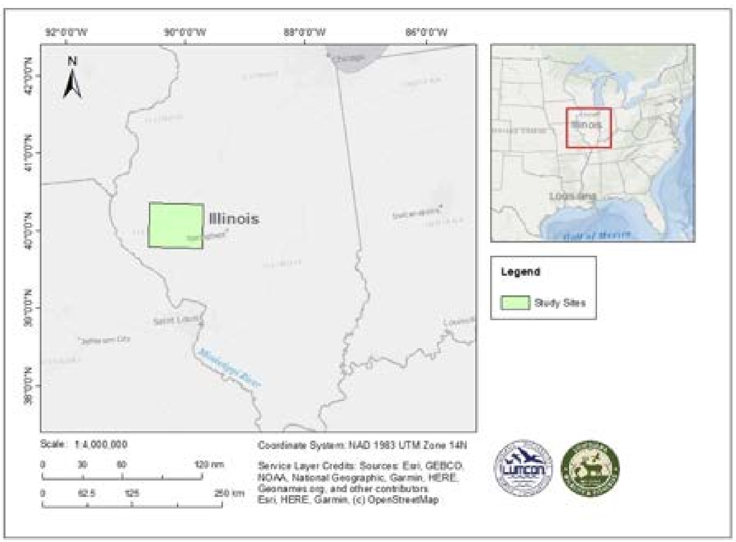

Figure 1. Location of the fish sample collection location near Beardstown, IL, USA.

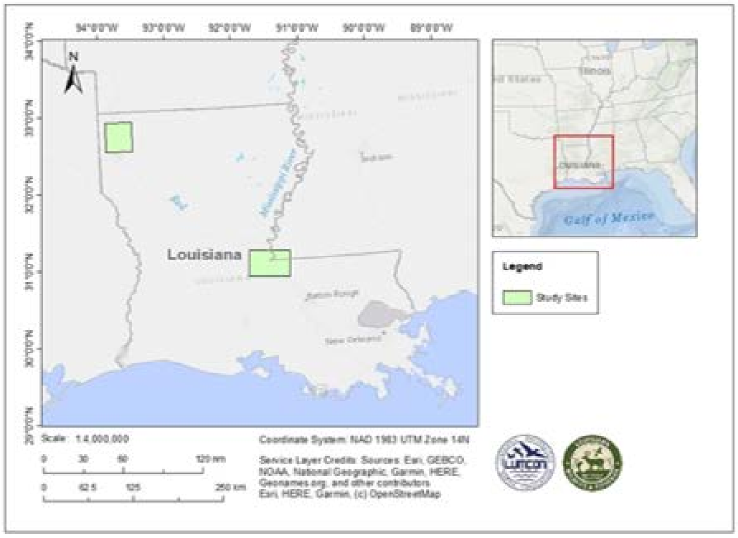

Figure 2. Location of the fish sample collection locations in Louisiana, USA.

Sample processing

A small cut of white muscle was taken from each thawed fish. Frozen samples were freeze-dried, split in half to create a replicate to lipid extract, and ground. Non-lipid-extracted samples were weighed [0.55 +/- 0.025] mg into tin capsules and stable isotope analysis was performed.

Lipid measurements

Lipids were chemically extracted using Soxhlet extraction overnight in a 2:1 chloroform-methanol solvent. After air-drying upon removal from the Soxhlet, samples were weighed [0.55 +/- 0.025] mg into tin capsules and stable isotope analysis was performed.

Stable isotope measurements

Each sample was then loaded into tin cups and flash-combusted using a Costech ECS4010 elemental analyzer. Then, these samples were analyzed for carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) using an interfaced Thermo Delta XP continuous flow stable isotope ratio mass spectrometer. Raw δ values were normalized on a two-point scale using glutamic acid reference materials with low and high values (i.e. USGS-40 ($\delta^{13}C$ = −26.4‰, $\delta^{15}N$ = −4.5‰) and USGS−41 ($\delta^{13}C$ = 37.6‰, $\delta^{15}N$ = 47.6‰)). Sample precision based on repeated sample and reference material was 0.1‰ and 0.2‰, for $\delta^{13}C$ and $\delta^{15}N$, respectively. Stable isotope ratios are expressed in δ notation in per mil units (‰), according to the following equation:

$$\delta X = [(R\ sample / R\ standard) - 1] \times 1000$$

where X is 13C or 15N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 for δ15N.

Statistical analyses

First we compared body size (standard length and mass) among collection sites (IL, LA-0101, LA-0103) and season (winter and spring) using separate, two-factor ANOVA with interactions between site and season. Next, we compared lipid content, C:N ratios (a proxy for lipid content), d13C and d15N values among collection sites using separate one factor ANOVAs. These data were only available for fish collected in the winter, so seasonal effects could not be examined. When significant differences were identified using ANOVA, we then conducted post-hoc analyses using Tukey honestly significant differences test. Significance of statistical test results were assessed at an alpha level of 0.05.

Results

We found that fish standard length differed among sampling sites ($F_{2,55}$= 196.82, p <0.0001), but did not differ between season ($F_{1,55}$= 0.90, p = 0.3467), or have a significant interaction between site and season ($F_{2,55}$= 0.23, p = 0.7934). Similarly, fish mass differed among sampling sites ($F_{2,55}$= 55.43 , p <0.0001), but did not differ between season ($F_{1,55}$= 0.0049, p = 0.9445), or have a significant interaction between site and season ($F_{2,55}$= 0.0635, p = 0.9386). Post-hoc analyses indicated that both standard length and mass were higher at the two Louisiana collection sites (LA-0101, LA-0103) relative to the Illinois collection site (Figure 2)

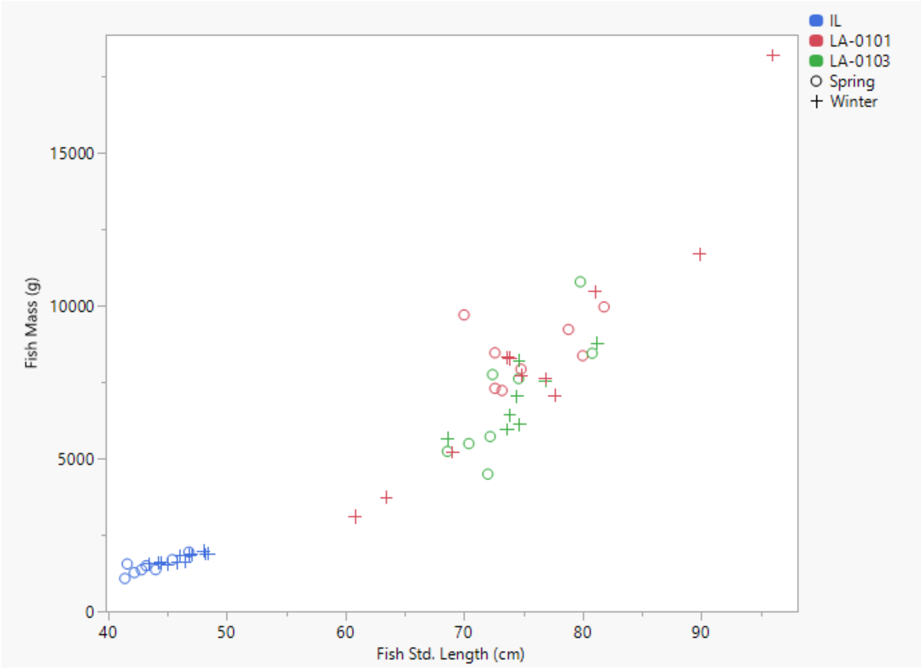

Figure 2. Relationship between standard length and mass of invasive carp collected from one site in Illinois (IL) and two sites in Louisiana (LA-0101 and LA-0103). Fish had larger body sizes in Louisiana relative to Illinois.

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We found that lipid content did not significantly vary among sampling sites ($F_{2,55}$= 1.0354, p = 0.3678). Likewise, there was no significant difference in C:N ratio (a common proxy for lipid content) among sampling sites ($F_{2,55}$= 0.3399, p = 0.7146). We found that (lipid-extracted) $\delta^{13}C$ values significantly differed among sampling sites ($F_{2,55}$= 8.6133, p = 0.0012). Additionally, non-lipid-extracted $\delta^{15}N$ values also differed among sites ($F_{2,55}$= 3.5806, p = 0.0408).

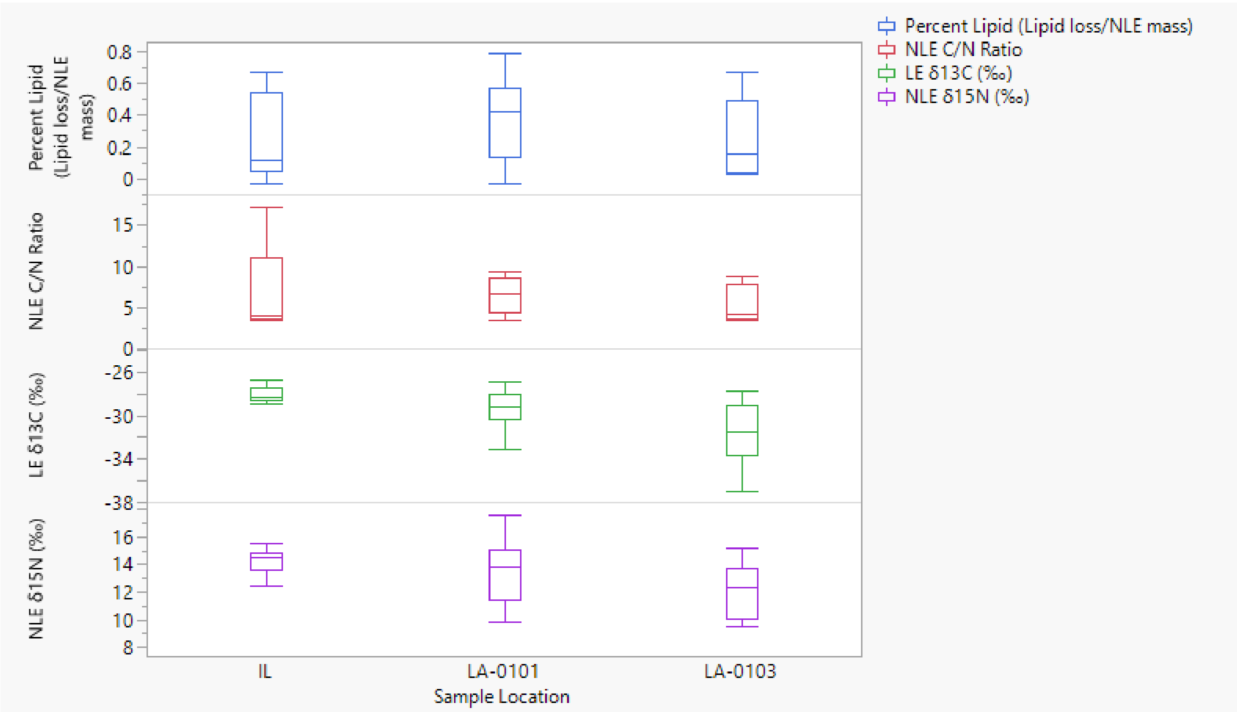

Figure 3. Relationships of lipid content (percent lipid), C:N ratio, d13C, and d15N values relative to sampling sites (IL, LA-0101, LA-0103). Values for d13C and d15N significantly varied relative to sampling sites, however lipid content and C:N ratio did not significantly differ among sampling sites.

Discussion

This research provides important first steps towards the goal of developing an Invasive carp-based aquaculture feed by assessing carp body size, lipid content, and diet proxies of fish harvested in Louisiana and Illinois. First, we found no significant difference in the muscle tissue lipid content between the Louisiana and Illinois invasive carp. This suggests that, at least in terms of lipid content, invasive carp from Louisiana and Illinois appear to be similar in respect to nutrient quality for aquaculture feed. However, we found that fish collected from Louisiana were significantly larger in both mass and length than those collected from Illinois. Specifically, Louisiana invasive carp were nearly twice as large on average. If this is not an artifact of the sample, harvesting fish in Louisiana could be advantageous to aquaculture feed production as larger fish will yield more product per fish.

Even so, there are concerns that larger carp could accumulate more toxins and heavy metals like lead, mercury, and cadmium. Previous studies indicate that the food chain transfer and direct transport across the gills are the primary pathways for heavy metals getting into carp tissue (Clements,1991; Yousafzai, et al., 2012). Heavy metals, organic pollutants, and other contaminants can bioaccumulate over time in fish as they grow and age (Coelho et al. 2013). This implied that tissues from larger fish often have higher concentrations of common pollutants relative to smaller fish. The larger fish collected from Louisiana therefore have a greater potential to have accumulated a higher contaminant burden relative to the smaller fish captured in Illinois.

In general, fish that occupy higher trophic levels often have higher contaminant burdens because of trophic magnification (Coelho et al. 2013). A prior study suggests that when concentrations of heavy metals in water are low, diet serves as the main pathway for heavy metals getting into carp tissue (Yousafzai, et al., 2012). We used stable isotope analysis as a proxy of trophic differences among invasive carp sampled for this research. Specifically, stable isotope values of nitrogen ($\delta^{15}N$) provide a proxy for trophic position (Minagawa and Wada 1984), while stable isotope values of carbon ($\delta^{13}C$) act as a proxy of habitat and basal resource use (Peterson and Fry 1987).

We found that both $\delta^{15}N$ and carbon $\delta^{13}C$ values of carp muscle tissue were slightly, but significantly, higher in Illinois relative to Louisiana. This suggests the possibility that Illinois carp, while smaller, occupy slightly higher trophic levels than invasive carp in Louisiana. If this is the case, it is possible that invasive carp in Illinois carp might have higher tissue contaminant concentrations relative to invasive carp in Louisiana, due to trophic magnification.

Future Plans

High concentrations of toxins might make carp from Louisiana unsuitable as fodder for aquaculture fish. The next step of this project will be in collaboration with partners at the Louisiana Universities Marine Consortium. They are analyzing these same muscle tissues for proximate composition (protein, lipid, energy, and moisture content), amino acid profiles, fatty acid profiles, and the presence of a suite of common contaminants (lead, mercury, cadmium, PCBs). Our body size and stable isotope-based dietary metrics will then be statistically compared to proximate composition and contaminant concentrations. These statistical comparisons will allow us to interpret the dietary drivers of fish quality and contaminant status between fish collection sites, as well as assess if the toxicity of the fish is low enough to be safe to use as fishmeal for aquaculture fish.

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